



SIGNIFICANCE OF DISINFECTION ON PHYSICAL PROPERTIES OF TREATED ENAMEL ON ORTHODONTIC BONDING

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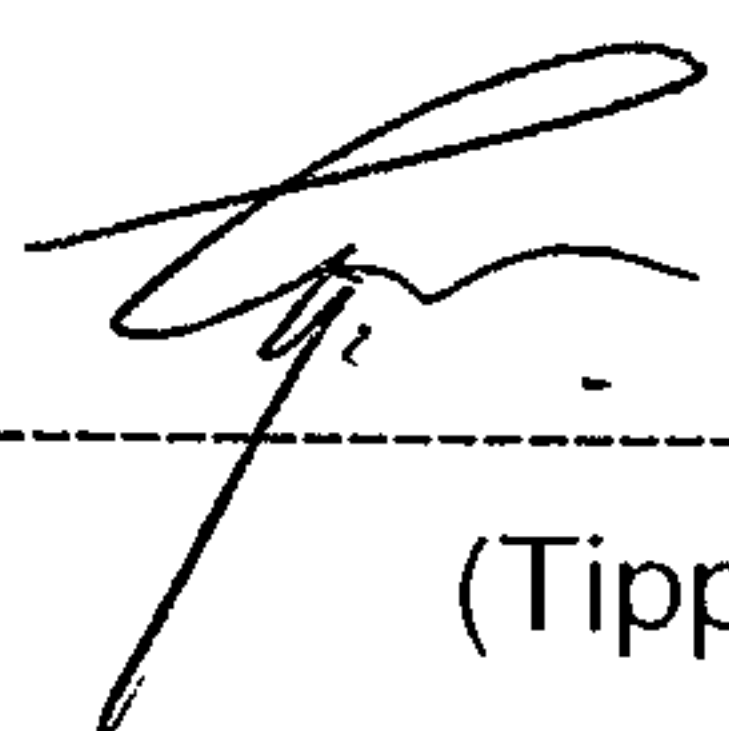
Abstract

Laboratory experiments on non-disinfected, extracted teeth provide the majority of reports in the literature. No information has been published on the changes of enamel properties under oral and laboratory situations when teeth must now be subjected to rigorous infection control treatments. It was hypothesised that the physical properties of the treated pre-disinfected enamel surfaces, in the *in vitro* laboratory study, should be different from those of the *in vivo* group where the samples were disinfected after bonding of brackets. The aim of this study was to determine the effect of a disinfectant, 1% sodium hypochlorite, on the properties of enamel to clarify whether or not the disinfection procedure had an effect on the physical properties of the enamel as used in studies dealing with bonding. After bonding a bracket on randomly selected right or left bicuspid of each patient (Group "b"), the bonded and the contralateral unbonded teeth were extracted and kept 24 hours in 1% sodium hypochlorite. 20 teeth from 10 orthodontic patients were used. After disinfection, another bracket was bonded on the contralateral bicuspid (Group "a"). Teeth were mounted in cold-cured acrylic resin following bracket removal. Before testing, the teeth were cut perpendicular to their long axis and polished to obtain parallel surfaces. The microhardness and modulus of elasticity were recorded with a UMIS-2000 machine, which is an ultra-microindentation system. Ten indentations were made on the buccal side of each tooth section at the resin-bonded area and measured at 30 μm from the enamel surface. Using a standard technique, specimens were examined with the scanning electron microscope at the indented areas and the enamel surfaces. For all samples investigated, the hardness was enhanced by the effect of 1% sodium hypochlorite.

Declaration

CANDIDATE'S CERTIFICATE

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



31.10.97

(Tippawan Leelahakriengkrai)

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Dedication

To my Dad, Mum and dear brothers, whom I love and admire, for their endless support and encouragement without which this Research Project would not have been possible.

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Chapter 1

Introduction

The bonding of orthodontic brackets to acid-etched enamel surfaces of teeth is a widely accepted clinical-orthodontic procedure (Sadowsky *et al.*, 1990) and the synthesis and development of resins have made the direct bonding of orthodontic attachments a practical clinical method (Retief and Denys, 1979), and many reports related to bonding enamel surfaces of extracted teeth were published.

According to the latest infection control protocols for reducing the risk of transmission of blood-borne pathogens, including the human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV), the extracted teeth must now be subjected to infection control treatment before testing in the laboratory. Extracted teeth used in dental studies often come from unknown sources; consequently, the users are not aware of the potential contamination of these teeth with bacteria, spores, and viruses. Pagniano *et al.* (1985) demonstrated that potentially pathogenic bacteria and fungi, such as *Aspergillus*, were recoverable following use of extracted human teeth in a dental teaching laboratory. Furthermore, since tooth preparation for teaching purposes is usually done without a liquid coolant, there is a greater probability of exposure to pathogenic organisms in the laboratory area (Belting *et al.*, 1964).

Several studies have recommended tooth disinfection/sterilization procedures on extracted teeth, including autoclaving, dry heat and chemical techniques, e.g. glutaraldehyde, ethylene oxide as well as sodium hypochlorite (Pantera and Schuster, 1990) before use in laboratory testing.

Sodium hypochlorite, which was first recommended by Dakin (1915), is one of the most common disinfectants currently employed in dentistry. It is known to be an excellent protein denaturant and antibacterial solution (Piskin *et al.*, 1995). It is recommended that a sodium hypochlorite dilution of 1:10 to 1:100 can decontaminate environmental surfaces, depending on the amount of organic material present (CDC, 1986, 1987). Senia *et al.* (1975) reported a one-minute immersion in 5.25 % sodium hypochlorite for sterilization of gutta-percha endodontic points. In addition, a five-minute immersion in 5.25% sodium hypochlorite was suggested for sterilizing dentures (Rudd *et al.*, 1984) and coronal surfaces of extracted teeth (Pantera *et al.*, 1988).

Future *in vitro* testing of bonding materials to tooth enamel will necessitate prior infection control procedures before the teeth can be used in the laboratory. These infection control measures may alter the physical properties of the tooth surface and enamel interprismatic regions. Any such changes may deleteriously affect the physical and bonding properties of the applied resins. Therefore, future laboratory testing of bonding hardness and stiffness properties will need to account for any physical changes resulting from obligatory infection control

procedures. Otherwise, the "*in vitro*" findings may not accurately represent the "*in vivo*" physical status. To date, the importance of infection control measures orthodontic bonding of brackets need to be further evaluated.

It was hypothesized that the physical properties of the treated pre-disinfected enamel surfaces, in the present *in vitro* laboratory study, should be different from those of the *in vivo* group where the samples were disinfected after bonding of brackets. The purpose of the present study was to determine the effect of disinfectant, 1% sodium hypochlorite, on the enamel properties to clarify whether or not the disinfection procedure had an effect on the physical properties of enamel as used in studies on bonding.

Chapter 2

Review of the Literature

3.1. *Structure and composition of enamel*

Enamel is the hardest of the mineralized tissues of the human body (Gwinnett, 1992). Embryologically it is derived from cells of the oral epithelium, which in turn originate in the ectodermal layer of the triplo-blastic embryo (Ten Cate, 1985).

Enamel covers the anatomical crown of the tooth. It is thickest over the cusps and thinnest at the base of pits, fissures, and the cervical region of the crown. Functionally, the tissue and its anatomical configuration provides a durable surface for the incision and communication of food and also affords protection to the underlying tissues, which in turn support the enamel. Enamel is composed of 95% by volume and approximate 96% by weight of inorganic substance (Gwinnett, 1992). This brittle tissue survives through its unique biophysical characteristics coupled with a more resilient tissue partner, the dentine.

3.1.1. Composition

Human tooth enamel consists of an inorganic matter approximately 93.6 - 98.5% by weight (Bowes and Murray, 1935, 1936; Bird *et al.*, 1948; McClure, 1948; Angmar *et al.*, 1963; Little, 1961; Lefevre and Manly, 1938; LeGeros *et al.*, 1983; Little *et al.*, 1962; Holager, 1972). The organic matter of the enamel is a minor component, ranging from 0.05 - 0.8% by weight, approximately 0.5% (Little, 1961; Little *et al.*, 1962; Armstrong and Brekhus, 1937; Weidmann and Eyre, 1967; Naujoks *et al.*, 1967; Eastoe, 1963; Savory and Brudevold, 1959; Losee and Hess, 1949; Anderson, 1949; Stack, 1967; Little and Casciani, 1966). The rest is composed of water.

3.1.1.1. The inorganic component

The inorganic component of enamel is principally apatite in its hydroxy, fluoro, or carbonate forms. Calcium and phosphate are the two major inorganic elements (Brudevold *et al.*, 1960). Minor variations occur in composition in which aluminum, barium, magnesium, strontium, radium, and vanadium, among others, can be found in the lattice. Enamel mineralization or maturation begins early in tissue formation (Crabb, 1959), in which nucleation centers (Rönnhlöm, 1962) produce a crystalline structure. Crystallites are embedded in an organic matrix.

3.1.1.2. The organic component

The enamel contains 1-2% of organic material (Eastoe, 1963; Mjör and Pindborg, 1973). The main organic components of enamel seem to be made up of two kind of proteins, a soluble glycoprotein and a more insoluble protein, which are of about equal size (Mjör and Pindborg, 1973). The amino acid composition of the organic material is characterized by a high level of proline. An x-ray diffraction or electron microscopic study of the enamel matrix cannot be performed to observe this element (Glas and Nylen, 1965; Sundström, 1968).

3.1.1.3. The water component

The structural water may amount to 1 to 2 % by weight according to Ferguson and Chestnut (1978) and Holcomb and Young (1980), or between 3 to 4% (Stack and Fearnhead, 1965). Generally, it is agreed that there are two different water components existing in dental enamel, loosely and firmly bound water (Little *et al.*, 1962; Angmar *et al.*, 1963; Myers, 1965; Little and Casciani, 1966; Carlström *et al.*, 1963; Dibdin, 1972; Davidson and Arends, 1977; LeGeros *et al.*, 1978; Ferguson and Chestnut, 1978; Holcomb and Young, 1980).

Loosely bound water is presumed to be present in the pores, adsorbed on the enamel crystallite surfaces, or associated with the organic matrix (Corcia and Moody, 1974) and can be removed by heating the enamel at 100 to 140° C or exchanged with D₂O or ethanol (Little *et al.*, 1962; Holager, 1972). The loss of the loosely bound water has no effect on the crystallographic properties of the mineral but results in the disappearance of a narrow resonance line in the proton nuclear magnetic resonance spectrum of enamel (Myers, 1965; Dibdin, 1972; LeGeros *et al.*, 1978; Holcomb and Young, 1980). The firmly bound water is accompanied by a change in the crystallographic properties of the enamel mineral and presumed to be associated with the mineral itself (Holcomb and Young, 1980; LeGeros *et al.*, 1978, 1983).

3.1.2. Micromorphology

3.1.2.1. The enamel prism

The basic microscopic component of enamel is the mineralized rod or prism. Ranging in size from 4-7 micrometers (μm), rods originate in the region close to the dentino-enamel junction and follow a tortuous, decussating course in the inner two-thirds of the tissue before arriving at their parallel alignment in the outer third of the enamel (Gwinnett, 1992). The prisms widen from a diameter of about 3 μm near the dentino-enamel junction to about 6 μm at the surface of the tooth (Osborne and Ten Cate, 1976). Enamel rods run slightly obliquely to a tangent to the outermost surface of the tissue. In cross section, enamel rods or prisms have

been described as keyhole shaped (Meckel *et al.*, 1965) and generally, a convenient way to describe the keyhole is to divide it into "head" and "tail" (Mjör and Pindborg, 1973) (Figure 1). The various patterns can be produced by changing the plane of sectioning (Figure 2). In earlier studies, the cross sectioned enamel rods were demonstrated to be five- or six-sided prisms (Mjör and Pindborg, 1973) and found in certain animals (Boyde, 1969). In human enamel, crystals are oriented parallel to the long axes of the "head" of rods and fan out at an angle of approximately 65° in the "tail" of rods (Figure 3). The arrangement of the prisms are different depending on the distance from the dentino-enamel junction. The interprismatic regions become very irregularly at the enamel surface (Osborne and ten Cate, 1976).

The course of prisms from the dentino-enamel junction to the enamel surface is not straight but S-curved (Mjör and Pindborg, 1973) and the geometric appearance would vary depending upon the plane of section (Gwinnett, 1992). The course of the prisms in the different layers are not parallel but intertwining which may help to resist against the breaking forces (Mjör and Pindborg, 1973). In many regions within approximately $12\ \mu\text{m}$ from the surface of the enamel, the crystallites are all parallel to the long axes of the prisms and the irregular, microporous, crystallite-free prism sheath no longer exists. The enamel in these regions therefore appears structureless in the light microscope. The parallel arrangement of the crystallites and the absence of prism sheaths are probably accounted for the observation that the surface enamel is the most highly mineralized layer (Osborne and Ten Cate, 1976). However, in the crowns of the primary teeth and commonly in the pit, fissure, and cervical regions of the permanent dentition, the rodless, prismless, or aprismatic enamel is also found (Gwinnett, 1967; Ripa, Gwinnett and Buonocore, 1966).

3.1.2.2. The prism sheath

Surrounding each prism is a sheath. The thickness is less than $0.5\ \mu\text{m}$ and in cross sections of the prism, the sheaths can be complete or incomplete (Osborne and Ten Cate, 1976) depending on the form of the prisms which vary according to the relation of the axes of adjacent prisms or groups of prisms (Gustafson and Gustafson, 1967). The prism sheath can be demonstrated by any histological method, in both decalcified sections and undecalcified ground sections or ground surfaces. The prism sheath was long considered to be a organic membrane but this view was abandoned some years ago. Electron microscopy has shown that enamel is composed of submicroscopic fibres and submicroscopic crystallites. Thus, the prism sheath is a region particularly rich in fibrils which, at low magnification of the light microscope, appear as a continuous layer of organic matter (Gustafson and Gustafson, 1967).

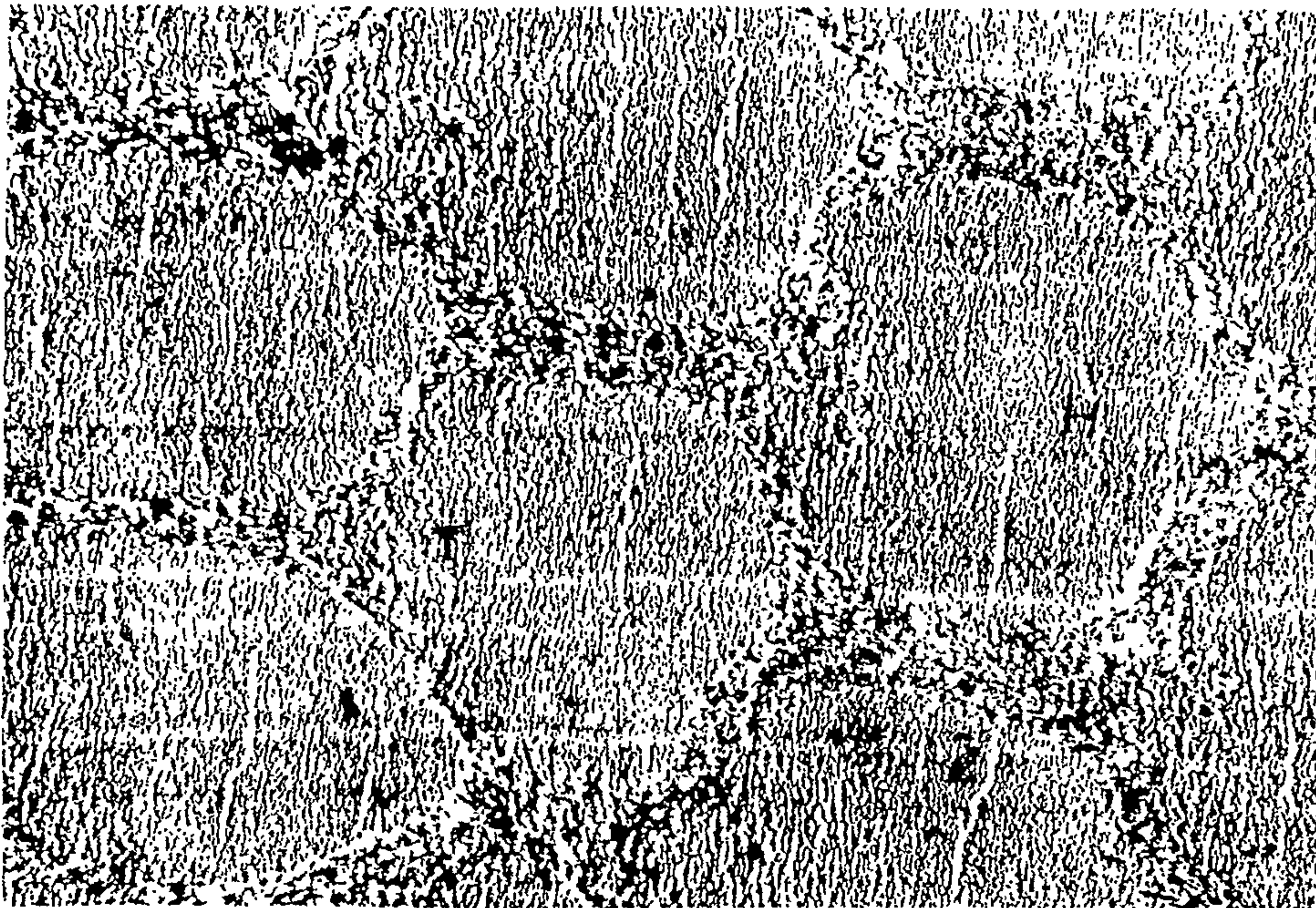


Figure 1: Electron micrograph of cross section of rods in mature enamel. Crystal orientation is different in "heads" (H) and "tails" (T) ($\times 5000$).

(From: Meckel A.H., Griebstein W.J., Neal R.J.: Structure of mature human dental enamels observed by electron microscopy. Arch. Oral Biol 10:775-83, 1965).

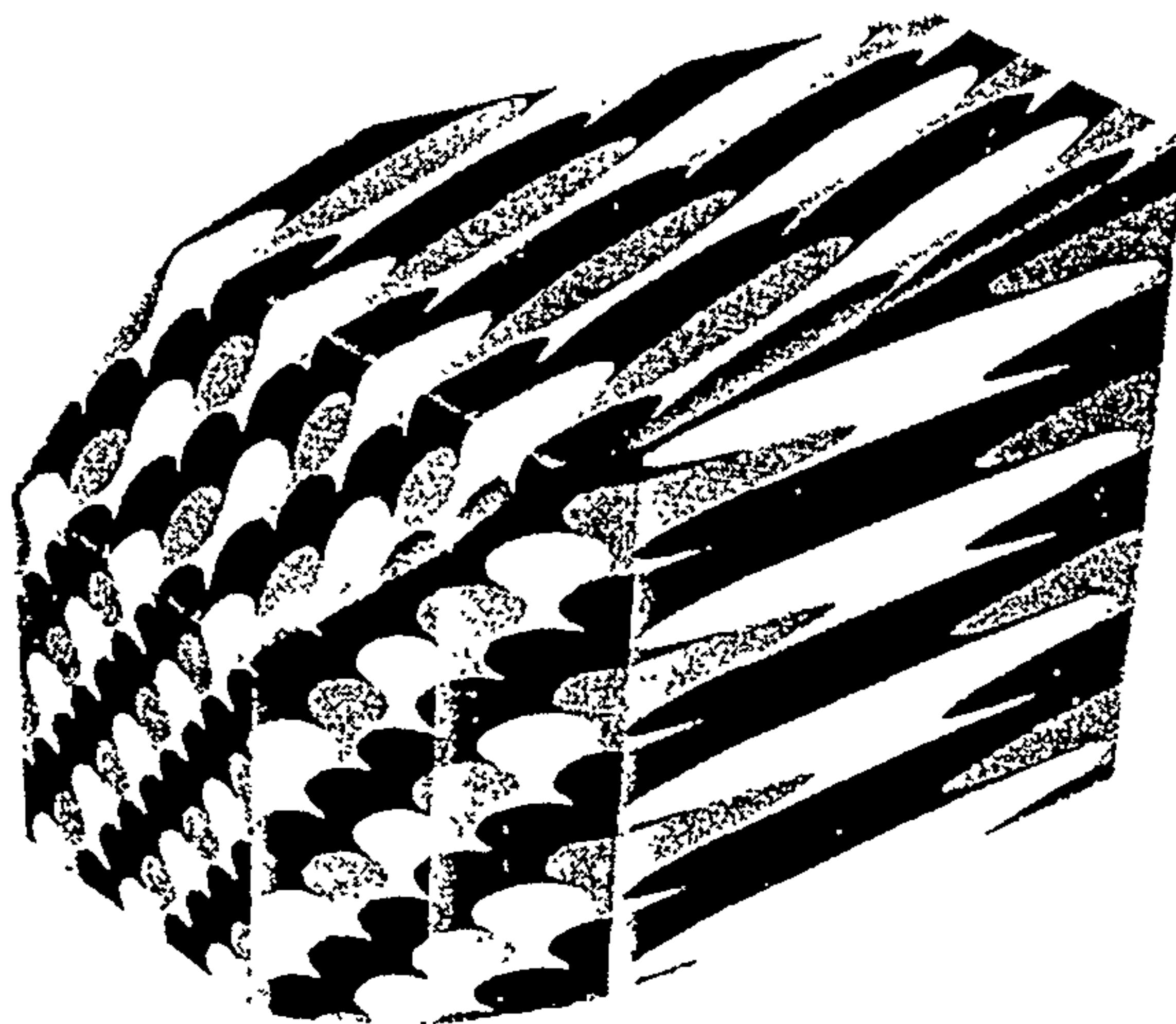


Figure 2: Model indicating packing of keyhole-shaped rods in human enamel. Various patterns can be produced by changing the plane of sectioning. (From: Meckel A.H., Griebstein W.J., Neal R.J.: Structure of mature human dental enamels observed by electron microscopy. Arch. Oral Biol. 10:775-83, 1965.)

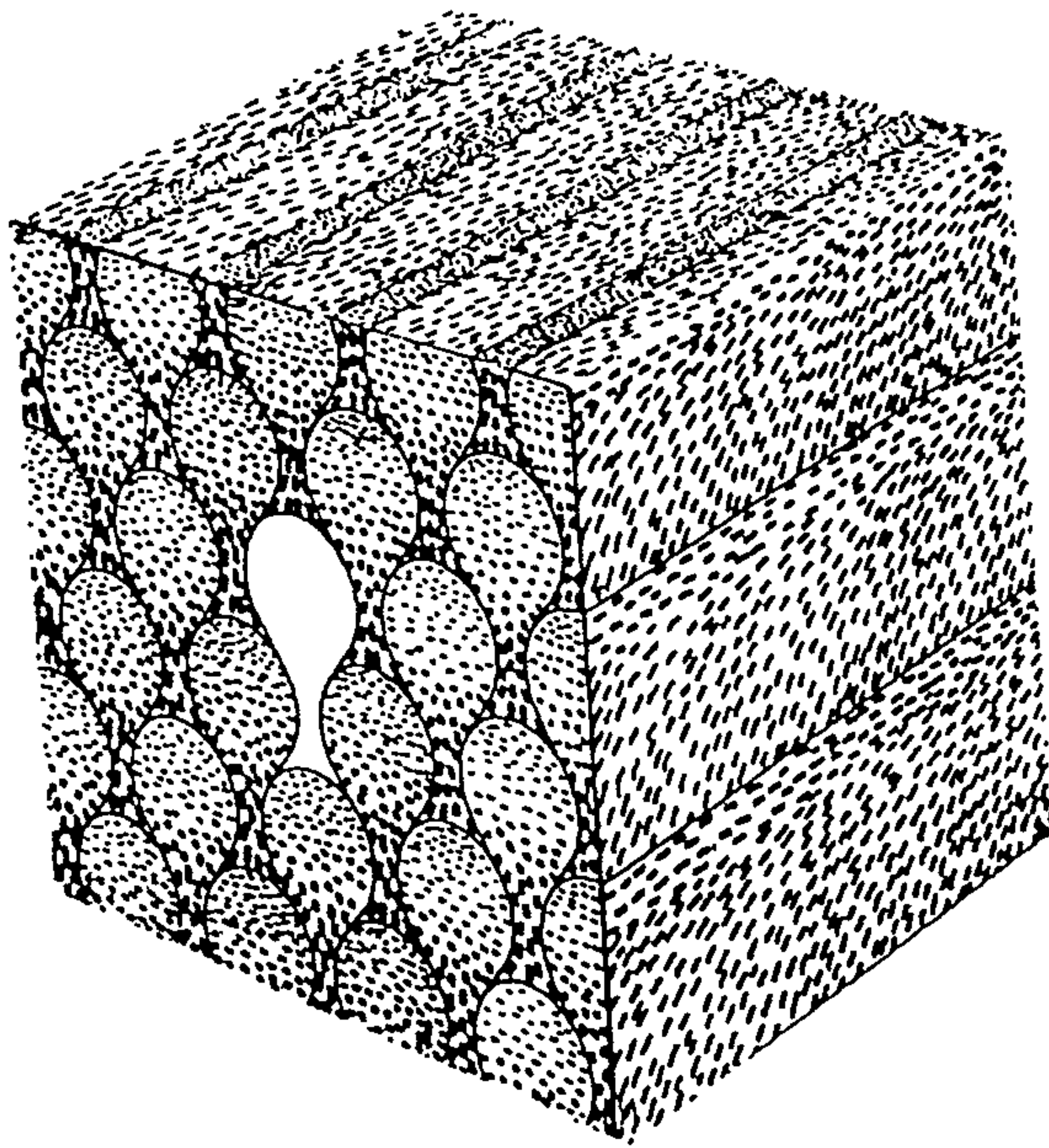


Figure 3: Drawing of keyhole pattern of human enamel indicating orientation of apatite crystals within individual rods. Crystals are oriented parallel to long axes of the "heads" of rods and fan out at an angle of approximately 65° in the "tails" of rods. (From: Griebstein W.J.: In Stack M.V., Fearnheard R.W., editors: *Tooth Enamel*. John Wright & Sons, Ltd., Bristol. p.p. 190, 1965)

3.1.2.3. The crystals

In mature human enamel, the hydroxyapatite crystals have short rods with the following approximate average dimensions: length 160 nm, width 40 nm and thickness 25 nm (Scott and Symon, 1971). The hydroxyapatite crystals in enamel are much larger than those found in dentine, cementum and bone (Mjör and Pindborg, 1973). The long axis of the crystals in the head is almost parallel to the length of the prism. In contrast, the direction of the crystals in the tail deviate, almost perpendicular at the tip of the tail and gradually become more longitudinally arranged (Mjör and Pindborg, 1973). It may thus be argued that the tail of the human keyhole prism may be the counterpart of the interprismatic enamel of other species (Glas and Nylen, 1965). The alignment of the long axis of the crystals has been observed from x-ray diffraction and electron microscopic studies (Mjör and Pindborg, 1973).

3.1.2.4. Cross striations and the Striae of Retzius

Cross striation appears in the single prism at approximately 4 to 6 μm intervals, and the striations in adjoining prisms are in phase. It can represent variations of the degree of mineralization along the prism, and the distance between them should indicate the daily increment of prism formation (Gustafson and Gustafson, 1967).

Striae of Retzius are incremental lines and they are spaced further apart than the cross striations, usually at intervals of about 20 to 80 μm . Generally, it is agreed that the Striae of Retzius are due to disturbed mineralization and present at the enamel surface as shallow furrows. In young teeth, they are particularly distinct in the cervical region of the crown. The enamel surface bulges in low ridges called perikymata. In elderly persons, the perikymata and the imbrication lines have worn away and the enamel surface may show scratches (Mjör and Pindborg, 1973).

3.1.3. Physical properties

3.1.3.1. Hardness

The hardness of tooth enamel is an essential physiological property (Frank, 1967).

The microhardness of teeth has been reported by Hodge and McKay (Hodge and McKay, 1933). In the past, two types of indentation experiments had been carried out:

- the indenter load was perpendicular to the surface (Philips and Swartz, 1948; Mühlemann, 1964; Koulourides, 1968; Koulourides *et al.*, 1974; Arends *et al.*, 1979; Gelhard and Arends, 1984), and
- the indenter load was parallel to the surface (Featherstone *et al.*, 1983; Scholtanus *et al.*, 1986).

Microhardness measurements of tooth material have only been regarded as an effective scientific technique since the advent of the Knoop unit of measurement (Knoop Hardness Number or KHN) (Knoop *et al.*, 1939). Hodge claimed that the earlier measuring instrument and techniques could not be adequately adapted for the measurement of enamel and dentine (Hodge, 1936).

Change of hardness of the enamel has been examined in the literature. It is measured either on the whole sample which is on the solution-exposed surface (Koulourides *et al.*, 1974), or on cross sections (Featherstone *et al.*, 1983). The former technique provides the advantage of being non-destructive and enabling a longitudinal study of the same specimen. Some studies related to this technique are carried out in order to find the correlation between the indentation length and the lesion depth (Arends *et al.*, 1979). However, it is claimed that this direct technique, where indentations are made on the outer enamel surface, cannot be used to provide details of the hardness changes below the surface nor in different regions of any enamel lesion (Featherstone *et al.*, 1983).

In the study of cross-sections, several experiments are reported using a Leitz miniload hardness tester with an interference contrast light system (Davidson *et al.*, 1974; Purdell-Lewis *et al.*, 1976). Davidson *et al.* (1974) noted that the microhardness achieved by this technique was a linear function of the local calcium content.

Purdell-Lewis *et al.* (1976), on the hardness study of sound premolar enamel and artificially demineralized white spot lesions, noted that this technique was reliable and the standard deviation obtained was less than 5% for enamel specimens. Furthermore, they also found that the hardness of sound enamel was different when measured at varying depth from the outer surface. In their study, the hardness of sound enamel at a depth of approximately 15 μm and 200 μm at the occlusal, midpoint and cervical areas were recorded. The results showed that the hardness at a depth of approximately 15 μm were 345, 330 and 319 KHN at the occlusal, midpoint and cervical areas, respectively. At a depth of 200 μm , the mean hardness seemed to have no difference; the hardnesses were 344, 346 and 345 KHN, respectively. However, the loading force of the indentation was not reported.

Collys *et al.* (1992), by using a Duramet 2 hardness tester (Leitz, Germany), reported that the KHN of sound human enamel was between 354-431 KHN with loads of 50-200 g.

The microhardness profiles are also produced to test the microhardness of dental mass. This technique is performed to compare changes during de- and remineralization of the tooth structure (Featherstone *et al.*, 1981, 1982, 1983).

There is a correlation between the mineral content of the enamel and the hardness. The study of Kodaka *et al.* (1992) revealed that there was a moderate correlation between the Vickers hardness and phosphorus concentration in the enamel ($r = 0.688$) but a low correlation with the calcium concentration ($r = 0.455$). However, they also found that the hardness did not directly reflect the various physical and chemical factors: apatite crystallization, porosity or enamel density, organic content or enamel protein.

In the study of time-dependency of microhardness indentation in human enamel, Herkströter *et al.* (1989) found that there was no change in microhardness indentation of sound human enamel within 5 days. Therefore, they concluded that enamel microhardness was not time-dependent.

By testing with an ultra micro-indentation system (UMIS), the Knoop Hardness Number of enamel was 343 KHN (Craig, 1993).

3.1.3.2. Elastic modulus

The elastic modulus of occlusal, side, and cusp enamel was reported to be 1.8, 6.0 and 8.2 GPa (Giga Pascal - see appendix 3), respectively. After improvement for precise results, Tyldesley (1959) determined mechanical properties of enamel by using a transverse type of loading system. The enamel modulus of elasticity was reported to be 19 GPa in bending.

In 1961, Craig *et al.* (1961, 1993) examined the modulus of elasticity of human enamel by compression and found that it was between 78.0 to 84.2 GPa.

3.2. Acid-etching procedure

3.2.1. The history of acid-etching procedure in orthodontics

Direct bonding of brackets is currently used by the majority of orthodontists throughout the world, being a direct consequence of the pioneering work of Buonocore and Bowen. Buonocore, in 1955, introduced the concept of acid etching with orthophosphoric acid which involved the alteration of the tooth surface by chemical treatment to provide a modified surface to which dental resin might adhere. He used 85% orthophosphoric acid (H_3PO_4) for 30 seconds and

reported that the bond strength of acrylic restorative resins was significantly increased by etching of the enamel surface.

3.2.2. The structural changes to enamel due to the etching procedure

It has been shown that the acid solution produces changes the enamel surface in two distinct ways; cleansing and producing micropores (Silverstone, 1974). In the first, a shallow layer of enamel is removed by etching, and plaque, surface and sub-surface cuticles are also removed effectively. In the second, the remaining enamel surface is rendered porous by the acid solution. It is in this porous region, or filamentous "tag-like" extensions that the resin is able to penetrate and so bond with the enamel (Gwinnett and Matsui, 1967; Gwinnett, 1971). The penetration of adhesive resins into treated enamel surfaces was described by Gwinnett and Matsui (1967). They found that "tag" lengths varied from material to material, from one tooth to another, and from one site to another (Gwinnett and Matsui, 1967). They also suggested that the formation of "resin tags" was the primary attachment mechanism of resin to phosphoric acid-etched enamel. With this procedure, acid-etching removed about 10 μm of the enamel surface and created a porous layer ranging from 5 to 50 μm deep. When a low-viscosity resin was applied, it flowed into the microporosities and channels of this layer and polymerised to form a micromechanical bond with the enamel. Etching also increased the wettability and surface area of the enamel substrate (Gwinnett, 1971; Retief, 1973; Silverstone, 1974).

Acid-conditioning creates and enhances existing enamel porosity. The depth changes in porosity and gross tissue loss depends on the type of acid used and its concentration. Porosity is enhanced to a greater depth in enamel as the strength of phosphoric acid is decreased. 0.5 N hydrochloric acid produced the most drastic change and the depth of micropores was more than 25 μm , while 50 % citric and 85 % phosphoric acid produced the least alteration, approximately 5 μm deep micropores (Gwinnett, 1971).

For the phosphoric acid, Silverstone (1974) reported that the tag length depended on the concentration of the acid, and the longer tags are observed in the 20 % to 30 % concentration range. It was also reported that a 50 % concentration produced enamel change of 19 μm compared to 30 μm . However, there is a critical concentration of approximately 30 %, below which an insoluble reaction product forms and may occlude the important enamel porosities (Chow and Brown, 1973). Phosphoric acid concentrations of 15 % and less produce changes similar to those of 70 % and greater (Silverstone, 1974). Diedrich (1981) reported that the distribution of various etching patterns on the same enamel surface may be considered the outcome of locally different acid sensitivity of the enamel structure, especially in the area of perikymata and imbrication lines.

With respect to etching patterns obtained, Silverstone *et al.* (1975) described three etching patterns in the acid concentration range 20-60 %. A Type I pattern in which there appeared to be a preferential removal of prism cores (Figure 4), a Type II pattern in which the prism peripheries were removed preferentially (Figure 5), and a Type III pattern, which occurred less often, in which the etched surface bore no resemblance to the enamel morphology (Figure 6). In another study, Silverstone (1975) found that a Type I etching pattern was observed when an enamel surface had been etched for 60 seconds with 30 % phosphoric acid, while a Type II was shown when using the higher acid concentration, 40 and 50 % (Figures 4 and 5).

In a later study, the acid concentration and etching times were considered according to permanent enamel loss (Hermesen and Vrifhoef, 1993). *In vitro*, a concentration of 50 % had been suggested by Retief (1975), while Silverstone (1974) and Gorelick (1977) considered a 30 % to 50 % concentration from the investigation for their effect on human enamel *in vitro*. Most commonly solution fell within the 30 % to 50 % range, with an etching of 60 seconds (Wei, 1970). There are a lot of studies to find the proper etching time. Many authors revealed that the enamel appearance is different when the etching time is changed (Brännström and Nordenvall, 1977). Marshall *et al.* (1975) noted that etching times needed to be longer in molars than in premolars for satisfactory bonds. Whittaker (1982) reported that the thickness of aprismatic enamel may explain the different etching time required in different teeth.

3.2.3. Etched-enamel and testing

The most common technique for the quantitative assessment of mineral content within enamel lesions is transverse contact microradiography (Collys *et al.*, 1992). This technique is not applicable to etched enamel because parts of the fragile etched surface are lost during sectioning. Furthermore, because of technical limitations, such as the slit width of the densitometer, changes in mineral content very near the enamel surface cannot be registered (de Josselin *et al.*, 1985).

Another sensitive technique to measure enamel demineralisation and remineralisation is the iodide penetrability method. The increased wettability and the roughness of etched surfaces enamel would influence the standardisation of this technique (Bakhos and Brudevold, 1982).

Several studies investigated the changes of mineral content and microhardness of enamel:

Davidson *et al.* (1974) studied the correlation of the change in microhardness of sound, decalcified and etched-tooth enamel and the calcium content. They found

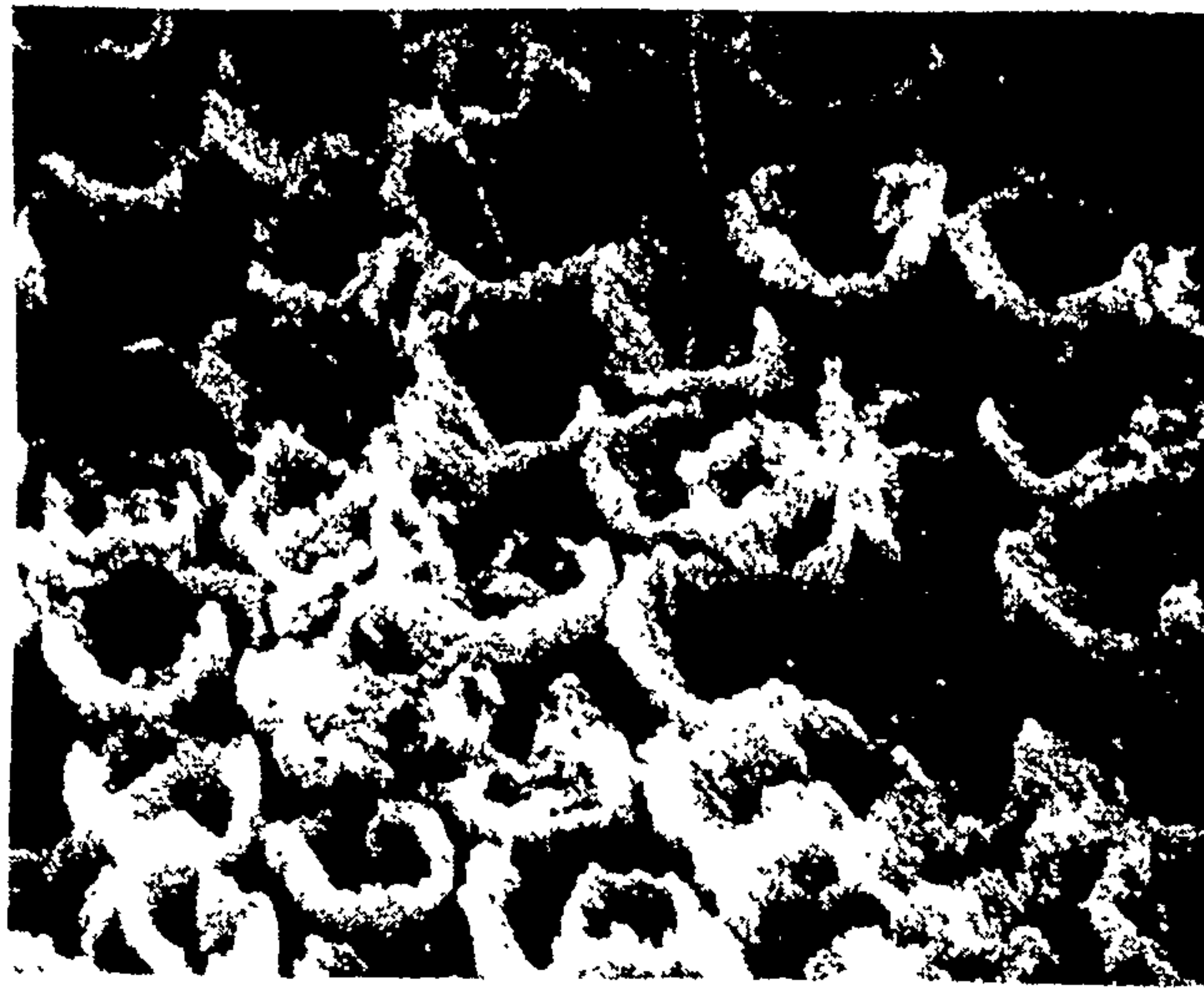


Figure 4: Scanning electron microscopy of an enamel surface that had been etched. A Type I etching pattern was seen in which prism centres had been removed preferentially. (From: Silverstone L.M. (1975): The acid etch technique: in vitro studies with special reference to the enamel surface and the enamel-resin interface. In *Proceedings of an International Symposium on The Acid Etch Technique*. Silverstone L.M. and Dogon I.L. North Central Pub Co, St Paul p.p. 13-39.)



Figure 5 Scanning electron microscopy of the etched enamel. A Type II etching pattern which was termed a preferential removal of prism peripheries was detected. (From: Silverstone L.M. (1975): The acid etch technique: in vitro studies with special reference to the enamel surface and the enamel-resin interface. In *Proceedings of an International Symposium on The Acid Etch Technique*. Silverstone L.M. and Dogon I.L. North Central Pub Co, St Paul p.p. 13-39.)

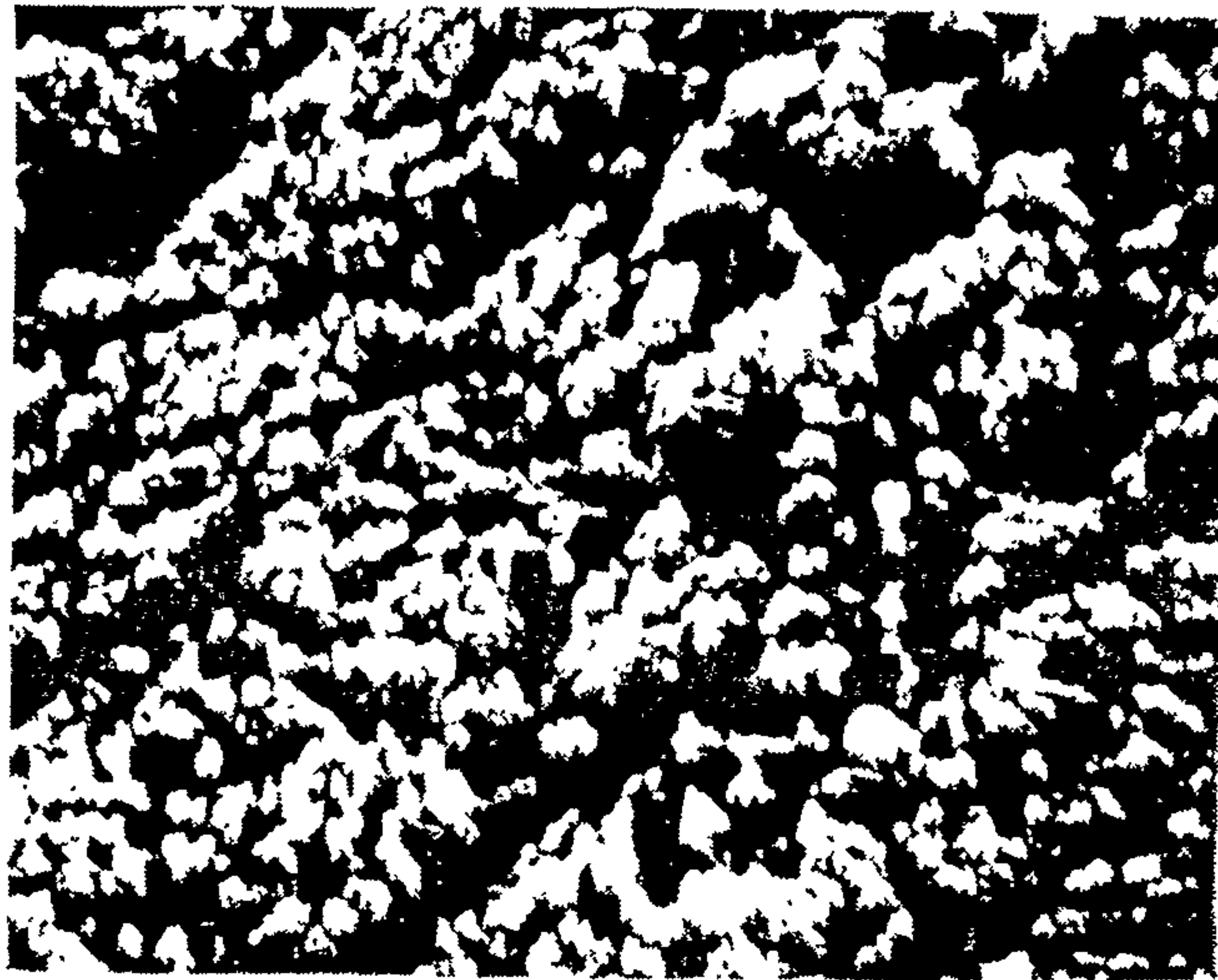


Figure 6: Scanning electron microscopy of the etched enamel. A Type III etching pattern which included areas resembling each of other patterns, as well as regions in which the etching pattern appears unrelated to prism morphology. (From: Swift E J, Perdigao J, Haymann H O. Bonding to enamel and dentin: A brief history and state of the art. 1995. *Quintessence Int* 26: 95-110, 1995).

that the local calcium content and the duration of decalcification were correlated with the microhardness of decalcified enamel. In the etched enamel, the microhardness in the acid-penetrated area and the calcium content were at a lower level compared with untreated enamel. Etched enamel possessed a KHN of 133.3, while it was 327-367 KHN in sound human enamel. In the decalcified lesion, the hardness was reduced to approximately 50% of its original value.

Collys *et al.* (1992), by using a Duramet 2 hardness tester (Leitz, Germany), examined the load dependency of hardness on demineralised enamel, both surface-softened and surface-etched, compared to sound enamel. They used different loads, and 20 indentations were made for each load. They found that with using 50-200 g of loads the hardness of sound enamel was 338.9-417.5 KHN while it was 87.8-171.4 KHN in the etched enamel. They also revealed that an increase in KHN was observed with the acid-treated specimen when indenting from the external surface while it was opposite in that of the sound enamel. This result was explained by a decrease in porosity as a function of depth.

3.2.4. Enamel/resin interface

The infiltration of resin monomer into enamel is a key to the retention of the bulk of resin, which remains superficially. Long "tags" of resin permeate into enamel and frequently exceed 50 μm in length (Gwinnett and Buonocore, 1965). The tag length depends on the concentration of phosphoric acid with the longest tags observed in the 20 to 30 % concentration range (Silverstone, 1974). The degree of resin penetration may influence bond strength and the enhanced retention value is related to tag length (Rock, 1974). More recently, Soetopo *et al.* (1978), reported that resin penetration may not be the only factor controlling bond strength. However, they noted that the resin penetration was still needed for a durable bond under clinical conditions.

3.2.5. Factors affecting bonding to enamel

Several factors may affect the establishment of adequate bonds in the clinical situation. The prophylaxis prior to acid conditioning has been needed since the reduction in bond-strength might occur if a prophylaxis is not performed (Miura *et al.*, 1973). Rinsing with a water spray for a minimum of 15 seconds to one minute can enhance the bond strength (Soetopo *et al.*, 1978). A dry field is another condition which is of paramount importance to bonding (Gwinnett, 1976).

The penetration of resin into enamel is also affected by the type of enamel. The resin penetration to approximately 5 μm is observed in a prismless enamel (Gwinnett, 1973).

3.2.6. The study on the mechanical properties of the enamel and composite resin

Shear bond strengths of composite resin to phosphoric acid-etched enamel were typically in the range of 20 MPa (Mega Pascal - see appendix 3) (Barkmeier *et al.*, 1986; Gilpatrick *et al.*, 1991; Gwinnett and Kanca, 1992). These bond strengths provided routinely successful retention of resins for a variety of clinical applications, including composite restorations, veneer prostheses as well as orthodontic brackets.

Many authors report that the reduction in tensile bond strength is caused from decreasing etching time (Mardaga and Shannon, 1982). Oppositely, some authors find no statistically significant differences in the bond strength for the different etching time (Barkmeier *et al.*, 1985; Legler *et al.*, 1989; Kinch *et al.*, 1986; Sadowsky *et al.*, 1990; Uno and Finger, 1995). Recently, a 37 % acid concentration applied for 15 seconds is recommended (Sadowsky *et al.*, 1990).

Apart from the laboratory tests on the acid-etching procedure, other findings related to the bracket-bonding process are also studied. The adhesion between bonding resin and orthodontic brackets are widely reported. *In vitro*, the tests are carried out to determine the effect on bond strength of brackets with resin (Sinha *et al.*, 1995; Mizrahi *et al.*, 1995; Bradburn and Pender, 1992; Whitlock *et al.*, 1994; Major *et al.*, 1995). The debonding procedure is also studied to search for the safest and most reliable technique, which is less harmful to the enamel after composite removal (Gwinnett and Gorelick, 1977; Retief and Denys, 1979; Howell and Weekes, 1990; Bishara and Trulove, 1990; Bishara and Jakobsen, 1993; Rueggeberg and Lockwood, 1990; Oliver, 1988).

In the literature previously presented, the tests were carried out in Bovine teeth (Rueggeberg and Lockwood, 1990; Lee *et al.*, 1974) or extracted human teeth (Gwinnett and Gorelick, 1972; Retief and Denys, 1979; Sheridan *et al.*, 1989; Bishara and Trulove, 1990; Howell and Weekes, 1990; Bradburn, 1992; Buonocore *et al.*, 1968; Silverstone, 1974; Sargison *et al.*, 1995), as well as on other material bases (Oliver, 1988; Whitlock *et al.*, 1994; Eliades *et al.*, 1995). There has been little documented on *in vivo* human teeth (Lee *et al.*, 1974).

The mechanical properties of the composite resin have also been reported. Craig (1993) reported that the hardness of the microfine composite was 23 to 36 kg/mm². Chardwick *et al.* (1990) studied on the effect of various storage media (distilled water, lactate (pH 4.0) and citrate (pH 6.0)) upon the surface microhardness and abrasion resistance of three composites and reported that there was no significant difference ($p > 0.05$) of the hardness of the specimens over one-week storage. However, over the one-year storage period, a reduction of the hardness of some composites was observed. In water storage, there was no significant difference ($p > 0.05$) between the wear factors of specimens after one

year or one week of storage period, while the slight increase of this factor was observed in other storage media.

3.3. Sterilisation of extracted teeth

The American Dental Association (ADA) (1988) and the U.S. Centres for Disease Control (CDC) (1986, 1987) as well as New South Wales (NSW) (1995) Health Department call for thorough removal of any organisms capable of transmitting disease from nondisposable items used in patient care. They recommended autoclaving as the best method of decontamination for material exposed to body fluids. However, it is difficult to sterilise the extracted teeth before being used in preclinical technique courses, clinical exercise, or other laboratory testing, because their structure may be damaged or altered by the sterilisation procedures (Pantera and Schuster, 1990).

From the reviewed literature, Pagniano *et al.* (1985) demonstrated that potentially pathogenic bacterial and fungi, such as *Aspergillus*, were recoverable, following use of extracted human teeth in a dental teaching laboratory. Furthermore, since tooth preparation in technique laboratories was generally done without a liquid coolant, there was greater probability of exposure to pathogenic organisms in the laboratory area and spread of communicable disease (Belting, 1964). However, infection associated with extracted human teeth could not be found (Pantera and Schuster, 1990).

For the sterilisation of extracted teeth before using in the laboratory, Pagniano *et al.* (1985) stored them in Roccal II 10 % for three weeks and reported that such storage was effective in reducing, but not eliminating, recoverable airborne microbes. They suggested autoclaving of teeth prior to storage in that disinfectant. The CDC (1987) recommended sodium hypochlorite dilutions of 1:10 to 1:100 for decontamination of environmental surfaces, depending on the amount of organic material present. Senia *et al.* (1975) reported that a one-minute immersion in 5.25 % sodium hypochlorite sterilised gutta-percha endodontic points contaminated by a variety of gram-positive, gram-negative, and spore-forming micro-organisms. Rudd *et al.* (1984) reported that a five-minute immersion in 5.25 % sodium hypochlorite was similarly effective in sterilising dentures. Pantera *et al.* (1988) also used a five-minute immersion in 5.25 % sodium hypochlorite for sterilisation of coronal surfaces of extracted teeth.

3.4. Sodium hypochlorite

3.4.1. History

Hypochlorites are the oldest and most widely used of active chlorine compounds in the field of chemical disinfection. The sodium hypochlorites in water solution are less stable, especially in products with higher chlorine concentrations. On exposure to air the hygroscopic-powdered hypochlorites attract water vapor and become less stable. Because hypochlorite solutions are widely accepted as disinfectants, they serve as standards for testing of other sanitizers (Dychdala, 1983).

The sodium hypochlorite solutions range in concentration from 1 to 15%, with 1 to 5% available chlorine products employed for domestic use and stronger solutions sold to industrial customers. Dakin's solution is one of the sodium hypochlorite solutions which has been used since World War I to disinfect open wound infections (Dakin, 1915). The concentration 0.1 to 1.25% is used for fungicidal purposes (Dychdala, 1983). Bunyan (1960) reported that rinsing with 0.2% hypochlorite solution stops postoperative bleeding within one minute after a tooth extraction or other oral surgery. The author also mentions that with its utilization as an effective hemostatic, there is also reduction of swelling of traumatized gingival tissues and diminution of the postoperative pain. Chlorine compounds are widely employed in hospitals to disinfect equipment, instruments, etc. (Sykes, 1963).

3.4.2. The effect of sodium hypochlorite on teeth

In dentistry, sodium hypochlorite is one of the most common disinfectant. It is also known to be an excellent protein denaturant, antibacterial and lubricative solution (Piskin *et al.*, 1995). Robinson and Kirkham also reported that it can increase penetration of mineral into carious enamel lesion (Robinson and Kirkham, 1990). Crane (1920) described the use of Dakin's solution, 0.5% sodium hypochlorite, in endodontic therapy. Nowadays, it is still the most favored endodontic irrigant in modern endodontic practice because of its antibacterial, lubricative, and tissue-dissolving properties (Penick and Osetek, 1970; Weine 1976; Cohen and Burns, 1987; Ingle *et al.*, 1985).

Due to the excellent protein denaturation of sodium hypochlorite, Holma *et al.* (1969) noted that the organic material of the enamel surface was removed by 10-12% sodium hypochlorite when treated for 2.5 hours.

Again, in 1970, Holma *et al.* investigated the action of sodium hypochlorite on organic substance and minerals of enamel. In this study, three bicuspid teeth were used and sectioned longitudinally in a buccolingual direction before testing. One

half of each tooth was decalcified and treated with a sodium hypochlorite solution (10-12% active chlorine, pH 10-11) to test the oxidation on the organic substance. The ultrastructure of the enamel surface of impacted and erupted teeth, with and without hypochlorite treatment, was observed. The results showed that the distal part of the hydroxyapatite crystallites was exposed by the oxidation procedure. The dentine part of the specimen was also examined for possible alteration due to the effect of sodium hypochlorite. The result revealed that the collagen was entirely oxidized and a similar result should also occur to the mineral content of the enamel (Holma *et al.*, 1970).

Kodaka *et al.* (1990) also used 10% sodium hypochlorite to remove the organic debris from the enamel surface. In this study, they found that the deproteinizing ability of sodium hypochlorite was still effective even with 30 minutes exposure time. In 1994, Kuroiwa *et al.* followed this procedure and confirmed the same result (Kuroiwa *et al.*, 1994).

Boyde and Lester (1968) used ethylene diamine in an extraction process for the removal of intermicrobial substances in studies of bacterial plaque in carious cavities. Ethylene diamine exerted a selective action on the bacterial cell walls. However, sodium hypochlorite appeared to be more effective than ethylene diamine in removing organic material from enamel.

Besten and Giambro (1995) examined the treatment of fluorosed and white-spot human enamel with calcium sucrose phosphate *in vitro*. In their study, the enamel was treated with 5.25% sodium hypochlorite for 20 minutes and placed in an artificial saliva. The result showed success in returning white-spot lesions to a normal enamel color. They explained that sodium hypochlorite can remove the surface organic material and result in a more ready diffusion of calcium and phosphate into the enamel. Thus, the diffusion of mineral into the lesion would be increased.

Galil (1975) attempted to find an *in vitro* enamel cleaning method for examining teeth by scanning electron microscopy. Human extracted teeth were used and immersed in 15 different agents to remove the acquired organic integument prior to examining the occlusal surface of enamel by scanning electron microscopy. The result showed that a solution containing 6% sodium hypochlorite gave the best outcomes, removing surface deposits but leaving the actual enamel intact. Even the treatment time for 30 minute was able to clean the enamel surface but 24 hours gave the total cleansing of occlusal surface, even in the fissure, leaving a white precipitate.

Venezie *et al.* (1994) reported that enamel pretreatment with 5% sodium hypochlorite for 1 minute can enhance bonding in hypocalcified amelogenesis imperfecta. However, it did not appear to alter the structure or mineral content of normal or hypocalcified enamel (Wright *et al.*, 1993).

Sodium hypochlorite was also used as the conditioning solution for increasing the mineral uptake for fluorosed enamel *in vitro*. Christensen *et al.* (1979) studied the effect of a mineralizing solution on sections of fluorosed human dental enamel *in vitro* by washing the specimens in a 15% hypochlorite solution for 30 seconds or 5 minutes before treating with a calcium phosphate solution and testing the mineral uptake. They found that sodium hypochlorite can increase the mineral uptake which 5 minutes showed the better results compared to those of 30 seconds. They clarified that it was because sodium hypochlorite removed protein which was densely packed in the pores between the enamel crystals. Consequently, this led to a much greater mineral uptake in sections exposed for 5 minutes than in those treated for 30 seconds.

It was reported that the several concentration levels and exposure times can also give the effect on soft-tissues or on dental impression materials (Piskin and Türkün, 1995, Bass *et al.*, 1992).

Although sodium hypochlorite has many advantages, the lack of chemical stability is an important disadvantage of this solution (Mark *et al.*, 1964; Hoffmann *et al.*, 1981; Dychdala, 1983; Johnson and Remeikis, 1993). The stability of sodium hypochlorite may be affected by temperature, concentration, pH, light, the presence of catalysts and organic material, and the presence of atmospheric carbon dioxide (The, 1979; Hoffmann *et al.*, 1981; Dychdala, 1983; Cottone and Molinari, 1987; Gerhardt and Williams, 1991; Johnson and Remeikis, 1993). However, from the study of Piskin and Türkün (1995), showed that 0.5% sodium hypochlorite stored at 4°C and 24°C and 5% solutions stored at 4°C showed satisfactory stability at 200 days.

3.5. Disinfectants and laboratory testing

According to the protocol for protecting and reducing the risk of transmission of blood-borne pathogens, including the human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV), the extracted teeth have to be disinfected before testing in the laboratory (Infection control policy, NSW Health Dept., 1995). Previous laboratory testing reported that extracted teeth could be disinfected externally and internally by exposure to formalin but not to iodophor, sodium hypochlorite, synthetic phenol or glutaraldehyde disinfectants due to the failure to disinfect (Tate and White, 1991). The results of others reported that autoclaving was an effective method of sterilizing extracted teeth (Pantera and Schuster, 1990).

Determining the properties of extracted teeth after disinfecting and sterilising, Shaffer *et al.* (1985) examined the effect of disinfection/sterilisation procedures on the enamel surface by scanning electron microscopy and bonding tests using 2%

glutaraldehyde for 24 hours, 1% sodium hypochlorite for 24 hours and steam autoclaving for 20 minutes at 260°F. They reported that there were no morphologically significant differences between intact enamel surfaces after disinfection/sterilization and those of intact, untreated controls. McGuckin and Pashley (1990) examined the effects of disinfection / sterilisation of intact *versus* prepared dentine surfaces using chemical disinfectants; phenolated 2% glutaraldehyde (Sporicidin) and 1% sodium hypochlorite; as well as autoclaving and found that there was no difference between the dentine bond strengths obtained on intact teeth exposed to any of these treatments *versus* non-treated control. Pashley *et al.* (1993) also reported that the sterilisation of teeth did not alter dentine permeability or bond strength.

At the present time, the direct bonding of brackets by using 37% orthophosphoric acid is now used by the majority of orthodontists. In etching procedure, the acid solution produces changes to the enamel surface, creates and enhances existing enamel porosity to which dental resin might adhere. The exposure of the enamel to different etching concentration can result in the dissimilar etching patterns; known as Type I, II and III in which the different parts of enamel prisms are preferentially removed.

Nowadays, several studies have advocated tooth disinfection/sterilisation procedures on extracted teeth before use in the laboratory testing. In the United Dental Hospital, a 24-hour immersion in 1% sodium hypochlorite is recommended and used for disinfecting the extracted teeth. Since sodium hypochlorite is known to be an excellent protein denaturant and antibacterial solution, these actions may alter the physical properties of the tooth surface and enamel interprismatic regions.

Considering the alteration due to the sodium hypochlorite on the enamel surface, prior infection control procedure before the teeth can be used in the laboratory may affect the complicated microstructures of the tooth enamel. Therefore, the laboratory testing on the properties of enamel may need to consider any physical changes resulting from the infection control procedure.

Chapter 3

Aims and Objectives

The specific aim of the research project therefore was:

To evaluate the effect of a clinical dental disinfectant (1 % sodium hypochlorite) on the tooth enamel surface by determining any changes in the physical properties of the acid etched and bonded enamel surface at the bracket-bonded areas.

The hypothesis of the research was:

The hardness of the acid etched and bonded non-disinfected enamel surfaces, in the *in vivo* group should be different from those of the *in vitro* laboratory study where the samples are disinfected before bonding the brackets.

Chapter 4

Materials and Methods

4.1. Selections and management of sample teeth

Premolar teeth, extracted for orthodontic reasons, were used in this study. Teeth were selected in this study to meet the following criteria:

1. The subjects were selected from the Anglo-Saxon patients being treated in the Orthodontic Department of the United Dental Hospital, University of Sydney, aged between 15-20 years, had been resident in the Sydney district and had no history of oral fluoride supplementation from the age of 2-3 years which was the time before enamel calcification of the upper permanent premolars.
2. Complete apexification of the premolar roots were present.
3. Opposing upper first or second premolars were used as the experiment and control sample because to their similar morphology.
4. Enamel with no detectable cracks or caries.
5. No recent history of bleaching treatment were present.
6. No significant clinical differences in size and morphology between two premolars were noted.

Once the patient was selected for this study, a patient information and a consent form for tooth donation were signed for the patient permission (see appendix 4). Then, an upper premolar was chosen as the experimental tooth (Group "a") and its opposite equivalent was designated as the control tooth (Group "b"). Contralateral teeth can be used to advantage in the investigation since the enamel of such teeth has proved to be identical at a microstructural level (Gustafson and Gustafson, 1955). This choice was predetermined using a random allocation to allow an equal number of left and right control teeth for the sample of ten teeth of each group.

The buccal surfaces of the experimental teeth were first subjected to an intraoral bonding procedure before extraction and decontaminated after extraction with the normal infection control protocol of the United Dental Hospital which was the placement of tooth for 24 hours in *Milton's* solution (1% sodium hypochlorite). The control teeth, after extraction, were immediately treated with the same infection control protocol and the buccal surfaces would receive the same bonding and debonding procedure to produce the extraoral (*in vitro*) bonding samples. The intraoral and extraoral bonding would be conducted as follows:

- A 37% orthophosphoric acid solution (3M *Scotchbond Etchant*, 3M *Unitek*®) was applied with a cotton pledget and left in place for 15 seconds. This duration of etching was recommended as the minimal duration which obtained insignificantly different effect on the retention of bonded orthodontic attachments

compared with the 60 seconds of equal acid concentration (Sadowsky *et al.*, 1990).

- The surface was washed in running water for 60 seconds and dried with a chip syringe.

- An unfilled resin (3M Unitek®) was coated on the tooth surface with a cotton pledget. Then, a Begg bracket (Dentaurum®) with a curved mesh-backing was attached to the tooth at the middle of the buccal surface, as shown in Figure 7, using the orthodontic bonding agent "Transbond XT Light Cure" (3M Unitek®) and then exposed to the curing light for 3 minutes. The time is sufficient for complete setting of the light-cured material. Then, the bracket was immediately debonded by squeezing with a bracket removing plier, leaving a thin resin layer on the surface (Figure 8).

Following these procedures, the teeth were washed in deionised water and stored at a low temperature (4° C) for not more than 2 months until the commencement of the experiment.

4.2 The specimen preparation prior to the testing with the UMIS-2000 (CSIRO®) machine

Each sample tooth was mounted by embedding the crown with cold-cured acrylic resin (Struers®) with its long axis perpendicular to the horizontal plane as shown in Figure 9. Then, the tooth was sectioned transversely at the middle of the coronal portion, and measured by a caliper from the cusp tip of the buccal surface to the cemento-enamel junction. The process was carried out by using a cutting machine (Isomet®) with de-ionised water as the coolant. The occlusal half was used in the experiment to avoid any enamel area which might have been injured during the extraction procedure (Figure 10).

The surface of the specimen was then be smoothed with a sand paper and finally polished to obtain a smooth and flat surface as required for the testing with the UMIS-2000 machine. The details of each process were as follows (Figure 11 and 12):

4.2.1. Grinding process

The surface was coarsely smoothed using sand paper (No.400) with de-ionised water as a coolant. The smoothing was performed only in the vertical direction and routinely checked by light microscope until the scratches from the

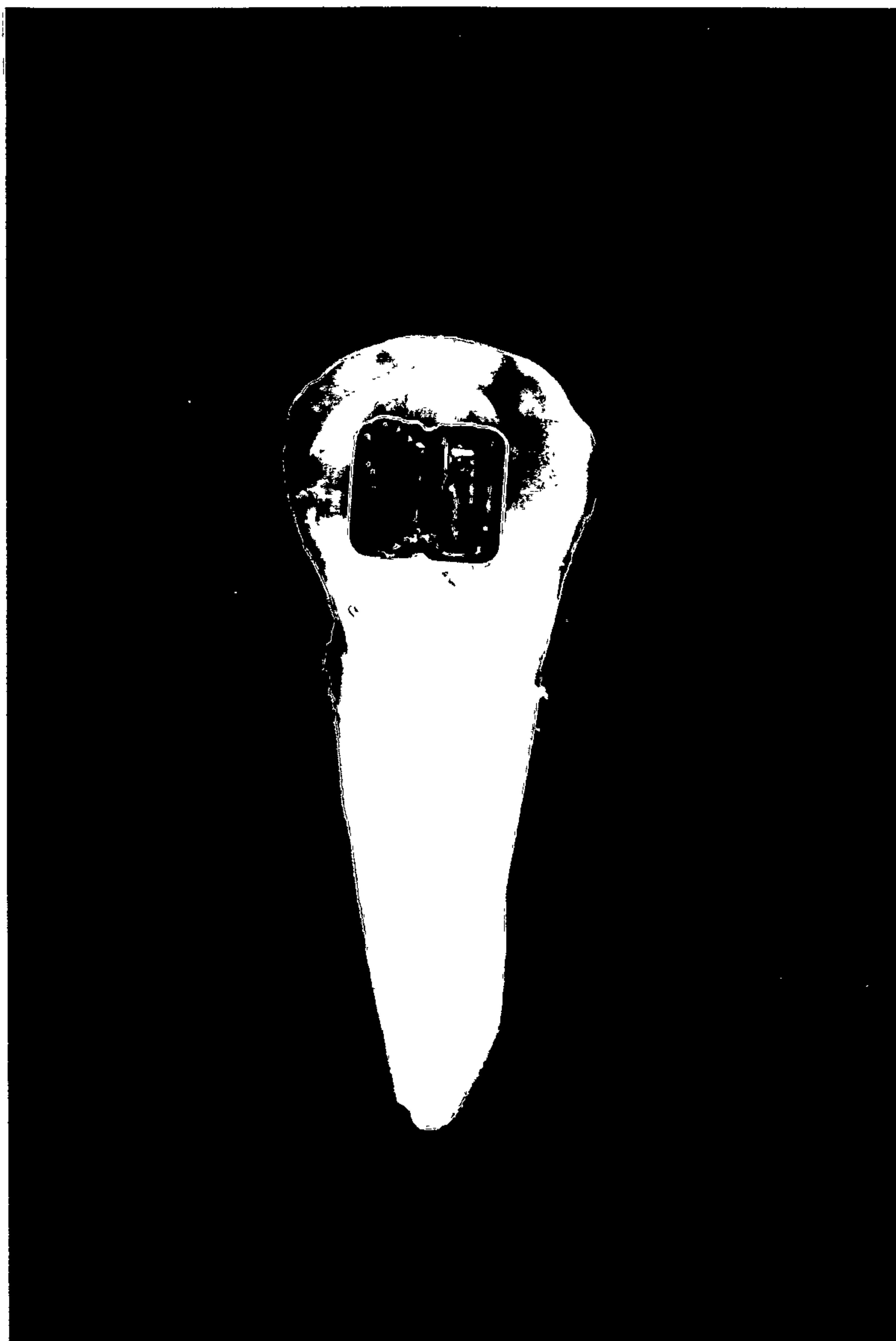


Figure 7: A Begg bracket attached in the middle area on the buccal surface of the sample tooth.

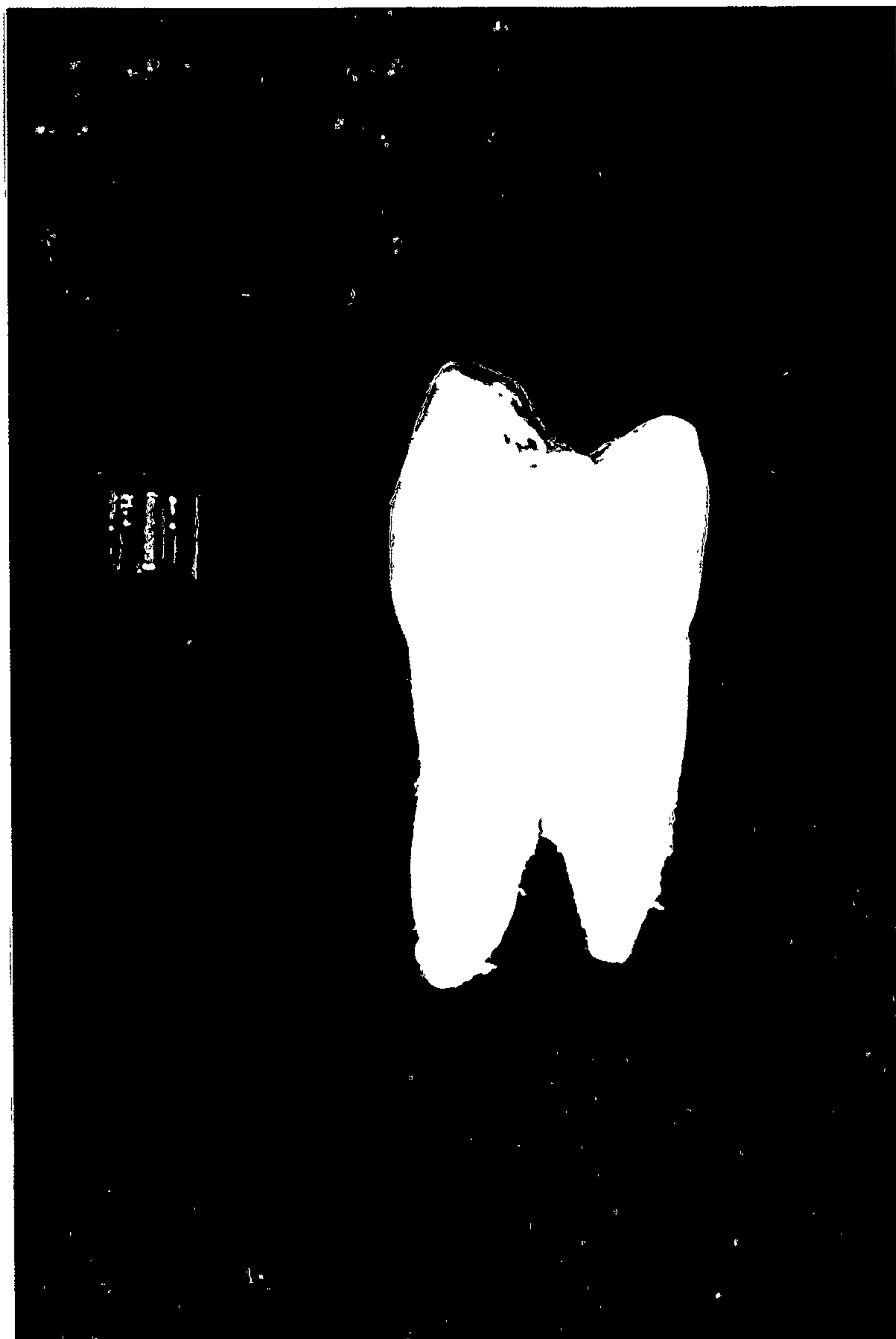


Figure 8: The bracket debonded, leaving only a thin resin layer on the tooth surface.

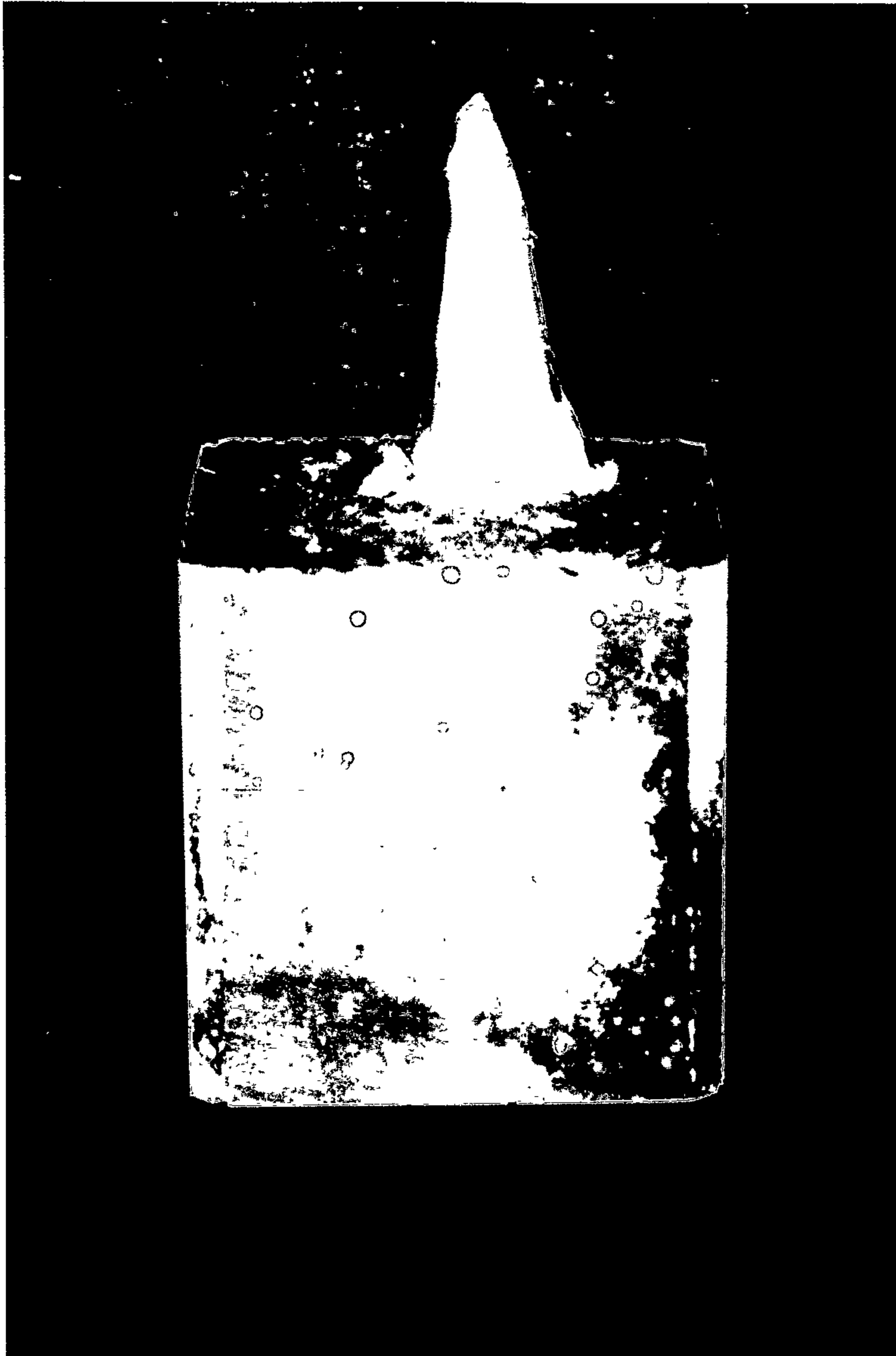


Figure 9: A sample mounted with cold-cured acrylic resin (*Struers*®) with its long axis perpendicular to the horizontal plane.



Figure 10: The tooth sectioned transversely at the middle of the coronal portion by using the cutting machine (*Isomet*[®]). The occlusal half was used in the experiment.

sand paper were in the same direction and had the same dimensions. Then, the specimen was turned 90° and received the same smoothing process until the scratches from the first stage disappeared. Then, a finer sand paper (No. 600) was applied in the same manner before a smoothing process with the machine was commenced (Figure 11).

The fine smoothing and polishing process with the machine (*Struers®*) was employed in the rotational direction (Figure 12). Fine smoothing ensured that the surfaces of all samples were in the same condition before the preparation continued, and that the surfaces of all samples in a sample holder were at exactly the same level. Furthermore, it would remove the deformations resulting from coarse grinding and produce a surface that was ready for polishing. Fine smoothing process was performed using the 15 µm abrasive grain size (*SAPNO P-15/DP-Suspension, Struers®*) with the specific lubricant (*LUGON/Green lubricant, Struers®*) for 10 minutes with 30 N of force pressure. Then, the specimen would receive the same smoothing procedure but with a reversed rotational direction to remove any deformations before passing through the ultrasonic cleanser for 10 minutes to remove the abrasive grains.

4.2.2 Polishing process

To remove deformations from fine smoothing and obtain a surface that was highly reflective, the samples must be polished before they could be examined under the microscope. Polishing was carried out on cloths of differing resilience with different abrasives. Two processes were used: diamond polishing and oxide polishing.

1. Diamond Polishing

The diamond polishing with 3 µm abrasive grain size (*SAPNO P-3/DP-Suspension, Struers®*) and 30 N of force pressure was employed for 10 minutes and followed with the reverse direction for 10 minutes with 15 N of force pressure. Then, the specimen was placed in the ultrasonic cleanser for 10 minutes to remove the abrasive grains.

2. Oxide Polishing

For the best possible finish, a final step using oxide polishing was required. The polishing action was achieved through a combination of chemical treatment and gentle abrasive action. For oxide polishing, colloidal silica with a grain size of approximately 0.04 µm and a pH of 9.8 (*OP-S Suspension, Struers®*) was used. The polishing required 30 N of force for 2 minutes before removing the residual polishing materials with an ultrasonic cleanser for 10 minutes.



Figure 11: The sample coarsely smoothed by a sand paper.

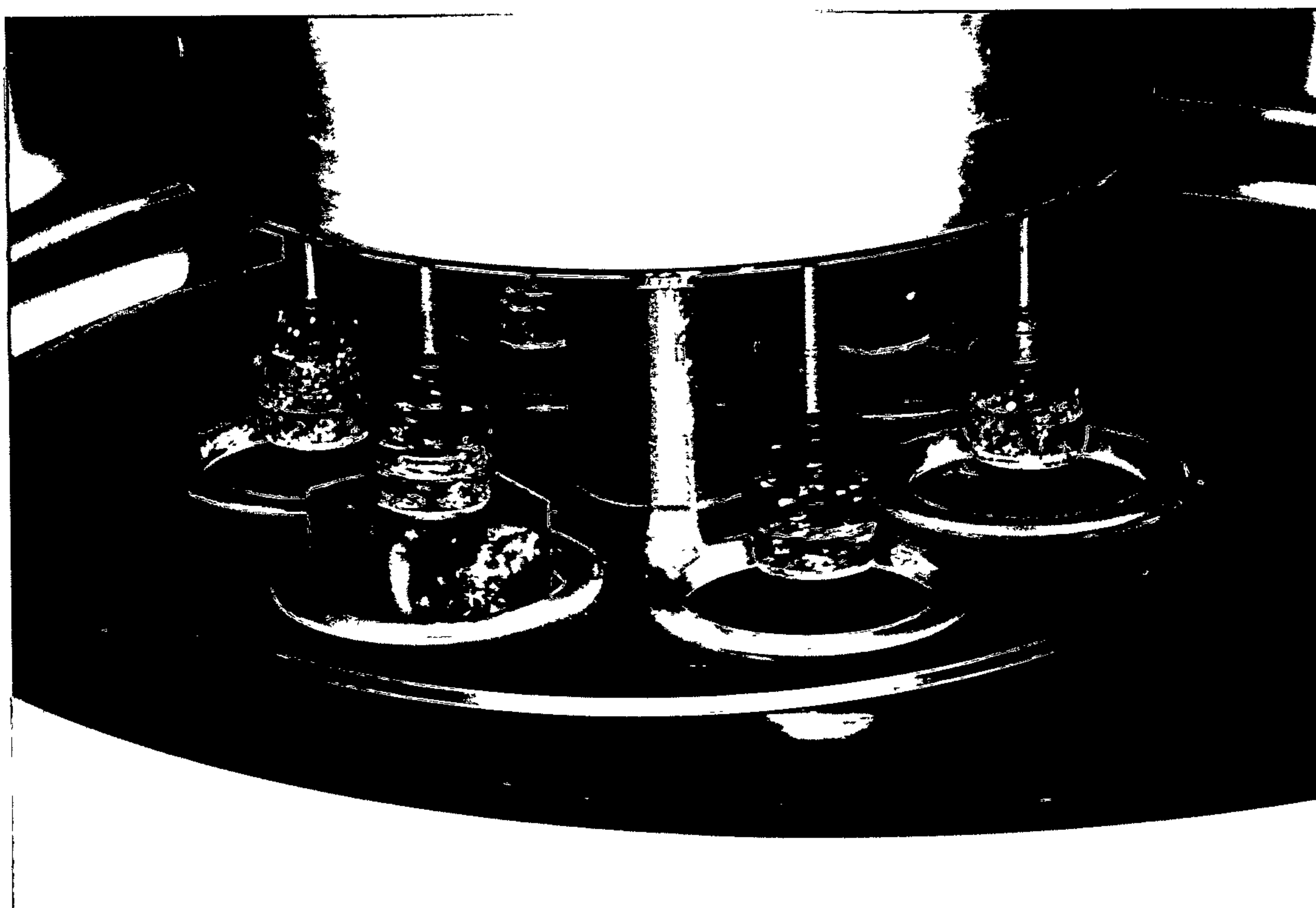


Figure 12: The fine smoothing and polishing process achieved using a rotational machine (*Struers*[®]).

4.3 The testing with the UMIS-2000 machine(CSIRO®) (see also appendix 2)

Before testing, the surface of each specimen was required to be parallel. To achieve this purpose, each specimen was attached to a metal base using wax and compressed by machine to provide the paralleling of the upper and the lower surfaces of the occlusal half of the specimen (Figure 13).

Ten indentations were made on the buccal side of each tooth section. All indentations were positioned approximately 30 µm from the enamel surface. This dimension is enough to allow the testing to occur within the resin-penetrated area and to avoid any crack which can occur from the pressure during indentating due to insufficient distance between the margin of the specimen and the indentating site. According to a pilot study, the width of the indentation was found to be approximately 7-10 µm. Therefore, distance between each indentation was set at 25 µm to avoid overlapping of each indentation.

The hardness of the enamel of the experimental and control surfaces was tested with a UMIS-2000 machine which was an ultra micro-indentation system (Figure 14). The hardness (H) measurements in GPa was made using a Berkovich indenter up to a maximum force of 200 mN. The contact force was 0.15 mN. Twenty load increments up to the maximum load for each indentation were performed.

The measurement of indentation hardness has long been recognized as a simple means of determining the depth distribution of hardness and elastic modulus of hard and soft materials (Frank, 1967).

The hardness in GPa, which were used in this study, is obtained by the manipulation of the data generated with the Berkovich indenter. Hardness values of enamel were measured under a maximum force of 200 mN.

4.3.1. Hardness measurement

A Berkovich indenter, which was defined as a diamond pyramid on an equilateral triangle base with face angles of 65.3° relative to the long axis, was used with load cycle analysis controlled by the UMIS operating computer software. After setting the initial contact on the surface, a maximum force of 200 mN was applied at each location site was achieved continuously until 20 increments were obtained and then unloaded in the same manner.

Hardness (H) was defined by a relationship between contact area (A) and indenter load (P) and is given by

$$H = P/A$$

where A was the area of contact which was related to the indenter penetration (h_p) into the material,

$$A = kh_p^2$$

so the hardness could be calculated by

$$H = P/kh_p^2$$

where h_p was the effective plastic depth of penetration and k was a geometric constant which for a Berkovich indenter was 24.5.

Hardness value was calculated at the maximum depth. Equivalent Vickers hardness value was also calculated from the average Berkovich hardness values.

4.3.2. Modulus of Elasticity

The elasticity of the indented material can be obtained from the unloading curve, provided the area of contact and the elastic modulus of the indenter are known. The slope of the unloading curve is given by

$$dP/dh = \alpha E^* \sqrt{A}$$

where the constant α is 1.167 for an indenter of triangular section and E^* is the effective modulus (the modulus of the indenter combined with the specimen).

The UMIS-2000 also provide the modulus of elasticity of the samples in $E/(1-\nu^2)$. To calculate the modulus of elasticity of the samples, this formula is given

$$E = x(1-\nu^2)$$

when x is the value which is provided by the UMIS machine and ν is the Poisson's ratio of the samples which is equal to 0.33 for the enamel and composite material.

The modulus of elasticity of each sample from each group was calculated and were then they were compared to each others.

The energy spent on loading (Wf) and irrecoverable energy (Wi) were also compared to confirm any alteration of the modulus of elasticity between both groups.

(Bell *et al.*, 1991/92).

4.4. The study by Scanning Electron Microscopy (SEM)

After UMIS testing, two samples of each groups were randomly selected and investigated at the indented areas. The teeth which were embedded in the resin were mounted on studs to present the prepared cross-sectioned surface for examination, coated with a thin layer of gold and palladium and examined in a scanning electron microscope (SEM) (*Philips, XL 30 and SEM 505*). Photographs were taken of each specimen at magnifications of x2000 and x5000, and compared.

Another pair of premolar teeth was used to confirm the etching pattern at the enamel surface of samples. Each of them was treated by 1% sodium hypochlorite for 24 hours and 37% orthophosphoric acid following the preparation for Group "a" and "b" samples. The teeth were coated with a thin gold layer. The buccal surfaces at the middle regions were studied by using SEM to evaluate the morphology of the etched enamel surface of each group. Photographs were taken at magnifications of x2000 and x5000, and compared.

4.5. Statistical Analysis

Student's paired *t*-test was performed to compare the difference in the hardness of the treated enamel between the control and the experimental groups. The following formulae were used:

$$t = \frac{\bar{D} - \mu_D}{\frac{S_D}{\sqrt{n}}}$$

where

$$\bar{D} = \frac{\sum D_i}{n}$$

$$S_D = \sqrt{\frac{\sum D_i^2 - n\bar{D}^2}{n-1}}$$

and

$\sum D_i^2$ = summation of the squares of each difference score

$n\bar{D}^2$ = samples size times the square of the sample mean difference

Significance levels (p values) were calculated. Results were declared significant if $p < 0.05$. The minitab statistical programme was used to perform all statistical calculation.

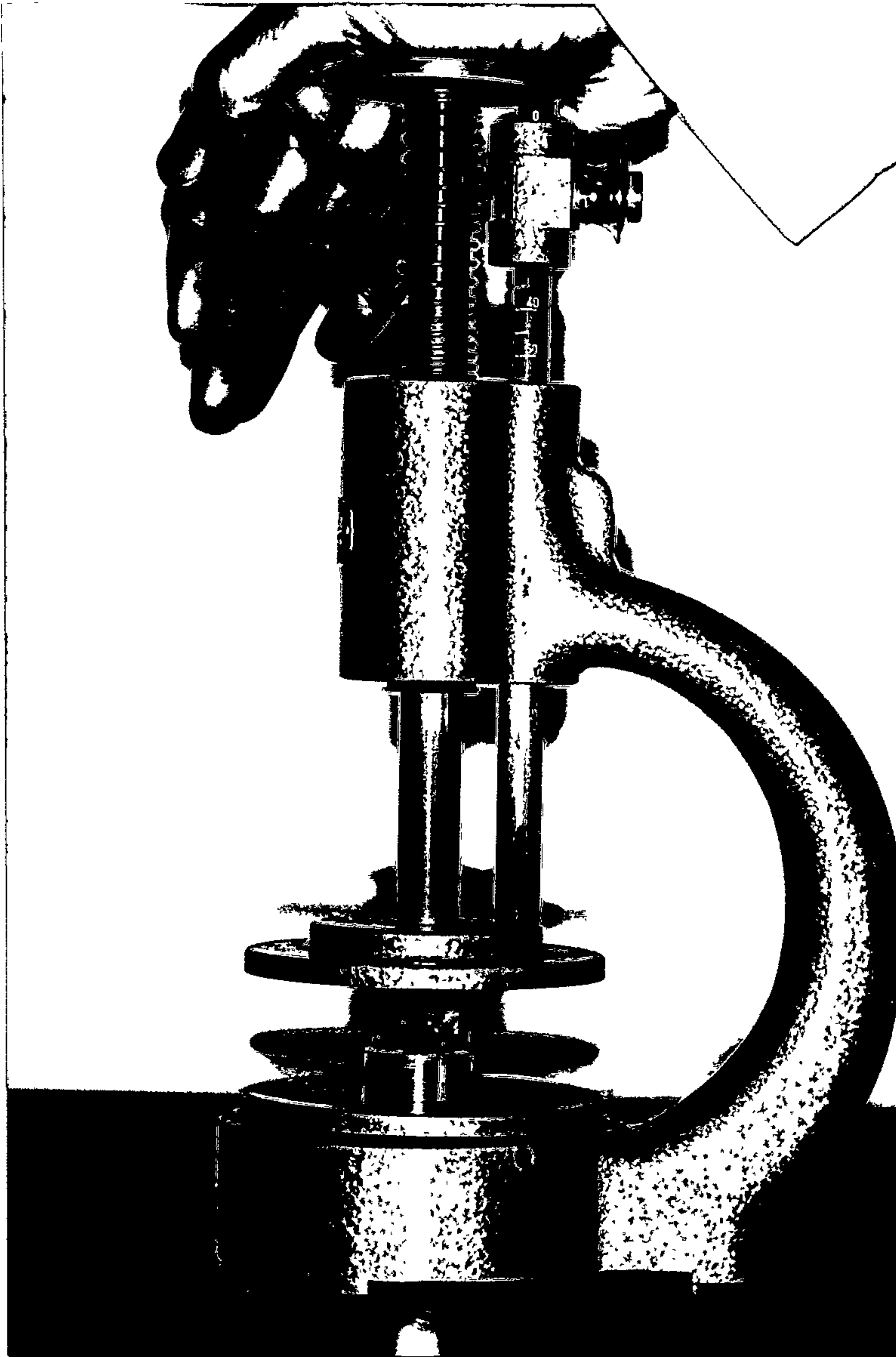


Figure 13: The specimen attached to a metal base using wax and pressed with a compressing machine to provide the paralleling of the upper and the lower surfaces before being tested with a UMIS-2000 (CSIRO®).

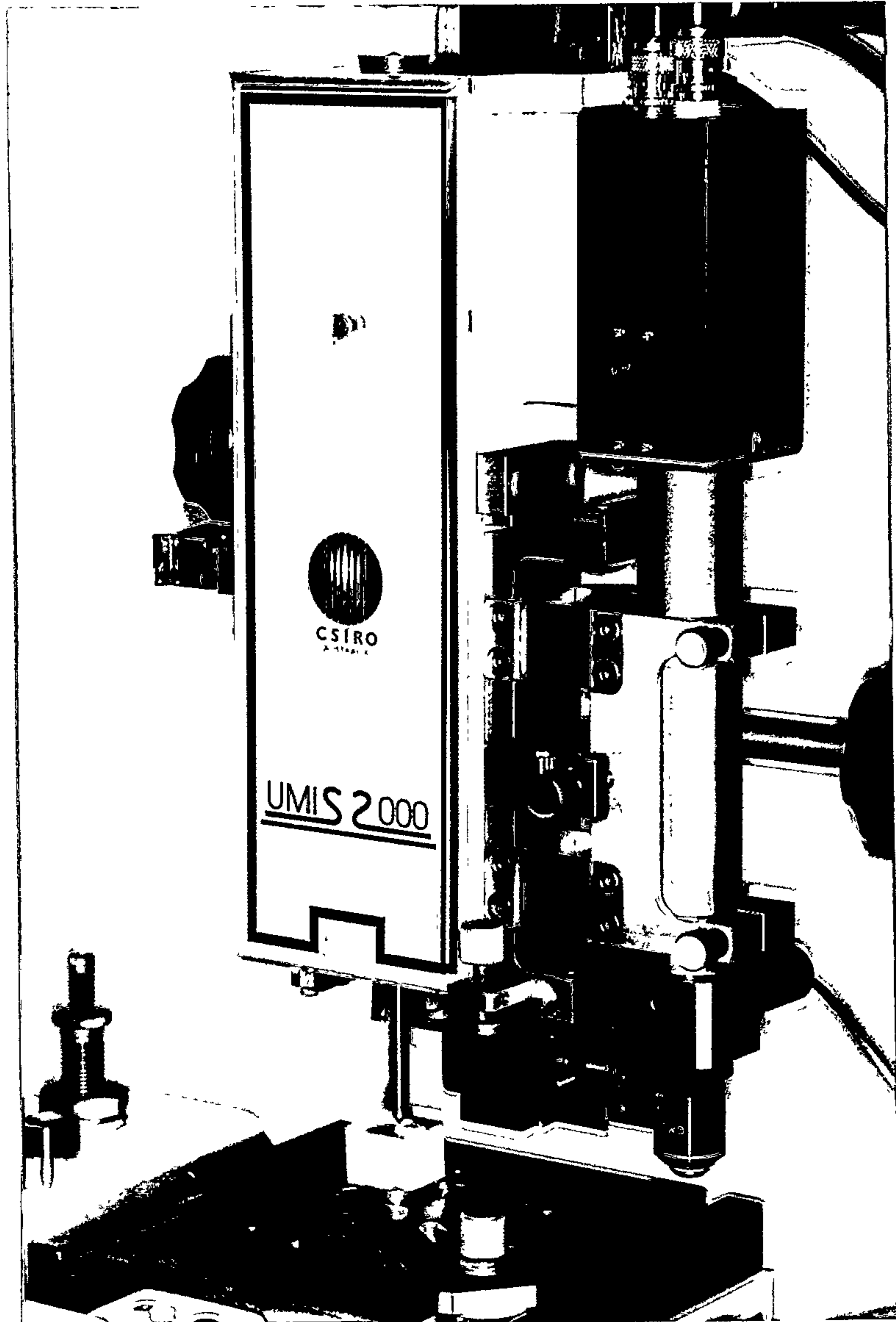


Figure 14: An ultra micro-indentation system UMIS-2000 (CSIRO®).

Chapter 5

Results

The data was displayed in the form of loading and unloading curve. Figure 15 illustrates the loading curve and unloading curve of the sample 1 in the present study. The calculation of hardness and the modulus of elasticity was described in appendix 2.

5.1. Hardness

The hardness values of ten-pairs of treated enamel, measured with UMIS-2000 machine, after (Group "a") and before (Group "b") the application of 1% sodium hypochlorite (1% NaOCl) are summarised in Table 1 and Figure 16. The number of indents were 8-10 with the number of increments were 20 for each indenter. The maximum force of 200 mN and the contact force of 0.1 mN were employed for indentating. The mean hardness of each pair of indenters in Group "a" and "b" were compared. From the data of each pair, the mean hardness of Group "a" was higher than that of Group "b", except the results from samples number 2, 3 and 7.

From the above data, the mean hardness values of the sample in Group "a" were significantly higher than those of Group "b" ($p = 0.000$) at $\alpha = 0.05$, statistically analyzed by Student's paired t -test at 95% level of confidence.

The equivalent Vickers hardness values of Group "a" and "b" were present and also compared to the mean Berkovich hardness in Table 2. These hardness values also showed a same trend to those of Berkovich hardness. The hardness values of sample a were higher than those of sample "b", except the results from sample number 2.

When the obtained equivalent Vickers hardness values were subjected to Student's paired t -test, the results showed that the hardness values of sample a were also significantly higher than those of sample "b" ($p = 0.022$) at 95% level of confidence.

Figure 15: The loading and unloading curve of each data.

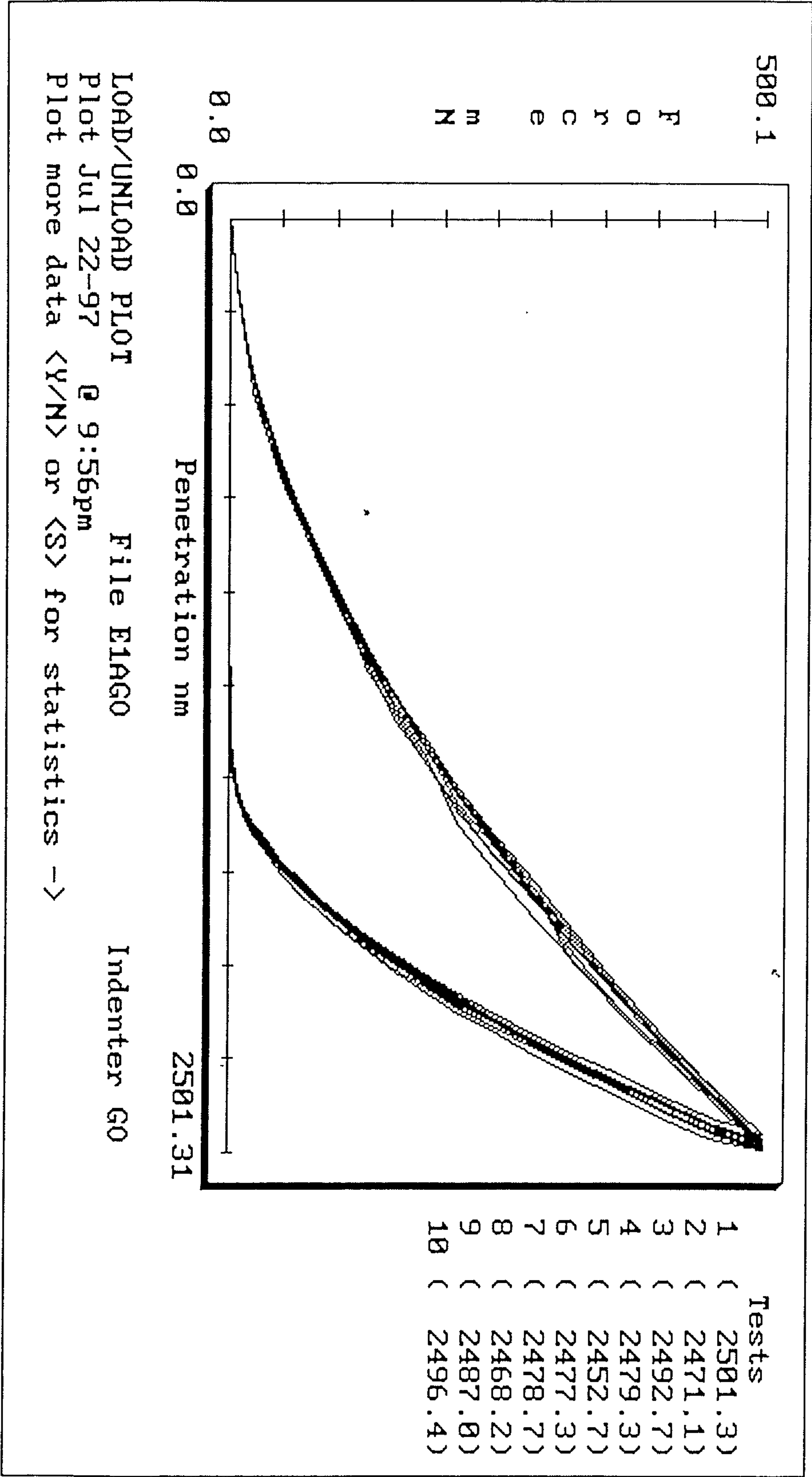


Table 1: Minimum, maximum and mean values of the hardness (GPa) of treated enamel from two groups: before (Group "a") and after (Group "b") being disinfected with 1% sodium hypochlorite.

Sample		Number of Indents	Hardness (GPa)			
			Min	Max	Mean	Standard deviation
1	a	9	5.1610	5.3587	5.2510	0.0650
	b	10	5.0039	5.4710	5.1878	0.1519
2	a	9	3.6750	4.4290	4.1351	0.2593
	b	10	4.2128	4.8914	4.6897	0.1840
3	a	9	2.0840	5.5380	4.8480	1.0470
	b	8	4.2960	5.4260	4.9060	0.3340
4	a	9	3.2280	4.7310	3.9950	0.4790
	b	10	3.0832	3.6074	3.3287	0.1763
5	a	9	4.8423	5.2509	5.0505	0.1348
	b	9	4.2600	5.2890	4.9040	0.3670
6	a	10	3.7480	5.0600	4.4460	0.4280
	b	9	2.9583	3.6577	3.2391	0.2009
7	a	10	3.2030	4.3920	3.9500	0.3550
	b	10	3.4590	4.7570	4.2010	0.3620
8	a	10	4.3782	5.1614	4.8223	0.2624
	b	9	2.6850	4.6690	4.0730	0.6780
9	a	9	3.3420	4.6790	4.0750	0.4010
	b	9	2.5960	3.9720	3.5260	0.4730
10	a	10	4.5696	5.0587	4.7792	0.1525
	b	10	2.7770	4.1180	3.6500	0.4240

Table 2: The comparison of Berkovich hardness values [mean (SD)] in GPa and equivalent Vickers hardness (kg/mm²) of treated enamel from two groups: before (Group “a”) and after (Group “b”) being disinfected with 1% sodium hypochlorite.

Sample	Mean Hardness (<i>Berkovich</i>) (GPa)		Equivalent Vickers hardness (kg/mm ²)	
	Group : a	Group : b	Group : a	Group : b
1	5.25 (0.065)	5.19 (0.1519)	479.1275	459.7914
2	4.14 (0.2593)	4.69 (0.184)	397.4851	430.0144
3	4.85 (1.047)	4.91 (0.334)	457.9508	438.5753
4	4.00 (0.479)	3.30 (0.1763)	308.5426	297.7499
5	5.05 (0.1348)	4.90 (0.367)	423.5973	334.3765
6	4.45 (0.428)	3.24 (0.2009)	328.3475	307.6831
7	3.95 (0.355)	4.20 (0.362)	377.4999	321.6366
8	4.82 (0.2624)	4.07 (0.678)	392.7918	334.6413
9	4.08 (0.401)	3.53 (0.473)	324.7934	319.7806
10	4.78 (0.1525)	3.65 (0.424)	407.0052	336.8409
mean	4.54	4.17	389.7141	358.109
S.D. of mean	0.45	0.68	56.6534	60.1259
difference	0.3958		31.6	
S.D. of diff	0.8055		36.2	
p value	0.0000		0.022	

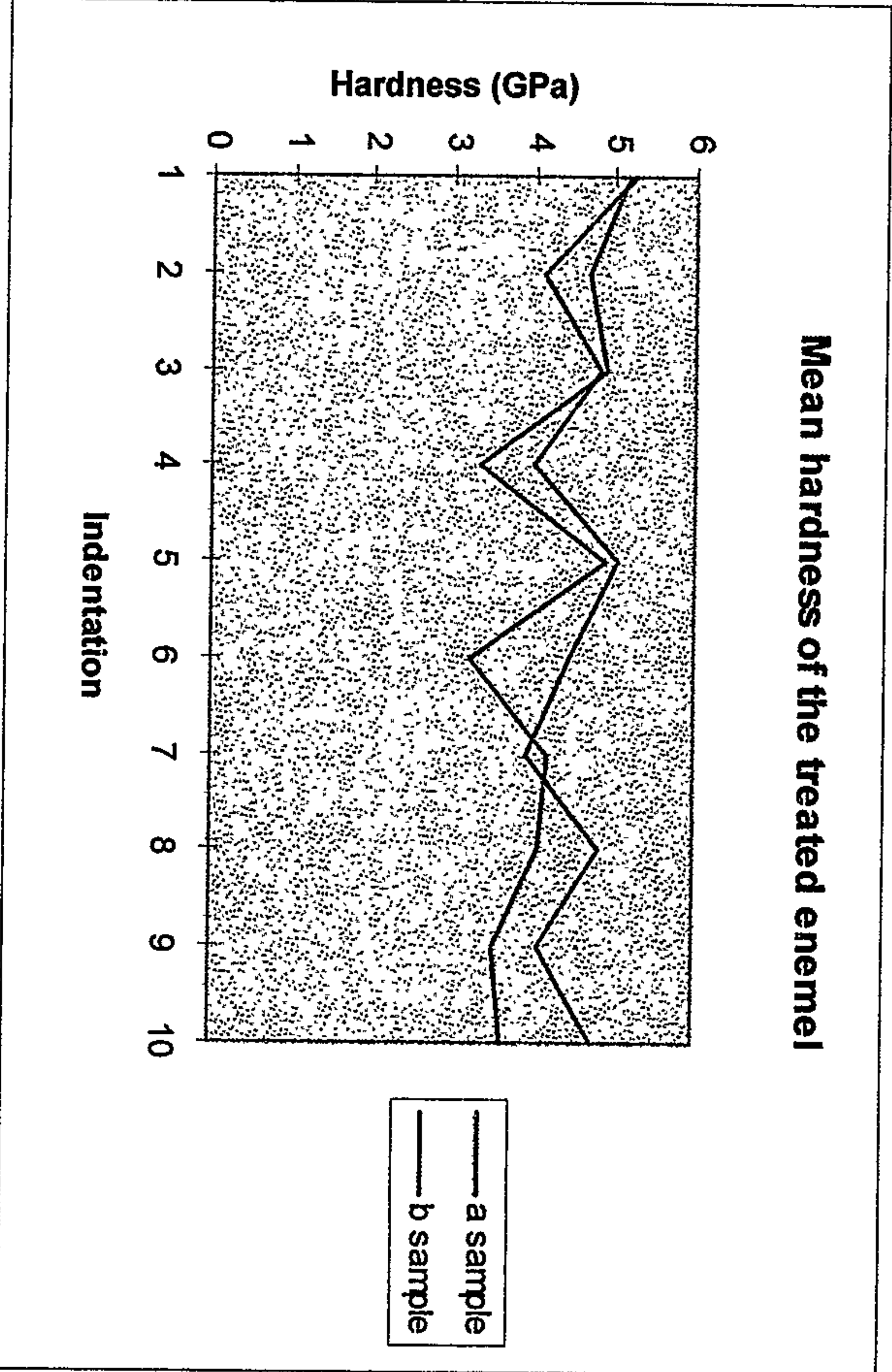


Figure 16: Mean Berkovich hardness values of the resin-bonded enamel of ten samples from each group : before (Group “a”) and after (Group “b”) being disinfected with 1% sodium hypochlorite.

5.2. Modulus of elasticity

The moduli of elasticity of the indenter and treated enamel (E^*) and those of the treated enamel themselves (E) are summarised in Table 3. For the composite materials (the indenter combined with the treated enamel), the moduli of elasticity were 74.64-96.6 GPa in range for sample "a" and 65.29-89.93 GPa for sample "b", respectively.

The moduli of elasticity (E^* and E) of the samples in Group "a" had a tendency to be higher than those of sample "b", except the values from the sample number 2 and 9. However, when all values were subjected to Student's paired t -test, there was no significant difference between the moduli of elasticity of Group "a" and of "b" (E^* : $p = 0.062$; E : $p = 0.067$) at 95 % level of confidence.

The energy spent on loading (Wt) and irrecoverable energy (Wi) of treated enamel are given in Table 4. The energy spent on loading (Wt) is defined as the area below the loading curve whereas the irrecoverable energy (Wi) is the area between the loading and unloading curve (Figure 17).

For the energy spent on loading (Wt), they were 95.42-123.29 nJ and 101.56-116.18 nJ for Group "a" and "b", respectively. The irrecoverable energy (Wi) of sample "a" and "b" were 57.21-80.11 nJ and 60.88-76.41 nJ, respectively. The data showed that the energy spent on loading values (Wt) of sample "a" were higher than those of sample "b", except the sample number 1, 5, 8 and 10. The irrecoverable energy (Wi) values of "Group "a"" were also higher than those of "Group "b"", however, the values from the sample number 1, 5, 8 and 10 showed a reverse tendency. Using the Student's paired t -test to analyze all data, the results showed that there was no significant difference neither in the energy spent on loading (Wt) nor in the irrecoverable energy (Wi) of treated enamel between both groups (Wt : $p = 0.97$; Wi : $p = 0.70$) at 95 % level of confidence.

Figure 17: The energy spent on loading (W_f) and the irrecoverable energy (W_i) defined as the different areas below the loading-unloading curve.

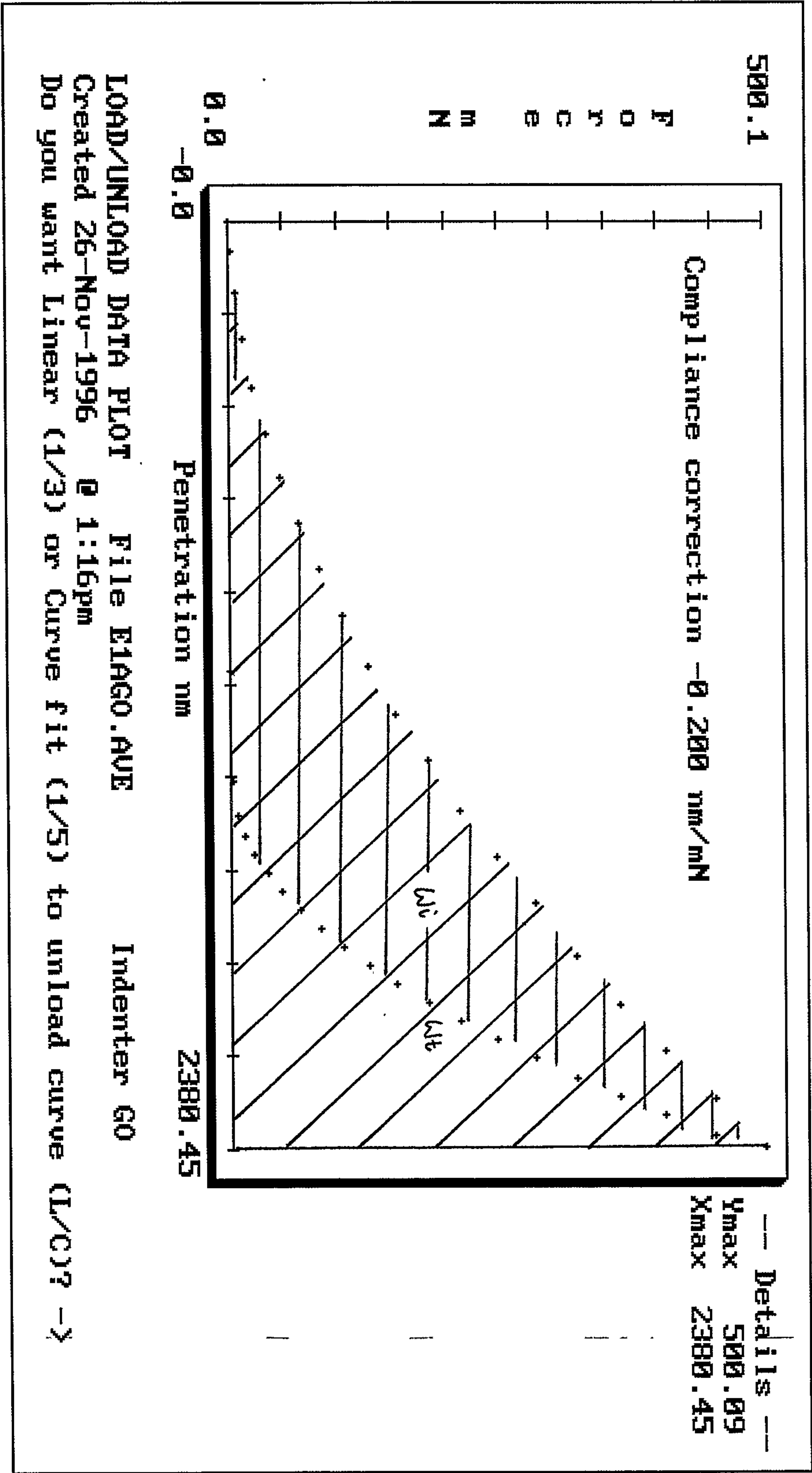


Table 3: The comparison of modulus of elasticity (E) of treated enamel from two groups: before (Group “a”) and after (Group “b”) being disinfected with 1% sodium hypochlorite.

	Modulus of elasticity of samples (E) (GPa)		Modulus of Elasticity of composite materials (indenter and samples :E*) (GPa)	
Sample	a	b	a	b
1	95.23506	86.02704	96.60017	88.07879
2	78.54703	80.79553	81.03796	83.16578
3	79.95965	78.4604	82.37592	80.95579
4	71.84693	68.365	74.63881	71.27814
5	84.14712	76.86298	86.31936	79.43785
6	78.23944	64.08032	80.74612	67.10921
7	82.66963	62.21756	84.93182	65.28514
8	93.50036	77.78534	95.00697	80.31493
9	72.66233	88.00815	75.42232	89.92564
10	94.09989	78.54337	95.55819	88.14204
mean	83.09074	76.11457	85.2638	79.3693
S.D. of mean	8.6120	8.6563	8.0760	8.7954
difference	6.98		5.89	
S.D. of diff.	10.61		9.68	
p value	0.067		0.062	

Table 4: The comparison of energy spent on loading (*W_f*) and irrecoverable energy (*W_i*) of treated enamel from two groups: before (Group “a”) and after (Group “b”) being disinfected with 1% sodium hypochlorite.

	Energy spent on loading (<i>W_f</i>) (nJ)		Irrecoverable energy (<i>W_i</i>) (nJ)	
Sample	a	b	a	b
1	95.4186	101.558	57.2066	60.8762
2	108.3658	106.6732	67.0425	65.2426
3	107.9905	106.2141	65.5451	64.0958
4	122.2833	113.8048	80.1028	72.147
5	106.2144	111.4734	65.4523	69.1449
6	116.5511	115.1573	74.4262	70.1629
7	115.275	113.8018	71.162	67.1478
8	111.1732	116.1818	71.0745	74.7436
9	123.2867	115.7383	79.9094	76.4063
10	109.3398	115.9526	69.3688	75.5984
mean	111.5898	111.6555	70.1290	69.5566
S.D. of mean	8.2216	5.0939	6.9565	5.2404
difference	-0.0657		0.5725	
S.D. of diff.	5.5088		4.6103	
<i>p</i> value	0.97074		0.70370	

5.3. Scanning Electron Microscopy (SEM)

The Scanning Electron Microscopy of indented areas is illustrated in Figure 19 (Group "a") and 20 (Group "b"). The SEM documentation of both groups showed clear prismatic patterns which indicated that the enamel surfaces were etched and the resin penetrated into the tag, involving the indented areas of both groups.

Ten indentations on the buccal side of the tooth section in Group "a" and Group "b" are given in Figure 18. Determining the areas of indentation, the fracture was detected around most of the indented areas in both groups. The SEM showed that the fracture had occurred within the prismatic area of the involved rods around the area of indentation.

The fracture patterns of both groups were also compared. In Figure 19a and 19b, the prisms broke along the border, spreading toward the centre of the prisms. The fracture was limited within the area of each involved prisms only. Some prisms cracked and were lifted up. In Figure 20a and 20b, the fracture spread out from the indentation area, and proceeded toward the border of the involved prisms without breaking their boundary.

When compared to the etching patterns from the study of Silverstone (1975), the etching pattern of sample "a" and "b" can be classified into Type II (Figure 5) and I, respectively (Figure 4).

In "Type I" pattern, the cores and "Type II" the peripheries; of the prisms are preferentially removed (Silverstone, 1975).

Determination of the microfeatures of the etched surfaces generated from both groups, Scanning Electron Microscopy was used. Figure 21a illustrated the enamel surface at the etched area of samples in Group "a" and Figure 21b illustrated Group "b".

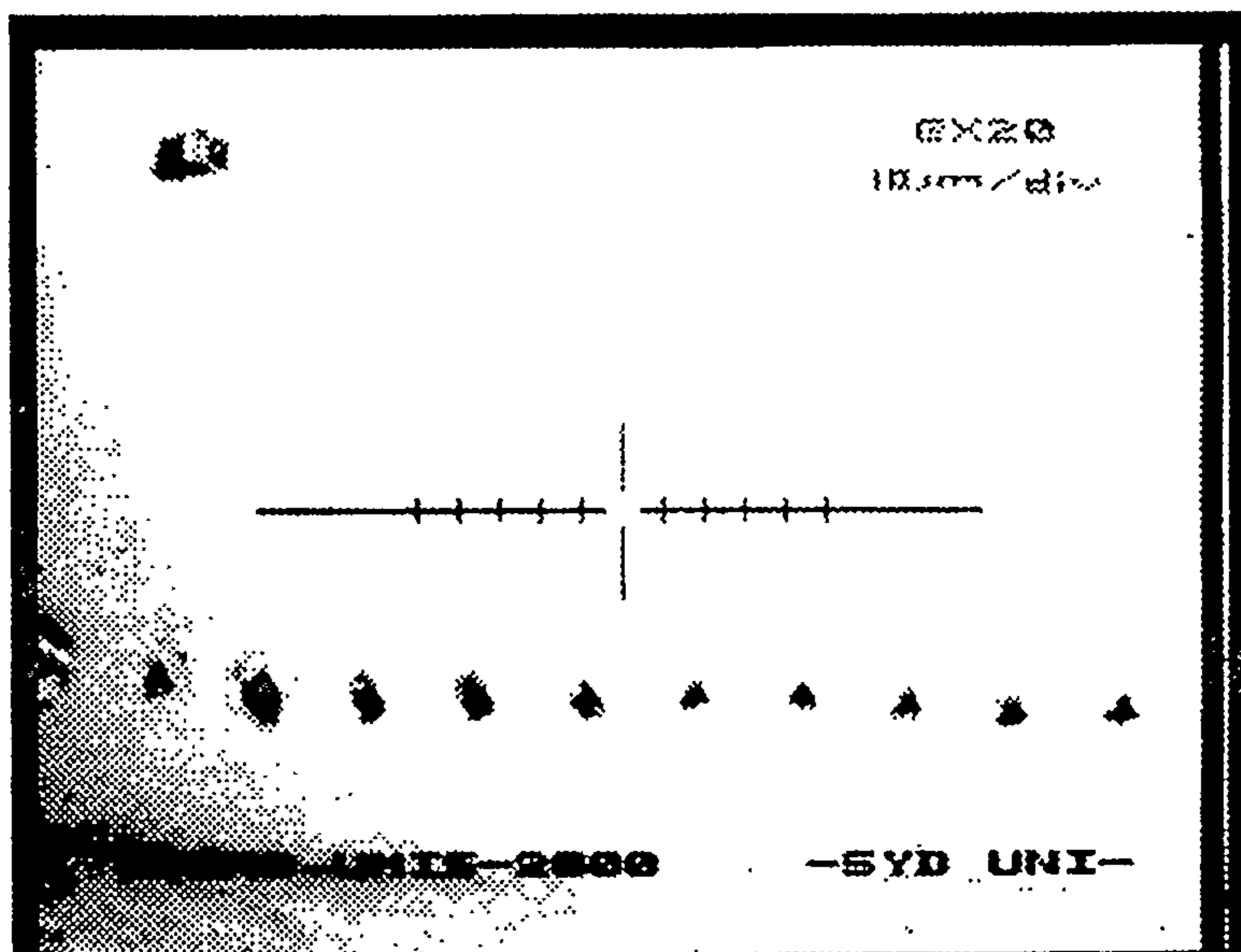
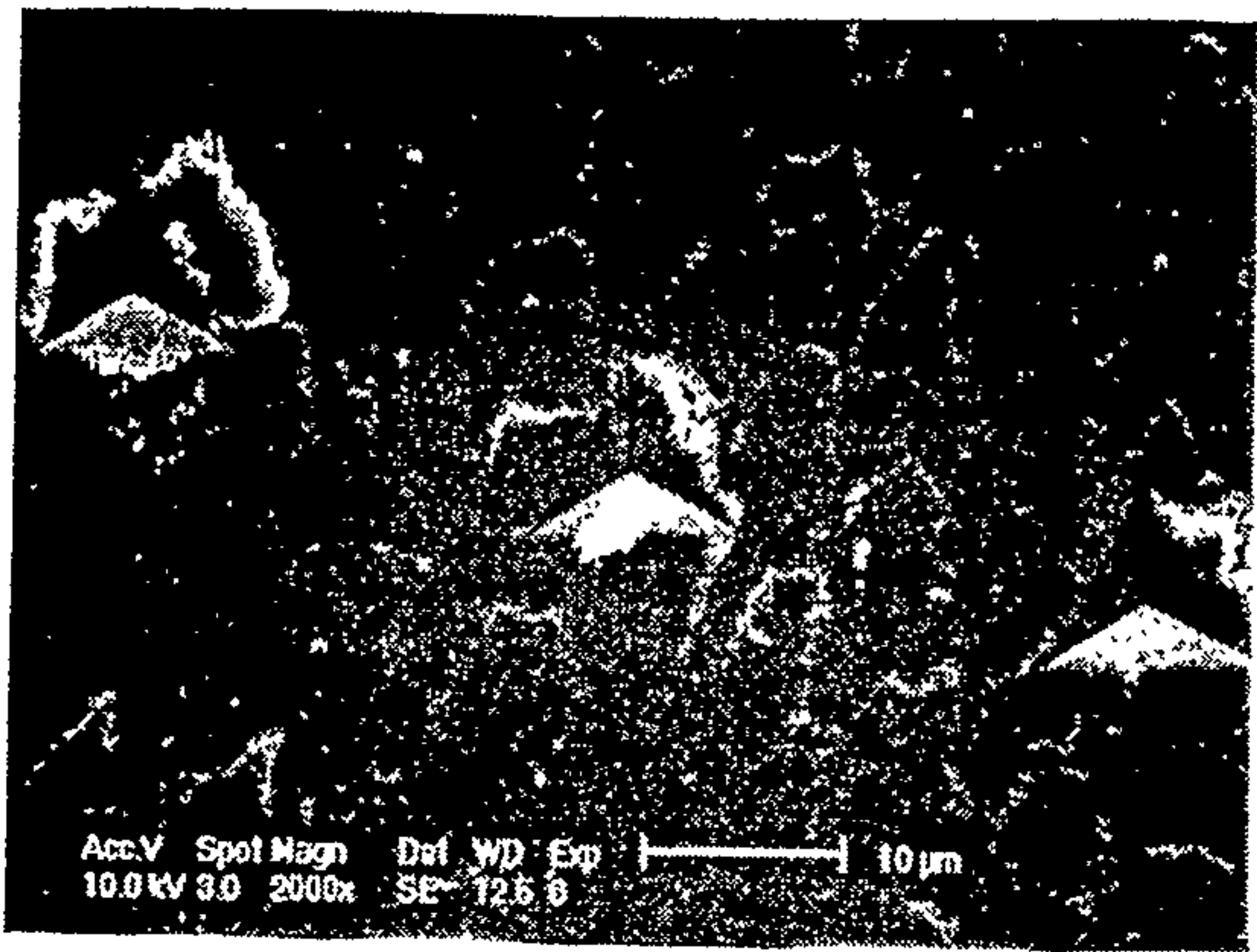
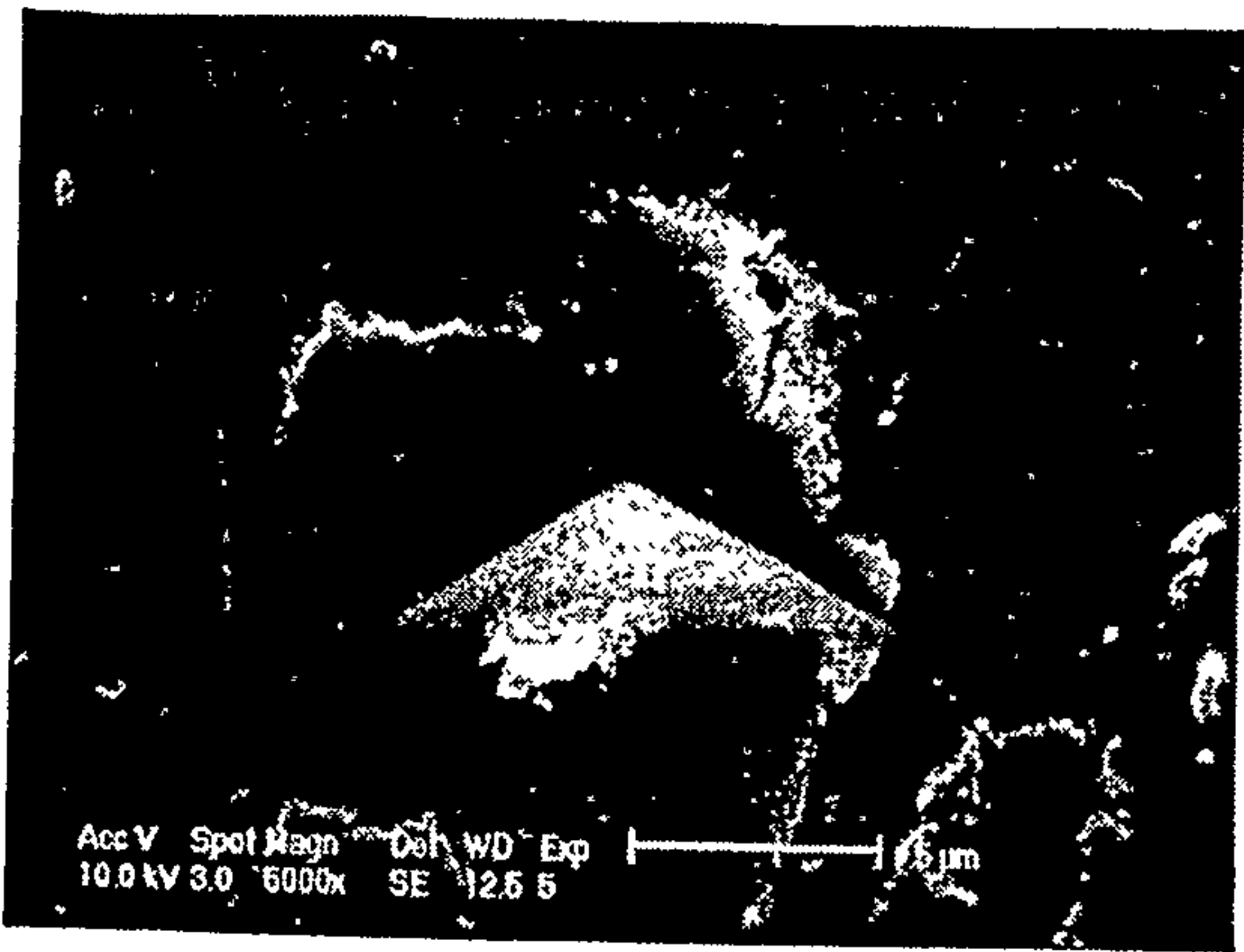


Figure 18: Ten indentations made on the buccal side of each tooth section, approximately 30 μm inside from the enamel surface and 25 μm between each indentation; Group "a" and Group "b" (x20).

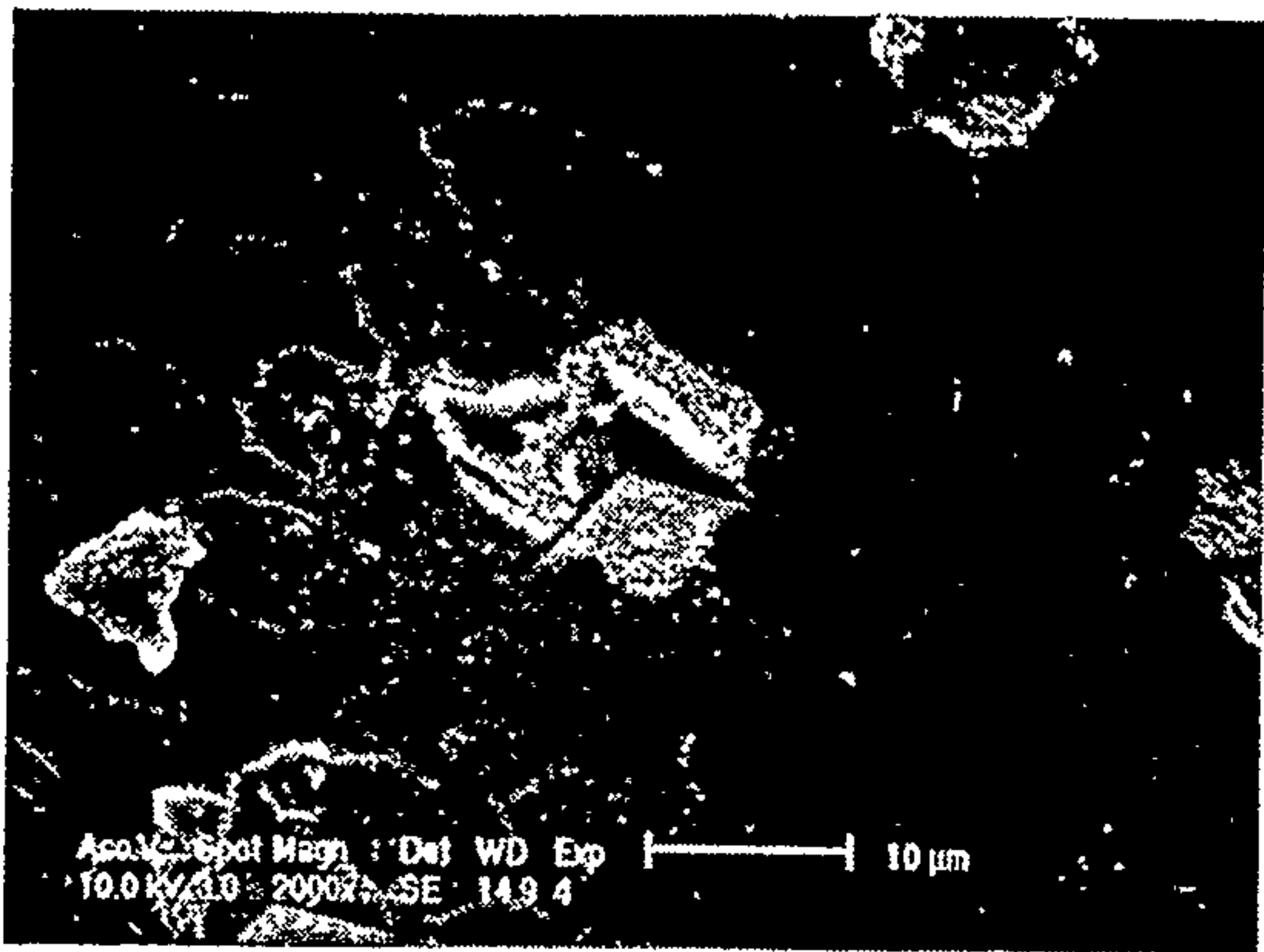


(a)

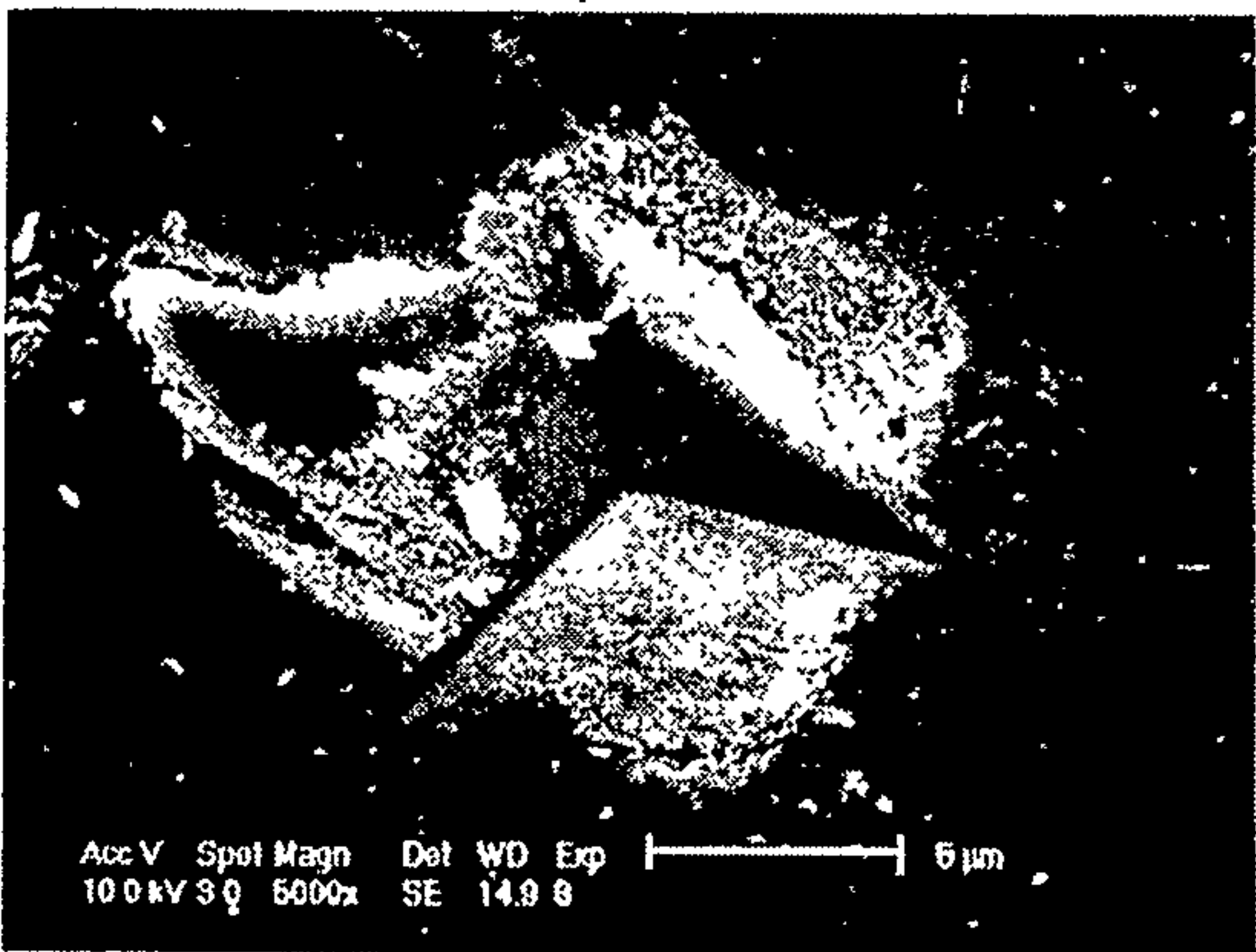


(b)

Figure 19: Scanning electron microscopy of indented area: from sample of Group "a".
(a x2000; b. x5000).

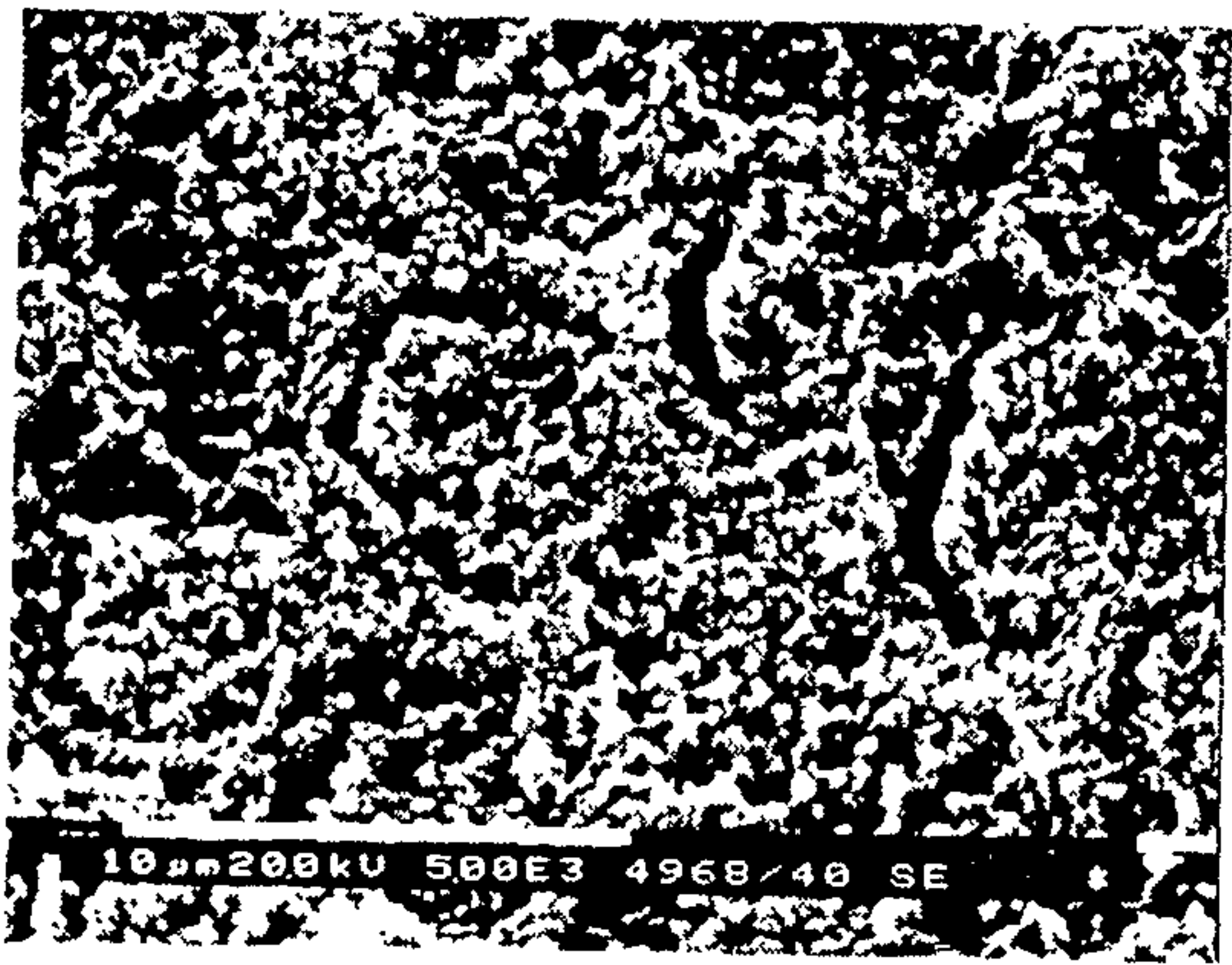


(a)

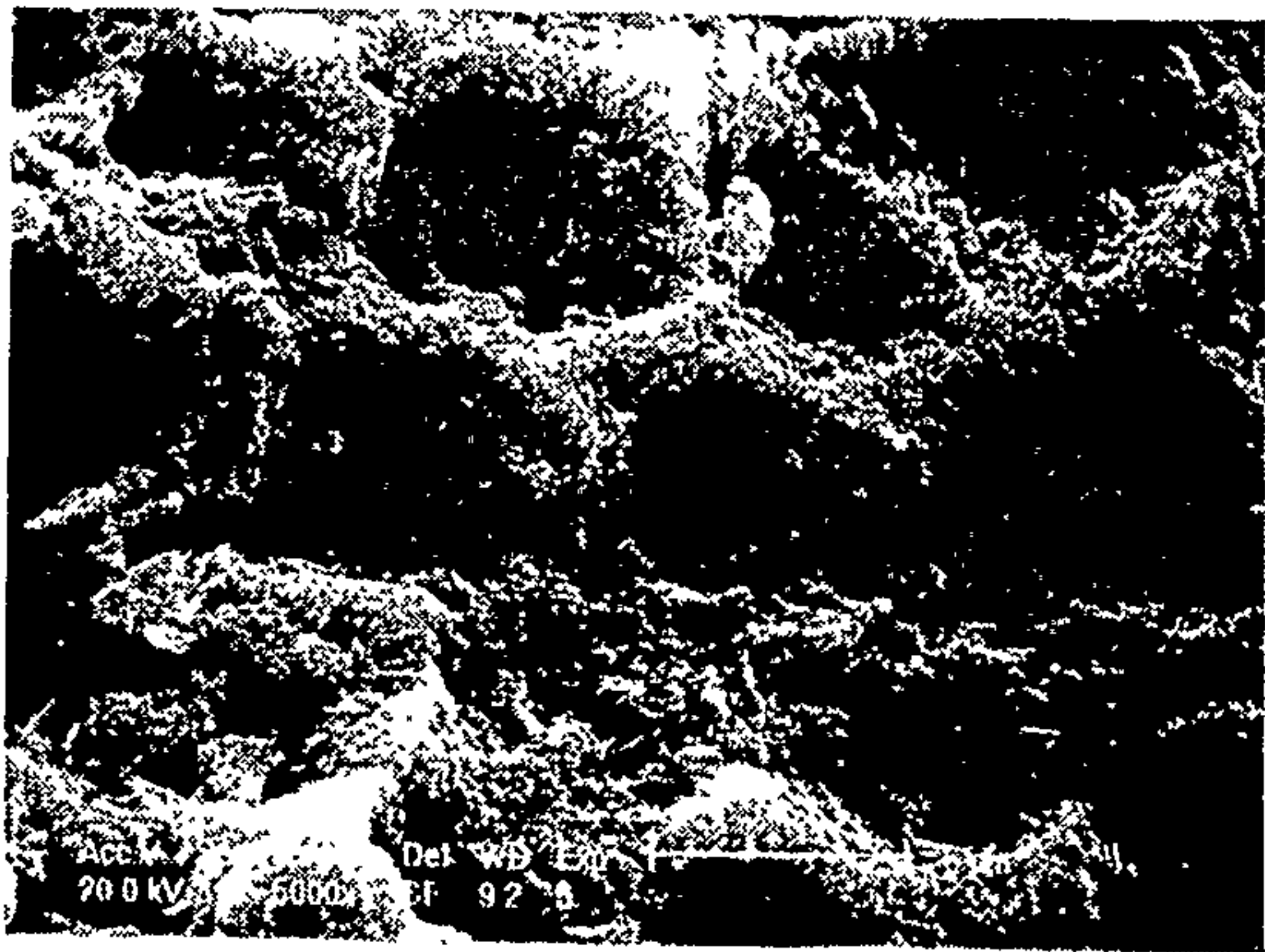


(b)

Figure 20: Scanning electron microscopy of indented area; from sample of Group “b” (a: x2000; b: x5000).



(a)



(b)

Figure 21: Scanning Electron Microscopy (SEM) showing the etched enamel surface of the samples in Groups “a” (a) and “b” (b).

Chapter 6

Discussion

Several studies have been undertaken to measure either the hardness of enamel or the bonding capabilities of the composite resin. However, with the increasing application of infection control procedure, extracted human teeth have to be disinfected before any laboratory testing. That is why the present study has been designed and the effects of the disinfection solution on the micro-hardness of the enamel has been evaluated. In this study, a commonly accepted disinfection method which was recommended and used in the United Dental Hospital; Sydney, Australia, was applied to treat extracted teeth. The results indicated that exposure to 1% sodium hypochlorite could significantly increase the hardness of acid etch treated enamel compared to the enamel exposed to 1% sodium hypochlorite after being treated with acid etchant.

6.1. Effect of 1% sodium hypochlorite on the hardness of the treated enamel.

The study indicated that hardness of the sound enamel treated with 1% sodium hypochlorite before the commencement of the etching and bonding procedure (Group "a") is higher than the hardness of the etched and resin-bonded enamel exposed to 1% sodium hypochlorite following the etching and bonding procedure (Group "b") ($p < 0.001$) (Table 1 and Figure 16).

In this study, the treated enamel of both groups are composite materials. Craig (1993) noted that the physical and mechanical properties of the composite materials present as the combination of two substances. Determining the obtained result, the mean hardness of the samples of enamel which were bonded with the resin after the application of 1% sodium hypochlorite (Group "a") is between 3.323-5.188 GPa (SD = ± 0.174 -0.678) (Table 1 and Figure 16) while the hardness of the samples in the other group (Group "b") is between 3.289 to 5.251 GPa (SD = ± 0.063 -1.047). The equivalent Vickers hardnesses are between 308.54 kg/mm² (3.02 GPa) to 457.95 kg/mm² (4.49 GPa) in Group "a" and between 297.75 kg/mm² (2.92 GPa) to 438.57 kg/mm² (4.30 GPa) in Group "b", respectively.

According to Craig 1993, the hardness of normal enamel was 343-430 kg/mm² in range. The hardness of the Bis-GMA, the unfilled resin which penetrates into the tag, is between 20 to 25 kg/mm². Compared to the findings of the present study and the results of the Craig's one, it might be possible to imply that these hardness values are lower than the hardness of the intact enamel since the samples in the present study are composed of the enamel and the unfilled resin. In addition, the results show that the amount of resin infiltration influenced the

hardness values obtained. The more the resin penetrates, the more the hardness value will be reduced. Therefore, this shows that Group "b" samples should contain more mass of resin than Group "a".

6.2. *The Cracking Pattern*

Indentation of brittle materials with a Berkovich indenter often produces cracks systems that emanate from the indentation corners (Dukino and Swain, 1992), as shown in Figure 22. In the present research, by studying the samples with Scanning electron microscopy (SEM), the cracking pattern of the etched enamel in each group was different from the illustration. Interestingly, the fracture of the treated enamel occurred only in the prismatic areas and was limited to within the enamel rods which were indented (Figure 19 and 20), not similar to the fracture types of brittle materials in the study of Dukino and Swain. One explanation may be that the human enamel is not a homogeneous material and being composed of many enamel rods or prisms (Gwinnett, 1992). The distribution of the loading force may be different from other homogeneous materials, such as glasses and ceramics, which are usually used to test the different pattern of fracture (John, 1983). Therefore, this may result in the different fracture appearance and occur at the weakest part of the materials, the resin regions, instead.

To compare the fracture patterns of different objects, the nature of fracturing may be explained by comparing the fracture patterns of different materials. Each material has a different fracture pattern. When an external stress is applied to a material, the material will be deformed, or strained. If the magnitude of the stress is increased, the material will eventually fail by fracturing. Not all materials fail in the same manner, and the type of fracture that occurs may be either brittle or ductile. In a ductile fracture, failure is preceded by a considerable amount of plastic deformation of the material but in a brittle or non-ductile fracture, there is little or no plastic deformation prior to failure. Figure 23a illustrates the types of fractures obtained when the materials are stressed to a failure point. Ductile materials begin to neck. The level of stress in the necked portion builds up to a high level and small cavities begin to form before the cup and cone type of fracture will be produced (John, 1983).

A failure of brittle material is also showed in Figure 23. The appearance of a brittle tensile failure may be of the shear type (Figure 23c) or the cleavage type (Figure 23d). A fracture of a brittle material, when stressed in compression, is normally by shear at 45° to the direct stress axis. The type of fracture obtained from a small cylindrical test-piece may be either of a double cone type or straight shear (Figure 23e) (John, 1983).

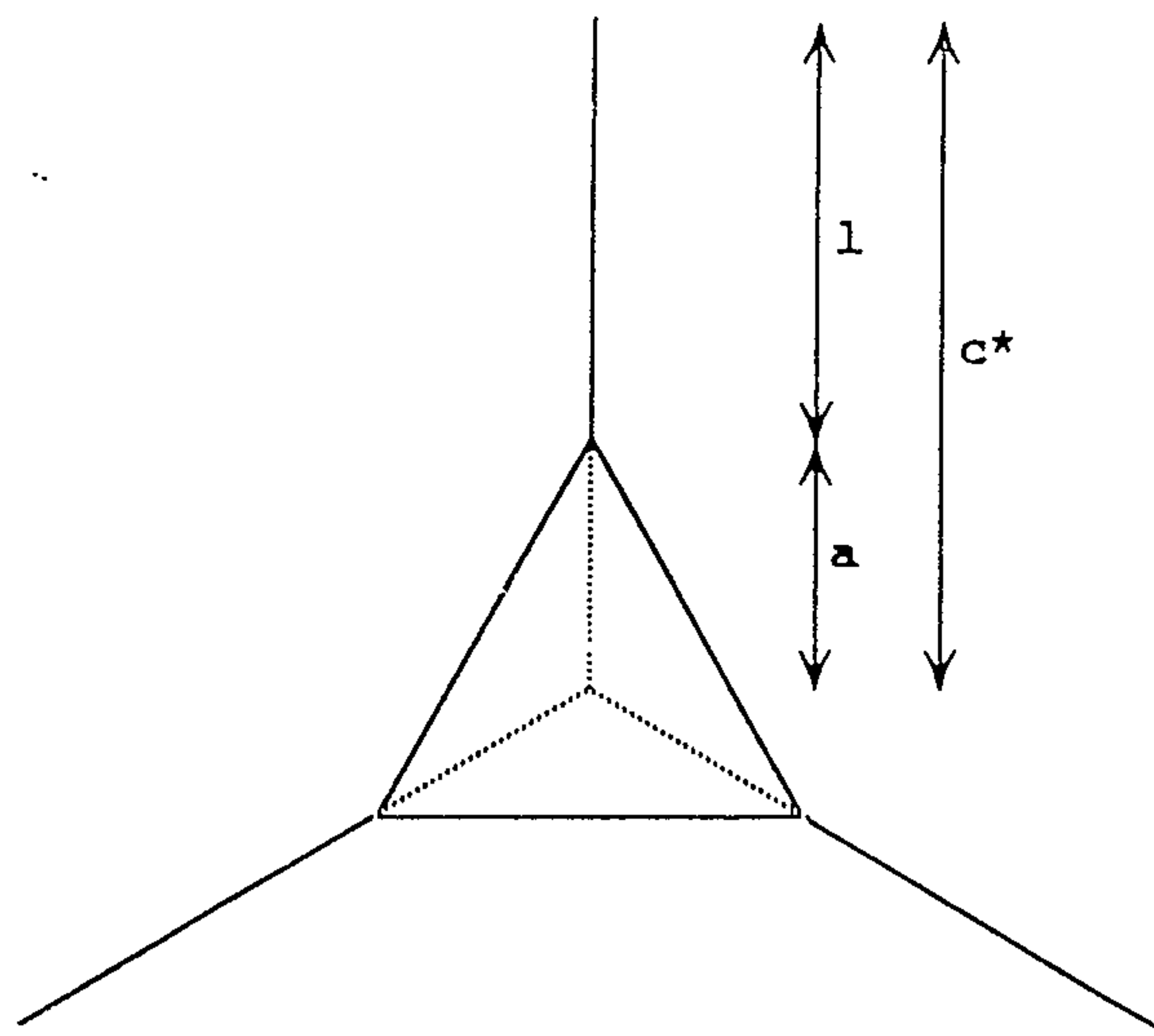


Figure 22: Illustration of indentation crack geometry of Berkovich impression (From: Dukino and Swain: The determination of surface plastic and elastic properties by Ultra Micro-indentation. Metrologia 28:463-9, 1992).

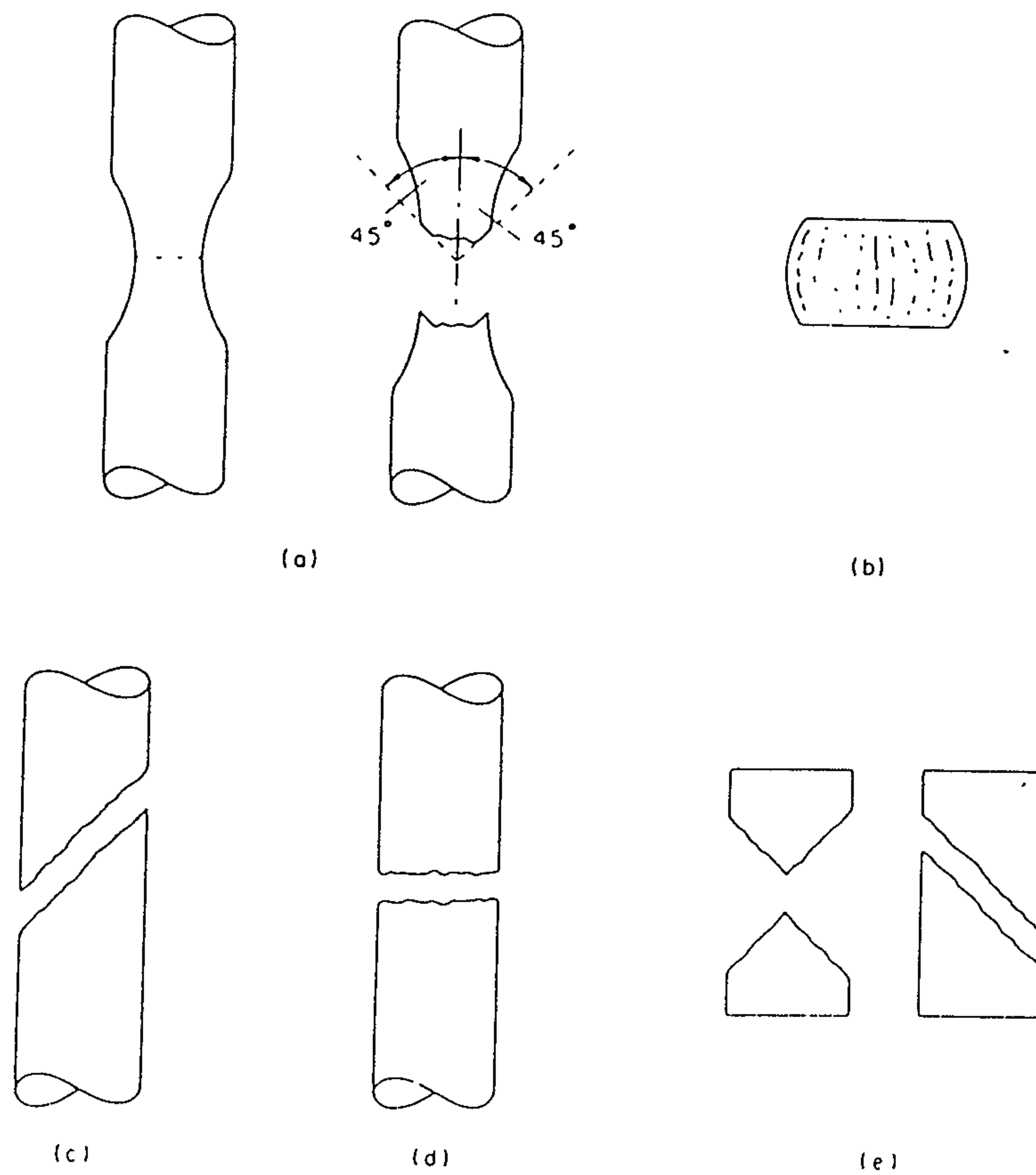


Figure 23: Fracture types. (a) Ductile tensile failure. Formation of cavities in necked area prior to fracture. (b) Ductile compressive failure. (c) Brittle tensile failure, shear type. (d) Brittle tensile failure, cleavage type. (e) Brittle compressive failure types (From. John V.B.: *Introduction to Engineering Materials*. 2nd ed, Macmillan Press, London, p p.273-5, 1983)

In the present study, the samples in Group "a" showed that the failure occurred along the border of the involved prisms. Dissimilarly, the fracture manner in the samples "b" began to form at the centre of prisms and traveled toward the border of the rods. As defined by Kelly and Macmillan (1986), a crack at the surface or in the interior of a material is a stress-concentrating flaw or weak point. The weak points of the prism should be at the border, in sample "a", and at the centre, in sample "b", respectively. This may be the reason why the fracture appearances of the samples are different.

6.3. Acid Etching Pattern

With respect to etching patterns obtained, Silverstone *et al.* (1975) described three patterns of etching patterns in the acid concentration range of 20-60 %. That is, a Type I pattern in which there appeared to be a preferential removal of prism cores (Figure 4), a Type II pattern in which the prism peripheries were removed preferentially (Figure 5), and a Type III pattern, which occurred less often (Figure 6), in which the etched surface bore no resemblance to the enamel morphology. In another study, Silverstone (1975) found that a Type I etching pattern was observed when the enamel surface had been etched for 60 seconds with 30 per cent phosphoric acid, while a Type II was shown when using a higher acid concentration, 40 and 50 %. In this study, 15-seconds was used for the acid exposure. From the study of Sadowsky *et al.* (1990), this duration of etching was recommended as a minimal duration. This period of time produced an insignificantly different effect on the retention of bonded orthodontic attachments compared with the 60 seconds in equal acid concentration (Sadowsky *et al.*, 1990). Uno and Finger (1995) also found that the application of 35% Orthophosphoric acid for 15 seconds was found to be sufficient. The etched enamel pattern was produced and provided an effective retentive pattern. When investigating the etching pattern by scanning electron microscopy (SEM), similar acid etching patterns were also detected which compared well with 60-seconds application of acid (Sadowsky *et al.*, 1990; Silverstone, 1975). Therefore, the 15-seconds application, compared to 60-seconds, should provide the identical acid-etching pattern.

From these previous studies (Silverstone *et al.*, 1975; Sadowsky *et al.*, 1990; Uno and Finger, 1995), if the enamel surfaces of both groups were changed by the acid etching, it might be possible to conclude that Type II etching pattern was matched for the samples in Group "a" whereas Type I pattern may happen in Group "b".

The SEM study confirmed that the results showed that the enamel prisms at the peripheries were preferentially removed in sample "a" (Figure 21a). Thus, Type II

etching pattern was observed. In sample "b", the majority of the cores of each prism were eliminated and Type I pattern was present (Figure 21b).

In 1975, Jörgensen studied the morphology of enamel surface etched with orthophosphoric acid using SEM and found that the acid pattern frequently varied significantly within the same tooth surface. However, the symmetrical areas on pairs of contralateral teeth showed almost identical etch patterns (Jörgensen, 1975).

In this study, contralateral teeth were used and tested in the same area, the middle of the buccal surface. Although, the etching pattern from both groups should be similar, SEM showed that the pattern of etched enamel of Group "a" and "b" were different even though the etching procedure for both groups was identical.

In the direct bonding procedure, the area of prism removal was the area where resin to penetrate (Gwinnett and Matsui, 1967; Gwinnett, 1971). This could infer that, with the resin infiltration, the region was weaker and can be easily cracked. According to Silverstone's study (1975), Type I was the common etching pattern when the enamel was etched with 37% orthophosphoric acid. This might explain the frequent finding of cracking pattern in sample "b". When the hardness value of the enamel and the resin was considered, the hardness of the resin was significantly low compared to the hardness of the enamel. Therefore, the area penetrated with the resin was weaker than any part of enamel and could be primarily cracked when loaded.

6.4. Effect of Sodium Hypochlorite and acid etching on the enamel.

In this study, the SEM investigation on the enamel surface showed the different etching and cracking patterns in samples "a" and "b". It might infer that the exposure to 1% sodium hypochlorite for 24 hours reduced the etching ability of the acid solution, or the enamel surface of both groups were affected by this disinfected solution.

Sodium hypochlorite is an effective deproteinizing agent (Piskin *et al.*, 1995). It was reported that 10-12% sodium hypochlorite can remove the organic material of the enamel surface within 2.5 hours (Holma *et al.* 1969, 1970). However, the removal of the organic material from the enamel surface is still possible even in different exposure time and concentrations.

The treatment with 10% sodium hypochlorite for 4 hours (Robinson *et al.*, 1990), or even 10 minutes was reported to be effective to remove organic debris from the enamel surface (Kodaka *et al.*, 1990; Kuroiwa *et al.*, 1994). Determining the lower

concentration, this ability is still reported. The enamel pretreatment with 5% sodium hypochlorite for 1 minute can enhance bonding success in hypocalcified amelogenesis imperfecta (Venezie *et al.*, 1994). They explained that the excess organic material at the enamel surface, which interfered with establishing a clinically successful acid etching pattern, was removed (Venezie *et al.*, 1994). The approach using 5.25% sodium hypochlorite for 20 minutes resulted in enhancing the calcium uptake due to the prior removal of organic substance (Besten and Giambro, 1995) and 6% could provide the best cleansing method for examining teeth (Galil, 1975). Galil also found that the deproteinization ability was maintained with a minimal timing; 30 minutes to 72 hours (Galil, 1975).

For the present study, it seems possible that the placement of teeth in 1% sodium hypochlorite for disinfecting purpose, for 24 hours, can remove the surface organic material and result in a ready intact enamel surface for etching procedures. Although the concentration was only 1%, the timing of exposure was quite long. Therefore, the ability to remove the organic substances should be still effective. This alteration before the commencement of the etching procedure may change the porosity resulting from the etching effect and also affect the resin penetration.

Silverstone (1975) noted that the acid solution cleaned and also effectively removed plaque, surface and sub-surface cuticle of enamel surface. Furthermore, it also produced a filamentous "tag-like" extension that resin could penetrate and bonded with the enamel (Gwinnett and Matsui, 1967; Gwinnett, 1971).

In this study, the teeth were exposed to the 1% sodium hypochlorite for 24 hours prior to etching procedure in the Group "a" samples. It is possible that the surface protein, including plaque and cuticles, will be eliminated and this may have affected the result obtained from the etching process. Therefore, the direct contact of the acid to the enamel surface without the protection of the protein cuticle may produce the similar effect as the etching with the stronger acid concentration and reduce the etching ability in result.

The sodium hypochlorite seems to initially etch the enamel surface and when the acid solution is carried out, the de-proteinized, intact surface enamel is etched. It is known that acid conditioning creates and enhances existing enamel porosity, and the depth to which changes in porosity and gross tissue loss occur depends on the type of acid used and its concentration (Gwinnett, 1971). For the phosphoric acid, Silverstone (1974) reported that tag length depended on the concentration of the acid. However, there is a critical concentration, approximately 30 per cent, may occlude the important enamel porosities (Chow and Brown, 1973). Phosphoric acid concentrations of 15 per cent and less produce changes similar to those of 70 per cent and greater (Silverstone, 1974). With the optimal concentration, Gwinnett and Buonocore (1965) indicated that long "tags" of resin permeate into enamel and frequently exceed 50 μm in length.

37% phosphoric acid is acceptably critical acid concentration for etching procedure (Sadowsky *et al.*, 1990). In this study, the enamel of the Group "a" samples may be double-etched by the effect of 1% sodium hypochlorite and 37% phosphoric acid. The resulting acid concentration which influences to the enamel surface may be stronger than common 37% phosphoric acid, which has to remove the surface protein before reaching the intact enamel. Therefore, the created tags are less than those only etched with 37% phosphoric acid in both amount of tag and tag length. In the result, created tag may not involve in the region at 30 μm from the enamel surface which was the area of study. Thus, the properties of treated enamel of sample "a" might be very similar to the normal enamel. This may explain why the hardness of Group "a" was more than the hardness of Group "b".

Naturally, the tooth surfaces are covered with the acquired pellicle, the organic structure which is formed by a selective adsorption of salivary proteins on the tooth surface. The study by Moreno (1975) indicated that the exposure of cleaned tooth surfaces to acid buffers induced a subsurface demineralisation whereas the surfaces with salivary pellicles on them remained intact. It might be showed the protective action of the pellicle influenced the etching capability. By application with 37% orthophosphoric acid, the surface pellicle might inhibit some etching ability before the acid can contact the enamel surface and produce the tag. Since sodium hypochlorite is the excellent protein denaturant, the prior exposure of the tooth to 1% sodium hypochlorite for 24 hours may remove this natural surface cuticle before the acid solution is applied.

6.5. The modulus of elasticity

The present study showed that there was no significant difference in the modulus of elasticity of both groups at 95% level of confidence. This result showed that the exposure of the teeth to the 1% sodium hypochlorite prior to the etching process did not make any alternation on the modulus of elasticity. To confirm the accuracy of the obtained finding, the energy spent on loading (Wf) and irrecoverable energy (Wi) of treated enamel were also compared. The results showed that there was no significant difference neither for the energy spent on loading nor the irrecoverable energy of both groups. These confirmed the previous results that there was no alternation in the modulus of elasticity when the teeth were exposed to the disinfection solution before using in the laboratory testing.

It is difficult to explain why the hardness values of the teeth were changed when they were subjected to the 1% sodium hypochlorite for 24 hours when this solution provided no effect on the modulus of elasticity. One explanation might be related to the environmental factors which may transform the properties of the teeth.

According to Craig (1993), the modulus of elasticity is related to the interatomic attraction forces within the material and usually is the same when the material is subjected to either tension or compression. In addition, the property is independent of any heat or mechanical treatments. However, he explained that the composition of the material is directly responsible for any changes in the value. Regarding the modulus of elasticity of Bis-GMA and enamel, Craig also reported that the values are of 2.1 GPa for Bis-GMA and 84.1 GPa for enamel (Craig, 1993). As the treated enamel of both groups was composed of Bis-GMA and enamel prisms, their modulus of elasticity should be equal. Although the etching pattern of Group "a" was Type II which only the peripheries of prisms were eliminated and the amount of resin infiltration should be less than that of Type I in Group "b", any alternation due to the resin mass might be very slight and make no difference in the modulus of elasticity.

Most results showed that the modulus of elasticity in Group "a" was higher than that in Group "b", except for samples 2 and 9. When confirmed the results with the energy spent on loading and irrecoverable energy of treated enamel, they revealed that the values obtained in Group "a" and "b" were almost the same. They also showed the Group "a" of sample 1, 5, 8 and 10 had less modulus of elasticity. These values exhibited considerable scatter and the trend of the results was difficult to locate.

Poolthong *et al.* (1997) studied the alterations of hardness and modulus of elasticity of a tooth in water and dry conditions. They found that dry conditions could significantly change the enamel modulus of elasticity whereas the enamel hardness could be maintained. In the present study, all samples were kept dry in the same container before testing. This dry environment might affect the values, thus, the trend of the results was indistinct. However, the hardness of the samples were unaffected by the dry environment.

Chapter 7

Conclusion

The results of this study showed that disinfecting the teeth with 1% sodium hypochlorite for 24 hours prior to the etching procedure can significantly influence the hardness values of the teeth in both groups; exposed to this disinfection before or after etching process. Teeth with a prior exposure to disinfection solution to the etching operation exhibited a higher hardness figures. These results confirm the hypothesis that "the hardness of the treated non-disinfected enamel surfaces, in the *in vivo* group should be different from those of the *in vitro* laboratory study where the samples are disinfected before bonding the brackets".

However, there was no significant difference in the modulus of elasticity which could be explained by the influence of the environmental factor, the dry condition.

Since the treated enamel in the present study were composed of enamel and unfilled resin, the hardness values were less than that of the intact enamel. Thus, the infiltrated resin of the treated enamel was considered. The amount of resin composition influenced the hardness and the increase in volume resulted in reduction of the hardness measures.

In determination of the indent area by using the scanning electron microscopy, it was showed that cracking pattern in both Group "a" and "b" were different from each other and from other brittle materials. The fracture of the treated enamel occurred only in the prismatic areas and limited within the enamel rods which were indented. On the *in vitro* teeth with a prior exposure of 1% sodium hypochlorite, the failure occurred along the border of the involved prisms. Dissimilarly, the fracture manner in the other Group "b" began to form at the centre of prisms and travelled toward the border of the rods.

When determining the etching pattern occurring in each group, the results showed that the enamel prisms at the peripheries were preferentially removed in the *in vitro* group whereas the cores of each prisms were mainly eliminated in the other group.

To explain the effect of 1% sodium hypochlorite on the alternation of the enamel surface prior to the etching process, the acquired pellicle and the deproteinizing ability of sodium hypochlorite were considered. As the surface cuticle has a protective action to the etching capability, the prior removal of this substances by the 1% sodium hypochlorite may provide the direct contact of the intact enamel to the etching solution, and lead to a different final result from that obtained with a 37% orthophosphoric acid solution.

The present study showed that the prior disinfection procedure can change one of the mechanical property, the indentation hardness, of the enamel.

Future laboratory testing of bonding hardness will need to account for any physical changes resulting obligatory infection control procedures. In addition, the accuracy of the *in vitro* findings on the representation of the *in vivo* physical status may need to be evaluated.

Chapter 8

Future Research

In the present study, it was found that disinfecting the human teeth with 1% sodium hypochlorite for 24 hours can significantly influenced enamel properties. To understand clearly on the effects of 1% sodium hypochlorite, the different exposure times should be employed and compared (for example, 6 hours, 12 hours and 24 hours) to investigate the alteration on enamel surface due to timing. In addition, the different concentration of sodium hypochlorite might also be applied with a specific time to determine the effects of concentration on the enamel properties.

In the future, the laboratory testing of human teeth will necessitate prior infection control procedures before the teeth can be used in the laboratory. These infection control procedures, apart from disinfecting with the 1% sodium hypochlorite for 24 hours, may also alter the physical properties of the teeth, at the enamel surface, cemental surface or deeper in the dentine. Therefore, it might be interesting to consider the mechanical properties, such as hardness and modulus of elasticity, of the intact enamel, before compared to after exposure to the sterilisation/disinfection procedures. In addition, the properties of dentine and cementum should not be abandoned. The testing of any alternation on their mechanical properties due to the effect of the sterilisation/and disinfection procedures should also be investigated. The examination on these alternations can be employed at the particular regions or it can be considered the changes from the outermost areas toward the inner parts of the tooth.

In the studies on the bond strength, Rock (1978) has shown that the degree of sealant penetration may influence bond strength. This might imply that the enhanced retention value is related to a tag length. It is equally important that the volume of resin in enamel plays a role, likely a major one, in retention. The bulk is confined to the outer 10 to 20 μm . Recently, Soetopo *et al.* (1978) obtained high bond strength in laboratory tests in the absence of identifiable tags. This does not necessarily preclude any penetration. They conclude that resin penetration may not be the only factor governing bond strength. However, they emphasised in saying that resin penetration is needed for a durable bond to be achieved under clinical conditions.

From the result of this research, the prior exposure to 1% sodium hypochlorite as the disinfection procedure can reduce the tag created by etching procedure. This might imply that the testing of bond strength on the disinfected teeth should be also affected. Thus, the future study on the effect of 1% sodium hypochlorite or other sterilisation/and disinfection procedures should be considered and further discussed.

Appendix 1

Hardness and modulus of elasticity

Hardness

Hardness is one of the most investigated properties and it is also one of the most complex to understand. Hardness may be broadly defined as the resistance to permanent surface indentation or penetration (Craig, 1993). The former hardness of a material can be measured by its resistance to scratching or to indentation. The latter type of test may be used only for materials that are capable of plastic deformation, namely metals and thermoplastic materials. The resistance of a material to indentation is not necessarily the same as its resistance to abrasion, but a hardness measurement obtained from an indentation test can be used as an empirical check for abrasion resistance. In general, metals possessing high hardness will have a high resistance to abrasive wear. Indentation-type hardness tests are widely used for the checking of metal samples as they are easy to make and yield information on heat treatment condition. There are also empirical relationships between the hardness of a metal and its tensile strength. The Hardness is therefore measured as a force per unit area of indentation.

The hardness of a ceramic material may be determined using a scratch test. Moh's scale of hardness, which was devised for assessing the relative hardness of minerals and rock, is based on ten naturally occurring minerals ranging from very soft talc to diamond (Table 5). In this test an attempt is made to scratch the surfaces of the standards with the material under test. The hardness of the unknown lies between the number of the mineral it just fails to scratch and that of the mineral it just scratches.

Table 5: Moh's scale of hardness

Number	Mineral
1	talc
2	gypsum
3	calcite
4	fluorite
5	apatite
6	orthoclase feldspar
7	quartz
8	topaz
9	corundum
10	diamond

(From: John V.B.: Introduction to Engineering Materials. 2nd ed, Macmillan Press, London, pp 331, 1983.)

Of the various type of indentation test available for the hardness testing of metals the most commonly used are Brinell hardness test, Knoop hardness test, Vickers hardness test, Rockwell hardness test and Shore A hardness.

• **Brinell hardness test.** The Brinell hardness test is among the oldest methods used to test metals and alloys used in dentistry. The methods depends on the resistance to the penetration of a small steel or tungsten carbide ball, typically 1.6 mm (1/16 inch) in diameter, when subjected to a weight of 123 N (27.7 lb). The resulting hardness values, known as the Brinell hardness number (BHN), is computed as a ratio of the load applied to the area of the indentation produced. the formula for computing the BHN is as follows:

$$\text{BHN} = \frac{L}{\frac{\pi D}{2} (D - \sqrt{D^2 - d^2})}$$

In the formula L is the load in kilograms, D is the diameter of the ball in millimeters, and d is the diameter of the indentation in millimeters, thus the units for BHN are kg/mm^2 . The smaller the area of indentation, the harder the material and the larger the BHN value. In testing the Brinell hardness of a material, the penetrator remains in contact with the sample tested for a fixed time of 30 seconds, after which it is removed and the indentation diameter is carefully measured. Tables have been developed from this formula for indentations of different diameters. From the table of values, the BHN is determined. The Brinell hardness test yields a relatively large indentation area, and as a result, this test is good for determining average hardness values and poor for determining very localised values (Craig, 1993).

• **Knoop hardness test.** The Knoop hardness test was developed to fulfill the needs of a microindentation test method. A load is applied to a carefully prepared diamond indenting tool with a pyramid shape, and the lengths of the diagonals of the resulting indentation in the material are measured. The shape of the indenter and the resulting indentation are illustrated in Figure 22. The Knoop hardness number (KHN) is the ratio of the load applied to the area of the indentation calculated from the following formula:

$$\text{KHN} = \frac{L}{l^2 C_p}$$

In this equation L is the load applied, l is the length of the long diagonal of the indentation, and C_p is a constant relating l to the projected area of the indentation. The units for KHN are also kg/mm^2 . Similar to the Brinell method, higher values for KHN represent harder materials.

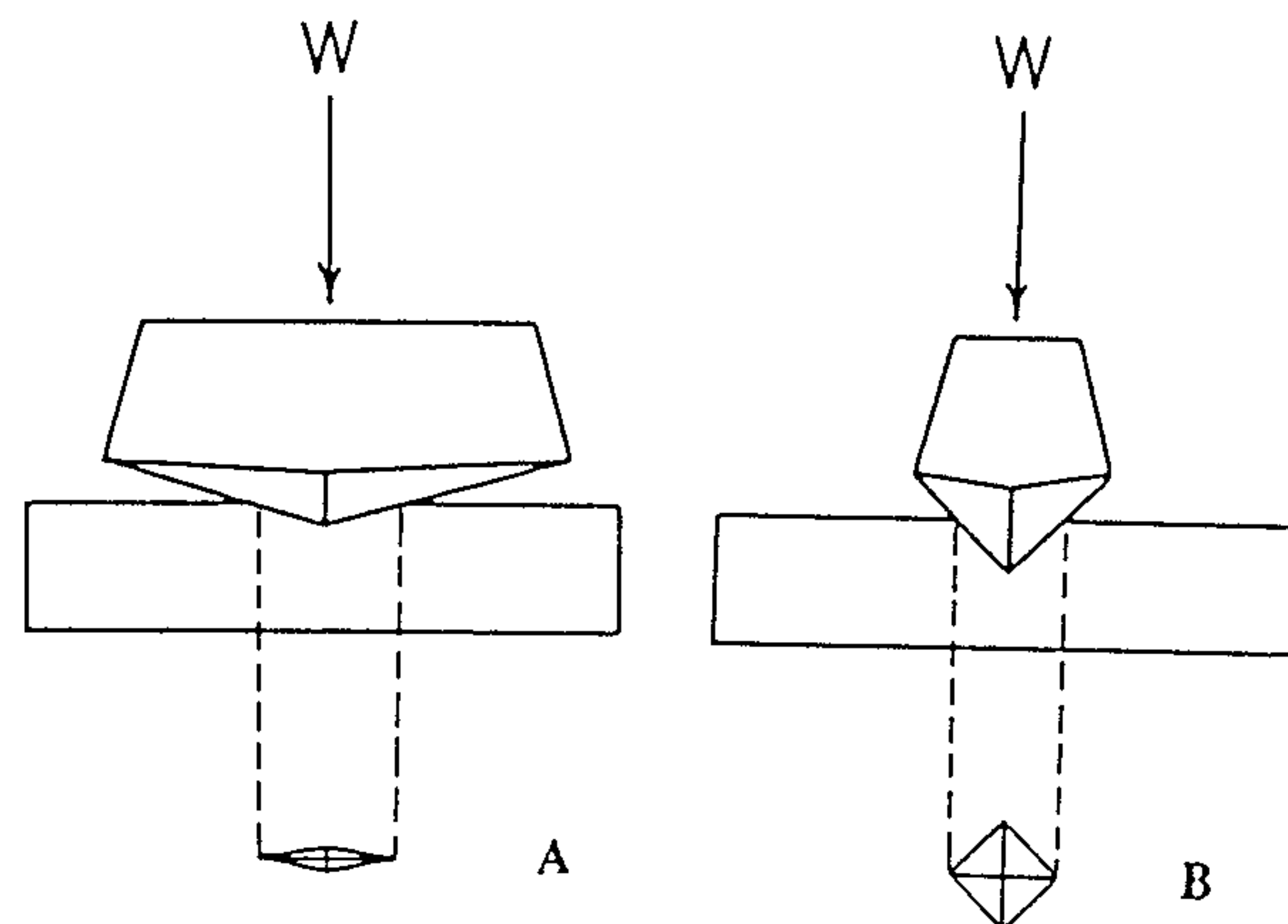


Figure 24: a: principle of the Knoop hardness measurement; b: diamond pyramid (Vickers) indentation test. (From: R.G. Craig: Restorative Dental Material. 9th ed, Mosby Year Book, St Louis, pp 89, 1993).

The Knoop method is designed so that varying loads may be applied to the indentation instrument. The resulting indentation area therefore varies according to the load applied as well as to the nature of the materials can be tested with a great range of hardness simply by varying the test load. Because very light load applications produce extremely delicate microindentations, this method of testing can be employed to examine materials that vary in area hardness. For example, the Knoop method has been used extensively in testing the hardness of both enamel and dentine in extracted teeth and in determining the hardness of metals and alloys that have isolated hard and soft phases throughout the material. The chief disadvantages of the method are the need for a highly polished and flat test sample and the time required to complete the test operation, which is considerably greater than that required for some other, less precisely controlled methods (Craig, 1993).

- **Vickers hardness test.** The 136-degree diamond pyramid, or Vickers hardness test, is also suitable for testing the surface hardness of materials. It has been used to a limited degree as a means of testing the hardness of restorative dental materials. The method is similar in principle to the Knoop and Brinell tests except that a 136-degree diamond pyramid-shaped indenter is forced into the material with a definite load application. The indenter produces a square indentation, the diagonals of which are measured as shown in Figure 22. In recent years the equipment for Knoop hardness testing has been adapted to use the 136-degree indenter. Loads are varied from 1 to 120 kg, depending on the hardness of the tested material. The Vickers test is especially useful in measuring the hardness of small areas and for very hard materials (Craig, 1993).

- **Rockwell hardness test.** The Rockwell hardness test was developed as a rapid method for hardness determinations. A ball or metal cone indenter is normally used, and the depth of the indentation is measured with a sensitive dial micrometer. The indenter balls or cones are of several different diameters, as well as different load applications (60 to 150 kg), with each combination described as a special Rockwell scale, Rockwell A-G, denoted R_A , R_B , etc.

The superficial Rockwell method has been used to test plastic used in dentistry. This method uses a relatively light (30 kg) load and a large-diameter (12.7 mm) ball in comparison with the standard Rockwell methods. The test is made by first applying a preload (minor load) of 3 kg. A major load of 30 kg then is applied to the sample for 10 minutes before a reading is taken. Because dental plastics are viscoelastic, recovery of the indentation occurs once the major load has been removed. The percent recovery can be determined on the same specimen by the following equation:

$$\text{Percent recovery} = \frac{A - B}{A} \times 100\%$$

where A is the depth of the indentation caused by application of the major load for 10 minutes, and B is the depth of the indentation after the major load has been removed for 10 minutes. The advantages of the Rockwell hardness test are that hardness is read directly and it is good for testing viscoelastic materials. The disadvantages are that a preload is needed, greater time is required, and the indentation may disappear immediately on removal of the load (Craig, 1993).

- **Shore A hardness.** The hardness measurements described previously cannot be used to determine the hardness of rubbers, since the indentation disappears after the removal of the load. An instrument called Shore A Durometer is used in the rubber industry to determine the relative hardness of elastomers. The instrument consists of a blunt-pointed indenter 0.8 mm ($\frac{1}{32}$ inch) in diameter that tapers to a cylinder 1.6 mm ($\frac{1}{16}$ inch). The indenter is attached by a lever to a scale that is graduated from 0 to 100 units. If the indenter completely penetrates the sample, a reading of 0 is obtained, and if no penetration occurs, a reading of 100 units results. Because rubber is viscoelastic, and accurate reading is difficult to obtain, since the indenter continues to penetrate the rubber as a function of time. The usual method is to press down firmly and quickly on the indenter and record the maximum reading as the Shore A hardness. The test has been used to evaluate soft denture liners, mouth protectors, and maxillofacial elastomers (Craig, 1993).

Modulus of Elasticity

The measure of elasticity of a material is described by the term elastic modulus or modulus of elasticity or Young's modulus, and denoted by the variable E . The elastic modulus represents the stiffness of a material within the elastic range. The elastic modulus can be determined from a stress-strain curve (Figure 23) by calculating the ratio of stress to strain or the slope of the linear region of the curve. The modulus is calculated from the equation

$$\text{Elastic Modulus} = \frac{\text{Stress}}{\text{Strain}} \text{ or } E = \frac{\sigma}{\epsilon}$$

Since strain is dimensionless, the modulus has the same units as stress, and is usually reported in MPa or psi.

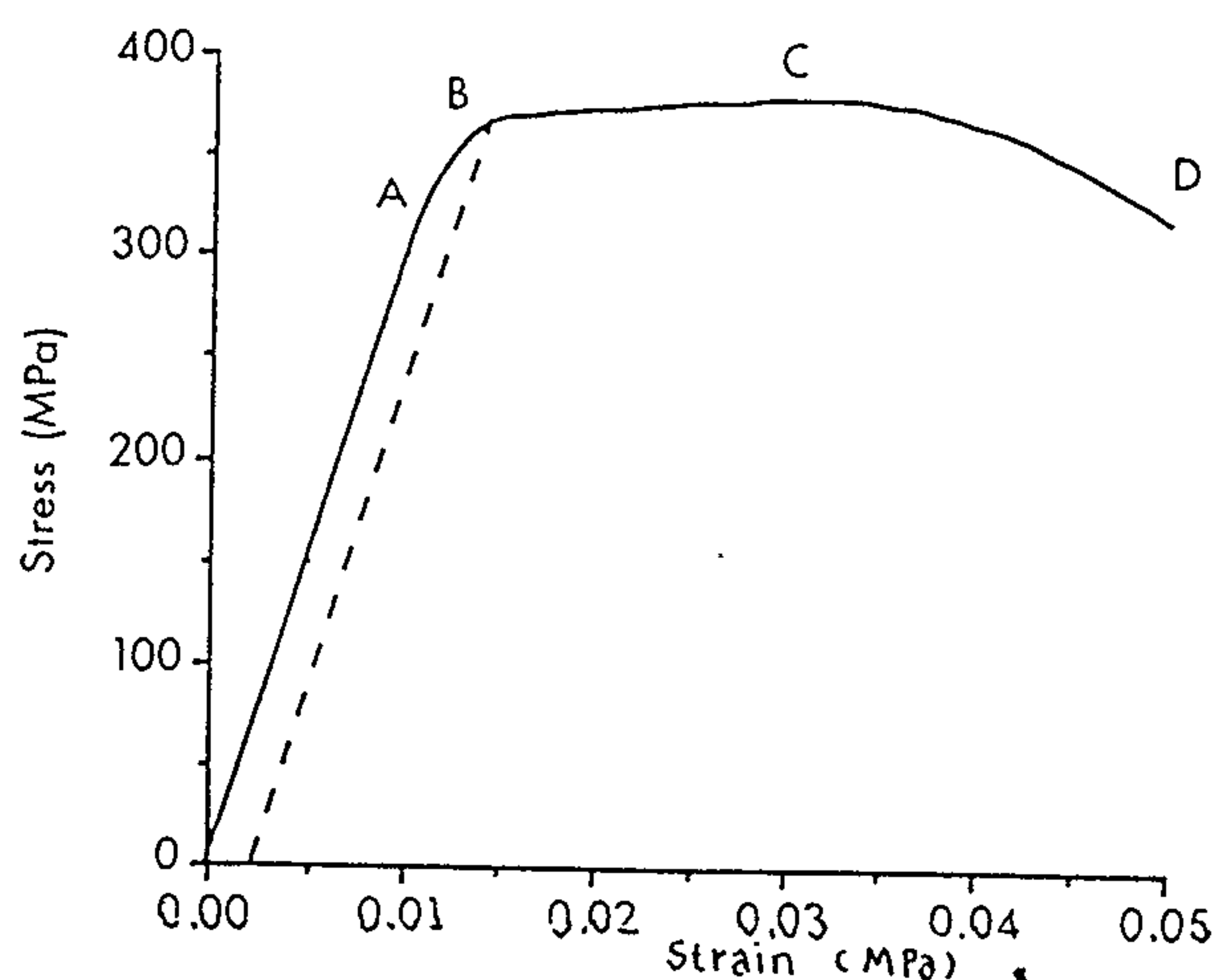


Figure 25: Stress-strain curve for a material subjected to a tensile stress (From: Craig R.G.: Restorative Dental Material. 9th ed, Mosby Year Book, St Louis, pp 63, 1993).

The elastic qualities of a material represent a fundamental property of the material. The interatomic or intermolecular forces of the material are responsible for the property of elasticity. The stronger the basic attraction forces, the greater the values of the elastic modulus and the more rigid the material. Because this property is related to the attraction forces within the material, it usually is the same when the material is subjected to either tension or compression. The property generally is independent of any heat treatment or mechanical treatment that a metal or alloy has received, but is quite dependent on the composition of the material.

Appendix 2

The UMIS (ultra micro-indentation system)

The measurement of indentation hardness previously mentioned are limited to the determination of macroproperties, with forces in the range 10 N to 30 kN, or in the case of microhardness, the loading in the range 0.1 N to 5 N, to characterizing coatings or more than approximately 3 μm in thickness. The thickness of applied surface layers or modified layers used in most high technology applications rarely exceeds a few micrometers and is often much less. Indentations produces for the purpose of investigating hardness and elastic behavior at these scales must be similarly small: too small for measurement by the usual optical methods. The forces required to press the indenter into the surface must also be well below those normally available on conventional microhardness testers. These conditions have led to produce the new instruments to replace the optical measurement of the surface impressions.

For the ultra micro-indentation systems, the initial forces are substantially less than 0.1 mN, the depths of penetration with resolutions less than 1 nm and force resolution less than 0.01 mN. The total depth of indenter penetration may be less than 100 nm.

The indenter used in this system for determining the depth distribution of hardness and elastic modulus is called Berkovich indenter. A Berkovich indenter, which is defined as a diamond pyramid on an equilateral triangle base with face angles of 65.3° relative to the axis, is used with load cycle analysis controlled by operating software. After setting the initial contact on the surface, a force of 250 mN is applied at each step continuously until 20 increments are obtained and then unloaded at the same rate.

The UMIS machine is operated with a high degree of automation under the control of a personal computer. The operation principle is as follows: a member carrying a specially developed elastic element is driven downwards by a long-range piezo driver. The elastic element supports a shaft with a diamond pyramid or spherical indenter attached. An LVDT (linear variable differential transformer) secures to the frame of the instrument recorded 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 indenting force. The depth LVDT output is zeroed when the indenter contacts the surface

The development of successful ultra micro-indentation instruments has required:

- (i) the achievement of high resolutions in the measurement of penetration

- depth and force;
- (ii) the realization of a precise depth measurement datum;
- (iii) a mechanically stable and robust design, with small compliance, which was not critically sensitive to thermal and vibrational disturbance;
- (iv) indenters with well defined tip geometry and a technique for correcting tip irregularity to uncertainties commensurate with the penetration depth resolution;
- (v) a model for the indenting process which allowed the derivation of reliable values for material properties from the observed data;
- (vi) a means of calibration of the depth measuring and force generation systems;
- (vii) a method of correcting for instrument compliance to allow the elastic properties of samples to be determined free of contamination by the elastic behavior of the instrument.

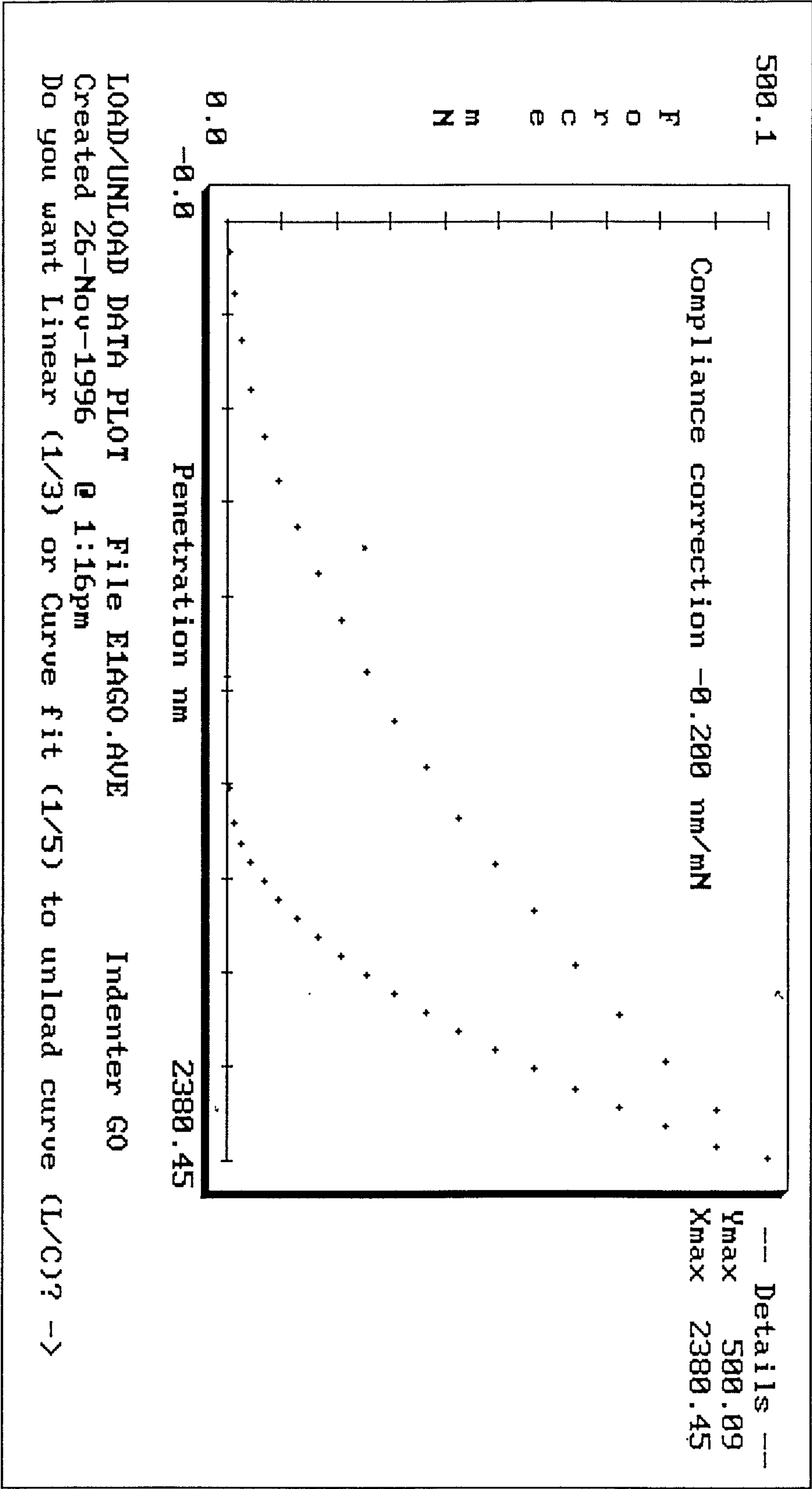
(Bell *et al.*, 1991/92)

In the present study, a Berkovich indenter was used with a load cycle system. Each data was displayed in the form of loading and unloading curve. The obtained data which were out of the indenter area function were marked and cutoff. Then, average data will be used for the final calculation (Figure 26). When analysing the data, the compliance correction was also computed since no diamond pyramid indenter is geometrically perfect and any imperfections present would be reflected in the calculated values of hardness and elastic modulus of test materials. This compliance correction is used to compensate for the sharpness of the indenter tip. The compliance correction or tip correction value which used in the present study was 0.02 nm/mN.

Data manipulation

The analysis calculates the initial penetration first. Initial penetration is estimated by fitting a power law regression to the first 10 points (as used in the present study) or selected by the user. The initial penetration is then added to the data and the result plotted. The approach ensures that plots start at zero. The origin (0,0) and two extra interpolation points are calculated from the regression to extrapolate between the first real data point to the origin.

Figure 26: Loading and unloading curve.



The unloaded approximation is calculated according to the selection; linear or curve fit, and superimposed over the new data which now includes the initial penetration. The plot for the simulated data described previously is shown in Figure 27. On the right hand side of the screen, the results are shown. The important values will be as follows:

- H_{max} = maximum penetration at maximum load,
- H_r = estimate of where the unload regression intercepts the X axis,
- X_i = estimate of where the projected slope at maximum force meets the X axis,
- H_p = estimate of the plastic penetration which is $X_i + 0.25 * (H_{max} - X_i)$

After selection and display of data with linear or curve regression fitted, a tip correction must be applied to the data. The analysis allows for area function correction or no correction at all. The detection of the correction and subsequent analysis is automatic from the contents of the files. A typical area function is shown in Figure 28. The area function expresses as a ratio. This ratio is based on the estimate of the effective area versus the theoretical area of a perfectly shaped Berkovich indenter at the same penetration.

Hardness values are calculated for each force step using the plastic penetration, H_p . The hardness values are plotted as a function of H_p , which is equal to the total penetration (Figure 29). In the case that the Poisson ration of the specimen is known, Young's modulus may be extracted from the $E/(1-\nu^2)$ term as shown in the summarized results (Figure 30).

Figure 27: Initial penetration estimated at the first 10 points of load curve and the curve fit selected for unload curve.

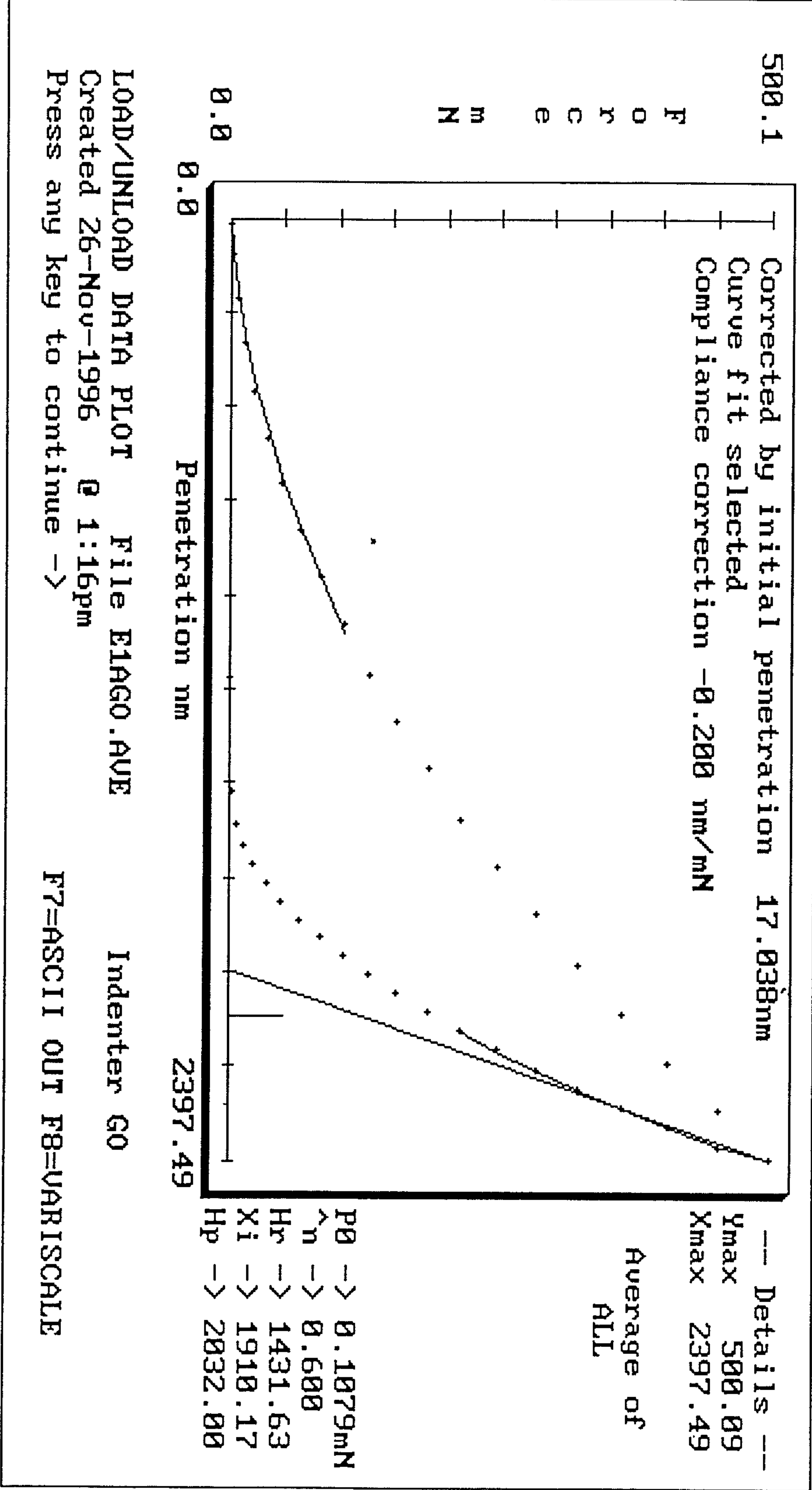


Figure 28: A typical area funtion.

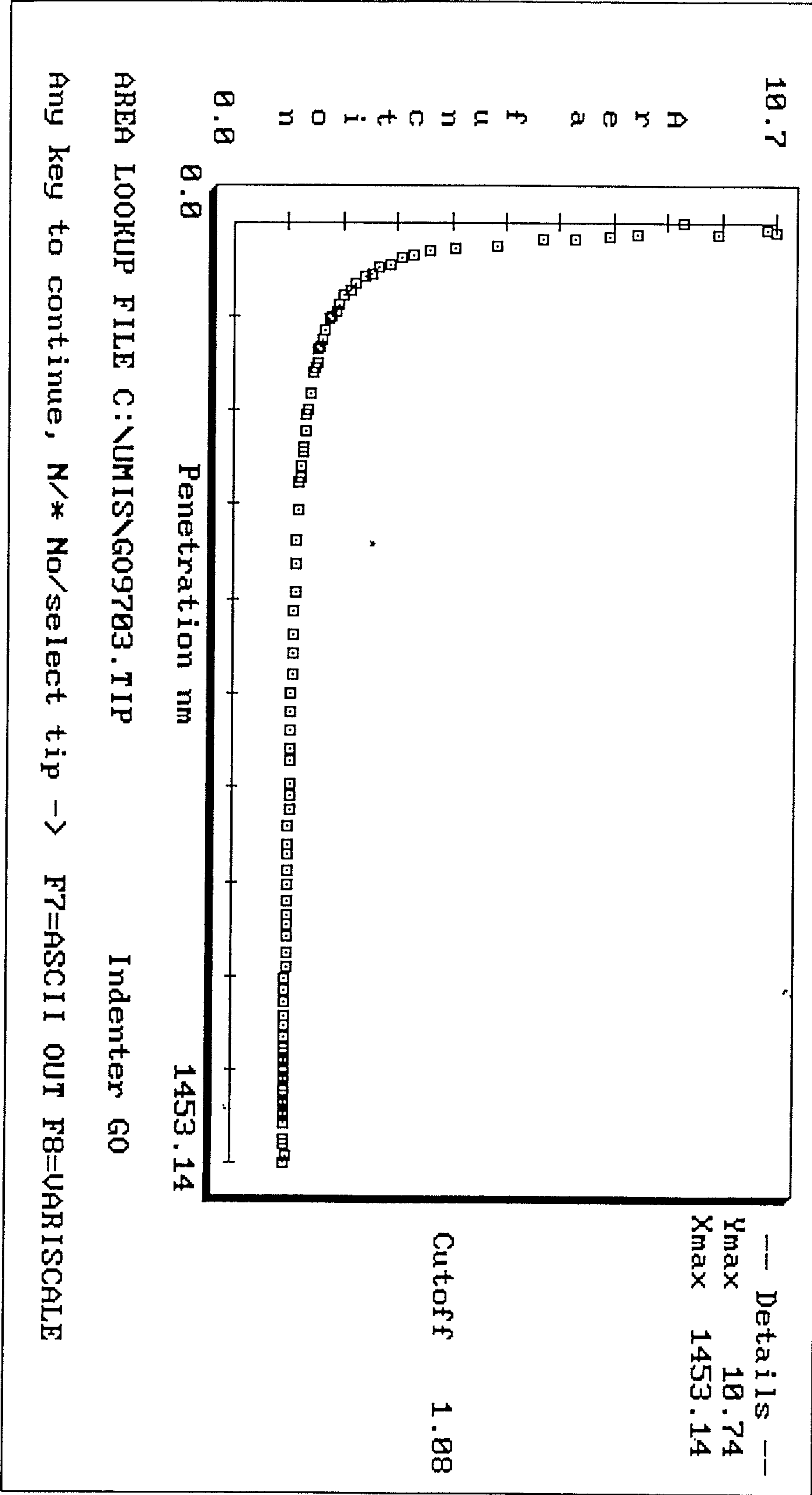


Figure 29: The hardness values plotted as a function of H_p

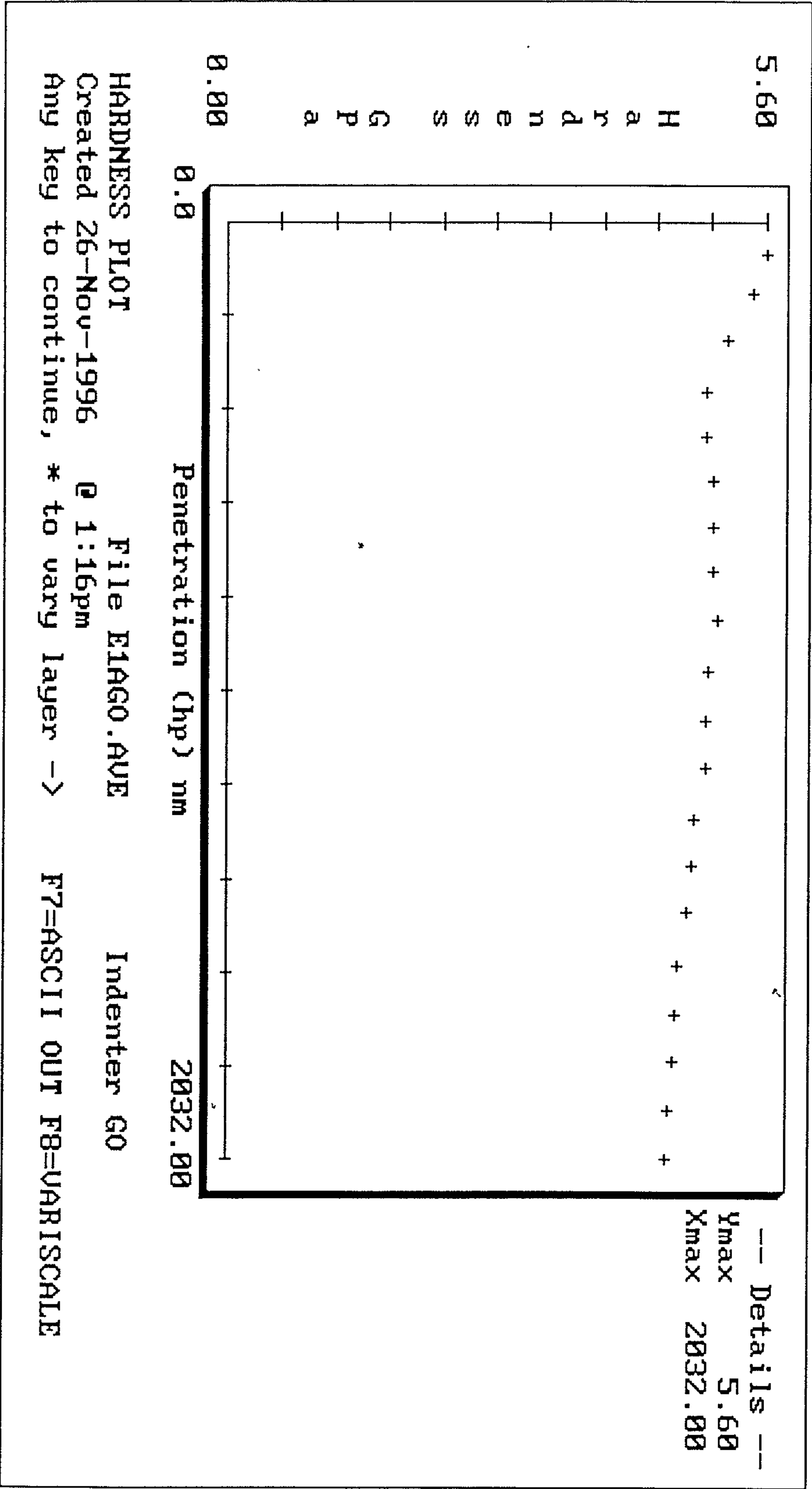


Figure 30: Summarized results.

Created 26-Nov-1996	SUMMARIZED RESULTS		✓ @ 1:16pm
Berkovich indenter			
File E1AG0.AVE			
Indenter G0			
Number of increments	20		
Maximum force	500.09 mN		
Contact force	0.15 mN		
Initial penetration	17.0 nm		
Adjusted total penetration	2397 nm		
Adjusted plastic penetration	2032 nm		
Elastic recovery	487 nm		
Elastic recovery rate	0.9745 nm/mN (curve fit)		
Exponent n in eqn $P = b(h-h_r)^n$	2.000		
Coefficient of correlation r	0.999296		
Hardness (at maximum depth)	4.57 GPa		
Equivalent Vickers hardness	420 HV eq		
E* (specimen & indenter)	84.11 GPa		
E/(1- ν^2) (specimen)	91.80 GPa		
E* /H (at maximum load)	18.39		
Returned energy ratio	0.38		
Revised plastic penetration	2032 nm	with	1.000 ratio
Hit a key to continue, <C> for hardcopy -> F7=ASCII OUT			

Appendix 3

Glossary of Terms

1 N/m ²	=	1 Pascal (Pa)	
1 kg/mm ²	=	100 kg/cm ²	
1 kg/mm ²	=	9806.65 kN/m ²	= 9806.65 kPa
1 kg/cm ²	=	14.223 lb/in ²	= 0.0981 MPa
1 lb/in ²	=	0.073 kg/cm ²	
1 lb/in ²	=	6.89476 kN/m ²	
10 ⁶ lb/in ²	=	6.89476 GN/m ²	
1 MPa	=	10 ⁶ Pascal	
1 GPa	=	10 ⁹ Pascal	
1 MPa	=	.001 GPa	

(From: Graig,R.G.: *Restorative Dental Materials*. 9th ed, Mosby Year Book Inc., pp 551-2 1993.)

Appendix 4

Patient information and inform consent

The importance of disinfection on the physical properties of tooth enamel

PATIENT INFORMATION

To Whom this may concern,

As part of your treatment plan, your clinician will have informed you of the need to remove two or four teeth to make the space needed to obtain the proper result.

I am involved with researching the properties of human tooth surfaces. To accomplish this study, some extracted teeth are required to be tested.

This study requires the attachment of an orthodontic bracket to the tooth surface. The bracket will remain in the mouth for only 10 minutes before the removal of the tooth will be carried out. The process will be similar to the real clinical situation and undertaken in the United Dental Hospital.

You are invited to assist us by allowing us to attach the bracket and collect the teeth after their extraction. This research will not in any way influence your orthodontic treatment, nor delay the commencement of treatment.

The experimental procedure and this form have been approved by the Ethics Committee of this hospital. Once the teeth are collected, they will be treated anonymously and cannot be identified individually.

Participation in this study is entirely voluntary: you are in no way obliged to participate. If you do participate, you can withdraw at any time.

If you agree to donate your teeth for this experimental research purpose, please complete the accompanying form.

If you would like to know more at any stage, please feel free to contact Dr. Tippawan Leelahakriengkrai, postgraduate student in the Discipline of Orthodontics, on 02 9282 0389.

Thanking you for your assistance.

Yours sincerely,

Dr. Tippawan Leelahakriengkrai

The importance of disinfection on the physical properties of tooth enamel

PATIENT CONSENT FORM

As your orthodontic treatment plan requires the extraction of teeth to facilitate treatment, this consent is to allow the extracted teeth to be subsequently used for the proposed research.

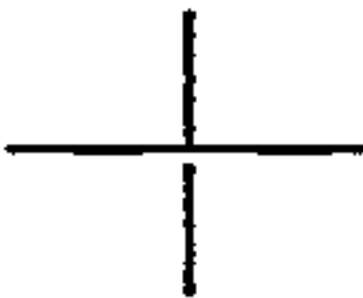
I.....of.....
(SURNAME) (GIVEN NAME) (FULL ADDRESS)

.....
.....
.....
have read and understood the Information for Participants on the above named research study and have discussed the study with Dr. Tippawan Leelahakriengkrai.

I do consent to donation and collection of such teeth as indicated below to be used for experimental research.

Teeth to be collected following extraction:

FDI annotation:

Palmer notation: 

Tooth description(s):
(e.g. upper right first premolar)

.....
.....
I freely choose to participate in this study and understand that I can withdraw at any time. I also understand that the research study is strictly confidential.

PARENT

Signed:.....
(Parent)

Name:.....
(Printed)

Witness:.....

Name:.....
(Printed)

Address:.....

.....
.....
Contact Phone: ().....

INTERPRETER (If interpreter used)

Name:.....

Contact address and Phone:.....
.....(Ph.).....

Signed:.....

References

Anderson D.J.: Nitrogen in human dental enamel. *Biochem J* 45:31, 1949.

Angmar B., Carlström D., Glas J.E.: Studies on the ultrastructure of dental enamel. IV. The mineralization of normal human enamel. *J Ultrastruct Res* 8:12, 1963.

Arends J., Schuthof J., Jongebloed W.L.: Microhardness indentations on artificial white spot lesions. *Caries Res* 13:290-97, 1979.

Armstrong W.D., Brekhuis P.J.: Chemical constitution of enamel and dentin. I. Principle components. *J Biol Chem* 120:677, 1937.

Bakhos Y., Brudevold F.: Effect of initial demineralization on the permeability of human tooth enamel to iodide. *Arch Oral Biol* 27:193-6, 1982.

Barkmeier W.W., Cooley R.L.: Laboratory evaluation of adhesive systems. *Oper Dent Sup* 5: 50-61, 1992.

Barkmeier W.W., Gwinnett A.J., Shaffer S.E.: Effects of enamel etching time on bond strength and morphology. *J Clin Orthod* 19:36-8, 1985.

Barkmeier W.W., Shaffer S.E., Gwinnett A.J.: Effects of 15 vs 60 second enamel acid conditioning on adhesion and morphology. *Oper Dent* 11:111-6, 1986.

Bass R.A., Plummer K.D., Anderson E.F.: The effect of a surface disinfectant on a dental cast. *J Pros Dent* 67:723, 1992.

Bell T.L., Bendeli A., Field J.S., Swain M.V., Thwaite E.G.: The determination of surface plastic and elastic properties by Ultra Micro-indentation. *Metrologia* 28:463-9, 1991/92.

Belting C.M., Habberfelde G.C., Juhl L.K.: Spread of organisms from dental air rotor. *JADA* 68:34-7, 1964.

Bell T.J., Bendeli A., Field J.S., Swain M.V., Thwaite E.G.: The determination of surface plastic and elastic properties by Ultra Micro-indentation. *Metrologia* 28:463-9, 1991/92.

Besten P.D., Giambro N.: Treatment of fluorosed and white-spot human enamel with calcium sucrose phosphate *in vitro*. *Pediatr Dent* 17:340-45, 1995.

Bird M.J., Gallup, H., Gaudino J., Hodge H.C.: A comparison of two methods of ashing enamel and dentin. *J Dent Res* 27:693, 1948.

Bishara S.E., Jakobsen J.R.: A comparative study of the debonding strengths of different ceramic brackets, enamel conditioners, and adhesives. *Am J Orthod Dentofac Orthop* 104:170-9, 1993.

Bishara S.E., Truelove T.S.: Comparisons of different debonding techniques for ceramic brackets: an *in vitro* study. Part II. Findings and clinical implications. *Am J Orthod Dentofac Orthod* 98:263-73, 1990.

Bowen R.L.: Dental filling material comprising vinyl silane treated fused silica and a binder conducting of the reaction product of bisphenol and glycidyl acrylate. U.S. Patent No. 3,066,112, 1962.

Bowes, J.H., Murray M.M.: The chemical composition of teeth. II. The composition of human enamel and dentine. *Biochem J* 29:2721, 1935.

Bowes, J.H., Murray M.M.: The chemical composition of teeth. III. The variation in chemical composition in relation to dental structure. *Biochem J* 30:977, 1936.

Boyde A.: Electron micorscopic observations relating to the nature and development of prism decussation in mammalian dental enamel. *Bull Grp Int Rech Sci Stomat* 12:151-207, 1969.

Bradburn G., Pender N.: An in vitro study of the bond strength of two light-cured composites used in the direct bonding of orthodontic brackets to molars. *Am J Orthod Dentofac Orthop* 102:418-26, 1992.

Bradley E.L.: Effects of etchant concentration and duration on the retention of orthodontic brackets: An *in vivo* study. *Am J Orthod Dentofac Orthop* 98:417-21, 1990.

Brännström M. Nordenvall K.J.: The effect of acid etching on enamel, dentine and the inner surface of the resin restoration: a scanning electron microscopic investigation. *J Dent Res* 56:917-23, 1977.

Brudevold F., Steadman L.T., Smith F.A.: Inorganic and organic components of tooth structure. *Annals of the New York Academy of Science* 85:110-32, 1960.

Bunyan J.: The use of hypochlorite for the control of bleeding. *Oral Surg* 13:1026-32, 1960.

Buonocore M.G.: A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. *J Dent Res* 34:849-53, 1955.

Buonocore M.G., Matsui A., Gwinnett A.J.: Penetration of resin dental materials into enamel surfaces with reference to bonding. *Archs Oral Biol* 13:61-70, 1968.

Carlström D., Glas J.E., Angmar B.: Studies on the ultrastructure of dental enamel. V. The state of water in human enamel. *J Ultrastruct Res* 8:24, 1963.

Centres for Disease Control: Recommended infection-control practices for dentistry. *MMWR* 35:237-42, 1986.

Centres for Disease Control: Recommendations of prevention of HIV transmission in health care settings. *MMWR* 36:1S-18S, 1987.

Chadwick R.G., McCabe J.F., Walls A.W.G., Storer R.: The effect of storage media upon the surface microhardness and abrasion resistance of three composites. *Dent Mater* 6:123-8, 1990.

Chow L.C. and Brown W.E.: Phosphoric acid conditioning of teeth for pit and fissure sealants. *J Dent Res* 52: 1158, 1973.

Cohen S., Burns R.C.: *Pathways of the Pulp*. 4th ed, St. Louis: CV Mosby, 1987.

Collys K., Slop D., Cleymaet R., Coomans D., Michotte Y.: Load dependency and reliability of microhardness measurements on acid-etched enamel surfaces. *Dent Mater* 8:332-5, 1992.

Corcia J.T., Moody W.E.: Thermal analysis of human dental enamel. *J Dent Res* 53:571, 1974.

Cottone J.A., Molinari J.A.: Selection for dental practice of chemical disinfectants and sterilants for hepatitis and AIDS. *Aust Dent J* 32:368-74, 1987.

Council on Dental Materials, Instruments, and Equipment; Council on Dental Practice; and Council on Dental Therapeutics. Infection control recommendations for the dental office and the dental laboratory. *JADA* 116:241-8, 1988.

Crabb H.S.M.: The pattern of mineralization of human dental enamel. *Proceedings of the Royal Society of Medicine* 52:118-22, 1959.

Craig R.G.: *Restorative Dental Materials*. 9th ed. Mosby Year Book Inc., pp 54-105, 260-3, 272, 1993.

Craig R.C., Peyton F.A, Johnson D.W.: Compressive properties of enamel, dental cements and gold. *J Dent Res* 40:936-45, 1961.

Crane A.B.: *A Practicable Root Canal Technique*. Philadelphia: Lea and Febiger, pp 69, 1920.

Cunningham W.T., Balekjian A.Y.: Effect of temperature on collagen dissolving ability of sodium hypochlorite endodontic irrigant. *Oral Surg* 48:175-7, 1980.

Dakin H.D.: On the use of certain antiseptic substances in the treatment of infected wounds. *Br Med J* 2:318, 1915.

Davidson C.L., Arends J.: Thermal analysis studies on sound and artificially decalcified tooth enamel. *Caries Res* 11:313-20, 1977.

Davidson C.L., Hoekstra I.J., Arends J.: Microhardness of sound, decalcified and etched tooth enamel related to calcium content. *Caries Res* 8:135-44, 1974.

Dibdin G.H.: The stability of water in human dental enamel studied by proton nuclear magnetic resonance. *Arch Oral Biol* 17:433-7, 1972.

Dogon I.L.: Studies demonstrating the need for an intermediary resin of low viscosity for the acid etch technique. *Proceedings of the International Symposium on Acid-Etch Technique*. St. Paul, Minn: North Central Publishing Co, pp 100-118, 1975.

Driessens F.C.M., Wöltgens J.H.M.: *Tooth Development and Caries*. Vol. I. Chapter 6, CRC Press, Inc., pp 96-152, 1986.

Dukino R.D., Swain M.V.: Comparative measurement of indentation fracture toughness with Berkovich and Vickers indenters. *J Am Ceram Soc* 75:3299-304, 1992.

Dychdala G.R.: Chlorine and chlorine compounds. In: Block S.S., (ed.) *Disinfection, Sterilization and Preservation*. Philadelphia: Lea and Febiger, pp 157-82, 1983.

Eastoe J.E.: The amino acid composition of proteins from the oral tissues:- The matrix proteins in dentine and enamel from developing deciduous teeth. *Archs Oral Biol* 8:633-52, 1963.

Eliades T., Eliades G., Breantley W.A., Johnston W.M.: Residual monomer leaching from chemically cured and visible light-cured orthodontic adhesives. *Am J Orthod Dentofac Orthop* 108:316-21, 1995.

Featherstone J.D.B., Cutress T.W., Rodgers B.E., Dennison P.J.: Remineralisation of early carious lesions *in vivo* by a self administered mouthrinse or paste. *Caries Res* 16:235-42, 1982.

Featherstone J.D.B., Rodgers B.E., Smith M.W.: Physicochemical requirements for rapid remineralization of early carious lesions. *Caries Res* 15:221-35, 1981.

Featherstone J.D.B., Ten Cate J.M., Shariati M., Arends J.: Comparison of artificial caries-like lesions by quantitative microradiography and microhardness profiles. *Caries Res* 17:385-91, 1983.

Ferguson S., Chesnut D.B.: A magnetic resonance spin label study of human dental enamel. *Arch Oral Biol* 23:85, 1978.

Frank R.M.: The ultrastructure of the tooth from the point of view of mineralisation, demineralisation and remineralisation. *Int Dent J* 17:661-83, 1967.

Galil K.A.: An *in vitro* enamel cleaning method for examining teeth by scanning electron microscopy. *J Canad Dent Assoc* 10:559-62, 1975.

Gelhard T.B.F.M., Arends J.: *In vivo* remineralization of artificial subsurface lesions in human enamel. *J Biol Buccale* 12:49-57, 1984.

Gerhardt D.E., Williams H.N.: Factors affecting the stability of sodium hypochlorite solutions used to disinfect dental impressions. *Quintessence Int* 22:587-91, 1991.

Gilpatrick R.O., Ross J.A., Simonsen R.J.: Resin-to-enamel bond strengths with various etching times. *Quintessence Int* 22:47-9, 1991.

Glas J.E., Nylen M.U.: A correlated electron microscopical and microradiographic study of human enamel. *Arch Oral Biol* 10:893-908, 1965.

Gordon T.M., Damato D., Christner P.: Solvent effect of various dilutions of sodium hypochlorite on vital and necrotic tissue. *J Endodon* 7:466-9, 1981.

Gorelick L.: Bonding metal brackets with a self-polymerizing sealant-composite: a 12-month assessment. *Am J Orthod* 71:542-53, 1977.

Gustafson A., Gustafson A.G.: The similarity between contralateral pairs of teeth. *Odont T* 63: 245, 1955.

Gustafson B., Gustafson A.G.: Microanatomy and histochemistry of enamel. In : A.E.W. Miles (ed.): *Structural and Chemical Organization of Teeth*. Vol.II, Academic Press, New York, pp 76-129, 1967.

Gwinnett A.J.: The ultrastructure of the "prismless" enamel of permanent human teeth. *Archs oral Biol* 12:381-87, 1967.

Gwinnett A.J.: Morphology of the interface between adhesive resins and treated human enamel fissures as seen by scanning electron microscopy. *Archs Oral Biol* 16:237-8, 1971.

Gwinnett A.J.: Histologic changes in human enamel following treatment with acidic adhesive conditioning agents. *Archs oral Biol* 16:731-738, 1971.

Gwinnett A.J.: Human prismless enamel and its influence on sealant penetration. *Arch Oral Biol* 18:441-4, 1973.

Gwinnett A.J.: The scientific basis of the sealant procedure. *J Prev Dent* 3:15-28, 1976.

Gwinnett A.J.: Structure and composition of enamel. *Oper Dent supp* 5:10-17, 1992.

Gwinnett A.J., Buonocore M.G.: Adhesives and caries prevention: A preliminary report. *Br Dent J* 119:77-80, 1965.

Gwinnett A.J., Matsui A.: A study of enamel adhesives. *Archs Oral Biol* 12: 1615-20, 1967.

Gwinnett A.J., Gorelick L.: Microscopic evaluation of enamel after debonding: Clinical application. *Am J Orthod Dentofac Orthop* 71:651-65, 1977.

Gwinnett A.J., Kanca J.: Micromorphology of the bonded dentin interface and its relationship to bond strength. *Am J Dent* 5:73-7, 1992.

Hand R.E., Smith M.I., Harrison J.W.: Analysis of the effect of dilution on the necrotic tissue dissolution property of sodium hypochlorite. *J Endodon* 4:60-4, 1978.

Helcomb D.W., Young R.A.: Thermal decomposition of human tooth enamel. *Calcif Tissue Int*, 31:189, 1980.

Haller B., Hofmann N., Klaiber B., Bloching U.: Effect of storage media on microleakage of five dentin bonding agents. *Dent Mater* 9: 191-97, 1993.

Herkströter F.M., Witjes M., Ruben J., Arends J.: Time dependency of microhardness indentations in human and bovine dentine compared with human enamel. *Caries Res* 23:342-4, 1989.

Hermesen R.J., Vrijhoef M.M.A.: Loss of enamel due to etching with phosphoric or maleic acid. *Dent Mater* 9:332, 1993.

Hodge H.C.: Hardness tests on teeth. *J Dent Res* 15:271-79, 1936.

Hodge H.C. and McKay H.: The microhardness of teeth. *J Am Dent Assoc* 12: 227-33, 1933.

Hoffmann P.N., Death J.E., Coates D.: The stability of sodium hypochlorite solutions. In: Collins C.H., Allwood M.C., Bloomfield S.F., *et al.*, (eds.): *Disinfectants: Their Use and Evaluation of Effectiveness*. London: Academic Press, pp 77-83, 1981.

Holager J.: Thermogravimetric experiments on tooth carbonates. *J Dent Res* 51:102, 1972.

Holma B.O., Granath L., Gustafson G.: A model for the study of tooth enamel by scanning electron microscopy. *Odontol Revy* 21:1-11, 1970.

Howell S., Weekes W.T.: An electron microscopic evaluation of the enamel surface subsequent to various debonding procedures. *Aus Dent J* 35:245-52, 1990.

Ingle J.I., Mullaney T.A., Grandich R.A., Taintor J.F., Fahid A.: Endodontic Cavity Preparation. In: Ingle J.I., Taintor J.F., (3rd eds.) *Endodontics*. Philadelphia: Lea and Febige, pp 178-85, 1985.

John V.B.: *Introduction to Engineering Materials*. 2nd ed, Macmillan Press, London, pp 273-5, 1983.

Johnson B.R., Remeikis N.A.: Effective shelf-life of prepared sodium hypochlorite solution. *J Endodon* 19:40-3, 1993.

Jörgersen K.D.: Contralateral symmetry of acid etched enamel surfaces. *Scand J Dent Res* 83:26-30, 1975.

de Josselin de Jong E., Ten Bosch J.J.: Error analysis of the microradiographic determination of mineral content in mineralized tissue slices. *Phys Med Biol* 30:1067-75, 1985.

Kelly A., Macmillan N.H.: *Strong Solids*. 3rd ed, Clarendon Press, Oxford, pp 57, 1986.

Kinch A.P., Taylor H., Warltier R.F.B., Oliver R.G., Newcombe R.G.: A clinical trial comparing the failure rates of directly bonded brackets using etch times of 15 or 60 seconds. *Am J Orthod Dentofac Orthop* 94:476-83, 1986.

Knoop F., Peters G.C., Emerson W.B.: A sensitive pyramidal tool for indentation measurements. *J Res Natn Bur Stand* 23:36-61, 1939.

Kodaka T., Debari K., Yamada., Kuroiwa M.: Correlation between microhardness and mineral content in sound human enamel. *Caries Res* 26:139-41, 1992.

Kodaka T., Kuroiwa M., Higashi S., Miake K.: Three dimensional observations of the relation between the natural enamel surface and the outermost layer in human permanent tooth. *Bull Tokyo Dent Coll* 31:105-15, 1990.

Kuroiwa M., Kodaka T., Kuroiwa M., Abe M.: Brushing-induced effects with and without a non-fluoride abrasive dentifrice on remineralization of enamel surfaces etched with phosphoric acid. *Caries Res* 28:309-14, 1994.

Koulourides T: Experimental changes of enamel mineral density: in Harris R.S. (ed.): *Art and Science of Dental Caries Research*. Chicago, University Press, pp 355-78, 1968.

Koulourides T., Phantumvanit J., Munksgaard E.C., Housch T.: An intra-oral model used for studies of fluoride incorporation in enamel. *J Oral Pathol* 3:185-95, 1974.

Lee H.L., Orlowski J.A., Enayda E., Rogers B.J.: *In vitro* and *in vivo* evaluation of direct-bonding orthodontic bracket systems. *JCO* 8: 227-38, 1974.

Lefevre M.L., Manly R.S.: Moisture, inorganic and organic contents of enamel and dentin from carious teeth. *J Am Dent Assoc* 25:233, 1938.

LeGeros R.Z., Bonel G., Legros R.: Types of "H₂O" in human enamel and in precipitated apatites. *Calcif Tissue Res* 26:111, 1978.

LeGeros R.Z., Silverstone L.M., Daculsi G., Kerebel L.M.: *In vitro* caries-like lesion formation in F-containing tooth enamel. *J Dent Res* 62:138, 1983.

Legler L.R., Retief D.H., Bradley E.L., Denys F.R., Sadowsky P.L.: Effects of phosphoric acid concentration and etch duration on the shear bond strength of an

orthodontic bonding resin to enamel: an *in vitro* study. *Am J Orthod Dentofac Orthop* 96:485-92, 1989.

Little M.F.: Studies on the inorganic carbon dioxide component of human enamel. II. The effect of acid on enamel CO₂. *J Dent Res* 40:903-14, 1961.

Little M.F., Casciani F.G.: The nature of water in sound human enamel. A preliminary study. *Arch Oral Biol* 11:565-71, 1966.

Little M.F., Cueto E.S., Rowley J.: Chemical and physical properties of altered and sound enamel. I. ash, Ca, P, CO₂, N, water, microradiolucency, and density. *Arch Oral Biol* 7:173-84, 1962.

Losee F.L., Hess W.C.: The chemical nature of the proteins from human enamel. *J Dent Res* 28:512-17, 1949.

Mackay F.: The effect of adhesive type and thickness on bond strength of orthodontic brackets. *Br J Orthod* 19:35-9, 1992.

Major P.W., Koehler J.R., Manning K.E.: 24-hour shear bond strength of metal orthodontic brackets bonded to porcelain using various adhesion promoters. *Am J Orthod Dentofac Orthod* 108:322-9, 1995.

Mardaga W.J., Shannon I.L.: Decreasing the depth of etch for direct bonding in orthodontics. *J Clin Orthod* 16:130-2, 1982.

Mark H.F., McKetta J.J., Othmer D.F., Standen A.: *Encyclopedia of Chemical Technology: Chlorine and Colors for Foods, Drugs and Cosmetics*. New York: Interscience Publisher, pp 10-7, 1964.

Marshall G.W., Olson L.M., Lee C.V.: SEM investigation of the variability of enamel surfaces after simulated clinical acid etching for pit and fissure sealants. *J Dent Res* 54:1222-31, 1975.

McClure F.J.: Fluorine in dentin and enamel of sound and carious human teeth. *J Dent Res* 27:287-98, 1948.

McGuckin R.S., Pashley D.H.: The effect of disinfection/sterilization treatments on Gluma-mediated dentin shear bond strength. *Am J Dent* 3:278-82, 1990.

Meckel A.H., Griebstein W.J., Neal R.J.: Structure of mature human dental enamel as observed by electron microscopy. *Archs Oral Biol* 10:775-83, 1965.

Miura F., Nakagawa K., Ishizaki A.: Scanning electron microscopic studies on the direct bonding system. *Bull Tokyo Med Univ* 20:245-60, 1973.

Mizrahi B., Mizrahi E., Cleaton-Jones P.F.: Shear bond strength of orthodontic brackets treated with Sebond. *Br J Orthod* 22:237-43, 1995.

Mjör I.A., Pindborg J.J.: *Histology of the Human Tooth*. Chapter 5. Munksgaard, Copenhagen, Denmark, pp 77-96 1973.

Moreno E.C.: The effect of acquired pellicle on enamel demineralization. In: *Proceedings of An International Symposium on The Acid Etch Technique.*, North Central Publishing Co., St Paul, Minn, pp 1-12, 1975.

Mühlemann H.R.: Storage medium and enamel hardness. *Helv Odontol Acta* 8:112-6, 1964.

Myers H.M.: Trapped water of dental enamel. *Nature (London)* 206:713, 1965.

Naujoks R., Schade H., Zelinka F.: Chemical composition of different areas of the enamel of deciduous and permanent teeth (the content of Ca, P, CO₂, Na and N₂). *Caries Res* 1:137-43, 1967.

NSW Health Department: Infection control policy, 1995.

Nishibori M., Kinoshita K.: Thin Solid Films. 48:325-31, 1979.

Oliver R.G.: The effect of different methods of bracket removal on the amount of residual adhesive. *Am J Orthod Dentofac Orthop* 93:196-200, 1988.

Osborne J.W., Ten Cate A.R.: *Advanced Dental Histology*. Chapter 16. 3rd ed, John Wright and Sons, Bristol, England, pp 109-18, 1976.

Pagniano R.P., Scheid R.C., Rosen S., Beck F.M.: Airborne microorganisms collected in a preclinical dental laboratory. *J Dent Educ* 49:653-5, 1985.

Pantera E.A., Schuster G.S.: Sterilization of extracted human teeth. *J Dent Educ* 54:283-5, 1990.

Pantera E.A., Zambon J.J., Shih-Levine M.: Indirect immunofluorescence for the detection of *Bacteriodes* species in human dental pulp. *J Endodon* 14:218-23, 1988.

Pashley E.L., Tao L., Pashley D.H.: Sterilization of human teeth: Its effects on permeability and bond strength. *Am J Dent* 6:189-91, 1993.

Penick E.C., Osetek E.M.: Intracanal drugs and chemicals in endodontic therapy. *Dent Clin North Am* 14:743-56, 1970.

Philips R.W., Swartz M.L.: Effect of fluorides on hardness of tooth enamel. J Am Dent Assoc 37:1-13, 1948.

Piskin B. and Türkün M.: Stability of various sodium hypochlorite solutions. J Endodon 21:253-5, 1995.

Poolthong S., Swain M.V., Mori T., Sumii T.: Mechanical properties of Tooth Following Different Storage Conditions. J Dent Res (In press), 1997.

Purdell-Lewis D.J., Groeneveld A., Arends J.: Microhardness and densitometric measurements of the effect of 4% SnF₂ Solution on artificial white spot lesions. Caries Res 10:216-26, 1976.

Raphael D., Wong T.A., Moodnik R., Borden B.G.: The effect of temperature on the bactericidal efficiency of sodium hypochlorite. J Endodon 7:330-4, 1981.

Retief D.H.: Effect of conditioning the enamel surface with phosphoric acid. J Dent Res 52:333-41, 1973.

Retief D.H.: Pulpal response to phosphoric acid and failure at the dental resin-etched enamel interface: in *Proceedings of an International Symposium on The Acid Etch Technique*: by Silverstone L.M. and Dogon I.L. North Central Pub Co., St. Paul, Minn, pp 213-9, 1975.

Retief D.H., Denys F.R.: Finishing of enamel surfaces after debonding of orthodontic attachments. Angle Orthod 49:1-10, 1979.

Ripa L.W., Gwinnett A.J., Buonocore M.G.: The "prismless" outer layer of deciduous and permanent enamel. Archs Oral Biol 11:41-8, 1966.

Robinson C., Hallsworth A.S., Shore R.C., Kirkham J.: Effect of surface zone deproteinisation on the access of mineral ions into subsurface carious lesions of human enamel. Caries Res 24:226-30, 1990.

Robinson C., Kirkham J.: The effect of fluoride on the developing mineralized tissue. J Dent Res 69:685-91, 1990.

Rock W.P.: The effect of etching human enamel upon bond strengths with fissure sealant resins. Arch Oral Biol 19:873-7, 1974.

Rönholm E.: The amelogenesis of human teeth as revealed by electron microscopy II. The development of the enamel crystallites. J Ultrastr Res 6:249-303, 1962.

Rosenfeld E.F., James G.A., Burch B.S.: Vital pulp tissue response to sodium hypochlorite. *J Endodon* 4:140-6, 1978.

Rossomando K.J., Wendt S.L.: Thermocycling and dwell times in microleakage evaluation for bonded restorations. *Dent Mater* 11:47-51, 1995.

Rudd R.W., Senia E.S., McCleskey F.K., Adams E.D.: Sterilisation of complete dentures with sodium hypochlorite. *J Prost Dent* 51:318-21, 1984.

Rueggeberg F.A., Lockwood P.: Thermal debracketing of orthodontic resins. *Am J Orthod and Dentofac Orthop* 98:56-65, 1990.

Sadowsky P.L., Retief D.H., Cox P.R., Hernandez-Orsini R., Rape W.G., Bradley E.L.: Effects of etchant concentration and duration on the retention of orthodontic brackets: An *in vivo* study. *Am J Orthod Dentofac Orthop* 98:417-21, 1990.

Sargison A.E., McCabe J.F., Gordon P.H.: *An vivo* study of self-, light-, and dual-cured composites for orthodontic bonding. *Br J Orthod* 22:319-23, 1995.

Savory A., Brudevold F.: The distribution of nitrogen in human enamel. *J Dent Res* 38:436, 1959.

Scholtanus J.D., Schuthof J., Arends J.: Influences of fluoridating varnishes on dentine *in vitro*. *Caries Res* 20:65-70, 1986.

Scott J.H., Symon N.B.B.: *Introduction to Dental Anatomy*. (8th ed.) Livingstone, Edinburgh and London, pp 175-212, 1971.

Senia S., Marroro R.V., Mitchell J.L., Lewis A.G., Thomas L.: Rapid sterilisation of gutta-percha cones with 5.25% sodium hypochlorite. *J Endodon* 1:136-40, 1975.

Senia S., Marshall F.J., Rosen S.: The solvent action of sodium hypochlorite on pulp tissue of extracted teeth. *Oral Surg* 31:96, 1971.

Shaffer S.E., Barkmeier M.S., Gwinnett A.J.: Effect of disinfection/sterilisation on *in vitro* enamel bonding. *J Dent Edu* 49:658-9, 1985.

Sheridan J.J., Brawley G., Hastings J.: Electrothermal debracketing. Part I. An *in vitro* study. *Am J Orthod* 89:21-7, 1986.

Shaffer S.E., Barkmeier W., Gwinnett A.J.: Effect of disinfection/sterilization on *in-vitro* enamel bonding. *J Dent Educ* 49:658-9, 1985.

Shih M., Marshall F.J., Rosen S.: The bacteriocidal efficiency of sodium hypochlorite as an endodontic irrigant. *Oral Surg* 29:613-9, 1970.

Silverstone L.M.: The acid etch technique: *In vitro* studies with special references to the enamel surface and the enamel resin interface. In: *Proceedings of the International Symposium on the Acid-Etch Technique*. St. Paul, Minn: North Central Publishing Co, pp 13-39, 1975.

Silverstone L.M.: Fissure sealants: Laboratory studies. *Caries Res* 8:2-26, 1974.

Silverstone L.M., Saxton C.A., Dogon I.L., Feferskov O.: Variation in the pattern of acid etching of human dental enamel examined by scanning electron microscopy. *Caries Res* 9:373-87, 1975.

Sim C., Neo J., Chua E.K., Tan B.Y.: The effect of dentin bonding agents on the microleakage of porcelain veneers. *Dent Mater* 10:278-81, 1994.

Sinha P.K., Nanda R.S., Duncanson M.G., Hosier M.J.: Bond strengths and remnant adhesive resin on debonding for orthodontic bonding techniques. *Am J Orthod Dentofac Orthop* 108: 302-7, 1995.

Soetopo, Beech D.R., Hardwich J.L.: Mechanism of adhesion of polymers to acid-etched enamel. *J Oral Rehab* 5:69-80, 1978.

Spangberg L., Engström B., Langeland K.: Biological effects of dental materials. 3. Toxicity and antimicrobial effect of endodontic antiseptics *in vitro*. *Oral Surg* 36:856-71, 1973.

Spangberg L., Rutberg M., Rydinge E.: Biologic effects of endodontic antimicrobial agents. *J Endodon* 5:166-75, 1979.

Stack M.V.: Chemical organization of the organic matrix in enamel; In: *Structural and Chemical Organization of Teeth*. Vol. 2, Chapter 20, Miles, A.E.W., Academic Press, New York, 20, pp 317-42, 1967.

Stack M.V., Fearnhead R.W.: *Tooth Enamel*. John Wright and Sons, Bristol, pp 113-6, 1965.

Stansford J.W., Paffenbarger G.C., Kumpula J.W., and Sweeney W.T.: Determination of some compressive properties of human enamel and dentin. *J Am Dent Assoc* 57:487-95, 1958.

Sundström B.: Histological decalcification using aqueous solutions of basic chromium (III) sulfate. *Odont Revy* 13: Suppl 13, 1968.

Swift E.J., Perdigao J., Heymann H.O.: Bonding to enamel and dentin: A brief history and state of the art, 1995. Quintessence Int 26: 95-110, 1995.

Sykes J.H.: Calcium hypochlorite for disinfection of hydrotherapy equipment. J Am Phys Ther 43:345-7, 1963.

Tate W.H., White R.R.: Disinfection of human teeth for educational purposed. J Dent Educ 55:583-5, 1991.

Ten Cate A.R.: *Oral Histology: Development, Structure, and Function*. 2nd ed. St Louis: C V Mosby, 1985.

The S.D.: The solvent action of sodium hypochlorite on fixed and unfixed necrotic tissue. Oral Surg 47:555-7, 1979.

Tsukamoto Y., Yamaguchi H., Yanagisawa M.: Thin Solid Films. 154:171-81, 1987.

Tyldesley, W.R.: The mechanical properties of human enamel and dentine. Bri Dent J 106:269-80, 1959.

Venezie R.D., Vadiakas G., Christensen J.R., Wright J.T.: Enamel pretreatment with sodium hypochlorite to enhance bonding in hypocalcified amelogenesis imperfecta: case report and SEM analysis. Pediatr Dent 16:433-6, 1994.

Wei S.: Electron microprobe analysis of remineralization. J Dent Res 49:621-5, 1970.

Weidmann S.M., Eyre D.R.: Amino acid composition of enamel protein in the fully developed human tooth. Caries Res 1: 349-55, 1967.

Whittaker D.K.: Structural variations in the surface zone of human tooth enamel observed by scanning electron microscopy. Archs oral Biol 27:383-92, 1982.

Weine F.S.: *Endodontic Therapy*. (2nd ed.), St. Louis: CV Mosby, pp 216-220, 1976.

Whitlock B.O., Eick D., Ackerman R.J., Glaros A.G., Chappell R.P.: Shear strength of ceramic brackets bonded to porcelain. Am J Orthod Dentofac Orthop 106: 358-64, 1994.

Wright J.T., Duggal M.S., Robinson C., Kirkham J., Shore R.: The mineral composition and enamel ultrastructure of hypocalcified amelogenesis imperfecta. *J Craniofac Genet Dev Biol* 13:117-26,1993.

Uno S., Finger W.J.: Effect of acid etchant composition and etch duration on enamel loss and resin composite bonding. *Am J Dent* 8:165-69, 1995.