Micromechanical and Structural Analysis of Compromised Dental Tissues

Erin Kathleen Mahoney
BDS (Otago) MDSc (Paediatric Dentistry) FRACDS

This thesis is submitted in fulfillment of the requirements for a degree of Doctor of Philosophy

Biomaterials
Faculty of Dentistry
University of Sydney
Australia
2005
ABSTRACT

Micromechanical and Structural Analysis of Compromised Dental Tissues

Completing dental treatment in young children can be very difficult. A more conservative surgical approach coupled with remineralisation of the residual compromised tissues could improve outcomes substantially. The remineralisation process associated with carious enamel is relatively well understood however the same is not true of the processes involved both in carious dentine nor in developmentally disrupted hypomineralised dental tissues. The aim of this thesis is to take a biomaterials science approach to exploring the properties and structure of compromised enamel and dentine. Three studies were completed.

Using a number of techniques, the mechanical and microstructural properties of in the first instance, carious dentine from primary incisors and then of both enamel and dentine in developmentally defective permanent molar teeth were determined using (amongst other techniques) an ultra-micro-indentation system (UMIS). These studies improve the biomechanical understanding of these compromised tissues such that it was then possible to develop a unique in vivo study to evaluate the changes in the mechanical and microstructural properties of tooth tissue brought about by some conventional restorative treatments. Together these investigations contribute to the understanding of the physical properties of compromised tissues and the effects of dental treatment on such properties.

In the caries free areas of primary incisors there was no change in the mechanical properties of dentine found between the pulp and the tooth surface whilst in the carious region there were two distinct decreasing patterns. Two of the teeth showed a constant reduction in hardness and modulus of elasticity from the pulpal region towards the surface of the lesion whereas the remaining teeth showed a similar decrease until the last 300 - 500 μm, when both the hardness and modulus of elasticity showed an obvious plateau and increase. It is speculated that this increase in mechanical properties at the surface is due to remineralisation of the carious dentine tissue from exogenous sources such as
saliva and this supports the concept that remineralisation of the so called infected layer may be possible.

Anecdotal clinical evidence suggests that the dentine of hypomineralised teeth is softer or of an 'unusual' consistency in comparison to unaffected teeth. The present study using the UMIS and SEM could find no difference in the mechanical properties or the microstructure respectively. This is in contrast to the investigation of the mechanical and microstructural properties of hypomineralised enamel of first permanent molar teeth. Using the UMIS, the hardness and modulus of elasticity were found to be statistically significantly lower (0.53 ± 0.31 GPa and 14.49 ± 7.56 GPa respectively) than normal enamel (3.66 ± 0.75 GPa and 75.57 ± 9.98 GPa respectively). Further microstructural analysis revealed that the fractured surface of the hypomineralised enamel was significantly more disorganised and the mineral content approximately 5% lower.

The final study investigated the effect of restorative treatment on the properties of carious dentine of hypomineralised permanent and primary molar teeth. This unique in vivo study failed to show any significant difference in the mechanical properties of the dentine irrespective of the restorative treatment provided. There was however a large inter-tooth variation in the hardness and modulus of elasticity of both treated and untreated carious lesions. As in the first investigation on carious primary incisor teeth, the changes in mechanical properties across each treated tooth revealed two distinct patterns; either a plateau behaviour with an increase in mechanical properties near the surface of the carious lesion or less commonly, a continual decrease in mechanical properties with no plateau behaviour evident. These trends appeared to be independent of the treatment provided. This study suggests that whilst progression of carious lesions may be halted as a result of restorative treatment/sealing the restoration itself in the short term (less than six months), does not appear to cause an alteration of the mechanical properties of carious dentine.

The present studies suggest that the mechanical properties of even the most severely denatured tissue such as 'infected' dentine can be improved with exposure to the oral environment. It is speculated that this is due to remineralisation potential of saliva. The final study has also shown that in the case of minimal intervention based treatment with a limited range of restorative materials that the restoration itself, in the short term, does not appear to increase
the mechanical properties of carious dentine. Further work is therefore required to understand why there is no apparent change in mechanical properties and whether a material and or procedure could be developed that would promote remineralisation and cause a substantial increase in mechanical properties of carious dentine.
Dedicated to
Rod and Finn
Declaration

This is to certify that the work presented in this thesis was carried out by the candidate in the Discipline of Biomaterials Science, Faculty of Dentistry, University of Sydney and has not been submitted to any other university or institution for a higher degree.

...........................................

Erin Kathleen Mahoney
ACKNOWLEDGMENTS

I had been told that completing a PhD would be stressful and traumatic. Instead because of the incredible support I have received from my supervisors and many others, I have had a fantastic three years.

None of this would be possible without the unwavering support and commitment from my supervisors, Professor Mike Swain and Associate Professor Nicky Kilpatrick. The biggest debt of gratitude goes to Professor Swain who has guided my research through two degrees beginning in 1999. I am continually amazed and appreciative of your enthusiasm for my research. Thank you also for your friendship, guidance in the academic world and your realistic expectations of me! Thank you to Associate Professor Kilpatrick for your support, friendship, proof reading and emergency flights to Sydney.

I owe a great debt to Dr Ramin Rohanizadeh who was never too busy to help me. Thank you also to Mr Ken Tyler for all your support, help and coffees in the past six years. I have no idea how I would have completed this thesis without both of your help.

This PhD would not have been completed without the financial help of the NHMRC for which I am very grateful.

I would like to thank Dr Linny Angker for her friendship and for sharing her enthusiasm in this area of research. I look forward to collaborating with you in the future. Thank you also to Daniel Soo and Shaira Ismail.

Thank you to the Department's of Paediatric Dentistry at Westmead Centre for Oral Health and the Sydney Dental Hospital for aiding in patient recruitment. Special thanks go to Dr's Cameron, Hibbert, Stephen and Malhi and Ms Melink for all their help during these three years.

I am also grateful to Tony Romeo at the EMU, University of Sydney for his patience and help during this PhD.
Thank you also is extended to Professor Kidd and Professor Watson of Guy's, King’s and St Thomas’ Dental Institute, who had me in their Research Units for a cold but wonderful winter.

Thank you to my Dad, Mum, and Patrick for their ongoing support and love.

This thesis is dedicated to the two men in my life. Thank you to Finn Alexander who grew inside me during the last year of this PhD and who has been the most wonderful, beautiful and rewarding gift that your dad and I have or will ever received. Thank you Rod, for your patience, love and never failing support throughout this PhD. I truly could not have done it without you. Isn’t life exciting!
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>TABLE OF CONTENTS</td>
<td>1</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>4</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>9</td>
</tr>
<tr>
<td>Chapter 1 INTRODUCTION</td>
<td>11</td>
</tr>
<tr>
<td>Chapter 2 LITERATURE REVIEW</td>
<td>15</td>
</tr>
<tr>
<td><strong>Part 1. Sound Enamel and Dentine</strong></td>
<td>16</td>
</tr>
<tr>
<td>Structure of Enamel in Health</td>
<td>16</td>
</tr>
<tr>
<td>Permeability of Enamel</td>
<td>18</td>
</tr>
<tr>
<td>Amelo-dentinal Junction (ADJ)</td>
<td>19</td>
</tr>
<tr>
<td>Structure of Dentine</td>
<td>19</td>
</tr>
<tr>
<td>Permeability of Dentine</td>
<td>24</td>
</tr>
<tr>
<td>Differences between Primary and Permanent Dentine</td>
<td>25</td>
</tr>
<tr>
<td>Mechanical Properties of Enamel and Dentine</td>
<td>26</td>
</tr>
<tr>
<td><strong>PART 2. Compromised Enamel and Dentine</strong></td>
<td>34</td>
</tr>
<tr>
<td>Enamel Hypomineralisation and Hypoplasia</td>
<td>34</td>
</tr>
<tr>
<td>Mechanical and Physical Properties of Hypomineralised/Hypoplastic First Permanent Molars</td>
<td>38</td>
</tr>
<tr>
<td>Dentine</td>
<td>43</td>
</tr>
<tr>
<td>Permeability of Carious Dentine</td>
<td>51</td>
</tr>
<tr>
<td>Mechanical Properties of Dental Caries</td>
<td>52</td>
</tr>
<tr>
<td>Treatment Options of Cavitated Carious Lesions in Dentine</td>
<td>55</td>
</tr>
<tr>
<td><strong>Part 3. The Remineralisation Process</strong></td>
<td>58</td>
</tr>
<tr>
<td>The Indentation Model</td>
<td>74</td>
</tr>
<tr>
<td>Chapter 3 METHODOLOGY</td>
<td>86</td>
</tr>
<tr>
<td>Obtaining Teeth for Experimentation</td>
<td>86</td>
</tr>
<tr>
<td><strong>Ultra-Micro-Indentation System (UMIS)</strong></td>
<td>87</td>
</tr>
<tr>
<td>Sample Preparation</td>
<td>89</td>
</tr>
<tr>
<td>Wax Mounting</td>
<td>92</td>
</tr>
<tr>
<td>Testing - UMIS</td>
<td>93</td>
</tr>
<tr>
<td>Positioning and Array Layout</td>
<td>94</td>
</tr>
<tr>
<td>Setting the Working Distance</td>
<td>94</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Loading and Unloading</td>
<td>96</td>
</tr>
<tr>
<td>Force</td>
<td>96</td>
</tr>
<tr>
<td>Dwell Time</td>
<td>98</td>
</tr>
<tr>
<td>Data Analysis</td>
<td>99</td>
</tr>
<tr>
<td>Scanning Electron Microscopy</td>
<td>102</td>
</tr>
<tr>
<td>Critical Point Drying</td>
<td>102</td>
</tr>
<tr>
<td>Specimen Coating</td>
<td>102</td>
</tr>
<tr>
<td>Operation of the SEM</td>
<td>103</td>
</tr>
<tr>
<td>Use of SEM for Other Investigation Techniques</td>
<td>103</td>
</tr>
<tr>
<td>Back Scattered Electron (BSE) Image</td>
<td>104</td>
</tr>
<tr>
<td>Energy Dispersive X-ray Spectrometer (EDS)</td>
<td>107</td>
</tr>
<tr>
<td>X-Ray Diffraction (XRD)</td>
<td>109</td>
</tr>
<tr>
<td>Chapter 4 MECHANICAL PROPERTIES OF CARIOUS DENTINE</td>
<td>112</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>114</td>
</tr>
<tr>
<td>Results</td>
<td>116</td>
</tr>
<tr>
<td>Discussion</td>
<td>124</td>
</tr>
<tr>
<td>Chapter 5 MECHANICAL PROPERTIES AND MICROSTRUCTURE OF</td>
<td>129</td>
</tr>
<tr>
<td>HYPOMINERALISED FIRST PERMANENT MOLAR TEETH</td>
<td></td>
</tr>
<tr>
<td>Chapter 5a Mechanical Properties of Hypomineralised Enamel</td>
<td>131</td>
</tr>
<tr>
<td>Material and Methods</td>
<td>132</td>
</tr>
<tr>
<td>Mechanical Properties</td>
<td>133</td>
</tr>
<tr>
<td>Scanning Electron Microscopy (SEM)</td>
<td>136</td>
</tr>
<tr>
<td>X-Ray Diffraction (XRD)</td>
<td>137</td>
</tr>
<tr>
<td>Energy Dispersive X-ray Spectrometer (EDS)</td>
<td>138</td>
</tr>
<tr>
<td>Back Scattered Electron (BSE) Image</td>
<td>138</td>
</tr>
<tr>
<td>Transmission Electron Microscope (TEM)</td>
<td>139</td>
</tr>
<tr>
<td>Results</td>
<td>140</td>
</tr>
<tr>
<td>Mechanical properties</td>
<td>140</td>
</tr>
<tr>
<td>SEM Images</td>
<td>147</td>
</tr>
<tr>
<td>X-Ray Diffraction</td>
<td>151</td>
</tr>
<tr>
<td>EDS</td>
<td>152</td>
</tr>
<tr>
<td>BSE Image</td>
<td>153</td>
</tr>
<tr>
<td>TEM</td>
<td>155</td>
</tr>
<tr>
<td>Discussion</td>
<td>157</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure 2-1. Single rod unit and relationship of enamel rod units to one another. .17
Figure 2-2. Showing Hunter Schreger Bands in cross section. .........................18
Figure 2-3. SEM photomicrograph of ADJ. ..................................................19
Figure 2-4. SEM photomicrograph of dentine................................................21
Figure 2-5. Permanent and primary molar teeth showing difference in size and
 thickness of dentine. ..............................................................................26
Figure 2-6. Molar-incisor hypomineralisation (MIH) .....................................37
Figure 2-7. Coronal section of hypomineralised first permanent molar showing
 accentuated Hunter Schreger Bands. Picture taken at 2 times magnification
 with optical microscope. ........................................................................39
Figure 2-8. Carious lesion in a primary molar tooth......................................44
Figure 2-9. a. Radiograph of enamel and dentine caries. ..............................45
Figure 2-10. The variation in hardness across carious teeth presented on a
 logarithmic scale vs. distance. ................................................................53
Figure 2-11. Relation of Knoop hardness number (KHN) to volume percent
 mineral (Vm).............................................................................................69
Figure 2-12. A typical load-displacement curve..........................................75
Figure 2-13. Schematic representation of the indenting process illustrating the
 total depth: h_t, contact depth: h_c, and the residual depth: h_r. ....................76
Figure 3-1. The Ultra-Micro- Indentation System ........................................88
Figure 3-2. The computer system associated with the UMIS.......................88
Figure 3-3. Low speed saw used in cutting samples.....................................90
Figure 3-4. Silicon carbide paper set up for initial polishing. .......................91
Figure 3-5. Automatic polishing machine used to polish samples.............. 91
Figure 3-6. Specially constructed impermeable container and metal base. ....93
Figure 3-7. Figure showing a sample at the indenting position on the left and
 viewing microscope on the right.............................................................94
Figure 3-8. Typical force vs penetration depth curve for enamel and dentine. .96
Figure 3-9. Graph illustrating the difference in the unloading curve (of dentine)
 with and without the dwell time (creep function) activated. ......................98
Figure 3-10. View through the microscope (associated with the UMIS) of an indentation with minimal cracking (left photograph) and an indentation with severe cracking (right photograph) .......................................................... 99

Figure 3-11. Force vs penetration graph showing an acceptable and an unacceptable indentation for dentine on a force vs displacement curve ...... 100

Figure 3-12. Data used for averaging from hardness vs penetration graphs for a single indentation in dentine .......................................................... 101

Figure 3-13. a. Image of carbon typical standard block used. b. Image of typical silicon-carbon standard block used .................................................. 105

Figure 3-14. Typical test sample of a hypoplastic tooth ........................................ 106

Figure 3-15. EDS system prior to its attachment to a SEM .................................. 107

Figure 3-16. Example of spectrum analysis of dentine showing the peaks for Ca, P, Mg and Na ................................................................. 109

Figure 3-17. Siemens Diffraktometer D5000 ......................................................... 111

Figure 3-18. Sample holder ................................................................................. 111

Figure 4-1. Diagrammatic representation of layout of indentations ..................... 115

Figure 4-2. a) Percent reduction in hardness across carious lesion and b) Percent reduction in modulus of elasticity across carious lesion ........................................... 120

Figure 4-3. a) Hardness of a single array in tooth 6 showing marked increase in hardness from the surface of lesion pulpally and b) Modulus of elasticity of single array in tooth 6 showing marked increase in modulus from the surface of lesion pulpally ................................................................. 121

Figure 4-4. a) Hardness of single array of tooth 8 showing increase in mechanical properties from 500 µm of the surface and b) Modulus of elasticity of single array of tooth 8 showing increase in mechanical properties from 500 µm of the surface ................................................................. 122

Figure 4-5. a) Hardness of caries free region of tooth 8 showing no change in mechanical properties from surface of lesion pulpally and b) Modulus of elasticity of caries free region of tooth 8 showing no change in mechanical properties from surface of lesion pulpally .................................................. 123

Figure 5-1. Optical cross section image of a prepared test tooth with indentation layout indicated ........................................................................... 134

Figure 5-2. Optical cross section image of prepared test tooth with indentation layout indicated ........................................................................... 136
Figure 5-3. Mechanical properties of control tooth from enamel at CEJ to a position level with the dentine cusp tip. A. Hardness and B. Modulus of elasticity ................................................................. 144

Figure 5-4. Mechanical properties parallel along ADJ from CEJ to dentine cusp tip of hypoplastic first permanent molar tooth ................................................................. 145

Figure 5-5. SEM photomicrographs of fractured surface. Upper left and right SEM photos are of unaffected, normal enamel showing orderly enamel rod structure. Lower left and right SEM photo are of hypomineralised enamel showing disorganised enamel rod structure with voids present .................. 148

Figure 5-6 a. SEM images of unaffected (upper images) and hypomineralised enamel (lower images) after 0 seconds etching (20 seconds dentine conditioner) and 5 seconds etching with phosphoric acid .................. 149

Figure 5-7. XRD patterns of hypomineralised and sound enamel .................. 152

Figure 5-8. BSE image of hypomineralised, unaffected enamel and dentine ...... 154

Figure 5-9. Average BSE intensities of test teeth tissues ............................ 155

Figure 5-10. Bright Field TEM micrographs (a) Ultrastructure of unaffected enamel (b) Ultrastructure of hypomineralised enamel .................. 156

Figure 5-11. Ring diffraction patterns of (A) unaffected and (B) hypomineralised enamel, taken using 5 second exposure time at a distance of 770mm ........... 157

Figure 5-12. Iso-stress versus Iso-strain model of E modulus .................. 163

Figure 5-13. Diagrams and picture showing regions of indentations of test teeth. Lower diagram modified from (12) ................................................................. 166

Figure 5-14. Diagram of areas in dentine on experimental teeth where EDS was conducted. 1: inner dentine, 2: inner 1/3 of dentine, 3: outer 1/3 of dentine, 4: outer dentine ................................................................. 168

Figure 5-15. Position of cut lines prior to fracturing .................................. 168

Figure 5-16. Location of regions investigated with SEM .................. 169

Figure 5-17. Scatter plots of the mechanical properties of control teeth. Distance normalised relative to the indentations location between the pulp and amelo-dentinal junction (ADJ) ................................................................. 170

Figure 5-18. Scatter plots of the mechanical properties of hypomineralised teeth. Distance normalised relative to the indentations location between the pulp and the amelo-dentinal junction (ADJ) ................................................................. 171
Figure 5-19. Lower region of dentine sample. A: sound dentine from control tooth, B: Hypomineralised dentine. .........................................................174
Figure 5-20. Middle region of dentine sample. A: sound dentine from control tooth, B: Hypomineralised dentine. .........................................................175
Figure 5-21. Top region of dentine sample. A: sound dentine from control tooth, B: Hypomineralised dentine. .........................................................176
Figure 6-1. Outline of arrays of indentations conducted in carious and unaffected dentine. .........................................................................................188
Figure 6-2. Position of SEM analysis along fractured surface. ....................190
Figure 6-3. Clinical photographs of test patients teeth prior to extraction. ......195
Figure 6-4. Hardness and Elastic Modulus from central array in tooth 6 (GIC restoration only) shown on linear vertical axis scale. ......................198
Figure 6-5. Hardness and Elastic Modulus from Figure 6-4 shown on logarithmic vertical axis scale. .................................................................198
Figure 6-6. Scatter plots of the hardness and modulus of elasticity of all arrays conducted in sound dentine of primary test teeth. .......................199
Figure 6-7. Scatter plots of the hardness and modulus of elasticity of all arrays conducted in sound dentine of permanent test teeth. ..............200
Figure 6-8. Comparison of hardness versus distance from a reference point for all teeth ......................................................................................202
Figure 6-9. Comparison of elastic modulus versus distance from a reference point for all teeth .................................................................203
Figure 6-10: Untreated specimen hardness versus distance from a reference point comparison for all teeth .........................................................204
Figure 6-11: Untreated specimen elastic modulus comparison versus distance from a referenced point for all teeth ..............................................204
Figure 6-12: Treated specimen hardness comparison ....................................205
Figure 6-13: Treated specimen elastic modulus comparison .......................206
Figure 6-14. Definition of [A] Distance from Plateau to lesion surface or lesion/restoration interface and [B] Distance from increase in mechanical properties to lesion surface or lesion/restoration interface. ............207
Figure 6-15. a. Hardness profiles of each indentation array from tooth 1 and ....209
Figure 6-16. a. Hardness profiles of each indentation array from tooth 7 and ....211
Figure 6-17. Graphical representation of Table 6-9. ......................................215
Figure 6-18. Graphical representation of Table 6-10. .................................216
Figure 6-19. SEM images of test teeth at lower magnification. .........................220
Figure 6-20. SEM images of test teeth at higher magnification. .........................222
<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-1</td>
<td>Enamel structural features</td>
<td>18</td>
</tr>
<tr>
<td>2-2</td>
<td>Classification of dentine by location, patterns of mineralisation and development</td>
<td>20</td>
</tr>
<tr>
<td>2-3</td>
<td>Organic components in dentine</td>
<td>24</td>
</tr>
<tr>
<td>2-4</td>
<td>Hardness and modulus of elasticity of enamel and dentine of permanent teeth</td>
<td>30</td>
</tr>
<tr>
<td>2-5</td>
<td>Hardness and Modulus of Elasticity of Primary Teeth</td>
<td>33</td>
</tr>
<tr>
<td>2-6</td>
<td>Causes of enamel hypoplasia</td>
<td>36</td>
</tr>
<tr>
<td>2-7</td>
<td>Prevalence of MIH</td>
<td>37</td>
</tr>
<tr>
<td>2-8</td>
<td>Mechanical properties of carious dentine</td>
<td>54</td>
</tr>
<tr>
<td>2-9</td>
<td>Fluoride concentrations used for demineralisation and remineralisation studies of dentine caries</td>
<td>83</td>
</tr>
<tr>
<td>3-1</td>
<td>Summary of the grinding and polishing steps</td>
<td>92</td>
</tr>
<tr>
<td>3-2</td>
<td>The parameters assigned to the testing procedure</td>
<td>95</td>
</tr>
<tr>
<td>3-3</td>
<td>Example of results produced by EDS system for enamel</td>
<td>109</td>
</tr>
<tr>
<td>4-1</td>
<td>The mean values ± standard deviation, median and the range of hardness and modulus in three specified regions of primary carious dentine</td>
<td>118</td>
</tr>
<tr>
<td>4-2</td>
<td>The mean values ± standard deviation, median and range of hardness and modulus of elasticity in three specified regions of primary sound dentine</td>
<td>119</td>
</tr>
<tr>
<td>4-3</td>
<td>Table comparing the mechanical properties of sound primary dentine in the present study with two recent studies conducted on primary teeth</td>
<td>128</td>
</tr>
<tr>
<td>5-1</td>
<td>Test parameters and conditions for Array Series 1</td>
<td>134</td>
</tr>
<tr>
<td>5-2</td>
<td>Test parameters and conditions for Array Series 2</td>
<td>135</td>
</tr>
<tr>
<td>5-3</td>
<td>Overall hardness and modulus of affected teeth</td>
<td>141</td>
</tr>
<tr>
<td>5-4</td>
<td>Mean overall hardness and modulus of elasticity of all three tissue types</td>
<td>142</td>
</tr>
<tr>
<td>5-5</td>
<td>Range of mechanical properties of test and control teeth</td>
<td>146</td>
</tr>
<tr>
<td>5-6</td>
<td>Results of EDS analysis of hypomineralised and normal enamel</td>
<td>153</td>
</tr>
</tbody>
</table>
Table 5-7. Average mechanical properties of inner, middle and outer dentine of hypomineralised and control teeth .......................................................... 172
Table 5-8. Results of EDS investigations ..................................................... 173
Table 5-9. Results comparing the mechanical properties of hypomineralised teeth in present study and sound primary dentine in study by Angker and colleagues (2003) ................................................................. 178
Table 6-1. Test conditions for indentation experiments in present study ........ 187
Table 6-2. Demographic and treatment details of patients and teeth used .......... 193
Table 6-3. Outline of numbering of test teeth and restorative treatment performed. ........................................................................................................ 196
Table 6-4. Summary of treated teeth and associated arrays and indentations performed .................................................................................................. 197
Table 6-5. Summary of range of mechanical properties of each treatment type provided to test teeth. Primary and permanent teeth combined ........ 201
Table 6-6. Distance of Plateau and increase in mechanical properties to lesion surface or lesion/restoration interface .............................................. 207
Table 6-7: Hardness and Elastic Modulus averages for Sound Primary Dentine 213
Table 6-8: Hardness and Elastic Modulus average for Sound Permanent Dentine ................................................................. 213
Table 6-9. Average hardness for carious regions, for all specimens ............... 214
Table 6-10. Average elastic modulus for carious regions, for all specimens ..... 215
Table 6-11: Hardness averages for Untreated and Treated Carious Specimens .. 217
Table 6-12: Elastic Modulus averages for Untreated and Treated Carious Specimens ........................................................................................................ 217
Table 6-13. Table comparing the mechanical properties of sound primary dentine in the present study with two recent studies conducted on primary teeth .. 228
Chapter 1 INTRODUCTION

Despite the fact that it is largely preventable, dental caries remains one of the most common chronic diseases of childhood and also one of the most costly (1). Dental services account for over 2.5 billion dollars or approximately 5% of total health system expenditure in Australia (AIHW, 2002). Whilst there have been substantial reductions in dental caries worldwide certain groups of the population still experience significant amounts of disease. Whilst 56% of 4 year olds in Australia are caries free the remaining 44% have a mean of 4.23 decayed, missing due to caries or filled teeth (Child Dental Health Survey; AIHW 1999). Of this disease over 80% remains untreated. The short-term sequelae of untreated decay in children’s teeth are pain, (12% of 5 years olds in Australia have experienced toothache) infection and abscesses (2). Once decay has reached this stage, it is often difficult to manage in the dental surgery and general anaesthesia (GA) and hospital admission may be required (3).

Large numbers of children develop first permanent molars that are associated with developmental defects which predispose them to caries in their permanent dentition. First permanent molars begin to calcify as early as 36 weeks in utero. The cells involved are susceptible to a wide range of systemic disturbances. Up to 25% of first permanent molars are affected by such disturbances which may include maternal illness, viral infections and periods of hypoxia around birth and early infancy (4). The manifestation of these disturbances can be seen clinically as hypoplastic and/or hypomineralised enamel. These teeth are more susceptible to plaque accumulation and dental caries not only because they are thought to be more porous but also because they can be very sensitive making effective oral hygiene difficult. Furthermore these teeth pose significant challenges to the clinician. The texture and composition of the residual tooth tissue is such that cavity preparation is difficult and subsequent bonding of conventional restorative materials prone to failure. In addition not only are these compromised teeth often exquisitely sensitive requiring robust local analgesia but they are also often difficult to access and isolate adequately in the young paediatric patient.

The traditional approach to restoring teeth has been essentially a surgical one. Caries is removed from the tooth (along with greater or lesser amounts of sound
tooth tissue) and is replaced with a restorative material. This approach is fraught with failure even if adhesive restorative techniques are adopted. In the primary dentition where the enamel and dentine are thin, not only is the pulp often irreversibly traumatized by the restorative procedure itself but also the residual tooth tissue is weak and breaks down easily in the oral environment. Furthermore in both the primary dentition and the compromised permanent molar, the quality and integrity of the interface between the restoration and tooth tissue is frequently inadequate resulting in microleakage, poor oral seal, re-infection and further caries. The current concept of caries is that of a chronic, initially reversible infectious disease in which bacterial acids demineralise tooth tissue causing loss of ions such as calcium and phosphate from the hard dental tissues followed eventually by destruction of the protein substructures. This concept implies that caries is a dynamic process of demineralisation and that the dental tissues affected have the potential to remineralise.

The demineralisation/remineralisation concept has been recognised and is relatively well understood with respect to enamel caries. However dentine is a more complex, vital structure which has significantly more protein than enamel. These proteins, predominantly type 1 collagen, are intimately associated with the mineralisation processes during development of dentine and in particular the calcium binding mechanisms. The current understanding of these processes and the effects of demineralisation and remineralisation of dentine is limited. Similarly there is a lack of information surrounding the same processes and their effects on developmentally defective hypoplastic/hypomineralised enamel and dentine.

With the exception of how sound enamel behaves, there is a paucity of information about the effect of demineralisation and subsequent remineralisation potential on tooth tissue. Despite this, the philosophy of Minimal Intervention is currently a popular clinical approach. The term Minimal Intervention (MI) evolved from the term ‘Atraumatic Restorative Technique’, (ART) which was used to describe the limited removal of ‘infected’ carious dentine whilst leaving non-infected ‘affected’ dentine. By removing the microbiological cause of caries it is believed that remineralisation can occur in the residual uninfected dentine. Certainly there is good clinical (5,6) and some microbiological evidence (7) that MI is successful. The landmark study by Mertz-Fairhurst showed that when frank untreated carious lesions were sealed beneath composite resin based restorations there was no enlargement of the lesions clinically or radiographically
over a 10 year period (8). Whilst undoubtedly the value of these studies are associated with favourable clinical outcomes, the existing evidence surrounding this more conservative approach to the management of caries lacks basic scientific support. There is little information on the micromechanical or structural effects of the Minimal Intervention philosophy particularly with respect to dentine caries and even less with respect to compromised (histologically altered) permanent teeth.

This thesis represents a series of exploratory studies that have been designed to forward understanding of the processes underpinning MI. The studies are a continuation of a programme of similar work in this area (9-12) in which the structure and composition of dental hard tissues are investigated and the relationship between the mechanical properties and remineralisation potential explored.

The second chapter of this thesis is a literature review and is presented in three sections. The first section outlines the structure and mechanical properties of sound enamel and dentine. The second section concentrates on the mechanical and structural properties of enamel hypomineralisation and hypoplasia and dental caries as examples of pathologically altered dental tissue. The third section discusses dentine remineralisation as this process is at present not well understood. This final section will also outline the various in vitro methods presently used to determine the remineralisation potential of dentine.

The present thesis utilises the Ultra-Micro-Indentation System (UMIS) to determine the mechanical properties of dental tissues. Chapter Three outlines the methodology of the UMIS and the other techniques used to describe the structural and mechanical properties of sound and compromised enamel and dentine in the present thesis.

Presently little is known about the mechanical properties of carious and sound dentine from primary teeth. Therefore, Chapter Four is a study conducted on advanced carious lesions in primary incisor teeth. This allowed baseline mechanical properties of carious and sound dentine in primary teeth to be established and further test the capabilities of the UMIS to test very soft tissues such as dentine caries. This chapter has been submitted for publication with the International Journal of Paediatric Dentistry.
Chapter Five is a series of exploratory experiments conducted on the enamel and dentine of hypoplastic/hypomineralised first permanent molar teeth. Baseline mechanical and structural properties are severely lacking in the dental literature on the enamel and dentine of these technically difficult teeth to treat. This series of experiments have recently been published in the European Journal of Oral Science and Biomaterials.

With a more thorough understanding of the baseline properties of carious and sound primary and permanent teeth, Chapter Six is a clinical assessment of the change in microstructural and mechanical properties of carious primary teeth and hypoplastic/hypomineralised first permanent molar teeth using common restorative materials. The teeth in this final exploratory study were restored without any attempt at caries removal and subsequently extracted after three to six months. Each tooth was then tested with the UMIS and scanning electron microscopy to further the present understanding of the effect of sealing frank dentine caries.

The final chapter in this thesis is a discussion of all the results of the present thesis.
Chapter 2 LITERATURE REVIEW

This literature review is divided into three general sections. The first section will briefly describe the structural and mechanical properties of sound enamel and dentine.

The second section will discuss the effect of pathological processes on the structure and mechanical properties of dental hard tissues. Dentine caries and enamel hypomineralisation/hypoplasia will be discussed as examples of destructive processes in dentine and enamel respectively. The histological structure and mechanical properties of dentine caries will be discussed and related to the current philosophies guiding clinical treatment. There will then be an overview of the aetiology and effect of developmental defects of enamel related specifically to the first permanent molar. From this section it will become apparent that our knowledge of the remineralisation capabilities of carious dentine requires further investigation and that prior to any research on the ability of enamel hypomineralisation/hypoplasia to be repaired, further basic biomaterial research is required on this increasingly common condition.

The final section will review the contemporary evidence surrounding the potential for remineralisation to occur in carious dentine. The methodologies used will be discussed, followed by a summary of the effect of glass ionomer cements and calcium hydroxide, two of the more common materials used to remineralise dental tissues, will complete this literature review.
Part 1. Sound Enamel and Dentine

Enamel and dentine are complex structures. Together these two unique tissues account for the majority of the dental hard tissues and can be compromised by a variety of disorders both developmental and acquired. In order to understand the pathological effects of conditions such as dental caries or developmental disturbances it is important to understand the structure and mechanical properties of healthy enamel and dentine.

Structure of Enamel in Health

Enamel is unique in the body as it not subject to ongoing renewal throughout life. The cells that are responsible for formation of enamel, the ameloblasts, are lost as the tooth erupts. As a result enamel has acquired a complex structural organization and a high degree of mineralisation to reduce its susceptibility to any destructive processes (13). Enamel is 96% by weight and 89% by volume, mineral. This high mineral content along with its other physical characteristics allow enamel to protect the dentine and pulp below it (14).

Mineral and Crystalline Components of Enamel

Although developing enamel crystals initially form as a carbonated apatite, the inorganic content of mature enamel is composed predominantly of hydroxyapatite crystals ($\text{Ca}_{10}\text{(PO}_4\text{)}_6\text{(OH)}_2$). In addition to hydroxyapatite, mature enamel contains carbonates and other trace elements such as magnesium, fluoride, strontium and lead (13). It is thought that the core of a mature enamel crystal contains more carbonate than in the peripheral regions and this carbonate rich core makes each crystal more susceptible to dissolution from the central region than its ends and sides (14).

The hydroxyapatite crystals in enamel are the largest in the body (30 nm x 70-90 nm vs. dentine; 3-6 nm x 60 nm) (13;14). The orientation of the crystals together form rod structures (prisms) a pattern that is established during formation of the enamel matrix (Figure 2-1) (14).
The enamel rod represents the 'mineralised trail' taken by the ameloblasts and their Tomes processes as they migrate along an undulating course from the amelo-dentinal junction (ADJ) to the enamel surface. Each rod is formed by the products of four ameloblasts (14) and, in the most part, the crystals are arranged along the longitudinal axis of the rod, along its central axis. The boundary between the rod and the inter-rod enamel is delineated by a narrow space containing organic material (rod sheath) (13).

Enamel is more susceptible to fracturing or separation along the boundaries between each rod (14).

![Figure 2-1. Single rod unit and relationship of enamel rod units to one another. Modified from Piesco and Simmelink 2002 (14).](image)

**Organic Matrix of Enamel**

The fact that there are much greater amounts of organic matter in developing enamel in comparison to mature enamel (which has less than 1% by weight 2% by volume) suggests that the primary role of the organic components of enamel is to direct the growth of enamel crystals. Although the function of the remaining organic component in mature enamel is unknown it may aid in cementation of individual enamel crystals as there is more organic component in the enamel rod sheaths (14).

The organic matrix is primarily composed of proteins and lipids. These predominantly originate from ameloblasts although exogenous substances (blood, saliva and oral flora) may also become incorporated (14).
Structural Features of Enamel

The structural features of enamel are summarised in Table 2-1.

<table>
<thead>
<tr>
<th>Structural Feature</th>
<th>Developmental Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enamel Rod</td>
<td>Secretory product of one ameloblast from the distal or interdigitating portion of Tomes' process.</td>
</tr>
<tr>
<td>Enamel Spindle</td>
<td>Extension of an odontoblasts process and tubule across the basal lamina during the initial stage of matrix formation.</td>
</tr>
<tr>
<td>Enamel Tufts</td>
<td>Hypomineralised areas of enamel near the ADJ formed during the initial stages of matrix secretion.</td>
</tr>
<tr>
<td>Enamel Lamellae</td>
<td>Hypomineralised areas of enamel extending from the ADJ for considerable distances into the enamel.</td>
</tr>
<tr>
<td>Cracks</td>
<td>May occur naturally, especially in hypomineralised areas between enamel rods; may be the result of lamellae; may be distinguished from lamellae in that they arise from the enamel surface and contain salivary proteins.</td>
</tr>
<tr>
<td>Hunter-Schreger Bands</td>
<td>Represent differences in the pattern of sectioning of enamel rods (Figure 2-2).</td>
</tr>
<tr>
<td>Gnarled Enamel</td>
<td>Twisting of enamel rods in the cusps of teeth due to the small radius of rotation of ameloblasts during secretion.</td>
</tr>
<tr>
<td>Enamel Pits</td>
<td>Found between cusps; represent thin areas of enamel matrix due to the crowding of ameloblasts during development.</td>
</tr>
<tr>
<td>Incremental Lines</td>
<td>Formed due to the cyclical activity of ameloblasts; represent hypomineralised areas or are due to small variations in rod orientation; during significant physiologic changes these lines are accentuated or hypomineralised.</td>
</tr>
<tr>
<td>Enamel Pellicle</td>
<td>Formed after the tooth is in the oral cavity; acquired from saliva and the oral flora.</td>
</tr>
</tbody>
</table>

Table 2-1. Enamel structural features.
Modified from Piesco and Simmelink (14).

![Dentine and Hunter Schreger Bands in enamel](image)

Figure 2-2. Showing Hunter Schreger Bands in cross section. (15).

Permeability of Enamel

The development and subsequent potential reversal of carious lesions in enamel is a dynamic process whereby substances are transported into and out of enamel.
(16). Whilst enamel is relatively impermeable in comparison to dentine (14), the fact that remineralisation and demineralisation occurs implies that healthy enamel must be a permeable structure. The permeability of enamel is relevant because of its assumed relation to caries susceptibility and resistance (17).

**Amelo-dentinal Junction (ADJ)**

The ADJ represents the interface between two very different mineralized matrices, enamel and dentine (Figure 2-3). It is a scalloped interface that allows the enamel and dentine to interlock. This region contains proteins which are thought to be centers of mineralisation (13;14).

![Figure 2-3. SEM photomicrograph of ADJ.](image)

**Structure of Dentine**

Dentine makes up the bulk of a tooth. On a weight basis it consists of 70% mineral in the form of a carbonate rich, calcium deficient apatite, 20% organic material, largely type I collagen and about 10% fluid (by volume it is 50%, 30% and 20% respectively) (18;19). Dentine is composed of tubules surrounded by a highly mineralised peritubular zone which is embedded in an intertubular matrix consisting largely of Type 1 collagen, apatite crystals and dentinal fluid. The organic substance consists of collagenous fibrils and a ground substance of mucopolysaccharides, whereas the mineral component consists mainly of hydroxyapatite (20). The major components of dentine are distributed into distinctive morphological regions to form a vital and complex hydrated composite.
in which the morphology varies with location and undergoes changes with age and disease (19;21). Table 2-2 summarises the classification of the fundamental dentine structures.

<table>
<thead>
<tr>
<th>Component</th>
<th>Pattern of mineralisation</th>
<th>Developmental pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intertubular dentine: found between dentinal tubules</td>
<td>Interglobular dentine: hypomineralised dentine between mantle and circumpulpal dentine; normally in coronal dentine</td>
<td>Primary dentine: formed prior to and during active eruption</td>
</tr>
<tr>
<td>Peritubular or Intratubular dentine: found and formed within the dentinal tubules</td>
<td>Tomes granular layer: hypomineralised layer in root dentine; similar to interglobular dentine in crown</td>
<td>Secondary dentine: formed after the tooth first comes into erupts by odontoblast processes</td>
</tr>
<tr>
<td>Mantle dentine: formed initially in the crown; outer coronal dentine</td>
<td>Sclerotic dentine: hypermineralised occluding dentinal tubules</td>
<td>Tertiary dentine: formed as a result of pathological response. May be reactionary or reparative.</td>
</tr>
<tr>
<td>Circumpulpal dentine: nearest to the pulp; formed in crown after mantle dentine has been deposited</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2-2. Classification of dentine by location, patterns of mineralisation and development.
Modified from Piesco 2003 (22).
Odontoblasts and Odontoblast Processes:

The odontoblasts are found on the pulpal surface of the dentine in the mature tooth. They are elongated cells with their nuclei at the basal end and they are responsible for the formation of dentine throughout the lifetime of the tooth. The odontoblast process, (a long protoplasmic process) passes within a narrow tube like structure, known as the dentine tubule, peripherally from the dental pulp. Considerable controversy exists as to how far the odontoblast process extends within the tubule (23). Many authorities maintain that it only extends about 25-30% of the tubule (24) whereas others claim that the process extends from the pulp to the enamel (25-27), a distance of between 3-5 mm (23;25). In their course from the pulp to the ADJ, the dentinal tubules describe an S shaped course, the first primary curvature having its convexity facing the root.
Dental Tubules

The dentinal tubules are approximately 1.5 μm in diameter but can vary between 1-4 μm (Figure 1.3). A highly mineralized collar referred to as peritubular dentine surrounds each of the tubules (Figure 2-4) (25). These parallel channels, that are orientated perpendicular to the ADJ, make the dentine a very porous structure. There are approximately 40,000 tubules per mm² (28) although this is not only site specific but also varies between the permanent and primary dentition (29-31). Pashley (1989) calculated that the percentage of the dentine surface area occupied by dental tubules varies between less than 1% just beneath the enamel to 22% of the area near the pulp (23). Fosse and colleagues (1992) utilised light microscopy to determine the distribution pattern of dentine tubules cut transversely near the ADJ, midway to the pulp, and near the pulp wall. They showed that the closer to the pulp, the greater number of dentine tubules per mm². Sumikawa and colleagues (1999), in their study of primary anterior teeth, found that the dentine tubule density near the ADJ was greater than in the permanent dentition. They also reported that the diameter of the tubules of primary teeth was greater than that of permanent tooth dentine (31). In contrast, Schilke and colleagues (2000) reported no difference in the numbers and diameter of tubules in the middle and deep layers of primary and permanent dentine (32). Finally some studies have suggested that the dentinal tubules in primary dentine are smaller in diameter with wider peritubular dentine compared with that seen in permanent teeth (33;34).

The dentinal tubules are not smooth, but rather have irregular walls with lateral branches and channels that allows connection between neighbouring tubules (23;35). The content of each tubule includes an odontoblast process, for all or part of their course, and fluid (19). The dentinal tubule however can become occluded with mineral during remineralisation or with aging (36).

Mineral and Crystalline Components of Dentine

As in enamel, the predominant mineral in dentine is hydroxyapatite, although it has trace amounts of Mg, Zn, F and other minerals present. The hydroxyapatite crystals in dentine are flattened plates however, unlike enamel, their dimensions are approximately 60–70 nm in length and 20-30 nm in width (22). The calcium and phosphate ions of hydroxyapatite have been found to attach directly to the
collagen organic matrix on a specific zone of the collagen termed the Ca-side (37).

Peritubular Dentine

The peritubular dentine is more radio-opaque and more electron dense than the intertubular matrix, showing it to be more highly mineralised (38). Peritubular dentine is not present in the predentine, first appearing some distance into the calcified dentine. The collar of peritubular dentine becomes wider as the tubule passes from the pulp towards the ADJ (29). This coincides with the gradual reduction in diameter of the odontoblast process as it passes in the same direction. The width of the peritubular dentine is approximately 0.5 um but can be as wide as 2.0 um. In demineralised ground sections, peritubular dentine appears to be composed almost entirely of granular crystals 2-3 nm in diameter (38). In demineralised sections, organic matrix is identified but makes up only a small amount of the overall composition of the peritubular dentine and consists of fibrils with no visible structure (25). It has been reported that the peritubular dentine in primary teeth is two to five times thicker than that found in permanent teeth (39).

Intertubular Dentine

The intertubular matrix is less mineralised than the peritubular dentine with the collagen fibrils running in a plane at right angles to the tubules (25). The apatite of the dentine is smaller than in enamel (35) and in the intertubular dentine the apatite crystals are arranged along the collagen fibrils with their long axes (c-axes) parallel to the long axes of the fibrils. The crystals are found in two main shapes. Plate-like crystals with widths of 15-30 nm as well as needle-shaped crystals approximately 3 nm thick and 40-60 nm long can be found (38).

Organic Matrix

Approximately 85-90% of the organic matrix of dentine consists of collagen, predominantly type I. There are minor amounts of type V and VI in mature dentine.

The collagen in sound dentine shows regular intermolecular cross banding of collagen (40). There are also a number of non-collagenous proteins present that are summarised in Table 2-3. The non-collagenous proteins cover the collagen fibrils and are associated with the mineral phase of dentine. Phosphoproteins are
believed to be critical for inducing mineral nucleation and for binding to the calcium phosphates of the apatite (41-45). The mineral in dentine is located either in the gaps between the collagen molecules or attached to the collagen fibrils themselves (46;47).

<table>
<thead>
<tr>
<th>Component</th>
<th>Comment</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen</td>
<td>Major organic component predominantly type 1 with small inclusions of type V and VI</td>
<td>May play a role in initiating mineralisation&lt;br&gt;Provides structural framework for dentine, while giving strength and resilience</td>
</tr>
<tr>
<td>Phosphoproteins</td>
<td>Major non collagenous protein</td>
<td>May play a role in mineralisation</td>
</tr>
<tr>
<td>Proteoglycans</td>
<td>Sermatan, chondroitin, and keratin sulfates; decorin and biglycan are present</td>
<td>Some inhibit mineralisation while others bind calcium</td>
</tr>
<tr>
<td>Y-carboxyglutamate containing proteins, matrix Gla and bone Gla proteins</td>
<td></td>
<td>Role in mineralisation is uncertain but they can bind calcium</td>
</tr>
<tr>
<td>Acidic glycoproteins</td>
<td>Osteopontin</td>
<td>May be associated with the odontoblast processes serving as link between cell membrane and matrix</td>
</tr>
<tr>
<td>Growth factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipids</td>
<td></td>
<td>Phospholipids may be involved in initiation of mineralisation</td>
</tr>
</tbody>
</table>

Table 2-3. Organic components in dentine. Modified from Piesco 2003 (22).

Permeability of Dentine

Given that dentine is a vital structure with direct communication with the pulpal tissues its permeability is of clinical significance. The placement of restorative materials as well as conditioners and etchants onto vital dentine may directly affect the dental pulp. Pashley and colleagues have conducted a large volume of the work in this area (48-53). Using hydraulic conductance (which is defined as the ease with which a fluid moves across a filtration barrier) they have shown that fluid can move in both directions across dentine (49). Factors such as size charge concentration, solubility of the diffusion species and the thickness of dentine will change its permeability (50;54). Whilst other dental components
contribute to its permeability, the dentinal tubules have been shown to be the most significant route for solute diffusion (55;56).

Pashley and colleagues have also found that dentine's permeability is not uniform. The greatest permeability is over the pulp horns and the lowest is in the centre of the dentine. This non-uniform pattern remains even when testing dentine discs of uniform thickness which suggests inherent regional differences in dentine permeability which cannot be explained solely by dentine thickness or tubule length (52). The lower permeability of the central dentine may be due to fewer tubules per unit area or because the central tubules have a smaller diameter as they are further away from the pulp. Tubules in the centre of the tooth may be more tortuous than the tubules over the pulp horns (52).

Other variations in permeability may arise from tubular irregularities associated with mineral deposits, organic components of the odontoblastic processes or intratubular deposits of collagen (57). These factors make the apparent functional size of the tubules less than the apparent anatomical size as seen in cross section in the SEM (35). Furthermore the presence of a smear layer of cut dentine decreases the permeability of dentine considerably and it has been suggested that 86% of the total resistance to fluid movement across dentine in vitro may be due to the smear layer (48;58). If the smear layer is removed by etching with dilute citric acid there is an increase in the filtration rate over un-etched controls. The increase in fluid flow was associated with the increase in size of tubule orifice as etching continued (48).

**Differences between Primary and Permanent Dentine**

Although thickness of dentine varies considerably between primary and permanent teeth the general structure is very similar (Figure 2-5). It has been suggested that dentine from primary teeth may be less calcified than permanent teeth (59) however there is relatively little information available in this area. Hirayama (1990) used an energy dispersive spectroscpe to show that the Ca and P concentrations in intertubular dentine were 24.9% (w/w) and 12.1% respectively whilst the peritubular dentine had 30.7% (w/w) Ca and 15.3% (w/w) P concentrations. In comparison the permanent dentine had Ca and P concentrations of 25.5% (w/w) and 12.5% respectively for intertubular dentine and 34.5% (w/w) Ca and 16.9% (w/w) P in the peritubular dentine (60). Lakomaa
and Rytomaa (1977) measured the differences in the element of primary and permanent dentine. In comparison to permanent dentine, primary dentine was found to contain more K and Mg although the levels were not statistically different and varied depending upon where geographically in Finland the teeth were collected (61). Furthermore the effect of differences in mineral content on the mechanical properties of permanent and primary dentine are unclear.

A number of studies have however suggested that the bond strength of various materials to primary dentine is significantly higher than permanent dentine (62-66), although this has not been found in all studies (64). Again, the reason for this is unknown.

\[ \text{Primary molar dentine} \]
\[ \text{Permanent molar dentine} \]

Figure 2-5. Permanent and primary molar teeth showing difference in size and thickness of dentine.

**Mechanical Properties of Enamel and Dentine**

The hardness and modulus of elasticity of dental tissues are fundamental mechanical properties that determine how tissues respond to the forces experienced in the oral environment. Hardness is defined as a materials' resistance to permanent indentation (67) and is considered to reflect its susceptibility to abrasive wear (68). The modulus of elasticity is the linear portion of the slope of the stress to corresponding strain below the proportional limit and indicates how a material will flex under loading (e.g. occlusal forces) (67).

**Permanent Teeth**

A summary of the available literature on the mechanical properties of permanent teeth is shown in Table 2-4.
The mechanical properties of dental enamel vary across the tooth (69-71). The modulus and hardness of enamel may be higher for surface enamel than for subsurface enamel (72;73). A comprehensive study by Cuy and colleagues (2002) compared the mechanical properties (hardness (H) and modulus of elasticity (E)) as determined by nano-indentation with the compositional variations (determined by electron probe microanalysis). They showed a significant decrease in H and E from the enamel surface to the ADJ, particularly on the lingual aspect of the crown of the teeth. These significant decreases were strongly correlated with reductions in the weight percent (wt.%) of P₂O₅ and CaO and increases in the wt.% of Na₂O and MgO. This study also showed a weak correlation between mechanical properties and the degree of prism alignment of the enamel tested (74). Other potential reasons for this decrease in mechanical properties include an increased porosity or an increase in water content near the enamel–dentine junction (75).

Xu and co workers (1998) used a Vickers diamond indenter in a modified microhardness tester with a range of forces (2, 3, 5, 10, 20 and 50 Newton), to measure the mechanical properties of teeth. The hardness and modulus of elasticity of the teeth tested were calculated from graphs of the loading and unloading cycles. The displacement was measured between the base of the Vickers indenter and the sample mounting plate using a pair of capacitive transducers, whereas the load was measured with a strain gauge. The hardness of enamel varied from 3.26 ± 0.21 GPa to 3.62 ± 0.26 GPa, depending on the orientation of the specimen. The hardness of dentine varied between 0.53 ± 0.02 GPa to 0.60 ± 0.02 GPa. The modulus of elasticity for enamel also varied between 78 ± 1 GPa to 98 ± 4 GPa depending on whether it was axial or occlusal enamel whilst that of dentine has been reported to vary between 18 and 22 ± 1 GPa (72). The studies report the composite average of the mechanical properties of both the intertubular and peritubular dentine. By contrast Kinney and colleagues (1996) were able to measure the individual hardness and modulus of elasticity of peritubular and intertubular permanent dentine using an atomic force microscope with a Berkovich diamond indenter. The hardness of fully hydrated peritubular dentine was independent of location and ranged from 2.23 to 2.54 GPa. The intertubular dentine hardness varied depending on location of indentation from 0.49 ± 0.02 to 0.52 ± 0.03 GPa near the amelo-dentinal junction to 0.12 ± 0.02 to 0.18 ± 0.02 GPa near the pulp. The modulus of elasticity for
peritubular dentine was $29.8 \pm 8.9$ GPa and $17.7 \pm 0.03$ to $21.1 \pm 1.13$ GPa for the intertubular dentine (76).

Primary Teeth

Until recently there has been very little work on the mechanical properties of primary teeth. Table 2-5 summarises the available information on the mechanical properties of primary enamel and dentine. Presently only one study compared the differences between primary and permanent enamel and dentine (77) and therefore most authors have attempted to compare their results with previous studies. This can be difficult as all reports use different testing methods and frequently report their findings in different units.

Some studies have reported on the average mechanical properties (77-80) whilst others have mapped the mechanical properties across different areas (72;74). Mahoney and colleagues reported a hardness and modulus of elasticity of primary enamel to be $4.88 \pm 0.35$ GPa and $80.35 \pm 7.71$ GPa respectively and concluded that the mechanical properties of primary enamel were similar to those found in permanent teeth (80). In contrast, Lussi et al., (2000), found that the average mechanical properties of primary tooth enamel were statistically significant lower than those of permanent teeth. Although they used a large number of teeth (60 each of primary and permanent teeth) the history of these teeth were not known and the times since extraction were not reported. The teeth used were found in a 'collection of extracted teeth' (77). Further work is needed to clarify the differences between the primary and permanent teeth.

Table 2-4 and Table 2-5 summarise the mechanical properties of permanent and primary enamel and dentine. The mechanical properties in both primary and permanent teeth are variable and site specific. It has been suggested that the decrease in hardness of intertubular dentine with distance from the ADJ may be due to different levels of mineralisation across dentine (76). It is further speculated that primary dentine may be less calcified than dentine from permanent teeth (59) and that the concentrations of calcium and phosphorus of primary dentine is less than that of permanent teeth (81). It is also possible that changes in tubule orientation and density between the ADJ and the pulpal surface may affect the mechanical properties (76). Schilke and colleagues (2000) have recently reported that there is no difference in the numbers and diameter of tubules in the middle and deep layers of primary and permanent dentine (32). In
contrast other studies have reported that primary dentine has smaller diameters of dentine tubules and wider peritubular dentine than permanent dentine (33;34) although presently these findings have not been verified.

More recent studies have confirmed the regional variation in mechanical properties across sound dentine. Angker and colleagues (2003) utilised a depth-sensing device (Ultra-Micro-Indentation system) to measure the mechanical properties of hydrated dentine. These authors reported that the mechanical properties of dentine decline significantly when approaching the pulp and speculated that this was due to a corresponding change in both inorganic content and the histological structure of dentine (82) (Table 2-5). A similar trend has been recently shown by other authors in primary (83;84) and permanent teeth (85;86). Kinney and colleagues (1996) have speculated that the decrease in mechanical properties of dentine upon approaching the pulp could be attributed to the increase in the number of dentinal tubules as well as an decrease in the hardness of the intertubular dentine (76).
<table>
<thead>
<tr>
<th>Author and Date</th>
<th>Dental tissue testing</th>
<th>Hardness</th>
<th>Modulus of Elasticity</th>
<th>Test method and scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinny et al., 2004 (87)</td>
<td>Hydrated Dentine</td>
<td>NM</td>
<td>24.4 GPa</td>
<td>Resonant ultrasound spectroscopy</td>
</tr>
<tr>
<td>Fuentes et al., 2003 (88)</td>
<td>Superficial Dentine</td>
<td>61.93 ± 6.57</td>
<td>NM</td>
<td>Vickers indenter, 300 g, Kg/mm²</td>
</tr>
<tr>
<td></td>
<td>Deep Dentine</td>
<td>64.01 ± 5.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cuy et al., 2002 (74)</td>
<td>Entire enamel from Surface to ADJ</td>
<td>Enamel surface &gt; 6</td>
<td>Enamel at ADJ &lt; 3</td>
<td>Enamel Surface&gt;115</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Enamel at ADJ &lt; 70</td>
</tr>
<tr>
<td>Hebelitz et al., 2001 (89)</td>
<td>Enamel rods in cusp region</td>
<td>Parallel to enamel rods: 3.9 ± 0.03</td>
<td>Perpendicular to enamel rods 3.3 ± 0.3</td>
<td>87.5 ± 2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>72.7 ± 4.5</td>
</tr>
<tr>
<td>Marshall et al., 2000 (90)</td>
<td>Enamel and Dentine adjacent to ADJ</td>
<td>Enamel: 3.51</td>
<td>Dentine: 0.83</td>
<td>Enamel: 63.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dentine: 19.65</td>
</tr>
<tr>
<td>Lussi et al., 2000 (77)</td>
<td>Enamel</td>
<td>346 ± 6.4 to 331.4 ± 13.9</td>
<td>NM</td>
<td>Knoop diamond indenter, 500 g, Knoop hardness number (KHN)</td>
</tr>
<tr>
<td>Giamalia et al., 1999 (91)</td>
<td>Enamel</td>
<td>291 ± 9.11</td>
<td>NM</td>
<td>Vickers Diamond Indentor, 300 g, Vickers Hardness Number (VHN), Kg/mm²</td>
</tr>
<tr>
<td>Kinney et al., 1999 (92)</td>
<td>Dentine</td>
<td>NM</td>
<td>Peritubular dentine: 30 GPa</td>
<td>Atomic Force Microscope, 400 µN, GPa</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Intertubular dentine: 15 GPa</td>
<td></td>
</tr>
</tbody>
</table>

Table 2-4. Hardness and modulus of elasticity of enamel and dentine of permanent teeth. NM—not measured. Modified from Mahoney 2001 (11).
<table>
<thead>
<tr>
<th>Reference</th>
<th>Material</th>
<th>Hardness</th>
<th>Modulus</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kielbassa et al., 1999 (83)</td>
<td>Enamel</td>
<td>350 KHN</td>
<td>NM</td>
<td>Knoop's diamond indenter, 30 g, Knoop hardness number KHN</td>
</tr>
<tr>
<td>Hannig et al., 1999 (94)</td>
<td>Enamel</td>
<td>356.7 ± 18.1 Vickers Unit</td>
<td>NM</td>
<td>Vickers diamond indenter, 100 g, Vickers units (HV 0.1)</td>
</tr>
<tr>
<td>Meredith et al., 1996 (73)</td>
<td>Enamel</td>
<td>273 KHN</td>
<td>299.5 GNm$^2$</td>
<td>Knopps diamond indenter, 4.9 N and 0.96 N for hardness and GNm$^2$ for modulus</td>
</tr>
<tr>
<td></td>
<td>Dentine</td>
<td>60.7 KHN</td>
<td>60.7 GNm$^2$</td>
<td></td>
</tr>
<tr>
<td>Willems et al., 1993 (71)</td>
<td>Enamel</td>
<td>3.39 ± 0.18 GPa</td>
<td>90.59 ± 16.13</td>
<td>Nano indentation, 10mN, GPa</td>
</tr>
<tr>
<td>Kodaka et al., 1992 (69)</td>
<td>Enamel</td>
<td>Outer: 462.0+/−28.9 VHN</td>
<td>NM</td>
<td>Vickers Hardness Number (VHN), Vickers shaped diamond indenter 25 gm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inner: 373.9+/−27.1 VHN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salama and Kinawi 1989 (95)</td>
<td>Enamel</td>
<td>229.32 to 238.5 VHN</td>
<td>NM</td>
<td>Vickers Hardness Number (VHN), Kg/mm$^2$, Vickers shaped diamond indenter 5-20 gm</td>
</tr>
<tr>
<td>Nishihara 1986 (96)</td>
<td>Enamel</td>
<td>206.7± 24 to 337.9 ± 62.5 VHN</td>
<td>NM</td>
<td>Vickers Hardness Number (VHN), Kg/mm$^2$, Vickers shaped diamond indenter 200 gm</td>
</tr>
<tr>
<td></td>
<td>Dentine</td>
<td>44.5 ± 9.9 to 76.9 ± 20.4 VHN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fosse et al., 1996 (97)</td>
<td>Enamel</td>
<td>111± 43 to 378 ± 109 Kg/mm$^2$ (depending on surface of tooth tested)</td>
<td>NM</td>
<td>Vickers Hardness Number (VHN), Kg/mm$^2$, Vickers shaped diamond indenter</td>
</tr>
</tbody>
</table>

Table 2-4 cont. Hardness and modulus of elasticity of enamel and dentine of permanent teeth.
NM = not measured. Modified from Mahoney 2001 (11).
<table>
<thead>
<tr>
<th>Authors</th>
<th>Material</th>
<th>Hardness</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purdell-Lewis et al., 1976 (98)</td>
<td>Enamel</td>
<td>$319 \pm 345$ (depending on surface of tooth tested)</td>
<td>Knoop Hardness Number (KHN), Knoop shaped diamond indenter, force not mentioned</td>
</tr>
<tr>
<td>Davidson et al., 1974 (99)</td>
<td>Enamel</td>
<td>$327 +/- 34$ to $367 +/- 17$ KHN</td>
<td>Knoop diamond indenter, 50 or 100 gm, Knoop hardness number (KHN)</td>
</tr>
<tr>
<td>Bowen et al., 1982 (100)</td>
<td>Dentine</td>
<td>NM</td>
<td>$2.8 \pm 0.79 \times 10^8$ psi</td>
</tr>
<tr>
<td>Ryge et al., 1961 (101)</td>
<td>Enamel</td>
<td>$123 - 355$ KHN</td>
<td>Knoop diamond indenter, 1-10000 gm Knoop hardness number (KHN)</td>
</tr>
<tr>
<td></td>
<td>Dentine</td>
<td>$242 - 348$ VHN</td>
<td>Vickers Hardness Number (VHN), Kg/mm$^2$, Vickers shaped diamond indenter 1-10000gm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$30 - 72$ KHN</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$65 - 80$ VHN</td>
<td></td>
</tr>
<tr>
<td>Tydesley 1959 (102)</td>
<td>Dentine</td>
<td>NM</td>
<td>$1.79 \times 10^6$ psi</td>
</tr>
<tr>
<td>Craig and Peyton 1958 (103)</td>
<td>Dentine</td>
<td>NM</td>
<td>$2.4$ to $2.7 \times 10^6$ psi</td>
</tr>
<tr>
<td>Craig and Peyton 1958 (104)</td>
<td>Enamel</td>
<td>$343 \pm 23$ KHN</td>
<td>Knoop diamond indenter, 50 gm, Knoop hardness number (KHN)</td>
</tr>
<tr>
<td></td>
<td>Dentine</td>
<td>$68 \pm 3$ KHN</td>
<td></td>
</tr>
</tbody>
</table>

Table 2-4 cont. Hardness and modulus of elasticity of enamel and dentine of permanent teeth. NM=not measured. Modified from Mahoney 2001 (11).
<table>
<thead>
<tr>
<th>Author and Date</th>
<th>Dental tissue testing</th>
<th>Hardness</th>
<th>Modulus of Elasticity</th>
<th>Test method and scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hosoya and Marshall 2004 (83)</td>
<td>Dentine</td>
<td>Outer: 56.8 – 60.0</td>
<td>2254 - 2597</td>
<td>Nano-indentation system, max force 10 mgf-100 gf</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle: 48.7 – 57.3</td>
<td>2249 - 2517</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inner: 30.6 – 40.6</td>
<td>1569 - 1702</td>
<td></td>
</tr>
<tr>
<td>Angker et al., 2003 (82)</td>
<td>Dentine*</td>
<td>Inner: 0.91 ± 0.15</td>
<td>16.91 ± 3.85</td>
<td>Ultra-Micro-Indentation System, max force 25mN, GPa</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle: 0.85 ± 0.19</td>
<td>17.06 ± 3.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Outer: 0.52 ± 0.24</td>
<td>11.59 ± 3.95</td>
<td></td>
</tr>
<tr>
<td>Hosoya et al., 2000(84)</td>
<td>Dentine</td>
<td>Inner: 30.3 ± 11.2</td>
<td>NM</td>
<td>Knoop diamond indenter, 15 g, Knoops hardness number (KHN),</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle: 48.2 ± 9.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Outer: 55.4 ± 17.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lussi et al., 2000 (77)</td>
<td>Enamel</td>
<td>313.4+/−18.6 to 331.4 +/− 16.2 KHN</td>
<td>NM</td>
<td>Knoop diamond indenter, 500 gm, Knoops hardness number (KHN)</td>
</tr>
<tr>
<td>Mahoney et al., 2000 (80)</td>
<td>Enamel</td>
<td>4.88 ± 0.35</td>
<td>80.35 ± 7.71</td>
<td>Ultra-Micro-Indentation System, max force 50 and 150mN, GPa</td>
</tr>
<tr>
<td></td>
<td>Dentine</td>
<td>0.92 ± 0.11</td>
<td>19.89 ± 1.92</td>
<td></td>
</tr>
<tr>
<td>Caldwell et al., 1957 (78)</td>
<td>Enamel</td>
<td>272+/−26 to 399 +/− 39 KHN</td>
<td>NM</td>
<td>Knoop diamond indenter, 500 gm, Knoops hardness number (KHN)</td>
</tr>
<tr>
<td>Saunsbury and Atkinson (1953) (79)</td>
<td>Enamel</td>
<td>917 (immature deciduous teeth)</td>
<td>NM</td>
<td>Relative Hardness number with Diamond shaped indenter 30 gm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>986 (mature deciduous teeth)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2-5. Hardness and Modulus of Elasticity of Primary Teeth.
(NM= not measured), * tested in hydrated conditions.
PART 2. Compromised Enamel and Dentine

As has been discussed, sound enamel and dentine are hard resilient tissues that may function satisfactorily throughout life. However, both tissues can be compromised by either developmental or acquired insults. Developmental disturbances resulting from either environmental or, genetic disturbances can cause enamel defects such as hypomineralisation and hypoplasia whilst the most common acquired disorder is dental caries. The following section will outline the physical and mechanical properties of enamel and dentine affected by developmental or acquired destructive processes. Emphasis will be placed on dentinal caries as enamel caries has been studied extensively and on developmental defects of enamel as these present an increasing challenge to clinicians.

Enamel Hypomineralisation and Hypoplasia

Enamel is ectodermally derived and is produced by ameloblasts which differentiate from inner enamel epithelial cells of the dental organ. In simplistic terms, enamel formation can be divided into three stages: matrix formation, calcification, and finally maturation. During matrix formation the enamel matrix is deposited and the necessary proteins are produced. Calcification is when the newly formed matrix becomes mineralised to approximately 30% and maturation is when the proteins are removed from the enamel matrix and the resulting enamel is fully mineralised (105-107).

Any local, systemic or genetic factor that disrupts the very sensitive ameloblasts at any stage can cause enamel defects. If the disruption occurs during the secretory phase then teeth are characterised by a deficiency of tooth substance that ranges from minor pits and grooves to total absence of enamel. This enamel defect is termed enamel hypoplasia (Figure 2-6) (108-110). Suckling and Thurley (1984) investigated enamel hypoplasia (of non genetic origin) and suggested that enamel hypoplasia resulted from a sudden severe insult to the ameloblasts while in their secretory phase (111). If disruption of ameloblasts occurs during either the calcification or maturation phase then the teeth will appear mottled and the
enamel will have a qualitative defect of enamel (111). This is termed enamel hypomineralisation and can present as an enamel opacity (Figure 2-6). Both of these terms are descriptive with no reference to a causative factor (112). Clinically and histologically, combinations of hypoplasia and hypomineralisation usually coexist (113).

There is debate in the literature as to the terms that should be used to describe hypomineralised or hypoplastic defects. Suckling and colleagues (1989 and 1984) defined developmental defects in enamel as enamel hypoplasia, demarcated opacities and diffuse opacities. They characterised hypoplasia as a break in the enamel surface, producing a reduced thickness of enamel, whereas a demarcated opacity is where there is an abnormality in translucency of the enamel but no alteration in its thickness. These lesions have clearly defined margins separating the abnormal from the normal enamel. A diffuse opacity is white on eruption and is similar in colour to a demarcated opacity, with no change in enamel thickness but there is no clearly defined margin of abnormal and normal enamel (108;111). Alaluusua and colleagues (2001) also entered the debate and stated that the term enamel opacity and enamel hypomineralisation with intact enamel surface may be used synonymously. An enamel opacity that breaks down secondary to masticatory forces is often inappropriately referred to as enamel hypoplasia. A more appropriate term for such defects might be post eruptive breakdown of a hypomineralised enamel lesion (114). There is no debate in the literature about fluorotic lesions which are predominantly diffuse. Such diffuse opacities will not be discussed further. In this literature review, the term enamel hypoplasia will be used when discussing a quantitative defect in enamel that is not caused by caries (or a genetic condition) and the term hypomineralisation will be used to discuss qualitative lesions not caused by dental caries.

Enamel hypoplasia and hypomineralisation can occur both in the primary and permanent dentitions (115). Often the exact cause of the defect is not obvious from the clinical history, however, a number of potential causes have been identified and are summarized in Table 2-6.
### Common factors reported to be associated with enamel hypoplasia

<table>
<thead>
<tr>
<th>Local</th>
<th>Systemic</th>
<th>Genetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trauma of primary predecessor</td>
<td>Severe metabolic disturbances including: Anti or neonatal infection, Vitamin D dependent rickets, Hypoparathyroidism</td>
<td>Amelogenesis imperfecta</td>
</tr>
<tr>
<td>Laryngoscopy</td>
<td>Premature birth and very low birth weight</td>
<td></td>
</tr>
<tr>
<td>Infection of primary predecessor</td>
<td>Ingestion of excess fluoride</td>
<td></td>
</tr>
<tr>
<td>Trauma due to extraction of primary predecessor</td>
<td>Nutritional deficiencies</td>
<td></td>
</tr>
<tr>
<td>Repaired cleft lip and palate</td>
<td>Brain injuries and neurological deficiencies, Kidney abnormalities such as nephritic syndrome, Allergies, Drugs and Chronic lead poisoning</td>
<td></td>
</tr>
<tr>
<td>Repaired cleft lip and palate</td>
<td>Radiation treatment, Maternal diabetes, Epidermolysis bullosa, Haematological disorders such as rhesus incompatibility and hyperbilirubinaemia, Dioxins in breast milk</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2-6. Causes of enamel hypoplasia.**
Modified from Mahoney 2001 (4).

Both enamel hypoplasia and/or hypomineralisation commonly and most severely affect first permanent molars. The aetiology is often unclear. Calcification begins at birth and the crown is complete between 2.5 and 3 years (116). Any disruption between these times can cause defects in the enamel of first permanent molars.

Recently the term molar-incisor hypomineralisation (MIH) has been introduced to describe isolated enamel defects in first permanent molars with or without hypomineralised defects on the upper or lower incisors (117,118) (Figure 2-6). Isolated defects of first permanent molar teeth may affect up to 25% of young children (119). The range in reported prevalence (Table 2-7) is probably due to variation in both the diagnostic criteria and the population examined.
Figure 2-6. Molar-incisor hypomineralisation (MIH).

A and B. Hypomineralised and hypoplastic defects of first permanent molars, C. Hypomineralised defects in incisors (patient also had affected first permanent molars).

<table>
<thead>
<tr>
<th>Study</th>
<th>Age in years of children examined</th>
<th>Number of children examined</th>
<th>Prevalence %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietrich et al., 2003 (120)</td>
<td>10-17</td>
<td>2408</td>
<td>5.6</td>
</tr>
<tr>
<td>Jälevik et al., 2001(121)</td>
<td>8</td>
<td>516</td>
<td>18.4</td>
</tr>
<tr>
<td>Leppäneni et al., 2001 (122)</td>
<td>7-13</td>
<td>488</td>
<td>19.3</td>
</tr>
<tr>
<td>Weerheijm et al., 2001 (123)</td>
<td>11</td>
<td>497</td>
<td>9.7</td>
</tr>
<tr>
<td>Alpöz and Ertugrul 1999 (124)</td>
<td>7-12</td>
<td>250</td>
<td>14.8</td>
</tr>
<tr>
<td>Aluluusua et al., 1996 (119;125)</td>
<td>6-7</td>
<td>102</td>
<td>17</td>
</tr>
</tbody>
</table>

Table 2-7. Prevalence of MIH.
Modified from Weerheijm and Mejäre 2003 (126).

Hypoplastic defects vary greatly in their size and shape and commonly present the clinician with a less than optimal crown form to restore. Unfortunately not only are affected teeth compromised by the hypoplastic and/or hypomineralised enamel but they are also more susceptible to dental caries, adding further complexity of their treatment (107;109;121;127-129). Along with an increase in overall treatment need, children with hypoplastic first permanent molar teeth have

37
been reported to have an increase in anxiety related to dental treatment which may be linked to the increased sensitivity reportedly associated with these teeth (114;122;130).

Mechanical and Physical Properties of Hypomineralised/Hypoplastic First Permanent Molars

To optimise the outcome of restoring a hypoplastic and/or hypomineralised molar, a better understanding of the extent of these lesions as well as their mechanical and physical properties is important (109). However, little is known to date due possibly to the difficulty obtaining adequate samples to study. Defective teeth may have only small portions of the enamel affected making sample preparation difficult, especially for conventional compressive and tensile tests which often require relatively large samples.

Enamel

Structure and Composition

Clinical and histological studies have shown that the disrupted or hypoplastic and/or hypomineralised enamel is most commonly localised to the cuspal portion of first permanent molars. Lesions tend to extend from the amelo-dentinal junction to the surface of the tooth (the amount of tissue can vary depending on the degree of tissue loss at time of examination) with the cervical third of enamel appearing normal (130;131). Kosián and Plačková (1962) reported that 40% of the defects \( n = 47 \) extended through the full thickness of enamel and were usually located on the sides and cusp tips of the crowns (132). One explanation for this may be that the longer the developing enamel remains in a partially mineralised porous state (a normal part of the mineralisation process of enamel), the greater the risk of damage (133-137). Enamel in developing human tooth germs has been shown to persist in this partially mineralised state in the incisal region for much longer than in the cervical region. Therefore, dental defects may be more likely to occur in the more slowly maturing incisal region (133-137).

Kostián and Plačková (1962) looked at the macroscopic appearance of hypoplastic enamel in ground section. They reported that the localisation and shape of the hypoplastic area varied greatly between teeth. Interestingly, they
also reported that the hypoplastic lesion was curved on its inner surface, parallel to the amelo-dentinal junction (ADJ). Most commonly the occlusal and cervical edges of the lesion run along the Hunter Schreger Bands or along the course of the prisms. In the hypoplastic region they found that the Hunter Schreger Bands and Striae of Retzius were often accentuated (Figure 2-7) (132).

![Accentuated Hunter Schreger bands](image)

Figure 2-7. Coronal section of hypomineralised first permanent molar showing accentuated Hunter Schreger Bands. Picture taken at 2 times magnification with optical microscope.

Jälevik and colleagues have investigated the content of chlorine, sodium, magnesium, potassium and strontium in the enamel of hypomineralised first permanent molars. They found that there was little difference in comparison to normal enamel (138;139). Using X-ray microanalysis, the relative concentration of calcium in the porous regions corresponded well to the morphological appearance. In severely hypomineralised enamel (determined by level of porosity) the concentration of calcium appeared to be close to zero. The concentration of phosphorus mimicked that of calcium although the degree of hypomineralisation did not affect the levels of phosphate as much as calcium. Interestingly, the outer most enamel layers had a markedly increased calcium level in both unaffected and porous/hypoplastic regions. The studies by Jälevik confirm an earlier study by Lodding and colleagues (1981) who utilised an ion probe and secondary ion mass spectrometry (SIMS) to investigate the element concentrations of hypomineralised permanent teeth. They also found that hypomineralised enamel had a higher porosity index and lower concentrations of
Mg, C and Na (140). The authors noted that the results of the microanalysis of the hypomineralised enamel resembled those generally found for sound dentine.

Secondary ion mass spectrometry (SIMS) and X-ray microanalysis (XRMA) were used by Jälevik and colleagues to determine the chemical composition of some inorganic elements of hypoplastic first permanent molars (139). In the hypomineralised area there was proportionally more carbon than in normal enamel and this corresponded to the degree of hypomineralisation determined by polarised microscopy (139). The authors did not determine whether this carbon was included in the enamel as carbonate or whether it was in the form of additional protein. Speculation on this may be drawn from studies on teeth affected with amelogenesis imperfecta (AI). Most of the studies on the protein content of teeth affected with amelogenesis imperfecta (AI) have concentrated on amelogenin which is the predominant matrix protein in developing enamel. Controlled removal of enamel proteins, such as amelogenin may provide the mechanism for regulating the rate of crystal growth as well as defining crystallite morphology (141-143). Hence it has been hypothesised that the inhibition of proteolysis leads to retention of protein, thereby interfering with the maturation process and growth of crystals (144). This may be the aetiology of several enamel defects such as amelogenesis imperfecta and dental fluorosis (145).

Fully developed enamel ultimately contains between 0.01-1% protein by weight (146). Teeth affected with Al generally show a substantial increase in protein content in comparison to normal enamel, although there is considerable variation between individual teeth and the type of Al (138;144). In the study by Wright and colleagues (1997), one of the teeth affected with hypoplastic Al had a protein content similar to normal enamel whereas in the teeth affected with hypomaturated Al, the protein content was between 4.4-6.8% which equates to a 20-30 fold increase in the protein content compared to normal enamel. Analysis of the composition of the proteins found that there were marked differences in both the quantity and quality of proteins found in different types of Al (144). The authors went on to hypothesize that retention of extracellular matrix materials could result from the synthesis of structurally altered proteins as well as from the defects in post-secretory processing of enamel extracellular matrices.
Mechanical and Physical Properties

Very little data are available on the mechanical and physical properties of hypoplastic first permanent molars. Suckling and colleagues (1989) did report on the physical appearance and hardness of defective enamel in permanent teeth, however only four of the 12 teeth investigated were molars. They used a Knoop diamond indenter and carried out a series of indents 30 μm from the anatomic surface to the ADJ. They showed that the hardness values varied between the hypoplastic lesions of individual teeth and also between the type and colour of the lesion. In general the hardness of the hypomineralised enamel was consistently lower than that of the control tooth (a single premolar), although teeth with demarcated opacities had the most consistently low subsurface enamel hardness values.

Jälevik (2001) in a larger study investigated the degree of porosity of hypoplastic/hypomineralised enamel using a polarised light microscope. The hypoplastic regions of the extracted teeth were found to be highly porous in comparison to a control, non affected premolar as well as the unaffected enamel in the cervical region. However in a number of teeth, the surface of the hypoplastic region was less porous than the body of the hypoplastic lesion. If the hypoplastic defect occupied the entire depth of enamel, the porous zones followed the bands of Hunter Schreger (Figure 2-7). If the hypoplastic defect did not affect the entire enamel thickness then the porous zone followed the lines of Retzius (107).

Mineral Content

At present there is very little literature available on the mineral content of hypoplastic first permanent molar teeth. Jälevik and colleagues have reported a mean Ca/P ratio in normal enamel of 1.8 and in the hypomineralised region to be on average 1.4 (139). Based on these results the authors concluded that hypomineralised enamel (analysed using SIMS and XRMA) had higher amounts of carbon and lower concentrations of calcium and phosphate compared with unaffected enamel (139). Kerebel and Kerebel (1981) and Rohanizadeh et al., (1998) report that in the condition known as odontodysplasia there was a reduction in the mineral content of enamel but the Ca/P ratio remained the same as in normal enamel (147;148). Clinically, teeth affected with odontodysplasia are often severely hypoplastic however the two conditions, enamel hypoplasia and regional odontodysplasia, are very different which may explain why the Ca/P
ratios are so different. Any alteration in the Ca/P ratios suggests either that the mineral phase is altered or that significant substitution (of ions such as Mg for Ca) may have occurred.

Wright and colleagues (1995) have attempted to clarify the mineral content of patients with AI. Using atomic absorption and spectroscopy to determine the calcium and phosphate content respectively they found that the Ca and P concentrations vary across different types of AI but that in only one of the three hypoplastic AI teeth was there a reduction in Ca or P. The mean calcium/phosphate ratio in the AI varied from 2.01 to 2.29 whereas the normal enamel it was 2.12 to 2.19 (145). Again it is difficult to compare these results to hypoplastic first permanent molars as the aetiology and pathogenesis are vastly different. In an attempt to determine the mineral content of hypoplastic and hypomineralised defects, Fearne and colleagues (1994) looked at developmental defects of enamel in primary teeth of infants who were born prematurely. They utilised dehydrated samples for both X-ray microtomography and digital backscattered electron imaging (DBSI) to determine the mineral content of affected teeth. Using X-ray microtomography they found that the mineral content of unaffected primary enamel was between 2.65 and 2.78 g cm\(^{-3}\), whereas the mineral content of the hypoplastic defects in the test teeth showed a 10% reduction in mineral content to between 2.30 and 2.50 g cm\(^{-3}\). The results from the DBSI were consistent with these results (149). DBSI has also been used to determine the mineral content of fluorosed enamel in deer. Kierdorf and colleagues (1997) utilised dried mandibular cheek teeth and observed little variation of mineral content in the control teeth with the exception of the most cervical enamel, which had slightly reduced mineral density. In comparison the fluorosed enamel showed approximately 9.5% reduction in mineral content as well as an increase in porosity and inhomogeneous mineralisation of the enamel (150;150).

Yanagisawa and colleagues (1984) used high resolution electron microscopy, microbeam electron diffraction and analysing electron microscopy on hypoplastic human teeth. There was however no mention in the article as to what the aetiology of the hypoplasia or which permanent teeth they were investigating. The hypoplastic region not only had relatively large crystal free voids but the enamel crystallites ranged from those of regular shape to irregular, large trapezoid, rhomboid and polygonal crystals. It was suggested that the regularly
shaped crystals were hydroxyapatite however the irregular, large crystals were strongly suggestive of whitlockite (151).

Despite the relatively small amount of literature available it is apparent that there are compositional and physical differences between sound enamel and that found in hypomineralised and/or hypoplastic first permanent molar teeth. The impact of these differences on the mechanical properties of the affected enamel is at present unknown.

**Dentine**

Systemic or local insults to first permanent molars are known to cause enamel hypoplasia or hypomineralisation. It is speculated that if the severity of the insult can cause damage to the ameloblasts, the same injury may damage the odontoblasts of affected teeth. At present there are no reports in the literature as to whether similar insults will alter the mechanical or physical properties of the dentine. Anecdotal clinical evidence suggests that the dentine of teeth affected with enamel hypomineralisation is softer or of an ‘unusual’ consistency in comparison to unaffected teeth. However there is no quantitative data to support this perception.

**Dental Caries**

Despite the fact that it is largely preventable, dental caries is the most common chronic disease of childhood (Figure 2-8) (1). Dental caries is a chronic, initially reversible infectious disease which begins with demineralisation of enamel, dentine or cementum by organic acids produced by the fermentation of dietary carbohydrates by oral odontopathic bacteria (152). When the bacteria in the oral cavity produce organic acids and the pH surrounding the dental hard tissue crystal surface drops below 5.5 the hydrogen ions react preferentially with the phosphate groups of the hydroxyapatite in the aqueous environment. This process converts the phosphate ion to HPO$_4^{2-}$, an ion that cannot contribute to the normal hydroxyapatite crystal, and the crystal dissolves (demineralisation) (153). As the carious processes proceeds there is progressive loss of mineral, cavitation of the tooth surface and destruction of the collagen support structures in dentine. This process is, however, not one way and remineralisation can occur.
Figure 2-8. Carious lesion in a primary molar tooth.

The pathophysiology of enamel caries has been studied extensively and the process of de- and re-mineralisation within enamel is relatively well understood (for a comprehensive review see Ferjerskov and Kidd (154)). By contrast little is known about dentinal caries. Further investigation into the de- and re-mineralisation processes in dentine is needed to enhance the knowledge base and also to aid the development of dentine caries prevention and treatment strategies.

Dentinal caries is a process that results in a number of layers within a carious dentinal lesion. These layers have been described in a variety of ways which can make classification confusing. Therefore the layers as defined by the histology will be discussed first followed by a description of the layers with respect to their remineralisation potential.
Figure 2-9. a. Radiograph of enamel and dentine caries.

Kindly donated by Dr P Dennison, Westmead Centre for Oral Health, Sydney.
b. Systematic illustration of progressive stages of carious lesion formation. 1) Tertiary dentine, 2) translucent zone, 3) zone of demineralisation, 4) zone of penetration and destruction and 5) indicates enamel rod direction, 6) body of lesion. Modified from (155).
Histological Classification of Carious Dentine

Non Cavitated Lesion
Despite the fact that a non cavitated lesion can result in large areas of demineralisation (Figure 2-9), until the enamel is cavitated there is no bacterial invasion of the dentine (25). However, the increase in porosity of the overlying enamel allows organic acids to penetrate and there is partial demineralisation of the peritubular and intertubular dentine (25). A non cavitated lesion can be remineralised in a favorable oral environment (154).

Cavitated Lesion
Once cavitation of the enamel occurs, bacteria invade into the dentine. The main pathway is through the dentinal tubules. The acid that these bacteria produce penetrates deep to the advancing bacterial front and which will cause demineralisation of the translucent zone. Towards the ADJ the bacterial species are mixed and proteolytic and hydrolytic enzymes add to the effect of acid production, resulting in destruction of the organic collagenous matrix as well as ongoing demineralisation (25).

A number of zones have been described in the cavitated carious lesion although there is disagreement in the literature as to the naming and division of these zones.

Translucent Zone
The translucent zone or zone of sclerosis in dentine is formed as a result of stimulation of the odontoblasts (Figure 2-9). Mineral is laid down by the odontoblast processes within the dentinal tubules to form a mineralised barrier of sufficient density to inhibit the diffusion of acids (25). It is called the translucent zone because when the caries is examined microscopically in ground sections with transmitted light it appears translucent.

Johnson and colleagues (1969) used light microscopy and SEM to describe the response of deciduous dentine to caries. They described the presence of a type of mineralisation in the translucent zone. This mineralisation is seen in the canals
which were obstructing the tubules, which in un-decalcified section is continuous with and indistinguishable from normal peritubular dentine and they described as sclerosis. It seems reasonable to postulate that sclerosis occurs by an acceleration of the normal physiological process of peritubular dentine formation in response to a mild stimulation such as might be expected at the periphery of the lesion (156). The ultrastructural appearance of such occluded canals is similar to that described in permanent teeth in response to caries (156) and similar to that described in translucent root dentine in response to ageing (157).

If bacteria have access to a continual supply of fermentable carbohydrates they will produce lactic acid. This acid causes dissolution of peritubular dentine, which is predominantly composed of hydroxyapatite. The calcium and phosphate liberated will greatly raise the ionic concentration in the dentinal tubule. Some of these ions will dissolve out of the lesion but some will diffuse down the lesion towards the pulp (23). As the hydrogen ions of the organic acid are buffered by trivalent phosphate in the walls of the tubules, the calcium and phosphate concentrations are increased to exceed the solubility product constant for various types of crystals (158). The deposition of these crystals represents a translocation of intrinsic dentine mineral rather than the transport of exogenous mineral into the tubules (23). Overall the structure of the intertubular dentine is normal with some patent tubules still apparent (38). However when observed by electron microscope, the apatite crystals of the peri- and intertubular dentine of this layer are markedly decreased in size and number, indicating demineralisation and softening (159). Although the tubules of the translucent zone are filled with new crystals they are not apatite but whitlockite which is much softer and has lower calcium content than apatite (159).

It has been speculated that the translucent zone would be harder than normal dentine because of the increased mineral in its dentinal tubules (19;160-162). However it has been found that whilst some parts of the transparent zone are harder than the surrounding dentine within other parts the reverse occurs (161). Using a modified version of the atomic force microscope (AFM) on hydrated carious dentine it has been showed that the elastic modulus (18.2 +/- 3.1 GPa) and nanohardness (0.8 +/- 0.2 GPa) of translucent intertubular dentine were both significantly lower than that of unaffected intertubular dentine (20.6 +/- 2.2 GPa; 1.0 +/- 0.1 GPa for elasticity and hardness respectively) (161;163). These findings confirm earlier work including that by Hosoya and colleagues who used a
Knoop indenter to show that the transparent zone was softer than sound dentine in 75% of specimens tested (84;164). From this evidence Marshall et al., 2001 concluded that the intratubular mineral deposits probably do not contribute significantly to the mechanical properties of the dentine particularly if intratubular crystals are loosely packed in the lumen as has been observed by some workers (161;165).

**Body of Lesion**

This layer is commonly further subdivided into three zones;
The zone of demineralisation, the zone of penetration and the zone of destruction

**Zone of Demineralisation**

The zone of demineralisation (Figure 2-9) is the deepest portion of the body of carious dentine and is usually bacteria free. Although this region is partially demineralised, Johnson and colleagues (1969) described several types of mineral deposits within the dentinal canals of this zone of primary teeth including crystals approximately 100 nanometers thick and between 400 and 1500 nanometers in length (38). Similar crystals have also been seen in studies on carious permanent dentine (25;166). From their morphology comparison it is believed that these plate like or leaf like crystals are octacalcium phosphates (165). The odontoblast process is continuous through this layer although it can show numerous minute holes or depressions in the cell membrane due to intra- and extra-cellular crystal depositions (159).

**Zone of Penetration**

This zone contains bacteria and many of the tubules in this region are enlarged due to the bacterial presence (Figure 2-9). Predominantly the bacteria are confined to the tubules (38). In this zone it is common to find large, dense rhomboidal crystals which are usually found at the periphery of the tubular space, often in association with bacterial remnants. As in the translucent zone, these crystals have been found to be whtlockite (38).

In the zone of penetration the intertubular dentin is extensively demineralised although the collagen fibers still show their normal typical banding (25).
The Zone of Destruction

This zone begins at the ADJ (Figure 2-9). It is heavily discoloured and little of the normal architecture of dentine remains. Little or no mineral remains and the microorganisms have moved out of the tubules and have invaded the peri and intertubular dentine. Aggregations of bacteria and necrotic tissue coalesce in the softened matrix to form areas known as liquefaction foci. Destruction of the tissue is often more advanced along the incremental lines of growth producing transverse clefts. Sarnate and Massler (1965) using scanning electron microscopy (SEM) investigated the microstructure of naturally occurring active and arrested carious lesions. They showed that in active carious lesions the peritubular dentine was entirely absent along with the majority of the mineral component of the intertubular region, although collagen was still present throughout. Conversely for arrested carious lesions the mineral was present in the intertubular region and even in the outer regions of the surface region there was peritubular material present (167). To date these findings have not been duplicated.

Odontoblast Processes

Yamada and colleagues (1983) used SEM to investigate the extent of the odontoblast process in carious dentine and found that it extended continuously from the pulp through the normal dentine, the translucent layer to the top of the zone of demineralisation. With ongoing carious attack the odontoblast processes disappear, collapsing with brush like ends (26). The tubule lumina of the zone of destruction are filled with rare remnants of the odontoblast process and the prevalence of bacteria increases superficially (168).

Tertiary Dentine

This is the layer of tubular dentine formed at the surface of the pulp chamber deep to the dentine carious lesion (Figure 2-9). This dentine, also referred to as reparative dentine, forms in response to an irritation, such as dental caries or other external stimuli. It is often atubular and contains less cellular components (168). It varies in structure from a well-formed tissue that is indistinguishable from adjacent normal dentine to severely dysplastic tissue, in which there are few tubules and many interglobular areas. With increasing stimulus there is greater chance of damage to odontoblasts and an associated increase chance of dysplastic dentine production. If the stimulus is sufficiently severe the odontoblasts die and no reactionary dentine is produced (25).
Clinical Definition of Layers of Carious Dentine

An alternative and more contemporary way to describe the carious dentinal lesion is to define the regions in relation to their potential to remineralise. This classification arose as the concept of minimal intervention developed and clinicians required guidelines for determining the amount of dentine that should be removed prior to placement of a successful restoration.

Once cavitation has occurred two layers of softened dentine can be defined depending upon their remineralisation potential; the inner and the outer layers (169;170). Fusayama and his research group have carried out a large number of studies on the physical and mechanical properties, biochemistry and remineralisation potential of both the inner and outer layers of carious dentine. They suggested that the distinction between the two layers can be determined using of 0.5% solution of basic fuchsin in propylene glycol applied to the carious dentine. The dye stains the outer dentine (which corresponds to the zone of destruction and zone of penetration and collagen denaturation) but not the inner carious dentine (zone of demineralisation and sound collagen fibers). The terms 'infected' and 'affected' have also been used to describe the outer and inner dentine respectively (169-171).

As described earlier, the outer or infected (zones of penetration and destruction) layer of carious dentine contains no odontoblast processes and is composed of denatured organic material and is highly infected with bacteria. Oghushi and Fusayama (1975) showed that the intertubular dentine of the outer carious dentine was highly demineralised and contained granular and leaf-like crystals scattered irregularly. Few collagen fibers remained and all had lost their normal distinct cross bands. Cross banding of collagen is thought to be essential for remineralisation of dentine (40). A marked difference in the number of intermolecular cross-links of collagen fibers has been demonstrated between this layer and sound dentine. In the outer layer the number of cross links decreased markedly, in comparison to sound dentine, which suggests an irreversible denaturation of collagen fibers has occurred causing a decrease in remineralisation potential (172).

The affected or inner layer of carious dentine (zone of demineralisation) is demineralised to some degree, but does contain some of the original collagen network and odontoblast processes and therefore has the potential to
remineralise (172;173). In this layer the apatite crystals are found like fringes bound to the collagen fibers which have cross-bands similar to those in normal dentine (40;171). The odontoblast process also remains similar to normal dentine. Although there were decreased numbers of cross-links on the collagen fibers in comparison to normal dentine, this change is thought to be reversible (172;174).

**Permeability of Carious Dentine**

Determining the permeability of carious dentine is also an important factor in determining the remineralisation potential of dentine. For remineralisation to occur, mineral ions such as Ca and P, must be able to penetrate. Carious dentine is much less permeable than sound dentine. This has been demonstrated by dye and stain studies (175), use of radioactive isotopes (176) and by determining differences in hydraulic conductance (177).

A number of theories have been suggested as to why carious dentine may be less permeable; these include tubule blockage by bacteria (50), and intertubular crystals and sclerosis of dentine (178). Arends and colleagues (1995) showed that the tubule diameter reduces as demineralisation progresses (178). Given that laminar flow in is proportional to diameter$^4$ (179), a decrease in diameter from 2.5 to 1.7 μm would decrease the flow rate by a factor of 4.7 (178). The decrease in diameter with increasing demineralisation time consequently decreases the permeability (or transport of potentially remineralising substances) of carious dentine (180).

Not all authors agree that carious dentine is less permeable than sound dentine. Haustein and colleagues have shown that the permeability of carbon-labeled alcohols, acids, sugars and drugs into carious dentine is similar to that found for sound dentine (180). This has been supported by other studies using the diffusion of resin monomers (181) although it appears from the work presently available that the dentine of active caries is less permeable than normal dentine to most solutes. This permeability of dentine is dependant upon the solute being tested and the permeability of each layer of the carious lesion. This is important because the ions essential for remineralisation must be able to penetrate each layer of the carious lesion.
Mechanical Properties of Dental Caries

Very little attention has been paid in the literature to the mechanical properties of carious dentine (Table 2-8). Understanding the mechanical properties of carious dentine is, however, important as the hardness of carious dentine may indicate its mineral content (182) and hence give an indication of its remineralisation potential. Furthermore hardness and texture are currently the most commonly used clinical parameters by which clinicians determine the extent of removal of infected, carious dentine (85). Individual clinical interpretation of the variations in mechanical properties (i.e. hardness) of dentine is subjective and may result in wide differences in the size of the cavities produced, the pulpal health beneath prepared cavities, and the integrity of the remaining tooth structure. In addition, the quality of the dentine remaining at the base of a cavity may have an effect on the bonding of some adhesive restorative materials (183).

Most of the studies conducted to determine the mechanical properties of carious dentine have been performed when the dentine is dry. Examination of demineralised dentine under dry conditions results in shrinkage, which can influence the recorded properties (19;163;184). The use of fully hydrated samples is therefore important to allow simulation of the in vivo situation.

Defining a single value for the hardness and modulus of elasticity of carious dentine is not feasible. The mechanical properties of hydrated carious dentine have been shown to vary considerably, depending upon the location within the lesion (Table 2-8). Marshall and colleagues (2001) developed a depth sensing atomic force microscope to determine both the composite and individual mechanical properties of a carious lesion and its components from unaffected dentine pulpally to the surface of the lesion. Although there is no indication as to whether the lesions were occlusal or proximal, the authors showed that each tooth had a unique profile of mechanical properties with both the hardness and modulus of elasticity decreasing significantly from the pulpal aspect of the lesion towards its surface (161). Similar findings were found by Angker and colleagues (2005) who recently mapped the mechanical properties across occlusal caries in primary molar teeth. They found that the hardness and modulus of elasticity decreased significantly and progressively towards the lesion cavity floor with the values ranging from 0.002 to 0.56 GPa and 0.015 to 14.55 GPa respectively. The reduction in mechanical properties followed two patterns, either a continuous
linear decrease or a similar decrease followed by a plateau and a slight increase at the lesion surface (Figure 2-10). The authors speculated that the plateauing and subsequent increase in properties at the surface of the lesion may be due to remineralisation occurring in the mouth as a result of saliva’s ability to cleanse open lesions (185).

Figure 2-10. The variation in hardness across carious teeth presented on a logarithmic scale vs. distance.
Note the slight increase in hardness near the cavity floor in (b). From Angker et al., 2005.
<table>
<thead>
<tr>
<th>Author and Date</th>
<th>Area of carious lesions tested</th>
<th>Technique used</th>
<th>Hardness (GPa)</th>
<th>Modulus of elasticity (GPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angker et al., 2004 (185)</td>
<td>Carious dentine* ^</td>
<td>UMIS</td>
<td>From lesion cavity floor to sound pulpal dentine 0.0001 to 0.52</td>
<td>From lesion cavity floor to sound pulpal dentine 0.015 to 14.55</td>
</tr>
<tr>
<td>Hosoya et al., 2004 (83)</td>
<td>From sound to affected carious dentine ^</td>
<td>Nano-indentation system</td>
<td>Unit: kgf/mm² Outer region: 48.9 – 57.5 Middle region: 40.7 – 42.9 Inner region: 33.6 – 34.8</td>
<td>Unit: kgf/mm² 2023 – 2174 1846 – 1996 1662 – 1776</td>
</tr>
<tr>
<td>Zheng et al., 2003 (183)</td>
<td>Apparently unaffected dentine to surface of carious lesion * (nm)</td>
<td>AFM and staining with caries detector dye to distinguish active and arrested lesions</td>
<td>‘Active lesions’: peritubular: 0.32 ± 0.07 – 1.08 ± 1.07 Intertubular: 0.02 ± 0.02 – 0.8 ± 0.03 ‘Arrested lesions’ peritubular: 0.21 ± 0.16 – 1.31 ± 0.08. Intertubular: 0.13 ± 0.02 – 0.97 ± 0.02</td>
<td>‘Active lesions’: peritubular: 0.97 ± 1.3 – 23.6 ± 1.3 Intertubular: 0.4 ± 0.5 – 18.9 ± 0.6 ‘Arrested lesions’ peritubular: 5.7 ± 2.4 – 25.0 ± 1.4 Intertubular: 2.0 ± 0.5 – 18.7 ± 0.5</td>
</tr>
<tr>
<td>Marshall et al., 2001 (161)</td>
<td>Apparently unaffected dentine to surface of carious lesion * #</td>
<td>AFM</td>
<td>0.08 – 0.8</td>
<td>1 – 15</td>
</tr>
<tr>
<td>Hosoya et al., 2000 (84)</td>
<td>Apparently unaffected dentine to surface of carious lesion and dentine surrounding carious lesion ^</td>
<td>Knoop indenter, visual inspection</td>
<td>Infected region not able to be measured as to soft for visual measurements Dentine under carious lesion: 27.6 ± 9.9 KHN</td>
<td>NM</td>
</tr>
<tr>
<td>Banerjee et al., 1999 (85)</td>
<td>Apparently unaffected dentine to surface of carious lesion #</td>
<td>Knoop indenter, visual inspection</td>
<td>13.64– 55.02 KHN</td>
<td>NM</td>
</tr>
</tbody>
</table>

Table 2-8. Mechanical properties of carious dentine.
- Types of teeth used:
  o ^:Primary teeth,
  o (nm): no mention of type (primary or permanent) of tooth utilised,
  o #: Permanent tooth
Treatment Options of Cavitated Carious Lesions in Dentine

When treating dentine caries, clinicians have traditionally removed all of the carious dentine including any stained tissue. Further (often sound) dentine and enamel was then removed in order to create mechanical retention within the cavity so that the defect could be restored with a non adhesive restoration. The aim being to remove all bacteria in the carious tissue and ensure that there was adequate bulk of restorative material. The more contemporary approach once a lesion has become cavitated is for clinicians to attempt to remove only the infected dentine biomass (heavily infiltrated with bacteria and their products), leaving affected (demineralised but not infected) dentine only. The rationale for this is that the affected dentine can be remineralised (and its mechanical properties improved) whereas the infected dentine cannot. With the advent of restorative materials that bond both chemically and/or micromechanically to enamel and dentine, cavity preparation is kept minimal, although inevitably between clinicians and between lesions, the amount of tooth tissue removed will vary. Minimal restorations are advantageous as removal of tooth structure weakens the tooth as a whole and increases the likelihood of future restorative re-treatment.

The placement of a restoration serves two purposes. First it replaces the hard tissue lost due to the carious process. Second it acts to as a seal, placing a barrier to the cariogenic bacteria gaining access to the pulpal tissues (186). Understanding what happens to the bacteria and the remaining carious dentine will guide the development of future caries treatment protocols. With current restorative techniques, it is impossible to provide a perfectly sealed restoration. Residual bacteria will inevitably remain. However, there is now evidence that the complete removal of all bacteria is not a prerequisite for a successful restoration. Arrest of the carious process may occur despite the presence of residual bacteria within the cavity (8). If fermentable carbohydrates are present, cariogenic bacteria will continue to produce acids leading to demineralisation of tooth tissue. However if fermentable carbohydrates are withdrawn, the bacteria cannot cause further demineralisation (187). This has led to the suggestion that it is not necessary to remove all the carious dentine, but only the softened (infected) dentine as long as the carbohydrate source has been withdrawn (188). Handleman and colleagues (1973) have reported that 6 months after the
placement of fissure sealants there was a 300 fold decrease in the recoverable microflora from small carious fissure lesions (189). Clinical and radiographic evidence from this paper also suggested that there was no progression of the carious lesions.

Clinical and radiographic examination in addition to scanning electron microscopy were used in a recent study to investigate the success, at one year of resin restorations on primary molar teeth (190). The control group (n=24) had all carious dentine removed and subsequently restored whereas the experimental group (n=24) had only the 'irreversibly infected' dentine removed. The removal was subjective and the amount of caries removed would have varied between teeth. Regardless of this the researchers showed at one year that the retention rate, marginal integrity and pulpal symptoms were identical in both groups with 75% of the experimental group failing to show any increase in size of the residual caries upon radiographic examination (190).

Mertz-Fairhurst and co-workers are responsible for a 10 year landmark clinical trial that strongly supported the notion that the carious process can be arrested beneath a restoration (8;191-199). They recruited 123 patients and placed 156 pairs of restorations in occlusal carious lesions extending into dentine. Half the teeth had sealed composite restorations placed directly over the carious lesion, with no caries removal. The other half had amalgam restorations placed either sealed with resin sealants or unsealed in which all softened carious tissue was removed in the traditional manner. At 10 years the sealed composite and amalgam restorations performed better (with respect to marginal adaption and other clinical criteria) than the non sealed amalgam and from standard radiographic examination, the composite restorations appeared to arrest the clinical progression of the carious lesion (8). However, apart from clinical and radiographic examination, no other more quantitative assessment was used to evaluate the dentine beneath the restorations. The degree to which remineralisation had occurred beneath these restorations was therefore not determined.

Treatment of Deep Carious Lesions

For many years indirect pulp capping has been advocated for the treatment of deep carious lesions in order to avoid pulpal exposure. The indirect pulp cap is a
technique in which the infected dentine is almost completely removed, leaving a layer of demineralised dentine (200;201). This term has recently been renamed 'the stepwise excavation technique' and involves the re-entry into the original cavity after varying intervals (usually a number of months) after treatment with various intracoronal restorations (traditionally non adhesive restorations such as zinc-oxide eugenol cement) and medicament (calcium hydroxide) (186;202-205). The aim of stepwise treatment is to promote sclerosis and the formation of reactionary dentine. In one large multi centre study conducted on permanent teeth, large carious lesions were treated initially with removal of the soft biomass plus the superficial portion of the necrotic and demineralised dentine. Soft dentine and carious enamel at the periphery of the lesion were removed and calcium hydroxide and a temporary restoration (type not specified) were placed. At periods of between 4 and 8 months the temporary restoration was removed along with the remaining carious dentine and the tooth was definitively restored. These authors found that only 5 of 94 teeth had pulp exposure upon re-entry. Interestingly, the researchers noted that most of the remaining carious dentine appeared not only to have become darker but also to have increased in hardness (186). It has been suggested that the increase in hardness represents an increase in mineral content. Therefore, dentine can, when sealed, remineralise or reharden itself. The findings in this study have been supported by similar studies (186;206), again using clinical assessment of 'hardness'.

The in vivo research conducted on the treatment of deep carious lesions has to date only been conducted on teeth that have had partial or full caries removal. With increasing evidence of the potential success of minimal intervention dentistry, less and less tooth tissue is being removed. Remineralisation of the complete carious lesions, both infected and affected regions, is a desirable goal. However currently there is little information on the mechanisms involved in the remineralisation of carious dentine. In the future, it would be ideal to be able to regenerate sound, healthy dentine (or an equivalent hard tissue) to replace the entire carious lesion; infected as well as affected components.
Part 3. Techniques to Determine Extent of Remineralisation

Remineralisation is the process of mineral re-deposition in enamel and dentine after mineral loss (207). The result of remineralisation may be reversal, reduction or arrest of a carious lesion (207;208). Studies suggest that both enamel and dentine have a greater potential to remineralise than bone (209;210). In enamel, mineral loss during caries results in increase porosity due to crystallite demineralisation (207). When the area in or around the carious enamel lesion becomes supersaturated with respect to calcium and phosphate, the enamel mineral is reformed either due to regrowth of existing crystallites or the formation/deposition of new crystallites (209;211).

The process of enamel remineralisation is well understood (155) and will therefore not be discussed further here. However remineralisation of dentine is more complex and less well understood (212). In dentine, demineralisation resulting from the drop in pH occurs initially around the inorganic components, followed by enzymatic degradation of the organic matrix (207;213;214). The composition of both mineral and the remaining organic matrix as well as its ultrastructure will influence the potential to remineralise (213).

Research on the ability of dentine to be remineralised has been conducted both in vivo and in vitro. Prior to reviewing the remineralisation potential of dentine it is important to have an understanding of the qualitative or quantitative methodologies that have been developed to determine the mineral content of unaffected or carious dentine. This is because changes in the amount of mineral in dentine provide an indication of the demineralisation and remineralisation processes (215). At present there is no in vivo assessment method available to determine the mineral content of dentine, although the use of in situ models have been used (216;217).

The following section will summarise the in vitro methods that have been developed to determine the mineral content of dentine and hence to evaluate remineralisation techniques.
Techniques for Assessing Remineralisation and Demineralisation of Dentine

**Microradiography (MR)**

MR is an x-ray absorption technology (218) and has been commonly used for determining the mineral content (demineralised and carious) of enamel and dentine. It has been used *in vitro* to determine the mineral content changes in demineralisation and remineralisation studies of both artificially created carious lesions (216;219-221) as well as naturally occurring lesions in dentine (219;222-225). There have been three ‘generations’ of MR developed (217):

i) Contact MR or transverse MR (TMR), in which thin sections (approximately 90 μm) are analysed. This method utilises a step-wedge of varying intensities that are irradiated along with the sample. Densitometry of the sample can be used to determine the mineral content utilising the Angmar formulae (226). From this method the lesion depth, mineral loss in comparison to sound tissue, and the mineral distribution can be determined reasonably accurately (218). This method results in three dimensional maps of mineral distribution in hard tissues and enables changes in mineral concentrations to be followed *in vitro*. It is said to combine the high resolution advantages of surface techniques and the quantitative aspects of volumetric measurements without the artifacts caused by specimen preparation (19).

ii) Longitudinal MR (LMR), in which thick (400 μm) tooth slices are analysed. This technique is non destructive and therefore allows the amount of mineral in dentine to be determined repeatedly, which allows several consecutive changes to be recorded longitudinally (218;227).

iii) Wavelength-independent MR (WIM) suitable for mineral quantification of whole teeth. This technique utilises polychromatic high-energy x-rays for non-destructive determination of the mineral content of whole teeth. When the whole tooth is used the detection limit is only 0.05 kg.m⁻² or 1500 vol%. WIM compares very accurately to TMR (228).

Although MR is the gold standard for quantification of mineral, there are disadvantages to each of the MR techniques. TMR and LMR require extensive
tooth preparation. As thin sections are required (100 μm or less) the preparation irreversibly destroys the sample. Furthermore, shrinkage can occur during preparation (229;230) which may lead to underestimation of quantitative parameters such as mineral loss and lesion depth, especially in highly demineralised tissues (219;231). To compensate for this, shrinkage must be accounted for when calculating the mineral content (231). It has been shown that TMR can be accurately carried out under wet conditions (230).

Another disadvantage of MR is that within 10 μm from the surface of the sample, mineral content can not be measured due to finite densitometer slit width and specimen curvature (218). The possibility of overlapping structures and non-planar surfaces makes quantitative measurements of the mineral densities prone to error with MR (19;36). Additionally the presence of ions with high x-ray absorption can lead to misinterpretation of the results. Strontium is present in some restorative materials (e.g. some glass ionomer cement (GIC)). If demineralised dentine is restored with such a material this may lead to inaccurate results (218). Finally WIM and LMR do not allow any determination of mineral distribution across a lesion (218).

**Polarised Light**

This technique involves very thin samples (approximately 80 μm) being observed in polarised light. The birefringence is calculated from the path of the differences in various intervals along a demineralised lesion. This technique can show qualitative mineral loss and gain due to changes in the birefringement. However quantifying mineral content using polarised light experiments is difficult (218;232). Most frequently, polarised light measurements can provide information on pore volume and lesion characteristics (such as depth and discrimination of the zones present in a dentine carious lesion) which have been related to remineralisation and demineralisation experiments of dentine (212;233-236).

**Iodide Permeability**

Iodide permeability has been used to detect early demineralisation changes in enamel by identifying permeability at the tooth surface. This technique measures the pore volume in a dental hard tissue and can give a sensitive measurement of the initial stages of de- and remineralisation (237).
A specimen of dental tissue is covered with a known concentration of iodide and then after a set period of time is washed away. The specimen is then blotted with deionised water allowing back diffusion and this solution is collected and transferred by blotting paper and a micro-sample dish. While under constant agitation the iodide content is analysed using an iodide selective electrode. The change in the amount of iodide present before and after exposure to a demineralising substance is related to a change in porosity (238). At present this technique has only been used on enamel which may be because although iodide permeability can determine the surface porosity it can not, at present, be used to monitor change in the depth of a lesion.

**Wet Chemical Analysis**

Methods have been developed to measure the small changes in calcium (Ca) and phosphate (P) dissolved from teeth by demineralisation processes. Initial Ca and P are required so that these values can be subtracted from the final Ca and P levels. A variety of scales and micro-methods exist for the determination of Ca and P in solution (239). Arends and ten Bosch suggest that this technique is not appropriate for clinical assessment as only large gains in mineral are measurable and only flat samples can be used with this technique (218). Another disadvantage of this technique is that each sample has to be destroyed for analysis and therefore long term analysis and multiple sampling cannot be carried out (240).

**Scanning Electron Microscopy (SEM) and Backscatter Intensities (BSE)**

SEM examination can be used to visualise directly the changes in the dental hard tissues. It has been used in several studies involving dentine (38;158;165;209;222;241) although quantitative mineral loss cannot be determined. It has the advantage of being a relatively easily accessible method with most research units having access to this technology. Also SEM allows the morphological details of the specimens to be determined.

Backscatter scanning electron microscopy (BSE) has received little attention in the dental literature although it provides similar information to that obtained from microradiographs with the potential for higher resolution (242;243). BSE can now be conducted on wet and non conducting specimens and does not require
extensive sample preparation (242). A disadvantage of this technique is that it requires a polished surface. Flat surfaces are necessary to remove topographical contrasts which can introduce artifacts (19).

BSE intensity in the SEM is measured in graylevels (0-255) and its intensity has been shown to correlate with calcium content (244;245), mineral content and mineral density in bone (244;246-250). A BSE image is created by different intensities recorded from pixel to pixel which reflect the difference in the atomic number and the contrast seen within an image is a function of the variations in the average atomic number of the sample (242;251). Additional factors that can affect the BSE signal include the accelerating voltage of the electron beam, the size of the filament and emission currents, the spot size of the beam and the focus, brightness and contrast settings (252). Therefore these factors must be kept constant when conducting tests.

A number of studies on dental calcified tissues have been reported using BSE imaging (150;241;242;245;253). Fearne and colleagues (1994) correlated the results of X-ray microtomographic and BSE imaging of hypomineralised and hypoplastic primary teeth from children born with very low birth weights (VLBW). Using sound enamel from control areas to calibrate their system, they tested dehydrated flat surfaces of sectioned primary teeth and found that the BSE images correlated well with X-ray microtomography. They demonstrated a 10% reduction in mineral content in the hypomineralised areas compared to the sound enamel in both X-ray microtomography and BSE technology. For quantitative results of total mineral content, calibration with other known (atomic number) standards must be conducted. Angker and colleagues (2004) quantified the BSE detector and allowed calibration of a number of elements with known atomic numbers. They noted that the changes in graylevels as a function of atomic number in this study were in agreement with the variation indicated in the BSE coefficient measured. This allowed the variation in graylevels to be used to measure the mineral content of sound and carious dentine. These authors found that there was a dramatic reduction in the mineral content across a carious lesion (241).

Most BSE studies have utilised dehydrated and coated samples. The effect of dehydration will be to underestimate the mineral loss and the apparent depth of carious lesion (219;230). Angker and colleagues (2004) captured the uncoated

62
BSE images as soon as the SEM chamber reached the reduced pressure (1.5 Torr). As shrinkage of the specimen can alter results, working at a reduced pressure minimised the shrinkage to 0-5% (241). Marshall and colleagues have also shown that a 'wet' backscattered technique potentially overcomes these limitations (242). This method appears to have promise in the determination of mineral content of carious dentine but has not yet been used to evaluate remineralisation of demineralised dentine.

**Confocal Laser Scanning Microscopy (CLSM)**

CLSM was introduced to obtain non-destructive microscopic topographies of the outer subsurface areas of dental hard tissue (221;254). This three dimensional technique is based on the elimination of stray light from out-of-focus planes by confocal apertures. Images are obtained by scanning over the sample with a spot-size light source and recording the light reflected from the in-focus plane. CLSM allows the visualisation of un-sectioned, natural teeth and can be observed under 'wet' conditions. Artifacts induced by drying or sample preparation can be eliminated (255). This method has been used extensively by Watson and colleagues in dental biomaterials with or without the use of fluorescent dyes (256-261). This method does not give mineral densities but is a visualisation tool (262). González-Cabezas and colleagues, using MR as the gold standard, evaluated the ability of CLSM to monitor the remineralisation of enamel (263). These authors showed that CLSM may provide a valid surrogate for MR when measuring enamel remineralisation. This was because CLSM was able to determine differences in remineralisation of enamel samples depending on the amount of fluoride used. There are a number of other studies that have used the ability of CLSM to give a qualitative assessment of mineral loss or gain in dentine (221;264). Büyükyılmaz and colleagues (1997) have shown that CLSM can be used as an adjunct to MR in remineralisation studies of dentine (221) and van der Veen and ten Bosch (1996) has shown that as mineral is lost from dentine that the confocal microscopic images show increasing fluorescence (264). Banerjee and Boyde (1998) investigated the relative mineral content as determined by BSE of carious dentine lesions relationship to the CLSM. These authors found that the autofluorescence signal correlated with the level of demineralisation but the depth of the lesion as seen by CLSM was significantly greater than seen by BSE. The authors concluded that the autofluorescence signal is not directly related to the mineral content (265).
Banerjee and colleagues have investigated the relationship between CLSM and the colour, hardness, infectivity, mineral content and the state of demineralisation of naturally carious lesions (85;265;268). A correlation existed between the zone of autofluorescence and the hardness of the affected tissue which highlighted the possibility that autofluorescence might be used as an in-vivo, objective histological marker for the softened, carious dentine requiring clinical excavation (85).

**Fourier Transformed Infrared Spectroscopy (FTIR)**

FTIR is a useful tool for analyzing chemical and phase changes in hard dental tissue. A disadvantage of FTIR spectroscopy is that it is easy to introduce technique artifacts such as alterations of the chemical structures by grinding procedures and mutilation of the surface during sampling (267). FTIR allows analysis of approximately 20 x 20 µm² regions of tissue sections.

FTIR as an analytical imaging method has been used to obtain both qualitative and quantitative information on both mineral and matrix of bone (268), cartilage (289), enamel (270) and dentine (271;272) with a special resolution of approximately 7 µm. FTIR can measure many parameters including the mineral:matrix ratio and crystallinity of dentine (271), protein conformation and content (273).

**Dyes**

The use of dyes is a simple way of identifying structures. Dyes are mainly used to distinguish between various layers of dentine rather than a change in mineral content (176). Mjör (1967) investigated the in vivo reaction of dentine to a variety of materials placed into prepared cavities using both dyes and microradiography. The dyes (toluidine blue and alcian blue) were of limited use as they failed to reveal differences (unlike the microradiographic material) between the test lining materials (168). Wei and colleagues (1968) investigated the penetration of toluidine blue and orange G into naturally occurring carious lesions that had or had not been treated with 10% stannous fluoride solution. They found that there was a marked decrease in the uptake of the dyes in the treated teeth in comparison to the untreated teeth (222). Although these studies have been conducted on dyes, at present they appear to be only moderately useful in
assessing the mineral content or change in mineral content in carious or demineralised dentine as it is a very subjective measurement.

**Photon Absorptiometry**

Many traditional methods of evaluation of mineral content do allow for repetitive measurements of the exact same region. Photon absorptiometry with a radioactive source (¹²⁵I) and a non-image-forming detector has been used to determine longitudinal measurements of the mineral content of enamel undergoing artificial demineralisation (274). The principle of this method is that during mineral loss the transmission of the radiation through the mineralised sample increases and this increase can be accurately recorded (275). Transmission of the radioactive source through the mineralised samples can be followed continuously over extended periods of time, and the change in mineral content can be calculated (276). Almqvist and colleagues (1988) validated this method in dentine by comparing it to atomic absorption. Specimens were placed in demineralising solutions and the calcium content calculated over time using atomic absorption and photon absorptiometry. The two methods correlated well. This methodology did not allow for the dentine to be fully hydrated throughout testing as each sample had to be removed from hydration for a minimum of 16 minutes to allow measurement. This method is not able to quantify the amount of remaining mineral, rather the rate of demineralisation. Other authors have shown that photon absorptiometry can be used to monitor gains in mineral in dentine root samples when subjected to remineralisation with varying fluoride levels (277).

**X-ray Tomographic Microscopy (XTM)**

This approach produces a quantitative, three dimensional map of mineral distribution in hard tissues and allows the mapping of changes in mineral concentrations *in vitro*. XTM uses monochromatic synchrotron radiation to measure the X-ray attenuation coefficient as a function of position in a sample. It is based on the same principles as computed tomography (CT). These results are directly related to the materials composition and microstructure can be used to provide a quantitative mapping of the mineral phases and their distribution (19). Kinney and colleagues have quantitatively mapped the mineral concentrations in naturally occurring carious dentine (232). This method has not
been validated against MR, but it appears a promising new method for mineral quantification.

**Microanalysis**

There are a number of different methods for elemental analysis. These include secondary ion mass spectrometry (SIMS), electron probe microanalysis (EPMA), proton probe microanalysis and energy dispersive x-ray spectrometer (EDS). Microanalysis is a valuable tool for tracking the de/re-mineralisation process through a carious lesion (215). The level of calcium can be used as an indicator of the level mineralisation of dentine (278). Therefore knowledge of local concentrations and mobility’s of elements is important for basic and clinical understanding of mineralised tissue (279) as well as determination of increased or decreased element presence in de and remineralisation studies.

SIMS has a detection limit of between $10^{-2}$ and $10^{2}$ atoms ppm for nearly all elements and is commonly used to determine the concentration of ions in a sample. It is also commonly used to image the element distribution within the microstructure of biomimetic tissues in three dimensions via in-depth profiling and surface imaging (280). Again this procedure requires thin sections to be prepared along with coating of the sample (279). The image sharpness can deteriorate gradually as the coating is penetrated over time (280). When coating dental caries, shrinkage can occur which may affect the outcome of testing.

SIMS can be used to quantify elements by determining concentrations (normalized to the matrix element Ca) by comparison with external standards such as synthetic or geological apatites of known composition (140). SIMS has been used to determine the distribution of different elements in enamel and dentine (280-285).

Electron probe microanalysis (EPMA) is a depth resolving technique that is far less sensitive than SIMS but it has been used to determine the elemental composition of enamel and dentine (140;286) as well as detection of trace elements such as fluoride in remineralisation studies (278). Interestingly when attempting to quantify Ca/P ratio, electron probe microanalysis has been shown to be more accurate than SIMS (140). Furthermore EPMA can analyse both the inorganic and organic materials. The principle of EPMA is that when electrons of appropriate energy bombard a sample, they cause the emission of x-rays whose
energy and relative abundance depends upon the composition of the sample. From this quantitative measurements can be made (215;286). Ngo and colleagues have shown that EPMA and TMR correlate well for demineralised dentine samples [Ngo et al., 1997].

EPMA does require a flat highly polished sample and the sample must be homogenous on the micro scale. Sound dentine is an un-homogenous structure with dentinal tubules and carious dentine is even more un-homogenous. What effect this has on EPMA is unknown [Ngo et al., 1997]. Another complication is that each sample must be stable under vacuum. Carious dentine will shrink if left under vacuum and again this is likely to affect the outcome (215).

EDS allows the determination of the most elements and commonly it is reported in a ratio of the chosen elements. Commonly the mean Ca:P ratio is commonly used when examining dentine (287-289). This method can be used to determine the presence of certain forms of calcium phosphate such as dicalcium phosphate or hydroxyapatite which have specific Ca/P ratios (288). For example the average of 1.97 from the dental literature sources has been suggested based on the best estimate of the calcium deficient, carbon rich form of dentine apatite (35). EDS can also be used to determine the presence of trace ions such as strontium, fluoride or magnesium (7;287;290-293).

**Laser Fluorescence**

Studies based on fluorescence observations of laser induced light scattering have shown the technique to be a quantitative measurement of mineral loss and remineralisation of enamel compared with longitudinal microradiography (294;295). Laser fluorescence utilises a low powered argon laser light and can detect differences in sound and carious tooth tissue. Although the exact nature of the fluorescing chromophores within the sound and demineralised tooth tissue is not known, it has been suggested that the fluorescence can be explained by the light-scattering phenomenon of tooth tissue. Hall and colleagues have found a linear correlation between laser fluorescence measurements and TMR for lesions in enamel up to 200 μm deep (296).

Laser fluorescence has been used extensively to identify early mineral loss in enamel (297;298), but much less has been studied in dentine. Wicht and

67
colleagues found a moderate correlation of laser fluorescence's and the depth of a demineralised lesion in root surfaces ($r = 0.5$) (298).

As well as this system's inability to quantify mineral loss, there are a number of other problems with this system. Firstly any staining, fluorosis or other developmental defects such as enamel hypoplasia present in test samples can alter the results of the laser fluorescence. Secondly it cannot be used adjacent to composite resin restorations and therefore may be of limited use in vivo or in situ (296).

**Mechanical Properties**

Although dentine is a structurally anisotropic biological composite (19;299), it is thought that its hardness will depend upon its state of mineralisation (85). A number of methods to determine the mechanical properties of dentine have been reported although indentation methods are the most common. These involve indenting the surface of the dentine using a specified force in a controlled and reproducible manner. Hardness is then determined by the size of the indent once the load is removed (300).

The hardness of a material is the ability of a material to resist permanent indentation. Hardness of carious dentine has been shown to be related to the level of infectivity by bacteria (301) and to its pH (302). Dentine hardness has also been used as a measure of the degree of mineralisation (163) and furthermore it has been suggested that microhardness measurements can provide measurement of mineral loss or gain (218). However the use of mechanical properties of dentine to reflect the mineral content of dentine is controversial. Herkströter and colleagues (1989) while investigating the effect of artificial demineralisation of dentine and enamel found no correlation between the hardness (using indentation with a Knoop indenter) and mineral content (LMR). Although they concluded that the hardness data are unlikely to directly reflect the mineral content of the tissue (303), more recent research has refuted this findings (182). Featherstone and colleagues (1983) developed an analytic expression relating the Knoop hardness to the volume percent of mineral (Figure 2-11) which, whilst inaccurate for very low concentrations of mineral appears to fit the experimental data over a range of mineral concentrations associated with normal and carious dentine (304).
At present there is no objective in vivo method for measuring hardness of dentine. Currently in clinical practice an assessment of hardness/texture of the dentine (or resistance to pressure of a dental probe) is used to determine the residual amount of demineralised carious dentine in a given lesion (82;188;305;306). Unfortunately this is a very subjective measure of both carious activity and mechanical properties (305).

The disadvantages of microindentation include the need for a finely polished surface for indentations to be accurately carried out. Until recently another major disadvantage was the shrinkage that occurred during testing as the specimen dried out (218). To overcome this problem, the mechanical properties of sound and demineralised dentine have been conducted under both wet conditions using atomic force microscopy (76;161;163;184) and the ultra-micro-indentation system (82;241). In vivo dentine is constantly wet, testing hydrated dentine or carious dentine may produce more realistic and valid results in comparison to dehydrated samples (82).

Young's elastic modulus of dentine is another fundamental property of a material (163) and is defined as the slope of the proportional part of the stress-strain curve (163). Currey has shown that the Young's modulus of bone has a strong positive relationship with calcium content (307) and there is strong evidence that the Young's modulus is also dependent on the amount of mineral (308). Recently, Angker and colleagues (2004) showed that the hardness and modulus of elasticity (measured using the UMIS) was dependant upon its mineral content as measured by backscattered scanning electron imaging (182).

\[ \sqrt{KHN} = 1.197V_m - 0.24 \]

**Figure 2-11. Relation of Knoop hardness number (KHN) to volume percent mineral (V_m).**
*From (304).*

From the above discussion it can be seen that measuring the mechanical properties of dental hard tissue can be used to determine the mineral content of both sound and carious dentine. Furthermore changes in mechanical properties can be used to evaluate remineralisation solutions or compounds.
The Use of Mechanical Properties in Dentine Remineralisation Studies

The majority of literature on the remineralisation potential of dentine has used microradiography (Table 2-9) although recording changes in hardness was reported as early as 1944 (309) when using microscopy and the Knoop indenter, dentine was remineralised using a solution of calcium salts with fluoride (309).

Forty years later Davina and colleagues (1986) investigated the ability of calcium hydroxide to remineralise dentine. Cavities were prepared in premolars of young patients and restored with composite resin either with a calcium hydroxide liner or without (controls). There was a significant increase in hardness under the calcium hydroxide liner in comparison to the controls (310). In another study Pereira and colleagues (1998) evaluated the acid inhibition zone that exists in dentine surrounding various glass ionomer cements (GIC). They postulated that the quality (hardness) of the inhibition zone was related to the degree of mineralisation. GIC was placed in prepared cavities and subsequently exposed to an artificial demineralisation system. The Knoop hardness in the inhibition zone surrounding the GIC’s was significantly greater than in the control dentine although the difference varied between GIC’s used (311). Akimoto and colleagues (2001) did something similar more recently using composite resin. Hardness measurements were conducted across the resin/restoration interface using a pyramidal indenter to identify if etched and demineralised dentine could be remineralised in vivo. They found that the non-resin infiltrated hybrid zone just beneath the resin-impregnated layer becomes harder following adhesive restorations. The increase in hardness correlated with the Ca content as shown by EDS (209).

The remineralisation of the inner carious dentine has been confirmed in human teeth (312). The fuschine stainable outer carious dentine was removed from pairs of bilateral vital human teeth with symmetrical cavities and the inner carious dentine preserved in the cavity floor. One of each pair was immediately extracted and the other was capped with carboxylate cement for three months. Sections of the pairs were compared for calcium content as determined by an electron probe microanalysis and Knoop indenter. The inner carious dentine increased markedly in calcium content and hardness, over time reaching the sound dentine level (312). This study has not been repeated due to ethical issues.
Kielbassa and colleagues investigated the effect of irradiation and fluoridation on the demineralisation and remineralisation patterns of root dentin. Knoop hardness numbers of 84 bovine incisors were determined before, as well as after demineralisation and after fluoride gel application. Irradiation resulted in a significant decrease in microhardness. There was a reduction in microhardness with increasing demineralisation time in all groups. Remineralisation with fluoride resulted in an increase microhardness (313).

An in vivo study by Mäkinen and colleagues investigated the ability of a chewing gum (containing xylitol and sorbitol) to arrest dentine caries in children. These authors got children to chew the gum over a 20-22 month period and showed clinically that all carious lesions had rehardened. These teeth were then removed and a Knoop indenter at a load of 200 gm was used to show that the rehardened surface layer (top 150 μm) was significantly harder than sound dentine and nearly as hard as sound enamel (314). Whilst this study did not include any objective measurement of the hardness of the carious teeth pre intervention it does suggest that hardness tests can be used to record remineralisation of dentine in situ.

Obviously, changes in hardness have been used to determine changes in mineralisation. At present there is no research on the effect or remineralisation on the modulus of elasticity of dentine.

Traditional Methods of Testing Mechanical Properties

The majority of available data on the mechanical properties of dental hard tissues have been generated by conventional compressive, tensile and bending tests (10). Conventional hardness testing methods are associated with some general limitations associated with the size and quality of the specimens obtainable from a tooth. For example, tensile tests not only require that a precisely shaped specimen be prepared but also that the specimen is precisely aligned (67;72;315;316). The very small dimensions required for homogeneous samples of dental hard tissue further compounds such difficulties (100;317). Preparation for such tests is difficult and time consuming and often results in only a few ideal specimens. Partially as a consequence of these problems the results reported demonstrate a wide variation.
In contrast to compressive, tensile and bending tests, the majority of hardness tests are relatively non-destructive and the specimen preparation relatively simple (317). Given that hardness is the ability of a material to resist permanent indentation (67), hardness testing is important for understanding how masticatory strains may be distributed throughout the tooth and for predicting how stresses and strains within the structures are altered due to restoration procedures, age and disease (19).

Most hardness tests involve making an indentation into the surface of the material to be tested. This is done at a specified force in a controlled and reproducible manner and for conventional hardness tests the results are determined by the size of the indent made into the material once the load is removed (300). The resulting indent is the product of the load and the elastic recovery of the material. Hardness tests are divided into two categories; macro (loads of over 1 kg, e.g. Brinell and Rockwell) and micro hardness (loads less than 1 kg, e.g. Knoop and Vickers) (10;318;319). These tests utilise a static indentation method where a load is applied to a material by a conical or pyramidal indenter. The relationship of the load to the area or depth of indentation is then calculated and a hardness value is given (10).

The most commonly used hardness tests used are the Brinell, Rockwell, Knoop and Vickers hardness (67;73;101). Each of these employs a different scale for the calculation of the hardness of a material, making direct comparison difficult. Each of the commonly used hardness tests also utilises a number of different shaped indenters and the hardness for each test is calculated in different ways depending on the shape of the indenter and the shape of indentation it leaves in the material it is testing. The Brinell system uses a steel or tungsten carbide ball typically 1.6mm in diameter. The size of the ball indenter and the associated load required to generate an impression makes it unsuitable for a small area characterisation such as enamel and dentine. For brittle materials, such large indenters introduce Hertzian cone cracks rather than plastic deformation (320). Both the Vickers and Knoop hardness tests utilise a diamond shape indenter but the Vickers hardness number is calculated by measuring the diagonals of the square and taking an average of the two, whereas the Knoop hardness number is calculated by measuring only the long diagonal of the non equal diamond (300).
There are a number of factors which influence the hardness test results. Hegdahl and Hagebø (1972) investigated the load dependence of micro indentation hardness testing in enamel and dentine and found that a slight but significant variation in hardness was obtained when the load was varied. These authors utilised relatively large loads (8 g to 64 g) but a load dependant relationship has also been noted by other authors (95;101;321).

Hardness tests can be correlated with a number of different mechanical properties. For example, indentation hardness has been used to study elastic modulus, creep and fracture of brittle materials (315;322). The modulus of elasticity (stiffness) is the ratio of stress to corresponding strain below the proportional limit. It is an indication of the amount of deformation that will occur in the tissue when a load is applied to it.

Ultra Micro-Indentation Hardness Test (UMIS)

When wishing to repeatedly test small areas of samples, visualising the impression with optical measurement is difficult using Knoop or Vickers indenters under conventional forces. Operator error is inevitable and inaccurate results can be obtained (323-326). To address this limitation a new generation of ultra micro-indentation system (UMIS) or nano-indentation instruments were developed (323;325;327). This approach involves recording the depth rather than the area of the indentation and has led the development of precisely controlled indentation systems. These are capable of indenting with initial forces substantially less than 0.1mN and progressively determining depths of penetration with resolution less than 1nm and force resolution better than 0.01mN (327). A further advantage of this system is that fully hydrated dentine samples can be tested, increasing the validity of the results (82;161;163).

The UMIS is controlled by a personal computer. The operating principle is as follows: a shaft carrying a specially developed elastic element is driven downwards towards the sample surface by a long-range piezo driver. The elastic element supports a shaft with a diamond pyramid or spherical indenter attached. A linear variable differential transformer (LVDT) secured to the frame of the instrument records the position of the shaft. When the indenter contacts the surface, the elastic element is deflected relative to the member carrying the elastic element and this deflection is monitored by a second LVDT. The deflection
of the elastic member generates the indentation force. The depth LVDT output is zeroed when the indenter contacts the surface, establishing a datum for the measurement of the indentation depth. The depth-sensing device continuously records changes in depth of an indentation during both the loading and unloading of the indenter and produces a unloading/loading curve (Figure 1.8) (11;327).

Although the UMIS can measure other mechanical properties, the two main mechanical properties determined are hardness and modulus of elasticity. The hardness is calculated from force and projected area. Unlike conventional micro-indentation hardness tests, the force displacement response is also measurable and the elastic modulus is determined from the relationship between force and depth of penetration, rather than from visual measurement of the indentation impression (10).

Due to the scale of operation, the UMIS requires mechanical and thermal stability and therefore must be well constructed and isolated to reduce both vibration and thermal variation (324;325;328). Precise knowledge of the indenter tip is crucial in order to know the correct projected area in the calculation. The tip calibration process is required individually for each indenter tip and is performed regularly to ensure continued accuracy of results (323;325).

**The Indentation Model**

To make reliable estimation of the hardness it is necessary to relate the depth of penetration, the force encountered and the indenter geometry. Each material indented, whether it is a dental material or not, will have a degree of plastic and a degree of elastic deformation of its surface as a consequence of the contact force. The magnitude of the deformation depends on:

- The sharpness of the indenter tip
- The hardness of the material surface
- The elastic modulus
- The contact force

The relationship between the depth of penetration and load is determined by the elastic deformation plus the behaviour of the material and typically results in a load displacement curve of the type illustrated in Figure 2-12. The indenting force
is increased in steps to a predetermined maximum and then decreased in the same steps back to the contact value. Outputs from this method are:

1. Hardness at every force step (presented graphically as a function of depth of penetration for each individual penetration step)
2. Elastic modulus of the material
3. A toughness index
4. The returned energy ratio

The analysis of this method is appropriate only for the Berkovich indenter. The diamond pyramid form of the Berkovich indenter is the most common in the determination of hardness because of its great stiffness and hardness [Poolthong 1998].

Figure 2-12. A typical load-displacement curve.

The quantities shown are:
- AB = loading curve
- BC = unloading curve
- Point A = first contact point between the indenter and the specimen surface
- Point B = point of maximum force and beginning of unloading
- Point C = last contact between the indenter and the specimen
- \( P_{\text{max}} \) = maximum load of the test
- \( h_t \) = maximum penetration depth of the indenter
- \( h_r \) = remnant depth of the indentation (length AC)

From the tangent of the unloading curve, the contact depth \( h_c \) can be determined, which is used in the determination of Young's modulus of elasticity. The depths are shown in diagrammatic form in Figure 2-13.

![Diagram](image)

**Figure 2-13. Schematic representation of the indenting process illustrating the total depth: \( h_n \), contact depth: \( h_c \), and the residual depth: \( h_r \).**

By knowing the contact force, the UMIS calculates the hardness by the force or load placed on a surface divided by the surface area of the indentation.

\[
H \text{ (hardness)} = \frac{\text{force/Projected surface area}}{}
\]

Or

\[
H = \frac{P}{A}
\]

where \( A \) is the area of contact and is defined as

\[
A = kh_c^2
\]

where \( k \) is a geometric constant which for a perfectly sharp Berkovich indenter is 24.5

The contact depth, \( h_c \), can be obtained from the load-displacement curve by fitting a line tangent to the unloading curve at maximum load and extrapolating to zero. The hardness is deduced from the value of \( h_c \), the maximum load and knowledge of the indenter geometry.

Both the Knoop and Vickers hardness tests utilise known force and the surface area to calculate the hardness. The UMIS on the other hand utilises a known load and the projected area of indentation in three dimensions to calculate the
hardness. As long as the indenter tip dimensions are known then any type of tip can be utilised. The UMI5S has the advantage of being able to calculate the Vickers hardness number as well as the unit of the UMI5S (GPa) because the geometry of the indentation tip is known [Mahoney 2001].

Young's modulus of elasticity of the indented material can be obtained from the unloading curve, provided the area of contact, Poisson's ratio and the elastic modulus of the indenter are known. It can be determined from the recovery rate on unloading at the maximum load. The relationship is given by:

$$E_m/(1-v^2) = \frac{1}{2}k_2G$$

- $E_m$ = Composite elastic modulus of the test material and indenter
- $C$ = Poisson's ratio of the test material
- $k_2$ = geometric constant
- $G$ = gradient of the linear regression at the maximum force of the unloading force-displacement data (323).

Dentine Remineralisation

The majority of research on remineralisation of dental hard tissues has been conducted in vitro on enamel by exposing it to calcifying solutions, supersaturated in calcium and phosphate salts or to saliva (329). It is well known that saliva has the ability to remineralise and repair carious enamel lesions (330;331) and there is increasing evidence to support the ability of other exogenous remineralising materials or solutions such as fluoride and CPP-ACP to remineralise and repair carious enamel (332;333). Much less is known about the ability of exogenous materials to remineralise or repair carious dentine.

The terms 'arrest' and 'remineralisation' of carious lesions are two terms that have been used synonymously to suggest remineralisation of carious dentine and although they both can limit the destruction caused by caries they are in fact different processes. Remineralisation is the re-deposition of mineral or mineral ions (e.g. Ca or P ions) onto demineralised tissue whereas arrest of a carious lesion is the when progression of the lesion is halted (155). Arrest of an active lesion can be caused by wear and polishing of the partly dissolved external microsurface and is always associated with the removal of the bacterial plaque
biofilm (155). Once a carious lesion has formed, arresting this lesion by adequate plaque control and other preventive strategies may halt its progression. Clinically the surface of arrested carious lesions will appear glossy and hard. There will be no further discussion of arrested carious lesions as this literature review will concentrate on the process of remineralisation.

There has been growing interest in the literature to determine whether carious dentine can be remineralised. The majority of this work has been conducted in vitro utilising artificially demineralised dentine rather than naturally occurring carious lesions (207;212;334-336). In addition to it being difficult to obtain appropriate carious teeth it is also difficult to eliminate the effect of the bacteria on the remineralisation potential of dentine. This can make translation of the in vitro research to the in vivo situation difficult.

The studies conducted in vitro have suggested that remineralisation of dentine is possible. Table 2-9 summarises the literature. In fact in vitro research has shown that artificially demineralised dentine can be hypermineralised. This is where the amount of mineral accumulated is in excess of sound dentine mineral value and this hypermineralised region is more acid resistant than sound or demineralised dentine (237;337;338). Studies have shown that demineralised dentinal tissues can acquire mineral up to 80-90 vol% within and on the surface of lesions if subject to in vitro remineralising solutions containing 1-10 ppm F- (healthy dentine is 50% vol% (18;19)) (339). It has also been suggested that hypermineralisation also occurs in naturally occurring lesions (339-341).

Most studies investigating remineralisation of dentine have utilised MR to show changes in lesion depth and accumulation of mineral following remineralisation of artificially demineralised lesions. Arends and colleagues (1990) measured the effect of varying fluoride concentrations on remineralisation of dentine. They showed that irrespective of concentration, fluoride caused decreases in lesion depth however the differences between concentrations were not statistically different (337). In contrast the amount of mineral accumulated was strongly influenced by the fluoride concentrations with higher fluoride concentrations resulting in increased mineral accumulation (337). Similar findings have been reported in other studies (Table 2-9). The in vitro remineralisation of carious dentine has also been demonstrated radiographically (222) by immersing ground sections of carious teeth in different fluoride remineralisation solutions. The
radiopacity of the samples increased upon submersion in the various solutions. Despite the *in vitro* evidence that carious dentine can be remineralised, the nature and process involved in this remineralisation and the reaction *in vivo* of the pulp to such remineralisation is currently unknown (342). It has been suggested that relatively intact organic fibrils within a carious lesion may act as sites of nucleation for new crystallites in the process of remineralisation (343).

It is difficult to compare different *in vitro* remineralisation studies not only because many different techniques are used but also many include air-drying of the demineralised dentine which strongly influences subsequent remineralisation (339). Furthermore there is no consensus in the literature as to what constitutes effective remineralisation solution for dentine (Table 2-9). Most studies have included fluoride as an aid to remineralisation however again there is a lack of consensus as to the most appropriate concentration that should be used both *in vitro* or *in vivo* (Table 2-9).

Whilst still quite rare there are a few *in vivo* studies that attempt to address the remineralisation potential of carious dentine (216;237;337;344). ten Cate and van Duinen (1995) used MR on dentine samples worn in partial dentures to investigate dentine remineralisation. They showed that artificially demineralised dentine samples could be hypermineralised when glass ionomer cements were placed adjacent to the samples. Interesting they were unable to use MR in 15% of their samples due to difficulty in result interpretation (216).

An important study by Eidelman and co-workers (1965) used a ‘half surface technique’ to study remineralisation of naturally occurring carious dentine. Patients with large occlusal lesions in molars had the superficial component of the dentine caries removed. Half of the remaining dentine was removed for immediate phosphorus content analysis and the other half was covered with amalgam with either a calcium hydroxide or wax liner for varying periods of time. Using phosphorus as an indication of the mineral content it was shown that there was an increase in mineral content (*increase phosphate*) at the base of the lesion as well as an increase in the clinical hardness of the remaining caries in the teeth treated with a calcium hydroxide but not in the wax lined teeth. The authors concluded that dentine remineralisation did occur in the test teeth (345).
Calcium hydroxide has been utilized for pulp protection at the base of a carious lesion and on carious dentine in indirect pulp treatment techniques. The calcium hydroxide is used to neutralize any remaining acidity in the depth of the lesion, to accelerate the obliteration of dentinal tubules, to facilitate the formation of secondary dentine and to cap possible undetected micro-exposures (346;347). As caries is a microbial infection, another advantageous property of calcium hydroxide is its high pH which makes it antimicrobial (348) even in deep carious lesions for extended periods of time (349-351).

Several investigators have studied the effect of calcium hydroxide on dentine in vivo. Klein (1981) reported that sclerosis of the underlying dentine occurred in 93% of teeth when calcium hydroxide was used as a base following complete caries removal (352). Similarly Mjör and colleagues (1981, 1967) showed an increase in microhardness and microradiography density of dentine following placement of calcium hydroxide in cavities prepared prior to tooth extraction. Moreover there was a further increase in dentine hardness immediately after extraction. The author speculated that although this increase in hardness was less than reported for vital teeth, it suggest that remineralisation of dentine may not be absolutely dependant upon tooth vitality (136;353).

The placement of calcium hydroxide on carious dentine has been studied in indirect pulp caps. Sowden (1956) and Law and Lewis (1961) observed that there was an increase in radiodensity of residual carious dentine after treatment with calcium hydroxide as early as 7 days after treatment (202;354). Tagger and colleagues (1975) investigated 11 teeth with large carious lesions that had CaOH₂ placed over the lesion. There was evidence of reparative dentine formation following calcium hydroxide placement in all teeth (355). However other studies have failed to confirm this finding (351;356). As a result of these conflicting results Ripa and colleagues (1972) investigated the effect of calcium hydroxide on extracted sound and carious teeth. Using microradiography they found no difference in the radiodensity of sound dentine up to 1 year after treatment with calcium hydroxide. However of the carious teeth treated with calcium hydroxide, six out of 16 showed an increase in radiodensity, although this change was well below the change in radiodensity reported for the in vivo situation. The authors reported that increase in radiodensity was limited to the discrete areas of the carious lesion and appeared to be the result of the physical penetration of the calcium hydroxide into the carious dentine (357).
Glass ionomer cements (GIC's) have also been used both in vitro and in vivo to remineralise dentine. This is because GIC's are frequently used as a restorative material for both permanent and deciduous dentitions. GIC's bond chemically to both enamel and dentine (358) and have been associated with relative low rates of secondary caries in the tooth surrounding such restorations (359-361). This is considered to be a result of the gradual release of fluoride (362-364). It is possible that GIC's prevent caries progression by favoring the remineralisation of dentine or by interfering with the growth or metabolism of the remaining cariogenic bacteria (216). This question currently remains unanswered.

Glass ionomer cements have been shown in vitro to remineralise dentine and produce a region surrounding the restoration that has a greater acid resistance than unaffected dentine. Pereira and colleagues (1998) investigated the hardness of this acid-inhibited zone and found that when this zone is subjected to an acid challenge, the hardness is statistically similar to normal dentine (311). ten Cate and van Duinen (1995) investigated the effect of GIC on dentine lesions in comparison to amalgam and composite resin restorations These authors utilised dentine discs placed in partial dentures with each restorative material. With the use of MR they found that adjacent to the GIC restorations that dentine exhibited hypermineralisation, whereas the samples with amalgam and composite resin restorations showed continual demineralisation (216).

An advantage of GIC's is their ability to seal restorations, blocking fermentable carbohydrates from the bacteria involved in dentine caries. An in vivo study by Weerheijm and colleagues (1993) investigated the effect of cariogenic microorganisms on carious dentine sealed with a GIC. Twenty permanent first molars with clear occlusal radiolucencies on radiographs were selected in children between ages 7 and 18. Samples were taken and then sealed with a GIC. After seven months the number of microorganisms had decreased 100 fold. This group also attempted to determine if any remineralisation of carious dentine had occurred. They used the resistance of a bur during sampling as a measurement of hardness. 45% of the sample teeth had apparently become harder after sealing with the GIC (365). This finding is however not exclusive to GIC’s (186;189;366-368).
This review of the literature has addressed several issues; firstly it has summarized current knowledge on the structure and mechanical properties of sound and compromised enamel and dentine. Whilst the properties of healthy tissues are reasonably well understood, it is evident that there is a general lack of knowledge surrounding the biomechanical properties of hypomineralised enamel and carious dentine. This is due in part at least to a difficulty of obtaining appropriate specimens. However given the increasing clinical challenges posed by both hypomineralised teeth and dental caries in very young children it is important that the biomechanical properties of these tissues are firmly established prior to the development of management protocols. The final section of this chapter reviewed the current state of knowledge surrounding the contemporary conservative approach to the management of carious teeth i.e. that based upon the remineralisation of demineralised tooth tissue. It included a review of the various approaches used to evaluate remineralisation of dentine and clearly demonstrates the need for further in vivo work. This thesis is part of ongoing research to address some of the deficiencies in the literature and to increase present understanding of the management of compromised dental tissues.
<table>
<thead>
<tr>
<th>Authors and date</th>
<th>Fluoride concentration used</th>
<th>Samples used</th>
<th>Measurement used</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mukai et al., 2001 (335)</td>
<td>Standard pH cycling with remineralisation solution containing 1.5 mM CaCl₂, 0.9 mM KH₂PO₄, 130 mM KCl and 20 mM HEPES at pH 7.0. 1,450 ppm toothpaste as a slurry for 5 minutes. 5 minute weekly treatment of 4000 ppm F or combined treatment of both.</td>
<td>Bovine root surfaces artificially demineralised at pH 5.0 with 0.1 and 0.5 ppm fluoride</td>
<td>MR</td>
<td>- In shallow lesions the 4000-ppm F solution and combined treatment enhanced mineral deposition at the lesion front and produced a hypermineralised area. 400-ppm solution may be effective for remineralisation of root dentin lesions even at 400μm.</td>
</tr>
<tr>
<td>Heilman et al., 1997 (212)</td>
<td>Remineralising solution with 1.5 mM Ca, 0.9 mM phosphate, 0.15 M KCl and 10 ppm and adjust to pH of 7 with addition of KOH at 24 hours, 3 and 7 days.</td>
<td>Artificially demineralised root surfaces. Demineralising solutions contained varying concentrations of NaF of 0, 0.25, 0.5, and 1 ppm</td>
<td>MR</td>
<td>Little remineralisation at 1 and 3 days. Remineralisation occurred on the remaining mineral and not on the organic matrix devoid of mineral. 1 ppm and 0.50 ppm gave greater remineralisation than 0.25 and 0.00 but not statistically significant. Even 0.00 ppm FL gave some remineralisation.</td>
</tr>
<tr>
<td>Inaba et al., 1995 (334;339)</td>
<td>Remineralising solution contained at 20mM HEPES, 1.5 mM Ca²⁺ as CaCl₂, 0.9 mM phosphate as KH₂PO₄ and 10 ppm F pH 7.0 for 2, 4 or 8 days.</td>
<td>Artificially demineralised</td>
<td>MR</td>
<td>Wet bulk samples showed less mineral accumulation than thin sections.</td>
</tr>
<tr>
<td>ten Cate et al., 1995 (336)</td>
<td>a) continuous presence of 3 μM F b) daily treatment with F toothpaste 1000 ppm</td>
<td>Bovine dentine artificially demineralised</td>
<td>MR</td>
<td>a) The effect of fluoride is greatest in demineralisation and overall there was net demineralisation of samples but remineralisation with group b caused greater remineralisation than group a b) overall caused losses of 24.6 +/- 1.8 μmol Ca²⁺.cm⁻² caused 2.3 +/- 3.4 μmol Ca²⁺.cm⁻²</td>
</tr>
</tbody>
</table>

Table 2-9. Fluoride concentrations used for demineralisation and remineralisation studies of dentine caries.

MR= microradiography, SEM= scanning electron microscopy, APF= Acidulated Phosphate Fluoride, F= Fluoride
<table>
<thead>
<tr>
<th>Authors and date</th>
<th>Fluoride concentration used</th>
<th>Samples used</th>
<th>Measurement used</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suge et al., 1995 (369)</td>
<td>DCPD in $H_3PO_4$ ([Ca]$= 1.0$ mol/L, [PO$_4]$= 3.0 mol/L and 1.0 mol/L used as a post treatment solution with NaF varied from 0 to 0.1 mol/L</td>
<td>Human dentine disks</td>
<td>SEM, permeability analysis, XMA-TEM</td>
<td>-Occluded dentinal tubules 10-15 μm. No difference in depth of penetration of mineral or change in permeability of dentine discs with or without fluoride used. The addition of fluoride the Ca/P ratio was higher. Increased change of deposit from DCPD to apatite with NaF concentration increase.</td>
</tr>
<tr>
<td>Almqvist and Lagerlöf 1993 (277)</td>
<td>Cycling de and remineralisation solutions. 0.02 ppm, 0.20 ppm and 2.0 ppm added to remineralisation solution of 1.0 mM calcium as CaCl$_2$, 2.0 mM phosphate as NaH$_2$PO$_4$, 0.01% Na$_2$S$_2$ and 50 mM KCl</td>
<td>Human molars</td>
<td>$\text{I}^{\text{125}}$ absorptiometry and MR</td>
<td>Overall loss of mineral seen but does dependant response on root hard tissue and remineralisation</td>
</tr>
<tr>
<td>Herkströter et al., 1991 (370)</td>
<td>Remineralisation solution 1.5 mM CaCl$_2$-2H$_2$O, 0.9 mM KH$_2$PO$_4$ and 20mM Hepes at pH 7.0 adjusted with KOH. Addition of 0.02, 0.2 and 2ppm fluoride added</td>
<td>48 extracted human premolars artificially demineralised. Teeth in pH cycling at 1:2 de: remineralisation periods</td>
<td>MR</td>
<td>-All fluoride levels caused an overall loss of mineral and no remineralisation was seen but demineralisation rate was significantly reduced for all samples where F was added. Increasing the fluoride concentration decreased the overall demineralisation</td>
</tr>
</tbody>
</table>
| Arends et al., 1990 (337) | 0, 0.5, 2 and 10 ppm fluoride added to remineralisation solution (1.5 mM Ca, 0.9 mM phosphate and 20 mM herpes at pH 7.0) | Artificially demineralised human premolars teeth | MR                                                              | -Lesion depth significant decrease at all F levels. 
- The efficacy of human remineralisation is approximately proportional to the square root of the F level in the remineralising solution 
- No statistical difference between different levels although there was the trend that as increase in fluoride level = increase in remineralisation and hyper-remineralisation |

Table 2-9. Fluoride concentrations used for demineralisation and remineralisation studies of dentine.
MR= microradiography, SEM= scanning electron microscopy, APF= Acidulated Phosphate Fluoride, F= Fluoride
<table>
<thead>
<tr>
<th>Authors and date</th>
<th>Fluoride concentration used</th>
<th>Samples used</th>
<th>Measurement used</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arends et al., 1989 (237)</td>
<td>0.02, 2, 10 ppm NaF added to remineralising solution of 0.5 mM Ca, 0.9 mM K$_2$HPO$_4$ phosphate at a pH of 7.0 for 8 or 21 days;</td>
<td>Bovine teeth artificially demineralised</td>
<td>MR, SEM</td>
<td>-Hypermineralisation of dentine occurred. F levels of 2 and 10 ppm showed hypermineralisation. -Remineralisation is fast initially and then levels off. -Distance into lesion that the mineral accumulated increased with increasing F concentration.</td>
</tr>
<tr>
<td>Shannon and Woolum 1978 (371)</td>
<td>2 minute tropical application of 0.4% SnF$_2$ followed by varying % of APF</td>
<td>Human premolar dentine</td>
<td>MH</td>
<td>0.4% SnF$_2$ increased microhardness by 2%. Use of APF produced softening overall by 4.1%</td>
</tr>
<tr>
<td>Levine and Rowles 1973 (372)</td>
<td>0, 5, 10, 20 and 100 ppm (added to remineralising solution of 100mg brushite to 6ml NaF solution in 0.5 M Na = K phosphate buffer, pH 6.0)</td>
<td>Carious human teeth</td>
<td>MR</td>
<td>5, 10 and 20 ppm F produced the greatest remineralisation</td>
</tr>
<tr>
<td>Levine 1972 (373)</td>
<td>Range of F from 0-100 ppm in 0.5 M phosphate buffer (pH 5.8-7.0) containing solid brushite (dicalcium phosphate dihydrate)</td>
<td>Human carious teeth</td>
<td>MR</td>
<td>F &lt; 5 ppm and F &gt; 50 ppm produced no desirable remineralisation</td>
</tr>
</tbody>
</table>

Table 2-9. Fluoride concentrations used for demineralisation and remineralisation studies of dentine.  
MR= microradiography, SEM= scanning electron microscopy, APF= Acidulated Phosphate Fluoride, F= Fluoride
The aim of this chapter is to describe the common approach and methods used in the series of experiments that comprise this thesis. Where methodology differs between experiments, this will be described individually in the respective chapters. All manufacturers of the various restorative materials and equipment are listed in Appendix 2.

Obtaining Teeth for Experimentation

The aim of this thesis is to describe the mechanical and physical properties of the carious and hypoplastic/hypomineralised process and to determine what effect minor restorative intervention has on these processes. At present in vitro assessment is the only objective method to determine the mechanical and physical properties. Therefore the teeth required for experimentation needed to be out of the mouth. The teeth were extracted on the basis of clinical need (severe dental caries or hypomineralisation/hypoplasia) as decided by an independent clinician. The teeth obtained for all experiments were obtained from the Department of Paediatric Dentistry at Westmead Centre for Oral Health. Ethical approval for the obtaining of teeth and any treatment performed on them was obtained from the ethics committee of the Western Sydney Area Health Service and Central Sydney Area Health Service (Appendix 3 and 4).

To determine what effect restorative materials have on the carious and hypomineralised/hypoplastic process, baseline physical properties of teeth needed to be established. Therefore for the first investigation, severely carious primary anterior incisors were used in order to provide baseline mechanical and physical properties. First permanent molar teeth affected with enamel hypoplasia and/or hypomineralisation were used in the second investigation in order to generate baseline mechanical and physical properties of this type of tooth. No demographic or identifying details of the donor were obtained for any of the teeth in the first two experiments. For the final study a combination of hypoplastic/hypomineralised first permanent molars with carious and carious primary molars were used. These teeth had been subject to specific treatments.
prior to extraction (See Chapter Six). Demographic details and the treatment performed were collected as shown in Appendix 5. Each tooth unless otherwise specified, was stored immediately after extraction in deionised water at 4°C, with a small number of thymol crystals to inhibit bacterial growth, until needed for testing. Although Poolthong (1998) has previously found that teeth can be dry for up to two days without influencing the hardness and modulus of elasticity of either dentine or enamel when using the UMIS, Habelitz and colleagues (2002) have found that storing sound enamel and dentine in deionised water can affect the recorded mechanical properties (374). The effect on the mechanical properties of storing carious dentine and hypomineralised enamel in deionised water is at present unknown. In each study, each test and control teeth were stored identically. It can therefore be predicted that any observed differences upon testing of hypomineralised or carious tissue will be similar to that of the sound tissue stored under the same conditions and therefore comparisons are valid.

Each of these exploratory studies in this thesis used different numbers of teeth. Finding appropriate teeth for each experiment was a limiting factor in all experiments.

**Ultra-Micro-Indentation System (UMIS)**

The UMIS is a nano-indentation technique originally developed for the indentation of thin films by the CSIRO of Australia (Figure 3-1 and Figure 3-2). This system enables multiple testing of a small area. The UMIS involves recording the depth of penetration rather than the contact area of the indentation visually. This instrument is capable of indenting with very low initial forces (substantially less than 0.1mN) and then progressive increasing load and determining depths of penetration with resolution less than 1nm and force resolution better than 0.01mN (327). An advantage of this system over conventional hardness measurements is that fully hydrated samples can be tested, increasing the relevance of the results and minimising the shrinkage especially of partially demineralised carious teeth (82;161;163).
Figure 3-1. The Ultra-Micro-Indentation System

Although the UMIS can measure a variety of mechanical properties, the two mechanical properties that were used in this thesis are the hardness and modulus of elasticity. The hardness is calculated from force and projected contact area, the latter determined from calibration of the tip geometry, and is the measure of a material's resistance to penetration. Unlike conventional micro-indentation hardness tests, the force-displacement response is also measurable and the elastic modulus is determined from the relationship between force and depth of penetration, rather than from visual measurement of the indentation impression (10).

Figure 3-2. The computer system associated with the UMIS
Sample Preparation

The UMIS requires a flat specimen preferably with a highly polished surface. The sample must be parallel to within 25 μm over the length of the testing surface. The following section summarises the preparation of the samples.

Resin Setting

Initially a small amount of alginate, mixed to manufacturer's instructions was placed over the carious or hypomineralised/hypoplastic region. This was to ensure that resin did not encroach upon the affected region. Each tooth was then entirely set in a cold-curing epoxy resin. This allowed for increased manageability and for the polishing and grinding system to hold the samples. The resin comprised of two constituents; resin and hardener. The required ratio for mixing these constituents was 25 parts of resin to 3 parts of hardener, by weight. The resin and hardener were mixed in a plastic cup for 2 minutes using a wooden stirrer. The teeth were placed in a rubber mould with the roots of the teeth resting on a small piece of re-useable adhesive (Blu Tack). The sides of the rubber mould were lined with a thin plastic film to allow easy removal. The resin mixture was slowly poured around the teeth in the mould so that the liquid sufficiently covered the tooth crown. The samples were given a minimum of 24 hours to set.

Sectioning

Once the resin had set, the embedded teeth were removed from their moulds and the region to be sectioned was determined. For all tests each tooth was sectioned through the centre of each lesion (carious or hypomineralised/hypoplastic region) in the mesial-distal axial plane. In the majority of the teeth this coincided with the centre of each tooth. The teeth were sectioned using a low speed saw under tap water irrigation to expose the testing surface (Figure 3-3).
Grinding and Polishing

To ensure the surface of the exposed teeth were free from scratches and deformation the specimens were polished prior to indenting. To achieve this result, the specimen was polished with successively finer sized abrasive particles. The grinding and polishing procedure involved four very similar steps undertaken in succession and was very similar to that used by Poolthong (1998) and Willems and colleagues (1993) (Table 3-1) (10;71).

Step One

The newly exposed surface was ground using grit #500 silicon-carbide paper in conjunction with a water lubricant/coolant. This was undertaken using hand motion on a specially designed polishing apparatus (Figure 3-4). The silicon carbide paper was attached to the polishing apparatus with the adhesive back. The sample was polished for 5 minutes in one orientation and then polished for a further 5 minutes in an orientation of 180 degrees to the original. The enclosed teeth were cleaned in the ultrasonic cleanser for 5 minutes to dislodge any silicon carbide sediment from the tooth surface.
Figure 3-4. Silicon carbide paper set up for initial polishing.

Step Two
The ground surface of the enclosed tooth was polished with a 9 µm diamond suspension, mixed with lubricant. This was undertaken using an automatic polishing machine. The grinding paper was attached to the polishing disc with the adhesive back. A polishing cloth was attached to the polishing disc with rotational speed of 150 rpm (Figure 3-5) was used in all polishing steps. The force applied to the samples was 5 N. The polishing disc and specimen holder rotated in the opposite direction for five minutes and then in the same direction for another five minutes - the force was increased to 10 N. The sample was cleaned in the ultrasonic cleaner for 5 minutes as before.

Figure 3-5. Automatic polishing machine used to polish samples.
Step Three
The exposed surface was polished with a 1 μm diamond suspension. A new polishing cloth was attached to the rotating disc and a different lubricant was administered.

<table>
<thead>
<tr>
<th>Step</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abrasive</td>
<td>Silicon-carbide</td>
<td>Diamond particle</td>
<td>Diamond particle</td>
</tr>
<tr>
<td>Grit/Grain size</td>
<td>#500</td>
<td>9μm</td>
<td>1μm</td>
</tr>
<tr>
<td>Lubricant</td>
<td>Water</td>
<td>DP-Green</td>
<td>DP-Red</td>
</tr>
<tr>
<td>Rotational speed [rpm]</td>
<td>-</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Force [N]</td>
<td>-</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Time [min]</td>
<td>5</td>
<td>5 in each direction</td>
<td>5 in each direction</td>
</tr>
<tr>
<td>Ultrasonic Cleaner Time [min]</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 3-1. Summary of the grinding and polishing steps

Each tooth sample was tested within 48 hours of polishing. Unless otherwise specified, while not being tested each sample was stored in deionised water with a small amount of thymol to inhibit bacterial growth at 4°C. When the tooth sample was ready for testing it was mounted on a metal base.

Wax Mounting
The finished teeth specimens were mounted on metal bases with wax by pressing with a paralleling machine (Figure 3-6). The mounting base contained a strong magnet to ensure adequate contact is obtained with the test base in the UMIS. The wax was slightly melted by heating with a hot plate prior to the pressing stage. The metal base was enclosed in a plastic impermeable container that allowed the sample to be surrounded by deionised water when testing or while the sample was being stabilised.
Figure 3-6. Specially constructed impermeable container and metal base. Lower picture: sample in place.

Testing - UMIS

The UMIS apparatus is enclosed in a cabinet and is installed in a small isothermal room (Figure 3-1). The temperature controller was started and ambient conditions of (23±1)°C and (50±10)% relative humidity were set throughout the indenting tests, similar to those used by Mencik and Swain (1994) and Pooithong (1998) (10,375). Before use the UMIS was allowed at least 30 minutes to stabilise to the conditions. Prior to testing the samples were removed from the refrigerator, and also given 30 minutes to reach a stable temperature in the UMIS cabinet. At all times the sample was encased in the specially constructed container covered with deionised water. This ensured that the dental tissues were fully hydrated at all times.

The UMIS instrument consists of three major components, the triangular pyramidal diamond indenter (or Berkovich indenter), an optical microscope, and an X-Y transport table. The transport table moves the specimen from the viewing position to the indenting position with a precision of approximately ± 1 μm [UMIS

**Positioning and Array Layout**

Specimens were placed on the 50 mm diameter work-table and the region to be tested was positioned under the microscope (Figure 3-7). The region was chosen so that it did not include any dentine or enamel imperfections. The selected region was displayed on the monitor connected to the UMIS and the location of the first indentation was set with an X-Y co-ordinate of 0-0. A series of indentation positions were then individually selected and the X-Y co-ordinates of each selected position were programmed into the computer following an array layout. The layout for each test run was individually programmed prior to testing. With indentation, the material surrounding the contact area is generally compressed, increasing the density of the region. Therefore each indentation is separated by approximately 1.5 times the area of indentation.

![Figure 3-7. Figure showing a sample at the indenting position on the left and viewing microscope on the right.](image)

**Setting the Working Distance**

After the positioning was complete, the specimen was translated from the viewing position to the indenting position (Figure 3.7). At the indenting position, the indenter was lowered until the tip was approximately 0.5-1 mm from the specimen surface. The option 'set working distance' was chosen from the software associated with the UMIS and the indenter was automatically lowered
until a contact force of 0.2 mN was encountered. If this contact force was not met then the graphic display indicated whether the indenter head needed to be raised or lowered. When the setting was correct it was known that the indenter had located the surface and this was set as the start position.

To ensure thermal stability, the working distance mode continuously measured the depth of the indenter as a function of time. This gave an indication of the stability of the system as changes in the contact position were measured in nm.

The UMIS is very sensitive to vibration and temperature changes and any alteration on the monitor indicated:

1. A drift due to environmental temperature instability or,
2. A drift due to specimen temperature instability or,
3. A fluctuation due to vibration and residual system noise.

When stability was acceptable (drift rate less than 0.2 nm/sec) the specimen was ready for testing. A hold segment of 30 seconds was inserted at the maximum load to monitor possible creep and/or mechanical stabilisation. A hold segment was also used in the research undertaken by Willems et al. (1993). The test parameters commonly used in this thesis are summarised in Table 3-2.

<table>
<thead>
<tr>
<th>TEST PARAMETERS</th>
<th>Berkovich (pyramidal triangular)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indenter type</td>
<td>0.2</td>
</tr>
<tr>
<td>Contact Force [mN]</td>
<td>Depended upon test</td>
</tr>
<tr>
<td>Max. Indentation Force [mN]</td>
<td>25</td>
</tr>
<tr>
<td>No. of increments</td>
<td>Square root</td>
</tr>
<tr>
<td>Increment progression</td>
<td>0.1 / 30.0 / 0.1</td>
</tr>
<tr>
<td>Dwell at Load/Max/Unload [sec]</td>
<td>17 – 35</td>
</tr>
<tr>
<td>Array column size</td>
<td>30</td>
</tr>
<tr>
<td>Delay between locations [s]</td>
<td>(23±1)°C and (50±10) %RH</td>
</tr>
<tr>
<td>Testing conditions</td>
<td></td>
</tr>
</tbody>
</table>

Table 3-2. The parameters assigned to the testing procedure.
Loading and Unloading

An indentation test consists of a series of loading and unloading steps. A loading consisted of 25 incremental steps proceeding every 0.1 seconds, followed by unloading in the similar manner (Figure 3-8 and Table 3-2). This method has been shown to produce reproducible and reliable data for the analysis of sound and carious enamel and dentine ((9;10;80;82;376).

![Graph showing force vs penetration depth for enamel and dentine](image)

**Figure 3-8. Typical force vs penetration depth curve for enamel and dentine.**

**Force**

The maximum force chosen for each test varied depending upon the dental hard tissue being tested. The UMIS software is unable to analyse the hardness and modulus of elasticity for any indentation that penetrates greater than approximately 18000 nm. Therefore preliminary tests were required to determine what the maximum force that could be used in each tissue type. The displacement operating range of the UMIS may be changed from nominally 2 to 20 μm (2000 to 20,000 nm). This range corresponds to the 0-10V output signal of the amplifier system used with the UMIS electronic system. Displacement in excess of this range are recorded as the maximum value and show no further changes as the amplifier has exceeded the operating range.

**Carious dentine**

Previous research in this laboratory has indicated that the mechanical properties of carious dentine of occlusal lesions of primary teeth varied considerably depending upon where the test is conducted. Angker and colleagues have
reported that the hardness varied between 0.002 to 0.56 GPa and therefore the resulting depth of penetration varied considerably (185). To allow the UMIS to calculate the mechanical properties of carious dentine maximum force used in these tests were 5 mN.

**Unaffected dentine**

Work conducted in our laboratory on the mechanical properties of unaffected, sound dentine has shown that its higher mechanical properties enabled a force of up to 50 mN to be used to produce reproducible results (80).

**Hypomineralised/Hypoplastic enamel**

A single paper has investigated the mechanical properties of hypomineralised teeth prior to the present studies (377). This study used a conventional Knoop diamond indenter at a maximum force of 25 gm. No research has been conducted using nano-indentation techniques therefore preliminary investigations in the maximum force that could be used with the UMIS on hypomineralised enamel were conducted. In the majority of trial teeth when 50 mN was used to test the mechanical properties, the results were not able to be calculated (due to very high maximum penetration depths and/or due to cracking around the indentation). The use of 20 mN allowed reproducible results to be obtained for all hypomineralised enamel in the preliminary investigation and therefore this value was used for all tests reported in this thesis.

**Sound Enamel**

Work conducted in our laboratory on the mechanical properties of unaffected enamel has shown that a force of between 50 and 150 mN could be used to obtain reproducible results (80). To ensure standardisation and allow direct comparison with hypomineralised enamel the maximum force used on sound enamel was 20 mN.

Although the forces used in the present thesis were relatively small in comparison to previous studies, the forces used will result in a composite average of the mechanical properties of all dentine and enamel structures for each indentation.
Dwell Time

To ensure minimal creep during the unloading, a 30 second delay at the maximum load was performed. This dwell time portion of the force-displacement data acquisition allows creep about the indentation contact site to occur to minimise the creep during the unloading curve, allowing a reliable elastic modulus value from the slope of the tangent to the unloading curve to be determined by the software associated with the UMIS (Table 3-2). An elastic material will not exhibit additional deflection if held at maximum load. In contrast, visco-elastic materials will continue to show ongoing displacement at maximum load. Dentine especially highly carious material does exhibit the latter behaviour. A very short dwell time for such material causes an additional creep displacement during unloading and may result in a negative slope of the initial unloading curve. As the modulus of elasticity is calculated from the unloading slope, the dwell time will minimise the error (378). Figure 3-9 shows the difference of a single indent in dentine with and without the creep delay. As can be seen from the graph the slope of the unloading curve is significantly different between each indentation. The resulting modulus also significantly varies from 17.11 GPa if the creep delay is used to 22.02 GPa if the creep function is not used.

![Single Indentation in Dentine with and without Creep Function](image)

Figure 3-9. Graph illustrating the difference in the unloading curve (of dentine) with and without the dwell time (creep function) activated.
Data Analysis

Although the specific analysis and ways of comparing the tests varied depending upon the experiment, the determination of an individual indentation hardness and modulus was similar for all tests.

Prior to analysis of each indentation, it was important to evaluate the quality of the indentations and determine whether any values needed to be discarded. For all experiments this was done immediately after testing by viewing under the attached microscope each indentation in the array allowing inspection of their geometry and position (Figure 3-10). The software associated with the UMIS also allowed elimination of unsuitable indentation values by production of individual load-unload curves recorded for each indentation. By observing these graphs, extraneous data could be deleted as required. Figure 3-11 shows a force versus penetration graph for an acceptable and unacceptable indentation in dentine. Figure 3.10 shows that not only does the rejected indent not reach the appropriate penetration depth for dentine, but also the shape of the curve is abnormal (80). The reason for this could be a surface imperfection on cracking of the tissue being tested (Figure 3-10).

Figure 3-10. View through the microscope (associated with the UMIS) of an indentation with minimal cracking (left photograph) and an indentation with severe cracking (right photograph).
Figure 3-11. Force vs penetration graph showing an acceptable and an unacceptable indentation for dentine on a force vs displacement curve. The unacceptable indentation shows an obvious distortion on the loading portion of the indentation.

The software associated with the UMIS also allows analysis of the test data. This program determines and adjusts the data for initial penetration under the contact force and makes corrections for indenter geometry. Each indentation was individually analysed using a curvilinear fit for the upper fifth portion of the unloading curve. This information was then calculated and displayed as required. The two main outputs retrieved were;

- Hardness as a function of contact depth
- Elastic modulus of the material determined at the maximum penetration depth in terms of the effective biaxial modulus \( E^* = E_m / (1 - \nu_m^2) \), where \( E_m \) is the uniaxial elastic modulus and \( \nu_m \) is Poisson’s ratio of the material

All indentations using the UMIS utilised a Berkovich indenter tip which was calibrated using fused silica and the technique developed by Oliver and Pharr (325). It is likely that there are minor errors present in that the values of \( E \) and \( H \) reported as the UMIS relies upon a methodology developed for elastic plastic materials whereas all the materials investigated, (except possibly sound enamel), displayed visco-elastic behaviour for which there is as yet no universally agreed
analysis algorithms. The approach adopted whereby the visco-elastic creep component was incorporated in the testing methodology has been widely used by authors for materials as diverse as polymers to skin and therefore was considered appropriate for generating results that enable comparisons between the (carious) states of materials to be measured. A discussion on this and other sources of error associated with instrumented or nano-indentation tests is given in a paper by Mencik and Swain (379).

How the values for each indentation hardness or modulus of elasticity were used varied between individual experiments therefore for further discussion refer to the individual experiments in relevant chapters.

A typical hardness as a function of penetration depth graph for dentine is shown in Figure 3-12. The mean hardness for each indent was reported by averaging all data points except the first three due to the surface roughness and tip irregularity, and the last one which is the creep induced hardness. Increasing the dwell time leads to an increase in the size of the indentation for a given load and results in an apparent reduction in the hardness. This effect (indentation creep) was eliminated by removing the first three and the last hardness values for each indentation. This method of deleting the first three data points and last point is similar to that used by Poolthong (1998) and has been published by Mahoney and colleagues (2000).

![Graph](image)

Figure 3-12. Data used for averaging from hardness vs penetration graphs for a single indentation in dentine.
For further discussion on the UMIS see the relevant section in the literature review (Literature review Part 3).

**Scanning Electron Microscopy**

A number of the investigations in this thesis used SEM to visualise enamel and dentine specimens. Although for each specimen the size and area investigated varied, overall the sample preparation was similar.

**Critical Point Drying**

Prior to SEM the carious dentine samples required dehydration by critical point drying. This technique has been recommended as it causes minimal shrinkage to the carious test specimens (380). Non carious enamel and dentine samples such as hypoplastic/hypomineralised enamel also require dehydration although this is obtained by the sample being left covered (to prevent dust contamination) on a clean surface of laboratory bench for at least 24 hours.

Critical point drying requires a number of steps:

*Initial Dehydration*

Each specimen is immersed in 100% Acetone for 30 minutes. The samples are then immersed in fresh 100% Acetone for a further 60 minutes.

*Critical Point Drying*

The dehydrated specimens were transferred directly from the acetone dehydrating solution to the critical-point drying apparatus, which utilizes CO₂ as the exchanging gas medium, and then dried according to the standard automated procedure.

**Specimen Coating**

There are many variables that can influence image quality, the most important being specimen coating. Specimen coating enables detailed features to be highlighted especially for detailed examination of biological samples. This is because biological tissues are non conductive materials. Without coating this can result in considerable electrical charging (image interference) and thermal loading (overheating of the specimen). A thin continuous layer of metal is a reasonably
good conductor which should alleviate much of the interference and thermal loading.

Magnetron Sputter Deposition

Initially a dehydrated sample is placed in the coating rig and a vacuum—pressure of less than $10^{-7}$ of atmospheric pressure is obtained. A closely controlled flow of argon raises the pressure in the chamber to the levels needed to generate the ionised argon plasma by the magnetron induced high frequency electric field. The resultant argon sputters metallic ions from a target electrode which coat the specimens. The combination of pumping to the high vacuum region and the continual introduction of process for gas ensures that coating conditions are both clean and reproducible. Either platinum or gold to an approximate thickness of 20 nm are used as the coating medias in this thesis.

Operation of the SEM

The SEM is a powerful tool used in all facets of science to observe and characterise heterogeneous organic and inorganic materials on the micrometre scale. As the SEM has been extensively reported in all science literature and therefore detailed description of this system is considered unnecessary.

The SEM used for enamel specimens was the XL-30 (Appendix 2). Digital images were taken at 20 kV with an electron beam spot size of 5 nm. The samples were examined in secondary electron mode. Studies on dentine used the 505. Digital images were taken at 20 kV with a spot size of 5 nm.

Use of SEM for Other Investigation Techniques

The SEM allowed the investigation of other physical properties of test samples. These techniques include Back Scattered Electron (BSE) Image and Energy Dispersive X-ray Spectrometer (EDS). For these techniques the flat, uncoated samples that were prepared for the UMIS were used. If additional samples were required then they were identically prepared.
Back Scattered Electron (BSE) Image

The operation of BSE is identical to that described previously using the Scanning Electron Microscope XL-30, although the detector was a solid-state backscattered electron detector. The SEM was operated at 20kV, a spot size of 5 nm, and a beam current of 1nA, magnification of 50 times, a constant working distance of 10 mm. The ‘variable pressure’ mode, using ambient air as the introduced gas, at a pressure of 1.5 torr in the chamber was used. These parameters were kept constant during the taking of BSE micrographs.

The aim of BSE imaging for the experiment on hypomineralised enamel was to determine if the mineral content of hypomineralised enamel was similar to sound enamel or dentine. Additional image and data analysis was therefore required. Additional details on the use of BSE in analysing the mineral content of hard tissues can be found in a series of studies by Angker and colleagues (182;241).

BSE is a technique which enables the investigation of the mineral density of samples via the detection of high energy backscattered electrons leaving the surface of the specimen (252). The intensity of the backscattered electron signal is dependent upon the total number of electrons from the primary beam that are scattered by the atoms in the target material and subsequently captured by the BSE detector. For a constant electron beam intensity, the number of scattered electrons is a logarithmic function of atomic number of the target material (381). The contrast in the BSE image is created by the different intensities recorded from pixel to pixel which reflects the differences in the atomic number of the target (251). Hence, the image contrast of the sample is a function of the variations in the ‘average atomic number’ of the area imaged (250;382;383). It has been shown therefore that the BSE intensity can be used to estimate the relative mineral content of a calcified tissue, as the mineral component has higher atomic number than the organic component (241). This technique allows the analysis of subtle changes in mineral levels found in carious dentine with resolution determined by the pixel size and approaching 1 μm³ (384).

Calibration of the System

To map the image sensitivity of the BSE detector, a sample of pure carbon was used. An image of the carbon coated glass slab was taken. Immediately afterwards a standard block (consisting of a finely polished sample of silicon and
pure carbon- atomic number 14 and 6 respectively) embedded in resin was used to standardise the graylevel spectrum for every specimen (Figure 3-13). The contrast and brightness were preset using the horizontal line profile tool on the SEM, in which the graylevel spectrum was extended to reach the top line of the profile tool on the enamel and down to the bottom line on the carbon and kept constant during the session. Images of the carbon sample were taken at the beginning and the end of the SEM session.

Figure 3-13. a. Image of carbon typical standard block used. b. Image of typical silicon-carbon standard block used.

Mineral Quantification

Each test sample was placed together with silicon-carbon standard block together on the specimen table in the SEM chamber. Immediately an image of the area of interest was taken ensuring that both the specimen and standard block were in each digital image (Figure 3-14).
Image Analysis

Each of the BSE images were then analysed using Digital Micrograph 3.3.1 on a Macintosh computer. The BSE intensity was measured in graylevels (0-255). The calibration was done in two steps. Firstly, the images of the carbon sample (A) were analysed to determine the BSE detector sensitivity response as a function of position over the area of the image used in the study. Any variation in the response function of the BSE detector was corrected by calculating a new image of the glass plate (B), in which each pixel value of the original image of the coated glass plate was divided by the image mean value (B = A/mean value). A new image of the glass plate (B) with floating value as a response function of the BSE detector was reproduced [B = A/mean value]. This image (B) would be subsequently used to correct the images of the specimen and the standard block (C) for the response function. The corrected image (D) was obtained from C/B.

The graylevel spectra of the silicone-carbon blocks were then re-ranged with the silicone graylevel at 255 and carbon at 0 to correct any variations of the experimental condition (241).

![Image Analysis Diagram](image)

Figure 3-14. Typical test sample of a hypoplastic tooth.

Data Analysis

The gray levels of all regions in a corrected image were determined by linear scans, 10 pixels by 10 pixels. The gray levels for each pixel in each region for all the three teeth were averaged and graphed to allow comparison between each
tissue type. Care was taken to ensure that in determination of the graylevels that the linear scans were not done in carious regions of dentine.

In the present study the actual mineral content of the specimens were not determined, rather the differences in the graylevels were used to hypothesise on the relative difference in mineral content between enamel, hypomineralised enamel and dentine.

**Energy Dispersive X-ray Spectrometer (EDS)**

EDS (Figure 3-15) enables the chemical analysis in the SEM to be performed by measuring the energy and intensity distribution of the x-ray signal generated by a focused electron beam. The x-rays from the SEM pass to the EDS detector. The signal is amplified and passed into a multi-channel analyser where the pulses are sorted by their voltages. The X-rays emitted from the sample atoms are characteristic in energy and wavelength not only for the element of the parent atom, but which shells of each atom lost electrons and from which shells electrons replaced them. From this a spectrum is produced (Figure 3-16). From the area under the peaks, analysis of the elements can be done. The computer programme associated with the EDS system will convert the area under the peak into weight or atomic percent (Table 3-3). From this information, qualitative measurements (relative percents) can be evaluated from the variation in mineral ion peaks (385). From this information the ratios of minerals such as Ca/P ratio can be reported along with presence of trace elements. In the present study the X-ray detector system attached to a SEM (SEM 505) was used.

![Figure 3-15. EDS system prior to its attachment to a SEM.](image)
Sample Preparation
The EDS testing is conducted in a full vacuum and although this system does not require coating, the samples in study four were coated to allow visualisation of the sample under secondary electrons. The sample is prepared identical to that for the UMIS and is coated in an identical way as for any SEM investigation. Carbon was chosen as the peak produced by carbon does not interfere with any peaks that the test was investigating, for example Ca, P, and Mg etc...

Data Acquisition
The EDS system was used in two sections of the present study. Firstly it was used to determine the percent component composition (such as Ca/P ratio) and the presence of trace elements in hypomineralised enamel. In this portion of the present study the system was operated at 20 kV, spot size of 5nm, specimen tilt of 15° and 15 to 20 mm working distance was used. The counting time was 100 sec. The X-rays were detected in a window approximately 1 x 1 mm. The counts can be conducted over various regions of test samples. The EDS system was also used to determine if there was a significant difference in the composition between sound dentine and dentine from teeth with enamel hypomineralisation. For this section of the study, the system was operated at 20 kV, spot size of 5nm, specimen tilt of 15° and 15 to 20 mm working distance was used. The counting time was 100 sec. Five readings in each area investigated were used at 2003 times magnification, each over an area of approximately 100 μm. The counts were conducted over various regions of test samples.
Figure 3-16. Example of spectrum analysis of dentine showing the peaks for Ca, P, Mg and Na. The two remaining peaks on the left (from left to right) of the image are for carbon and oxygen respectively and are not used in analysis.

<table>
<thead>
<tr>
<th>Element</th>
<th>Atomic %</th>
<th>Weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>60.49</td>
<td>66.67</td>
</tr>
<tr>
<td>P</td>
<td>37.55</td>
<td>31.99</td>
</tr>
<tr>
<td>Na</td>
<td>1.52</td>
<td>0.96</td>
</tr>
<tr>
<td>Mg</td>
<td>0.23</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Table 3-3. Example of results produced by EDS system for enamel.

**X-Ray Diffraction (XRD)**

Some of the most sophisticated physical methods available have been used to study synthetic, biological and mineral apatites. One of these methods, XRD is a major tool used to investigate structures at the atomic scale dimension (386;387). Initially, it was used for the determination of crystal structure although it is now also commonly used to determine the identification, purity, crystallite size and type of apatite. The Ca:P ratio, presence of mixed phases and the amount and type of substitution of the structure can also be determined by XRD (388). XRD measures atomic spacing in crystals using diffraction. When an X-ray strikes an atom, it is scattered in all directions. In crystalline materials most of the beams scattered by the atoms interfere destructively. However, under some conditions the interference is constructive resulting in these reflected X-ray beams being
detected in diffraction experiments (389). The radiation used is copper k-alpha with a wavelength of 1.5418 Angstroms. Traditionally, powdered samples are used in XRD although it can be used to characterize solid samples ranging in size from about 1 millimeter square up to intact four-inch wafers.

XRD analysis enables the volume (vol) and/or weight (wt) percent (%) of the various crystalline phases present in a specimen to be determined. Typically the average phase values of biological tissues and therefore its main limitation lies in the detection limit of these phases (1 wt% of crystalline phase and higher for poorly crystalline phases) (388). This method if used in combination with other methods can give a comprehensive picture of the physiochemical properties of synthetic and biological apatites.

Sample Preparation

Sample preparation for XRD is very different from other methods used in the present studies. It is advantageous to have a flat sample of the dental tissue although this may not always be applicable due to specimen size constraints.

In the present study both sound and hypomineralised samples were used to analyse the mineral phase. Attempts were made to carry out analysis in each enamel specimen by covering the unwanted dental tissue with a number of different coatings (aluminum tape, tinfoil, various clays etc...). None of the coating used were able to block diffraction measured from the unwanted tissue or the coatings had similar diffraction patterns as hydroxyapatite or other biological apatites. Therefore samples of sound and hypomineralised enamel were fractured away from the surrounding dental tissues. This was done by making cuts above and below the area of interest with a conventional dental bur. From these indentations the piece of dental tissue for investigation was prepared. Soflex discs were used to remove any unwanted enamel and dentine from the sample. The samples were then washed in distilled water and left to dry.

Operation of XRD

In the present study a Siemens XRD machine was utilised (Figure 3-17). The computer software associated with the XRD enabled the recording and analysis of the x-ray diffraction pattern results obtained from each samples.
The prepared sample is placed in a sample holder and held in place by a small amount of clay (Figure 3-18).

![Sample holder/goniometer](image)

Figure 3-17. Siemens Diffraktometer D5000.

![Sample holder](image)

Figure 3-18. Sample holder.

The sample holder slides into the fixture normally installed on the goniometer and is supported on its lower and upper surface under spring pressure. The sample is then tested. The X-ray detector was scanned from 3° to 40°, with 0.01° step size, and 10 seconds analysis time per step. CuKα radiation working at 40kV and 40 mA was used in this study. Each test took approximately 6 hours.
Chapter 4 MECHANICAL PROPERTIES OF CARIOUS DENTINE

Dental caries is a destructive infectious process which results in a deterioration of the mechanical properties of dentine through a process of demineralisation (211;390). Historically management of dental caries has involved the mechanical removal of the infected, degraded tooth tissue and its replacement by an inert restorative material. Commonly the amount of tooth tissue that needs to be removed prior to the placement of the restorative material is determined subjectively based upon clinical assessment of the hardness of the remaining tissue. When such methods are used the amount of dentine that is removed has been shown to vary considerably between clinicians (211;390) resulting in equally variable amounts of carious tooth tissue being left behind.(391;392). The effect of placing restorations over residual dentinal caries is relevant as, if successful, it could reduce the interventive nature of dental treatment a fact that would be of value to both clinicians and patients alike. The potential benefit of this approach has caused researchers to investigate the success of restorations placed directly over frank caries (8;190). Mertz-Fairhurst and co-workers are responsible for a 10 year landmark clinical trial that strongly suggests that the carious process can be arrested beneath a restoration (8;191;192;194-199;393). However apart from the relatively subjective clinical and radiographic assessment of the dentine beneath the restorations, the study had no objective method of assessing whether the carious process had been reversed or merely arrested as a result of the placement of the restorations. In short, the degree of remineralisation of the carious dentine could not be determined. Similarly Ribeiro and colleagues (1999) used clinical, radiographic and scanning electron microscopy to investigate the effect of partial (experimental group) and complete (control group) removal of infected carious dentine in primary teeth. The retention rate, marginal integrity and pulpal symptoms were identical between the groups at 1 year with 75% of the experimental teeth failing to show radiographically any increase in the size of the residual carious lesion (190). Again there was no quantitative evaluation of changes in mineral content of the carious lesions.

In addition to this clinical research, in vitro studies have evaluated the effect of dentinal caries on the bond strength and sealing ability of various dental
materials (394-397). Yoshiyama and colleagues have consistently shown the bond between sound dentine and resin to be superior to that between caries affected dentine (hard dentine stainable with a caries detector dye) and resin which in turn is superior to the bond between resin and infected dentine (denatured, active carious dentine) (395;396). It has been noted, however that these lower bond strengths potentially may arise from lower tensile strengths of the carious dentine itself as opposed to an actual reduction in the adhesive bond between resin and the carious tooth tissue (395), although at present there is very little data to support this hypothesis.

The hardness of a tissue may be defined as its ability to resist permanent indentation (67) and the modulus of elasticity (stiffness) is the ratio of stress to corresponding strain below the proportional limit. The modulus of elasticity provides an indication of the amount of reversible deformation that may occur in a tissue when a load is applied (100). Measuring hardness has been shown to be a reasonable method of examining the inorganic content of a calcified tissue including tooth tissue (69;182;218;304). To date the bulk of research in this field has concentrated on the changes in hardness across carious dentine in permanent teeth (161;164;390;398). However recently decreases in the hardness and modulus of elasticity (measured using an ultra-micro indentation system -UMIS) have been shown to be directly related to the mineral content (measured using scanning electron microscopy backscattered electron microscopy, SEM-BSE) of occlusal carious lesions in the dentine of primary molars (182). To date there has been no attempt to record the mechanical properties of smooth surface lesions in primary teeth despite that fact that smooth surfaces of anterior primary incisors are particularly common sites for caries in young children. This type of dental decay in infants or toddlers is termed early childhood caries (EEC) (399;400). Restoration of primary incisors has been shown to be relatively unsuccessful due in part to the challenges of bonding restorative materials to small amounts of compromised dental hard tissues (401-406). Therefore the aim of this study was to describe the mechanical properties across carious lesions in primary incisors with the intention being to improve understanding of the carious process in these sites.
Materials and Methods

Eight primary central incisors which had been extracted due to dental decay were collected for this study. The inclusion criteria was (i) teeth needed to be from children less than 4.5 years old at time of extraction, (ii) there should be no history of dental abscess associated with any of the teeth and (iii) there must be at least one carious lesion on the labial surface of each tooth.

The teeth were stored and prepared as described in Chapter 3. After polishing, a grid reference was scored on the polished surface with a scalpel blade to allow orientation of the specimen for the experiments. Once prepared and while waiting testing, the samples were kept fully hydrated in deionised water with a small number of thymol crystals. The effect of storage of carious dentine in deionised water is unknown although it has been shown to affect enamel and dentine of sound teeth (374).

The use of the UMIS to evaluate the hardness and modulus of elasticity of small specimens of enamel and dentine has been reported previously (80;82;376). When each specimen was ready for testing it was placed on the work table of the UMIS with the polished surface upwards. The area to be tested was then determined with the aid of the microscope associated with the UMIS. Depending on the size of the carious lesion, between four and six lines of indents were conducted in the carious lesion of each tooth (Figure 4-1), with the first series starting at the incisal region of the each tooth with each subsequent array approximately 500 µm apically. The first indentation in an array began in sound or minimally affected dentine, 200 µm from the most pulpal edge of the discolouration front with each subsequent indentation in each series 100 µm labially to first indent. The last indent was within the last 100 µm of the tooth surface. Attempts were made to follow the path of the dentinal tubules. The number of indents per series depended on the depth of the carious lesion but varied between 10 and 25 indentations per line. For all teeth, a single series of indentations was also carried out in unaffected dentine apical to the lesion by way of a control. Unlike the indentations conducted in the carious regions, these indentations were conducted under sound enamel. Therefore the last indentations in sound dentine were conducted within 100 µm of the enamel dentine junction. Each indentation was carried out in 25 increments to a maximum force of 5 mN using the Berkovich indenter. Due to the small sample
sizes, at all times the dentine was kept 100% hydrated by the placement of
droplets of distilled water over the entire carious lesion, rather than completely
being completely submerged in water. The UMIS was allowed at least 20 minutes
to stabilise after the water was placed on the sample.

![Diagram](image)

**Figure 4-1. Diagrammatic representation of layout of indentations.**

The software associated with the UMIS calculates the hardness as a function of
the depth of penetration of each indentation. The UMIS calculates the hardness
at each of 25 increments to a maximum force of 5mN and from this the mean
hardness is calculated to give the overall hardness for each indentation. The
modulus of elasticity for each indentation is calculated as a function of the
unloading curve at the maximum depth of penetration (9;80).

The hardness and modulus of elasticity for the outer, middle and inner third of the
carious lesion of each tooth were determined by combining and averaging the
values obtained for the four to six arrays conducted for each tooth. The inner and
outer dentine regions combined all indentations within 500 µm from the pulp and
within 500 µm from the carious lesion surface respectively. The middle region
was all indentations between these two regions. Additionally, results of the
change in the mechanical properties (hardness and modulus of elasticity) were
graphed for each tooth from which general trends across the carious lesion could be determined. These results were compared with the series of indents carried out in unaffected dentine of each tooth.

**Results**

A single value for the hardness and elastic modulus of the carious lesion for each tooth could not be determined as these properties varied significantly throughout each lesion. Therefore the mechanical properties of the inner, middle and surface third of all test teeth were combined and an average, median and range for each third was calculated and shown in Table 4-1. This table shows that the mechanical properties of carious lesions in the primary dentition decreased from the inner region to the surface (as shown by the median and average values for each region) although the actual values vary significantly between each specimen (as shown by the wide range of values).

Table 4-2 shows the mechanical properties of the three regions for sound dentine. The average percentage reductions in hardness and modulus of elasticity of the eight test teeth at the surface, middle and inner third of the carious lesions were compared to those of the unaffected dentine in the same regions (Figure 4-2). These graphs demonstrate the significant reduction in hardness of carious dentine from the inner aspect through to the surface of the lesion. The outer surface third of the carious lesions had, on average, only 10% of the mechanical properties of sound dentine. From these graphs it is also obvious that although the indentations began 200 μm pulpal to the discoloration front (i.e. to the apparent front of the carious lesion) there was already a reduction in both the hardness and modulus of elasticity of 10 and 20% respectively in this region.

Closer examination of the changes in mechanical properties across individual arrays reveals two distinct patterns. Two of the teeth (teeth 1 and 6) showed a constant reduction in hardness and modulus of elasticity from the 'unaffected dentine' towards the surface of the lesion (Figure 4-3). The remaining teeth showed a consistent decrease in the mechanical properties from the 'unaffected dentine' to the surface of the lesion for 1000 to 1200 μm. This was followed by an increase in both the hardness and modulus of elasticity in the most superficial
300 - 500 μm (Figure 4-4). This second pattern was seen consistently across all the arrays in teeth 2-5, 7 and 8.

In the caries free control regions there was no significant change in mechanical properties (Figure 4-5).
<table>
<thead>
<tr>
<th>Dentine regions</th>
<th>Hardness (GPa) mean ± SD</th>
<th>Hardness range (GPa)</th>
<th>Median (GPa)</th>
<th>Modulus of elasticity (GPa) mean ± SD</th>
<th>Modulus of elasticity range (GPa)</th>
<th>Median (GPa)</th>
<th>Number of indentations (measurements)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer/surface region of carious lesion</td>
<td>0.07 ± 0.1</td>
<td>0.0007 - 0.30</td>
<td>0.01</td>
<td>0.99 ± 1.45</td>
<td>0.01 - 4.88</td>
<td>0.12</td>
<td>379</td>
</tr>
<tr>
<td>Middle of carious lesion</td>
<td>0.15 ± 0.14</td>
<td>0.01 - 0.47</td>
<td>0.10</td>
<td>3.50 ± 3.67</td>
<td>0.07 - 15.3</td>
<td>2.16</td>
<td>385</td>
</tr>
<tr>
<td>Inner portion of carious lesion</td>
<td>0.29 ± 0.16</td>
<td>0.05 - 0.72</td>
<td>0.28</td>
<td>7.77 ± 5.05</td>
<td>2.07 - 28.33</td>
<td>5.98</td>
<td>390</td>
</tr>
</tbody>
</table>

Table 4-1. The mean values ± standard deviation, median and the range of hardness and modulus in three specified regions of primary carious dentine.
<table>
<thead>
<tr>
<th>Sound dentine regions</th>
<th>Hardness (GPa) mean ± SD</th>
<th>Hardness range (GPa)</th>
<th>Median (GPa)</th>
<th>Modulus of elasticity (GPa) mean ± SD</th>
<th>Modulus of elasticity range (GPa)</th>
<th>Median (GPa)</th>
<th>Number of indentations (measurements)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer/surface</td>
<td>0.40 ± 0.18</td>
<td>0.22 - 0.65</td>
<td>0.32</td>
<td>8.96 ± 3.14</td>
<td>5.6 - 13.2</td>
<td>8.10</td>
<td>60</td>
</tr>
<tr>
<td>Middle</td>
<td>0.45 ± 0.18</td>
<td>0.39 - 0.73</td>
<td>0.39</td>
<td>10.98 ± 2.53</td>
<td>6.45 - 13.50</td>
<td>11.39</td>
<td>64</td>
</tr>
<tr>
<td>Inner</td>
<td>0.39 ± 0.16</td>
<td>0.16 - 0.62</td>
<td>0.37</td>
<td>11.14 ± 4.35</td>
<td>6.95 - 12.82</td>
<td>9.53</td>
<td>57</td>
</tr>
</tbody>
</table>

Table 4-2. The mean values ± standard deviation, median and range of hardness and modulus of elasticity in three specified regions of primary sound dentine.
Figure 4-2. a) Percent reduction in hardness across carious lesion and b) Percent reduction in modulus of elasticity across carious lesion.
Figure 4-3. a) Hardness of a single array in tooth 6 showing marked increase in hardness from the surface of lesion pulpally and b) Modulus of elasticity of single array in tooth 6 showing marked increase in modulus from the surface of lesion pulpally.
Figure 4-4. a) Hardness of single array of tooth 8 showing increase in mechanical properties from 500 μm of the surface and b) Modulus of elasticity of single array of tooth 8 showing increase in mechanical properties from 500 μm of the surface.
Figure 4-5. a) Hardness of caries free region of tooth 8 showing no change in mechanical properties from surface of lesion pulpally and b) Modulus of elasticity of caries free region of tooth 8 showing no change in mechanical properties from surface of lesion pulpally.
Discussion

Although there has been a report on the mechanical properties of sound and mildly affected dentine surrounding a caries lesion in primary incisors (84), this is the first report describing the mechanical properties of the entire carious lesions in primary incisors. Under conditions of constant hydration the hardness and elastic modulus of carious lesions have been compared with those of unaffected dentine in the same teeth. The inner portion of the carious dentine lesion had an average hardness and modulus of elasticity of 0.29 ± 0.16 GPa and 7.77 ± 5.05 GPa respectively. The teeth chosen for this study had severe caries which resulted in the dentine in the inner region being demineralised. Therefore the inner portion of the carious dentine had lower mechanical properties than would be expected in sound teeth. From the inner region, the mechanical properties deteriorated progressively toward the lesion cavity floor where the lowest values for hardness and modulus of elasticity were found (0.07 ± 0.1 GPa and 0.99 ± 1.45 GPa respectively). In contrast, the mechanical properties of sound dentine from the pulpal (inner) to surface of the dentine did not show the same deterioration, with similar hardness and modulus of elasticity’s recorded for the inner (0.39 ± 0.16 GPa) and surface regions (0.40 ± 0.18 GPa).

Although in general the mechanical properties of carious dentine of the smooth surface lesions decrease from the inner to the surface of the carious lesion, two distinct trends are seen. Two of the teeth tested showed a constant reduction in hardness and modulus of elasticity from the inner dentine towards the surface of the lesion. This finding is consistent with the view that the dentine at the surface of a carious lesion will be completely demineralised with the remaining collagen severely denatured. This region is thought to be ‘infected dentine’ and unable to be remineralised (172). Conversely, six of the teeth showed a decrease in the mechanical properties from the inner dentine towards the surface of the lesion until the final 300 - 500 μm towards the surface, when the mechanical properties increased. With the exception of one very recent study (185) who reported a similar finding in occlusal lesions of primary teeth, this has not previously been reported. It is possible that this increase in mechanical properties is evidence of a natural remineralisation process that might take place in the mouth resulting from the capacity of saliva to promote mineral deposition in open lesions (185). It is expected that the capacity for remineralisation is greater in smooth surface
lesions compared with occlusal lesions due to relative ease of exposure to saliva and other remineralising factors such as fluoride.

At present there is limited information in the dental literature on the mechanical properties of carious lesions particularly those in the primary dentition. Hosoya and colleagues [2000] investigated the mechanical properties of carious dentine on deciduous anterior teeth. This study utilised seven teeth (four primary canines and three primary anterior incisors) that were dehydrated and tested with a conventional microhardness tester at a 15 g load. However the very soft nature of the ‘infected zone’ of the carious lesion meant that accurate measurement of hardness in this zone was impossible so the discussion was limited to hardness values of dentine under and surrounding the carious lesion (84). The same authors have used more recently a depth-sensing nano-indenter to investigate primary canine teeth (83). Using three extracted or exfoliated carious primary canines, indentations were made with a Berkovich indenter on various positions around the tooth including in the carious lesion. The mechanical properties of the affected carious region and the dentine surrounding the carious lesion were determined. No attempt was made to determine the mechanical properties of the actual infected caries in this study and the study was conducted on dry samples (83). Comparison of the current study with those by Hosoya and colleagues [2000 and 2004] are difficult not only because different areas of the teeth were studied but also the current study used hydrated samples (which is clinically more realistic) whereas the earlier work used dehydrated samples. The effect of hydration on the mechanical properties of primary tooth dentine is significant with a 10 fold increase in both hardness and elastic modulus being recorded when specimens were dehydrated (12). Marshall and colleagues have investigated the mechanical properties of carious lesions in permanent teeth (type not specified) (161). They used the atomic force microscope (AFM) to determine the hardness and modulus of elasticity of fully hydrated carious peritubular and intertubular dentine. They showed graphically that the mechanical properties (hardness and modulus of elasticity) of carious lesions decreased significantly from the apparently normal dentine into the discoloured regions, consistent with the present study. Marshall et al., (2001) did not report any increase in mechanical properties at the surface of the lesions in their teeth. However it was not clear in their study whether they were investigating occlusal or smooth surface carious lesions (161). It is difficult to compare the results from these studies because not
only were the permanent teeth investigated but also the actual values for the mechanical properties were not reported.

The values reported in the present study for the mechanical properties of sound dentine are low in comparison to previous studies on either primary or permanent teeth (10;72;80;82;83;83;90). Hosoya and Marshall (2004) and Angker and colleagues have recently published studies on the mechanical properties of sound dentine in primary canines and primary molar teeth respectively and their findings along with those of the present study are summarised in Table 4-3. The study by Hosoya and Marshall investigating the hardness and modulus of elasticity of the sound dentine surrounding carious dentine in primary canines found similar values for the hardness reported in the present study although the modulus of elasticity reported were substantially higher (83). Angker and colleagues (2003) utilising the UMIS and primary molar teeth with large carious lesions, reported the hardness and modulus of elasticity of the inner, middle and surface of sound dentine to be much higher than in the present study. The reason for the lower reported values of the present study and that reported by Angker and colleagues (2003) is unknown with both studies being conducted on the same apparatus on hydrated primary teeth. Other than the obvious differences in the type of tooth used, another difference between the studies is the variation in orientation of indentation with the present study being conducted in the lingual-labial direction in contrast to the mesial-distal axial section. Finally the present study used very carious primary incisors whereas although not reported in the study by Angker and colleagues, the extent of the carious process of decay may have been lower.

Although the present study found the mechanical properties of sound dentine were lower overall than in other studies, the hardness and modulus of elasticity in the dentine regions adjacent to the ADJ (outer dentine) and the pulp (inner dentine) were on average lower than in the middle region. This is similar to at least three other studies (86;164;245). Angker and colleagues (2003) and Hosoya and Marshall (2004) however found only the average mechanical properties near the pulp to be lower (82;83).

Primary incisors in very young children are commonly affected with dentine caries. Traditionally, the treatment for such carious lesions is to restore them with a resin or glass ionomer restorative material, after removal of the carious dentine,
or, if the carious process is severe, to extract the affected tooth. Restoration of primary incisors can be clinically challenging not least as a result of the limited cooperation shown by some young children. As a result residual caries may remain and a temporary restoration placed. Furthermore the carious process itself may well result in a significant loss of good quality enamel as well as demineralising the underlying dentine. Both resin and glass ionomer cement based materials rely predominantly on the formation of a good bond to tooth structure which in turn may be significantly compromised by the carious process. The results of the present study show that the mechanical properties of carious dentine change dramatically throughout the lesion which will have implications on the quality/dependability of the resulting bond with any adhesive tooth colored restorative material. Yoshiyama [2002] has postulated that the lower tensile strengths of caries-infected dentine itself will affect the bond strength for resin materials (395). Furthermore the dramatic reduction in modulus of elasticity seen in dentine caries may mean that it will flex more than any restorative material placed upon it when loaded under occlusal function. These differential flexural properties may cause further deterioration in the integrity of the bond and may explain why some restorations ultimately fail if placed over carious dentine.

The present study has shown that in the majority of carious teeth tested that the surface 300 – 500 μm of a carious lesion has the ability to re-harden. This is most likely to be due to redeposition of mineral from saliva resulting in minor remineralisation although this was not investigated in the present study. If remineralisation is occurring even in severely carious primary incisor teeth then future treatment of these teeth may be preventive and palliative rather than surgical.
<table>
<thead>
<tr>
<th>Study</th>
<th>Outer- near ADJ</th>
<th>Middle</th>
<th>Inner- near pulp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hardness</td>
<td>E*</td>
<td>Hardness</td>
</tr>
<tr>
<td>Present study</td>
<td>0.40 ± 0.18</td>
<td>8.96 ± 3.14</td>
<td>0.45 ± 0.18</td>
</tr>
<tr>
<td>Angker et al., 2003 (82)</td>
<td>0.91 ± 0.15</td>
<td>16.33 ± 3.83</td>
<td>0.85 ± 0.19</td>
</tr>
<tr>
<td>Hosoya and Marshall, 2004 (83)</td>
<td>0.57 ± 0.12</td>
<td>24.27 ± 3.93</td>
<td>0.56 ± 0.12</td>
</tr>
</tbody>
</table>

Table 4-3. Table comparing the mechanical properties of sound primary dentine in the present study with two recent studies conducted on primary teeth.

E*: modulus of elasticity, ADJ: amelo-dentinal junction
Chapter 5 MECHANICAL PROPERTIES AND MICROSTRUCTURE OF HYPOMINERALISED FIRST PERMANENT MOLAR TEETH

Enamel hypoplasia is characterised by a deficiency of tooth substance that ranges from minor pits and grooves to total absence of enamel caused by a disruption to the ameloblasts during matrix secretion (108;109;131). If the disruption occurs during either the calcification or maturation phase of enamel formation then upon eruption portions of the teeth will appear opaque. This is a qualitative defect termed enamel hypomineralisation. Both of these terms are based solely on descriptive criteria and no association is made with aetiology (112). For the purposes of this thesis the term hypomineralisation will be used to refer to the affected teeth.

To date the research on hypomineralised teeth has focused on its prevalence and cause. There has been little work on the structure and composition of affected molars. The aim of this chapter is to investigate the mechanical and physical properties of hypomineralised enamel and the dentine of affected teeth in a series of experiments.

The first series of experiments was conducted on hypomineralised and sound enamel. The mechanical properties of unaffected and hypomineralised enamel and dentine were initially compared using the UMIS. The mechanical properties of hypomineralised enamel were found to be significantly lower than unaffected enamel. To investigate this further a series of experiments using Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Spectrometer (EDS) and X-Ray diffractometer (XRD) and BSE analysis were completed and a pilot study using TEM was also conducted. The majority of this work has recently been published (Appendix 1).

The second series of experiments was conducted on the dentine of first permanent molar teeth affected with enamel hypomineralisation. This was done because it was hypothesised that if the severity of an insult was sufficient to
cause damage to the ameloblasts resulting in enamel hypomineralisation, the same injury may also damage the odontoblasts. This could potentially result in changes to the structure and composition of the dentine beneath the affected enamel. In the second part of this study the mechanical properties of the dentine of affected molars were investigated with the UMIS and the findings were verified with SEM and EDS.
Chapter 5a  Mechanical Properties of Hypomineralised Enamel

Isolated enamel defects are commonly seen in first permanent molar teeth and are usually a combination of enamel hypoplasia and hypomineralisation (113). To date the research on hypomineralised teeth has focused on the prevalence and the aetiology. There has been little work on the structure and composition of affected molars. Such information may assist in defining better treatment strategies for these teeth, which traditionally pose a significant clinical challenge. The crowns of affected teeth are often compromised in terms of both the quantity and the quality of the remaining tooth tissue. Not only does this make choosing the appropriate restorative material difficult but also defining the most durable restorative technique (i.e. cavity form and/or adhesive mechanism) can be challenging. The defective enamel can fracture under normal occlusal forces leading to excessive chipping and wear (117;122). Understanding the structure and properties of hypomineralised enamel is fundamental to improving the restorative outcomes for these teeth.

Knowledge of the mechanical properties of teeth such as, the hardness and modulus of elasticity may lead to an improved understanding of the behaviour of hypoplastic teeth both in function (i.e.: why they chip and wear) and to what extent this structure must be removed prior to restoration to minimise the likelihood of recurrent failure. Unfortunately little is known about the mechanical properties of hypomineralised enamel. This may reflect the difficulty in obtaining samples as well as the limitations of conventional compressive and tensile tests which require large specimen samples for adequate testing. Commonly hypomineralisation tends to affect only the cusp tips or the sides of cusp tips in first permanent molars (131), which severely limits the potential size of the specimen. The Ultra-Micro-Indentation System (UMIS), a nano-indentation system allows multiple tests on a small areas of hydrated material to be conducted (80;82;376).

It has been suggested that the mechanical properties of a calcified tissue are generally linked to its mineral content (69;76;218;304). Incorporation of additional
phases such as carbonate, magnesium, sodium and fluoride into the apatite structure of enamel crystals cause changes in the physico-chemical and mechanical properties of enamel. For example a higher concentration of carbonate in a crystal structure leads to a lower crystallinity and therefore higher solubility of hard dental tissues, whereas, fluoride substitution results in bigger crystals and better crystallinity (fewer crystal defects), which in turn reduces the enamel solubility (388). Understanding the nature and content of the mineral is also important when trying to bond dental materials to tooth tissue. Some materials, such as glass ionomer cements, which are used to restore hypomineralised defects, rely on bonding to the mineralised components of the tooth substrate. Energy Dispersive X-ray Spectrometry (EDS) and X-Ray diffraction can be used to acquire information on the biological apatites present in enamel. These methods cannot however, provide quantitative measurement of mineral content. Backscattered Scanning Electron (BSE) imaging has been used to study the calcification state of mineralised tissues such as bone and dental hard tissues (241;242;245;253). The graylevel intensity in the BSE image has been reported to be highly correlated with calcium content (244), mineral content, mineral density and atomic number of bone (246;247). This system is similar to microradiography but has better resolution and has the additional advantage of simpler specimen preparation (242).

Understanding the structure and properties of hypomineralised enamel is fundamental to improving the restorative outcomes for these teeth. The aim of this study was to determine the mechanical properties of hypomineralised first permanent molar teeth (with the UMIS), determine the morphological structure of hypomineralised defects and the effect standard etching has using scanning electron microscopy (SEM), determine the chemical composition and crystalline structure of the hypomineralised areas using Energy Dispersive X-ray Spectrometer (EDS) and X-Ray diffraction and finally to measure the mineral content of the hypomineralised tissue in comparison to unaffected enamel using BSE images.

**Material and Methods**

Eight first permanent molar test teeth extracted due to severe enamel hypomineralisation with or without enamel hypoplasia and two control teeth (first
Premolars extracted for orthodontic reasons) were used in this study. A further five hypomineralised teeth and a single premolar tooth were used for the etching experiment for SEM. Premolars were used as control teeth as there was difficulty in obtaining caries free first permanent molar teeth. There was no information available on the history of the teeth or the patients from which they were extracted.

Upon extraction each tooth was placed into deionised water with a small number of thymol crystals to inhibit bacterial growth and stored at 4°C. All specimens were prepared for indentation as described in Chapter 3. After each specimen was enclosed in resin, the teeth were axially sectioned through the centre of the tooth which coincided with the centre of the hypomineralised/hypoplastic lesion. Each of the teeth was polished and stored as describe in Chapter 3. Tests were typically conducted within 2 days of preparation.

**Mechanical Properties**

When each specimen was ready for testing, it was placed on the work table of the UMIS with the polished surface upwards.

Two series of indentations were conducted in various regions on each of the test teeth and the control teeth. All indentations were conducted on the prepared surface to a maximum force of 20 mN. The maximum force was reached with 25 increments and the displacement recorded at each step. The force at each increment is given according to the formula, \( F_i = (i/n)^2 F_m \) (where \( F_i \) is the force at the \( i \)th step, \( n \) is the total number of steps and \( F_m \) is the maximum force). All indentations were made using a Berkovich indenter. At all times the test surface was kept 100% hydrated with the specially constructed impermeable container (Chapter 3).

**Array series one:**

As the hypomineralised region of the test teeth is more opaque than the unaffected enamel, the areas to be tested could be determined visually with the aid of the microscope associated with the UMIS. The first indentation was between 200 and 400 µm from the external tooth surface. The subsequent indentations were made 100 µm from the previous indentation (Table 5-1) in a 3
by 3 arrangement. To determine the mechanical properties of 'normal', unaffected enamel, 10 indentations to a maximum force of 20 mN, were conducted in the (visually) unaffected enamel in the cervical region of each test tooth (Figure 5-1). Each indentation in this region began 100 to 200 μm from the enamel surface. Two sets of nine indentations were also carried out in identical regions of the enamel of the control teeth to a maximum force of 20 mN. The layout of the indentations and test conditions of the control teeth and the unaffected enamel were identical to the indentations carried out in the hypomineralised region (Table 5-1 and Figure 5-1).

<table>
<thead>
<tr>
<th>Test parameters</th>
<th>100 μm apart in both x and y axis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indentation layout</td>
<td>3 x 3</td>
</tr>
<tr>
<td>Maximum force</td>
<td>20 mN</td>
</tr>
<tr>
<td>Number of increments per indentation</td>
<td>25</td>
</tr>
<tr>
<td>Incremental Progression</td>
<td>Square Root</td>
</tr>
<tr>
<td>Dwell at load/max/unload (sec)</td>
<td>0.1/30/0.1</td>
</tr>
<tr>
<td>Test conditions</td>
<td>(23 ± 1)°C and (50 ± 10) % RH</td>
</tr>
</tbody>
</table>

Table 5-1. Test parameters and conditions for Array Series 1.

Figure 5-1. Optical cross section image of a prepared test tooth with indentation layout indicated.
The results were obtained by calculating the hardness at each of the 25 increments up to the maximum force of 20 mN, assuming the elastic modulus remained constant, these values were then averaged. Two way ANOVA tests were conducted to compare mean hardness and elasticity to (P<0.001). The overall mechanical properties were determined for the affected and unaffected enamel in the test teeth and the enamel in the control teeth by averaging all indentations of each tooth.

Array series two:

Each test and control tooth had a single line of indents parallel to the amelodental junction (ADJ) with the first indent starting at between 200 and 400 μm from the ADJ. Care was taken to ensure that each indentation in series one and two were at least 200 μm apart. Each subsequent indent was made 100 μm occlusally from the previous one with the last indent in enamel parallel to the dentine tip (Figure 5-2). Although the line of indents began at different distances from the CEJ, each indent on all teeth (test and control) was carried out 250 μm parallel to and along the entirety of the ADJ (Table 5-2). Depending on the size of the tooth and where the indents began, there were between 45 and 60 indents per tooth. The placement of the final indentation varied. In a large number of the experimental teeth, the cusp tip of the hypomineralised region had fractured away (commonly due to enamel hypomineralisation) prior to extraction. Therefore, the final indent was carried out either 100 μm from the edge of the remaining hypomineralised enamel or at the dentine cusp tip as shown in Figure 5-2.

<table>
<thead>
<tr>
<th>Test parameters</th>
<th>100 μm apart 250 parallel to ADJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indentation layout</td>
<td>20 mN</td>
</tr>
<tr>
<td>Maximum force</td>
<td>25</td>
</tr>
<tr>
<td>Number of increments per indentation</td>
<td>Square Root</td>
</tr>
<tr>
<td>Incremental Progression</td>
<td>0.1/30/0.1</td>
</tr>
<tr>
<td>Dwell at load/max/unload (sec)</td>
<td>(23 ± 1)°C and (50 ± 10) % RH</td>
</tr>
</tbody>
</table>

Table 5-2. Test parameters and conditions for Array Series 2.
Figure 5-2. Optical cross section image of prepared test tooth with indentation layout indicated. Also shown is the transition region that occurs between unaffected and hypomineralised enamel.

The range in mechanical properties across the enamel specimens was determined and the results graphed for both test and control teeth. Differences in both hardness and modulus of elasticity were identified.

**Scanning Electron Microscopy (SEM)**

Following micro-indentation tests, nine specimens were prepared for SEM. Two SEM investigations were undertaken.

*Experiment One; to compare fractured surfaces of affected and unaffected enamel.*

Fractured surfaces of unaffected and hypomineralised enamel were examined from one hypomineralised and one control tooth to reveal enamel microstructure. This was done by cutting a notch above and below the region to be investigated with a water cooled diamond impregnated circular saw. The notched specimens were then fractured through the sound and hypoplastic enamel. Care was taken to not cut into the affected region. Each specimen was then left to dry for 24 hours. The dehydrated specimens were then sputter coated with gold and observed using the SEM (Philip's 505) at 20 kV with a spot size of 5 nm. The
samples were examined in secondary electron mode from the same surface as was used for testing the mechanical properties.

_Experiment Two; to describe the effect of etching on affected and unaffected enamel._

Five hypomineralised molars (not used in the above indentation experiments) and one control tooth were enclosed in resin as described in Chapter 3. Each tooth was then sliced in 3 mm sections through the centre of the hypomineralised region with a water cooled diamond impregnated circular saw. To standardise the sections, each slice was polished as for indentation analysis as described in Chapter 3. Two sections of each tooth were each etched for 0, 5, 20, 40 and 60 seconds with 37% phosphoric acid. The same etching procedure was conducted for a single section of the control tooth. After the appropriate etching period the tooth sections were rinsed with distilled water for 60 seconds. For the sections that were not etched (0 seconds), to remove the smear layer caused by the polishing process, the tooth was conditioned with standard dentine conditioner (10% aqueous solution of copolymer of acrylic acid and maleic acid) for 20 seconds and then rinsed with distilled water for 60 seconds. Each specimen was then left to dry for 24 hours. The dehydrated specimens were then sputter coated with gold and observed using the SEM (Philip's 505) at 20 kV with a spot size of 5 nm. The same surface that was used for the testing the mechanical properties samples were examined in secondary electron mode.

**X-Ray Diffraction (XRD)**

To determine the crystal structure and crystallinity of hypomineralised enamel, XRD was carried out using a Siemens machine (Diffraktometer D5000, Karlsruhe, Germany) as described in Chapter 3. A small piece of hypomineralised enamel from the affected areas was fractured and placed in the XRD sample holder. The X-ray detector was scanned from 3° to 40°, with 0.01° step size, and 10 seconds analysis time per step. CuKα radiation working at 40kV and 40mA was used in this study. As a control, the same procedure was also carried out on a fracture of sound human enamel to compare the diffraction patterns between pathologic (hypomineralised) and sound enamel. Attempts were made to fracture the sound and hypomineralised enamel in a similar orientation. Both samples were orientated in a flat plane when analysed.
A qualitative estimate of the crystallinity of samples was determined by measuring the broadening of peaks. The broadening of XRD diffraction peaks reflects crystal size and/or perfection or strain: the broader the peak, the smaller or less perfect or more strained the crystals. To measure the broadening, the width of the peak was measured at the half point of the peak’s height. The height of the peaks is the distance from highest point of the peak to the base line of the peak (388).

**Energy Dispersive X-ray Spectrometer (EDS)**

A single, flat, carbon coated sample that had been used in the testing of mechanical properties was used for the EDS analysis as described in Chapter 3. The system was operated at 20 kV, spot size of 5nm, specimen tilt of 15° and 15 to 20 mm working distance was used. The counting time was 100 sec.

Five counts were conducted, three in the hypomineralised region and two in sound enamel of the same tooth. The counts in hypomineralised enamel were conducted in three areas half way between the amelo-dentinal junction and the surface of the sectioned test tooth. The unaffected enamel counts were conducted in the centre of the sound enamel in the cervical region of the test tooth. For all of the five measurements, the X-rays were detected in a window approximately 1 x 1 mm. This allowed the relative amounts of calcium (Ca), phosphorus (P), sodium (Na), potassium (K) and magnesium (Mg) to be determined.

**Back Scattered Electron (BSE) Image**

Following the UMIS tests, three uncoated test specimens were observed and photographed with back scatter electron detector using a Scanning Electron Microscope XL-30 (FEI, Eindhoven, Netherlands) with a solid-state backscattered electron detector (FEI, Eindhoven, Netherlands). The SEM was operated at 20kV, a spot size of 5 nm, and a beam current of 1nA, magnification of 50 times, a constant working distance of 10 mm and a pressure of 1.5 torr in the chamber. These parameters were kept constant during the taking of BSE micrographs. Image and data analysis is described in Chapter 3.
Transmission Electron Microscope (TEM)

TEM offers the ability to view the ultrastructural organisation of hypomineralised enamel crystallites. As part of the overall investigation of the physical and mechanical properties of hypomineralised/hypoplastic enamel, a pilot TEM study on a section of control and hypomineralised enamel was investigated. The primary investigator was an engineering undergraduate student at the University of Sydney and was supervised by this candidate, Dr Ramin Rohanizadeh and Professor Michael Swain. A full description of TEM sample preparation and operation of machinery operation can be found in (407).

Affected and unaffected enamel specimens from a single first permanent molar were cut using a standard sterile dental diamond drill. The two samples were enclosed in epoxy resin. For TEM observations, very thin samples are required for minimising the absorption of the electrons in the material to ensure electron transparency. A tripod polishing method was used to obtain a TEM cross section down to around 5-10μm. Attempts were made to thin the sample further with a microtome but due to limitations of the instrument at the University of Sydney and the friability of the hypomineralised enamel, this was unsuccessful. Therefore, standard ion beam thinning was performed to obtain a specimen thin enough to be studied on the TEM. An open slot grid (2 X1 mm) was used. A small amount of epoxy was applied to fix slot grid to the surface of specimen. The sample was left to cure for 30 minutes at 100°C. The specimen was removed from the attached grid by dissolving in acetone over night. The two TEM sections, one of hypomineralised enamel, one of sound enamel were observed using Philips CM120 Biotwin TEM operating at 120 kV.
Results

Mechanical properties

Array series one:
The depth penetrated by the indenter was on average, 300-500 nm in the sound dentine and between 1000-1500 nm in the hypomineralised region. The average hardness and modulus of elasticity for the test and control teeth is shown in Table 5-3. This table shows that the hardness of hypomineralised enamel varies from 0.10 ± 0.05 to 0.95 ± 0.12 GPa which was significantly lower (P<0.001) than that of unaffected enamel (2.89 ± 0.92 to 5.35 ± 1.10 GPa). The modulus of elasticity of the hypomineralised region (3.45 ± 1.08 to 23.81 ± 4.34 GPa) was also significantly lower (P<0.001) than unaffected enamel of the test teeth (60.90± 3.51 to 89.91 ± 7.67 GPa). The hardness and modulus of elasticity of the enamel of the control teeth were similar to the unaffected enamel of the test teeth. A summary of the overall mechanical properties of each tissue is shown in Table 5-4.
<table>
<thead>
<tr>
<th>Tooth Number</th>
<th>Unaffected enamel (GPa)</th>
<th>Hypomineralised Enamel (GPa)</th>
<th>Reduction in Mechanical Properties (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hardness</td>
<td>Modulus of Elasticity</td>
<td>Hardness</td>
</tr>
<tr>
<td>1</td>
<td>5.35 ± 1.10</td>
<td>80.36 ± 27.01</td>
<td>0.32 ± 0.16</td>
</tr>
<tr>
<td>2</td>
<td>3.48 ± 0.62</td>
<td>60.90 ± 3.51</td>
<td>0.67 ± 0.31</td>
</tr>
<tr>
<td>3</td>
<td>3.54 ± 0.43</td>
<td>85.70 ± 3.56</td>
<td>0.34 ± 0.18</td>
</tr>
<tr>
<td>4</td>
<td>3.73 ± 0.48</td>
<td>89.91 ± 7.67</td>
<td>0.73 ± 0.19</td>
</tr>
<tr>
<td>5</td>
<td>3.70 ± 0.79</td>
<td>70.50 ± 16.10</td>
<td>0.85 ± 0.40</td>
</tr>
<tr>
<td>6</td>
<td>2.89 ± 0.92</td>
<td>64.64 ± 9.16</td>
<td>0.10 ± 0.05</td>
</tr>
<tr>
<td>7</td>
<td>3.52 ± 0.39</td>
<td>77.90 ± 2.99</td>
<td>0.95 ± 0.12</td>
</tr>
<tr>
<td>8</td>
<td>3.48 ± 1.06</td>
<td>74.68 ± 7.84</td>
<td>0.31 ± 0.09</td>
</tr>
<tr>
<td>Control 1</td>
<td>3.09 ± 0.48</td>
<td>75.02 ± 7.72</td>
<td>NA</td>
</tr>
<tr>
<td>Control 2</td>
<td>3.31 ± 0.57</td>
<td>79.48 ± 7.48</td>
<td>NA</td>
</tr>
</tbody>
</table>

Table 5-3. Overall hardness and modulus of affected teeth.
<table>
<thead>
<tr>
<th></th>
<th>Average Hardness (GPa)</th>
<th>Average Modulus of Elasticity (GPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unaffected enamel of test tooth</td>
<td>3.66 ± 0.75</td>
<td>75.57 ± 9.98</td>
</tr>
<tr>
<td>Hypomineralised enamel of test tooth</td>
<td>0.53 ± 0.31</td>
<td>14.49 ± 7.56</td>
</tr>
<tr>
<td>Enamel from control teeth</td>
<td>3.2 ± 0.16</td>
<td>77.25 ± 3.15</td>
</tr>
</tbody>
</table>

Table 5.4. Mean overall hardness and modulus of elasticity of all three tissue types.
Array series two:
The mechanical properties of a control tooth are shown in Figure 5-3. These graphs, which were identical for both control teeth, show that the hardness and modulus of elasticity of the enamel from the CEJ region to the cusp across the enamel does not vary significantly. In comparison, Figure 5-4 shows an example of the hardness and modulus of elasticity for an affected specimen. The mechanical properties of the enamel closest to the CEJ are similar to those seen in the control teeth however as the indentations move more coronally towards the cuspal region both the hardness and modulus of elasticity decrease significantly until the mechanical properties are approximately 20% that of unaffected cervical enamel or that of the control teeth. Near identical patterns were found in all 8 affected teeth. Examination of these graphs show that the reduction in mechanical properties that occurs across the hypomineralised region is essentially linear through the area designated a ‘transition zone’ (Figure 5-2 and Figure 5-4), until the mechanical properties plateau. Because of this, defining a single value for either hardness or modulus of elasticity is inappropriate. Table 5-5 however summarises the ranges of values and medians recorded across these regions for all test specimens compared to the controls. The wide variation in mechanical properties across the hypomineralised regions may be due in part to the difficulties defining exactly where the hypomineralised region starts. Macroscopically the start of the linear decrease in mechanical properties appeared to coincide with the clinical appearance of the opaque lesion of the hypomineralised defect. However using the UMIS microscopic viewer the decrease in mechanical properties actually occurred between 400 and 800 μm cervical to the visual demarcation of the hypomineralised defect, which is approximately equivalent to the transition area of 500-600 μm (Table 5-5).
Figure 5-3. Mechanical properties of control tooth from enamel at CEJ to a position level with the dentine cusp tip. A. Hardness and B. Modulus of elasticity.
Figure 5-4. Mechanical properties parallel along ADJ from CEJ to dentine cusp tip of hypoplastic first permanent molar tooth. 
A. Typical Hardness and B. Modulus of Elasticity. Horizontal dashed lines drawn through the sound and hypomineralised enamel to assist with the determination of the width of the transition region.
<table>
<thead>
<tr>
<th>Tooth number</th>
<th>Width of transition area (µm)</th>
<th>Cervical enamel (GPa)</th>
<th>Hypoplastic region (GPa)</th>
<th>Modulus of elasticity range (GPa)</th>
<th>Modulus of elasticity (GPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hardness</td>
<td>Medians</td>
<td>Hardness</td>
<td>Medians</td>
</tr>
<tr>
<td>Control 1</td>
<td>NA</td>
<td></td>
<td>2.71 - 4.15</td>
<td>3.43</td>
<td>NA</td>
</tr>
<tr>
<td>Control 2</td>
<td>NA</td>
<td></td>
<td>2.86 - 4.13</td>
<td>3.42</td>
<td>NA</td>
</tr>
<tr>
<td>Hypomineralised 1</td>
<td>500</td>
<td>3.64 - 4.99</td>
<td>3.83</td>
<td>0.27 - 1.74</td>
<td>0.73</td>
</tr>
<tr>
<td>Hypomineralised 2</td>
<td>600</td>
<td>3.42 - 5.53</td>
<td>3.53</td>
<td>0.48 - 1.38</td>
<td>0.65</td>
</tr>
<tr>
<td>Hypomineralised 3</td>
<td>500</td>
<td>2.94 - 4.41</td>
<td>3.34</td>
<td>0.32 - 1.36</td>
<td>0.87</td>
</tr>
<tr>
<td>Hypomineralised 4</td>
<td>500</td>
<td>2.45 - 4.46</td>
<td>3.09</td>
<td>0.52 - 1.05</td>
<td>0.64</td>
</tr>
<tr>
<td>Hypomineralised 5</td>
<td>500</td>
<td>2.10 - 4.18</td>
<td>2.93</td>
<td>0.07 - 0.26</td>
<td>0.49</td>
</tr>
<tr>
<td>Hypomineralised 6</td>
<td>500</td>
<td>2.03 - 3.82</td>
<td>2.84</td>
<td>0.28 - 0.97</td>
<td>0.50</td>
</tr>
<tr>
<td>Hypomineralised 7</td>
<td>500</td>
<td>2.16 - 4.70</td>
<td>2.98</td>
<td>0.09 - 1.35</td>
<td>0.87</td>
</tr>
<tr>
<td>Hypomineralised 8</td>
<td>600</td>
<td>2.22 - 3.53</td>
<td>2.82</td>
<td>0.37 - 1.20</td>
<td>0.58</td>
</tr>
</tbody>
</table>

Table 5.5. Range of mechanical properties of test and control teeth. NA = not applicable. For construction of this table the hypoplastic region was defined as the area with that was discoloured to the naked eye. The rest of the indents were deemed unaffected cervical enamel. The minimum and maximum values of hardness and modulus were used to determine the range in each region.
SEM Images

SEM experiment one:
Typical fractured surface SEM images are shown in Figure 5-5. The images of normal enamel show an amorphous, orderly rod appearance. In contrast the hypomineralised enamel is disorganised, with variable rod widths and there is a loss of distinct boundaries between the enamel rods. In the hypomineralised enamel there are also obvious voids.

SEM experiment two:
Representative SEM images of etched hypomineralised and unaffected enamel are shown in Figure 5-6 a and b. The images of unaffected enamel at 5, 20, 40 and 60 seconds (etching) show increasingly the expected typical etching pattern, where the peripheral regions of the rods are dissolved preferentially leaving the cores intact. As expected, the images of etched unaffected enamel at 0 (dentine conditioning only) and 5 seconds show less demineralisation of rod boundaries, although with no etching there appears to be a roughening of the enamel surface, consistent with the beginnings of outlines of enamel rod structure. In contrast the images of the hypomineralised enamel do not show the typical etching pattern. As etching times increase the affected enamel becomes increasingly disorganised and the prism boundaries in the hypomineralised specimen are not clearly demarcated. The hypomineralised enamel does not show any preferential dissolution of the rod boundaries as seen in unaffected enamel.
Figure 5-5. SEM photomicrographs of fractured surface. Upper left and right SEM photos are of unaffected, normal enamel showing orderly enamel rod structure. Lower left and right SEM photo are of hypomineralised enamel showing disorganised enamel rod structure with voids present.
No etch: Dentine conditioner only  
Etching: 5 seconds

![SEM images of unaffected (upper images) and hypomineralised enamel (lower images) after 0 seconds etching (20 seconds dentine conditioner) and 5 seconds etching with phosphoric acid.](image_url)
Figure 5-6. b. SEM images of unaffected (upper images) and hypomineralised enamel (lower images) after 20, 40 and 60 seconds etching.
**X-Ray Diffraction**

XRD pattern obtained from hypomineralised enamel demonstrated that calcium hydroxyapatite was the only calcium phosphate phase present with no peaks associated with any other additional phases (e.g. tricalcium phosphate, TCP; Dicalcium Phosphate Dihydrate, DCPD; Octacalcium Phosphate, OCP) (Figure 5-7). The apatite peaks of (002) and (112) lattice planes were located respectively at \(2\theta = 25.8\) and \(2\theta = 32.2\) degree. The XRD pattern of hypoplastic enamel showed that the intensity of (002) plane to that of (300) was about 5, while from the standard pattern of hydroxyapatite powder (JCPDS# 09-0432), without any preferred orientation, this ratio is about 0.7. Higher intensity of (002) lattice plane demonstrates the preferred orientation of (002) in the hypomineralised enamel. It should be noted that the apatite crystals in enamel are highly oriented and the preferred orientation varies as a function of the sample orientation. As we can see from XRD pattern of sound enamel, different preferred orientation was obtained between sound and hypomineralised enamel specimens. This could be due to different orientation between the samples of sound and hypomineralised enamels used for XRD analysis.

No shift of any of the diffraction peaks were detected from the pattern of hypomineralised enamel (Figure 5-7). Incorporation of additional ions such as carbonate, sodium, and magnesium into apatite structure can cause changes in crystalline parameters, leading to the shift of one or more peaks. The effect of this incorporation is expected to be small. No significant difference in crystallinity between hypomineralised and sound enamels was observed from the XRD patterns (Figure 5-7).
Figure 5-7. XRD patterns of hypomineralised and sound enamel. Apatite was the only phase detected from XRD pattern of hypomineralised enamel. No significant difference in crystallinity of enamel apatite crystals was observed between sound and hypomineralised enamels. Patterns from sound and hypomineralised enamel demonstrated different preferred orientation of crystals.

EDS

The results of EDS analysis of three points in hypomineralised enamel and two points in sound enamel are shown in Table 5-6. The mean calcium to phosphate ratio for hypomineralised enamel was 2.07 (calculated from Wt%) or 1.60 (calculated from At%). This was not significantly different from means ratios for that of sound enamel from the same sample of 2.07 and 1.74 respectively.
<table>
<thead>
<tr>
<th>SPECIMEN AREA</th>
<th>Calcium</th>
<th>Phosphorus</th>
<th>Magnesium</th>
<th>Sodium</th>
<th>Potassium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wt%</td>
<td>At%</td>
<td>Wt%</td>
<td>At%</td>
<td>Wt%</td>
</tr>
<tr>
<td>Hypo 1</td>
<td>64.95</td>
<td>58.07</td>
<td>30.65</td>
<td>35.45</td>
<td>1.28</td>
</tr>
<tr>
<td></td>
<td>2.70</td>
<td>4.21</td>
<td></td>
<td></td>
<td>0.42</td>
</tr>
<tr>
<td>Hypo 2</td>
<td>66.16</td>
<td>59.75</td>
<td>31.68</td>
<td>37.03</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>1.48</td>
<td>2.34</td>
<td></td>
<td></td>
<td>0.23</td>
</tr>
<tr>
<td>Hypo 3</td>
<td>65.05</td>
<td>58.53</td>
<td>32.09</td>
<td>37.37</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>1.62</td>
<td>2.54</td>
<td></td>
<td></td>
<td>0.48</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>65.39</strong></td>
<td><strong>58.78</strong></td>
<td><strong>31.47</strong></td>
<td><strong>36.62</strong></td>
<td><strong>0.83</strong></td>
</tr>
<tr>
<td></td>
<td><strong>1.93</strong></td>
<td><strong>3.03</strong></td>
<td></td>
<td></td>
<td><strong>0.38</strong></td>
</tr>
<tr>
<td>Norm 1</td>
<td>66.67</td>
<td>60.49</td>
<td>31.99</td>
<td>37.55</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>0.96</td>
<td>1.52</td>
<td></td>
<td></td>
<td>0.23</td>
</tr>
<tr>
<td>Norm 2</td>
<td>66.17</td>
<td>59.86</td>
<td>31.98</td>
<td>37.44</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>1.42</td>
<td>2.24</td>
<td></td>
<td></td>
<td>0.31</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>66.42</strong></td>
<td><strong>60.18</strong></td>
<td><strong>31.99</strong></td>
<td><strong>37.50</strong></td>
<td><strong>0.14</strong></td>
</tr>
<tr>
<td></td>
<td><strong>1.19</strong></td>
<td><strong>1.88</strong></td>
<td></td>
<td></td>
<td><strong>0.27</strong></td>
</tr>
</tbody>
</table>

Table 5-6. Results of EDS analysis of hypomineralised and normal enamel. Wt% = weight percent, At%: Atomic ratio percent, Hypo = hypomineralised enamel, Norm = unaffected enamel.

**BSE Image**

An example of a BSE image of a single hypomineralised sample is shown in Figure 5-8. The average values for the graylevels of the hypomineralised enamel, unaffected enamel and (for comparison) dentine is shown in Figure 5-8. As calibration was done with just silicon and carbon standards relative values only for mineral content rather than absolute values can be determined. From Figure 5-8 it can be seen that the BSE intensity of the hypomineralised region is only slightly less intense than for the normal enamel. This is reflected in Figure 5-9 where it can be seen that for hypomineralised enamel, the graylevels were approximately 5% less than for the unaffected enamel. In comparison the graylevels of dentine were approximately 25% lower than unaffected enamel, which is consistent with the dentine having approximately 25% less mineral (by weight) than unaffected sound enamel (14;18;19).
Figure 5-8. BSE image of hypomineralised, unaffected enamel and dentine.
Figure 5-9. Average BSE intensities of test teeth tissues.

**TEM**

Due to the difficulties in the TEM sample preparation, very few specimens were available with the required thickness (~100nm). Figure 5-10 (a) shows an area of sound enamel. The hydroxyapatite crystals appear uniform and closely packed with little disorganisation or pores. In comparison, Figure 5-10 (b) shows the enamel crystals are disorganised with increased porosity between the crystals. In addition there appear to be porous or organic rich regions, the arrowed feature, which appears as a planar like defect within the hypoplastic enamel.
Figure 5-10. Bright Field TEM micrographs (a) Ultrastructure of unaffected enamel (b) Ultrastructure of hypomineralised enamel.
The red outlines a localised region in hypomineralised enamel where the enamel crystals appeared smaller and disorganised. The arrow points to a porous or organic rich region which appears as a planar like defect within the hypomineralised enamel.

Selected area electron beam diffraction patterns from the crystalline components of normal and hypomineralised enamel are shown in Figure 5-11.
Figure 5-11. Ring diffraction patterns of (A) unaffected and (B) hypomineralised enamel, taken using 5 second exposure time at a distance of 770mm. Note the non uniformity of the intensity of the spot patterns signifying parallel or orientated crystals especially in the sound dentine. The absence of defined spots for the hypomineralised enamel which indicates that the crystals are less perfect.

The ring diffraction pattern in Figure 5-11a (unaffected enamel) consists of small distinctive diffraction spots in comparison to Figure 5-11b (affected enamel) which shows a more continuous ring pattern. The diffraction pattern of sound enamel demonstrated a larger crystal size (Figure 5-10) than the finer grain size observed in the hypomineralised enamel (Figure 5-10). The presence of two semi-circles in the electron diffraction of both hypomineralised and unaffected enamel demonstrated preferred orientation of the enamel crystals which is consistent with the reported structure of the mineral phase in sound enamel (388).

Discussion

The most striking finding from this study is the very dramatic reduction in the hardness and modulus of elasticity of hypomineralised enamel. This study has shown that hypomineralised enamel from first permanent molar teeth has a hardness and modulus of elasticity of $0.53 \pm 0.31$ GPa and $14.49 \pm 7.56$ GPa respectively. This is significantly lower than the hardness and modulus of elasticity of unaffected enamel of the test teeth; $3.66 \pm 0.75$ GPa and $75.57 \pm 9.98$ GPa respectively (P<0.001).

This study has also reinforced previous studies which have reported that the cervical third of enamel appears, at the ultra structural level to be normal
(107;131). In the present study the mechanical properties of the cervical enamel appear to be indistinguishable from those of sound control teeth. The minor undulations seen in the graphs of the control tooth and unaffected region of the test teeth are to be expected as it has been previously shown that the mineral content and mechanical properties of unaffected permanent enamel are intra tooth dependant (74;377). However this study shows that the hardness and modulus of elasticity of such teeth decreases from the clinically normal cervical enamel to the hypomineralised region occlusally and that this decrease is predominantly linear.

There is limited information available on the mechanical properties of hypomineralised first permanent molar teeth. Suckling and colleagues (1989) investigated the physical appearance and hardness of hypoplastic enamel of 12 permanent teeth (four of which were permanent molars). They used a Knoop diamond indenter, at a force of 25gm, to conduct a series of indents beginning 30 μm from the anatomic surface of the tooth moving towards the amelo-dentinal junction (377). Consistent with the present study, these authors found that the hardness values varied between individual affected teeth. These authors noted that the hardness of the hypoplastic enamel was however consistently lower than the control. Closer comparison with this earlier study is difficult because the results were presented as a range of Knoop Hardness Number (KHN) (e.g. KHN<50) for each indent in each specific region rather than giving a single value for each series of indentation.

The present study revealed marked differences between SEM photomicrographs in the sound enamel and the hypomineralised enamel. These differences include an increase in porosity and consistently disorganised rod structure in the hypomineralised enamel. The increase in porosity and disorganization may contribute to the reduction in mineral content, although it is unlikely that this can entirely account for the very large reduction in the mechanical properties of affected teeth found in this study. The SEM investigation of etched, hypomineralised enamel convincingly showed that the classical pattern of etching as seen in the control enamel is not present in hypomineralised enamel, regardless of the etching time. Whilst other SEM studies have been reported for hypoplastic and hypomineralised enamel most have involved conditions such as amelogenesis imperfecta (AI) or fluorosis (408-413). Suckling and colleagues (1989) examined polished isolated hypomineralised defects under SEM and
found a similar lack of clarity in the prism boundaries as noted in the present study (377).

The SEM images have shown that the unaffected enamel shows the classical etching pattern while this is absent in the hypomineralised specimens. As the etching times of the hypomineralised specimens increased the affected enamel becomes increasingly disorganised and the prism boundaries less defined and demarcated. The hypomineralised enamel does not show any preferential dissolution of the rod boundaries as seen in unaffected enamel. It is possible that there is an increase in protein content (and consequently a decrease in mineral) in hypomineralised enamel. If this protein is located in the inter-rod regions it may limit the preferential dissolution by the etchant of the mineral at the rod boundaries. Given that etching the less organised hypomineralised enamel does not produce the classic etch pattern it is likely that the bond formed between any restorative/adhesive material and such teeth will be compromised. There do not appear to be any studies that have assessed this in the past however clinical experience would suggest bonding to compromised enamel can pose challenges with orthodontists in particular experiencing debonding of attachments more commonly from hypomineralised teeth.

In bone the modulus of elasticity has been positively correlated with apatite content (307) and although the mineral content of the hypoplastic teeth has not been examined to date, the low modulus of elasticity and hardness seen in this study suggests that the mineral content is reduced. In order to further speculate on the mineral content in hypomineralised enamel this study utilised BSE imaging. A number of studies on dental calcified tissues have been reported using BSE imaging (150;241;242;245;253) although no studies have been carried out on hypomineralised permanent molar teeth. Fearne and colleagues (1994) correlated the results of X-ray microtomographic and BSE imaging of hypomineralised and hypoplastic primary teeth from children born with very low birth weights (VLBW). Using normal enamel from control areas to calibrate their system, they dehydrated flat surfaces of sectioned primary teeth and found that the BSE images correlated well with X-ray microtomography. They demonstrated a reduction in mineral content of almost 10% in both X- Ray microtomography and BSE images in the hypoplastic regions compared to sound regions (134). In the present study, although the mechanical properties showed a dramatic reduction (up to 96%), the BSE was only reduced by approximately 5%. As in the
present study, calibration was done using silicon and carbon standards only. For more quantitative results, further calibration with other known (atomic number) standards would have to be conducted. Regardless of this, it is evident that there is a relatively small reduction in overall mineral content of hypomineralised enamel and this small reduction itself cannot explain the significantly lower mechanical properties recorded in the present study.

Multiple studies on developing enamel organs have shown that the Ca/P molar ratio is constant along the developing tooth organ, although it varies between individual teeth (134;414). Both EDS and the Ca/P ratio are used to determine the calcium content in hard tissues. If the ratio is altered from the stoichiometry of calcium hydroxyapatite, it can suggest that the mineral phase is altered or that significant substitution (of ions such as Mg for Ca) may have occurred. In the present study Ca/P ratio for hypomineralised enamel was determined using the EDS and was found to be 2.07 which was not significantly different to that of the control tissue. Whilst several other studies (414-416) have also failed to demonstrate any significant difference in the Ca/P ratio in compromised enamel. Jälevik and colleagues (2001) did find that median Ca/P ratio of hypomineralised enamel was significantly lower (1.4) than the unaffected enamel (1.8) (139). There are a number of reasons that the findings in this study are not consistent with that of Jälevik and colleagues. Firstly, in the present study only one sample was analysed with EDS, whereas in the study by Jälevik, 17 samples were analysed. Secondly Jälevik and colleagues used the EDS to analyse line scan from the EDJ to the enamel surface. Although they did report that the EDS results were fairly consistent throughout the length of the scans, there may be some variations in the Ca/P ratio at different parts of the hypomineralised regions that were not scanned in this current study. It is difficult to make further comments on the differences between the studies as both the EDS study and the X-ray diffraction findings in the present study are consistent, and have been conducted on separate samples.

Similarities between the Jälevik study (139) and the present study were found however in the analysis of the minor elements present in enamel. The EDS analysis consistently showed a higher percentage of magnesium in the hypomineralised regions of the tooth compared with sound enamel whilst the content of Na and K did not change. Magnesium may be substituted into the core of the enamel crystals and may represent errors during hard tissue
formation (139;417). It is unknown what effect these substitutions or inclusions may have on the mechanical and physical properties of hypomineralised teeth.

X-ray diffraction is a popular methodology for examining the dental tissues or materials (130). In the present study X-ray diffraction was used to aid in determination of the chemical composition and crystalline structure of hypomineralised areas in comparison to unaffected enamel. The XRD pattern indicates that the only calcium phosphate phase present in the hypomineralised enamel was calcium apatite. This was unexpected as the very significant reduction in mechanical properties of affected enamel suggested that the calcium mineral phases may have altered. The pilot TEM study confirms these findings. The diffraction pattern seen by TEM showed no additional rings in the diffraction pattern of hypomineralised enamel. This suggests that despite an increase in concentration of ions such as magnesium the concentrations were not high enough to modify the X-ray diffraction of crystalline in hypomineralised enamel and the mineral phase in hypomineralised enamel matches that of sound enamel. The TEM analysis also found the hypomineralised sample had localised areas of loosely organised smaller grain particles with an increase in porous spaces. This was not found in the unaffected enamel.

The low hardness and elastic modulus seen in the hypoplastic region tested indicates that these mechanical properties of hypoplastic enamel are similar to, or lower than those of dentine (80;82). The present study has shown using EDS, X-Ray diffraction and TEM that the mineral phase seen in hypomineralised enamel is an apatite as found in unaffected enamel, although hypomineralised enamel has an increase in substitutions of Mg ions. The effect this minor substitution has on the mechanical properties is at present unknown. BSE imaging has also suggested that the mineral content of hypomineralised teeth is reduced in comparison to unaffected enamel.

If we consider hypoplastic enamel to be composed of hydroxyapatite and protein then it should be possible to predict the elastic modulus from the volume fraction of these two phases. From the present study it was found that hypoplastic enamel has 5 to 10% less mineral phase than sound dentine and we shall assume this difference is composed of protein, whereas for sound teeth the protein component is typically less than 1%. 

161
The elastic modulus of composite materials maybe estimated based upon whether these composites are treated an iso-stress or iso-strain type materials. That is they exhibit uniform stress or strain through them upon being loaded. Composites composed of individual particles in a matrix, such as filler particles in a dental restorative composite resin may be considered as iso-stress material whereas continuous fiber reinforced composite resin would be considered an iso-strain material. In the latter case the strain on both the fibers and the resin matrix is the same, whereas for the former the stress on both the resin matrix and the embedded particles are the same. The resultant expressions for the elastic modulus of such composites is as follows (316):

Iso-stress model (Rule of Mixtures);

\[ E_c = V_fE_m + (1-V_f)E_p \]

Where \( E_c \) is the elastic modulus of the composite, \( E_m \) is the elastic modulus of the matrix, \( E_p \) is the elastic modulus of the particles and \( V_f \) is the volume fraction of the matrix.

Iso-strain model;

\[ \frac{1}{E_c} = V_f\frac{1}{E_m} + (1-V_f)\frac{1}{E_p} \]

Figure 5-12 is shown a comparison of the elastic modulus of a composite based upon the two models. The values of the elastic modulus of the protein and mineral were chosen as 1 and 100 GPa respectively. The iso-stress model, or rule of mixtures model, predicts a linear variation with composition whereas the iso-strain model predicts a sharply increasing change of properties as the volume fraction of the protein phase approaches zero. If we consider sound enamel, which has an E modulus between 60 and 80GPa, then the iso-strain model would predict that less than 1% of the enamel was protein. Whereas in the case of hypoplastic material, which typically had E modulus values between 10 and 20 GPa, the volume fraction of protein is estimated at between 4 and 10% which is consistent with the BSE image analysis of the present study.
The present study has found that the mechanical properties of hypomineralised first permanent molar teeth are significantly lower than unaffected enamel. Additionally affected teeth have a lower mineral content, higher protein content and micro-structurally the enamel rods are more disorganised and there appears to be more porosity than in unaffected enamel. Furthermore, SEM imaging of etched hypomineralised enamel show that a typical enamel etchant does not create the traditional etching pattern. It is possible that the expected increase in organic content i.e. protein within hypomineralised enamel may act to inhibit etching. Together these findings help to explain why traditionally hypomineralised teeth are extremely difficult to restore with clinicians reporting loss of both restorative material and tooth tissue (130). Attempts to elucidate any further differences between sound and hypomineralised enamel are continuing with investigations using transmission electron microscope and Infrared spectroscopy.
Chapter 5b  Mechanical Properties of the Dentine of Hypomineralised Teeth

It is generally thought that enamel hypomineralisation and/or hypoplasia is caused by an insult resulting in damage to the ameloblasts. It is possible that the same injury may damage the odontoblasts of affected teeth. This damage could potentially result in a disruption to dentine and consequently there may be changes in the mechanical and physical properties of the dentine in hypomineralised teeth. This theory is further supported by anecdotal clinical evidence indicating that the dentine of teeth affected by enamel hypomineralisation 'feels' softer or is of an 'unusual' consistency in comparison to unaffected teeth. Although there is an increasing amount of literature on the mechanical properties of sound and carious dentine (12;84;85;161;163;182), at present there is no literature on the mechanical properties of dentine in hypomineralised teeth.

The aim of this experiment was to determine the hardness and modulus of elasticity of dentine from hypomineralised/hypoplastic first permanent molar teeth and to relate this to the physical properties as determined by scanning electron microscopy (SEM) and energy dispersive X-ray spectrometer (EDS).

Material and Methods

Seven first permanent molar teeth extracted due to severe enamel hypoplasia and/or hypomineralisation were used along with two caries free premolars, extracted as part of orthodontic treatment, as controls. No information was available on the treatment or age of the patient from whom the teeth were extracted.

The study teeth were either caries free or had only small carious lesions that were hard and arrested. This allowed all testing to be conducted away from the area of previous caries. Premolars were used as control teeth as there was difficulty in obtaining caries free first permanent molar teeth. All specimens were prepared for indentation as described in Chapter 3. After each specimen was
enclosed in resin, the teeth were axially sectioned in the mesial-distal direction through the centre of the tooth. This coincided with the centre of the hypomineralised lesion. Each of the teeth was polished and stored as describe in Chapter 3.

**Mechanical Properties**

All indentations were carried out on the polished surface of each specimen to a maximum force of 20 mN using the Berkovich indenter. The area of dentine tested lay directly beneath the hypomineralised enamel. Therefore each specimen had two or three (depending upon the size of the hypomineralised region) arrays conducted between 200 and 400 μm from the pulp chamber wall to the amelo-dentinal junction (ADJ) following the dentinal tubules on opposite cuspal corners (Figure 5-13). If the enamel lesion was limited to only one cuspal corner, the two arrays of indentations were conducted at least 200 μm apart, in the same corner. Each indentation was 100 μm apart and the number of indentations for each specimen varied depending upon the size of the teeth and their pulp chamber. The average number of indentations was 35 per array. The arrays ran approximately parallel to the tubule direction in that part of the tooth. To ensure that the indentations were directly under the hypomineralised region and followed the tubule direction all arrays radiated from the pulp chamber wall to the mesial and distal corners of each tooth (Figure 5-13).
During testing, the specimen was kept immersed in deionised water in a specially constructed holder for the UMIS (Chapter 3). The data collection and analysis of all indents was carried out as described in Chapter 3. Each indentation was conducted with a 25 loading increments with a dwell time at maximum force for 30 seconds and 25 unloading decrements.

**Data analysis**

Each indentation was analysed as described in Chapter 3. This allowed the hardness and modulus of elasticity to be determined for each individual indentation of each array. The results were presented graphically as well as averages of values for the inner, middle and outer dentine regions as previously
described by Angker and colleagues (82). The mean mechanical properties of each region were also determined. As all teeth were of different sizes, determination of the inner, middle and outer dentine regions were determined by normalising relative to the indentation location between the pulp and the ADJ and then dividing this distance into thirds.

**Energy Dispersive X-ray Spectrometer (EDS)**

A first permanent molar teeth extracted due to severe enamel hypoplasia and/or hypomineralisation were used along with a single caries free control tooth (premolar), previously used for testing with the UMIS. No information on the treatment or age of the patient whose teeth were used in this study was obtained.

Each tooth was prepared as described in Chapter 3. Each tooth was dehydrated and coated in carbon. The system was operated at 20 kV, spot size of 5nm, specimen tilt of 15° and 15 to 20 mm working distance was used. The counting time was 100 seconds. Five readings per area investigated were used at 2000 times magnification, each over an area of approximately 80 μm². The counts were conducted over four regions on each test sample as shown in Figure 5-14. The regions selected were along a similar line as the indentation array conducted with the UMIS. Care was taken to ensure that the EDS analysis was done at least 100 μm away from any indentations in the area being examined. The composition of Ca, P, Mg and Na were determined for each area was determined (Wt%). To generate a single value for each region (as in Figure 5-14) the five readings per region were averaged and reported for the ratio of Ca/P, Ca+Mg+Na/P, Ca/Mg and Ca/Na.
Figure 5-14. Diagram of areas in dentine on experimental teeth where EDS was conducted. 1: inner dentine, 2: inner 1/3 of dentine, 3: outer 1/3 of dentine, 4: outer dentine.

**Scanning Electron Microscopy (SEM)**

A single first permanent molar tooth affected with enamel hypomineralisation and an unaffected premolar tooth, were used for SEM analysis. The dentine was fractured by the cutting of grooves above (in the enamel) and below (in the root dentine) using a slow speed saw under tap water irrigation (Figure 5-15). A lacron carver was then inserted into the cut surface and the sample was fractured with finger pressure. The teeth were dehydrated and coated for SEM analysis as discussed in Chapter 3. The system was operated at 20 kV, spot size of 5nm, specimen tilt of 15° with a working distance of 10-15 mm was used.

Figure 5-15. Position of cut lines prior to fracturing.
Digital images were taken at a variety of magnifications in three areas along the fractured surface corresponding to the top, middle and lower region of the dentine investigated with the UMIS (Figure 5-16).

![Diagram of tooth cross-section with labeled regions](image)

**Figure 5-16.** Location of regions investigated with SEM.

### Results

#### Mechanical properties

Graphically the results of the mechanical properties for the groups of teeth are presented in Figure 5-17 and Figure 5-18 and the average mechanical properties for each region are presented in Table 5-7. The mechanical properties of the premolars used as controls showed an increase in both the hardness and modulus of elasticity from the pulpal region and then a plateau after approximately 500 microns (Figure 5-17). The mechanical properties decreased in the final 500 microns beneath the ADJ. From the graphs it is evident that the mechanical properties of dentine from hypomineralised first permanent molars were not noticeably different to those of the control teeth (Figure 5-18). There was more scatter of the values for hardness and modulus of elasticity of hypomineralised teeth than the control although this is to be expected with the larger number of teeth studies. These results are mirrored in the results of the average mechanical properties of the dentine regions in Table 5-7.
Figure 5-17. Scatter plots of the mechanical properties of control teeth. Distance normalised relative to the indentations location between the pulp and amelo-dentinal junction (ADJ).
Figure 5-18. Scatter plots of the mechanical properties of hypomineralised teeth. Distance normalised relative to the indentations location between the pulp and the amelo-dentinal junction (ADJ).
Table 5-7. Average mechanical properties of inner, middle and outer dentine of hypomineralised and control teeth.

<table>
<thead>
<tr>
<th>DENTINE REGION</th>
<th>Number of indentations</th>
<th>Mean Hardness (GPa)</th>
<th>Mean Modulus of Elasticity (GPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control teeth</td>
<td>Hypomineralised teeth</td>
</tr>
<tr>
<td>Inner Dentine</td>
<td>254</td>
<td>0.65 ± 0.10</td>
<td>0.71 ± 0.10</td>
</tr>
<tr>
<td>Middle Dentine</td>
<td>217</td>
<td>0.76 ± 0.21</td>
<td>0.72 ± 0.15</td>
</tr>
<tr>
<td>Outer Dentine to ADJ</td>
<td>208</td>
<td>0.69 ± 0.09</td>
<td>0.57 ± 0.08</td>
</tr>
</tbody>
</table>

|                       |                        | Control teeth       | Hypomineralised teeth          |
|                       |                        | 17.22 ± 3.19        | 18.29 ± 1.20                   |
|                       |                        | 20.48 ± 0.69        | 18.08 ± 1.19                   |
|                       |                        | 18.43 ± 1.97        | 14.00 ± 1.58                   |

Table 5-8 summarises the results of the EDS investigations in weight ratios.

There is little variation between the different dentinal regions with respect to the Ca+Mg+Na/P and Ca/P ratios in both the hypomineralised or control tooth. Both teeth tested showed a slight increase in these ratios as the readings moved closer to the enamel. The average values for the Ca/P ratio was consistently lower for the dentine from hypomineralised enamel. The Ca/Mg ratio was consistent between the two teeth although the absolute values were lower in the hypomineralised tooth. This is consistent with the hypomineralised tooth having a lower level of magnesium than the control tooth. The results also showed that the dentine from the hypomineralised tooth had a lower Ca/Na ratio than the control tooth. This indicates that the dentine of the hypomineralised teeth showed a higher level of sodium than the control teeth.

**SEM**

Representative digital images are shown in, Figure 5-19, to Figure 5-21. The dentine from the control tooth shows dentinal tubules, intertubular dentine and peritubular dentine is intact and regular in all regions investigated. These images also show that there is no obvious structural difference in the regions examined between the dentine of the control and the tooth affected. The dentinal tubules in both samples are of similar size and orientation.
<table>
<thead>
<tr>
<th>Tooth</th>
<th>Regions</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wt%</td>
<td>At%</td>
<td>Wt%</td>
<td>At%</td>
<td>Wt%</td>
</tr>
<tr>
<td>Hypo</td>
<td>1.40 ±</td>
<td>1.08 ±</td>
<td>1.41 ±</td>
<td>1.09 ±</td>
<td>1.42 ±</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.1</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Control</td>
<td>1.83 ±</td>
<td>1.23 ±</td>
<td>1.89 ±</td>
<td>1.28 ±</td>
<td>1.86 ±</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>0.01</td>
<td>0.05</td>
<td>0.03</td>
<td>0.04</td>
</tr>
</tbody>
</table>

i) Ca/P ratio

<table>
<thead>
<tr>
<th>Tooth</th>
<th>Regions</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wt%</td>
<td>At%</td>
<td>Wt%</td>
<td>At%</td>
<td>Wt%</td>
</tr>
<tr>
<td>Hypo</td>
<td>1.60 ±</td>
<td>1.32 ±</td>
<td>1.59 ±</td>
<td>1.27 ±</td>
<td>1.63 ±</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>0.02</td>
<td>0.04</td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>Control</td>
<td>1.97 ±</td>
<td>1.37 ±</td>
<td>1.98 ±</td>
<td>1.34 ±</td>
<td>1.96 ±</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>0.02</td>
<td>0.05</td>
<td>0.01</td>
<td>0.02</td>
</tr>
</tbody>
</table>

ii) Ca+Mg+Na/P ratio

<table>
<thead>
<tr>
<th>Tooth</th>
<th>Regions</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wt%</td>
<td>At%</td>
<td>Wt%</td>
<td>At%</td>
<td>Wt%</td>
</tr>
<tr>
<td>Hypo</td>
<td>15.39 ±</td>
<td>9.33 ±</td>
<td>17.85 ±</td>
<td>10.83 ±</td>
<td>17.66 ±</td>
</tr>
<tr>
<td></td>
<td>0.86</td>
<td>0.51</td>
<td>2.00</td>
<td>± 1.21</td>
<td>0.60</td>
</tr>
<tr>
<td>Control</td>
<td>15.00 ±</td>
<td>10.99 ±</td>
<td>24.25 ±</td>
<td>14.25 ±</td>
<td>26.60 ±</td>
</tr>
<tr>
<td></td>
<td>1.73</td>
<td>1.02</td>
<td>0.93</td>
<td>± 0.93</td>
<td>4.49</td>
</tr>
</tbody>
</table>

iii) Ca/Mg ratio

<table>
<thead>
<tr>
<th>Tooth</th>
<th>Regions</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wt%</td>
<td>At%</td>
<td>Wt%</td>
<td>At%</td>
<td>Wt%</td>
</tr>
<tr>
<td>Hypo</td>
<td>15.77 ±</td>
<td>9.04 ±</td>
<td>18.11 ±</td>
<td>10.38 ±</td>
<td>15.93 ±</td>
</tr>
<tr>
<td></td>
<td>0.87</td>
<td>0.56</td>
<td>1.11</td>
<td>± 0.63</td>
<td>1.24</td>
</tr>
<tr>
<td>Control</td>
<td>33.97 ±</td>
<td>38.30 ±</td>
<td>52.63 ±</td>
<td>66.49 ±</td>
<td>53.79 ±</td>
</tr>
<tr>
<td></td>
<td>6.49</td>
<td>± 4.98</td>
<td>40.98</td>
<td>± 40.0</td>
<td>25.76</td>
</tr>
</tbody>
</table>

iv) Ca/Na ratio

Table 5-8. Results of EDS investigations.
Results are in weight ratios. Regions correspond to regions show in Figure 5-14 (1 closest to pulp and 4 at ADJ). Hypo= hypomineralised tooth.
Figure 5-19. Lower region of dentine sample. A: sound dentine from control tooth, B: Hypomineralised dentine.
Figure 5-20. Middle region of dentine sample. A: sound dentine from control tooth, B: Hypomineralised dentine.
Figure 5-21. Top region of dentine sample. A: sound dentine from control tooth, B: Hypomineralised dentine.
Discussion

The present study has shown that the mechanical properties of the dentine of minimally carious hypomineralised first permanent molar teeth are similar to that of control/unaffected teeth. This is surprising given the anecdotal but consistent evidence from clinicians that the dentine of these is soft or ‘unusual’ in consistency. The SEM photomicrographs of fractured surfaces of dentine from teeth with enamel hypomineralisation/hypoplasia also failed to show any obvious microscopic variation in structure compared to sound controls.

A number of authors have reported that there is variation in the dentine of various regions of the tooth (76;82;83;83;86;88;245). Consistent with the present study, Angker and colleagues (2003) have recently shown in a methodologically similar study that the hardness and modulus of elasticity of primary molars increases from the dentine at the pulp towards the middle portion of the crown (12;82). Table 5-9 is a summary of the mechanical properties of the hypomineralised teeth found in the present study and the sound dentine in the study by Angker and colleagues (2003). The control teeth in the present study were not included as they mirror the findings for hypomineralised teeth. It can be seen that the mechanical properties of hypomineralised teeth in the present study appear to have similar mechanical properties in the middle portion of the sound primary dentine but significantly lower in the region close to the pulp. The explanation for this is unclear but it should be noted that dentine from primary teeth may be less calcified in general than permanent teeth (59). In addition neither Angker and colleagues (2003) nor Hosoya and co workers showed a reduction in the mechanical properties at the ADJ (Table 5-9) (82;84). In contrast, Tesch and colleagues (2001) measured the mineral content and mechanical properties of sound dentine from the ADJ towards the pulp in permanent molars. Whilst it is difficult to discern the position of the test sites, it does not appear that dentine near the pulp was tested. A reduction in the mineral content, thickness of mineral crystals and the hardness and modulus of elasticity of dentine below the ADJ was reported (245). This is consistent with the present study and other studies (86). It therefore appears that the dentine of hypomineralised teeth has very similar mechanical properties from the pulp to the ADJ in sound permanent teeth but may differ near the pulp in primary dentine.
<table>
<thead>
<tr>
<th>DENTINE REGION</th>
<th>Mean Hardness (GPa)</th>
<th>Mean Modulus of Elasticity (GPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ANGKER*</td>
<td>Hypomineralised teeth- present</td>
</tr>
<tr>
<td>INNER DENTINE</td>
<td>0.52 ± 0.24</td>
<td>study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.59 ± 3.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HYPOMINERALISED teeth- present</td>
</tr>
<tr>
<td></td>
<td></td>
<td>study</td>
</tr>
<tr>
<td>MIDDLE DENTINE</td>
<td>0.85 ± 0.19</td>
<td>17.06 ± 3.09</td>
</tr>
<tr>
<td></td>
<td>0.72 ± 0.15</td>
<td>18.06 ± 1.19</td>
</tr>
<tr>
<td>OUTER DENTINE TO</td>
<td>0.91 ± 0.15</td>
<td>16.91 ± 3.85</td>
</tr>
<tr>
<td>ADJ</td>
<td>0.57 ± 0.08</td>
<td>14.00 ± 1.58</td>
</tr>
</tbody>
</table>

Table 5-9. Results comparing the mechanical properties of hypomineralised teeth in present study and sound primary dentine in study by Angker and colleagues (2003). *Study by Angker and colleagues (82).

If the elemental composition is altered from the stoichiometry of calcium hydroxyapatite, it is possible that the mineral phase has changed or that significant substitution may have occurred. The EDS analysis of dentine has shown that there was a slight increase in Ca+Mg+Na/P, Ca/P, Ca/Na and Ca/Mg ratios as the readings moved closer to the enamel. Interestingly the mechanical properties do vary from the pulp towards the ADJ although the variation is not an overall increase; instead the mechanical properties increase from the pulp then decrease slightly towards the ADJ. Although only a single hypomineralised and control tooth was used in this study, it does suggest that these ratios do not directly correlate with the mechanical properties of dentine. Furthermore the overall Mg and Na level also does not appear to correlate well with the mechanical properties of dentine in hypomineralised teeth. Whilst the hypomineralised tooth had an overall lower Mg level and a higher Na level than the control tooth their mechanical properties were not significantly different. It is the lack of a significant difference in both the structure (as seen on SEM) and the mechanical properties (UMIS) of the dentine from hypomineralised teeth which suggests that it is possible the differences in the various ratios seen with the EDS may be eliminated if a larger sample size is used. In other words these differences may be accounted for by experimental protocol. Experimental protocol may also account for the Ca/P ratio of the dentine from the hypomineralised tooth was consistently lower than found for the unaffected tooth. It is also possible that the amount of calcium seen in the dentine of hypomineralised teeth is lower and warrants further investigation. Further research with the dentine of hypomineralised teeth using the EDS will be conducted in the future by this research Unit.
Dental caries in the primary and permanent dentition remains a significant public health problem worldwide. Similarly developmental defects of enamel, particularly hypomineralisation/hypoplasia are a significant problem, being the most common developmental dental disorder observed in children and adolescents (418;419). Unfortunately not only is the enamel compromised but these hypomineralised teeth are also more susceptible to dental caries, adding further complexity of their treatment (107;109;121;127-129). These conditions frequently lead to pain, infection and abscess formation and are associated with significant morbidity in children and adolescents.

The traditional management of dental caries is to remove all of the carious dentine including any stained dentine. Further dentine and enamel would also be removed so that the defect that remained could be restored with a non bondable restoration. With this traditional cavity preparation the aim was to remove all bacteria in the carious tissue, to ensure that there was sufficient bulk for the restoration and preserve pulp vitality.

Recently the amount of carious tissue removal that is required for a successful restoration have been questioned. It is now accepted clinical practice that upon lesion cavitation, clinicians will remove only the infected dentine biomass (heavily infiltrated with bacteria and their products), leaving affected (demineralised but not infected with bacteria) dentine only. The rationale being that this can prevent extensive pulp injury by leaving firm but stained carious dentin at the cavity floor during excavation. With the advent of restorative materials that bond both chemically and/or mechanically to enamel and dentine, cavity preparation is kept minimal although inevitably between clinicians and between lesions, the amount of tooth tissue removed will vary. Minimal restorations are advantageous as removal of tooth structure weakens the tooth as a whole and increases the likelihood of future restorative treatment.
The stepwise excavation technique has continued the debate on the amount of tissue required for a successful restoration. This technique involves the re-entry into the original cavity after varying treatment intervals with an assortment of intracoronal restorations (traditionally non adhesive restorations such as zinc-oxide eugenol cement) and medicaments (calcium hydroxide) (186;202-205). The aim of stepwise treatment is to promote dentine sclerosis, encourage the formation of reactionary dentine, support remineralisation and provide pulp protection (205). In one large multi centre study conducted on permanent teeth, large carious lesions were treated initially with removal of the soft biomass plus the superficial portion of the necrotic and demineralised dentine. Soft dentine and carious enamel at the periphery of the lesion were also removed and calcium hydroxide and a temporary restoration (type not specified) were placed. At periods of between 4 and 8 months the temporary restoration was removed along with the remaining carious dentine and the tooth was definitively restored. These authors found that only 5 of 94 teeth had pulp exposure upon re-entry. Interestingly the researchers noted that most of the remaining carious dentine appeared to have increased in hardness as well as darkening (420). It has been suggested that the increase in hardness is reflective of an increase in the dentine mineral content. The hypothesis is therefore that dentine can, when sealed remineralise. Whilst the findings in this study has been supported by similar studies (186;191) there has yet to be any quantitative support for this hypothesis.

Mertz-Fairhurst and co-workers are responsible for a 10 year landmark clinical trial that strongly supported the notion that the carious process can be arrested beneath a restoration without any frank caries removal with a resin or amalgam/resin restoration (8;191-199). They recruited 123 patients and placed 156 pairs of restorations, in occlusal carious lesions extending into dentine. Half the teeth had sealed composite restorations placed directly over the carious lesion, with no caries removal. The other half had amalgam restorations placed either sealed with resin sealants or unsealed in which all softened carious tissue was removed in the traditional manner. At 10 years the sealed composite and amalgam restorations performed better (with respect to marginal adaption and other clinical criteria) than the non sealed amalgam and from standard radiographic examination, the composite restorations appeared to arrest the clinical progression of the carious lesion (8). Their data demonstrates beyond doubt that bonded and sealed restorations placed on soft carious dentine arrest the progress of these lesions and that these restorations last in excess of 10
years (421). However apart from clinical and radiographic examination, no other more quantitative assessment was used to evaluate the dentine beneath the restorations. The occurrence of remineralisation was therefore not determined.

In order to obtain accurate information on the physical, biological and mechanical characteristics of sealed carious lesions, it is advantageous to be able to place restorations *in situ* and allow the restorations to remain undisturbed for a period of time prior to extraction. Quantitative laboratory based studies can then be performed on which may help to elicit the effect the sealing on the physical and mechanical properties of affected dentine and enamel. Accurate information such as this is, at present, rare in the dental literature due to the difficulty of obtaining samples.

In paediatric dental clinics, young patients frequently present with multiple carious lesions in their primary teeth. Similar lesions are not uncommon in hypomineralised/hypoplastic first permanent molar teeth. The restoration of these two groups of teeth is often difficult especially in the very young child due to limited cooperation and as a result extraction is often the treatment plan of choice (4;422;423). Prior to their extraction, the placement of temporary restorations is common to limit pain and to allow time for additional treatment planning discussion to be completed (4). These temporarily restored and definitively extracted carious primary teeth and compromised first permanent molar teeth provide an ideal opportunity to study the effect of conservative sealed restorations on the mechanical and physical properties of carious dentine.

Chapters 4 and 5 in this thesis allow comparison to be made between the results of the teeth used in the present study with known restorative histories and those of unrestored carious primary molars and compromised first permanent molars. Chapter 4 confirmed that severely carious primary teeth show a dramatic decrease in mechanical properties across the carious region although many did show a 'rehardening' at the surface 300 – 500 µm. Chapter 5 indicated that the dentine of first permanent molar teeth has very similar mechanical properties to that of unaffected teeth but the mechanical properties of the enamel in hypomineralised teeth are significantly lower than what is found in unaffected teeth.
The aim of the present dentine study is to investigate the change in properties of carious dentine in hypomineralised/hypoplastic permanent molars and primary molar teeth after isolation from the oral cavity with standard restorative materials. This work should further inform the protocols provided to clinicians as to the efficacy of minimal invasive treatment using different common restorative materials.

**Materials and Methods**

**Patient Recruitment**

When a child patient is referred to Westmead Centre for Oral Health (WCOH) or the United Dental Hospital (UDH) they are seen by one of the specialist paediatric dentists for a regular consultation. Consultation clinics are run at both hospitals for screening and formulation of treatment plans of all these patients. If these patients do not have an acute condition requiring immediate treatment, they are placed on a waiting list for treatment. There are three waiting lists:

- Treatment under local anaesthetic: where patients who are able to cope are treated in the dental chair under local anaesthetic. This waiting list is between one and three months.
- Sedation (IV) waiting list: where all the treatment that is required is carried out in a single session with the aid of intravenous sedation. This treatment is only available at WCOH. Waiting list is between two and six months.
- General Anaesthetic (GA) waiting list: where all treatment is carried out in a single session under general anaesthetic. Waiting list for non acute treatment is between three and nine months at both WCOH and UDH.

Potentially eligible patients for this study were referred by the treating specialist after screening, to the principle investigator for the placement of the temporary restorations. Eligible children were:

- Patients that required extraction of one or more carious primary or permanent teeth due to dentine caries.
- Less than 16 years old.
- Were fit and healthy with ASA (American Society of Anesthesiologists Score) 1 (fit and healthy) or 2 (mild systemic disease e.g. mild asthma).
Patients were excluded if they:

- Required immediate removal of teeth due to pain or dental abscess (i.e. within one month).
- If patient was ASA 3 (disease with functional impairment), 4 (severe disease with threat to life) or 5 (moribund).
- Finally patients were excluded if any of the clinicians who examined them felt that they were uncooperative for even the most simple of treatment needing to be carried out in this study.

At this point the nature of the study was explained verbally and by way of patient information sheet and a consent form was signed. This study was approved by the Ethics committee of the Western Sydney and Central Sydney Area Health Services Ethics committees (Appendix 3 and 4). If the child or parent/guardian did not want to participate in the study at this time they were informed that they will receive a letter in the mail when they were eligible for their complete treatment as on one of the waiting lists.

Approximately 15 patients were recruited for this study although teeth were only eventually collected for six patients. From these six patients, 14 teeth were eventually collected and analysed. The following reasons for nine patients teeth not being collected include; tooth exfoliating or extracted by dentist outside of hospital or patient not turning up for eventual definitive treatment under local, IV GA.

Each patient that was included in the study often required a number of restorations and extractions of primary and/or permanent teeth. Not all teeth in each patient were appropriate for inclusion as test teeth. The test teeth that were to be included in the study had to be:

- Primary molars or permanent molar teeth with macroscopically evident dental caries.
- No clinical and radiographic evidence of abscess formation.
- Sufficient residual enamel to allow GIC restorations to bond and eliminate microleakage.
Treatment Procedures

Patients that were recruited for this study had an initial examination with simple demographic details collection. Other details that were collected at this time and recorded on a specially designed sheet (Appendix 5) were the oral health status of patient including teeth present, decay and oral health status, radiographs present and any other treatment required. If cooperation was adequate clinical photographs were taken of the test teeth in situ.

All treatment was completed in a single visit by the principle investigator. At this time attempts were made to treat all carious teeth that had open lesions. This was to reduce the pain the young patient felt while awaiting definitive treatment even if the tooth treated was not to be included in this study. Unfortunately as a number of the patients were very young and had a limited attention span not all carious teeth were treated at the treatment session. The teeth that were not able to be treated due to limited cooperation were still included in the study if they were to be extracted.

The treatment provided for all teeth were identical except for the restorative material eventually placed. Treatment provided:

- All teeth were cleaned with a new toothbrush and tap water. No toothpaste was used. Special attention was paid to the test carious teeth where each tooth was cleaned for approximately 10 seconds. This was to remove any plaque over the carious lesion.
- Each tooth to be treated was isolated with cotton rolls and salivary aspirator. As the patients that were included in the study were predominantly young, nervous patients who were not having definitive treatment, rubber damn was not used.
- Caries in enamel was removed with either hand instruments or slow speed hand pieces on both teeth. This is to ensure a clean/caries free margin. No dentine caries was removed from any test teeth.
- The cavity will be cleaned with a standard cavity conditioner (Ketac Cavity Conditioner)
- The tooth was restored with a glass ionomer cement (GIC) (Ketac Molar) with or without a base of calcium hydroxide (Ca(OH)_2). The glass ionomer cement was covered with Vaseline.
• If the patient has a single tooth that was to be included in the study then it received a GIC restoration with no base.

• If the patient had two or more teeth that were to be included in the study then the decision as to which tooth should have a Ca(OH)₂ base was made with a flip of the coin- Heads meant that the tooth on the left side of the mouth had a GIC only and the tooth on the right had a GIC restoration with a Ca(OH)₂ base. If the coin was tails then it was the reverse.

• If the patient had three or more teeth that were to be included in the study then the coin was flipped and the most posterior test teeth were chosen and the remaining teeth had a GIC restoration placed without a base.

• If there were teeth that were not to be included in the study required temporary restorations to eliminate pain whilst awaiting definitive treatment, standard temporary restorations were placed.

• Each patient was given standard oral hygiene instruction and instructed to telephone the chief investigator if the restorations fell out or they had any concerns.

Each patient was identified by a unique research number. A separate list was kept of all participants’ dental record number and their unique research number. This list was kept at a separate secure location to their record sheet and their dental files at all times. The chief investigator was the only person with access to the locked filing cabinet where records were stored. However once the teeth were extracted the teeth were identified by their unique research number only. Information about the treatment provided was recorded in the patients unit file as is normal procedure at both Dental Hospitals.

**Sample Collection**

Weekly, the chief investigator monitored the waiting list to determine when the participating patients were to have their teeth removed. As each patient completed treatment, either under IV sedation, local or general anaesthetic, the teeth were collected by the treating clinician and placed directly into previously prepared sterile containers. These containers contained Hank’s balanced salt solution with a few crystals of thymol to inhibit bacterial growth.
Each tooth was then prepared for UMIS analysis by enclosing each sample in resin, sectioned and polished as described in Chapter 3.

The teeth were then tested in the UMIS and SEM analysis. When the teeth were not being prepared or were not being tested they were stored in Hank's balanced salt solution with a small number of thymol crystals.

**Laboratory Testing**

**Mechanical Properties**

Upon extraction each test tooth was placed into Hank's balanced salt solution with a small number of thymol crystals to inhibit bacterial growth and stored at 4°C. All specimens were prepared for indentation as described in Chapter 3. After each specimen was enclosed in resin, the teeth were axially sectioned in the mesial-distal direction through the centre of the tooth which coincided with the centre of the carious lesion. Each of the teeth was polished and stored as describe in Chapter 3. Tests were typically conducted within 5 days of preparation.

When each specimen was ready for testing, it was placed on the work table of the UMIS with the polished surface upwards and the tests performed on the polished surface. For all experiments conducted on enamel or dentine, the maximum force was reached with 25 increments and the displacement recorded at each step. The force at each increment is given according to the formula, $F_i=(i/n)^2 F_m$ (where $F_i$ is the force at the $i$th step, $n$ is the total number of steps and $F_m$ is the maximum force). All indentations were made using a Berkovich indenter (See Chapter 3). At all times the test surface was kept 100% hydrated by submergence of each sample in a specially constructed holder (Chapter 3).

After the indentations were complete the data was analysed. Table 6-1 summarises the test parameters for the indentations conducted in carious and sound dentine.
<table>
<thead>
<tr>
<th></th>
<th>Carious Dentine</th>
<th>Sound Dentine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Indenter Type</strong></td>
<td>Berkovich</td>
<td>Berkovich</td>
</tr>
<tr>
<td><strong>Contact Force</strong></td>
<td>0.1 mN</td>
<td>0.1 mN</td>
</tr>
<tr>
<td><strong>Maximum Force</strong></td>
<td>5 mN</td>
<td>10 mN</td>
</tr>
<tr>
<td><strong>Number of increments</strong></td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td><strong>Incremental progression</strong></td>
<td>Square root</td>
<td>Square root</td>
</tr>
<tr>
<td><strong>Dwell at load/max/unload (sec)</strong></td>
<td>0.1 / 30 / 0.1</td>
<td>0.1 / 30 / 0.1</td>
</tr>
<tr>
<td><strong>Delay between indentations (sec)</strong></td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td><strong>Testing conditions</strong></td>
<td>(23±1)°C and (50±10) %RH</td>
<td>(23±1)°C and (50±10) %RH</td>
</tr>
</tbody>
</table>

Table 6-1. Test conditions for indentation experiments in present study.

After sample preparation in the centre of the carious region, a reference vertical scratch with made with a sharp scalpel blade (Figure 6-1). This line was used as a reference point when setting up the various lines of indents. Each specimen had between three and six arrays carried out in the carious region and two arrays in unaffected dentine (Figure 6-1). The number of arrays varied depending upon the size of the tooth and the carious lesion.

The arrays conducted in the macroscopically carious region began between 200 and 250 μm from the pulp chamber wall and following the dentinal tubules was continued to the lesion cavity floor. The arrays in the carious region were spread evenly over the carious lesion (Figure 6-1). The two arrays conducted in the unaffected/sound dentine were conducted in macroscopically caries free dentine beginning between 200 and 400 μm from the pulp chamber wall following the dentinal tubules to the ADJ at the cuspal corners (Figure 6-1). For the arrays in carious and unaffected dentine, each indentation was 100 μm apart and was carried out to a maximum force of 5 and 10 mN respectively using the Berkovich indenter.
Figure 6-1. Outline of arrays of indentations conducted in carious and unaffected dentine.

a. Outline of arrays of indentations conducted in carious and unaffected dentine. Blue lines represent arrays conducted in carious dentine, red lines are those conducted in unaffected/sound dentine. The number of arrays per tooth varied depending upon size of carious lesion and tooth being tested.

b. Diagrammatic representation of terminology used for description of dentine regions. Tooth depicted is a permanent tooth with enamel hypoplasia. Not all teeth used in this study were permanent teeth—the primary test teeth did not have hypomineralised enamel. Diagram modified from (12).
During testing, each specimen was kept immersed in deionised water in a specially constructed holder for the UMIS (Chapter 3). When each specimen was not in use for testing it was stored at 4°C in Hanks Balanced solution with a small number of thymol crystals. Each indentation was conducted with 25 loading increments with a dwell time at maximum force for 30 seconds and 25 unloading indentations.

Data analysis
Each indentation was analysed as described in Chapter 3. Due to the number of arrays conducted both qualitative and quantitative analysis of the indentations was conducted.

Qualitative:
The hardness and modulus of elasticity determined for each series of indentations for each array is presented graphically to allow visualisation and description of any evident trends in the sound and carious regions of each tooth. Finally, the graphs for the two centre arrays were normalised to a reference point (the point at the onset of a noticeable decline of mechanical properties) and superimposed on one another in groups of all teeth, untreated teeth and treated teeth to determine if the types of treatments had any affect on the mechanical properties of the test teeth.

Quantitative:
The average mechanical properties of the two centre arrays (the arrays on either side of the scratch line) of the carious lesions were utilised for quantitative analysis. The arrays from the sides of the carious lesion were excluded to ensure a reliable analysis, as the data from this region might have been compromised by sound dentine due to complexity in distinguishing precisely the boundary of the lesion. This allows the comparison of mechanical properties between individual teeth and treatments. For both the sound and carious dentine, the dentine was categorized into three regions based on the position of the indent (Figure 6-1). Due to the different sizes of the dentine region in primary and permanent teeth the values varied between primary and permanent teeth. For primary and permanent teeth these regions were: (1) the inner dentine: indentations in the region within 400 µm and 600 µm respectively above the pulp, (2) the middle dentine: indentations between inner and outer dentine and (3) the outer dentine:
indentations in the region within 400 μm and 600 μm respectively below the surface of the carious lesion or lesion/restoration interface.

Scanning Electron Microscope Analysis
Following indentation three specimens used for the indentation experiments were used for SEM analysis. All of these teeth were primary molars from the same patient (Patient B- Table 6-2 and Table 6-3) and had the following treatments:

- Tooth 3: GIC only
- Tooth 4: GIC and Ca(OH)$_2$
- Tooth 5: No treatment i.e. carious lesion that did not have any restorative treatment

Fractured surfaces of dentine were used. Fracturing of the dentine was done by the cutting of grooves above (in the enamel) and below the caries (in the root dentine) with a slow speed diamond saw under tap water irrigation (Figure 3.3). A lacron carver was then inserted into the cut surface and the sample was fractured with finger pressure. The area of dentine revealed corresponded to similar areas that were used for UMIS investigation (Figure 6-1 and Figure 6-2).

The teeth were dehydrated with 100% acetone, critically point dried, and coated for SEM analysis as discussed in Chapter 3. The system was operated at 20 kV, spot size of 5 nm, specimen tilt of 15° with a working distance of 10-15 mm was used.

![Figure 6-2. Position of SEM analysis along fractured surface. Diagram modified from (12).](image-url)
Digital images were taken at a variety of magnifications in three areas along the fractured surface to correspond to the surface, middle, lower and pulpal region of the carious dentine (Figure 6-2).

Results

Patients and Treatment Provided

Table 6-2 and Table 6-3 summarises the demographic details and treatment provided for each patient. Six patients were included in the study and ranged from age 3 to 11. Four of the patients allowed clinical photographs to be taken of one or more of the test teeth prior to the prescribed treatment (Figure 6-3). The other two patients included in this study did not consent for photographs due to limited cooperation.

Four patients were in the mixed dentition from which nine permanent teeth were included in this study. All of these teeth were extracted due to caries in hypomineralised/hypoplastic first permanent molars, except in a single case. Patient C had a balancing extraction of tooth 16 as part of their overall treatment and this tooth was included in the study. The remaining two patients were in the primary dentition from which six first primary molar teeth were included in the study.

All patients had bitewing and/or OPG radiographs taken at the time of their initial examination, which together with their clinical examination showed that along with the test teeth, patient 1, 2, 4 and 5 had a number of other carious teeth.

GIC restorations were placed on three primary teeth and five permanent first molar teeth. GIC restorations with Ca(OH)$_2$ as a liner, were placed in one primary molar and one permanent molar. Two teeth (one primary and one permanent molar tooth) that had caries with no treatment were also included in the study. Two permanent molars with composite restorations were also included in this study as at the time of treatment there was no Ketac Molar restorative material available to the treating clinician. One of the permanent molars upon sectioning did not appear to have caries but was still included as a dentine control.
Although not specifically asked at the time of treatment, there was no mention in the dental notes of treated patients that there were any treatment complications.
<table>
<thead>
<tr>
<th>Patient letter</th>
<th>Age at first appointment (yrs)</th>
<th>ASA Level</th>
<th>Waiting list</th>
<th>Number of primary teeth carious (max=20)</th>
<th>Number of permanent teeth with Caries (C) and/or Hypomineralisation (HM)</th>
<th>Teeth treated and treatment provided</th>
<th>Other teeth included in study from patient</th>
<th>Time from first appointment to teeth extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3</td>
<td>I</td>
<td>GA</td>
<td>7</td>
<td>0</td>
<td>54: GIC only 84: GIC only*</td>
<td>54: no treatment*</td>
<td>6 months</td>
</tr>
<tr>
<td>B</td>
<td>4</td>
<td>I</td>
<td>GA</td>
<td>9</td>
<td>0</td>
<td>64: GIC only 74: GIC and CaOH$_2$</td>
<td>54: no treatment*</td>
<td>4 months</td>
</tr>
<tr>
<td>C</td>
<td>9</td>
<td>I</td>
<td>IV</td>
<td>0</td>
<td>H/C- 4</td>
<td>26: GIC and CaOH$_2$ 46: GIC only 36: GIC only</td>
<td>16: no treatment*</td>
<td>3 months</td>
</tr>
<tr>
<td>D</td>
<td>10</td>
<td>I</td>
<td>IV</td>
<td>0</td>
<td>H/C- 2</td>
<td>16: GIC only</td>
<td>3 months</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>11</td>
<td>I</td>
<td>GA</td>
<td>8</td>
<td>H/C- 3</td>
<td>26: GIC only 46: GIC only*</td>
<td>4 months</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>8</td>
<td>I</td>
<td>IV</td>
<td>2</td>
<td>H/C- 3</td>
<td>16: composite only 46: composite only</td>
<td>4 months</td>
<td></td>
</tr>
</tbody>
</table>

Table 6-2. Demographic and treatment details of patients and teeth used.

* Due to patient group selected, cooperation was very limited in these patients. Therefore either not affected teeth had treatment carried out but the teeth were still included in the study or only the simplest treatment possible was conducted.

Waiting lists: GA- general anaesthetic, IV- intravenous sedation, T- local anaesthetic treatment waiting list.
Patient C

Patient D

Figure 6-3. Clinical photographs of test patients' teeth prior to extraction. Note not all patients allowed clinical photographs to be taken.
<table>
<thead>
<tr>
<th>Tooth</th>
<th>Patient</th>
<th>Type of tooth</th>
<th>Restoration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>Primary</td>
<td>GIC</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>Primary</td>
<td>GIC</td>
</tr>
<tr>
<td>3</td>
<td>B</td>
<td>Primary</td>
<td>GIC</td>
</tr>
<tr>
<td>4</td>
<td>B</td>
<td>Primary</td>
<td>GIC + Ca(OH)₂</td>
</tr>
<tr>
<td>5</td>
<td>B</td>
<td>Primary</td>
<td>no treatment</td>
</tr>
<tr>
<td>6</td>
<td>C</td>
<td>Permanent</td>
<td>GIC only</td>
</tr>
<tr>
<td>7</td>
<td>C</td>
<td>Permanent</td>
<td>no treatment</td>
</tr>
<tr>
<td>8</td>
<td>C</td>
<td>Permanent</td>
<td>GIC + Ca(OH)₂</td>
</tr>
<tr>
<td>9</td>
<td>C</td>
<td>Permanent</td>
<td>GIC</td>
</tr>
<tr>
<td>10</td>
<td>D</td>
<td>Permanent</td>
<td>GIC</td>
</tr>
<tr>
<td>11</td>
<td>E</td>
<td>Permanent</td>
<td>GIC</td>
</tr>
<tr>
<td>12</td>
<td>E</td>
<td>Permanent</td>
<td>no treatment</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>Permanent</td>
<td>composite</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>Permanent</td>
<td>composite</td>
</tr>
</tbody>
</table>

Table 6-3. Outline of numbering of test teeth and restorative treatment performed. Three types of restorative materials, Calcium Hydroxide Ca(OH)₂, Glass Ionomer Cement (GIC) and composite Z-100, were used for treatment.

**Description of Carious Lesions**

Appendix 6 has the summary data for the mechanical properties of all arrays conducted and associated with these is an image of each carious lesion in section. Although the size of the carious lesion varied between teeth, the average size of the carious lesions as determined by the decrease in mechanical properties was 1550 µm for the carious untreated teeth and 1450 µm for the carious treated teeth. No attempt was made to determine the activity of the carious lesion as at present there is no accurate test for a lesions activity.

**Mechanical Properties**

Both qualitative and quantitative results of the mechanical properties were determined from the dentine from test teeth with or without restorative treatment. Qualitative assessment was determined from examination of the arrays whereas quantitative
assessment was conducted by comparing the average mechanical properties of the various regions (Figure 6-1) conducted for each test tooth.

<table>
<thead>
<tr>
<th>Tooth</th>
<th>No. of Arrays</th>
<th>No. of Indentations</th>
<th>Type of Tooth</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>136</td>
<td>Primary</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>109</td>
<td>Primary</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>123</td>
<td>Primary</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>94</td>
<td>Primary</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>123</td>
<td>Primary</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>228</td>
<td>Permanent</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>119</td>
<td>Permanent</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>239</td>
<td>Permanent</td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>185</td>
<td>Permanent</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>255</td>
<td>Permanent</td>
</tr>
<tr>
<td>11</td>
<td>7</td>
<td>166</td>
<td>Permanent</td>
</tr>
<tr>
<td>12</td>
<td>8</td>
<td>192</td>
<td>Permanent</td>
</tr>
<tr>
<td>13</td>
<td>6</td>
<td>203</td>
<td>Permanent</td>
</tr>
<tr>
<td>14</td>
<td>3</td>
<td>114</td>
<td>Permanent</td>
</tr>
<tr>
<td>Total</td>
<td>91</td>
<td>2286</td>
<td></td>
</tr>
</tbody>
</table>

Table 6-4. Summary of treated teeth and associated arrays and indentations performed.

Qualitative Assessment

There was a large amount of data available for each tooth (Table 6-4) making it impractical to describe each set of arrays for each tooth. Each array for each tooth is presented in Appendix 6. For qualitative assessment of the arrays the logarithmic scale on the Y axis was chosen. This was because when the liner scale (Figure 6-4) for the hardness and modulus of elasticity values was used, the most significant changes when the indentations near the carious lesion surface, are not clearly defined (Figure 6-5).
Figure 6-4. Hardness and Elastic Modulus from central array in tooth 6 (GIC restoration only) shown on linear vertical axis scale.

Figure 6-5. Hardness and Elastic Modulus from Figure 6-4 shown on logarithmic vertical axis scale.

**Sound Dentine**

The hardness of sound primary and permanent dentine as determined from all test teeth, ranged from 0.17 to 1.14 GPa and 0.35 to 1.15 GPa respectively. The modulus of elasticity of sound primary and permanent dentine ranged from 8.61 to 21.8 and 8.60 to 22.21 GPa respectively.

Figure 6-6 and Figure 6-7 show the results for the hardness and modulus of elasticity of primary and permanent dentine respectively, normalised with respect to the distance between the pulp and ADJ. The mechanical properties of the arrays conducted in sound dentine of primary teeth generally showed relatively constant mechanical properties from the pulp towards the ADJ, although there was a large degree of scatter of results (Figure 6-6). The arrays conducted in permanent dentine show less scatter than the results for primary dentine most likely due to the larger number of arrays conducted (Figure 6-7). The scatter plots for permanent dentine indicate that the mechanical properties show a definite decreasing trend towards the ADJ. It is evident
from the scatter plots of the modulus of elasticity data that the modulus adjacent to the pulpal region is also lower than the central region (Figure 6-7). This decrease near the pulpal region is less evident for the hardness data.

**Hardness Sound Dentine Primary Teeth**

![Graph showing hardness data](image)

**Modulus of Elasticity of Sound Dentine Primary Teeth**

![Graph showing modulus of elasticity data](image)

Figure 6-6. Scatter plots of the hardness and modulus of elasticity of all arrays conducted in sound dentine of primary test teeth.
Figure 6-7. Scatter plots of the hardness and modulus of elasticity of all arrays conducted in sound dentine of permanent test teeth.

Carious Dentine
The range in mechanical properties for each of the treatments provided to the carious test teeth are summarised in Table 6-5.
<table>
<thead>
<tr>
<th>Treatment type</th>
<th>Hardness Range (GPa)</th>
<th>Modulus of Elasticity Range (GPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>0.002 – 0.98</td>
<td>0.02 – 20.06</td>
</tr>
<tr>
<td>GIC restoration only</td>
<td>0.0008 – 1.10</td>
<td>0.01 – 21.84</td>
</tr>
<tr>
<td>GIC restoration with and Ca(OH)₂</td>
<td>0.001 – 1.10</td>
<td>0.009 – 19.25</td>
</tr>
<tr>
<td>Composite resin restoration</td>
<td>0.07 – 1.05</td>
<td>1.61 – 20.07</td>
</tr>
</tbody>
</table>

Table 6-5. Summary of range of mechanical properties of each treatment type provided to test teeth. Primary and permanent teeth combined.

All arrays conducted in carious dentine showed a decrease in hardness and modulus of elasticity from the apparently unaffected dentine adjacent to the pulp towards the lesion surface. In the outer region of the carious lesions two main trends in the mechanical properties were noted, although within each tooth there were often mixtures and slight variations to each.

**Trend one:**

The first trend which is seen to some degree in almost all teeth is; after the continuous linear decrease in mechanical properties there is a plateauing, followed commonly by an increase in mechanical properties. An example of this is seen in Figure 6-15, which shows the arrays conducted in Tooth 1, a primary tooth that had GIC restorative treatment only. These arrays show the typical plateauing of the mechanical properties (Array LHS 1) followed by either a small or very large increase in mechanical properties. Also seen in Figure 6-15, there were arrays that after the plateauing and increase in mechanical properties showed a further small reduction in the most superficial three or four indentations (Array LHS 1). Overall this first trend, with all the variations was seen in both the primary and permanent teeth regardless of treatment type.

**Trend two:**

Figure 6-16 summarises the arrays conducted on tooth 7 (permanent tooth with no restorative treatment) and indicates that all arrays conducted in this tooth show a continuous reduction in mechanical properties to the surface of the lesion. The trend was seen in the majority of arrays in Tooth 4 (primary tooth with GIC and calcium hydroxide) and in all arrays in Tooth 7.
To allow comparison of these trends between the different restorative treatments provided, data from the most central array for each tooth was chosen. The central array was chosen because it was the most standard of arrays between each tooth. As the dentine thickness between specimens was different, the plots began from a reference point, 650 µm from the pulp. This point coincided within the region of the linear decrease in mechanical properties in the carious region for all teeth.

- All test teeth central arrays:
The hardness and modulus of elasticity of all test teeth (except tooth 14 which appeared carries free upon testing) are shown in Figure 6-8 and Figure 6-9 respectively. These graphs show a linear reduction in hardness and elastic modulus and in most teeth the first trend with a plateau and increase in mechanical properties is also seen with proximity to the top (floor) of the lesion. Overall the width of the linear reduction in mechanical properties is seen to be between 1000 to 1500 µm.

![All Teeth Hardness Comparison](image)

Figure 6-8. Comparison of hardness versus distance from a reference point for all teeth.
Figure 6-9. Comparison of elastic modulus versus distance from a reference point for all teeth.

- Untreated carious specimens:

From the reference point of 650 μm from the pulp, Figure 6-10 and Figure 6-11 outline the hardness and elastic modulus of the central arrays from untreated specimens. Other than a linear reduction in mechanical properties as the indentations move closer to the pulp, there is no other common trend to describe the untreated primary or permanent teeth behaviour.
Figure 6-10: Untreated specimen hardness versus distance from a reference point comparison for all teeth.

Figure 6-11: Untreated specimen elastic modulus comparison versus distance from a referenced point for all teeth.
- Carious specimens with restorative treatments (all types of treatments pooled for analysis):

Figure 6-12 and Figure 6-13 illustrate the hardness and modulus of elasticity of all specimens that had treatment of any type from the referenced point (650 μm). Arrays with both Trend one and two, outlined above are evident in the treated specimens and appear independent of treatment type or whether the treatment was provided in a primary or permanent tooth.

---

**Figure 6-12:** Treated specimen hardness comparison.
Figure 6-13: Treated specimen elastic modulus comparison.

Further Plateau Analysis
The average distance of the beginning of the plateau and the commencement of the increase in mechanical properties (if present) from the lesion surface or lesion/restoration interface is determined for each array, in each tooth as indicated by locations A and B in Figure 6-14 and the average for each tooth is presented in Table 6-6. For all teeth combined, the average distances to the plateau and the increase in mechanical properties from the lesion surface was 881 ± 357 µm and 427 ± 161 µm respectively. Table 6-6 reinforces the finding that the presence of plateauing and an increase in mechanical properties in the outer region of the carious lesion was independent of treatment and occurred in untreated teeth also.
Figure 6-14. Definition of [A] Distance from Plateau to lesion surface or lesion/restoration interface and [B] Distance from increase in mechanical properties to lesion surface or lesion/restoration interface.

<table>
<thead>
<tr>
<th>Tooth number</th>
<th>[A] Distance from Beginning of Plateau to Lesion surface (µm)</th>
<th>[B] Distance from Beginning of Mechanical Property Increase - to Lesion Surface (µm)</th>
<th>Restoration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1200</td>
<td>500</td>
<td>GIC</td>
</tr>
<tr>
<td>2</td>
<td>1000</td>
<td>400</td>
<td>GIC</td>
</tr>
<tr>
<td>3</td>
<td>700</td>
<td>400</td>
<td>GIC</td>
</tr>
<tr>
<td>4</td>
<td>400</td>
<td>No increase noted</td>
<td>GIC + Ca(OH)₂</td>
</tr>
<tr>
<td>5</td>
<td>300</td>
<td>300</td>
<td>No treatment</td>
</tr>
<tr>
<td>6</td>
<td>1100</td>
<td>600</td>
<td>GIC</td>
</tr>
<tr>
<td>7</td>
<td>No plateau</td>
<td>No increase</td>
<td>No treatment</td>
</tr>
<tr>
<td>8</td>
<td>1100</td>
<td>600</td>
<td>GIC + Ca(OH)₂</td>
</tr>
<tr>
<td>9</td>
<td>700</td>
<td>400</td>
<td>GIC</td>
</tr>
<tr>
<td>10</td>
<td>1300</td>
<td>700</td>
<td>GIC</td>
</tr>
<tr>
<td>11</td>
<td>No plateau</td>
<td>200</td>
<td>GIC</td>
</tr>
<tr>
<td>12</td>
<td>1300</td>
<td>400</td>
<td>No treatment</td>
</tr>
<tr>
<td>13</td>
<td>600</td>
<td>200</td>
<td>composite</td>
</tr>
<tr>
<td>14</td>
<td>NA- caries free tooth</td>
<td>NA-caries free tooth</td>
<td>composite</td>
</tr>
</tbody>
</table>

Table 6-6. Distance of Plateau and increase in mechanical properties to lesion surface or lesion/restoration interface.
Summary of Qualitative Assessment

The following can be summarised from the qualitative data analysis:

1. Sound dentine:
   
   a. The range in the mechanical properties of the sound primary and permanent dentine was very similar.
   
   b. The mechanical properties for sound dentine of primary teeth were generally linear from the pulp to the ADJ.
   
   c. Sound permanent and primary dentine showed slightly lower mechanical properties in the regions near the pulp and ADJ.

2. Carious Dentine:

   a. All arrays conducted in carious teeth show a linear decrease in mechanical properties as the indentations enter the carious region and this decrease was between 1000 – 1500 μm across dentine tissue.

   b. Two trends were seen after the linear decrease in mechanical properties. Trend one (the most common) includes an extensive minimum plateau region and a slight increase in mechanical properties in the most superficial part of the carious lesion. Trend two shows a continuous decrease in mechanical properties towards the lesion surface. The trend seen was independent of the treatment provided.

   c. The minimum plateau when present commenced at approximately 881 μm from the lesion surface. The presence and distance from lesion surface of the plateau was independent of treatment provided and also appeared in untreated teeth.
Tooth information
Tooth 1:
Restorative placed: GIC only
Time with restoration in situ: 6 months
Primary tooth

Figure 6-15a: Hardness profiles of each indentation
Red lines on diagram: Indentations in caries
Blue lines on diagram: Indentations in normal dentine
Tooth information

Tooth 1:
Restorative placed: GIC only
Time with restoration in situ:
6 months
Primary tooth

Figure 16-15b: Modulus of elasticity profiles of each indentation
Red lines on diagram: Indentations in caries
Blue lines on diagram: Indentations in normal dentine
Tooth information
Tooth 7:
No restorative placed
Permanent tooth

Figure 6-16a: Hardness profiles of each indentation
Red lines on diagram: Indentations in caries
Blue line on diagram: Indentations in normal dentine
Tooth information
Tooth 7:
No restorative placed
Permanent tooth

Figure 6-16b: Modulus of elasticity profiles of each indentation
Red lines on diagram: Indentations in caries
Blue line on diagram: Indentations in normal dentine
Quantitative Analysis

For each tooth the average hardness and modulus of elasticity for the inner, middle and outer regions (see Figure 6-1) of the sound and carious regions was calculated and presented. From these results the average mechanical properties of treated teeth were pooled and compared to the teeth that did not have any restorative treatment.

- **Sound Dentine**

The data from each array in sound dentine for all teeth was pooled to allow an overall hardness and modulus of elasticity for primary and permanent dentine to be determined for each region (Table 6-7 and Table 6-8). Total number of arrays used for calculation of primary and permanent dentine mechanical properties was 9 and 17 respectively.

<table>
<thead>
<tr>
<th>Hardness average (GPa)</th>
<th>Sound Primary Dentine</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Inner Dentine</td>
<td>0.64 ± 0.07</td>
<td>Middle Region</td>
<td>0.75 ± 0.06</td>
</tr>
<tr>
<td>Outer Dentine</td>
<td>0.59 ± 0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elastic Modulus average (GPa)</td>
<td>14.13 ± 2.44</td>
<td>16.45 ± 0.51</td>
<td>13.38 ± 1.08</td>
</tr>
</tbody>
</table>

Table 6-7: Hardness and Elastic Modulus averages for Sound Primary Dentine

<table>
<thead>
<tr>
<th>Hardness average (GPa)</th>
<th>Sound Permanent Dentine</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Inner Dentine</td>
<td>0.71 ± 0.04</td>
<td>Middle Region</td>
<td>0.71 ± 0.06</td>
</tr>
<tr>
<td>Outer Dentine</td>
<td>0.62 ± 0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elastic Modulus average (GPa)</td>
<td>15.63 ± 2.09</td>
<td>17.33 ± 2.27</td>
<td>15.03 ± 0.97</td>
</tr>
</tbody>
</table>

Table 6-8: Hardness and Elastic Modulus average for Sound Permanent Dentine

From Table 6-7 and Table 6-8, it is evident that there is no significant difference in the mechanical properties of sound dentine from primary or permanent dentine. The overall hardness and elastic modulus values for primary dentine were found to be lower in the...
inner and outer region in comparison to the middle region. In contrast, for permanent
dentine the average mechanical properties of the inner and middle regions were
consistently higher than the average of the outer dentine adjacent to the ADJ.

- Carious regions- individual teeth

The average hardness and modulus of elasticity for the inner, middle and outer carious
region for each tooth is presented in Table 6-9 (Figure 6-17) and Table 6-10 (Figure
6-18) respectively.

<table>
<thead>
<tr>
<th>Tooth no.</th>
<th>Inner Dentine</th>
<th>Middle Region</th>
<th>Outer Dentine</th>
<th>Restoration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.09</td>
<td>0.02</td>
<td>0.01</td>
<td>GIC</td>
</tr>
<tr>
<td>2</td>
<td>0.26</td>
<td>0.04</td>
<td>0.003</td>
<td>GIC</td>
</tr>
<tr>
<td>3</td>
<td>0.49</td>
<td>0.11</td>
<td>0.03</td>
<td>GIC</td>
</tr>
<tr>
<td>4</td>
<td>0.265</td>
<td>0.04</td>
<td>0.01</td>
<td>GIC + Ca(OH)₂</td>
</tr>
<tr>
<td>5</td>
<td>0.74</td>
<td>0.34</td>
<td>0.07</td>
<td>No treatment</td>
</tr>
<tr>
<td>6</td>
<td>0.69</td>
<td>0.05</td>
<td>0.01</td>
<td>GIC</td>
</tr>
<tr>
<td>7</td>
<td>0.76</td>
<td>0.13</td>
<td>0.03</td>
<td>No treatment</td>
</tr>
<tr>
<td>8</td>
<td>0.86</td>
<td>0.04</td>
<td>0.01</td>
<td>GIC + Ca(OH)₂</td>
</tr>
<tr>
<td>9</td>
<td>0.86</td>
<td>0.06</td>
<td>0.01</td>
<td>GIC</td>
</tr>
<tr>
<td>10</td>
<td>0.68</td>
<td>0.10</td>
<td>0.12</td>
<td>GIC</td>
</tr>
<tr>
<td>11</td>
<td>0.73</td>
<td>0.09</td>
<td>0.05</td>
<td>GIC</td>
</tr>
<tr>
<td>12</td>
<td>0.17</td>
<td>0.04</td>
<td>0.01</td>
<td>No treatment</td>
</tr>
<tr>
<td>13</td>
<td>0.79</td>
<td>0.05</td>
<td>0.01</td>
<td>composite</td>
</tr>
<tr>
<td>14#</td>
<td>0.62</td>
<td>0.76</td>
<td>0.75</td>
<td>composite</td>
</tr>
</tbody>
</table>

# Apparently caries-free tooth

Table 6-9. Average hardness for carious regions, for all specimens.
Figure 6-17. Graphical representation of Table 6-9.

<table>
<thead>
<tr>
<th>Tooth no.</th>
<th>Inner Dentine</th>
<th>Middle Region</th>
<th>Outer Dentine</th>
<th>Restoration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.22</td>
<td>0.08</td>
<td>0.14</td>
<td>GIC</td>
</tr>
<tr>
<td>2</td>
<td>4.73</td>
<td>0.62</td>
<td>0.02</td>
<td>GIC</td>
</tr>
<tr>
<td>3</td>
<td>8.41</td>
<td>2.61</td>
<td>0.20</td>
<td>GIC</td>
</tr>
<tr>
<td>4</td>
<td>5.98</td>
<td>1.21</td>
<td>0.24</td>
<td>GIC + Ca(OH)_2</td>
</tr>
<tr>
<td>5</td>
<td>15.42</td>
<td>9.19</td>
<td>1.74</td>
<td>No treatment</td>
</tr>
<tr>
<td>6</td>
<td>15.26</td>
<td>0.64</td>
<td>0.03</td>
<td>GIC</td>
</tr>
<tr>
<td>7</td>
<td>16.98</td>
<td>3.79</td>
<td>0.60</td>
<td>No treatment</td>
</tr>
<tr>
<td>8</td>
<td>17.61</td>
<td>0.77</td>
<td>0.18</td>
<td>GIC + Ca(OH)_2</td>
</tr>
<tr>
<td>9</td>
<td>16.53</td>
<td>1.18</td>
<td>0.05</td>
<td>GIC</td>
</tr>
<tr>
<td>10</td>
<td>15.20</td>
<td>1.11</td>
<td>1.18</td>
<td>GIC</td>
</tr>
<tr>
<td>11</td>
<td>17.14</td>
<td>2.30</td>
<td>0.44</td>
<td>GIC</td>
</tr>
<tr>
<td>12</td>
<td>4.94</td>
<td>1.04</td>
<td>0.08</td>
<td>No treatment</td>
</tr>
<tr>
<td>13</td>
<td>16.87</td>
<td>0.65</td>
<td>0.08</td>
<td>Composite</td>
</tr>
<tr>
<td>14*</td>
<td>16.86</td>
<td>16.07</td>
<td>15.35</td>
<td>Composite</td>
</tr>
</tbody>
</table>

\* Apparently caries-free tooth

Table 6-10. Average elastic modulus for carious regions, for all specimens.
Figure 6-18. Graphical representation of Table 6-10.

Except for tooth 14 (caries free permanent molar), the tables and graphs of the average mechanical properties show a dramatic decrease in mechanical properties from the inner to outer dentine. It can also be seen from these tables that the hardness of a carious lesion- treated or untreated is consistently less than 0.05 GPa in the outer carious region. The modulus of elasticity is less constant in this region. The treatment provided to each test tooth appeared to have no affect on the hardness and modulus of elasticity of any region.

- Carious regions- overall
To give the overall average hardness and modulus of elasticity of the carious regions of the test teeth that had had restorative treatments and those that did not, the results for the primary and permanent were pooled. Table 6-11 and Table 6-12 are a comparison of the hardness and modulus of elasticity respectively of the teeth that had no restorative treatment (carious untreated teeth) and the arrays conducted in teeth that had had GIC, calcium hydroxide/GIC or composite resin restorations (carious treated teeth).
<table>
<thead>
<tr>
<th></th>
<th>Inner Dentine</th>
<th>Middle of Caries</th>
<th>Outer Dentine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carious untreated teeth</td>
<td>0.56 ± 0.33</td>
<td>0.19 ± 0.15</td>
<td>0.04 ± 0.03</td>
</tr>
<tr>
<td>Carious treated teeth</td>
<td>0.57 ± 0.26</td>
<td>0.17 ± 0.21</td>
<td>0.09 ± 0.22</td>
</tr>
</tbody>
</table>

Table 6-11: Hardness averages for Untreated and Treated Carious Specimens.

<table>
<thead>
<tr>
<th></th>
<th>Inner Dentine</th>
<th>Middle of Caries</th>
<th>Outer Dentine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carious untreated teeth</td>
<td>12.45 ± 5.85</td>
<td>5.11 ± 3.47</td>
<td>0.80 ± 0.77</td>
</tr>
<tr>
<td>Carious treated teeth</td>
<td>11.99 ± 5.85</td>
<td>2.12 ± 3.57</td>
<td>1.25 ± 3.96</td>
</tr>
</tbody>
</table>

Table 6-12: Elastic Modulus averages for Untreated and Treated Carious Specimens.

From these tables it can be seen that both the untreated and treated carious lesions show a similar overall decline in mechanical properties from the inner to outer carious dentine. Overall the mechanical properties of the untreated carious lesions are higher (except in the most superficial portion) than the treated teeth although due to the limited number of untreated carious teeth in the present study, statistical significance was not determined (n=3).

Summary of Quantitative Assessment
The following can be summarised from the qualitative data analysis:

1. Sound dentine:
   a. In the present samples there was no significant difference in the overall mechanical properties of sound dentine of primary or permanent dentine.
2. Carious dentine:
   a. The treatment provided to each test tooth appeared to have no affect on the hardness and modulus of elasticity of any region tested.
SEM

Figure 6-19 and Figure 6-20 are representative SEM images of the three test teeth with different restorative treatments;

- Tooth 5: without any restorative treatment
- Tooth 3: with GIC restoration only
- Tooth 4: with GIC and calcium hydroxide liner

The images show lower (Figure 6-19) and higher magnifications (Figure 6-20) of the pulpal, lower, middle and surface region of the fractured surfaces of the test teeth (Figure 6-2).

Regardless of treatment, all test teeth showed a continual deterioration of the physical properties from the pulpal aspect to the carious lesion surface. Although in all images dentinal tubules can be distinguished, they are progressively less defined and the boundary between a tubule and the surrounding intertubular dentine is less obvious closer to the lesion surface. Tooth 5 which had no restorative treatment shows the most severe destruction of normal architecture on the surface of the carious lesion. Interestingly of the three teeth, the lesion in tooth 5 has the smallest overall size, with the beginning of the carious destruction not becoming obvious until the middle images of the carious lesion. Teeth 3 and 4 show obvious destruction at the images taken in the lower region, yet the destruction on the surface or surface region is less than seen in tooth 5.

For all three teeth, the images taken in sound dentine (close to the pulp) show regular dentinal tubules with well defined intertubular and peritubular dentine. The image of the 'sound' pulpal dentine of the tooth 3, with a GIC restoration is already showing signs of mild destruction in this area. Interestingly although this tooth has a large carious lesion, macroscopically the carious lesion appears to be smaller than the very large lesion of tooth 4 (GIC and calcium hydroxide). An obvious explanation for this is that when tooth 3 was fractured, the fracture line went through a mildly carious region rather than a heavily 'infective' region as may have happened in tooth 4.

At the most superficial region of each test tooth (surface region) there are a number of common features noted. Firstly the tubules and peritubular dentine are almost completely destroyed. The intertubular dentine appears more granular and is presumably predominantly collagen or its breakdown products. There are also
differences in this region between each tooth. In the image of tooth 5, (tooth with no treatment) there are faint tubule outlines in the centre of the image although there is no normal tubular structure, with amorphous plaque like material predominating the image. In the surface region, tooth 3 also appears to have a plaque present although in comparison to tooth 5, this has more regular dental tubular outlines. In contrast the outlines of dentinal tubules on the surface section of tooth 4 are very faint. In tooth 4, the intertubular dentine appears very grainy and coarse. The grainy nature of tooth 4 is apparent in the middle and to some extent in the lower region also. Another interesting feature of this tooth (tooth 4) is the obvious dense encasement of the dentinal tubules with presumably peritubular dentine. It appears to be present in the lower region (and to a lesser extent in the middle region) and yet the surrounding intertubular support for it has disappeared leaving the tubular structure vulnerable. The tubules therefore appeared fractured and weak.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Tooth 3</th>
<th>Tooth 4</th>
<th>Tooth 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progressive destruction of intertubular, peritubular and tubule structure</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Grainy/porous appearance of intertubular dentine</td>
<td>No</td>
<td>Yes- from lower to surface region</td>
<td>No</td>
</tr>
<tr>
<td>Enlargement of dentinal tubules</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Plaque present in surface region</td>
<td>Slight</td>
<td>No</td>
<td>Yes- predominant feature</td>
</tr>
</tbody>
</table>
Tooth 3: GIC restoration only

Tooth 4: GIC restoration with Calcium hydroxide

Tooth 5: No treatment

Figure 6-19. SEM images of test teeth at lower magnification. The top images are from the dentine from pulpal aspect of test teeth (Note the difference in magnification of image from tooth 3). The lower images are from the lower region of carious dentine.
Figure 6-19. SEM images of test teeth at lower magnification.
Top photos are from the middle section of the carious dentine. The lower line is from the surface region of carious dentine.
Tooth 3: GIC restoration only
Tooth 4: GIC restoration with Calcium hydroxide
Tooth 5: No treatment

Figure 6-20. SEM images of test teeth at higher magnification.
Top photos are from the pulpal section of the carious dentine. The lower line is from the lower region of carious dentine.
Figure 6-20. SEM images of test teeth at higher magnification. Top photos are from the middle section of the carious dentine. The lower line is from the surface region of carious dentine.
Discussion

The aim of this study was to determine if treatment of severely carious lesions would alter the mechanical and microstructural properties of carious dentine. This study was conducted by comparing the mechanical and microstructural properties of five primary and nine permanent teeth then had known treatment histories using the UMIS and SEM respectively. The microstructural analysis conducted with SEM allows visualisation of any differences in the physical nature of treated and untreated carious specimens. Determination of the hardness and modulus of elasticity of carious and sound dentine allows the qualitative and quantitative assessment of these fundamental biomaterial properties.

The hardness of a tissue may be defined as a measure of a material's resistance to deformation by surface indentation (67). The modulus of elasticity (stiffness) is the ratio of stress to corresponding strain below the proportional limit. The modulus of elasticity provides an indication of the amount of deformation that may occur in a tissue when a load is applied (100). The hardness and modulus of elasticity of dental tissues are fundamental mechanical properties in determining how the tissues will react in the oral environment. These mechanical properties have also been shown to be a reasonable method of examining the inorganic content of calcified tissues including tooth tissues (69;182;218;241;253;304), which is useful when attempting to assess the amount if any of remineralisation of the carious lesions.

Limited sample size is the greatest weakness of the present study. Obtaining an adequate number of appropriate specimens for a study of this nature is difficult. Comparison between untreated and treated carious dentine included 3 different types of treatments. The restorative materials used were Glass Ionomer Cement (GIC), Calcium Hydroxide Ca(OH)\textsubscript{2} and a composite Z100. Due to the limited number of specimens, we collated the data from all treated teeth, irrespective of restorative type. Further work on a larger number of teeth treated with each restorative material will be required before more definitive outcomes are possible. Ethically teeth were extracted when necessary which results in a lack of standardisation of the time restorations were in situ. This variation ranged from 3 to 6 months. Regardless of this however, this study is unique in a number of ways:
1. Ethical approval was obtained to allow treatment of carious lesions and the collection of these teeth after a known time interval. Obtaining ethical approval for a study such as this is rare in Australasia. Standard restorative materials and procedures were used and therefore approval was less difficult that initially anticipated. It is expected that obtaining ethical approval for the present study will allow further work on other known and novel restorative procedures and materials to be tested in a similar way.

2. Although there has been one study evaluating the effect of sealing carious lesions without any attempt to remove any of the infected or affected carious dentine (8), it did not quantitatively assess the properties underlying dentine. This study does.

3. The present study investigated three common dental materials, a resin composite, GIC and Ca(OH)$_2$, of which the latter two have been generally considered to promote ‘remineralisation’ of softened dentine. Preliminary data from this study does not support this however, the mechanism by which remineralisation occurs and its effectiveness still needs further scientific assessment.

4. Finally the design of this study has utilised both primary and permanent teeth using identical study protocol. This allows direct comparisons of their mechanical properties. Previous studies have generally investigated a single tooth type only (80;83;88;185).

The present study utilised qualitative and quantitative assessment of the mechanical properties of carious lesions treated with GIC with or without calcium hydroxide lining to further investigate the findings by Mertz-Fairhurst and colleagues (8). In the present study regardless of treatment types, all test teeth showed that once the indentations entered the carious region that the hardness and modulus of elasticity fell over a distance of approximately 1000 µm. From this point two trends were evident; either the mechanical properties displayed a minimum plateau with a slight increase in mechanical properties or conversely the linear decrease continued. As multiple arrays were conducted for each tooth, most test teeth showed a combination of both trends although the presence of a plateau predominated. The absolute values of the mechanical properties for each array and the presence of a plateau and the amount of the increase in mechanical properties after the plateau region was independent of the treatment conducted. Both trends were also present in teeth that did not have any
treatment performed i.e. teeth with carious lesions that had been continually exposed with no restorative treatment.

The present investigation has shown that the mechanical properties of the 'infected', highly denatured layer of carious dentine can be rehardened, with the surface of the carious lesion in the test teeth showing an increase with or without a plateauing of mechanical properties. This increase was independent of any treatment provided and indeed occurred in the teeth with no restorative treatment also. Angker and colleagues (2005) using the UMIS conducted a series of indentations in a similar layout as the present study on eight untreated carious primary molar teeth with unknown histories. Similar to the present study, these authors report that the hardness and modulus of elasticity decreased significantly towards the cavity floor and had hardness and modulus of elasticity values that varied from 0.001 to 0.56 GPa and 0.15 - 14.55 to GPa respectively. These values for the hardness and modulus of elasticity of untreated primary teeth are similar to those reported in the present study of 0.002 – 0.98 GPa and 0.02 – 20.06 respectively (185). Interestingly, Angker and colleagues (2005) also noted in the untreated teeth that the change in mechanical properties exhibited two distinct trends which were identical to the trends noted in the outer region in the present study. Angker and colleagues attributed the increase in mechanical properties of the outer region noted in most teeth to indicated the natural remineralisation process that may take place in a mouth as a result of the increase capacity of saliva to cleanse open lesions (185). If this explanation is accurate, then it is likely that the increase in mechanical properties seen in the outer surface of the treated teeth in this study occurred prior to any restoration being placed and that the restoration itself did not alter the mechanical properties of any portion of the carious lesion in any observable way. Regardless of the reason for this increase in mechanical properties, none of the values for hardness and modulus of elasticity recorded reached those found for sound dentine or enamel.

Although the mechanical properties did not appear to be altered with restorative treatment, there were noticeable differences in the microstructure of the primary molar teeth analysed with SEM. All teeth examined showed a continual deterioration of the physical properties from the pulpal aspect to the carious lesion surface of each test tooth where the tubules and peritubular dentine are almost completely destroyed. There are a number of differences between each
tooth with the untreated tooth showing complete breakdown of normal architecture at the surface of the lesion and amorphous plaque like material predominating. This is not unexpected for an untreated tooth to have a large amount of plaque present as it was exposed for the longer period to the oral environment. In contrast the tooth treated with a GIC and calcium hydroxide, the intertubular dentine appeared grainy and has lost its normal architecture throughout the carious region. As this grainy nature was not seen in the tooth treated with just a GIC, it can be speculated that the calcium hydroxide has altered the structure of the carious tissue. However as only a single tooth was examined this is purely speculation at this stage. Another interesting feature of this tooth is the obvious dense encasement of the dentinal tubules with (it is presumed), peritubular dentine. The surrounding intertubular support for the tubules has disappeared leaving the tubular structure vulnerable, fractured and weak. Again whether this is due to the nature of the calcium hydroxide it is unknown. Further SEM work will need to be conducted to determine if these differences could be attributed to the treatments conducted.

As primary teeth are reportedly less well calcified (59) there is debate in the dental literature as to whether the mechanical and physical properties of primary and permanent dentine are similar. In the present study, the indentations conducted in sound dentine ran from the pulpal (inner dentine) to the dentine at the ADJ (outer dentine) in all test teeth. The average hardness of the dentine in the inner, middle and outer region of primary teeth was 0.64, 0.75 and 0.59 GPa respectively. This was in contrast to the average hardness for permanent dentine of 0.71, 0.71 and 0.62 GPa respectively. The findings for the modulus of elasticity for the same regions were 14.13, 16.45 and 13.38 GPa for primary teeth dentine and 15.63, 17.33 and 15.03 GPa respectively for permanent dentine. From this data it can be concluded that there is no significant difference in the mechanical properties of sound primary and permanent teeth.

Comparison between the present study with previous studies conducted on the mechanical and physical properties of sound dentine is difficult due to the varying study protocols and units reported. The absolute values are, however, within the limits of previous studies on permanent teeth using the UMIS (10) and other instrumented hardness apparatuses (76;245). Recently a small number of studies have been conducted within our research unit on the mechanical properties of sound dentine in primary teeth. Angker and colleagues (2003) investigated the
mechanical properties of primary molars using the Ultra Micro-Indentation System on primary dentine and reported that the hardness and modulus of elasticity to ranged from 0.52 – 0.91 GPa and 11.59 – 16.33 GPa respectively (82) which is comparable with the present study (Table 6-13). In contrast, Hosoya and Marshall (2004) have also recently reported on the mechanical properties of sound dentine in primary canines and found the hardness values were lower overall than the present study, yet higher for the modulus of elasticity (83). Table 6-13 summarises the results from the present study and the studies by Angker and colleagues (2003) and Hosoya and Marshall (2000). Further analysis of this table reveals, that similarly to other studies on permanent teeth (86;164;245) the hardness and modulus of elasticity of dentine adjacent to the ADJ (outer dentine) and the pulp (inner dentine) were on average lower than in the middle region. In the studies by Angker and colleagues (2003) and Hosoya and Marshall (2000) found only the average mechanical properties near the pulp to be lower (82;84). This is similar to what was found in the present study for permanent teeth. There is little doubt in the dental literature that the mechanical properties of sound dentine adjacent to the pulp are lower than the middle region, although further research will be required to determine if in fact the mechanical properties of sound dentine are also lower in the region adjacent to the ADJ.

<table>
<thead>
<tr>
<th>Study</th>
<th>Outer- near ADJ</th>
<th>Middle</th>
<th>Inner- near pulp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hardness</td>
<td>E*</td>
<td>Hardness</td>
</tr>
<tr>
<td>Present study</td>
<td>0.64 ± 0.07</td>
<td>14.13 ± 2.44</td>
<td>0.75 ± 0.06</td>
</tr>
<tr>
<td>Angker et al., 2003(82)</td>
<td>0.91 ± 0.15</td>
<td>16.33 ± 3.83</td>
<td>0.85 ± 0.19</td>
</tr>
<tr>
<td>Hosoya and Marshall 2004(83)</td>
<td>0.57 ± 0.12</td>
<td>24.27 ± 3.93</td>
<td>0.56 ± 0.12</td>
</tr>
</tbody>
</table>

Table 6-13. Table comparing the mechanical properties of sound primary dentine in the present study with two recent studies conducted on primary teeth.

E*: modulus of elasticity, ADJ: amelo-dentinal junction

Although the numbers of teeth used in the present study is limited, from the present investigation the following conclusions can be drawn:

1. The mechanical properties of sound dentine adjacent to the pulp are lower than the middle region in primary and permanent teeth.
2. Further research will be required to determine if the mechanical properties of sound dentine in primary and permanent teeth are lower in the region adjacent to the ADJ.

3. The highly infected surface region of carious lesions has the ability to reharden.

4. The mechanical properties of infected carious dentine were not significantly altered when treated with calcium hydroxide, GIC or a resin restoration.

5. The microstructure of primary teeth was altered depending upon the treatment provided but further work will be required in this area.

6. Further research will be required using alternate methodologies and larger numbers of teeth to determine if restorative treatments can affect the mechanical and microstructural properties of carious dentine.
Chapter 7  DISCUSSION

This thesis is made up of a series of three experiments. The first two experiments establish the baseline properties of carious primary incisor and hypomineralised first permanent molar teeth. With this information, the final experiment describes the effect of treatment on the mechanical and microstructural properties of treated primary and carious teeth.

Very young children often present with carious primary incisors. Such teeth may be either extracted (which usually involves the use of a general anaesthetic) or restored. The traditional approach to restoration involves the removal of the carious enamel and dentine and replacement of the missing tooth tissue with either a resin based material or a glass ionomer cement. However restoration of primary incisors is associated with a high failure rate due both to development of further caries and problems with retention and integrity of the restoration itself (401-406). A more conservative approach would involve less or no removal of carious tooth tissue followed by remineralisation of the residual carious tissue. Presently there is minimal understanding of the ability of dentine to be remineralised and the effect this remineralisation will have on the integrity of the tooth. Furthermore there is a lack of understanding of baseline mechanical and microstructural properties of carious dentine. The first experiment (Chapter 4) reported on the mechanical properties of sound and carious dentine from primary incisors. Under conditions of constant hydration the hardness and elastic modulus of the carious lesions were compared with those of unaffected dentine in the same primary incisors. This study not only allowed comparison with previous studies but also provided further insight into the mechanical properties of carious lesions. The inner portion (that closest to the pulp) of the carious dentine lesion had an average hardness and modulus of elasticity of $0.29 \pm 0.16 \text{ GPa}$ and $7.77 \pm 5.05 \text{ GPa}$ respectively. The overall mechanical properties of the inner, unaffected region (under the carious lesion) was lower that had been previously reported for primary teeth (82;83). It is possible that the primary teeth used in this study were very carious and even the macroscopically unaffected dentine adjacent to the pulp (inner region) was already slightly demineralised resulting in lower mechanical properties. From the inner region, the mechanical properties deteriorated progressively toward the lesion cavity floor where the lowest values
for hardness and modulus of elasticity were found (0.07 ± 0.1 GPa and 0.99 ± 1.45 GPa respectively). Although the mechanical properties of sound dentine from the pulpal (inner) to the surface of the dentine did not show the same deterioration, with similar hardness and modulus of elasticity recorded for the inner and surface regions, the mechanical properties of the sound dentine in the primary incisors were also low in comparison to previous reports (82-84). The results for primary incisors for the inner, middle and outer region of sound dentine were 0.40, 0.45 and 0.39 GPa respectively whereas Angker and colleagues (2003) using the same UMIS system, found the hardness of sound dentine in primary molars ranged from 0.52 – 0.91 GPa (82). It is difficult to explain the lower values found in primary incisors. It is possible that incisor teeth do differ innately from molar teeth in their structure and mechanical properties. The differences may also have arisen from the fact that the orientation of indentations in the present study was in the lingual - labial direction in contrast to the mesial-distal axial section of the Angker study. If in fact the hardness and modulus of elasticity of primary teeth are lower than posterior primary teeth, this may result in increased wear and increased flexing on loading respectively and this may help to explain some of the clinical problems associated with the restoration of primary incisors. Further modification of restorative materials may be required to match the mechanical properties of primary anterior teeth more appropriately.

As in previous studies in carious primary (185) and permanent teeth (161), a dramatic linear decrease in mechanical properties upon entering the carious region in the primary incisors was demonstrated. This width in the drop in mechanical properties was consistently 1000 to 1200 μm in length. The majority of teeth in this study showed an increase in hardness (and elastic modulus) in the most superficial, 300 – 500 μm of the carious lesions. A similar finding has also been recently reported for occlusal lesions of primary teeth (185). It is speculated that this increase in mechanical properties is due to natural remineralisation processes and therefore suggests that remineralisation of smooth surfaces in primary incisors is possible even in severely carious lesions. This surface rehardening is also frequently encountered clinically. Mäkinen and colleagues (1998) investigated the ability of a chewing gum (containing xylitol and sorbitol) to arrest dentine caries in children. After 20-22 months they showed clinically that all carious lesions had rehardened and this was confirmed by hardness testing upon the teeth removal (314). Extrapolating this further, in the future if the entire depth of the carious lesion could be rehardened not only would this result in significantly
smaller restorations being placed but the tooth integrity that was lost with cavitation could be restored. However further work is required to establish the basis of this increase in hardness, whether it is related to remineralisation and to further explore ways to enhance such “natural” or non-restorative induced remineralisation of severe lesions. The final study (Chapter 6) was designed with this in mind.

In addition to dental caries, increasing numbers of children are presenting with compromised first permanent molars. These teeth are not only susceptible to plaque accumulation and dental caries but also pose significant restorative challenges to the clinician. The texture and composition of the residual tooth tissue is such that cavity preparation is difficult and subsequent bonding of conventional restorative materials is prone to failure. As a result these teeth often need to be extracted. There is a dearth of information on the microstructural and mechanical properties of the hypomineralised enamel and a complete absence of information on the dentine of such teeth. Improved understanding of these baseline mechanical properties will allow for the development of informed clinical protocols and improve outcomes. Therefore Chapter 5 comprised of a series of experiments on both the enamel and dentine of compromised first permanent molars. The most striking finding from the enamel study was the very low hardness and modulus of elasticity of the hypomineralised enamel (0.53 ± 0.31 GPa and 14.49 ± 7.56 GPa respectively) in comparison to unaffected enamel (3.66 ± 0.75 GPa and 75.57 ± 9.98 GPa respectively). In contrast the studies on dentine found that the mechanical properties and the microstructure of the dentine of minimally carious hypomineralised first permanent molar teeth appear similar to that of control/unaffected teeth.

The very poor mechanical properties found in enamel explain why traditional restorative options for hypomineralised first permanent molar teeth are fated to fail. Not only is the enamel of affected teeth soft but will flex more on loading. Although the affected enamel appears to have similar apatite composition to unaffected enamel, the affected enamel has an overall lower mineral content and the enamel crystals, seen in TEM (Transmission Electron Microscopy) and the enamel rods, seen by SEM (Scanning Electron Microscopy) appear more disorganised. This is likely to lead to problems when restored teeth are exposed to occlusal loads in function. Furthermore the SEM images of etched hypomineralised enamel indicate that the traditional etching patterns seen for
sound enamel was absent. Upon increased etching times, the hypomineralised enamel becomes increasingly disorganised and there is no preferential dissolution of the rod boundaries seen. It is speculated that there is a higher level of protein present in the inter-rod area of hypomineralised enamel which would help to explain the limited dissolution in this area. If the amount of protein is increased and the characteristic etching pattern is absent, the traditional adhesive materials such as composite resin are unlikely to bond adequately at the microstructural level. Together with the significantly reduced mechanical properties of affected tissue this may explain why many restorations in these teeth fail.

Clinicians are frequently forced to carry out treatment on hypomineralised first permanent molar teeth when a child is very young. When affected teeth require restoration there are three options to ensure longevity of the restoration. Firstly to maximise the tooth integrity, stainless steel crowns can be placed. These full coverage restorations are relatively time consuming and young children can find this type of treatment difficult especially on such sensitive teeth (130). A second option is to remove (surgically) the hypomineralised enamel and any carious tissue. Although this will ensure there is unaffected enamel and dentine available for bonding with an adhesive material, the hypomineralised defects can often be large and will not follow traditional cavity outlines. This again creates very large, difficult, and time consuming restorations which are likely to require further more complex treatment in both the short and long term. The final option, which minimises the amount of affected tooth tissue that removed, is to use a restoration such as a glass ionomer cement. This material will bind chemically to the affected enamel, although presently it is not known if this bond is adequate to prevent loss of the restoration. Furthermore as this type of restorative material cannot contribute significantly to supporting the residual hypomineralised tissue, these teeth continue to chip and fracture on loading resulting in further restorative treatment. To limit the amount of surgical treatment required for hypomineralised first permanent molars, remineralisation of both the residual compromised tooth would be advantageous. As a result of the increase in mineral it can be predicted that there will be a simultaneous increase in mechanical properties which may be associated with improved longevity for the restored tooth.

Anecdotally clinicians report that the dentine beneath hypomineralised enamel is soft or cheesy and yet the hardness and modulus of elasticity are apparently no
different to that of sound dentine. The underlying reason for this is unclear. It is possible that clinicians are unable to determine enamel from dentine during cavity preparation. They may confuse the soft, unusual consistency of the hypomineralised enamel with the sound dentine. As hypomineralised teeth are more prone to caries it is also possible that the unusual consistency of the dentine is in fact caries. Finally it is possible that there is in fact a difference in the consistency and mechanical properties of dentine from hypomineralised teeth that the present study has been unable to detect. Further research using other techniques such as atomic force microscopy and TEM will be required to determine if there is in fact a difference in the dentine of sound and hypomineralised teeth.

Dental caries is not only one of the most common chronic diseases of childhood but also one of the most costly (1). There has recently been a shift in treatment planning philosophy with the goals now being aimed at initially reducing the causative microbiota and contributing risk factors in an endeavour to halt the carious process and then stimulating remineralisation of the residual damaged tissues (424). This is essentially the Minimal Intervention (MI) approach. Understanding the changes that take place in dental hard tissues when exposed to this approach will help in the understanding of our ability to use, stimulate, or augment natural regenerative processes of the pulp-dentine complex (424). With remineralisation of compromised dental tissues it is expected that the outcomes of restorative treatment can be optimised. With improved (re)mineralisation there will be an increase in the bulk of the residual tooth, less surgical removal of tooth tissue, smaller restorations, decreased sensitivity in, and potentially less damage to, the pulpal-dentine complex. This has clear positive implications from all perspectives; clinically, professionally, socially and economically. In line with this there is increasing interest in the ability of compromised dental tissues, such as dental caries, to be replaced, regenerated or remineralised using tissue engineering (424) or exogenous substances such as fluoride or CPP-ACP.

Numerous in vivo methods have been used in an attempt to quantify the progression of carious lesions after minimal restorative treatment. The simplest method is to assess the clinical outcome, usually only through clinical examinations. This has been reported in a number of studies conducted on the ART (Atraumatic Restorative Technique) in developing countries (425-429). Inevitably such studies will underestimate the disease experience as they rely
solely on clinical examination with no radiographs and as a result there will be little understanding of the effect of this approach on the carious dentine itself. Other *in vivo* approaches have involved monitoring the microbial load (186;206;301;351;430), the colour (301) or wetness (188) of remaining carious dentine after treatment. Assessment of both colour and wetness of a lesion are essentially subjective and examiner dependant whilst monitoring microbial loads requires the removal of carious dentine which will result in a change in the environment which is difficult to quantify. Mertz-Fairhurst and colleagues (1998) in their study of sealed permanent teeth utilised standard intra-oral radiographs to evaluate the progression of the sealed carious lesions over a 10 year period (8;191). This method has also been used in other studies (186) but the assessment of the progression of the carious lesions using radiographs is still very subjective. Work is continuing on the use of other mechanisms of *in vivo* diagnosis and mineral quantification tools such as Quantitative Light-Induced Fluorescence (431-433). Although this method is promising it has generally been used to quantify mineral loss in enamel rather than deep dentine lesions in which the increased water content of the tissues under investigation has given rise to problems calibrating the technology. There is currently no reliable and valid *in vivo* technique for measuring the mineral content of dentine hence it is not possible to monitor the remineralisation that is said to occur beneath restorations. Therefore *in vitro* assessment of the remineralisation capabilities of dentine dominates the literature. Most of the studies focus on artificially demineralised dentine (212;217;337;344). It is obvious why artificially demineralised dentine is studied however the lack of bacteria in such a model will undoubtedly affect the results of such remineralisation and demineralisation studies. Therefore the final study in this thesis used naturally occurring carious lesions in an attempt to overcome these limitations.

The final study in this thesis utilised a micromechanical approach to assess the properties of carious dentine with known treatment histories. This approach has a number of advantages. Firstly it allows both quantitative and qualitative data to be obtained allowing direct comparison of different treatment regimes. Multiple measurements over the whole sample can be conducted without it being destroyed, allowing the sample to be used for other tests such as SEM analysis. Micromechanical methodology also allows the intrinsic integrity of teeth to be described which is directly relevant to the clinical situation. If the tooth is to function mechanically in a manner similar to its intact state then any treatment
that "repairs" a carious lesion or hypomineralised defect, should aim to achieve comparable mechanical properties to that intact state. The advantage of the micromechanical approach were highlighted in Chapter 5, where for the first time a significant reduction in the mechanical properties of hypomineralised enamel was identified. Micro-radiographical techniques (such as BSE or microradiography) cannot be used in isolation as they can significantly underestimate the clinical situation of compromised tissues. The results of BSE in Chapter 5 showed that whilst there is only a 5% reduction in mineral content of hypomineralised enamel, these teeth are not only clinically but also mechanically (as established using the UMIS) much softer and more flexible than unaffected teeth. The use of micro-radiographical techniques in isolation in remineralisation investigations must therefore be viewed with some caution and it has therefore been suggested that a more appropriate endpoint in remineralisation studies should be the establishment of mechanical properties consistent with the normal tissue (434).

The final study investigated the mechanical properties of carious lesions treated with a variety of restorative materials. This study is unique. Ethical approval was obtained to allow treatment of carious lesions and the collection of these teeth after a known interval. Obtaining ethical approval for a study such as this is very rare in Australasia. Obtaining ethical approval for this type of study not only allows objective assessment of the change in carious dentine with known treatment histories, but will also allow further work on other restorative procedures and materials to be tested in a similar way. In this investigation, although the mechanical properties of the individual teeth varied, all teeth (regardless of treatment provided) showed a dramatic linear decrease in mechanical properties upon entering the carious region. Similar to the primary teeth tested in the first experiment of this thesis, the linear decrease was consistently 1000 to 1500 µm in length. Two further trends were evident in the surface region of the carious tissue. One involved two of the test teeth and consisted of a continual reduction in the mechanical properties right to the surface of the lesion whereas the majority of teeth showed a plateauing followed by an increase in mechanical properties in the final 800 µm. Again these trends appeared to be independent of how the teeth had been treated.

This study is the first to use a biomechanical approach to the effect of restorative treatment on tooth tissue. The protocol for the final study specified that no caries
was to be removed prior to restoration. As a result both infected and affected
dentine remained. It has been reported that the infected region of carious dentine
cannot be remineralised, whereas the affected region in an appropriate
environment can be (171). To date there appears very little evidence to support
this claim. For remineralisation to occur in the infected region of dentine caries, it
can either be initiated from the pulp outwards or from the surface of the lesion
inwards. The first study in this thesis has shown that advanced smooth surface
lesions show an increase in mechanical properties in the surface region even
with no treatment (Chapter 4). This has also been reported for the infected region
in occlusal lesions in primary molar teeth (12) and in the testing of the mechanical
properties of untreated teeth in the final study (Chapter 6). Given that the
mechanical properties of carious dentine have been shown to correlate with its
mineral content (182) it is possible to hypothesise that remineralisation of the
infected region can occur from the external environment. The final study explored
the possibility that the mechanical properties of the carious lesions could be
further increased by placement of a restoration. No differences in the mechanical
properties were found between the untreated teeth and treated teeth irrespective
of the materials used. Increases in the mechanical properties at the surface of the
lesions were found to be no different to those found in the previous studies of
untreated carious teeth (185)(Chapter 4). Simply sealing the carious dentine from
the oral cavity did not appear to enhance the mechanical properties nor
potentiate remineralisation of the infected outer region, although it did not result
in any reduction either. It is possible that the increase in mechanical properties
seen in the surface region was due to remineralisation prior to any restorative
procedure conducted in this study. The SEM analysis of treated primary molar
teeth have shown that regardless of treatment, all teeth showed a continual
deterioration of the physical properties from the pulpal aspect to the carious
lesion surface with the dentine tubules, peritubular and intertubular dentine
becoming progressively less defined. Although there were differences in the
microstructure of each tooth examined, these differences did not appear to have
any affect on the mechanical properties of these teeth when tested with the
UMIS.

The final study suggested that whilst it may be possible to arrest a carious lesion
for over 10 years, by placing a sealed restoration directly over it (8), at least in the
short term the enhancement of the mechanical properties does not occur. If we
speculate that an increase in mechanical properties is due to the deposition of
mineral, for an increase in mechanical properties to occur upon sealing of carious lesions the addition of rechargeable bioactive materials with or without bactericidal properties to current restorative materials may enhance this process. Further research is needed.

The present study has a limited sample size and larger number of teeth will be required to confirm the findings of this study. Larger numbers of teeth will allow more comprehensive quantitative information on the microstructural and mechanical properties using UMIS and SEM respectively of treated carious dentine. Finally prior to any definitive conclusion, a method of measuring the mechanical properties in vivo needs to be developed. This would facilitate accurate measurement of the hardness and modulus of elasticity of carious dentine prior to and after restorative treatment has been completed. It would also allow assessment of any remineralisation strategy clinically. This is the future direction of research in our unit.
Chapter 8    APPENDIX

Appendix 1.
Published Papers arising from this thesis.

Appendix 2.
Manufacturers of Equipment and Materials used in present thesis.

Appendix 3.
Ethics acceptance from Western Sydney Area Health Service (WSAHS) and Central Sydney Area Health Service (CSAHS) Ethics committees.

Appendix 4.
Consent form and patient information sheet for WSAHS.
As these forms were almost identical for the submission to each ethics committees, the CSAHS forms were not included.

Appendix 5.
Form used in final study (Chapter 6) to collect information during clinical treatment sessions.

Appendix 6.
Full graphical results for all test teeth from final study (Chapter 6).
APPENDIX ONE

Articles:
Mechanical properties and microstructure of hypomineralised enamel of permanent teeth
Mechanical properties across hypomineralised/hypoplastic enamel of first permanent molar teeth

Statement of contribution
Erin Mahoney- primary researcher and writer

FS Ismail- Assisted with indentation of hypomineralised enamel and dentine

R. Rohanizadeh- Assisted with operation of X-ray diffraction and SEM machinery and analysis

NM Kilpatrick- Supervisor

MV Swain- Supervisor
Mechanical properties and microstructure of hypomineralised enamel of permanent teeth

Erin K. Mahoney a,*, R. Rohanizadeh a, b, F.S.M. Ismail c, N.M. Kilpatrick d, M.V. Swain e

a Biomaterials Unit, 8th Floor, United Dental Hospital, University of Sydney, 2 Chalmers Street, Surrey Hills, Sydney NSW 2010, Australia
b Bone and Skin Research Group, Department of Physiology, University of Sydney, NSW 2010, Australia
c Department of Aerospace, Mechanical, and Mechatronics Engineering, University of Sydney, Sydney NSW 2010, Australia
d Murdoch Children's Research Institute, Royal Children's Hospital, Flemington Road, Melbourne, VIC 3055 Australia
e Department of Oral Sciences, School of Dentistry, University of Otago, PO Box 647, Dunedin, New Zealand

Received 3 December 2003; accepted 13 February 2004

Abstract

Isolated enamel defects are commonly seen in first permanent molar teeth but there has been little work on the physical and morphological composition of affected molars. The aim of this study was to determine the mechanical and morphological properties of hypomineralised first permanent molar teeth, utilising the Ultra-Micro-Indentation System (UMIS) and scanning electron microscope, respectively. Further investigations using Energy Dispersive X-ray Spectrometry (EDS), Back Scatter Electron (BSE) Imaging, and X-ray diffraction were employed to attempt to determine the chemical composition, mineral content and crystalline structure of the hypomineralised tissue, respectively, of eight first permanent molars with severe enamel hypomineralisation. The hardness and modulus of elasticity were found to be statistically significantly lower (0.53 ± 0.31 and 14.49 ± 7.56 GPa, respectively) than normal enamel (3.66 ± 0.75 and 75.57 ± 9.98 GPa, respectively). Although the fractured surface of the hypomineralised enamel was significantly more disorganised and the relative mineral content was reduced by approximately 5% in comparison to sound enamel, the mineral phase and Ca/P ratio was similar in hypomineralised and sound enamel. The dramatic reduction in the mechanical properties of first permanent molar teeth has ramifications when clinicians are choosing restorative materials to restore the defects. The reason for the dramatic reduction in mechanical properties of hypomineralised first permanent molar teeth is at present unknown.

Keywords: Mechanical properties; Elasticity; Teeth; Enamel; Hypoplasia

1. Introduction

Isolated enamel defects are commonly seen in first permanent molar teeth and are usually a combination of enamel hypoplasia and hypomineralisation [1]. Enamel hypoplasia is characterised by a deficiency of tooth substance that ranges from minor pits and grooves to total absence of enamel caused by a disruption to the ameloblasts during matrix secretion [2-4] (Fig. 1). If the disruption occurs during either the calcification or maturation phase of enamel formation, then the teeth will appear opaque and this is a qualitative defect termed enamel hypomineralisation or enamel opacity (Fig. 1). Both of these terms are based solely on descriptive criteria and no association is made to aetiology [5]. For the purposes of this paper the term hypomineralisation will be used to refer to the affected teeth investigated in this study.

To date most of the research in this area has focused on the prevalence and the cause of enamel hypomineralisation. There has been little work on the physical and morphological composition of affected molars. Such information may assist in defining better treatment strategies for these teeth, which traditionally pose a significant challenge to clinicians. The crown form is compromised in terms of both the quantity and the quality of the remaining tooth tissue. This makes choosing not only the appropriate restorative material difficult but also defining the most durable restorative technique (i.e. cavity form and/or adhesive mechanism) also challenging. The defective enamel can fracture under normal occlusal forces leading to excessive chipping and wear [6,7]. Furthermore, these defective
molars are more susceptible to dental caries and tend to be hypersensitive, which leads to increased levels of anxiety for children during dental treatment [7–9]. Understanding the structure and properties of hypomineralised enamel is fundamental to improving the restorative outcomes for these teeth. Previous histological investigations have shown that the disturbed enamel shows hypomineralisation localised to the cuspal region, with the cervical third of the enamel having a normal morphological and histological appearance [10].

Knowledge of the mechanical properties of teeth such as, the hardness and modulus of elasticity may lead to an improved understanding of the behaviour of hypoplastic teeth in occlusion. The modulus of elasticity is the linear portion of the slope of the stress to corresponding strain below the proportional limit and indicates how a material will flex under loading. The hardness of a material is a measure of its ability to resist a permanent indentation [11] and is considered to reflect susceptibility to abrasive wear [12]. Presently little is known about these properties of hypomineralised enamel probably because of the difficulty in obtaining samples as well as an inability to test the small sample area common in hypomineralised teeth. The Ultra-Micro-Indentation System (UMIS), a nano-indentation system allows the multiple tests on a small areas of hydrated material to be conducted [13–15].

It has been suggested that the mechanical properties of a calcified tissue are generally linked to its mineral content [16–19]. Incorporation of additional phases such as carbonate, magnesium, sodium and fluoride into the apatite structure of enamel crystals results in changes in physico-chemical and mechanical properties of enamel. For example a higher concentration of carbonate in crystal structure leads to a lower crystallinity and therefore higher solubility of hard dental tissues, whereas, fluoride substitution results in bigger crystals and better crystallinity (fewer crystal defects), which in turn reduces the enamel solubility [20]. Understanding the nature and content of the mineral is also important when attempting to bond dental materials to tooth tissue. Some materials, such as glass ionomer cements, which are used to restore hypomineralised defects, rely on bonding to the mineralised components of the tooth substrate. Energy Dispersive X-ray Spectrometry (EDS) and X-Ray diffraction can be used to acquire information on the biological apatites present in enamel. These methods cannot however, provide quantitative measurement of mineral content. Backscattered Scanning Electron (BSE) imaging has been used to study the calcification state of mineralised tissues such as bone and dental hard tissues [21–24]. The graylevel intensity in the BSE image has been reported to be highly correlated with calcium content [25], mineral content, mineral density and atomic number of bone [26,27]. This system is similar to microradiography but has better resolution and has the additional advantage of simpler specimen preparation [23].

Understanding the structure and properties of hypomineralised enamel is fundamental to improving the restorative outcomes for these teeth. The aim of this study was to determine the mechanical properties of hypomineralised first permanent molar teeth (with the UMIS), determine the morphological structural of hypomineralised defects using scanning electron microscopy (SEM), determine the chemical composition and crystalline structure of the hypomineralised areas using Energy Dispersive X-ray Spectrometer (EDS) and X-ray diffraction and finally to measure the mineral content of the hypomineralised tissue in comparison to unaffected enamel using BSE images.

2. Material and Methods

2.1. Sample preparation

Eight first permanent molar test teeth extracted due to severe enamel hypomineralisation with or without
enamel hypoplasia and two control teeth (first premolars extracted for orthodontic reasons) were used in this study. Upon extraction each tooth was placed into deionised water with a small number of thymol crystals to inhibit bacterial growth and stored at 4°C. Poulthong (1998) has previously found that teeth can be dry for up to 2 days without influencing the hardness and modulus of elasticity of either dentine or enamel when using the UMIS [28]. The effect of storage hypomineralised enamel in deionised water is unknown although it has been shown to affect enamel of normal teeth [29]. It is expected therefore that any reduction in mechanical properties of the hypomineralised or sound enamel will be similar and therefore comparisons are still valid.

Preparation of the specimens has been described in detail previously [14]. Each tooth was encased in cold cured epoxy resin and sectioned through the centre of the lesion in the mesial-distal axial plane using a water cooled diamond impregnated circular saw (Isomet, Buehler Ltd., Lake Bluff, USA). The cut surface was then polished with successively finer grade silicon carbide paper and finally with 9 and 1 μm polycrystalline diamond suspension. Each sample was placed in an ultrasonic bath between each and immediately following the polishing steps. Once prepared and while waiting testing, the samples were kept fully hydrated in deionised water with a small number of thymol crystals. Tests were typically conducted within 2 days of preparation.

2.2. Mechanical properties

When each specimen was ready for testing, it was placed on the work table of the UMIS with the polished surface upwards. As the hypomineralised region of the test teeth is more opaque than the unaffected enamel, the area to be tested could be determined visually with the aid of the microscope associated with the UMIS. To determine the mechanical properties of the hypomineralised region of affected teeth, nine indentations were made in the affected region of the prepared surface to a maximum force of 20 mN. The maximum force was reached with 25 increments and the displacement recorded at each step. The force at each increment is given according to the formula, \( F_i = \frac{i}{n} F_\text{max} \) (where \( F_i \) is the force at the \( i \)th step, \( n \) is the total number of steps and \( F_\text{max} \) is the maximum force). The first indentation was between 200 and 400 μm from the external tooth surface. The subsequent indentations were made 100 μm from the previous indentation (Table 1) in a 3 x 3 arrangement. To determine the mechanical properties of ‘normal’, unaffected enamel, 10 indentations to a maximum force of 20 mN were conducted in the (visually) unaffected enamel in the cervical region of each test tooth. Each indentation in this region began 100–200 μm from the enamel surface. Two sets of nine indentations were also carried out in identical regions of the enamel of the control teeth to a maximum force of 20 mN. The layout of the indentations and test conditions of the control teeth and the affected enamel were identical to the indentations carried out in the hypomineralised region (Table 1). All indentations were made using a Berkovich indenter (for more detailed description of the UMIS techniques, refer to Mahoney et al. [14]). At all times the test surface was kept 100% hydrated by the placement of droplets of distilled water over the test region.

The software associated with the UMIS was used to calculate the hardness and modulus. Prior to testing the Berkovich indenter tip was calibrated using fused silica and the technique developed by Oliver and Pharr [30]. The modulus of elasticity for each indentation was calculated as a function of the unloading curve at the maximum depth of penetration. The hardness was calculated at each of the 25 increments up to the maximum force of 20 mN, assuming the elastic modulus remained constant, these values were then averaged (Table 2). Two-way ANOVA tests were conducted to compare mean hardness and elasticity to \( P < 0.001 \). The overall mechanical properties were determined for each tissue in the test teeth and the enamel in the control teeth by averaging all indentations of each tooth.

2.3. Scanning electron microscopy (SEM)

Following micro-indentation tests, three specimens were prepared for SEM. To reveal the enamel microstructure, fractured surfaces of unaffected and hypomineralised enamel were examined. This was done by cutting a notch above and below the region to be investigated with a water cooled diamond impregnated circular saw (Isomet, Buehler Ltd., Lake Bluff, USA). The notched specimens were then fractured through the sound and hypoplastic enamel. Care was taken to not cut into the affected region.

Each specimen was then left to dry for 24 h. The dehydrated specimens were then sputter coated with

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Test parameters and conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test parameters</td>
<td>Indentation layout</td>
</tr>
<tr>
<td></td>
<td>100 μm apart in both x- and y- axis</td>
</tr>
<tr>
<td></td>
<td>3 x 3</td>
</tr>
<tr>
<td>Maximum force</td>
<td>20 mN</td>
</tr>
<tr>
<td>Number of increments per indentation</td>
<td>25</td>
</tr>
<tr>
<td>Incremental progression</td>
<td>Square</td>
</tr>
<tr>
<td>Dwell at load/max/unload (s)</td>
<td>0.1/30/0.1</td>
</tr>
<tr>
<td>Test conditions</td>
<td>23 ± 1°C and 50 ± 10% RH</td>
</tr>
</tbody>
</table>
Table 2
Overall hardness and modulus of affected teeth

<table>
<thead>
<tr>
<th>Tooth number</th>
<th>Unaffected enamel (GPa)</th>
<th>Hypomineralised enamel (GPa)</th>
<th>Reduction in mechanical properties (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hardness</td>
<td>Modulus of elasticity</td>
<td>Hardness</td>
</tr>
<tr>
<td>1</td>
<td>5.35±1.10</td>
<td>80.36±27.01</td>
<td>0.32±0.16</td>
</tr>
<tr>
<td>2</td>
<td>3.48±0.62</td>
<td>60.90±3.51</td>
<td>0.67±0.31</td>
</tr>
<tr>
<td>3</td>
<td>3.54±0.43</td>
<td>85.70±3.56</td>
<td>0.34±0.18</td>
</tr>
<tr>
<td>4</td>
<td>3.73±0.48</td>
<td>69.91±7.67</td>
<td>0.73±0.19</td>
</tr>
<tr>
<td>5</td>
<td>3.70±0.79</td>
<td>70.50±16.10</td>
<td>0.85±0.40</td>
</tr>
<tr>
<td>6</td>
<td>2.89±0.92</td>
<td>64.64±9.16</td>
<td>0.10±0.05</td>
</tr>
<tr>
<td>7</td>
<td>3.52±0.39</td>
<td>77.90±2.59</td>
<td>0.95±0.12</td>
</tr>
<tr>
<td>8</td>
<td>3.48±1.06</td>
<td>74.68±7.84</td>
<td>0.31±0.09</td>
</tr>
<tr>
<td>Control 1</td>
<td>3.09±0.48</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Control 2</td>
<td>3.31±0.57</td>
<td>79.48±7.48</td>
<td>NA</td>
</tr>
</tbody>
</table>

Two-way ANOVA tests showed that there was a significant difference ($P<0.001$) between the mean hardness and elasticity of the hypomineralised and sound enamel.

gold and observed using SEM (SEM 505, Phillips, Eindhoven, The Netherlands) at 20 kV with a spot size of 5 nm. The samples were examined in secondary electron mode.

2.4. X-Ray diffraction (XRD)

To determine the crystal structure and crystallinity of hypomineralised enamel, XRD was carried out using a Siemens machine (Diffractometer D5000, Karlsruhe, Germany). A small piece of hypomineralised enamel was fractured from the affected areas and placed in the XRD sample holder. The X-ray detector was scanned from 3° to 40°, with 0.01° step size, and 10 s analysis time per step. CuKα radiation working at 40 kV and 40 mA was used in this study. As a control, the same procedure was also carried out on a fracture of sound human enamel to compare the diffraction patterns between pathologic (hypomineralised) and sound enamel. Attempts were made to fracture the sound and hypomineralised enamel in a similar orientation. Both samples were orientated in a flat plane when analysed.

The crystallinity of samples was determined by measuring the broadening of peaks. The broadening of XRD diffraction peaks reflects crystal size and/or perfection or strain: the broader the peak, the smaller or less perfect or more strained the crystals. The broadening of peaks were measured at half height of the deconvoluted diffraction peak. The height of the peaks is the distance from highest point of the peak to the base line of the peak.

2.5. Energy dispersive X-ray spectrometer (EDS)

A single, flat, carbon coated sample that had been used in the testing of mechanical properties was used for the EDS analysis. For the analysis of the percent component composition of hypoplastic and sound, enamel the X-ray detector system (EDAX, P-505, SUJTW-Sapphire) attached to a SEM (SEM 505, Phillips, Eindhoven, The Netherlands) was used. The system was operated at 20 kV, spot size of 5 nm, specimen tilt of 15° and 15-20 nm working distance was used. The counting time was 100 s.

Five counts were conducted, three in the hypomineralised region and two in sound enamel of the same tooth. The counts in hypomineralised enamel were conducted in three areas half way between the amelodentinal junction and the surface of the sectioned test tooth. The unaffected enamel counts were conducted in the centre of the sound enamel in the cervical region of the test tooth. For all of the five measurements, the X-rays were detected in a window approximately 1 x 1 mm. This allowed the relative amounts of calcium (Ca), phosphorus (P), sodium (Na), potassium (K) and magnesium (Mg) to be determined.

2.6. Back scattered electron (BSE) image

Following the UMIS tests, three uncoated test specimens were observed and photographed with back scatter electron detector using a Scanning Electron Microscope XL-30 (FEI, Eindhoven, Netherlands) with a solid-state backscattered electron detector (FEI, Eindhoven, Netherlands). The SEM was operated at 20 kV, a spot size of 5 nm, and a beam current of 1 nA, magnification of 50 times, a constant working distance of 10 mm and a pressure of 1.5 torr in the chamber. These parameters were kept constant during the taking of BSE micrographs.

A glass plate coated with a thin film of evaporated carbon was used to map the sensitivity of the BSE detector, whilst a fine polished sample of silicon and pure carbon embedded in resin was used to standardise the graylevel spectrum for every specimen. The contrast and brightness were preset using the horizontal line profile tool on the SEM, in which the graylevel spectrum was extended to reach the top line of the profile tool on the enamel and down to the bottom line on the carbon and kept constant during the session.
The BSE images were analysed using Digital Micrograph 3.3.1 on a Macintosh computer. The BSE intensity was measured in greylevels (0–255). The calibration of the system was done first by analysing the carbon sample (A) to detect the characteristic response function of the BSE detector over the area of the image used in the study. A new image of the carbon glass plate (B) with floating value as a response function of the BSE detector was reproduced [B = A/mean value]. This image (B) was subsequently used to correct the images of the test specimens and the standard block (C) for the response function. The corrected image of the test specimens (D) was obtained from C/B. The graylevel spectra of the enamel-carbon blocks were then re-ranged with the enamel graylevel at 255 and carbon at 0 to correct any variations of the experimental condition. Detailed information on this methodology has been reported elsewhere [21,31].

The graylevels were determined for the hypomineralised region, unaffected region and for dentine by linear scans, 10 pixels x 10 pixels. The graylevels for each pixel in each region for all the three teeth were averaged and graphed to allow comparison between each tissue type. Care was taken to ensure that in determination of the graylevels that the linear scans were not done in various regions of dentine.

3. Results

3.1. Mechanical properties

The depth penetrated by the indenter was on average, 300–500 nm in the sound dentine and between 1000 and 1500 nm in the hypomineralised region. The average hardness and modulus of elasticity for the test and control teeth is shown in Table 2. This table shows that the hardness of hypomineralised enamel varies from 0.10±0.05 to 0.95±0.12 GPa which was significantly lower (P<0.001) than that of unaffected enamel (2.89±0.92 to 5.35±1.10 GPa). The modulus of elasticity of the hypomineralised region (3.45±1.08 to 23.81±4.34 GPa) was also significantly lower (P<0.001) than unaffected enamel of the test teeth (60.90±3.51 to 89.91±7.67 GPa). The hardness and modulus of elasticity of the enamel of the control teeth were similar to the unaffected enamel of the test teeth. A summary of the overall mechanical properties of each tissue is shown in Table 3.

3.2. SEM images

Typical fractured surface SEM images are shown in Fig. 2. The images of normal enamel show an amorphous, orderly rod appearance. In contrast the hypomineralised enamel is disorganised, with variable rod widths and there is a loss of distinct boundaries between the enamel rods. In the hypomineralised enamel there are also obvious voids.

3.3. X-ray diffraction

XRD pattern obtained from hypomineralised enamel demonstrated that calcium hydroxyapatite was the only calcium phosphate phase present with no peaks associated with any other additional phases (e.g. tricalcium phosphate, TCP; Dicalcium Phosphate Dihydrate, DCPD; Octacalcium Phosphate, OCP) (Fig. 3). The apatite peaks of (002) and (112) lattice planes were located, respectively, at 2θ=25.8° and 2θ=32.2°. The XRD pattern of hypoplastic enamel showed that the intensity of (002) plane to that of (300) was about 5, while from the standard pattern of hydroxyapatite powder (JCPDS# 09-0432), without any preferred orientation, this ratio is about 0.7. Higher intensity of (002) lattice plane demonstrates the preferred orientation of (002) in the hypomineralised enamel. It should be noted that the apatite crystals in enamel are highly oriented and the preferred orientation varies as a function of the sample orientation. As we can see from XRD pattern of sound enamel, different preferred orientation was obtained between sound and hypomineralised enamel specimens. This could be due to different orientation between the samples of sound and hypomineralised enamels used for XRD analysis.

No shift of any of the diffraction peaks were detected from the pattern of hypomineralised enamel (Fig. 3). Incorporation of additional ions such as carbonate, sodium, and magnesium into apatite structure can cause changes in crystalline parameters, leading to the shift of one or more peaks. The effect of this incorporation is expected to be small. No significant difference in crystallinity between hypomineralised and sound enamels was observed from the XRD patterns (Fig. 3).

3.4. EDS

The results of EDS analysis of three points in hypomineralised enamel and two points in sound
enamel are shown in Table 4. There was no significant difference between the means for the calcium to phosphorus ratio for hypomineralised enamel (2.07 wt%, 1.60 at%) and that of sound enamel from the same sample (2.07 wt%, 1.74 at%).

3.5. BSE image

An example of a BSE image of a single hypomineralised sample is shown in Fig. 3. The average values for the graylevels of the hypomineralised enamel, unaffected enamel and (for comparison) dentine are shown in Fig. 4. As calibration was done with silicon and carbon standard, relative values only for mineral content rather than absolute values could only be determined. From Fig. 4 it can be seen that the BSE intensity of the hypomineralised region is only slightly less intense than for the normal enamel. This is reflected in the graphs (Fig. 5) where it can be seen that for hypomineralised enamel, the graylevels were approximately 5% less than for the unaffected enamel. In comparison the graylevels of dentine were approximately 25% lower than unaffected enamel, which is consistent with the dentine having approximately 25% less mineral (by weight) than unaffected sound enamel [32–34].

4. Discussion

The most striking finding from this study is the very dramatic reduction in the hardness and modulus of elasticity of hypomineralised enamel. From the present research, the aetiology of this decrease is unclear. This study has shown that hypomineralised enamel from first permanent molar teeth has a hardness and modulus of elasticity of 0.53 ± 0.31 GPa and 14.49 ± 7.56 GPa, respectively. This is significantly lower than the hardness and modulus of elasticity of unaffected enamel of the
Table 4
Results of EDS analysis of hypomineralised and normal enamel

<table>
<thead>
<tr>
<th>Specimen area</th>
<th>Calcium wt%</th>
<th>Calcium at%</th>
<th>Phosphorous wt%</th>
<th>Phosphorous at%</th>
<th>Magnesium wt%</th>
<th>Magnesium at%</th>
<th>Sodium wt%</th>
<th>Sodium at%</th>
<th>Potassium wt%</th>
<th>Potassium at%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypo 1</td>
<td>64.95</td>
<td>58.07</td>
<td>30.65</td>
<td>35.45</td>
<td>1.28</td>
<td>1.88</td>
<td>2.70</td>
<td>4.21</td>
<td>0.42</td>
<td>0.39</td>
</tr>
<tr>
<td>Hypo 2</td>
<td>66.16</td>
<td>59.75</td>
<td>31.68</td>
<td>37.03</td>
<td>0.45</td>
<td>0.67</td>
<td>1.48</td>
<td>2.34</td>
<td>0.23</td>
<td>0.21</td>
</tr>
<tr>
<td>Hypo 3</td>
<td>65.05</td>
<td>58.53</td>
<td>32.09</td>
<td>37.37</td>
<td>0.75</td>
<td>1.12</td>
<td>1.62</td>
<td>2.54</td>
<td>0.48</td>
<td>0.44</td>
</tr>
<tr>
<td>Sound 1</td>
<td>66.67</td>
<td>60.49</td>
<td>31.99</td>
<td>37.55</td>
<td>0.15</td>
<td>0.23</td>
<td>0.96</td>
<td>1.52</td>
<td>0.23</td>
<td>0.21</td>
</tr>
<tr>
<td>Sound 2</td>
<td>66.17</td>
<td>59.86</td>
<td>31.98</td>
<td>37.44</td>
<td>0.12</td>
<td>0.18</td>
<td>1.42</td>
<td>2.24</td>
<td>0.31</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Hypo = hypomineralised enamel, Sound = sound, unaffected enamel.

Fig. 4. BSE image of hypomineralised, unaffected enamel and dentine.

Average Backscatter Intensities

```
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Intensities</th>
</tr>
</thead>
<tbody>
<tr>
<td>sound enamel</td>
<td>200</td>
</tr>
<tr>
<td>hypomineralised</td>
<td>150</td>
</tr>
<tr>
<td>enamel</td>
<td></td>
</tr>
<tr>
<td>dentine</td>
<td>100</td>
</tr>
</tbody>
</table>
```

Fig. 5. Average BSE intensities of test tooth tissues.

test teeth; 3.66 ± 0.75 and 75.57 ± 9.98 GPa, respectively (P<0.001). There is limited information available on the mechanical properties of hypomineralised first permanent molar teeth. Suckling and colleagues [2] investigated the physical appearance and hardness of hypoplastic enamel of 12 permanent teeth (four of which were permanent molars). They used a Knoop diamond indenter, at a force of 25 gm, to conduct a series of indents beginning 30μm from the anatomic surface of the tooth moving towards the amelo-dentinal junction [35]. Consistent with the present study, these authors found that the hardness values varied between individual affected teeth. These authors noted that the hardness of the hypoplastic enamel was however consistently lower than the control tooth used in their study. Closer comparison with this earlier study is difficult because the results were presented as a range of KHN for each
indents rather than giving a single value for each indentation.

The present study revealed marked differences between SEM photomicrographs in the sound enamel and the hypomineralised enamel. These differences included an increase in porosity and consistently disorganised rod structure in the hypomineralised enamel. The increase in porosity and disorganization may contribute to the reduction in mineral content, although it is unlikely that this can entirely account for the very large reduction in the mechanical properties of affected teeth found in this study.

This study utilised BSE to speculate on the calcification state of the hypomineralised region of first permanent molar teeth. A number of studies on dental calcified tissues have been reported using BSE imaging [21–24,36] although no studies have been carried out on hypomineralised permanent molar teeth. Fearne and colleagues [37] correlated the results of X-ray microtomographic and BSE imaging of utilising hypomineralised and hypoplastic primary teeth from children born with very low birth weights (VLBW). Using normal enamel from control areas to calibrate their system, they utilised dehydrated flat surfaces of sectioned primary teeth and found that the BSE images correlated well with X-ray microtomography. They demonstrated a reduction in mineral content of almost 10% in both X-ray microtomography and BSE images, in their hypoplastic regions compared to sound regions [37]. In the present study, although the mechanical properties showed a dramatic reduction (up to 96%), the BSE was only reduced by approximately 5%. As in the present study, Fearne and colleagues calibrated using silicon and carbon standards only. For more quantitative results, further calibration with other known (atomic number) standards would have to be conducted. The present study does however support the use of BSE as a simple method for the analysis of quantitative differences in mineral content of hypomineralised tooth tissue [38].

X-ray diffraction is a popular methodology for examining the dental tissues or materials [39,40]. In the present study X-ray diffraction was used to aid in determination of the chemical composition and crystal-line structure of hypomineralised areas in comparison to unaffected enamel. The XRD pattern indicates that the only calcium phosphate phase present in the hypomineralised enamel was calcium apatite. This was unexpected as the very significant reduction in mechanical properties of affected enamel suggested that the calcium mineral phases may have altered.

Multiple studies on developing enamel organs have shown that the Ca/P molar ratio was constant along the developing tooth organ, although it varies between individual teeth [41–43]. Both EDS and the Ca/P ratio are used to determine the calcium content in hard tissues. If the ratio is altered from the stoichiometry of calcium hydroxypatite, it can suggest that the mineral phase is altered or that significant substitution (of ions such as Mg for Ca) may have occurred. In the present study Ca/P ratio for hypomineralised enamel was determined using the EDS and was found to be 2.07 which was not significantly different to that of the control tissue. Whilst several other studies [43–46] have also failed to demonstrate any significant difference in the Ca/P ratio in compromised enamel Jālevik and colleagues [44] did find that median Ca/P ratio of hypomineralised enamel was significantly lower (1.4) than the unaffected enamel (1.8). There are a number of reasons that the findings in this study are not consistent with that of Jālevik. Firstly, in the present study there only one sample was analysed with EDS, whereas in the study by Jālevik, 17 samples were analysed. Secondly Jālevik and colleagues used the EDS to analyse line scan from the EDJ to the enamel dentine junction. Although they did report that the EDS results were fairly consistent throughout the length of the scans, there may be some variations in the Ca/P ratio at different parts of the hypomineralised regions that were not scanned in this current study. It is difficult to make further comments on the differences between the studies as both the EDS study and the X-ray diffraction findings in the present study are consistent, and have been conducted on separate samples.

Similarities between the Jālevik study [44] and the present study were found however in the analysis of the minor elements present in enamel. The EDS analysis consistently showed a higher percentage of magnesium in the hypomineralised regions of the tooth compared with sound enamel whilst the content of Na and K did not change. Magnesium may be substituted in to the core of the enamel crystals and may represent errors during hard tissue formation [44,47]. It is unknown what effect these substitutions or inclusions may have on the mechanical and physical properties of hypomineralised teeth.

5. Conclusion

From the present study it is obvious that the mechanical properties of hypomineralised first permanent molars are significantly lower than the unaffected enamel. This has ramifications when clinicians are attempting to restore affected teeth as the matching of restorative material mechanical properties with that of hypomineralised enamel is difficult. The reason for the dramatic difference in mechanical properties of hypomineralised enamel is at present unknown. Investigation of the mineral content, and mineral phases in the present study have yet to provide a definitive basis for why the mechanical properties of hypomineralised enamel are so
significantly compromised. In an attempt to further elucidate the differences, investigations are continuing using transmission electron microscope and Infrared spectroscopy.

References


Mechanical properties across hypomineralized/hypoplastic enamel of first permanent molar teeth


The aims of the present study were to investigate the mechanical properties of first permanent molars affected with enamel hypomineralization or hypoplasia, and to describe the appearance of these lesions under scanning electron microscopy. Eight first permanent molar test teeth and two unaffected premolars (controls) were enclosed in resin, then sectioned axially and polished. The hardness and modulus of elasticity was determined from a single array of indentations made parallel to the amelodentin junction using an Ultra-Micro-Indentation system. The teeth were then examined using the scanning electron microscope. The mechanical properties of the test teeth in the unaffected cervical region (hardness and modulus range, 2.03–4.99 GPa and 50.39–96.87 GPa, respectively) were similar to those of the control enamel (hardness and modulus range, 2.71–4.15 GPa and 62.06–95.77 GPa, respectively). Between the unaffected cervical enamel and the hypomineralized region there was a transitional area of 500–600 µm where the mechanical properties in the experimental teeth decreased linearly. The mechanical properties of the hypomineralized region of each experimental tooth were significantly lower than those of the control or cervical regions (hardness and modulus range, 0.01–1.74 GPa and 3.26–40.96 GPa, respectively). The scanning electron microscopy views revealed disorganized enamel with poorly demarcated prism boundaries in the affected regions. In conclusion, the hardness and modulus of elasticity of hypomineralized enamel in first permanent molars is significantly less than in unaffected areas of the same tooth. The reason for this is unclear but may be related to the lack of organization of the enamel crystals.

Demarcated developmental enamel defects are commonly seen in first permanent molars (1). These defects are usually a combination of enamel hypomineralization and hypoplasia caused by an insult to the ameloblasts during amelogenesis. Enamel hypoplasia is a qualitative defect in enamel, whereas hypomineralization is a qualitative defect that presents as clearly identifiable demarcated defects in the translucency of the enamel, which is in contrast to the more diffuse lesion typical of fluorosis (2). For the purposes of this article, the term hypomineralization will be used to refer to the affected teeth used in this study. Hypomineralized defects vary greatly in their size and shape. Frequently defective enamel can fracture under normal occlusal forces, leading to excessive chipping and wear of the dentition, requiring restoration (2, 3). Hypomineralized defects present the clinician with a challenge. The crown form is often compromised in terms of both the quantity and the quality of the remaining tooth tissue, which makes defining an appropriate cavity form very difficult. Furthermore, these defective molars are more susceptible to dental caries and tend to be hypersensitive, which leads to increased levels of anxiety for children during dental treatment (3–5). A previous investigation has shown that hypoplastic first permanent molars have greater carbon incorporation and lower calcium and phosphorus concentrations, as well as a different Ca to P ratio, than the adjacent normal enamel (6). The authors went on to speculate that the increased carbon content, as determined from X-ray microanalysis, could be the result of an increased carbonate concentration or an increase in the organic component of the enamel. Understanding the structure and properties of hypomineralized enamel is fundamental to improving the restorative outcomes for these teeth.

The hardness of a material is a measure of its ability to resist a permanent indentation (7) and is considered to reflect susceptibility to abrasive wear (8). Modulus of elasticity is the linear portion of the slope of the stress to corresponding strain below the proportional limit and indicates how a material will flex under loading. Understanding these two aspects of defective enamel may lead to an improved understanding of the behaviour of hypoplastic teeth, both in function (i.e. why they chip...
and wear) and to what extent this structure must be removed prior to restoration to minimize the probability of recurrent failure. Unfortunately, little is known about the mechanical properties of hypomineralized enamel. This may reflect the fact that conventional compressive and tensile tests require large specimen samples for adequate testing. Commonly, hypomineralization tends to affect only the cusp tips or the sides of cusps tips in first permanent molars (9), which severely limits the potential size of the specimen. The development of a nano-indentation technique to measure mechanical properties allows multiple testing of very small areas of material, thus enabling accurate determination of mechanical properties of small regions of dental tissues (10-12).

The aim of this study was to determine the hardness and modulus of elasticity of a hypoplastic first permanent molar from unaffected enamel at the cemento-enamel junction (CEJ) to the hypomineralized region at the cusp using the Ultra-Micro-Indentation System (UMIS) and to examine the polished surface under scanning electron microscopy (SEM).

**Material and methods**

**Mechanical properties**

Eight first permanent molar test teeth, extracted because of severe enamel hypomineralization, with or without enamel hypoplasia, and two control teeth (first premolars extracted for orthodontic reasons) were used in this study. Premolars were used as control teeth as there was difficulty in obtaining caries-free first permanent molar teeth. Upon extraction, each tooth was placed in deionized water, containing a small number of thymol crystals to inhibit bacterial growth, and stored at 4°C. The effect of storage of hypomineralized enamel in deionized water is unknown although it has been shown to affect the enamel of normal teeth (13). However, it is predicted that any effect on mechanical properties of water storage will be the same across both sound and hypomineralized enamel; thus, comparisons are still valid.

Sample preparation and use of the UMIS on enamel and dentine specimens have been previously reported (10). Each tooth was encased in cold-cured epoxy resin and sectioned through the centre of the lesion in the mesial-distal axial plane using a water-cooled diamond-impregnated circular saw (Isomet; Buehler, Lake Bluff, IL, USA). The cut surface was then polished with successively finer grade silicon carbide and finally with 9- and 1-μm polycrystalline diamond suspension. Once prepared and while awaiting testing, the samples were stored fully hydrated in deionized water containing a small number of thymol crystals.

When each specimen was ready for testing, it was placed on the work table of the UMIS with the polished surface upwards. The area to be tested was then determined with the aid of the microscope associated with the UMIS. Each test and control tooth had a single line of indents parallel to the amelo-dentinal junction (ADJ) with the first indent starting between 200 and 400 μm from the CEJ. Each subsequent indent was made 100 μm occlusally from the previous one, with the last indent in enamel parallel to the dentine tip (Fig. 1). Although the line of indents began at different distances from the CEJ, each indent on all teeth (test and control) was carried out 200 μm parallel to and along the entirety of the ADJ. Depending on the size of the tooth and where the indents began, there were between 45 and 60 indents per tooth. The placement of the final indentation varied. In a large number of the experimental teeth, the cusp tip of the hypomineralized region had fractured away (commonly as a result of enamel hypomineralization) prior to extraction. Therefore, the final indent was carried out either 100 μm from the edge of the remaining hypomineralized enamel or at the dentine cusp tip, as shown in Fig. 1. Each indentation was carried out in 25 increments to a maximum force of 20 mN using the Berkovich indenter. [For a more detailed description of the UMIS technique, the reader is referred to Mahoney et al. (10).] At all times the test surface was kept 100% hydrated by the placement of droplets of distilled water over the whole test region.

The software associated with the UMIS calculated the hardness as a function of the depth of penetration of each indentation. Both hardness and elastic modulus are determined from analysis of the unloading component of the force-displacement curve to determine the plastic penetration depth at maximum load, \( h_p \). The hardness is represented by \( H = \frac{F_{max}}{A} \), where \( A \) is the area of contact which is related to \( h_p \). On the other hand, the elastic modulus is given by \( E = \frac{(dP - dH) + A^2}{A^2} \), where \( dP/dH \) is the slope of the unloading curve at \( F_{max} \). The hardness was calculated for each indent by averaging the hardness values for the 25 increments (to the maximum force of 20 mN). The modulus of elasticity for each indentation was calculated as a function of the unloading curve at the maximum depth of penetration. The Berkovich indenter tip was calibrated using fused silica and the technique developed by Oliver & Pharr (14). A discussion on this and other sources of error associated with instrumented or nano-indentation tests is given in a publication by Menckik & Swain (15). The range in mechanical properties across the enamel specimens was determined and the results graphed for both test and control teeth. Differences in both hardness and modulus of elasticity were identified.

**Scanning electron microscopy**

Following microindentation tests, the specimens were prepared for SEM. To reveal the enamel microstructure, the specimens were etched for 2 min in a 1 : 5 dilution of 35%
phosphoric acid and then allowed to air dry for 48 h. The area investigated using SEM was on the same surface on which the indentations were conducted, but was approximately 500 μm occlusally from the line of indentations in both the experimental and control teeth. The dehydrated specimens were sputter-coated with gold and observed using the scanning electron microscope (SEM 505; Phillips, Eindhoven, the Netherlands) at 20 kV with a spot size of 50 nm. The samples were examined in secondary electron mode from the same surface as used for testing the mechanical properties.

Results

Mechanical properties

The mechanical properties of a control tooth are shown in Fig. 2. These graphs, which were identical for both control teeth, show that the hardness and modulus of elasticity of the enamel from the CEJ region to the cusp across the enamel does not vary significantly. In comparison, Fig. 3 shows an example of the hardness and modulus of elasticity for an affected specimen. The mechanical properties of the enamel closest to the CEJ are similar to those seen in the control teeth. However, as the indentations are placed more coronally towards the cuspal region, both the hardness and modulus of elasticity decrease significantly until the mechanical properties are ≈20% that of unaffected cervical enamel or of the control teeth. Near-identical patterns were found in all 8 affected teeth.

Examination of these graphs shows that the reduction in mechanical properties which occurs across the hypomineralized region is essentially linear through the area designated a "transition zone" (Figs. 1 and 3), until the mechanical properties plateau. Because of this, defining a single value for either hardness or modulus of elasticity is inappropriate. Table 1, however, summarizes the ranges of values and medians recorded across these regions for all test specimens compared with the controls. The wide variation in mechanical properties across the hypomineralized regions may be a result, in part, of the difficulties in defining exactly where the hypomineralized region starts. Macroscopically, the start of the linear decrease in mechanical properties appeared to coincide with the clinical appearance of the opaque lesion of the hypomineralized defect. However, using the UMIS microscopic viewer, the decrease in mechanical properties actually occurred between 400 and 800 μm cervical to the

---

Fig. 2. Mechanical properties of the control tooth from enamel at the cemento–enamel junction (CEJ) to a position level with the dentine cusp tip. (A) Hardness. (B) Modulus of elasticity.

Fig. 3. Mechanical properties parallel along the amelo–denticinal junction (ADJ), from the cemento–enamel junction (CEJ) to the dentine cusp tip, of the hypoplastic first permanent molar tooth. (A) Hardness. (B) Modulus of elasticity. Horizontal dashed lines are drawn through the sound and hypomineralized enamel to assist with determining of the width of the transition region.
Table 1. Range of mechanical properties of test and control teeth

<table>
<thead>
<tr>
<th>Tooth number</th>
<th>Width of transition area (μm)</th>
<th>Cervical enamel (GPa)</th>
<th>Hypoplastic region (GPa)</th>
<th>Modulus of elasticity range (GPa)</th>
<th>Modulus of elasticity (GPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hardness ranges</td>
<td>Medians</td>
<td>Hardness ranges</td>
<td>Medians</td>
</tr>
<tr>
<td>Control 1</td>
<td>NA</td>
<td>2.71-4.15</td>
<td>3.43</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Control 2</td>
<td>NA</td>
<td>2.86-4.13</td>
<td>3.42</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Hypomineralized 1</td>
<td>500</td>
<td>3.64-4.39</td>
<td>3.83</td>
<td>0.27-1.74</td>
<td>0.73</td>
</tr>
<tr>
<td>Hypomineralized 2</td>
<td>600</td>
<td>3.42-5.33</td>
<td>3.53</td>
<td>0.43-1.38</td>
<td>0.65</td>
</tr>
<tr>
<td>Hypomineralized 3</td>
<td>500</td>
<td>2.94-4.41</td>
<td>3.34</td>
<td>0.32-1.36</td>
<td>0.87</td>
</tr>
<tr>
<td>Hypomineralized 4</td>
<td>500</td>
<td>2.45-4.46</td>
<td>3.09</td>
<td>0.52-1.05</td>
<td>0.64</td>
</tr>
<tr>
<td>Hypomineralized 5</td>
<td>500</td>
<td>2.10-4.18</td>
<td>2.93</td>
<td>0.07-0.26</td>
<td>0.49</td>
</tr>
<tr>
<td>Hypomineralized 6</td>
<td>500</td>
<td>2.03-3.82</td>
<td>2.84</td>
<td>0.28-0.97</td>
<td>0.30</td>
</tr>
<tr>
<td>Hypomineralized 7</td>
<td>500</td>
<td>2.16-4.70</td>
<td>2.98</td>
<td>0.09-1.35</td>
<td>0.87</td>
</tr>
<tr>
<td>Hypomineralized 8</td>
<td>600</td>
<td>2.22-5.53</td>
<td>2.82</td>
<td>0.37-1.20</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>67.19-85.96</td>
<td>76.71</td>
<td>61.40-95.77</td>
<td>81.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>54.40-72.50</td>
<td>59.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>64.72-90.04</td>
<td>88.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50.96-92.85</td>
<td>77.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50.15-89.12</td>
<td>68.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>59.59-79.87</td>
<td>63.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50.39-70.34</td>
<td>62.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50.39-70.30</td>
<td>68.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>61.90-96.87</td>
<td>73.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14.73-29.74</td>
<td>16.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.26-37.51</td>
<td>11.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9.32-21.63</td>
<td>14.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9.32-21.53</td>
<td>23.36</td>
</tr>
</tbody>
</table>

NA, not applicable. For construction of this table the hypoplastic region was defined as the area that was discoloured to the naked eye. The rest of the incisors were deemed unaffected cervical enamel. The minimum and maximum values of hardness and modulus were used to determine the range in each region.

The visual demarcation of the hypomineralized defect, which is approximately equivalent to the transition area of 500–600 μm (Table 1).

Discussion

The clinical appearance of hypomineralized or hypoplastic enamel has been shown to be typically localized to the cuspal or side portion of the crown of first permanent molars, extending from the ADJ to the surface of the

Fig. 4. Top figures on left and right - SEM images of hypomineralized enamel. Lower figures left and right - SEM images of unaffected cervical enamel. Images on the left (top and lower) are magnified ×5430. Images on the right are magnified ×356. Bar is 10 μm.
Mechanical properties of hypomineralized enamel

The present study confirms the findings at the histological level, as the mechanical properties of the cervical enamel appear to be indistinguishable from the mechanical properties of the control teeth. The minor undulations seen in the graphs of the control tooth and unaffected region of the test teeth are to be expected, as it has been shown that the mineral content and mechanical properties of unaffected permanent enamel are intra-tooth dependent (17, 18). However, this study shows that the hardness and modulus of elasticity of such teeth decrease from the clinically normal cervical enamel to the hypomineralized region occlusally, and that this decrease is predominantly linear.

There is limited information available on the mechanical and physical properties of hypomineralized or hypoplastic first permanent molars. SUCKLING and colleagues (18) investigated the physical appearance and hardness of hypoplastic enamel in permanent teeth. Using a Knoop diamond indenter, a series of indents, 30 μm from the anatomic surface to the ADJ, were completed. They found that the hardness values varied between individual affected teeth, which is consistent with the results of the present study. As these authors did not test ‘unaffected’ enamel, they were unable to show the dramatic drop in the mechanical properties when the indents entered the hypoplastic region, as in the present study. They also noted that the hardness of the hypoplastic enamel was consistently lower than that of the control tooth, although the teeth with demarcated opacities had the most consistently low subsurface hardness values.

The present study has shown that the hypomineralized enamel did not show the classical etching pattern seen in control enamel. It is possible that upon etching, there was uniform removal of the hypomineralized enamel, rather than the differential etching patterns seen in unaffected enamel. Whilst other SEM studies have been reported for hypoplastic and hypomineralized enamel, most have involved conditions such as amelogenesis imperfecta (AI) or fluorosis (19-24). SUCKLING and colleagues (18) examined polished isolated hypomineralized defects under SEM and found a similar lack of clarity in the prism boundaries as noted in the present study. By contrast, in a large study by SEOW & AMARTUNGE (23), the etching patterns were shown to be dependent upon the variant and mode of inheritance of each AI type, with the smooth hypoplastic variant showing none of the typical etching patterns. It is possible that etching a less organized enamel structure may not result in the classic etch pattern, which in turn may have a detrimental effect on bonding between restorative/adhesive and the affected first permanent molar enamel. We are unaware of any investigations of the bonding behaviour of hypomineralized tissue and the role of etching.

In the present study, the hypomineralized region of the test teeth had markedly inferior mechanical properties in comparison to normal enamel. The reason for this is currently unclear. It is possible that the reduction is partly related to the amount of mineral present in the hypomineralized tissue. CURRERY (25) has shown that the modulus of elasticity of bone is positively correlated with the apatite content, and ANGER et al. (26) have recently shown that the mechanical properties of carious dentine are dependent on its mineral content. Although the mineral content of the hypoplastic teeth has not been examined to date, the low modulus of elasticity and hardness seen in this study suggests that the mineral content is reduced. Further speculation on the amount of mineral in affected teeth can be drawn from studies of patients with AI. In an attempt to determine the mineral content of hypoplastic and hypomineralized defects, FEARN and colleagues (27) investigated developmental defects of enamel in the primary teeth of patients who were born prematurely. These authors utilized dehydrated samples for both X-ray microtomography and digital backscattered electron imaging (DBSI) to determine the mineral content of affected teeth. Using X-ray microtomography, they found that the mineral density of unaffected primary enamel was between 2.65 and 2.78 g cm⁻³, whereas that of the hypoplastic defects in the test teeth showed a 10% reduction in density to between 2.30 and 2.50 g cm⁻³. The results from the DBSI were consistent with these results. Further work, investigating the mineral content and ultrastructure of these teeth, would be useful to understand the present observations and aid in the choice of restorative material.

The dramatic reduction in hardness of the hypoplastic enamel shown in this study may help to explain why traditionally hypoplastic teeth are difficult to restore, with clinicians reporting loss of both restorative material and tooth tissue (4). Hardness is known to be related to cumulative surface removal processes, such as abrasive wear (8). The low hardness and elastic modulus seen in the hypoplastic region tested indicates that these mechanical properties of hypoplastic enamel are similar to, or lower than, those of dentine (10, 12). This significant reduction in hardness and modulus creates a very challenging situation for the clinician in choosing the most appropriate restorative material and technique with which to manage this problem condition. Further studies investigating the mineral content and ultrastructure of these teeth would be beneficial, not only to increase our understanding of the present observations, but also in forming the development of more effective restorative approaches.

Acknowledgements – The authors wish to thank Mr Tony Romeo for his patience and help with the SEM.

References


APPENDIX TWO

Equipment used

Berkovich indenter
Syntone BA,
Switzerland

BSE
Solid-state backscattered electron detector FEI,
Eindhoven,
Netherlands

Critical-point drying apparatus
Bal-tec 030 Critical Point Dryer,
Balzers of Lichtenstein

EDX
X-ray detector system
EDAX, P-505,
SUTW-Sapphire

Paralleling machine
Leitz, Wetzlar,
Germany

Polishing Machine
Rotoforce-4 and Multidoser,
Struers
Copenhagen,
Denmark

Scanning Electron Microscope
1. Philips XL-30 FEI,
Eindhoven, Netherlands
2. Phillips 505,
Eindhoven, Netherlands

TEM
Philips CM120 Biotwin
Eindhoven, Netherlands

X-Ray Diffraction
Siemens machine
Diffraktometer D5000
Karlsruhe, Germany
Materials Used

Acid etchant:
37% Orthophosphoric Acid
SDI Ltd
Bayswater, Victoria
Australia

Alginate:
Unijet-II, Fast set.
3M Unitek,
Monrovia, California,
USA

Blu Tack,
Bostik Pty. Ltd.
Victoria,
Australia

Dentine conditioner
GC Dentine Conditioner
76-1 Hasunuma-cho
Itabashi-ku
Tokyo
Japan

Diamond Suspension, polishing discs and Lubricant
DP-Pan and DP- Lubricant- Green and Red
Struers,
Copenhagen,
Denmark

Epoxy:
TEM epoxy: Epoxy Bond 110
Ted Pella, Inc. and PELCO International,
P.O. Box 492477,
Redding,
CA, USA

UMIS epoxy resin:
Epofix, Struers,  
Copenhagen,  
Denmark

Hanks’ Balanced Salts  
SIGMA R  
SIGMA-ALDRICH CO.  
PO Box 14508 St. Louis MO,  
USA 63178

Ketac™ - Molar Quick Aplicap™  
3M ESPE America Inc  
Plymouth Meeting, PA  
USA

Thymol  
ICN BIOMEDICALS INC  
1268 South Chillicothe Road  
Aurora, Ohio  
USA 44202

Z100  
3M ESPE America Inc  
Plymouth Meeting, PA  
USA
APPENDIX THREE

Ethics acceptance from Western Sydney Area Health Service (WSAHS) and Central Sydney Area Health Service (CSAHS) Ethics committees.
September 15, 2003

Dr Erin Mahoney
Department of Paediatric Dentistry and Orthodontics
Westmead Centre for Oral Health
Westmead NSW 2145

Dear Dr Mahoney,

Research Proposal: ‘The effect of temporary restorations on the mechanical properties of carious dentine’

Thank you for your letter dated 4 September 2003 enclosing revised Participant Information and Consent Forms Version 2 dated 8 September 2003 in accordance with the requests of the Human Research Ethics Committee letter dated 5 August 2003.

As the Committee’s ethical concerns have now been satisfied, approval of the study is confirmed and it may now commence. An approved copy of the revised Participant Information and Consent Forms Version 2 dated 8 September 2003 is enclosed for your records.

Could you please sign and return to the Research Office the letter of acceptance which was forwarded to you with our letter dated 5 August 2003.

The Committee wishes you well with the study and looks forward to receiving progress reports in due course.

Yours sincerely

Dr Howard Smith
Secretary
Western Sydney Area Health Service
Human Research Ethics Committee
3 September 2003

Dr E Mahoney
Department of Paediatric Dentistry and Orthodontics
Westmead Centre for Oral Health
WESTMEAD NSW 2145

Dear Dr Mahoney,

Re: Protocol No X03-0159 - “The effect of temporary restorations on the mechanical properties of carious dentine”

The Executive of the Ethics Review Committee, at its meeting of 28 August 2003, considered your correspondence of 27 August 2003. In accordance with the decision made by the Ethics Review Committee, at its meeting of 11 June 2003, approval is now granted to proceed.

- This approval is valid for four years, and the Committee requires that you furnish it with annual reports on the study’s progress beginning in September 2004.

- This approval relates to the ethical content of the study only, and you are responsible for the following:

  - negotiating individual arrangements with the Heads of service departments in those situations where the use of their resources is involved, and

  - arranging an identity pass for any researcher who is not employed by the Central Sydney Area Health Service. You and the researcher should present yourselves at the Security Department, Building 12, Royal Prince Alfred Hospital with a copy of this approval letter.
• if appropriate, informing the study sponsor that the membership and
procedures of the CSAHS Ethics Review Committee (RPAH Zone) comply
with the National Statement on Ethical Conduct in Research Involving
Humans.

• If you or any of your co-investigators are University of Sydney employees or have
a conjoint appointment, you are responsible for informing the University’s Risk
Management Office of this approval, so that you can be appropriately indemnified.

Yours sincerely,

Lesley Townsend
Secretary
Ethics Review Committee (RPAH Zone)
HERC:EXECOR03-09
Consent form and patient information sheet for WSAHS.
As these forms were almost identical for the submission to each ethics committees, the CSAHS forms were not included.
CONSENT TO PARTICIPATE IN RESEARCH

Title of Research Project: The effect of temporary fillings on decayed teeth.

Name of Researcher: Dr Erin Mahoney

1. I understand that the researcher will conduct this study in a manner conforming with ethical and scientific principles set out by the National Health and Medical Research Council of Australia and the Good Clinical Research Practice Guidelines of the Therapeutic Goods Administration.

2. I acknowledge that I have read, or have had read to me the Participant Information Sheet relating to this study. I acknowledge that I understand the Participant Information Sheet. I acknowledge that the general purposes, methods, demands and possible risks and inconveniences which may occur to me during the study have been explained to me by ______________________ (“the researcher”) and I, being over the age of 16 years or over the age of 14 years but under the age of 16 years (delete as applicable), acknowledge that I understand the general purposes, methods, demands and possible risks and inconveniences which may occur during the study.

3. I acknowledge that I have been given time to consider the information and to seek other advice.

4. I acknowledge that refusal to take part in this study will not affect the usual treatment of my condition.

5. I acknowledge that I am volunteering to take part in this study and I may withdraw at any time.

6. I acknowledge that this research has been approved by the Western Sydney Area Health Service Human Research Ethics Committee.

7. I acknowledge that I have received a copy of this form and the Participant Information Sheet, which I have signed.

8. I acknowledge that sponsoring pharmaceutical companies and any regulatory authorities may have access to my medical records to monitor the research in which I am agreeing to participate. However, my identity will not be disclosed to anyone else. (Please delete this paragraph if not applicable)

Before signing, please read ‘IMPORTANT NOTE’ following.

Name of participant __________________________________________ Date of Birth ______________________

Address of participant __________________________________________

Name of parent or guardian (where applicable) ____________________________

Address of parent or guardian (where applicable) ____________________________

Signature of participant __________________________________________ Date: ______________________

Signature of parent or guardian (where applicable) __________________________ Date: ______________________

Signature of researcher __________________________________________ Date: ______________________

Signature of witness __________________________________________ Date: ______________________
IMPORTANT NOTE

This consent should only be signed as follows:
1. Where a participant is over the age of 16 years, then by the participant personally.
2. Where the participant is between the age of 14 and 16 years, it should be signed by the participant and by a parent or guardian.
3. Where the participant is under the age of 14 years, then the parent or guardian only should sign the consent form.
4. Where a participant is under a legal or intellectual disability, eg unconscious, then particular consent should be sought from the Human Research Ethics Committee as to whether the person should take part in the research.

INDEPENDENT WITNESS:

I, ____________________________________________ (name of independent witness)

of ___________________________________________ hereby certify as follows:

1. I was present when ____________________________________ ("the participant") appeared to read or had read to him / her a document entitled Participant Information Sheet; or
   I was told by ____________________________________ ("the participant") that he/she had read a document entitled Participant Information Sheet (*Delete as applicable)

2. I was present when ____________________________________ ("the researcher") explained the general purposes, methods, demands and the possible risks and inconveniences of participating in the study to the participant. I asked the participant whether he/she had understood the Participant Information Sheet and understood what he/she had been told and he/she told me that he/she did understand.

3. I observed the participant sign the consent to participate in research and he/she appeared to me to be signing the document freely and without duress.

4. The participant showed me a form of identification which satisfied me as to his/her identity.

5. I am not involved in any way as a researcher in this project.

6. (Delete this clause if not applicable) I was present when ____________________________________ ("the interpreter") read the Participant Information sheet to the participant in the __________________________ (here insert appropriate language) language. I certify that when the researcher explained the general purposes, methods, demands and possible risks and inconveniences of participating in the study that what was said by both the researcher and the participant was translated by the interpreter from the English language into the __________________________ language and vice versa. When I spoke to the participant what I said and what the participant said was translated by the interpreter from the English language into the __________________________ language and vice versa.

Name of independent witness ____________________________________________

Address ____________________________________________

Signature of independent witness ____________________________ Date: ________________

Relationship to participant of independent witness ____________________________________________
CONSENT TO PARTICIPATE IN RESEARCH

INTERPRETER:

If an interpreter is used, the following addition is necessary –

I ____________________________________________________________ (name of interpreter)
of ______________________________________________________________________ certify as follows:

1. I am qualified to translate speech and writing from the English language into the ______________ language and vice versa.

2. I read the Participant Information Sheet to the participant in the ______________ language and he/she appeared to understand it.

3. I was present when the researcher explained the general purposes, methods, demands and possible risks and inconveniences of participating in the study to the participant and I translated all that was said by the researcher and by the participant from the English language into the ______________ language and vice versa.

4. I was present when the independent witness spoke to the participant and I translated all that was said by the independent witness and by the participant from the English language into the ______________ language and vice versa.

Signature of Interpreter ___________________________ Date ___________________________
PARTICIPANT INFORMATION

"THE EFFECT OF TEMPORARY FILLINGS ON DECAYED TEETH"

Investigators:

Dr Erin Mahoney
Department of Paediatric Dentistry and Orthodontics
Westmead Centre for Oral Health
Westmead NSW 2145

Associate Professor Nicky Kilpatrick
Royal Children’s Hospital
Parkville, VIC 3502
Ph: 9345 5462
Ph: 9845 7450

You have a number of very decayed teeth, some of which are going to be taken out under a general anaesthetic, intravenous sedation or in the dental clinic in the future. There is currently a waiting list of between 2 and 6 months for this. During this time we try to reduce the chance of you getting toothache by placing temporary fillings in to these large decay holes, which we believe may produce some healing in your tooth. There are a number of different filling materials that we can use however we don’t really know which one will produce more healing. What we would like to do is to look at the teeth that have been filled under a microscope once they have been extracted.

The study is being conducted by Dr Erin Mahoney and Associate Professor Nicky Kilpatrick who are both Specialist Paediatric Dentists. If you agree to participate in this study, Dr Mahoney will place temporary white fillings in your teeth that will be removed later. Dr Mahoney will be using 2 different ways of filling your teeth; one which involves the placement of just a white filling that sticks to teeth and the other that involves the placement of a cream beneath the white filling. Both ways are used widely by dentists and therapists around Australia. These temporary fillings are placed very easily without causing pain and without using local anaesthetic (the needle). Dr Mahoney is very gentle and experienced, however if your become agitated during treatment it will be stopped and no temporary restorations will be placed. Similarly if you get toothache whilst on the waiting list we will help you contact the Department of Paediatric Dentistry at Westmead who will see and look after your child as an emergency.

In the future when you attends the hospital for your dental treatment to have the tooth removed we would like to keep the teeth that were filled and that have now been removed. Dr Mahoney will then study these teeth under the microscope to see what has happened beneath the fillings. No teeth will be removed other than those identified as part of your dental care.

Participation in this study is entirely voluntary; you are in no way obliged to participate and - if you do participate - you can withdraw at any time. Whatever your decision, please be assured that it will not affect your treatment or your relationship with the staff at Westmead Centre for Oral Health. Once the teeth have been given to Dr Mahoney they will not be identified, so it will not be possible to withdraw from the study after that point.

All aspects of the study, including results, will be strictly confidential and only the investigators named above will have access to information on participants. A report of the study may be submitted for publication, but individual participants will not be identifiable in such a report.
PARTICIPANT INFORMATION

"THE EFFECT OF TEMPORARY FILLINGS ON DECAYED TEETH"

Complaints
Any person with concerns or complaints about the conduct of a research study can contact the Patient Representative, Ms Jillian Gwynne Lewis, Telephone No 9845 7014 or email jillian_lewis@wsahs.nsw.gov.au

Contact Information
If you have any problems while on the study, please contact Dr Erin Mahoney
Working hours Telephone No: 9845 7450 or
After hours Telephone No: 0409922233
CONSENT TO PARTICIPATE IN RESEARCH

Title of Research Project: The effect of temporary fillings on decayed teeth.

Name of Researcher: Dr Erin Mahoney

1. I understand that the researcher will conduct this study in a manner conforming with ethical and scientific principles set out by the National Health and Medical Research Council of Australia and the Good Clinical Research Practice Guidelines of the Therapeutic Goods Administration.

2. I acknowledge that I have read, or have had read to me the Participant Information Sheet relating to this study. I acknowledge that I understand the Participant Information Sheet. I acknowledge that the general purposes, methods, demands and possible risks and inconveniences which may occur to me during the study have been explained to me by ___________________ ("the researcher") and I, being over the age of 16 years or over the age of 14 years but under the age of 16 years (delete as applicable), acknowledge that I understand the general purposes, methods, demands and possible risks and inconveniences which may occur during the study.

3. I acknowledge that I have been given time to consider the information and to seek other advice.

4. I acknowledge that refusal to take part in this study will not affect the usual treatment of my condition.

5. I acknowledge that I am volunteering to take part in this study and I may withdraw at any time.

6. I acknowledge that this research has been approved by the Western Sydney Area Health Service Human Research Ethics Committee.

7. I acknowledge that I have received a copy of this form and the Participant Information Sheet, which I have signed.

Before signing, please read ‘IMPORTANT NOTE’ following.

Name of participant ____________________________ Date of Birth ____________________________

Address of participant ________________________________

Name of parent or guardian (where applicable) ________________________________

Address of parent or guardian (where applicable) ________________________________

Signature of participant ________________________________ Date: ____________________________

Signature of parent or guardian (where applicable) ________________________________ Date: ____________________________

Signature of researcher ________________________________ Date: ____________________________

Signature of witness ________________________________ Date: ____________________________
WESTERN SYDNEY AREA HEALTH SERVICE
WESTMEAD NSW 2145

"The effect of temporary fillings on decayed teeth"

IMPORTANT NOTE

This consent should only be signed as follows:

1. Where a participant is over the age of 16 years, then by the participant personally.

2. Where the participant is between the age of 14 and 16 years, it should be signed by the participant and by a parent or guardian.

3. Where the participant is under the age of 14 years, then the parent or guardian only should sign the consent form.

4. Where a participant is under a legal or intellectual disability, eg unconscious, then particular consent should be sought from the Human Research Ethics Committee as to whether the person should take part in the research.

INDEPENDENT WITNESS:

I, ________________________________________________ (name of independent witness)
of ________________________________________________ hereby certify as follows:

1. I was present when ______________________________________ (“the participant”) appeared to read or had read to him / her a document entitled Participant Information Sheet; or
   I was told by ______________________________________ (“the participant”) that he/she had read a document entitled Participant Information Sheet
   (*Delete as applicable)

2. I was present when ______________________________________ (“the researcher”) explained the general purposes, methods, demands and the possible risks and inconveniences of participating in the study to the participant. I asked the participant whether he/she had understood the Participant Information Sheet and understood what he/she had been told and he/she told me that he/she did understand.

3. I observed the participant sign the consent to participate in research and he/she appeared to me to be signing the document freely and without duress.

4. The participant showed me a form of identification which satisfied me as to his/her identity.

5. I am not involved in any way as a researcher in this project.

6. (Delete this clause if not applicable) I was present when ______________________________________ (“the interpreter”) read the Participant Information sheet to the participant in the ______________________________________ (here insert appropriate language) language. I certify that when the researcher explained the general purposes, methods, demands and possible risks and inconveniences of participating in the study that what was said by both the researcher and the participant was translated by the interpreter from the English language into the ______________________________________ language and vice versa. When I spoke to the participant what I said and what the participant said was translated by the interpreter from the English language into the ______________________________________ language and vice versa.

Name of independent witness ________________________________________________
Address ________________________________________________

Signature of independent witness _____________________________________________ Date: ________________

Relationship to participant of independent witness ____________________________
CONSENT TO PARTICIPATE IN RESEARCH

"The effect of temporary fillings on decayed teeth"

INTERPRETER:

If an interpreter is used, the following addition is necessary —

I ___________________________________________________________________________ (name of interpreter)

of ___________________________________________________________________________ certify as follows:

1. I am qualified to translate speech and writing from the English language into the __________ language and vice versa.

2. I read the Participant Information Sheet to the participant in the ______________ language and he/she appeared to understand it.

3. I was present when the researcher explained the general purposes, methods, demands and possible risks and inconveniences of participating in the study to the participant and I translated all that was said by the researcher and by the participant from the English language into the ______________ language and vice versa.

4. I was present when the independent witness spoke to the participant and I translated all that was said by the independent witness and by the participant from the English language into the ______________ language and vice versa.

Signature of Interpreter __________________________ Date __________________________
APPENDIX FIVE

Form used in final study (Chapter 6) to collect information during clinical treatment sessions.
Demographic details

Dental hospital patient being treated at (circle):

UDH

Westmead

Patients study identification number: __________________________

Patients DOB: __________________________

Date of first appointment after referral to hospital: __________________________

Age at first appointment: __________________________

ASA level (circle): I II III+

Waiting list (circle): Treatment IV Sedation General Anaesthetic

Letter of explanation of project given (circle): Y N

Consent obtained (circle): Y N

Intra oral examination

Teeth present (circle):

[Diagram of teeth conditions]
Caries present:

Hypoplastic teeth (list and indicate which surface of tooth has the hypoplasia):

Radiographs present:

Teeth identified as eligible for inclusion in the study:
Control tooth:
Treatment tooth:

Treatment provided
Date: ____________
Tooth number and treatment provided:
Further treatment required:   Y   N
If yes: what and why?

At time of extraction
Date:
Original restoration present at time of extraction? (circle):   Y   N
APPENDIX SIX

Full graphical results for all test teeth from final study (Chapter 6).
Primary tooth
6 months
Time with GIC in situ:
Restorative placed: GIC only
Tool 1:
Tooth Information

Denulne
Blue lines on diagram: Indentations in normal
Red lines on diagram: Indentations in caries

Figure 1: Hardness profiles of each indentation

1000
100
10
1
0
Distance from pulp chamber
Log Needle Hardness
Distance from pulp chamber
Log Needle Hardness
Distance from pulp chamber
Log Needle Hardness
Distance from pulp chamber
Log Needle Hardness
Distance from pulp chamber
Log Needle Hardness
Distance from pulp chamber
Log Needle Hardness
Distance from pulp chamber
Log Needle Hardness
Primary Tooth
6 months
Time with GIC in situ:
Restorative placed: GIC only
Tooth 1:
Tooth Information

Figure 1: Modulus of elasticity profiles of each
Figure 2b: Module of Elasticity profiles of each tooth.

Primary tooth

Time with GC in situ:
Restoration placed: GC only

6 months

Red bars on diagram: Indentations in caries
Blue line on diagram: Indentations in normal

Denute
Figure 3: Hardness profiles of each indentation.

Dentine
Blue lines on diagram: Indentations in normal
coronal dentine. Red lines on diagram: Indentations in cervices

Primary tooth
4 months.
Time with GC in situ:
Restoration placed: GC only.

Tooth 3:
Tooth Information
Primary tooth
4 months
Time with CIC
+ C(\text{OH})_2 in situ
Restoration placed: CIC + C(\text{OH})_2
Tool: 4

Figure 4: Hardness profiles of each indentation

Dentine
Blue lines on diagrams: Indentations in normal
Red lines on diagram: Indentations in caries

Figure 4a: Hardness profiles of each indentation
Primary tool:

4 months
Time with Ca + C4(OH)² in situ
 Restoration placed: Ca + C4(OH)²

Tool I:

Tool II:

Tool III:

Tool IV:

Figure 4b: Modulus of Elasticity profiles of each}

Denute
Blue lines on diagram: Indentations in normal
Red lines on diagram: Indentations in erutes
Figure 5b: Modulus of Elasticity profiles of each indentation.

Primary Tool
+ 4 months
Time in situ:
No Restorative Placed
Tool 5:
Tool Information
Figure 6: Hardness profiles of each indentation.

Permanent tool:
3 months

Time with Ca(OH)₂ in situ:
Recovery phase: Ca(OH)₂ only

Tool 6:

For each indentation.
Permanent tooth

3 months
Time with Ca(OH)² in situ

Restoration procedure: Ca(OH)² only

Tool G:

Tool Information

Indentation
Blue lines on diagram: Indentations in normal
Red lines on diagram: Indentations in caries

Figure 6b: Modulus of elasticity profiles of each
Figure 7a: Hardness profiles of each indentation.

Permanent tooth

3 months

Time in situ:

No restorative placed.

Tooth Information
Figure 7: Modulus of Elasticity profiles of each denning
Blue line on diagram: Indentations in normal
Red lines on diagram: Indentations in cuttes
Indentation

Permanent Tooth
3 months
Time in situ:
No Restorative placed
Tool T:
Figure 6a: Hardness profiles of each indentation

Denote blue lines on diagram: Indentations in normal
Red lines on diagram: Indentations in caries

Removable tooth
3 months
Time with GIC + Ca(OH)_2 in situ:
Resorbative phase: GIC + Ca(OH)_2

Tooth B:
Initial Information
Figure 8b: Modules of elasticity profiles of each

Permanent tooth

3 months

Rinses with GIC + Ca(OH)² in situ

Resiniferous placed: GIC + Ca(OH)²

Tooth Information
Decontamination
Blue lines on diagrams: Indentations in normal
Red lines on diagram: Indentations in cut
Figure 9a: Hardness profiles of each indentation
Permanet tool
3 months
Time with GIC in situ
Restoration placed: GIC only
Tool 9:
Tool Information
Permanant Tooth

3 months

TIME WITH GC IN SITU

RESECTION DENTIN: GC only

TOOTH 16:

TOOTH INFORMATION

Figure 10b: Modulus of Elasticity profiles of each

Dentine
Blue lines on diagram: Indentations in normal
Red lines on diagram: Indentations in caries

Module of Elasticity

Log Modulus (GPa)

Log Modulus (GPa)

Log Modulus (GPa)

Log Modulus (GPa)

Log Modulus (GPa)
Figure 11: Hardness profiles of each indentation.

Tooth Information

4 months

Time with GIC in situ:
Restoration placed: GIC only
Tooth 1:

Permanent tooth

Blue lines on diagram: Indentations in normal
Red lines on diagram: Indentations in cases

Denote
Permanent tool.

Time with GC in situ.

Reid lines on diagram: Indentations in carbons

Denton

Blue lines on diagram: Indentations in normal

Indentation

Recreate II: Modulus of Elasticity profiles of each

Figure 11b: Modulus of Elasticity.
Figure 12a: Hardness profiles of each indentation

Denine
Blue line on diagram: Indentations in normal
Red line on diagram: Indentations in areas

Permanant tooth
4 months
Time with GIC in situ:
Restoration placed: GIC only
Tooth 12:
Tooth Information
Periapical Tooth

Time with GIC in situ:
Restoration placed: GIC only

Tooth Information

4 months

Figure 12: Modulus of elasticity profiles of each permanent tooth.
Permanent tooth
4 months
Time with Composite Z100 in situ:
Resorption present: Composite Z100
Tooth 13
Figure 13b: Modulus of Elasticity profiles of each pilot tool

Permanent tool

4 months

Time with Composite Z100 in situ

Resinization present: Composite Z100
Figure 1a: Hardness profiles of each indentation

Dentine
Blue line on diagram: Indentations in normal
Red line on diagram: Indentations in caries

Permanent tooth
No Restorative Placed
Tooth 4:
Tooth Information
Dentine
Blue line on diagram: Indentations in normal
Red lines on diagram: Indentations in caries
Indentation

Figure 14: Modulus of elasticity profiles of each

Modulus of Elasticity

Permanent tooth
No restoration placed

Tooth I4
REFERENCES


(9) Holt A. The hardness of paediatric teeth and the influence of acidic drinks. Faculty of Mechanical Engineering, University of Sydney, 1998.


Ref Type: Electronic Citation


(102) Tyldeley WR. The mechanical properties of human enamel and dentine", , British Dental Journal 1959; 106:269-278.


(166) Lester KS, Boyde A. Some preliminary observations on caries (remineralization) crystals in enamel and dentine by SEM. Virchows Arch Abt A Pathol Anat 1968; 17:196-212.


(176) Barber D, Massler M. Permeability of active and arrested carious lesions to dyes and radioactive isotopes. ASDC Journal of Dentistry for Children 1964; 31:26-33.


(238) Zero DT, Rahbek I, Fu J, Proskin HM, Featherstone JDB. Comparison of the iodide permeability test, the surface microhardness test, and the mineral distribution of bovine enamel following acid challenge. Caries Research 1990; 24:159-164.


(251) Lloyd GE. Atomic number and crystallographic contrast images with the SEM: a review of backscattered electron techniques. Mineralogical Magazine 1987; 51:3-19.


260


261


(404) Berkowitz RJ, Moss M, Billings RJ, Weinstein P. Clinical outcomes for nursing caries treated under general anaesthesia. ASDC Journal of Dentistry for Children 1997; 64(3):210-211.


