ASSESSMENT OF ENAMEL CHANGES
DURING FIXED ORTHODONTIC
TREATMENT WITH AND WITHOUT
OZONE

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requirement for the degree of
PhD

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Dedication

To

my deceased Father and Brother

my Mother
for her endless love and support

my sisters, brothers and friends
for making life
more beautiful

and finally

to my country the
UAE
for their generous support

Amna
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Declaration

I declare that

(i) the thesis is not one for which a degree has been or will be conferred by any other university or institution;

(ii) the thesis is not one for which a degree has already been conferred by this University;

(iii) the work for the thesis is my own work and that, where material submitted by me for another degree or work undertaken by me as part of a research group has been incorporated into the thesis, the extent of the work thus incorporated has been clearly indicated.

(iv) the composition of the thesis is my own work.

Signed:                                                                                                  Date:
Abstract

Assessment and management of enamel changes during fixed orthodontic treatment in permanent teeth have been widely discussed in the literature. The use of validated detection methods and assessment of caries risk factors before starting treatment is required. These should examine the effect of the different caries preventative methods and help clinicians in decision making for caries susceptible orthodontic patients. The overall aim of this study was to assess enamel changes during fixed orthodontic treatment and to manage changes using ozone gas treatment.

The repeatability of a 3-D laser scanning machine was tested *in-vitro*. The reproducibility of detection methods, (ICDAS II, the DIAGNOdent and the photographic method) in measuring early enamel lesions in smooth surfaces *in-vitro* and *in-vivo* were tested. In addition, the ICDAS II, the DIAGNOdent and the QLF were evaluated for the purpose of caries detection on non-cavitated carious lesions on approximal surfaces of premolar teeth and were validated by histological analysis. The changes on teeth after debonding of the orthodontic brackets were evaluated *in-vitro* 3-dimensionally. The shear bond strength of two types of orthodontic adhesive systems subjected to air was also tested and compared with the shear bond strength of a group subjected to ozone gas. Moreover, the ability of ozone treatment to prevent white spot carious lesions around orthodontic brackets in permanent teeth was assessed *in-vivo*, using the ICDAS II, the DIAGNOdent and a digital camera imaging system. The Mutans streptococci (Ms) level in saliva, (at baseline and after 12 months of starting the orthodontic treatment), and in plaque, (on ozone treated teeth and a control air group), was assessed. In addition, the salivary buffer capacity
was assessed at baseline and after 12 months of starting orthodontic treatment.

Using the one-way ANOVA test, the repeatability of the laser scanner was 6.6 μm. There was a significant correlation between the lesion depth and the detection methods scores ($p < 0.01$). The Spearman rank correlation coefficient between the lesion depth and the ICDAS II *in-vitro* was 0.88. The correlation between the lesion depth and the DIAGNOdent scores was 0.73, and 0.75 between the QLF scores and the ICDAS II. The weighted kappa value for the ICDAS II *in-vivo* was 0.94 and 0.69 for the DIAGNOdent scores.

The Mann-Whitney test showed significant differences in adhesive thickness and enamel loss between tested groups ($p < 0.001$). The mean (±SD) adhesive thickness (after debonding) and enamel losses (after finishing the enamel surfaces) for groups 1 and 2 were respectively 31.2 μm (±26.5 μm), 102.7 μm (± 79.7 μm) and 22.8 μm (± 18.1 μm), 50.5 μm (± 31.3 μm). The Mann-Whitney test showed that the mean (±SD) bond shear strength of 8.1 MPa (±2.7) for group 1 was significantly lower than the mean (±SD) shear bond strength of 10.8 MPa (±2.4) for group 2 ($p < 0.001$). However, The Mann-Whitney test revealed no significant differences in shear bond strength between the group subjected to ozone and the control air group ($p = 0.337$). The mean (±SD) shear bond strength of the group subjected to ozone was 11.7 MPa (±2.3) and 10.8 MPa (±2.4) for the group not subjected to ozone.

The ANOVA test showed a significant effect of time between the ozone treated group and the control air group upon the mean ICDAS II scores and DIAGNOdent scores ($p < 0.05$). With DIAGNOdent, the number of teeth which had a score zero
was 280, score 1 was 79 and score 2 was 11 teeth. With ICDAS II, 289 teeth showed score zero, 64 showed score 1 and 17 of teeth showed score 2.

In the ozone group, 154 teeth with DIAGNOdent and 151 teeth with ICDAS II showed score zero. Twenty nine teeth with DIAGNOdent and 27 teeth with ICDAS II showed score 1. Two teeth with DIAGNOdent and 7 teeth with ICDAS II showed score 2. In the control air group, 126 teeth with DIAGNOdent and 138 teeth with ICDAS II showed score 0. Fifty teeth with DIAGNOdent and 37 teeth with ICDAS II showed score 1. Nine teeth with DIAGNOdent and 10 teeth with ICDAS II showed score 2. The number of teeth with white spot lesions at the end of the treatment, using photographic assessment, was 41 (28.5%) for the control air group and 13 (9%) for the ozone group ($p = 0.006$, $p < 0.05$). The Spearman’s rank correlation coefficient between the changes in the DIAGNOdent and the ICDAS II scores at 1 year was 0.72.

The Mutans streptococci level in the plaque of the control air group was not significantly different than that of the ozone treated group using the Wilcoxon Signed Rank test ($p = 0.527$, $p > 0.05$). There was a significant increase in the level of Ms in saliva and the buffer capacity at 12 months compared to the baseline ($p = 0.004$) in both groups. The weighted kappa value of the repeated and rescored photographs was 0.94.

The results suggest that it can be suitable to use 3-D laser scanning, ICDAS II, DIAGNOdent and a digital camera for longitudinal monitoring of non-cavitated carious lesions on enamel smooth surfaces. These methods proved valid and reliable.
The results of these *in-vivo* studies could help clinicians in decision making in regard to caries susceptible orthodontic patients and could help to detect the effect of new treatment modalities during orthodontic recall visits. In addition, the present study adds new knowledge for the orthodontist with regard to ozone treatment as a part of orthodontic therapy in order to reduce the incidence of carious lesions around orthodontic brackets.

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Publications

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CHAPTER ONE

LITERATURE REVIEW

1

ASSESSMENT OF ENAMEL CHANGES IN ORTHODONTIC BONDING

Macchi et al, 2006
1.1 Orthodontic bonding materials

The term bonding is defined as “Uniting of an adhesive to an adhered”, and can be achieved by adhesion. Adhesion is defined as “The attraction between molecules of different materials, when their surfaces are brought into contact” (Buonocore 1955).

The most commonly used orthodontic adhesives are composite resin and Glass Ionomer Cements (GICs).

1.1.1 Composite resin

Composite resins have been the most widely used adhesives for orthodontic bonding procedures. A major drawback of this technique is that the resin has to be applied in a completely dry clinical situation. Bond failure with composite resin has largely been attributed to moisture contamination arising from saliva, gingival crevicular fluid and water from a dental spray.

Composition

The composition of the composite is based on the Bisphenol Glycol Metha Acrylate (Bis-GMA) monomer which is a viscous liquid. This was synthesised by Bowen at the National Bureau of Standards, U.S.A (Bowen 1962). In order to render the resin suitable for formulating into a composite, a less viscous monomer is used, namely Triethylene Glycol Dimeth Acrylate (TEGDMA). In an effort to improve the properties of composite resin, some manufacture’s replace part or all of the Bis-GMA with urethane dimethacrylate.
The properties of the unfilled resin are improved by the addition of filler particles. Conventional composites contain particles, (glass or ground quartz), of 10-30 micrometres diameter. The particles are treated with a saline coupling agent to enable bonding to occur between the particles and the resin (Bowen 1962). The conventional composites have filler particles of 80% w/w. Microfilled resins contain 50% fillers w/w and have filler particles of 0.04 micron diameter or less. Hybrid type composites have a mixture of the two filler particles and can achieve 87% w/w filler incorporation. According to the type of inorganic filler, composite resins are classified as highly-filled composite or low-filled composite. The smaller the particle diameter the less filler that can be included into the matrix.

**Setting reaction**

In a chemically cured composite, a two paste system is used. One paste contains benzoyl peroxide while the other paste contains the activating amine. In light cured composite systems, two components are involved in the initiating systems, namely a ketone and amine. The ketone, camphorquinone, is sensitive to blue light at wavelengths in the region of 470 nm. Free radicals are produced which initiates the additional polymerisation.

1.1.2 *Fluoride releasing composites*

These materials contain fluoride and have the properties of both polyalkenoate and composite resin. Incorporation of fluoride into the resins can create problems including particle separation and loss of mechanical integrity because of the highly polar nature of fluoride salts and the low polarity of dental resins (Underwood et al.
Mechanism of fluoride release

Fluoride release from resin composites is proceeded by a stress-corrosion effect. This involves hydroxyl ions from the surrounding water breaking up the outer silica particles of the composite network which in turn opens deeper layers of the material to further attack. This can also lead to discolouration of the materials. A fluoridated—releasing resin has been developed by Rawls and Zimmerman (1983) in which the fluoride ion was incorporated as a mobile ion charge in an anion-exchanging system. In the latter system, fluoride release occurs when the fluoride ions are actively exchanged for other anions in the oral environment rather than by a passive dissolution process.

Research by Geddes and McNee (1982) studied the antimicrobial effect of fluoride ions. The authors demonstrated that fluoride ions interfered with the initial bacterial adhesion and colonization and so affected bacterial metabolism. Minimum inhibitory concentrations of 100-200μg/ml sodium fluoride were required to inhibit the growth of oral streptococci, while concentrations of up to 30-fold this figure were required to be bactericidal (Maltz and Emilson 1982).

1.1.3 Adhesive for precoated brackets

The composite used to precoat the Adhesive Precoated Bracket (APC) is a version of Transbond XT composite (3M Unitek) modified by increasing its viscosity. The difference between the adhesive used on precoated brackets and that used for bonding uncoated brackets is the percentage of the ingredients incorporated in the material (Bishara et al. 1997). The Transbond XT contains 14% BisGMA, 9% Bis
EMA, and 77% filler (Silicate quartz and submicron silica). The corresponding values for the APC adhesive on the precoated brackets are 12%, 8% and 80%, respectively (Bishara et al. 2002). Cooper et al (1992) reported the advantages of the adhesive APC system over conventional light-cured system to be as follows:

- Consistent quality and quantity of light-cured adhesive.
- Easier clean-up following bonding.
- Reduced waste.
- Improved asepsis.
- Better inventory control.

The bond strength of APC brackets have been evaluated in several studies (Bearn et al. 1995; Bishara et al. 1997; Bishara et al. 2002; Sfondrini et al. 2002). The results of in-vitro studies that have compared the bond strength of APC and conventional brackets have been contradictory. Bearn et al (1995) and Bishara et al (2002) compared the in-vitro shear bond strength of metallic APC brackets with that of identical brackets bonded with Transbond and found no significant differences between the two types. However, APC metal brackets have been shown to produce lower bond strengths than uncoated brackets (Bishara et al. 1997; Sfondrini et al. 2002).

Light-cured adhesives have become increasing popular for bonding orthodontic attachments because they offer several advantages over chemically cured adhesives. Advantages include ease of use, extended working time, improved bracket placement, easier cleanup of excess adhesive and better physical properties because air is not incorporated during mixing (Pollack and Blitzer 1982; Tavas and Watts
The major disadvantage of visible light-cured adhesive is the time required to expose the adhesive to the curing device.

### 1.1.4 Glass ionomer cement

Wilson and Kent (1972) formulated new translucent cement for dentistry - the Glass Ionomer Cement (GIC). This was a hybrid of silicate and polycarboxylate cements and could bond physiochemically to both enamel and dentine. The actual formulation of GIC varies between manufacturers but the amount of resin in the final set restoration may be between 4.5% to 6%, and perhaps slightly more in the lining materials (Sidhu and Watson 1995). Research dealing with the hydrophilic GICs confirmed their advantage in releasing fluoride, but conversely showed poor bond strength compared to composite resin (Wiltshire 1994; Itoh et al. 1999).

Improvements in orthodontic bonding materials have led to the advent of Resin Modified Glass Ionomer Cements (RMGIC). These are a hybrid of GIC and composite resin. The true RMGI cement is a two-part system characterized by an acid-base reaction (which is critical to its cure) a diffusion-base adhesive between the tooth surface and the cement allowing fluoride release (Sidhu and Watson 1995). Initially commercial RMGIC were light cured such as Zionomer (DENMAT), Geristore (DENMAT), Vitabond (3M), XR Ionomer (Kerr), Fuji LC (GC) and Photac-Bond (ESPE). Blight and Lynch (1995) found that the in-vitro bond strength of Geristore glass ionomer was greater than that of the non-resin modified glass ionomer cement. Bishara et al (1998) concluded that with etched enamel, and in a wet environment, the light-cured resin-reinforced glass ionomer adhesive system had comparable shear bond strength to that of the traditional light cured composite resin.
system.

Most clinical orthodontic bonding requires an estimated bond strength of 6 to 8 MegaPascals (MPa) or 60-80 kg/cm² (Reynolds 1975). Although bond strengths for RMGICs have been reported to range from 5.39 to 18.9 MPa (McCourt et al. 1991; Ewoldsen et al. 1995; Meehan et al. 1999; Shammaa et al. 1999; Graf and Jacobi 2000; Summers et al. 2004), they remain clinically satisfactory. Positive qualities of RMGICs include their ability to bond to teeth in the presence of moisture. In addition, the release of fluoride from RMGIC lining materials has been reported to be continuous without affecting their physical properties. Penetration of fluoride into dentine was recorded at 100μm (micrometer) or greater (Mitra 1991). It has also been found that RMGICs release significant amounts of fluoride throughout a 28-day period (Tam et al. 1991). However, the restorative materials that have demonstrated a high initial fluoride release declined slowly over a period of 4 months (Garcia-Godoy and Jesen 1990). The results of one study showed that the rate of fluoride release from a RMGIC (KetacFil®) began with a fast burst of fluoride which quickly diminished to low levels in 3 days. Under neutral pH conditions the rate of fluoride release at 72 hrs was significantly slower than at pH 4 (Carey et al. 2003).

Fluoride release from GICs can be influenced by several factors, for example, fluoride content of the material, solubility (Forsten 1977), nature of the dissolving medium (El Mallakh and Sarkar 1990), and temperature (Forsten 1990). A number of studies have been conducted on the fluoride release from GICs. Those studies reported that greater quantities of fluoride were released during the first few days and after that the level of fluoride release fell to a constant level (Forsten 1990;
Hatibovic-Kofman and Koch 1991; Creanor et al. 1994; Weidlich et al. 2000). Donly and Segura (2002) have measured the release of fluoride over 30 days from a RMGICs loaded with different levels of fluoride and then evaluated the adjacent dentine demineralisation inhibition relative to these fluoride levels. The results were that as sodium fluoride additions increased, fluoride release increased. Evaluation of demineralisation indicated the RMGIC inhibited adjacent demineralisation in a direct relationship with sodium fluoride concentration. In fact 3%, 2% and 1% fluoride exhibited significantly less adjacent demineralisation than the non-loaded RMGIC and non-fluoridated resin-based composite ($p < 0.05$).
1.2 Effect of orthodontic bonding and debonding techniques on the enamel surface

1.2.1 Bonding technique

Some clinicians have experienced problems with bonding and debonding techniques (Wertz 1980). One of the most common cited problems in this regard is the damage to the enamel surface during orthodontic treatment.

The bonding procedure of orthodontic brackets is based on four main steps; cleaning, enamel conditioning, sealing and bonding. Failure to perform each of these steps meticulously may create problems which may compromise the desired result.

1.2.1.1 Cleaning

Cleaning of the tooth surface before bonding is an essential step. This process aims to remove the plaque and the organic pellicle that normally covers all enamel surfaces. Rotary instruments are normally required for the procedure which requires a rubber cup or a polishing brush. Pumice prophylaxis does not appear to affect the bonding procedure adversely, and cleaning the teeth may be advisable to remove plaque and debris that might otherwise remain trapped at the enamel-resin interface after bonding (Graber et al. 2000).
1.2.1.2 Etching

Etching with acid

Conventional method

Direct bonding brackets on etched enamel surfaces have been widely evaluated in the orthodontic literature and are reported to be clinically successful (Buonocore 1955; Sadowsky et al. 1990). Etching of the enamel surface with phosphoric acid leads to dissolution of the hydroxyapatite crystals producing microporosities into which fluid monomer can penetrate (Beech and Jalaly 1980; Beech et al. 1985).

The effect of etching enamel surfaces has been evaluated by Silverstone (1974). The findings were that the average loss of the fluoride rich surface enamel, etched with phosphoric acid concentrations ranging from 30% to 50%, following a 60 seconds etch and ranged from 7-12μm. A study by Bishara et al. (2000) estimated that enamel surface loss following etching was between 10 and 30μm.

Retief et al. (1985) reported that the depth of etch and the amount of surface enamel lost during the etching procedure was dependent on the type of acid and its concentration, the duration of etching and the chemical composition of enamel. Studies and clinical experience indicate that longer acid etching periods provide no more, and probably less, retention because of the loss of surface structure (Powers et al. 1997; Fricker 1998). It is recommended to etch with 30-40% orthophosphoric acid liquid for 30 seconds prior to bonding of orthodontic brackets. That time should be enough to obtain adequate bond strength for the required clinical performance.
**Crystal growth method**

An alternative method of preparing the enamel surface for direct bonding of orthodontic brackets was suggested by Smith and Cartz (1973). They transpired that a deposit of white spherulitic crystalline calcium sulphate was produced when polyacrylic acid reacted with the enamel. In addition, sulphate polyacrylic acid has been investigated for its potential for crystal growth by Maskeroni *et al* (1990). Sulphated polyacrylic acid solution when applied to the enamel resulted in the growth of CaSO₄ H₂O₂ (gypsum) crystals. The crystal form improved surface retention to which brackets may be bonded. Some studies have detailed several advantages of the crystal growth bonding technique over the phosphoric acid etch technique. There are as follows: (1) there is no significant damage of the enamel surface, (2) it is easy to debond the brackets and to clean the enamel surface, (3) there is minimum loss of the outer fluoride rich enamel layer and (4) after debonding there are fewer residual adhesive tags (Smith and Cartz 1973; Maijer and Smith 1986).

**Etching with Laser**

The potential application of lasers in dental practice has been studied for over 40 years (Featherstone and Nelson 1987). The first application of a laser in the dental field was reported by Sognnaes and Stern (1965). They used the laser for the purpose of inhibiting caries by increasing the resistance of enamel to demineralisation. Multiple laser etching techniques have been introduced for bonding of orthodontic adhesive to enamel and the application of a CO₂ laser showed surface roughening at 9.32µm and surface glazing at 10.59µm (Kuroda and Fowler 1984). The authors claimed that etching occurred at power levels low enough to avoid damage to the
tooth surface.

The effects of laser have been widely studied and possible applications include enamel etching, caries prevention, caries removal, as well as involvement in endodontic and periodontal procedures (Willenborg 1989; Zakariasen et al. 1991). Some studies have compared the use of CO2 lasers for preparing the enamel with the using of conventional acid etching technique. The authors of these studies concluded that the conventional acid etching method was superior (Varma and Tandon 1997; Obata et al. 1999; Fuhrmann et al. 2001).

**Bonding of enamel without etching or conditioning**

Some materials have the ability to bond to the unetched enamel surface. Cyanoacrylate bonding agents were examined by Ajlouni et al (2004). They concluded that the agent was unsuitable for clinical use as it resulted in a weak bond strength. Glass ionomer cements have been found to be suitable for direct bonding of orthodontic brackets to teeth without acid etching (White 1986). An added advantage of these materials was the slow release of fluoride ions.

**1.2.1.3 Role of sealants and adhesive**

Sealants in orthodontic practice are necessary to achieve required bond strength and to improve resistance to microleakage. In contrast some researchers have concluded that the intermediate resin may not be necessary at all (Prevost et al. 1982; Wang and Tarng 1991). Ceen and Gwinnett (1981) found that light-polymerized sealants protected the enamel adjacent to the brackets from dissolution and subsurface lesions, whereas chemical cured sealants polymerized poorly, exhibited drift and had
low resistance to abrasion (Dickinson et al. 1991; Axelsson and Zachrisson 1992).

1.2.1.4 Excess adhesive removal

It is normal clinical practice to ensure that excess adhesive is removed after bonding the orthodontic bracket to prevent or minimize gingival irritation and plaque build-up around the periphery of the bonding base. This reduces potential periodontal damage and the possibility of enamel decalcification. In addition, removal of excess adhesive can improve aesthetics, not only by providing a neater and cleaner appearance, but also by eliminating exposed adhesive that might become discoloured in the oral environment (Graber et al. 2000).

There are two basic types of adhesive namely acrylic and diacrylic. Both types exist in either filled or unfilled forms. Some composite resins contain large filler particles, other contain minute filler particles. Adhesives with larger particle fillers are recommended for extra bond strength, but careful removal of the excess is necessary because excess adhesive promotes plaque more easily than do others (Zachrisson and Brobakken 1978).

1.2.1.5 Bracket types

The types of brackets used in orthodontic practice have the same role in potential plaque accumulation. The clinical implications are that as the ceramic bracket surface is rougher and more porous than steel brackets it will more easily attract plaque and stain to the surrounding enamel. On the other hand, the corrosion susceptibility of stainless steel brackets may lead to enamel discolouration and roughness around the brackets which in turn may lead to plaque accumulation (Maijer and Smith 1982; Matasa 1998).
Other factors, such as bracket base design, may lead to demineralisation around the periphery of the bracket base which is smaller than the bracket wings. The uses of elastic ligatures around the bracket could lead to plaque accumulation. Although those elastics are time saving, stainless steel wires are safer and more hygienic (Zachrisson and Brobakken 1978; Forsberg et al. 1991).

1.2.2 Debonding technique

The clinical debonding procedure is divided into two stages namely bracket removal and residual adhesive removal. Caution and care should be taken during both stages to avoid any damage to tooth enamel.

1.2.2.1 Bracket removal

The original method of metal bracket removal involved placing the tips of a twin-beaked plier against the mesial and distal edges of bracket base. The pliers were gently squeezed on the bracket wing mesiodistally and the bracket was removed with a “peel” force. A peeling force is effective in breaking the adhesive bond by creating peripheral stress concentrations that cause bonded metal brackets to fail at low force values (Oilo 1993).

In ceramic bracket removal, enamel fracture and loss, during debonding, has been reported in some studies (Redd and Shivapuja 1991; Artun 1997). The ceramic bracket will not flex when squeezed with debonding pliers. As a consequence, the method required to remove the bracket is to lift the bracket off with a peripheral force application. Some ceramic bracket parts may be left on the tooth which requires removal with a dental bur. Removing the residual part of a ceramic orthodontic bracket using the tip of twin-beaked pliers is not recommended since it
might introduce horizontal enamel cracks (Redd and Shivapuja 1991). The use of low-speed grinding of ceramic brackets, with water coolant, is recommended but such treatment in the absence of water may cause permanent damage or necrosis of dental pulps (Vukovich et al. 1991).

1.2.2.2 Residual adhesive removal

Residual adhesive on the enamel surface can be removed using several clean-up methods. Rotary instruments are often used to remove residual adhesive after orthodontic bracket removal. Some clinicians use a high-speed Tungsten Carbide (TC) bur and green rubber wheel bur, but the majority uses a low-speed TC bur. Others use an ultrasonic scaler, or debonding pliers. It has been reported that the greatest enamel loss occurred following the use of an ultrasonic scaler or high-speed TC bur and the least with the slow-speed TC bur (Ireland et al. 2005).

Thermal debonding and the use of lasers have the potential to be less traumatic and involve less risk of enamel damage (Hayakawa 2005), but these techniques are still at a preliminarily stage (Graber et al. 2000). There is also the potential thermal pulpal damage being caused during laser application. Therefore, the method and duration of laser pulses must be precisely controlled and appropriate to the adhesive being used (von Fraunhofer et al. 1993; Ma et al. 1997). Enamel loss following residual adhesive removal was reported to be between 55.9μm (Fitzpatrick and Way 1977) and 149.87μm (Krell et al. 1993) dependent on the method of adhesive removal.
1.2.3 Amount of enamel lost in bonding and debonding

How much enamel is removed during orthodontic therapy bonding and debonding has frequently been discussed in the literature. Several factors are related to the amount of enamel loss. These include the type of adhesive resin used and the instruments used for prophylaxis and debonding (Zachrisson and Arthun 1979; Diedrich 1981; Thompson and Way 1981; van Waes et al. 1997).

Research by Pus and Way (1980) and Thompson and Way (1981) reported that an initial prophylaxis with a bristle brush for as little as 10 to 15 seconds may abrade away as much as 10μm of enamel, whereas only about 5μm may be lost when a rubber cup is used. They also reported that from 5 to 8μm of the amount of enamel is lost during the cleanup of unfilled resins using hand instruments.

Rotary instruments are required for adequate removal of filled resin. Indeed 10 to 25μm of enamel may be lost with those procedures. Using a high-speed bur and a green rubber wheel removed about 20μm of enamel, while a low-speed TC bur removed approximately 10μm of enamel (Pus and Way 1980). Several studies have reported that, depending on the instruments used for prophylaxis and debonding, total enamel loss for filled resins was estimated to be 30 to 60 μm (Pus and Way 1980; Thompson and Way 1981; Bishara and Fehr 1997). Research by van Waes et al (1997) found approximately 7μm of enamel loss when using a TC bur and indicated a more limited loss and damage of enamel when TC burs were used carefully. Deep-reaching enamel tearouts, down to a depth of 100 μm have been reported by Diedrich (1981), with localised enamel loss of 150 to 160 μm.
1.3 *In-vitro bond strength testing*

Bond strength is the force *per* unit area required to break a bonded assembly with failure occurring in, or near, the adhesive/adherent interface (International Organization for Standardization 2003).

Proffit and Fields (1983) found that the maximum occlusal force was in the range of 31 to 35 N, while orthodontic forces never exceeded 1 pound (4.4 N) *per* tooth (Newman 1965). Adequate bond strength ranges has been reported in several studies. Low and von Fraunhofer (1976) found bond strength to be in the range of 0.6 to 0.8 kg/cm², (Maijer and Smith 1979) -10kg, (Odegard and Segner 1988) - 20.4 to 26.6 Megapascal (MPa), (Joseph and Rossouw 1990) -13.88 MPa. The difference in opinion regarding adequate bond strength may be related to variations in the experimental procedures.

Beech *et al.* (1985) stated that bond strength testing is notorious for producing results with wide variations. This may be due to variations in the enamel surface and bracket base nature, the thickness and continuity of the materials beneath the bracket and the accuracy of the material mixing. Cook and Youngson (1988) reported a value of 60.9 N, when using glass polyalkenoate cement to bond metal orthodontic brackets, while Fox *et al* (1991) reported a figure of 33.1N.

The use of shear loading is recommended in orthodontic bracket bond strength testing due to the relative simplicity of the experimental configuration and the presumably increased reliability of simulating the debonding that occurs during treatment (Eliades and Brantley 2000).
1.3.1 Factors to be considered during orthodontics in-vitro bond strength testing

Orthodontic bonding materials are being continually developed. The development of these materials has always focused on the values of the bond strength as an indicator of its improvement.

1.3.1.1 Type of tooth and nature of enamel surface used for bonding
The most common teeth used in these studies are human premolar teeth, human incisors, bovine incisors, human molars and human deciduous molars. Baboon incisors have also been used. In the absence of evidence that different enamel types affect orthodontic bond strength, it would seem preferable to use premolars for all future studies, as these teeth are often extracted from patients for orthodontic purposes (Fox et al. 1994). The enamel surface and its fluoride content change with age, and this could affect bond strength and etch pattern (Weatherell et al. 1972).

Recent evidence has shown that significant differences in bond strength exist between different teeth and it has also been suggested that different tooth morphologies may show variations in shear bond strength. Whittaker (1982) proposed that an increase thickness of prismatic enamel may affect bond strength. Mattick and Hobson (2000) have shown that the nature of the etched enamel surface varies between different teeth. Hobson et al (2001) concluded that there were significant differences ($p < 0.001$) in bond strengths between different tooth types and that future bond strength studies of surface enamel should use one tooth type or...
an equal number of different tooth types in order to achieve stratification. Linklater and Gordon (2001) concluded that canine and premolar teeth exhibited significantly higher shear bond strengths ($p < 0.001$), and significantly lower probability of failure at given levels of applied stress, than incisor teeth.

### 1.3.1.2 Storage medium before bonding or debonding

Various storage solutions have been investigated in several studies (Retief et al. 1989). Addition of physical and/or chemical microbial inhibitors to the storage medium is reported to have altered the dentine shear bond strength (McGuckin and Pashley 1990; Goodis et al. 1993). Table 1-1 details the variety of storage media used between bonding and testing. Fox et al (1994) suggested that the timing between bonding and testing was probably not critical, as long as this period was not less than 24 hours.

<table>
<thead>
<tr>
<th>Nature of storage medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water 37°C 24 hours</td>
</tr>
<tr>
<td>Water 37°C 24 (other times)</td>
</tr>
<tr>
<td>Water room temperature 24 hours</td>
</tr>
<tr>
<td>Water room temperature (other times)</td>
</tr>
<tr>
<td>Saline 37°C 24 hours</td>
</tr>
<tr>
<td>Saline refrigerated</td>
</tr>
<tr>
<td>Thermocycled then 100% humidity for up to 60 days</td>
</tr>
<tr>
<td>water 37°C for 1 week</td>
</tr>
<tr>
<td>Artificial saliva 37°C 24 hours</td>
</tr>
<tr>
<td>Acid phosphate buffer for times up to 1 week</td>
</tr>
</tbody>
</table>

**Table 1-1:** Storage medium used between bonding and testing (Fox et al. 1994).
1.3.1.3 Tooth surface preparation

Three ordinary steps are usually applied before bonding the brackets namely prophylaxis, surface etching, washing and drying. These steps are summarized in to one term namely “prophylaxis”.

Prophylaxis

Rotary instruments are required for this procedure which involves either a rubber cup or a polishing brush. The majority of studies use pumice applied with a rubber cup (Winchester 1991). The importance of the duration of prophylaxis has not been formalised, but commonly it varies from 15 seconds to 30 seconds.

Surface etching

- Acid etching

The acid concentration and the etch time is still controversial. The effects of variations in acid concentration have been evaluated in several studies. Some studies have reported that the shear bond strengths were not significantly influenced by acid concentration (Beech and Jalaly 1980; Barkmeier et al. 1987; Sadowsky et al. 1990). Sadowsky et al (1990) found that reducing the etching time of 37% phosphoric acid from 60 to 15 seconds had no significant effect on the retention of bonded orthodontic attachments. Therefore, it is recommended to use 37% phosphoric acid solution for 30 seconds.

- Alternative to acid etching

The crystal growth method reduces the bond strength to enamel by 50% with ceramic brackets compared to conventional methods. Clinically, this method showed that the
bond strength was more than adequate (6.71 MPa) (Maskeroni et al. 1990). Other studies using the same method have reported lower bond strength which was not adequate for clinical performance (Artun and Bergland 1984; Farquhar 1986; Howells and Jones 1989).

Blight and Lynch (1995) investigated a 2.5% nitric acid etch and compared it to 37% phosphoric acid for the bonding of ceramic brackets with composite resin *in-vitro*. Nitric acid has been found to produce a more uniform, less deep etching pattern, with no significant difference in bond strength or enamel fracture. In addition, conditioning with 10% of polyacrylic acid had bond strength similar to that of acid etching with phosphoric acid.

Generally, the use of crystal growth method resulted in a reduced bond strength when compared with the use of phosphoric acid. Nevertheless, the bond strength was still above the minimum bond strength of 60 kg/cm² recommended by Reynolds (1975).

- **Laser etching**

The application of Nd:YAG laser (Neodymium: yttrium-aluminum-garnet) irradiation to dental enamel has been studied *in-vitro* by (von Fraunhofer et al. 1993). Laser etching of enamel by an Nd: YAG laser was used for 12 seconds at the maximum power of 3 Watt (W). The bond strengths (1.28 ± 0.24 Kg/mm²) resulting from this method were clinically and scientifically acceptable. The mean shear bond strengths obtained with laser power setting of 1 W and 2 W in the same study were lower (*p* < 0.01) than that achieved with acid etching. Nevertheless more research is required
before an agreed consensus is achieved.

Also, an erbium, chromium, yttrium, scandium, gallium, garnet (Er, Cr: YSGG) hydrokinetic laser system was investigated by Usumez et al. (2002). Their conclusion was that enamel conditioning with an Er, Cr: YSGG laser cannot be considered a successful alternative to the conventional methods of increasing bond strengths of orthodontic brackets to enamel.

**Washing**

Washing with water is essential to remove the etching agent and to remove any deposit from the enamel surface. It has been suggested that over-rinsing may remove the crystals and consequently reduce bond strength (Maijer and Smith 1986). However, insufficient rinsing will not totally remove the polyacrylic acid solution and this will negatively affect the bond strength. Bishara et al. (1995) has suggested that approximately 1 minute of rinsing is enough to ensure the total removal of the polyacrylic acid and still preserve the enamel crystals. In general, a 20 second wash has been found to be sufficient to remove deposits during routine etching procedures.

**Drying**

Drying for 15 seconds with an oil-free air stream is essential after washing to produce a frosted appearance in the enamel. An-oil free air stream is preferable to avoid contamination of the freshly etched enamel.
1.3.1.4 Method and direction of debonding

Many testing machines have been used in orthodontic bond strength studies. These have included a pair of specially designed opening pliers, a Howden universal testing machine, a Chantillon Model DTC Universal Tester, a MTS testing apparatus, and, the most commonly used one, the Instron testing machine (Fox et al. 1994).

1.3.1.5 Site of failure

The most common failure site, according to the previous studies was adhesive failure between bracket and adhesive. Obviously no failure is the most desirable clinical outcome but if failure happens the most desirable failure site is between the adhesive and the enamel as this would make polishing much easier and consequently produce less damage to the enamel surface.

1.3.1.6 Number of specimens used per test

In order to generate meaningful data from in-vitro bond strength testing, at least 20 and preferably more specimens should be used per test (Beech et al. 1985; Welbury et al. 1988). Fox et al (1994) concluded that it is preferable to use 30 specimens per test, presumably on the basis of statistical advice.

1.3.1.7 Bond strength units and values

There is a wide variation in the bond strength values reported in the literature largely due to differences in the testing methods and materials employed. Also many different units are used to express bond strengths, namely pounds or pounds per square inch (p.s.i), kilogram force or kilogram per square centimetre (kg/cm²), Newtons or MegaNewton per square metre (MN/m²) and MPa. The accepted S.I. (International System of Units) unit for force is the MPa. These units provide an
indication of the force per unit area required to dislodge the bracket. This would mean that a bracket with twice the surface area of the one tested would require twice the surface force to dislodge. As long as dimensions of the bracket base are quoted, the use of either N or MPa is appropriate in quoting bond strength (Fox et al. 1994). The MPa unit is considered the accepted unit for bond strength measurement, which is equivalent to one MN/m².

1.3.1.8 Cross-head speed

A wide variation of crosshead speeds was reported when using the Instron testing machine. Alexandre et al (1981) tested at 0.05 inches per minute, Knoll et al (1986) and Winchester (1991) at 2mm per minute. Faster testing speeds tend to yield decreasing bond strengths (Rider 1977). Slowing the crosshead speeds of the testing machine during shear bond testing of orthodontic brackets from 5 to 0.5 mm/min significantly increased the mean shear bond strength from 7 to 12.2 MPa, an increase of by 57% (Bishara et al. 2005). For consistency, the cross-head speed of the loading plate is usually set at 0.5 mm/min in shear bond testing (Eliades et al. 1991; Kao et al. 1995).

1.3.1.9 Quality of the materials and how it is handled

The physical and chemical properties of the materials can be indicated by the failure mode of the adhesive. For adhesive failure (located in the adhesive interference), many point to the wetting properties, or chemical reactions within the substrate, which is necessary to improve the bond strength. A cohesive failure (a fracture in one of the materials to the side of the interface), indicates that the physical properties of the material has limited the bond strength of the assembly. Failure mode observations indicates how the system is working and pointing out its weakest link (Oilo 1993).
1.3.1.10 Light curing

The shear bond strength may be influenced by the light curing technique used for resin polymerisation during bracket bonding. High-power LED light source ($\geq 1000$ Mw/cm$^2$) has been reported to produce higher contraction strain during resin polymerisation and contraction stress may contribute to insufficient clinical shear bond strength. However, low-intensity lights followed by a final exposure with high-intensity light (soft start polymerisation) has been demonstrated in several studies (Yoshikawa et al. 2001; Halvorson et al. 2002; Oberholzer et al. 2003). The polymerisation strains were significantly reduced and the material properties were also improved when using the soft start polymerisation technique (low intensity curing technique). It also has been suggested that the soft start LED polymerisation mode should produce higher orthodontic bracket shear bond strengths than the fast-mode LED or halogen-light polymerisation (Yoshikawa et al. 2001; Deb and Sehmi 2003; Oberholzer et al. 2003; Turkkahraman and Kucukesmen 2005).

1.3.1.11 Adhesive thickness

Buonocore (1963) reported that increasing adhesive thickness produced a weaker adhesive joint. Chemically cured and one-paste resin also produced similar results (Evans and Powers 1985). However, another study by Mackay (1992) showed that increasing the adhesive thickness from 0 to 0.26 mm had statistically no significant difference. The light-cured RMGI cement has been reported to have its highest mean bond strength at the 0.25 mm thickness in both tensile and shear test modes. This was indicated in a study undertaken on 120 human premolar teeth (Arici et al. 2005).
This result showed that the adhesive layer thickness of the light-cured RMGI cement could be less than 1 mm to gain clinically more reliable bond strength during orthodontic treatment.
1.4 Quantitative measurements in clinical dentistry

To date, most studies assessing the tooth condition after debonding have used linear contact measuring devices. Quantitative measurements were performed by assessing enamel surfaces before and after bonding with a miniaturized boley gauge (Brown and Way 1978), or by optical profilometric techniques (Pus and Way 1980; Krell et al. 1993; Zhang et al. 2000). Both these techniques allow only a limited number of reference points per tooth surface which may influence the final result. In addition, the mechanical measuring methods have their limits, such as being time consuming, subject to operator fatigue and measurement error due to human inconsistencies.

To reduce the variability of such assessments many measurements must be performed on each tooth surface to obtain an accurate result. The need to collect accurate data relating to three-dimensional shapes and surfaces has led to an increased interest in the science of such measurements namely metrology. Anthony (1986) defined measurement as: “the process of finding the value of a physical quantity experimentally using an instrument or system”. Any such measuring system is used primarily to extend the abilities of the human senses which can discover and recognise differences and distinguish between degrees of texture, size, shape, sound, colour and smell.

In an industrial situation, the science of metrology has evolved to allow measurement techniques of high accuracy and reproducibility to be applied in quality control particularly within the automotive industry. For dental applications, surface profiling sensitive to changes at the micron level is necessary (McDowell et al. 1988) and current methods involving the use of three-dimensional co-ordinate measuring
machines are designed to meet these specifications.

In the field of dental research, and mainly in the field of operative dentistry, some of these systems have been used to quantify the loss of tooth substance caused by abrasion of restorations or opposing teeth (Jorgensen and Asmussen 1978; McDowell et al. 1988; Rignon-Bret et al. 2002).

In orthodontic research there is an increasing demand for techniques that make use of 3-Dimensional measurements (3-D). Over the years, as 3-D technologies have improved, the 3-D applications have become more innovative. The application of 3-D techniques used for modeling analysis include general applications to clean up 3-D data, merge, and register to create an accurate model. Measuring tools utilize and rapidly form automeasuring functions of 3-D models and have permitted construction of the estimated result before treatment and to measure the actual result of treatment. Clinical orthodontic applications of 3-D measurement include orthodontic model analysis, diagnosis of anteroposterior symmetry of the dental arch, and the diagnosis of the length discrepancy of the dental arch.

The majority of in-vitro studies which have been conducted to investigate the enamel changes during orthodontic procedure have used Scanning Electron Microscopy (SEM) (Pang et al. 1999; Zarrinnia et al. 1995; Zhang et al. 2000; Schuler and van Waes 2003; Tufekci et al. 2004; Fjeld and Ogaard 2006).
In orthodontic research, a number of quantitative techniques have been described for research purposes. These include stereomicroscopy, profilometry, stereophotogrammetry and three-dimensional digitising. In the present study three-dimensional digitising was used to quantify the changes on the enamel surface during bonding and debonding of the orthodontic brackets.

1.4.1 Three-dimensional digitizing

Halazonetis (2001) explained four basic common methods for acquiring 3-D shapes from images:

- **Stereo analysis.** This method uses two cameras to capture the image of the subject in a binocular vision system. Two images are produced where every point is projected onto two corresponding points in the two images. The disadvantage of this system is the difficulty in having adequate resolution.

- **Shape from shading (SFS).** This method depends on the brightness and the direction of the light sources coming from the surface of the imaged subject. The problem here is the same brightness can be the result of the subject not having constant reflecting properties. In addition, real objects do not have constant reflecting properties.

- **Photometric stereo.** This is similar to the first method described but in this system, one camera is used with two light sources to get two images.

- **Structured lighting:** This method projects a known pattern of light on an object to infer its shape. Generally the light source could be a spot or line of laser light. This light strip has to scan the object by sweeping the laser or moving the object itself.
In dentistry, the first use of three-dimensional co-ordinate measuring machines was reported by Oashi et al (1973). Their study used a Mitutoyo-MX203 co-ordinate measuring machine and digitiser with a 9800 series Hewlett-Packard computer system. That system interfaced with a computer in an interactive on-line mode for surface measurement of teeth and restorative materials in the x, y and z axes. In this modified form these programmes provided data storage and recall.

The Computer Graphic Co-ordinate Measuring System, which is capable of measuring up to 50,000 points on a tooth surface and gathering x-axis data at intervals of 10 µm is accurate and reproducible of surface topography and has been used for tooth surface assessment (McDowell et al. 1988). The Michigan Computer Graphic Co-ordinate Measuring System and similar systems (Sakaguchi et al. 1986) involved the use of highly accurate touch-trigger probes, which contacted the surface of replicas of the areas to be studied. By necessity such replicas have to involve materials of an abrasion resistant nature, usually epoxy resin.

van Waes et al (1997) assessed loss of enamel caused by orthodontic bracket debonding using a mechanical computerized three-dimensional scanner with resolution of 1µm. A total of 2646 measurements were performed on six human premolars. The result showed an average loss of enamel of 7.4µm, with a range between 1 and 52µm.

A further development of digital scanning and associated software has been the use of non-contact laser probes for surface analysis. The laser light-scanning probe records data from a series of closely separated points on a surface to define a feature.
These optical systems determine the co-ordinate points on a surface by ‘triangulation’, that is, directing light towards a surface in one direction and collecting the scattered light in a different direction. Providing the surface to be scanned is non-reflective, such machines are able to scan many types of material. These include tooth or replicas or impression materials such as addition-cured polysiloxanes. The wear of restorations was investigated by Roulet et al (1980), and involved non-contact laser probes. During that study, up to 5,000 measuring points were assessed to an accuracy of 6.6 µm and profiles of the samples were produced from this data at 100 µm intervals. The materials being used to study the wear of dental porcelain in an artificial mouth situation through image superposition and subtraction (DeLong et al. 1985).

In 1992, Quick et al developed a scanning ruby laser digitiser, which was used to scan and measure dental impressions and casts. It was found that such a system was accurate enough to measure differences in the order of 40 µm. The standard deviation of repeated measurements of a stud collar, of approximately 1 mm width was 14 µm. However, they commented that such systems were expensive to use and maintain as they required the services of both an engineer and a computer programmer.

Recently, a faster and dentally dedicated, non-contacting laser probe instrument has been developed (Laserscan 3-D Pro, Willytec GmbH, Gräfelfing, Germany). The 3-D scan volume (single scan) is 16 mm (width) x 38 mm (height) x 150 mm (length). A “multiscan” function allows several single scans to be added to give a total width of 150 mm. The scan rate is 8000-14000 measured points per second, depending upon the surface topography which allows enhanced visualisation and evaluation of
the results. The scanning machine can also use international metrology systems software. The package of software available allows researchers to perform their own analyses, using the central facility for acquisition of data (Seymour et al. 1996, Yeganeh et al. 1999; Jovanovski and Lynch 2000; Cherukara et al. 2002; Baysan and Lynch 2003; Forrester-Baker et al. 2005).

The computerised co-ordinate metrology was used to investigate the stress distribution within the labial marginal region associated with three common designs of marginal preparation for porcelain veneers, with a variety of marginal and incisal configurations (Seymour et al. 2001). They reported that metrology gave the most accurate possible two-dimensional representation of the ‘real’ situation. Measurements of maximum labial reduction along the mid-labial plane during preparation for porcelain veneers were taken and analysed using the metrology technique (Cherukara et al. 2002). The study showed that the use of small round burs (D001-012), when used side on at an angle of 45 degrees to the tooth surface to produce dimples as depth guides, resulted in the greatest frequency of tooth reductions closer to the ideal depth chosen for this study, within the 0.4 mm to 0.6 mm range. The computerised co-ordinate metrology was also used to determine changes in gingival margin contour and swelling after the placement of metal ceramic crowns (Marashdeh 2003). The results of that study showed that the changes in gingival profile involved an increase in gingival thickness of 0.190 mm from the original baseline data. The conclusion from that study was that the co-ordinate measurement machine was able to detect post-treatment changes in gingival profile reliably. The gingival volume changes in drug-induced gingival over growth also were analysed, before and after gingivectomy, using a 3-D imaging laser scanning
Thomason et al. 2005. The mean vertical tissue reduction was found to vary from 1.58 to 2.56mm. They concluded that this method will measure changes in gingival tissue to within 60µm (30% of the mean measured change) in one plane, making it suitable for the assessment of small changes in gingival contour. This method would be ideal for assessment of longitudinal changes in gingival contour. In another study, the shoulder region of the implant abutment and corresponding regions of both impressions and dies were scanned and measured using co-ordinate metrology (Forrester-Baker et al. 2005). Ten impressions of a metal implant abutment were made with each of three different types of impression materials. They found that the technique was capable to measure any change in measured dimensions occurring during impression making.

The further development in Computer Tomography (CT) scans was based on the occlusion being visualised from tooth models. Macchi et al (2006) itemized several functions of CT scanning in the field of dentistry. The system allows 3-D superimposition of the anatomic teeth before and after the set up to view the amount of movement before and after treatment. This allowed viewing of the aligned 3-D crowns and roots, surrounding bone and assessment of bone thickness and any fenestrations. It was also possible to change the torque of a single tooth or group of teeth and evaluate the amount of bone present before fenestration became obvious.
1.4.2 Conventional visual/tactile examination

Detection of early carious lesions were made in the 19th century and over the last 20 years there have been many attempts to improve the methods used to detect and diagnose the presence of carious lesions.

Ismail (2004b) has discussed the reasons why early non-cavitated dental lesions should be included in new diagnostic systems of dental caries. Firstly, there is evidence that non-cavitated lesions are more prevalent than cavitated lesions in economically developed countries (Amarante et al. 1998). Secondly, non-cavitated caries lesions are more likely to be restored compared with those with a sound tooth surfaces (Ismail and Gagnon 1995). Thirdly, non-cavitated lesions, especially in smooth surfaces in young children, may serve as indicators of caries activity (Domoto et al. 1994; Grindefjord et al. 1995; Imfeld et al. 1995). Fourthly, inclusion of early signs of the caries process improves the precision of clinical trials of preventive agents (Howat et al. 1981).

A combination of a visual inspection method and use of a probe has been used for many years to detect dental caries. Using a dental probe is not recommended anymore in Europe due to its potential damage to dental tissue and the possibility of transferring cariogenic bacteria from one tooth site to another. In addition, probing with a sharp instrument does not add to the accuracy of the detection of caries and it may damage the enamel surface covering early carious lesions (Bergman and Linden 1969; Ekstrand et al. 1987). A recent study has evaluated the effects of dental probing on occlusal surfaces of 40 molar teeth (20 sound and 20 with initial caries) using scanning electron microscopy in clinical study (Kuhnisch et al. 2007b).
Probing-related surface defects, enlargements and break-offs of occlusal pits and fissures were observed on all occlusal surfaces with initial carious lesions and on two sound surfaces, respectively. Whereas no traumatic defects whatsoever were visible on unprobed occlusal surfaces.

To facilitate direct visual diagnosis of interproximal surfaces, an elastic temporary tooth separation was employed. Elastic orthodontic rings were used in this technique by inserting these rings between the contact surfaces of teeth for three to four days. After removal of the ring, the created space allowed visual detection of approximal caries. This method may serve as a supplement to conventional and radiographic examination in the improved management of approximal carious lesions.

Diagnostic methods to detect early demineralisation \textit{in-vivo} include visual inspection, tactile examination with a dental probe and radiographic examination, Electronic Caries Monitoring (ECM) and Fibre-Optic Transillumination (FOTI). All of these methods have limitations affecting either their diagnostic performance or their practicality in a clinical setting. These methods cannot be correlated with actual mineral loss as they produce random data. Transverse Microradiography (TMR) methods for detection of very early lesions are destructive and cannot be used \textit{in-vivo} (de Josselin de Jong \textit{et al.} 1987). Decay is difficult to detect in radiographs unless lesions are larger than 2mm to 3mm deep into dentine, or involve one third of the bucco-lingual distance (Rock and Kidd 1988).
Review of the literatures revealed the following as the most practical and widely tested diagnostic methods.

### 1.4.2.1 Clinical severity index

Ekstrand et al (1995) showed a connection between the severity of carious lesions and their histological depth. White spot lesions, which other require air-drying to visualise are mostly limited to the outer 1/2 of the enamel. The obvious white or brown spot lesion evident without air-drying, indicates that the lesion is located between the inner 1/2 of the enamel and the outer 1/3 of the dentine. Localised enamel breakdown due to caries, with no visible dentine involvement indicates that the lesion extends to the middle 1/3 of the dentine. A greyish, brownish or bluish shadow of the dentine shining up through apparently intact enamel also indicates a lesion extending to the middle 1/3 of dentine. Frank cavities with visible dentine indicate that a lesion has been extended to the inner 1/3 of dentine. Drying is very important because it removes the water from the intercrystalline spaces and fills it with air. Enamel has a refractive index of 1.62 while the refractive index of water is 1.3. Clinically the white spot lesion appears white because of alterations in the refraction of light through the enamel. The difference in refractive index between the air and enamel is greater than between water and enamel so the lesion after drying becomes more obvious.

### 1.4.2.2 The International Caries Detection and Assessment System (ICDAS)

The International Caries Detection and Assessment System (ICDAS I and II) presents a new model for the measurement of dental caries. The ICDAS criteria (Appendix) incorporate concepts from the research conducted by Ekstrand et al
(1995, 1997) and from a systematic review of the literature on clinical caries detection system (Ismail 2004a) and other sources (Fyffe et al. 2000; Ekstrand et al. 2001; Chesters et al. 2002; Ricketts et al. 2002; Ekstrand et al. 2005).

The ICDAS system was developed to bring forward the current understanding of the process of initiation and progression of dental caries to the fields of epidemiological and clinical research. If dental caries is classified using agreed upon criteria and systems, then comparison of findings by epidemiologists and clinicians from different countries would be feasible (International Caries Detection and Assessment System Coordinating Committee 2005a).

The ICDAS model provides more clarity for lay and non-dental audiences as well as continuity with traditional measures, while also reflecting the current research evidence from cariology. Key changes are to carefully avoid the use of the misleading and widely misunderstood term “caries free” and to explicitly acknowledge whether or not initial lesions clinically confined to the enamel are included or excluded in examinations (International Caries Detection and Assessment System 2005b). The ICDAS measures the surface changes and potential histological depth of carious lesions by relying on surface characteristics.

A number of studies have been performed to study the relationship between shadowing or grey discoloration and the presence or absence of caries. Some have reported that there was a statistically significant relationship (Kidd et al. 1994; Rudolphy et al. 1995) whereas others have found no such relationship (Kidd et al. 1995; Rudolphy et al. 1996).
To apply the ICDAS system, the teeth should be clean and dry. To enhance visual inspection, it is recommended to remove plaque and debris using a ball-ended explorer and it is highly advisable to clean the teeth with a tooth brush or a prophylaxis head/cup before the examination.

The new criterion for the detection and assessment of dental caries is referred to as ICDAS II. This system attempts to achieve integration and coordination of the emerging field of caries assessment. The criteria of ICDS II includes assessment of coronal primary caries code, pits and fissures, smooth surface (mesial and distal), free smooth surface (buccal and lingual) and direct examination of mesial and distal surfaces (with no adjacent teeth).

The reliability of the ICDAS was measured from data collected on training of examiners in the Detroit Dental Health Project. The study found good to very good inter-examiner reliability among dentists who were trained over a period of 1 week. The kappa coefficients for inter-examiner agreement ranged between 0.74 and 0.88. The intra-examiner kappa coefficients for the two main examiners were around 0.78. One secondary examiner had an intra-examiner reliability of 0.77 and a fourth secondary examiner who worked only on Saturdays had an intra-examiner kappa of 0.50 (International Caries Detection and Assessment System 2005b).

1.4.3 Radiographic examination

Radiographs are considered an essential technique for interproximal caries detection. Bitewing radiographs were useful indicators of dentinal caries on occlusal surfaces, and it was well recognised that the prevalence of occlusal caries may have been
underestimated without such images (Richardson and McIntyre 1996). Radiographs have good diagnostic efficacy in detection of large cavitated lesions and/or proximal lesions, however radiographs cannot detect the early stage of the disease.

Radiographic technique has several limitations including its relative insensitivity and its user dependence in terms of technical performance and interpretation (Marthaler and Germann 1970). Patients are exposed to a high amount of ionizing radiation. This is in contrast to the current trends in safety standards (as low as reasonably achievable), which advise that every effort be made to reduce the exposure of patients to ionizing radiation. Furthermore, it causes environmental pollution and the cost associated with the use of radiographs is another consideration.

1.4.4 Histological diagnosis of ‘white spot lesion’

By sectioning the enamel perpendicular to the surface, it is possible to create 80-100mm thick ground sections and examine the white spot lesions by microradiography and polarized light microscopy. When the section is examined after being air dried, the porous lesion (pore volume exceeding 1%) appears as a wedge shaped defect with its base at the enamel surface. On examination of the same section, with the intercrystalline space filled with water, there was more than 5% pore volume in the tissue mainly beneath the enamel surface (Thylstrup and Fejerskov 1986).
The inactive white spot lesion has microcavities. The rod pattern of this lesion is clearly identified under the polarising microscope (Kidd and Joyston-Bechal 1997) (Figure 1-1). As for the small enamel lesion, it has been divided into four zones based upon its histological appearance when longitudinal ground sections are examined with the light microscope. There is a translucent zone at the inner advancing front of the lesion. A dark zone may be found just superficial to this. The body zone lies between the dark zone and the apparently undamaged surface enamel. The relatively unaffected surface zone superficial to the lesion is the fourth zone.

Figure 1-1: Light microscope appearance of the white spot lesion on a smooth surface (Kidd and Joyston-Bechal 1997).

Histological studies have played an important role in challenging the perception that dental caries is not simply a process of progressive demineralisation. Clinical studies show that there are microscopic signs of dissolution of the outer enamel surface after one week of placing an orthodontic band on teeth, which in turn creates plaque...
stagnation areas (Ogaard et al. 1983; Holmen et al. 1985). These areas cannot be seen clinically even after careful air drying but can only be found by using a scanning electron microscope on extracted teeth (Diedrich 1981; Holmen et al. 1985).

1.4.5 Diagnostic aids

1.4.5.1 FOTI

Fibre-optic transillumination (FOTI) is a qualitative method that has been used since the 1970s. In this method, white light from a cold-light source is passed through a fibre to an intra-oral fibre-optic light probe that is placed on the buccal or lingual side of the tooth. The surface is examined using transmitted light and viewed occlusally. A carious lesion appears darker compared with the neighbouring sound tissue. The contrast between sound and carious tissue is then used for the detection of lesions.

A number of studies have been undertaken to evaluate the FOTI performance to detect posterior approximal carious lesions (Stephen et al. 1987; Peers et al. 1993). Their results showed low-to-good sensitivity and good specificity. It was reported that the FOTI specificity value was the highest of all detection systems including radiographs. FOTI would often miss caries (low sensitivity) but when it predicted caries, demineralisation was usually present (high specificity) (Pine and ten Bosch 1996). In an in-vitro study, it was reported that a combination of FOTI and visual inspection was useful for determining the occlusal of lesions depth (Cortes et al. 2003).
To improve the performance of FOTI, Digitized Fiber Optic Transillumination (DI-FOTI) was introduced. The tooth image was viewed from the occlusal aspect during transillumination through computer image analysis. The results of an *in-vitro* study, that involved imaging of extracted teeth, indicated that the device exhibited superior sensitivity for the detection of caries as compared to radiographic imaging (Schneiderman *et al.* 1997). Another study has also indicated that both the sensitivity and specificity are very high (Vaarkamp *et al.* 1997). However, more research is required before applying DI-FOTI routinely in clinical situation.

### 1.4.5.2 ECM

The Electrical Caries Monitor (ECM) was developed for the detection of non-cavitated early lesions. This method is based on detecting the increase in electrical conductivity, which is measured from the probe tip in the fissure through the dental pulp to a hand held earth (Pine and ten Bosch 1996). During the caries process porosities are formed. These are enlarged and filled with water and ions from saliva. These form an interconnecting pathway, which allows the passage of an electrical current and causes the resistance value to fall and this can be measured.

The reproducibility of this ECM method has been reported to be fair to good. The accuracy of ECM was compared *in-vitro* with visual detection for the detection of occlusal dentine caries in primary teeth (Ashley 2000). It was reported that both methods were excellent at detection occlusal caries extending into dentine and postulated that visual detection should be considered the system of preference due to its comparative ease of use.
The electrical conductivity of the ECM was measured to assess the suitability of the device in occlusal caries detection (Lussi et al. 1995). It was concluded that the device was suitable in occlusal caries detection but with its low specificity.

It was reported that ECM device was a good predictor of clinically relevant carious lesions on the occlusal surface of permanent teeth that could develop caries during an 18-month period (Ashley et al. 2000).

A new in-vitro study was undertaken to compare the diagnostic performance and reproducibility of two electrical methods (Electronic Caries Monitor III, ECM and Cariometer 800, CRM) on the occlusal surfaces of molar teeth (Kuhnisch et al. 2006). The intra- and interexaminer reproducibility was 0.69/0.62 for ECM and was 0.79/0.74 for CRM. The CRM showed at least equivalent diagnostic performance to the ECM. They concluded that improvement in the techniques was desirable.

Ricketts et al (1997) reported some advantages of the ECM, including sensitivity and the provision of an objective reading, which had the potential for monitoring lesion progression, arrest or remineralisation. It also reported some disadvantages of the ECM for example low specificity, which leads to a substantial number of sound teeth being filled unnecessarily due to the false positive readings. A different measurement technique, with an improved probe contact, appears to be advisable (Huysmans et al. 2005).

1.4.5.3 QLF

The laser fluorescence method measures the fluorescence of the tooth that is induced
after light irradiation to discriminate between carious and sound enamel. The term Quantitative Laser Fluorescence (QLF) has been applied to the research method of measuring induced tooth fluorescence after using laser light, at or near the 488 nm range, to quantify tooth demineralisation and lesion severity. This method has been evaluated in several studies, which have shown a strong correlation between a decrease in fluorescence and the degree of enamel demineralisation (Emami et al. 1996; Hall et al. 1997). The QLF method is based on the principle that the autofluorescence of the tooth changes with the mineral content of the dental hard tissue. Carious lesions radiate less fluorescence than sound tissue due its reduced mineral content. Therefore, this method is best suited to longitudinal detection of early lesions of the enamel on accessible smooth surfaces. Many investigations have involved the monitoring of white spot lesions around orthodontics brackets.

A number of clinical investigations have been undertaken to detect and monitor early carious lesions using QLF. The first of these studies monitored the changes in the white spot lesions following the removal of the fixed orthodontic appliances (Al-Khateeb et al. 1998). It was reported that remineralisation of the lesions was achieved within a few weeks. Moreover QLF detected five to ten times more early lesions than conventional detection methods, and was particularly useful for occlusal pit and fissure cases, and furthermore was reproducible (Ferreira-Zandonà et al. 2000).

QLF had better sensitivity, but poorer specificity, than visual examination alone or radiographic examination alone. The intra- and inter-examiner reliability of QLF on sixteen teeth lesions of varying severity were examined by ten examiners on three
occasions. Both intra and inter examiner agreement was good (Pretty et al. 2002). QLF allows for longitudinal analysis of tissue in-vitro and in-vivo (Higham et al. 2005).

1.4.5.4 DIAGNOdent system

DIAGNOdent a device designed for detection and quantification of hypomineralisation of occlusal and smooth surfaces lesions, was introduced to the market in 1998. It is a laser based device using a diode laser light source and emitting light at 655-nm wavelength from a fibre optic bundle. The light is directed onto the occlusal surface of a tooth at 1 mW peak power (Keller et al. 1998). A fibre optic cable transmits the light to a hand-held probe with a fibre optic eye in the tip. Organic and inorganic materials in the tooth substance absorb the light and infrared fluorescence occurs. The emitted fluorescence is collected at the probe tip, passes into a ascending fibre, is processed and is presented and displayed as an integer value, in the range of 0 to 99. A laser probe is used to scan the fissure area in a sweeping motion. Two values are displayed, a current value for the probe position and a maximum value for the whole surface examined. Increased fluorescence indicates the presence of carious tooth substance.

The DIAGNOdent device has been used in a number of in-vitro studies and a few in-vivo studies. It was suggested that the device could be utilized in a longitudinal control study of lesions as well as to observe the outcome of preventive treatment (Lussi et al. 1999). DIAGNOdent was shown to be an accurate system for detection of occlusal caries in-vitro when compared to visual examination and radiography (Attrill and Ashley 2001; El-Housseiny and Jamjoum 2001; Lussi and Francescut
The results of in-vitro studies also indicate that the readings are influenced by several variables, which include the degree of dehydration of the lesion, the presence of dental plaque and the presence of various types of stains.

The DIAGNOdent device showed in an in-vitro study a higher diagnostic accuracy in the detection of dentinal caries than enamel caries (Shi et al. 2000). The authors proposed that DIAGNOdent values were dependent on the volume of the caries rather than on the depth of the lesion. They concluded that overall correlation between DIAGNOdent and microradiography results were moderate but the device appeared superior to conventional radiography. They also reported that the device was very sensitive to the presence of stain, deposits and calculus, which led to erroneous reading. So, any changes in the physical structure of the enamel, including disturbed tooth development or mineralisation could lead to erroneous readings. Second repeat sets of DIAGNOdent measurements showed better correlation with the microradiography standard, which was construed as improved operator learning and skill development. Clinical experience was, therefore a "fundamental prerequisite" for using the device.

In an in-vivo study undertaken by Lussi et al (2001), it was reported that the high sensitivity of the DIAGNOdent was achieved by detecting occlusal caries. Another study by Tranaeus et al (2002) concluded that, in the clinical situation, the DIAGNOdent device showed excellent intra-operator agreement for measurements of carious lesions on smooth surfaces and good intra- and inter- operator agreement for lesions on occlusal surfaces. It was recommended that the device should be used throughout the longitudinal dental measurements on a patient. DIAGNOdent
readings were repeatable on intact sound and carious pit and fissures (Abu-Naba’a 2003). To date, there is only one published clinical study evaluating the reliability of the DIAGNOdent for measuring orthodontically induced white spot lesions (Aljehani et al. 2006). The device showed excellent intra-examiner reliability and good inter-examiner reliability.

The instructions for the DIAGNOdent system specified that the occlusal area to be diagnosed should be clean because plaque, tartar and discolouration may give false readings. All debris and stain should also be removed. The instructions also suggested that numeric data between 5 and 25 indicated initial lesions in the enamel and values greater than this range indicated early dentinal caries. Advanced dentine caries were said to yield values greater than 35 (the ProphyFlex System, Kavo, Germany). Cleaning using air-abrasives improved the diagnostic ability of the device especially for deep lesions. Rinsing with water or a three-in-one syringe was recommended after use after air-abrasion.

A new version of the DIAGNOdent device was introduced in 2006. The DIAGNOdent pen is a new device based on the same principle as the original DIAGNOdent system. The main unit measures 21 cm with two sapphire tips, wedge shaped and tapered shaped. The thickness of the wedge shaped tip is only 4 mm with a width of 1.1 in an attempt to facilitate the access to proximal surfaces whereas the thickness of the tapered shaped is 0.7 mm which can be used for smooth surfaces. The device is cordless and easy to operate. The DIAGNOdent pen seems to be as effective as the original DIAGNOdent device in regard to its validity and reliability for quantification of smooth surface caries in-vitro, and gives excellent agreement
with the original DIAGNOdent device (Aljehani et al. 2007). However, when comparing both devices in an in-vitro study by Kuhnisch et al (2007a), the DIAGNOdent pen indicated significantly lower values than the original DIAGNOdent. They concluded that both devices showed an imperfect reproducibility and suggested their usage as an adjunct tool only in clinical practice.

**Limitations and advantages of DIAGNOdent**

Many concerns regarding the DIAGNOdent system remain. The DIAGNOdent device uses a 680-nm filter and detects caries by measuring changes in fluorescence intensity rather than analyzing spectral differences (Keller et al. 1998). The scientific evidence shows a direct correlation between the numeric DIAGNOdent reading and the severity of the disease. A number of studies have shown that the correlation with the degree of enamel demineralisation is limited in depth (Emami et al. 1996; Al-Khateeb et al. 1997). It is of considerable concern that the DIAGNOdent is unable to distinguish between superficial and dentinal decay in-vitro.

The DIAGNOdent technique has many positive attributes. It is more reliable in detecting dentinal caries lesions, if proper cut off points are used. At present, some clinical studies have shown that the device seems to have good sensitivity (0.75-0.96) although a quite low specificity (0.68-0.86) (Lussi et al. 2001; Heinrich-Weltzien et al. 2002; Lussi and Francescut 2003; Anttonen et al. 2003).

The DIAGNOdent device is simple to use in everyday practice, is not complicated and provides qualitative measurements. Studies suggest good sensitivity and producibility. It is also helpful where radiography is not suitable or was refused by
the patient. It gives instant feedback to both clinician and patient and provides information in relation to patient management.

1.4.5.5 Digital photography

Digital images are made up of picture elements; the basic unit of image detail (pixels) comprising red, green, and blue light, each set at a level between 0 and 255 of shade colours. If all three colours are set at 255 white is the result, while if all are set at zero, black is the result. There are 256 grey shades that result from all three colours being set at the same number. Varying the level of each of the three colours results in the range of 16 million colours. Numerical values for each of these colours are stored on the Charged Couple Device (CCD). This is made up of pixels, the number of which, combined with the degree of compression, determines the quality of the final output.

According to the number of pixels a digital camera has its usefulness can be divided into three degrees of resolution (Hutchinson and Williams 1999):

- Low resolution (e.g. 320 x 240), these are no longer available.
- Mid-range (e.g. 649 x 480), generating a file size of approximately 350 KB.
- Megapixel (e.g. 1280 x 960), producing files of approximately 600 KB.

Storage of images

Depending on camera model and manufacturer, the digital images can be stored onto a compact flash card. The most commonly used memory cards can store between 4MB (Mega Byte) and 1000MB; 1GB (Giga Byte) = 1000MB. Smart media cards, recordable CDs which can store up to 650MB, or floppy disks which can store up to
1.4MB are often used. Recently, portable flash memory devices (USB flash and USB card) with multi-gigabyte capacities are available and widely used for storage the data. After storing the images, various peripherals, such as a computer card adaptor or floppy disk adaptor, are available to enable direct connection of the card to computer, allowing the images to be downloaded.

Images are stored as JPEG (Joint Photographic Expert Groups), which store between 300 and 500 KB, so careful consideration need be given to the disk size needed to store a reasonable number of images. These images can be archived using appropriate software or can be edited using one of the standard image editing software such as Paint Shop or Adobe Photoshop software.

**Limitations and advantages of digital camera**

There are three factors mentioned by Hutchinson and Williams (1999) that can limit the use of digital images (Table 1-2).

<table>
<thead>
<tr>
<th>Factors</th>
<th>Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>The optical resolution of the image</td>
<td>The finer the CCD grid the finer the details of the image and the higher the number of pixels per unit area.</td>
</tr>
<tr>
<td>The amount of memory in the camera</td>
<td>The larger the number of pixels the greater is the amount of data that need to be stored: more pixels mean fewer images.</td>
</tr>
<tr>
<td>Resolution of output device</td>
<td>If the printer’s resolution is less than that recorded by the camera, the final quality of the image will be determined by the resolution of the printer, and <em>vice versa</em>.</td>
</tr>
</tbody>
</table>

Table 1-2: Main factors which may limit use of digital images (Hutchinson and Williams 1999).
Sandler and Murray (2001) have listed some of the advantages of a digital camera as shown in Table 1-3.

<table>
<thead>
<tr>
<th>Advantages of a digital camera</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Rapid turn-around.</td>
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<tr>
<td>• Checkable exposure accuracy.</td>
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<tr>
<td>• No ageing of photos.</td>
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<tr>
<td>• Dust and scratches are irrelevant.</td>
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<tr>
<td>• Built in white balance.</td>
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<td>• Immediate viewing.</td>
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<td></td>
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<tr>
<td>• No film or processing costs.</td>
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<tr>
<td>• Inexpensive storage.</td>
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<tr>
<td>• Easy retrieval.</td>
</tr>
<tr>
<td>• Duplication easy.</td>
</tr>
<tr>
<td>• Transmission around the world rapidly is entirely feasible.</td>
</tr>
</tbody>
</table>

**Table 1-3:** Advantages of a digital camera (Sandler and Murray 2001).

**Dental photography**

Clinical dental photography is a very helpful tool in dentistry. It may be used for cases including both patient and professional education and medico-legal purposes. A computer imaging system can allow patients to see the potential outcome following treatment and reduce misunderstanding (Bonner 1992). In orthodontic practice, photographs can be used to show the appearance of enamel decalcification and what a particular appliance looks like. It also gives an estimation of the facial changes resulting from orthognathic surgery. Photographs are usually taken before, during and after dental treatment.

Previous studies into decalcification during and after orthodontic therapy have been mainly cross-sectional in design (Gorelick *et al.* 1982; Mizrahi 1982; Mizrahi 1983; Artun and Brobakken 1986; Geiger *et al.* 1988). Assessment has mostly been made by direct visual inspection, classifying the extent of the lesion in terms of thirds of
the tooth surface (Mitchell 1992). However, mostly the operator made no distinction between idiopathic enamel opacities and decalcification.

The first time photographs were utilised to assess the carious changes occurring in enamel was reported by Hill and Geddes (1975). Mitchell (1992) developed a quantitative method of assessing decalcification during orthodontic therapy using a photographic technique. The method gave a magnified permanent record, which could be recorded and allowed the size of the lesions to be quantified. In addition, it allowed a longitudinal comparison before and at the end of treatment (Mitchell 1992; Willmot et al. 2000). It has been also reported that the white spot lesions can be measured reliably from photographic slides converted into digital images (Willmot et al. 2000). The images from digital cameras are claimed to be accurate and reproducible (Benson et al. 2005).

Computerised digital photography has been boosted recently with advancement in computing, telecommunication and the internet. Digital images can be taken and stored using recently developed digital cameras. An attempt was made to investigate the use of computer scanning of photographic images to trace the area of white spot lesions around orthodontic brackets. The area was calculated by a computer, which was about 1.8 times the actual site (Harazaki et al. 2001).

The reproducibility of a standardised photographic technique was demonstrated for recording opacities by a group of epidemiologists as part of a large multicentered European study (Cochran et al. 2004). Their method involved taking two transparencies of the permanent maxillary central incisor of 8-year-old children, the first after 8 seconds while the teeth were wet and the second after 105 seconds when
the teeth had been allowed to dry out naturally. The conclusion of that study was that the photographic method was mostly robust and reproducible when used by epidemiologists from seven study sites.

A number of factors could contribute to variations in measurement of enamel demineralisation from photographic images. One possible source of error was the subjective nature of the index used to record the demineralisation. The digital images were analysed using a computer programme, which is able to objectively differentiate many more shades than the human eye (Benson et al. 2000). Another source of variation suggested was the angle at which the camera was placed relative to the buccal surface of the tooth was important (Benson et al. 1998). A change in the angle may alter the perspective of the images, which would affect the size of the area of demineralisation. It has been suggested that the camera should be angled no more than 20º to the perpendicular of the buccal surface. Incorporation of a calibrating grey scale with each image should improve the standardization of photographs for longitudinal studies (Benson et al. 2000).

In general, the errors in clinical photography can be divided into two groups. The first comprises errors due to inappropriate choice or use of equipment including the camera, lens, flash, retractors, mirrors or suction, or a lack of understanding of digital camera technology. The second group of errors relates to any recording medium and involves inappropriate positioning of the subject (McKeown et al. 2005).
2.1 Enamel structure

Enamel is a highly mineralised tissue, consisting of about 96% mineral, an impure form of hydroxyapatite, which is a crystalline calcium phosphate, and 4% water and organic materials.

Mannerberg (1960) classified the stages of enamel structure changes according to age. At 8 years of age all teeth show evident perikymata on one third to two thirds of the tooth surface; at age of 13, the number is reduced from 80% to 70%; and at age 18, 25% to 50% of teeth demonstrate such an appearance. The reason behind the changes on superficial enamel layers is that the tooth surface is not in a static state. Mechanical forces such as tooth brushing habits and abrasive food may lead to the dynamic changes that take place throughout life in the enamel layers. Using Scanning Electronic Microscopy (SEM), the open enamel prism ends are recognized as small holes, while in adult teeth cracks are seen. SEM shows no evidence of prism ends and deep and finer scratches run across the surface. Teeth in an adolescent reflect an intermediate stage. Mannerberg (1960) found the normal tooth enamel surface wear ranged from 0 to 2 μm per year.

Wet enamel has a higher translucency than dry enamel. Therefore, as the enamel becomes dry the amount of light which passes through will be reduced and the tooth will appear whiter. The translucency of human enamel therefore, strongly influences the clinical colour of the tooth (O'Brien 1985).
O'Brien and Fanian (1984) measured the translucency of human enamel and found that the translucency of enamel was a function of the wavelength. Their conclusions were that the higher the wavelength the higher the value of translucency. Therefore the enamel was more translucent under a light richer in yellow and red such as a tungsten light.

### 2.2 Factors affecting development of caries

There is a delicate balance between health and disease and it is essential to consider the multi-factorial aetiology of disease. Frequency of carbohydrate intake is a major contributing factor in most cases of dental caries.

Pathological factors involved in caries development include plaque acid, which results from diet and plaque, reduction in salivary flow, low buffering and oral clearance of acidic saliva. In contrast, there are protective factors to caries development which include saliva buffering capacity, calcium and phosphate levels, buffering and remineralisation, oral clearance and topical fluoride application. These can be regarded as the factors that maintain the demineralisation and remineralisation of teeth. Featherstone (1999) summarised these factors in a schematic diagram (Figure 2-1).
2.2.1 Risk factors

Bacterial flora and plaque

- Bacterial flora

Two groups of bacteria that have been implicated in dental caries are the Mutans streptococci (Ms) and Lactobacilli species. Acidogenic bacteria, which can produce acid from carbohydrate, are the mutans group (S mutans and S sobrinus). When the acids are produced by these bacteria, they diffuse into the tooth enamel or dentine and dissolve the minerals from crystals down inside the tooth. Since Ms are generally considered to be one of the major aetiologic agents for the initiation of caries, the obvious consequence of an increased Ms level would be an increase in the incidence of caries.

Figure 2-1: Schematic diagram of the balance between pathological factors and protective factors in the dental caries process (Featherstone 1999).


- **Plaque retention**

The plaque biofilm is a complex and dynamic environment and its relation with dental enamel is more than its adhesion on the surface. The interaction between the plaque biofilm and the enamel crystals is the first stage of the carious process and is important for our understanding of the mechanism of early enamel caries (Hashizume et al. 2002).

Fermentation of carbohydrates from food and beverages, caused by bacterial plaque, leads to the production of acid ions on the tooth surface. The effectiveness of the salivary buffering of this acid is inversely proportional to the plaque thickness. Thick plaque is held in deep fissures and grooves, between interproximal surfaces and around rough surfaces and over-contoured restorations. Mechanical oral hygiene procedures are not very effective in removing plaque from these sites, which are, therefore, the most common areas for caries initiation.

- **Effect of plaque on pH**

Fermentable carbohydrate entering the oral environment dissolves in saliva and become available to plaque microorganisms, which metabolise them and causes an immediate 2-4 pH drop at the tooth surface. The degree of this fall depends on the plaque thickness, the number and mix of plaque bacteria and the efficiency of salivary buffering. The recovery to normal resting pH takes from 20 minutes for the average patient, to several hours for those with a high susceptibility to caries. A very high salivary flow rate may return the pH towards neutrality quite rapidly, but local retention of sticky foods may delay the rise in pH until the food is dissolved or removed.
**Other acid sources**

Acids are available from a variety of extrinsic sources, such as carbonate in carbonated soft drinks, citrus fruit juices and gastric reflux or regurgitation. Rapid demineralisation after prolonged exposure to extrinsic sources can turn mild caries into a rampant attack.

### 2.2.2 Protective factors

**Protective dietary factors**

Some foods contribute to the protective factors which decrease demineralisation. Plaque is less able to attach to the tooth surface in the presence of fat. Other foods may themselves act as buffers. Foods that require vigorous chewing can be considered protective because such chewing markedly increases salivary flow and therefore buffering capacity. This factor alone can return pH in plaque to neutrality quite rapidly. Recently, some studies have been conducted *in-vivo* on the use of sugar-free chewing gum containing Casein Phosphopeptide-Amorphous Calcium Phosphate nanocomplexes (CPP-ACP). The results showed that, in the enamel subsurface lesion, remineralisation was increased with gum containing either 18.8 or 56.4mg CPP-ACP by 101 and 151 per cent respectively relative to the control sugar-free gum (Shen *et al.* 2001). The sugar-free gum containing CPP-ACP increases salivary flow rate after two minutes of consumption by 2.48 ± 0.98ml/min (Cai *et al.* 2003) and by 2.92ml/min (Dawes and Macpherson 1992).
**Saliva**

The major volume of saliva (93%) is secreted by the major salivary glands and the remaining 7% by the minor glands. These glands are located in every region of the mouth except for the gums and the anterior part of the hard palate. Saliva is sterile when it leaves the salivary glands but ceases to be so as soon as it mixes with the crevicular fluid, remains of food, microorganisms and desquamated oral mucous cells (Llena-Puy 2006).

Saliva is mostly produced by the submandibular and sublingual glands and its daily secretion rates range between 500 and 700 ml and the average volume in the mouth is 1.1 ml. Ninety-nine percent of saliva is water and the other 1% is composed of organic and inorganic molecules. Saliva plays a major role in protecting the teeth against acid challenge. Assessment of salivary parameters has been proposed as an essential element of the diagnosis and management of severe demineralisation and caries (Fejerskov and Manji 1990).

The salivary components perform a series of specific functions and are summarised in **Table 2-1** by Sreebny *et al* (1992).
### Functions of the Salivary Components

<table>
<thead>
<tr>
<th>Functions</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lubrication</td>
<td>Mucin, praline-rich glycoproteins, water</td>
</tr>
<tr>
<td>Antimicrobial action</td>
<td>Lysozyme, lactoferrin, lactoperoxides, mucins, cystins, histatins,</td>
</tr>
<tr>
<td></td>
<td>immunoglobulins, proline-rich glycoproteins</td>
</tr>
<tr>
<td>Maintaining mucosa integrity</td>
<td>Mucins, electrolytes, water</td>
</tr>
<tr>
<td>Cleansing</td>
<td>Water</td>
</tr>
<tr>
<td>Buffer capacity and remineralisation</td>
<td>Bicarbonate, phosphate, calcium, staterin, proline-rich anionic proteins,</td>
</tr>
<tr>
<td></td>
<td>fluoride</td>
</tr>
<tr>
<td>Preparing food for swallowing</td>
<td>Water, mucins</td>
</tr>
<tr>
<td>Digestion</td>
<td>Amylase, lipase, ribonucleases, proteases, water, mucins</td>
</tr>
<tr>
<td>Taste</td>
<td>Water, gustin</td>
</tr>
<tr>
<td>Phonetics</td>
<td>Water, mucin</td>
</tr>
</tbody>
</table>

**Table 2-1:** Functions of the salivary components (Sreebny *et al.* 1992).

**Main protective factors of saliva**

- The calcium and phosphate ions in saliva are supersaturated when the enamel apatite is at neutral pH. The phosphate provides a significant buffering capacity at resting pH and in the early stages of the acidic challenge.

- Pellicle, derived from saliva, provides a high level of protection against an acid challenge. It acts as a barrier against the diffusion of acid ions into the tooth. It may also inhibit mineralisation of apatite to form calculus from the supersaturated levels of calcium and phosphate ions in saliva.

- There is a very effective bicarbonate buffering system in stimulated salivary flow that contributes to a high level of protection against both organic and erosive acids on the tooth surface.
Salivary flow and oral clearance rate influences removal of food debris and microorganisms.

The fluoride ion content of saliva is low (0.03 p.p.m or 1.6 mmol/l on average) but will still contribute to the overall protection and repair of the tooth enamel.

**Salivary flow**

There is strong evidence that salivary flow rates influence both caries risk and caries activity (Papas et al. 1993). An increase in salivary flow helps the physical cleansing action of saliva, increases its buffering capacity and anti-bacterial activities and accelerates clearance of substance. Saliva provides the major source of natural protection and repair of teeth following an acid challenge. The normal salivary flow rate is > 1.0 ml/min and a reduction of salivary flow to less than 0.7 ml/min may increase the caries risk.

Some factors cause reduced amount of saliva. These factors include bulimia, Sjogren’s syndrome, rheumatoid arthritis, diabetes mellitus and pernicious anaemia. In addition, medications, such as antihistamines, decongestants, pain killers, diuretics and antidepressants, can also impair salivary flow as can head and neck irradiation (American Dental Association website 2005).

**Salivary pH and buffer capacity**

Saliva contains specific buffer mechanisms such as bicarbonate, phosphate and some protein systems which not only have a buffer effect but also provide an ideal environment for eliminating certain bacterial components that require a very low pH
to survive. The carbonic acid – bicarbonate buffer acts best when the stimulated salivary flow rate rises. The phosphate buffer plays an important role when salivary flow is low. The pH of unstimulated saliva can be less than 6, rising exponentially to pH 8 at very high flow rates. At a pH of greater than 6 the saliva is supersaturated with phosphate and calcium. When the pH falls below the critical level (5.5) the HA begins to dissolve thereby freeing phosphates that attempt to restore the pH balance. In the final analysis, the buffer mechanism depends on the phosphate and calcium ion content of the surrounding medium.

A low pH in the oral environment favours colonization by aciduric bacteria, particularly Ms, whereas a higher salivary pH retains a higher buffering capacity. The effect of buffer mechanisms is greater on the free surfaces, which are covered by a thin layer of bacterial plaque, than on interproximal surfaces. There is a significant negative correlation between the salivary buffering capacity and the frequency of caries (Russell et al. 1990).

**Fluoride**

**Effect on enamel**

Fluoride plays a highly significant role in the demineralisation – remineralisation process. In an acid environment, the fluoride ion reacts strongly with free Ca$^{++}$ and HPO$_4^{-}$ ions, forming fluorapatite crystals, in which fluoride substitutes for some hydroxyl ions.

Fluorapatite is less soluble than pure hydroxyapatite because of tighter subunit stacking. Fluorapatite crystals are unable to be dissolved by acid ions above pH 4.5,
the critical pH for fluorapatite, with the result that the mineral is more resistant to acid dissolution.

Fluoride ions are present within tooth structure in concentrations as high as 2500-4000 p.p.m (132-210 mmol/l) at the surface of enamel but the concentration in saliva may be as low as 0.03 p.p.m (1.6 mmol/l) as stated earlier. The incorporation of fluoride into teeth during development or the use of topical fluoride after eruption enhances the availability of these ions leading to increased inhibition of demineralisation and enhancement of remineralisation when acid ions interact with the tooth surface.

Fluoride inhibits the development of caries by inhibiting the demineralisation process and enhancing the normal remineralisation process by preferentially reacting with hydroxyapatite breakdown products to form fluorapatite or fluoride-enriched apatite. The optimal level of fluoride necessary to inhibit caries will vary for each person according to the level of acid ions present and its relationship to the level of the balancing protective agents. The fluoride ion can also react on established lesions and it can contribute to remineralisation of incipient enamel caries. It can partly remineralise carious dentine and thus slow down or arrest the carious process. Fluoride ions also remineralise root carious lesions to the extent that they may not need restoration. Topical fluoride is effective in inhibiting smooth surface caries and in aiding remineralisation of enamel. It is less effective in fissure or approximal caries because of the difficulty of removing mature plaque.
2.3 Assessment of risk factors prior to orthodontic bonding

Assessment of caries susceptibility prior to orthodontic treatment seems reasonable in order to identify patients with an increased risk of the disease. Data obtained to evaluate caries activity can be obtained from the case history, clinical and radiographic examination and supplementary tests which may include assessment of salivary flow rate, plaque scores and bacterial monitoring using salivary Ms and lactobacillus counts. These factors must be put together to obtain an individual profile. Orthodontic treatment is considered as one possible aggravating factor in the development of carious activity as orthodontic appliances may act as a risk factor.

2.3.1 Bacterial assessment

Orthodontic patients have oral environmental changes that lead to increased numbers of Ms in saliva and plaque (Lundstrom and Krasse 1987a; Lundstrom and Krasse 1987b). Several studies have revealed that Ms colonization was higher in teeth with fixed orthodontic appliances compared with the control teeth without appliances (Jenatschke et al. 2001; Attin et al. 2005). Therefore patients at risk should be allocated at the beginning of the treatment to a regime to prevent and monitor caries progression during their orthodontic therapy.

To determine the plaque and saliva Ms level, the site-specific Strip mutans technique is often used. This technique was developed by Jensen and Bratthall (1989) and was modified by Wallman and Krasse (1993).
In spite of the clinical convenience of this method and the ease and simplicity of its use, and the accessibility of saliva and the non-invasive manner of obtaining the specimen, few reports have evaluated the potential usefulness of this site-specific method for screening individuals for caries risk.

2.3.2 Salivary assessment

Saliva have been used to estimate Ms infection in the oral cavity, as Ms are considered the main aetiological factor for dental caries initiation in humans (Hamada and Slade 1980; Loesche 1986)

The relationship between the numbers of Ms and caries occurrence is a good indicator for caries-susceptible patients. It was shown that Ms levels in stimulated saliva correctly reflect those in plaque even with the known limitations of utilizing saliva (Togelius et al. 1984).

It has been reported that the wearing of fixed orthodontic appliances results in an increase in the numbers of Ms during active orthodontic treatment (Forsberg et al. 1991). The increased levels of Ms may subsequently become a factor in the higher incidence of enamel demineralisation observed in some orthodontic patients.
2.4 Orthodontic treatment and caries progression

2.4.1 Plaque accumulation

The presence of orthodontic appliances and other devices such as wires, elastics and bands in the oral cavity creates a potential environment for plaque to accumulate around orthodontic brackets. Accumulations of plaque have been seen on composite surfaces adjacent to adhesive retention elements, on interfaces between composite and enamel (Gwinnett and Ceen 1979) and particularly beneath a band from which the cement has been lost (Mizrahi 1982). This environment changes the biological balance of the mouth and increases the patient’s risk of caries. Orthodontic therapy requires high compliance and there is a need to continuously monitor patients’ oral hygiene.

The presence of orthodontic attachments makes tooth cleaning more difficult, and predisposes to plaque accumulation on the tooth surface both around the attachment and between it and the gingival margin. In addition, fixed appliances may restrict the ability of the tongue to remove food particles from the mouth (Chang et al. 1997).

2.4.2 Demineralisation

Demineralisation and remineralisation results first in a sub-clinical lesion and eventually in the production of a white spot lesion (ten Cate and Duijsters 1982).

The earliest visual clinical presence of caries is known as “white spot lesion”. It is best be seen on a dried surface. The lesion appears as a small, opaque, white area and the colour of the lesion distinguishes it from adjacent translucent sound enamel. The
colour change is based on the increased porosity of the tissue, which alters the way in which the light is scattered. If air-drying reveals a white spot in the enamel, the change in enamel porosity is slight. If the porosity is clinically visible as a white spot without air-drying the porosity is larger.

As a consequence of the rapid plaque accumulation around brackets, decalcification and white spot lesions have occurred within a few weeks of wearing brackets (Gwinnett and Ceen 1979; Gorelick et al. 1982; O'Reilly and Featherstone 1987). Melrose et al (1996) reported that early enamel carious lesions can form in areas of plaque retention, associated with orthodontic bands, in periods as short as 4 weeks and mineral loss has been recorded after only 4 weeks (O'Reilly and Featherstone 1987).

Actually, the carious phenomenon is a dynamic process of lesion progression and lesion repair. An earlier study found a correlation between the duration of treatment and the appearance of minor and deep demineralisation. The probability of lesions formation in patients, who had fixed appliances for more than 2 years was higher than 1 year (Geiger et al. 1988). The progression of demineralisation was not dependant on plaque volume but on differentiation of microflora which leads to a greater concentration of acid-forming bacteria (Balenseifen and Madonia 1970). At the same time, the concentration of free calcium and phosphate ions that is required for remineralisation decreases.
2.4.3 **Bacterial proliferation**

The demineralisation of dental enamel is caused by acids produced from the fermentation of dietary carbohydrates by dental plaque bacteria. *Mutans streptococci* and *lactobacilli* are among the acid-forming microorganisms that proliferate. Ms are considered as important cariogenic plaque organisms (Tanzer 1987; van Houte 1994), and Ms are implicated as a potent caries-conductive micro organism in man (Gibbons 1968).

Patients undergoing orthodontic therapy have more Ms and *lactobacillus* in their plaque than normal patients. A study by Sakamaki and Bahn (1968) showed a five fold increase in lactobacillus counts in patients undergoing active orthodontic treatment. Many growth sites were located on gingival margins and on the edges of orthodontic bands. Another study by Corbett *et al* (1981) showed that caries-free patients with orthodontic bands had far more Ms in buccolingual and proximal sites than patients without bands. A study by Liu *et al* (2004) has been conducted to clarify the genotypic stability of Ms longitudinally during orthodontic therapy. Plaque samples were obtained from the supragingival surface of upper right teeth of 17 patients and were recorded. The isolated *streptococci* were identified on the basis of their morphological and biochemical properties. DNA was prepared from strains of Ms and was then identified. Primed polymerase chain reaction fingerprinting was applied in determining the genotypes of Ms. The result of this study indicated that a maximum of 3 different genotypes of Ms were found in an individual and that the Ms clones were very stable during orthodontic therapy. The amount of Ms in the saliva increased exponentially with the number of bands and brackets. This was shown in a study by Rosenbloom and Tinanoff (1991) who compared Ms values in the saliva of
patients in the active orthodontic treatment phase with those subjects in retention, subjects who were post-retention and a control group with no orthodontic appliances. Only those patients with fixed appliances had increased Ms levels. All other subjects demonstrated only small numbers of Ms. Cariogenic bacteria, and the Ms group in particular, had sufficient time to form carious lesions.

2.4.4 Caries

The white spot lesion is cariogenically active and may be progressing. The time required for dental caries to progress from a white spot lesion to an incipient lesion is not known exactly but the time required for dental caries to progress from the incipient phase to a clinical enamel lesion is well reported and averages three to four years in permanent teeth (Woodward and Leake 1996).

*Mutans streptococci* are strongly implicated in the initiation and progression of dental caries. Besides being acidogenic and acid tolerant, Ms possess the ability to synthesize extracellular glucans from sucrose. This process may increase the cariogenicity of plaque by enhancing plaque mass, promoting the colonization of Ms, and changing the diffusion properties of the plaque matrix. Retentive areas of solid surfaces are the prefered colonization sites for Ms and their presence on this surface at high levels is an indicator of increased caries risk (Klock and Krasse 1979). In patients with orthodontic appliances, the approximal spaces and the smooth surfaces around brackets are exposed to the greatest risk of caries (Gorelick *et al.* 1982).
2.4.5 Prevalence of decalcification during fixed orthodontic treatment

Several clinical studies have confirmed the susceptibility of patients under going orthodontic therapy to dental caries (Chang et al. 1999; Batoni et al. 2001). The incidence of decalcification following a course of fixed appliance therapy, that lasts approximately 2 years, has been reported to be as high as 50% (Gorelick et al. 1982; Artun and Brobakken 1986; Ogaard 1989a). A significant increase in the prevalence and severity of demineralisation was shown, after orthodontic therapy, compared with controls, and the overall prevalence among orthodontic patients ranged from 2 to 96 percent (Mizrahi 1982; Mitchell 1992). However, the decalcification was reported to increase for both the control and experimental teeth (Mattick et al. 2001).

Zachrisson and Zachrisson (1971) investigated the increase in the number of carious lesions in fully banded patients who had been treated on average for 19 months. To ensure correct diagnosis of caries, bitewing radiographs were taken before and after the completion of therapy. The results showed no significant increase in the incidence of caries in fully bonded patients, but a shift in location of caries from approximal to buccal and lingual sites was found. Teeth on which attachments had been placed buccally or lingually had higher rates of caries. Other studies also confirmed that there was an increased incidence of carious lesions on the facial and lingual aspect of the teeth during treatment with fixed appliances (Gorelick et al. 1982; Artun and Brobakken 1986; Ogaard 1989a).

The appearance and distribution of sites of demineralisation are highly variable. White spot lesions have been reported as occurring most frequently on the upper lateral incisors and first permanent molar (Artun and Brobakken 1986). Gorelick et
al (1982) reported that the highest incidence occurred in the maxillary incisors and the lowest was in the maxillary posterior segment. However, 50% of the patients demonstrated resistance to white spot formation. They suggested a relationship between resistance to white spot formation and the rate of salivary flow. In general, decalcification most commonly occurs on maxillary teeth more than mandibular teeth (Artun and Brobakken 1986; Ogaard 1989b).

Repetition of the cementing procedure, due to detachment of brackets or bands, produces more decalcification. Dincer and Erdinc (2002) in an in-vivo study found that “recemented teeth” showed more decalcification. Decalcification lesions were found as follow: 17 recemented teeth, (36.1%), in the upper jaw and 8 recemented teeth (25.8%) in the lower jaw. The distribution pattern of sites of demineralisation may be explained by these affected tooth surfaces lying some distance from the regions adjacent to major saliva gland output. However, after debonding the orthodontic brackets the white spot lesions can be seen clinically, where the enamel surface appears matted in appearance (O'Reilly and Featherstone 1987) (Figure 2-2).
Figure 2-2: Appearance of enamel surface with white spot lesions after debonding of orthodontic brackets.

2.5 Trials to eliminate white spot lesion during orthodontic therapy

The obvious degree of iatrogenic enamel damage during orthodontic treatment suggests the need for preventive programmes. Several preventative methods have been applied to overcome the enamel demineralisation problem during orthodontic treatment. Table 2-2 summarises some of the clinical trials which have been conducted over the last 20 years, in an attempt to eliminate, or prevent, the degree of enamel damage associated with fixed orthodontic appliance therapy.
Table 2-2: Clinical trials to eliminate or reduce white spot lesions during fixed orthodontic therapy using different preventative methods (from 1998-2007).

<table>
<thead>
<tr>
<th>Authors</th>
<th>Design</th>
<th>Index used/ assessment method</th>
<th>Prevention methods</th>
<th>Prevalence and result</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Al-Khateeb et al. 1998)</td>
<td>Clinical trial assessment of white spot lesions after orthodontic therapy</td>
<td>Quantitative laser fluorescence method</td>
<td>The regular use of a fluoride dentifrice</td>
<td>During a 1-year follow-up period, the areas of the lesions decreased and the enamel fluorescence lost was partly regained indicating that a remineralisation process had occurred</td>
</tr>
<tr>
<td>(Millett et al. 1999)</td>
<td>Longitudinal clinical comparative randomized controlled trial.</td>
<td>Modified DD index/ Standard photograph technique assessment</td>
<td>Comparison of prevalence in orthodontic patients between glass ionomer and resin composite</td>
<td>Baseline to debond on glass ionomer side mean ± SD (2.8± 0.4), resin composite (2.7± 0.6)</td>
</tr>
<tr>
<td>(Gaworski et al. 1999)</td>
<td>Clinical comparative controlled trial</td>
<td>Enamel decalcification scale</td>
<td>Comparison of 2 adhesives with fluoride (Fuji Ortho LC) and without fluoride (Concise)</td>
<td>298 teeth, 96 teeth were evaluated for decalcification</td>
</tr>
<tr>
<td>(Wenderoth et al. 1999)</td>
<td>Clinical randomized controlled trial</td>
<td>Photos evaluated blindly by 7 observers for white spot formation</td>
<td>Fluoride-releasing sealant</td>
<td>No significant difference ($P &gt; 0.05$) between the decalcification rates of the treatment or control groups</td>
</tr>
<tr>
<td>(Millett et al. 2000)</td>
<td>Longitudinal clinical comparative randomized controlled trial A split-mouth design</td>
<td>Caries index 4-point scale/assessed using clinical photographs</td>
<td>Componer versus composite for bonding.</td>
<td>There was more decalcification related to brackets bonded with resin adhesive than with compomer ($P = 0.0075$). No statistically significant (OR 0.22; 95% CI 0.02–1.07) difference between the materials</td>
</tr>
<tr>
<td>(Banks et al. 2000)</td>
<td>A prospective controlled clinical trial</td>
<td>Index by direct clinical observation</td>
<td>Fluoridated versus non-fluoridated elastics.</td>
<td>The overall reduction in score per tooth produced by the fluoride-releasing elastomerics was 49%, a highly significant difference ($P &lt; 0.001$)</td>
</tr>
<tr>
<td>(Gillgrass et al. 2001)</td>
<td>Clinical comparative randomized controlled trial. A split-mouth design</td>
<td>Assessment of White Spot Lesions (WSL) was by visual inspection 4-point scale</td>
<td>Componer versus GIC for banding.</td>
<td>No significant difference in the proportion of patients with new WSL between the two materials ($p = 0.16$)</td>
</tr>
<tr>
<td>(Ogaard et al. 2001)</td>
<td>Clinical randomized controlled trial</td>
<td>White spot lesion index (Gorelick et al. 1982)</td>
<td>Fluoride and antimicrobial varnish versus fluoride varnish</td>
<td>Significant differences between the control and experimental group in the proportion of patients with WSL ($p &lt; 0.01$).</td>
</tr>
<tr>
<td>(Mattick et al. 2001)</td>
<td>Clinical randomized controlled trial. A split-mouth design</td>
<td>Modification of the Enamel Defect Score (EDS) (Artun and Brobakken, 1986). Standardised photographs</td>
<td>The experimental teeth were ligated to the archwire using fluoride releasing elastomeric modules</td>
<td>Statistically significant difference in the degree of decalcification between the two groups at the end of treatment ($P = 0.0002$). The experimental side experienced fewer defects than the control side</td>
</tr>
<tr>
<td>(Harazaki et al. 2001)</td>
<td>Clinical controlled trial</td>
<td>Photographic tracing and area of lesion were calculated by computer</td>
<td>Treated group received laser treatment and acidulated phosphate fluoride solution (APF)</td>
<td>Result showed increases in white spots: laser-irradiated group, 1.41 times; controls, 2.87 times. The difference was statistically significant</td>
</tr>
<tr>
<td>Study Authors</td>
<td>Study Design</td>
<td>Methods</td>
<td>Findings</td>
<td></td>
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<td>(Anderson et al. 2002)</td>
<td>Clinical comparative controlled trial</td>
<td>The teeth were sectioned and examined under polarized light microscopy</td>
<td>Acid-etched with 30% phosphoric acid for 30 seconds, and treated for 60 seconds with laser; and group 4 (laser only), teeth were treated for 60 seconds with laser. The average lesion depth in the laser-only group was reduced by 94.1% and the average lesion area was reduced by 94.4% when compared with the control group. In the pumice-etch-laser group, the average lesion depth was reduced by 89.1% and the average lesion area was reduced by 92.2% when compared with the control group.</td>
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<tr>
<td>(Gorton and Featherstone 2003)</td>
<td>Clinical randomized controlled trial</td>
<td>Cross-sectional microhardness testing</td>
<td>Comparison between fluoride releasing glass ionomer cement and composite resin (no fluoride). Significantly more demineralisation around the brackets of the control group (p &gt; 0.05)</td>
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<tr>
<td>(Van Miller and Donly 2003)</td>
<td>Clinical comparative randomized controlled trial</td>
<td>The sections were photographed with polarized light microscopy in an imbibition media of water. The body of each lesion was measured with a computerized imaging system</td>
<td>Comparison between fluoride releasing glass ionomer cement and polyacid-modified resin-based composite cement, in comparison to non-fluoride releasing zinc phosphate cement. Significantly less demineralisation adjacent to the glass ionomer cement group compared with the polyacid-modified resin cement group and non-fluoride releasing zinc phosphate control group (P &lt; 0.05). There was no significant difference in adjacent demineralisation inhibition between the zinc phosphate cement group and polyacid-modified resin cement group (P &lt; 0.05)</td>
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<td>(Pascotto et al. 2004)</td>
<td>Clinical, randomized controlled trial</td>
<td>Assessed by cross-sectional microhardness.</td>
<td>Comparison between fluoride releasing glass ionomer cement (Fuji Ortho LC) and without fluoride (Concise) (control group). Significant reduction in enamel demineralisation in tested group (p &lt; 0.05)</td>
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<td>(Zimmer and Rottwinkel 2004)</td>
<td>Longitudinal prospective randomized comparative clinical trial.</td>
<td>W S L index (Gorelick et al. 1982)</td>
<td>Extended prophylaxis regimen. Although extended prophylaxis significantly reduced the decalcification frequency in the risk group (P ≤ 0.05) compared with the control group. Decalcification frequency did not reach the low rate found in the low-risk group (P ≤ 0.05).</td>
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<tr>
<td>(Willmot 2004)</td>
<td>Clinical comparative randomized controlled trial. Double-blind design. Postorthodontic</td>
<td>Computerised image analysis</td>
<td>Daily use the low fluoride formulation of mouthrinse/toothpaste. The average difference in WSL percentage reduction of white spot size between tested and control group after 6 months was approximately half of the size (54.3%).</td>
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<tr>
<td>(Benson et al. 2004)</td>
<td>Prospective, longitudinal, clinical, randomized controlled trial</td>
<td>Photograph, Computerised image analysis</td>
<td>Fluoridated elastomers. Fluoridated elastomers do not affect the quantity of disclosed plaque around an orthodontic bracket.</td>
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<tr>
<td>(Hu and Featherstone 2005)</td>
<td>In-vitro study</td>
<td>Sectioned and evaluated quantitatively by cross-sectional microhardness testing</td>
<td>Applying a light-cured filled sealant. Teeth treated with fluoride varnish exhibited 30% less demineralisation than the control teeth, the enamel-etched teeth, and the teeth treated with a light-cured, unfilled sealant (P &lt; 0.05).</td>
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Several clinical and laboratory trials have been conducted to try to explain white spot lesion development around orthodontic brackets. Those trials have identified certain criteria for the patients selection before starting orthodontic treatment. Certain oral and/or written instructions should be given to the patients after starting the treatment and certain preventative methods should be applied during the treatment.

### 2.5.1 Patient selection and education

A high level of oral hygiene is required before initiating fixed orthodontic therapy. However, there will always be a proportion of patients whose ability to maintain good oral hygiene will be improved as treatment progresses. Regular communication between the patients and the dental hygienist is essential to coordinate dental care.
education, diet and tooth cleaning. These procedures can help reduce dental complications with patients with fixed orthodontic treatment, but will not eliminate them. Using microbial monitoring is important to identify those patients most at risk of decalcification as evidenced by high salivary Ms and *Lactobacilli* counts.

### 2.5.2 Oral hygiene instruction

Professional oral hygiene instruction, for the duration of orthodontic therapy, has been shown to be effective in reducing decalcification (Artun and Brobakken 1986). However, this approach is very labour intensive and costly.

**Mechanical plaque removal**

Many designs of toothbrushes and cleaning aids are available to the public. These can be obtained over the counter in pharmacies and indeed may be sold by dental practitioners themselves. Special orthodontic tooth brushes are also available for orthodontic patients. These tooth brushes have a groove along the length of the head designed to fit over the brackets and arch wire. In addition, supplemental interdental cleaning aids are essential, *e.g.* a single tufted toothbrush, to clean between the teeth and under the arch wire.

Manufacturers are heavily promoting oral irrigation systems and powered toothbrushes. It does seem however that oral irrigation device should only be used as an adjunct to the other methods of tooth cleaning (Attarzadeh 1986). Studies which have examined the efficiency of an electric over a manual toothbrush, for orthodontic patients, have been inconclusive (Boyd *et al.* 1989).
In orthodontic patients it was demonstrated that the ultrasonic toothbrush was better at reducing gingival inflammation but plaque scores were lower on buccal surfaces of teeth with orthodontic brackets. Ms counts were significantly decreased in the electric and ultrasonic groups, which should reduce the risk of oral disease (Costa et al. 2007).

**Chemical plaque removal**

Hogg (1990) postulated that to be effective a chemical plaque removal agent must be capable of reducing the oral flora by 99.9%. The chemical agent must not affect the balance of the oral microflora or promote the emergence of resistant microorganisms and clearly must be non-toxic. Chlorhexidine is the most effective agent due to its absorption onto the acquired pellicle which prolongs its presence and effect in the mouth (Hogg 1990). However, chlorhexidine will be less effective with orthodontic patients unless used at the completion of appliance therapy after removing the orthodontic attachments (Brightman et al. 1991).

**2.5.3 Protection of the enamel surface around the orthodontic attachment**

Two approaches have been proposed in an attempt to reduce potential detrimental enamel changes during orthodontic therapy. The first approach is aimed at reducing acid solubility by the application of topical fluoride before, during and after treatment. The second approach is to protect the enamel surface by using different materials and providing a protective coating.
2.5.3.1 Fluoride

Fluoride can help reduce the formation of caries, helping remineralisation during pH fluctuations and inhibiting glycolysis of plaque bacteria (Levine 1991). Fluoride reacts with the enamel forming calcium fluoride and fluoroapatite which in turn acts as a slow-releasing agent enhancing the remineralisation of the etched enamel and making it more resistant to acid dissolution (Bohrer and Gedalia 1980; Bryant et al. 1985).

Different applications of fluoride have been used to protect the enamel during orthodontic treatment.

**Toothpaste and mouth rinsing**

The results of a review study of published data on preventative measures during orthodontic therapy have shown that toothpastes with a concentration of 1,500 and 5,000 ppm fluoride had a better preventative effect than those with a concentration of 1,000 ppm. Supplemental use of a brush-on gel with 5,000 ppm fluoride once a day had a better preventative effect compared with conventional fluoride toothpaste alone (Derks et al. 2004).

Salivary fluoride concentrations have been reported to reach a significantly higher level compared with the baseline values following approximately two weeks use of sodium fluoride mouth rinse (100, 250, 1000 ppm F), with one rinse per day (Duckworth et al. 1987). A strong linear relationship exists between salivary and plaque-fluid fluoride, suggesting that plaque-fluid fluoride is influenced by the concentration of salivary fluoride. However, plaque-fluid fluoride exhibits larger
inter-site and inter-subject variations (Vogel et al. 1992).

Several studies have shown that daily rinsing with sodium fluoride (0.5% or 2%) and/or weekly acidulated phosphate fluoride rinses (1.2%) are effective in reducing the prevalence of enamel decalcification during orthodontic therapy (Zachrisson 1975; O’Reilly and Featherstone 1987; Saloum and Sondhi 1987; Ogaard 1988; Ogaard et al. 2006). However, it was suggested that visible white spot lesions on labial surfaces should not be treated topically with concentrated fluoride agents since this procedure may prevent complete repair (Ogaard 1988). The benefit from this approach is highly dependent on patient cooperation and patient compliance has been reported in other studies to be poor (Geiger et al. 1992).

**Professionally applied fluoride**

The application of topical fluoride before etching has been evaluated in several studies. Some studies have shown a reduction in bond strength between enamel and adhesive resin (Low et al. 1975; Meng et al. 1998) while others found no significant effect upon bond strength (Wang and Sheen 1991; Cacciafesta et al. 2005). Application of fluoride varnish immediately following bonding has shown a reduced decalcification by up to 50% (Todd et al. 1999). Application of professional fluoride gel around orthodontic brackets is also effective in reducing decalcification (Zachrisson 1975; O’Reilly and Featherstone 1987). Another study concluded that topical application of 0.4% stannous fluoride gel in orthodontic patients did not cause a significant decrease in Ms in the saliva or biofilm (Bretas et al. 2005). The drawback to the latter approach is that it is expensive and time consuming in terms of operator time.
**Fluoride containing etchant**

The mechanism by which fluoride reduces either demineralisation or caries is multifactorial. Certainly fluoride increases the resistance of the enamel to acid dissolution, increases the maturation rate of the enamel and interferes with the metabolism of microorganisms (Bryant et al. 1985; Meng et al. 1997). The formation of reaction products on the enamel surface because of the acidic fluoride treatments has been investigated in several studies. Some studies showed controversial results as etchant dissolves the enamel and is then rinsed away thereby producing little or no protective effect (Thornton et al. 1986).

The topical application of fluoride has been reported to decrease the bracket bond strength (Thornton et al. 1986; Garcia-Godoy et al. 1991; Meng et al. 1997; Meng et al. 1998). In contrast, a study by Takahashi et al (1980) reported that the application of 30% H₃PO₄ solution containing 0.02% NaF resulted in an increase in the fluoride content in the enamel surface without decreasing the bond strength. Other studies have indicated that a mixture of the 37% phosphoric acid and an acidulated phosphate fluoride gel (50% and 66% fraction) would be used as an etchant to minimize the loss of sound enamel during the etching as well as the debonding procedure without compromising the required bracket bond strength (Kim et al. 2005).

**Fluoride containing cement for bonding**

The development of fluoride containing cements for cementation of orthodontic bands has been widely reviewed. The polyalkenoate cements have been shown clinically to cause less decalcification than zinc phosphate or zinc polyacrylate
(Maijer and Smith 1988; Mizrahi 1988). Zinc polycarboxylate and RMGI demonstrated less demineralisation than the zinc phosphate in-vitro (Foley et al. 2002). Whether this is due to the increased fluoride content of glass ionomer cements, or their increased adhesion and better band retention is still unclear.

**Fluoride containing bonding adhesive**

Fluoride has been added to orthodontic bonding materials in the hope that its presence could prevent or minimise decalcification. The widely used types of fluoride containing materials are glass ionomer and composite resin. Research, dealing with the GICs, confirmed the advantage of releasing fluoride and thereby protecting enamel from decalcification but, conversely, have been shown to have poor bond strength compared to composite resin (Wiltshire 1994; Itoh et al. 1999).

Improvements in orthodontic bonding materials have led to the advent of resin modified glass ionomer adhesives (RMGA). Bishara et al (1998) concluded that with etched enamel and in a wet environment the light-cured resin-reinforced glass ionomer adhesive system had comparable shear bond strength to that of the traditional light cured composite resin systems. Bond failure with composite resin has been attributed to moisture contamination arising from saliva, gingival fluids and water from dental syringes. The studies on fluoride releasing composites have shown that the bond strength was less than that of the conventional type (Chan et al. 1990). However, Bishara et al (1991) concluded that this was due to inadequate polymerisation rather than the addition of sodium fluoride.
**Fluoride releasing modules**

A number of studies have been conducted on the use of fluoridated elastomers around orthodontic brackets. In *in-vitro* studies, fluoridated elastomers have been shown to release fluoride for up to 6 months (Wiltshire 1996). That and previous studies have suggested that teeth with fluoridated elastomers had harder surface enamel (Wilson and Love 1995), which is probably more resistant to enamel demineralisation. They also indicated that *in-vivo* that the use of fluoridated elastomers reduced the severity, and probably the incidence, of demineralisation during orthodontic treatment (Banks *et al.* 2000; Mattick *et al.* 2001).

In their assessment of bacterial colonisation, Wilson and Love (1995) confirmed a significant reduction after one week in the salivary Ms count of patients using fluoridated elastomers. However, Ms counts returned to baseline values after two weeks. A clinical study by Benson *et al.* (2004) investigated the effect of fluoridated elastomers on disclosed plaque. The conclusion of that study was that fluoridated elastomers do not affect the quantity of disclosed plaque around an orthodontic bracket and that the individual patient’s level of oral hygiene was the most important factor determining the area of the buccal surface covered with disclosed plaque.

Recently, some *in-vitro* studies investigated the effect of the stretch ability of fluoridated elastomeric ligatures. A study by O'Dwyer *et al.* (2005) concluded that fluoridated elastomeric ligatures released appreciable amounts of fluoride over a 196-day period when placed in distilled water and incubated at 37°C. This release could theoretically inhibit demineralisation *in-vivo.* Also, stretching fluoridated elastomerics produced a statistically significant increase in the amount of fluoride
released *in-vitro*. Storie *et al* (1994) examined the fluoride release from 5 samples of 4 loop sections of fluoridated elastomeric chain that had been stretched by 50% of their original length. They found that over half the total fluoride was released in the first 24 hours and 90% by the end of the first week. A study by Eliades *et al* (1999) showed that the structure of polyurethane was altered by the deformation caused by stretching elastomeric chains. They demonstrated a honeycomb pattern of filament detachment that corresponded to the strained areas. This might explain the greater availability of fluoride for release. However, the fluoridated elastomers developed well-mineralised, proteinaceous films after 3 weeks in the mouth (Eliades *et al*. 1999). This would subsequently affect the rate of fluoride release. Moreover, complications, such as differences in diet, salivary flow rates and oral-hygiene regimens might produce differences in fluoride release between patients. In addition, the crossover in such trials may allow fluorides from elastomers to be absorbed onto the control teeth and thus will bias the results. Clinical trials have shown that the fluoridated elastomers do not eliminate the incidence of decalcification after orthodontic therapy (Banks *et al*. 2000; Mattick *et al*. 2001). However, that may be due to the crossover design of such trials which may allows and thus biasing the results fluoride from elastomers to be absorbed onto the control teeth prove to be a weak point especially since the slow release of fluorides from elastomers may be absorbed onto the control teeth preferentially thereby biasing the results. In addition, complications, such as differences in diet, salivary flow rates and oral-hygiene regimens might produce differences in fluoride release between patients.
2.5.3.2 Sealant and varnish

Painting sealant resin around orthodontic bonds has also been employed, but the oxygen inhibition of polymerisation limits the protective effect (Ceen and Gwinnett 1981; Saloum and Sondhi 1987). Clinical trials have shown that the application of light-cured resin sealants to the labial enamel surface has been reported to decrease the amount of demineralisation by 13% (Banks and Richmond 1994). However, many sealants fail to provide enamel protection for smooth surfaces adjacent to bonded brackets (Ceen and Gwinnett 1981).

It has also been reported that chlorhexidine (CHX) application in a varnish formulation resulted in longer lasting suppression of Ms compared with other forms of application (Emilson 1994; Attin et al. 2003). Varnish containing 45% CHX has been reported to decrease the numbers of Ms by a factor of 10, 22 weeks after a single application to the occlusal fissures of test teeth (Schaeken et al. 1989).

The optimal CHX varnish concentration suggested for effective suppression of Ms is 40% CHX (EC40®, Explore, Nijmegen, Netherlands). The most potent CHX treatment was effective in high-risk non orthodontic subjects, but failed in Ms highly colonised teeth in orthodontic patients (Jenatschke et al. 2001; Attin et al. 2005). Whether this varnish would provide a protective coating around orthodontic bonded attachments remains speculative. An in-vitro application of a fluoride varnish has been shown to prevent demineralisation (Kindelan 1996; Todd et al. 1999; Gillgrass et al. 2001).
2.5.3.3 Laser irradiation

Many methods of irradiation have claimed to be effective in eliminating pain in teeth and in preventing dental caries (Harazaki and Isshiki 1997; Harazaki et al. 1998).

The laser was first reported to increase the resistance of tooth enamel to acid attack (Sognnaes and Stern 1965). Argon laser irradiation has been reported to be effective in the control of dental caries in the laboratory situations (Powell et al. 1993; Hicks et al. 1995; Westerman et al. 1996). It has also been reported that the use of an argon laser at low energy density, \((10.74 \text{ J/cm}^2)\), significantly increased the fluoride retention in lasered enamel that had approximately 400 times more fluoride than the unlasered enamel (Nammour et al. 2005).

In a clinical study, 10 patients undergoing orthodontic treatment were subjected to a combination of Nd-YAG and acidulated phosphate fluoride treatment. The changes, (increase), in the areas of white spot for the laser irradiated group, was 1.41 times and 2.87 times for the control group which was statistically significant \((p < 0.05)\) (Harazaki et al. 2001). However, laser application, solely for caries prevention, would not appear to be sensible under the prevailing conditions (Apel et al. 2002).

Anderson et al (2002) investigated the in-vivo effects of argon laser irradiation on enamel decalcification during orthodontic treatment. Their results showed that argon laser irradiation was effective in reducing enamel decalcification during orthodontic treatment. The argon laser was also used for curing of the orthodontic bonding adhesive in an attempt to confer demineralisation resistance to enamel and to save chair time. Noel et al (2003) found, in-vitro, that brackets cured with the argon laser
for five seconds yielded bond strengths similar to a 40 seconds conventional light-cured control group. They concluded that argon lasers used for bonding orthodontic brackets would save a significant amount of chair time while possibly conferring demineralisation resistance upon the enamel.

2.5.4 Reducing plaque accumulation by the orthodontic appliance

Plaque retention may be reduced by using small size brackets, using stainless steel ligature wires instead of elastomeric rings, minimal employment of looped arch wires, careful removal of excessive resin during bonding of the brackets and regularly checking the cement bond under orthodontic band.
2.6 Ozone “alternative treatment”?

2.6.1 Background

Joseph Priestly (1733-1804), the discoverer of oxygen, noted that electrical sparks fired in a closed volume of air resulted in some compression. In 1786, Martinus Van Marum (1750-1837), subjecting oxygen to electrical discharges noted “the odour of electric matter,” and the accelerated oxidation of mercury. In 1840 Christian Friedrich Schoenbein (1799-1868) repeated these experiments and concluded that this odour was due to gas which he named ozone (O₃) from the Greek-ozein (odorous), and described several of its properties.

A native American, when fishing, recognised a correlation between a successful catch of fish and a strong odour released by the action of lightning following an electric storm. These people therefore preferentially fished subsequent to electric storms, a custom which still prevails today. Since the upper layer of lake water is enriched with dioxygen this phenomenon is explained by an elevated level of ozone generation in this biosphere. It should be noted that ozone in lake water arises as a consequence of air diffusion in its upper layer rather than as a product arising from chemical reactions therein.

Jean-Luis Soret, in 1865, was one of the first people to prove that ozone oxidises a variety of organic compounds by interacting with chemical double bonds. The first production of a water purification plant, with the use of ozone, was undertaken by Baron Hendrik Pieter Tindal (1852-1902) in Amsterdam in 1888. The first major city to use this technique was Paris in 1898, and thereafter its use spread widely. In 1903-
1906 ozone was introduced in drinking water treatment plants in Europe and was used to disinfect the water. Hundred of years after ozone was discovered, it was installed in 1940 for taste and odour control of drinking water.

Ozone was first suggested as a disinfectant for drinking water in the 19th century in view of its powerful ability to inactivate micro-organisms. Certainly, many researchers have emphasised that the utilisation of this oxidant over limited time periods can effectively disinfect water supplies with no side effects such as taste and odour which are characteristic of other disinfecting agents (Fenter and Ingols 1956; Broadwater et al. 1973). Ozone disinfects by destroying, neutralising or inhibiting the growth of pathogenic micro-organisms, and has been found to be an effective alternative biocidal agent to chlorine. However, high levels of O₃ were found to be insufficient for disinfection in view of the limited solubility of this gas in water and adverse toxicological problems arising from the employment of such high concentrations (Guinvarc’h 1959).

In the past, O₃ has been the agent of choice for the disinfection of public water supplies, rather than chlorine, in the United States of America. In the 1990’s, ozone installations have been incorporated in swimming pools, hot tubs and spas. However, one major disadvantage is that O₃ is that it is stable for only a short period of time and decomposes to form molecular oxygen which is utilised for sustenance of aquatic life and hence it disappears very quickly from the system. Its high reactivity means removal from the system quickly and no remnants of ozone are found in water distribution systems (Savoye et al. 2001).
Humans are often exposed to $O_3$ during their daily lives. Brauer and Brook (1995) measured personal exposures to $O_3$, (personal monitoring conducted with a nitrite-coated filter passive $O_3$ sampler), in two groups of 25 healthy people. It was postulated that regular outdoor activities increased this exposure. In addition, lung function parameters were measured in subjects exposed to an equivalent distribution of elevated and low $O_3$ concentrations for irritation of the eyes and/or airways respectively. It was reported that on days with moderately elevated levels of $O_3$ in the environment that this powerful gas exerted only a minor influence on pulmonary responses when expressed relative to that of other constituents of the air in selected locations (e.g., outdoor forestry or indoor domestic environments). Such constituents could be reaction products of $O_3$ which could occur with motor chainsaw exhausts, in the case of forestry workers and also from components originating from $O_3$ deposition on the surfaces of indoor locations (Hoppe et al. 1995).

Bocci (1994) emphasised that the potential toxicity of $O_3$ should not preclude utilisation for medical purposes. Certainly, carefully selected dose levels of this agent are of potential therapeutic value in the management of circulatory disorders, viral diseases and cancer. The permissible concentration of ozone, to which workers may be exposed, is 0.1 ppm over 24 hours. The short-term exposure limit was 0.3 ppm for 15 minutes. A concentration of 10 ppm ozone in air is generally accepted as “Immediately Dangerous to Life or Health” (IDLH). Ozone can be detected by its odour at a concentration of about 0.04 ppm (Lippmann 1993).
2.6.2 Ozone producing

Ozone is a natural component and can be produced by ultra-violet rays of the sun reacting with the earth’s upper atmosphere, which creates a protective ozone layer, or it can be produced by different types of ozone generators, which are used for clinical applications.

In recent times, with the use of electronics, systems have been devised for the production stream of O$_3$ from highly purified medical oxygen, at a precise concentration. Monitoring equipment has become available for continuous stream testing of the O$_3$ gas.

2.6.4 Medical uses

Ozone is a powerful oxidizing agent. It is also a very reactive and unstable gas with a short half-life before it reverts back to oxygen. Ozone can attack many biomolecules such as the cysteine, methionine and the histidine residues of proteins. There is also evidence that O$_3$ produces OH radicals in aqueous solution which are extremely reactive species that contribute to tissue injury (Hoppe et al. 1995).

The first use of O$_3$ in the medical field is attributed to Nikola Tesla in 1900 (medical ozone therapies). In the First World War, ozone bactericidal properties were used to treat infected wounds, burns and fistulas. Ozone first use as a coagulant and microflocculant was in 1960.
Ozone is widely used in medicine due to the wide spectrum of its biological effects. It can be used as an antimicrobial agent against bacteria, fungi, protozoa and virus. The effect of O₃ in the destruction of micro-organisms is well described. Moore et al. (2000) indicated that low levels of ozone (2 ppm for 4 hours) has a significant (P < 0.05) greater bactericidal effect on the micro-organisms that are responsible for causing common foodborne illnesses and infections. They concluded that the efficacy of ozone coupled with its relatively low running costs, known deodorising properties, lack of environmentally sensitive residues and its ability to kill resistant bacteria should lead to an increase in the use of ozone as a disinfectant.

Regular drinking of ozonated water may have a major benefit in preventing diseases and ozonating the water kills all the viruses and bacteria and removes all contaminants, such as fluorine (Chevrier et al. 2004; Brooke et al. 2006).

**Autohaemotherapy**

The minor technique of autohaemotherapy involves drawing 5-10 ml of venous blood from the patient and immediately mixing it with an equal volume of O₃ and injects it intramuscularly. This method was used for the treatment of asthma, cancer and herpetic infections. The major technique of auotohaematherapy is carried out 2-3 times weekly where 60-100 ml of blood is collected in citrate-phosphate-dextrose, then slightly ozonated (Bocci 1996).

There are several mechanisms by which ozonised blood was thought to improve the circulation and oxygenation of hypoxic tissues. Erythrocytes were probably ozone’s major target cells. Ozone exerts its oxidising actions in a matter of seconds, and can
generate reactive species, which were firstly quenched by antioxidant compounds in plasma, secondly by cell membrane phospholipids, glycolipids and glycoproteins and thirdly, intracellular components such as enzymes and DNA (after exhausting the reserve of intracellular reduced glutathione) (Bocci 1996). A review by Wells et al (1991) reported that ozone inactivates HIV-1 virions in a dose dependant manner. The data indicate that the antiviral effects of ozone include viral particle disruption, reverse transcriptase inactivation, and/or a perturbation of the ability of the virus to bind to its receptor on target cells. Ozone treatment offers promise as a means to inactivate human retroviruses in human body fluids and blood product preparations.

That ozone was a potential inducer of Tumour Necrosis Factor, (TNF-alpha), in human blood and Ficoll-Purified Blood Mononuclear Cells (FBMC) was studied by Paulesu et al (1991). The applied O3 was found to be critical in terms of TNF production and cell mitogenesis. Furthermore, in blood with a high O3 concentration, it was found to be more effective than FBMC. A significant release of transforming growth factor beta (TGF-beta 1), in volunteers’ blood following exposure to O3, was also found. This data supported the theory that autohaemotherapy may provide a valuable therapeutic approach to achieve immune-regulatory effects.

Ozone therapy can activate the antioxidant system, influencing the level of glycaemia and some markers of endothelial cell damage. The therapeutic efficacy of ozone was studied by Martinez-Sanchez et al (2005) to treat patients with type 2 diabetes. Their result show that Ozone treatment (local and rectal insufflation of the gas) improved glycaemic control, prevented oxidative stress, normalized levels of organic peroxides, and activated superoxide dismutase. The pharmacodynamic effect
of ozone in the treatment of patients with neuroinfectious diabetic foot can be ascribed to the possibility of it being a superoxide scavenger. In addition, there were no side effects. They concluded that medical ozone treatment could be an alternative therapy in the treatment of diabetes and its complications.

**Ozonated olive or sunflower oils**

Ozonised sunflower oil of concentration ranging from 1.18 to 9.5 mg/ml⁻¹, (Oleozón), has been shown to have antimicrobial effects against viruses, bacteria and fungi (Sechi et al. 2001). Oleozón is a substance produced by the reaction of ozone with unsaturated fatty acids present in sunflower oil. This reaction occurs almost exclusively with carbon-carbon double bonds, and produces several compounds, such as hydrogen peroxide (Santrock et al. 1992). Ozonated olive oil is used in skin lesions, burns, acne, fungal infection, leg sores, cold sores, gingivitis and in many other conditions. When ozone is bubbled through virgin olive oil, it is claimed to become safe to breathe. This therapy is claimed to be beneficial for asthma, bronchitis and pneumonia. Ozonated gel application is painless and is extremely cost effective for both physicians and patients (Sechi et al. 2001).

**O₃ “bagging”**

This method involves isolating the area to be treated with a bag. A mixture of O₃ and O₂ is pumped into the bag and absorbed through the skin (Church 1980). It was first used in Germany as a bactericidal agent, particularly on staphylococcal, streptococcal and protean infections, and had been employed in the treatment of open wounds. It has also been used on ulcers, such as varicose, diabetic and pressure sores, burns and any wounds that are infected, or slowly healing, or those that refuse
to heal. A sedative action on sensory nerve endings, and a stimulation of superficial blood flow was also noticed (Dolphin and Walker 1979).

**2.6.4 Ozone in dentistry**

Towards the end of the 19th century, O₃ was used for the disinfection of working surfaces in hospitals and dental clinics. It was also proposed for the treatment of dental unit waterlines in the last decade and for the sterilization of endodontic instruments (Brunel et al. 1965). Filippi et al (1991) reported that the microbial effect of O₃ (10 μg O₃/ml water) on dental treatment units lasted longer than conventional methods such as hydrogen peroxide/silver ion. In addition, the *Pseudomonas aeruginosa*, a potentially pathogenic micro-organism, frequently found in dental treatment units was eliminated after O₃ treatment. Under the precondition that the dental chair had been thoroughly sanitised, the system showed a good disinfecting effect. Moreover, there was no evidence of air pollution related to the use of O₃ in the treatment area and no O₃ was detected in water taken from units. Later, air lines were tested after variable concentrations of O₃ in water was introduced. The 5 minute (O₃ 2100 ppm) application achieved sterile water. The biofilm in the tube walls was removed after 15 minutes of application and flushing (Al Shorman et al. 2002). Ozone is approximately 10 times more soluble in water than O₂. Mixed into aqua bidestillata (pyrogen-free) water, the half-life of ozone is nine to ten hours (at pH 7 and 20°C), and at 0°C this value is doubled.

Ozone bubbles, (O₃ concentration approximately 10 ppm), was used as a disinfectant for dental instruments and removable dentures. The effectiveness of this cleaner against *Candida albicans* was investigated and levels of the fungus were found to
decrease to about 1/10 of their initial value after 30 minutes, and to 1/103 after a 60 minute period of exposure (Murakami et al. 1996). A study by Oizumi et al (1998) showed that direct exposure to 20 mg/h gaseous ozone could be clinically useful for denture disinfection and more effective than ozonated water (1 ppm and 3 ppm).

An in-vitro study was conducted to investigate the adsorption of amino-alcohol to the titanium implant surfaces, and the possibilities to chemically remove the adsorbed alcohols in order to recover a pristine titanium surface. The amino-alcohol solution was supplied to the sample surfaces and four different methods were subsequently used in order to remove the adsorbed alcohol molecules. It was shown that rinsing in water, saline solution and 5% H₂O₂ did not remove the amino-alcohol from the surface. However, exposure to ozone produced by using a commercial mercury lamp in ambient air resulted in complete removal of the adsorbed amino-alcohol. The results show that the amino-alcohol used forms a stable and dense film at the implant surface in-vitro (Krozer et al. 1999). They suggested that the presence of such a film most likely prevents re-integration to occur at the implant-tissue interface in-vivo.

A study by Haimovici et al (1970) showed that O₃ could be used as a local therapeutic agent when applied for disinfection of the root canal system after pulp disease. Ozonated water was tested on the buccal flora and after successive rinses, a substantial reduction in the number of colonies of bacteria was found. The antimicrobial activity lasted for 20 minutes in the open water and then decreased substantially after 30 minutes (Minguez et al. 1990).

Curozone (USA) Inc, in conjunction with Professor Edward Lynch and his team at Queens University, Belfast have developed the HealOzone technology to treat dental
caries. Ozone could therefore be applied to all the teeth every 3 to 4 months which would dramatically reduce the possibility of decay (Abu-Naba’a 2003; Holmes 2003). This treatment protocols does not require removal of diseased or carious tissue, which causes morphological changes in the tooth surface.

It has been proven that ozone has the capability of changing niche environments by pH alteration from acidic to alkaline and demineralisation being replaced by remineralisation. The process of remineralisation occurs following the change in pH. Minerals from saliva enter the enamel and/or dentine which lead to enamel remineralisation.

Eighty percent of new tooth decay starts in the fissures on the tooth surface and the application of ozone to these areas kills bacteria, viruses and fungi in fissures and thus stops the decay process and allows the tooth to seal itself permanently (Abu-Naba’a 2003).

**Effect of ozone on oral microorganisms and oral cells**

Recently, a number of studies have been undertaken on the effects of ozone on treating dental caries and the reduction of oral microorganisms (Baysan et al. 2000; Baysan 2002; Baysan and Lynch 2003; Abu-Naba’a 2003; Holmes 2003; Baysan and Lynch 2004; Abu-Salem 2004; Nagayoshi et al. 2004a; Nagayoshi et al. 2004b; Arita et al. 2005; Huth et al. 2005; Celiberti et al. 2006; Dahnhardt et al. 2006; Huth et al. 2006; Huth et al. 2007; Estrela et al. 2007; Baysan and Beighton 2007; Baysan and Lynch 2007).
Some studies have investigated the effects of ozone on oral microorganisms. Ozone gas application, for a period of 10 seconds, was found to be capable of reducing the number of Ms and *streptococcus sobrinus* on saliva-coated glass beads. Forty sterile saliva-coated glass beads were randomly divided into two groups for each microorganism. One glass bead was put into a bijou bottle with 3 ml of Todd-Hewitt broth. Ms and *streptococcus sobrinus* were incubated anaerobically overnight. Each glass bead then washed with 2 ml of Phosphate Buffer Saline (PBS) and immediately, 10 seconds of ozone gas was applied to the glass beads in the test groups. The use of PBS eliminated the effect of reductants present in culture media. There was a significant (*p* < 0.001) reduction (mean ± SE) in ozone-treated samples for Ms (log10 1.01 ± 0.27) and *streptococcus sobrinus* (log10 1.09 ± 0.36) compared with the control samples (log10 3.93 ± 0.07) and (log10 4.61 ± 0.13) respectively. This *in-vitro* treatment was found to be effective, quick and simple (Baysan *et al.* 2000).

It was speculated that in Primary Root Carious Lesions (PRCLs), ozone clearly interacted with the abundant organic material in the lesions, which may reduce the anti-microbial effect of ozone. However, there were less organic materials from some salivary proteins on the glass beads than in PRCLs. In another *in-vivo* study by Baysan and Lynch (2004), the application of ozone either for 10 or 20 seconds was found to significantly reduce most of the micro-organisms in PRCLs without any side effects recorded at recall intervals between 3 and 5.5 months (*P* < 0.001). The difference (mean ± SE) in total micro-organisms in the ozone-treated samples after either a 10 seconds treatment was log10 4.35 ± 0.49 or a 20 seconds treatment was log10 0.46 ± 0.26 compared with the control samples at log10 7.00 ± 0.24 and was log10 6.00 ± 0.21 respectively. Out of the 65 PRCLs reviewed, 33 lesions had become hard, 27 lesions reversed to severity index 1 from severity index 2, and five
lesions remained the same following ozone application for a period of either 10 or 20 seconds.

In a study by Kamali et al (2003) a strip of Ms was placed in the subject’s mouth and rolled on the tongue. A part of the surface of the sticks was treated with ozone for 20 seconds. The result of that study showed a significant difference, \( p = 0.022 \), in the number of Ms in the saliva and plaque before and after ozone treatment. The study also showed that ozone effectively penetrated into the lesions and killed the majority of microorganisms. Another study was conducted to investigate the efficiency of an ozone delivery system on lactobacilli in saliva. It was reported that ozone application for 20 seconds brought about a significant reduction in the growth of lactobacilli. The conclusion of this study was that ozone had an antimicrobial effect on lactobacilli in saliva (Hedberg et al. 2003).

A recent in-vitro study was undertaken to evaluate the antibacterial effect of the HealOzone device on Ms and to compare it with the already proven activity of two dentine-bonding systems (Polydorou et al. 2006). The teeth were left in broth cultures of \( 10^6 \) colony-forming units (CFU) ml\(^{-1} \) of Ms at 36ºC for 48 hours. The total number of microorganisms was determined from the dentine chips for the ozone group and bonding system group. All treatments significantly reduced the number of Ms present compared with the control group. The antimicrobial effect of both bonding systems and treatment with 80 seconds of ozone was significantly higher than the 40 seconds ozone treatment. They concluded that HealOzone treatment and the bonding systems show striking antimicrobial effects against Ms.

However, the antimicrobial potential of 60 seconds ozone gas was assessed in an in-
vitro study and compared with Photodynamic Therapy (PDT); (methylene blue in combination with or without a diode soft laser, and a soft laser alone) and antimicrobial solutions (2% chlorhexidine or in 0.5 and 5% hypochlorite solution) (Muller et al. 2007). Their result showed that only the 5% hypochlorite solution was able to totally eliminate the microorganisms in the biofilm. The observed reduction of viable counts by vacuum-ozone application and PDT was less than one $\log_{10}$ step. They concluded that under the conditions of the current study, gasiform ozone and PDT had a minimal effect on the viability of microorganisms organized in a cariogenic biofilm. However, they did not bubble the ozone into the biofilm unlike the application method on caries where the ozone tip is placed against the caries surface, thereby fushing ozone into the lesion. Also the biofilm contained growth media full of reductants which would have formed a redox reaction with the ozone before the ozone could have killed the bacteria. In another study by Baysan and Beighton (2007), the ability of ozone to kill micro-organisms associated with non-cavitated occlusal caries, with a DIAGNOdent score of between 11 and 30 and clinical severity score of 3, was examined in-vitro. The occlusal surfaces were treated with ozone ($n = 53$) or air ($n = 49$) for 40 seconds, and the underlying infected dentine was exposed and treated also with ozone for 40 seconds. In the occlusal surface, they found that there was no significant difference between the ozone group and the air group ($p < 0.01$). The difference (mean ± SE) in micro-organisms count in the ozone-treated group was $\log_{10} 2.81 ± 1.39$ compared with the control air group $\log_{10} 2.95 ± 1.74$. Treatment of the exposed dentine with ozone for 40 seconds resulted in a significant reduction in bacterial counts in the ozone group ($p = 0.044$). The difference (mean ± SE) in micro-organisms count in the ozone-treated group in exposed dentine was $\log_{10} 2.79 ± 0.14$ compared with the control air
group $\log_{10} 3.07 \pm 0.18$. They speculated that the interaction between ozone gas and the micro-organism/dentine matrix was so strong so as to reduce the effective diffusion gradient of ozone within the tissue. Ozone gas might have difficulty in reaching a sub-surface non-cavitated carious lesion. Thus, they suggest the ozone doses should be applied for a longer time, more than 40 seconds, to reach deep lesions. The antimicrobial effect could be achieved in such experiments with application of ozonated water. However this study was flawed as the clinical measurements for recording the severity of the lesions was not a validated index. In addition, the ozone was not used according to the recommended technique, ozone is not recommended for treating infected dentine beneath non cavitated fissures. Ozone is only recommended to treat $\leq 1$mm of infected dentine and no attempt was made in this study to quantify this. Extracted teeth dry out easily and the method used treated relatively dry caries which is not recommended as ozone is very soluble in moisture. The method of applying the ozone was also not accurate as the tube blowing out the ozone was not pressed against the infected dentine which is the recommended technique. Kavo state that DIAGNOdent readings of 11 to 23 are not associated with infected dentine so it is strange that lesions with these readings were entered when many of these will have no infected dentine. However, ozone gas was reported to significantly reduce the number of bacteria in small lesions ($1\text{mm}^2$) while in large lesions (which is contraindicated) the effects were small or non-existent (Baysan and Lynch 2004).

A study by Nagayoshi et al (2004a) demonstrated that ozonated water (4 mg/l) for 10 seconds was effective in killing gram-positive and gram-negative oral microorganisms and oral *Candida albicans* in pure culture and had strong
bactericidal activity against the bacteria in plaque biofilm ($p < 0.01$). Also Arita et al. (2005) found that aqueous ozone (2-4 mg/l for 1 min.) reduced the number of *Candida albicans* on denture plates significantly ($p < 0.01$).

A recent study by Huth et al. (2006) investigated whether gaseous ozone ($4 \times 10^6 \mu$g/ml) and aqueous ozone (1.25-20 $\mu$g/ml) exerted any cytotoxic effects on human oral epithelial (BHY) cells and gingival fibroblast (HGF-1) cells compared with established antiseptics [chlorhexidine digluconate, sodium hypochlorite (NaOCl) and hydrogen peroxide (H$_2$O$_2$), over a time of 1 minute. The study also compared the antimicrobial, metronidazole, over 24 hours. Cell counts, metabolic activity, Sp-1 binding, actin levels, and apoptosis were evaluated. Ozone gas was found to have toxic effects on both cell types. Essentially no cytotoxic signs were observed for aqueous ozone. CHX (2%, 0.2%) was highly toxic to BHY cells, and slightly (2%) and non-toxic (0.2%) to HGF-1 cells. NaOCl and H$_2$O$_2$ resulted in markedly reduced cell viability (BHY, HGF-1), whereas metronidazole displayed mild toxicity only to BHY cells. Overall, aqueous ozone revealed the highest level of biocompatibility of the tested antiseptics. The aqueous ozone revealed a high level of biocompatibility to fibroblasts, cementoblasts and epithelial cells (Nagayoshi et al. 2004b; Huth et al. 2006). Aqueous ozone (20 ppm for 15 min.) also was reported to have inhibitory effects on the NF-$\kappa$B, suggesting that it has anti-inflammatory and immune-modulatory capacities on the periodontal disease (Huth et al. 2007).

The antimicrobial efficacy of ozonated water, gaseous ozone (a constant 50 mL/min flow for 20 min), sodium hypochlorite and chlorhexidine was determined in human root canals infected by *Enterococcus faecalis* (Estrela et al. 2007). The result showed that no solution used as an irrigant over a 20 minutes contact time demonstrated an
antimicrobial effect against *Enterococcus faecalis*. They concluded that the irrigation of infected human root canals with ozonated water, 2.5% NaOCl, 2% chlorhexidine and the application of gaseous ozone for 20 minunte was not sufficient to inactivate *Enterococcus faecalis*. However, the gaseous O₃ was not bubbled in as recommended and the aqueous O₃ dose was low.

**Effect of ozone on carious lesion**

The results of the study by Baysan (2002) on the clinical management of primary root caries lesions using ozone, showed a reduction in the number of colony forming units from \( \log_{10} 6.8 \) to \( \log_{10} 3.6 \) after 10 seconds, and from \( \log_{10} 6 \) to less than \( \log_{10} 1 \) after 20 seconds. The ozone treatment was also performed within a clinical trial on 214 lesions (Baysan 2002). These were treated for 10 seconds with ozone, ozone and fissure sealant, fissure sealant only and controls. The results were significant within the lesions in the treatment group in terms of the number of lesions which became hard 64.9% of the lesions in the treatment group compared to 7.5% in the control group. A further reduction in DIAGNOdent readings was also found. The fissure sealant groups gave better results than the treatment group, where 68.5% of the sealants were retentive whilst the retention was 38.5% in the control group.

The result of study by Holmes (2003) showed that at 18 months of recall visits, 87 (100%) of ozone-treated PRCL's had arrested, whilst in the control group, 32 lesions (37%) of the PRCL's had worsened from hard to soft \( (p < 0.01) \), 54 (62%) PRCL's remained hard and only one of the control PRCL's had reversed \( (p < 0.01) \). He concluded that non-cavitated primary root caries can be arrested non-operatively with ozone and remineralising products. Ozone, being a strong oxidant, would oxidize PRCL bimolecules and hence open dentine channels in the lesions, helping
the diffusion of calcium and phosphate ions throughout the depth of the lesions. Surface hypermineralisation is less likely to occur following the application of ozone (Baysan 2002).

Ozone treatment has been investigated in another study to assess its effect on fissure caries of permanent teeth (Abu-Naba’a 2003). Results showed that the ozone treatment for 10 seconds produced significant remineralisation in fissure caries regardless of lesion type or location.

The High field Proton Nuclear Magnetic Resonance (1H NMR) system used for the detailed analysis of biomolecules, was used to determine the effect of ozone treatment on the biomolecules in plaque, saliva and root carious lesions. Amongst these molecules are formic and pyruvic acids. These were proved to contribute substantially to the decreased pH values associated with active carious lesions in root caries. By doing so, the precipitation of the minerals was inhibited (Silwood et al. 1999). In a recent study by Grootveld et al (2006), the 1H NMR system was employed to simultaneously evaluate the oxidising actions of ozone (4.48 mmol) towards a wide range of salivary biomolecules in view of its applications in dental practices. This treatment revealed that reactive oxygen species gave rise to the oxidative consumption of pyruvate, lactate, carbohydrates, methionine and urate. Further, minor O3-induced modifications included the oxidation of trimethylamine and 3-D-hydroxybutyrate, the fragmentation of salivary glycosaminoglycans to NMR-detectable saccharide fragments and the conversion of polyunsaturated fatty acids to their ozonides. Moreover, evidence for the ability of ozone to induce the release of selected low-molecular-mass salivary biomolecules from macromolecular binding-sites was also obtained.
The use of gaseous ozone for 40 seconds without the use of mineralising solutions was reported to reduce caries progression, when compared with untreated control lesions on non-cavitated fissure carious lesions in permanent molars (Huth et al. 2005).

A recent study was conducted to assess the hardness, and laser fluorescence values, for carious lesions in children treated with ozone gas, and compared with a control group (Dahnhardt et al. 2006). The results showed a significant improvement of the hardness value for the ozone treated group after 4, 6 and 8 months ($p < 0.05$), compared with the baseline values. The control lesions showed no change at any recall visits. An average reduction of 13% of the laser fluorescence values immediately after ozone treatment was also reported. In addition, 94% of children were treated and 93% had their dental anxiety reduced.

Resently, Baysan and Lynch (2007) carried out a clinical study on the effect of 10 seconds of ozone either with or without a root sealant for management of leathery root caries. They demonstrated that 38.4% of non-cavited lesions and only 5.7% of cavitated lesions became hard in the ozone group while none of the lesions became hard in the control group ($p < 0.001$). After 1, 3 and 6 months, the ECM and DIAGNOdent readings showed improvement in the ozone only group when compared to the control group ($p < 0.001$) and in the ozone and sealant group ($p < 0.05$). They concluded that ozone is capable of clinically reversing leathery primary root caries lesion and capable of helping to retain better the root sealant. This treatment regime may be considered an alternative to the traditional invasive approach for management of these lesions.
Ozonated water has lots of applications in oral medicine and oral surgery. It acts as a haemostatic and anti-inflammatory agent after tooth extraction, enhances local oxygen supply and inhibits bacterial proliferation. The application of ozonised water (concentration 11-12 μg ozone/ml water), clearly showed an acceleration of wound healing within the first 48 hours, resulting in earlier epithelial wound closure after 7 days ($p < 0.01$) (Filippi 1997). A study by Homutinnikova and Durnovo (1999) aimed to determine the efficiency of ozone therapy (ozonised 0.9% NaCL solution at 102-196 mg/1 ozone locally at 237 mg/1 ozone concentration) in the treatment of the open fractures of the mandible. They concluded that ozone therapy method was a perfect method of conservative therapy of open fractures of the mandible and in preventing the development of inflammatory complications by stabilization of membrane lipid peroxidation processes in the body as well as in the oral cavity.

Safety of the handpiece system and the design of the cup were tested during the application of ozone on teeth with primary root caries, for 10 and 20 second treatments. The detector was placed 2 mm from the edge of the cup. Levels of ozone, within the patients’ mouths, were lower than the recommended EU and FDA regulation for ozone concentration permissible in the air (Baysan 2002).

Recently, a study by Millar and Hodson (2007) was conducted to evaluate the safety of an ozone gas device. HealOzone were used in a clinical simulation, using a phantom head, while recordings of ozone levels were made in the pharyngeal and nasal regions of the patient and near the mouth of the operator. The clinical simulations included ozone application for caries management for 10 seconds and endodontic treatment for 40 seconds. All recorded ozone levels with the HealOzone
device were zero and so they concluded that the HealOzone was safe to use and fit for purpose of dental treatment.

2.6.5 Effect of ozone on bond strength and surface hardness of dental bonding materials

To date, there are few published studies on the effect of ozone on bond strength. A recent study evaluated the influence of a direct high-dose gaseous ozone application, 2100 ppm for 60 seconds, on dentine and enamel shear bond strength to composite. That study showed that a high-dose of ozone gas for 60 seconds did not affect the shear bond strength value of bovine enamel and dentine samples to composite resin. In contrast, bleaching using H₂O₂ resulted in significant decreased bond strength ($p < 0.05$) on enamel specimens (Schmidlin et al. 2005).

The effect of a 40 seconds ozone application on sealant tag length and microleakage was evaluated on intact and prepared sound molar fissures (Celiberti et al. 2006). The results indicated that ozone did not influence the enamel physical properties and neither enhanced nor was harmful to the sealing ability. Prepared fissures exhibited a statistically significantly lower microleakage compared to intact fissures. They concluded that ozone dehydrates enamel and consequently enhance its microhardness, which was reversible. It was also reported that the application of ozone, for 10 seconds to restorative materials, did not significantly affect the surface hardness of the materials tested ($p = 0.15$), (without ozone -116.4 N, with ozone -128.6 N) (Campbell et al. 2003). It was also reported that the predominant failure mode in all treatment groups was adhesive between resin and teeth. Conversely, another study evaluated the bond strength of glass-ionomer cement to dentine after
HealOzone treatment (Czarnecka et al. 2004). It concluded that the HealOzone treatment alone had a tendency to weaken the shear bond strength of glass ionomers bonded to bovine enamel, but this was eliminated by the use of ozone reductant (aqua, sodium fluoride xylitol, citric acid). The group subjected to ozone exhibited lower values of shear bond strength than that of the group subjected to ozone and reductant liquid.

Studies have been published on the use of ozone for the treatment of carious dental tissue but none have investigated the effect of ozone treatment in the proximity of orthodontic brackets on the initiation of new caries.

The Cochrane Database of Systemic Reviews reported on a systemic review of studies which had been conducted on the effect of ozone treatment to arrest or reverse the progression of dental caries (Rickard et al. 2004). The Cochrane report felt that more research was needed in order to reverse existing carious lesions with ozone but the Cochrane report did not investigate the prophylactic use of ozone to prevent the development on new carious lesions. Moreover, only three trials were included in their report and they considered these studies to be biased as the same operator applied the ozone and performed the assessment criteria. In addition, these three studies have not applied air to the control lesions. In the studies in this thesis all of the above conforms to the criteria requested by Cochrane. Since the Cochrane report other research has been published which also conforms to the above (Holmes, 2003).
Advantages of ozone in dental field

In addition to the multiple uses of ozone treatment in dental practice we have listed many advantages:

- Less need to remove healthy tooth structure.
- Tooth brush bristles are too large to clean in the fissures.
- Less drill, less restorative, no difficult treatment.
- Less need to replace the filling at regular intervals.
- Less need for fissure sealants which commonly leak over time.
- Less dental anxiety for patients when compared with regular dental treatment.
- Reduction of the use of fluoride and calcium containing pastes and sprays.
- No injections.

Disadvantages of ozone

Up until now, the studies which have been conducted on the uses of ozone in the dental treatment did not report any side effect from the use of low or high dosages of ozone. However, in other medical fields, there are some reports on the side effects of ozone on humans.

In 1980 the German Medical Society for ozone therapy reported that 644 therapists were polled regarding their 384,775 patients, comprising a total of 5,579,238 ozone treatments administrated. There were only 40 cases of side effects noted out of this number, which represents the incredibly low rate of 0.000005 %. Ozone has thus claimed to be the safest medical therapy ever devised. However, there are some side
effects which can be occurring with high ozone concentration or with mishandling of ozone generators:

- Some detox symptoms have been reported such as low-grade fever, nausea, sore lungs, faintness, flue-like symptoms or tiredness when starting to use ozone. Prolonged exposure in a room full of ozone can cause coughing and light headedness. These symptoms usually subside within a few days. When inhaled, even at very low levels, ozone can cause acute respiratory problems, aggravate asthma, significant temporary decreases in lung capacity of 15 to over 20 percent in some healthy adults, inflammation of lung tissue and impair the body’s immune system defenses, making people more susceptible to respiratory illnesses, including bronchitis and pneumonia (U.S Environmental protection agency E P E).

- Hepatitis C and HIV infections have also been reported following ozone autohaemotherapy (Daschner 1997). A more recent cross sectional study demonstrated that transmission of HIV infection due to cross contamination occurred amongst 6 out of 31 patients who were exposed to autohaemotherapy or intramuscular injection in a out-patient department of a hospital in Italy (Faustini et al. 2005). Clearly this problem was caused by the cross infection procedures and not by ozone. Acute bilateral visual loss after intra-discal and peri-ganglionic injection of ozone-oxygen gas mixture for lumbar disk herniation has been reported (Lo Giudice et al. 2004).

- Cost of ozone medical generators from £ 400 upwards for total set up.

- Requires skill to operate; operator performing ozone treatment must be well trained to prevent ozone escaping in large amounts.
Daily changes in ambient O₃ exposure are linked to premature mortality, even at very low pollution levels. Bell *et al* (2006) found strong evidence of this relationship between O₃ exposure and mortality when they used data that included only O₃ levels nearing background concentrations, which typically range from 10 to 25 ppb. Therefore, any anthropogenic contribution to ambient O₃, however slight, still presents an increased risk for premature mortality.
Significance of the study

The foremost goal of orthodontic treatment is to improve function and aesthetics. To achieve this goal, the orthodontist must do extensive preventive treatment to maintain tooth tissue. In spite of optimal oral hygiene measures, there is a chance of developing white spot lesions during orthodontic treatment with fixed appliances, which may present subsequent aesthetic problems that need to be considered.

Most studies assessing the tooth condition after debonding have used linear contact measuring devices. The evolution of digital scanning, together with superpositioning software, has improved the accuracy of assessment.

Previous trials in decalcification around orthodontic brackets mainly evaluated the use of fluoride. Different methods of orthodontic fluoride administration have been presented, but the increase in the enamel resistance to caries was probably not too significant. More fluoride may tend to precipitate calcium phosphate onto the enamel surface and block the surface pores. This limits remineralisation to the superficial part of lesions, and the optical appearance of white spot lesions is not reduced. Also fluoride tooth paste did not inhibit the formation of white spot lesions. Although sufficient clinical evidence proves that the process of white spot formation can be reversed, more information is necessary before an optimal remineralisation program for orthodontic patients can be established (Grabber and Vanarsdall 2000).

Recently, many studies have been carried out on the effects of ozone on treating dental caries. Ozone gas application, for a period of 10 seconds was capable of
reducing the number of *s.mutans* and *s.sobrinus* on saliva coated glass beads and this treatment was reported to be effective, quick and simple (Baysan *et al.* 2000). A study by Abu-Naba’a (2003) on assessment of ozone’s effect on fissure caries of permanent teeth showed that ozone treatment produced significant remineralisation in lesions regardless of lesion type or location.

This study may prove, or disprove, the capability of ozone treatment to reduce the development of white spot lesions during fixed orthodontic treatment. It might also add new knowledge for orthodontists to consider regarding ozone treatment as part of orthodontic therapy. It may also demonstrate that ozone has no adverse effect or has an adverse effect on the bond strength of the orthodontic brackets.
Hypothesis

Use of a glass ionomer cement as an orthodontic bonding lute leads to less damage to enamel at debonding compared to a conventional orthodontic composite resin bonding lute, whilst the shear bond strength of both systems would be adequate for clinical use; the enamel surface changes associated with orthodontic bonding and debonding and the adhesive thickness remaining on the tooth surface are quantifiable using a 3-D laser scanning technique; the use of clinical detection tools, the DIAGNOdent and ICDAS II *in-vitro* and *in-vivo*, are repeatable and can detect and quantify smooth surface carious lesions; the use of QLF *in-vitro* can also detect and quantify smooth surface carious lesions; ozone gas would have no adverse effect on the shear bond strength of orthodontic brackets bonded with an orthodontic composite resin adhesive resin; ozone application would be associated with a reduced incidence of white spot lesions around orthodontic brackets over 12 months and these lesions can be detected by detection tools (the DIAGNOdent, ICDAS II and digital camera); the digital camera images are repeatable.
3.1 Introduction

The detection of smooth surface caries in the early stages allows a greater chance for the arrest of caries and avoids further treatment (Shi et al. 2001b; Pinelli et al. 2002). The main limitation in caries management strategies is the lack of assessment methods which can reliably establish the extent of the subsurface decay (Pitts 1996; Featherstone 2000; Stookey 2000). Clinically sensitive methods for the detection and quantification of early carious lesions are essential for clinical practice and caries research. Better detection and diagnostic methods would help and guide the clinician in decision making.

The DIAGNOdent system is proposed as a suitable tool for caries detection and quantification of hypomineralisation lesions and it is widely employed in laboratory and clinical studies. It is based on the principle that an increase of fluorescence is related to bacterial metabolism, rather than to crystalline dissolution (Lussi et al. 2004). The auto-fluorescence phenomenon of a sound tooth is still not clear but might result from combining an inorganic matrix with light absorbing organic molecules (Hibst et al. 2001). The laser fluorescence light is less absorbed and scattered by enamel than light of shorter length, so that it penetrates the tooth more deeply (Hibst et al. 2001). For that reason, quantification of fluorescence from carious dentine is possible. The DIAGNOdent device was found to perform better in detection of carious tissue than sound tissue (Lussi et al. 2004).
The International Caries Detection and Assessment System coding criteria (ICDAS) were developed by an international expert committee in the field cariology (Pitts 2004) which was based on the Ekstrand et al (1997) criteria that have been validated using histology. The ICDAS was designed to meet the following criteria: (i) measure stages of the carious process, rather than just the ‘decayed’ stage; (ii) exclude noncarious lesions (staining, fluorosis, opacities and (iii) define the terms and descriptions used to measure the carious process (Ismail et al. 2007).

The ICDAS has been recently re-evaluated by a broad spectrum of experts at a two day workshop sponsored by the National Institute for Dental Research, the American Dental Association and the International Association for Dental Research (2005). They concluded that the ICDAS is an appropriate system and can be considered as a new clinical classification system for dental caries. The clinical criteria, now referred to as ICDAS II, have been modified based on the consent agreement at the workshop. The current version of ICDAS does not include an assessment of lesion activity which will be added in near future (Ismail et al. 2007). The severity criteria used in the ICDAS II diagnostic system are as follow:

0 = sound tooth surface.

1 = first visual change in enamel.

2 = distinct visual change in enamel.

3 = localised enamel breakdown due to caries with no visible dentine.

The Quantitative Light-induced Fluorescence (QLF) method is based on the principle that the auto-fluorescence of the tooth changes with the mineral content of the dental hard tissue. Carious lesions radiate less fluorescence than sound tissue due to less
mineral content. Therefore, this method is best suited for longitudinal detection of early lesions of the enamel on accessible smooth surfaces. Many investigations have used this technique in the monitoring of white spot lesions around orthodontic brackets (Hall et al. 1997; Al-Khateeb et al. 1998; van der Veen and de Josselin de Jong 2000; Tranaeus et al. 2001; Aljehani et al. 2006; Meller et al. 2006).
3.2 Materials and methods

3.2.1 Study 1

The Laser Scan System

The measurement technology of the laser scan 3-D (Laserscan 3-D Pro, Willytec GmbH, Gräfelfing, Germany) (Figure 3-2-1) was employed in this study to assess feeler gauge thickness and enamel morphology in chapter four studies. The software package (Accudat, International Metrology Systems Ltd., Livingstone, UK) was used in conjunction with the metrology hardware. The latter was capable of measuring and digitising free-form surfaces and analysing the acquired data based on the High Tech Basic (HTBasic) programming language.

Study 1: Repeatability of laser scanning machine

Aim

To investigate the repeatability of measurement on enamel using a computerised co-ordinate measurement machine and to assess its suitability as a tool for the study of some enamel surface changes associated with orthodontic procedures, e.g. adhesive thickness remaining and enamel loss after bracket debonding.
The measuring process takes place according to the following technical principle: the object to be measured is scanned from different angles, creating and saving a 3-D image of the surface. The views of the object surface, which overlap, are then collated by the system software. As its name implies, the software matches and compares the data captured by each view creating a final 3-D image of the object surface.

Each laser scan software component works on the basis of “clouds of points”, which are represented as surfaces or views. The software creates the visual impression of completed surfaces. Laser scan 3-D needs only a short amount of time to capture and save hundreds of thousands of surface points.
Laser scan 3-D comes with two software modules:

- **Scan 3-D**: The three-dimensional measurement and digitisation of object surfaces.
- **Match 3-D**: Segments of a 3-D-surface are combined and matched to give a larger completed surface. Surface analysis, surface subtraction and file-management are also performed using this software.

**Technical data (according to manufacturer's catalogue)**

- Accuracy after 3-D matching: 8 µm.
- Reproducibility: 2 µm.
- 3-D scan volume (single scan): 16 mm (width) x 38 mm (height) x 150 mm (length).
- “Multiscan” function allows adding several single scans to a total width of 150 mm (e.g. complete jaws).
- Scan time: 8000-14000 measured points per second, depending upon the surface topography of the scanned surface.

Four standardised feeler gauges (Anti stated control centre, Rs component Pty Ltd, Smithfield, NSW, Australia) of 0.03 (±0.0025), 0.04 (±0.0030), 0.08 (±0.0051), 0.50 (±0.0127) mm (± SD) thickness were used. Impressions were taken for the feeler gauges using a light bodied poly-vinyl-siloxane impression material (Blend a Gum, light N, DENTSPLY, DeTrey GmbH, D- 78467 Konstanz, Germany). These impressions were then poured using die stone (Silky-Rock-Whip Mix, Corporation, Louiville, Kentucky 40217, USA) (**Figure 3-2-2**).
Prior to the sequence of scans, the scanner was calibrated automatically, (built in procedure), to comply with the manufacturer’s recommendations. The stone models then were scanned ten times for each feeler gauge. The replicas were scanned at intervals of 30 μm uni-directionally in the negative x-axis (from left to right). The resulting images were manipulated using modified analytical software (Accudat, International Metrology Systems Ltd., Livingstone, UK) to construct three profiles (Jovanovski et al. 1996). This software gives three decimals places for the thickness and zero decimal places for the angle measurements. The thickness of each gauge was measured from the image of every scan (Figure 3-2-3). Consequently forty readings in total were taken, ten readings per gauge.
Figure 3-2-3: The feeler thickness measurement. Thickness is shown here at 0.503mm.
Study 2: Accuracy of ICDAS II, DIAGNOdent and QLF for detection and quantification of smooth-surface caries: an in-vitro study

Aim

To correlate the readings of the DIAGNOdent, ICDAS II and QLF with tooth carious lesion depth and to compare the accuracy of the detection methods against histological examination.

3.2.2 Study 2

3.2.2.1 Teeth preparation

Thirty-two extracted premolar teeth were used in this study with the patients’ informed consent. The teeth were selected on visual observation of the caries and white spot lesions on approximal surfaces. The teeth were cleaned with a tooth brush and rinsed under tap water, then stored in distilled deionised water, (pH 7), prior to preparation and testing. The teeth had been extracted for reasons unrelated to the objective of this study and with the informed consent of the patients.
3.2.2.2 Clinical presentation

The teeth were sprayed with water and dried with oil-free compressed air. Photographs of the lesions were taken using a digital camera (FinePix 6900 Zoom, Fujifilm, Japan). The teeth were dried for 5 seconds under a constant pressure using a three in one syringe. The most severe carious lesion on the approximal surface was chosen, by the visual detection method, using ICDAS II to stratify the carious lesions.

3.2.2.3 DIAGNOdent measurement

A light fluorescence device was used, (DIAGNOdent, Kavo, Biberach, Germany). The teeth were dried for 5 seconds using a three in one syringe. The B probe tip, for smooth surfaces, was selected. The laser device was calibrated against a porcelain reference object before the assessment. It was then recalibrated after 10 teeth had been evaluated. Readings were taken over the length of the lesion by scanning the whole surface and the peak value was read. The readings were recorded manually and were then classified into scores according to the recommended cut-off points as stated by the manufacturer.

After the DIAGNOdent measurements, the sites of the peak reading values were indicated on a photographic image for orientation and subsequent histological study of the tooth (Figure 3-2-4).
Chapter 3

3.2 Materials & methods

3.2.2.4 QLF measurement

Images of the test site were captured and recorded. The images were stored, processed, and analysed with the QLF software (Inspektor QLF 1.97, Inspektor Research System, Amsterdam, The Netherlands).

QLF looks at two phenomena. Firstly it examines the green auto-fluorescence of enamel and the dentine. Disturbances in these lesions are due to initial caries (observed as fluorescence loss) or developmental defects (Figure 3-2-5a). The pseudocolours of the green fluorescence varies from blue (5%) via pink (10%) to red (20%) then orange (25%) and to yellow (30%) (Figure 3-2-5b). The second phenomenon is the red fluorescence from the metabolic products of bacteria, plaque, calculus and advanced caries.

Figure 3-2-4: The site of the DIAGNOdent peak reading value marked on the photographic image.
The present study classified the pseudocolours of the green fluorescence, according to the size of the yellow pseudocolour, into 4 grades; grade 0: no pseudocolours, grade 1: the yellow colour < 25% of the pseudocolours size, grade 2: the yellow colour ranged from 25% to 50% of the pseudocolours size, grade 3: the yellow colour ranged from > 50% to 75% of the pseudocolours size, and grade 4: the yellow colour was > 75% of the pseudocolours lesion size. Those grades were produced by comparing the lesion depth from the microscopic images and the QLF pseudocolour images. The QLF index was matched to correlate the QLF pseudocolour phenomenon with the histological scales (This index was performed after the histological examination mentioned below).

**Figure 3-2-5:** The QLF images and the morphology of the lesions; (a) green auto-fluorescence of the dental hard tissues enamel and dentine, (b) the pseudocolours for the green fluorescence.
3.2.2.5 **Histological examination (gold standard)**

The teeth were sectioned perpendicular to the occlusal surface, through the most severe carious area. Each approximal tooth surface was cut in the middle of the scribed mark using a 0.3 mm thick water cooled slow speed diamond saw (Buehler, Lake Bluff, Illinois, USA). The tooth slices were manually polished with silicon carbide paper (200, 400, 600, 1000 and 1200 grits in sequence).

The tooth slices were examined under a light microscope (Leica stereomicroscope, Wild M3Z, Heerbrugg, Switzerland) with a magnification of forty times (40X). A five point scale, 0 - 4, was used to stratify the sites according to histological evidence of penetration by caries of the dental hard tissues. The microscopic images of each tooth slice were taken using a digital camera (Coolpix 950, Nikon Japan) with a MDC lens 0.82-0.29X (*Figure 3-2-6*).

*Figure 3-2-6:* Light microscopic appearance of a white spot lesion on a smooth surface of a premolar tooth with the lesion extending into the inner half of enamel (Magnification 40X).
The clinical and laboratory detection criteria used in the present studies 1 and 2, with the scoring system, are presented in Table 3-2-1.

<table>
<thead>
<tr>
<th>Scores</th>
<th>ICDAS II codes</th>
<th>DIAGNOdent readings</th>
<th>QLF pseudocolours</th>
<th>Histological scales (Gold standard)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No evidence of caries and no change in enamel translucency after air drying for 5 seconds.</td>
<td>&lt; 10 Sound.</td>
<td>No pseudocolours</td>
<td>No caries.</td>
</tr>
<tr>
<td>1</td>
<td>Carious opacity is visible after air drying for 5 seconds.</td>
<td>10-14 Enamel caries in the outer half.</td>
<td>Yellow colour &lt; 25% of the pseudocolours lesion</td>
<td>Caries limited to the outer half of the enamel.</td>
</tr>
<tr>
<td>2</td>
<td>There is a carious opacity or discolouration that is not consistent with the clinical appearance of sound enamel. The lesion is located in close proximity (in touch or within 1mm) of the gingival margin.</td>
<td>15-20 Caries in enamel up to DEJ.</td>
<td>Yellow colour 25%-50% of the pseudocolours lesion.</td>
<td>Caries extending into the inner half of the enamel.</td>
</tr>
<tr>
<td>3</td>
<td>Once dried for 5 seconds there is carious loss of surface integrity without visible dentine.</td>
<td>21-30 Caries in the enamel reaching DEJ with some dentinal demineralisation.</td>
<td>Yellow colour &gt; 50-75% of the pseudocolours lesion.</td>
<td>Caries limited to the outer half of the dentine.</td>
</tr>
<tr>
<td>4</td>
<td>This lesion appears as a shadow of discoloured dentine visible through the enamel surface beyond the white or brown spot lesion, which may or may not show signs of localised breakdown.</td>
<td>≥ 31 Deep dentinal caries</td>
<td>Yellow colour &gt; 75% of the pseudocolours lesion.</td>
<td>Caries involving the inner half of the dentine.</td>
</tr>
</tbody>
</table>

Table 3-2-1: Detection criteria applied, in the present study, for visual inspection (ICDAS II), DIAGNOdent, QLF and histological examination (gold standard).
Study 3: In-vivo reliability and correlation of DIAGNOdent and a visual detection method on smooth surfaces caries

3.2.3 Study 3

Background and Aims

The International caries detection and assessment system (ICDAS) coding criteria were developed to investigate the process of initiation and progression of dental caries in the fields of epidemiological and clinical research. The ICDAS system assesses the surface changes and potential histological depth of carious lesions by relying on surface characteristics. It is recommended that when applying the ICDAS system, the teeth being studied are clean and dry. It is also recommended that a ball-ended explorer be used as a visual aid for removing any remaining plaque and debris and for checking surface contour, minor cavitations or the presence of sealants.

DIAGNOdent readings may be affected by the plaque components together with external stains and calculus. Plaque and stains must be removed by an efficient system to allow readings to produce an accurate rank of the site. Toothbrushing or brushes mounted in handpieces can be used on smooth surfaces to remove any contamination. Air-abrasive systems are recommended to be used on the occlusal surfaces before DIAGNOdent readings are taken. The aims of this study were to assess ICDAS II and DIAGNOdent reliability and to examine any correlation between the two detection methods.
**Device**

DIAGNOdent is a laser based device which uses a diode laser light source and emits light at 655-nm wavelength from a fibre optic bundle. The emitted fluorescence is collected at the probe tip, passes into an ascending fibre which is then processed and presented on the display as value in the range of 0 to 99. In fact, two values are displayed, a current value for the probe position, ‘moment’, and a maximum value for the whole surface examined the "peak" (Figure 3-2-7). Increased fluorescence indicates the presence of carious tooth substance with higher numerical values. As recommended by the manufacturer, the calibration is performed using a porcelain disc or by initiating each measurement by touching sound buccal enamel from an individual’s dentition (Figure 3-2-8).

![Figure 3-2-7: DIAGNOdent device.](image1)

![Figure 3-2-8: Tip “B” used for smooth surface diagnosis. The “B” is placed on the calibration disc.](image2)
3.2.3.1 Subjects and Measurements

Five patients (2 female and 3 male) mean ± SD age of (16.6 ± 1.1) years participated in this in-vivo study. The study was ethically approved by the Research Ethics Committee at Rashid Hospital, Dubai, United Arab Emirates. All patients were in a retention period following orthodontic therapy with fixed appliances. All participants and their parents were informed about the study and written consent was obtained from them. Teeth with fluorosis, stains and restorations were excluded.

Buccal smooth surfaces of the teeth were cleaned using a rotating bristle brush and water and then dried for 5 seconds using a three-in- one syringe. The buccal surfaces were further checked for the presence of minor cavitations or sealants using a ball-ended explorer. Visual clinical caries assessment was performed, under clinical lighting, using the ICDAS II coding criteria for the free smooth surface (Ismail 2004a). Criteria for the ICDAS II are presented in Table 3-2-1.

Eighty four intact, sound and carious lesions were included. These comprised thirty-seven teeth with a clinical severity score of zero, twenty-seven teeth with a score of one, sixteen teeth with a score of two and four teeth with a score of three.

Photographs were taken of the tested surfaces of all teeth using a digital camera (D70s Nikon digital SLR camera, 2.5/2.5A Nikon Corp., Japan) with micro lens (AF Micro-Nikkor 105mmf/2.8D, Nikon Corp., Japan). The images were saved on a PC to facilitate the comparison with further DIAGNOdent measurement after a one week interval.
The DIAGNOdent device was calibrated against the porcelain reference object and then on a sound surface of each tooth prior to the examination of the suspected site. The DIAGNOdent readings were taken over the length of the lesion, by scanning the whole surfaces, and recording the highest value using the “B” tip. The DIAGNOdent readings were classified into scores according to the manufacturer’s recommended cut-off points (DIAGNOdent manual, 1999) (Table 3-2-1). The percentages of the teeth categorised by the ICDAS II baseline score and DIAGNOdent baseline readings are presented in Figures 3-2-9 a & b.
Figure 3-2-9: Pie charts representing the percentage of teeth categorised by (a) baseline DIAGNOdent scores and (b) by baseline ICDAS II scores.

The DIAGNOdent readings and their exact sites were recorded on the print out photographs along with the ICDAS II values (Figure 3-2-10). One week later, the teeth were cleaned and air dried and readings were repeated on the same surfaces.
Figure 3-2-10: Digital photograph representing the sites corresponding to where DIAGNOdent readings were taken.
3.2.4 Statistical analyses

The SPSS (Statistical Package for Social Sciences), version 13 was used to analyse the data.

**Study 1**

The descriptive statistics, including the mean, standard deviation and minimum and maximum values were calculated for the feeler gauges. A one-way ANOVA was used to determine whether significant differences existed between the repeated measures ($p < 0.01$). Minitab was applied to determine the repeatability of the laser scanning machine measurements.

**Study 2**

The correlation tests were performed using the non-parametric Spearman’s correlation coefficient. The level of agreement between the detection methods was made using the linear weighted kappa statistic. To look for bias, the level of significance was measured using the Sign test ($p < 0.01$).

**Study 3**

The reliability was tested for ICDAS II and DIAGNOdent scores using weighted kappa values. The correlation tests were performed using non-parametric Pearson correlation coefficient.
3.3 Results

3.3.1 Study 1

The results of the study are presented in Table 3-3-1.

<table>
<thead>
<tr>
<th>Scan No.</th>
<th>Feeler 1</th>
<th>Feeler 2</th>
<th>Feeler 3</th>
<th>Feeler 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.033</td>
<td>0.041</td>
<td>0.082</td>
<td>0.551</td>
</tr>
<tr>
<td>2</td>
<td>0.035</td>
<td>0.043</td>
<td>0.086</td>
<td>0.513</td>
</tr>
<tr>
<td>3</td>
<td>0.031</td>
<td>0.043</td>
<td>0.081</td>
<td>0.510</td>
</tr>
<tr>
<td>4</td>
<td>0.031</td>
<td>0.040</td>
<td>0.082</td>
<td>0.550</td>
</tr>
<tr>
<td>5</td>
<td>0.029</td>
<td>0.045</td>
<td>0.085</td>
<td>0.497</td>
</tr>
<tr>
<td>6</td>
<td>0.036</td>
<td>0.041</td>
<td>0.084</td>
<td>0.562</td>
</tr>
<tr>
<td>7</td>
<td>0.030</td>
<td>0.040</td>
<td>0.089</td>
<td>0.521</td>
</tr>
<tr>
<td>8</td>
<td>0.034</td>
<td>0.041</td>
<td>0.084</td>
<td>0.530</td>
</tr>
<tr>
<td>9</td>
<td>0.028</td>
<td>0.042</td>
<td>0.085</td>
<td>0.485</td>
</tr>
<tr>
<td>10</td>
<td>0.032</td>
<td>0.043</td>
<td>0.083</td>
<td>0.503</td>
</tr>
</tbody>
</table>

Table 3-3-1: Measurements of ten repeated scans of the feeler gauge models

(1: 0.03 mm; 2: 0.04 mm; 3: 0.08 mm; and 4: 0.50 mm).
The repeated thickness measurements of the scans of the feeler gauges gave standard deviations of 0.003 mm (3 µm), 0.002 mm (2 µm), 0.002 mm (2 µm) and 0.025 mm (25 µm) of feelers 1, 2, 3 and 4 respectively (Table 3-3-2).

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeler 1</td>
<td>10</td>
<td>0.028</td>
<td>0.036</td>
<td>0.031</td>
<td>0.003</td>
</tr>
<tr>
<td>Feeler 2</td>
<td>10</td>
<td>0.040</td>
<td>0.045</td>
<td>0.041</td>
<td>0.002</td>
</tr>
<tr>
<td>Feeler 3</td>
<td>10</td>
<td>0.081</td>
<td>0.089</td>
<td>0.084</td>
<td>0.002</td>
</tr>
<tr>
<td>Feeler 4</td>
<td>10</td>
<td>0.485</td>
<td>0.562</td>
<td>0.522</td>
<td>0.025</td>
</tr>
<tr>
<td>Valid N (listwise)</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3-3-2: Descriptive statistics of the feeler gauge thicknesses (in mm).

From the One-Way ANOVA, there was no significant difference between the measurement repeatability in each group of feeler gauges. Using Minitab for the ANOVA test result, the error mean square was 0.0000055mm.

The repeatability of the measurements can be defined as:

\[
\text{Repeatability} = 2.8 \times S, \quad \text{where } S = \sqrt{\text{error mean square}}
\]

\[
S = 0.002349.
\]

\[
\text{Repeatability} = 2.8 \times S = 0.0066 \text{ mm} = 6.6 \mu\text{m}.
\]
3.3.2 Study 2

The microscopic examination of lesion extension showed that for thirty-two lesions, 37.5% were limited to the outer half of the enamel (scale 1), 40.6% had lesions extending into the inner half of the enamel (scale 2), 15.6% had caries in the outer half of the dentine, (scale 3) and 6.3% had deep dentinal carious lesions (scale 4) (Figure 3-3-1).

Figure 3-3-1: Pie chart representing the percentage of teeth categorised by the histological scale.
The result of the correlation test is shown in Table 3-3-3. There was a significant correlation between the lesion depth and the detection methods scores, \((p < 0.001)\).

The Spearman’s rank correlation coefficient between the histological and the ICDAS II was 0.88. Between the histological and the DIAGNOdent scores the correlation was 0.77, and was 0.75 between the QLF scores and histological scales. The correlation coefficient between the DIAGNOdent and ICDAS II was 0.57, between the DIAGNOdent scores and the QLF was 0.61, and was 0.69 between the QLF scores and ICDAS II.

<table>
<thead>
<tr>
<th></th>
<th>DIAGNOdent</th>
<th>ICDAS II</th>
<th>QLF</th>
<th>Histological</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIAGNOdent</td>
<td>1.000</td>
<td>0.573</td>
<td>0.609</td>
<td>0.775</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.</td>
<td>0.001</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>N</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>ICDAS II</td>
<td>0.573</td>
<td>1.000</td>
<td>0.692</td>
<td>0.881</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.001</td>
<td>.</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>N</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>QLF</td>
<td>0.609</td>
<td>0.692</td>
<td>1.000</td>
<td>0.750</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.000</td>
<td>0.000</td>
<td>.</td>
<td>0.000</td>
</tr>
<tr>
<td>N</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Histological</td>
<td>0.775</td>
<td>0.881</td>
<td>0.750</td>
<td>1.000</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>.</td>
</tr>
<tr>
<td>N</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>32</td>
</tr>
</tbody>
</table>

**Table 3-3-3:** Correlations between diagnostic tests.

The distribution of teeth with their categories according to the detection tools and the “gold standard” scores is presented in Tables 3-3-4, 3-3-5 and 3-3-6.
### Table 3-3-4: Cross tabulation showing the distribution of teeth according to the ICDAS II scores and “gold standard” scores.

<table>
<thead>
<tr>
<th>Gold standard</th>
<th>Score 1: Outer half of enamel</th>
<th>Score 2: Inner half of enamel</th>
<th>Score 3: Outer half of dentine</th>
<th>Score 4: Inner half of dentine</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score 1</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Score 2</td>
<td>0</td>
<td>11</td>
<td>2</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Score 3</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Score 4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>12</strong></td>
<td><strong>15</strong></td>
<td><strong>4</strong></td>
<td><strong>1</strong></td>
<td><strong>32</strong></td>
</tr>
</tbody>
</table>

### Table 3-3-5: Cross tabulation showing the distribution of teeth according to the DIAGNOdent scores and the “gold standard” scores.

<table>
<thead>
<tr>
<th>Gold standard</th>
<th>Score 1: Outer half of enamel</th>
<th>Score 2: Inner half of enamel</th>
<th>Score 3: Outer half of dentine</th>
<th>Score 4: Inner half of dentine</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score 1</td>
<td>7</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Score 2</td>
<td>2</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Score 3</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Score 4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>9</strong></td>
<td><strong>15</strong></td>
<td><strong>6</strong></td>
<td><strong>2</strong></td>
<td><strong>32</strong></td>
</tr>
</tbody>
</table>

### Table 3-3-6: Cross tabulation showing the distribution of teeth according to the QLF grades and the gold standard scores.

<table>
<thead>
<tr>
<th>Gold standard</th>
<th>Score 1: Outer half of enamel</th>
<th>Score 2: Inner half of enamel</th>
<th>Score 3: Outer half of dentine</th>
<th>Score 4: Inner half of dentine</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score 1</td>
<td>9</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Score 2</td>
<td>1</td>
<td>7</td>
<td>4</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>Score 3</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Score 4</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>10</strong></td>
<td><strong>12</strong></td>
<td><strong>8</strong></td>
<td><strong>2</strong></td>
<td><strong>32</strong></td>
</tr>
</tbody>
</table>
The linear weighted kappa value was 0.73 between the gold standard and the DIAGNOdent indicating a moderate to good agreement. A figure of 0.75 was recorded between the gold standard and ICDAS II, indicating a good agreement. The value was 0.59 between the gold standard and the QLF and indicated a moderate agreement. The Sign test revealed no significant differences between lesion depth and detection methods scores ($p > 0.01$) (Table 3-3-7).

<table>
<thead>
<tr>
<th></th>
<th>Linear weighted kappa values</th>
<th>Sign test</th>
<th>Spearman's rank correlation coefficients values</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICDAS II</td>
<td>0.75</td>
<td>$p = 0.69$</td>
<td>0.88</td>
</tr>
<tr>
<td>DIAGNOdent</td>
<td>0.73</td>
<td>$p = 0.29$</td>
<td>0.77</td>
</tr>
<tr>
<td>QLF</td>
<td>0.59</td>
<td>$p = 0.39$</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Table 3-3-7: The agreement values for the detection methods used in the present study.

The DIAGNOdent system could not distinguish between lesions in the outer half of the enamel and lesions in the inner half of the enamel. Three teeth which showed score 1 histologically were scored 2 (two teeth) and 3 (one tooth) by DIAGNOdent. The DIAGNOdent system gave the same result as the histological result in detecting dentine caries.

Histologically, lesions in the outer half of the enamel did not differ from the result obtained by ICDAS II. In deeper lesions, ICDAS II appeared to be slightly less than that from the histological result.
3.3.3 Study 3

Thirty-seven tooth surfaces which were examined in this study were coded as sound by the ICDAS II scoring system while the DIAGNOdent scores detected forty-one to be so, at both visits.

The number of teeth which had not changed in the ICDAS II score between the two visits was 80 out of 84 (95.2%). Four out of 84 (4.8%) changed by one category. There was no reading with two and three category change.

The number of teeth which had not changed in the DIAGNOdent score between the two visits was 65 out of 84 (77.4%). Fourteen out of 84 (16.7%) changed by one category, 4 out of 84 (2.8%) changed by two categories, and one out of 84 (1.2%) had a three category change (Figure 3-3-2). The least agreement occurred in the DIAGNOdent score 3 ranges. The distribution of teeth with their categories according to the ICDAS II scores and the DIAGNOdent scores, in two visit intervals, is presented in Table 3-3-8.
Figure 3-3-2: Pie chart representing the percentage of teeth categorised by DIAGNOdent scores in both visits.

Table 3-3-8: Cross tabulation showing the distribution of teeth with their categories according to the ICDAS II scores and the DIAGNOdent scores, in two visit intervals.
The weighted kappa value for the ICDAS II was 0.94, indicating excellent agreement between the two visits. In contrast, it was 0.69 for the DIAGNOdent scores, indicating good agreement between the two visits.

The Pearson correlation coefficient value between the visual scores and the DIAGNOdent scores for the first visit was 0.96 with 0.19 of measurement error, indicating a strong relationship. For the second visit, the Pearson correlation coefficient value was 0.84 still indicating a very good agreement.
3.4 Discussion

3.4.1 Study 1

In the present study poly-vinyl-siloxane impression material was used as this material had least influence on the 3-D laser scanner performance (Rueda et al. 1996). In a study by DeLong et al. (2001) into factors influencing optical 3-D scanning, the digitizing performance of poly-siloxane impression materials was high. It was also reported that the elastomeric impression materials did not affect the accuracy of the definitive cast (Brosky et al. 2003). It has been suggested that the least amount of dimensional change and long term stability, in the region of 0.1%, occurs when an elastomeric impression material, such as poly-vinyl-siloxane is used (Millstein et al. 1998).

It was essential to calibrate the laser scanner prior to each experiment, because the actual accuracy of the laser scanner is an inherent property peculiar to each machine. Ten readings per feeler gauge, with a total of forty readings for the thickness would be a suitable number for this study since there was no significant difference between readings. The maximum standard deviation of repeated measurements of the same profile was \( \pm 3 \, \mu m \) for feelers 1 and 4. The repeatability of this laser scanner machine was 6.6 \( \mu m \). This value was found to conform within the stated accuracy of 8 \( \mu m \) and reproducibility of 2 \( \mu m \). It also found to be similar to the accuracy of \( \pm 6.4 \, \mu m \) reported by Seymour et al. (1996) which involved scanning the buccal surface of
teeth. Profilometry methods investigated by other researchers, (Mitchem and Gronas 1982; Lutz et al. 1984) used a diamond or emerald stylus to traverse across an epoxy surface which gives a maximum difference of \( \pm 4 \, \mu m \). Consequently, these methods would have been possibly destructive to the samples under investigation (Taylor et al. 1993). The laser probe, used in this study was of a non-contact type, so the technique was totally non-destructive to the human tooth surface. The data compared positively with those from Quick et al (1992) who used a similar non-destructive technique and obtained a standard deviation, from repeated scans of a metal stud collar, of \( \pm 14 \, \mu m \). It was also comparable with the result obtained by Marashdeh (2003) who obtained a standard deviation, from repeated width measurements, of gauge blocks of \( \pm 5 \, \mu m \) and a laser scanner machine repeatability of \( 11 \, \mu m \).
3.4.2 Study 2

The difficulty in caries detection is less associated with advanced lesions than with early lesions. The detection of early caries is not simple and needs accurate diagnostic tools for quantification. The foremost goal in detection methods is to provide flexibility for clinicians and researchers to determine the phase of the carious progress.

In \textit{in-vitro} studies, caries detection is undertaken in more ideal conditions than in \textit{in-vivo} conditions, as there is no bacterial plaque acquired pellicle, no saliva and no soft tissues present. Nevertheless, laboratory studies allow histological validation to assess more accurately the lesion extent (Ekstrand \textit{et al.} 1997; Nyvad 2004).

The results of the \textit{in-vitro} studies indicate that the readings of laser fluorescence are influenced by several variables including the degree of dehydration of the lesion, the presence of dental plaque and the presence of various types of stains (Lussi \textit{et al.} 1999; Shi \textit{et al.} 2000). Another possible variable is the storage medium which may influence the laser fluorescence readings. Saline solutions could cause minor changes in the organic content of carious lesions whilst formalin and chloramine storage medium may increase the fluorescent response (Shi \textit{et al.} 2001b). The DIAGNOdent reading was almost 1.5 times higher for teeth stored in formalin than for those stored in thymol saline (Shi \textit{et al.} 2001b). In the present study, the teeth were stored in distilled deionized (pH 7) water in order to minimise the changes in the carious lesions organic content.
The laser fluorescence instrument measures changes in the organic content of carious lesions rather than the mineral loss (Iwami et al. 2004; Iwami et al. 2005) and this could explain the slight variation in laser fluorescence readings under in-vitro and in-vivo settings.

Most of the published studies used the Spearman's rank correlation coefficient test to compare the diagnostic performance of the detection methods. However, this test does not measure the agreement between the detection methods (Bland 2000) and researchers do not appear to realise this. Systematic reviews are usually accompanied by meta-analyses for the purpose of calculating weighted averages of an effect or a performance estimate across studies. There are however several situations in which meta-analyses are not appropriate (Bader and Shugars 2004). In diagnostic studies it has been claimed that “meta-analysis should only be considered when the studies have been recruited from clinically similar populations, use comparable experimental and reference tests and are unlikely to biased. Even when these criteria are met, there may still be such heterogeneity between the results of the studies that it is inappropriate to summarise the performance of a test as a single number.” (Deeks 2001).

Two statistical analyses were applied in this study. Spearman's rank correlation coefficient is a non-parametric method for measuring the degree of association between two variables. The linear weighted kappa statistic provides a measure of agreement between two variables. Minor disagreement would be penalised less severely than major disagreements. The method applies a weight of disagreement by just one category or when the disagreement is by two categories and so on, and a weight of zero when the maximum agreement occurs.
The aim of this study was to evaluate the ICDAS II, the DIAGNOdent, and the QLF for *in-vitro* detection and quantification of carious lesions on smooth approximal surfaces. The methods were validated by histopathogy, as a gold standard, in the determination of the lesion depth. The linear weighted kappa values, (0.75 and 0.73 respectively), showed that the association between the ICDAS II and DIAGNOdent were comparable with the lesion depth indicating a good correlation with the gold standard. The QLF value (0.59) was lower than the other two methods values but it was also comparable with the lesion depth, indicating a moderate correlation with the gold standard. In the present study, the Sign test, which is based upon the direction of differences between two measures rather than quantitative data, was applied. This test is applicable in the case of two related samples when the experimenter wishes to establish that the conditions are different. In the present study, the Sign test revealed no significant differences between the three methods and the gold standard ($p > 0.01$). Most of the previous studies assessing the accuracy of the detection tools did not report the significance level.

The QLF classification in the current study was performed after sectioning the teeth and was based on the histological evaluation rather than clinical evaluation. This created a clinical index for the QLF system relying on the pseudocolour images. This would facilitate direct visual examination of the smooth surface carious lesions from the changes in the size of the yellow pseudo-colour. However, this classification needs to be valid to establish a base for the direct visual assessment of the lesion from the pseudocolour images. In the current study, 2 weeks after creating this index, the QLF images were scored according to this classification and were correlated with the histological scores in order to evaluate how it is comparable with the lesion
depth. During the creation of the QLF index, the correlation value was in perfect association with histological scores. However, after 2 weeks, the correlation value (0.59) was in moderate association with the histological scores confirming that such visual assessment is based on subjective assessment which led, as shown in this study, to the variations among the same operator in different occasions.

The values revealed by the Spearman's rank correlation coefficients were slightly higher than that revealed by the linear weighted kappa statistic. However, correlations values of 0.88, 0.75 and 0.77 respectively for the ICDAS II, the QLF and the DIAGNOdent were in good agreement with the gold standard. The ICDAS II trend was more with lesion depth measurement than the laser fluorescence device. The correlation between the ICDAS II and QLF (0.69), and the QLF and DIAGNOdent (0.61) were good and were moderate between DIAGNOdent and ICDAS II (0.57). However, all methods were found to be comparable.

Most of the published studies investigated the performance of the DIAGNOdent to detect occlusal caries and few studies have been undertaken on the detection of early carious lesions on smooth surface enamel. The earliest studies into the performance of the DIAGNOdent in the detection of occlusal caries in permanent teeth were performed on extracted teeth. The device performed better than bitewing radiography and it had a sensitivity of 0.76-0.84 and a specificity of 0.79-0.87 (Lussi et al. 1999).

The performance of the device was not, however, statistically different from that of visual inspection (Pereira et al. 2001; Reis et al. 2006). The result of the present study is in agreement with the results from previous studies which indicated a strong
correlation with lesion depth (Shi et al. 2001a, b; Aljehani et al. 2006).

The DIAGNOdent seems to be more suitable for the detection of small superficial occlusal carious lesions rather than deep dentinal lesions (Alwas-Danowska et al. 2002) and it has high diagnostic validity in the detection of the initial carious process in permanent teeth (Lussi et al. 1999). It was also reported that the device provided good performance in the detection and quantification of very early enamel carious lesions in primary teeth (Mendes et al. 2005). As a result of its good reproducibility it could also be used to monitor caries regression or progression on approximal surfaces (Lussi et al. 2006).

The Spearman's rank correlation coefficients value of 0.77 for lesion depth using the DIAGNOdent method, was similar to the value of 0.76 and 0.78 found in earlier studies in the detection of early lesions in smooth carious surfaces (Mendes et al. 2005; Aljehani et al. 2006). Other studies observed a higher correlation between the DIAGNOdent laser fluorescence values (0.78 - 0.86) and lesion depth on smooth surface carious lesions of permanent teeth (Shi et al. 2001a, b). This finding was probably due to the use of a more accurate method as they used a polarised light microscope. The conclusions may also have been influenced by the differences in the storage medium and in the calibration of the device.

With DIAGNOdent, we observed that the device did not distinguish between the carious lesions in the outer and the inner half of enamel and the performance was better in the deeper lesions. This is in agreement with previous studies which reported that the device did not achieve a good performance in differentiating
between outer and inner enamel occlusal carious lesions in permanent teeth *in-vivo* (Lussi *et al.* 2001) and with smooth surfaces of primary teeth *in-vitro* (Mendes *et al.* 2005).

In the present study, a new index was performed for the QLF method to facilitate quantification of carious lesions on smooth surfaces by observing the changes in the size of the yellow pseudocolour. When comparing the lesion depth with changes in pseudocolours of the QLF green fluorescence, we observed that there was a parallel correlation between the lesion depth and the size of the yellow pseudocolour. The values obtained in the present study for the Spearman's rank correlation coefficient test and the linear kappa test, 0.75 and 0.59, respectively, were comparable with lesion depth, indicating a good correlation with the gold standard. However, this new method needs further investigation.

The QLF method has been evaluated in several studies which have shown a strong correlation between a decrease in fluorescence and the degree of enamel demineralisation (Emami *et al.* 1996; Hall *et al.* 1997; van der Veen and de Josselin de Jong 2000; Boersma *et al.* 2005; van der Veen *et al.* 2007).

The QLF method has many scientific advantages as it offers closer correlation with changes in enamel mineral content. On the other hand, it has some disadvantages in its image reconstruction software and its hard- and software components which render it a rather expensive technique. Moreover, the QLF technique cannot be applied to any white spot lesion on the smooth tooth surfaces in orthodontic patients. If the lesion is close to the gingival margin and/or to the orthodontic bracket, the reconstruction of the light fluorescence becomes rather difficult due to insufficient
sound tissue between the lesion, bracket and gingiva (Aljehani 2006).

The result of this study showed that the ICDAS II method had a higher correlation coefficient than the fluorescence methods. This implied that the ICDAS II is a better method for evaluating the carious lesions in the enamel smooth surfaces. This finding is in agreement with the results from previous studies which reported a better performance of visual inspection methods over the laser fluorescence methods (Verdonschot et al. 1999; Cortes et al. 2003; Kordic et al. 2003). However, ICDAS still lacks validated definitions of caries activity which currently limits its use for clinical practice and reliability of carious detection on specific tooth surfaces such as smooth approximal surfaces (Ismail et al. 2007). More scientific study is required to support the present study before recommending the ICDAS for quantification of smooth surface lesions.

In general, the visual inspection methods have relatively poor diagnostic performance in dental practice. Therefore, it is preferable for this to be accompanied by diagnostic aids, such as laser fluorescence systems, particularly in the case of early carious lesions.
3.4.3 Study 3

The intra-examiner reliability is the consistency of the measurements reproduced by one examiner on different occasions. The reliability of any diagnostic method is important in both epidemiological and clinical studies as it reveals how well the method fulfils the requirement of providing consistent and standardised readings (Nyvad et al. 1999).

Care should be practiced when visual inspection alone is applied to evaluate the clinical appearance of tooth stain. Fluorosis may reflect on presentation as a structural variation rather than a surface change due to an active biofilm (Ekstrand et al. 1998). Therefore, teeth with fluorosis and stains were excluded in this study as they may have given false positive readings.

The ability of the laser used by the DIAGNOdent to penetrate into the tooth structure could be limited by external configurations (plaque, calculus, stains or fluorescent materials). Therefore, the buccal surfaces of the teeth were cleaned using a rotating brush and water to remove any barriers which may produce false positive results. In order to standardise the humidity of teeth and to prevent over-dehydration, the teeth were dried for 5 seconds in a standardised way prior to testing. It was reported that the more dehydrated the teeth (over 15 seconds), the higher the DIAGNOdent values (Mendes et al. 2004).
Patient selection was based on inclusion of teeth of all severities in the clinical situation. The enamel decalcification on smooth surfaces is more significant with orthodontic patients than in patients who have never received any kind of dental appliances due to the prolonged time of treatment. In this study, selection of orthodontic patients, after fixed orthodontic appliance therapy, allowed a check amongst the least three white spot lesions severities in smooth surfaces which may be difficult to obtain with non-orthodontic patients.

At present only a few clinical studies have been published, mostly on occlusal surfaces, which have shown that the DIAGNOdent seems to have good sensitivity, (0.75-0.96), although with quite a low specificity (0.68-0.86) in the detection of carious lesions (Lussi et al. 2001; Lussi and Francescut 2003; Anttonen et al. 2003).

In this study, clinical intra-examiner agreement was tested for ICDAS II and DIAGNOdent measurement of buccal smooth surface lesions. The weighted kappa value for the ICDAS II was 0.94, showing excellent agreement and was 0.69 for the DIAGNOdent thereby showing good agreement at both visits. Both methods demonstrated a good to excellent reliability in the detection of buccal smooth surface lesions in permanent teeth. The weighted kappa value for the ICDAS II in this study was in the same range with the values reported by Eggertsson et al (2005) in-vivo. Those values ranged from 0.63 to 0.90 for the occlusal surface and from 0.55 to 0.85 for the buccal pits/lingual grooves. It was also similar to the values which ranged from 0.73 to 0.90 for smooth surface which have been reported in the in-vitro study by Ferreira-Zandona et al (2007).
In comparing the result of this study with the results of the *in-vitro* studies, the DIAGNOdent correlation value of this study was lower than the correlation value, (0.90-0.98), of studies conducted on occlusal lesions of permanent teeth (Alwas-Danowska *et al.* 2002; Kuhnisch *et al.* 2004). A number of variables such as humidity and the presence of barriers on the tooth surface may adversely affect the reproducibility of the DIAGNOdent technique (Shi *et al.* 2000). These variables are more controlled under *in-vitro* conditions.

Shi *et al.* (2001a) conducted a study involving quantification of smooth surface caries using DIAGNOdent under clinical conditions. The reliability value was 0.75 (kappa) which is less in agreement with the present study value of 0.69 (weighted kappa). In other clinical trials on permanent smooth tooth surfaces, the intra-operator agreement was 0.94. This indicates excellent agreement and the inter-operator agreement ranged from 0.79-0.87, indicating a very good agreement (Tranaeus *et al.* 2004). The latter of those results are in less agreement with the current study result. This may be due to variations in the clinical application of the techniques, as over-dehydration of teeth which may produce false positive DIAGNOdent values.

The reliability value of the present study was in good agreement with the value of 0.62 which was the result of an *in-vivo* study testing the reliability of the DIAGNOdent in the occlusal surface of permanent teeth (Abu-Naba’a 2003).

To date there is only one published clinical study on the use of the DIAGNOdent device for detection and monitoring white spot lesions on smooth surfaces following orthodontic therapy (Aljehani *et al.* 2006). Their results have shown an excellent
intra-operator agreement (Intra-class Correlation ICC value = 0.95) and good inter-operator agreement (ICC value=0.75).

In comparison between intra and inter-operator agreement, the values varied from one study to another. This may be due to several factors such as operator effect, subject effect and improper calibration of the device.
3.5 Conclusions

- The computerised co-ordinate measurement technique can be used as a research tool for clinical applications. It can provide data relating to dimensions of adhesive thickness or enamel loss. The results are graphic, reproducible and accurate, thus making the machine appropriate for use in studies of the morphology of actual changes on the enamel surface.

- The ICDAS II, DIAGNOdent and QLF are comparable for the quantification \textit{in-vitro} of lesion depth in enamel on smooth surfaces of permanent teeth. Visual inspection (ICDAS II) had the highest correlation with histological observations of carious lesions on smooth surface enamel caries. The methods can be used to monitor the progression of suspected carious lesions which would be contributing to the decision-making process concerning appropriate preventive and operative strategies in caries management.

- The ICDAS II and the DIAGNOdent were reliable detection methods for the detection of buccal smooth surface lesions of permanent teeth \textit{in-vivo}. Both methods correlated well with each other statistically.
4.1 Introduction

Direct bonding of orthodontic brackets to etched or conditioned surfaces is achieved by the use of resin adhesives or cements. Recently, adhesive systems have been modified from acrylic and epoxies to epoxy-acrylates and from glass ionomer fluoride releasing cements, to the current resin-modified glass ionomer cements. Composite resins have been the most widely used adhesives for orthodontic bonding procedures. A major drawback of this technique is that the resin must be applied in a completely dry field.

Resin-modified glass ionomer cements can be used for bonding in the presence of moisture. Their fluoride releasing ability is significantly greater than that of fluoride-releasing composites and sufficient to both inhibit demineralisation and promote remineralisation of tooth structures adjacent to glass ionomer restorations (Donly 1994). Moreover, the fluoride level and the subsequent release of fluoride from these materials increases when they are exposed to fluoride ions from external sources (Diaz-Arnold et al. 1995). In addition, the bond strength of resin-modified glass ionomer cements was reported to be clinically successful for orthodontic bonding (Graf and Jacobi 2000; Summers et al. 2004).

The removal of attachments and all adhesive resin from tooth surfaces without iatrogenic damage is the main objective of bracket debonding. Improper debonding techniques can cause enamel damage and be more time consuming (Pus and Way 1980; Yamada et al. 2002; Ireland et al. 2005).
Quantitative measurement methods for assessing tooth surfaces during orthodontic bonding and debonding procedure have some limitations which have been reported in previous studies (Brown and Way 1978; Pus and Way 1980; Krell *et al.* 1993).

To improve the accuracy of assessment, digital scanning with a non-contacting laser probe and associated software were performed. This method allowed measurement of many points on the tooth surfaces, performed volumetric calculations of the total loss of substance and enhanced visualisation and evaluation.

In the current study we used the laser scanning machine (Laserscan 3-D Pro, Willytec GmbH, Gräfelfing, Germany) for digitising the tooth surface and the international metrology systems software (Accudat, International Metrology Systems Ltd., Livingstone, UK) to process the data. The scanner’s linear accuracy (6.6 μm) of the laser scanning machine was assessed in *study 1* with reference feeler gauges and found to conform to its stated accuracy of 8 μm and reproducibility of 2 μm. These values are in line with the evaluation of Mehl *et al.* (1997).

The purpose of this chapter was to measure the 3-D changes on tooth surfaces after removal of orthodontic brackets and after removal of residual adhesive and finishing. Two orthodontic adhesive systems were used: a resin-modified glass ionomer cement (Ortho LC, Fuji, GC Corp, Tokyo, Japan) and an adhesive resin bonded to an adhesive precoated bracket (3M Unitek, Monrovia, CA, USA).
Aim

The purpose of this study was to evaluate, 3-dimensionally, the changes on tooth surfaces after debonding orthodontic brackets and after removing residual adhesive and finishing in-vitro.

4.2 Materials and methods

4.2.1 Study 4

4.2.1.1 Teeth

The teeth were prepared in accordance with the guidelines of the International Organization for Standardization (ISO, 2003).

Sixty freshly extracted human maxillary premolars teeth were used for this study. They had been extracted for reasons unrelated to this study and were used with the patients’ informed consent. The teeth were selected based on visual observation of the soundness of the coronal portion, no caries, no cracks on the buccal surfaces and
not having been subjected to any chemical agents. The teeth were stored in distilled, deionized (pH 7), water before preparation and testing.

The roots of the teeth were removed with a slow-speed saw (Buehler, Lake Bluff, Ill) (Figure 4-2-1) to allow the teeth to be placed centrally in the moulds with the buccal surfaces uppermost. The lingual area of each tooth was embedded in self-cured acrylic resin in a circular metal mould. The moulds were placed in room-temperature water to minimize any temperature rise from the exothermic setting reaction of the resin.

Figure 4-2-1: Slow speed saw cutting the root of the premolar teeth.

The teeth were cleaned with a non-fluoride pumice and rubber prophylaxis cups applicators for 10 seconds, sprayed with water and dried with oil-free compressed air.

First impressions were taken of the teeth in light bodied poly-vinyl-siloxane material (PROVIL novo, GmbH and CoKG, Hanau, Germany). First models were produced
in dental stone (Silky-Rock-Whip Mix Corp., Louisville, Ky) and scanned in the 3-D imaging instrument (Laserscan 3-D Pro, Willytec GmbH, Gräfelfing, Germany) to establish baseline data. Stone models are preferred for scanning because it was found, through previous use of the instrument, that surfaces such as teeth, plastics, metals, and ceramics cause scattering of the laser beam and consequent loss of resolution.

The teeth were randomly divided into 2 groups of 30.

In group 1, premolar metal brackets (Victory Series, 3M Unitek, Monrovia, CA, USA), with 0.022-in slots were used. The teeth were etched with Fuji Ortho LC conditioner (10% polyacrylic acid solution) for 20 seconds and rinsed with water for 20 seconds and the enamel surface was kept moist. Light-cured Fuji Ortho adhesive was mixed and applied according to the manufacturers’ instructions.

In group 2, light-cured APC adhesive-coated metal orthodontic brackets (3M Unitek, Monrovia, CA, USA) with 0.022-in slots were used. The enamel was etched with 37% orthophosphoric acid for 30 seconds, thoroughly rinsed with water for 30 seconds, and dried with oil-free compressed air. The brackets for both groups were positioned, seated and light-cured according to the manufacturers’ instructions. The excess adhesive was removed from the margins of the brackets with a dental probe before light curing.

The brackets for both groups were positioned on the least curved part of the buccal enamel surface and under a standard load of 3 kg, supplied by a plunger-type loading device (Figure 4-2-2) to standardise the procedure as described by Bishara et al (2002).
Figure 4-2-2: Bracket bonding under a constant load of 3 kg.

The specimens were placed in distilled water at 37°C and stored for 24 hours before testing (Figure 4-2-3).

Figure 4-2-3: The specimens in water at 37°C.
Debonding of the brackets was performed on a testing machine (2000S, Lloyds Instruments, Fareham, United Kingdom) by using a loop in stainless steel wire (0.018 × 0.025) at a crosshead speed of 1 mm per minute. The wire passed beneath the bracket wing with the buccal surface perpendicular to the horizontal plane (Figure 4-2-4).

Figure 4-2-4: Bonded tooth set in acrylic block.

After debonding of the brackets, second impressions were taken of the buccal surfaces of the teeth, and these were again replicated in dental stone. The second models were also scanned on the 3-D imaging instrument. All visible residual adhesive was then removed from the surface of the teeth with a tungsten carbide finishing bur (Komet 0197 H21 R012, 8-bladed, Lemgo, Germany) in a slow-speed handpiece at 7400 rpm, under normal clinical conditions, and were cleaned with pumice using a rubber prophylaxis cup for 10 seconds. The teeth were then cleaned with water spray for 20 seconds. Third impressions were taken of the bonding surface and the third models were produced.
4.2.1.2 3-D measuring principle function

The measuring technology of the Laserscan 3-D system is based upon optical reflection. A laser line is projected onto the object to be measured. Taken from a side perspective, the beam appears as a slice of the object profile. As the object moves under that laser line the profile changes. The profile, or rather slices of the profile, are captured and saved at regular intervals. When the object has completed its movement under the beam a full model of the object’s surface will have been created from all captured slices. Technically, viewing using this method takes place through an optical lens system that is set at a specific angle. The camera chip behind this lens system registers the slices of the profile in short intervals and sends these data to the software, which saves each point (Figures 4-2-5). Due to the physics of the optical effect, the object surface left and right of the contoured line appears unfocused. Only the produced contour is completely in focus (Figure 4-2-6). The software then saves this information. Once all the points of one contour line have been captured, the object being measured is moved one step (to the left) in order to catch the next contour. For each contour line, the measuring process takes less than a fraction of a second to complete.

During this experiment, the calibration of the scanner was kept in the same configuration at an interval distance of 30 μm.
Figure 4-2-5: The laser line projection creates a plane in the 3-D space. Through the patented lens system, the photo plane of the camera is focused on the aforementioned plane, even though, as seen in the photo, this is at a steep angle to the object.

Figure 4-2-6: As seen in the photomicrograph, the laser line describes the exact contour of the object being measured.
A total of 180 models were scanned with the 3-D laser scanning instrument. The resulting scanned images—(1) before bonding, (2) after debonding of the brackets, and (3) after removing of residual adhesive—were processed and superposed by using the modified analytical software (Accudat, International Metrology Systems, Ltd., Livingstone, UK). The second and third images were superposed on the first image by defining similar selected unchanged regions. The software displayed the transformed representative points of the debonded tooth surface (Figures 4-2-7 a & b).
Figure 4-2-7: Scanned images with 4,000 representative points (heart shape) for superposing the baseline (a) with the same tooth after bracket debonding (b).
After superposing both images, three profiles of three planes of each surface mid-third, mesial-third, and distal-third were produced. Each plane was chosen to comprehensively evaluate surface changes (Figure 4-2-8). Measurements of adhesive thicknesses and enamel losses were made 3 times for each plane and the average readings were recorded. The differences in surface thickness were recorded for the tooth surfaces and compared with the baseline measurements (Figures 4-2-9 a & b).

Figure 4-2-8: Scanned images after superposing the baseline (a), and the debonded tooth surface (b) with a proposed plane of measurement.
Figure 4-2-9: (a) The difference in the tooth surface (residual adhesive of APC bracket) is shown here as 0.030 mm. (b) No differences in tooth surface morphology before bonding the bracket and after removal of Fuji Ortho LC adhesive.
Study 5: Shear bond strength and residual adhesive after orthodontic bracket debonding

Aims

- To compare the shear bond strength and determine the area of residual adhesive on teeth after the debonding of brackets bonded with two types of orthodontic adhesives \textit{in-vitro}.
- To evaluate the effect of 10 seconds of ozone application on the shear bond strength of the orthodontic brackets \textit{in-vitro}.

4.2.2 Study 5

4.2.2.1 Teeth

Selection criteria for the teeth, preparation and bonding procedure was the same as the procedures undertaken in \textit{study 4}.

Ninety freshly extracted upper premolar teeth were used for this study. These teeth had been extracted for unrelated reasons to the objective of this study and with the informed consent of the patients.
4.2.2.2 Bonding procedure

The teeth were randomly divided into three groups of 30 for bracket attachment.

**Group 1**: Premolar metal brackets (Victory Series, 3M Unitek GmbH, ESPA Platz, 82229 Seefeld, Germany) with 0.022 inch slots were used. The measurement of the area of the bracket bases was made with digital calipers with an accuracy of 0.01 mm and determined to be 10.5 mm$^2$. The teeth were etched with Fuji Ortho LC conditioner, (10% polyacrylic acid solution), for 20 seconds, rinsed with water for 20 seconds and a thin film of water was brushed one time on the enamel surface. Fuji Ortho LC adhesive was mixed and applied according to manufacturers’ instructions.

**Group 2**: Premolar adhesive coated metal orthodontic brackets (APC™ bracket, Victory Series, 3M Unitek GmbH, ESPA Platz, 82229 Seefeld, Germany) with 0.022 inch slots were used. The enamel was etched with 37% orthophosphoric acid for 30 seconds, thoroughly rinsed with water for 30 seconds and dried with oil-free compressed air. The buccal surface of each tooth was subjected to air from the HealOzone unit (Kavo, Biberach, Germany) for 10 seconds after etching using a 5mm delivery cup.

**Group 3**: The buccal surface of each tooth was subjected to ozone from the HealOzone unit for 10 seconds after etching using a 5mm delivery cup. The same brackets system as in *group 2* was used in this group.

Identification was attached to each specimen in order to provide a basis for blinded testing. The specimens for all groups were stored in distilled water at 37°C for 24
hours before testing as in study 4.

4.2.2.3 Shear bond strength test

Debonding of the brackets was performed, 24 hours after bracket bonding, on a testing instrument (2000S, Lloyds Instruments, Fareham, England), as reported in study 4. This was not pure shear as the load was applied some distance from the bonding interface. This method has been described by Fox et al (1994). The bond force were recorded in Newtons and then divided by the bracket base area, 10.5 mm², and converted to Megapascals (MPa), (1MPa = 1N/mm²).

4.2.2.4 Bond failure assessment

After bracket debonding, impressions of the teeth were taken, for the three groups, in a light bodied polyvinyl-siloxane material (PROVIL® novo, GmbH, Bödefeld-Hunau, Germany) and poured in die stone (Silky-Rock Whip Mix, Corporation, Lousiville, Kentucky 40217, USA). The resulting models were scanned using the 3-D laser scanner and the resulting images were examined to view the bond failure interface.

The adhesive remnants left on the enamel surface were scored and classified using a modified Adhesive Remnant Index (ARI). Score 0: > 75% of adhesive was left on the tooth image; score 1: > 50% - ≤ 75% of adhesive left on tooth image; score 2: > 25% - ≤ 50% of adhesive left on tooth image; score 3: > 0% - ≤ 25% of adhesive left on tooth image; score 4: > 0% - < 25% of adhesive left on tooth image and score 5: no adhesive left on the tooth image (Figure 4-2-10). The modified ARI was expanded from the original ARI scale (Artun and Bergland 1984) which considered
adhesive on the tooth surface as follow: score 1 = 75% of adhesive left on tooth; score 2 = 50% of adhesive left on tooth; score 3 = 25% of adhesive left on tooth; score 4 = less than 25% of adhesive left on tooth and score 5 = no adhesive left on the tooth. Excess resin outside the bracket base area was not considered.

Figure 4-2-10: Scanned images showing enamel surfaces with modified ARI scores of 0 through 5. (1) ARI=0, (2) ARI=1, (3) ARI=2, (4) ARI=3, (5) ARI=4, (6) ARI=5.
4.2.3 Statistical analysis

The descriptive statistics, including the mean, standard deviation, and minimum and maximum values were calculated for tested groups. The Mann-Whitney test was used to determine whether significant differences existed between the 2 groups.

Bond strength of the tested groups was compared using the Mann-Whitney U test. The residual adhesive was compared using the Chi-square test. A significance level of 0.05 was used.
Flow chart for study 4

Sixty extracted human teeth (upper premolars)

Baseline: First impression model
(Polyvinyl-siloxane material)

Bracket bonding under constant load of 3 k

Debonding + second impression + model

Finishing and cleaning + third impression model

Scanning using 3-D laser scanner
(Laser scan 3-D Pro, Willytec BmbH, Germany)

Result analysis using modified analytical software
(Accudat, International Metrology Systems, UK)
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4.2 Materials & methods

Flow chart for study 5

Ninety extracted human teeth (upper premolars)

Baseline: First impression model (Polyvinyl-siloxane material)

Bracket bonding under constant load of 3 k

Group 1 (30 teeth) Light-cured Fuji Ortho adhesive

Group 2 (30 teeth) Light-cured adhesive-coated metal orthodontic brackets (APC) Air application 10 s before etching and 10 s after bonding

Group 3 (30 teeth) APC brackets Ozone application 10 s before etching and 10 s after bonding


Scanning using 3-D laser scanner (Laser scan 3-D Pro, Willytec BmbH, Germany)

Result analysis
4.3 Results

4.3.1 Study 4

The means (± SD) of adhesive thickness for groups 1 and 2 and the standard deviations are shown in **Table 4-3-1**. The mean (± SD) thickness for Fuji Ortho LC adhesive (**group 1**) was 31.2 µm (± 26.5 µm) and the mean (± SD) thickness for adhesive resin of the APC bracket (**group 2**) was 102.7 µm (± 79.7 µm). The Mann-Whitney test showed significant differences in adhesive thickness between the 2 groups (**p** < 0.05).

<table>
<thead>
<tr>
<th>Adhesive thickness</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>30</td>
<td>0</td>
<td>100</td>
<td>31.2</td>
<td>26.5</td>
</tr>
<tr>
<td>Group 2</td>
<td>30</td>
<td>10</td>
<td>470</td>
<td>102.7</td>
<td>79.7</td>
</tr>
</tbody>
</table>

**Table 4-3-1**: Adhesive thickness (in µm) in both groups.

This study showed that the enamel loss after finishing and polishing was not evenly distributed and that some composite remained on the tooth surface. The readings were taken from the enamel loss areas only (**Figure 4-3-1**).
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4.3 Results

Figure 4-3-1: Typical result of differences in tooth surface showing areas of enamel loss and residual adhesive. The residual adhesive thickness is shown here as 0.045 mm.

The mean (± SD) enamel loss after finishing and polishing the enamel surface of the teeth bonded with Fuji Ortho LC adhesive was 22.8 µm (± 18.1 µm). The least enamel loss measured was zero and the maximum loss was 73.5 µm. The mean (± SD) enamel loss for the APC brackets was 50.5 µm (± 31.3 µm), with the least enamel loss measured at 10 µm, and the maximum loss was 120 µm (Table 4-3-2). The Mann-Whitney test showed significant differences in enamel loss between the 2 groups (p < 0.05).
In one case, with the precoated brackets, enamel was fractured during bracket removal. A total of six readings (including the deepest fracture point) were taken from the fractured area (Figure 4-3-2), and the mean fracture depth was 0.12mm = 120 µm.

<table>
<thead>
<tr>
<th>Enamel loss</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>30</td>
<td>0</td>
<td>73.5</td>
<td>22.8</td>
<td>18.1</td>
</tr>
<tr>
<td>Group 2</td>
<td>30</td>
<td>10</td>
<td>120</td>
<td>50.5</td>
<td>31.3</td>
</tr>
</tbody>
</table>

**Table 4-3-2:** Enamel loss (in µm) in both groups.
*Figure 4-3-2:* (a) Scanned image showing enamel fracture, (b) Maximum fractured depth is shown here as 0.184 mm.
4.3.2  **Study 5**

4.3.2.1  **Shear bond strength**

The shear bond strength of the orthodontic adhesives and the testing conditions are shown in Table 4-3-3. The Mann-Whitney test revealed a significant difference in shear bond strength amongst the two groups ($p < 0.001$). The mean shear bond strength (8.1 MPa) of group 1 (Fuji Ortho LC in a wet condition), was significantly lower than the mean shear bond strength (10.8 MPa) of group 2 (adhesive bonding agent in a dry condition).

<table>
<thead>
<tr>
<th>Group</th>
<th>Condition</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Wet</td>
<td>30</td>
<td>4.4</td>
<td>11.6</td>
<td>8.1</td>
<td>2.7</td>
</tr>
<tr>
<td>2</td>
<td>Dry</td>
<td>30</td>
<td>5.4</td>
<td>17.1</td>
<td>10.8</td>
<td>2.4</td>
</tr>
</tbody>
</table>

**Table 4-3-3:** Descriptive statistics for the shear bond strength test (MPa).

The shear bond strength of the orthodontic adhesives and the testing conditions are shown in Table 4-3-4. The Mann-Whitney-U test revealed no significant difference in shear bond strength between the two groups ($p = 0.337$). The mean shear bond strength (10.8 ± 2.4 MPa) of group 2 (not subjected to ozone) was not significantly different than the mean shear bond strength (11.7 ± 2.3 MPa) of group 3 (subjected to ozone).
### Table 4-3-4: Descriptive statistics of shear bond strengths (in MPa) of the two groups.

<table>
<thead>
<tr>
<th>Shear Bond Strength</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 2</td>
<td>30</td>
<td>5.41</td>
<td>17.1</td>
<td>10.8</td>
<td>2.4</td>
</tr>
<tr>
<td>Group 3</td>
<td>30</td>
<td>7.48</td>
<td>16.05</td>
<td>11.7</td>
<td>2.3</td>
</tr>
</tbody>
</table>

#### 4.3.2.2 Bonding failure interface

**Figure 4-3-3** and **Table 4-3-5** show example of the failure site for both groups and the results. Most of the adhesive resin in group 2 exhibited a cohesive failure (90%) while only 7% exhibited bracket/adhesive failure.

In one case an enamel fracture was observed. All of group 1 failed at the enamel/adhesive interface (100%).

**Figure 4-3-3**: Scanned images showing bond failure interface.
4.3.2.3 Comparison of ARI

Group 1 and 2

Table 4-3-6 shows the distribution of ARI scores for the two adhesives. The adhesive left on the enamel in group 1 had an ARI score of 5. The adhesive left on the enamel in group 2 had ARI scores ranging from 1-4. The Pearson Chi-square test showed a significant difference in the ARI score amongst the two groups ($p = 0.02$).

<table>
<thead>
<tr>
<th>Group</th>
<th>Enamel/Adhesive</th>
<th>Adhesive failure</th>
<th>Bracket/Adhesive</th>
<th>Enamel fracture</th>
<th>Cohesive failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0%</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>27</td>
<td>2</td>
<td>1</td>
<td>90%</td>
</tr>
</tbody>
</table>

Table 4-3-5: Failure interface for the two groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Enamel/Adhesive</th>
<th>Adhesive failure</th>
<th>Bracket/Adhesive</th>
<th>Enamel fracture</th>
<th>Cohesive failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0%</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>90%</td>
</tr>
</tbody>
</table>

Table 4-3-6: Distribution of the Modified Adhesive Remnant Index (ARI) scores.
Group 2 and 3

The ARI scores for the failed brackets are presented in Table 4-3-7. The Pearson Chi-square test indicated that there were no significant differences between the two groups \((p = 0.817)\).

<table>
<thead>
<tr>
<th>Modified ARI Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
</tbody>
</table>

Table 4-3-7: Distribution of the Modified Adhesive Remnant Index.
4.4 Discussion

4.4.1 Study 4

As shown in this study, coordinate laser metrology was able to measure the changes of the buccal enamel surface after orthodontic bonding and debonding procedures. The laser scan Pro system was designed specifically for dental applications and was considered appropriate to study the changes in tooth surfaces, because these are large in relation to the accuracy of the measuring system. The stated accuracy of the system was better than 8 µm and its reproducibility was stated to be 2 µm. Several studies have shown that this methodology of analysing digitised replicas was capable of providing objective and accurate assessment of morphological change in dental hard tissue (Seymour et al. 1996; Mehl et al. 1997; Yeganeh et al. 1999; Cherukara et al. 2002) and in dental soft tissue (Jovanovski and Lynch 2000; Thomason et al. 2005).

The scanning was performed using optical reflection from a laser line projected onto the object rather than a laser point (as from an optical probe). This gives a very high acquisition speed (8,000 - 14,000 points per second) thus significantly reducing the scanning time to around 45 seconds for the tooth surface. Adjustments of the scanning parameters were performed to increase the resolution of the image. To obtain finer resolutions, the point spacing of 30 µm in the x-y dimension was recommended for a single tooth.

The software used in this study was able to rotate the scanned image to a chosen plane. This can then be superposed onto other images of the same tooth, which was
particularly useful when using replicas from long term studies where a great amount of alteration of tooth position or morphology had taken place. The software allows for choosing the region of interest and excluding all non-studied areas. A measure of accuracy of the matching method was given by the standard deviation between the superposed surfaces, so it was generally desirable that the 'stable region' be as large as possible to minimise the standard deviation to the suggested range (7-25 microns).

Clinical studies could be conducted by scanning impressions of the dentition to assess the effect of orthodontic treatment on enamel surfaces with this same technique. Moreover, the tooth with the fractured enamel, shown in Figure 4-3-2, demonstrated the ability of co-ordinate metrology in measuring fracture depth. The accuracy of these measurements could make the dental application of such metrology system versatile. This method is highly repeatable for hard tissues, as seen in this study, and sufficient to permit reliable detection of changes (Jovanovski and Lynch 2000; Baysan and Lynch 2003).

Pus and Way (1980) and Thompson and Way (1981) reported that initial prophylaxis with a bristle brush for 10 to 15 seconds can abrade as much as 10 µm from the enamel, whereas only 5 µm might be lost when a rubber cup is used. They also reported that from 20 to 40 µm of total enamel is lost when removing unfilled resin. The total amount of enamel loss for filled resin was estimated to be 30 to 60 µm (Thompson and Way 1981; Bishara et al. 1997). van Waes et al (1997) found 7 µm of enamel loss with a tungsten carbide bur at low speed but less loss and damage of enamel when those burs were used carefully. Deep enamel fractures, down to a depth of 100 µm, were reported by Diedrich (1981) as well as localised enamel loss of 150 to 160 µm.
The mean enamel loss for filled resin bonding was reported by Pus and Way (1980) to range from 29.5 to 41.2 µm and by Tufekci et al (2004) to range from 35 to 54 µm. These findings are similar to ours; the enamel loss for both groups was 22.8 µm to 50.5 µm.

In the present study, there was a significant difference in enamel loss between the 2 groups. In group 1, the mean (± SD) enamel loss was 22.8 µm (± 18.1µm). In group 2, the mean (± SD) enamel loss was 50.5 µm (± 31.3 µm). This finding suggests that, because the failure site in group 1 was at the enamel/adhesive interface with all of the adhesive left on the bracket, the resin clean-up was easier than in group 2 with 90% of the adhesive left on the tooth. Therefore, more enamel was lost and more time was consumed during removal of the adhesive resin in group 2.

The Mann-Whitney test showed a significant difference of adhesive resin thickness in both groups, with less adhesive resin left on the enamel surface after debonding the Fuji cement. This difference might be explained not only by the bonding of the adhesive resin in wet conditions but also by the etching with 10% polyacrylic acid for 20 seconds; this might cause a reduction in the bond strength between the enamel and the adhesive resin. Consequently, this led to less adhesive resin on the enamel surface.

Several studies showed that the loss of enamel thickness during orthodontic procedures is the result of the clinical practice of etching and bonding. Fitzpatrick and Way (1977) found a 55.6 µm loss after chemical etching for 90 seconds, bracket placement, bracket removal and clean-up. They attributed a mean loss of 9.9 µm of enamel due to etching. Although this is a small amount, it reduced the fluoride-rich layer which declines rapidly in the first 20 µm of enamel thickness.
The enamel lost during orthodontic procedures is not significant in terms of total thickness of enamel (excluding enamel fractures or gouges from injudicious use of hand instruments or burs). The claim that removal of the outer layer of enamel (which is particularly caries resistant and fluoride rich) might be harmful does not agree with recent views on tooth-surface dynamics and with clinical experiences over many years (Graber et al. 2000). The dynamic balance between demineralisation and remineralisation, as determined by pathologic and protective factors, influences the end result.

Brackets bonded with acid etching and composite resins require more oral hygiene care to avoid demineralisation around the brackets (Gorelick et al. 1982; Ogaard 1988). In general, the research reported agrees that orthodontic bonding materials cause iatrogenic effects. Residual adhesive on the enamel after debonding can cause plaque retention, colour changes, and enamel surface loss. Loss of the enamel surface layer, which is rich in fluoride, can lead to decalcification, a commonly cited problem. In addition, studies showed that composite resin bonding is a mechanical risk to the enamel during debonding and removal of excess adhesive (Zachrisson et al. 1980; Farquhar 1986). However, the amount of tooth loss in clean-up procedures is largely determined by the tactile ability of the operator and the instruments used.

The results of the present study show that both the remnant adhesive and the enamel loss are not consistently distributed over the tooth surfaces. This indicates that previous studies probably gave an average value, whereas this study determined the maximum and the minimum on the surfaces.

The method of superposing images allowed us to choose an area of interest containing enamel loss and remnant adhesive. The average of the enamel loss was
slightly higher than that reported in previous studies. A possible explanation for this, as previously suggested, might lie in the method of measurement in the previous studies. In group 2, the analysis showed that the adhesive was incompletely removed, pointing to the clinical difficulty of comprehensive tooth clean-up. The result also showed a case of enamel fracture, and that agrees with the findings of earlier studies (Yamada et al. 2002).

Our study showed that 3-D laser scanning, with modified software, can successfully be used to determine the effect of orthodontic therapy on enamel loss. The system is convenient and could be applied in the clinical setting. The results were found to be accurate and reproducible. However, other computer based 3-D analysis methods have earlier been described as expensive, subject to super positioning errors and impractical for large clinical studies (Bayne et al. 1994).
4.4.2 Study 5

The mean ± SD bond strength values, for both groups, in study 5 were lower than that determined by Sunna and Rock (1999) (22.08 ± 3.41 MPa), Sfondrini et al (2001) (13.2 ± 0.9 MPa), and Ip and Rock (2004) (13.6 ± 2.5 MPa). The shear bond strength (10.8 ± 2.4 MPa) for APC adhesive bracket in this study was similar to the shear bond strength (11.03 ± 3.05 MPa) reported by Newman et al (1994) for metal brackets bonded with light cured composite with similar properties to the precoat resin.

It has been reported that the minimum bond strength of 6-8 MPa was adequate for most clinical orthodontic needs (Reynolds 1975). In the current study, the shear bond strength of the groups tested ranged from 8.1 to 10.8 MPa. The Fuji Ortho LC is a light-cured resin-reinforced glass ionomer and is formulated to bond orthodontic brackets in a wet environment. This eliminates the need to maintain the teeth in a completely dry condition during the bonding procedure (Bearn et al. 1995).

The results of this study indicate that the Fuji Ortho LC adhesive, when used on conditioned enamel, in a wet environment, has similar bond strength (8.1 ± 2.7 MPa) to the traditional light-cured composite adhesive. It was also found that all mode of bracket failure for the group 1 adhesive was at the enamel/adhesive interface. This suggests that the bond to the bracket is stronger than the bond to enamel. This is in agreement with previous reports (McSherry 1996; Summers et al. 2004). The weaker bonding between the Resin Modified Glass Ionomer (RMGI) and the enamel should
make it easier for clinicians to clean up the adhesive on the enamel surface after debonding. This finding may make Fuji Ortho LC more desirable for use in orthodontic therapy.

With the precoated metal brackets, Bishara et al (1997) found a greater frequency of ARI score 1, (all the adhesives remained on the enamel surface), suggesting a relatively weaker bond between the adhesive and the bracket. It was found for group 2 in our study that the cohesive failure occurred within the resin with the resin remaining on both the tooth and the bracket. Consequently, cohesive failure can be considered undesirable as the removal of remnant adhesive from the tooth surface may lead to enamel damage and may increase chair-side time.

A study by O'Brien et al (1988) suggested that the ARI score depended on many factors including the bracket base design and the adhesive type and not only the bond strengths at the interfaces. The ARI was useful in determining the percentage of bond-failure sites by ranking the amount of resin remaining on scanned tooth images after debonding. The ARI scores for this study displayed enamel fracture on debonding for the adhesive resin (group 2) on one tooth. Rix et al (2001) indicated that the increase of enamel fracture might be related to the extraction force. It was previously reported that enamel failure occurred when the bond strength exceeded 13.5 MPa (Retief et al. 1970). The results of this study agreed with this observation in that the enamel fracture occurred with teeth at higher bond strength. That might be due to the micromechanical bond nature between the composite resin and the etched enamel. The bond strength, when using the polyacrylic acid, showed clinically acceptable bond strength for use in orthodontic treatment.
On the basis of the above observation, Fuji Ortho LC adhesive may be ideal for orthodontic bonding purposes as it provided adequate bond strength and the most desirable location for bond failure at the enamel-adhesive interface. In addition this material can be used in a wet environment.

Caution should be taken by dental clinicians when undertaking novel treatments in order to avoid compromising other factors of their overall treatment. This study aimed to assess the possible acceptance of the clinical practice of using ozone gas therapy around orthodontic brackets to prevent formation of white spot lesions and other forms of decalcification. 10 seconds of ozone gas application was capable of reducing the number of colony forming units from $\log_{10} 6.73 \pm 0.27$ to $\log_{10} 3.36 \pm 0.48$ and from $\log_{10} 6.30 \pm 0.28$ to $\log_{10} 1.17 \pm 0.62$ when applied for 20 seconds, \textit{in-vivo} (Baysan 2002).

Ozone shows potential to be an alternative caries management method. A clinical study demonstrated that ozone gas and ozonated water were effective in killing oral microorganisms in pure culture or in carious lesions and had strong bactericidal activity against the bacteria in plaque biofilm (Baysan 2002; Kamali \textit{et al}. 2003; Nagayoshi \textit{et al}. 2004a; Baysan and Lynch 2004). In addition, Nagayoshi \textit{et al} (2004a) study showed that ozonated water inhibited the accumulation of experimental dental plaque \textit{in-vitro}. Therefore, the application of ozone gas around orthodontic brackets could be described as a new protocol in an attempt to reduce enamel demineralisation. There should be little fear of influencing the shear bond strength to brackets, as the results of this study demonstrated.
Previous studies reported that fluoride could be incorporated into phosphoric acid etchant before bonding the orthodontic brackets. Fluoride reacts with the enamel forming calcium fluoride and fluoroapatite and this reaction has been reported to reduce bond strength of composite resin (Aasenden et al. 1972). Also fluoride application before conditioning and bonding significantly lowers bond strength values of resin modified glass-ionomer (Cacciafesta et al. 2005).

Although an increase in fluoride concentration adjacent to an orthodontic bonding agent is desirable, the clinical relevance remains unclear as to the ideal level of fluoride in enamel required to confer protection from demineralisation (Chadwick and Gordon 1995).

Sealants are recommended on the bracket bases to enhance bond strength, to enhance marginal adaptation and eliminate interfacial permeability and to protect enamel from dissolution. However, the application of some sealants has failed to provide consistent protection against white spot lesions formation on smooth tooth surfaces due partly to the effect of oxygen inhibition of polymerisation (Ceen and Gwinnett 1981).

To date, a few studies assessing the effect of ozone on bond strength have been published. A new study showed that a high-dose of ozone gas for 60 seconds did not affect the shear bond strength value of bovine enamel and dentine samples to composite resin, while bleaching resulted in a significantly decreased bond strength ($P < 0.05$) on enamel specimens (Schmidlin et al. 2005). The mean ±SD bond strength value (30.5 ± 2.3 N) for the ozone group was similar to the control group value (30.9 ± 6.6 N). Another study found that 40 seconds ozone application on sealant tag length and microleakage did not influence the enamel physical properties
and neither enhanced nor was harmful to the sealing ability (Celiberti et al. 2006). It was also reported that a 25 second application of ozone had no detrimental effect on the bond strength of the resin composite tested on either dentine or enamel. That study also found that the predominant failure mode in all treatment groups was adhesive between resin and teeth.

Another study reported that the application of ozone for 10 seconds to restorative materials did not significantly affect the surface hardness of materials tested ($p = 0.15$). The mean ±SD bond strength without ozone (116.4 ± 50.1 N) and with ozone (128.6 ± 49.4 N) (Campbell et al. 2003). A non-published study also investigated the possibility that ozone may have an adverse effect on the bond of composite resin to tooth structure and also the surface hardness of restorative materials. They reported that ozone application for 25 seconds had no detrimental effect on the bond strength of the resin composite tested to enamel ($p = 0.29$) and dentine ($p = 0.15$). They also found a slight reduction in hardness of materials used, but that was not significant ($p > 0.05$) (Cunningham et al. 2007).

Conversely, another study by Czarnecka et al (2004) evaluated the bond strength of glass-ionomer cement to dentine after HealOzone treatment. That study concluded that the HealOzone treatment alone had a tendency to weaken the shear bond strength of glass ionomers bonded to bovine enamel, but this is eliminated by the use of ozone reductants. The group subjected to ozone exhibited lower values of shear bond strength compared with the group subjected to ozone and reductant liquid. It is also flawed as the ozone will have dried out the enamel surface which will have reduced the “bonding” ability of the glass ionomer cement to enamel. Conversely was also reported that the use of reductant liquid should be avoided because it may
negatively influence bond strength and therefore the quality of the restoration (Schmidlin et al. 2005). The differences in the bond strength results of previous studies may be due to variation of enamel type (human or bovine) and the surface layer of enamel used (surface enamel or ground enamel).

The results of this study however agree with those of other investigations in that no significant reduction of bond strength values was observed when ozone was applied before the bonding procedure. The bond strength of 11.7 MPa for the ozonated group in this study is considered sufficient to withstand most clinical orthodontic needs, and was above the minimal requirement for adequate bond strength reported, as 6 to 8 MPa, by Reynolds (1975).
4.5 Conclusions

Study 4

- Enamel loss due to orthodontic procedures can successfully be measured *in-vitro* with a 3-D laser scanner.
- This study method could be easily applied clinically in an *in-vivo* study.
- Bonding in wet conditions using the method in the current study, resulted in little or no adhesive left on the enamel surfaces after debonding of the brackets, thus greatly reducing the risk of enamel damage.
- The variability of tooth clean-up among operators or different techniques could be examined by a study with a number of clinicians.

Study 5

- Both bonding systems provide adequate bond strengths. There was a significantly greater shear bond strength between brackets and enamel after 37% orthophosphoric acid etching and composite bonding (*group 2*) compared to 10% polyacrylic acid etching and a RMGI cement (*group 1*).
- Fuji Ortho LC could be useful when the enamel surface is contaminated with water before the application of bonding materials. The ARI result for Fuji Ortho LC showed that all brackets were failed at the enamel-adhesive interface.
- The weaker chemical bonding between the RMGI and the enamel should make it easier for clinicians to clean up the adhesive on the enamel surface after debonding.
- The residual adhesive assessment and the bonding failure interface examination can be quantified by this novel laser scanning technique.
- The study suggests a benefit in using Fuji Ortho LC cement as the coating on precoated brackets. This may be considered by the manufacturers of orthodontic brackets.
- Ozone application for 10 seconds after etching did not affect the shear bond strength values of the orthodontic brackets 24 hours after bracket bonding.
- Tested groups showed clinically acceptable bond strengths.
5.1 Introduction

The practice of clinical dentistry has changed from restorative treatment towards prevention of caries progression and from the quote from G.V. Black’s (1914) "extension for prevention" to "minimally invasive". The aim of the new concept in modern dentistry is to preserve healthy, natural tooth structure and to focus on maximum conservation of demineralised, non-cavitated enamel and dentine. The development of detection methods for early caries, allows for the application of the new approaches to managing dental caries. Moreover, the development in the caries assessment protocols allows the dentist to distinguish between patients with different levels of caries risk.

Prevention of white spot lesions is important as repair of a carious lesion, once it occurs, has still not been perfected. The detection of carious lesions in the early stages, and the monitoring of their development, should provide us with a measure of the effectiveness of preventive treatments used in longitudinal studies. Moreover, quantification of the lesion area, enamel loss and depth may improve the evaluation of carious lesions. Other parameters, such as salivary assessment, are also considered important elements in the diagnosis and management of patients with carious lesions.

The presence of fixed orthodontic appliances in the oral cavity increases the clinical risk factors for enamel demineralisation. Plaque accumulation around brackets and an increase in the level of mutans streptococci and lactobacilli were detected in the oral cavity after bonding orthodontic appliances (Forsberg et al. 1991; Jenatschke et
Early enamel carious lesions are detected clinically as a white opaque spot and appear chalky in active lesions. White spot lesions are softer than the surrounding enamel. Quantification of these lesions requires accurate detection methods.

Clinicians and researchers have used several new diagnostic techniques for detection of early carious lesions. Among these, the DIAGNOdent light fluorescence method has produced a good deal of interest in the early detection of carious lesions on occlusal (Shi et al. 2000; Shi et al. 2001a, b) and smooth surfaces (Staudt et al. 2004; Mendes et al. 2005; Aljehani 2006). In contrast, conventional methods, such as visual inspection, are based on subjective evaluation which may lead to variations among different operators. However, the ICDAS provides a new criterion model for the quantification of carious lesions and gives more clarity for lay and non-dental audiences as well as continuity with traditional measures, while also reflecting the current research evidence from cariology (ICDAS Coordinating Committee 2005). A few clinical investigations have been conducted to detect and monitor carious lesions using ICDAS but, up to now, no studies have investigated the enamel changes around fixed orthodontic brackets.

Most of the previous studies, assessing enamel changes during orthodontic treatment, have involved direct visual assessment of the white spot formation and distribution. Photography has been employed in studies of aetiology of caries to assess, and to quantify, the extent of the surface changes (Hill and Geddes 1975). Photographic methods, for assessment of decalcification during orthodontic therapy, provide a
permanent record which could be re-scored and thus allow longitudinal comparisons at the beginning and at the end of treatment (Mitchell 1992).

The uses of photography to assess enamel surfaces have been reported to offer several advantages over clinical examination methods. These include randomness, objectivity, remote examination, subject and examiner comfort and the application of different approaches in the use of the same materials (Nunn et al. 1992; Cochran et al. 2004; Wong et al. 2005). It was concluded that standardised photographic methods are a valid and reliable method to diagnose and record acquired defects of enamel (Mitchell 1992; Wong et al. 2005).

Enamel demineralisation, associated with orthodontic treatment, can be eliminated or prevented by good oral hygiene or fluoride application. However, the benefits gained from these elements are variable and depend on patient compliance.

A few studies reported that white spot lesions improved following debonding of orthodontic brackets (Ogaard and Ten Bosch 1994; Al-Khateeb et al. 1998). However, in contrast, Mattousch et al (2007) reported that white spot lesions did not disappear after debonding of orthodontic brackets. The majority of lesions were considered to be stable, while 15% had got worse after 2 years of retention. In another study, naturally occurring white spot lesions regression was reported to be small and the prevalence of caries found to be high. This might require restorative intervention for aesthetic or cariology reasons (van der Veen et al. 2007). Long term monitoring of white spot lesions is limited, as most research studies were discontinued one year after bracket debonding.
Recently, ozone has been considered in dentistry as a potential antibacterial agent. It was found to have an antimicrobial effect as well as being associated with a reduced severity of caries. The effect of gaseous ozone and aqueous ozone have been investigated in several studies (Baysan et al. 2000; Baysan 2002; Baysan and Lynch 2003; Abu-Naba’a 2003; Holmes 2003; Baysan and Lynch 2004; Abu-Salem 2004; Nagayoshi et al. 2004a; Nagayoshi et al. 2004b; Arita et al. 2005; Huth et al. 2005; Celiberti et al. 2006; Dahnhardt et al. 2006; Huth et al. 2006; Huth et al. 2007; Estrela et al. 2007; Baysan and Beighton 2007; Baysan and Lynch 2007).

Ozone does not damage enamel. However, it was shown to dehydrate enamel and as a result reduce its microhardness, but this was reversible (Celiberti et al. 2006). The effect of ozone on non-cavitated fissure carious lesions in permanent teeth was reported to reduce the lesions significantly in patients at high caries risk (Huth et al. 2005).

The application of ozone gas for 10 seconds around orthodontic brackets during intervals visit should prevent or reduce the potential of demineralisation and development of white spot lesions. Consequently, the cosmetic appearance of the labial tooth surface should not be affected. The best approach, during orthodontic treatment, is to prevent the occurrence of caries by the least invasive methods. Ozone treatment is considered as being such a non-invasive method and so its practice should be considered.
Study 6: Assessment of the use of ozone to influence caries around orthodontic brackets

**Aims**

- To assess using ICDAS II, DIAGNOdent and a digital camera imaging system, *in-vivo*, for the detection of carious lesions around orthodontic brackets.
- To assess, *in-vivo*, the ability of ozone or air treatment to prevent white spot carious lesions around orthodontic brackets in permanent teeth.
- To assess the immediate effect of 10 seconds application of ozone or air on the Ms level in plaque on the Ms site strips.
- To assess the Ms level in plaque on ozone treated teeth and control air treatment teeth.
- To assess the Ms level in saliva and the salivary buffering capacity at baseline and after 12 months of starting the orthodontic treatment.
- To measure the digital image reproducibility.
5.2 Materials and methods

5.2.1 Study 6 (Part A): Assessment by ICDAS II and DIAGNOdent

5.2.1.1 Inclusion and exclusion criteria

A total of fifty-seven patients were assessed for eligibility. All patients had to meet a predetermined standard of oral health, i.e. no caries, pre-existing white spot lesions or dental restoration on the labial/buccal surface of teeth, no gingival hyperplasia, no visible plaque deposits by naked eye examination and no gingival bleeding on blunt probing around the gingival margins. Patients with impacted teeth or missing teeth were excluded. Patients who were using an antimicrobial mouthwash, were diabetic and had a history of antibiotic use in the last 2 months were excluded. Nine patients refused to participate. Eight patients were excluded based on the following reasons:

- Five patients did not meet the predetermined standard of oral health; four of them had dental restorations or caries on the labial surface of the teeth and one had gingival hyperplasia.
- One patient had an impacted upper canine tooth.
- Two patients required functional appliance therapy.
5.2.1.2 Subject and study design

Forty patients (21 female and 19 male) with a mean ± SD age of 16.3 ± 2.6 years fulfilled the inclusion criteria and were enrolled into the trial. The subjects were on the orthodontic treatment waiting list for provision of fixed appliance therapy. Patients and parents were given written information about the study prior to signing the informed consent form. Ethical approval for the study was obtained from the Research Ethics Committee at Rashid Hospital, Dubai, UAE.

A split mouth technique was implemented to examine the upper labial/buccal segment, where one side (left or right) was randomly allocated to the test group, and the other side served as a control. The randomisation was performed using random number tables. The allocation was concealed in consecutively numbered, sealed envelopes, which were opened just before assessment.

5.2.1.3 Caries risk assessment (salivary and plaque assessment)

For the baseline examination, the subjects refrained from all oral hygiene measures for 24 hours. The salivary Ms levels were assessed at baseline for all subjects using a commercially available test kit (Dentocult® SM Strip mutans, Orion Diagnostica, Espoo, Finland) as per manufacturer’s instructions. The strips were placed on patient’s tongue and were then removed and immersed in a tube with a liquid medium (Figure 5-2-1). The tubes were incubated in air, with 5% CO₂, for 48 hours. The composition of the medium was similar to that of mitis salivarius agar, with the sucrose concentration increased to 30 percent. For each strip, counts were performed
after the incubation period by the operator and scores were taken.

![Image](image_url)

**Figure 5-2-1:** The Dentocult® SM Strip *mutans* used in the study in a tube with a liquid medium.

The salivary buffer capacity was also monitored for all subjects using Dentobuff® Strips, (Orion Diagnostica, Espoo, Finland), as per manufacturer’s instructions. Before sampling, the patients were instructed to avoid eating, drinking and tooth brushing for 2 hours. During sampling, the patients were instructed to chew a paraffin pellet for 5 minutes during which any saliva produced was collected in a suitable vessel. One drop of stimulated saliva was applied to the pH pad, using a disposable pipette supplied in the kit (**Figure 5-2-2**). After 5 minutes, the colour of the pH pad was compared with the colour chart provided with the kit. Three colours are presented in the *mutants streptococci* colour chart as follows: Blue; indicates pH $\geq 6.0$ (high salivary buffering capacity), Green; pH $4.5 - 5.5$
(intermediate salivary buffering capacity), Yellow; pH ≤ 4.0 (low salivary buffering capacity).

Figure 5-2-2: Application of the saliva drop to the pH pad using a disposable pipette.

The Ms level in plaque was recorded, (at the baseline before bracket bonding and at the last follow up visit, one year after starting orthodontic and ozone treatment), for all patients using the site strip (Dentocult® SM Strip mutans, Orion Diagnostica, Espoo, Finland). The immediate effect of ozone or air application for 10 seconds on the Ms level in plaque was also recorded. Before sampling, the patients were instructed to refrain from tooth brushing for 24 hours. The maxillary lateral incisor
and the corresponding tooth in the opposite arch of each patient were examined, isolated with cotton rolls and dried. The tip of a sterile probe was carefully applied to the labial sites by gently moving the probe in a standardised way on the tooth along the entire gingival border of the bonded bracket and at baseline at approximately the same site without the bracket present. The sampled plaque was instantly spread on the roughened sides of the plastic site strips and was then immersed in a tube with a liquid medium. The tubes were incubated in air with 5% CO₂ for 48 hours. The plaque samples were recorded at baseline to observe if any differences existed between the quadrants and these were recorded at the end of treatment to compare the ozone-treated side and the control air side.

At baseline, the plaque of thirty seven teeth (lateral incisor teeth) was also sampled and each was spread on two strips (for each tooth). Half of the strips (one for each tooth) were immediately subjected to ozone for 10 seconds from the HealOzone unit (Kavo, Biberach, Germany) while the other half was treated with air for 10 seconds. The strips were then immersed in a tube with a liquid medium. The tubes were incubated in air with 5% CO₂ for 48 hours. The plaque samples were recorded to observe if there was an immediate effect of 10 seconds application of ozone on the number of Ms in plaque. The parameters for caries risk used in this study are summarised in Table 5-2-1.
5.2 Materials & methods

### 5.2.1.4 Bonding and decalcification assessment

Prior to bonding, the teeth were cleaned using a prophylaxis paste without fluoride (NUPRO®, Dentsply DeTrey GmbH, Konstanz, Germany) in a rubber prophylaxis cup applicator for 15 seconds on each tooth. The teeth were then thoroughly sprayed with water for 20 seconds and dried with oil-free compressed air for 15 seconds.

All patients had light-cured APC™ adhesive coated metal orthodontic brackets with 0.022-inch slots applied (3M Unit™, Monrovia, CA, USA). The enamel was etched with 37% orthophosphoric acid gel for 30 seconds, thoroughly rinsed with water for 30 seconds and dried with oil-free compressed air for 30 seconds. The brackets were

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<table>
<thead>
<tr>
<th>Risk indicators</th>
<th>Caries risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>Ms count of saliva and plaque</td>
<td>Score 0: &lt; 10,000 CFU/ml</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Ph values of saliva</td>
<td>≥ 6</td>
</tr>
<tr>
<td>Buffering capacity of saliva</td>
<td>Blue</td>
</tr>
</tbody>
</table>

**Table 5-2-1:** Caries risk parameters used in this study according to the chair-side strips manufacturer’s instructions.

Twelve months after starting the orthodontic treatment, the salivary assessment was repeated for all subjects.
positioned and seated and light-cured as *per* manufacturer’s instructions. Excess bonding materials were removed with a dental probe before light curing. The upper labial/buccal surface was considered as right and left halves each on the central incisor, lateral incisor, canine and one of the premolars (either first or second).

After bracket bonding, the assigned teeth were scored by one clinician (second operator) using the International Caries Detection and Assessment System (ICDAS II) as explained before in chapter three (*Table 3-2-1*). Before taking the ICDAS II scores and the DIAGNOdent readings, all teeth were cleaned using a non-fluoride prophylaxis paste for 15 seconds, thoroughly sprayed with water for 20 seconds and air dried with oil-free compressed air for 5 seconds. A dental light was used during the scoring for all assessment visits. The effect of the prophylaxis paste on the DIAGNOdent reading was examined before prophylaxis the teeth and found that it is have no effect on the DIAGNOdent readings.

The DIAGNOdent device was calibrated against the porcelain reference object and the sound surface of every tooth prior to the examination of the suspected site. Baseline readings were taken from around the orthodontic bracket (*Figure 5-2-3*).

During all assessments visits with ICDAS II and DIAGNOdent, the teeth were washed and dried under a constant pressure, distance and duration and the scores were taken under a standard dental light. The same dental chair was kept at one position during all assessment visits (in the same clinic).
Figure 5-2-3: DIAGNOdent readings taken from the mesial side of the maxillary left central using the “B” tip.

The readings were taken from one point at 1mm from bracket margins for each zone i.e. mesial, distal, incisal/occlusal and gingival (Figure 5-2-4). The readings were taken from the same points during the subsequent assessment visits at the first recall and at the 6, 9 and 12 months visits. The readings of the four zones were converted to the DIAGNOdent scores according to the manufacturer’s cut-off point as explained before in chapter three (Table 3-2-1). The mean readings and scores of the four zones was calculated and used in the statistical analysis.
Figure 5-2-4: The dot’s circle on the maxillary right central represents the tooth surface which was exposed to the ozone dose from the 6mm delivery cup, while the dots on the maxillary left central represents the reference point (at 1mm from bracket margins) on which the DIAGNOdent tip “B” was placed at the same point at each recall visits.
5.2.1.5 Ozone application

Ozone delivery system

The ozone delivery system is a portable device with an ozone generator to deliver ozone at a concentration of 2,100 ppm ± 10%. The vacuum pump pulls air through the generator at a flow rate of 615 cc/minute to supply ozone to the lesion and purges the system of ozone after ozone treatment. A disposable removable silicone cup attached to the handpiece is provided for receiving the gas and exposing a selected area of the tooth to the gas. The tightly fitting cup seals the selected area on the tooth to prevent escape of ozone. The ozone is drawn out of the sealing cup through an ozone neutraliser that converts the ozone to oxygen. A suction system then removes any possible remaining ozone whilst the cup is still adapted to the treated area (the suction system passed the gas from the delivery system through manganese (II) ions). A switch on the HealOzone system allows for the unit to function as normal but to only deliver air in order to blind the patient.

Overdoses of ozone were prevented as the ozone dose is designed to be maintained by the device by a constant voltage of the generator and the constant airflow through it. Lower doses could be possible if there is failure to regularly change a desiccant especially if extra humidity is present in the air. Desiccants were changed in this study before each recall visits (each 3 months). The device was calibrated once from the company before starting this study.

The quadrants treated with ozone or air were treated by a second dentist using a random approach and this was recorded and stored in a locked filing cabinet and the
second dentist was the only person who has access to these records. The code was only broken at the end of the study. All assessments were carried out by the other dentist. The maxillary labial/buccal teeth surface of the test side (either the right or the left side) was subjected to ozone from the HealOzone unit for 10 seconds after bonding the bracket. A 6mm delivery cup was used for all teeth. A further time of 10 seconds was allowed for the suctioning system to remove any ozone remaining inside the cup, while the cup was still adapted to the tooth surface. The other quadrant was subjected to all the same treatment except that the HealOzone unit only produced air and this side served as a control.

**Figure 5-2-5** shows the enamel surface area which is exposed to the ozone dose using a silicone cup size of 6 mm. The size of the cup was chosen after several assessments to adapt different cup sizes around brackets on central, lateral, canine and premolar teeth. The cup size 6 mm was the most suitable size.

![Image of ozone treatment](image)

**Figure 5-2-5:** Ozone treatment using the silicone cup size 6mm, sealed around the orthodontic bracket and the ozone delivered through the handpiece.
The time required for the ozone or air application and the suctioning was set on the HealOzone unit for each patient at each time of application of ozone treatment.

For data collection, each patient was seen at baseline, 3, 6, 9 and 12 months intervals. Ozone or air application, DIAGNOdent readings and enamel scoring for decalcification were repeated at 3, 6 and 9 months. At 12 months, all the assessment measures, without ozone or air application, were repeated.

Standardised dietary and oral hygiene instruction was given to all patients verbally by the orthodontist and written by means of a locally produced booklet in Arabic. All patients were instructed to brush twice daily with orthodontic brushes and a fluoridated tooth paste.

5.2.1.6 Blinding

A second dentist applied the ozone or air and the other operator who carried out all the measurements was blinded. The DIAGNOdent readings and the ICDAS II scores were registred in a separate form than the ozone or air application form. Therefore, the operator who took the DIAGNOdent readings and scored the teeth was not aware which quadrant had been treated with ozone. The patient was also blinded to the side of ozone or air application.
5.2.2 Study 6 (Part B): Assessment by digital camera

5.2.2.1 Patients

The same patients in section A were included in this part of the study. After bonding the brackets, the teeth were cleaned using a fluoride-free prophylaxis paste (NUPRO® Dentsply, DeTrey GmbH, Kanstanz, Germany) and a rubber prophylaxis cup applicator for 15 seconds. The teeth were then sprayed with water for 20 seconds and dried with oil-free compressed air for 15 seconds prior to image capture.

5.2.2.2 Image capture and analysis

To ensure the quality of images, several photographs were taken before assessment with different set ups of the camera. The set up of the camera and the method of taking the photograph was performed following reading of the camera manual supplied by the manufacturer and from training from a professional photographer. The method of photograph capture relied on previous studies which had been undertaken on assessment of enamel defects (Mitchell 1992; Benson et al. 2000; Benson et al. 2004; Wong et al. 2005; Benson et al. 2005).
The patients were seated upright on a straight backed chair and asked to look directly to the ala-tragal plane which was approximately parallel to the floor. A pair of cheek retractors was then inserted into the patient’s mouth. The coloured photographs of the teeth were taken at 1:1 magnification using a Nikon camera (D 70s Nikon digital SLR camera, 2.5/2.5A Nikon corporation, Japan) with a Nikon SU-800 wireless speedlight flash and with a micro lens (AF Micro-Nikkor 105mmf/2.8D, Nikon corporation, Japan) which had a built-in ring with two flashes, SB-R200, set to the left and right of the mouth. The flash head was fitted with a colour filter holder and with a close-up positioning device. The camera with attachments is illustrated in Figure 5-2-6.

The camera was set to manual operation with a shutter speed of 1/125 of a second and with an aperture value (f) of 22. It was recommended to keep the f at a 22 setting as this would increase the depth of field compared to smaller values. The image size was set at 1504 x 1000 pixels, image quality fine and an ISO sensitivity of 400. Each flash was tested prior to the study to ensure consistency of light output.
Chapter 5

5.2 Materials & methods

The Micro-Nikkor lens is a fixed barrel lens once the aperture of magnification is set and clicked into place. The length of the barrel does not change when the lens tilts forward. Hence, the distance from the focal plane of the camera to the teeth was standardised when the teeth were in focus. The photographic images were saved onto a personal computer and the presence of white spot lesions were then recorded, for each individual tooth, using Nikon camera software (Picture Project, Ver.1.5, Nikon Corporation, Japan).

The photographic recordings were repeated at the last follow up visit one year (± one week) after starting orthodontic treatment. At the end of the assessment a total of 747

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**Figure 5-2-6:** (a) Front view of the camera with the ring attached to two flashes with two close-up positioning adapters. (b): Geometry drawing illustrates the direction of light toward the object (from camera manual).
photographic images were recorded and the accurate images, with no reflection or shading, were selected by one examiner for scoring.

5.2.2.3 Assessment of decalcification

The outcome measurements were recorded from the coloured photographic images at baseline and at twelve months after starting orthodontic treatment. The assigned teeth were scored using the modified Enamel Decalcification Index (EDI) (Banks and Richmond 1994), which was expanded from the original EDI (Artun and Brobakken 1986). In the EDI each tooth facial surface is divided into four zones: G = gingival; M = mesial; D = distal; and O = occlusal. A score was recorded for each zone: 0 = no decalcification or White Spot Lesion (WSL), 1 = decalcification < 50% of area, 2 = decalcification > 50% of area, 3 = decalcification covering 100% of the area or decalcification with cavitations. In order to be precise each zone was scored to obtain an accurate score and the total score per tooth was measured from the average scores of the four zones, (gingival, mesial, distal and incisal/occlusal) as illustrated with the clinical example in Figure 5-2-7.
Figure 5-2-7: The circle in the photograph represents the area within ozone application in which the white spot lesions have been scored according to the modified EDI. A clinical example of the use of an EDI for the upper left central tooth is shown: gingival and distal zones shows severe decalcification > 50%; score = 2, mesial < 50% of decalcification; score = 1 and incisal score = 0 with no decalcification. The white spot lesions (traced area) around the bracket covers more than half of the tooth surface; score = 2. The score for the upper left central incisor tooth = 2.
To test the intra-examiner reproducibility of the photographic method, 50% of the patients from the 12 month assessment visit were re-photographed one week after the last images were taken. One hundred and sixty four photographic images were produced and 144 were selected for scoring. Those photographic images were numbered randomly and saved in separate files than files including patient’s identification. The new photographs were scored according to the EDI scores and were then compared with the previous scored images for the same patient. The scores for the two visits were compared statistically.

**Final subjects’ intervention**

At the end of the assessment and before the statistical analysis three patients were excluded; one patient did not attend any recall visits and two patients were excluded because of repeated bracket failure (4 brackets, canine and premolar brackets). In *part A*, a total of 37 patients (20 female and 17 male) of mean ± SD age of 16.2 ± 2.5 years completed the treatment and the assessment and were included in statistical analyses. In *part B*, a total of 36 patients (20 female and 16 male) were included in statistical analyses (one patient’s photographs were deleted by accident).
5.2.3 Statistical analyses

The SPSS version 15 was used to analyse the data. The significance level was set at 0.05 (2-tailed).

For salivary measurement (Ms levels and buffer capacity), a non-parametric Wilcoxon Signed Rank test was performed to determine subjects with low and high caries risk. The Wilcoxon Signed Rank test was also used to analyse whether significant differences of Ms levels in plaque existed between the ozone group and the control air group. The DIAGNOdent readings, DIAGNOdent scores and ICDAS II scores were analysed using Univariate Analysis of Variance (ANOVA).

The correlation tests were performed using the non-parametric Spearman’s correlation coefficient.

A logistic regression model was used to examine the effect of ozone treatment on the formation of white spot lesions around the orthodontic brackets and to compare the carious lesion development between the ozone group and the control air group. The intra-examiner reproducibility of the photographic method was measured using the weighted kappa statisti
Flow of participants through each stage of study 6
CONSORT (Consolidated Standards of Reporting Trials) Diagram 2005
(www.consort-statement.org)

Assessed for eligibility (N = 57)

Enrolment

Excluded (N = 17)
Not meeting inclusion criteria (N = 9)
Refused to participate (N = 8)
Other reason (N = 0)

Eligible patients (N = 40)

No. of Patients randomised to split mouth (Upper labial/buccal segment)

Assessment by means of:
DIAGNOdent, the International Caries Detection and Assessment System coding criteria (ICDAS II) and Photography
Application of ozone (10 seconds on each tooth) (N = 40)

Allocation

Assessment by means of:
DIAGNOdent, the International Caries Detection and Assessment System coding criteria (ICDAS II) and Photography
Application of air (10 seconds on each tooth) (N = 40)

Follow up at 3, 6 and 9 months + O3 (N = 40)

Follow up at 3, 6 and 9 months + air (N = 40)

Lost to follow up; did not attend (N = 1)
Excluded; bracket failure (N = 2)

Follow up at 12 months
Assessment by detection methods
No O3, (N = 37)

Follow up at 12 months
Assessment by detection methods
No air, (N = 37)

Analysis

Analysed (N = 37 with DIAGNOdent readings, N = 22 with DIAGNOdent scores, N = 19 with ICDAS II)
Excluded from analysis; no changes in the scores during follow up visits (N = 15 with DIAGNOdent and N = 18 with ICDAS II)
Flow chart for salivary assessment
Study 6 (A)

At baseline: before bracket bonding
(N= 40)
Ms level assessment using Dentocult® SM strips and buffer capacity assessment using Dentobuff® strips

- The strips were placed on patient’s tongue, immersed in a tube with a liquid medium and incubated for 48 hours
- Patients were instructed to chew a paraffin pellet for 5 minutes, saliva was collected and one drop of stimulated saliva was applied to the pH pad, using a disposable pipette supplied in the kit. After 5 minutes, the colour of the pH pad was compared with the colour chart provided with the kit

After one year of starting orthodontic, ozone or air treatment
(N=37)
Repeat the assessment

Data analysis
Flow chart for Ms assessment in Plaque Study 6 (B)

At baseline: before bracket bonding
(Maxillary lateral incisor tooth, N= 80 teeth)

Control air group (N= 40 teeth)                     Ozone group (N=40 teeth)

Tooth was isolated and dry, sample was taken from approximately the same site of without the bracket present along the entire gingival border bracket using sterile probe, spread on the Dentocult® SM strips, strips immersed in a tube with a liquid medium and incubated for 48 hours

Plaque sampling was repeated after one year of starting orthodontic, ozone and air treatment (N=74 teeth)
Sample was taken from gingival side of bracket

Control air group                      Ozone group
(N= 37 teeth)                      (N= 37 teeth)

Data analysis
Flow chart for teeth preparation during assessment
Study 6 (C)

At baseline: after brackets bonding
(N= 320 teeth)
Assessed teeth were cleaned using a rubber cup and prophylaxis paste without fluoride for 15 seconds, sprayed with water for 20 seconds and dried with oil-free compressed air for 5 seconds prior to ICDAS II and DIAGNOdent measurements

DIAGNOdent, ICDAS II, photographic assessment and ozone or air application

At 3, 6 and 9 months recall visits
Cleaning steps were repeated before DIAGNOdent, ICDAS II, photographic assessment and ozone or air application

At 12 months recall visits
Cleaning steps were repeated before DIAGNOdent, ICDAS II, photographic assessment with no ozone or air application
5.3 Results

5.3.1 Part A

5.3.1.1 Caries risk assessment

The result of the chair-side Dentocult® SM Strip and scoring is illustrated in Figure 5-3-1.

Figure 5-3-1: Scores of the colony density on the test strips; Score 0: < 10,000 CFU/ml, Score 1: < 100,000 CFU/ml, Score 2: 100,000-1,000,000 CFU/ml, Score 3: > 1,000,000 CFU/ml.

CFU = Colony-Forming Unit.
The majority of the patients had scores 1 and 2 both at baseline and at the end of the treatment.

The level of Ms in saliva at baseline and after 1 year of treatment is presented in Table 5-3-1. At baseline, 13.5% of patients showed a score of zero, 40.5% of patients showed score one, 37.8 % scored two and 8.1% showed score three. At 1 year, 8.1% of patients showed score zero, 35.1% of patients showed score one, 37.8% score two and 18.9% showed score three.

<table>
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<tr>
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<tr>
<td>Ms 1 yr</td>
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<tr>
<td></td>
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<td>12</td>
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<tr>
<td></td>
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<tr>
<td>Total</td>
<td>5</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 5-3-1: Cross tabulation showing number of the subjects with the changes in the salivary Ms levels at baseline and after 1 year.

Wilcoxon Signed Rank test showed positive differences of 5.00 in the mean rank of the Ms in saliva between baseline and after 1 year (Table 5-3-2). There was a significant increase in the proportion (% total CFU) of Ms at 12 months compared to the baseline counts ($p = 0.004$, $p < 0.05$).
Table 5-3-2: The mean rank of the Ms in saliva between baseline and after 1 year.

<table>
<thead>
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<th></th>
<th>N</th>
<th>Mean Rank</th>
<th>Sum of Ranks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Ranks</td>
<td>0 a</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Positive Ranks</td>
<td>9 b</td>
<td>5.00</td>
<td>45.00</td>
</tr>
<tr>
<td>Ties</td>
<td>28 c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. MS1yr < Ms baseline
b. MS1yr > Ms baseline
c. MS1yr = Ms baseline

The results of the salivary pH at baseline and after 1 year are presented in Table 5-3-3. At baseline, 48.7% of patients showed a pH value ≥ 6, 40.5% of patients showed a pH value of 4.5-5.5 and 10.8% had a value of ≤ 4. At 1 year, 70.3% of patients showed a pH value of saliva ≥ 6, 29.7% of patients showed a pH value of 4.5-5.5 and no value was observed ≤ 4.

Table 5-3-3: Cross tabulation showing pH values of saliva at baseline and after 1 year.
Chapter 5

5.3 Results

The Wilcoxon Signed Rank test showed positive and negative differences of 8.5 in the mean rank of salivary pH values between baseline and 1 year after starting the treatment (Table 5-3-4). The salivary pH values at baseline differed significantly from the salivary pH values at 1 year ($p = 0.004$, $p < 0.05$).

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean Rank</th>
<th>Sum of Ranks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 0 Negative Ranks</td>
<td>2$^a$</td>
<td>8.50</td>
<td>17.00</td>
</tr>
<tr>
<td>Positive Ranks</td>
<td>14$^b$</td>
<td>8.50</td>
<td>119.00</td>
</tr>
<tr>
<td>Ties</td>
<td>21$^c$</td>
<td>8.50</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. $1 < 0$

b. $1 > 0$

c. $1 = 0$

Table 5-3-4: The mean rank of the salivary pH between baseline and at one year.
5.3.1.2 Plaque sample assessment

Example of the chair-side Dentocult® SM Site Strips is illustrated in Figure 5-3-2.

![Figure 5-3-2: Mutans streptococci colonisation scores in plaque on the Dentocult® SM Site Strips.](image)

The Wilcoxon Signed Rank test showed negative differences of 10.50 in the mean rank of the Ms in plaque after immediate ozone application for 10 seconds (Table 5-3-5). The proportion (% total CFU) of Ms in plaque was reduced significantly compared to the group subjected to air ($p < 0.001$).
Ms in ozone group – Ms in control group

<table>
<thead>
<tr>
<th>Ms in ozone group – Ms in control group</th>
<th>N</th>
<th>Mean Rank</th>
<th>Sum of Ranks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Ranks</td>
<td>20</td>
<td>10.50</td>
<td>210.00</td>
</tr>
<tr>
<td>Positive Ranks</td>
<td>0</td>
<td>.00</td>
<td>.00</td>
</tr>
<tr>
<td>Ties</td>
<td>17</td>
<td>.00</td>
<td>.00</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. Ms in ozone group < Ms in control group
b. Ms in ozone group > Ms in control group
c. Ms in ozone group = Ms in control group

Table 5-3-5: The mean rank of the Ms in plaque between ozone and control groups.

The level of Ms in plaque for the control air and ozone groups is presented in Table 5-3-6. In the control air group, 29.7% of plaque samples showed a score of zero, 40.5% of plaque samples showed score one, 24.3% scored two and 5.4% showed score three. While, in the ozone group, 62.2% of plaque samples showed score zero, 35.1% showed score one, 2.7% of patients showed score two and no score three was recorded.
The result of the mean ranks of the Ms in plaque for ozone and control air groups between baseline and 12 months recall visits are presented in Table 5-3-7. In the ozone group, the Wilcoxon Signed Rank test showed negative differences of 5.0 and positive differences of 5.56 in the mean rank of the Ms level in plaque. There was a significant increase in Ms level in plaque within the ozone group between baseline and at 12 months ($p = 0.013$). In the control air group, the Wilcoxon Signed Rank test showed positive and negative values of 7.0. There was a significant increase in the Ms level in the plaque between baseline and at 12 months ($p = 0.002$). However, at 12 months, the Ms level in plaque of the control air group was not significantly different than that of the ozone treated group ($p = 0.527, p > 0.05$).

### Table 5-3-6: Cross tabulation showing number of the teeth with the changes in the Ms levels in plaque after 10 seconds ozone application.

<table>
<thead>
<tr>
<th>Ms Scores</th>
<th>Ozone group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Control group</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>13</td>
</tr>
<tr>
<td>Ms in ozone group (baseline-1yr.)</td>
<td>N</td>
<td>Mean Rank</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>--------</td>
<td>-----------</td>
</tr>
<tr>
<td>Negative Ranks</td>
<td>1 a</td>
<td>5.00</td>
</tr>
<tr>
<td>Positive Ranks</td>
<td>9 b</td>
<td>5.56</td>
</tr>
<tr>
<td>Ties</td>
<td>27 c</td>
<td>5.00</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>50.00</td>
</tr>
<tr>
<td>Ms in control group (baseline – 1yr.)</td>
<td>N</td>
<td>Mean Rank</td>
</tr>
<tr>
<td>Negative Ranks</td>
<td>1 d</td>
<td>7.00</td>
</tr>
<tr>
<td>Positive Ranks</td>
<td>12 e</td>
<td>7.00</td>
</tr>
<tr>
<td>Ties</td>
<td>24 f</td>
<td>7.00</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>84.00</td>
</tr>
</tbody>
</table>

a. Ms in ozone group at 1yr. < Ms in ozone group at baseline.

b. Ms in ozone group at 1yr. > Ms in ozone group at baseline.

c. Ms in ozone group at 1yr. = Ms in ozone group at baseline.

d. Ms in control group at 1yr. < Ms in control group at baseline.

e. Ms in control group at 1yr. > Ms in control group at baseline.

f. Ms in control group at 1yr. = Ms in control group at baseline.

Table 5-3-7: The mean rank of the Ms in plaque between ozone and control groups (between baseline and 12 month).

The level of Ms in plaque at baseline and after 1 year of treatment for the ozone group is presented in Table 5-3-8. At baseline, 29.7% of patients showed a score of zero, 40.5% of patients showed score one, 24.3% scored two and 5.4% showed score three. At 1 year, 21.6% of patients showed score zero, 37.8% of patients showed score one, 29.7% score two and 10.8% showed score three.
The level of Ms in plaque at baseline and after 1 year of treatment, for the control air group, is presented in Table 5-3-9. At baseline, 27.1% of patients showed a score of zero, 37.8% of patients showed score one, 29.7% scored two and 5.4% showed score three. At 1 year, 16.2% of patients showed score zero, 40.5% of patients showed score one, 27.1% score two and 16.2% showed score three.

Table 5-3-8: Cross tabulation showing number of the subjects with the changes in the plaque Ms levels at baseline and after 1 year in the ozone group.
Table 5-3-9: Cross tabulation showing number of the subjects with the changes in the plaque Ms levels at baseline and after 1 year in the control group.

<table>
<thead>
<tr>
<th>Ms Scores in control group</th>
<th>Ms 1 yr</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Ms baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>15</td>
</tr>
</tbody>
</table>
5.3.1.3 DIAGNOdent and ICDAS II assessment

During statistical analysis, fifteen subjects (40.5%) were removed from the analysis with DIAGNOdent scores, and eighteen subjects (48.6%) with ICDAS II. That was because their scores were always zero.

The overall changes in the scores from the follow up visits were compared with the baseline. The mean ± SE DIAGNOdent readings, DIAGNOdent scores and ICDAS II scores were analysed using Univariate Analysis of Variance (ANOVA).

Patients and ozone treatment had a fixed effect, while visits had a random effect. For the analysis of DIAGNOdent readings changes, there was a significant effect of time and treatment upon the mean DIAGNOdent readings between the ozone treated group and the control air treatment group ($F = 123.1$, $p < 0.001$). The estimated marginal mean ± SE for the control air group was $8.53 ± 0.60$ and was $7.27 ± 0.60$ for the ozone group (Table 5-3-10).

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Std. Error</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
</tr>
<tr>
<td>Control</td>
<td>8.561</td>
<td>0.60</td>
<td>8.397</td>
</tr>
<tr>
<td>Ozone</td>
<td>7.269</td>
<td>0.60</td>
<td>7.150</td>
</tr>
</tbody>
</table>

Table 5-3-10: Estimated marginal means for control and ozone groups.
In the ozone treated group there was a significant increase in DIAGNOdent readings over the visits ($F = 15.450$, $p = 0.004$). The estimated marginal mean value at baseline was 6.101 and was 8.568 at the last recall visit (Table 5-3-11 & Figure 5-3-3). In the control air group, there was also a significant effect of time and treatment upon the mean DIAGNOdent readings ($p < 0.001$). The estimated marginal mean value at baseline was 6.588 and was 10.520 at last recall visit (Table 5-3-12 & Figure 5-3-3).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Visits</th>
<th>Mean</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
</tr>
<tr>
<td>Control group</td>
<td>Baseline</td>
<td>6.588</td>
<td>6.322</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7.345</td>
<td>7.079</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>8.574</td>
<td>8.303</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>10.520</td>
<td>10.254</td>
</tr>
<tr>
<td>Ozone group</td>
<td>Baseline</td>
<td>6.101</td>
<td>5.835</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6.581</td>
<td>6.315</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>7.169</td>
<td>6.903</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>7.926</td>
<td>7.660</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>8.568</td>
<td>8.301</td>
</tr>
</tbody>
</table>

Table 5-3-11: Change in the mean of the DIAGNOdent readings for the ozone treated group and the control group from the baseline over the time of the follow up visits.
Figure 5-3-3: Changes in the mean of the DIAGNOdent readings for the ozone treated group and the control group from the baseline over the time of the follow up visits.

The change in DIAGNOdent readings was considered clinically significant when a difference between visits was more than three DIAGNOdent readings (DIAGNOdent manual, 1999). As presented in Table 5-3-12 and Table 5-3-11, the mean difference in DIAGNOdent readings between the recall visits for the ozone group was 0.542 and the difference between baseline and last recall visit was 2.47 which is less than three different readings. According to the DIAGNOdent manual that difference in reading, between baseline and last recall visit, is considered clinically not significant (less than 3). While, the mean difference in DIAGNOdent readings between the recall visits for the control air group was 1.089, the difference between baseline and last recall visit for the control air group was 3.932, which is clinically significant according to the DIAGNOdent manual.
For the analysis of DIAGNOdent scores changes, there was a slight increase in DIAGNOdent scores for the ozone group over the visits. As presented in Table 5-3-13 and Figure 5-3-4, the estimated marginal mean value at baseline was 0.284 and was 0.420 at the end of treatment. In the control air group there was a significant increase in scores over the visits and the estimated marginal mean value at baseline was 0.307 and 0.966 at the end of the treatment. There was a significant effect of time upon the mean DIAGNOdent scores ($F = 7.527; p = 0.009, p < 0.05$).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean differences in readings over visits (B)</th>
<th>t</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
</tr>
<tr>
<td>Control</td>
<td>1.089</td>
<td>20.685</td>
<td>0.985</td>
</tr>
<tr>
<td>Ozone</td>
<td>0.542</td>
<td>10.304</td>
<td>0.646</td>
</tr>
</tbody>
</table>

Table 5-3-12: Regression coefficient for the differences in DIAGNOdent readings between control and ozone groups over treatment time.
### Table 5-3-13: Change in the mean of the DIAGNOdent scores for the ozone treated group and the control group from the baseline over the time of the follow up visits.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Visits</th>
<th>Mean</th>
<th>95% Confidence Interval</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
<td>Upper Bound</td>
</tr>
<tr>
<td>Control group</td>
<td>Baseline</td>
<td>.307</td>
<td>.209</td>
<td>.404</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>.432</td>
<td>.334</td>
<td>.529</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>.523</td>
<td>.425</td>
<td>.620</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>.705</td>
<td>.607</td>
<td>.802</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>.966</td>
<td>.869</td>
<td>1.063</td>
</tr>
<tr>
<td>Ozone group</td>
<td>Baseline</td>
<td>.284</td>
<td>.187</td>
<td>.381</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>.227</td>
<td>.130</td>
<td>.325</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>.239</td>
<td>.141</td>
<td>.336</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>.341</td>
<td>.244</td>
<td>.438</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>.420</td>
<td>.323</td>
<td>.518</td>
</tr>
</tbody>
</table>

**Figure 5-3-4:** Changes in the mean of the DIAGNOdent scores for the ozone treated group and the control group from the baseline over the time of the follow up visits.
The result of analysis of the ICDAS II changes is presented in Table 5-3-14 and Figure 5-3-5. In the ozone group there was no change in ICDAS II scores from baseline until 6-month recall visit. The scores then increased at the 12 month visit. The estimated marginal mean value at baseline was 0.592 and was 0.829 at end of the treatment. In the control air group, there was a slight increase in ICDAS II scores from baseline until 6-month recall visit, but the scores dramatically increased after that to the end of the treatment. The estimated marginal mean value at baseline was 0.579 and was 1.250 at the end of the treatment. There was a significant effect of time upon the mean ICDAS II scores ($F = 42.874, p = 0.017, p < 0.05$).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Visits</th>
<th>Mean</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
</tr>
<tr>
<td>Control group</td>
<td>Baseline</td>
<td>.579</td>
<td>.528</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>.579</td>
<td>.528</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>.605</td>
<td>.554</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>.750</td>
<td>.698</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1.250</td>
<td>1.199</td>
</tr>
<tr>
<td>Ozone group</td>
<td>Baseline</td>
<td>.592</td>
<td>.541</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>.592</td>
<td>.541</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>.592</td>
<td>.541</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>.625</td>
<td>.573</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>.829</td>
<td>.788</td>
</tr>
</tbody>
</table>

Table 5-3-14: Change in the mean of the ICDAS II scores for the ozone treated group and the control group from the baseline over the time of the follow up visits.
The result of this study revealed that 40.5% of subjects showed no change in DIAGNOdent scores and 48.6% of subjects showed no changes in ICDAS II scores, over the 12 month period.

With DIAGNOdent, the number of teeth which had a score zero was 280, score 1 was 79 and score 2 was 11 teeth. With ICDAS II, 289 of teeth showed score zero, 64 showed score 1 and 17 teeth showed score 2 (Table 5-3-15).

**Figure 5-3-5:** Changes in the mean of the ICDAS II scores for the ozone treated group and the control group from the baseline over the time of the follow up visits.
Chapter 5  

5.3 Results

<table>
<thead>
<tr>
<th>DIAGNOdent scores</th>
<th>ICDAS II scores</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>0</td>
<td>267</td>
<td>12</td>
</tr>
<tr>
<td>1</td>
<td>22</td>
<td>51</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>289</td>
<td>64</td>
</tr>
</tbody>
</table>

**Table 5-3-15**: Distribution of the teeth according to the ICDAS II and DIAGNOdent scores over the recall visits.

In the ozone group, 154 teeth with DIAGNOdent and 151 teeth with ICDAS II showed score zero. Twenty nine teeth with DIAGNOdent and 27 teeth with ICDAS II showed score 1. Two teeth with DIAGNOdent and 7 teeth with ICDAS II showed score 2. In the control group, 126 teeth with DIAGNOdent and 138 teeth with ICDAS II showed score 0. Fifty teeth with DIAGNOdent and 37 teeth with ICDAS II showed score 1. Nine teeth with DIAGNOdent and 10 teeth with ICDAS II showed score 2 (**Table 5-3-16 & Figure 5-3-6**).
Table 5-3-16: Distribution of the teeth according to the ICDAS II and DIAGNOdent scores in both groups over the recall visits.

<table>
<thead>
<tr>
<th></th>
<th>ICDAS II scores</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Control group</td>
<td>138</td>
<td>37</td>
</tr>
<tr>
<td>Ozone group</td>
<td>151</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>289</td>
<td>64</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>DIAGNOdent scores</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Control group</td>
<td>126</td>
<td>50</td>
</tr>
<tr>
<td>Ozone group</td>
<td>154</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>280</td>
<td>79</td>
</tr>
</tbody>
</table>

Figure 5-3-6: Bar charts showing the numbers of the teeth with their scores according to the DIAGNOdent and ICDAS II measurements between ozone and control groups.
The result of the correlation test is shown in Table 5-3-17. There was a significant correlation between the detection methods scores ($p < 0.001$). The Spearman’s rank correlation coefficient between the changes in the DIAGNOdent and the ICDAS II scores over the recall visits was 0.75.

<table>
<thead>
<tr>
<th>Change in scores at 1 year</th>
<th>DIAGNOdent</th>
<th>ICDAS II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation Coefficient</td>
<td>1.000</td>
<td>.751</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.</td>
<td>.000</td>
</tr>
<tr>
<td>N</td>
<td>370</td>
<td>370</td>
</tr>
</tbody>
</table>

Table 5-3-17: Correlations between the detection methods over the recall visits.

5.3.1.4 Salivary assessment for excluded and included subjects

The subjects that were excluded from the analysis of the DIAGNOdent scores were compared with those that were included in the analyses. At the 1 year recall of starting the treatment, the Ms level in saliva for the excluded subjects exhibited a significantly lower mean rank of 13.13 compared with the mean rank of 23 for the included subjects (Table 5-3-18). The Mann-Whitney test showed significant differences in the Ms level of saliva between the excluded and included subjects ($p = 0.006$). The mean (± SE) Ms level for the excluded subjects was 1.07 (± 0.19), and mean (± SE) Ms level for the included subjects was 1.97 (± 0.15) (Table 5-3-19).
<table>
<thead>
<tr>
<th>Subjects for analysis</th>
<th>N</th>
<th>Mean Rank</th>
<th>Sum of Ranks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Ms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excluded subjects</td>
<td>15</td>
<td>13.13</td>
<td>197.00</td>
</tr>
<tr>
<td>Included subjects</td>
<td>22</td>
<td>23.00</td>
<td>506.00</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5-3-18: The mean rank of the Ms in saliva after 1 year of starting the treatment.

<table>
<thead>
<tr>
<th>Subjects for analysis</th>
<th>N</th>
<th>Mean</th>
<th>Std. Error Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Ms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excluded subjects</td>
<td>15</td>
<td>1.07</td>
<td>.19</td>
</tr>
<tr>
<td>Included subjects</td>
<td>22</td>
<td>1.97</td>
<td>.15</td>
</tr>
</tbody>
</table>

Table 5-3-19: Descriptive statistic for the salivary Ms level of the excluded and included subjects after 1 year of treatment.

In testing the pH value between the excluded and included subjects, the excluded subjects showed a mean rank of 24.33, which was significantly higher than the mean rank of 15.36 for the included subjects (Table 5-3-20). At 1 year, 73.3% of excluded subjects showed a pH value ≥ 6, 20% of subjects showed a pH value of 4.5-5.5 and 6.7% had a value of ≤ 4. While with included subjects, 31.8% of subjects showed a pH value of saliva ≥ 6, 54.5 % of subjects showed a pH value of 4.5-5.5 and 13.6 of subjects had a value of ≤ 4. The Mann-Whitney test showed significant differences in salivary pH values between the excluded and included subjects ($p = 0.013$).
### Table 5-3-20: The mean rank of the salivary pH after 1 year of starting the treatment.

<table>
<thead>
<tr>
<th>Subjects for analysis</th>
<th>N</th>
<th>Mean Rank</th>
<th>Sum of Ranks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean pH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excluded subjects</td>
<td>15</td>
<td>24.33</td>
<td>365.00</td>
</tr>
<tr>
<td>Included subjects</td>
<td>22</td>
<td>15.36</td>
<td>338.00</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chapter 5

5.3 Results
5.3.2 Part B

A total of 389 photographs were taken for 36 patients and a total of 576 teeth were scored (288 teeth for the baseline photographs and the same for the 12 month photographs). The teeth which were scored using the photographic images are the same teeth which have been assessed in part A using the DIAGNOdent and ICDAS II.

At the end of the treatment, white spot lesions were recorded on the labial/buccal surface of twenty two patients (61.1%), while fourteen patients (38.9%) did not show any white spot lesions on their teeth (Table 5-3-21).
### Table 5-3-21

The number of counted teeth with/without WSL around the orthodontic brackets one year after starting the orthodontic treatment in both groups.
The number of white spot lesions that developed on the maxillary anterior and premolar teeth at the end of photographic assessment is given in Table 5-3-22. The number of teeth with white spot lesions at the end of the treatment was 54 out of 288 teeth (18.7%) in both groups.

<table>
<thead>
<tr>
<th>Tooth number</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>WSL No</td>
<td>62</td>
</tr>
<tr>
<td>WSL Yes</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
</tr>
</tbody>
</table>

Table 5-3-22: Distribution of WSL according to tooth type and photographic assessment.

The proportion of white spot lesions on the central incisors at the end of the treatment was 13.9%, 30.5% on the lateral incisors, 18% on canines, and 12.5% on premolars. The lateral incisors had the highest prevalence of white spot lesions. The Wald’s test revealed no significant difference in the distribution of white spot lesions between the right side and the left side of the mouth ($p = 0.478$, $p > 0.05$).

The number of teeth with white spot lesions at the end of the treatment was 41 (28.5%) for the control air group and 13 (9%) for the ozone group (Table 5-3-23). There was a significant difference in WSL between the ozone treated group and the control air group (Wald’s test) ($p = 0.006$, $p < 0.05$).
Table 5-3-23: The number of teeth with WSL at the end of the treatment in the control and the ozone treated groups.

The distribution of teeth with their categories according to the EDI scores at the two visit intervals is presented in Table 5-3-24. The weighted kappa value of the repeated and rescored photographs for 144 teeth was 0.94, indicating excellent agreement between the two visits.

Table 5-3-24: Cross tabulation showing the distribution of repeat photographs for 144 teeth with their categories according to the EDI scores of the two visit sessions.


5.4 Discussion

Study 6: Part A

To avoid the possibility of pre-treated scores, subjects with pre-existing white spot lesions or decalcification were excluded. A split-mouth technique is a common design used to compare the two sides of the patient’s mouth in clinical trials of orthodontic treatment. Implementation of this design would ensure an equal number of right and left test sides and would reduce the inter-participant differences and increase the power of a study. Moreover, the design of the present study was a double blind design which eliminated subjective bias on the part of both subjects and the operators. Double blind experiments achieve the highest standard of scientific basis. In the present study, neither the individuals nor the operators knew which side belonged to the ozone treated group or the control air group. Only after all the data were recorded did the researchers know which side was treated with ozone and which was treated with air.

Only maxillary labial and buccal teeth were assessed in this study, as decalcification occurs more frequently on maxillary teeth than mandibular teeth (Artun and Brobakken 1986; Ogaard 1989b).

There was no safety concern in using the ozone delivery system in the present study. The suction system passed the gas from the delivery system through manganese [II] ions to neutralise it. There was no possibility of ozone leakage from within the cup to
the oral cavity because the ozone delivery system was designed so that if the seal is broken within the cup, the system switches off ozone delivery immediately and sucks away any remaining ozone in the cup. The assessment of the safety of ozone during the treatment of root caries with the ozone delivery system was performed in-vitro (Baysan 2002). The tip of the ozone analyser’s sensor was held within 2 mm of the edge of the cup both during and after the application of ozone for 10 seconds. The maximum ozone detectable level in the air around the cup during and after the 10 seconds of ozone application was $0.0012 \pm 0.0003$. The mean (0.0012 ppm) ozone level in the air conformed to the EU and FDA regulations. These limit values are exceeded in the air quite frequently all over Europe. A recent study also confirmed the safety of using the HealOzone in dentistry (Millar and Hodson 2007). The HealOzone was set to a 10 seconds ozone application on the lower right second molar tooth. The readings were recorded every 5 seconds at three sites; patient’s pharynx, patient’s nasal orifice and near the clinical operator’s mouth. The mean of five readings were calculated and all recorded ozone levels were zero.

Some salivary parameters indicate a strong protective effect against dental caries. Evaluating the causative factors in saliva of patients at risk to dental caries can allow recommendations to be made appropriate to each patients needs. The salivary buffering capacity prevents a reduction in pH by neutralising acid in the oral cavity after fermentable carbohydrate intake. Therefore, it has the capacity to protect the tooth from dental caries (Leone and Oppenheim 2001). In the present study according to the salivary pH result, we observed that at baseline 48.7% of subjects had a high buffer capacity of saliva and 40.5% had an intermediate buffer capacity, and 10.8% had a low buffer capacity. At 1 year, 70.3% of subjects had a high buffer
capacity of saliva and 29.7% had an intermediate buffer capacity. The buffer capacity had increased from baseline to a 1 year interval in 21.6% of subjects. This finding is similar to a previous study which showed a significant ($p < 0.001$) increase in buffer capacity observed after three months of active orthodontic treatment (Chang et al. 1999). The findings can be explained by the evidence that insertion of a foreign body in the mouth will increase salivary flow. Therefore, the presence of the orthodontic appliances in patients’ mouths would stimulate salivary gland secretion, and subsequently the salivary pH and buffer capacity would change as they follow the rate of saliva secretion (Andersson et al. 1974). Chang et al. (1997) reported that the pH of unstimulated saliva can be less than 6, rising exponentially to approximately pH 8 at very high flow rates. Previous studies reported a tendency for buffer capacity to increase with salivary flow rate (Klock and Krasse 1977; Chang et al. 1999).

During fixed orthodontic treatment, the highest incidence of decalcification occurs in the upper anterior teeth, a site with little exposure to saliva. This reflects a relationship between resistance to decalcification and salivary flow rate. Salivary buffer capacity correlates directly with caries (Russell et al. 1990).

In the present study a significant increase in the Ms of saliva was observed when comparing the baseline values to the values obtained after 1 year of bonding the brackets. This finding is in agreement with previous studies in that the number of Ms in saliva increased significantly following insertion of fixed orthodontic appliances (Forsberg et al. 1991; Rosenbloom and Tinanoff 1991; Jordan and LeBlanc 2002).
Plaque acts as a physical barrier for the diffusion of acid away from the enamel surface and it can also prevent remineralisation by calcium and phosphate ions from the saliva. Moreover it is a source of acid production in the presence of fermentable substrate attaching directly to enamel (Chang et al. 1997). For that reason, the density of Ms in plaque indicates almost certainly, an increased risk of enamel caries.

The labiogingival area of the maxillary lateral incisors had the highest incidence of white spot lesions as reported in previous studies (Gorelick et al. 1982; Ogaard 1989a; Millett et al. 1999; Benson et al. 2004). Ogaard (1989b) postulated that the most likely explanation for this observation was that the brackets were placed closer to the gingival margin because of the anatomical shape of the lateral incisors, causing difficulties in tooth cleaning and increasing the likelihood of plaque accumulation. Therefore, in this study, the lateral incisor teeth were selected as the donor site for the plaque samples site.

The patients were instructed to avoid eating, drinking and tooth brushing for 2 hours as these factors could affect the salivary secretion rate and buffering capacity of saliva. Several studies have confirmed that eating, drinking and medication could affect the salivary secretion rate and should be avoided (Heintze et al. 1983; Lenander-Lumikari and Loimaranta 2000).

Several studies have revealed that Ms colonisation in plaque was higher in teeth with fixed orthodontic appliances compared with control teeth without appliances (Lundstrom and Krasse 1987b; Chang et al. 1999; Jenatschke et al. 2001; Attin et al. 2005). However, the Ms and lactobacilli levels in plaque was reported to reduce after
6 weeks of debonding the orthodontic brackets, indicating that fixed appliance therapy increases the risk of caries (Boersma et al. 2005). They also reported that this reduction was more pronounced for lactobacilli, which may indicate that Ms levels need more time to return to normal, or that the natural balance between these bacteria is shifted during orthodontic treatment.

In the present study, there was a significant increase in the Ms level of plaque in both groups ($p < 0.001$). Although the number of subjects which showed an increase in the Ms counts in plaque, after one year of bonding the brackets, was lower ($n = 9$) in the ozone group than in the control air group ($n = 12$) but that was not significant statistically ($p > 0.05$). The increased levels of Ms in plaque is important as Ms is one of the major aetiological factors for the high risk of enamel demineralisation in orthodontic patients and this correlates with the findings in the present study. The findings showed that the DIAGNOdent and the ICDAS II, exhibited significant differences between the ozone treated group and the control air group ($p < 0.05$). A higher incidence of demineralisation or white spot lesions was observed on the tooth surfaces of the control air group and this shows the beneficial effect of ozone application in the prevention of demineralisation around the orthodontic brackets.

Previous studies on the effect of ozone on the primary root caries showed that 10 seconds of ozone gas application was capable of reducing the number of colony forming units from $\log_{10} 6.73 \pm 0.27$ to $\log_{10} 3.36 \pm 0.48$, and from $\log_{10} 6.30 \pm 0.28$ to $\log_{10} 1.17 \pm 0.62$ when applied for 20 seconds, in-vivo (Baysan 2002). Also, ozonated water was capable of reducing the number of Streptococcus mutans and Streptococcus sobrinus on saliva-coated glass beads in the ozone group ($\log_{10} 3.57 \pm 0.37$) compared to the control group ($\log_{10} 5.91 \pm 0.15$) ($p < 0.001$), in-vitro.
(Baysan et al. 2000). Another in-vivo study has shown that 20 seconds application of ozone gas produced a significant difference \( (p = 0.022) \) in the numbers of Ms in saliva compared to the control group and also showed that ozone effectively penetrates into the lesion and kills the great majority of micro-organisms (Kamali et al. 2003). The results observed in the present study showed a significant decrease in the level of Ms in plaque \( (p < 0.001) \) after immediate application of ozone gas for 10 seconds on the Ms strips. But the level of Ms in plaque was increased significantly from the baseline to the 12 months recall visits. This result showed that ozone has an immediate effect on the Ms level in plaque and this effect decreases over the time. However, the immediate effect of 10 seconds ozone application during the recall visits seems to have the ability in reducing the number of WSLs around the orthodontic brackets compared to the control air group. That was quantified by the detection tools which showed a significant difference between the two groups. The efficiency of ozone treatment in reducing WSLs around orthodontic brackets can be partly explained by the fact that ozone reduced the Ms in plaque resulting in a delayed recolonisation compared with the control air group. The re-application of ozone could slow the recolonisation pattern and achieve long-term suppression. Other factors may play an important role in the prevention or reduction of enamel demineralisation such as maintaining good oral hygiene practices, diet or nutritional changes and professional tooth cleaning. However, such factors depend on patient motivation and compliance.
In this study, 40.5% of subjects were excluded from the analysis for the DIAGNOdent scores, and 48.6% of subjects with ICDAS II. All subjects were included in the analysis for DIAGNOdent readings. The reason for this exclusion was that their scores were always zero and that mean it have no variations. Therefore, they were removed because they added no informations to the analysis.

When comparing the amount of Ms in saliva and salivary pH value for the subjects that were excluded from the analysis with those included in the analyses, it was found that the excluded subjects showed a lower amount of Ms and a higher value of salivary pH than the included subjects. The subjects who had a lower level of Ms and a higher value of salivary pH are considered to be at low caries risk. This suggests that in patients who have high salivary levels of Ms and low salivary pH before treatment, the application of appropriate preventive measures should be used to decrease the amount of Ms and that may help in the prevention of incipient caries formation during orthodontic treatment.

It was observed in this study that the subjects with an elevated risk of demineralisation, with higher levels of Ms and a lower salivary pH, showed a higher incidence of white spot lesions. In contrast, subjects with a lower risk of demineralisation, with low levels of Ms and high salivary pH, showed a resistance to demineralisation during the study period. The assessment of caries risk factors before treatment would allow target preventative treatment to the patients. Therefore, employing caries risk assessment before starting orthodontic treatment would help target subjects at high risk who should be selected for a comprehensive preventive regimen and thus would limit the number of subjects who require intense care during
orthodontic treatment and would reduce preventative treatment costs for the patients at low risk. By caries risk assessment, prediction of carious lesions before treatment is possible in patients with an elevated risk of caries.

The results of this study showed that the DIAGNOdent scores were increased for 40.5% of patients and the ICDAS II scores were increased for 48.6% of patients while the rest of the patients demonstrated resistance to white spot formation. Indeed 37.8% of the same patients showed an increase in scores for both the DIAGNOdent and the ICDAS. This is in agreement with previous studies which have reported that the incidence of white spot lesions following a course of fixed appliance therapy was nearly 50% (Gorelick et al. 1982; Artun and Brobakken 1986).

For the analysis of DIAGNOdent changes, there was a steady increase in DIAGNOdent readings over the visits for both groups which is considered statistically significant (Figure 5-3-3). However, according to the DIAGNOdent manual, if the difference between the readings was less than three readings that is considered not significant clinically. In the ozone group the change in the DIAGNOdent readings was less than three which claimed to be clinically not significant. While in control air group the changes were clinically significant as it was more than three readings changes. For the analysis of DIAGNOdent scores, there was a slight increase in DIAGNOdent scores for the ozone group over the visits. As presented in Figure 5-3-4, the estimated marginal mean value at baseline was 0.285 and was 0.420 at end of the treatment. In the control air group there was a significant increase in scores over the visits where the estimated marginal mean in value at baseline was 0.307 and was 0.966 at end of the treatment. There was a significant effect of time upon the mean
DIAGNOdent scores \((p < 0.05)\). This finding is in agreement with previous studies on the effects of ozone on treating dental caries and the reduction of oral microorganisms (Baysan et al. 2000; Baysan 2002; Baysan and Lynch 2003; Abu-Naba’a 2003; Holmes 2003; Baysan and Lynch 2004; Abu-Salem 2004; Nagayoshi et al. 2004a; Arita et al. 2005; Huth et al. 2005; Dahnhardt et al. 2006; Baysan and Lynch 2007).

An interesting finding was that, in the ozone treated group, the lesions had an overall negative change in DIAGNOdent mean scores at the 3 and 6 month recall but there was an immediate slight positive change in the mean scores after 6 month and at the 12 month recall visit. In the control air group, there was a shifting toward higher DIAGNOdent scores over all recall visits which meant steady deterioration. Negative changes imply an improvement while positive changes imply an increase in caries. The ozone treated teeth showed a slight decrease in DIAGNOdent fluorescence (negative change) at the first two recall visits. After that, fluorescence increased gradually until the last recall visit. This might be explained as ozone can influence on the carious process when the lesion is small or just started. But over time, when the lesion is established and becomes large, 10 seconds of ozone applications may be less effective to influence the carious lesion. A study by Baysan and Lynch (2004) reported that ozone gas for 10 seconds was capable of reducing the number of bacteria in small lesions \((1\text{mm}^2)\) significantly, whilst in large lesions the effects were small or non-existent. Ozone treatment might be more effective if it was applied in higher doses (20, 40 or 60 seconds) during monthly recall visits.
The influence of ozone on laser fluorescence was investigated after 2, 4, 6 and 8 months of ozone treatment (Dahnhardt et al. 2006). Eighty two lesions in 28 children with at least two open single-surface lesions were assessed. They found that the use of ozone resulted in an average reduction of 13% of the laser fluorescence values immediately after the ozone treatment. They concluded that the ozone reversed caries in the surface lesions by shifting the chemical balance promoting remineralisation of tooth structure. The result of the present study indicates that the DIAGNOdent device can be used for monitoring lesions progression as the fluorescence increased for both groups and is correlated with the ICDAS II scores precisely at the last two recall visits. Our finding is in agreement with a similar clinical study on the use of the DIAGNOdent device to evaluate the effect of two preventative programs on incipient carious lesions around orthodontic brackets for the detection and monitoring of white spot lesions on smooth surfaces following orthodontic therapy (Aljehani et al. 2006). They suggested that it might be feasible to use DIAGNOdent for longitudinal monitoring of carious lesions on smooth surfaces. For longitudinal quantification, there was a significant difference in the DIAGNOdent readings between the first and final evaluation ($p = 0.025$).

When the analysis of the ICDAS II for the ozone treated group was conducted, the results revealed no change in the mean scores at the 0, 3 and 6 month recall visits but it revealed an increase in the mean scores at the 9 and 12 month recall visits. In the control air group, the ICDAS II scores revealed no change at the 0 and 3 month recall visits, a slight positive change at the 6 and 9 month recall visits and a significant positive change at the 12 month visit. This can be explained as the progression of white spot lesions needs a period of time to be obvious visually. The scores at the
end of the study indicate a slight change in enamel translucency to opaque which is evidence of a carious lesion after air drying for 5 seconds.

It was reported that cariogenic bacteria, and the Ms group in particular, need sufficient time to form carious lesions. Studies have suggested, on average, it takes one year for a carious lesion to be established, and three years before it reaches the dentine (Berman and Slack 1973; Newbrun 1989). That is partly in agreement with our observation of the development of white spot lesions around orthodontic brackets after one year of starting the active orthodontic treatment and would explain the sudden positive changes in ICDAS II scores after 9 months of starting the treatment. Although the DIAGNOdent readings implied that the demineralisation process was active with positive changes from the first recall visit and until the last recall visit, this was not recorded visually by ICDAS II until the fourth recall visit. The formation of ‘normal’ carious lesions is a usually a slower process, which takes at least 6 months (Ekanayake and Sheiham 1987).

In the control air group, the higher DIAGNOdent and ICDAS II scores gave an indication that the lesions had developed with further loss of mineral. In the ozone group, the zero score for DIAGNOdent and ICDAS II gave an indication of no progression in the enamel surface. The overall change recorded by the detection methods during the present study suggest that the ozone treatment was capable of influencing caries around orthodontic brackets in permanent maxillary teeth.

In order to understand the influence of ozone on the carious process and to monitor its efficiency over the time, Lussi and Francescut (2004) carried out a preliminary
experiment in which the DIAGNOdent readings were measured after 40 seconds of ozone application on the occlusal surface of 18 teeth at regular periods of 2, 5, 15, 30 and 60 minutes. The teeth showed a sudden decrease in fluorescence in the first minutes after ozone application (2 to 15). After that time, fluorescence increase gradually but did not reach the value obtained before ozone application. Thus the effect of ozone was reduced gradually after a period of time. Therefore, for accurate follow up of mineralisation over time, it is recommended to take the measurement with DIAGNOdent prior to the ozone application (Lussi and Francescut 2004; Dahnhardt et al. 2006). The follow-up measurements should be carried out on the following visits and not immediately after ozone application. Therefore, in the present study, the measurements were taken before ozone application at each recall visit.

An essential aspect of detection methods is to provide consistent and a standardised measurement within the methods used. In the present study, the result of the correlation test indicated a good association between the ICDAS II and the DIAGNOdent with a value of 0.75. This finding of good correlation indicates consistent performance of the detection methods used in the present study. As a consequence, the method may be suitable for longitudinal studies for caries monitoring, provided that these methods can detect small changes in carious lesions over time.
**Study 6: Part B**

The photographic images were analysed and the teeth were scored using camera software to produce an enlarged computer image. The camera software was able to enlarge the photo size and was able to objectively distinguish many more shades than the human eye. In this study a professional camera based on the single lens reflex design was used. The digital images for this study were 1504 pixels wide and 1000 pixels high which allowed high-resolution images. Moreover, the camera allows full flexibility with the micro lens for close-up work and with multipoint flash units to produce differences in enamel colour.

Dental caries generally occurs in a bilaterally symmetrical pattern in the arch and displays a non-random or aggregated distribution pattern of white spot lesions within the quadrant (Hujoel *et al.* 1994). In this study the distribution pattern was found equally in both quadrants. This would not agree with previous studies which found a higher amount of plaque on the right side of right-handed tooth brushers than on the left with orthodontic patients (Benson *et al.* 2004) and with non-orthodontic patients (Addy *et al.* 1990). Although all of the patients in this study were right-handed, the distribution of white spot lesions was equal on both sides. This may be due to the instructions to maintain good oral hygiene and to use the orthodontic brushes properly for both sides of the mouth and may be due to the effect of fluoridated toothpaste.
The highest EDI score recorded in this study was two while other studies have recorded higher scores (Marcusson et al. 1997; Banks et al. 2000). This might be because the teeth included in the present study had no caries or pre-existing white spot lesions while other studies did not mention this inclusion criterion in their studies. Moreover, the result of salivary assessment at baseline showed that the majority of the patients were at low or intermediate caries risk and that would also help explain the limited changes in EDI scores. In addition, the assessment in this study was undertaken for a period of one year while most of the reviewed studies ranged between 18-22 months. It was reported that the white spot formation increased during longer treatment times (Marcusson et al. 1997).

In a previous study the highest percentage (15.3 %) of white spot lesions was found in the anterior maxillary segment (Gorelick et al. 1982). In addition, white spot lesions occurred in 61% of cases during orthodontic therapy despite a comprehensive preventive programme involving topical fluoride application (Ogaard et al. 2001). In this study the incidence of new lesions in the treatment group was lower (9%) with no extra prophylactic programme compared with the Gorelick and Ogaard studies. This fits in with the preventative effect of the ozone treatment. When comparing the control air and ozone treated groups, fewer white spot lesions developed on the teeth in the ozone group (9 %) in contrast with the control air group (28.5%).

The reproducibility value reported in this study was better than that reported in other published papers when assessing the white spot directly from photographic images (Mitchell 1992) or on projected images (Linton 1996). It was also better than the value of 0.79 obtained when converting the slide scanner to digital Tagged Image
File Format (TIFF) images and using computerised image analysis software for the measurement of plaque area surrounding the orthodontic brackets *in-vivo* (Benson *et al.* 2004). The reproducibility value of 0.94 for this study was within the range of values, from 0.94 to 0.99, obtained from computerised TIFF images analysis for the measurement of demineralisation on the buccal surface of teeth *in-vitro* (Benson *et al.* 2000), and for the measurement of WSLs surrounding orthodontic brackets *in-vivo* (Benson *et al.* 2005). The use of a professional digital camera in this study, although more expensive, helped in reducing the errors associated with some photographic techniques. The use of ring flashes and colour filter holders to create desirable lighting effects when taking close-up shots and to prevent irregular flash lightning ensured a good result. Reflected light may mask an area of decalcification and may lead to an overestimate of the area of decalcification (Benson *et al.* 2000). This was diminished by the design of this study.

The use of digital camera images provides an alternative assessment approach for quantification of clinical white spot lesion around orthodontic brackets.
5.5 Conclusions

- The International caries detection and assessment system coding criteria (ICDAS II) and the DIAGNOdent readings were reliable detection methods for the detection of labial/buccal enamel carious lesions of permanent teeth \textit{in-vivo}.
- Both methods correlated well with each other statistically.
- Assessment of the caries risk factors before orthodontic treatment will help in decision-making by using an appropriate preventative regime for the patients at high caries risk. This approach will help the dentist to design treatment strategies and provide knowledge for the patients to improve their dental care.
- The white spot lesion formation associated with fixed orthodontic treatment can be assessed accurately and reproducibly using a professional digital camera under clinical conditions.
- White spot lesion formation associated with fixed orthodontic treatment can be assessed using the detection methods, the ICDAS II, DIAGNOdent and appropriate digital camera. Ozone treatment was capable of significantly reducing white spot lesion formation around orthodontic brackets.
- Ozone did not totally prevent WSLs around the orthodontic brackets.
Summary

Early enamel loss and the changes on the enamel surface led to the introduction of a proper treatment approach to prevent the progression of lesions and proposed suitable detection methods to assess lesions. However, decalcification associated with fixed orthodontic therapy still exists in spite of the multi-preventative methods which have been demonstrated to influence its occurrence. Ozone was introduced as one of the treatment approaches to manage dental caries but has not been reported to manage the decalcification around orthodontic brackets. Validity of the detection methods to assess lesion progression and regression have been introduced widely but are still posing questions regarding their accuracy.

The 3-D laser scanning has a prospect of being implemented in measuring lesion depth and enamel changes associated with orthodontic bonding and debonding procedures. The repeatability of the laser scanner in the present study was very good, which confirmed the findings of previous studies. DIAGNOdent and the ICDAS II systems have the potential to be used in assessing the early changes in the enamel surface. The two systems were found to have good reproducibility in both in-vitro and in-vivo studies. They also produced good correlation with lesion depth when compared to histological scores and were also found to be correlated to each other. The detection tools provide a sound method by which lesions could be quantified.

Glass ionomer cement was introduced as an orthodontic bonding lute and was found to be better than conventional composite resins in term of producing less damage to enamel surface at debond. The glass ionomer cement used in this study was
found to have minimum damage to the enamel surface compared to the conventional composite resins detected by the 3-D laser scanning. In addition, the shear bond strength for both orthodontic adhesive systems was found to be adequate for clinical use. The 10 second dose of ozone showed no adverse effect on the shear bond strength of the orthodontic brackets in-vitro.

The detection methods, ICDAS II and DIAGNOdent, confirmed that ozone had a significant influential beneficial effect on the reduction of the development of WSL compared to the control air group. The 10 second dose of ozone reduced the amount of the decalcification but was not able to totally prevent its occurrence.
Future work

The studies of this thesis have postulated some areas that merit further work:

- To measure 3-dimensionally the enamel changes associated with fixed orthodontic appliances using the laser scan machine in the clinical situation.
- Further work is required to validate the QLF index, which has been developed and used in study 2, and to support the validity of this index in quantification of the carious lesion depth *in-vivo*.
- More *in-vitro* and *in-vivo* scientific studies are required to support the recommendations of the ICDAS II for quantification of smooth surface lesions.
- More longitudinal studies are required to monitor regression or progression of the white spot lesions after removal of orthodontic brackets using the valid detection methods.
- A study of the application of ozone gas for more than 10 seconds (20, 40 or 60 seconds), to determine the optimal dosage to obtain the maximum benefit from ozone in preventing decalcification around orthodontic brackets.
- Assessment of other means of delivery of ozone to orthodontic patients such as ozonated water rinses.
- A study on monthly application of ozone on orthodontic patients during the recall visits.
- To assess, via a longitudinal study, the ability of ozone gas to remineralise white spot lesions after the removal of fixed orthodontic brackets.
- To examine, *in-vitro*, any chemical changes on metal or ceramic orthodontic brackets and attachments due to the application of ozone gas.


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References


References


References


References


APPENDICES
Consent Form for the *in-vitro* studies (2, 4 and 5)

“Assessment of enamel surface changes during orthodontic bracket bonding and debonding”

A PhD student, Miss Amna Al Shamsi B.D.S., is currently carrying out research on premolar teeth at the Royal Victoria Hospital, School of Dentistry. Amna has requested that my practice collect some extracted premolar teeth so she can complete her research.

Your tooth/teeth are suitable for this research and therefore I would ask you to kindly consent to this/these teeth being used for this purpose. Your tooth/ teeth will not be used for any other purpose.

If you have no objections could you please sign the form below.

**Thanking you**

I agree to my tooth/teeth being used for this specific research project

Signature…………………………………………

Name (print)……………………………………………Ref.

NO…………...

Date……………………

Presents Signature…………………………………

Witnessed…………………………………………
### Form for histological examination of teeth

#### Study 2

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<th>Tooth no.</th>
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Ethical Approval for the in-vivo study
(Study 6)

Date: 24/05/05

Prof. Phillip-John Lamey,
Professor of Oral Medicine
School of Dentistry,
Royal Victorian Hospital
Queen’s University, Belfast

Sub: Approval of a Research Project

Dear Professor Lamey,

I was asked by Dr. Amna H. Al-Shamsi to write to you informing about her research proposal titled: “An Assessment of the use of Ozone to influence caries around orthodontic brackets”. The proposal was reviewed by The Research Ethics Committee of Rashid Hospital on January, 23rd, 2005, and the committee forwarded some comments and enquiries about certain points. After receiving the required justified reasons and clarifications from Dr Al-Shamsi, the committee has finally approved the proposal and the consent form its meeting on 15/03/2005.

Kind Regards,

Dr. Khalil I. Qayed
Chairman,
Medical Research Ethics Committee
Rashid Hospital
Information letter to patients and their parents/guardians.
Evaluation of the safety and efficacy of the use of ozone for the prevention of dental decay (holes in teeth) around orthodontic brackets

Study purpose:

Your child is being asked to participate in a research study being conducted by Prof. J P Lamey, Prof. E Lynch, Dr. Saif Al-Wali and Miss. Amna Al-Shamsi of Queen’s University Belfast, Division of Restorative Dentistry and the Orthodontic Department at Rashid Hospital, Dubai.

The objective of this feasibility clinical study is to investigate the safety and efficacy of an investigational ozone treatment regime to prevent dental decay around orthodontic brackets. The ozone will be administrated through the use of the HealOzone™ ozone delivery device. Your child is being invited to participate in a clinical study as he/she has the potential to develop some demineralisation around orthodontic brackets on his/her teeth.

A maximum of 40 patients will be needed in this study site to achieve the number of lesions indicated. The duration of the study will be 12 months from the date of initial orthodontic treatment. Information will be collected just before treatment and at follow-up assessments taken at baseline, 3, 6, 9 and 12 months.

This form is to inform you and your child about the nature and risks of the clinical study in which your child has been invited to participate. You and your child should carefully read this Informed Consent (IC) form before you decide to grant approval or not for your child’s participation. You will be given copy of this IC form if you decide to participate in this study.

Background:

Ozone was first suggested as a disinfectant for drinking water in the 19th Century in view of its powerful ability to kill bugs. The modern development of ozone’s application to medicine began in the 1950s in Europe and gradually spread throughout the continent and then to Australia, Cuba, Brazil and Columbia. In World War 1, ozone was used medically to treat wounds and other infections. It is used in water purification and sewage treatment.

Benefits of participation:

No benefits from the study will be guaranteed. This study will provide important knowledge regarding the value of the ozone delivery device and the ozone treatment regimen for preventing dental decay around orthodontic brackets. A possible benefit is that this treatment may prevent dental decay around orthodontic brackets. In
future, orthodontic patients may benefit from information gained as a result of this clinical study.

Possible risks/discomforts:

Ozone is known as a harmful gas but it is one of nature’s most powerful oxidants. When applied in large amounts and for a short time, it is proven that ozone is a useful gas. While the level of ozone to be used in the study is high, up to 2,100 ± 100 parts per million (ppm), the gas will be applied around the bracket for a very brief period of only 10 seconds. Precautions, which should limit potential ozone exposure, include:

- The tight fitting design of the delivery device made by the cup and tooth should contain the ozone treatment.
- Ozone is well known to be toxic to living tissue. The dentist performing the treatment will be trained to prevent any large amounts of ozone escaping.
- The device operates by suction only; the pathway for ozone being under negative pressure, meaning ozone should not leak out. In the event of leak, only air should leak in- no significant ozone should leak out.
- If an incomplete seal occurs or if a leak arises, a flow sensor will shut down the ozone generator.
- After 10 seconds of delivery of ozone, the suction will remain on for an additional 10 seconds to purge away any remaining ozone.
- Ozone is stable for only a very brief time. It decomposes to form oxygen and free radicals and hence disappears very quickly.

In principle, the potential toxicity of ozone should not prevent its use as a therapeutic agent. At the correct dose, ozone can be useful as a therapeutic agent.

While the potential risks of ozone exposure have been considered and are felt to be minimized in the design of the delivery device, this study does not involve an investigational device and may have risks, which are currently unforeseen. While the probability of ozone exposure is low, 100% capture of ozone delivered is not assured and certain risks do exist. Your exposure will be for only 10 seconds.

You/your child will be informed of any new findings that may affect your willingness to continue to participate.

Participation/alternatives:

You/your child’s participation in this study is strictly voluntary and it is your right to refuse participation or to withdraw from the study without penalty or loss of benefits to which you/your child is entitled. You/your child is not obligated to participate in this study.
Study procedure:

The follow-up assessments will be conducted over the course of 1 year. All participants will need to come for follow-up visits monthly for baseline, 3, 6, 9 and 12 months. Each appointment will take approximately 30-60 minutes. You will be asked to complete a separate medical consent form and a separate medical form to confirm current medical status during your first visit. Your dentist has explained the specific dental test and procedure required to be performed.

The following procedures will be performed:

- Salivary and plaque assessment will be carried out using special salivary strips at baseline and at 12 months. Plaque sample will be taken from the gingival side of the orthodontic brackets (the maxillary lateral incisor teeth).
- Prior to clinical assessments measurements will be carried out using the DIAGNOdent device and photography which will measure the decay around the brackets. Subsequently, clinical examination of the lesions will be performed using a special clinical index (ICDAS II) describing the severity of the decay. Ozone will be applied for a period of 10 seconds around each bracket inside a sealing cup, which will be carefully placed to seal against the tooth and cover the whole lesion. Any residual ozone will then be purged with suction for an additional 10 seconds on each tooth. The ozone application will be on one quadrant of the mouth (randomly left or right) and the other quadrant will receive air application. The same procedures will be performed at time points of 3, 6, 9 and 12 months but no ozone or air will be used at 12 months.
- Measurements, readings of each tooth and clinical criteria used to detect demineralisation lesions will be carried out. Each tooth will be followed up for a period of up to 12 months at the intervals of 3, 6, 9 and 12 months.

You will also receive preventive advice including extensive oral hygiene and dietary advice.

It is very important for study purposes that you/your child returns for all evaluation visits. If you agree to participate in this study, you are expected to return for all follow-up visits and remain in contact with the medical center/clinical for 1 year to monitor your progress.

Responsibility/compensation:

You will not be provided with any financial compensation for voluntarily participating in this study. You or your insurance company will be responsible for any routine treatment cost.

In the unlikely event physical injury occurs as a result of participating in this study, the necessary facilities, emergency treatment and professional medical services will be available to research patients, just as they are to general community. If you/your child are injured you should immediately contact Miss. Amna Al-Shamsi 0506263146. Further information can be obtained from the hospital/clinic Division of Orthodontics.
Ethical committee approval:

You understand that approval for this study and approval to initiate patient enrolment has been obtained from Ethical Committee at Rashid Hospital. If you have any questions regarding patient rights as a research subject you can call the Ethical Committee representative on telephone number 04 337111.

Termination of study:

This study may be terminated by any of the study principal investigators, Prof. J P Lamey, Prof. E Lynch and Dr. Siaf al-Wali at any time.

Record/report:

You understand that the data collected from you/your child’s participation will be used solely to evaluate the HealOzone™ Ozone delivery device. You/your child will not be identified in any publication or photograph without your expressed written permission. By participating in this study, you will allow officials from regulatory agencies to have access to and inspect you/your child’s dental/medical records to verify the accuracy or validity of the data. You will also allow them to photocopy information from your dental/medical records for study purposes. Your participation in this study will remain confidential.
Consent form for the *in-vivo* study 6

Study to evaluate the safety and efficacy of the use of ozone for the prevention of dental decay (holes in teeth) around orthodontic brackets

Statement of consent:

Your and your child’s signature on this form indicates that you both have understood to your satisfaction the information regarding participation in this research project and agree to have your participation as a subject. In no way does this waive you or your child’s legal rights nor release the investigators, sponsors, or involved institutions from their legal and professional responsibilities. You understand that your participation is voluntary and that he/she is entitled to receive ozone treatment around the orthodontic brackets on one side of his/her mouth and to receive air on the other side. You should feel free to ask for clarifications or new information throughout your research. For any further questions or your rights as a research subject, please contact Miss. Amna Al-Shamsi 050 6263146.

If you or your child have any questions concerning your rights as a possible participant in this research, please contact the Ethical Committee representative at telephone number 04 337111.

Participant’s Name (please print):……………………………………………… Date……

Participant’s Signature:…………………………………………………………

Parent/Guardian’s Name (please print):……………………………………..Date……

Parent/Guardian’s Signature:…………………………………………………..

Investigator/Delegate’s Name (please print):…………………………….. Date……

Investigator/Delegate’s Signature:…………………………………………

A copy of this form has been given to you to keep for your record and reference.
Clinical examination form 1 (Study 6)

Patient name: ...........................................  File no: .................

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<tr>
<th>Measurements</th>
<th>Photograph</th>
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<tbody>
<tr>
<td><strong>Salivary &amp; plaque assessment</strong></td>
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<td><strong>Colour of the pH pad:</strong></td>
<td>Baseline:</td>
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<td>At 12 month:</td>
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<td>At 12 month: ................................</td>
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<td><strong>Mutans streptococci level in saliva:</strong></td>
<td>Baseline:</td>
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<td>Baseline: ..................................</td>
<td>At 12 month:</td>
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<td>At 12 month: ................................</td>
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<td><strong>Mutans streptococci level in plaque:</strong></td>
<td>Baseline:</td>
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<td>At 12 month: ................................</td>
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<td>At 12 month:</td>
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Comments:..........................................................................................................................
Clinical examination form 2 (Study 6)

Patient Name: ........................................................................................................ File no.........

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<th>ICDAS II Scores / Visit</th>
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- Visits: 0; baseline, 3; 3m-recall, 6; 6m-recall, 9; 9m-recall, 12; 12m-recall.
- m; mesial, d: distal, i; incisal, g; gingival.
Clinical examination form 3 (Study 6)

Patient Name: ................................................................. File no. .......

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- Visits: 0; baseline, 3; 3m-recall, 6; 6 m-recall, 9; 9 m-recall, 12; 12 m-recall.
- m; mesial, d; distal, i; incisal, g; gingival.
Clinical examination form 4 (Study 6)

Patient name: .............................................. File no: ..............
Registration no.: ........................................ Clinical examiner: ........
Date of birth: ............................................. Date: .................
Sex: ........................................................
Contact no.: ..............................................
Nationality: ................................................

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<td>4th visit (9 months) ................................................ ..</td>
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Comments: ........................................................................