DENTINE PERMEABILITY, ADHESIVE PENETRATION
AND INTERFACIAL STRESS:
A CONFOCAL MICROSCOPE STUDY.

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ABSTRACT

Dentine has a heterogeneous structure and is inherently wet due to the presence of fluid filled tubules. Bonding to dentine has not met with the same success as enamel bonding, and dentine bonding systems continue to be developed. *In vitro* evaluation of these systems centres around interfacial morphology; SEM or TEM (involving considerable sample preparation), microleakage and bond testing. Dynamic interfacial performance with minimal sample preparation has not been evaluated.

To address this, techniques were developed to record the appearance of dentine/restorative interfaces in real time, during and after the placement of restorations, in addition to interfacial micropermeability and dynamic performance under load.

Teeth, maintained in near physiologic conditions, were restored with a dentine bonding system. In different cavity configurations and interfacial regions examined using fluorescence confocal microscopy (tandem scanning and laser scanning microscopes). Fluorescent dyes were added to the components of the dentine bonding systems and pulpal fluid to clarify the location of the components within the dentine and highlight any micro-permeability. Images were captured on 35mm film and/or video. In addition, a range of computer software programmes were used to capture, edit and store video rate image sequences. Fracture experiments were conducted in shear mode with tooth samples held in a custom made jig with load cell and computer controlled servo-motor pusher to load the sample. Real time images of the interfaces during failure were recorded along with synchronised load data. This allowed the dynamic patterns of interfacial failure to be recorded and categorised for the first time.

The fluorescence confocal microscopy techniques provided an *in vitro* evaluation of dentine permeability, adhesive penetration and interfacial performance under stress, and enabled video rate recording of events at the interface. These techniques have been used in the comparison of performance of dentine bonding systems and have also been influential in the development stage of new materials.
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STRUCTURE OF THE THESIS

The thesis is structured so that the first chapter is concerned with the literature, the second chapter outlines the materials and methods common to the studies in the thesis. Chapters 3-7 refer to these general methods, but otherwise stand alone with their own introduction, materials and methods, results discussion, and conclusions. Chapter 7 should be read in conjunction with the enclosed video tape. The still photographs do not easily convey the dynamic events recorded in the video. However, the thesis may be read without the video tape. Chapter 8 summarises the findings of the whole thesis and gives areas of future study.
## ABBREVIATIONS

<table>
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<th>Polymer chemistry:</th>
<th>Compound</th>
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<tr>
<td>bisGMA</td>
<td>adduct of bisphenol-A and glycidyl methacrylate</td>
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<td>DMA</td>
<td>dimethacrylate</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylene diamine tetra acetic acid</td>
</tr>
<tr>
<td>GPDM</td>
<td>glycophosphonic acid dimethacrylate</td>
</tr>
<tr>
<td>2-HEMA</td>
<td>2 hydroxyethyl methacrylate</td>
</tr>
<tr>
<td>4 META</td>
<td>4 methacryloxyethyltrimellitic acid</td>
</tr>
<tr>
<td>MMEP</td>
<td>mono (2 methacryloxy) ethyl phthalate</td>
</tr>
<tr>
<td>MPDM</td>
<td>methacryl propane diol monophosphate</td>
</tr>
<tr>
<td>5-NMSA</td>
<td>N methacrylol 5-aminosalicylic acid</td>
</tr>
<tr>
<td>phenyl-P</td>
<td>2-(meth acryloxy) ethyl phenyl hydrogen phosphate</td>
</tr>
<tr>
<td>PMDM</td>
<td>pyromellitic acid dimethacrylate</td>
</tr>
<tr>
<td>TEGDMA</td>
<td>triethylene glycol dimethacrylate</td>
</tr>
<tr>
<td>UDMA</td>
<td>urethane dimethacrylate</td>
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### Dentine bonding systems

- CFLB2: Clearfil Liner Bond 2, Kuraray
- OB: Optibond, Kerrs
- SBMP: Scotchbond Multipurpose, 3M
- SBMP+: Scotchbond Multipurpose plus, 3M

### Microscopy

- CLSM: Confocal laser scanning microscope
- TSM: Tandem scanning microscope
- OI: Oil immersion
- NA: Numerical aperture
Chapter 1

LITERATURE REVIEW

1.1 Introduction

There is a clinical demand for versatile tooth coloured, aesthetic, adhesive restorative materials which do not prejudice the long term health of the tooth. The advantages of developing a restorative material which adhere to dentine are that:

- a conservative approach to cavity preparation can be adopted, as sound tooth tissue is not sacrificed to provide retention.
- the cavity is sealed and so risk of sensitivity or recurrent caries is eliminated.
- functional stresses can be transmitted across the restorative dentine interface and may reinforce the weakened tooth.

Clinically, modern dentine adhesives are required for composite restorations, especially where cavities are confined to dentine, but also for the cementation of veneers, onlays and inlays. There will be advantage in the use of an adhesive cavity liner with a variety of non-adhesive restorative materials and dentine adhesives also have applications in the placement of preventive restorations, endodontics and in the treatment of dentine hypersensitivity.

Dentine is a vital, wet tissue and adhesion of restorative materials to dentine is more difficult than adhesion to enamel. Adhesive restorative materials must be able to attach to dentine and then be stable in a wet environment.

To date, developments are needed not only in the dentine adhesives, to improve clinical success, but also in the in vitro assessment of adhesive systems during their development and following their release into the market, to improve prediction of clinical performance. The factors affecting the success of bonding restorative materials to dentine relate to the substrate for bonding - the dentine, its structure and...
permeability, and also to the properties of the adhesive resins and restorative materials. Together they are responsible for the final interface between the dentine and the restorative material. The interfacial morphology, the sealing ability and the response of the interface to stresses imparted by the setting restoration and the application of load, therefore form the subject of the literature review for this thesis.

1.2 Dentine permeability

Dentine is a heterogeneous tissue containing tubules with their odontoblast processes. The morphology of the dentine varies from site to site in the same tooth, from tooth to tooth within individuals, and also between species. Throughout life the dentine structure will alter with the production of secondary dentine, in response to physiological and pathological conditions, such as attrition, abrasion, erosion and caries. Changes in the dentine structure will influence the dentine permeability, and clinically may effect the success of bonding techniques for adhesive restorative materials.

In contrast to the wet dentine, composite restorative materials are hydrophobic. Dentine permeability is therefore an important factor when considering placement and longevity of these types of adhesive restorations. As a result, the treatment of the dentine surface prior to bonding, the method of bonding and the effectiveness of the seal of the restoration have all attracted considerable attention. Dentine permeability also plays an important role in the aetiology and treatment of dentine sensitivity, as pain may be induced by a variety of stimuli to exposed dentine causing fluid to move within the tubules. The stimuli include thermal changes, the application of hypertonic solutions and air streams.

Permeability, expressed as fluid movement or hydraulic conductance, has been extensively studied using a variety of in vitro and in vivo models (Pashley et al., 1981a,b, 1984, Brännström and Åström 1972, Vongsavan and Matthews 1992, Ciucchi et al 1995). The techniques involved have included the tracing of radio
active isotopes and the measurement of a single meniscus, air bubble or fat droplet in fluid filled systems connected to the coronal or radicular dentine.

Movement of the fluid in the dentine tubules was likened to capillary fluid movement by Brännström and Åström (1972). The direction and rate of fluid flow in response to thermal stimuli and application of an air spray applied to coronal dentine in vitro were recorded by Brännström et al (1967). The method involved the observation of movement of the meniscus of saline in a glass capillary tube attached to the pulp cavity of an extracted tooth. It was estimated that the tubular contents moved 5 -10 μm in the first second of an air blast. A rapid fluid flow in response to stimuli was related to dental pain in vivo (Brännström 1966, Brännström and Åström 1972). The maximum flow rate recorded was 2-4 mm sec⁻¹ (Berggren and Brännström 1965). The rapid outward flow of fluid in response to air drying has been confirmed by other studies (Pashley et al 1984 and Ciucchi et al 1995).

Variations in dentine permeability and fluid flow in different regions of the tooth have also been studied. The structure of dentine is arranged such that the tubular size and spacing changes from the amelodentinal junction (ADJ) to the pulp surface. SEM studies of fractured coronal dentine were used to determine tubular density and diameter (Garberoglio and Brännström 1976). Near the pulp the tubules were very close together, with 45000 tubules mm⁻², and a mean diameter of 2.5 μm, whereas near the ADJ tubules were widely spaced with 20000 mm⁻², and a diameter of 0.9 μm. The inner dentine will therefore be more permeable than the outer dentine. Dentine permeability will increase as the area of exposed dentine increases or as the dentine thickness decreases.

Pashley et al (1981b, 1984) described a closed fluid system attached to a dentine sample or pulp chamber for use in vivo and in vitro. The movement of an air bubble in a micropipette within the system was used to measure the fluid flow or hydraulic conductance of the dentine sample or tooth. Hydraulic conductance, defined by Pashley (1990) as the ease with which fluid flows across a filtration barrier, is dependent on the fluid flow with time, surface area, pressure outside the tooth and
pressure inside the tooth. The apparatus described has been developed over the years and extensively used to measure hydraulic conductance of dentine under a variety of experimental conditions. In addition to the confirmation that fluid flow of inner dentine was greater than outer dentine, this technique has been used to map other areas of the tooth. The dentine over the pulp horns was more permeable than the occlusal dentine, buccal dentine more permeable than occlusal dentine and coronal dentine more permeable than radicular dentine (Pashley 1990).

When dentine is cut, a smear layer is produced which reduces the dentine permeability. This layer has been examined by scanning electron microscopy and transmission electron microscopy TEM (Boyde 1964, Brännström and Johnson 1974, Pashley 1984). It is composed of the cutting debris and may contain amorphous dentine debris (mineralised collagen matrix), bacteria and elements of the cutting or grinding medium. The smear is adherent to the dentine surface and occludes the dentine tubules with plugs of smear material. The thickness of the smear (0.5 - 15 μm) is dependent on the method of cutting, the speed and the presence or absence of an efficient water irrigation (Gwinnett 1984, Pashley 1984). The smear layer can be reduced by polishing with a fine abrasive paper, and can be removed by treatment with acid or chelating solutions which dissolve the inorganic material. The morphology of the mineralised dentine is addressed in section 1.4.

Although the presence of the smear layer significantly reduces the dentine permeability, clinically, it does not provide a sufficient seal to the tubules to prevent pulp inflammation (Pashley 1984, 1990). In addition, adhesives bonded directly to the smear layer tend to fail between the dentine and the smear layer. The treatment of the smear layer in preparation for dentine bonding is addressed in section 1.4.

As already described, fluid flow through the tubules is, in part, a function of the pulp pressure. This has been measured by a number of workers using in vivo models with animals or humans. Measurements vary according to the species, but more importantly the method and animal used 15 cm H₂O in cats (Vongsavan and Matthews 1972) 32.6 cm H₂O in dogs (Pashley et al 1981b), 14.1 cm H₂O in humans
In vivo the use of local anaesthetic solutions with vasoconstrictors reduce the pulpal blood flow (Kim et al 1984, Pitt Ford et al 1993) and so it might be expected that the dentine pulpal pressure and outward fluid flow would be similarly reduced (Ciucchi et al 1995). The removal of the smear layer after cavity preparation in vivo has resulted in an outward flow of fluid of 0.36 µl min⁻¹ in humans (Ciucchi et al 1995). In dogs a variety of fluid flow rates were recorded according to the location of the dentine, and in addition a reduction of flow rate was recorded with time. This was related to leakage of plasma proteins into the pulp fluid, which subsequently occluded the dentine tubules (Pashley 1990). The clinical importance of the pulpal pressure and outward flow of pulp fluid is in the potential wetness of the dentine surface and in the design of hydrophilic dentine bonding agents which may work in this hostile environment. Pashley (1990) also speculated that the outward flow of dentinal fluid may also have a protective role in restorations with microleakage, decreasing the ingress of bacterial toxins and reducing clinical dentine sensitivity. However, this has not been demonstrated.

Pashley and co-workers have also studied the factors which may affect the diffusion of molecules across dentine (Pashley 1985). As dentine behaves as an impermeable solid transversed by water filled tubules most substances move across dentine by simple diffusion. Once placed on the dentine surface, the solute saturates the adjacent dentinal fluid and begins diffusing down the concentration gradient. The diffusion is therefore not only affected by the patency of the dentine tubules and the pulp pressure, but also the thickness of the dentine to be traversed, and the nature, concentration and molecular weight of the solute. This is important in considering applications of dentine bonding systems, especially those which are designed to ‘chase’ water (see section 1.3).

In all the models described, dentine permeability (fluid flow, rate of diffusion or hydraulic conductance) has been measured by considering the dentine as a whole tissue irrespective of events within individual tubules. The interaction of a fluid with individual tubules has not been observed. There are also limitations in using a single meniscus or an air bubble in fluid systems to detect small pressure changes. A single
meniscus in a capillary (Brännström et al 1967) will be subject to capillary flow in addition to pressure changes in the fluid and the surface tension of an air bubble (Pashley 1984) has to be overcome before there is any movement. These produce inaccuracies in the fluid flow measurements, with a tendency to under record. As the internal diameter of the capillary tube is reduced in an attempt to improve the sensitivity of the system, the effects of the surface tension are increased. The surface tension effect was therefore reduced by the use of a milk fat droplet within a Ringer’s solution, fluid system (Vongsavan and Matthews 1992).

It would therefore be advantageous to develop a model which would allow the direct observation and measurement of fluid movement within the dentine tubules. In addition, such a model would enable the interactions between fluid components of dentine bonding systems and the dentine surface and tubules to be observed.

### 1.3 Dentine bonding

#### 1.3.1 Mechanisms of Dentine Bonding

An adhesive may be defined as a material which when applied to substrate surfaces, can join them together and resist separation (Kinloch 1980). It is clear that intimate interfacial contact is needed for strong adhesive joints. The adhesive must be able to spread easily over the surface of the substrate, have low contact angle and viscosity on application and be able to displace entrapped air in the surface of the substrate. Mechanisms of adhesion are not fully understood. Kinloch (1980) described the following mechanisms: mechanical interlocking, diffusion theory, electron theory and adsorption theory. All but the electron theory are applicable to dentine/adhesive interfaces.

The original concept of resin adhering to dentine was based on a chemical reaction whereby the adhesive would react with the dentine either with the inorganic portion via calcium ions or with the organic portion via amine (-NH) or hydroxyl groups (-OH). Dentine adhesives may be represented $\text{CH}_2=\text{CH-R -X}$, where $R$ is a spacer molecule such as a methacrylate and $X$ is reactive to the dentine for example a
phosphonate. Chemical bonding between the adhesive and the dentine were widely disputed as the practical bond strengths obtained were much lower than theoretically expected (Asmussen and Hansen 1993) and spectroscopic studies have not demonstrated chemical adhesion of resin molecules to dentine (Eliades et al 1990, Spencer et al 1992). Despite their potential weakness, van der Waals forces might contribute to the final bond due to the intimate contact between the adhesive resin and the conditioned dentine (Spencer et al 1992).

A micro-mechanical interlocking mechanism is currently considered the major method of adhesion between the dentine and the adhesive. The dentine is demineralised by acid treatment to expose a collagen network which can be penetrated by the adhesive resin to form a hybrid layer or resin/dentine interdiffusion zone (Nakabayashi et al 1982, Erickson 1989, Van Meerbeek et al 1992). The hybrid layer forms the focus of this thesis and the details of the hybrid layer are presented in later sections.

1.3.2 Dentine Bonding Systems

Classification of dentine bonding systems is not straight forward due to the different treatment regimes for the dentine and the array of chemicals used.

The development of dentine bonding systems has often been described in terms of ‘generations’ (Asmussen and Hansen 1993). The ‘generations’ give a historical perspective and are primarily designated by manufacturers to herald a new direction in product development. As the industry is so competitive, these developments sweep through the companies and result in a batch of similar products being released within a short time period. The generations therefore contain a heterogeneous group of materials bound by a developmental idea rather than primarily a chemical similarity. It is also difficult to define the beginning and the end of the generations.

Morphological classifications based on the treatment of the smear layer were suggested by Pashley (1991b) and Van Meerbeek et al (1992). Three morphological categories were proposed: removal of the smear layer, preservation or modification
of the smear layer and partial dissolving of the smear layer and dentine impregnation. The smear layer removal group was subdivided into two groups; in Mode 1 the resin inter-diffusion zone was limited to the inter-tubular dentine, in Mode 2 the inter-diffusion zone extended into both inter and peritubular dentine. Mode 2 therefore included the more aggressive acids. In a study of ten dentine adhesive systems, Van Meerbeek et al (1994) stated that generally one of two adhesive strategies was followed: either to infiltrate and modify the smear layer, or to demineralise the dentine surface layer and form a hybrid or inter-diffusion zone. Perdigao (1995) proposed a similar classification for dentine bonding systems, based on the treatment of the smear layer and the constituents of the bonding systems.

A further classification using bond strength was proposed by Eick et al (1991). This classification is complicated by the range of bond strength results. Information on the morphology of the systems was also included.

The diverse chemistry and the multiple stages included in the preparation of the dentine dictate that classification of dentine bonding systems should include a combination of morphological and chemical categorisation.

**Preservation or modification of the smear layer**

Little clinical success was achieved with the early dentine bonding systems which ignored the presence of the smear layer and were designed to chemically bond to the dentine. These systems include the first materials developed by Buonocore in 1956, using a methacrylate-based material with a phosphate group, and the later phosphonate-ester-based materials such as Scotchbond (3M). These materials were the ‘first’ and ‘second’ generation systems. The interface between the dentine and phosphate was unstable in water (Huang and Soderholm 1989) and the clinical performance was poor. The smear layer was the site of failure, with the resin unable to penetrate the smear layer to attach to the underlying dentine (Eick 1991). In addition, there was no evidence of the chemical bonding claimed (Eliades et al 1990, Spencer et al 1992).
This group also contains those systems in which the smear layer was modified using ‘primers’ before the application of the bonding resin. The primers included NPG-GMA (for example All Bond, Bisco) or PENTA (for example Prisma Universal Bond) (van Meerbeek et al 1992).

Partial dissolving of the smear layer and impregnation

Van Meerbeek et al (1992) stated that the action of this group lay between the complete removal and the preservation/modification of the smear layer. The smear layer was partially dissolved, creating a limited resin impregnated dentine layer, without completely removing the smear plugs. There were relatively few systems in this category, but included within this group was XR bond (Kerr) which contained a phosphonated dimethacrylate (Van Meerbeek et al 1992).

Also included in this category was Scotchbond 2 (3M) which used a primer containing maleic acid and HEMA (Perdigao 1995). This primer was designed to both demineralise and infiltrate the smear layer, and the degree of smear layer removal was difficult to assess. Indeed this system was categorised in the ‘removal of the smear layer’ group by Van Meerbeek et al (1992). This category was named Group 1 mode 1, whereby the inter-tubular but not the peri-tubular dentine was dissolved and infiltrated. It was given ADA approval, but still had disappointing clinical results. Nevertheless, Scotchbond 2 was seen as an important development in dentine bonding with in vitro bond strengths in the region of 22.9 MPa (Eick et al 1991).

Later in the 1990s systems with self etching primers were developed and these have also been included in this category (Perdigao 1995). The acidic primer in these materials demineralised the smear layer and the underlying dentine, but also contained the adhesive monomer which was polymerised in the within the dentine. The action of these materials was also described by Inokoshi et al (1997) and compared to the acid etch group, which completely removed the smear layer. Examples of this type of system were the experimental conditioners containing phenyl-P and HEMA (Watanabe et al 1994, Nakabayashi and Saimi 1996) and also
the commercially available Clearfil Liner Bond 2 (Kuraray) containing phenyl-P HEMA and 5-NSMA. The latter system has also been classified as a ‘fourth generation’ dentine bonding system.

**Removal of the smear layer**

A variety of chelating agents and acids have been used to remove the smear layer. Acid treatment of the dentine prior to bonding was introduced in the late seventies (Fusayama *et al* 1979), but did not gain immediate acceptance. The acid demineralised dentine was expected to provide mechanical retention for the restorative composites by penetration of the resin into the dentine tubules. The hydrophobic nature of the resin and increased wetness of the dentine, due to the removal of the smear layer, meant that initially acid treatments did not produce the significant improvement in the bond strengths expected with this approach (Torney 1978, Van Dijken and Horstedt 1986).

Systems have since been developed to aid the formation of a hybrid zone. These systems can be subdivided according to the acid/chelating agent and the primer used. The primers were designed to fix the dentine, to provide bonding sites for the adhesive or to improve the adhesive penetration. This resulted in the wide diversity of the ‘third generation’ materials, which included the following systems.

- EDTA was used in GLUMA, Bayer Dental. This system was used in combination with a HEMA and glutaraldehyde primer. Shear bond strength results with this system were favourable (Munksgaard and Asmussen 1985, Finger 1988). The toxicity of glutaraldehyde has been the cause of concern, although Cox *et al* (1988) showed no adverse pulpal reactions.

- Acidic solutions of ferric oxalates were followed by the application of acetone solutions of 5% NTG-GMA and 5% PMDM (Bowen *et al* 1982) and nitric acid preparation was combined with the use of aluminium oxalate in Tenure (Den-Mat). These salts entered the smear layer and were referred as ‘mordants’ to fix the smear layer and provide bonding site for the adhesive resin. The problems
reported with oxalic acids were that precipitates found on the dentine surface and indeed within the dentine which interfered with subsequent penetration of the adhesive resins (Simpson et al 1992, Pashley et al 1993a).

- A 10% citric acid / 3% ferric chloride solution used in the removal of smear layer was reported by Nakabayashi et al (1982). This was followed by the application of 4-META which was designed to promote infiltration of the adhesive monomer into the dentine.

In a variety of systems phosphoric or maleic acid demineralisation of the dentine has been combined with a range of primers designed to promote the entry of adhesive resin into the dentine. The systems, developed in the 1990s, were named ‘fourth generation’ systems. The essential features were: an acid etchant, a primer and an adhesive resin.

**Acid Etchants**

An acid is used to etch both enamel and dentine in the so called ‘total’, ‘all’ or ‘uni’ etch. The effect of acid on the dentine is to remove the smear layer, open the tubules, demineralize the intertubular and peritubular dentine, exposing a network of collagen fibres. The simultaneous preparation of the enamel and dentine with a ‘total etch’ avoids the inadvertent contamination with components designed for use on only one substrate. Phosphoric acid has traditionally been used as an etchant and is most commonly, but not exclusively, used for dentine etching. A variety of other acid solutions or gels have been used in the fourth generation systems, including, nitric and maleic. Several gel preparations have been formulated to aid the application of acid to the desired location. Acid concentration, its presentation (solution or gel) and duration of application have been the cause of much debate and the effect of these parameters on the dentine morphology are presented in the morphology section of this chapter.
**Primers**

Demineralisation is followed by the application of a volatile, hydrophilic primer in either an acetone, ethanol or aqueous solution. The primers are designed as a wetting agent to infiltrate the etched dentine and promote penetration of the adhesive resin subsequently applied to the dentine surface.

Primer molecules have two functional groups: one with an affinity with the dentine, one with the resin (Erickson 1992). The use of primers with hydrophilic and hydrophobic components, dramatically increased the bonding efficiency of resin materials to dentine. This was thought to be by improving the wettability of the dentine and thus the ability of the adhesive to penetrate the demineralised surface of the intertubular and peritubular dentine. Details of the primer action are given in the morphology section of this chapter.

Organic solvents usually acetone and/or ethanol are commonly included in the primers, whereas other primers are in aqueous solution. The primers are applied to the dentine surface and the volatile components allowed to evaporate, prior to adding the adhesive resin. HEMA is also a component of most primers, due to its wetting behaviour, affinity with dentine and ability to polymerise. A range of other monomers are incorporated in primers, and these include GMA, PMDM, PMGDM, and PENTA (Perdigao 1995).

**Adhesive Resins.**

Adhesive resins consist of hydrophobic monomers such as bis-GMA, but in addition, the hydrophilic material, HEMA, is often included to facilitate the wetting of the dentine (Erickson 1992). The adhesive resins bond to dentine by entering the porous etched dentine and subsequent polymerisation of the resin results in a micromechanical interlocking between the resins and the dentine. An interdiffusion zone or hybrid layer is formed (Nakabayashi et al 1982, Erickson 1989). The performance of the interface will be dependent properties of the adhesive resin layer within and above the dentine. These include the ability of the resin adhesive to penetrate and polymerise within the demineralized zone, the thickness of the adhesive layer, the degree of...
polymerisation and the elastic modulus of the resin adhesive (Eliades 1994, Van Meerbeek et al 1992, 1994, Davidson and Abdalla 1994). The properties may be altered by the polymer chemistry or by the inclusion of filler particles. The terminology is therefore rather misleading as the terms resin and adhesive refer to monomers and the terms ‘unfilled’ and ‘filled’ adhesive resins are in common usage.

Since the start of this study (1992) a wide variety of ‘fourth generation’ products have been rapidly introduced into the market. Although it can be simply stated that this generation relies on a total etch, primer and adhesive resin, there are numerous inconsistencies in such things as terminology, the number of different components, and in the number of different clinical stages prior to the placement of the resin composite restoration. This has led to confusion among dental practitioners, with resulting poor acceptance and compliance with manufacturers instructions. The systems include etchants or conditioners which are always/sometimes washed off, primers which may require light curing, adhesive resins which usually require curing but may be self curing and require mixing (Watson and Bartlett 1994).

Despite confusion caused by the extensive range of products manufactured, the fourth generation systems have met with more success than previous generations of materials, producing bond strengths up to 20 MPa in vitro. These results were comparable with bond strengths for composite/acid etched enamel interfaces (Barkmeier et al 1986). In addition, cohesive failure of the dentine during bond strength testing has been reported, a finding seldom seen with the ‘third generation’ adhesives.

Since the experimental work for this thesis was completed, ‘single bottle’ systems have been developed with pressure from clinicians to revert to a ‘simple’ bonding system. The first to be marketed were Bisco One Step (Bisco) and Prime and Bond (Dentsply). Several other manufacturers have followed with products along the same lines. The systems may require the dentine to be etched and, although there is a single component to apply, generally a double application is required. In short, several stages are still required and, as in the fourth generation systems, the systems
vary in their mode of application. These new systems have yet to be fully evaluated in independent studies. Their greater apparent simplicity may lead to better clinical acceptance, but laboratory studies so far reported have shown that the morphology of the interface with these materials is highly dependent on the presence of hydrated dentine, and the removal of excess water on the dentine surface (Tay et al. 1996e).

This thesis describes the use and development of in vitro testing methods to evaluate dentine bonding systems. During the time of the study, time ‘fourth generation’ systems were in use and being developed for release, and it is this generation which are therefore utilised in the studies described.

1.4 Morphology of the interface

The morphology of the interfacial region has been studied by microscopy techniques, and additional information has been gained from surface chemical analysis and nano-indentation. A summary of these techniques are given below and this is followed by a review of interfacial morphology studies using these techniques.

1.4.1 Techniques

Imaging of dentine and dentine/restorative interfaces

A variety of imaging techniques have been used and developed for the examination of dental hard tissues and dentine/restorative interfaces. The optimal requirements are to be able to image the interfacial region in near normal conditions, without disruption or stress induced by preparation techniques. However, in order to examine hard biological tissues it is often necessary to cut the sample to produce a flat surface, a thin section or in the case of dentine/restorative interfaces to expose the interface. These procedures will produce a surface smear layer consisting of amorphous cutting debris, pressure welded to the underlying issue (Boyde 1964). Other features of dental hard tissues, which need consideration when selecting a method of preparation and examination, are the translucency of the dentine, the lack of contrast between the dentine and resin composites or adhesives, and the difficulty of producing ultra-thin sections.
**Conventional light microscopy (LM)**

Transmitted light microscopy is not a practical option for the examination of restorative interfaces with dental hard tissues, as it can only be used if a sample is sufficiently thin to allow the transmission of light. In the case of dental hard tissues with restorations, the sections would be too fragile and would risk separation of the sample components.

The dentine/restorative interface can be revealed by a single section through the tooth. The polished surface of such a sample may be examined with reflected (epi-illumination) LM. However, this gives sparse information as the light interacts with the specimen through a considerable depth and thus is reflected from a large vertical ‘slice’. Image detail is limited as an optically thick section is produced, the greater part of which is out of focus.

**Transmission Electron Microscopy**

TEM has been used to examine the ultrastructure of demineralised dentine and the resin dentine interface (van Meerbeek 1993a). Tooth samples were decalcified with EDTA before making ultrathin (90 nm) sections. Preparation may also involve the samples being embedded in epoxy resin. Preparation of calcified tissues samples for examination with a TEM was described by Goret-Nicaise and Dhem (1987). The difficulties of this technique are the preparation of the thin sections and the interpretation of the photomicrographs, bearing in mind artefacts due to fixation.

**Scanning Electron Microscopy**

The SEM has been widely used to image dental hard tissues and to evaluate restorative materials and techniques (Boyde 1964, van Meerbeek et al 1992, Titley et al 1994). Surface characteristics of samples can be rapidly evaluated and it has the advantages of a large depth of field and high resolution. For examination of the interfacial region samples need to be sectioned and polished. Samples need to be sputter coated (generally with gold) in a vacuum prior to examination. Dehydration shrinkage during preparation results in contraction stresses and crack artefacts, particularly in the dentine with its high water content. This problem can be reduced by, environmental
SEM (ESEM), which allows the examination of wet samples (Gwinnett 1994a, Cowan et al 1996, Inokoshi et al 1997) or by freeze drying or critical point drying (CPD) techniques, or by drying with hexamethyldisilazane (HMDS) or in Pedri II (Perdigao et al 1995, Carvalho et al 1996a) prior to sputter coating. Caution has been advised in measuring, for example hybrid layer or gap size, without a correction factor to allow for sample shrinkage.

Further information about the interfacial region may be gained by the differential removal of the layers which make up the dentine/restorative interface. Argon beam etching improves the contrast of the different layers at the interface, by preferentially removing the resin impregnated dentine layer which has low resistance to argon ion bombardment (Boyde and Stewart 1962). This technique was used for polished resin embedded dentine samples by Van Meerbeek et al (1992). They reported that the structure of the interface was enhanced with a 30 second etch on a circular target with an argon ion beam without cooling. The adhesive resin layer and resin tags were highly resistant to etching and appeared dark when examined with an SEM.

Replication techniques using vacuum stable materials such as dental impression addition silicones overcome the dehydration shrinkage problem of sample preparation for SEM examination (Grundy 1971, Barnes 1978, 1979). However, such techniques are generally used for evaluation of marginal adaptation of restorative materials in vitro and in vivo, and do not provide sufficient detail for the study of an interdiffusion zone.

**Atomic Force Microscopy**

AFM operates on non-conductive specimens in atmospheric conditions and is able to produce high resolution 3D images of surface topography of dental hard tissues (Marshall et al 1993, Grayson and Marshall 1993). Images are produced via a scanning Si$_3$N$_4$ probe tip which, with the aid of a laser beam, photo detector and piezo tube, maps the topography of the sample under examination. The probe may operate in contact or tapping mode. In contact mode, the probe exerts a force in the region of $10^{-4}$ N. It is influenced by adhesive and frictional forces, and may damage soft, hydrated tissues. These problems are reduced by the tapping mode, as it does not exert shear.
forces on the sample and so produces few artefacts and allows reproducible
measurements. This development has been advantageous in the study of dentine even
when demineralized (Marshall et al 1993). The use of this technique has been reported
for the examination of etched dentine (Marshall et al 1993) and dentine/restorative
interfaces (Grayson and Marshall 1993). Only small samples (1mm thick) were
examined in these studies and fieldwidths were 20 μ m^2 -100 μm^2.

Confocal Microscopy
The main advantage of confocal microscopy is that it offers the possibility of
sub-surface imaging of translucent mineralised tissues, including dentine, in near
normal conditions.

Confocal scanning microscopes eliminate scattered, reflected or fluorescent light from
out of focus planes within a sample. Although surface images may be produced, the
main benefit is to produce thin subsurface optical sections. A series of images of
increasing depth within the sample can be made. This technique is ideal for semi­
transparent samples such as dentine or other mineralised tissues. Images of up to 80 μm
below the surface of a tooth can be produced with confocal microscopy. Illumination
sources may be incoherent white light from a mercury arc or coherent light from a laser
source. The microscopes may be operated in fluorescence or reflection mode. The
labelling of components of dentine bonding systems and glass ionomer cements with
fluorescent dyes for improved contrast of components at the dentine /restorative
interface has been reported (Watson 1989).

Unlike other microscopy techniques, very little sample preparation is required.
Samples can be kept hydrated and although a flat surface is advantageous, a surface
smear layer is of little importance, as the subsurface of the sample is imaged. Thus
extensive sample preparation, such as thin sectioning or embedding, is avoided.
However, reduction of the smear layer by gentle polishing will improve the quality of
an image because the smeared material acts as a light scattering medium.
In some confocal microscopes, rapid scanning of the sample produces a fast enough frame rate to be used to observe high speed events such as the cutting of enamel at video rate (25 frames sec\(^{-1}\)) (Watson 1990). *In vivo* confocal microscopy has also been developed to image oral tissues including soft tissues, teeth and restorative materials (Watson *et al* 1992). Further information on these techniques is given in the section on confocal microscopy (1.8).

**Chemical Analysis**

*Micro Raman Laser Spectroscopy (MRLS)*

MRLS is a surface analysis technique and has been used for obtaining both chemical and structural information in minute areas of dental samples (Suzuki *et al* 1991). Raman measurements are made in normal atmospheric conditions without the need for the application of a high vacuum. No specific sample dimensions or translucency are required, but surface grinding and polishing are needed. The samples can be further used for SEM examination.

A laser beam with small spot size (1 \(\mu\)m) is focused on a sample. The Raman spectra produced are used to determine the structure of molecules, and for chemical analysis; producing high lateral resolution maps of the molecular composition of the sample surface.

Raman spectroscopy has been used as a microprobe for the chemical characterisation of the resin/dentine interdiffusion zone, and has provided additional information about morphology (van Meerbeek 1993). An argon-ion gas laser with 514.5 nm wavelength was used in these studies and agreement was reported between the information on the interdiffusion zone revealed by MRLS and SEM.

*Fourier transform Infra red (FTIR) Photo-acoustic Spectroscopic Analysis*

In photo-acoustic spectroscopy the heat generated by the absorption of light at the surface of a solid sample is measured (Spencer *et al* 1992). FTIR spectroscopy can be used to study solid/aqueous interfaces, such as hydrated dentine samples (Eliades *et al* 1997). The technique provides a non-destructive method of determining the molecular
composition of the surface layer of a sample. The chemical analysis of the interfacial region by FTIR technique has not provided evidence of any primary chemical adhesion between the resin molecules and the dentine. The technique has been used following AFM to investigate the effect of acid conditioners on dentine morphology, molecular composition and collagen conformation (Eliades et al 1997). The drawbacks of the technique are the spectral interference from water in the sample and the limited lateral resolution. (Edler et al 1991, Spencer et al 1992).

Physical Analysis

**Nano hardness**

The use of a computer controlled nano-indentation technique has been described to measure the Young’s modulus and hardness of the interdiffusion zone (Van Meerbeek et al 1993c). Minute indentations were made with a triangular tip indenter, a few microns in diameter, using loads of a 1-20 μN. Nano hardness measurements were calculated by measuring the distance from the surface of the specimen to the depth of the indentation. Van Meerbeek et al (1993c) reported that the advantages over the Vickers or Knoop hardness tests were the small size of the indenter, which allowed narrow areas of material to be assessed, and that the technique used gave an assessment of both hardness and elasticity. It may be possible that results were dependent on the nano-indenter contacting the polymerised resin or the filler particles in the adhesives and restorative materials, in addition, establishing the relationship between the test site and the anatomical site can be a problem with this type of measuring technique. Accurate data regarding the elasticity and hardness are valuable when considering the interfacial behaviour in function, with polymerisation stresses or under load.

Having considered the techniques available for investigating interfacial morphology, the results of previous studies are now presented.

1.4.2 **Review of interfacial morphology - Results of previous studies**

The morphology of the dentine/resin interface is dependent upon the preparation of the dentine, (which may involve acids and primers or self etching primers), and the
composition of the adhesive resins applied. This part of the literature review summarises the appearance of the dentine after acid treatments and the effect of primers and resins on the appearance of the interdiffusion zone (hybrid layer) and the adhesive resin interface.

**Effect of acids on the dentine.**

Acids totally or partially remove the smear layer from the dentine and open the dentine tubules. In addition, the intertubular and peritubular dentine is demineralised to expose a network of collagen fibres (Pashley 1992, Eliades 1994). Between the unaltered dentine and superficial collagen network is a zone of partially demineralised dentine (van Meerbeek *et al* 1992, Tay *et al* 1996d). The permeability and wetness of the dentine is increased, as is the potential micro-porosity of the demineralised dentine surface (Pashley *et al* 1992, Pashley *et al* 1993b, van Meerbeek *et al* 1992).

The appearance of the demineralised dentine is dependent on the type of acid, concentration, application time and type of thickening agent. Commercially available etchants have included phosphoric, nitric, maleic or citric acids. Concentration and application times in total or uni etch systems are designed so as to etch the enamel sufficiently without extensively demineralising the dentine. In general concentrations range from 10-40%, with an application time of 15-20 seconds.

Decalcification depths measured by SEM techniques have been reported as 0.5 μm with EDTA, 1 μm with <4% maleic acid, 6 μm with 2.5% nitric acid and 7.5 μm with 10% phosphoric acid (Van Meerbeek *et al* 1992). Two demineralisation patterns were reported in this study, according to the type of acid. Preparation with EDTA, or 2.5 - 4% maleic acid resulted in a ‘mode 1’ appearance with demineralisation of the intertubular but not peri-tubular dentine, whereas more aggressive preparation with nitric, phosphoric acid or oxalic acid gave a ‘mode 2’ appearance with decalcification of the surface and peritubular dentine. A later study using TEM concluded that the deep demineralized dentine probably contained residual mineral particles (Van Meerbeek *et al* 1993a). It was shown by applying varnish to part of the dentine surface that a surface
layer of the substrate is completely removed by the acid solutions and so comparisons of depth of demineralization and depth of resin infiltration or interdiffusion zone need to be interpreted with care (Van Meerbeek et al 1992, Perdigao 1995).

Acids are generally presented as coloured gels. The colour ensures that the etchant can be seen clearly when applied to the dentine and gel preparation gives better control of application than the original fluid preparations. These gels use colloidal silica or polymers as thickeners. Overall results of studies examining the effect of the different thickening agents have been inconclusive. The silica micro-particles leave a residue on the dentine which cannot be removed by vigorous washing, whereas the polymer thickened gels were generally reported to leave a clean surface after rinsing, and were associated with a deeper demineralisation zone (Kubo 1991, Perdigao et al 1994, 1996). More recently, Eliades et al (1997) found evidence of residual polyvinyl alcohol (PVA) particles using FTIR studies. Kanca (1993) also highlighted the potential problem of silica thickeners being adherent to the dentine surface and reported an associated reduced bond strength, but similar reductions in bond strengths have not been reported elsewhere (Swift 1993, Uno and Finger 1995). Thus although much has been written about the merits or otherwise of the various thickening agents, the significance of these agents remains unclear.

Further characterisation of the decalcified dentine surface was presented by Pashley et al (1993b) and Gwinnett (1994a). The collagen matrix is normally supported by hydroxyapatite crystals. When this support is removed during decalcification the collagen matrix may collapse and the spaces between the collagen fibres decrease, especially if the dentine is desiccated in preparation for SEM studies or air dried as part of a clinical procedure. Evidence of this collapse was presented as the collagen network or 'cushion' bulging out laterally and narrowing the tubules when examined in longitudinal section by SEM (Van Meerbeek 1992). Coalescence and collapse of the outer fibres of the collagen network was also described by Pashley et al (1993b) and Gwinnett (1994a). In the latter study, demineralised dentine surfaces which had been desiccated in SEM preparation, or by air drying in an ESEM had a smooth surface with
open funnellled tubules, whereas the fibrous integrity of the collagen network was preserved by critical point drying (CPD).

The effect of acids on dentine have also been recorded with AFM, illustrating that the intertubular matrix begins to collapse during the initial stages of demineralisation (Grayson and Marshall 1993). An increase in dentine surface roughness has similarly been reported by Eliades et al (1997). In the latter study denaturation of the surface collagen was also demonstrated by FTIR.

These studies illustrate the delicate nature of the hydrated demineralised collagen network and the importance of its treatment both in sample preparation for examination and clinical procedures prior to bonding.

**Primers.**

An open collagen network is required if adhesive monomers are to be able to infiltrate the demineralised dentine surface to form a hybrid layer (Nakabayashi et al 1982). The primers are thought to penetrate and expand the collagen network, opening the interfibrillar spaces into which the adhesive resin may then penetrate. However, if the dentine surface is air dried after etching, evaporation of water from the collagen matrix may increase the collapse of the fibrillar network. This reduces the interfibrillar spaces and hinders the infiltration of monomers (Pashley 1993b, Perdigao 1995). Denatured surface collagen and the residues of the smear layer which remain on the surface of the demineralised dentine, together with the collapsed fibre network, are thought to be responsible for the 'electron dense' layer described in TEM studies at the interface (van Meerbeek et al 1993a, Tay et al 1996d). It has been speculated that the penetration of the primers may be through the open tubules and the superficial layer of the peritubular dentine, following a lateral path to the intertubular dentine. The primers may then raise the collagen matrix, increasing the interfibrillar pore size for resin infiltration (Perdigao 1995).

A variety of methods have been used in an attempt to prevent or overcome the collapse of the collagen network. Stabilising or re-expanding the collagen matrix has been seen
as one of the functions of primers. Ferric chloride has been used to try to stabilise the matrix (Nakabayashi et al 1982) and an aqueous solution of HEMA or salicylic acid used to re-expand the matrix (Tay et al 1996d). It would appear that a dehydrated surface of collagen fibres may be restored to an open network by re-wetting the dentine surface (Gwinnett 1994a, Tay et al 1996d). The use of ‘wet bonding’ techniques, where the dentine is not dried (Kanca 1992) or where the dentine is re-wetted (Gwinnett 1994b) have also attracted a great deal of attention.

The difficulty of the wet bonding techniques is the clinical interpretation of ‘wet’ or ‘moist’ dentine. Excess water on the dentine surface has been shown to result in a globular appearance at the interface as primer droplets are included in the residual water and are then trapped at the interface (Tay et al 1996 a,b,c).

Acetone is commonly used in primers as it readily evaporates, and is also considered to ‘chase’ water in the collagen matrix (Gwinnett 1992, Gwinnett and Kanca 1992a). Monomer in the primer is thought to replace water between the collagen fibres as the acetone evaporates along with the water. Primers based on acetone or alcohol solvents may be more dependent on a wet bonding technique. Conversely, primers in aqueous solution may contain sufficient water to re-wet and re-expand the collagen fibres. This may be a better approach to ensuring that the demineralised dentine has an open, but not over wet collagen network. When water free primers were applied to dry demineralised dentine a thin hybrid zone was observed, whereas a thicker hybrid zone and complete wetting of the fibrils with the resin was observed with SEM and TEM when the primers were applied to moist dentine (Tay et al 1996d). In the dry conditions, the area of demineralised dentine below the hybrid zone not infiltrated by resin was referred to the ‘hybridoid’ region.

From these studies, it may therefore be concluded that the dentine should not be excessively desiccated with an air jet after rinsing the etchant, and the primer should be based on an aqueous solution so that the water may re-wet the demineralised dentine, where necessary, without the danger of inducing the ‘over wet’ phenomenon.
It may be summarised that, although, there is agreement that the quality of the hybrid layer is dependent on the effective infiltration of the primer, in many cases the conclusions reached have been deduced via the appearance of the final hybrid layer and performance of the interfacial region, for example in bond strength studies. Direct observation of changes in the morphology of the dentine as a result of primer application and the distribution of the primer within the interface are generally lacking.

There is therefore a need to observe the interface with minimal preparation, in near normal conditions, to record the behaviour of primer on application to the dentine and record the distribution of the primer within the interdiffusion zone.

**Hybrid or Interdiffusion zone**

Infiltration of resin into conditioned intra and inter tubular dentine was first illustrated by Nakabayashi *et al* (1982), using a conditioner with 10% citric acid and 3% ferric chloride in conjunction with a 4-META to promote both monomer infiltration and adhesion at the dentine surface. This infiltrated dentine layer was and has been named the ‘hybrid zone’. There is general agreement that the polymerisation of the infiltrated monomer results in micro-mechanical adhesion of the resin to the dentine (Nakabayashi *et al* 1982, Erickson 1989, Pashley 1991b).

The appearance of dentine/resin interfaces of a variety of bonding systems in use in the early 1990’s was evaluated by van Meerbeek *et al* (1992) (see section 1.3). The assessment was made using SEM with argon etching and the interfaces were characterised according to the changes in the smear layer. Group 1 - removal of the smear layer, Group 2 - preservation of the smear layer and Group 3 - partial dissolution of the smear layer.

The group 1 dentine bonding systems involving the use of an acid conditioner followed by a monomer with hydrophilic and hydrophobic groups have gained most attention during recent years. Van Meerbeek *et al* (1992) referred to the conditioned dentine infiltrated with resin as the ‘resin-dentine inter-diffusion zone’. This zone
had low resistance to argon-ion bombardment and varied in width (0.5-2.5 μm) with
the different bonding systems.

TEM studies (van Meerbeek et al 1993a) and Raman profiles (Suzuki et al 1991, van
Meerbeek et al 1993b) supported the conclusions of the SEM studies. Three layers in
the interdiffusion zone were identified. An upper denatured collagen layer
completely incorporated by resin, a mid collagen meshwork with resin in the
interfibrillar spaces, and in the partially demineralised dentine at the base of the
interdiffusion zone hydroxyapatite crystals encapsulated by resin. A gradual
transition of the resin to dentine was identified both morphologically and chemically.
This gradient was also identified in nano-indentation hardness studies (van Meerbeek
et al 1993c).

Concern has been expressed about two areas of the interdiffusion zone: namely the
dentine surface and the demineralisation front in the deeper dentine (Erickson 1992,
Pashley 1992). At the surface it is speculated that a dense mat of collapsed and
denatured collagen may not be easily penetrated by the resin monomers.

Deeper in the dentine, there may be a failure of monomers to penetrate the full depth
of the demineralised zone, thereby leaving a permeable zone of partially
demineralised dentine above the deeper sound dentine (van Meerbeek et al 1992,
Eick 1993). Pashley et al. (1993b) suggested a schematic model of resin penetration with
the highest concentration above the dentine surface, lowest in the demineralized,
denatured collagen surface, and intermediate below the surface, with the resin ideally
filling the spaces between the collagen fibres. Evidence for lack of penetration to the
full depth of demineralisation will be considered in the section on micropermeability.

**Self etching primers**

Systems using self etching primers have continued to be developed along side the
acid/primer systems. The rationale is to combine the conditioning and priming stages
to simplify the clinical procedures, but also to ensure that there is no discrepancy
between the depth of demineralisation and the depth of resin infiltration as both
processes occur together. These primers have included phenyl-P, 5-NMSA, and HEMA. In theory, the primers are designed to have an intrinsic acidic activity enabling penetration of the smear layer to demineralise the underlying dentine, creating porosities for the simultaneous entry of monomers. As the demineralisation and monomer penetration result from a single application to the dentine it is considered that the problems of collagen collapse and disparity in the depth of demineralisation and monomer penetration may be minimised. SEM and TEM studies have provided evidence for the production of a hybrid layer with these formulations in bovine teeth and have concluded that diffusion channels were created into the dentine, permitting the monomer to fully infiltrate into the dentine to the depth of the acid demineralisation (Chigira et al 1994, Watanabe et al 1994, and Nakabayashi and Saimi 1996). The depth of the hybrid layers reported was reduced compared to those produced by ‘total etch’ systems and also dependent on the duration of the primer application (2.1- 4.1 μm in bovine samples Nakabayashi and Saimi 1996).

1.4.3 Summary

From the extensive literature published, a picture is emerging of the morphology of the interface with a variety of dentine adhesives and more importantly, the influence of the components of the systems on the interfacial morphology.

Although many techniques reported have allowed examination of interfaces at high magnification, they have also involved considerable sample preparation. In addition, specimen dehydration radiation damage and charging effects induced in the dentine by high energy electron and vacuum systems may lead to important artefacts, of most concern being the dehydration of the dentine and the restorative materials. Confocal microscopy techniques allow the subsurface examination of undisturbed interfaces with little preparation and fluorescence confocal microscopy has been used to examine dentine/restorative interfaces. It was therefore proposed to develop these techniques to examine and record the dynamic events at cavity surfaces and the morphology of dentine/restorative interfaces.
1.5 Micropermeability

In vitro assessment of dentine/restorative interfaces may include an evaluation of interfacial seal, in addition to studies of the interfacial morphology. This has been investigated on two levels; traditional leakage studies to assess gap formation, and more recently, nanoleakage studies, where the interfacial seal is examined at a higher magnification to detect interfacial porosities even in the absence of marginal gap formation.

In the short term, a lack of interfacial seal may be responsible for sensitivity to thermal stimuli and in the longer term caries and staining at the margins of restorations. The ingress of bacteria and bacterial toxins may also set up an inflammatory reaction in the pulp (Pashley 1990). The hydrolysis of the bond between the restorative and the dentine in the presence of water at the interface has previously been cited as a problem. More importantly, the biocompatibility of the material is reduced if monomer is able to leach from incompletely polymerised resin into the dentine tubules and pulp (Cuicchi and Bouillaguet 1997).

The ingress of fluid from cavity margins along gaps between the dentine and restorative material has been evaluated in microleakage studies. A variety of methods have been used to measure the extent or depth of penetration of dye or other markers along the restorative/dentine interfaces (Bauer 1984). A single section or multiple sections of the restored tooth may be used in the assessment, but generally, such methods give only a gross assessment of the quality of the interface. It is recognised that microleakage studies have only a limited value in the prediction of clinical performance of restorations in vivo. However, it is generally accepted that with an enhanced in vitro sealing ability, an increased rate of clinical success may be anticipated. The dentine permeability and the reaction of the pulp will determine the clinical response to microleakage (Pashley 1990).

Morphological studies suggest a good adaptation between the restorative material and the dentine via a hybrid layer with the newer dentine bonding systems (Watson 1989, van Meerbeek et al 1992, Sano et al 1994a). Even in the absence of gap formation, it is
important to assess the micropermeability of an interface and to establish the site of any porosity and failure of seal within the interface. Recent studies have examined dentine/adhesive interfaces at high magnification to assess ‘nanoleakage’ through the porosities in the interdiffusion zone (Sano et al 1994 a, Sano et al 1995 a,b). Porosities in the interfacial region may result from incomplete infiltration of the primer and adhesive resin into the conditioned demineralised dentine, and/or shrinkage of the resin away from the dentine during polymerisation. The clinical significance of nanoleakage has not been evaluated, but it is hypothesised that interfacial failure may progress if fluid is able to penetrate the interface and cause degradation of the demineralised collagen fibres and adhesive resin molecules.

Nanoleakage has been assessed by the penetration of silver nitrate directly through a sectioned interface or from the outer surface of a tooth (Sano et al 1994a, Sano et al 1995a). In the absence of gap formation as detected by morphological studies, penetration of silver nitrate into the hybrid zone was demonstrated by SEM studies using restored bovine teeth. High magnification revealed that the silver nitrate had penetrated between the adhesive resin and the mineralised dentine. This zone corresponded to the demineralised zone as identified by cryo SEM studies.

The chemistry of adhesives and restorative materials and the technique employed in their application to the dentine will influence the interfacial seal. Some primers and adhesives have been designed to reduce dentine permeability and may be used also as sealers in the treatment of hypersensitivity (Pashley et al 1988). The ‘wet bonding technique’ may result in a residue of water at the interface where priming or bonding has taken place in over wet conditions (Tay et al 1996b): this will have a detrimental effect on the interfacial seal (Inokoshi et al 1997). A summary of the results of a selection studies examining the microleakage and nanoleakage of ‘fourth generation’ dentine bonding systems examined in this thesis is given in Table 1.1.

The problems with the present methods of assessment of interfacial seal are the lack of detail in the microleakage studies and extensive preparation of the samples required for the nanoleakage studies. It may be possible to gain further information regarding the
interfacial seal with fluorescence confocal microscopy with the interface examined at high magnification in near normal conditions after minimal preparation.

The studies to date have examined the ingress of fluid from the external surface of the tooth, or the uptake of silver nitrate by the interfacial area when exposed by sectioning (Sano et al 1995a). The seal to pulpal fluid has not been investigated. Outward flow of fluid from the pulp to exposed dentine surfaces has been confirmed by a variety of studies (Pashley and Pashley 1991). Interfacial seal to pulp fluid would provide an excellent method of assessing interfacial porosity and loss of attachment between the adhesive and the tubule wall, and would not be reliant on fluid reaching the interface from the external surface of the tooth. Indeed the pulp fluid may be responsible for the degradation of the exposed collagen and resin, and the continued breakdown of the interface, even in the absence of a communication with the external surface of the tooth.
Table 1.1 Summary of leakage studies

Examples of methods and results of microleakage/nano leakage studies using 4th generation dentine bonding systems used in this thesis.

<table>
<thead>
<tr>
<th>Authors &amp; date</th>
<th>Cavity preparation</th>
<th>Storage</th>
<th>Testing method</th>
<th>Materials tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sano et al. 1995b</td>
<td>Class V cervical saucer shaped cavities.</td>
<td>Tested after 24 hours at 37°C.</td>
<td>Nano leakage detected with silver nitrate from the outer tooth surface. Multi sections were made for examination with SEM.</td>
<td>✓ silux (KB200) - silux</td>
</tr>
<tr>
<td>Ferran &amp; Davidson 1996</td>
<td>Class II restorations, cervical margins below CEJ. In vivo and in vitro.</td>
<td>In vivo extracted after 2-3 months. In vitro - thermocycled 5-55°C 250 times.</td>
<td>Microleakage detected with 2% methylene blue solution from the outer tooth surface.</td>
<td>✓ +Z100 - -</td>
</tr>
<tr>
<td>May et al. 1996</td>
<td>Box shaped Class V restorations.</td>
<td>Samples thermocycled 5-55°C 500 times.</td>
<td>Microleakage detected with silver nitrate from the enamel surface.</td>
<td>✓ +Z100 - +XRV</td>
</tr>
<tr>
<td>Swift et al. 1996</td>
<td>Wedge shaped Class V cavities, into enamel above, denture below.</td>
<td>Filled with a single increment Z100. Samples thermocycled 5-500°C 800 times.</td>
<td>Microleakage detected with silver nitrate.</td>
<td>✓ +Z100 +Z100 +Z100</td>
</tr>
</tbody>
</table>
1.6 Polymerisation stresses

The polymerisation shrinkage of resin composites is well recognised (Bowen et al 1983). The shrinkage occurs in the adhesive and resin composite during the conversion of the monomer to polymer. It results in the development of stresses within the restorative material and at the dentine/adhesive resin interface. The magnitude of the stresses are dependent on the amount of shrinkage and the ability of the materials to yield (Feilzer et al 1990, Lutz et al 1991). Stress relief may be afforded by the flow of composite in the unpolymerized areas and so, the ability of the composite to flow has a significant role to play in the ultimate stress development (Feilzer et al 1993). Composites exhibit a viscoelastic response, with the behaviour being dependent on the rate of the force applied.

The shrinkage stresses are in competition with the adhesive bond at the composite dentine interface. If these stresses are too great they will contribute to interfacial gap formation and micropermeability, if the interface is disrupted; or to cusp deflection if the interface remains intact (Pearson and Hegarty 1989, Meredith and Setchell 1997).

Composite Restorative Materials

Commercially available composite materials are composed of organic polymers based on bis-GMA or urethane dimethacrylate (UDMA), an inorganic filler phase, a coupling agent and an initiator/activator system for polymerisation (Rees and Jacobsen 1989). The monomers undergo free radical addition polymerisation and are capable of cross linking. The viscosity of the monomers is relatively high, and so diluent monomers (eg TEGDMA) are added to improve the handling properties. Unfortunately, the addition of these monomers also increases the polymerisation shrinkage. Composites have been characterised according to filler particle size, filler loading, Young’s modulus of elasticity and Vickers hardness (Willems et al 1992).
**Polymerisation**

Polymerisation of composites may be activated by the mixing of two pastes one containing an initiator (usually benzoyl peroxide) and the other an activator (a tertiary amine) (Watts 1992). These are the so called 'chemically activated' or 'auto curing' composites. Alternatively, polymerisation may be activated by an external energy source, using an intense visible (blue) light around 470nm from a curing unit (VCL). These composites employ photosensitised free radical initiators and an amine reducing agent (Watts 1992). They are commonly referred to as 'light activated' or 'light cured' composites. The nomenclature is somewhat misleading as in both cases the polymerisation involves a chemical reaction. However, in this thesis the terms 'light activated' and 'chemically activated' will be used.

**Setting stress**

During polymerisation the composite changes in its physical properties and therefore its response to stress. Plastic flow may occur during the pre-gelation stage and this may compensate for the effects of shrinkage. However in the post-gelation phase rigid contraction results in stress development, and is of clinical importance. Its magnitude, relative to strain, is dependent upon the visco-elastic response of the composite. Compliance of the surrounding structures may afford further stress relief, or alternatively there will be adhesive or cohesive failure and a loss of interfacial integrity (Watts 1992, Feilzer et al 1995). The shrinkage stress is influenced by the rate of polymerisation. The shorter the period where flow of the composite can occur the greater the stress. Light activated composites reach the gel point rapidly, generally at 15% conversion.

A method of measuring setting stress using samples of composite resin between two discs, one attached to a load cell and the other to a tensometer, was used in a series of investigations by Davidson et al (1984) and Feilzer et al (1987, 1993). Any axial contraction of the restoration was counteracted by displacement of the cross head to re-establish the original inter-disc distance and shrinkage stress was recorded continuously.
**Degree of Shrinkage**

The final polymerisation shrinkage is dependent on monomer composition, degree of final polymerisation, filler type and composition. A range of 24 hour shrinkage values of 2.6-7.1% were reported by Feilzer et al (1988), and Watts and Cash (1991) reported a similar range of 0.65% to 7.9% for visible light activated materials using a ‘deflecting disk’ technique. Currently there are no resin composite restorative materials which exhibit low (or 0%) shrinkage or expansion during polymerisation. However, there are investigations into the use of spiro orthocarbonates (SOCs) which expand during polymerisation (Thompson et al 1979, Stansbury 1990, Eick et al 1993).

Where the composite is imperfectly polymerised the shrinkage will be correspondingly reduced. The degree of polymerisation (conversion) is a measure of the number of C=C bonds converted to C-C bonds. It is generally in the region of 50-70%, (Ferracane and Greener 1986) and may be measured by infra red spectroscopy (IR) or nuclear magnetic resonance (NMR) (Watts 1992, Watts et al 1996).

**Porosity and air inhibition**

The mode of activation and porosity both influence polymerisation rate and setting stress (Feilzer et al 1993, Alster et al 1992). Chemically activated composites are generally characterized by relatively slow polymerisation which takes place almost uniformly throughout the material and is independent of the depth of the restoration, as long as the material has been correctly mixed. This results in a lower polymerisation shrinkage stress as compared with that of light activated composites (Feilzer et al 1993). The mixing of materials introduces porosity. Chemically activated composites are mixed by definition, but increasing the porosity of light activated composites, by pre-polymerisation ‘mixing’ spatulation, has been shown to decrease setting stress. Porosity may reduce the rate of stress development and degree of stress by either oxygen inhibition due to the admixed air or by increasing the free surface area associated with the pores in the bulk of the composite (Alster et al 1992, Feilzer et al 1993).
Oxygen interferes with the polymerisation of resin restorative materials and adhesives by reacting preferentially with the free radicals produced to activate the polymerisation process. This results in an air inhibited layer of variable thickness according to the monomers present, values of 7-84 \( \mu \text{m} \) were reported by Rutyer (1981). The potential of the monomers to polymerize is not lost if supplied with sufficient free radicals and initiators diffuse into the un-polymerised material (Elaidès and Caputo 1989). This is of importance in the mixing and subsequent polymerisation of adhesive resins at the resin composite interface.

**Light intensity**
Polymerisation of light activated composites is not uniform throughout the depth of the composite restoration as it is dependent on the light activating the release of free radicals to initiate polymerisation. The variable depth of polymerisation arises from attenuation of light transmission in these materials (Watts 1992) and this variation of depth of activation may be studied by examining microhardness profiles (Watts *et al* 1984a) or degree of conversion Ferracane (1985). Watt and Cash (1994) quantified the amount of light transmitted through dental biomaterials and found that there was considerable reflection from the surface of composite materials (30-90%), depending on the material examined.

Light curing units for light activated composites were reviewed by Shortall and Harrington (1996). Wavelength, intensity, irradiation time, distance from light source to composite, the composite filler, shade and restoration thickness were considered important variables in obtaining adequate polymerisation. A small change in light intensity has been reported to cause a significant change in the degree of conversion. The extent of polymerisation 2mm below the surface of the restoration was reported as being dependent upon; the exposure duration, and intensity of the light, and further reduced by increasing the distance between the light source and the composite (Rueggeberg and Jordan 1993). It was suggested that a light intensity of 233mW/cm\(^2\) would provide sufficient energy to adequately cure a layer composite 1mm thick (Rueggeberg *et al* 1993) and later >300mW/cm\(^2\) was recommended (Shortall and Harrington 1996).
Without complete polymerisation of the composite, the mechanical properties of the restoration are compromised. Ferracane and Greener (1986) reported a relationship between the increased mechanical properties (tensile strength, compression strength, hardness and flexural modulus and strength) and higher degrees of conversion.

There has been interest in developing high intensity lights to produce rapid polymerisation, with increased depth of activation. However, concern has been expressed that with a high intensity light the rate of polymerisation and of shrinkage is increased and may allow insufficient opportunity for composite flow and stress relief (Uno and Asmussen 1991, Feilzer et al 1995). Indeed high light intensities were reported to negatively affect interfacial integrity when a high output light intensity (650 mW cm$^{-2}$) was compared to a low light intensity (250 mW cm$^{-2}$) cure in shallow class V cavities (Feilzer et al 1995). Similar findings were reported by Uno and Asmussen (1991). It would also appear that mechanical properties (fracture toughness and flexural strength) of restorations are not decreased by curing at lower light intensity as long as the irradiation time was increased (Miyazaki et al 1996). There is therefore support for the use of moderate light intensity, with recognition that the degree of conversion is also dependent on other factors such as exposure time, type of resin and depth of restoration.

**Cavity configuration**

Polymerisation stress in resin composites has also been studies as a function of restoration shape. Initial studies by Davidson et al (1984) speculated on the importance of cavity design on composite flow allowing stress relief and interfacial integrity being maintained only when the bond strength exceeded the shrinkage stress. Later the restoration shape was described by the configuration factor (C-factor)- the ratio between bonded and non bonded (free) surfaces of the restoration (Feilzer et al 1987). A class I occlusal cavity is described with a ratio of 5:1 and thus a high C-factor, whereas a class V abrasion cavity, a ratio of 2:1 and thus a low C-factor. Values of C<1 refer to composite layers applied to a flat surfaces, or those with shallow curves. The shrinkage stress for cavities was determined by the use of disc samples with diameter and height ratios to simulate the c-factor of clinical
restorations (Feilzer et al 1987). The polymerisation stress was greater in those cavities where there was a greater ratio of bonded to un-bonded surfaces, due to the greater constraints of the cavity and greater resistance to flow of the restorative material in response to polymerisation shrinkage.

**Elastic moduli and elastic cavity wall concept**

The elastic modulus may be envisaged as the stiffness of unit thickness (Watts 1994). In biomaterials this will vary with temperature, and the mode and geometry of the force applied. One objective of designing restorative materials is to match the moduli of the dentine and the restorative material. A more realistic objective may be to reduce the differential between the elastic behaviour of the tooth and the restorative material to reduce interfacial stress which may result from thermal, mechanical, or shrinkage strain. The modulus of resin composite restorative materials and adhesives may be altered by designing chemical changes in the polymer to increase cross linking or by the addition of fillers. A more controversial ways of changing the elastic behaviour would be to use a low light intensity to produce a low degree of conversion. This needs to be weighed against the mechanical properties of the restoration (Watts 1994) and the risk of leaching of free monomers (Craig 1997).

During polymerisation it is particularly important to minimise the interfacial stresses, to avoid disruption of the interface or distortion of the tooth. The speed of the polymerisation process has been discussed, but where an adhesive layer is placed on the dentine prior to the restoration it may be possible for this layer to absorb some of the stresses applied by the polymerisation of the overlying restorative material.

Polymerisation contraction stress was reported to be reduced by 20-50% by the application of an intermediate layer of unfilled resin between the dentine and the restorative material (Kemp-Scholte and Davidson 1990a,b). It was suggested that the strain capacity of the low modulus resin layer would compensate for the shrinkage stress of the polymerising resin composite restoration. A gradient of elasticity is built up from the relatively stiff dentine, through the interdiffusion zone, adhesive resin,
low viscosity resin to the resin composite restorative material (Van Meerbeek et al. 1993c). Thus the hybrid layer and the adhesive resin form an artificial elastic wall between the shrinking restoration and the rigid dentine. This was termed the elastic wall concept (Kemp-Scholte and Davidson 1990a, b).

The elastic wall concept also has implications for the interfacial seal. A high correlation was found between the modulus of elasticity of the restorative material and the marginal leakage factor. The lower the Young’s modulus of the restorative resin, the better the marginal integrity as defined by marginal leakage (Kemp-Scholte and Davidson 1990b). The Young’s modulus and flow capacity of the restorative material will depend on the polymer chemistry (molecular weight and degree of polymer cross linking) in addition to the filler content (Braem et al. 1986, Willems et al. 1992).

Further support for the elastic wall concept was provided by nano-indentation data for dentine, interdiffusion zone adhesive resins and low viscosity resin by Van Meerbeek et al. (1993c). This work confirmed the presence of a gradient of elasticity across the dentine composite interface. There was evidence of the mixing of the air inhibited layer of the resin with the composite and of the greater elasticity of the dual activated compared to light activated resins tested. Nano hardness measurements reported were inter-tubular dentine 496 MPa, interdiffusion zone 148 - 196 MPa, low viscosity resin 196 MPa adhesive 102-113 MPa and a significant difference was found between the Young’s modulus measurements of the different layers (Van Meerbeek et al. 1993c). Pearson and Hegarty (1989) found that cuspal flexure was not detected until the final increment of restorative material was placed in contact with the enamel, indicating that although the dentine is relatively stiff that it is able to absorb stress exerted by polymerisation shrinkage.

**Summary**

The effects of polymerisation stress have been studied by a range of different methods, and these have resulted in the hypotheses and observations summarised above. It is suggested that the interdiffusion zone, or hybrid layer, not only provides...
micro mechanical retention between the dentine and the composite restoration, but
together with the overlying resin adhesive may provide an elastic layer which may
have sufficient strain capacity to compensate for the restoration setting shrinkage,
thereby helping to preserve the integrity of the bond.

Clinically, disruption of the interfacial region is one of the most important effects of
excess stress. Morphological studies or assessment of the interfacial seal therefore
offer an appropriate method of assessment. However, it is important that the
interfacial region is not unduly disrupted in the sample preparation or method of
observation. New techniques involving confocal microscopy would therefore offer a
suitable method of study. The detection of the loss of interfacial seal needs to be at
the level of micropermeability rather than microleakage. Such a method would
provide a suitable model to compare the effect of the polymerisation or setting stress
of a variety of materials and of cavity design using a single adhesive resin which can
be light or chemically activated.

1.7 Bond strength

The mechanics and mechanisms of joint failure and the measured joint strength are
dependent on joint geometry, the mechanical properties of the adhesive and the
substrate, the temperature and the rate of force application (Kinloch 1982). Again,
these factors are of relevance when considering the failure of dentine/adhesive
interfaces. Prediction of bond strength at failure necessitates a knowledge of the
stress distribution at the interface, and the mechanical properties of the materials
involved, including the presence of flaws (Kinloch 1982). In dentistry it is
particularly difficult to obtain this data, but these principles form a useful
background when considering the failure of dentine adhesive interfaces and
interpreting the studies published in the dental literature.

The bond strengths of dental materials are generally measured by shear or tensile
bond tests, with a force applied parallel or perpendicular to the dentine/adhesive
interface. Other tests described are torsion, cleavage, pull or extension (Oilo 1993, Pashley et al 1995a).

Bond strength is the force per unit area required to break a bond assembly, with failure occurring in or near the adhesive/adherent interface. In testing dentine adhesives, the stress at failure has been calculated as the load at failure divided by the nominal area of the bonded surface.

Large inter and intra laboratory variations in bond strength results have highlighted that different methods, or small modifications of a single method, can give 2-4 times the difference in bond strength values (Oilo 1993). The lack of standardisation of bond strength testing methods is now well recognised. There have been repeated calls for standardisation and a number of recommendations for the substrate and the testing methods (Söderholm 1991 and ISO TR 11405). However, the development of an appropriate in vitro test has been hampered by an inadequate knowledge of the interfacial stresses during loading of restorations in vivo, and the factors which may influence the outcomes of any laboratory tests. It is recognised that the results of dentine bonding tests may be influenced by dentine substrate; etching, priming, bonding methods and components; storage and testing variables (Pashley et al 1995).

1.7.1 Specimen variables
Bonding substrates

The use of human teeth for in vitro tests is accepted as being preferable to bovine teeth (Retief et al 1990). Other substrate factors which have been investigated are remaining dentine thickness (Suzuki and Finger 1988, Tagami et al 1990), dentine permeability (Tao and Pashley 1989, Prati and Pashley 1992) and age of the dentine (Tagami et al 1993). These factors appear to be decreasing in significance with the development of new bonding systems as effectiveness of these systems increases. Whilst using earlier bonding systems, some decreases in bond strengths were reported with deep as compared to superficial dentine. This may be related to the deep dentine structure or to the relative increased wetness of the deep dentine (Prati and Pashley 1992). More recent dentine bonding systems, however, appear to be more tolerant of the presence of water, indeed some have reported no significant
differences in bond strengths when bonding to moist or deep dentine (Sano et al 1994 b). This change may relate to the better infiltration of the primer and adhesive into the demineralised dentine.

These results were mirrored by the studies comparing bonding site (occlusal vs gingival dentine). Again the variables were the density and size of the tubules which, in turn, affected the area of dentine available for bonding and the wetness of the dentine surface once the smear layer had been removed.

Bonding to sclerotic dentine is also required clinically and has been the subject of interest of in vitro studies. Sclerotic dentine is less etchable (Duke and Lindemuth 1991, Gwinnett 1994, van Meerbeek et al 1994), its tubules commonly occluded by mineral crystals and so lower dentine bond strengths have been reported (Yoshiyama et al 1996).

**Dentine Bonding Systems**

The morphology of the interfacial region and the modulus of elasticity of the dentine bonding components and the dentine substrate will have a potential effect on the results of the bond strength tests. The factors to be considered are the method of preparation of the dentine including: removal of the smear layer, the degree of infiltration of the demineralised dentine with resin, and the compatibility of the adhesive components both with the primer and the composite. Asmussen and Uno (1993) hypothesised that the adhesive resins should have specific wetting characteristics to match the prepared dentine surface for maximum bond strength, and concluded in their studies that both the solubility parameter and the polarity of resin were important variables. The moduli of elasticity of the components of the interface has been presented earlier in the chapter. The stiffness of the interfacial layers, together with thickness and gradient of change in the moduli of elasticity across the interface are all likely to affect the response of the interfacial region to stress.
The relationship between bond strength tests and the performance of the interfaces tested by other parameters has also been examined. No relationship was found between the shear bond data and microleakage (Fortin et al. 1994), and similarly no relationship was found between thickness of the interdiffusion zone and the micro tensile test (Sano et al. 1995c).

**Specimen Size**

The size of the interfacial area to be tested also influences bond strength results. Models of failure probability such as the Griffiths defect theory developed for uniform brittle materials and the Weibull risk of rupture analysis have been applied to adhesive/dentine interfaces (Sano et al. 1994b, Pashley et al. 1995 and Kelly 1995). It can be expected that larger the bonded area of the test specimen, the larger the number of flaws and the lower the bond strength. This has led to the development of micro-tensile test techniques which are discussed below.

**Storage**

A variety of storage times and conditions, including cyclic fatigue or thermal stress have been advocated. These were reviewed by Rueggeberg (1991). There is agreement that the specimens should be kept wet, but other standards have not been agreed, as there is no evidence as to which, if any, offer the most appropriate methodology (Eliades 1994). However, as bond strength tests are designed as preliminary screens it would appear that the simpler the method the better. This approach would favour testing after 24 hour storage and no cyclic stressing. The ISO standards recognise a short term 24 hour storage and long term 6 month storage time in water (ISO TR 11405).

### 1.7.2 Testing methods and variables.

**Rate of loading**

Bond strength is influenced by the speed of force application. Due to the visco elastic response of the adhesive the bond strength is dependent on the test rate. The application rate of the force is generally set by arbitrary agreement at 0.75 (+/- 0.3) mm min⁻¹ (ISO TR 11405), although studies report a range of rates (see Table 1.2).
Dentine bonding studies have not analysed the relationship between force application speed and bond strength results.

**Test geometry**
The test methods which have developed over the years have intended to measure shear or tensile bond strengths. A variety of models have been described for each method. It is well recognised that both the mean bond strength results and the standard deviations of these types of experiments vary widely, and it is unlikely that mechanical bond strengths will ever yield consistent data even for one bonding agent under controlled conditions (DeHoff *et al* 1995).

**Shear test**
Tests which were designed to apply loads in shear test are relatively easily performed, but there is a tendency to develop a bending moment. Loads can be applied in push mode by a blunt end shear bar or by a knife edge, or in pull mode by a wire loop.

**Tensile test**
Models intended to test tensile bond strength include a point or uniform application of load, by virtue of the geometric configuration of the sample. Thus the load is applied by incorporating a hook within the restorative material to attach the loading apparatus, but this arrangement causes stress concentration. However even if the whole restorative material is secured in a clamp arrangement the distribution of the stress along the interface will not be uniform.

Results of examples of both shear and tensile bond strength tests for the fourth generation materials used in the *in vitro* studies in this thesis are included in Table 1.2.

**Finite Element Analysis (FEA)**
Studies using finite element analysis have demonstrated that the stresses at the interface in either shear or tensile tests are not uniform, but are highly dependent
upon the test geometry and loading configuration (van Noort 1989). Indeed he concluded that the measurement of bond strength could only give a nominal bond strength value and not the true stress at fracture because of this non uniform distribution of stress along the interface. Using a 2D finite element model of a block of composite adhering to a flat dentine surface, it was demonstrated that in tensile mode the stress pattern is dependent on the height of the restoration. The maximum stress appeared within the bulk of the restoration for composite blocks less than 3 mm, but at the edge of the block when the height was greater than 3mm. Different stress patterns resulted when using point and uniform tensile loads.

In shear mode the same model demonstrated that the stresses were tensile on the side of load application and compressive on the far side away from the load. The interfacial stresses increased as the distance between the point of application and the dentine surface is increased and related to bending moments generated. Planar shear bond tests were further examined by 3D FEA with a cylindrical restoration adherent to a dentine substrate (De Hoff et al 1995). The patterns of stress concentration and the underestimation of the true interfacial bond strength by conventional shear tests were confirmed.

In addition, using the 2D model described above, finite element analysis has been used to demonstrate that high modulus composites have higher stress at the edge of the restoration (van Noort 1989) and that the extension of the adhesive in a flash or fillet beyond the interface will result in an artificially high value for dentine bond strength (van Noort et al 1991).

Whilst the FEA studies have highlighted the dangers of conventional testing techniques and explained some of the disparity in the results of published studies, FEA methods themselves provide but a theoretical method of analysis. The difficulty of model construction limits the complexity and detail of the models used. The analysis is further hampered by both the lack of data on the modulus of elasticity of the components of the interfacial region and the lack of knowledge of interfacial stress patterns in vivo. This technique, at present, may therefore be used most
appropriately to confirm and explain failure patterns with differing loads and geometry \textit{in vitro}, rather than to predict interfacial behaviour under differing conditions in the clinical environment.
### Table 1.2 Summary of bond strength studies.

Examples of methods and results of bond strength studies, using 4th generation dentine bonding systems used in this thesis.

<table>
<thead>
<tr>
<th>Authors &amp; date</th>
<th>Test</th>
<th>Dentine Bonding System</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barkmeier &amp; Erickson 1994</td>
<td>Shear bond strength</td>
<td>25.5±7.5 MPa</td>
<td>3.66mm diameter cylinder of P50. Stored in water at 37°C for 24hrs. Slightly reduced by extreme air drying of primer, significantly reduced by aggressive thinning of adhesive.</td>
</tr>
<tr>
<td>Fortin et al 1994</td>
<td>Shear bond strength</td>
<td>10.5±3.5 MPa</td>
<td>OB significantly better than SBMP. No correlation between shear bond strength and microleakage.</td>
</tr>
<tr>
<td>Tam &amp; Pilliar 1994</td>
<td>Fracture toughness and SEM study</td>
<td>-</td>
<td>Examined morphology of fractured interface; bond failure occurred in the unsupported collagen layer and overlying resin-modified layer.</td>
</tr>
<tr>
<td>Sano et al 1994</td>
<td>Microtensile test</td>
<td>38 MPa</td>
<td>Stored in water at 37°C for 24 hours, load rate 1mm min⁻¹. Increasing tensile bond strength with decreasing size of the bonded area.</td>
</tr>
<tr>
<td>Mason et al 1996</td>
<td>Shear bond strength</td>
<td>-</td>
<td>5 mm diameter sample. Stored in water at 37°C for 24 hours. Load rate 5 mm min⁻¹. Bond strength to enamel (28.2 ±4.9) significantly better than dentine.</td>
</tr>
<tr>
<td>Mason et al 1996</td>
<td>Shear bond strength</td>
<td>In vitro 18.7 MPa</td>
<td>6 mm diameter cylinders, stored for 10 days, thermal cycled. Load rate 0.5 mm min⁻¹. Significant difference between SBMP and OB in vitro.</td>
</tr>
<tr>
<td>Plasmans et al 1996</td>
<td>Microtensile test</td>
<td>27.8-12.8 MPa</td>
<td>Stored in 30-95% humidity, at 23-27°C for 24 hours. Load rate 2 mm min⁻¹. Shear bond strength dependent on humidity.</td>
</tr>
<tr>
<td>Yoshayama et al 1996</td>
<td>Microtensile test</td>
<td>Occlusal 17.8 MPa</td>
<td>Stored in water at 25°C for 24 hours. Load rate 1 mm min⁻¹. Gingival and occlusal dentine- no significant difference. Sclerotic and 'normal' dentine - significant difference.</td>
</tr>
<tr>
<td>Tam &amp; Yim 1997</td>
<td>Fracture toughness</td>
<td>0.45 MN m⁻³/² (SD 0.23)</td>
<td>24 hour storage at 37°C in water. Load rate 0.5mm min⁻¹. Bovine teeth, miniature short rod specimen geometry.</td>
</tr>
</tbody>
</table>
1.7.3 Developments in bond strength testing methods

**Micro tensile tests**

The relationship between the bonded surface area and the tensile strength of adhesive materials was demonstrated by (Sano et al 1994b). Composite resin restorations bonded to dentine were sectioned and trimmed to produce specimens with a bonded area of 1-3 mm$^2$. The specimens were cemented to a testing jig and loaded at a cross head speed of 1 mm min$^{-1}$. This method is referred to as a microtensile test. The smaller specimens had a higher tensile bond strengths and a smaller scatter, and had adhesive rather than cohesive failures. Sano et al (1994b) suggested specimens with a bonded surface area of 1.6-1.8 mm$^2$. Prior to this work, testing newer dentine bonding agents with conventional tensile techniques was tending to produce frequent cohesive dentine failures. A wide range of failures due both to the stress concentration patterns and interfacial flaws. The preparation techniques for microtensile test samples are difficult and must also stress the interface. In addition, specimen hydration has not been investigated. However, these tests do offer the possibility of testing the regional bond strengths of various portions of a cavity or tooth (Sano et al 1995c).

**Fracture toughness tests**

Plane strain fracture toughness ($K_{IC}$) reflects the ability of a material or adhesive interfaces to resist crack initiation and propagation. $K_{IC}$ is the critical stress intensity factor and is calculated from the maximum fracture load, thickness of the bar specimen, the line load and the minimum stress intensity factor coefficient. The stress intensity factor must calculated for each specimen anatomy and crack length. This can be achieved using finite element analysis (Lin and Douglas 1994). The method may involve the use of miniature short rod toughness specimens with chevron interfaces loaded in tensile mode. These tests were introduced to test the dentine adhesive interfaces by Tam and Pilliar (1993) suggesting that the results would characterise the interface fracture resistance by reflecting both the interfacial bond strength and inherent defects at or near the interface.
The results indicated that ranking of materials mirrored that obtained from tensile bond strength tests, but that failures occurred along the interface rather than cohesively within the dentine (Tam and Pilliar 1993, 1994). Again the relevance of this type of test to the clinical condition is not understood.

**Morphology post fracture**

Some studies have published the morphology of the fractured surfaces in addition to the shear or tensile bond strength values. The examination needs to be of sufficient detail as to be able to locate the site of failure and SEM studies have been most commonly employed (Pashley 1995). However, preparation of the post fracture samples for examination may introduce stresses and additional cracks. The information can only be derived from the surface of the sample and therefore the amount of information about the fracture pattern is limited and it will be difficult to determine exactly where the fracture occurred.

With conventional techniques, cohesive dentine failures are frequently reported in association with recorded bond strengths of > 10-15 MPa (Brown *et al* 1996) or 20-30 MPa (Pashley *et al* 1995). This actually precludes the measurement of interfacial bond strength and so the development of new testing methods has been advocated. Concern has been expressed about the validity of these results, the bond strength of dentine has been reported as considerably greater than the reported bond strengths of bonded specimens (Pashley 1995). A summary of dentine bond strength is included in Pashley (1995). Shear strengths of 78-91 MPa were reported by Watanabe *et al* (1996), depending on the tubular orientation and location within the tooth. This indicates that the presence of interfacial flaws have had an influence on the path of the crack and that the techniques employed produce areas of unrecorded stress concentration.

Although studies to date have recorded the interfacial appearance post fracture, the ‘live’ examination and recording of a failing interface has not been reported.
1.7.4 Summary
To date, there is little knowledge of the clinical interfacial stresses in a restored tooth when a load is applied. The existing bond strength tests act as part of an in vitro screening process, and as such, simplicity is advantageous. However, it would be unwise to predict clinical performance on the results of these tests alone. Standardisation of the existing bond strength tests is needed, but it will still be difficult to compare the results from different laboratories as failures are so dependent on the load and test geometry. Any differences in handling the materials and samples will also change the properties of the materials at the interface and therefore may change the response of the interface to load. The present tests report nominal bond strengths, which are the result of the production of a critical stress at the most vulnerable place along the bonded area, and do not reflect stress patterns along the interface.

Techniques are required to give more information about the response of the dentine/adhesive interfaces to load. Visualisation of the interface at high magnification as the failure occurs would give further insight into interface failure, especially if the load and failure patterns could be recorded simultaneously.

1.8 Confocal scanning microscopy

Basic Principles
The physical principle of any scanning microscope is that the sample is scanned with a radiation probe which illuminates only one point in or on the specimen at one time. The resultant signals are used to reconstruct an image when the probe is scanned point by point over the sample. In confocal scanning microscopy the scattered reflected or fluorescent light from out of focus planes is eliminated and only one point in the sample in the focal plane is illuminated and imaged at a time. Again, by scanning the sample an image can be formed.

An aperture is placed in the illuminating beam to produce a narrow beam of light. This is focused by the lens, so that a point in the focal plane in the specimen is illuminated.
Figure 1.1  Simplified ray diagram of the confocal microscope principle. The lens represents the microscope. Light from the light source is focused in the sample by the objective lens. Only light returning from the focused-on plane returns via the lens and aperture. Light from planes above or below the plane of focus is blanked off by the opaque disc around the aperture.
Only light returning from the focused-on plane in the specimen is able to pass a second (conjugate) aperture and is then detected (Fig. 1.1). The second aperture prevents the transmission of light scattered from other planes in the specimen and in the microscope. The apertures are placed 160 mm behind the objective (the tubelength of a standard RMS objective is 160 mm). In this image plane an aperture will be imaged on the focused-on plane in the specimen and light returning from this spot will also be brought to focus at the same level. The optical pathway of the illuminating and reflected light are separated and scanning is achieved by moving the specimen or the illuminating beam (Watson and Boyde 1987, 1991).

Development

The history of confocal microscopes spans four decades. The first recorded confocal optical instrument (used to determine brain neuron pathways) is attributed to Minsky (1957). Developments in the 1960’s resulted in the Tandem Scanning Reflected Light Microscope (TSRLM or TSM) (Petran et al 1968). General recognition of the advantages of this type of scanning microscope was slow. The TSM continued to be developed in Czechoslovakia (Petran 1985) then in the UK (Boyde 1984, 1989). Using the same design principles another TSM was manufactured in America by Tracor Northern and released in 1987. In addition, a unilateral TSM has been developed and reported (Boyde et al 1990). This was easier to align, but required extra optical components to remove stray reflected light. Low light levels made reflection images more difficult to record than with the two sided TSM (Boyde et al 1990). This system has not met with the same popularity as the two sided TSM.

The first laser scanning microscope was reported by Davidovits and Egger (1971). The next stages in development were the improvement in

- image quality (Brakenhoff et al 1979, Van der Voort et al 1985 and Wilson and Shepard 1984). Scanning was by sample movement, producing high quality images, but with a slow frame rate.
- scanning rate to 4 frames per second using galvanometer mirrors to move the illuminating beam in x and y (Carlsson et al 1985, White et al 1987).
scanning at video rate using acousto-optical deflection to move the scanning beam (Draaijer and Houpt 1988).

**Tandem Scanning Microscopes (TSM)**

Real time, direct view TSMs are described by Watson and Boyde (1987, 1991). The apertures for illuminating and reflected light are arranged on either side of a disc. The optical pathways for the illuminating and reflected light are separated. The disc is positioned 160mm behind the objective lens at the image plane, so that the lens forms an image of the illuminated holes in the focused-on plane in the specimen. The scanning disc is designed so that the thousands of apertures are paired and at an unique distance from the centre of the disc. When the disc rotates each describes a single scanning line across the specimen, and the image is made up of all the scanning lines. A rotation speed as low as 100 rpm produces a steady real time image.

**Confocal Laser Scanning Microscopes (CLSM)**

A laser beam is introduced from a black box through the head of a conventional microscope and scanned over the specimen. Development in scanning methods allow the production of video rate images. In these microscopes the aperture size and thus the ‘confocality’ of the image is variable. The reflected or fluorescence signal from the sample is taken back to the box for de-scanning, collection and subsequent image manipulation. The CLSM is dependent on computer programmes for image processing, frame storage and display.

The greatest use of these microscopes is in fluorescence microscopy as coherent light from a laser source as in a CLSM tends to produce ‘speckle’ in reflection images. This phenomenon can be reduced by frame averaging, but the facility to view and record events in the sample at video rate is then lost.

**Image quality**

In light microscopy, resolution can be a function primarily of the numerical aperture of the optical system and the wave length of the light (Watson 1997). The greater the numerical aperture of the objective and the lower the wavelength of the light the
greater the resolution. The numerical aperture (NA) is the light gathering capacity of the objective. The larger the lens (and more expensive) the greater the light gathering capacity. There are practical limitations to reducing the wavelength of the incident light because of the difficulties of working with ultra violet light.

A suitable coupling medium between the objective lens and the sample needs to be used, in order to produce images below the surface of the sample under examination. Generally, a coverslip is used to protect the objective, and the medium is placed above and below. Ideally the refractive index of the medium should be matched with the sample, coverslip and the NA of the objective. Oil, glycerine and water are possible media for use with biological mineralised tissues. The refractive indices for these media are oil 1.5, glycerine 1.4 and water 1.3. Oil immersion media are favoured as the NA of the immersion objectives is the highest. Within the dental field the disadvantages of using oil are that it can only be used in in vitro samples and that it tends to ‘clear’ the image with time. Clearing occurs as the oil penetrates the dentine tubules, displacing the water within them and altering their refractive index, thus making them more difficult to image. Glycerine is used in preference to water in in vivo samples or where the experimental method necessitates the medium being washed off to resume the experiment. There are significant z or optical axis measurement errors for the unwary if oil immersion objectives are used to focus at great depth in tissues containing a large amount of water (eg cells).

**Fluorescence confocal microscopy**

Fluorescent materials (fluorophores) absorb light at one wavelength and emit light at a longer wavelength. This phenomenon can be used to ‘optically’ label biological samples. Fluorescence imaging has found widespread application in cell biology, especially with the development of immunological labelling of samples. In the dental field, fluorescent dyes have been used to label the components of dentine bonding systems and restorative materials, which, not only increases the contrast between the dentine and the restorative, but also gives an insight into the distribution of the materials at the interface (Watson and Boyde 1987b, Watson 1989, Boyde et al 1990b).
A number of fluorescent dyes have been used in these applications including, rhodamine B which is activated by light at 546\textmu m (green) and emits light at 600\textmu m (red), and fluorescein which absorbs at 450-490\textmu m (blue) and emits at 520\textmu m (yellow) (Watson 1989). As excitation and emission wavelengths for fluorescein were shorter and did not overlap with those of rhodamine B it was possible to dual label samples. In this case two components of the bonding system were labelled with different dyes and viewed independently.

It is important that the fluorescence seen is labelled bonding agent at the interface and penetrating the dentine tubules, rather than unattached fluorophore. This can confirmed by comparing images of the dentine/restorative interfacial region and resin tags, prior to, and after dissolving the dentine and enamel with HCl and NaOCl. Such studies have demonstrated the presence of fluorescent labelled resin tags after removal of the dentine. These tags which had penetrated the dentine tubules, remained as fronds floating in the immersion water and confirmed that the fluorescent dye had labelled the resin (Watson 1989).

The list of possible fluorophores is extensive (Conn’s Biological Stains and Sigma catalogue 1997). Several already used in biological applications have been identified to have similar absorption and emission wavelengths as either rhodamine B or fluorescein. A table of the fluorophores used in the thesis are given in Tables 2.4.

The choice of fluorophore depends not only on operating at convenient wavelengths, but also solubility in the media to be labelled, in this case components of dentine bonding systems. The stability of the label once those components are bonded to the tooth and overlying restorative material should also be considered.

In the TSM either glass or interference filters are positioned in the path of the illuminating and returning emitted light. In the CLSM a variety of fluorescent channels provide laser lines of different wavelengths, with dichroic mirrors to filter the emitted light.
Fluorophores are subject to photobleaching. This is an irreversible process in which the dye molecules are photo-chemically changed to a non-fluorescent form. The rate of photobleaching is dependent on the intensity of the light and the duration of the illumination. This presents more of a problem with CLSMs as they operate at greater light intensity.
STATEMENT OF THE PROBLEM

An ideal dentine adhesive system has yet to be developed, although many systems have been manufactured and released onto the market. Reliable in vitro methods of assessment are required to screen both recently released systems and to help in the development of new systems.

In order to assess the application and performance of dentine bonding systems methods need to be established of imaging not only the static interface, but also dynamic events at the interface (for example during application of the adhesive components, or the performance of the interface under load). It is important that the interface is not disturbed during sample preparation, that the samples are viewed in near normal conditions without the introduction of artefacts. Quality images at high magnification are required, both of the static interface and of dynamic events at the interface in real time (at video rate). The methods used previously are described in the literature review and highlight their inability to allow such an interfacial examination and evaluation.

It was therefore proposed that a variety of in vitro testing methods be developed and used to assess the morphology of the interfacial region, and to make an assessment of the interface in function with regard to application of components, micropermeability and behaviour during loading. Fluorescence confocal microscopy had previously been used to image dentine restorative interfaces, as samples could be viewed in real time, in near normal conditions and with little sample preparation. Advances in computer technology, especially in the fields of computer image acquisition and data management, and in the confocal microscopes gave potential for the development of confocal microscopy techniques in the examination of dentine restorative interfaces.

The broad aims of the study were therefore:
- To develop a method of visualising fluid movement in mineralised samples, by developing a method of coupling a sample to a coverslip to provide a ‘window’ for observing real time events.
• To examine the subsurface morphology of dentine/restorative interfaces and investigate the influence of components and handling characteristics of dentine bonding systems.
• To develop a method of assessing the effectiveness of dentine/adhesive interfacial seal, and to use this to compare the performance of a variety of dentine adhesives.
• To evaluate the effect of restorative materials with differing setting shrinkage on the dentine/adhesive interfacial seal.
• To develop a method to investigate the behaviour of dentine/adhesive interfacial region when composite restorations are loaded in shear mode by: microscopically imaging the interfacial region whilst it is loaded until bond failure, recording the load at failure and the crack geometry, recording changes in load with time.

The specific objectives are given at the beginning of each chapter.
Chapter 2

GENERAL MATERIALS AND METHODS

2.1 Objectives

To avoid repetition in later chapters, the materials and methods used and developed in this thesis, and common to several chapters, are presented in this section. The topics included are:

- dentine adhesives,
- restorative materials,
- tooth preparation,
- fluorescent dyes,
- microscopy,
- image recording and analysis.
2.2 Dentine Adhesives

Dentine Bonding Systems - Test systems

The following fourth generation dentine bonding systems were used in the studies described in Chapters 3-7. Details of the components as described by the manufacturers and the mode of application are given in Tables 2.1 and 2.2.

Scotchbond Multi Purpose [SBMP] (3M Dental Products, Minn USA). This system employed an aqueous solution of 10% maleic acid in poly (vinyl alcohol) to etch both the enamel and dentine, a dentine primer containing HEMA (2-hydroxyethylmethacrylate) and a polyalkenoic acid copolymer (as in Vitrebond glass ionomer liner base 3M) and an unfilled resin adhesive containing HEMA and bis-GMA (adduct of bisphenol-A and glycidyl methacrylate) as well as photoinitiators for light activation at 470/480 nm. SBMP was one of the first ‘4th generation’ systems released in the UK and was used for all the pilot studies in the thesis.

Scotch Bond Multipurpose Plus [SBMP+] (3M Dental Pdts, St Paul, Minn, USA). The 10% maleic acid of the original SBMP was replaced by 35% phosphoric acid in the ‘plus’ version. As in SBMP, the primer contains an aqueous solution of HEMA, polyalkenoic acid copolymer and is not light cured. The adhesive in this system is an unfilled resin containing bis-GMA and HEMA and contains initiators for both photo and chemical polymerisation.

Chemical polymerisation initiators are required where light activation cannot be utilized: e.g. under an amalgam restoration. In these cases the manufacturers refer to the use of an ‘activator’ on the dentine prior to the primer and the mixing of the adhesive with a ‘catalyst’ prior to application. As the chemical formula of the ‘catalyst’ is changed in the polymerisation reaction it would be more appropriately named an initiator. The components are given in Table 2.2. The activator is an ethanol based solution of a sulfinic acid salt and a photoinitiator compound. The ‘catalyst’ contains bis-GMA and HEMA as in the adhesive, but also the peroxide component found in a chemically activated system.
Optibond (Kerr Manufacturing, European Division, Peterborough, UK). The manufacturers recommend that a phosphoric acid etch is applied to both enamel and dentine. The primer contains MMEP, which has been reported to improve bonding efficiency and decrease contraction compared to HEMA containing systems (Chigira et al 1989). The adhesive resin was a low viscosity dual activated filled resin supplied as liquid A and filled accelerator paste B, which require mixing. The filler is 0.6 mm barium aluminium borosilicate.

Clearfil Liner Bond 2 [CFLB 2] (Kuraray Co. Ltd., Osaka, Japan, European distributor Cavex, Haarlem, Holland). This system was initially supplied as a sample system KB 200, and was later released commercially as Clearfil Liner Bond 2. The primer (which conditions the dentine) is supplied as a two bottle liquid system and contains phenyl-P, HEMA and 5-NMSA in ethanol and water. The primer both déminéralises and primes the dentine and is not washed off the dentine after application. The adhesive resin is light cured and lightly filled, it includes HEMA and 10-MDP (10-methacryloyloxydecyl dihydrogen phosphate). It can be used in combination with a micro-filled low viscosity resin composite, Protect F Liner, reported to release fluoride.

EBS and Experimental primers (ESPE Seefeld, Germany). The EBS system is used with a 35% phosphoric acid etch, primer and unfilled adhesive resin. The EBS primer was evaluated during its development to assess the influence of: including photoinitiators in the primer, light activating the primer after its application and agitating the primer during the application time. Within the thesis the primers E1, E2 and E3 were evaluated (Table 2.1 and 2.2). The E3 primer was later released as EBS with the E3b application instructions. These primers were used with Pertac Universal Bond resin.
Control Dentine Bonding System

*Pertac Universal Bond* (ESPE, Seefeld, Germany). This single stage adhesive resin, used without dentine conditioning, was chosen as the control system. It contained carboxylic acid and methacrylate. This resin is also used in EBS as the resin adhesive following the priming stage, and with the experimental primers E1, E2 and E3 above.

2.3 Restorative Materials

Light activated composites were used for the majority of the studies in this thesis. A large variety of materials in this category have been manufactured. The materials chosen were Herculite XRV (Kerr), Z100 (3M) and Pertac Hybrid Unifil (ESPE) were used with their related dentine adhesives. However, in the fracture experiment (Chapter 7) it was decided to use the same composite for all systems as any differences in moduli of elasticity and viscoelastic properties would influence the behaviour of the restoration under load, and therefore might influence the interfacial failure. Herculite is an ultrafine midway filled composite with <60% volume filler of size <3 μm, Z100 is an ultrafine compact-filled composite with >60% volume filler and a particle size <3 μm and Pertac Hybrid Unifil is a fine mid-way filled composite with <60% volume of filler with particle size >3 μm (Willems *et al* 1992). Details are given in Table 2.3.

A chemically activated composite (Adaptic, Johnson and Johnson, East Windsor NJ USA) was used for the polymerisation stress study (Chapter 6). This material is reported as an ultrafine compact-filled composite with >60% volume filler and a particle size <3μm (Willems *et al* 1992). As described in the literature review, the rate of polymerisation and of shrinkage of composites which require mixing is slower and less than that found in the light activated composites.
<table>
<thead>
<tr>
<th>Product</th>
<th>Code</th>
<th>Etchant</th>
<th>Primer</th>
<th>Adhesive</th>
<th>Liner</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pertac Universal Bond</td>
<td>-</td>
<td></td>
<td></td>
<td>HEMA, Bis methacrylate</td>
<td></td>
</tr>
<tr>
<td>Tripton</td>
<td></td>
<td></td>
<td>Polyhexanide</td>
<td>TEG-DMA, UDMA, phosphate methacrylate (chloride free), aerosil, camphorquinone, di-butyl tin dilaurate.</td>
<td></td>
</tr>
<tr>
<td>XR Bond</td>
<td></td>
<td></td>
<td>Phosphonated DMA</td>
<td>UDMA, TEGDMA, phosphonated DMA</td>
<td></td>
</tr>
<tr>
<td>Scotch Bond Multipurpose</td>
<td>SBMP</td>
<td>maleic acid</td>
<td>HEMA, polyalkenoic acid copolymer, water</td>
<td>HEMA, bis-GMA, photo-initiators</td>
<td></td>
</tr>
<tr>
<td>Scotch Bond Multipurpose plus</td>
<td>SBMP</td>
<td>phosphoric acid</td>
<td>HEMA, polyalkenoic acid copolymer, water (+ activator - sulfinic acid salt)</td>
<td>HEMA, bis-GMA, photo-initiators (+catalyst - HEMA, bis-GMA, peroxide initiator)</td>
<td></td>
</tr>
<tr>
<td>Optibond</td>
<td>OB</td>
<td>phosphoric acid</td>
<td>HEMA, GPDM, MMEP, ethanol, water</td>
<td>bis-GMA, HEMA, Barium Aluminium Borosilicate glass Disodium hexa fluoro Silicone Fumed silica</td>
<td></td>
</tr>
<tr>
<td>Clearfill Liner Bond 2. (+KB 200)</td>
<td>CFLB2</td>
<td></td>
<td>Phenyl P, HEMA, 5-NMSA, ethanol, water</td>
<td>bis-GMA, HEMA, bis-GMA, F release monomer, photoinitiators</td>
<td></td>
</tr>
<tr>
<td>Experimental Primer 1</td>
<td>E1</td>
<td>phosphoric acid</td>
<td>HEMA, acidic methacrylate difunctional methacrylate water, photoinitiators</td>
<td>HEMA Bis methacrylate</td>
<td></td>
</tr>
<tr>
<td>Experimental Primer 2</td>
<td>E2</td>
<td>phosphoric acid</td>
<td>HEMA, TEGDMA, acidic methacrylate, photoinitiators, water</td>
<td>2-HEMA Bis methacrylate</td>
<td></td>
</tr>
<tr>
<td>Experimental Primer 3 (= E3a, E3b, EBS)</td>
<td>E3</td>
<td>phosphoric acid</td>
<td>HEMA, methacrylic magnesium chelate, water</td>
<td>HEMA Bis methacrylate</td>
<td></td>
</tr>
</tbody>
</table>

* According to manufacturers' data
Table 2.2: Dentine Bonding Systems - application mode.

<table>
<thead>
<tr>
<th>Product code</th>
<th>Etchant</th>
<th>Primer</th>
<th>Adhesive</th>
<th>Liner</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pertac Universal Bond</td>
<td></td>
<td></td>
<td>light cure 20 s.</td>
<td></td>
</tr>
<tr>
<td>Tripton</td>
<td>30 s application, air dry.</td>
<td></td>
<td>light cure 60 s.</td>
<td></td>
</tr>
<tr>
<td>XR bond</td>
<td>30 s application, air dry</td>
<td>light cure 10 s.</td>
<td>light cure 60 s.</td>
<td></td>
</tr>
<tr>
<td>SBMP</td>
<td>15 s etch, rinse dry</td>
<td>30 s application, air dry</td>
<td>light cure 20 s.</td>
<td></td>
</tr>
<tr>
<td>SBMP+</td>
<td>15 s etch, rinse dry</td>
<td>30 s application, air dry</td>
<td>light cure 20 s, or mix with catalyst</td>
<td></td>
</tr>
<tr>
<td>OB</td>
<td>15 s etch, rinse, dry.</td>
<td>30 s application with agitation, air dry, light cure 20 s.</td>
<td>mix A &amp; B, light cure 30 s.</td>
<td></td>
</tr>
<tr>
<td>CFLB2</td>
<td>mix 2 liquids A &amp; B. 20 s application, air dry</td>
<td></td>
<td>light cure 20s.</td>
<td>light cure 20s.</td>
</tr>
<tr>
<td>E1</td>
<td>15s etch, rinse, dry.</td>
<td>30 s application, air dry not light cured.</td>
<td>light cure 20 s.</td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>15s etch, rinse dry.</td>
<td>30 s application, air dry light cured 20 s.</td>
<td>light cure 20 s.</td>
<td></td>
</tr>
<tr>
<td>E3a</td>
<td>15s etch, rinse dry.</td>
<td>30s application, air dry not light cured.</td>
<td>light cure 20s.</td>
<td></td>
</tr>
<tr>
<td>E3b</td>
<td>15s etch, rinse dry.</td>
<td>30s application with agitation, air dry, not light cured.</td>
<td>light cure 20s.</td>
<td></td>
</tr>
</tbody>
</table>
Table 2.3 Description of composite restorative materials.

<table>
<thead>
<tr>
<th>COMPOSITE</th>
<th>DESCRIPTION</th>
<th>% VOLUME FILLER</th>
<th>MEAN PARTICLE SIZE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herculite XRV (Kerr)</td>
<td>Ultra fine midway filled</td>
<td>55%</td>
<td>1.0 μm</td>
</tr>
<tr>
<td>Z100 (3M)</td>
<td>Ultra fine compact filled</td>
<td>64%</td>
<td>1.0 μm</td>
</tr>
<tr>
<td>Pertac Hybrid Unifil (ESPE)</td>
<td>Fine midway filled</td>
<td>53%</td>
<td>4.2 μm</td>
</tr>
<tr>
<td>Adaptic (Johnson and Johnson)</td>
<td>Ultra fine compact filled</td>
<td>65.5%</td>
<td>3.2 μm</td>
</tr>
</tbody>
</table>

Details from Willems et al 1992.
2.4. Tooth Preparation.

Storage
Recently extracted non carious lower third molar teeth were used throughout the study, they were stored for no longer than one month from extraction in a refrigerator prior to preparation. The storage medium was isotonic saline in the earlier studies and water in the later studies. The teeth were used with the consent of the patient, as approved by the institution ethical committee. Care was taken to keep the teeth hydrated at all times, by keeping the teeth in water until required for preparation and using all drills and saws with water cooling.

Cavity Preparation
Cavities were prepared with tungsten carbide burs (#57 fissure bur: Smartbur; Precision Rotary Instruments Inc, Bridgewater Corners, Vermont, USA), at high speed with water cooling. Two cavity designs were employed throughout the study:
- **Approximal** wedge shaped cavities extending into dentine, were prepared with standard dimensions as far as tooth size would allow, in the range occlusal wall 1.5 mm, pulpal wall 2.5 mm and with a 90° internal angle. The upper finishing line was in enamel and lower in cementum. The dentine tubules opening on the pulpal wall connected with the pulp and those on the occlusal wall connected with the occlusal enamel (Figure 2.1a).

- **Occlusal cavities** 4 mm long x 3 mm wide x 4 mm deep were prepared to extend into dentine (Figure 2.1b). The cavities were prepared to a standard depth in an attempt to standardise the dentine morphology at the base of the cavity and to allow the use of ‘thin’ and ‘thick’ restorations.

In addition, restorations were placed on flat dentine surfaces to remove the effect of both cavity line angles and the effect of an enamel bond. These teeth were prepared by sectioning horizontally at a level equivalent to the floor of the 4 mm deep occlusal cavities (Figure 2.1c) or vertically to expose approximal dentine surfaces in the case of the fracture studies (Figure 7.1). Details of the sectioning technique are given below.
Figure 2.1 Diagram of longitudinally sectioned teeth illustrating the cavity designs employed and the schematic orientation of the dentine tubules to the cavity surfaces.

a. Approximal wedge shaped cavities.

b. Occlusal cavity, cavity floor was 4 mm deep.

c. Restoration placed onto the horizontally sectioned tooth. The section was at an equivalent level as the floor of the occlusal cavities, a glass slide was used as the matrix for the restoration.
Application of bonding systems

All components were applied according to the manufacturer's instructions. The components of the systems employed, and their mode of application are given in Tables 2.1 and 2.2.

Placement of composite resin restorations

Restorations were made with a light cured resin composite restorative material either: Z100 or Herculite XRV. An incremental technique was used for all restorations, unless otherwise stated. Each increment was less than 2 mm thick and was cured for 40 s with a blue light source from a visible curing light unit (VCL): Heliolux II (Vivadent, Liechtenstein)- 200 mW cm\(^2\) with a 7 mm tip, in the earlier experiments (Chapters 3, 4.1 and 5.1) and Optilux (Demetron Research Co., CT, USA) - 470 nm, 800 mW cm\(^2\) with a 10 mm tip, in the later work (Chapters 4.2, 5.2, 6 and 7). Confirmation of the light intensity was made with a light meter (Demetron Inc Co).

Sectioning and polishing

A low speed diamond saw rotating under water (Labcut, Agar Scientific, Stanstead, Essex, UK) was use to section teeth. In addition, a wire saw with 300 \(\mu\)m diamond impregnated wire (Well Wire Saw 3241, DR Bennett Ltd., Leicester UK) was used with water cooling for some sectioning for the fracture studies (Chapter 7). The geometrical arrangement of the wire saw facilitated sectioning in the planes required to prepare these particular samples. The diamond wheel, however, produced a flatter surface than the wire saw and was therefore used for all sections which were to be microscopically examined.

A longitudinal section in a mesio/distal plane through the mid portion of lower third molar teeth was found to produce samples with the major tubular orientation parallel to the cut surface. This enabled the path of tubules to be traced in prepared samples from a dentine/restorative interface to the pulpal surface and thus aided the evaluation of fluid movement along tubules and penetration of dentine adhesive components into the
dentine. Sectioning to expose dentine/restorative interfaces produced two samples separated by a 300 μm saw cut, and the interface of both samples was examined in the studies reported.

Polishing after sectioning was carried out with fine caborundum paper (grade P1200), by hand or using a rotary polishing machine (D.R. Bennett, Leicester UK). Sectioned samples were washed with water or placed in an ultrasonic bath containing water and a detergent for 5 minutes. The polishing and cleaning procedures used, reduced the smear layer produced by sectioning and improved the image quality considerably. Diamond polishing laps (1-5 μ) were avoided as the lap particles became lodged in the dentine tubules and interfered with image quality by producing speckled aberrant reflections.

2.5 Use of Fluorescent Dyes

To facilitate the examination of the dentine/restorative interfaces, the features of the interfacial regions were highlighted by the addition of fluorescent labels to components of the bonding systems or to fluids introduced into the dentine.

In studies of the subsurface interfacial morphology, fluorescence confocal microscopy was used to evaluate the distribution of the components of the bonding systems and their relationship to the dentine. Minute quantities of the fluorescent dyes were added to the different components of the dentine bonding systems (activator, primer and bonding agents) in sufficient quantity to provide a fluorescence signal when viewed microscopically with the appropriate filters. Initial samples revealed that very few grains of dye were required to produce an adequate signal. Grains were transferred to the solution to be labelled by dipping a dry resin applicator tip into the dye required. In single labelling experiments a dye was added to one component, and in dual labelling studies two components were labelled to demonstrate the distribution of the components in relation to one another and to the dentine.

A variety of dyes were used during the course of the study period, including rhodamine B, fluorescein, auramine O and Lucifer yellow. Details of these are given
in Table 2.4. Fluorescein and rhodamine B had been used in previous studies (Watson 1989). They had contrasting fluorescence colours and were readily available and relatively inexpensive.

Appropriate filters on the tandem scanning and laser scanning microscopes were available for these dyes (Table 2.5). For the dual labelling studies the wavelengths of the light emitted from the dyes needed to be sufficiently different to avoid false positive signals (cross talk).
Table 2.4  Details of the fluorescent dyes used to label components of dentine bonding systems.

<table>
<thead>
<tr>
<th>Fluorescent Dye</th>
<th>Presentation</th>
<th>Molecular Formula</th>
<th>Solubility</th>
<th>Absorption (nm) (Max λ)</th>
<th>Manufacturer and Batch No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auramine O</td>
<td>Yellow Powder</td>
<td>C_{17}H_{21}N_{3}HCl (FW 303.8)</td>
<td>Slightly soluble in water (10 mg/ml)</td>
<td>432</td>
<td>Sigma 303</td>
</tr>
<tr>
<td>Fluorescein</td>
<td>Orange Powder</td>
<td>C_{28}H_{10}Na_{2}O_{5} (FW 376.28)</td>
<td>Very water soluble (40 mg/ml)</td>
<td>490</td>
<td>Sigma 64 H3414</td>
</tr>
<tr>
<td>Lucifer Yellow</td>
<td>Orange Powder</td>
<td>C_{13}H_{9}N_{2}O_{5}S_{2}K_{2} (FW 521.6)</td>
<td>Very water soluble. Soluble in ethanol</td>
<td>428</td>
<td>Sigma 23 H3657</td>
</tr>
<tr>
<td>Rhodamine</td>
<td>Green Powder</td>
<td>C_{2n}H_{3n}CIN_{2}O_{3} (FW 479.02)</td>
<td>Soluble in water (30ml/ml) Soluble in ethanol (50mg/ml)</td>
<td>543</td>
<td>34142-57212608</td>
</tr>
</tbody>
</table>

Details from: Green (1991)

Table 2.5  Filters and laser lines used in fluorescence confocal microscopy studies.

<table>
<thead>
<tr>
<th>FLUORESCENCE</th>
<th>TSM FILTERS</th>
<th>CLSM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Short Pass</td>
<td>Barrier</td>
</tr>
<tr>
<td>Blue/yellow</td>
<td>450 +/- 20 nm wide band pass</td>
<td>520 +/- 5 nm band pass (for dual labelling)</td>
</tr>
<tr>
<td></td>
<td>490 +/- 5 nm narrow band pass</td>
<td>520 + 100 nm long pass (for single labelling)</td>
</tr>
<tr>
<td>Green/red</td>
<td>546 +/- 5 nm narrow band pass</td>
<td>600 + 80 nm long pass</td>
</tr>
</tbody>
</table>


2.6 Confocal Microscopy

Both a tandem scanning microscope (TSM) and a confocal laser scanning microscope (CLSM Odyssey): both Noran Instruments, 2551 W Beltline Highway, Middleton, WI, USA) were used throughout the study. Both microscopes were located on anti vibration tables to improve image quality.

Tandem scanning reflected light microscope (TSM)

The development of the microscope was presented in Chapter 1.6 and its use in examining dentine/restorative interfaces was first presented by Watson and Boyde in 1987.

The microscope is used in the same way as a conventional epi-illumination microscope, the directly viewed image is steady, but as only a thin optical section is viewed with the TSM, the structural details are much clearer than the image produced with a conventional microscope. This is illustrated in Figure 2.2. The z plane of the optical section is changed by fine focusing and a series of images at different planes of focus can be combined by photographic or computer methods to provide a ‘extended focus image’. This is illustrated with a CLSM image in Figure 2.3, where only a part of each dentine tubule can be seen in a single optical section, as it weaves into and out of the plane of focus, but the whole tubule length is seen when extended focus image is made.

The illumination source for the TSM used in these studies was a 100 W mercury arc lamp. The aperture disc was a silicon wafer, blackened to decrease reflectivity and minimise stray light, and had an aperture pattern inscribed by photolithology. The apertures were square and arranged in a spiral pattern. The disc was coupled to a motor by magnets, and was rotated to give scanning speeds of 8 - 600 frames per second, depending on the speed of the motor. This allowed samples to be viewed in real time ie at video rate (25 frames per second). The alignment of the microscope was important to ensure good quality images.
The motorised objective stage with piezo electric translators could be controlled in x and z planes using an external computer or by simple control box with a joy stick. The movement was accurate to 0.2 μm (calibration data for TSM). This system failed in 1992 and was replaced by a more versatile stepper motor controlled stage for control of movement in x, y, and z. The accuracy of this system was equivalent to the piezo system. The objectives used were oil immersion objectives (OI) with high numerical aperture (NA), for the reasons explained in the review of the literature section (Chapter 1.8). Those most commonly used in the studies in this thesis were x20 /0.8 NA, x60/1.4 NA and x100/1.4 NA. The samples were viewed through a glass coverslip, with immersion oil as a coupling medium above and below the coverslip. Samples were mounted on glass slides with plasticine (Harbutts Ltd, Bath, Somerset) and levelled using a levelling device before being viewed. The levelling device compressed the sample onto the plasticine in the desired orientation. The plasticine and the coverslip acted as safety devices against damage to the objective. The use of the microscope with the rigidly held specimens in the fracture studies (Chapter 7) demanded a high level of accuracy to align the specimens to avoid damaging the very expensive (£2000) objectives.

Image quality was ensured by basic procedures which included: checking the microscope was aligned and dust free, using an anti vibration table. The contrast of the images was improved by the use of the field stop when viewing bright images. This decreased the field of view, but had the advantage of improving contrast and definition by decreasing stray light and by only using the centre of the lenses, which have better optical proportion (Watson personal communication).
Figure 2.2  Illustration of the image quality of an enamel (e), dentine (d), and restorative (a) interface viewed with a reflected light microscope (a) and a confocal scanning light microscope (b). The optical tomography is well illustrated by the detail of the enamel and dentine structure and the appearance of the air bubble in the restoration. x25 0.75NA oil immersion objective (OI). Field width 200 μm (With thanks to TFWatson).

Figure 2.3  Illustration of a single optical tomogram (a) through a dentine sample (tubules labelled with an experimental fluorescent dye ‘APSS’) compared to an extended focus view (b) comprising optical tomograms over a 20 μm z range. The extended focus view allows the tubules length to be imaged as it follows a spiralling pattern through the dentine. The better the sectioned surface of the dentine is aligned with the tubular orientation, the less the extended view is required to be able to follow the tubules along their course. x60 1.4NA CSLM, 488/520 nm. Olympus fluoview Microscope. Scale bar 20 μm. (With thanks to TF Watson).
Confocal Laser Scanning Microscope (CLSM)

In this system, laser light from the video scan module (black box) entered the microscope through a camera relay port, the operation of the system and image processing were via Windows™ based computer programmes. Further details of these systems are given in later sections.

Illumination was provided by an Argon-ion laser with 50 mW maximum power. An acoustic optical deflector (AOD) and galvanometer provided high frequency scanning of the laser beam. The laser illumination could be varied according to: the laser intensity, the slit size (the smaller the slit the more confocal the image), the excitation wavelength and the barrier filter. Laser line (exciter) filters provide illumination at 488 or 515 nm. Three channels were available reflection, fluorescence for red/green fluorophores (rhodamine) and fluorescence for blue/yellow fluorophores (fluorescein) (see Table 2.5). Combined reflection and fluorescence images were also possible.

The CSLM was used in combination with the oil immersion objectives described above. The magnification of the monitor was greater than with the systems associated with the TSM, and could be increased further with an electronic ‘zoom’ facility. The laser speckle visible in the reflection images was reduced by summing frames. However, this resulted in events not being recorded in real time, and in difficulties in controlling the position of the sample as movement of the sample and the image on the screen were no longer synchronised. There was evidence of photo-bleaching of dyes in the samples examined, especially when using high magnification objectives with high NA. This could be avoided by decreasing the laser intensity and turning the laser to the idle mode whenever the sample was not being viewed.

Calculation of field widths

Field widths viewed with the objectives with the microscopes were measured using a glass slide with engraved grid. Images of this grid were made with the various combinations of cameras and monitors described below so that of the field width with each system could be measured.
2.7 Image recording

Still images

Images were recorded on colour transparency (Elite II 400, Kodak, UK) and black and white 35 mm film (TMAX 400, Kodak), using an Olympus (OM2) camera with an automatic exposure time. With the TSM the images were recorded directly with the attachment of the camera via a x10 ocular photo-tube. With the TSM operating in fluorescence mode, long exposure times (3-4 minutes) were required when light levels were low.

In the case of the CSLM images were captured and stored as described below and the images photographed directly from the video monitor, using the 35 mm film and OM2 camera on a tripod.

Video cameras.

Images were made with the TSM coupled to sensitive video cameras: SIT (Silicon Intensified Tube, JAI, Copenhagen, Denmark) or CCD (Charged Couple Device, COHU). The SIT camera was able to operate at very low light levels, but suffered from some ‘pin cushion’ distortion at the periphery of the field of view (Inoue 1986). The CCD camera was not able to operate at such low light levels, but gave a more linear image and gave a greater magnification of the image on the screen, because of the smaller detector array (1/2” vs 1”). The CCD camera was only available after the studies described in Chapter 3.

Video recording.

Video recording rate in the UK is 25 frames second\(^{-1}\), or one frame every 40 ms. The rate of scanning is important as without a fast enough scan rate, recording of events cannot be made in real time, that is at video rate. In studies for this thesis, images were captured on VHS video tape (ordinary or sVHS) to be replayed and analysed. The retrieval and editing of these tapes was rather cumbersome and time consuming compared to the computer software which later became available. However, the video
tapes were a useful back up for these computer images. Video images could be made from the computer images for presentations and theses etc. by using a video converter.

**Computer Image Acquisition and Storage**
A range of computer hardware and software programmes assisted image capture, processing and storage as the work described in the thesis progressed.

**MISIS 3D plus (St Etienne, France).**
Using the CCD or SIT cameras with the TSM, this system allowed the acquisition and storage of single images or series of multiple images. The latter were recorded at specific time intervals or made as a series of optical 'cuts' through a pre-determined 'z' range (generally 10-30 mm, accurate to 0.5 μm). Acquired images could be processed, for example to determine linear measurements between chosen points on an image. This software was available for studies described in Chapters 3, 4.1, 5.1. Unfortunately, the 386 computer used to run it failed and the programme could not be replaced.

**Metamorph (Universal Imaging Corp., West Chester, PA. USA)**
For the laser scanning microscope, live images were captured and displayed using the 'Metamorph' image processing system and it was possible to record, store and process single image frames. Image brightness and contrast could be controlled. One aspect of the processing allowed images to be superimposed and colour coded. Several images of a single field of view could be recorded with different laser lines and long pass filters in order to make comparisons of reflection and fluorescence images and to highlight the distribution of different fluorescent dyes incorporated within the sample examined. However, the pseudo colouring had limited value and was by no means comparable to the colour images recorded with the TSM, because of the limitations of the software. Hard copy of the recorded images was made by taking photographs of the monitor as described above.
Figure 2.4  Photograph of the computer screen illustrating a single frame from a fracture sequence and the surrounding computer screen controls to replay the fracture sequence.
Real time images recorded with the CCD or the SIT camera were digitised using Tempus software, initially running on a 486 PC (HP), (Chapter 7.2). Using the Kinetic Imaging ‘Acquisition Manager’ programme within the software, live images were viewed as ‘videos’ and could be recorded as single frames (‘snaps’) or as a video sequence (‘grabs’ of up to 4000 frames). The latter could be replayed and edited using the image processing programme -Kalcium Analyse. Fig. 2.4. illustrates the view on a monitor with a single frame from a fracture sequence and the surrounding on screen ‘video’ controls. The image quality could be improved by adjusting brightness and contrast. The image processing using this programme was also used in these studies for the calculation of surface area of samples (Chapter 7.2). Measurements of the areas were repeated to check the reproducibility.

A Random Array of Independent Discs (RAID) was used as the data storage medium for the video rate images, because at the time, (1994) none of the hard disks available were capable of transferring and storing data at the rates required. Storage of images in the grab sequences required an enormous memory capacity. Images were stored directly onto the RAID - a mass storage unit of hard disks running in parallel. This gave 9 Gb of capacity equivalent to 24 minutes of 512 x 512 x 8 bit deep video rate (25 frames per second) data. The transfer rate was at 12 Mb s⁻¹. Subsequent hardware developments now allow similar results to be achieved using high specification hard disks.

The Tempus software along with the RAID was developed specifically for the microscopy and imaging facility at Guy’s Dental School, to allow the recording of dynamic events within tooth samples such as tooth cutting and interfacial failure (EPSRC grant GRJ01035). The technology for handling and storing the large volume of data involved was not developed and available until the end of 1995. This system was developed along with a miniature straining stage (incorporating a motorised pusher with load cell and optical encoder) and a data acquisition unit (DAU) to allow the synchronised recording of images with load and position. This was ideal for recording the events when interfaces are placed under load. The advance in technology
can be measured by comparing the amount of information reported in Chapters 7.1 and 7.2.

**Image quality**

As can be seen in the thesis, the image quality was best when recorded with the TSM on 35mm film, but this technique did not allow the recording of real time images. However, the reduction in image detail when images were digitised and displayed, was outweighed by the ability to make real time recordings of the dynamic events at the interface, the easy access to stored images and the convenient storage on CD.

2.8 **Statistical Analysis**

The majority of results are presented as qualitative data - descriptions and images of interfaces. The micropermeability data (Chapters 4 and 6) was analysed using the Kolmogorov-Smirnov test (Lehmann 1975) and the shear bond stress data (Chapter 7) analysed using one way analysis of variance in conjunction with the Bonferroni test for multiple comparison of means test, in addition to a Kaplan Meier Survival analysis. Details of the analysis are given in the relevant chapters.
Chapter 3

DEVELOPMENT OF A METHOD OF OBSERVING MOVEMENT OF FLUID THROUGH DENTINE TUBULES IN REAL TIME

3.1 Introduction

Many procedures in operative dentistry may subject the fluid content of dentine to rapid changes. Fluid movement occurs within the dentine tubules and is implicated as a possible mechanism of dentine sensitivity as well as profoundly influencing the efficacy of many dentine adhesives used in restorative dentistry. In addition, the dentine bonding systems are applied to dentine surfaces as a series of fluid components which interact both with the dentine surface and with the dentine tubules.

To examine fluid movements in dentine, the tubules and their contents need to be observed and recorded in real time and at high resolution. Subsurface images of the dentine are required, and so an immersion medium is needed to optically couple the objective lens with the sample. The sample needs to be separated from the coupling medium by a coverslip so that the coupling medium does not interfere with fluid movement in the tubules and so that dyes introduced into the tubules to map fluid movement do not contaminate the coupling medium and interfere with image quality. Consequently, this sort of experimentation is not feasible with non-coverslip immersion objectives for low refractive-index immersion media (for example water, saline or methyl cellulose solutions).

Images with the highest resolution are produced by objectives with high numerical aperture (NA - light gathering ability). The high NA objectives are used with a coverslip to avoid inadvertent damage to the objective and the working distance is generally less than 200 μm including the coverslips thickness. Immersion oil (with the same refractive index as a glass coverslip) is generally used as both the coupling medium above the coverslip and the mounting medium between the sample and the coverslip (Watson 1997).
However, in order to record fluid movement within the dentine tubules an alternative
non fluid medium of appropriate refractive index has to be found as the mounting
medium, as a fluid medium would interfere with events in the tubules.

3.2 Objectives

To develop a method of coupling a sample to a coverslip in order to visualise fluid
movement within a mineralised sample. The requirements of the coupling medium
were, that it did not interfere with the quality of the image, nor with the sample
structure. It was envisaged that this would lead to the production of a durable
cementation system for attaching teeth to coverslips so enabling fluid/tissue inter­
reactions to be studied and recorded using real time confocal scanning microscopy. The
proposal was to investigate a variety of dentine bonding agents as possible adhesives
for attaching coverslips to dentine after first setting criteria for the suitability of such
adhesives as coupling media for dentine samples.

A further aim was to trace the movement of the different components of a dentine
bonding system as they were applied to a dentine cavity surface.

3.3 Materials and method

Suitability of dentine adhesives for attaching coverslips to teeth.

The following criteria were employed:

1. The bond between the coverslip and tooth must allow handling of the
   specimen and storage in water or a humid environment.
2. The adhesive should not penetrate the sectioned surface of the dentine
   into the tubules where fluid movement was to be observed.
3. The adhesive must not detract from the quality of the image produced.
4. The thickness of the coverslip should permit the objective lens to be
   focused within the dentine.
Figure 3.1 Diagram of prepared tooth sample illustrating the cavity design.

Figure 3.2 Prepared tooth sample with approximal cavities cemented to a plastic coverslip.
Table 3.1

<table>
<thead>
<tr>
<th>Method of attaching plastic coverslips to dentine samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Primer (where indicated) and adhesive applied to the coverslip and dentine surface according to the manufacturer’s instructions.</td>
</tr>
<tr>
<td>2. Second layer of adhesive applied to the dentine surface. Prepared coverslip positioned on the dentine surface. Finger pressure applied to the coverslip to remove entrapped air. Light activation of adhesive through the coverslip.</td>
</tr>
</tbody>
</table>
Tooth preparation.
Recently extracted human lower third molar teeth were sectioned longitudinally in a mesio-distal plane, in the mid portion of the tooth, so that the major tubular orientation was parallel to the cut surface (see Chapter 2). The pulp contents were left in situ unless fluid movement from the pulp chamber was to be observed. In the latter case the pulp was removed with a pair of college tweezers to avoid the creation of a smear layer on the surface of the pulpal dentine, the roots were amputated with a diamond bur in an air turbine handpiece under a water spray.

Cavities were cut into the mesial and distal aspects of the tooth sections (Figure 3.1). A chamber was created for the introduction of test fluids once a coverslip was attached to the sectioned tooth surface. Fluid/dentine interactions could then be examined along the walls of the cavity, just below the sectioned tooth surface, using a confocal microscope.

Cementation of the coverslip.
A variety of adhesives were investigated with glass and then with plastic (polyester) coverslips (Agar Scientific, Stanstead, Essex) to find a combination which would give a reliable bond. In initial studies glass coverslips were used without preparation, later samples were prepared by first coating the coverslip with a silane coupling agent or sandblasting with fine alumina grit prior to cementation.

Prior to investigating the dentine adhesives as the mounting medium domestic type cyanoacrylate adhesives were evaluated, but results with these were disappointing, because the bond was hydrolytically unstable.

The original dentine adhesives (see Table 2.1) investigated (from 1991) were Tripton (ICI Dental Macclesfield, UK), XR bond (Kerr's Dental Mfg., Romulus, Mich), Pertac Universal Bond (ESPE, Seefeld, Germany) (1992). Later in the project, as the former adhesives became superceded by newer developments, the use of Prime and Bond, PSA (Dentsply, Weybridge, Surrey UK) was investigated. The stages of attaching the coverslips are given in Table 3.1. To avoid an air inhibited layer of non polymerised
adhesive resin at the periphery of the sample, the junction between the periphery of the sample and the plastic coverslip was sealed with Air Block (Dentsply, Weybridge, Surrey UK). This ensured the sample was more robust, by helping to prevent the coverslip from peeling away from the tooth.

**Assessment of the penetration of the adhesive into the dentine.**

To assess the penetration of the adhesives into the dentine, samples were cemented to a flat matrix of thin acetate sheet using the trial dentine adhesives and then sectioned at a 90° angle to the plane of the matrix. The stages in attaching the matrix were identical to those used for attaching the coverslips.

The dentine adhesives were labelled by adding fluorescent dyes to the components of the bonding systems (Watson 1989): rhodamine B in the primer (where used) and auramine O in the light activated resin. The penetration of the labelled bonding system components into the dentine was then assessed with a TSM using a x60/1.4 NA oil immersion objective and appropriate filters. A few grains of the dyes were added to the components of the adhesive systems in sufficient quantity to obtain a fluorescence signal. Images were recorded using 35 mm film.

**Observation of fluid movement**

Observation of fluid movement within the dentine was dependent on several factors. Most important in the method was ensuring that the tubules opening onto the cavity wall were not sealed by the cementing dentine adhesive. This could occur if the adhesive formed a meniscus at the junction of the dentine and the coverslip during the cementation process. This problem was addressed by exploring a variety of methods of cementation. These included coating the cavity with nail varnish, which was peeled off following adhesion; obturation of the cavity with an addition curing silicone impression material; or by re-defining the cavity with a high speed tungsten carbide fissure bur with water cooling following adhesion to the coverslip.
Tracking of fluid along the dentine tubules.

Once a suitable cementation technique was established, the effectiveness of the technique within an experimental situation was verified. Prepared samples were examined immediately or stored for 1 week in a humid environment at 4°C prior to examination. The humid conditions were obtained by storing each sample in a sealed glass container with water. This gave a relative humidity of 90%, as measured with a calibrated digital relative humidity meter (Sidu et al. 1997). Test cavities were re-defined with the high speed bur to remove any excess adhesive at the junction of the dentine and the coverslip. The dentine was etched with phosphoric acid for 15 s and the cavity filled with fluorescent dye (0.5 g rhodamine B in 50 ml normal saline). In other samples fluid movement was observed from the pulp chamber. The fluorescent dye solution was introduced through polythene tubes sealed into the pulp chambers with a glass ionomer cement (Fig 3.2). Images demonstrating the progress of the dye along the tubules were viewed with x20/0.8NA oil immersion objective in the TSM and recorded using a SIT camera in conjunction with a MISIS Image 3Dplus image analysis system or recorded on 35 mm film. The 3Dplus programme was used to acquire through focus images with a range of 5 -15 μm. Images were captured at 0, 1, 2, 3, 5, 10 and 15 minute intervals and the distance the dye had travelled along the tubules could be measured.

The quality of the images of the moving dye within the dentine tubules was evaluated subjectively. A comparison with fluorescent images obtained in experiments where hydrated samples were mounted with oil above and below the coverslip was made.

Tracking SBMP primer and adhesive resin components in the dentine.

Teeth were prepared as described above to track the primer and adhesive components of SBMP in the dentine when applied to a cavity surface. After adhesion of the coverslip, the cavities were re-defined with the high speed bur to remove any excess adhesive at the junction of the dentine and the coverslip and the cavities were prepared with maleic acid, rinsed and dried. Samples were viewed with a TSM using a x60/1.4 NA oil immersion objective, whilst the cavity surface was treated with the SBMP primer and adhesive. The primer was labelled with rhodamine or the adhesive
with fluorescein to aid the assessment of the distribution of the components. The stages of dentine preparation were recorded on 35 mm film as the components were applied. The effect of an air jet and brushing the components was examined.
Figure 3.3  Combined reflection (LHS) and fluorescence image (RHS) of acetate sheet bonded to a tooth with XR Bond. The longitudinally sectioned sample is imaged at the enamel dentine junction. Enamel prisms (E) and fine lateral branching dentine tubes (D) can be seen in the reflection image. The distribution of the dentine primer (labelled with rhodamine B) is highlighted in the fluorescence image. Diffuse mixing of the primer in the adhesive can be seen. x60/1.4N/A oil immersion above and below the coverslip. 546/600 nm. Scale bar 20 μm.

Figure 3.4  Combined reflection and fluorescence image of acetate sheet bonded to a tooth with Pertac Universal Bond. The longitudinally sectioned sample shows the dentine tubules in the reflection image and the adhesive layer (labelled with auramine O) in the fluorescence image. There has been virtually no penetration of the dentine or the tubules. x60/1.4N/A oil immersion above and below the coverslip. 450/520 nm. Scale bar 20 μm.

Figure 3.5  Combine reflection and fluorescence image of the interface between a cavity cut in dentine (D) and a resin composite restorative material (C), using Tripton as the dentine adhesive. The Tripton primer (P), labelled with rhodamine B, has infiltrated the smear layer, but not the underlying dentine. The diamond bur has produced a much thicker smear layer than in Figures 3.3 and 3.4 which were cut with a fine grit low speed diamond saw. x60/1.4N/A oil immersion above and below the coverslip. 546/600 nm. Scale bar 20 μm.

Figure 3.6  Combined reflection and fluorescence image of acetate sheet bonded to a tooth with Prime and Bond. The longitudinally sectioned sample shows the dentine tubules in the reflection image and the distribution of the primer, labelled with rhodamine B, in the fluorescence image. x60.1.4N/A oil immersion above and below the coverslip. 546/600 nm. Scale bar 20 μm.
3.4 Results

Adhesion of coverslips.
Figure 3.2 illustrates a completed sample. A durable bond between a glass coverslip and the tooth sample could not be obtained with the adhesive systems. The bond between the resin and the glass was sensitive to water storage, but was sufficiently strong to withstand careful handling of the glass/adhesive sample at the time of placement. Plastic coverslips with dentine adhesives were the preferred combination, because of the requirements for long term stability. Tripton bond and Prime and Bond gave the most reliable cementation. The use of the air barrier gel allowed the resin at the periphery of the samples to be adequately cured, and so reduced the likelihood of a 'peeling' type failure at the weak air inhibited edge of the sample.

Prime and Bond PSA proved a useful alternative to the earlier dentine adhesives. A durable bond was obtained which was sufficiently robust to allow immediate use of the samples or storage in 90% humidity. However, after 24 hours storage it was not possible to focus the objective within the dentine and the plastic coverslips had to be thinned by grinding to allow the dentine to be visualised.

Penetration of the coupling medium adhesive into the dentine.
Figures 3.3 - 3.6 illustrate the interface between the tooth surface and the dentine bonding resins. Comparing reflection and fluorescence images XR Bond (Fig. 3.3) is seen to have penetrated the dentine surface, being confined to an erratic distribution within the thin dentine smear layer produced by the low-speed diamond saw. Pertac Universal bond (Figure 3.4) did not penetrate the intertubular dentine or the tubules. A similar appearance was seen with Tripton (Figure 3.5). This image is of a section through a tooth restored with Tripton and a light-cured resin composite restoration. The fluorescence image illustrates the penetration of the adhesive system into the thicker smeared layer, which is always produced when hard tissues are cut with coarse cut diamond burs. Figure 3.6 illustrates the interface between the tooth surface and the Prime and Bond. This material was used in later samples when Tripton bond was no longer available. Again no penetration of the resin into the dentine surface can be seen.
Observation of fluid movement.

Re-defining the cavity with a tungsten carbide fissure bur in an air turbine with water cooling was the most successful way of preparing the dentine surface to be examined, and was easiest to perform when a plastic coverslip was used. This technique ensured that the dentine surface was free from excess adhesive. Although cutting the dentine and meniscus of cementing adhesive adjacent to the coverslip resulted in damage of the coverslip in some samples, relatively few samples were lost in this way. The disadvantages of the other techniques were the difficulties in manipulating the masking materials to give the desired results.

The thickness of the adhesive and plastic coverslip (250 μm thick) allowed subsurface dentine to be imaged. However, after storage of the samples prepared with Prime and Bond the objective could not be focused within the dentine. This indicated that the adhesive layer had increased in thickness by taking up water during the storage period. Once the coverslip had been polished to an appropriate thickness, these samples were made more robust by the addition of resin composite to seal the periphery, rather than using the air barrier gel.

Tracking of fluid along the tubules.

Figure 3.7 shows a timed sequence following the passage of fluorescent dye (rhodamine B in saline) through dentine tubules. This sequence of images, produced in a sample cemented with Tripton to a plastic coverslip, compares favourably with the images seen in Figure 3.5, recorded with the conventional arrangement of oil above and below a glass coverslip. The resins did not interfere significantly with the quality of the images and the dentine tubules at the cavity surface are patent and not sealed off by the resin.

Fluid movement through the tubules was rapid. In this sequence the fluid moved in the region of 80 μm in 2 minutes. The progression of the fluorescent dye along the tubules was erratic with bursts of activity. The reservoir of dye emptied with time and so fluid movement was also dependent on a reservoir of dye remaining in the cavity. The
distance the dye travelled along the tubules with time was measured using the 3Dplus MISIS programme, as seen in Figure 3.8.

**Tracking of the primer and resin in the dentine.**

Figures 3.9 - 3.12 illustrate the penetration of the SBMP primer (P) and adhesive (A) along the dentine tubules (T) following application with a fine sable hair brush. The influence of air drying was to increase the tubular penetration of the primer (Figs. 3.9 and 3.10). The effect of air-blowing the adhesive was to increase the thickness of the resin in the line angle, a finding common to all the samples (Figs. 3.11 and 3.12).
Figure 3.7 Timed sequence following the passage of fluorescent dye (rhodamine B in saline) through dentine tubules from the cavity surface (allowed) after demineralisation with a 10 s phosphoric acid etch. This sequence of images, produced in a sample cemented to a plastic coverslip, compares favourably with the images seen in Figure 3.5, recorded by conventional means with oil above and below the coverslip. x60.1.4N/A oil immersion above the coverslip, sample bonded to the coverslip with Tripton. 546.600 nm. Scale bar 20 μm.
Figure 3.8 Timed sequence of images as in Figure 3.7, but fluid tracking from the pulp cavity and recorded as images from the monitor whilst using the 3D+ computer programme. Illustrating the use of the system in recording through focus images and for measuring the progression of the fluorescent dye along the tubules. x60/N/A oil immersion above the coverslip, sample bonded to the coverslip with Tripton. 546.600 nm. Scale bar 20 μm.
Figure 3.9 Illustrating the passage of SBMP primer, labelled with rhodamine B, into the dentine and dentine tubules immediately after application to the cavity surface. x60/1.4N/A oil immersion above the cover slip, sample bonded to the coverslip with Tripton. 546/600 nm. Scale bar 20 μm.

Figure 3.10 The same field of view as in Figure 3.9 with further penetration of primer (P) into the dentine tubules (T) and reduction in the pooling at the cavity angle following air thinning. x60/1.4N/A oil immersion above the coverslip, sample bonded to the coverslip with Tripton. 546/600 nm. Scale bar 20 μm.

Figure 3.11 Illustrating the passage of SBMP adhesive, labelled with florescein, into the dentine tubules and superficial dentine to form a hybrid zone, immediately after application to the cavity surface. x60/1.4N/A oil immersion above the coverslip, sample bonded to the coverslip with Tripton. 450.520 nm. Scale bar 20 μm.

Figure 3.12 The same field of view as in Figure 3.11 with further penetration of the adhesive (A) into the dentine tubules (T) following application of an air stream to try to thin the adhesive layer. x60/1.4N/A oil immersion above the coverslip, sample bonded to the coverslip with Tripton. 450/520 nm. Scale bar 20 μm.
3.5 Discussion

Prior to this study assessments of permeability have been made by the observation of a bubble in a fluid filled sealed system in communication with the pulp chamber or with a section of dentine (Pashley et al 1988). Images of the fluid moving through the tubules have not been reported. In this study recording of the fluid movement was only possible because the tubules below the surface of the sample could be visualised in near normal conditions, without interference from the mounting medium. This technique therefore reported the tracking of fluid along individual tubules rather than giving overall results of permeability through the dentine sample as a whole. The erratic way in which the fluid travelled through the tubules had not been reported previously. The fluid progressed on a relatively even front through the dentine (within one field of view - 300 \( \mu \text{m} \)). If the smear layer on the dentine surface was not removed with acid treatment, no fluid movement was traced from the cavity surface within the 15 min observation time.

The model could be adapted to provide a positive pressure with the addition of the longer tubes and an appropriate column of water.

This window technique overcame the reported difficulties of tracking the movement of fluid in a capillary tube at a distance from the tooth, especially when needing to record small changes in flow and pressure. In the past a meniscus or air bubble has commonly been used as discussed in the literature review. However, the dynamics of bubble movement within thin bore tubes meant that these techniques had potential measurement errors (Vongsavan and Matthews 1992). The latter co workers have reduced these problems with the use of fat droplets produced by diluting milk in Ringer’s solution.

The \textit{in vitro} morphological assessment of dentine bonding systems and restorative materials has been dependent on the examination of the static dentine/restorative interface, rather than dynamic interaction between the substrate and the restorative material during the placement of restorations. By using dentine bonding system
components labelled with fluorescent dyes, the current model has potential in being used to observe and record the dentine/adhesive interface during placement. This technique could thus give further information on the interaction of the components with each other and with the dentine and enable comment on the handling properties of the materials. In this case the effect of air drying and thinning the components was recorded. The thickness of the SBMP adhesive previously reported as 54 μm with brush application, 32 μm with light air blow, 9 μm with a heavy air blow and 22 μm with brush thinning (Fundingsland personal communication 1995).

The most durable adhesion of coverslips to dentine after storage of samples in water or 90% humidity at 4°C for a week was obtained with the dentine adhesives and plastic coverslips. The cyanoacrylate adhesives proved less reliable especially after storage (proprietary versions of these adhesives are not normally recommended for applications subject to soaking in water). Similar problems were encountered with the glass coverslips, even if the glass were first treated by grit blasting with 50 μm alumina or treated with a silane coupling agent used in dental adhesive techniques to improve the affinity of ceramics for hydrophobic resins. Short term use of epoxy resins and glass has also been reported by Watson (1987).

The dentine bonding agents used in this study were selected because they have been reported to preserve or only modify the smear layer (Nakabayashi et al 1982, van Meerbeek et al (1992). Those adhesives which remove the smear layer were not included, as the resin bonding agent would be likely to penetrate the dentine surface and tubules where fluid movement was to be observed. This was undesirable as it would interfere with fluid movement within the tubules.

The results showed that Tripton, Pertac Universal Bond and Prime and Bond did not penetrate the dentine even when the tubules opened directly onto the surface where the adhesives were placed. The Tripton system consisted of a polyhexanide primer and a bonding resin. The Pertac Universal Bond, however, had no primer, but relied on a bonding agent. Figure 3.5 illustrated the penetration of the Tripton bond agent into the smear layer but not into the underlying dentine. This is further highlighted by the
thicker smear layer in this sample, produced by preparation with a high speed diamond bur rather than with a fine diamond wheel. When ICI withdrew from the dental market the Tripton adhesive system was sold to the optics industry as a means of encapsulating and bonding the ends of optic fibres (Watson personal communication).

The XR Bond system also consisted of a primer and bonding agent. However, this system is reported to partially dissolve the smear layer (van Meerbeek et al 1992). The images of this bonding agent (Figure 3.3) show patchy penetration into the dentine surface, but no penetration into the subsurface dentine or dentine tubules. For this reason Tripton or Pertac Universal Bond were considered to be the most appropriate bonding agents for this application.

During the period of this study there has been considerable development of dentine bonding agents, and several have been removed from the market. Those dentine bonding agents which leave the smear layer unaltered, (including Tripton and Pertac Universal Bond) have tended to be superceded by newer ('fourth') generation systems which remove the smear layer with a 15 s acid etch ('total etch'). Developments were in this direction because the smear layer is the weak link with the adhesive and resin composite. However, to help acceptance in General Dental Practice, a variety of 'single bottle' dentine adhesives have been designed. These employ a single component-a combined primer and adhesive, to prepare the dentine. A search for a currently available product, suitable for coupling dentine samples to coverslips has resulted in the use of Prime and Bond PSA. This system was simple to use, having only one component, and was found to meet all the criteria required, except that, the adhesive swelled after storage in 90% humidity. This made focusing within the dentine sample impossible, but was overcome by polishing the coverslip to reduce its thickness. This process further stressed the adhesion between coverslip, adhesive and tooth. The coverslip also became more flexible as its thickness was reduced. This problem was overcome by placing a light activated resin composite around the periphery of the sample rather than using the air barrier gel. The increase in thickness of this adhesive with time, had not been previously documented.
For future studies this experimental set up, using the TSM and an image analysis programme to measure the distance from the surface of the sample to the inferior surface of the coverslip, would provide a method of measuring the changes in thickness of adhesives in wet and dry conditions with time.

The effect of different immersion media used to couple the dentine sample to the coverslip did not appear to degrade the lateral microscopic resolution of the samples under examination in this study. The optimisation of the microscopic operating conditions may be compromised by mixing immersion media of different refractive indices below a coverslip. In fact this may happen much more frequently than is appreciated when biologic hydrated samples are examined using oil immersion oil objectives with oil on the specimen (Watson 1997). This could have an effect on the apparent and actual z distances observed and measured; an error which may be significant for through focus reconstructions.

In theory, the combined thickness of the plastic coverslip (250 μm) and the film of adhesive resin should have been greater than the working distance of the conventional oil immersion objectives that were used. However, we found no problem focusing up to 50 μm within samples as measured by the microscope, except in samples where Prime and Bond had been used as the adhesive and the samples stored in 90% humidity as outlined above. It could be that the intimate relationship between the resin-impregnated interface and the polyester coverslip show improved optical properties because of the close match in refractive index, effectively reducing the optical distance between the end lens of the objective and the surface of the sample. It is probable that the refractive index of the resin and polyester coverslip were coincidentally very similar: this was illustrated by the removal of a reflecting surface from the imaging path, thus maintaining high quality imaging.
3.6 Conclusions

This method of attaching teeth to coverslips enabled fluid movements within the dentine tubules to be studied with the confocal light microscope in real time. Tripton and Prime and Bond PSA dentine adhesive systems provided a durable cementation system for attaching teeth to plastic coverslips. A chamber, formed by the coverslip and cavity cut into the dentine, allowed injection of fluorescent dye into the chamber and the observation of fluid movement in the dentine tubules. The fluid movement along the tubules, marked by the fluorescent dye, was erratic, taking place in bursts of activity.

The microscopic visualisation of the application of primer and adhesive to a cavity surface was also observed through a plastic coverslip using the same model. It enabled the dynamics of primer and adhesive application to the dentine to be observed and highlighted the beneficial influence of air thinning on the progression of the primer and adhesive along the dentinal tubules.

The dentine adhesives could be used as a coupling medium to adhere to other mineralised tissues - both biological and geological, thus enabling fluorescent dyes to be introduced in a similar way to highlight the structure of the mineralised tissue.
Chapter 4

MORPHOLOGICAL STUDIES OF DENTINE/RESTORATIVE INTERFACES

4.1 PRELIMINARY INVESTIGATION - SCOTCHBOND MULTI PURPOSE

4.1.1 Introduction

One method of assessing the potential performance of dentine bonding systems is to study the microscopic form of the dentine/resin interface. Many of the methods described require extensive preparation of the dentine/restorative sample. Fluorescence confocal microscopy has been used to examine the interfacial region of dentine and enamel with third generation bonding systems (Watson 1989). The techniques described in this chapter were therefore developed to make a preliminary assessment of a fourth generation bonding system.

4.1.2 Objectives

The aim of this preliminary study was to use and develop fluorescence confocal microscopy techniques to investigate the morphology of the resin/dentine interface of a dentine bonding agent: Scotchbond Multi Purpose [SBMP]. The details of this bonding system are given in Chapter 2. At the time of the study this system was one of the first fourth generation systems marketed in the UK.

The objectives of this study were:

1. To examine the subsurface morphology of dentine/restorative interfaces in longitudinally sectioned samples with restorations in situ.

2. To investigate changes in operator technique on the appearance of the interfacial region.
3. To observe the dentine tubules *en face*, cut in transverse section, whilst curing the dentine adhesive.

All investigations were made with the aid of fluorescent labels incorporated in the components of the SBMP system (Watson 1989).

### 4.1.3 Materials and Methods

40 approximal wedge shaped cavities were prepared in twenty teeth and the cavities were prepared with the SBMP system according to the manufacturer’s instructions (Table 2.2). Details of cavity preparation, restoration, and examination used and developed for this preliminary study are included in Chapter 2 - General Materials and Methods.

Single or dual fluorescent labelled samples were prepared to aid the examination of the interfacial region. The components of the bonding system were labelled: primer with rhodamine B and adhesive with fluorescein. Restorations were made with Z100: a light activated composite restorative material. The teeth were then longitudinally sectioned in a mesial / distal plane through the restorations. Samples were prepared by thinning the adhesive with an air jet or mini application brush. All samples were sectioned longitudinally within 3 hours of placing the restoration.

The interfacial region was examined using a TSM. Reflection and fluorescence images were recorded on 35 mm film or with a SIT or CCD camera, digitised and image processed or reconstructed with the MISIS 3Dplus computer programme. Samples were also examined using a CLSM with images recorded by taking photographs of the monitor.

Two further samples were produced with the crown of the tooth horizontally sectioned to remove the occlusal enamel, thus presenting the dentine tubules at 90 ° to the cut surface of the remaining tooth. This flat plane was then etched with the 10% maleic acid and fluorescent labelled primer applied as per the manufacturer’s instructions. The adhesive resin was then placed under dark, safe-light, operating conditions. A glass coverslip placed on the resin acted in a similar manner to a clear matrix strip.
The sample was then examined under the microscope using red illumination in order to find the etched and primed cut surface. The illumination colour was then changed to blue and the adhesive resin allowed to polymerise using the microscope light source. Images of primer distribution were recorded using 35 mm film.

4.1.4 Results

Images of the dentin/restorative interface (Figures 4.1 and 4.2) illustrate the morphology of the interfacial region. Inclusions of primer (P) within the adhesive (AO were found frequently when the adhesive was thinned by brushing, rather than air thinned. However, use of an air thinning technique had a tendency to cause pooling of the adhesive in irregularities in the dentine surface and at the angle of the cavities (Figure 4.3). Resin tags (T) were formed where the adhesive entered the dentine tubules. Penetration of the dentine tubules tended to be greater on the occlusal surface rather than the pulpal aspect of the cavity. A hybrid layer (H) was clearly visible indicating that the inter-tubular dentine had been penetrated by the adhesive (Figure 4.3).

These features were also visible when viewed with a CLSM. The distribution of the primer and adhesive could be visualised within the dual labelled samples by illuminating the samples with the appropriate laser lines. However with the TSM, there appeared to be some cross talk between the fluorescein and rhodamine signals, with the available filters.

Even when the teeth were sectioned within 3 hours of placing the restoration, the bond appeared to be intact; there was only one sample out of eight where gap formation was seen, indicating a marked failure of the dentine resin bond.

Distribution of the primer in the dentine tubules was also seen by examining the cut and prepared surface of the dentine (Figure 4.4). Primer (P) labelled with rhodamine B can be seen lining the periphery of the majority of dentin tubules, showing as an orange ring. Where the tubules have been penetrated by resin adhesive (A) they appear dark
within this ring, where the resin has failed to penetrate the orifices remain plugged with reflective debris (D), which is a light green colour. Observation of the curing adhesive resin showed marked movement of the resin.
Figure 4.1  Resin composite (C) dentine (D) interface where the primer has been inadequately air dried and the adhesive brushed to thin rather than air thinned. Adhesive (A) has pooled at the cavity angle and inclusions of the primer (P), labelled with rhodamine B, can be seen within the adhesive. 546/600 nm. x60/1.4NA OI. Scale bar 100 μm.

Figure 4.2  Dentine/restorative interface as in Figure 4.1, but adhesive labelled with fluorescein. The fluorescein labelled adhesive appears green whilst the rhodamine labelled primer is yellow/red in colour. 450/-nm. x60/1.4NA OI. Scale bar 100 μm.

Figure 4.3  Fluorescence image illustrating a resin composite (C)/dentine (D) interface. Adhesive (A), labelled with fluorescein is seen filling irregularities in the cavity surface, resin tags (T) within the dentine tubules and a well defined hybrid zone (3-4 μm wide) are also visible. 450/520 nm. x60/1.4NA OI. Scale bar 20 μm.

Figure 4.4  Dentine surface with tubules sectioned at 90°, after preparation with etch, primer (labelled with rhodamine B) and adhesive. Primer (P) can be seen at the periphery of the majority of the dentine tubules. Those tubules with dark centres are filled with adhesive (A), those with bright green reflective centres are filled with debris. 546/-nm. x60/1.4NA OI. Scale bar 20 μm.
4.1.5 Discussion

Scotch Bond Multi Purpose was selected for this preliminary investigation as it was a new generation dentine bonding agent at the time of the study (1992). Unlike many of the contemporary total etch systems it used maleic rather than phosphoric acid, and had a co-polymer unique to the 3M dentine adhesive systems.

The morphology of the interfacial region was imaged with the aid of fluorescence confocal microscopy. Dual labelling of the components revealed further information about the relative distribution of primer and adhesive components, compared to single labelling. However, the simplicity of single labelling would be advised for initial evaluations of interfacial morphology, especially if there is the possibility of cross talk. The problem of cross talk with the TSM was later overcome by the use of a band pass filter for fluorescein, however this decreased the strength of the fluorescent signal. A search was begun for a more appropriate dye, as discussed in Chapter 2.

A distinct hybrid layer (3-4 μm deep) as described by Nakabayashi et al (1982) was visible in the images of the subsurface morphology of the interface. The formation of a hybrid layer or interdiffusion zone when dentine is prepared with the SBMP system has also been reported by van Meerbeek et al (1993a) with SEM and TEM studies. They reported a depth of decalcification with 10% maleic acid of approximately 3 μm and that the low viscosity resin was able to penetrate to this depth. Raman spectroscopy demonstrated effective penetration of resin into the decalcified dentine, but failed to demonstrate any chemical bond. It was therefore concluded that adhesion between the dentine and resin composite is a function of micro-retention by resin impregnation of the residual collagen matrix left after decalcification, rather than chemical reaction.

Although this system uses a relatively simple technique with three stages, the influence of operator technique was seen and attention to detail during each stage must not be underestimated. The manufacturer’s instructions did not include specific details on the appropriate thickness or of modifying the thickness of the primer and resin layers. In an attempt to avoid pools of primer or adhesive at the cavity angles, a brushing or air thinning technique is commonly used in clinical practice. In this
study, inclusions of primer within the adhesive were most notably seen with brush thinning rather than air thinning. The adhesive was considerably more viscous that the primer and so was more liable to be displaced when thinned with a brushing technique. This underlines the need for specific instructions with products, rather than leaving technique to the discretion of the operator.

Primer displacement of this material has also been described in other studies (van Meerbeek *et al* 1996, Perdigao 1995). The displaced primer fragments have generally been associated with poor penetration of the primer into the dentine surface and tubules, as seen in this study. This may reflect the effect of the demineralising and drying of the dentine surface and the ability of the primer to re-wet and penetrate this surface. It may be possible for a layer of exposed surface collagen to shrink and collapse after etching, washing and lightly drying. The labelled primer may attach to this layer, but not be able to penetrate it, thus making it more liable to be displaced. An alternative explanation of primer displacement may be that there is penetration but not polymerisation of the co-polymer below the surface of the hybrid layer, or indeed pre-polymerisation of the co-polymer on the surface of the dentine, allowing the surface primer layer to be easily detached (Perdigao 1995). The co-polymer is specific to SBMP (and SBMP+ see Chapter 7).

Further investigation of the SBMP interfacial morphology was undertaken with TEM (van Meerbeek 1996). An ‘amorphous electron dense layer’ with ‘electron lucent globules’ was noted on the surface of the hybrid layer. The chemical nature of this layer could not be determined because of its sub-micron dimensions. However, when modified primer (without co-polymer) was used this amorphous layer was not seen, indicating that the co-polymer was responsible. It was further considered that HEMA in the primer could infiltrate the demineralised dentine whilst the carboxyl groups of the copolymer react with calcium on the dentine surface. These calcium polyalkenoic complexes may prevent infiltration of the resin into the dentine. Whatever the mechanism that is involved, the displacement of this layer of material is unique to this type of 3M adhesive. Van Meerbeek *et al* 1996 reported that where the
amorphous layer was lacking, the hybrid layer was more uniformly intact, which again concurred with the findings in this study.

Using the cavity design employed by Watson and Wilmot (1992), a clear distinction could be made between tubules connected to the occlusal enamel and those in connection with the pulp (Figure 2.1a). Greater penetration of the primer and adhesive into the dentine was noted in those tubules connected to the occlusal surface. This finding concurred with the findings of Watson and Wilmot (1992) for Syntac adhesive (Ivoclar Vivadent, Leichtenstein) and indicates the possible influence of the pulp fluid pressure and hence dentine wetness, on the performance of dentine adhesives. The formation of resin tags and micro-mechanical attachment to the tubule walls was also described in TEM studies (Titely et al 1995 and van Meerbeek et al 1996).

Gaps between restoration and dentine were rarely seen. The cavity design had a ratio of free to constrained surfaces of 1:2 and the gap formation compared favourably with the restorations with 5 constraining surfaces (Chapter 6).

Additional information was gained by examining the dentine tubules en face through a glass coverslip and layer of adhesive resin as it cured. The maleic acid was effective in removing the smear layer and the majority of dentine plugs, the primer was visible as an annulus lining the tubule walls. It was possible to observe movement of adhesive materials during light polymerization, by excitation with the illuminating light source of the microscope. The adhesive components were placed on top of the sectioned tooth sample, under a coverslip, with safe lighting conditions. Once the material was activated with light of 470 nm wavelength considerable movement of the coverslip and resin was seen as the polymerisation progressed. Such images clearly indicate the complexity and stresses of the adhesive/tooth. The recording methods developed for the fracture studies (Chapter 7) may in the future be used to record the resin movement during polymerisation.
4.1.6 Conclusions

The methods employed in this study were considered suitable for evaluation of the morphology of the interfacial region for teeth restored with SBMP, a fourth generation bonding system available at the time of the study.

A simple preparation technique allowed the interface to be visualised under near normal conditions, unlike many other methods generally employed to determine interfacial morphology.

Methods of applying the dentine bonding system, relating to its likely clinical usage resulted in an alteration in the appearance of the interfacial region. In particular, the distribution of the primer and adhesive at the dentine interface was technique sensitive. Penetration of the primer and adhesive into the dentine tubules, a distinct hybrid zone and a thin layer of adhesive were observed with adequate drying of the primer and air thinning of the adhesive. The delicate primer layer on dentine was easily displaced by the viscous adhesive often giving rise to a bizarre appearance.

Examination of the interface en face during curing gave further information as to the distribution of the components and the dynamics of the adhesive layer during curing.

This system has since been superseded by Scotchbond Multi Purpose Plus which has a phosphoric acid etching gel. Results of investigations with this system can be seen in Chapter 6.
4.2 THE INFLUENCE OF COMPONENTS AND HANDLING CHARACTERISTICS OF DENTINE BONDING SYSTEMS

4.2.1 Introduction

Both the formulation and the handling of dentine bonding systems may influence the morphology of the dentine resin interface. With the ever expanding manufacture and marketing of dentine bonding systems, there is a need for a relatively simple method of evaluating the new dentine bonding systems so as to advise manufacturers during the development stage and practitioners of new systems when commercially released. The method outlined in the preliminary investigation was used to compare a variety of dentine bonding systems both available commercially and at the development stage.

4.2.2 Objectives

The aim of this study was to investigate the influence of conditioners, primers and resins of dentine bonding systems on dentine interfacial morphology using the fluorescence confocal microscopy as described in the preliminary investigation.

The study employed 3 commercial dentine bonding systems, in common use, and an experimental system, under development at the time of the study. The primers in the experimental system, used in conjunction with a phosphoric acid etch and an unfilled adhesive resin, were used to evaluate the influence of photo-initiators, light-activation, and application method of primers on the interface.

4.2.3 Materials and Methods

The dentine bonding systems selected and the composition of their components, as given by the manufacturers, are shown in Table 2.1. Pertac Universal Bond (ESPE, Seefeld, Germany), a single stage adhesive resin, was chosen as a control, as it employs
neither etchant nor primer and is unfilled. The other commercial systems were Optibond (Kerr UK Ltd, MI, USA) with a filled adhesive resin, and Clearfil Liner Bond 2 (Kuraray, Cavex, Haarlem, Holland) a self etching primer with a filled resin. Products E1-E3 were experimental primers requiring conditioning with phosphoric acid, used in combination with an unfilled resin adhesive.

The application mode of the adhesives tested and the restorations used for each product are listed in Table 2.2. The effect of light curing on the experimental primers when used in combination with an unfilled resin was examined by comparing E1 with E2 treatments. The influence of the primer application method on dentine, simple application or agitation, was conducted by comparing E3a with E3b treatments.

Approximal wedge shaped cavities were prepared in 10 teeth for each bonding system and the dentine was treated according to the manufacturers' instructions (Table 2.2). The different components of the dentine bonding systems were labelled with rhodamine B and lucifer yellow to aid the examination of the interfacial region. Samples were either single labelled with dye in one component, or dual labelled with a different dye in the primer and adhesive. Following adhesive treatments, restorations were made with an appropriate light cured resin composite restorative material (Table 2.1) and then sectioned longitudinally within three hours of placing the restoration.

The dentine/adhesive interfacial region was examined using a TSM with a x20/0.8 NA, x60 or x100 1.4/NA oil immersion objective. Reflection and fluorescence images were recorded on 35mm film or with a SIT or CCD camera, digitised and image processed or reconstructed with MISIS 3Dplus computer software. In addition, the CLSM and Metamorph computer software were used to produce reflection and fluorescence images (see Chapter 2). Both single and composite colour encoded images were produced of samples dual labelled with lucifer yellow in the primer and rhodamine B in the adhesive resin.
4.2.4 Results

The appearance and depth of the interfacial zone was dependent on the etchant or conditioner used. There was little alteration of the dentine or penetration of the tubules with adhesive in the control Pertac Universal Bond. This appearance was also seen when Pertac Universal Bond was used as an adhesive to cement coverslips to dentine (Chapter 3, Figure 3.6).

All systems using phosphoric acid: Optibond and the experimental materials, demonstrated aggressive preparation of the dentine surface with funnelling of the tubular orifices and well defined lateral tubules. These samples showed no evidence of bond failure *i.e.* gap formation and separation of the restorative material from the dentine, even when sectioned directly after completion of the restorations. The fluorescence confocal images gave evidence of good primer and adhesive penetration into the widened dentinal tubules and lateral tubules and also into the demineralised surface of the intertubular dentine to form a hybrid layer (Figures 4.5-4.12). The hybrid layer was in the region of 4.5-6 μm.

The dual labelled samples (E3b Figures 4.13 to 4.16) illustrated the penetration of the primer (labelled with lucifer yellow) into the dentine surface and its distribution around the periphery of the tubules, and with the adhesive filling the centre of the tubules. Some discontinuity in the tubular filling with primer or adhesive was seen in several single labelled samples at high magnification (Figure 4.6).

The quality of the reflection images of the CLSM lacked the detail of the TSM images, in the main due to the speckling effect of the laser. This can be seen when comparing Figure 4.15 with TSM reflection images.

There was evidence of mixing of the labelled primer with the adhesive and the labelled adhesive with the composite prior to polymerisation. The primer can be seen in Figs. 4.7-4.8 and the air inhibited adhesive can be seen in Figs. 4.10 and 4.12. The adhesives had a tendency to pool at the angle of the cavities (Fig. 4.8) or in irregularities in the
penetration of the adhesive into the dentine was minimised if a very thin layer of adhesive was applied or aggressive air thinning was employed (with a prolonged air blast with the nozzle tip positioned close to the adhesive).

The length of the resin tags was dependent on whether the tubules connected with the pulp. On the pulp side of the cavity the tubules ran at right angles to the cavity surface. The primer penetration was regularly seen 45-60 μm along the tubules and the resin generally penetrated to a lesser extent. On the occlusal aspect, the tubules ran at an acute angle to the cut dentine surface (Figure 4.5). The dentine was characterised by wide funnelled tubules (Figures 4.11-4.12) and long resin tags which, in some samples, could be traced over long distances as far as the amelo-dentinal junction. The microscopic appearances of Optibond and of the experimental systems were similar.

The Clearfil system demonstrated a hybrid layer with less funnelling of the tubules than with the more aggressive phosphoric acid total-etch systems. Alteration of the dentine surface was visible even in reflection images at low magnification (Figure 4.17). The adhesive in this system had a tendency to become disrupted and inclusions of labelled adhesive were seen within the Protect Liner layer.
Figure 4.5 Optibond: primer + rhodamine B. Fluorescence image illustrating the cavity angle with pulpal wall (P) and occlusal wall (O). The tubules of the pulpal side are at right angles to the dentine surface, the full extent of the primer penetration can be seen in this image and there is a well defined hybrid layer. The tubules of the occlusal side are at a more acute angle, the full extent of the primer penetration is not seen on the image, with some tubular penetration extending to the amelodentinal junction. x20/0.8NA OI TSM, 540/600 nm. Scale bar 100 µm.

Figure 4.6 Optibond: adhesive + rhodamine B. Fluorescence image illustrating some discontinuity in the filling of the tubules with the adhesive. X100.1.4NA, OI TSM, filters 546/600 nm. Scale bar 10µm.

Figure 4.7 ESPE primer E3 + rhodamine B. Primer has penetrated the dentine tubules and mixed with the adhesive and resin composite. The tubules have been enlarged as a result of the preparation with phosphoric acid. x100/1.4NA, OI TSM, filters 546/600 nm. Scale bar 10µm.

Figure 4.8 ESPE E3 primer + rhodamine B. In this reflection image the tubules are in connection with the pulp. The primer has penetrated into the dentine tubules and superficial dentine at the cavity surface to form a hybrid zone. The adhesive has pooled at the angle of the cavity and there is evidence of mixing of the primer with both the adhesive and the resin composite. x100/NA, OI TSM, filter 546/-.. Scale bar 10 µm.

Figure 4.9 ESPE E1, Adhesive + rhodamine B has entered the demineralised and primed tubules and superficial dentine. The pooling of the adhesive in irregularities of the cavity surface is illustrated in this image. x00/1.4NA, OI TSM, no filters. Scale bar 10 µm.

Figure 4.10 E2, adhesive + rhodamine B. Reflection image funnelling of the tubular orifices is evident with good penetration of the adhesive into the tubules and their lateral branches. A well marked hybrid zone (H) is visible. x100/1.4NA, OI TSM, filter 546 nm. Scale bar 10 µm.

Figure 4.11 ESPE E3. This combined image show the typical primer distribution on the occlusal side of the cavity. The primer (+ rhodamine B) has penetrated the demineralised dentine surface and the tubules. Reflection 540/-nm, 4.19 fluorescence image. x100/1.4NA, OI TSM, 540/600 nm. Scale bar 10 µm.

Figure 4.12 ESPE E3. Adhesive + Rhodamine B. The adhesive has entered the tubules and superficial dentine to form a hybrid zone. The adhesive has also mixed with the overlying resin composite. Reflection x 100/1.4NA, OI TSM 540/-nm. Scale bar 10 µm.
Figures 4.13-4.16  A single field of dentine adhesive interface restored with E3b, dual labelled with lucifer yellow in the primer and rhodamine B in the adhesive.

Figure 4.13  Fluorescence image demonstrating the distribution of E3b primer at the interface. Primer labelled with lucifer yellow. x60/1.4NA, OI CSLM fluorescence channel 488/515 nm. Scale bar 10 μm.

Figure 4.14  Fluorescence image demonstrating the distribution of adhesive at the interface. Adhesive labelled with rhodamine B fluorescence image demonstrating the distribution of E3b primer at the restorative dentine interface. Primer labelled with lucifer yellow. x60.1.4NA, OI CSLM fluorescence channel 488/515 nm. Scale bare 10 μm.

Figure 4.15  Reflection image illustrating the tubular anatomy lost in the fluorescence images. The image quality is poor compared to the TSM reflection images due to the speckling effect. x60/1.4NA, OI CSLM reflection channel 488 nm. Scale bar 10 μm.

Figure 4.16  Colour encoded image, composed of 3 computer superimposed images (Figures 4.13-4.15) demonstrating the combined distribution of E3b primer and adhesive at the interface, the hybrid layer is marked H. Blue-reflection image; yellow-fluorescence image for primer (P) labelled with lucifer yellow; red-fluorescence image for adhesive (A) labelled with rhodamine B. x60.1.4NA, OI CSLM. Scale bar 10 μm.

Figure 4.17  Reflection image of Clearfil Liner Bond 2. An alteration in the dentine with loss of tubule definition is seen at the cavity surface. Pooling of the protect liner is visible at the angle of the cavity. x20/0.8NA OI TSM, 540/600 nm. Scale bar 100 μm.
4.2.5 Discussion

The subsurface morphology of the interfacial region was imaged with the aid of fluorescence confocal microscopy. Rhodamine B could be incorporated in the primers and adhesives; lucifer yellow was taken up by the primers only. This enabled either single labelling or dual labelling of samples, for example with lucifer yellow in the primer and rhodamine B in the adhesive.

The most important factor in determining the appearance and depth of the interdiffusion zone was the method of conditioning the dentine. A distinct hybrid layer as described by Nakabayashi et al. (1982) and van Meerbeek et al. (1992) was visible in the phosphoric acid total-etch systems but to a lesser extent with the CFLB2 preparation. The surface dentine and tubules were well penetrated by all primers and adhesives when handled according to the manufacturers’ instructions, indicating good wetting ability and compatibility of the components. A hybrid layer was noted around the walls of the tubules as well as at the dentine surface and was in agreement with the findings of TEM studies for Optibond (van Meerbeek et al. 1996). However, there was some apparent discontinuity in the filling of the tubules seen at high magnification with single labelled samples. The reason for this appearance may be that the space is taken up by the unlabelled component, the tubular contents prevent continuous filling, or that the adhesive or primer has contracted on polymerisation. The latter phenomenon has been reported by (Goracci 1997).

The tubular etch patterns with the phosphoric acid etch varied according to the tubular pattern and orientation (Figures 4.7 -4.8 compared to Figures 4.11-4.12). The tubules generally were cut at an acute angle to the dentine surface on the occlusal aspect and at 90° on the pulpal aspect of the cavities used in this study. This may be of importance in other aspects of the success of the interfacial region as measured by micro-permeability or bond strength.

The distribution of the different components of the dentine bonding systems in the interface is not easily determined by other methods of studying interfacial morphology.
In this study fluorescence images at high magnification (x600-1000) demonstrated the distribution of the primer at the periphery of the tubules and the depth of the hybrid layer. In the dual labelled CLSM studies the primer was best detected around the funnelled necks of the tubules, the appearance suggested that the primer lined the demineralised internal surface of the tubule and the resin filled the central part of the tubule. (Figures 4.13-4.16).

Penetration of the primer and adhesive into the conditioned surface dentine and tubules in the CFLB2 was less readily demonstrated, although alteration of the surface dentine with lack of tubular definition was evident in the reflection images (Figure 4.8). A demineralisation depth of 2-3 μm had been reported for a 60 s application time of a similar product (Watanabe et al 1994), and 0.6 μm by Perdigao (1995) for a 30 s application of KB 200 (the experimental version of CFLB2). Inclusions of adhesive within the Protect Liner in CFLB2 occurred when brushing the viscous Protect Liner onto the air inhibited layer of the adhesive, which may relate to the difference in the viscosity between the thin air inhibited layer of the resin which remains on the dentine surface and the thick Protect Liner which was then applied to the surface. This was not dissimilar to SBMP, but in this system, it was the primed surface which was displaced. Although this system does not remove the smear layer, penetration of the tubules by the resin has been noted in this and previous studies (Watanabe et al 1994, Burrow et al 1994).

Pooling of adhesive at cavity angles and within the cavity wall irregularities occurred with all the adhesives but could be limited by avoiding use of excess adhesive and removing any visible excess at the angles of the cavities with a small sponge before polymerisation. The macroscopic assessment of this layer during adhesive application was improved by labelling the adhesive with fluorescent dye. Images of the interface revealed that if too little adhesive was applied or the layer air-thinned too aggressively the resin tags and hybrid zone were poorly developed.

The dentine bonding systems were applied according to the manufacturers' instructions. The application of the etchant is described in the general methods section.
and was not a wet bonding technique (Kanca 1992, Gwinnett 1992). The experimental primer showed excellent wetting of the dentine substrate, suggesting that collapse of the etched collagen and failure of infiltration of the collagen network did not occur with this experimental primer/adhesive system (Gwinnett 1994a).

4.2.6 Conclusions

The conditioner employed had the greatest influence on the interfacial morphology, with the phosphoric acid producing the most aggressive preparation with widest hybrid zone and greatest funnelling of the tubules, Clearfil Liner Bond 2 showing a narrower hybrid zone, and the Pertac Universal control showing no penetration of the smear layer. The morphology of the hybrid zone of the test commercial and experimental systems with a phosphoric acid etch were all similar, indicating good penetration of dentine by the adhesive components. However, this needs to be confirmed by micropermeability studies.

The influence of the different formulations and application techniques of the experimental primers was not sufficient to be detected by changes in the interfacial morphology alone. However, the method gave valuable information as to the distribution of the different components of the bonding systems within the interface.
Chapter 5

MICROPERMEABILITY STUDIES OF
DENTINE /RESTORATIVE INTERFACES

5.1 PRELIMINARY INVESTIGATION-SCOTCHBOND MULTI PURPOSE

5.1.1 Introduction

Even in the absence of gap formation detected by high resolution microscopy it is necessary to detect the presence of pores within the interfacial region and to test the interfacial seal to tracer fluids and dyes. Nano leakage studies (Sano et al 1994a, 1995a,b) were designed to detect such leakage, however, the samples required a great deal of preparation. The aim of this study, therefore, was to evaluate micropermeability of dentine/adhesive interfaces to pulp fluid, using a confocal microscopy technique which involved considerably less sample preparation than TEM and SEM.

5.1.2 Objectives

To develop a method of assessing the effectiveness of the dentine/adhesive interfacial seal provided by dentine bonding systems, to pulp fluid labelled with fluorescent dye.

To investigate the sealing ability of SBMP to fluids from the pulpal surface.

5.1.3 Method

Approximal cavities were prepared in 8 lower third molar teeth in accordance with the method described in Chapter 2 (Fig. 2.1a). Dentine surfaces to be bonded were prepared with the SBMP system according to the manufacturer's instructions (see Chapter 2 Table 2.1). A selection of cavities were restored with the fluorescent labelled primer and adhesive (either rhodamine B or fluorescein), whilst for comparison, others were unlabelled. All cavities were restored with Z100. The roots of
the restored teeth were amputated with a high speed diamond bur with water cooling. Access was gained to the pulp chamber with the bur through floor of the pulp chamber and the pulp removed with a barbed broach. The teeth were inverted and secured in a tray of water with Plasticine® (Harbutts, Bath, Somerset). The water level was sufficient to keep the crowns of the teeth wet. Saline labelled with a contrasting fluorescent dye (rhodamine B 0.5 g in 50 ml saline) was dripped into the pulp cavities with a syringe. A cover was placed over the tray of teeth and the dye solution was left in the pulp chambers for three hours and the dye solution replenished as required. The teeth were rinsed under running water, sectioned longitudinally and polished as outlined earlier. The permeability of the interfaces was assessed using confocal fluorescence microscopy, both the TSM and CLSM with x20 and x60 oil immersion objectives. Images were recorded on 35 mm film.

5.1.4 Results

Three hours was sufficient time for the fluorescent dye solution to reach the interfacial region in all teeth examined. With shorter soaking times this could not be guaranteed. It was necessary to refill the pulp chambers with dye solution during the 3 hour period. Leakage of the interface at the level of the hybrid layer was seen in all samples, with dye solution migrating from the pulp chamber down the tubules and escaping into the hybrid layer and along the interfacial region between the dentine and resin (Figures. 5.1-5.2). It was much easier to interpret the leakage in the samples with unlabelled primers and adhesives. With the TSM, rhodamine B in the pulp chamber gave superior images, as compared to any other combination of dyes and labelling.
Figure 5.1 Laser print of composite (C) / dentine (D) interface (as captured with image processing system and displayed on monibot). Fluorescein labelled saline has penetrated the dentine tubules from the pulpal aspect into the hybrid zone (H) - combined reflection/fluoresence CLMS image. x60/1.4NA Oil. Scale bar 10 μm.

Figure 5.2 Laser print of composite (C) / dentine (D) interface as in Figure 5.1. Fluorescence CLSM image 488/515 nm. x60/1.4NA Oil. Scale bar 10 μm.
5.1.5 Discussion

Although the pilot morphology study in Chapter 4 indicated that the bond between SBMP resin and dentine was intact, this micropermeability study illustrated the inability of the SBMP to create a seal to fluid flow from the pulpal aspect. This finding is in agreement with Degrange (1993) who illustrated leakage from the outer surface of the tooth toward the pulp.

The cavity design resulted in a clear distinction between the tubules which had no connection with the pulp chamber on the occlusal aspect and those with a connection with the pulp on the pulpal wall (Figure 2.1). Each cavity was sectioned and so this enabled two measurements of micropermeability to be made, separated by the 300 μm saw cut. To make an assessment by this method, it was important that the tubules were parallel to the sample surface. It was therefore not practical to make further samples from a single tooth.

Initially it was hypothesised that dual labelling of the adhesive and pulp fluid with contrasting fluorescent dyes would enhance the examination of the interfacial region and assessment of the sealing ability of the hybrid layer to pulpal fluid. However, at a practical level the sealing ability was more easily assessed using a single labelled sample with dye solution from the pulp chamber, as visually the image was more easily interpreted and there was no possibility of cross talk.

This method allowed a more detailed assessment of the seal or permeability of the interface than a conventional microleakage study with percolation of dye from the external surface of the restoration. The term ‘microleakage’ was therefore not considered to be appropriate and a new term ‘micropermeability’ was used to describe this technique.

At this stage the results are presented as a visual record of the interface, a qualitative assessment. The next stage was to develop a quantitative assessment of the micropermeability, by measuring the length of the interface, with and without a seal to pulpal fluid.
5.1.6 Conclusions

This confocal microscopy technique using rhodamine B labelled saline to highlight areas of micropermeability provided a method of assessing the interfacial seal provided by a dentine bonding system. The SBMP hybrid layer, used in wedge shaped approximal cavities, did not form an efficient seal to fluorescent labelled saline from the pulp.
5.2 THE INFLUENCE OF COMPONENTS AND HANDLING CHARACTERISTICS OF DENTINE BONDING SYSTEMS ON MICROPERMEABILITY

5.2.1 Introduction

The earlier studies of dentine/adhesive interfacial morphology with multi-stage 4th generation bonding systems (Chapter 4) indicated that, in the systems tested, the interfacial shape was primarily determined by the conditioner used and that there was good penetration of the demineralised dentine by the primers and adhesives. However, it may be hypothesised that differences in the formulation and application techniques of these systems may result in differences in the porosity or seal of the interface: this may be detected by an assessment of micropermeability as outlined in the pilot study (section 5.1).

5.2.2 Objectives

This study aimed to investigate the interfacial micropermeability of 3 commercial dentine bonding systems and 3 versions of an experimental system. The systems used were those studied in Chapter 4.2.

Thus the influence of the following parameters on interfacial morphology were assessed:

- dentine conditioning
- degree of filler in the adhesive resin
- influence of photo-initiators, light-curing, and application method of the primer on the interface (using the experimental primers in conjunction with phosphoric acid and an unfilled adhesive resin).

A further aim of the study was to compare the morphological appearance of the interface with that of its permeability to pulpal fluids.
5.2.3 Materials and Method

The dentine bonding systems selected were as in Chapter 4.2, and details of their composition and mode of application are given in Tables 2.1 and 2.2. In total there were six test groups (Optibond, Clearfil Liner Bond 2, E1, E2, E3a, E3b-2commercial and 4 experimental) and again Pertac Universal Bond, a single stage adhesive resin, was chosen as a negative control, as it employs neither etchant nor primer and is unfilled.

The effect of light curing on the experimental primers when used in combination with an unfilled resin was examined by comparing E1 with E2 treatments. The influence of the primer application method on dentine, simple application or agitation, was conducted by comparing E3a with E3b treatments.

The sealing ability to pulpal fluids, was evaluated on 10 teeth for each group. The teeth were prepared as described in the pilot study, with approximal cavities and no fluorescent labelling of the dentine bonding components. Restorations were made with appropriate resin composites as indicated in Table 2.2 and according to the methods given in Chapter 2. Saline labelled with rhodamine B (0.5 g in 50 ml) was syringed into the pulp chamber with minimal pressure and after 3 hours the teeth were rinsed under running water, sectioned longitudinally and examined as outlined in the pilot study.

To establish the site of micropermeability within the interface samples all of the dentine bonding systems were examined using oil immersion objectives (up to x1000 overall magnification). The TSM was used in fluorescence mode with 546 nm excitation and 600 nm pass filters. Images were recorded on 35 mm film. In addition, fluorescence images of the entire length of the pulpal interface were made field by field, using a x20/0.8 NA oil immersion objective. The images were stored using the MISIS 3Dplus computer programme. Examining each field width, the length of interface with and without micropermeability was measured directly on the computer monitor. Calibration slides were used to find the fieldwidth as seen on the monitor and
a paper scale was made to make the measurements on the monitor. To test the method, inter examiner agreement of measurements was sought with other researchers in the department. The percentage of the entire pulpal axial wall interfacial micropermeability was then calculated for each sample.

The interfacial micropermeability data was analysed using the Kolmogorov-Smirnov test (Lehman 1975). The parameter of interest was the pattern or distribution of the data rather than the central tendency, such as the median. The null hypothesis is that the pattern of micropermeability for all systems is the same, and the significance was set at $\alpha=0.05$. Because of the small sample size it was necessary to use exact non-parametric inference techniques, and the data was analysed using StatXact Version 3 (StatXact 3 for Windows, Cytel Software Corporation, Cambridge MA 02139). A Bonferroni correction (Everitt 1995) enabled multiple group comparisons to be made.
Figure 5.3  Fluorescence image of a dentine/adhesive interface illustrating areas where the interface is sealed (S) and areas with micropermeability (M) to rhodamine B dye. The dye has migrated along the dentine tubules from the pulp chamber. x20/1.8NA OI TSM FW 700 μm, filters 546/600 nm. Scale bar 100 μm.

Figure 5.4  Fluorescence image illustrating interfacial micropermeability (M) with rhodamine B passing along the tubules through the hybrid zone and along the interface (I) and interfacial sealing, with migration of the dye around the tags of adhesive (T). Cavity restored with E3b. x100/1.4NA OI TSM, filters 546/600 nm. Scale bar 10 μm.

Figure 5.5  Fluorescence image illustrating a sealed interface with passing migration of the rhodamine B dye around the tags of adhesive (T), but not reaching the dentine surface of hybrid zone. Cavity restored with E3b. x100/1.4NA OI TSM, filters 546/600 nm. Scale bar 10 μm.
5.2.4 Results

The appearance of a sealed and micropermeable interface can be seen in Figures 5.3-5.5. When viewed with the x100 objective it was possible to detect the site of micropermeability within the interface. For example, in the sample imaged in Figure 5.4, micropermeability could be detected at the tubular orifice and along the surface of the dentine, or through the hybrid zone itself. The possible routes of micropermeability identified in the present study are illustrated in Figure 5.6. The present study detected no association between the bonding system and the site of permeability within the interface.

The micropermeability data for each dentine bonding system is shown in Figure 5.7, together with an overlying box plot to indicate the data range and quartiles. The results of the Kolmogorov-Smirnov test analysis for all system pairs are summarised in Table 5.1. There was no significant difference in the micropermeability patterns for Optibond, Clearfil Liner Bond 2, and E3b, and no significant difference between E1, E2 and E3a. However, the former experimental group was superior in that it exhibited lower micropermeability values.

The control system Pertac Universal bond was the most prone to micropermeability to fluid from the pulp, with all samples examined exhibiting 100% leakage along the smear layer. In comparison, 60% of Optibond restorations were sealed; the maximum micropermeability exhibited for this system was 50% of the interface, whereas, 33% of cavities restored with Clearfil Liner Bond 2 were sealed.

The most variable micropermeability results were obtained with the experimental system E1, where the primer containing photoinitiators and was not light initiated before the application of the unfilled adhesive resin (see Table 2.2). These results were moderately improved, but not significantly, by light curing the primer directly (E2), but with this system there was a trend for the micropermeability to be 0% or 100%. These patterns of micropermeability results are illustrated in Figure 5.7. In contrast, when a primer, without photoinitiators was used with an unfilled resin (E3a & b) the
micropermeability was improved (Fig. 5.7). There was no significant difference in the micropermeability of E3b compared with the commercial Clearfil Liner Bond 2 and the more complex Optibond system; all of the failure patterns were similar.

Table 5.1  Summary of the exact probabilities calculated from the Kolmogorov-Smirnov test for each system tested

<table>
<thead>
<tr>
<th></th>
<th>CFLB2</th>
<th>E1</th>
<th>E2</th>
<th>E3a</th>
<th>E3b</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>0.03</td>
<td>0.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>0.01</td>
<td>0.01</td>
<td>0.24</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0.03</td>
<td>0.03</td>
<td>0.48</td>
<td>0.27</td>
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</tr>
<tr>
<td>E3b</td>
<td>0.07</td>
<td>0.4</td>
<td>0.14</td>
<td>0.03</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Optibond  CFLB2  E1  E2  E3a
Figure 5.6 Diagram of micropermeability of resin dentine interfaces: Routes of fluid flow from the pulp.

1. No micropermeability. The interface is sealed by resin impregnation of the hybrid zone (h), and by the resin tag (r) in the dentine tubule (t) and lateral tubules (l).

2. Micropermeability around the resin tags, through the lateral tubules and hybrid zone.

3. Micropermeability through the porous base of the hybrid zone (*).

4. Micropermeability around the resin tag and along the interface (*) between the hybrid zone and the adhesive resin (a), with resin tag pulled out of the tubule.

Micropermeability may be developed through any part of the hybrid zone, or along the interface between the resin and the hybrid zone to involve tubules sealed to pulp fluid by their resin tags.
Figure 5.7  Graph illustrating the percentage of each interface exhibiting micropermeability to pulp fluid.

- Optibond
- Clearfil Liner Bond 2
- E1
- E2
- E3a
- E3b

0%  Percentage Micropermeability  100%
5.2.5 Discussion

The micropermeability experiments revealed important information regarding the interfacial porosity, especially in samples apparently free of interfacial gaps as assessed in the morphology studies.

Micropermeability is dependent upon the extent of penetration of the adhesive components into etched dentine and the development of any porosities or gap formation which may result from polymerisation shrinkage of the primer, adhesive or resin components. The use of a control system which retained a modified smear layer confirmed that the fluorescent dye could penetrate the porosities within the smear layer and at the interface. The method also gave an indication of the site of failure within the interface.

Nanoleakage studies using silver nitrate staining techniques and SEM or TEM evaluation have similarly demonstrated the presence of porosities within the resin-dentine interdiffusion zone when using a variety of dentine bonding systems (Sano et al 1994a, and 1995a), but these methods required considerable sample preparation and have not been used to demonstrate leakage from the pulpal aspect.

In this study, the failure of the seal at the interfacial region was located either at the tubule orifice or within the hybrid zone. The possible routes for micropermeability are drawn in Figure 5.6. The link between the peritubular dentine and the resin tags may be of prime importance when determining the micropermeability of the restoration. It is possible that polymerisation shrinkage may pull out the resin tags from tubules, creating a microscopic gap of sufficient size to make the interface permeable. Evidence for this was seen at high magnification (Figure 5.4) with the rhodamine B dye permeating around the resin tags. Likewise, the impregnation of the primer and adhesive into the conditioned surface intertubular dentine is of relevance not only for the strength of the bond but also for the interfacial sealing.
The least micropermeability was seen with the commercial systems, with Optibond and Clearfil Liner Bond 2. These systems were comparable despite differences in the dentine preparation and interfacial morphology. These micropermeability results were matched by the E3b experimental system and its associated method of handling. This indicated that when using the unfilled resin the primer should be agitated on its application to the dentine and not polymerised with photo-initiation. There was a danger that the resin, being less viscous than the filled resin, could be excessively thinned by aggressive air drying. The critical minimal resin thickness may be related to oxygen inhibition of the surface layer.

It may be postulated that inferior micropermeability results were obtained by including photoinitiators in the experimental primers, because of the greater shrinkage of the unfilled resin and so a greater stress on the seal formed by the polymerised primer. The trend for the micropermeability to be 0% or 100% when light activated directly (see E2 Figure 5.7) indicates that the primer seal may be effective if not displaced after polymerisation. By using a non light activated primer and unfilled resin better micropermeability results were obtained, perhaps because of residual flexibility within the primed surface.

Interestingly, this system did not include a volatile solvent, but still showed excellent wetting of the dentine substrate; suggesting that collapse of the demineralised collagen and failure of infiltration of the collagen network was not a problem with this experimental system, with an aqueous primer rather than an acetone solution (Gwinnett 1994a).
5.2.6 Conclusions

The fluorescence confocal microscopy technique described in the present study enabled both the degree and the site of interfacial micropermeability to be assessed.

Although the morphology of the dentine restorative interface of the test commercial and experimental systems tested showed good penetration of dentine by the adhesive components, testing interfacial micropermeability revealed that none of the bonded systems formed a complete seal to fluid from the pulp.

Differences in the formulation and application techniques of the systems resulted in differences in the porosity or seal of the interface.

The micropermeability was not a function of the conditioner used and the thickness of the hybrid layer: Optibond and Clearfil Liner Bond 2 had comparable interfacial micropermeability.

The experimental system, with an unfilled resin, demonstrated low interfacial micropermeability (comparable to Optibond and Clearfil Liner Bond 2 with filled resins) when a primer without photoinitiators was applied on the dentine surface with agitation. However, inclusion of photoinitiators and photo polymerisation of the same experimental primer had a detrimental effect on micropermeability.

It is important that the primer and adhesive the viscoelastic behaviour are compatible to avoid polymerisation stresses disturbing the interfacial seal.
Chapter 6

EFFECTS OF DIFFERING SETTING STRESS ON
INTER-FACIAL MICROPERMEABILITY

6.1 Introduction

The competing stresses set up within the dentine/adhesive resin interface during restoration polymerisation were described in the literature review. Some of these stresses will be inherent to the polymerisation of adhesive components, others may be indirectly transmitted to the interface by dimensional changes in the overlying restoration. As discussed earlier, the preparation of the dentine in many dentine bonding systems is a multistage process, involving the use of an acidic conditioner, surface activator, priming agent and adhesive resin bond, prior to the placement of the filling material. Some commercially available adhesives give the dentist the option for self curing or light curing the adhesive itself. This gives the opportunity to use different versions of the adhesive with chemically activated and light activated restoratives and so assess the effects of the differing polymerisation stresses of these materials on the integrity of the restorative interface.

Scotch Bond Multipurpose Plus (SBMP+) adhesive is such an adhesive and is not dependent on light for polymerisation. It may be used with a variety of restorative materials, including light and chemically activated resin composites, but also amalgam restorations. These materials show shrinkage during setting and so impart different stresses to the dentine/restorative interface whilst setting.
6.2 Objectives

The aim of this study was to evaluate the effect of restorative materials with differing setting shrinkage on the sealing ability of the interfacial region using a dentine bonding agent with chemically and/or light-activated mechanisms (SBMP+). The influence of the cavity configuration, bulk of the restoration and intensity of the curing light were also assessed.

6.3 Materials and Methods

Third molar teeth were divided into three groups and each prepared with the adhesive system according to the manufacturer’s instructions (Chapter 2, Tables 2.1 and 2.2). Restorative materials were selected to include a variety of setting mechanisms, capable of imparting different stresses to the adhesive interface:

- **Group 1. Amalgam (Dispersalloy, Dentsply, USA).** This allowed the adhesive to be polymerised via the chemically activated reaction only. Polymerisation of all components was dependent on the activator and ‘catalyst’ and visible curing light was not used to promote curing. This group was selected to give minor dimensional changes on setting and a thin film of chemically polymerised adhesive.

- **Group 2. Chemically activated composite (Adaptic, Johnson and Johnson, East Windsor, NJ, USA).** Polymerisation was initiated by the mixing of the two pastes. This was selected as a slow polymerising composite, where polymerisation commence throughout the composite, independent of light transmission.

- **Group 3. Light-activated composite restorative material (Z100, 3M Dental Pdts., St Paul, Minn, USA) selected to impart maximum stress to the adhesive interface.** The adhesive film was placed thinly and both light and chemically activated. A curing light of either low (Group 3a) or high intensity (Group 3b) was applied for 40 s through the composite. A Heliolux II (Vivident, Liechtenstein)- 200 mW cm² with a 7 mm tip with a well used bulb was used for the lower intensity light studies, and
an Optilux (Demetron Research Co., CT, USA) - 470 nm, 800 mW cm\(^2\) with a 10 mm tip for the higher intensity light studies. Confirmation of the light intensity was made with a light meter (Demetron Inc Co).

**Morphology of the dentine / adhesive / restorative interface.**

Occlusal cavities 4 mm long x 3 mm wide x 4 mm deep, extending into dentine, were prepared as described in Chapter 2 - General Materials and Methods, Fig. 2.1b. The cavity surfaces of occlusal cavities extending into dentine were prepared with the SBMP+ system prior to restoration. The different components of the dentine bonding system were labelled with rhodamine B or lucifer yellow, whilst dye was added to the ‘catalyst’ for the chemically activated adhesive resin option.

Three teeth in each group were restored with one of the three main components labelled with rhodamine B. A further six teeth were restored with pairs of components double labelled with lucifer yellow in the primer and rhodamine B in the adhesive so that the mixing of the components could be visualised. The teeth were restored with either Z100, Adaptic or Dispersalloy as outlined above, sectioned longitudinally through the restoration after 24 hours and then examined with a TSM and CLSM with x20-x100 oil immersion objectives.

**Sealing ability against pulpal fluids with conventional cavities; 'realistic' operating conditions.**

A further 55 teeth with occlusal cavities were divided into the three restorative groups (1, 2, 3a and 3b see Table 6.1) and each prepared with the different polymerisation methods for the adhesive system and thicknesses of restorative materials.

‘Thick’ restorations (4 mm deep) were placed in 10 teeth in Group 1 amalgam, Group 2 ‘chemically activated’, Group 3a light cured with high intensity light and Group 3b ‘light activated’ with low intensity light. ‘Thin’ restorations (2 mm deep) were placed in five Group 2, 3a and 3b samples. All light activated composites were
placed and polymerised in a single increment. The visible light curing units were described in Chapter 2.

Following cavity restoration, the roots of the teeth were amputated and fluorescent labelled pulpal fluid was introduced to the pulp cavity to assess the micropermeability (see below).

**Sealing ability to pulpal fluids with flat dentine surfaces; 'ideal' operating conditions.**
These dentine samples were configured to remove the effect of cavity walls and line angles and also the effect of an enamel bond. 15 teeth were prepared by sectioning horizontally to produce a flat dentine surface at an equivalent level to the floor of the occlusal cavities in the previous experiments (Figure 2.1c). The effect of adhesive bonding to enamel was removed by carefully masking this structure with nail varnish at the periphery of the horizontal section. The roots were amputated and hydration of the pulpal dentine was maintained by placing a pledget of wet cotton wool in the pulp chamber.

The 15 teeth were assigned to three groups of five. Ten were restored with a thin (<1 mm thick) layer of Adaptic and five with Z100. Half of the Adaptic restorations were placed with the adhesive chemically activated and the other half with the adhesive also light activated. The composites were placed using a glass slide as a rigid clear matrix (Figure 2.1c) and the samples were then left on the slide until the end of the experiment, when they were gently removed following soaking in water.

**Measurement of micro-permeability.**
The pulp chambers were soaked with rhodamine B dye solution and then sectioned. All sections were examined with TSM and CLSM, and micropermeability measured as described in Chapter 4.2.

To take account of the dentine depth of the cavity floor, the remaining dentine thickness over the pulp chamber was measured for all of the teeth following
sectioning and examination. Readings were taken in the middle of the pulpal floor, at each cavity line angle, and then averaged.

**Statistical Analysis**

The micropermeability data was analysed using the Kolmogorov-Smirnov test (Lehmann 1975).

### 6.4 Results

**Morphology of the adhesive bond**

The morphology of the interface between the adhesive and dentine showed good penetration of the etched dentine by the primer and adhesive resin (Figures 6.1 and 6.2). The fluorescent labelled primer often left a distinctly labelled surface zone in the hybrid layer (Figures 6.1 and 6.2). The primer infiltration dentine to a consistent depth of 10 μm, whilst the resin tag penetration was more variable, with generally less penetration into tubules which communicated with the pulp (Figures 6.3 and 6.4). The activated and primed surface layer was prone to displacement by the adhesive resin, if the adhesive application brush was allowed to touch the primer when it was being placed. These primer fragments were visible as fluorescent labelled inclusions within the adhesive layer (Figures 6.1 and 6.5). In group 1 the unpolymerised adhesive mixed intimately with the overlying amalgam, filling any voids with which it communicated (Figure 6.6). In group 2 the adhesive film thickness was very variable when placing the chemically cured composite (Figure 6.7), but this was not a problem with the amalgam when the restoration was very well condensed (Figure 6.6).

The chemically activated adhesive and composite showed a diffuse mixing at the interface (Figure 6.7), and, in the light activated system, the air inhibited layer at the surface of the adhesive allowed mixing of the adhesive and composite. In cavities where the Z100 had been placed as a thick layer and light activated in one increment there was evidence of disturbed features in the interfacial region with the tooth, suggestive of incomplete polymerisation in this region (Figure 6.8). In samples
prepared with the high intensity light the base of the restoration had a tessellated appearance and gap formation at the dentine/adhesive was a common occurrence, especially at the cavity angles (Figures 6.9 and 6.10).

**Micropermeability studies.**

The samples which were subjected to leakage studies were unlabelled apart from the dye solution from the pulp chamber. The micropermeability results are summarised in Table 6.1. The effect of resin impregnating the demineralised dentine layer could be discerned as an alteration in the reflectivity of this region in the back-scattered reflection images. The distribution of dye could also be seen in fluorescence mode with the correct excitation and pass filters. Where the adhesive sealing ability was optimal, the progress of the dye toward the composite restoration was prevented by the resin impregnated dentine tubules and inter-tubular zone. However, leakage of dye along the interface could be quite marked if there was microscopic de-lamination in this region. This was particularly apparent in samples where polymerisation stresses had resulted in gap formation or the light-activated resin composite was inadequately polymerised at the base of the cavity (Figures 6.6, 6.8-6.10). The leakage of the dye in these areas served to highlight the interfacial morphology and the polymerisation problems in Group 3a and 3b samples.

Samples where the materials were placed against a flat dentine surface and a glass matrix, produced a more consistent microscopic appearance than those where the materials were placed in occlusal cavities.

Data was analysed using the Kolmogorov-Smirnov test with exact non-parametric inference and the results are presented in Table 6.2. Z100 showed significantly more leakage than the amalgam and Adaptic placed in bulk (p<0.001 for the low intensity light and p<0.001 for the high intensity light). The use of the low and high intensity light did not influence the micropermeability, all comparisons being non significant at p=0.05. However, the difference between large and small increments of Z100 with the low intensity light were significant (p=0.042). When placed in bulk there was
evidence of incomplete composite polymerisation near the cavity floor for these restorations. The least leakage for the light-activated adhesive and composite was achieved on a flat surface with masked enamel and thin composite, and these restorations showed significantly less micropermeability than the thin Z100 restorations. Chemically-activated composite performed well when the surface of the restoration was freely exposed, and better as a thick rather than thin restoration, as a conventional cavity rather than constrained under a glass matrix.

The thickness of the remaining dentine had no significant effect on the results in this study, even though there was quite a large variation in this dimension (1.84 mm median, range 0.9 - 2.8 mm).
Figure 6.1  Fluorescence image to illustrate the distribution of primer (labelled with lucifer yellow) at the dentine restorative interface. The cavity was restored with SBMP+ and Z100. The primer is distributed in the hybrid zone (H) and at the periphery of the tubules (T), giving them a 'ghost-like' appearance. x60/1.4NA lens CLSM laser 488 nm, barrier filter 515 nm. Scale bar 10 μm.

Figure 6.2 Fluorescence image of the same field of view as Figure 6.1, but to illustrate the distribution of adhesive (labelled with rhodamine B) in the hybrid zone (H), and penetrating the tubules (T). x60/1.4NA OI CLSM laser 514 nm, barrier filter 550 nm. Scale bar 10 μm.

Figure 6.3  Reflection image of a cavity line angle, filled mainly with adhesive (S). A gap can be seen along the interface with the pulpal dentine (arrowed). Activator labelled with rhodamine B. x20/0.8NA TSM OI, filter 546/- nm. Scale bare 100 μm.

Figure 6.4 Fluorescence image of same region as Figure 6.3. The excellent tubular penetration by the adhesive on the 'dry side' of the cavity can be seen, with limited penetration pulpally. Some of the dentine tubules communicate with the pulp (P), whilst the shape of the cavity causes others to be separated from pulpal fluid. x20/0.8NA OI TSM filters 546/600 nm. Scale bar 100 μm.

Figure 6.5 Combined reflection (LHS) and fluorescent (RHS) image of adhesive interface with an amalgam restoration (A). Primer labelled with rhodamine B. Displaced fluorescent labelled fragments (open arrows in the reflection image) can be seen within the adhesive layer above the dentine (D). x20/0.8NA OI TSM filters 546/600 nm. Scale bar 100μm.

Figure 6.6 . Fluorescence image of an amalgam restoration. The amalgam was packed rapidly, prior to polymerisation of the adhesive. The adhesive was labelled with rhodamine B and shows significant tubular penetration and has mixed intimately with the overlying amalgam, filling any voids within it. x20/8.0NA TSMN lens, filter 546/600 nm. Scale bar 100 μm.
Figure 6.7  Reflection image of an Adaptic restoration (C), adhesive (S), with the primer labelled with lucifer yellow. Adhesive film thickness varied greatly with the chemically cured composite but was generally <300 μm. The chemically cured adhesive and composite showed a diffuse mixing at the interface (open arrow). x20/0.89NA OI TSM, filter 450/- nm. Scale bar 100 μm.

Figure 6.8  Combined reflection image (LHS) and fluorescene image (RHS) of a Z100 restoration with an incomplete curing at the base of the cavity. Rhodamine B dye solution has moved along the dentine tubules from the pulp chamber to the dentine/restorative interface and leaked into the overlying adhesive and inadequately cured resin composite (C). x20/0.8NA OI, TSM filter 546/- nm. Scale bar 100 μm.

Figure 6.9  Fluorescene image of Z100 in a thin (2 mm deep) restoration polymerised with a high intensity light. The resin composite at the base of the cavity (C) has a tessellated appearance, suggesting a cohesive failure whilst incompletely polymerised. The dye solution from the pulp has progressed across the dentine/restorative interface and then in the gaps between the islands of composite. x100/1.4NA OI, TSM, filter 546/600 nm. Scale bar 10 μm.

Figure 6.10  Fluorescene image of Z100 from the same sample as in Figure 6.9, but from the cavity angle, showing a fracture of the dentine (D) and hybrid zone (h) marked with arrows. In addition, the adhesive at the base of the cavity has taken up rhodamine B dye from the pulp and there is evidence of gap formation (*) with tearing of the adhesive (s). x100/1.4NA OI, TSM, filter 546/600 nm. Scale bar 10 μm.
Table 6.1   Summary of micropermeability results.

Micropermeability expressed as a percentage of the cavity floor interface.

<table>
<thead>
<tr>
<th>Conventional Class I restoration</th>
<th>Number of samples</th>
<th>Median % Micropermeability</th>
<th>Mean Dentine thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC SBMP+ / Amalgam</td>
<td>10</td>
<td>9.25</td>
<td>1.7</td>
</tr>
<tr>
<td><strong>Group 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC SBMP+ / thick Adaptic</td>
<td>5</td>
<td>12</td>
<td>1.4</td>
</tr>
<tr>
<td>CC SBMP+ / thin Adaptic</td>
<td>5</td>
<td>50.25</td>
<td>1.9</td>
</tr>
<tr>
<td><strong>Group 3a</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LC SBMP+ / thick Z100</td>
<td>10</td>
<td>56.4</td>
<td>1.6</td>
</tr>
<tr>
<td>LC SBMP+ / thin Z100</td>
<td>5</td>
<td>80.3</td>
<td>1.8</td>
</tr>
<tr>
<td><strong>Group 3b</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LC SBMP+ / thick Z100</td>
<td>10</td>
<td>70</td>
<td>1.9</td>
</tr>
<tr>
<td>LC SBMP+ / thin Z100</td>
<td>5</td>
<td>83.1</td>
<td>1.7</td>
</tr>
<tr>
<td><strong>Restoration of flat occlusal surface</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC SBMP+ / Adaptic</td>
<td>5</td>
<td>55</td>
<td>1.85</td>
</tr>
<tr>
<td>LC SBMP+ / Adaptic</td>
<td>5</td>
<td>70</td>
<td>2.3</td>
</tr>
<tr>
<td>LC SBMP+ / Z100</td>
<td>5</td>
<td>12.5</td>
<td>2.2</td>
</tr>
</tbody>
</table>
Table 6.2

Summary of the exact probabilities from the Kolmogorov-Smirnov test for SBMP+ combinations.

<table>
<thead>
<tr>
<th>Conventional Class I restoration</th>
<th>Group 2 Thick</th>
<th>Thin</th>
<th>Group 3a Thick</th>
<th>Thin</th>
<th>Group 3b Thick</th>
<th>Thin</th>
<th>Adaptic CC SBMP+</th>
<th>LC SBMP+</th>
<th>Z100 LC SBMP+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 CC SBMP+ / Amalgam</td>
<td>NS</td>
<td>0.001</td>
<td>0.007</td>
<td>0.01</td>
<td>0.002</td>
<td>0.003</td>
<td>0.0003</td>
<td>0.0003</td>
<td>NS</td>
</tr>
<tr>
<td>Group 2 CC SBMP+ / thick Adaptic</td>
<td>0.01</td>
<td>0.007</td>
<td>0.008</td>
<td>0.03</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
<td>NS</td>
</tr>
<tr>
<td>CC SBMP+ / thin Adaptic</td>
<td>NS</td>
<td>0.014</td>
<td>NS</td>
<td>0.08</td>
<td>NS</td>
<td>0.04</td>
<td>NS</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>Group 3a LC SBMP+ / thick Z100</td>
<td>0.042</td>
<td>NS</td>
<td>0.002</td>
<td>NS</td>
<td>NS</td>
<td>0.01</td>
<td>NS</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>LC SBMP+ / thin Z100</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.04</td>
<td>NS</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 3b LC SBMP+ / thick Z100</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.04</td>
<td>0.04</td>
<td>NS</td>
<td>0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LC SBMP+ / thin Z100</td>
<td>NS</td>
<td>0.041</td>
<td>0.04</td>
<td>0.004</td>
<td>0.013</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Restoration of flat occlusal surface</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adaptic CC SBMP+</td>
<td>NS</td>
<td>0.004</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LC SBMP+</td>
<td>NS</td>
<td>0.013</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Key: LC light activated
CC chemically activated
6.5 Discussion

The development of adhesives with both chemical and light activation widens their application in restorative dentistry. The newer chemically activated version of SMBP+ can be used with restorative materials which do not allow light transmission.

The adhesive examined in this study had been available as a light-activated material for some time, and its interfacial characteristics when used in wedged shaped cavities have been reported in Chapter 4. It might be expected that the conversion of the primer would be improved by the use of the chemical activator on the etched dentine and that the durability of this layer would be improved. However, it was still common to see displacement of the primer layer into the overlying adhesive. The possible explanations of this phenomenon seen only in the SBMP range of products were offered in Chapter 4.

The chemically-activated adhesive system had quite a rapid initial polymerisation which could result in thick layers of adhesive in the line angles and floor of the cavities, thus the handling properties were not ideal, and displacement of excess adhesive could not always be guaranteed. Rapid, vigorous condensation of the amalgam enabled thin adhesive films to be formed as the adhesive was pushed out of the cavities. Any voids within the set amalgam were filled with adhesive which also formed a complete seal between the restoration and the tooth.

The use of adhesive materials as directed by the manufacturers' is important, but it was considered of interest to evaluate how well this chemically activated adhesive system worked when light-activated composite thickness' were in excess of recommended practice and when visible curing lights of lower than ideal intensity were used. The recommended light intensity is >300 mW cm\(^{-2}\) and lights with reduced intensity may commonly be used unwittingly within general dental practices (Shortall and Harrington 1996).
The restorative materials were selected to transmit different stresses to the underlying adhesive. Amalgam and Adaptic gave the best results in conventional occlusal cavities. The amalgam would be expected to show the smallest dimensional changes (0.15 - 0.2% shrinkage within 1 hour, ISO 1995) and so would be relatively passive with the adhesive. Similar results were produced by the chemically activated composite: these materials are generally characterised by relatively slow polymerisation and by lower polymerisation shrinkage stress as compared with that of light activated composites (Alster et al 1992, Feilzer et al 1993). The mixing-in of porosity has been reported to reduce the rate and degree of stress by either oxygen inhibition due to the admixed air or by increasing the free surface associated with the pores in the bulk of the composite (Feilzer et al 1993). The poorer permeability results with the thin layer of Adaptic could have been due to difficulty in handling this 'sticky' material in small quantities in a relatively deep cavity, causing disruption of the chemically polymerising adhesive. The occlusal cavity design gave a high configuration factor (Feilzer et al 1987) with just one free surface to allow the composite to shrink towards its bulk. When the composite was of the light-activated type with a greater polymerisation shrinkage, this cavity configuration would therefore facilitate maximum disruption at the adhesive interface.

Interestingly, the intensity of the curing light and the thickness of the composite did not significantly influence the micropermeability results and where leakage occurred, there was evidence of significant failure at the adhesive interface and within the immediately overlying resin composite. Both the intensity and the spectral nature of the low intensity light output would have been less than ideal as the bulb had been well used over a number of years. The intensity measured did not portray the full extent of the inefficiency of the lower intensity curing light.

The morphology of the interfacial region was not ideal in any of the class I cavities restored with the light activated material. In the case of the low intensity light very inadequately polymerised material was observed at the base of the cavities. The un-polymerised base of the restoration failed to seal the interface from dye from the pulp. Dye also permeated into the composite (Figure 6.6). However, gap formation
was not seen in these samples as the incompletely polymerised composite allowed relaxation of polymerisation stresses which developed in the composite nearer the light source. With the higher intensity light there was evidence that the restorative material at the base of the cavity was polymerised to a greater degree, but that the polymerisation stresses caused cohesive failure of the composite at the base of the cavity. Figure 6.9 illustrates the tessellated failure pattern which was highlighted by the permeation of the fluorescent dye around the islands of composite. In addition, interfacial failure with gap formation and in some cases cohesive failure of the dentine was seen at the cavity angles (Figure 6.10).

Factors determining failure patterns due to polymerisation shrinkage will include the magnitude and direction of the stress generated, the degree of polymerisation and cohesive strength of the restorative material at the base of the cavity, and the adhesive strength of the components in the interfacial region. Interfacial tearing and failure of the hybrid zone was seen most frequently at the angles of the cavities, whereas the cohesive composite failure occurred more extensively adjacent to the cavity floor. At the cavity angles it is likely that the polymerisation stress is greater due to the larger volume of composite (Davidson et al 1984). There may also be some stress breaking component due to the pooling of the adhesive (Kemp-Scholte and Davidson 1990a). At the cavity angles failures were sited at the dentine/resin interface indicating that the viscoelastic behaviour of the adhesive was sufficient to overcome the shrinkage to allow initial conversion of the deep composite, with a later failure of the adhesive dentine interface. Alternatively, the interfacial failure may have been early and due to the increased stress at the angles and this resulting in conversion of the deep composite without disruption. The former theory is supported by the interfacial morphology observed elsewhere along the cavity floor where the adhesive layer was thinner, and it may be speculated that the early stresses were sufficient to disrupt the deep composite.

The results support the view that even with a high intensity light, this practice of placing restorations in bulk should not be condoned as a clinical procedure. Neither is the development of higher intensity lights for more rapid conversion supported.
The final degree of conversion is also important and there is a balance to be struck between residual elasticity of the adhesive and composite and the long term stability of the restoration.

To help overcome handling problems associated with cavity line angles and high configuration, samples were made with a flat dentine surface and with the enamel masked, so as to remove the effect of stresses to the dentin interface because of a superior enamel bond. This 'ideal' experimental design could only be used with the composites, because to place amalgam in that way would have been technically difficult.

Studies involving composite resins in the 1980's suggested that a durable composite dentine bond could only be formed with a discus shaped restoration placed within a shallow cavity or on flat dentine surface (Hansen 1984, Davidson et al 1984). It was suggested that the contraction forces are greater along the longest arc of the restoration, that is tending to lift the margins of a restoration on a flat surface (Lambrechts and Vanherle 1982).

Somewhat surprisingly, the chemically activated composite gave poor results in the current experiments, even when the adhesive was light-activated prior to adding the Adaptic. A possible explanation for these results may be that the rigid glass slide acted as a constraining influence, not considered in the design models above. This may cause the polymerisation stresses to be strongly directed to the interface which did not have ample opportunity to develop sufficient bond strength. The amount of free surface area was quite small, being restricted to the periphery of the composite layer.

Conversely, this thin flat configuration gave the best results with the light-activated composite. This can most readily be explained by the optimal polymerisation of both the adhesive and the composite with a higher degree of conversion than the chemically activated composite.
Many experimental testing regimes use flat surfaces for determining properties such as bond strength. These experiments have shown that the same adhesive will give very different results depending on the combinations of materials used and how they are placed on the tooth. Further experiments may be designed to observe the restoration and interfacial region during polymerisation with different cavity configurations using the 'window' technique described in Chapter 3.

It was difficult to assess pulpal position when cutting occlusal cavities and so there was quite a variation in residual dentine thickness for all the samples under examination. (0.9 - 2.8 mm). By implication, the deeper cavities could be expected to be wetter, and so present more of a problem for bonding. However, this variable did not effect our results, indicating that the Scotchbond Multi Purpose + has a good tolerance of wet dentine.

6.6 Conclusions

In summary, the remaining dentine thickness (and hence dentine wetness) had an insignificant effect on the results and the primed dentine surface was prone to displacement by the adhesive. The adhesive gave an excellent seal under amalgam restorations. When using an chemically activated composite it was important for the operator to work quickly and control of adhesive film thickness could be a problem.

The integrity of the bond was highly dependent upon the stresses imparted to it by the overlying restoration and the degree of polymerisation. The interfaces in amalgam and chemically activated thick restorations were significantly less permeable than the light activated restorations. The chemically activated composite gave a better seal when used in bulk, whereas the light activated composite performed best in thin section in a low configuration cavity arrangement with the use of a glass matrix. In the class I restorations, the difference in the light intensities used made no significant difference to the interfacial micropermeability. The use of a bulk filling technique with light activated restorations cannot be condoned in a high configuration cavity as this may result in residual unpolymerised resin at the base of the cavity, even with a high
intensity light source in 4 mm deep cavities. In the 2 mm restorations, with high intensity curing light, cohesive failure of the composite and even interfacial and dentine fracture at the cavity angles were observed.
Chapter 7

FRACTURE STUDIES OF DENTINE /RESTORATIVE INTERFACES.

7.1 PRELIMINARY INVESTIGATION SCOTCHBOND MULTI PURPOSE

7.1.1 Objectives

Previous studies have investigated the failure of resin/dentine interfaces by a variety of methods including tensile and shear bond tests, and by examining the surfaces of fractured samples with conventional light or scanning electron microscopy. These were reported in the literature review (Chapter 1.7). None have recorded the propagation of the crack along the interface in real time. The objectives of this study were to gain further information on interfacial failure using fluorescence confocal microscopy. This would be achieved by:

- examining and recording the interface under load in real time, until the point of failure
- examining the interface of the reassembled fractured sample.

7.1.2 Method

Lower third molar teeth were included in auto-polymerising denture base acrylic (Simplex Rapid, Associated Dental products Ltd., Kemdent Works, Swindon UK) in cylindrical moulds (internal dimensions: 30 mm diameter, 25 mm height, Model 813-019 Leco, USA). The samples were sectioned with a slow speed, water cooled diamond saw to expose an approximal dentine surface. Samples were stored in water,
even once the teeth had been included in acrylic, to ensure that the dentine remained hydrated. In order to produce a very small crack or deficiency within the interface, thin PTFE tape (plumber’s jointing tape) was draped and adapted over the most coronal 2 mm of the sectioned approximal dentine. A matrix for a resin composite bobbin was made from a section of a drinking straw (external diameter 4 mm). This was positioned so that the margin of the PTFE tape produced a cord of a circle across the edge of the drinking straw and secured to the dentine surface with modelling putty (Figure 7.1). The dentine within the matrix was then prepared with the SBMP system according to the manufacturer’s instructions (Table 2.2), but with a fluorescent label in the adhesive, either rhodamine B or auramine O. A 2 mm deep Z100 restoration was then packed in the matrix and cured. The teeth were sectioned longitudinally through the restoration as indicated in Figure 7.1 to expose an interface on a flat surface which could be examined using a TSM. The remainder of the acrylic sample was sectioned to produce a block of suitable size (8 mm wide x 23 mm long x 15 mm high) for mounting in a jig on a straining stage (Figure 7.2).
Figure 7.1  Line diagram illustrating the positioning of the straw matrix, PTFE tape and composite bobbin on the tooth sample in the resin block, (b) buccal, (l) lingual aspect. The line x-x illustrates the final section through the block and composite bobbin.

Figure 7.2  Diagram of a sample prepared for loading, mounted in the miniature jig.
Figure 7.3 Photograph of a sectioned tooth sample included in an acrylic resin block, with composite bobbin bonded to the approximal dentine. The coronal aspect of the bobbin was trimmed with a razor blade to make a flat surface for the application of the load. The load was applied in the direction indicated by the arrow.

Figure 7.4 An image of a composite bobbin and the dentine/adhesive interface prior to loading. The defect in the resin composite (C) / dentine (D) interface formed by the PTFE tape (*) is illustrated. The adhesive (A) is labelled with fluorescein. 450/520 nm. x20/0.8NA OI. Scale bar 100 µm.

Figure 7.5 Illustrates the disadvantages of the PTFE tape in some samples, with the polishing debris lodged within the defect site. 450/520 nm. x20/0.8NA OI. Scale bar 100 µm.

Figure 7.6 Approximated fracture surfaces following failure of the dentine/restorative interface, illustrating failure between the hybrid zone (H), and the adhesive (A), a cohesive failure of the composite (C), and a crack within the resin composite (*). The dotted line indicates the original relationship of the fractured surfaces. The adhesive was labelled with fluorescein 450/520 nm. x20/0.8NA OI. Scale bar 100 µm.

Figure 7.7 Approximated fractured surface following failure of the dentine/restorative interface, illustrating failure between the adhesive (A) and the composite (C). The primer (P) is labelled with rhodamine B. 546/600 nm. x20/0.8NA OI. Scale bar 100 µm.

Figure 7.8 Approximated fractured surfaces following failure of the dentine/restorative interface, illustrating failure within the resin composite (C), the adhesive (A), and the dentine (D). Cracks within the resin composite are also visible (*). The dotted line indicates the original relationship of the fractured surfaces. The adhesive is labelled with fluorescein. 450/520 nm. x20/0.8NA OI. Scale bar 100 µm.
The coronal aspect of the sectioned restoration was trimmed using a razor blade in order to produce a flat surface for loading. This produced a slight step in the dentine surface at the margin of the restoration giving an excellent reference point for relocating the composite bobbin on the dentine following fracture (Figure 7.3). The samples were mounted in a miniature straining device which could be positioned on the TSM stage (Watson 1994). A servo motor driven pusher (or blade 0.7 mm wide) was used to load the resin composite button. The pusher was aligned to run parallel to the interface less than 300 μm the dentine surface (Figure 7.2). This jig was placed on the microscope stage and the position of the sample adjusted to lie parallel with the focal plane of the objective as the stage travelled in the x and y plane. The pusher was also adjusted to travel in this plane. This enabled the whole length of the interface between dentine adhesive and resin composite to be examined and avoided damage to the objective during loading of the sample. The interface was examined through a glass coverslip using a x20/0.8 NA oil immersion objective. The rate of application of the load to the samples was varied so that once a crack was seen, the rate was reduced to zero. This allowed cracks to be generated and then to propagate with their own energy before the blade was advanced again. The interface under load and during failure was recorded on video with a CCD camera and on 35 mm film after the fracture event.

7.1.3 Results

Figure 7.4 shows a TSM image of an interface prior to loading, illustrating the resin composite restoration (C) and the deficiency in the restoration interface formed by the PTFE tape (*). Some problems arose in the application of the PTFE tape and it was difficult to form a consistent defect. Some samples de-bonded during or following the preparation prior to applying the load, and it was also noted that the adhesive overlying the PTFE had a gel like consistency. The appearance of the samples was marred by the polishing debris which became lodged within the defect site (Figure 7.5).

Development of the crack could be followed at video rate as the bond between the adhesive and the hybrid zone became 'unzipped' in a slip/stick failure pattern with tears extending into the adhesive. Propagation of the failure could later be examined frame by frame.
Further information with regard to the fractured surfaces could be gained by repositioning the displaced composite button close to the dentine surface, post fracture. A variety of failure sites were observed when the samples were loaded (Figures 7.6-7.8). Fractures can be seen between the adhesive (A) and hybrid zone (H); adhesive and resin composite; and within the composite, adhesive or dentine (D). Subsidiary cracks propagated from the adhesive into the resin composite in some samples (Figure 7.8). These were characteristically at a 45° angle to the interface and propagated in the opposite direction to that of the load. Several modes of failure were often to be seen within a sample, but the majority were between the adhesive and the hybrid zone.

7.1.4 Discussion

Observation of dynamic fracture using video-rate confocal fluorescence microscopy provided a wealth of information on the fracture process for each sample. In addition to the video recordings, which could be studied frame by frame, a record of the pre and post fracture surface could be made on 35 mm film. Fluorescent dye labelling of the adhesive helped to highlight the distribution of failure within the interface. This technique, therefore yielded far more information on the dynamics of interfacial failure under load, when compared with conventional fracture studies. It was possible to begin to describe and categorise the dynamic failure patterns. The repositioning of the composite bobbin and the ability to examine both fracture interfaces, also gave a better understanding of the complexity of the failure site within the interfaces.

It has been shown that sub-surface evaluation of fracture interfaces is very important when determining where tooth/restoration bond failure has occurred. Failures which appeared to be adhesive in nature by surface examination (SEM or low resolution light microscopy), were frequently shown to be cohesive in nature when examined with the confocal microscope (Wilmot et al 1994).

The use of the PTFE tape provided a known site of weakness within the sample from where the crack started, but it was difficult to control the appearance of the defect and the application of the tape. Subtle stepping of the dentine surface with a razor blade
ensured that the tooth and restoration could be realigned accurately following the fracture.

Finite element analysis has been used to investigate the importance of sample and loading geometry, and their effect on the stresses generated within the sample once loaded (van Noort 1989). In order to standardise the geometry and loading, further developments in the method were needed for the main study:

- Omission of the PTFE tape, as its position was not easily controlled and unnecessarily complicated the sample geometry.

- Rhodamine B was selected as the fluorescent label for the adhesive as it gave the stronger signal and more dissolved readily in the adhesive.

- The pusher needed to load the bobbin as close to the adhesive interface, and thus as close to the dentine surface, as possible.

- The bobbin height and bonded surface area needed to be carefully controlled.

- To avoid pooling of the primer and adhesive at the angle between the dentine and the matrix, these components needed to be applied prior to placing the matrix. However it was equally important to avoid a flash of resin around the composite bobbin as this has been shown to influence bond strength data (van Noort 1989).

- The rate of application of the load needed to be constant and standardised, rather than intermittent and governed by the interfacial appearance. It was decided to advance the pusher at a rate of 0.2 mm min⁻¹ as this rate generally allowed the crack tip to be followed with the microscope.

The next stage in the development of this method was to simultaneously record load and position data and to link these with the real time images of the interfacial failure. Advances in image capturing and analysis were required to allow these developments and a greater interpretation of the fracture patterns.
7.1.5 Conclusions

The method enabled the failure of adhesive interfaces to be observed and recorded at video rate. The development of a crack could be followed along the interface.

In this preliminary study, failure of the dentine/restorative bond under load occurred in a variety of sites, but was most commonly observed between the adhesive and the hybrid zone.

Following this preliminary study, it was recognised that a method of capturing and storing digitised image sequences of the interfaces under load was needed. In addition, it was envisaged that future studies would simultaneously record images together with load and displacement data. This would facilitate the comparison of interfacial failure of different dentine bonding systems and enable more quantitative data to be collected.
THE INFLUENCE OF DENTINE ADHESIVES ON INTERFACIAL FAILURE UNDER LOAD - A STUDY USING REAL TIME IMAGING AND LOAD PROFILES.

7.2.1 Objectives

Following on from the preliminary study, the aim of this study was to develop a shear test model which could simultaneously give real time information on sample loading and morphology of the interface. This would allow information regarding not only the critical shear stress, but also the loading profiles together with the mode and site of interfacial failure.

The objectives were firstly, to investigate the behaviour of the dentine/adhesive interfacial region when composites are loaded in shear mode by:

- microscopically imaging the interfacial region as it is loaded until bond failure.
- recording the load at failure and the crack geometry
- recording changes in load and position with time.

And secondly, to compare the site and mode of bond failure with different dentine adhesives under a variety of conditions: adhesives with different filler content, on dentine prepared with phosphoric acid and primer compared with a self etching primer.

7.2.2 Materials and Method

The three dentine adhesive systems selected with a phosphoric acid preparation were Optibond, EBS and SBMP+. Optibond had a filled resin whereas SBMP+ and EBS had unfilled resins. The systems were used according to the manufacturer’s instructions (Table 2.2). For all systems this involved a phosphoric acid gel etch (15 s application and 30 s wash) the appropriate manufacturers’ primer and adhesive, and an XRV resin composite bobbin. The self etching primer system selected was Clearfil Liner Bond 2
(CFLB2), which employs a primer with phenyl-P and was used with a resin adhesive and Protect Liner (Chapter 2).

**Sample preparation**

Lower third molar teeth were mounted in embedding epoxy resin (Scandiplex, Scandia, Germany) in cylindrical moulds (internal dimensions: 30 mm diameter, 25 mm height, Model 813-019 Leco, USA). The resin cylinders produced were sectioned using a low speed diamond saw under water to produce blocks with an exposed surface of approximal dentine as described in the preliminary study (Figure 7.1). However, the samples were prepared without PTFE tape. The blocks were then stored in water at 4°C until required.

Five blocks were prepared for each adhesive system to be tested. The dentine was prepared with the appropriate etch, primer, and adhesive as outlined previously. Adhesives were labelled with a few grains of rhodamine B dye as in the preliminary study. A composite restoration was placed on the exposed approximal dentine surface, using a section of drinking straw (4 mm external diameter) as a matrix. The restoration was positioned in the midline of the exposed dentine, with its superior margin just below the occlusal enamel. The straw matrix was held in position with plasticine and a single portion of XRV resin composite was dispensed and condensed into the matrix to make a bobbin approximately 1.5 mm high. The composite was cured with a blue light (Optilux, Kerrs Mfg, UK) applied for 40 seconds with the plasticine *in situ*, and a further 20 seconds after its removal to ensure exposure of the composite to the curing light. The blocks were then sectioned longitudinally through the midline of the bobbin with a wire saw under water with minimal loading thus producing 10 samples for each adhesive system. In order that samples were loaded after a consistent short storage time, they were stored in water for 24 hours at room temperature prior to the fracture experiment.

In addition, control samples, without fluorescent dye in the adhesive, were prepared for each adhesive system tested. Initially, two samples were prepared for each of the systems, but additional samples were required for the CFLB2 system.
Immediately before fracturing the sectioned surfaces of the composite, and dentine were polished by hand with fine emery paper (grade P1200). The composite bobbin was polished with a coarse then fine emery paper to reduce its height to 1 mm. The surface adjacent to the enamel was trimmed with a razor blade to provide a flat surface for the application of the load and to ensure that the composite bond was only to dentine and not to enamel. Any ‘flash’ of composite or adhesive extending onto the dentine beyond the base of the bobbin was also removed with a scalpel blade. The effectiveness of this method of removing adhesive flash was confirmed on microscopic examination of the sample. The samples were all prepared in the same way and the risk of damage was minimised by the careful handling of the samples, for example by hand rather than machine polishing.

**Loading and failure of samples**

During load application the tooth was aligned in a miniature straining gauge as in the preliminary study. The interface was viewed with a TSM whilst a shearing load was applied to the composite bobbin. The experimental set up is illustrated in increasing detail in Figures 7.9 to 7.11.
Figure 7.9 Illustration of the experimental set up, with the miniature straining stage (S) holding the tooth sample under the x20/0.8 OI objective in the TSM. Illustrated are: the motorised microscope (M) stage for adjustment in x and y and the motor encoder (E).

Figure 7.10 Illustration of the experimental set up, with the tooth sample held in the jig (J) on the straining stage with adjustment screw visible and the blunt pusher (P) with load cell (L).

Figure 7.11 Close up of the mounted tooth sample to show the relationship of the blade (P) to the composite bobbin (C). The sample is covered with a glass slide and the meniscus visible relates to the immersion oil between the sample and the coverslip. The dentine has been strained with rhodamine B to highlight the tooth structure.
Load and position data recording

A blunt blade tip (as in a miniature lathe parting off tool) mounted on a precision linear stage was driven by a highly geared DC micro-motor, with shaft encoder, to apply a shearing load to the composite bobbin at a constant speed of 0.2 mm min⁻¹. The blade was blunt, designed to avoid a peel effect. A 100 N load cell was placed in series behind the blade (Figure 7.10). The load was applied parallel to the dentine restorative interface and as close as possible to the base of the composite bobbin to limit the amount of horizontal rotation, or bending moment, of the bobbin. The maximum distance was 300 μm as this permitted the blade to be seen in the same field width as the interface.

Load and position data were sourced directly from the load cell and shaft encoder on the linear actuator respectively. These were relayed via a Data Acquisition Unit (DAU) to the same PC as used for image manipulation and storage. The load and position data were synchronised with the computer images. There was also a visual display of load and position data. The load cell was calibrated with masses of 5, 10 and 20 g prior to each experiment.

Image Recording

Video rate recording of the interface

Reflection and fluorescence images of the interface produced using a x20/0.8NA oil immersion objective in the TSM, were recorded in real time using a CCD camera and captured simultaneously both on VHS video tape and digitised using the RAID. A video of the interface prior to loading was recorded for each sample. This was used to estimate the thickness of the adhesive layer. Measurements were made on the monitor using a paper scale as described in Chapter 2. The distance between the interface and the dentine surface (see Figure 7.14) was also measured in the same way.

During loading, propagation of the crack tip was followed along the length of the interface. The fracture image sequences captured on the RAID were replayed for a frame by frame assessment. The frames of interest for each fracture were stored on the
RAID and on optical discs as a ‘grab’ sequence which could be replayed as a video loop on the computer.

*Post fracture images*

After the fracture, the blade position was returned to its start position and the composite bobbin was re-located to its pre-fracture position, whilst viewed with the TSM to confirm the bobbin position. The site of the failure was recorded for each field width along the interface, using a x60/1.4 NA oil immersion objective for confirmation were necessary. Images were recorded on 35 mm film.

*Analysis of results*

The parameters recorded were: shear bond stress, dynamic pattern of failure and site of failure, and load profile.

*Shear bond Stress*

The shear stress of each sample was calculated from the area of attachment of the bobbin and the peak failure load.

The area of the interface was calculated by measuring the de-bonded surface the composite bobbin using the TSM with a x2.5 dry objective and the Kalcium Analyse programme (Kl). If the bobbin had been lost during failure, the dentine surface where the bobbin had been attached was identified and measured in a similar manner. The mean bonding area was calculated.

Univariate analysis of the shear bond results was performed. In addition, the shear stress results were analysed using a one way analysis of variance in combination with a Bonferroni test, and a Kaplan Meier Survival analysis was performed for each group, and inter group were comparisons made.

Comparisons of the shear bond stress of the test samples (with fluorescent dye) and control samples (with no dye) were made using Hodges-Lehmann estimates of shift parameters. The effect of adhesive thickness and distance between the blade and the
interface were made using the Kruskall Wallis test. This analysis determined whether the shear bond stress populations for the four materials were identically distributed.

Pattern of failure

The dynamics of the failure pattern were analysed and characterised according to the appearance of the interfacial failure as far as possible by replaying the fracture image sequences stored on the RAID. The categories of failure pattern ascribed were peel, slip/stick, shock wave and snap.

Site of failure

After replacing the composite bobbin, the site of the failure along the whole interface was recorded. The fracture description was characterised as: dentine; dentine/adhesive; adhesive; adhesive/composite or composite. A record was made of tearing of the adhesive and of supplementary cracking of the dentine.

Load Profile

The load and position readings during the loading and failure of the sample were recorded and later transferred to a spreadsheet, (Microsoft Excel version 5.0). Shear stress was plotted against time a ‘stamp trace’ and the shapes of the plots compared to assess whether differences in the material groups or the pattern of could be detected. At the start of the study load/time traces were recorded with a pen recorder to confirm the computer generated profiles.

7.2.3 Results

The raw data are given in Table 7.1. These are examined and illustrated in the following results sections. The mean of the interfacial area was 4.6 mm² (standard deviation 0.98).

Shear bond stress.

Univariate summary statistics for each system are given in Table 7.2. The materials can be ranked: EBS, SBMP+, Optibond and CFLB2 in order of decreasing mean shear
stress. However, the results of the analysis of variance and Bonferroni analysis (Tables 7.3 and 7.4) revealed no significant difference between the three adhesives with phosphoric acid preparation, but there was a significant difference between the EBS and the CFLB2 with self etching primer and filled Protect Liner (Table 7.4). The mean failure was dependent on the dentine bonding system. The materials showed a range of shear bond values as indicated by the 95% confidence intervals (Table 7.2), the most consistent was the CFLB2.

The log-rank test for equality of survivor functions are given in Table 7.5. and the results of the Kaplan Meier survival analysis are shown in Figure 7.12. The 50% survival rate was highest for the EBS, and lowest for the CFLB2.

Shear bond stress results of the control samples, without dye in the adhesive, were comparable to the test samples with dye. The analysis with the Hodges-Lehmann analysis, exact inferential, gave no significant difference between the groups (p= 1.0). The result of the first control sample of the CFBL2 was much larger than expected, therefore 10 samples were completed for this material.

Likewise the Kruskall-Wallis analysis demonstrated that there was no statistically significant difference between the four material populations according distance between the blade and the dentine surface (p= 0.4).

A similar test showed there to be a significant difference between adhesive thickness p=0.04. Subsequent pair wise analysis showed that the source of this difference was between Optibond and EBS, p=0.04. There were no other differences.
### Table 7.1
Results of fracture experiments: optibond, SBMP+, EBS and CFLB2.
Shear stress, bonded area, adhesive thickness, blade to dentine distance.

<table>
<thead>
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<th>Shear Stress</th>
<th>Bonded area</th>
<th>Adhesive thickness</th>
<th>Blade/dentine dist. (fdw)</th>
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<td>15.3</td>
<td>5.2</td>
<td>17</td>
<td>0.25</td>
</tr>
<tr>
<td>F84</td>
<td>15.3</td>
<td>5.5</td>
<td>25</td>
<td>0.25</td>
</tr>
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<td>F85</td>
<td>21.5</td>
<td>3.6</td>
<td>30</td>
<td>0.25</td>
</tr>
<tr>
<td>F86</td>
<td>12.5</td>
<td>5.5</td>
<td>25</td>
<td>0.3</td>
</tr>
<tr>
<td>F87</td>
<td>15.1</td>
<td>4.3</td>
<td>17</td>
<td>0.25</td>
</tr>
<tr>
<td>F88</td>
<td>10.2</td>
<td>4.3</td>
<td>20</td>
<td>0.25</td>
</tr>
<tr>
<td>F89</td>
<td>19.4</td>
<td>6.19</td>
<td>20</td>
<td>0.25</td>
</tr>
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<td>Control</td>
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<td></td>
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</tr>
<tr>
<td>F104</td>
<td>21.2</td>
<td>4.1</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>F105</td>
<td>14.2</td>
<td>3.8</td>
<td>0.25</td>
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<td>Clearfil</td>
<td></td>
<td></td>
<td></td>
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<td>Liner B 2</td>
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</tr>
<tr>
<td>F64</td>
<td>8.3</td>
<td>4.4</td>
<td>6</td>
<td>0.5</td>
</tr>
<tr>
<td>F65</td>
<td>11.5</td>
<td>4.9</td>
<td>12</td>
<td>0.25</td>
</tr>
<tr>
<td>F66</td>
<td>6.4</td>
<td>6.1</td>
<td>17</td>
<td>0.2</td>
</tr>
<tr>
<td>F67</td>
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<td>3.4</td>
<td>12</td>
<td>0.25</td>
</tr>
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<td>F68</td>
<td>9.7</td>
<td>3.9</td>
<td>17</td>
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<td>5.9</td>
<td>17</td>
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<tr>
<td>F70</td>
<td>7</td>
<td>4.4</td>
<td>13</td>
<td>0.3</td>
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<tr>
<td>F71</td>
<td>10</td>
<td></td>
<td></td>
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<td>F72</td>
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<td>4.9</td>
<td>3</td>
<td>0.25</td>
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<td>F73</td>
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<td>3</td>
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<tr>
<td>F102</td>
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<td>4.6</td>
<td>0.25</td>
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</tr>
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<td>F111</td>
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<td>3.9</td>
<td></td>
<td></td>
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<td>F115</td>
<td>14</td>
<td>4.3</td>
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</tr>
<tr>
<td>F117</td>
<td>8</td>
<td>5.9</td>
<td></td>
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</tr>
</tbody>
</table>

**KEY**

fdw = field width = 450um
Table 7.2  Univariate summary statistics of shear bond strength for dentine bonding systems.

<table>
<thead>
<tr>
<th>Dentine Bonding System</th>
<th>n</th>
<th>Mean</th>
<th>95% Confidence Interval</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optibond</td>
<td>10</td>
<td>11.4</td>
<td>8.1 to 14.6</td>
<td>4.6</td>
</tr>
<tr>
<td>SBMP+</td>
<td>10</td>
<td>12.9</td>
<td>9.5 to 16.4</td>
<td>4.8</td>
</tr>
<tr>
<td>EBS</td>
<td>10</td>
<td>15.9</td>
<td>13.5 to 18.3</td>
<td>3.3</td>
</tr>
<tr>
<td>CFLB2</td>
<td>10</td>
<td>8.6</td>
<td>7.4 to 9.9</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Table 7.3  Results of the analysis of variance of sheer bond strength for dentine bonding systems.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Mean Square</th>
<th>F value</th>
<th>Prob&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>3</td>
<td>88.6</td>
<td>6.01</td>
<td>0.002</td>
</tr>
<tr>
<td>Within groups</td>
<td>35</td>
<td>14.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 7.4 Summary of probabilities associated with the Bonferroni test of shear bond strength for dentine bonding systems tested.

<table>
<thead>
<tr>
<th>Dentine Bonding System</th>
<th>Optibond</th>
<th>SBMP+</th>
<th>EBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBMP+</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBS</td>
<td>0.06</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>CFLB2</td>
<td>0.8</td>
<td>0.1</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 7.5 Long-rank test for survivor functions for shear bond strength.

<table>
<thead>
<tr>
<th>Dentine Bonding System</th>
<th>Events observed</th>
<th>Expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optibond</td>
<td>10</td>
<td>7.8</td>
</tr>
<tr>
<td>SBMP+</td>
<td>10</td>
<td>11.4</td>
</tr>
<tr>
<td>EBS</td>
<td>10</td>
<td>16.7</td>
</tr>
<tr>
<td>CFLB2</td>
<td>9</td>
<td>3.0</td>
</tr>
</tbody>
</table>

$X^2 = 16.95 \ p = 0.001$
Figure 7.12
Kaplan Meier survival estimates for all dentine bonding systems tested.
Figure 7.13  Histogram: site of failure expressed as a percentage of the interface.
Site of Failure.
The histogram (Figure 7.13) illustrates the distribution of the site of failure along the interfaces expressed as a percentage along the x axis. The dentine/adhesive interface was the most common failure site in the Optibond and SBMP plus samples. In contrast the EBS failures were distributed equally between all components of the interface. The CFLB2, without the phosphoric acid etch, has the greatest percentage of interfacial failure at the dentine/adhesive interface.

The Optibond failures were accompanied by small fractures of the dentine, thus the fracture line frequently involved the hybrid layer. SBMP+ failures occurred at the level of the detached primer described in Chapter 5 and the hybrid layer was generally left intact. Failures of this system were accompanied by tearing of the adhesive in some of the samples. The tears extended only part way into the adhesive layer and appeared in high density along the whole or part of the interface. In contrast, failures of the EBS adhesive involved the whole width of the adhesive from dentine to composite and were more widely spaced. These failures are illustrated in Figures 7.14a-f, the complexity of the failure site and the wealth of information provided by repositioning the composite bobbin is illustrated in these figures. Frequently, a fracture through the composite from the point of contact of the blade, progressing to the interfacial region was seen in the first fieldwidth as illustrated in Fig 7.14a. Fractures deep into the dentine substrate were seen furthest from the blade application, generally about 3 fieldwidths from the end of the bobbin (Figure 7.14b). The crack tip in these fractures was seen returning to the interface just before the tail end of the bobbin.

Dynamic Patterns of failure.
Four patterns of failure were identified; catastrophic ‘snap’, peel, stick/slip and shock wave.

Catastrophic snap failures.
These failures, once started, progressed very rapidly along the interface. Three features were commonly seen.

- a flash of light seen progressing along the interface.
• the crack progressed too rapidly to be captured on more than a single frame even when recording at video rate (one frame every 40 ms).
• fluid movement in the dentine tubules heralding a visible fracture, as air or immersion oil entered the tubules from the fracture interface.

Peel.
This slower failure occurred with a steady peeling of the interface. The locus of the failure was between the dentine and the adhesive, and the failure was not accompanied tearing of the adhesive.

Slip/stick
These fractures were accompanied by the tearing of the adhesive and had the appearance of unzipping of the interface. The slip/stick was slower than a catastrophic snap fracture and in some samples occurred in the initial failure zone, preceding a rapid snap failure further along the interface.

Shock wave.
This pattern was characterised by initial cracks opening up in the adhesive. The tears or cracks started as narrow slits, which opened to develop into rhomboid spaces involving the whole width of the adhesive. The line of failure then progressed to involve all components of the interface; forward along the dentine/adhesive interface and backward along the composite/adhesive interface, as well as entering into the dentine and composite.

The results for the different dentine bonding systems were that 60% of Optibond samples had a snap failure, 60% of SBMP+ had a slip/stick failure and EBS had a shock wave failure pattern. Individual frames taken from the failure sequences are shown in Figs.7.15 - 7.17 to illustrate the failure patterns. These patterns are shown in real time in the video which is presented as part of this thesis.
As initial failures commonly started with a small fracture into the composite at the point of contact of the pusher blade, the recording of the fractures was commenced about three fieldwidths from this edge of the bobbin.

Load profiles

Examples of plots shear bond stress against time for the different bonding systems are given in Fig. 7.18. The shape of the plots were similar for all materials tested.
Figure 7.14  Reflection TSM images illustrating the variety of dentine/restorative interfacial failure sites. The composite bobbins have been repositioned against the dentine in their pre fracture location. The adhesive in all systems has been labelled with rhodamine B. The images are arranged so that the load has been applied to the bobbin surface at the top of the images.

a. The pusher (P) was advanced in the direction indicated by the arrows to load the sample. The distance between the pusher and the dentine surface (—). The composite (c) has a cohesive failure indicated by the small arrows. The separation of the bobbin from the dentine is indicated with the large arrows, with a failure between the adhesive and the dentine. Above this the failure includes some failure of the hybrid zone. 450/520 nm, x20/0.8NA OL. Scale bar 100 μm.

b. SBMP+ sample, near the end of the bobbin furthest from the load. A complex failure illustrating tears in the adhesive layer (a), failure between the composite (c) and the adhesive, and a fracture of the dentine (f). 450/520 nm. x20/0.8NA OL. Scale bar 100 μm.

c. Same sample as in (b), illustrating the typical failure site in this material, with failure between the dentine and adhesive accompanied by frequent tears of the adhesive, extending only part way into the adhesive layer. The hybrid layer (h) has only minor areas of failure. x60/1.4NA OL. Scale bar 10 μm

d. Same sample as in (c), illustrating the failure at higher magnification, at a point where there has been a failure of the hybrid layer (arrowed). The detail includes a fracture through a resin tag (lower arrow) x100/1.4NA OL. Scale bar 10 μm.

e. CFLB2 which has failed between the composite and the filled adhesive layer. Both the adhesive resin (a) and the protect liner (p) are visible. This sample had failed in peel mode and there are very minor tears of the protect liner which help to confirm the pre-fracture location of the bobbin (—). x20/0.8NA OL. Scale bar 100μm.

f. EBS sample illustrating a typical site of failure following a shock wave failure pattern. Slits are visible through the whole adhesive layer. The first failure (1) has extended forward to a dentine/adhesive failure. The second failure to appear (2) demonstrates the progress of the crack tip back towards the loaded surface of the bobbin as a cohesive failure of the composite. The bobbin is not visible in this image. x100/1.4NA OL. Scale bar 10 μm.
Figure 7.15 Rapid slip/slick progressing to a snap pattern of failure in an Optibond sample, with the composite bobbin (c), adhesive (a) and dentine (d). The reflection images are single images from a fracture sequence digitised and stored with the aid of the Kalcium Analyses programme and the RAID. The direction of the load application is indicated. The rapidly advancing crack tip between the dentine and adhesive is arrowed and the third image illustrates the interface being followed by moving the microscope stage to follow the crack tip along the interface. (TSM x20/1.4NA OI. Scale bar 100 μm).
Figure 7.16  Slip/stick pattern of failure in a SBMP+ sample, with the composite bobbin (c), adhesive (a) and dentine (d). The reflection images are single images from a fracture sequence digitised and stored with the aid of the Kalciom Analysers programme and the RAID. The direction of the load application is indicated. The advancing crack tip between the dentine and adhesive with accompanying tears into the adhesive is arrowed. (TSM x20/1.4NA OI. Scale bar 100 μm).
Figure 7.17. Shock wave pattern of failure in an EBS sample, with the composite bobbin (c), adhesive (a) and dentine (d). The reflection images are single images from a fracture sequence digitised and stored with the aid of the Kalcium Analyse programme and the RAID. The direction of the load application is indicated.

Frame 1: the failure in the whole thickness adhesive layer is marked with an arrow.

Frame 2: this failure opens and a new failure line opens.

Frame 3: the failures continue to open to form rhomboid spaces and the failure tips progress forward along the dentine adhesive interface and backward between the composite and the adhesive. (TSM x20/1.4NA OL. Scale bar 100 mm).
Figure 7.18  Graphs of load against time for all dentine adhesives tested.
7.2.4 Discussion

This new technique of fracture study enabled the failure of adhesive interfaces to be observed and recorded at video rate, but also to record both position and load data from the pusher. This would not have been possible without the advances in image data capture and storage.

Shear bond tests using a blunt pusher were chosen for these in vitro experiments, as they were the most easily configured to allow examination of the interface at the time of the fracture. The sample geometry was designed to take into account of the findings of FEA studies with the pusher as near to the dentine surface as possible, removal of any flash around the bobbin, standardised methods of bobbin preparation and bobbin dimensions. The bobbins had an average bonding area of 4.6 mm² which was smaller than many reported cylinders used in shear bond tests, but larger than those used in micro-tensile test specimens (Sano et al 1994b). The ISO standard is 3 mm diameter specimens (TR 11405). In this study 4 mm diameter specimens were sectioned, the final sample size was dependent on the saw cut with the 300 μm diamond wheel. The size was selected to allow ease of handling and also reflected the present move toward samples with a smaller surface area and therefore less flaws.

The distance of the blade from the pusher varied from 0.2 to 1 fieldwidths (equivalent to 90-450 μm). This variation was not sufficient to influence the shear stress results.

The load was applied at a rate of 0.2 mm min⁻¹ following the preliminary study. This was more with the faster speed of 5 mm min⁻¹ (commonly used for shear bond tests) or the ISO standard speed of 0.75 mm min⁻¹ (TR 11405), but allowed the crack tip to be followed in the majority of samples.

The shear stress data was in a similar range as reported by previous studies, (see summary Table 1.2). Although the bond strength for materials in this study could be ranked EBS>SBMP+>OPTI>CFLB2, there was only a significant difference between EBS and CFLB2.
It was important to establish whether the inclusion of the fluorescent dyes within the adhesive influenced the performance of the bonding system. The comparison of test and control samples, with and without fluorescent dye in the adhesive, confirmed that the inclusion of fluorescent dye did not influence the bond strength. The inclusion of the dye, was important in facilitating the observation of fracture events at the interface and the analysis of the post fracture surfaces.

Differences in the behaviour of the dentine adhesive systems under load were not manifest in the shear stress profiles with time. The site and pattern of failure, however, differed for the adhesive systems investigated.

The shock wave failure of the EBS, commencing within the unfilled adhesive, indicated that the adhesive layer was able to absorb a considerable amount of energy before the critical shear stress was reached. The lines of failure were equally distributed through the different components of the interfacial region (dentine, adhesive, composite and their interfaces). This indicated that the components of the system are well matched. Further evidence for this was found in the results of micropermeability studies in Chapter 5, where the E3 system (later marketed as EBS) performed well and provided a good seal to fluids from the pulp.

The mismatch of the primer and adhesive components of the SBMP+ system noted previously in morphological and micropermeability studies (Chapters 4 and 5) was emphasised in this study by the failure line being sited at the dentine/adhesive interface, leaving the hybrid layer intact. In motion this was seen as a stick/slip pattern with a zig zag tearing pattern of the adhesive (seen in 70% of samples).

The Optibond adhesive was filled and had a snap failure in the majority of samples. These results indicated a stiff interface. The tearing pattern of the adhesive (seen in 40% of samples) was similar to the SBMP+, but chips of dentine were pulled out of the dentine surface during crack propagation. Despite the different failure patterns of the Optibond and EBS systems, there was no significant difference in the shear bond
results: it should also be remembered that they had also been equally matched when tested for micropermeability (Chapter 5).

The CFLB2 system had a peeling fracture. There was some minor adhesive tearing and localised dentine fractures with this material. Studies presented in Chapters 4 and 5 indicated that this material also had the thinnest hybrid layer, being prepared with salicylic rather than phosphoric acid, and low micropermeability results. These results would indicate that this hybrid layer, although relatively thin, was of high quality.

Analytical studies and finite element analyses have highlighted the presence of areas of stress concentration at or near the interface. In this study the real time images illustrate the local interfacial behaviour and failure as a result of the build up stress along the interface. A complex array of fracture patterns were detected. The differences in the failure patterns were too subtle to be graphically displayed in the plots of load and position with time, and also beyond the capabilities of FEA at this stage.

The reasons for the differences between the dentine bonding systems observed in these fracture studies warrants further investigation. The chemical composition of the components was responsible for the interfacial morphology, (influencing the width of the hybrid zone, degree of penetration of the primers and adhesives), but was also responsible for the visco-elastic properties of the interfacial region both of the hybrid layer and the adhesive layer. The speed of load application was constant for all tests, but at different rates of load application the visco-elastic behaviour of the interfacial region would expect to change (Aubrey and Sherriff 1980).

Further information is needed on both the loading of restorative interfaces in clinically and on the visco-elastic properties of interfaces. On first examination the degree of filler in the adhesive may be held responsible for the differences in behaviour, however, as the adhesives are not chemically identical, it is not possible to predict the difference in visco-elastic properties based on whether the adhesive is said to be filled or unfilled.
7.2.5 Conclusions

This method not only enabled failing dentine/adhesive interfaces to be imaged and recorded at video rate, for the first time, but also allowed the simultaneous recording of the load applied to the sample and the distance travelled by the blade applying the load.

This enabled failure sites and dynamic failure patterns to be described for the systems tested. For these materials, differences in the failure behaviour of the interfaces were observed in both the dynamic failure pattern and site of failure. The addition of the fluorescent dye did not influence behaviour of the interface. The bond stress at failure and load/position profiles alone were not able to highlight these differences.

The site and dynamic pattern of interfacial failures were dependent on the dentine bonding system.

Four patterns of failure were seen:

- Optibond a snap failure at the dentine adhesive interface with cracks into the hybrid zone, indicating a stiff interface.
- SBMP+ a stick/slip pattern at the dentine adhesive interface with small frequent adhesive tears, indicating stress breaking relating to flaws.
- EBS a shock wave pattern with failure equally distribute in all parts of the interface, indicating well matched components.
- CFLB2 a peeling pattern with relative ease of separation of the dentine and adhesive.

Further investigation is required into the visco-elastic properties of the interfacial region, the influence of the adhesive chemistry including filler content, and the effect of rate of load application.
Chapter 8

CONCLUSIONS AND FURTHER STUDIES

The studies reported in this thesis describe a range of methods to evaluate the appearance and performance of dentine/resin adhesive interfaces. The developments in microscope technology during the time of the study allowed the scope of the interfacial evaluation to be broadened to include a record not only the static interface at specific times, but also dynamic real time events at the dentine/adhesive interface. The most important development was in digital image data transfer and storage capabilities. Development of real time (video rate recording) confocal laser scanning microscopes enabled coherent illumination sources to be directly compared with incoherent (white light) sources.

Fluorescent dyes were used to highlight the distribution of the dentine bonding system components within the interface (Chapters 3, 4 and 7). The more traditional roles of fluorescent dyes in fluid tracking and detection of leaks was applied to evaluate dentine permeability and dentine/adhesive interfacial micropermeability (Chapters 3, 5 and 6).

Development of a 'window' model for the observation of events within the dentine enabled the assessment of dentine permeability to be made by observing the passage of fluid movement through individual dentine tubules. This is in contrast to previous methods which evaluated overall tissue permeability; by monitoring the progress of an air bubble, or fat droplet, in a fluid filled system. The model reported in this thesis was dependent on the development of a suitable mounting technique which would allow optical coupling of the sample to the coverslip, but at the same time would not interfere with the patency of the dentine tubules below the sample surface.

This 'window' technique was used to record the distribution of the components of dentine bonding systems during their application to the dentine surface and the effect of different application techniques. In the future, this model could be used to observe...
the interface during polymerisation of composite restorations. In a wider context, the method of attaching coverslips to samples to produce an observation window could also be used in the study of other biological and geological mineralised samples.

The morphology studies (Chapter 4) gave valuable information as to the distribution of the different components of the bonding systems within the interface. The relatively simple method of sample preparation maintained the hydrated state of the samples and the interfacial region was examined in near normal conditions, giving detail of the distribution primer and adhesive resin in the superficial dentine and dentine tubules. The importance of matching the components of the systems was highlighted. Good penetration of dentine by the adhesive components was seen in the dentine bonding systems examined; the greatest influence on the interfacial morphology was the type of conditioner employed.

Assessment of the interfacial seal in the micropermeability studies (Chapter 5) was a more critical test of the interface and revealed that none of the bonding systems tested formed a complete seal to fluid from the pulp. The fluorescence confocal microscopy technique enabled both the degree and the site of micropermeability within the interface to be assessed. In addition, differences in the formulation and application techniques of the systems resulted in differences in the porosity or seal of the interface. The micropermeability was not a function of the conditioner used and the thickness of the hybrid layer.

The integrity of the interfacial bond was dependent upon the type of restorative material, the cavity design and the degree of polymerisation (Chapter 6). The restorative materials which were studied were selected to impart different stresses to the interface. Gap formation, with cohesive failure of the composite and even interfacial and dentine fractures at the cavity angles, was observed with the occlusal cavity configuration, in contrast to the good marginal adaptation seen in the wedged shaped cavities in the earlier studies (Chapter 4). Both interfacial seal and the depth of polymerisation were compromised with a bulk filling technique in deep class I cavities, when using either a high or low intensity curing light. The remaining
dentine thickness (and hence dentine wetness) had an insignificant effect on the results.

Observation of interfaces under load (Chapter 7) enabled failing dentine/adhesive interfaces to be imaged and recorded at video rate, for the first time. Samples were mounted in a miniature straining stage. This enabled a load with known force and rate to be applied to an adhesive restoration whilst the interface was viewed with the confocal microscope. In a preliminary study sample geometry was established, together with the method of video rate recording and the analysis of the reassembled sample post fracture with high magnification TSM. In the main study in the method of image computer recording storage and analysis was developed along with the simultaneous recording of the load. The latter gave additional information, from which shear stress and load profiles were calculated.

Interfacial failure was characterised for the materials tested, both for the site of failure and the dynamic failure pattern. Four dynamic patterns of failure were seen: shock wave, snap, slip/stick and peel. The failures were of a complex nature, most frequently sited the interface between the dentine and the adhesive, but also included cohesive failures: tears of the adhesive and cracks of the dentine and hybrid layer. The site and dynamic pattern of interfacial failures were dependent on the dentine bonding system.

The bond stress at failure and load profiles (the data normally collected in bond strength studies) were not able to reveal the differences in dentine bonding systems highlighted by recording images of the interface in real time. The use of control samples without fluorescent dye in the adhesives confirmed that the addition of fluorescent dye did not alter the performance of the bonding systems tested.

In the future, further studies are required to provide more information on the behaviour of the interfaces under load and the factors which influence the failure pattern, including: the viscoelastic behaviour of the various parts of the interface, the effect of increasing the filler content of resin adhesive, the thickness of the resin layer and the effect of changing the modulus of elasticity of the restorative material. As in the micro-tensile testing method which has been reported, it would be possible to produce
different cavity designs and section the samples to enable the performance at various site in the cavity walls to be tested. Only one geometric configuration, speed of loading and blade design were used in this thesis. These variables all deserve further investigation.

Together the studies have produced new and effective methods of evaluating dentine adhesive interfaces, giving a better overall view of how these materials might function in clinical practice. The morphology studies can be considered as an initial screening, determining adhesive penetration. The micropermeability studies provide further information as to the seal of the restoration even in the absence of gap formation, and can indicate the response of the interface to setting stresses imparted by the overlying restoration. A further indication to performance is given by the response of the interface to applied load.

Already in vitro methods of assessing dentine adhesives described in this thesis have been used not only in the evaluation of existing dentine bonding systems, but also in the development of new materials. As so many dentine adhesive systems are available, it is not surprising that there is confusion amongst dental practitioners as to their relative merits and to the application techniques required. Materials are often released onto the market with little information, other than company advertising. To help practitioners and to reduce expensive clinical failures, it is important that rapid and realistic in vitro screening methods are available to assess dentine adhesive systems, when they are released and before the start of clinical trials. The screening methods also need to be able to compare the performance of new materials with those already in clinical use.

Adhesive systems are being developed which use one component - the 'single bottle' systems. Experience would suggest that attempting to prepare the dentine with one component would result in a reduction in performance. The techniques described in this thesis are now being used to assess the performance and influence the development of these systems.
References


Archs Oral Biol;37: 175-185


Watanabe I, Nakabayashi N, Pashley DH. (1994). Bonding to ground dentine by 

Watson TF. (1989). A confocal optical microscope study of the morphology of the 
tooth/restoration interface using Scotchbond 2 dentin adhesive. J Dent Res;68:124-
1131.

Watson TF. (1990). The application of real-time confocal microscopy to the study of 

Watson TF. (1994). Applications of high speed confocal imaging techniques in 


Watson TF and Boyde A. (1987b). The use of fluorescent markers for studying the 
distribution of a dentine bonding agent between a composite restoration and tooth. 
Clinical Materials;2:45-53.


EXACT NON-PARAMETRIC INFERENCE

The basis of statistical hypothesis testing is to summarise data in terms of a probability or "p-value" and then use this to either accept or reject a specified hypothesis, and traditionally the value of p, which determines acceptance, or rejection is 0.05. Conventional statistical testing is based on large sample theory that assumes specific underlying distributions resulting in an asymptotic p value. If these assumptions are not fulfilled, as in most real research, then the resulting p values are incorrect and incorrect decisions will be made. The situation is resolved by the use of "exact nonparametric inference". This uses permutational techniques to generate the exact distribution of the test statistic. R.A. Fisher suggested this approach to hypothesis testing in 1925, but its use in all but the simplest problems, was limited by computational difficulties. However this problem has now been eliminated due to the efficient algorithms developed by Mehta and implemented in StatXact 3 for Windows. Exact tests have been used where appropriate in this project.

Statistical tests used:

1. The Wilcoxon-Mann-Whitney test (also known as the Wilcoxon ranksum test) is one of the most popular two-sample tests. It is generally used to detect shift alternatives. This test has an Asymptotic Relative Efficiency of 95.5% compared to the student t-test when the underlying populations are normal. The Hodges-Lehmann procedure extends the Wilcoxon-Mann-Whitney test to provide a median unbiased point estimate and an exact confidence interval for the magnitude of the shift between two populations.

2. The Kolmogorov-Smirnov two-sample test is a test for the equality of two distributions against the general alternative that they are different. Because
this test attempts to detect any possible deviation from the null hypothesis, it is not as powerful as the Wilcoxon-Mann-Whitney test if the alternative is that one distribution is shifted with respect to the other. However, the one-sided forms of the Kolmogorov-Smirnov test can be specified, and these tests have good power against the one sided alternative that one distribution is stochastically larger than the other is.


1 StatXact 3 For Windows. CYTEL Software Corporation. 675 Massachusetts Avenue, Cambridge MA 02139, USA.
Publications resulting from the studies in this thesis


