NEODYMIUM:YAG LASER INDUCED PULPAL ANAESTHESIA: A STUDY INVESTIGATING CLINICAL EFFICACY AND EFFECTS ON TEETH

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A thesis submitted in fulfilment of the requirements for the degree of

MASTER OF SCIENCE IN DENTISTRY

Discipline of Oral & Maxillofacial Surgery

Faculty of Dentistry

University of Sydney

January, 1997

A much lower pulsed CO_2 laser irradiation energy fluence 50-60 J/cm^2 (Stern *et al.*, 1972) can produce localised melting, surface roughening and crystal fusion (Kantola *et al.*, 1973; Featherstone and Nelson, 1987), as well as microscopic fissure formation, leading to reduction in enamel acid solubility (Nelson *et al.*, 1986) with relatively minimal surface destruction. These results have been attributed to the highly efficient absorption of CO_2 laser by the dental hard tissues (Featherstone and Nelson, 1987).

The Nd:YAG laser has a characteristic wavelength of 1064 nm, which is negligibly absorbed by enamel and water (Spitzer and TenBosch, 1975), allowing it to penetrate deeply into the interior of the tooth (Fried *et al.*, 1993). Even at high energy fluences (up to 10,000J/cm^2), studies using Scanning Electron Microscopy has shown that Nd:YAG irradiation caused no crater formation and no discernible effect on the apatite (Meurman *et al.*, 1992). At this wavelength, it is also weakly absorbed and strongly scattered by dentine (Spitzer and TenBosch, 1975). Because of the absorption and the scattering coefficient of dentine being considerably higher than those of enamel, laser exposure can lead to relatively higher maximum surface temperature in dentine (White *et al.*, 1992) with subsequent subsurface heating (Seka *et al.*, 1994) and could

direct the energy towards the pulp (Koort and Frentzen, 1991;1992). Launey *et al*. (1987) confirmed that the pulsed Nd:YAG laser (200-2,000 J/cm^2) energy did not fuse the surface of enamel, but diffused to the pulp and caused pulpal overheating. They also concluded that Nd:YAG laser is the wrong instrument for the treatment of dental hard tissues.

The introduction of the pulsed infrared laser systems with pulse durations ranging within a few hundred microseconds (the typical time of flashlamps pulsed YAG lasers is 100-800 microseconds), has been claimed to provide a way for increasing the peak power density while keeping the pulse energy density constant. This would allow for tissue cooling and avoid heat expansion between bursts of laser pulses (Nelson *et al*., 1986), so that the laser effect is confined to the surface region without affecting the underlying dentine and the pulp (Leighty *et al*., 1991; Khosrovi *et al*., 1991).

For example, the pulsed Nd:YAG laser (dLase300) has a relatively low average power (0.3-3.0W), but can achieve peak power approximately 1,000W and create instantaneous surface temperatures in excess of

1,200°C, which is sufficient to cause localised ablation of organic tissue and melting of the inorganic surface of enamel and dentine (White *et al.*, 1992). The Er:YAG laser (200-400 microseconds pulse), in the presence of water, can ablate enamel effectively (Hibst and Keller, 1993) without causing a great rise in intrapulpal temperature (Hibst and Keller, 1990; Steven *et al.*, 1996).

Due to the fact that Nd:YAG laser is poorly absorbed by dental hard tissues absorption and has a pulse duration (100-150 microseconds) which is relatively longer than the thermal relaxation time of enamel (approximately 60microseconds) (Fried *et al.*, 1994), therefore, despite the pulsing ability of the Nd:YAG laser, the possible detrimental thermal damages to the dental hard tissues and the adjacent tissues still cannot be avoided (Kramer *et al.*, 1990; Koort and Frentzen, 1991; 1992).

The Nd:YAG laser radiation operating in a photodisruptive mode, for instance with mode-locked Nd:YAG laser, has very short pulse durations of some pisoseconds, which will cause an optical break-down of hard tissue in a nonthermal and nonlinear fashion, accompanied by shock wave and plasma formation (Koort and Frentzen, 1991). As a side effect,

the high pressure wave can cause cracks and microcracks and zone of debris at the cavity surface (Frentzen and Koort, 1990).

Yamamoto and Sato (1980a), Kimura *et al*. (1983), Tagomori and Morioka (1989), Oho and Morioka (1990), Myers (1990), Myers and Riddle (1990) and Hess (1990, 1991) painted absorption materials on the enamel surface enabling more efficient absorption of the laser's energy, enhancing the laser interaction with the enamel with low energy level. This energy level is far below the 4494 J/cm^2 level that Adrian (1977) reported was necessary to produce pulpal necrosis.

The morphologic changes of the coated enamel surface induced by pulsed Nd:YAG laser at low energy density (eg., 95 J/cm^2) were described as being many small bubble-like inclusions making up a pock marked surface with indistinct craters, accompanied with pitting and surface flaking (Myers and Riddle, 1990; Hess, 1990). At a relatively higher energy density (eg., 239 J/cm^2), the irradiated surface was described as being covered by multiple overlapping disk-shaped impact craters (Tagomori and Morioka, 1989) with the periphery raised and having the appearance of molten enamel, but round and glassy-like. In addition, the thin smooth fused surface layer sometimes flaked off, revealing the

underlying rougher enamel surface (Nelson *et al.*, 1986; Nelson *et al.*, 1987). The occasional higher peak power of the pulse produced the beaded enamel appearance which resulted from a superheated enamel surface that underwent subsequent cooling at room temperature (Arcoria *et al.*, 1991).

Besides crater formation, cracking (0.1-0.5 micron in width) occurred throughout the entire lased area, but did not extend into the adjacent unlased enamel. Cracking remained constant in quantity and width despite increasing energy density (Hess, 1990; 1991). The surface features such as cracks, surface flaking and craters typical of infrared laser irradiated enamel are explained by stresses in enamel due to expansion and contraction through localised heating and shock waves associated with the beam's interaction with the tooth (Ferreira *et al.*, 1989). Hess (1990) pointed out these bubble-like inculsions, cracks and fissure features produced on the enamel surface greatly increase surface area, and may possibly be used for mechanical retention for dental restorative materials.

4.1.1. Laser induced enamel resistance to demineralisation.

Stern and Sognnaes (1964; 1972) demonstrated, by means of ruby and CO_2 lasers, that the lased enamel surfaces were more resistant to

subsurface demineralisation than the unlased enamel. They also postulated that the mechanism behind the laser's ability to decrease demineralisation on lased enamel was due to the reduction of permeability through the fusion and sealing of its natural irregularities on the surface (Stern *et al.*, 1972). Since then, many investigators have attempted to transfer the result of these basic studies to clinical research to prevent dental caries (Kantola, 1972; Kantola *et al.*, 1973).

Yamamoto and Ooya, (1974) and Yamamoto and Sato (1980a;1980b) studied the Nd:YAG laser's ability to alter demineralisation of enamel and showed that the Q-switched Nd:YAG laser at low energy density of 20 J/cm^2 induced melting of apatite crystals with subsequent recrystallistion. This leads to the formation of other calcium phosphate phases within the melts; phases other than apatite (tetracalcium diphosphate monoxide that has reduced carbonate content) could be identified (Nelson *et al.*, 1987). This altered surface was less susceptible to demineralisation. They speculated that the resistance may be due to the physical alterations induced by the laser. Yamamoto and Sato (1983) in an *in vitro* study, further demonstrated that subsurface lesions caused by demineralising agents or artificial plaque could be prevented in areas of laser-treated enamel. This laser induced enamel acid resistibility is

dependant upon the laser absorption of the enamel (Morioka *et al.*, 1989). The resistance is increased proportionally with the energy density (Altshuler *et al.*, 1996) and this can be achieved by the combined effects of Nd:YAG laser radiation with fluoride (APF) application (Watanabe *et al.*, 1989). The optimum energy density seemed to be at 65J/cm^2 (Yamada, 1996). Morioka *et al.* (1989) observed that the acid resistance was dramatically higher only if the sample was irradiated prior to APF application, indicating that the laser-modified enamel has an enhanced uptake of APF. The use of low energy Nd:YAG laser (371-3785 J/cm^2) on an etched enamel surface can reduce the surface reflection and enhance laser absorption, causing reduction in the roughness of the surface enamel, as measured with a profilometer. They suggested that this alteration may be related to the increased enamel resistance to demineralisation (Quintana *et al.*, 1992).

Although there are several possible explanations, the reasons for the observed inhibition of carious lesion formation after laser modification are still not clear.

4.1.2. Laser-etching of enamel surface.

The morphological appearance of the enamel treated with the infrared lasers is that of melted, recrystallised or fused enamel crystal structure. The degree of physical changes in crystal size and shape that occurs as melting and fusion progresses is a function of surface temperature changes. Below the temperature threshold, the surface changes can be observed but have a non-uniform pattern (McCormack *et al.*, 1995).

When the CO_2 laser is used at low energy densities not exceeding 50 J/cm^2, it has the capacity to roughen tooth enamel in a manner similar to the acid-etch technique used in restorative dentistry (Stern *et al.*, 1972; vonLenz *et al.*, 1976; Nelson *et al.*, 1986; Featherstone and Nelson, 1987; 1989; Liberman *et al.*, 1984).

It has been demonstrated that pulsed Nd:YAG laser, at low energy density, (range between 95-239 J/cm^2) on photoabsorbing dye coated enamel surface, can produce numerous microcavities and microfissures (Hess, 1990), and also can induce subsurface roughening with partitions up to 10 micron in height (Hess, 1996) which may provide space for the retention and penetration of dental resin. White *et al.*, (1991e) found *in vitro* that Nd:YAG (186 J/cm^2) etching of coated enamel improves bond

strength to metal orthodontic brackets. Roberts-Harry (1992) in a clinical study, has reported that Nd:YAG ($103J/cm^2$) etching for orthodontic bracket placement took considerably longer time (10 times), was less reliable in terms of bond strength, and produced more discomfort than conventional acid etching. Dederich and Tulip (1991) investigated the use of the Nd:YAG laser to modify dentine as a pretreatment for bonding efficiency. They found that the bond strength was less with the laser than without. Arcoria *et al*. (1991) have demonstrated by using profilometery that the coaxial use of CO_2/Nd:YAG laser can produce more profound enamel surface roughness which is comparable with the acid-etched surface.

4.2. Neodymium:YAG laser effects on dentine.

The effects of the Nd:YAG wavelength on dentine have not been fully defined. In theory, laser energy can be used to alter the structure and chemistry of dentine surfaces (Featherstone and Nelson, 1987). It has been reported that when dentine is irradiated with the Nd:YAG laser energy, depending on the power (Dederich *et al*., 1984; Featherstone and Nelson, 1987) and energy density (White *et al*., 1993; Altshuler *et al*., 1996), it may cause either no effect on dentine or melting with

subsequent recrystallisation into a nonporous and continuous "glazed" surface which may partially or totally obliterate dentinal tubules (Dederich *et al.*, 1984; Dederich *et al.*, 1988; Khayat *et al.*, 1991). This surface appearance can conceivably demonstrate reduced permeability to fluids and can explain the mechanism of laser induced desensitisation.

White *et al.* (1991d) in an *in vitro* study, investigated the effects of the pulsed Nd:YAG laser on dentine and demonstrated that laser energy causes dentinal surface roughening and partial closing of the dentinal tubules. The greatest amount of surface modification and occlusion of the dentinal tubules was observed at the highest power setting (greater than 1W, 10Hz) and longer exposure time (ranging between 30-240sec). There was a trend towards a decrease in hydraulic conductance at higher powers and longer exposure time (50% reduction in hydraulic conductance occur at levels of 2W and higher). They also postulated that the fluid within dentinal tubules contains proteins which could be coagulated within the tubules by the laser energy.

Dentinal permeability is defined as the ability of fluids and substances to move through dentine. The smear layer is a mineralised dentine matrix which adheres tenaciously to the dentine surface and is produced whenever dentine is cut (Eick, 1992); it can be quickly degraded by acids

(Pashley, 1984). There is evidence to show that the presence of a smear layer on cut dentine can reduce dentine permeability (Pashley *et al*., 1984; Pashley, 1981; 1984). When there is an exposure of dentine or opening of dentinal tubules as in cutting a cavity, or any factors which might lead to a potential increase in dentinal permeability, it will lead to an increase in dentine sensitivity. (Pashley, 1986, 1990; Brannstrom *et al*. 1968, 1991).

Lilja (1979; 1980) has reported that there are differences in sensitivity between crown and root dentine. These differences may be attributed to several factors. In the root there is a lower density of tubules as well as nerve fibres, including intratubular fibres, than in coronal dentine. Additionally, in the root fewer fibres are intimately associated with odontoblasts and there is more evidence of nerve degeneration.

Stabholz *et al*. (1992) and Miserendino *et al*. (1995) in *in vitro* studies, investigated the effect of Nd:YAG on dentinal permeability, by assessing the amount of 5% methylene blue dye penetration on the root dentinal wall, and reported that the application of Nd:YAG laser reduced the permeability of root dentine. Forrest-Winchester and Walsh (1993) in an *in vitro* study, measured the radioactivity of the thymidine's eluate

content before and after lasing, and demonstrated that pulsed Nd:YAG laser (70mJ/pulse, 10Hz., 84J/cm^2, for 2min) irradiation can effectively (96% of cases) reduce fluid flow through dentine. Goodis *et al*. (1992) in study using fetal calf serum to simulate dentinal fluid concluded that the pulsed Nd:YAG laser is effective in reducing dentine permeability. They also found that this laser-induced reduction of dentinal permeability increased as the firing frequency of the laser increased.

Liu and Lan (1996), in an *in vitro* study of human extracted teeth, have demonstrated under Scanning Electron Microscopy, that the application of pulsed Nd:YAG laser even at a very low energy output of 30mJ/10pps for 2min on root dentine (with cementum being removed) with exposed tubules, can cause melting of dentine and closure of tubules up to 2-3micron depth without surface cracking. Also, the lased layer resisted abrasion by electrical tooth brushes.

The combinations of changes in dentinal permeability, protein coagulation within tubules and dentine surface modification by the laser energy, may explain the clinical result of temporary desensitisation of dentine.

White *et al*. (1993) pointed out that the Nd:YAG laser imposed changes on dentine; increases with the increases in pulse energy and energy density, and also as the inherent laser wavelength increases. They also defined the energy threshold for dentine physical modification from single pulses of Nd:YAG laser as 167mJ energy per pulse with energy density of 207J/cm^2, using a 320micron contact optical fibre. White *et al*. (1994) reported that in the presence of both water and air cooling, pulsed Nd:YAG laser with energy of 2W and 10Hz., has the ability of ablating dentine (rate of 103micron/sec) without causing carbonisation.

Recent studies demonstrated the free-running pulsed Nd:YAG laser has the potential to sterilise the root canal (Hardee *et al*., 1990) and seal the dentinal walls (Miserendino *et al*., 1995), to produce a pressure wave effect when interacting with the dentinal wall in the presence of water medium (Miserendino and Miserendino, 1992) and to enhance the debridement and sterilisation of dentine (Levy *et al*., 1993).

However, the effects of the Nd:YAG wavelength on dentine are unreliable and inconsistent from one location to the next due to the presence of intrinsic variations, such as the pigment variations (Dederich, 1993), different dentine types (Koopah *et al*., 1996), difference tubular

diameters (Pashley, 1990), tubule density (TenBosch and Zijp, 1987) and nerve fibre (Lilja, 1979; 1980) distributions. These laser induced dentinal changes are further complicated by the variation in peak power of each burst of laser pulses and this can contribute to the irregularity of lasing (Dederich *et al*., 1984). Moreover, the presence of charred-dentine which acts as a photoabsorbing substance, can alter the surface absorption and lead to inconsistency (Cernavin, 1995).

4.3. Neodymium:YAG laser effects on the pulpal integrity.

The tissue interaction of infrared lasers is caused by thermal conduction and convection of heat (Koort and Frentzen, 1991;1992). Therefore, one of the main concerns is the effect of infrared lasers on the pulpal tissue and the adjacent tissues. Usually, continuous-wave lasers and pulsed lasers with pulse durations in the microsecond range generate considerable heat in the region of the pulp chamber during the irradiation process (Stern and Sognnaes, 1972; Frame, 1985). This is because, during pulse duration, heat diffusion plays an important role in this type of thermal inducing laser-tissue interaction (Boulnois, 1986), especially when the interaction times are in excess of the thermal relaxation time for the dental hard tissue.

Myers and Myers (1985a), demonstrated that a free running pulsed Nd:YAG laser (The American Dental Laser) can effectively remove and sterilise carious lesions that had not yet penetrated the dentino-enamel junction. White *et al.* (1993), in a three-year follow-up clinical study of after patients who received caries removal and restoration, has confirmed the pulpal integrity of the lased teeth is unchanged.

McCarthy (1990), investigated the vitality of lased teeth clinically by measuring the electrical pulp testing readings before and after each laser treatment with pulsed Nd:YAG laser. He suggested that treatment with the pulsed Nd:YAG laser clinically would not compromise the pulpal vitality of the treated tooth.

Despite the amount of published information on the clinical application of the pulsed Nd:YAG laser in dentistry (Myers and Myers, 1985b; Myers and McDaniel, 1991; Myers *et al.*, 1992; Hardee *et al.*, 1990; White *et al.*, 1991c; Yamamoto and Sato, 1980) the degree of pulpal changes (ie. the overall viability of the odontoblastic layer and the intrapulpal cellular structure) subsequent to surface irradiation and the intrapulpal heating are ongoing concerns of researchers; specifically, the effects of prolonged

elevation of pulpal temperature during pulsed Nd:YAG laser irradiations, including when used for the clinical induction of pulpal anaesthesia.

4.3.1. Laser induced pulpal warming.

Wavelengths greater than approximately 390nm (Adrian, 1977) and/or with pulse widths greater than 350 microseconds (Lin *et al.*, 1992) have been shown to possess inherent heat-producing characteristics and to cause pulpal warming (Serebro *et al.*, 1987).

There is little possibility for the pulp to conduct heat significantly to dentine and enamel, although the mineralised structures act poorly as heat sinks. A rise in pulpal temperature results in a hyperaemic reaction of the pulpal blood flow (Hibst and Keller, 1990). This intensified blood circulation is reversible if the intrapulpal temperature rise ranges between 6-12°C. If more than 12 °C rise, an irreversible pulp necrosis occurs (Keller and Hibst, 1991).

Until recently, the enamel prisms and the intertubular substances of dentine were considered as a system of tightly packed quasi-colinear optical waveguides. These tissue-waveguides are non-uniform in nature, but can transport light energy from the enamel and dentine surfaces

directly to the dentino-pulpal junction. The difference in the surface area between the enamelo-dentinal junction and the pulp chamber surface causes a light field compression effect, which create an increase in light flux density whenever light is propagated from the enamel to the pulp (Altshuler *et al.*, 1991). Based on this hypothesis, Altshuler *et al.* (1992) developed a thermophysical model to correlate the hard tissue ablative effect and the pulp chamber overheating with different laser irradiations. Using this model, Altshuler *et al.* (1993) demonstrated that pulsed Nd:YAG laser has a very shallow processing depth on enamel before its radiation caused pulp overheating.

Previous animal studies have demonstrated that pulpal tissue cannot survive in elevated intrapulpal temperatures above 5°C for long time periods when irradiated with visible and infrared lasers (Stern *et al.*, 1969; Adrian *et al.*, 1971; Serebro *et al.*, 1987; Melcer *et al.*, 1985; Renneboog-Squilbin *et al.*, 1989; Arcoria *et al.*, 1991).

Because the odontoblasts are highly differentiated cells which form dentine, normal odontoblastic cellular structure and function can be adversely affected by the elevation of intrapulpal temperature (Zach and Cohen, 1962; Zach, 1973).

Miserendino *et al*. (1989) in an *in vivo* study, have demonstrated that the odontoblastic layer may be destroyed if the pulpal temperature is raised beyond 5°C level. According to the *in vivo* study by Zach and Cohen (1965), an increase in intrapulpal temperature below 2.2°C was observed to fall within a safe range of thermal effects, whereas a rise in pulpal temperature of 5.5°C caused pulp necrosis 15% of the time. Temperature increases of 11°C and 16°C resulted in pulpal necrosis 60% and 100% of the time, respectively. However, their experimental method of continuously applying heat by a hot soldering iron to the crowns of monkey teeth for a length of time, is not comparable to the methods used to measure the temperature changes induced by laser irradiation, which takes into account the energy density, the pulsing ability of the laser source and the tooth thickness.

Allen (1993) in an *in vitro* study measured the temperature changes during etching of dental enamel with pulsed Nd:YAG laser for 12sec on extracted human teeth, with energy between 1-3W, by placement of thermocouples at the dentinal pulp opposite the irradiation site through an occlusal opening. He concluded that the rise of intrapulpal temperature at this power level appeared to be of sufficient magnitude to cause at least localised pulpal inflammation and possible irreversible pulpal damage.

White *et al.* (1991d), in an *in vitro* study, measured the pulpal temperature changes during pulsed Nd:YAG laser irradiation, using a 200 micron non-contact fibre, at 5mm from the coronal dentine surface. They concluded that for lasers with power of 1W or less the pulpal temperature rise was less than 6°C, for up to 120sec of laser treatment, when the remaining dentine thickness was 1mm or greater. Three years later, White *et al.* (1994) re-examined *in vitro* the rise in intrapulpal temperature on a much smaller surface area of $2mm^2$, but on root dentinal surface, using the same laser with a 320 micron contact fibre instead. They drew a similar conclusion except that the temperature rises were within the thermal safety limit when the laser power was within 1W, 10Hz and the lasing time was less than 30sec, with 2mm remaining dentine thickness.

Levy (1991) proposed the concurrent use of a coolant (air and water mixture) at adjustable pressures to control the elevation of temperature during pulsed Nd:YAG laser application and found it can provide adequate heat protection to the pulp; equivalent to that of the dental drill (Miserendino *et al.*, 1993; Abt *et al.*, 1992). Rizoiu *et al.* (1994) have demonstrated that even at laser power of 3-4 times higher than 3W, the

rise in the intrapulpal temperature is limited to 2.5°C, as compared with 12°C without air-water-cooling. In a histological study, Miserendino *et al*. (1994) confirmed that the use of such a concurrent cooling with the pulsed Nd:YAG laser irradiation on enamel, with an exposure of 12W for 5sec, caused no damage to the pulp.

4.3.2. Laser effects on pulpal histology.

Adrian (1977) was the first to study the effects of Nd:YAG laser exposure on dental pulp. He reported that above the threshold energy density of 4494J/cm^2, coagulation necrosis of the pulpal content occurred. This energy level was 2-3 times more intense than other types of lasers studied and the resultant pulpal damage was limited to the area of irradiation. The pulpal effects induced by the Nd:YAG laser were found to be much less severe than those with the ruby laser (Adrian *et al*., 1971). From these results Adrian concluded that the pulp was more resistant to injury by the Nd:YAG laser than by the ruby laser.

In animal studies (Ohkubo and Yamamoto, 1981; Melcer *et al*., 1985), it has been reported that Nd:YAG irradiation can stimulate calcified tissue formation and induce reparative dentine formation in rats. This

phenomenon of Nd:YAG laser induced pulpal calcified tissue formation in rats has been demonstrated (Shigeru and Hiroshi, 1989) to be related to the increase of alkaline phosphatase activity after laser irradiation. However, Arcoria *et al.* (1991; 1994), studying laser induced dentinogenesis, pointed out that the reparative dentine formation is a natural phenomenon in rat pulp due to occlusal wearing. Thus, the search for new dentine formation has not always been positive in rat studies with laser treatment.

Recent histological evidence suggests that the overall viability of the odontoblastic layer and intrapulpal cellular structure (that is the presence of odontoblastic nuclei, normal odontoblastic layer without disruption, viable stroma and contiguous cell matrix etc.), in conjunction with the degree of new dentine formation, is a more reliable indicator of pulp response to laser irradiation (Arcoria *et al.*, 1991; 1994). Furthermore, these responses can be used to determine the damage threshold energy densities following laser irradiation (Arcoria and Miserendino, 1995).

Because the potential for transmission through enamel and dentine into the pulp and the potential for inducing heat production are dependent on the overall interaction with the tissue with darkened pigmentation, the application of the 1064nm Nd:YAG laser may have somewhat limited

indications at higher pulse energy (Arcoria *et al.*, 1994) and higher energy density (Koort and Frentzen, 1992).

The pulsed Nd:YAG laser, at low energy densities not exceeding 50 J/cm^2, and at high-repetition rate has been shown to remove carious tooth structure utilising a photoabsorbing effect in the discoloured lesion (Arcoria *et al.*,1992) and to remove calculus (Arcoria and Vitasek-Arcoria, 1992) without the degree of pulpal damage exhibited at higher energy levels (Arcoria *et al.*, 1994).

In an *in vitro* study on freshly extracted human third molars, White *et al.* (1991d) demonstrated that exposure of enamel or dentine to pulsed Nd:YAG laser, with power up to 2W, 20Hz, energy density up to 318 J/cm^2 (200 micron fiber) and total exposure up to 240J and 120 second, with remaining dentine thickness more than 1mm, caused no significant immediate pulpal disruption.

Similarly, Goodis *et al.* (1992), in an *in vivo* study, examined the pulpal effects of the pulsed Nd:YAG laser (360J) applied to enamel of third molars, for up to 120sec with energy density of 124 J/cm^2 and remaining dentine thickness of 2.8$\pm$ 0.3mm, at one week and one month after laser treatment. They found that it is possible to use a pulsed Nd:YAG laser on

enamel without affecting the pulp. Later, White *et al*. (1994), in an *in vivo* clinical study confirmed that the pulpal response of pulsed Nd:YAG laser exposure on dentine during caries removal, with power less than 2W at 10Hz, with energy density less than 165 J/cm^2 (300 micron fiber), exposure up to 240J (120 sec) and remaining dentine thickness greater than 2mm, caused no immediate or late (one week) adverse histological pulpal effects.

Parkins *et al.* (1991), in an *in vivo* histological study of the pulpal response of pulsed Nd:YAG laser exposure on premolar teeth, at 2 day, 5 day, and one week after clinical induction of pulpal anaesthesia (with energy density of 62J/cm^2 and total energy dose of 180J) and cavities cut 2mm into the dentine and filled, found no pulpal changes. Later, Parkins *et al.* (1992), investigated the histological pulpal response to various energy dosages of the pulsed Nd:YAG laser exposure on premolar teeth and reported that the threshold energy density of 124J/cm^2 (1.5W, 15Hz), with total energy dose of 360J (for 240sec), caused no variation in vitalometer readings and there was normal pulpal histology.

4.4. Laser induced pulpal anaesthesia.

Pulsed Nd:YAG laser induced anaesthesia is one of the therapeutic applications in clinical dentistry which has been claimed to offer a painless, simple and safe alternative in managing needle-phobic and paediatric patients (Nagasawa, 1988; Myers and McDaniel, 1991; Parkins and Miller, 1991).

The anaesthetic effect of Nd:YAG laser during dental treatment was first reported by Nagasawa in 1984. He described a technique where a patient's tooth crown was lased repeatedly with continuous Nd: YAG laser at a power density between 100-200mW/cm^2, under the pain threshold. The tooth became insensitive, resulting in surgical analgesia for dental drilling in more than 95% of cases, without causing pain or any observable histological damage to the pulp. Under photomicrographic analysis, the dentinal tubules in the surface layer were observed to have disappeared. He postulated that such an obliteration presumably blocked the conduction of sensory impulses to the pulp.

In recent years, the introduction of a free running pulsed Nd:YAG laser (American Dental) in dentistry, was claimed to have the ability to induce surgical anaesthesia for cavity cutting without resorting to supplementary local anaesthesia (Myers and McDaniel, 1991). Myers and McDaniel

(1991), described a procedure which requires lasing the entire crown surface of the tooth in a continuous flowing motion for 120-240sec, at a power setting between 0.75-1.5W (energy density range between 62-124J/cm^2). The duration of the pulpal anaesthesia following such a procedure varied from 10-60min, and seemed to be more effective on teeth where the dentinal thickness is less than 2mm. In addition, the anaesthetic effect couls also be extended by relasing.

Parkins and Miller (1992) in a clinical study with premolars that were extracted for orthodontic reasons, demonstrated that the sensitivity of a tooth to a cutting instrument can be eliminated with exposure to pulsed Nd:YAG laser energy of 0.75W, 15Hz. (energy of 62J/cm^2) for 240sec over the entire coronal pulp. Interestingly, electric pulp test readings (Analytic Technology Vitality Scanner) did not vary significantly from pretreatment values recorded. They suggested the effect of laser energy is confined only to the dentine.

Matsumoto (1994) investigated the anaesthetic potential of this laser, with a much higher energy setting of 2W and 20Hz, but varied the energy density by keeping the laser about 5cm or more away from the tooth surface without producing pain sensation. The exposure time was 120sec/surface on both buccal and lingual or palatal surface of the

coronal portion of the dental pulp. He reported clinical effectiveness of pulsed Nd:YAG laser induced surgical anaesthesia allowing cavity or crown preparation without pain in 60% cases investigated.

4.4.1. The mechanisms of laser induced pulpal anaesthesia.

The mechanisms involved on the so called " Laser analgesia in lieu of anaesthesia" is not well understood. This is partly because the mode of conduction of impulses from dentine to pulpal nerves, in spite of various theories and scientific findings is not clearly known (Brannstrom *et al.*, 1967; Anderson *et al.*, 1970; Dubner *et al.*, 1978).

Matsumoto and co-workers have studied the gallium-aluminium-arsenide lasers which emit radiation with a wavelength near to that of Nd:YAG lasers (904nm vs 1060nm, respectively) and reported it to be effective for desensitisation (Matsumoto *et al.*, 1985). The mechanisms for desensitisation were thought to operate at the level of the pulp (Wakabayashi *et al.*, 1992; 1993), but not at the level of the exposed dentinal tubules (Sato *et al.*, 1989; Hoji, 1990). A greater degree of effectiveness of pain reduction was found with Nd:YAG laser irradiation when compared with GaAlAs laser in dentine hypersensitivity.

Matsumoto (1994) postulated the pain reduction with pulsed Nd:YAG laser in treatment of dentine hypersensitivity (Matsumoto *et al*., 1989; Midda *et al*., 1992) was due to the anaesthetic effect of the laser. That raises the important possibility that pulpal nerves or cells may be influenced directly by Nd:YAG energy irradiation (Forrest-Winchester and Walsh, 1993).

Funato *et al*. (1991) investigated *in vivo* the effects of Nd:YAG laser on the microcirculation, using the "rabbit ear chamber" method, and demonstrated that low energy of Nd:YAG irradiation (<40J/cm^2) can cause dilatation of arterioles and transient increase of blood flow, but has no observable morphological changes in the microcirculation. They concluded that the low Nd:AYG energy irradiation may have anaesthetised the nerve fibres innervating the wall of the arterioles.

White *et al*. (1993) in an *in vitro* study removed the smear layer from the cut dentine surface and demonstrated that the pulsed Nd:YAG energy threshold for morphological change in dentine is above 207 J/cm^2, with a 0.3mm fibre. Similarly, Altshuler *et al*., (1996) *in vitro*, investigated laser induced modification peculiarities of enamel and dentine surfaces and reported that with energy density between 20J-200 J/cm^2 can attenuate the dentinal canals.

Even though the energy density (range of 62-124J/cm^2) being used clinically for laser induced anaesthesia (Myers and McDaniel, 1991) is well below the energy density threshold (207 J/cm^2) for dentinal modification (White *et al.*, 1993), the laser energy may be enough to reduce dentine permeability (Forrest-Winchester and Walsh, 1993; Stabholz *et al.*, 1992; Miserendino *et al.*, 1995). This is possibly through photocoagulation of proteins within the tubules (White *et al.*, 1991d) and it may also cause some attenuation and obturation of dentinal tubules (Liu and Lan, 1996; Altshuler *et al.*, 1996).

It is apparent that other mechanisms may also be operating beyond the level of the dentine (Parkins and Miller, 1992) in laser induced pulpal anaesthesia.

Studies that investigated the responses of intradental nerves to pulsed Nd:YAG laser energy in animals, have claimed that pulsed Nd:YAG laser energy either irradiated on the whole crown (Suda *et al.*, 1996) or on exposed dentine (Orchardson and Whitters, 1996; Friedman *et al.*, 1994), can elicit concurrent activity of intradental nerve fibres (A-delta and C-fibre). In addition, it can suppress intradental nerve (A-delta fibre) responses to mechanical stimulation, but not to electrical stimulation

(Friedman *et al*., 1994) after lasing. The level of anaesthesia can be increased with increased laser energy dosage (Orchardson and Whitters, 1996), but the doses required to achieve this may cause damage to the nerve (Orchardson *et al*., 1994) and the pulp (Suda *et al*., 1996).

Saski *et al*. (1996) in an *in vitro* study investigated the optical properties of freshly extracted human pulp at different laser emission wavelengths, using an integrating sphere spectrophotometry and reported that at the emission wavelength of Nd:YAG (1064nm), less than 1% is absorbed, 87% is transmitted, and 31% is reflected by the human pulp. Hence the poor absorption of the Nd:YAG energy by the pulp and enamel coupled with high scattering in dentine, raises the possibility that the distribution of the Nd:YAG laser energy within a human tooth may be preferentially at the dentino-enamel and dentino-pulpal interfaces. This preferential deposition of energy not only may cause subsurface heating and pulpal warming, but also suggests that the mechanisms of laser inducing anaesthesia may operate within the neuro-odontoblastic complex at the dentino-pulpal interfaces.

Early studies (Frank, 1966; 1968; Arwill, 1967; 1968) have disclosed the existence of bare nonmyelinated intradentinal nerves which lie in close relationship to the odontoblastic process, in the pulpal third of the human dentine. In addition, structures suggestive of synaptic or attachment sites

have been recorded (Bennett and Goodenough, 1978; Larsen, 1977) in the neural-odontoblastic complex, and it has been postulated that odontoblast has receptor activity (Anderson *et al.*, 1970; Dubner *et al.*, 1978).

In a morphological study of dentinal nerve endings, Ochi and Matsumoto (1988) examined the relationship between pulpal nerves and odontoblastic processes in human teeth using Scanning Electron Microscopy, on an ultrathin-frozen section, and observed a space of about 20nm between nerve fibres and odontoblastic processes with no special structures between them. They postulated that a morphological change such as momentary swelling or contraction of the odontoblastic process occurs when a stimulus is applied to the dentine, and the resulting stimulation is transmitted to the nerve fibres and is felt as pain. They also found the presence of coated pits on odontoblastic processes at points of close proximity to the nerve fibres, and hypothesised that some type of substance transfer was involved in the stimulus transmission mechanism. It is believed that coated pits are involved in endocytosis and exocytosis, in the absorption and secretion of proteins and other electrolytes and ions, and in the secretion of neurotransmitters at

synapses between the nerve fibres and odontoblastic processes (Ritch and Philpott, 1969).

4.4.2. Pulpal innervation within the dental pulp.

Pulp innervation includes afferent neurons which conduct sensory impulses as well as efferent sympathetic fibres which provide neurogenic modulation of pulpal blood flow (Dorscher and Kim, 1990).

Sensory fibres of the pulp consist of myelinated A-fibre (A-beta and A-delta) and unmyelinated C-fibre (Byers, 1984; Johnsen, 1985; Hirvonen, 1987; Nair *et al.*, 1992) Nerve fibres are classified according to their diameter and conduction velocities.

The myelinated A-beta fibres have a larger axon diameter (>5micron) and a faster conduction velocity (of >30 m/s), compared with A-delta fibre, which have an average 3.5micron diameter and less than 30m/s conduction velocity. The unmyelinated C fibres have an axon diameter of less than 0.5micron and conduction velocities of less than 2m/s (Narhi, 1985; Nair *et al.*, 1992; Virtanen, 1991). Each of these fibres play a different role in the experience and perception of pain.

A-beta fibre can respond to vibration and are stimulated at a lower electrical threshold (Dong *et al*., 1985). A-delta fibre (93% of the pulpal myelinated fibers) are activated by hydrodynamic stimuli, such as drilling, air, cold, sweetness and rapid heat, which leads to rapid fluid movement within the dentine tubules (Narhi *et al*., 1982; Ahlquist *et al*., 1984; Brannstrom, 1982; 1986; Brannstrom and Astrom, 1972; Narhi *et al*., 1982), and which stimulates the mechanosensitive nerve endings located on close proximity to the odontoblast cell body (Nair, 1993; Trowbridge, 1986) and at the pulp-dentine border (Jyvasjarvi, 1986; Jyvasjarvi and Kniffki, 1987).

4.4.2.1. Thermal transmission.

Stimulus-evoked pain can occur when the stimulus is of sufficient intensity. When a thermal stimulus is applied to a tooth, the heat is transported through enamel and dentine by conduction. Temperature distribution in the tooth is governed by the thermal diffusivity of the dental tissue, which is a function of the thermal gradient and the tooth thickness (Mumford, 1979). It has been shown that diffusibility of enamel and dentine is relatively low (Braden, 1976). A crucial factor in thermal stimulation of teeth is the rapidity with which the temperature change

occurs, as slow heating of the tooth does not produce a response (Ahlberg, 1978; Narhi *et al*., 1982d).

The response of pulp nerves to heat seems to be two-phased, with an immediate A-fibre response followed by a C-fibre response, usually 10-20 second later (Narhi *et al*., 1982d).

The sensory response to thermal stimulation occurs before there is a temperature change in the region of the pulpo-dentinal junction where the sensory nerve endings are located. A rapid temperature change causes either a thermal expansion or contraction of dentine; this could produce a narrowing or widening of the dentinal tubules, and an increase or a decrease in the volume of fluid within the dentinal tubules and thus contribute to the hydrodynamic forces produced within the dentine (Brannstrom and Astrom, 1972; Horiuchi and Matthews, 1973). This fluid flow results in activation of the mechanosensitive nerve endings at the pulpo-dentinal junction (Brannstrom *et al*., 1976; Brannstrom and Johnson, 1970). The nerve fibres that are activated seem to be the A-fibre; pulpal C-fibre do not respond to heat until a considerable change in pulp temperature has occurred (Narhi *et al*., 1982b; Narhi and Haegerstam, 1983; Trowbridge *et al*., 1980; Trowbridge, 1985). On the other hand, some responses of A-fibre to heat seem to be a result of direct irritation of the nerve axons (Matthew, 1977). Consequently, it

seems that several activation mechanisms of pulpal nerves in response to heat stimulation may function.

It seems that heat stimulation of human teeth is capable of producing two different types of pain; initial sharp, localised pain followed by a dull poorly localised pain and mediated by the fast and slowly conducting interdental nerve fibres (Narhi *et al*., 1992). Hensel and Mann (1956) showed that sharp pain was perceived when the temperature at the enamelo-dentinal junction had been elevated to 48°C, and in the pulp only 0.6°C. With greater elevation in pulp temperature a dull and poorly localised pain sensation was induced.

Narhi *et al*. (1982), using a dentinal temperature recording technique, have recorded the mean threshold temperature, in the cat, to be 43.8 $\pm$ 3.4°C (nerve unit with conduction velocity less than 3.5m/s). The estimated threshold temperature is not absolute due to variation on the sites of measurement and applied stimulation; therefore this temperature is not decisive.

4.4.2.2. Electrical transmission.

When monopolar electric stimuli are applied to teeth the current is mainly along the path of least resistance. Electrical stimuli are amplified by the current being preferentially conducted along the dentinal tubules and the line of any nerve fibre rather than through the more solid intertubular part of the dentin, so the current density is greater at the pulpo-dentinal junction than might be expected (Munford, 1979). A minority of the current also passes through the apical foramen and outwards through the periodontal membrane (Suzuki, 1941; 1953). The threshold voltages required to activated C-fibre are three times higher than that of A-delta fibre (Newton and Munford, 1972; Anderson and Pearl, 1975).

When an electrical stimulus is applied as in clinical Electrical Pulp Testing, only the A-delta fibers are normally activated (Fuss *et al.*, 1985; Trowbridge, 1985). Therefore it provides information solely related to the activation and competent functioning of A-delta fibre and no information regarding C-fibre or about the blood supply to the pulp tissue.

CHAPTER II EXPERIMENTAL STUDIES

1. Pulpal anaesthesia induced by Nd:YAG laser and EMLA 5% topical anaesthetic cream. Part I: A randomised, double-blind, comparative study.

1.1. Introduction and aims

"After exposure to Nd:YAG laser energy, the tooth became insensitive" the resulting surgical anaesthesia allowed tooth cutting with no pain and without the need for supplementary local anaesthesia (Nagasawa, 1984). This phenomenon offers a therapeutic application of the Nd:YAG laser in dentistry in managing paediatric patients and patients who suffer from needle-phobia and haemophilia (Myers and McDaniel, 1991).

The effectiveness of the clinical induction of surgical anaesthesia by pulsed Nd:YAG laser energy have been reported by a number of authors (Nagasawa, 1984; Parkins and Miller, 1992; Matsumoto, 1994). However, the results that have been reported from these studies varied from 60% (Matsumoto, 1994) to 95% (Nagasawa, 1984) of success. This inconsistency is partly due to the variation of the methods used in assessing the degree of surgical anaesthesia, and partly due to the

variation of energy dosages (per irradiated surface area) that were used. There has been also a lack of control to of the placebo effects in these studies.

The purpose of this study was to investigate and quantify the clinical anaesthetic potential of Nd:YAG laser by comparing it with a potent topical anaesthetic, EMLA 5% cream, on premolar teeth with single or double roots, from maxilla or mandible, in a paired, double blind, randomised manner.

1.2. Materials

a) Study group

Prior approval from the relevant Ethical Committee and consent from the volunteer/parent/guardian were obtained. Forty four subjects, with a male to female ratio of 1:1, with an age range of 14-18 years, requiring extraction of bicuspid teeth for orthodontic reasons, formed the study group. Subjects were in good health and had no pre-existing history of any allergy to local anaesthetic agents. Those with pacemakers, hearing aids and other attached electronic devices were excluded. The teeth undergoing investigation were vital, restoration and caries-free, and in good periodontal heath.

b) Equipment

i) The pulsed Nd:YAG American Dental Laser [1] (model, dLase300) was used in this study. It has an emission wavelength of 1064nm and a pulse width of 150 microseconds. It is equipped with a 320micron contact fibreoptic delivery system and a He:Ne aiming beam (P1-1). Appropriate Laser protective goggles and head phones were also utilized.

ii) Electric pulp tester[2] (Analytic Technology, USA). The instrument has a display counter that indicates a scale from 0-80 and a rate control dial which is set at 1. It provides an electrical stimulus which increases from 0-80 (15-300volts) over a period of 48secs (P1-2).

iii) A power meter [3]- Ophir model S/N 7523, Head: 30A150 (P1-3) to measure the average power output at the fibre end before every application of the laser.

iv) EMLA[4] 5% topical anaesthetic cream (P1-4) which is Eutectic Mixture of lignocaine (2.5%) and prilocaine (2.5%) Local Anaesthetics.

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[1] Sunrise technologies Inc., California, USA.

[2] Analytic Technology, USA.

[3] Ophir Optics, Jerusalem, Israel.

[4]Astra Pharmaceuticals, NSW, Australia.

When lignocaine and prilocaine bases are combined in eutectic form, the melting point of the mixture is lowered and this allows the anaesthetic agents to form an oil at oral temperature and consequently facilitates increased mucosal absorption of the local anaesthetic agents.

v) Highspeed drill (speed of 200,00 rpm or above) with water and air cooling plus a flat fissure diamond bur to prepare the standardised Class V cavity of 2mm diameter and 1mm depth (P1-5).

vi) Placebo[5] EMLA cream (P1-6) and Orahesive bandage[6] (P1-7).

1.3. Methods

The study was random, double-blind in design with the subject unaware of the test agent, and an Electrical Pulp Tester, providing a stimulus which was free of operator influence. The selection of the type of laser emission was controlled by a person other than the operator, using a foot-pedal.

In each subject, a pair of 1st or 2nd premolars, of the mandible or maxilla, were randomly selected to receive a combination of test agents, namely EMLA 5% cream and He:Ne aiming beam or placebo cream and pulsed

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[5]Astra Pharmaceuticals, NSW, Australia.

[6]Convatec, Victoria, Australia.

Nd:YAG laser (American Dental). The degree of anaesthesia was estimated by applying an electrical stimulus using the Electric Pulp Tester (EPT) and by cutting of a standardised Class V cavity at the centre of the buccal surface with a bur at highspeed. In all cases, the assessment was terminated when the sensitivity was first felt and the sensitivity experienced was immediately recorded using a visual analogue scale (0-100mm). The application of test agents, the use of Electrical Pulp Testing and the cavity preparation were carried out by different person in order to maintain the double blindness of the study.

a) Each subject was dressed up with a pair of ear headphone (without sound) and a pair of laser protective goggles to maintain double blindness and for laser safety. The subject was asked to indicate by raising his or her hand when the sensitivity was first felt.

b) A baseline Electrical Pulp Testing measurement (P1-8) was taken on the selected tooth before the application of the paired agents. The centre of the buccal surface of the tooth was selected as the area for Electrical Pulp Testing.

c) After the buccal sulcus was dried, a 0.5 mg of EMLA 5% or placebo cream in a syringe was applied to the buccal sulcus adjacent to the test

tooth (P1-9) and was secured and isolated with an Orahesive bandage. The agent was allowed to stay for 20 mins.

d) After 15 mins of placement of the EMLA or Placebo cream, the Laser (pulsed Nd:YAG) or the sham treatment (He:Ne aiming beam only) was applied in a roving contact motion, on the entire cervical halves of both buccal (P1-10) and lingual/palatal surfaces (P1-11) for 120sec/surface, with a 320 micron fibre. The mean power was 1.12W (range: 0.88-1.3W), at 15Hz, the energy density ranged between 73-107J/cm^2 and total energy dose ranged between 106-156J/surface.

e) Electrical Pulp Testing reading was taken almost immediately after Laser or the sham application.

f) Five mins later, a standardised Class V cavity was prepared at the centre of the buccal surface, with a rotary highspeed handpiece, under proper water and air coolent. The cavity preparation was terminated when the subject first felt sensitivity and a pain score was obtained. Then the procedure was repeated on the tooth from the contralateral side.

1.4. Results

The complete experimental data from EMLA and Laser group are listed in Appendix I.a. and Appendix I.b. respectively. Results showed a mean increase of EPT readings of 11.7 unit for EMLA group and 10.1 unit for

Laser group. The mean EPT change in relation to jaw and root types are presented in table (Table 1.a) and in graph (Fig.1a). Using 2-samples t-test, there was no significant association between jaw or root types and the EPT rise after EMLA application, whereas in Laser group, the rise in EPT readings were significantly higher in single than double rooted teeth ($p<0.05$).

Twenty six cavities were completed in the EMLA group (59%) and 30 cavities were completed in the Laser group (68%) and the percentage of cavity completed in relation to jaw and root types are presented in Fig.1b. The mean pain scores at the termination of drilling were 22.95mm and 21.7mm respectively (Table 3a). Using the Chi-squared test of independence, the EMLA group cavity completion rate was significantly dependent upon both the jaw (Maxilla 75%, mandible 31%, $p=0.005$) and the root types (single 42%, double 83%, $p=0.007$), whereas in the Laser group the cavity completion rate was independent of both the jaw (maxilla 71%, mandible 62%, $p=0.5$) and the root types (single 65%, double 72%, $p=0.6$).

Statistically, using the Logistics Regression Analysis (EMLA $p=0.7$, Laser $p=0.2$), there was no significant association between the size of the rise in EPT readings and the chance of completing the cavity, independent of jaw or root types. Furthermore, using the paired t-test

showed no significant difference between the groups, in terms of cavity completed and increase in EPT readings. However, the increase in EPT readings from the baseline after application (Fig. 1c & d) of either agents (EMLA p=0, Laser p=0), regardless either single (Laser, p=0, EMLA,p=0) or double (Laser, p=0.03, EMLA, p=0.03) rooted, or from maxilla (Laser, p=0.002, EMLA, p=0.003) or mandible (Laser, p=0.001, EMLA, p=0.002) was statistically significant (Fig. 1c).

1.5. Discussion

Studies had evaluated the clinical efficacy (Holst and Evers, 1985; Vickers and Punnia-Moorthy, 1992; Svensson and Petersen, 1992) and safety (Pere *et al.*, 1992) of intraoral use of EMLA 5% anaesthetic cream in producing anaesthesia of gingival tissue as well as inducing periodontal (Donaldson and Meechan, 1995) and pulpal anaesthesia (Vickers and Punnia-Moorthy, 1993).

The results of this study have demonstrated the anaesthetic potency of oro-mucosal application of EMLA 5% topical anaesthetic cream, in increasing pulpal threshold to electric stimulation and allowing highspeed bur cavity (Class V) preparation, at a completion rate of 59%, in single or double-rooted, maxillary or mandibular premolar teeth.

The statistical difference of the cavity completion on premolar teeth between the maxilla and mandible is possibly related to the intrinsic difference in bone density between two arches. It may suggest that the anaesthetic ability of EMLA is dependant upon the mucosal absorption and osseous diffusion.

Due to the low viscosity of EMLA cream, it is difficult to maintain it in one position (Holst and Evers, 1985). In this study, the cream was secured with the use of Orahesive oral bandage (Vickers and Punnia-Moorthy, 1993; Svensson and Petersen, 1992), but it had been suggested that the use of custom-made splints to occlude the cream on the oral mucosa might further increase efficacy (Donaldson and Meechan, 1995) of application.

For ethical reasons, no allowance has been made in this study to quantify the amount of local anaesthetic absorption in the circulation through venous blood sampling. However, the amount of the EMLA cream used and the application duration employed were based on previous findings which would produce the peak level of pulpal anaesthesia. The maximum anaesthetic effect of 0.5mg of 5% EMLA is between 15-30min oro-mucosal application (Vickers and Punnia-Moorthy, 1993).

In the Laser group the overall cavity completion rate was 68%, statistically independent of both the jaw (maxilla 71%, mandible 62%) and the root types (single 65%, double 72%). The EPT readings were significantly higher in single rooted teeth. These may suggest the anaesthetic effects induced by laser on teeth are a function of the exposure dose per pulpal surface area (volume), rather than the rate of bony diffusion as in EMLA cream.

There was no complaint of sensitivity during the application of laser using the energy density which was described in this study. However, in one patient, the test tooth became hyperalgesic after the lasing, so that the cavity testing could not be performed. It has been shown in animal studies that pulsed Nd:YAG laser irradiated on the whole crown (Suda *et al*., 1996) can cause concurrent activity of intradental nerve (A-delta and C) fibres (Orchardson and Whitters, 1996; Friedman *et al*., 1994). This induction of hyperalgesia by the laser may possibly be caused by the rapidity with which the temperature change occurs within the tooth (Ahlberg, 1978; Narhi *et al*., 1982d).

The results of this study show there is a statistically significant increase in EPT readings from the baseline value after Laser application. When a monopolar electric stimulus is applied as in clinical Electrical Pulp Testing, only the A-delta fibres are normally activated (Trowbridge,

1985), so it raises the important possibility that pulsed Nd:YAG laser radiation may have direct influence in the sensory thresholds of the intradental nerves in teeth (Orchardson and Whitters, 1996; Suda *et al.*, 1996).

A 1mW Helium Neon (He:Ne) laser was used as an aiming beam for the infrared Nd:YAG laser; it has a wavelength of 632.8nm. It has been shown to be effective clinically for treatment of dentinal hypersensitivity (Matsumoto *et al.*, 1986), and has the ability to cause hyperpolarisation of the membrane potential (Iwase *et al.*, 1988). However, studies have claimed that lower power He:Ne laser irradiation (0.1-5mW) did not affect nerve transmission (Jarvis *et al.*, 1990) especially in healthy teeth (Wakabayashi *et al.*, 1986). Orchardson and Whitters (1996) had reported that the He:Ne aiming beam alone had no effect on interdental nerve responses. Strang *et al.* (1994) in an *in vivo*, double blind, crossover trial have confirmed the anaesthetic potential of Nd:YAG laser on upper central incisors when assessed by the change in Electrical Pulp Test reading, and have reported that the Nd:YAG laser (plus the He:Ne aiming beam) increases the pain thresholds to electrical stimulus significantly higher than the sham treatment group (He:Ne aiming beam only), but only for 5min period after application.

It had been considered to include both placebo arm and He:Ne laser groups in the study design, but unfortunately this proposal has been forbidden on ethical grounds by the local human ethics committee.

Despite numerous investigations, the mechanism of the laser clinical induction of pulpal anaesthesia is not fully understood. It has been suggested the clinical phenomenon of laser induction of anaesthesia operates at the dentine level (Nagasawa, 1984; 1988; Parkin and Miller, 1992). Nagasawa (1988) suggested that the dentine canals in the surface layer disappeared as a result of laser irradiation for clinically induced dental analgesia.

The ability of Nd:YAG energy to cause a reduction in dentinal permeability has been reported by a number of authors (Stabholz *et al.*, 1992; Miserendino *et al.*, 1995; Forrest-Winchester and Walsh, 1993; Goodis *et al.*, 1992). In theory, the combination of protein coagulation within tubules and the partial closure of the dentine tubules through surface melting by the laser energy, may explain this observation. However, the effects of Nd:YAG energy on dentine can be very inconsistent. It is partly due to the effects of laser on dentine being dependent on the overall interaction with the tissue with dark pigmentation and partly to the irradiated laser energy density. Therefore,

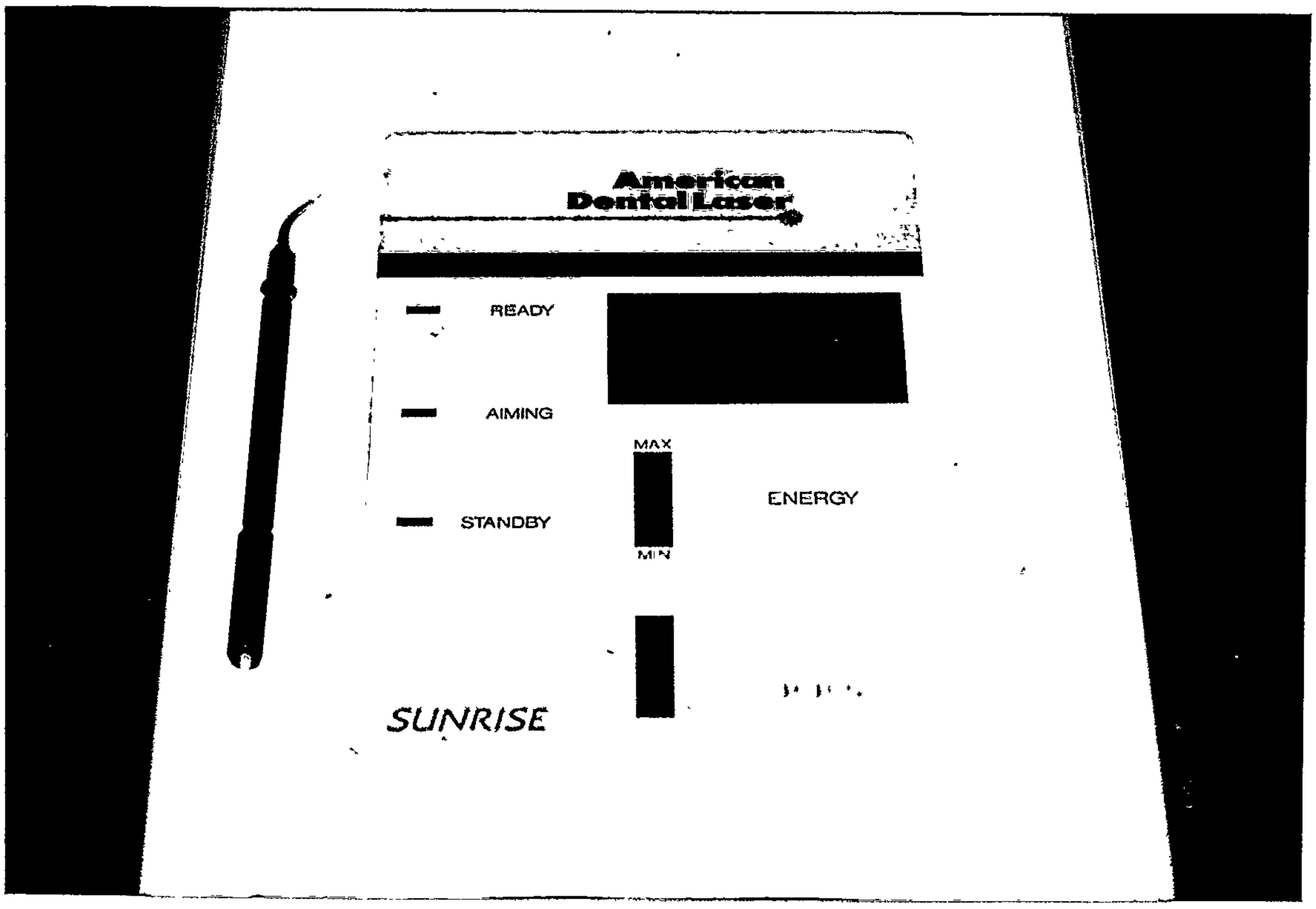
the final clinical manifestations can be unpredictable and are difficult to reproduce and quantify in a clinical study.

It seems the mechanism of pulsed Nd:YAG laser induced surgical anaesthesia may be operating at both dentinal and pulpal levels, of varying degrees, which is largely dependant upon the total exposure of laser energy dose and the irradiated surface area (volume) of the coronal pulp, which in turn is determined by the laser parameters such as power, energy per pulse, duration of exposure and the intrinsic variations of dentine at various sites. Further studies are required to determine these factors to which such an effect may contribute to the phenomenon of so called "Laser analgesia in lieu of anaesthesia".

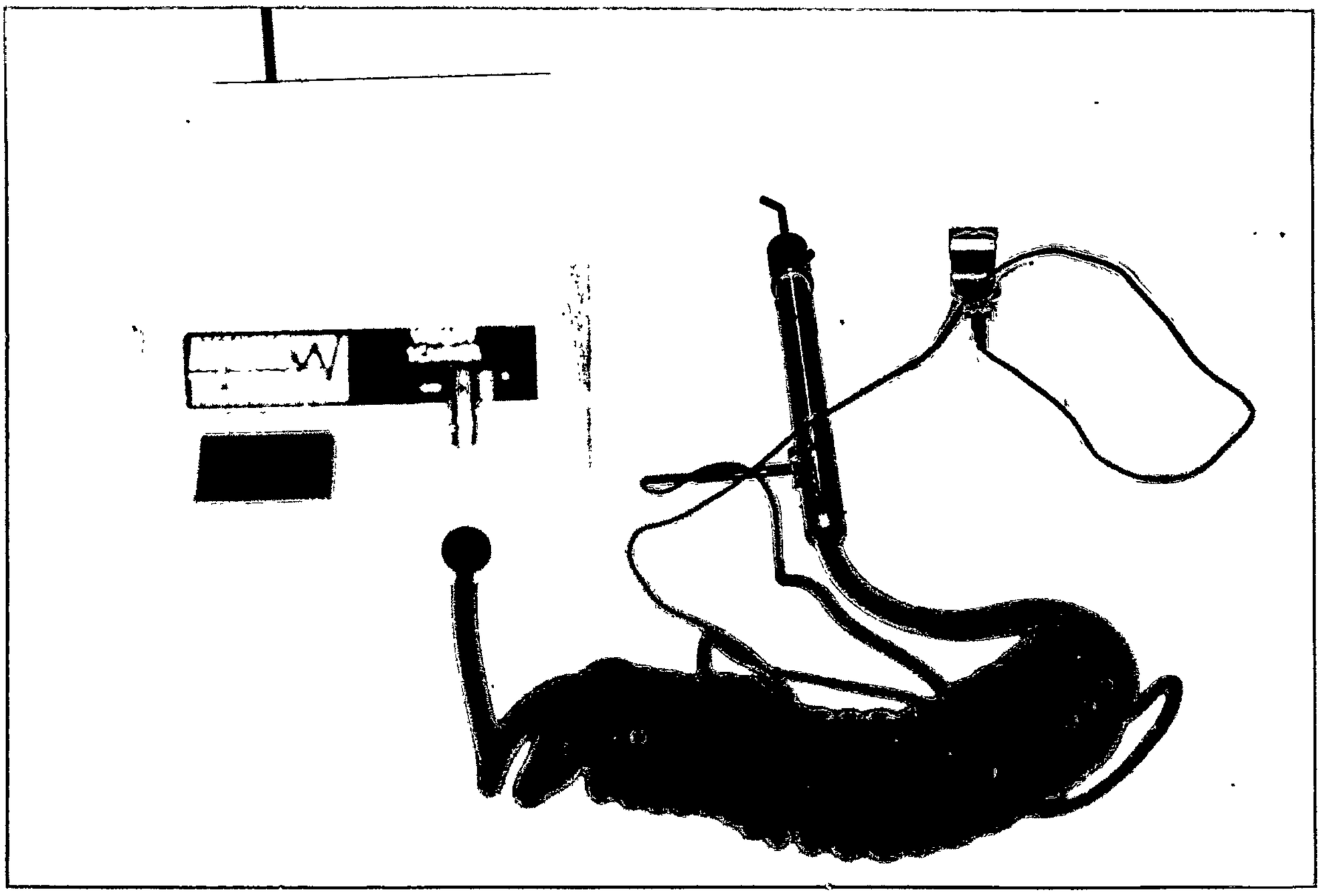
1.6. Conclusion

It is concluded that this clinical study confirms the effectiveness of pulsed Nd:YAG laser treatment for the induction of pulpal anaesthesia on premolar teeth. The depth of anaesthesia induced was comparable to that produced by a potent topical anaesthetic, EMLA 5% cream. It is suggested that pulsed Nd:YAG laser energy can suppress the response of intradental nerves to an electrical stimulus. It also postulated that the mechanisms of so called "Laser analgesia in lieu of anaesthesia" may involve local effects on dentine as well as changes to neural functions

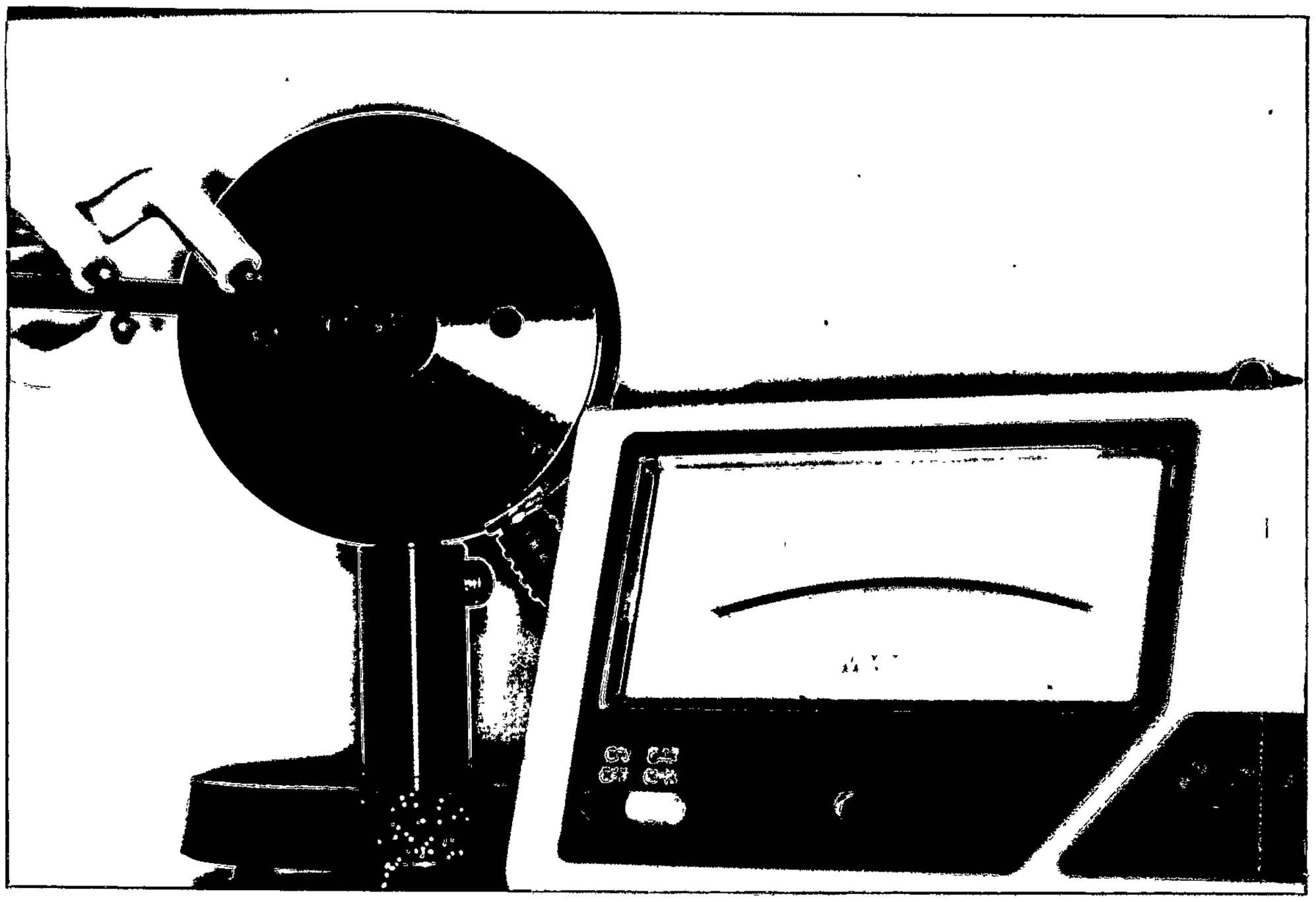
operating or initiating within the neuro-odontoblastic complex, at the dentino-pulpal interface.



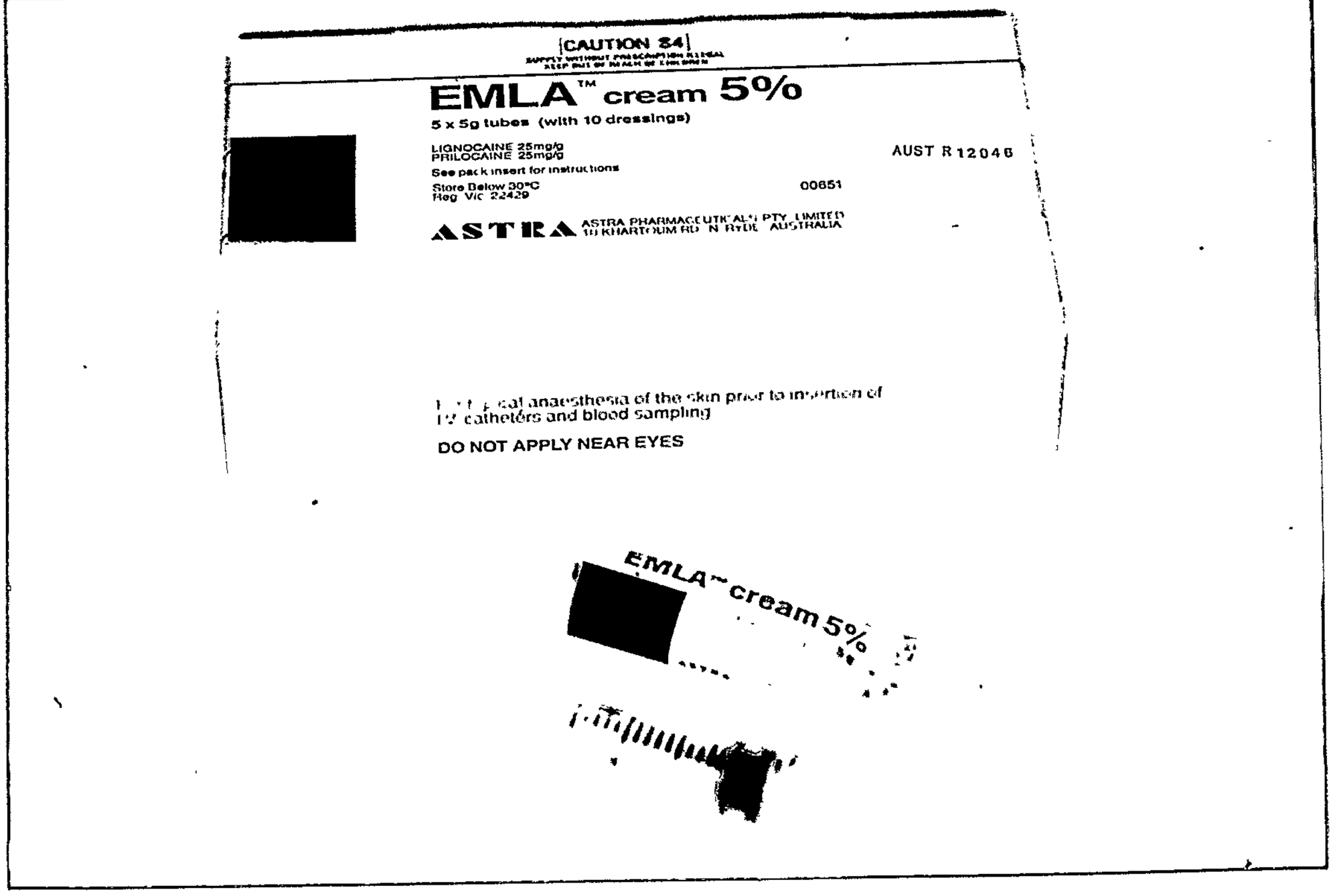
P1-1. This photograph illustrates a pulsed Nd:YAG American Dental [1] Laser(dLase300).



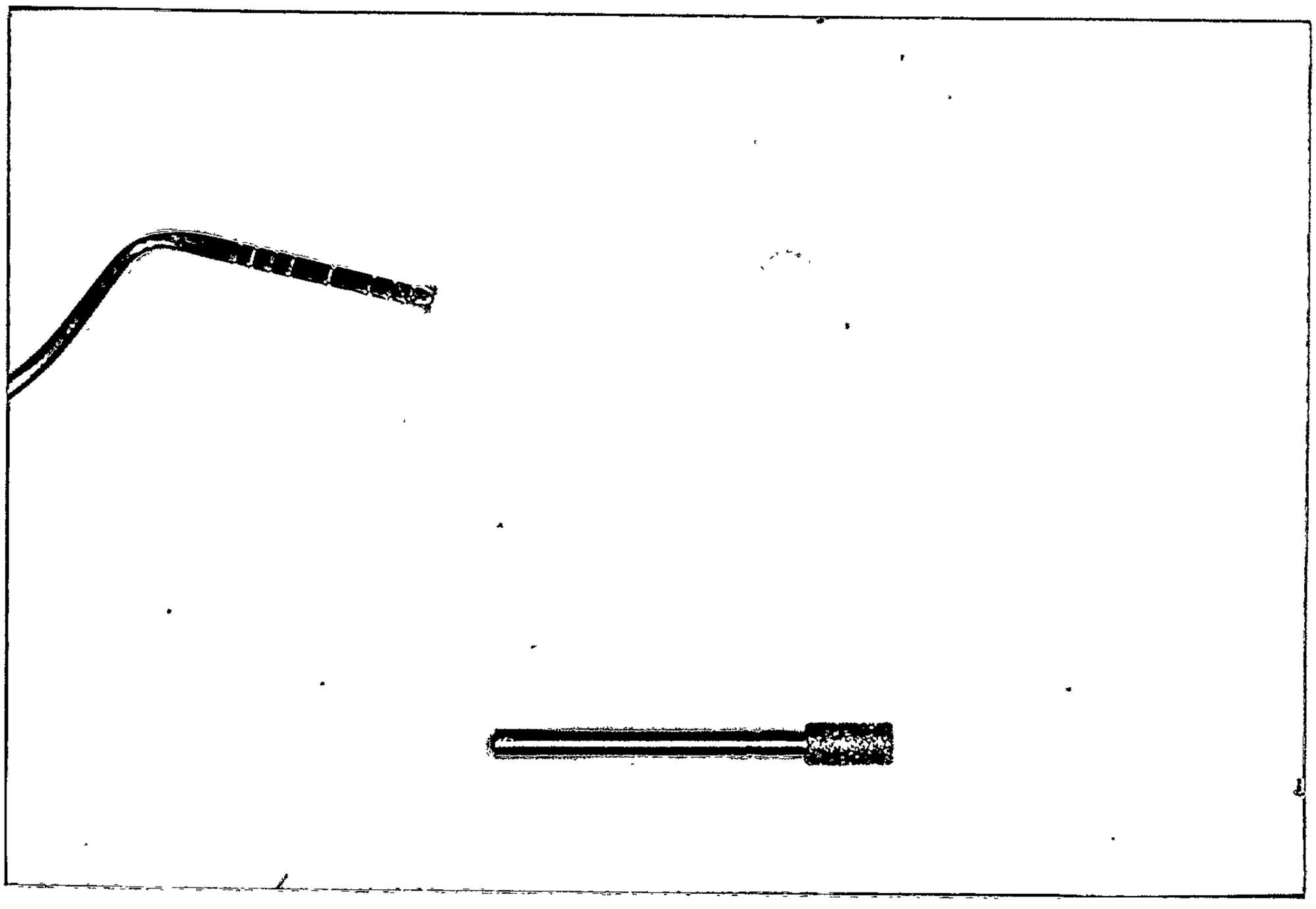
P1-2. This photograph illustrates a Electric PulpTester [2] (Analytic Technology, USA)



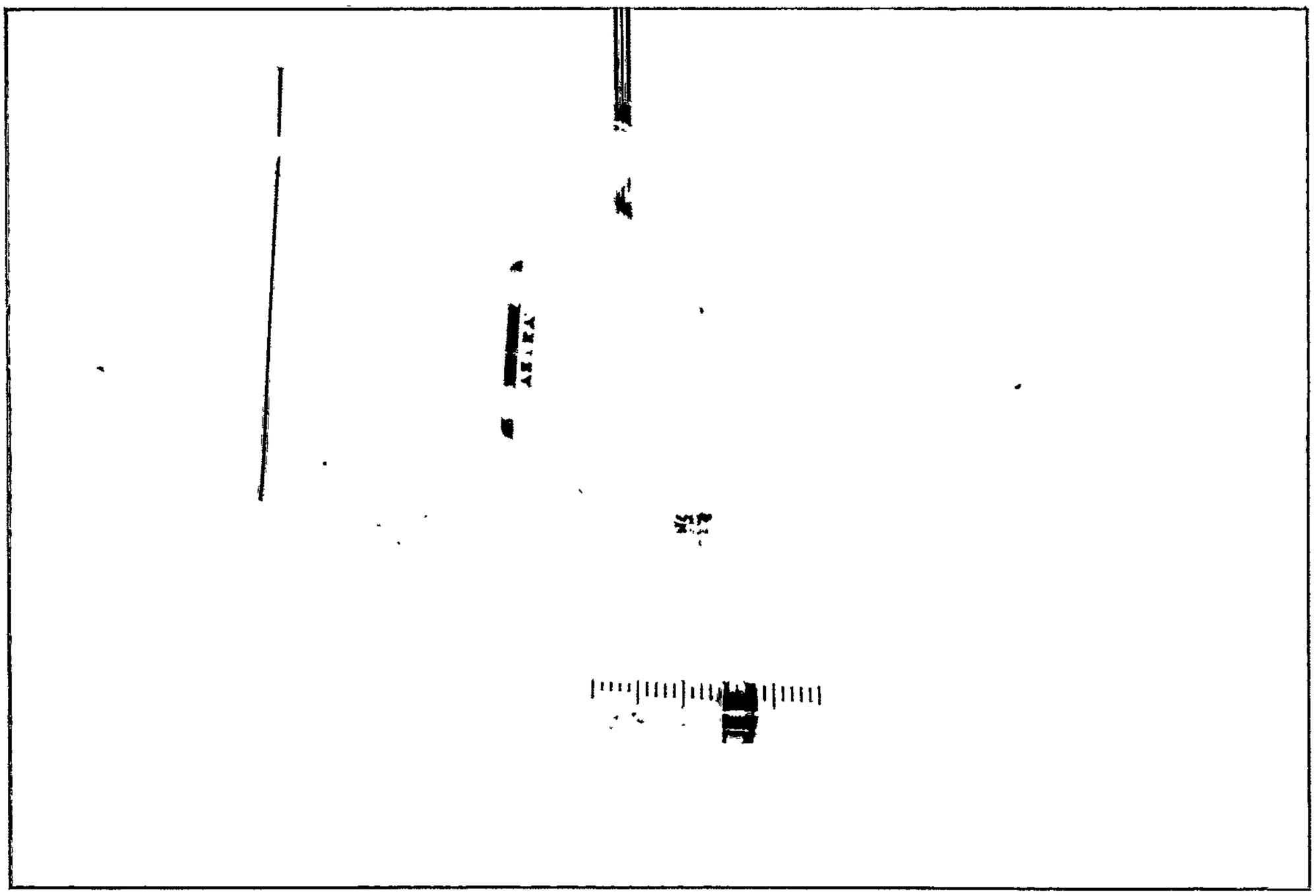
P1-3. This photograph illustrates a power meter [3] (Ophir model, S/N7523, head:30A150).



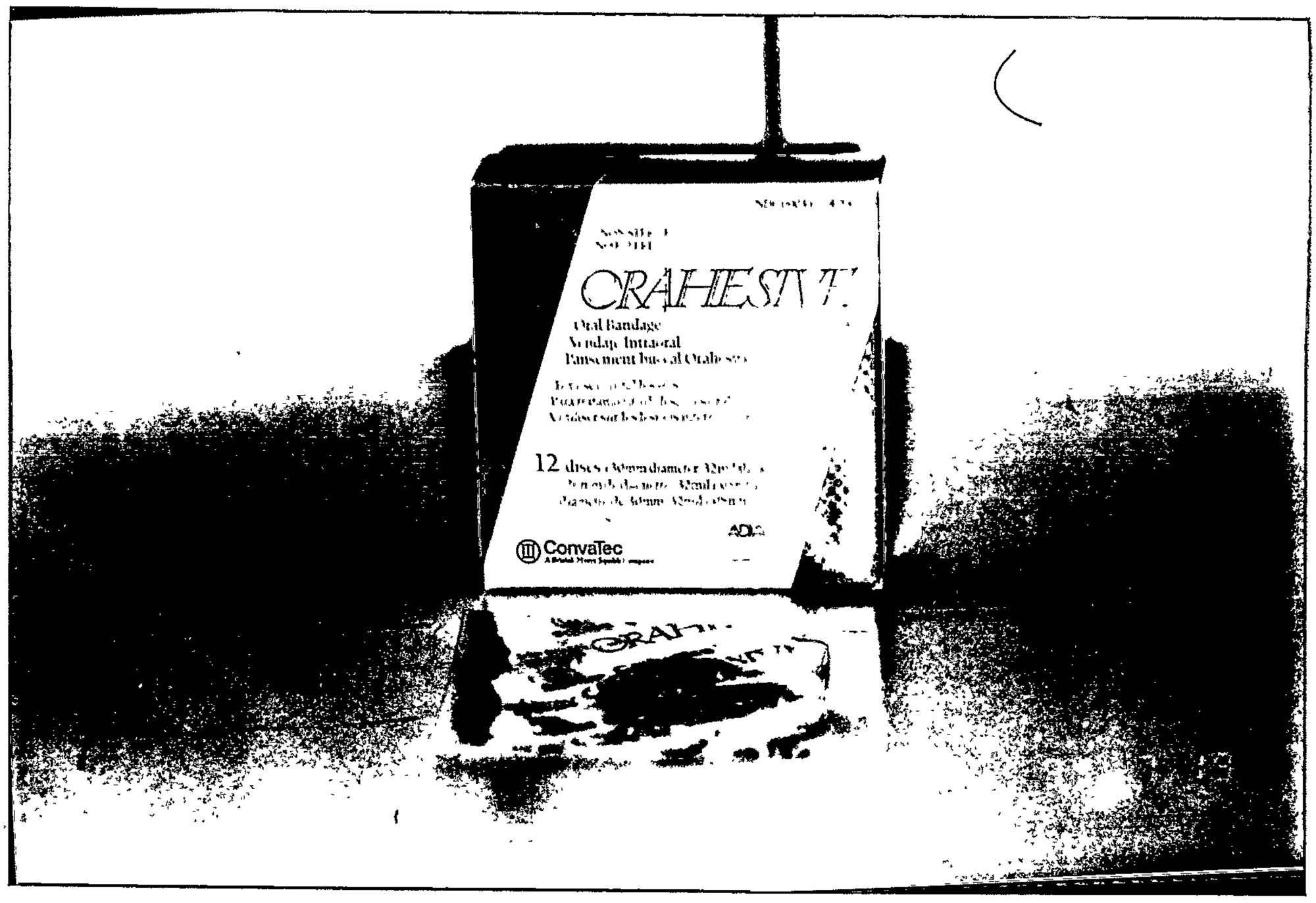
P1-4. This photograph illustrates the EMLA [4] 5% topical anaesthetic cream, with a loading syringe.



P1-5. This photograph illustrates the standardised Class V cavity (2mm by 1mm depth) & a diamond flat fissure bur.



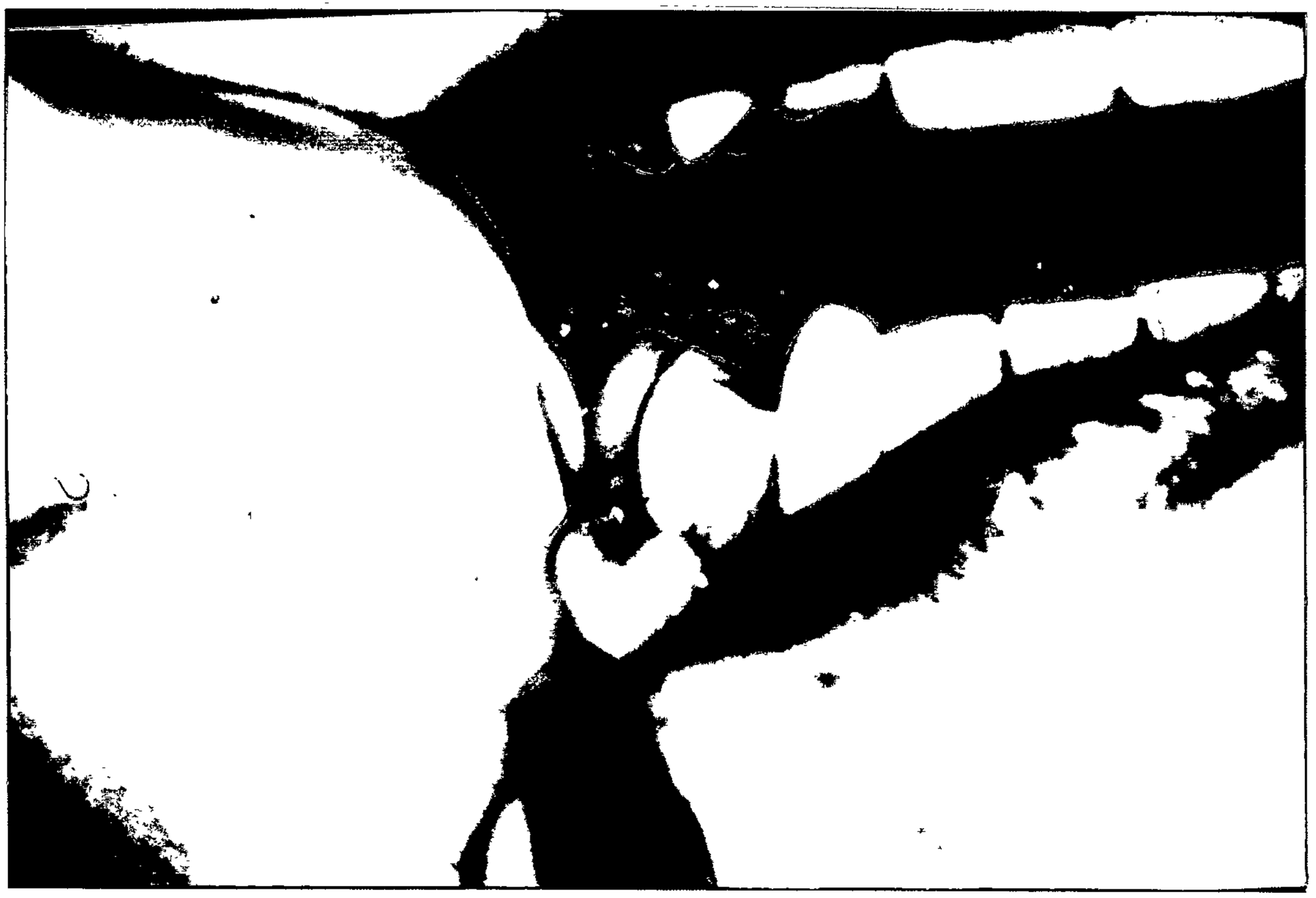
P1-6. This photograph illustrates The Placebo [5] EMLA cream in individual package.



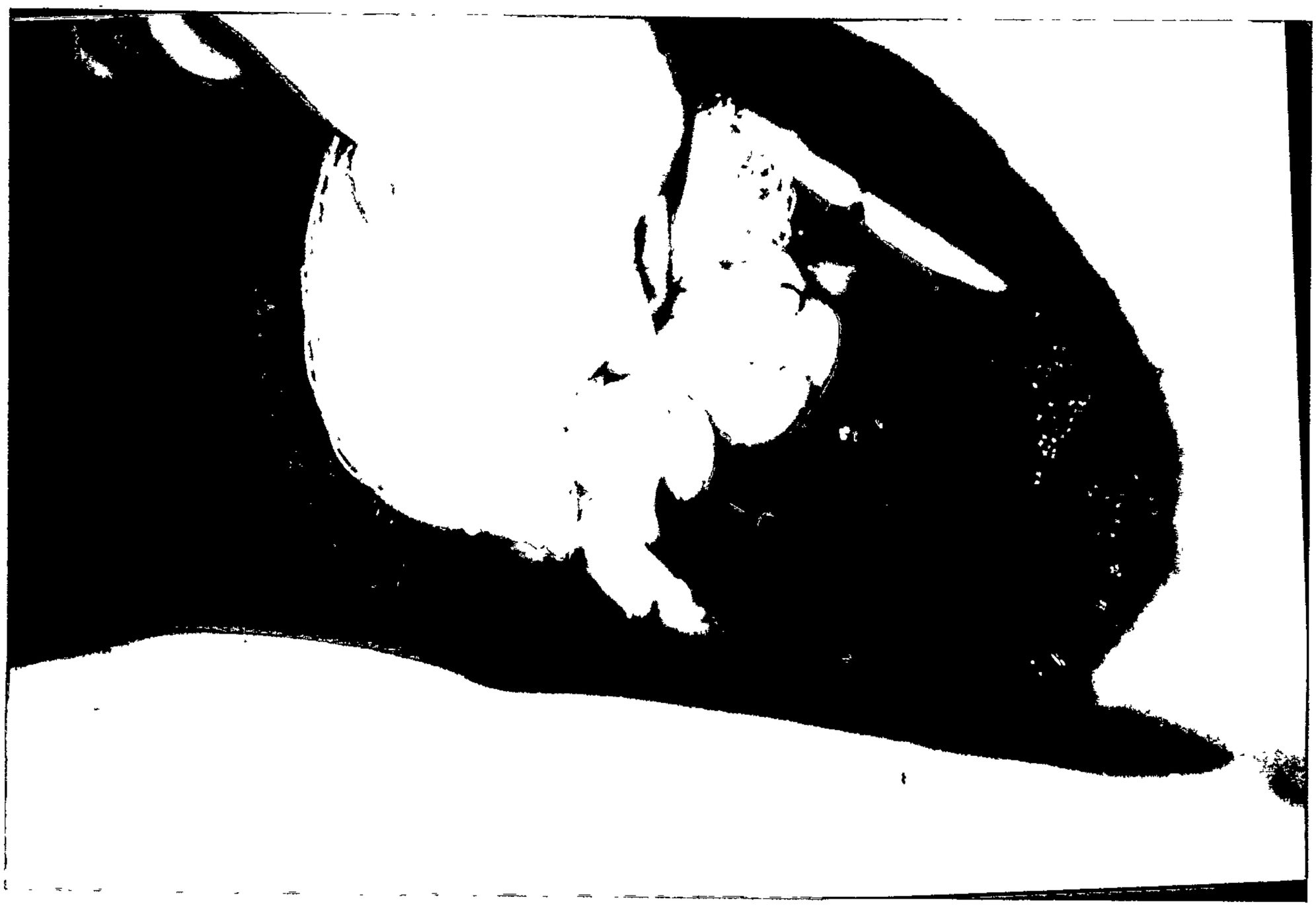
P1-7. This photograph illustrates the Orahesive [6] oral bandage (Convatec, Victoria, Australia) for cream isolation.

P1-8. This photograph illustrates the baseline EPT reading being taken at the centre of the tooth surface.

P1-9 .This photograph illustrates the oral application of either EMLA 5% or Placebo cream.



P1-10. Oral application of either the Laser or the sham treatment, in roving contact motion, on the cervical half of buccal surface.



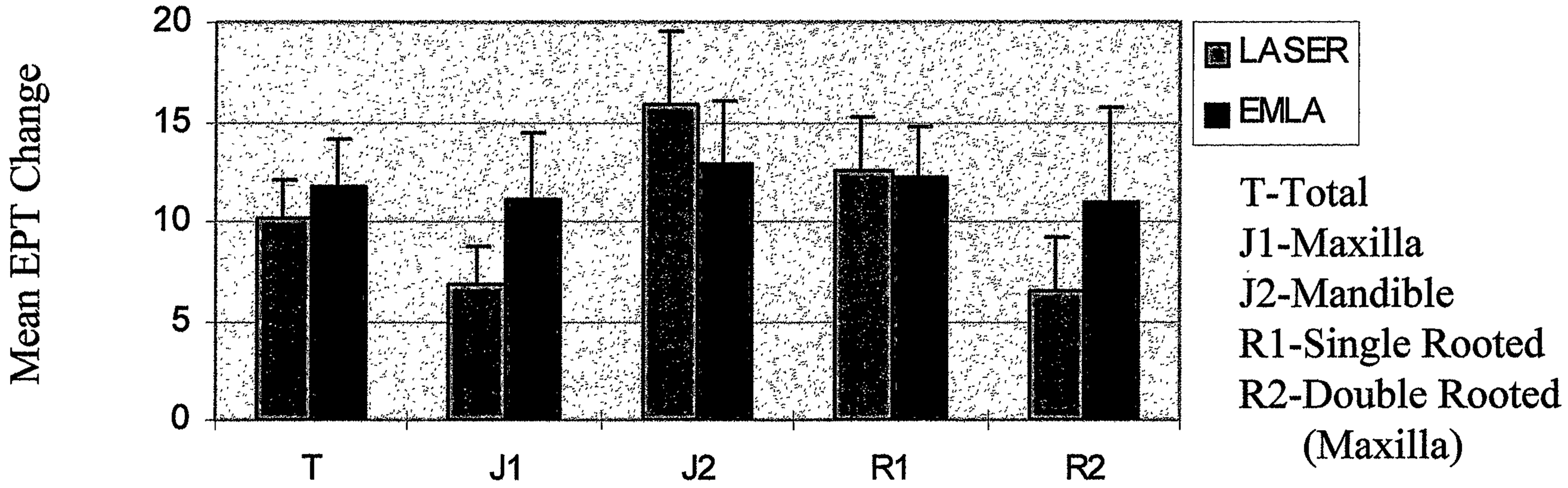
P1-11. Oral application of either the Laser or the sham treatment, on the cervical half of lingual surface.

Table 1.a. Mean value (with standard deviations) of EPT readings, cavity complete (%) and pain scores, in relation to jaw and root types, from Laser and EMLA groups.

	Overall	Maxilla	Mandible	1-Root	2-Root
E-cavity	59±5%	75±4%	315%	42±5%	83±4%
E-Pre	48.6±13.2	45.7±14.4	53.6±8.8	52.4±10.9	43.0±14.4
E-Post	60.3±16.1	56.8±16.5	66.5±13.9	64.7±15.0	54.0±16.2
E-Pain	23.0±18.4 mm	20±19.5m mm	27.4±15.9 mm	29.0±19.2 mm	14.1±13.1 mm
L-cavity	68±5%	71±5%	63±5%	65±5%	72±5%
L-Pre	48.8±14.2	48.0±14.0	50.2±14.9	52.2±15.8	43.9±9.9
L-Post	58.9±14.4	54.9±14.6	65.9±11.5	64.8±12.2	50.4±13.4
L-Pain	22.0±21.1 mm	20.0±18.7 mm	25.7±24.9 mm	26.5±22.5 mm	15.4±17.3 mm

E= EMLA, L=Laser, Pre=pre-application EPT reading,
P=pain score, Post= post-application EPT reading,
1-Root=single-rooted teeth, 2-Root=double-rooted maxillary teeth.

Fig.1.a. Mean EPT Change in relation to Jaw & Root types Laser vs EMLA



T-Total
J1-Maxilla
J2-Mandible
R1-Single Rooted
R2-Double Rooted (Maxilla)

Fig.1.b. Percentage of Cavity Completed in relation to Jaw & Root types Laser vs EMLA

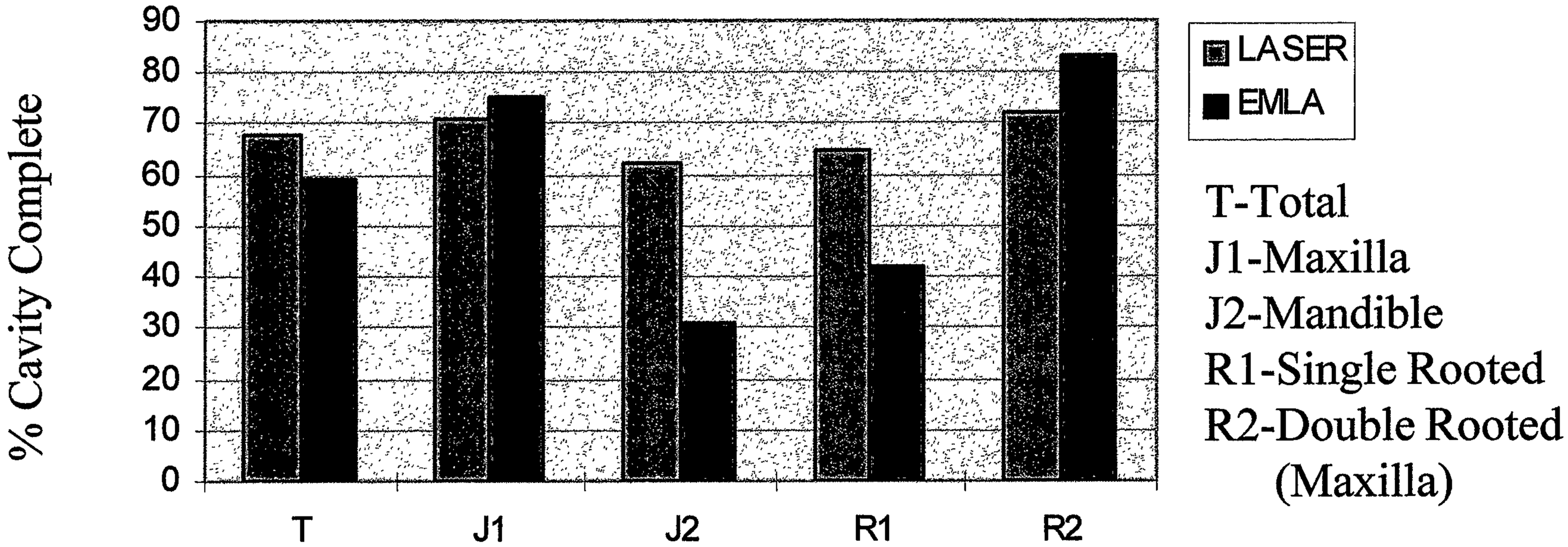


Fig.1.c. Mean EPT Score in relation to Jaw & Root types before and after Application of Laser

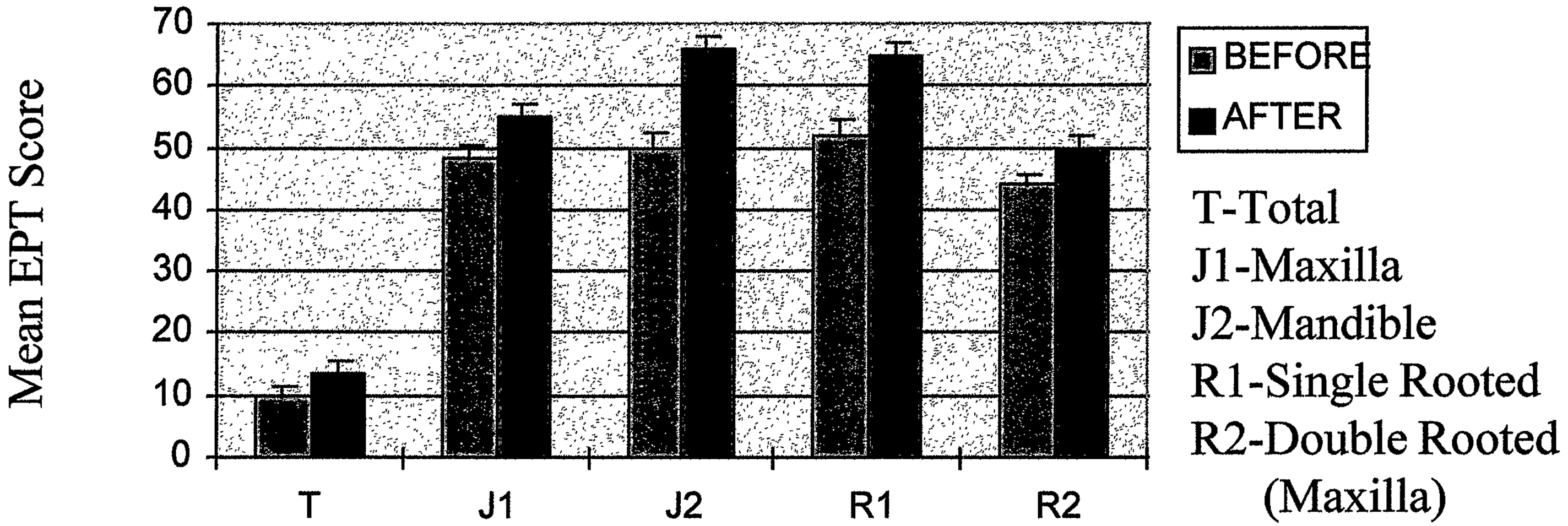
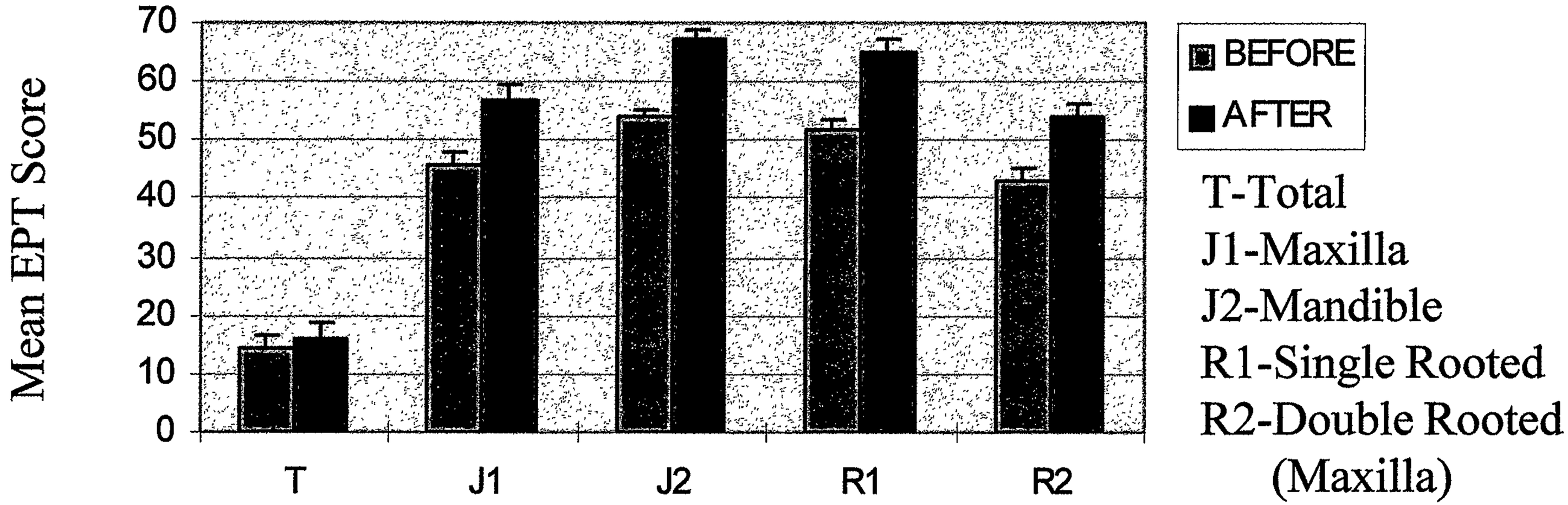


Fig.1.d. Mean EPT Score in relation to Jaw & Root types, before and after Application of EMLA



2. Pulpal anaesthesia induced by Nd:YAG laser and EMLA 5% topical anaesthetic cream. Part II: A morphological and histological study.

2.1. Background and aims

A double blind, randomised study to evaluate the clinical efficacy of pulpal anaesthesia induced by pulsed Nd:YAG laser and EMLA 5% topical anaesthetic cream on premolar teeth was carried out. In 44 subjects, a pair of single or double rooted premolar teeth, from the same arch received a combination of the test agents: EMLA 5% cream and He:Ne aiming beam or placebo cream and pulsed Nd:YAG laser. After the application of the paired agents, the degree of anaesthesia was estimated by an Electric Pulp Tester and by drilling of a standardised Class V cavity. Results showed that there is clinical evidence of pulpal anaesthesia with the application of pulsed Nd:YAG laser. The clinical efficacy of pulp anaesthesia induced by laser is comparable to that produced by EMLA 5% topical anaesthetic cream. Subsequently, the teeth were extracted and stored in Karnovsky's fixative solution (2% formaldehyde and 2.5% glutaraldehyde in 0.1M sodium cacodylate at pH of 7.4) for two days and then subjected to morphologic and histological investigations.

The aim of these investigations was to observe any morphological changes on enamel and dentine surfaces, to detect any alterations in dentinal permeability, and to observe any immediate superficial pulpal disruptions, on specimens that had been irradiated by the pulsed Nd:YAG laser for clinical induction of pulpal anaesthesia, at the energy dose recommended by the manufacturer.

2.2. Morphological study: Scanning Electron Microscopy (Laser/cavity cut versus EMLA/cavity cut).

2.2.1. Introduction

The morphological changes on the dental hard tissues induced by laser irradiation can be studied topographically under Scanning Electron Microscopy (SEM). The morphologic changes of the coated enamel surface induced by pulsed Nd:YAG laser at low energy density (eg., 95 J/cm^2) were described as being many small bubble-like inclusions making up a pock marked surface with indistinct craters, accompanied with pitting and surface flaking (Myers and Riddle, 1990; Hess, 1990). At a relatively higher energy density (eg., 239 J/cm^2), the irradiated surface was described as being covered by multiple overlapping disk-shaped impact craters (Tagomori and Morioka, 1989) with the periphery raised and having the appearance of molten enamel, but round and glassy-like.

The occasional higher peak power of the pulse produced the beaded enamel appearance which resulted from a superheated enamel surface that underwent subsequent cooling at room temperature (Arcoria *et al.*, 1991).

Besides crater formation, cracking (0.1-0.5 micron in width) occurred throughout the entire lased area, but did not extend into the adjacent unlased enamel. Cracking remained constant in quantity and width despite increasing energy density (Hess, 1990; 1991).

2.2.2. Materials and methods

Six paired-tooth specimens (from 44 pairs of extracted teeth from study 1) were randomly selected, and dehydrated in a graded series of ethanol prior to undergoing critical point drying. After critical point drying, the specimens were attached to aluminium stubs and coated with a thin conducting layer of gold (Robinson *et al.*, 1987). All samples were then examined in a JSM-840A Scanning Electron Microscope (operating at 15kV).

The tooth was positioned with the long axis vertically and magnified until the palatal/lingual cervical half of the tooth surface area filled the entire viewing screen of the SEM. This was then accepted as the central point of the lased area. Photographs were taken between 33x-550x

magnifications of the area to show the overall pattern and the detail of surface changes.

Rating of the surface appearance was accomplished without knowledge of treatment conditions. The enamel of the cervical half of the palatal/lingual surface of the tooth crown was rated using the following criteria:

1- Absence of surface cratering and melting (Fig.2.2. a &b).

2- Presence of disk-shaped impact craters and melting with finger-like projections to the adjacent surface (Fig.2.2. c &d).

2.2.3. Results and discussion

Results (Table 2.2.a) showed there was no observable enamel surface cratering and melting from both Laser (Fig.2.2.e &f) and EMLA (Fig.2.2.g &h) groups. But, there were observable surface roughening features such as cracks, pits and flakes from either group. However, there was no statistically significant difference between the Laser and EMLA groups in terms of surface roughening, using the Wilcoxon test ($P>0.05$).

The results of this morphological study under Scanning Electron Microscopy is concurrent with that reported by Meurman *et al*. (1992), that low level Nd:YAG irradiation does not cause crater formation or discernible effect on the apatite. However, the presence of surface

cracks, flakings and pitting on tooth enamel (but not actual melting or crater formation) can occur (Ferreira *et al.*, 1989; Pogrel *et al.*, 1993). Unfortunately, when forceps extraction is the method of removal of teeth as was the case in this study, surface roughening such as cracking and flaking can occur. Also, the surface effects of forceps extraction may destroy any possible surface effects caused by the laser and this requires further investigation. Therefore the interpretation of the results in this study should be done with a degree of caution.

Table 2.2.a. Rating scale for enamel surface changes- under Scanning Electron Microscopy.

1- Absence of surface cratering and melting.
2- Presence of disk-shaped impact craters and melting with finger-like projections to the adjacent surface.

Specimen no.	1-Ratings	2-Ratings	Ave. Ratings
94071A(L)	1(SR)	1(SR)	1(SR)
94071B(E)	1(SR)	1(SR)	1(SR)
94072A(L)	1(SR)	1(SR)	1(SR)
94072B(E)	1(SR)	1(SR)	1(SR)
94073A(L)	1(SR)	1(SR)	1(SR)
94073B(E)	1(SR)	1(SR)	1(SR)
94076A(L)	1(SR)	1(SR)	1(SR)
94076B(E)	1(SR)	1(SR)	1(SR)
94077A(L)	1(SR)	1(SR)	1(SR)
94077B(E)	1(SR)	1(SR)	1(SR)
94078A(L)	1(SR)	1(SR)	1(SR)
94078B(E)	1(SR)	1(SR)	1(SR)

(SR)= surface roughening, (E)= EMLA group, (L)= Laser group

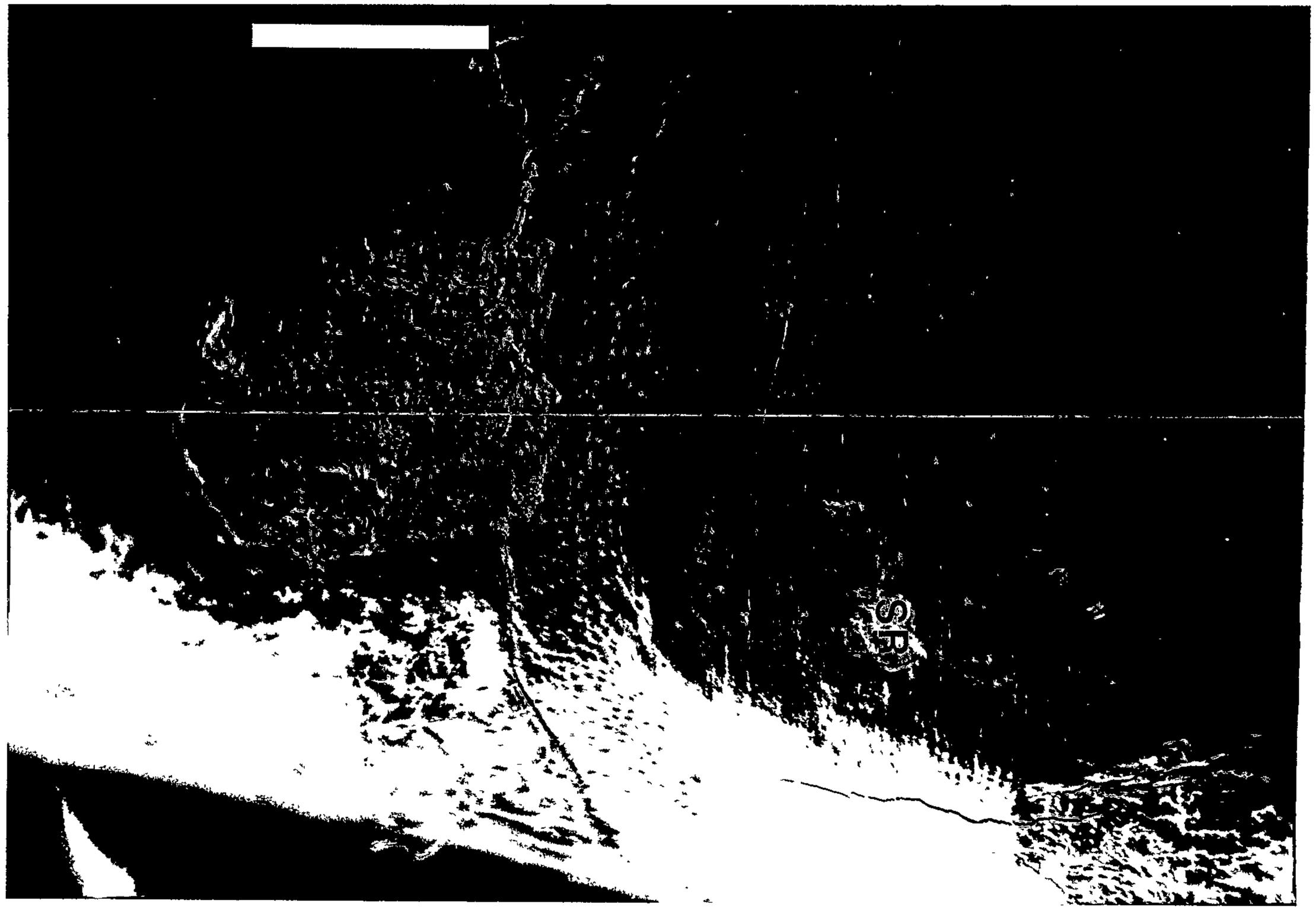


Fig.2.2.a. SEM(35x) of the palatal cervical half, enamel surface, from a control specimen- absence of surface melting and crater formation, and surface roughness (SR) possibly due to forcep extraction. Bar=1mm.

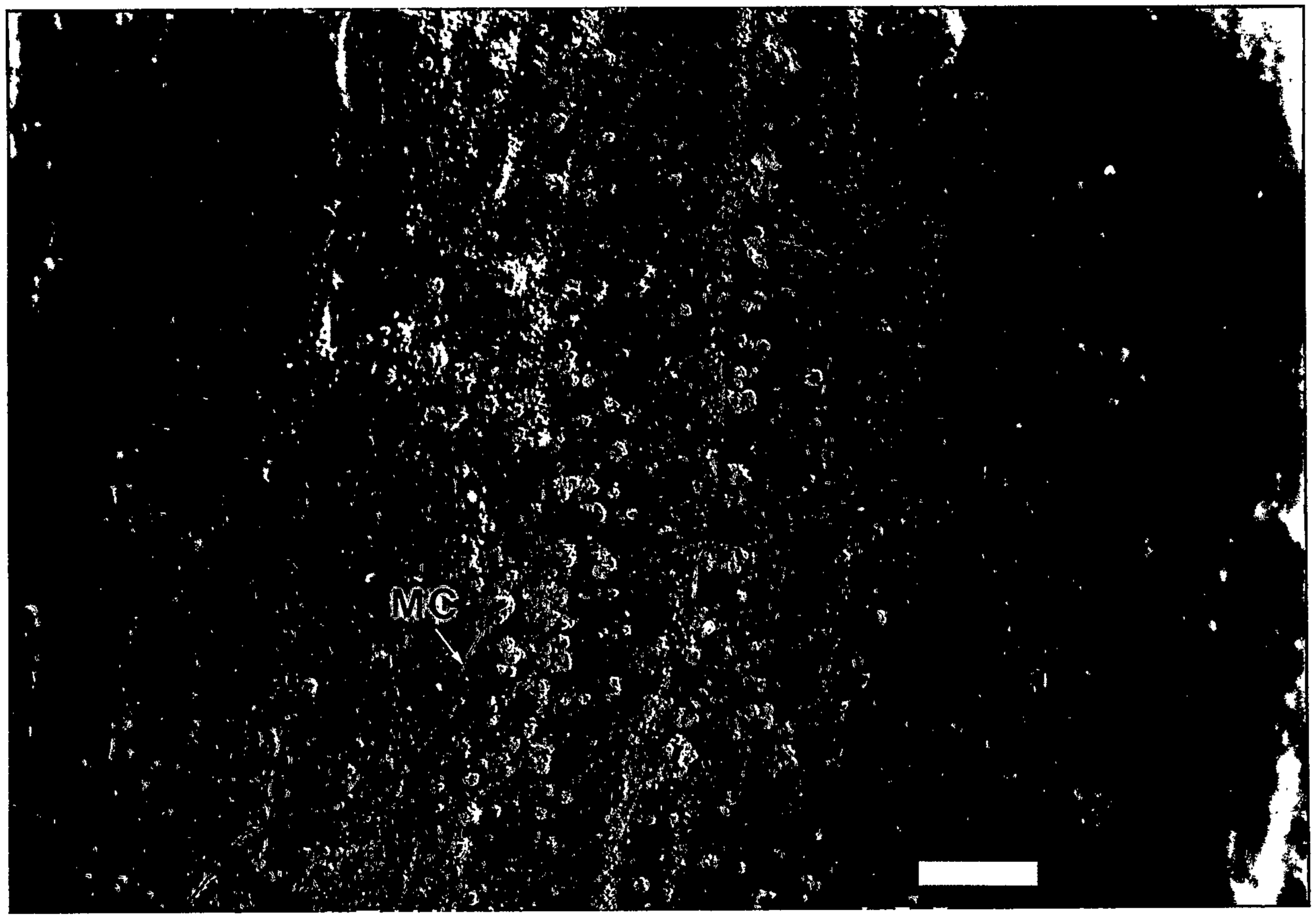


Fig.2.2.b. SEM(135x), close up examination in Fig. 2.2.a.- absence of surface cratings and melting. Note presence of microcracks (MC). Bar =100um.

Fig.2.2.c. SEM(33x) of the buccal cervical half, enamel surface from a Laser control specimen (irradiated with Nd:YAG pulsed energy at 8W, 30pps, 2 mins with 320 microns fibre and no dye), demonstrates the presence of disk-shaped impact craters (CR). Bar = 1mm.

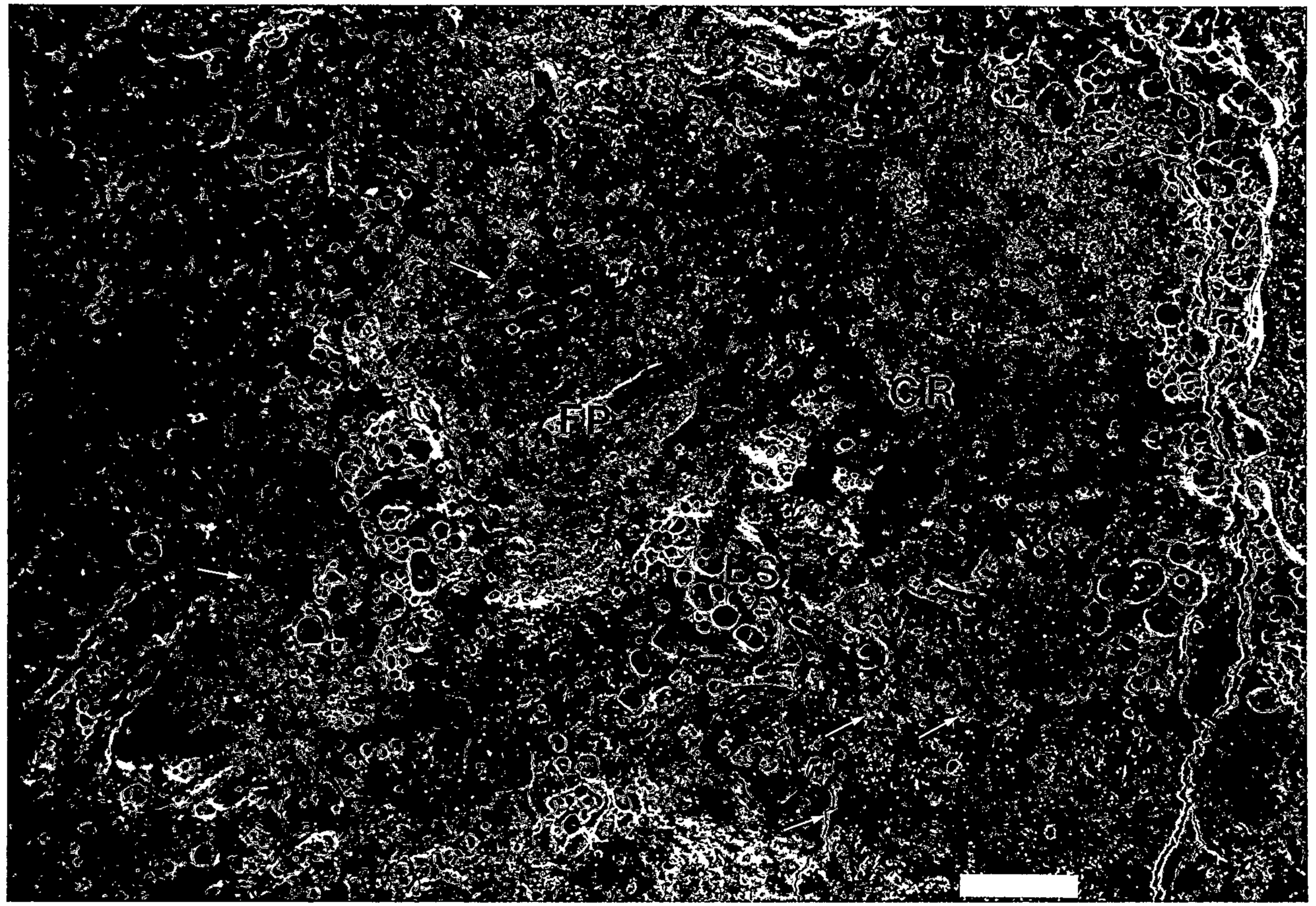


Fig.2.2.d. SEM(135x), close up examination in Fig. 2.2.c.- multiple impact craters (CR); bubble-like structures (BS) within smooth elevation; finger-like projections from the periphery(FP); numerous pittings and microcracks (↑). Bar = 100um.

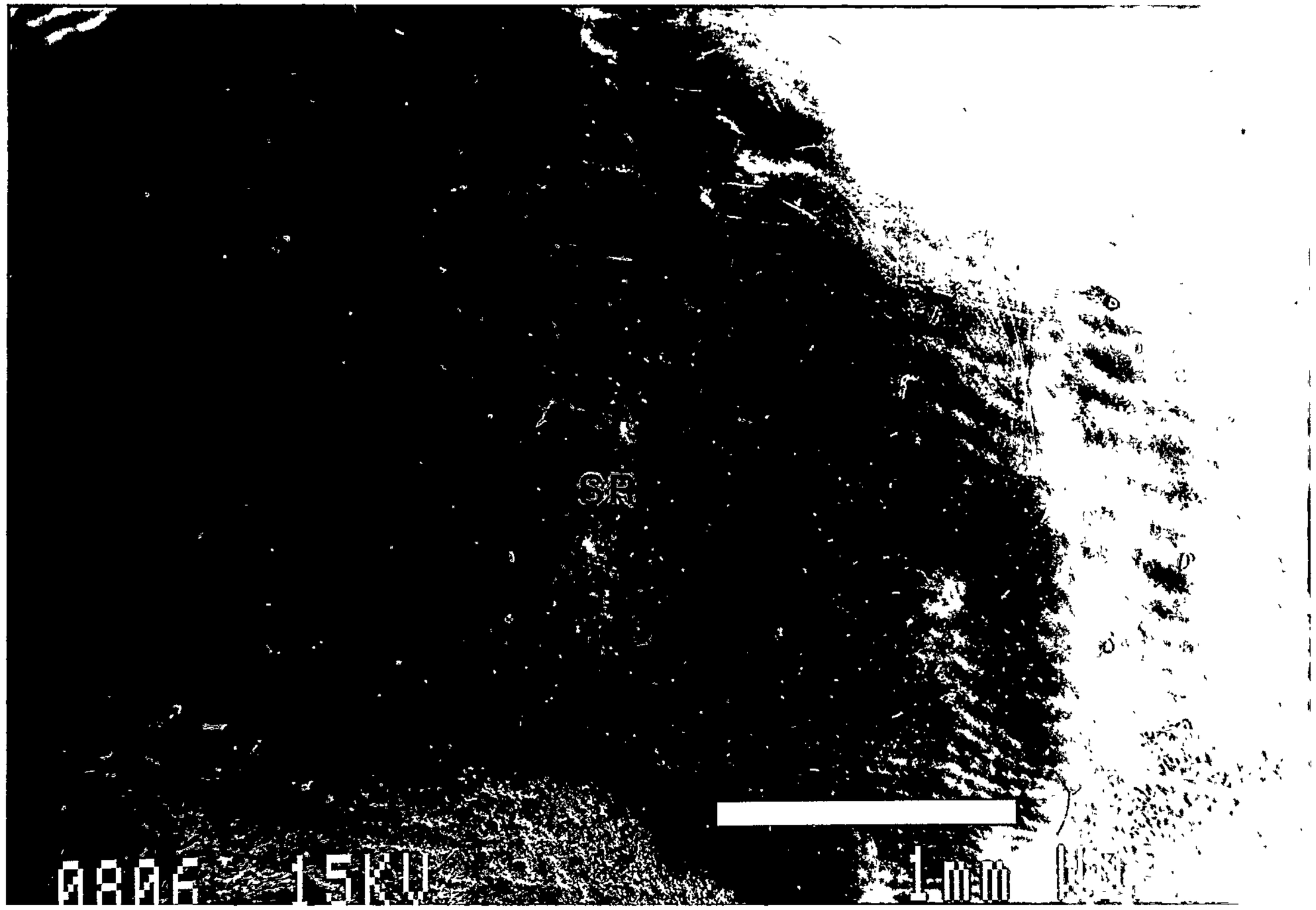


Fig.2.2.e. SEM(35x) of the palatal cervical half, enamel surface, from a typical Laser-treated specimen- no melting or cratering. Surface roughness (SR). Bar = 1mm.

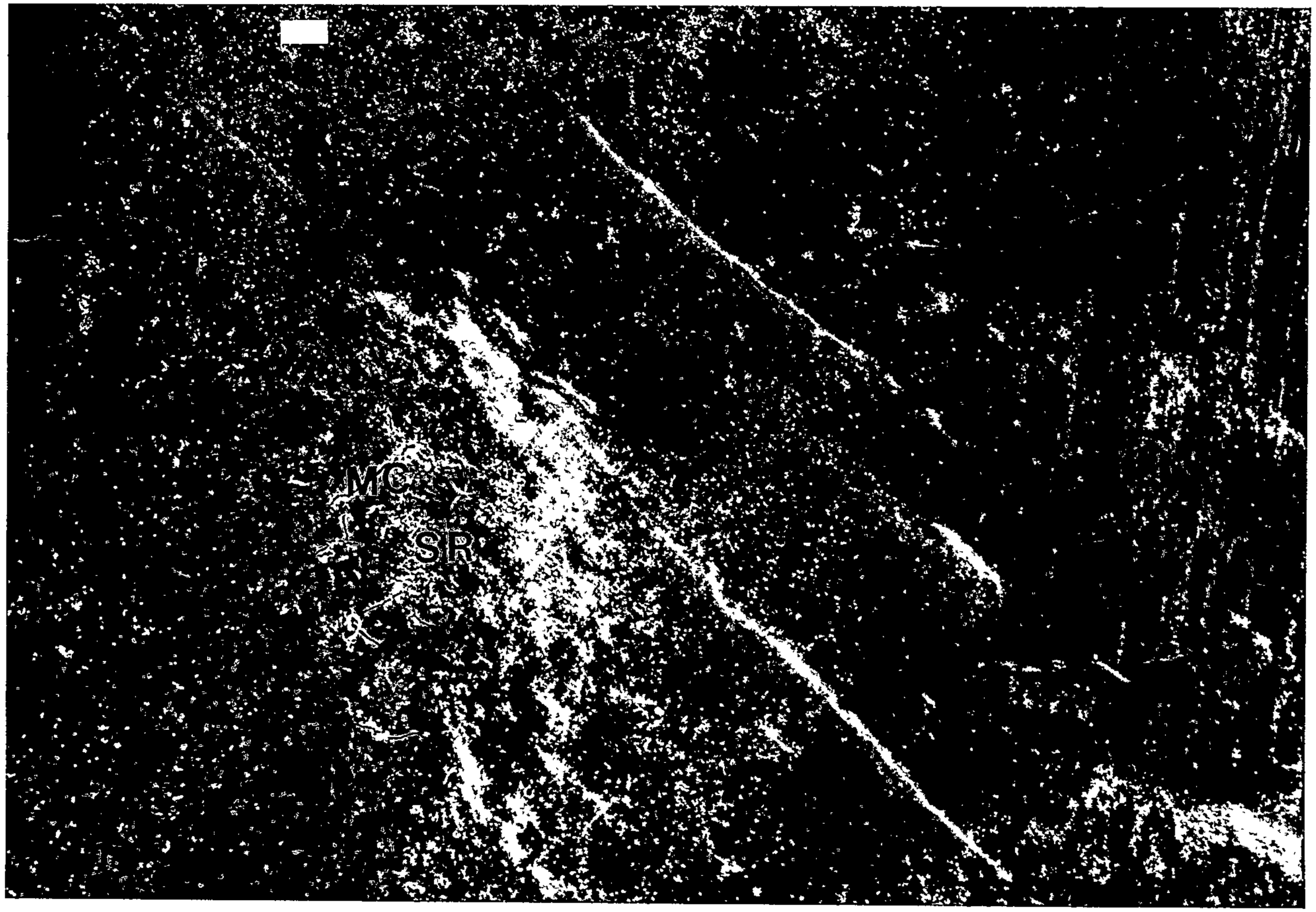


Fig.2.2.f. SEM(550x), close up examination in Fig. 2.2.e.- surface roughness (SR) and microcracks (MC), but not melting or cratering. Bar = 100um.

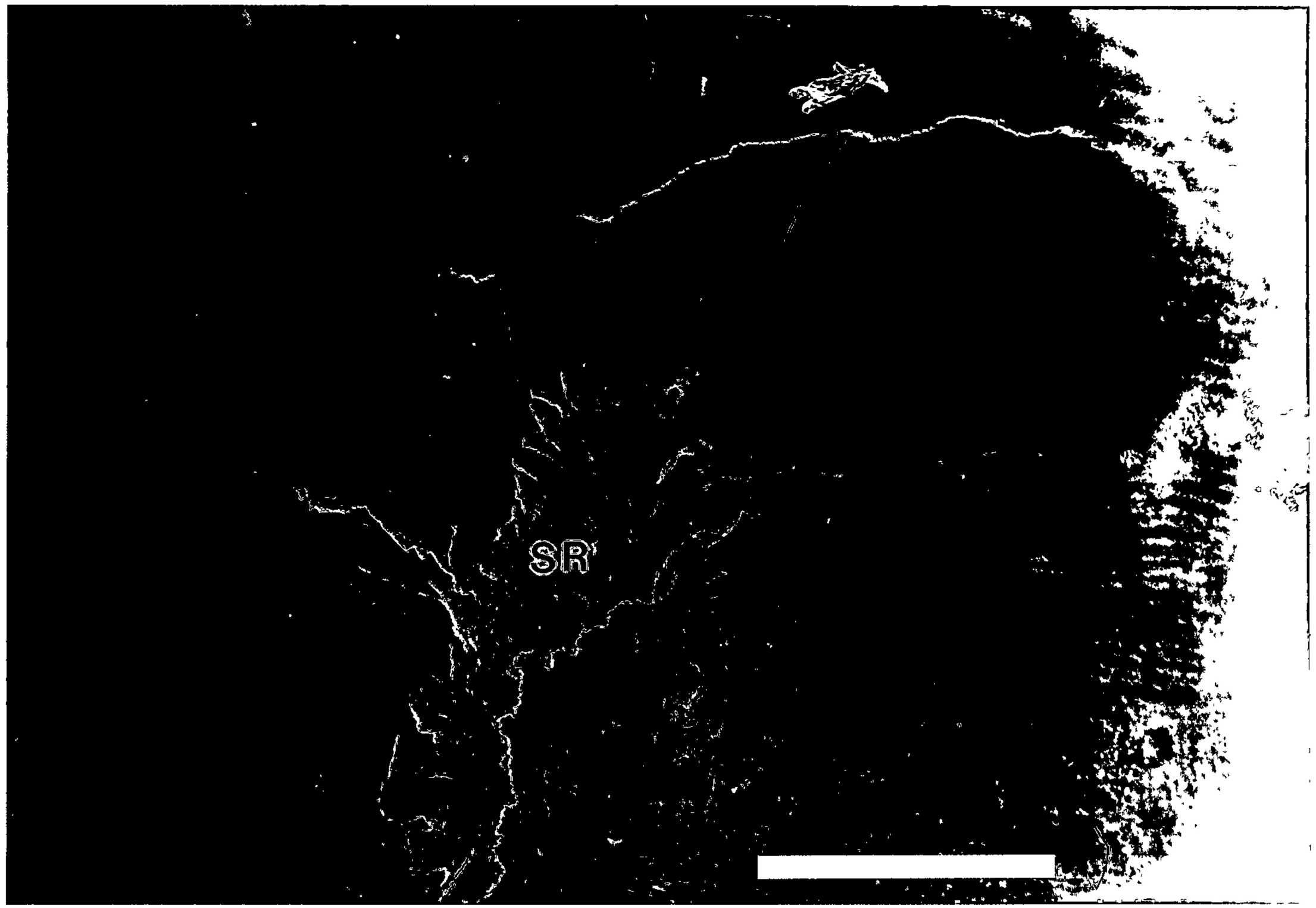


Fig.2.2.g. SEM(35x) of the palatal cervical half, enamel surface, from a typical EMLA-treated specimen- absence of surface melting and crater formation, and surface roughness (SR) possibly due to forcep extraction. Bar=1mm.

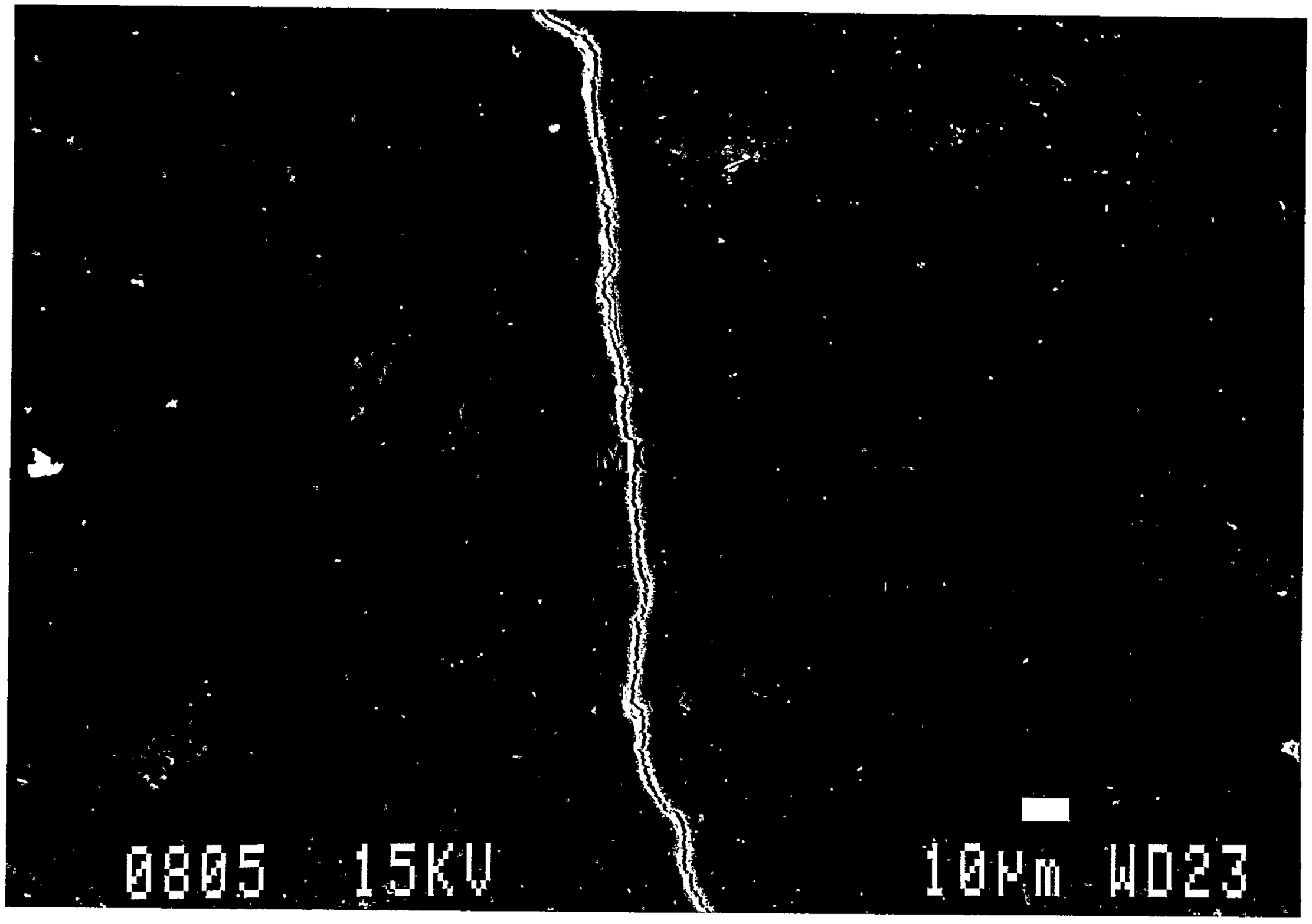


Fig 2.2.h. SEM(550x), close up examination in Fig. 2.2.g.- microcrack (MC) with a 0.8 microns in width. Bar = 10um.

2.3. Morphological study under Scanning Electron Microscopy (Laser/control).

2.3.1. Materials and methods

In order to demonstrate the laser surface effects on enamel without the artefacts caused by forceps extraction, the occlusal surface of six freshly extracted premolars were selected for further investigations. After the occlusal surfaces of the specimens were cleaned by prophy brush with water, the palatal/lingual occlusal table was subjected to 1.30W, 15Hz pulses of Nd:YAG laser for 2mins. The buccal occlusal table was unlased and taken as the control surface. The specimens were prepared similarly, as previously described, for Scanning Electron Microscopy examination.

The rating of enamel surface appearance was accomplished without knowledge of treatment conditions. The enamel of the occlusal surface of the tooth crown were rated using the following criteria:

0- Normal and smooth enamel surface.

1- Presence of pitting, flaking and microcracks, but not actual surface cratering and melting.

2.3.2. Results and discussion

The results (Table 2.3.a) showed that there was no observable pitting, flaking and cracking on the enamel surface irradiated with 1.30W, 15Hz pulses of Nd:YAG laser for 2mins. Using the Wilcoxon test (P=1), there was no statistically significant differences between the Laser (Fig.2.3.a &b) treated surfaces and the control (Fig.2.3. c &d) surfaces.

This pilot study (2.2 and 2.3) suggested that exposure to plain Nd:YAG laser pulses for clinical induction of pulpal anaesthesia, caused no enamel surface alterations when observed under Scanning Electron Microscopy.

Table 2.3.a. Rating scale for enamel surface changes-under Scanning Electron Microscopy.

0- Normal and smooth enamel surface.
1- Presence of pitting, flaking and microcracks, but not actual surface cratering and melting.

Specimen no.	1-Ratings	2-Ratings	Ave. Ratings
96049A(C)	0	0	0
96049A(L)	0	0	0
96049B(C)	0	0	0
96049B(L)	0	0	0
96049C(C)	0	0	0
96049C(L)	0	0	0
96049D(C)	0	0	0
96049D(L)	0	0	0
96049E(C)	0	0	0
96049E(L)	0	0	0
96049F(C)	0	0	0
96049F(L)	0	0	0

(C)= Control group, (L)= Laser group

Fig.2.3.a. SEM(80x) of the buccal incline enamel surface, from a typical Laser-treated specimen- the absence of pitting and microcracks formation. Bar = 100um.

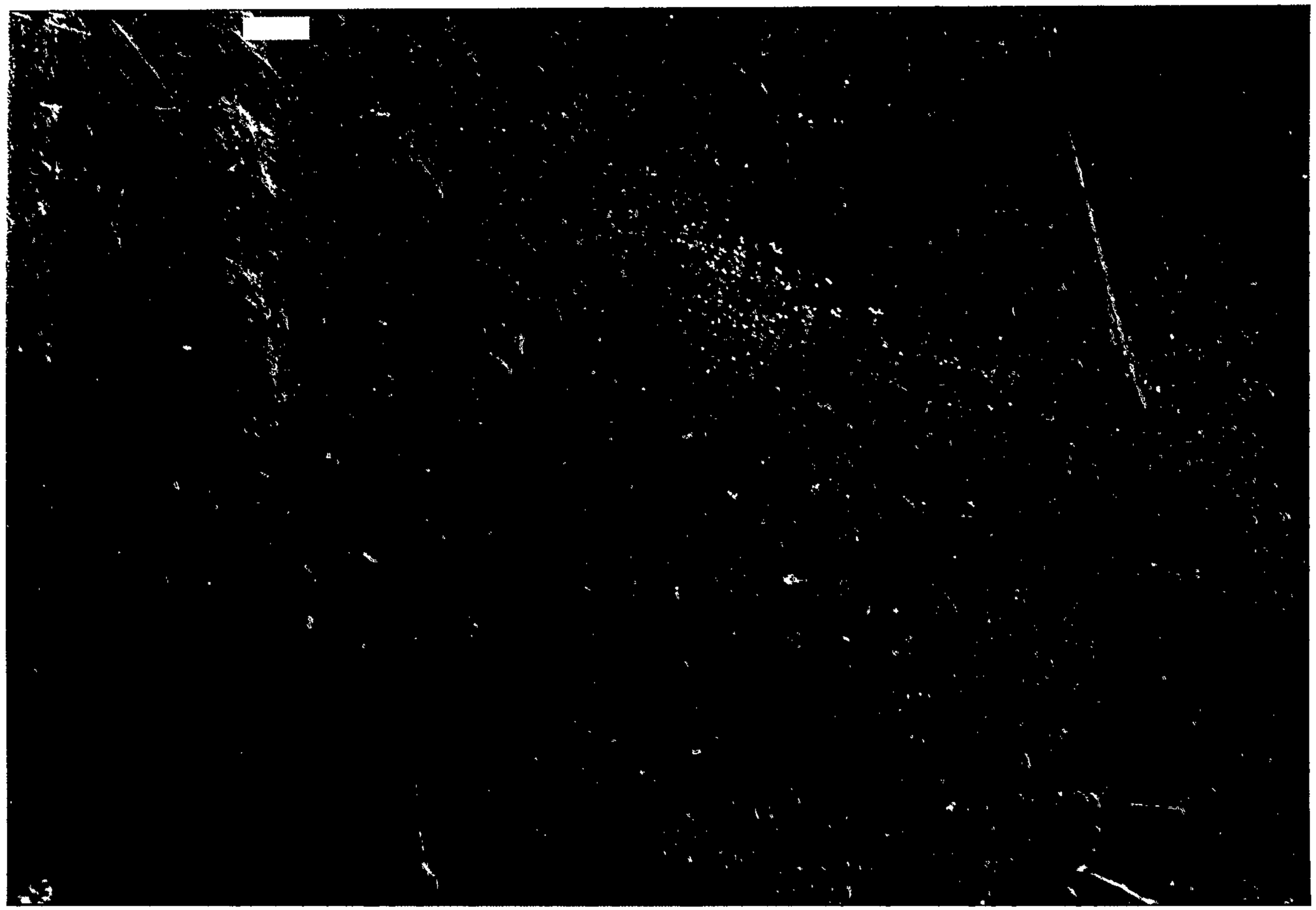


Fig 2.3.b. SEM(880x), close up examination in Fig. 2.2.a.- normal enamel surface. Bar = 10um.

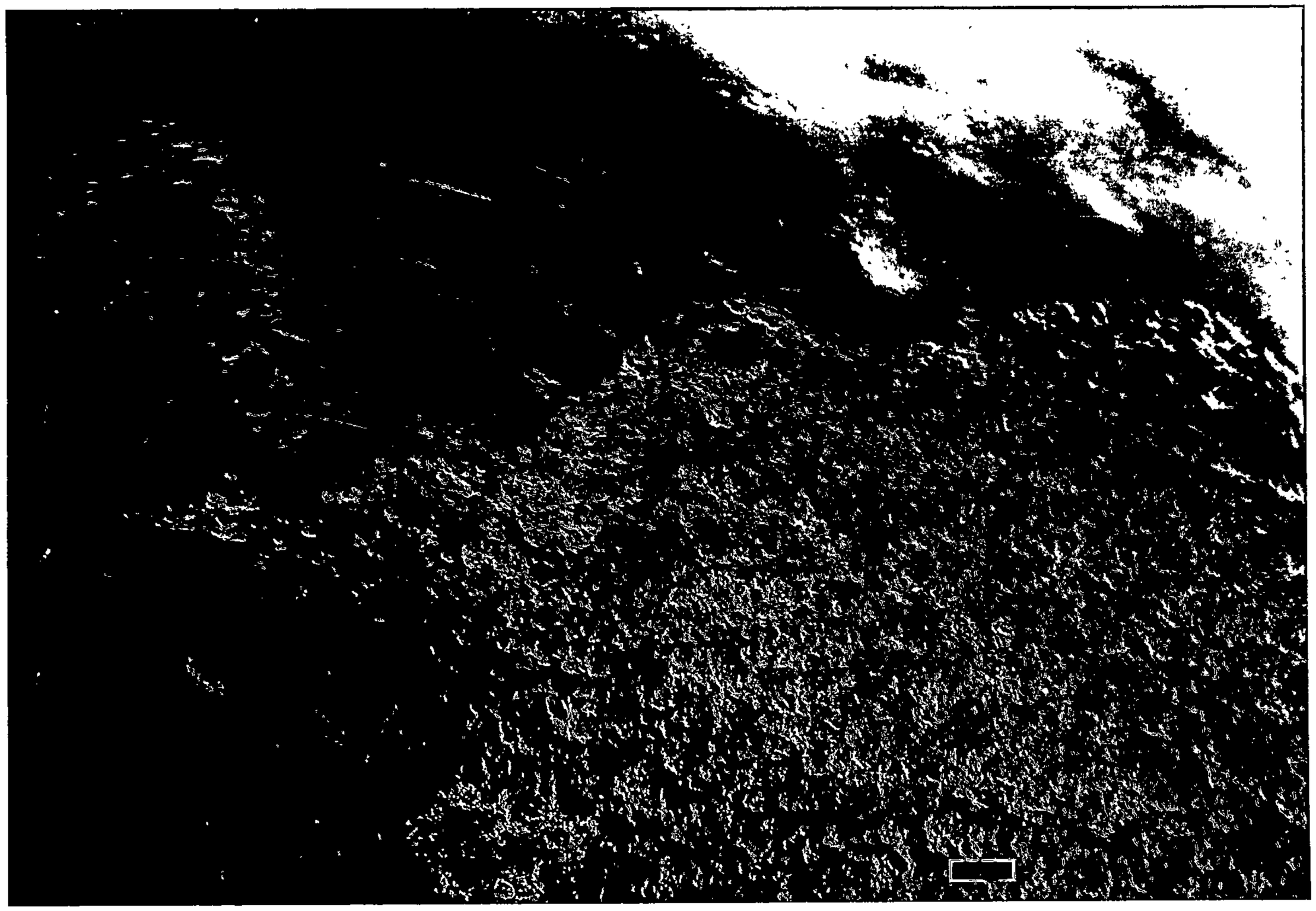


Fig.2.3.c. SEM(80x) of the palatal incline enamel surface, from a typical control specimen- absence of pitting and microcracks formation. Bar = 100um.

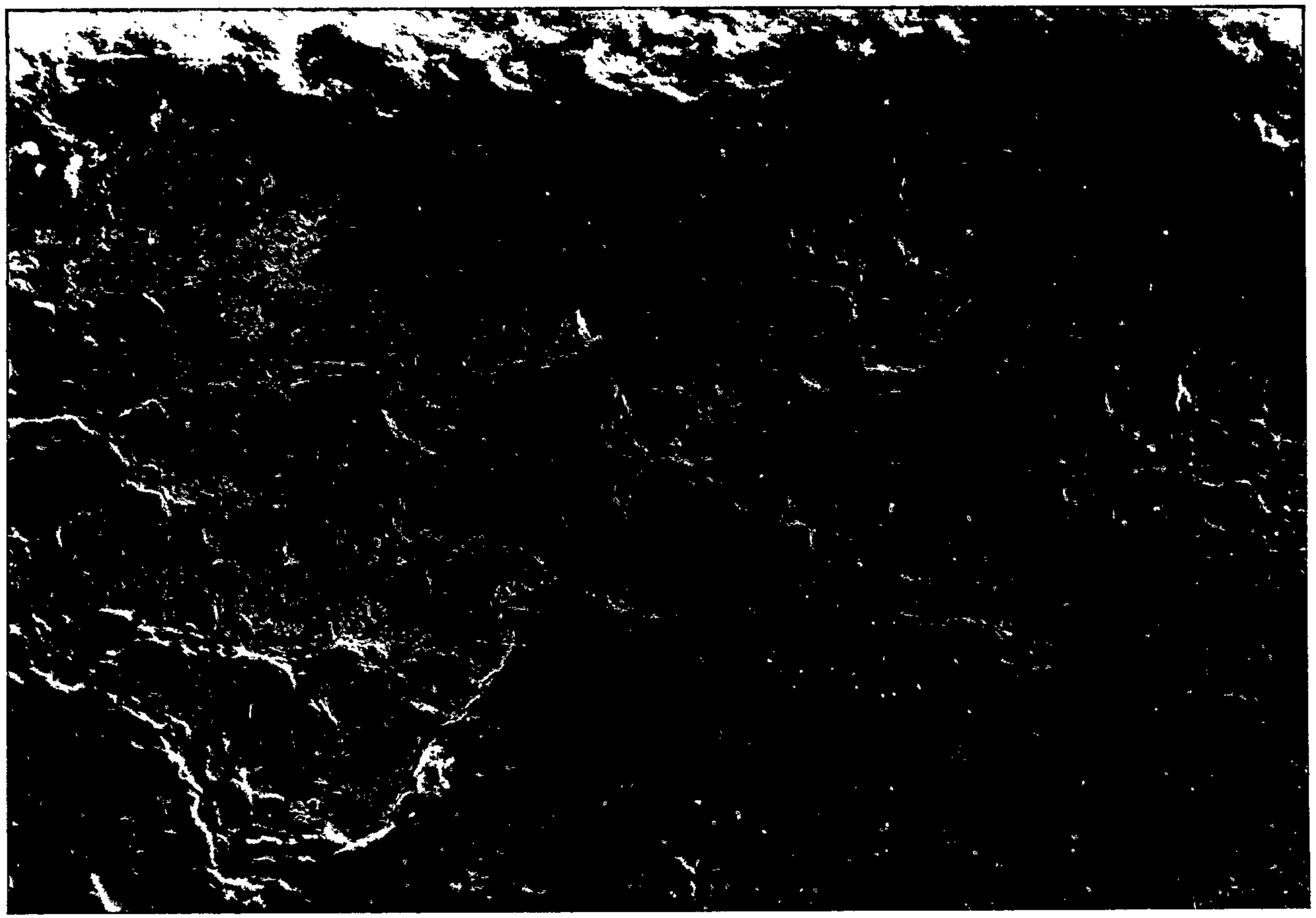


Fig.2.3.d. SEM(880x), close up examination in Fig. 2.2.c- normal enamel surface. Bar = 10um.

2.4. Longitudinal undecalcified ground-sectioning.

2.4.1. Introduction and aim

Laser effects on the surface of mineralised tissues can be studied by SEM techniques or top-view light microscopy. Artefacts (ie. cracks) caused by the preparation process are a special problem with SEM techniques (Pogrel *et al.*, 1993). Longitudinal, undecalcified, ground-sectioning provides a useful technique to study the effects of lasers on tissues beneath the surface of the irradiated samples. The extent (i.e. the depth) of any laser-induced alterations in the mineralised tissues can also be precisely determined (Frentzen *et al.*, 1990; Koort and Frentzen, 1991).

Koort and Frentzen (1992) employed a modified sawing and grinding technique for undecalcified sections, and demonstrated zones of debris and carbonisation on enamel with an underlying area of necrosis (Fig.2.4a), even when using low pulsed Nd:YAG energy. They also reported that microcracks always appeared when using energies above a threshold of more than 100 J/cm^2.

The histological method used in this study has been used on dental hard tissues, and is modified from previous undecalcified ground-sectioning studies on lased teeth (Donath, 1982; Frentzen *et al.*, 1990; Koort and Frentzen, 1992; Rohrer, 1992; Koort and Frentzen, 1993; Lars, 1994).

The purpose of this investigation was to detect any alterations and loss of morphology in enamel and dentine layers on the cervical half of palatal (or lingual) portion of the crown in longitudinal sections.

2.4.2. Materials and methods

Ten paired-tooth specimens (from 44 pairs of extracted teeth from study 1) were randomly chosen and prepared for undecalcified ground-sectioning. After extraction, the root apices were cut off with a highspeed bur, to permit entry of fixative to the pulp, to minimise shrinkage artefact. The teeth were then stored in Karnovsky's fixative (2% formaldehyde and 2.5% glutaraldehyde in 0.1M sodium cacodylate at pH 7.4) solution for two days at room temperature.

These specimens were dehydrated at room temperature, using increasing ethanol concentrations (30mins for each step): 70%, 80%, 90% and 3X100%. Plastic infiltration was undertaken with hydroxyethyl-methacrylate (London White Resin) and 100% alcohol, at a ratio of 1:1 for 1hr, and then placed in 100% London White Resin for 24hrs in a fridge (4°C), in a light-proof container to avoid premature polymerisation. After the penetration of the whole specimen with the plastic embedding medium, any old resin was discarded and new 100% resin was added.

Then it was left in an oven at a temperature of 60°C for 48hrs, to ensure that the medium in the entire mineralised specimen was fully hardened.

In a microsawing machine, parallel sections of 100-200 micron thickness were cut from the plastic embedded specimen with a 0.2mm/D64 Diamond saw. The desired final thickness of the specimen was obtained with a microgrinding system using sandpaper of 800-400 grit. Without removal of the plastic embedding medium, the final sections were stained with 2% Toluidine-Blue solution for two minutes before being examined by light microscopy (at magnifications of 10, 25 and 50x).

The rating was done by two trained observers without knowledge of treatment conditions using the following criteria:

0- No disruption, normal enamel surfaces (Fig. 2.4.b).

1- Presence of microcraks in enamel layer.

2- Disruption of enamel surface, including areas of carbonisation and necrosis.

3- Disruption of dentine surface, including areas of carbonisation and necrosis.

2.4.3. Results and discussion

Results (Table 2.4.a) showed no observable alterations on both enamel and dentine layers including areas of carbonisation and necrosis from

both Laser (Fig. 2.4.c) and EMLA (Fig. 2.4.d) groups. However, there were microcracks present in the enamel layer, in 10% (1/10) cases of the Laser (Fig. 2.4.e & f) group. The microscopic appearance of these microcracks could be easily mistaken for normal enamel lamella, which usually run from the enamel surface all the way to the dentino-enamel junction. Instead, these microcracks originated from the enamel surface and terminated with mini-branches within the enamel layer.

This result was concurrent with the claim by Koort and Frentzen (1992) that microcracks appeared always when using pulsed Nd:YAG laser energies above a threshold of more than 100 J/cm^2. Unfortunately, when forceps extraction is the method of removal, tooth surface cracking can commonly occur. The surface effects of forceps extraction may be misinterpreted as the surface effects caused by the Laser and this uncertainty requires further investigation.

The findings in this study were not concurrent with the observations in the dentine layer reported by Nagasawa (1988) that the dentine canals in the surface layer disappeared as a result of laser irradiation for clinically induced dental analgesia.

This pilot study suggested that exposure to plain Nd:YAG laser pulses, with the energy density range of 73-107J/cm^2, for clinical induction of

pulpal anaesthesia, caused no enamel and dentine layer alterations, as demonstrated by longitudinal undecalcified ground-section studies.

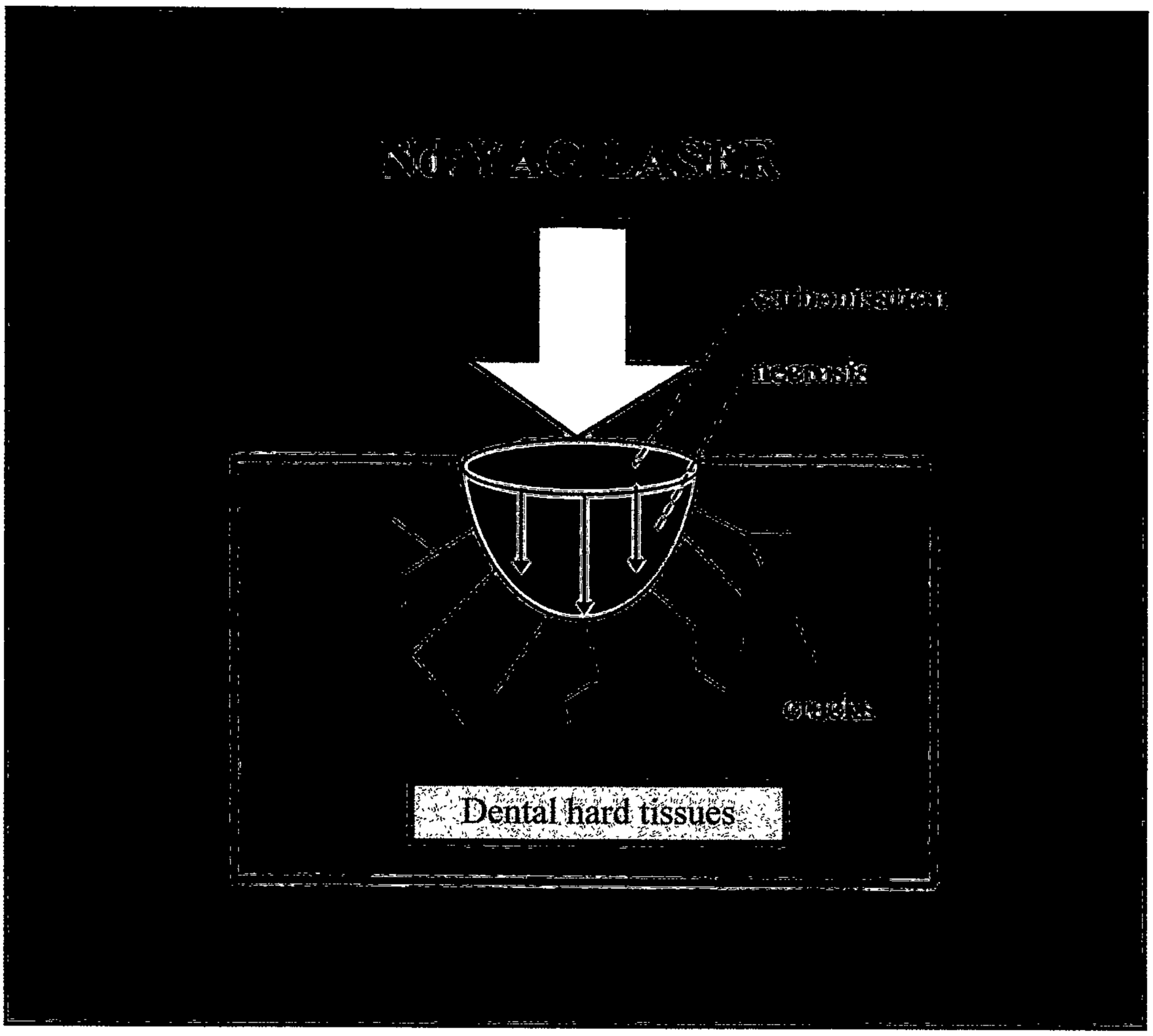


Fig. 2.4.a. Model of interaction of Nd:YAG laser radiation with dental hard tissues: demonstrates zones of debris and carbonisation on enamel with underlying area of necrosis and microcracks. (From Koort and Frentzen, 1992).

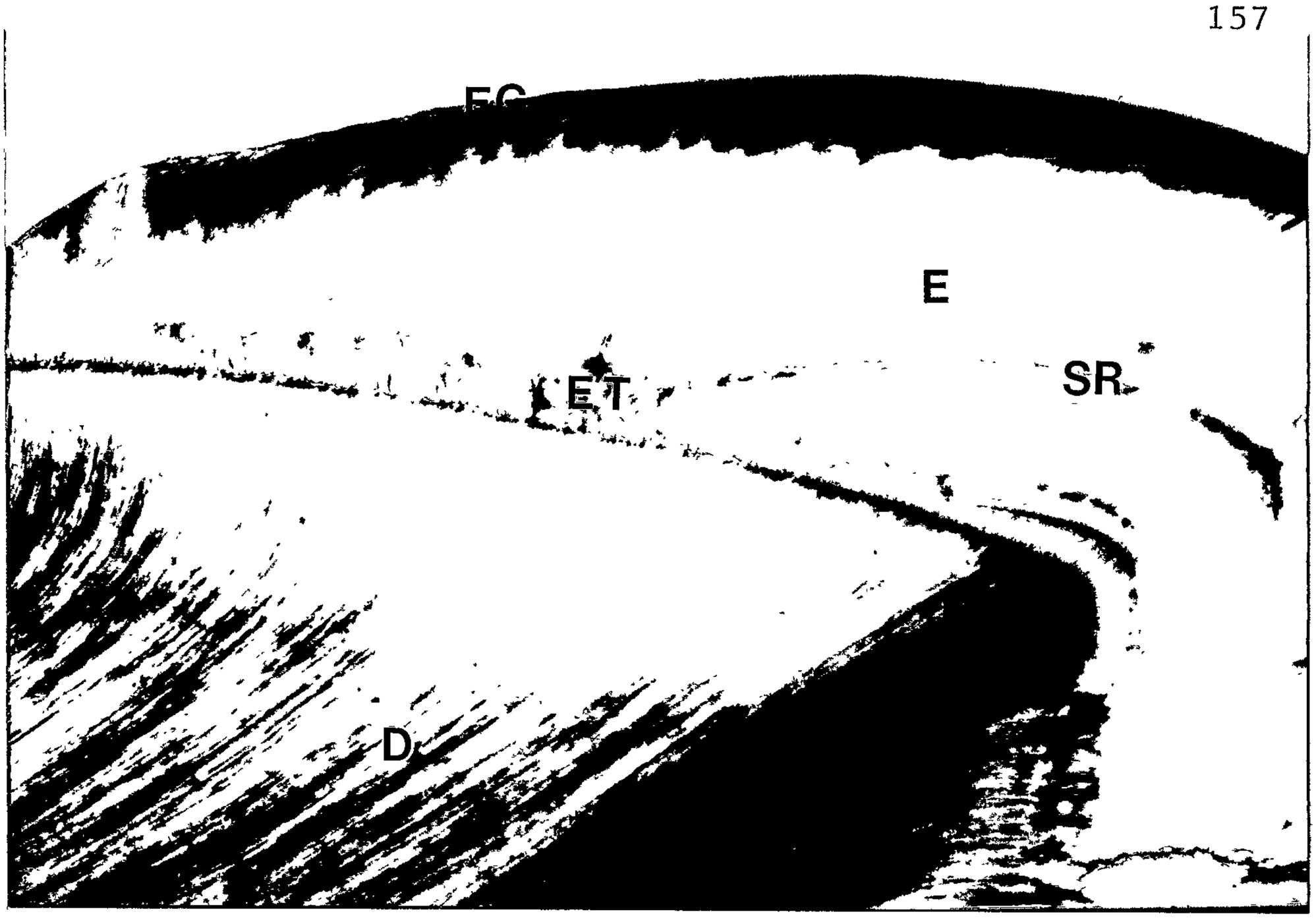


Fig.2.4.b. LM(10x), ULGS, of the palatal surface, from a control specimen- normal enamel (E), dentine (D) layer: enamel cuticle (EC), striae of Retzius (SR) and enamel tufts (ET).

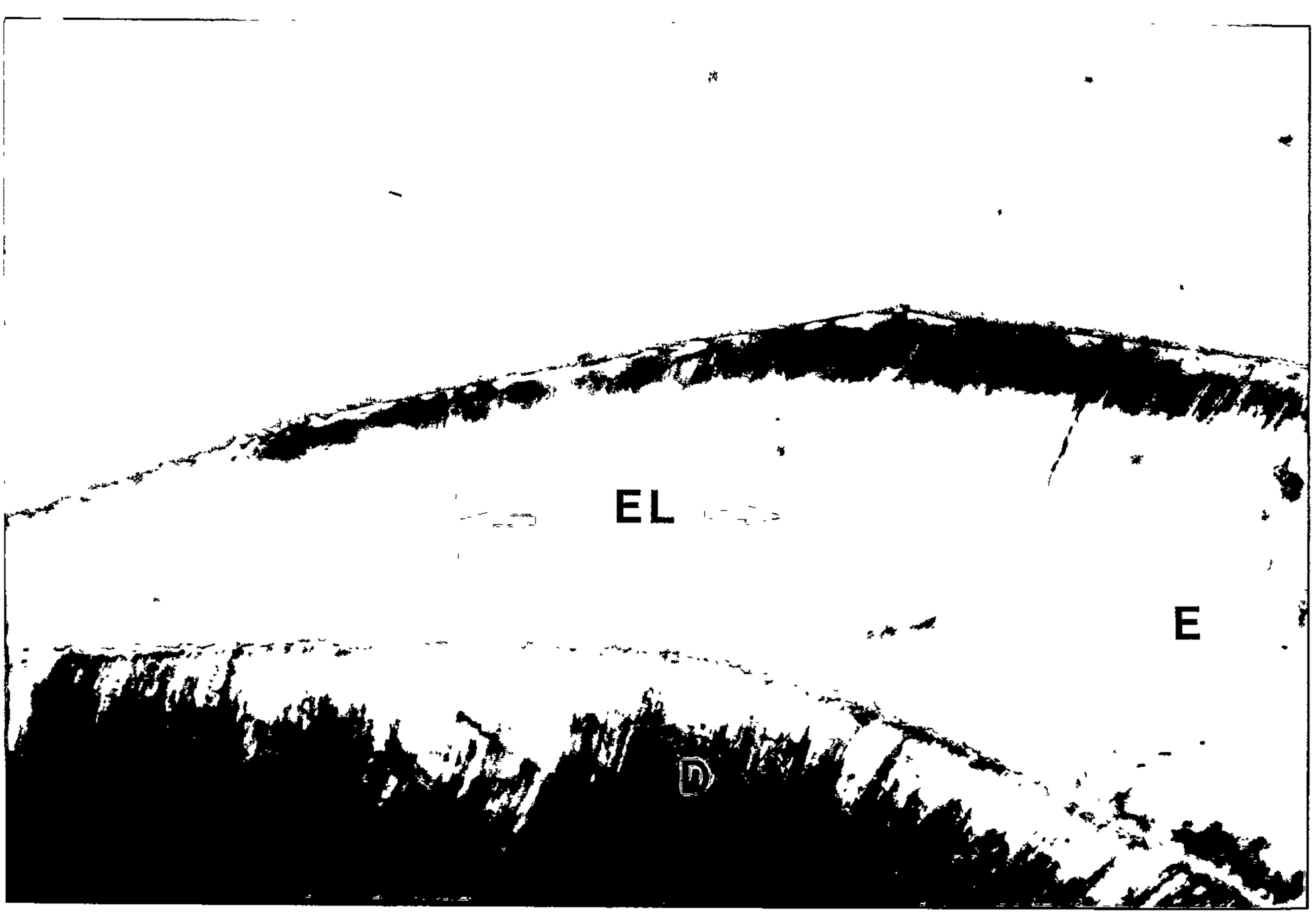


Fig.2.4.c. LM(10x), ULGS, of the cervical half of the palatal surface, from a typical Laser-treated specimen-normal enamel (E) and dentine (D) layer: enamel lamella (EL) can easily be mistaken as microcracks.

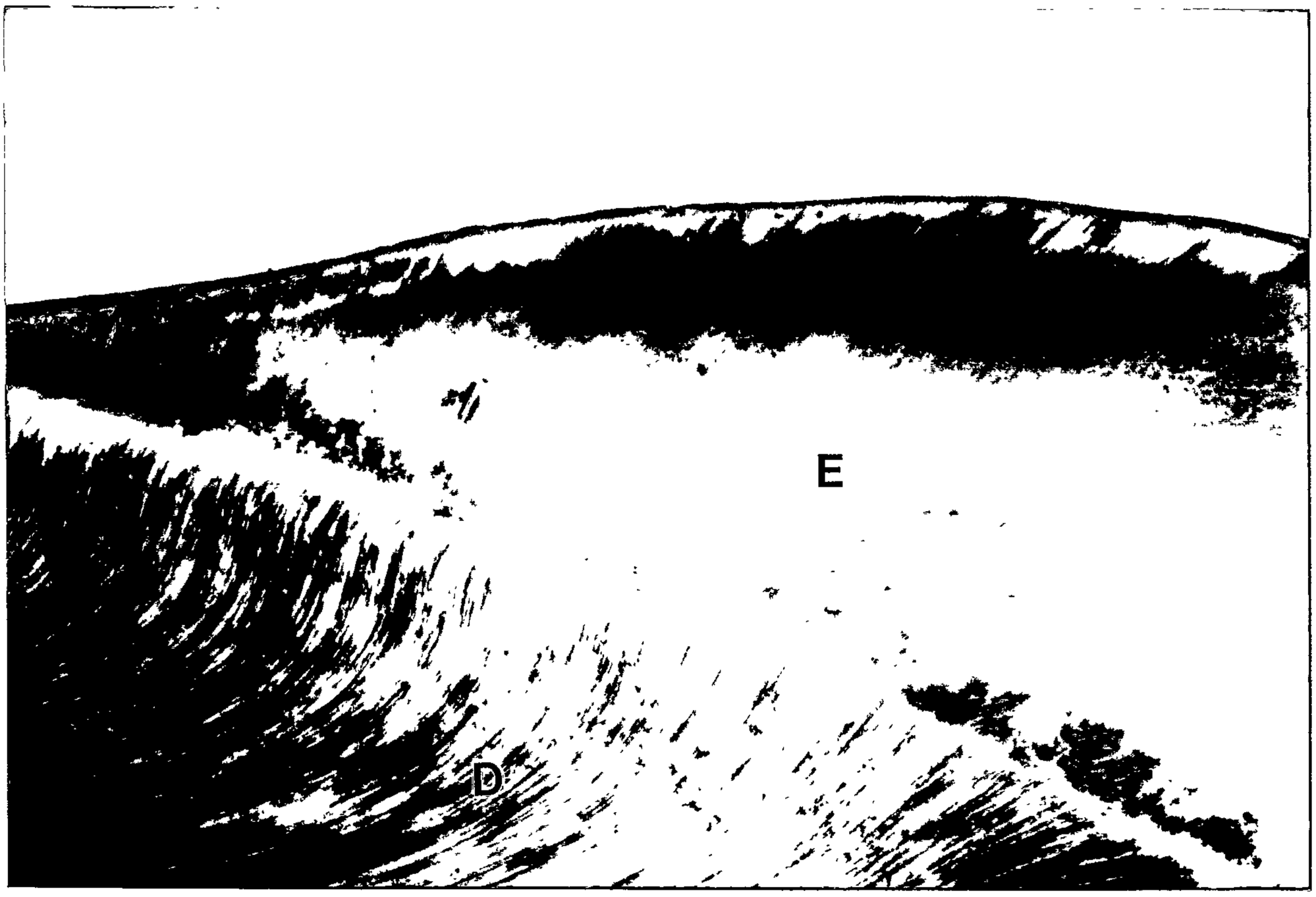


Fig.2.4.d. LM(10x), ULGS, of the cervical half of the palatal surface, from a typical EMLA-treated specimen-normal enamel (E) and dentine (D) layer.

Fig.2.4.e. LM (10x), ULGS, of the cervical half of the palatal surface, from a typical Laser-treated specimen- normal enamel and dentine layer: enamel lamella (EL) and a suspected microcrack (MC).

Fig.2.4.f. Under close up examination in Fig. 2.4.e., LM (25x)- the enamel lamella (EL) and a suspected microcrack (MC) which appears to disappeared within the enamel layer.

Table 2.4.a. Rating scale of morphological changes in enamel and dentine layers-under longitudinal undecalcified ground-sectioning.

0- No disruption, normal enamel surfaces.
1- Presence of microcracks in enamel layer.
2- Disruption of enamel surface, including areas of carbonisation and necrosis.
3- Disruption of dentine surface, including areas of carbonisation and necrosis.

Specimen no.	1-Ratings	2-Ratings	Ave. Ratings
94064A1(L)	0	0	0
94064A1(E)	0	0	0
94064A2(L)	0	0	0
94064A2(E)	0	0	0
94064A3(L)	0	0	0
94064A3(E)	0	0	0
94064B(L)	0	0	0
94064B(E)	0	0	0
94074C1(L)	0	0	0
94074C1(E)	0	0	0
94074C2(L)	0	0	0
94074C2(E)	0	0	0
94074C3(L)	0	0	0
94074C3(E)	0	0	0
94074D(L)	1	1	1
94074D(E)	0	0	0
940135(E)	0	0	0
940145(L)	0	0	0
940314(E)	1	1	1
940324(L)	0	0	0

Energy density ranged between 73-107J/cm^2,
(E)= EMLA group, (L)= Laser group

2.5. Dye penetration- dentinal permeability.

2.5.1. Introduction and aim

Dentinal permeability is defined as the ability of fluids and substances to move through dentine. In theory, laser energy can be used to alter the structure and chemistry of dentine surfaces (Featherstone and Nelson, 1987) by melting with subsequent recrystallisation into a nonporous and continuous "glazed" surface which may partially or totally obliterate dentinal tubules (Dederich *et al.*, 1984; Dederich *et al.*, 1988; Khayat *et al.*, 1991). This surface appearance can conceivably demonstrate reduced permeability to fluids. White *et al.* (1991d) in an *in vitro* study, further demonstrated that pulsed Nd:YAG laser energy can decrease hydraulic conductance at higher powers and longer exposure time. They also postulated that the fluid within dentinal tubules contains proteins which could be coagulated within the tubules by the laser energy.

Previous studies (Forrest-Winchester and Walsh, 1993; Goodis *et al.* 1992) have demonstrated the effectiveness of pulsed Nd:YAG laser energy in reducing dentine permeability. It has been suggested that by assessing the amount of 5% methylene blue dye penetration on the root dentinal wall, the effect of Nd:YAG on dentinal permeability can also be investigated (Stabholz *et al.*, 1992; Miserendino *et al.*, 1995).

The purpose of this study was to investigate the effect of Nd:YAG laser treatment on dentinal permeability by assessing the distance of 5% methylene blue dye penetration from the pulp towards the dentino-enamel junction, at the region of cervical half of palatal/lingual surface (non-cavity cut) of the tooth.

2.5.2. Materials and methods

Ten paired-tooth specimens (from 44 pairs of extracted teeth from study 1) were selected randomly for this study. After fixation, the teeth were washed and rinsed in distilled water. Then the teeth were secured horizontally with greenstick compound material onto aluminium stubs and sectioned longitudinally across the cut cavity into halves using a diamond saw, under water cooling. The pulpal content was removed with a hand excavator to ensure the pulp chamber was completely clean. Then 5% methylene blue was carefully dispensed only onto the pulp chamber space, and no excess dye was allowed to spill over the rest of the surface. Both the specimen and the stub were mounted onto the base of a container and stored for 24hrs in an aqueous 2% thymol solution to inhibit bacterial growth. The depths of dye penetration of the specimens, were examined microscopically (Wild Leitz light microscope) at 25x and 32x magnifications, by two trained observers, in a blind manner, using

the previously prepared negative and positive control specimens as the standard criteria.

At the region of the cervical half of palatal/lingual surface (non-cavity cut) of the tooth crown, the penetration of the dye, from the pulp towards the dentino-enamel junction was rated as follows:

0- No dye penetration, or penetration no more than half the depth of the dentine.

1- Dye penetration beyond half the depth of the dentine, but below the level of the dentino-enamel junction (Fig. 2.5.a).

2- Dye penetration up to the dentino-enamel junction (Fig. 2.5.b).

2.5.3. Results and discussion

Results (Table 2.5.a) showed that in all specimens in the EMLA group (Fig. 2.5.c) the dye had reached the level of the dentino-enamel junction. Whereas, in the Laser group (Fig. 2.5.d), 3/10 (30%) of the specimens had dye penetrated beyond half depth of dentine, and below the level of the dentino-enamel junction, demonstrating a reduction of dentinal permeability. This finding is concurrent with previous studies (Stabholz *et al.* ,1992; Miserendino *et al.*, 1995; Goodis *et al.*, 1992) which reported that Nd:YAG energy pulses reduce dentinal permeability. However, statistical analysis using the paired t-test (P=0.28), showed that

there was no significant difference between the Laser and EMLA groups in terms of the observed depth of dye penetration in dentine.

This pilot study suggest that exposure to Nd:YAG laser pulses for the clinical induction of pulpal anaesthesia, with the energy density ranging between 73-107J/cm^2, causes little reduction in dentinal permeability as demonstrated by dye penetration method. It is apparent that other mechanisms may also be operating beyond the level of the dentine, in laser induced pulpal anaesthesia.

Table 2.5.a. Observers' rating of dye penetration.

0- No dye penetration, or penetration no more than 1/2 depth of dentine.
1- Dye penetration beyond 1/2 depth of dentine, but below the level of dentino-enamel junction.
2- Dye penetration up to dentino-enamel junction.

Specimen no.	1-Ratings	2-Ratings	Ave. Ratings
1715(L)	2	1	1.5
1725(E)	2	2	2
2115(E)	2	2	2
2125(L)	2	1	1.5
2914(E)	2	2	2
2924(L)	2	2	2
3014(E)	2	2	2
3024(L)	2	2	2
3115(E)	2	2	2
3125(L)	2	2	2
3214(L)	2	2	2
3224(E)	2	2	2
3435(E)	2	2	2
3445(L)	1	1	1
3535(L)	2	2	2
3545(E)	2	2	2
3835(E)	1	1	1
3845(L)	1	1	1
3935(E)	1	2	1.5
3945(L)	2	2	2

(E)= EMLA group, (L)= Laser group

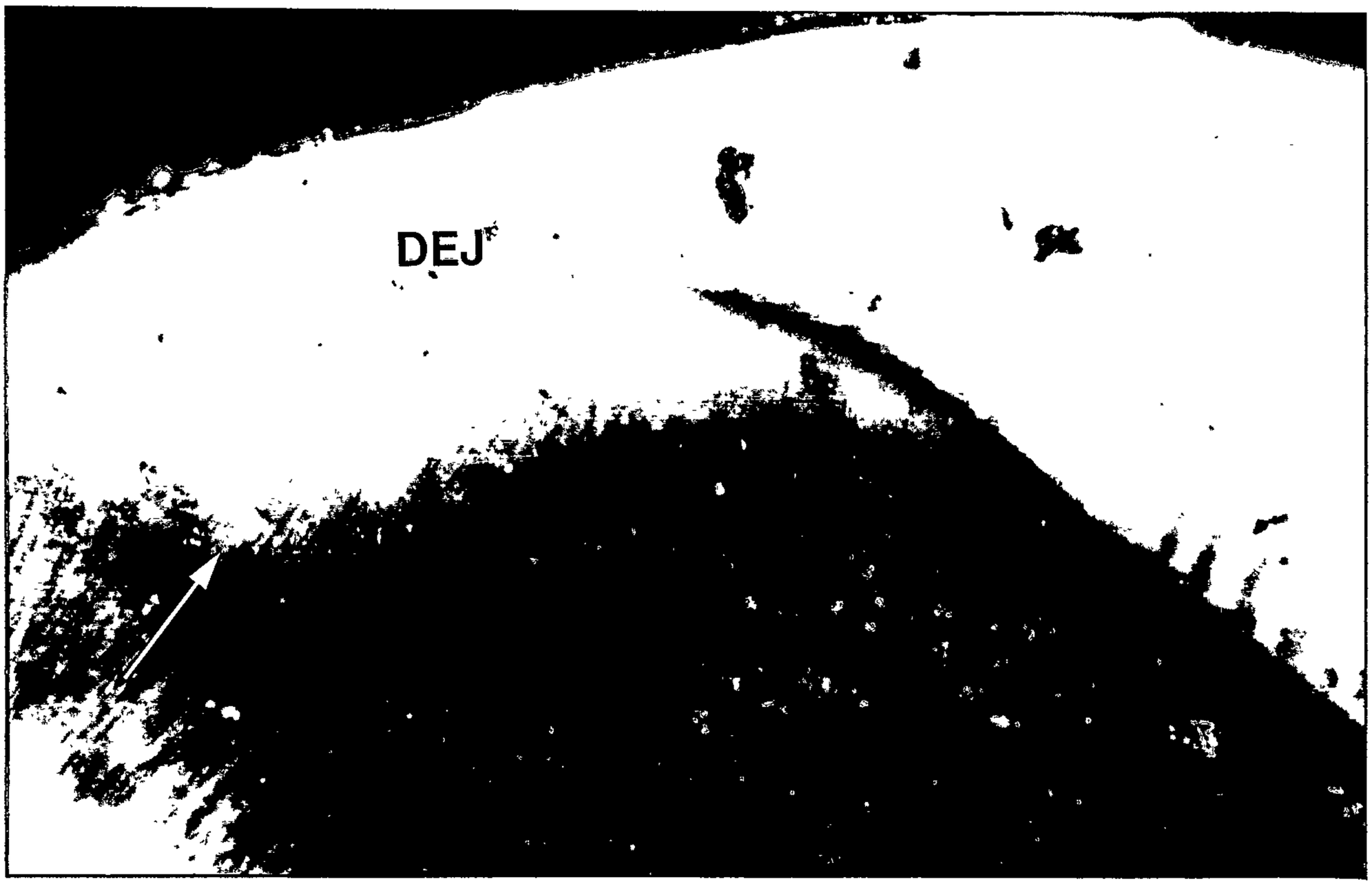


Fig.2.5.a. LM(32x) of longitudinal section of cervical half of tooth section from a Laser control specimen (Nd:YAG pulses, at 2.0W, 30pps, 2 mins, 200 microns fibre)- the level of dye penetration (↑), the dentino-enamel junction (DEJ).

Fig.2.5.b. LM(32x) of longitudinal section of cervical half of tooth section from a control specimen (normal non-lased).

Fig.2.5.c. LM(25x) of longitudinal section of cervical half of palatal surface, from a typical EMLA-treated specimen, demonstrates dye penetration (↑) up to the dentino-enamel junction.

Fig.2.5.d. This light micrograph (25x) of longitudinal section of cervical half of palatal surface, from a typical Laser-treated specimen- dye penetration (↑) beyond half depth of dentine.

2.6. Decalcified H&E staining-pulpal histology (Laser/cavity cut versus EMLA/cavity cut)

2.6.1. Introduction and aims

There have been numerous publications on the therapeutic application of the pulsed Nd:YAG laser in dentistry (Myers and Myers, 1985b; Myers and McDaniel, 1991; Myers *et al.*, 1992; Hardee *et al.*, 1990; White *et al.*, 1991c; Yamanoto and Sato, 1980). Nevertheless, because its emission wavelength (1064nm) possesses inherent heat-producing characteristics in dental hard tissues (Adrian, 1977; Fried *et al.*, 1993; Spitzer and TenBosch, 1975), its effects on the pulpal integrity (i.e. the overall viability of the odontoblastic layer and the intrapulpal cellular structure) subsequent to surface irradiation remain major concerns of researchers; in particularly, the effects of prolonged elevation of pulpal temperature during Nd:YAG laser anaesthesia.

The purpose of this study was to investigate the effects (immediate) of pulsed Nd:YAG laser on the pulp after being irradiated for clinical induction of pulpal anaesthesia and to define the histological variation between EMLA and Laser treated teeth.

2.6.2. Materials and methods

Eighteen paired-specimens (from 44 pairs of extracted teeth from study 1) were placed in a 10% EDTA decalcifying solution for four weeks. The decalcifying solution was changed weekly. A radiograph was taken to confirm complete decalcification. After decalcification the specimens underwent dehydration and infiltration processes and finally were embedded in paraffin before sectioning. The process was deemed complete when the specimen was easily bent, with little resistance. The specimens were dissected and placed in tissue cassettes, sectioned at 5 micron in thickness, stained with Haematoxylin & Eosin, and then examined microscopically at 100x magnification using previously established positive and negative control specimens as the standard criteria. Multiple histological sections belonging to each treatment condition, from different teeth, were presented in a blind manner, to two trained observers.

The superficial pulpal responses, at the cervical half of palatal/lingual (non-cavity cut) surface of the tooth crown, were rated, using the criteria modified from previous pulp biology studies which are as follows: (Sayegh and Reed, 1974; Adrian, 1977; Stanley and Bethesda, 1968; Stanley *et al*. 1975; White *et al*., 1991; 1992; 1993; 1994; Arcoria *et al*., 1991; 1994; Arcoria and Miserendino, 1995).

0- Normal cellular elements in the pulp area underlying the zone of laser irradiation (Fig.2.6.a).

1- Disruption of the odontoblastic cell layer underlying the zone of laser irradiation, including: elongation, irregular cytoplasmic membranes, vacuolisation and subodontoblastic oedema (Fig.2.6.b).

2- Disruption of the odontoblastic cell layer underlying the zone of laser irradiation, including: loss of stroma and viable epithelial root sheath of the odontoblastic layer, cytoplasmic changes, and aspirated odontoblasts (Fig.2.6.c).

2.6.3. Results and discussion

Results (Table 2.6.a) showed that there were 22% (4/18) specimens of the Laser(Fig.2.6.b) group and 28% (5/18) specimens from the EMLA (Fig.2.6.d) group with observable superficial disruption which included the elongation, irregular cytoplasmic membrane, vacuolisation and subodontoblastic oedema, of the odontoblastic cell layer. Interestingly, there was an observable increase in vascularity of the pulp from both groups in 22% (4/18) cases. Statistical analysis using the paired t-test (P=1), showed there was no significant difference between the Laser and the EMLA groups in terms of the observable pulpal disruption. The mean remaining dentine thickness for both groups was 1.84±0.18mm.

When forceps extraction is the method of tooth removal, cellular displacement or aspiration and haemorrhage can result from forceps pressure (Sayegh and Reed, 1974). Conventional cavity preparation can cause dehydration, evaporation, and mechanical and thermal injury to the tooth structure (Seltzer and Bender, 1984). It is possible that the pulpal disruption seen in this sample was due to the highspeed bur cutting of ClassV cavity, the Laser treatment, or both. The data from this study show that the effects of the Nd:YAG pulsed laser are indistinguishable from pulpal disruption effects of the EMLA group.

A critical factor in the response of the pulp to thermal and mechanical irritations is the remaining dentine thickness (Stanley and Swerdlow, 1964). It is possible that the pulpal disruption seen in this sample was due to highspeed bur Class V cavity cutting, the Laser treatment, or both. However, it has been shown that for mechanical and thermal trauma, such as highspeed bur cutting (Stanley and Bethesda, 1968) and laser irradiation (White *et al*., 1991; Goodis *et al* 1992; White *et al* 1994), 2mm of remaining dentine thickness was sufficient barrier to prevent adverse pulpal sequelae. The mean remaining dentine thickness in this study was 1.84mm. Therefore the possibility of the observable pulpal changes being caused by fixation artifacts or technical difficulties

(Stanley and Swerdlow, 1964; Stanley and Bethesda, 1968) can not be discounted when interpreting the results in this study.

Perhaps, these preparation artefacts could be minimised by initial microscopic scanning of a large sample size so that enough specimens still remain for analysis (Stanley and Bethesda, 1968).

However, because of the effects of the conventional cavity preparation, one cannot determine whether or not there is any atypical pulpal response to the pulsed Nd:YAG laser treatment at the energy level used for clinical induction of pulpal anaesthesia on human premolar teeth. Furthermore, if this study was to measure the acute pulpal inflammatory response to laser exposure, then a larger sample size (n>70) would be needed to identify and quantify the degree of vascular dilatation and congestion (eg. counting the number of red blood cells), oedema, vacuolisation and degree of alterations (elongation) of the odontoblastic layer.

However, this pilot study suggest that exposure to Nd:YAG laser pulses for clinical induction of pulpal anaesthesia, with the energy density range of 73-107J/cm^2 (320 micron fibre), with total energy dose range of 106-156J/surface, followed by highspeed bur cavity cutting, induced pulpal changes comparable with that of EMLA (with highspeed cavity cutting).

Table 2.6.a. Rating scale for cell disruption on decalcified sectioning with H&E staining

0- Normal cellular elements in the pulp area underlying the zone of laser irradiation.
1- Disruption of the odontoblastic cell layer underlying the zone of laser irradiation, including: elongation, irregular cytoplasmic membranes, vacuolisation and subodontoblastic oedema.
2- Disruption of the odontoblastic cell layer underlying the zone of laser irradiation, including: loss of stroma and viable epithelial root sheath of the odontoblastic layer, cytoplasmic changes, and aspirated odontoblasts.

Specimen no.	RDT(mm)	1-Ratings	2-Ratings	Ave. Ratings
18A(L)	2.2	0	0	0
18B(E)	2.0	0	0	0
12A(E)	1.75	0	0	0
12B(L)	1.7	0	0	0
10A(L)	1.7	0	0	0
10B(E)	1.9	0	0	0
19A(E)	1.96	0(IV)	0	0(IV)
19B(L)	1.94	0	1(IV)	0.5(IV)
8A(E)	1.5	0	0	0
8B(L)	1.7	0	0	0
20A(E)	1.96	0(IV)	1(IV)	0.5(IV)
20B(L)	1.96	0	0	0
6A(E)	1.7	0	0	0
6B(L)	1.7	0	0	0
28A(E)	2.0	0	0	0
28B(L)	1.96	0	0	0
5(E)	1.8	0	0	0
5B(L)	1.7	0	0	0
40A(E)	1.8	1	1(IV)	1(IV)
40B(L)	1.7	1	1(IV)	1(IV)
1A(L)	2.2	0	0	0
1B(E)	2.0	0	0	0
15A(E)	1.96	0	0	0
15B(L)	1.94	0	0	0

41A(E)	1.7	0(IV)	0(IV)	0(IV)
41B(L)	1.7	1	1(IV)	1(IV)
14A(E)	1.96	0	0	0
14B(L)	1.96	0	0	0
42A(E)	1.5	1	1	1
42B(L)	1.7	0	0	0
43A(L)	1.7	0	0	0
43B(E)	1.9	0	1	1
13A(E)	2.0	0	0	0
13B(L)	1.96	0	0	0
44A(E)	1.75	1	0(IV)	0.5(IV)
44B(L)	1.7	1	1	1(IV)

(IV)= Increase vascularity,
RDT= Remaining dentine thickness (mean thickness of 1.84± 0.18mm),
(E)= EMLA group, (L)= Laser group.

Fig.2.6.a LM(100x), of the pulp underlying cervical half of the palatal surface, from a control specimen- normal pulpal histology: dentine (D), predentine (P), odontoblastic cell layer (OD), cell-free zone of Weil (CFZ) and plexus of Raschkow (PR).

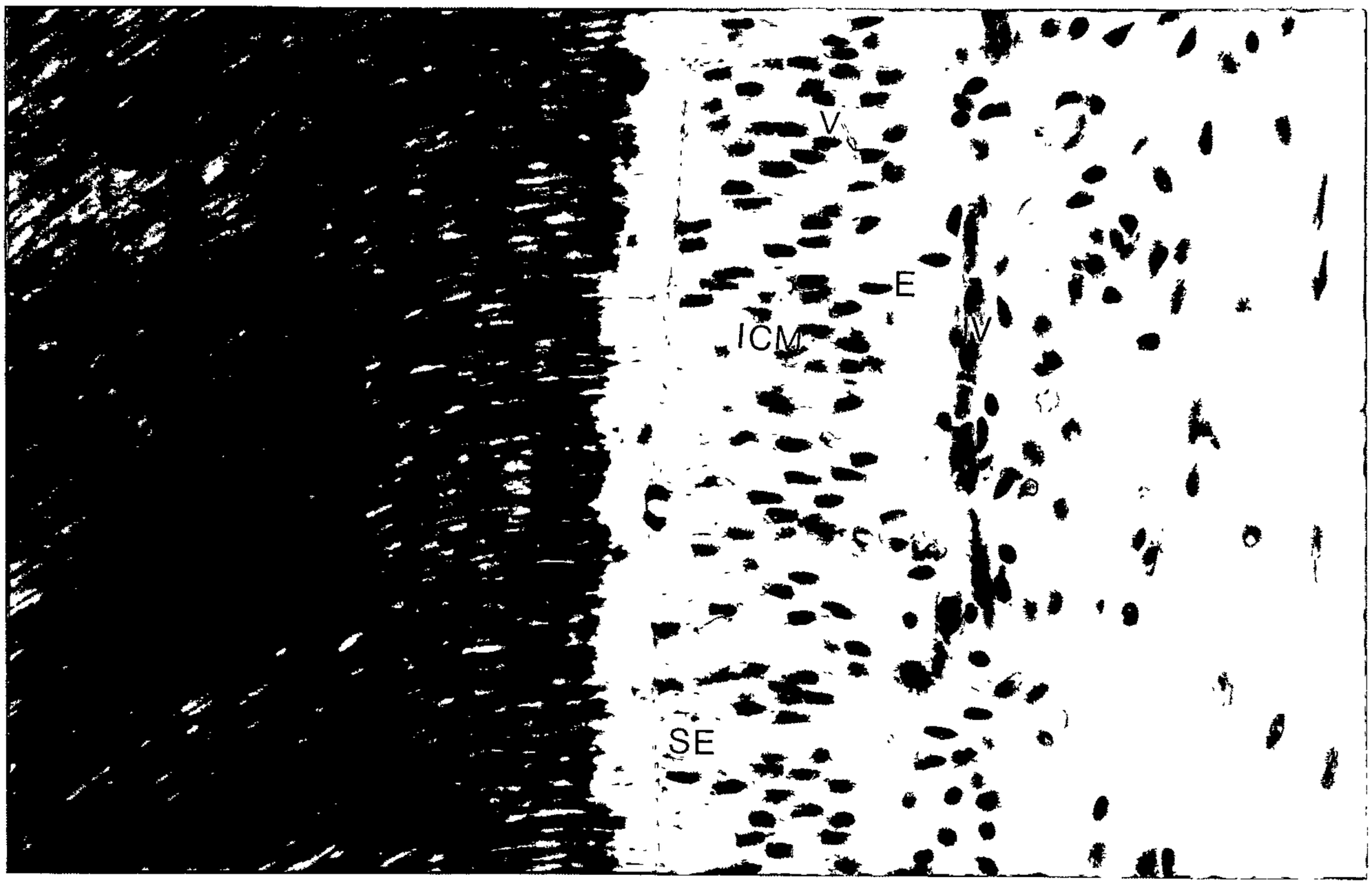


Fig.2.6.b. LM(100x), of the pulp underlying the zone of irradiation, from a typical Laser-treated specimen-disruption including: the elongation (E), irregular cytoplasmic membrane (ICM), vacuolisation (V) and subodontoblastic oedema (SE) of the odontoblastic cell layer and increase vascularity (IV).

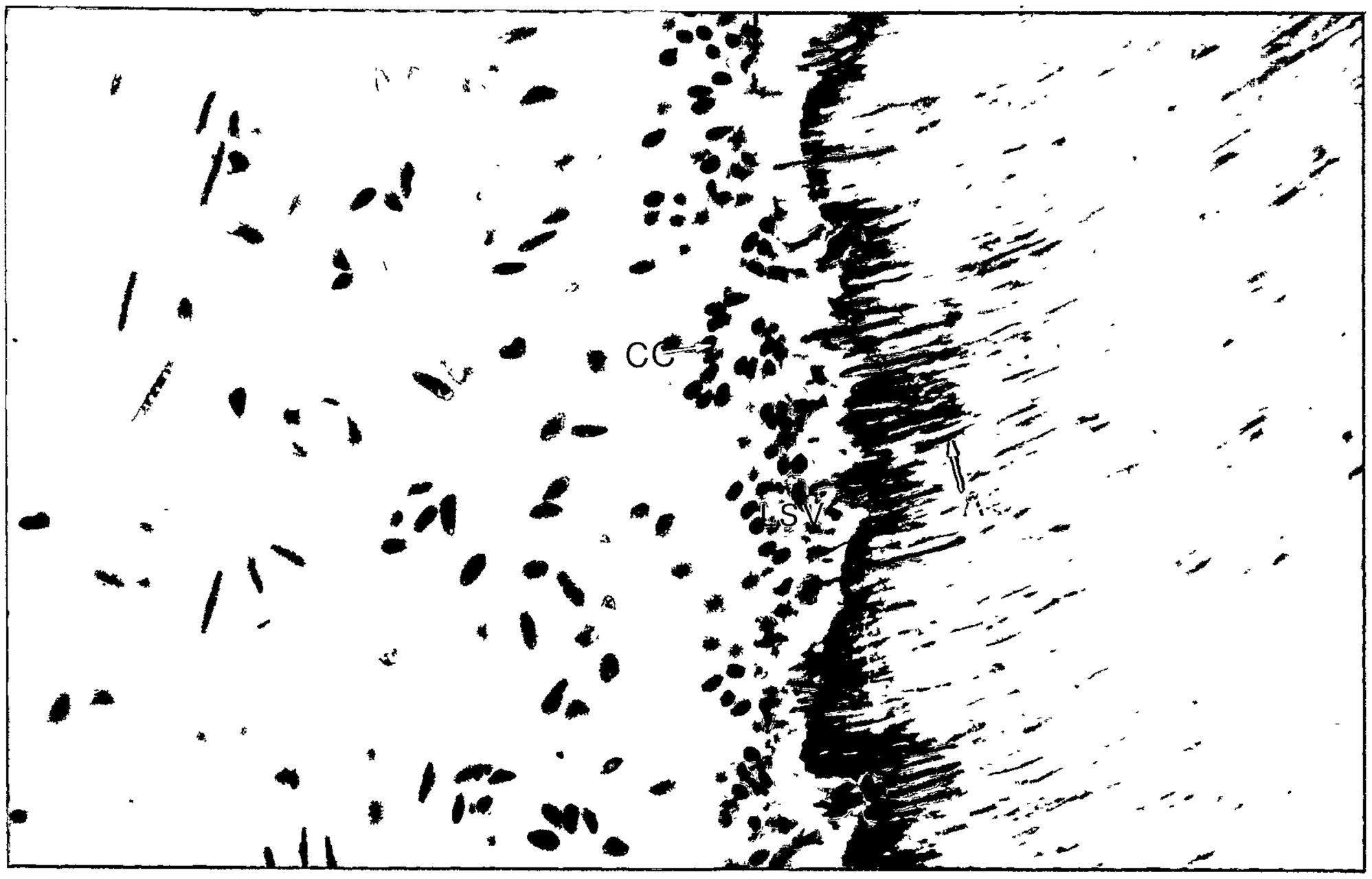


Fig.2.6.c. LM(100x) of the pulp underlying the zone of irradiation, from a positive Laser specimen (Nd:YAG pulses, at 2.0W, 30pps, 2 mins, 200 microns fibre)- loss of stroma and viable epithelial root sheath (LSV), cytoplasmic changes (CC), and aspirated odontoblasts (AO).

Fig.2.6.d. LM(100x), of the pulp underlying the cervical half of the palatal surface, from a typical EMLA-treated specimen- elongation (E), irregular cytoplasmic membrane (ICM), vacuolisation (V) and subodontoblastic oedema (SE) and increase vascularity (IV).

2.7. Decalcified H&E staining-pulpal histology (Laser versus control)

2.7.1. Aim

The aim of this study was to determine whether there is any atypical pulpal response to the pulsed Nd:YAG laser treatment at the energy level used for clinical induction of pulpal anaesthesia on human premolar teeth, in the absence of the effects of the conventional cavity preparation.

2.7.2. Materials and methods

Six paired-premolars from the same arch were selected and one from each pair of premolars was subjected to 1.30W, 15Hz pulses of Nd:YAG laser, for 120secs/surface on buccal and lingual/palatal surfaces, with a total energy dose of 156J/surface, using roving motion as used for *in vivo* clinical induction of pulpal anaesthesia. The contralateral premolar was used as the control, and both teeth were extracted by forceps after 30mins.

After extraction, the specimens were rinsed and apical one-fourth of the root were removed with highspeed bur under water cooling. Then the specimens were fixed in Karnovsky's solution for two days before being placed in a nitrous acid and sodium citrate decalcifying solution for one and a half weeks. The decalcifying solution was changed weekly. After decalcification, the specimens underwent dehydration and infiltration

processes and finally were embedded in paraffin before sectioning. The process was deemed complete when the specimen was easily bent, with little resistance. The specimens were dissected, placed in tissue cassettes, sectioned at 5 micron in thickness, stained with Haematoxylin & Eosin, and then examined microscopically at 100x magnification using previously established positive and negative control specimens as the standard criteria.

To define the histological variation between the control and the laser treated teeth, multiple histological sections belonging to each treatment condition, from different teeth were presented in a blind manner, to two trained observers.

The superficial pulpal responses at the cervical half of the tooth crown were rated, using the criteria modified from previous pulp biology studies which is as follows: (Sayegh and Reed, 1974; Adrian 1977; Stanley *et al.* 1975; White *et al.*, 1991; 1992; 1993; 1994).

0- Normal cellular elements in the pulp area underlying the zone of laser irradiation.

1- Disruption of the odontoblastic cell layer underlying the zone of laser irradiation, including: the elongation, irregular cytoplasmic membrane, vacuolisation and subodontoblastic oedema.

2- Disruption of the odontoblastic cell layer underlying the zone of laser

irradiation, including: loss of stroma and viable epithelial root sheath of the odontoblastic layer, cytoplasmic changes, and aspirated odontoblasts.

2.7.3. Results and discussion

The results (Table 2.7.a) show that there was normal pulp histology in both control (Fig. 2.7.a) and Laser (Fig. 2.7.b) groups. With one-paired specimens, one from Laser (Fig. 2.7.c) and one from control (Fig.2.7.d) group, there was mild elongation and subodontoblastic spacing of the odontoblastic cell layer underlying the zone of laser irradiation. No observable increase of vascularity from either group was found. Due to the small sample size of this study no statistical analysis was carried out.

White *et al.* (1991a) examined extracted third molars which were treated with pulsed Nd:YAG laser within three minutes of removal. It should be noted that any laser energy transmitted to the pulp could not be decreased by an intact circulation, which would be expected to occur in an intact, vital pulp (Kim *et al.*, 1983; 1992). Furthermore, it cannot be confirmed if any acute or chronic inflammatory response was caused by this laser irradiation. In this present study, because the teeth were exposed to laser energy for about 30mins before forceps removal, any thermal effect to the vital pulp could be decreased by the intact pulpal circulation. This study design can therefore demonstrate the immediate superficial pulpal

changes following the laser treatment of inducing pulpal anaesthesia under *in vivo* clinical conditions.

It is because of the potential for transmission through enamel and dentine into the pulp and the potential for inducing pulpal warming, that the applications of the Nd:YAG laser have been somewhat limited to lower energy density (Koort and Frentzen, 1992; Arcoria *et al.*, 1994). Using histologic methods, *in vivo*, it has been defined that laser induced pulpal damage is a function of the energy density of laser irradiation (Adrian *et al.*, 1971; Adrian, 1977; Powell *et al.*, 1989; Powell *et al.*, 1993; Melcer *et al.*, 1987).

Goodis *et al.* (1992) and White *et al.* (1994) have defined the energy density safety threshold to exposure of pulsed Nd:YAG laser on enamel or dentine, as power less than 2W at 10Hz, (320 micron fibre), with energy density less than 165 J/cm^2, for 120secs (total energy dose of 240J), with remaining dentine thickness more than 1mm. However, the presence of minor vacuolisation within the odontoblast cell layer has been reported (White *et al.*, 1991a).

Similarly, Parkins *et al.* (1991) and Parkins *et al.* (1992), investigated *in vivo* the histological pulpal response to various energy dosages of the pulsed Nd:YAG laser after clinical induction of pulpal anaesthesia on

premolar teeth, at 2days, 5days, and one week, and reported that the threshold energy density of 124J/cm^2 (1.5W, 15Hz), with total energy dose of 360J (for 240secs), caused no variation in clinical vitalometer readings, and pulpal histology was normal.

Findings of this pilot study (2.6, 2.7) are concurrent with the findings of previous studies that exposure to plain Nd:YAG laser pulses for clinical induction of pulpal anaesthesia, with the energy density range of 73-107J/cm^2 (320 micron fibre), with a total energy dose range of 106-156J/surface (for 120secs), caused no immediate atypical superficial pulpal changes, under *in vivo* clinical conditions.