MARGINAL ADAPTATION OF COMPOSITE RESIN RESTORATIONS *IN VITRO*

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I Coli, P., Blixt, M. & Brännström, M. (1993) The effect of cervical grooves on the contraction gap in class II composites. *Operative Dentistry* **18**: 33-36.

II Blixt, M. & Coli, P. (1993) The influence of lining techniques on the marginal seal of Class II composite resin restorations. *Quintessence International* **24**: 203-210.

III Coli, P. & Brännström, M. (1993) The marginal adaptation of four different bonding agents in Class II composite resin restorations applied in bulk or in two increments. *Quintessence International* **24**: 583-591.

IV Garberoglio, R., Coli, P. & Brännström, M. (1995) Contraction gaps in Class II restorations with self-cured and light-cured composite resins. *American Journal of Dentistry* **8**: 303-307.

V Coli, P., Derhami, K. & Brännström, M. (1997) In vitro marginal leakage around Class II composite resin restorations with glass-ceramic inserts. *Quintessence International* **28**: 755-760.

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VII Coli P. & Karlsson, S. (1999) In Vitro microleakage of Class II composite resin restorations after different dentin pretreatments. Submitted for publication.

ABSTRACT

Marginal adaptation of composite resin restorations in vitro

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The use of composite resin as a restorative material implies the risk of gap formation at the margins between the restoration and cavity walls, due to polymerisation contraction, particularly in restorations with the cervical wall in dentin/cementum. As a consequence, postoperative hypersensitivity, leakage of bacterial products, secondary caries, pulpal inflammation and necrosis may occur.
The objective of the present work was (i) to verify *in vitro* the hypothesis that some of the direct techniques available today can give optimal marginal adaptation and/or prevent microleakage in class II restorations; (ii) to test the hypothesis that dentin roughness is dependent on pretreatments and correlates to shear bond strength and microleakage; (iii) to study the influence of a hybrid layer, resin tags and interfacial contacts between the adhesive resin and the dentin on shear bond strength and microleakage.
Class II cavities with cervical margins in dentin/cementum were prepared with different cavity designs and dentin was pretreated in different ways. The restorations were performed with different combinations of liners, bonding agents and composite resins. Different application techniques of the composite resins were used and a post-restorative "rebonding" technique was also tested. The marginal adaptation was studied with disclosing solutions penetrating the marginal gaps between the restorative materials and the tooth tissues. Restoration sections were examined under a light microscope and replicas under a scanning microscope. Pretreated dentin surfaces were studied by scanning electron microscopy, atomic force microscopy and optical profilometry. Shear bond strength tests were also performed.
The findings demonstrated that cavity design may affect marginal adaptation since retention grooves at the cervical wall improved the marginal seal. Several bonding agents did not ensure a complete seal of the cervical margins in a predictable way, neither did the use of a glass-ionomer cement, whereas All Bond and All Bond 2 often resulted in a predictable good marginal adaptation. The use of liners did not reduce microleakage towards the pulp with the exception of a polystyrene-based one. Different application techniques of the composite resins did not affect the quality of marginal adaptation and the inclusion of glass ceramic inserts in the restoration did not reduce microleakage. The dentin roughness was partly dependent on surface pretreatment and correlated to shear bond strength, which appeared mainly to depend on the formation of interfacial contacts between adhesive resin and dentin, rather than on hybrid layer formation. Resin tags increased the bond resistance to shearing forces. None of the studied bonding mechanisms appeared to give improved marginal adaptation compared to the others. Hygroscopic expansion resulted in a significant improvement of the marginal seal and resin infiltration of the marginal gaps once the restorative material had set ("rebonding") resulted in good marginal adaptation. The results clearly showed the unpredictability of several materials and techniques. Thus, a cautious attitude to the use of composite resins should be adopted in view of the great variations in the marginal adaptation results within the same product.

Key words: adhesion, composite resin, dental materials, dentin, gap, marginal adaptation, microleakage, roughness

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To Maria and Vasco, my parents.
for giving me life and, more important,
for teaching me what is of worth in it.

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PREFACE

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INTRODUCTION

For almost a hundred years amalgam has been the most widely used material for posterior restorations. It has proved to be an inert material toward the pulp (Cox et al. 1987), insuring a long term good prognosis for the restoration (Roulet 1997).
Concerns regarding possible health-threatening properties and environmental problems related to amalgam, the need to remove large amounts of sound tooth structure to be removed for good cavity form and retention and the increasing esthetic demands in society strongly favour the use of alternative restorative materials.
The dental practitioner has nowadays the possibility to choose among a wide range of materials and techniques for posterior restorations.
Unfortunately, most of the techniques are more time consuming and therefore more expensive than amalgam restorations (Roulet 1997).
Among the direct restorative materials, the composite resins have become the most widely used as amalgam substitutes because of their good esthetics. Their use in anterior teeth goes back to the 50s, when Buonocore introduced the enamel-etch technique (Buonocore 1955), whereas their accepted use in posterior restorations is not older than 10-15 years.
In the following part a background of the chemistry of the composite resins and of the adhesion to tooth structures is given.

Chemistry of composite resin.

Composite resin consists of a mixture of three main components:
a) a resin matrix,
b) an inorganic filler and
c) a coupling agent: a silane responsible for the establishment of a chemical bond between the resin matrix and the inorganic filler.

The resin matrix often consists of diacrylates like the Bisphenol-glycidyl methacrylate resins (BisGMA) or urethane dimethacrylate resins (UDMA).
The polymerisation reaction of the matrix consists of the formation of monomer chains reacting with each others. The monomer relationship transforms from intermolecular distances of 0.3-0.4 nm into primary covalent bonds with lengths of about 1.5 nm (McMurry 1986). As a consequence, the distance of the chemical bond decreases and the resin shrinks linearly and volumetrically (Hedahal & Gjerdet 1977).
In order to improve the mechanical properties of the composite resins and to reduce the amount of polymerisation shrinkage, filler particles are added to the formulation.
There are three main types of fillers with regards to the size of the particles: macrofillers (glass, quartz, silicates) with a size of 0.1-100 microns, hybrids and microfillers (silica dioxide) with a size of 40-150 nm (Lutz et al. 1987).
An increased filler content improve the strength, the stiffness and the coefficient of thermal expansion of the composite resin (Peutzfeldt 1997).
To reduce the high viscosity of BisGMA in order to incorporate fillers, dimethacrylates of lower molecular weight like ethyleneglycol dimethacrylate (EGDMA) and triethyleneglycol dimethacrylate (TEGDMA) are often added to the formulation (Peutzfeldt 1997).
The lower the viscosity of the monomer mixture, the more filler may be incorporated into the mixture. There are, however, limits to the amount of filler that can be incorporated. These limits are due to clinical handling of the composite resins as well as the fact that by lowering the viscosity of the mixture the polymerisation shrinkage of the composite resin increases (Asmussen 1975).
Furthermore, Young's modulus of the composite resin increases with increase of the filler content, thus decreasing the flow of the composite resin during polymerisation.
An excessive increase of the filler content therefore reduces the possibility of relieving part of the contraction stresses by flow relaxation (Swift et al. 1995).
The currently available composite resins still shrink 1.5 to 7.1 % by volume during polymerisation (Davidson et al. 1984, Jensen & Chang 1985, Feilzer et al. 1988), generating shear and tensile stress up to 20-24 MPa (Feilzer et al. 1990).

Due to the polar nature of the monomers, the polymeric matrix absorbs water and undergoes hygroscopic expansion, thus reducing the stress induced in the composite resin and in the surrounding tooth structure by the polymerisation shrinkage (Asmussen & Jorgensen 1972).
On the other hand, hygroscopic expansion of the composite resins weakens the bonding between the filler and the matrix, with consequent loss of filler particles and therefore reduction of the mechanical properties of the composite resins as well as wear resistance (Söderholm 1981).

Adhesion of composite resin.

Adhesion principles
A basic concept of adhesion is that the adhesive liquid must come into close contact with the substrate to facilitate molecular attraction and allow either chemical adhesion or penetration for micromechanical attachment (Beech 1982). For such a close contact to occur, it is necessary that the liquid "wets" the surface well, that is a drop spreads across the surface rather than remaining beaded on the surface. The critical surface tension of wetting is a value, γ_c, for a solid surface, representing the surface tension of a liquid that allows a drop to spread across the surface so that the contact angle of the drop with the surface is zero. Liquids with surface tension less than γ_c will spread spontaneously, while those with greater surface tension will form contact angles greater than zero. For good "wetting", the surface tension of the adhesive liquid should be equal to or less than the critical surface tension for the solid substrate (Erickson 1992). Thus, to improve the wettability, the critical surface tension, γ_c, of the solid may be increased by pretreatment of the surface and/or the adhesive liquid should have low surface tension values or, in other words, have low viscosity.

Adhesion to enamel
The composite resins are well retained against the enamel thanks to the mechanical retention achieved by acid etching.
The adhesion to unetched enamel is poor since in the oral environment the enamel is covered by an organic pellicle which reduces the surface reactivity (Stanford 1985). Jendresen & Glantz (1981) reported a low critical surface tension of such enamel surfaces, around 28 dynes/cm, as well as of the cut enamel because of the presence of a smear layer. The etching of the surfaces with 40% H_3PO_4 transforms them to high-energy surfaces of 72 dynes/cm. The increase in surface energy as well as the increase in surface roughness and bonding area make the bonding of hydrophobic resins possible since they can wet and penetrate the microporosities of the dry etched enamel (Van Meerbeek et al. 1992a). The adhesion forces to enamel are greater than 20 Mpa and superior to the stress generated by composite shrinkage (Asmussen & Munksgaard 1985, Roulet 1997). For this reason, the composite resin shrinkage occurring during the polymerisation often does not lead to the detachment of the restorative material from the cavity walls surrounded by enamel.
The adhesion to dentin surfaces, dentin being a more variable and complex substrate than enamel, has been revealed to be much more complicated.

Dentin
Dentin forms the bulk of the tooth and consists of 70% inorganic material (hydroxyapatite), 20% organic material (type I collagen) and 10% water by weight. It is slightly harder than bone and softer than enamel (Ten Cate 1994).
Dentin is transversed in its entire thickness by closely packed dentinal tubules which extend from the enamel-dentin junction to the pulp with a diameter of 0.6-0.9 μm at the enamel-dentin junction and 2.5 μm near the pulp.
In the middle of the dentin there are approximately 30000 tubules/mm^2, whereas at the pulpal surface the number ranges from 59000 to 76000/ mm^2. For this reason, deeper dentin shows a reduced solid substrate compared to superficial areas as the portion of dentin represented by

tubule apertures increases (Brännström & Garberoglio 1972, Brännström et al. 1974, Thomas & Payne 1983, Olsson et al. 1993).
The dentinal tubules contain fluid which constantly flows from the pulp toward the outer surfaces, due to a pulpal pressure of about 30 mmHg (Beveridge & Brown 1965). This flow increases the wetness of the dentin surface.
Depending on the dentin portion considered, dentinal tubules may be differently oriented, ranging from cross-cut to parallel to the dentin surface; the degree of mineralisation of the dentin may be very different depending on the age and condition of the tooth (if decayed, abraded etc.) and the dentinal tubules may be widened as in case of acid attack, or completely obliterated because of sclerotic processes (Jokstad & Dahl 1996).

Smear layer
In addition to the dentin variables previously mentioned, bonding to dentin is further complicated by the presence of a smear layer produced whenever dentin is cut or ground.
The smear is a layer of debris which covers the normal structural components of the dentin and penetrates several micrometres into the tubules to form smear plugs. It is composed of a mixture of partly denaturated collagen and mineral (Gwinnett 1984).
Since the smear layer occludes the tubules, it greatly reduces the dentin permeability and therefore the communication between the pulp and the oral cavity (Pashley 1984).

Adhesion to dentin
The development of adhesives able to produce reliable bonding of composite resins to dentin has been a difficult challenge for the last 40 years.
Buonocore and coworkers published the first attempt to bond to dentin in 1956 (Buonocore et al. 1956). However, not until the early 80s could bonds of clinical relevance be obtained (Asmussen & Hansen 1993).
The concept behind this generation of dentin bonding agents was that components of the unfilled resins should react with inorganic or organic components of the dentin.
Ionic interactions between the dentinal calcium and bonding resin groups containing phospate, amino acid, amino alcohol or dicarboxylic acids were expected.
Similarly, chemical interactions between the organic portion of the dentin and isocyanate, aldehyde, acid chloride and carboxylic acid anhydride groups of the bonding agents were suggested (Asmussen & Hansen 1993).
The bond strengths obtained with such dentin bonding systems were quite low and the clinical performances relatively poor (Ziemecki et al. 1987, Tyas 1991).
One reason for these results is that these adhesives were bonding to the smear layer rather than to the dentin itself, therefore depending on the cohesive bonding of the smear layer or on the adhesion between the smear and the dentin surface (Swift et al. 1995).
As the smear layer inherent bond strength to dentin is estimated to be about 5 MPa (Pashley 1992), it is not surprising that the composite resin polymerisation contraction tended to pull off the bonded smear layer from the dentin surface.

In order to increase the possibility of chemical interactions of the adhesives with the dentin itself or to allow resin penetration into the underlying dentin, removal or modification of the smear layer was suggested (Munksgaard & Asmussen 1984, Erickson 1989, Joynt et al. 1991).
Another important reason for at least partial removal of the smear layer is that bacteria entrapped in the smear layer can survive and multiply under restorations, thus inducing pulp inflammation and necrosis (Brännström 1984).

The smear layer may be removed by means of acid solutions, whereas water or mild agents such as 3% and 30% hydrogen peroxide or 95% alcohol do not have any effect on the smear layer (Brännström & Johnson 1974).
It must be considered that with removal of the smear layer, the wetness of the dentin surface increases due to the fluid flow from the dentinal tubules. In fact, removal of the smear plugs increases the surface of the wet tubules by 10-25% (Garberoglio & Brännström 1976).
This increase in wetness tended to dilute the components of the bonding systems and reduced the possibilities of chemical bonds between dentin surface and unfilled resins as well as the possibilities of micromechanical retention for the hydrophopic resins used in earlier bonding

attempts (Nordenvall & Brännström 1980, Mitchem et al. 1988, Andraeus et al. 1988, Tao & Pashley 1989). Therefore, earlier generations of dentin bonding systems have not been able to establish a sufficient bond to the dentin and the results obtained have been quite unpredictable (Prati 1993, Swift et al. 1995).
The occurrence of a chemical bond between adhesive materials and dentin surface has not yet been corroborated by experimental data.

Hybrid layer
Most of the current dentin bonding systems are now based on acid etching of the enamel and dentin at the same time and infiltration of the tooth surfaces with highly hydrophilic resins.
Beside the removal of the smear layer and increase in dentin wetness, another effect of the use of acid solutions is decalcification of the solid dentin substrate.
The amount of dentin decalcification is dependent on the concentration, pH and time of application of the acid (Van Meerbeek et al. 1992b).
This process produces a layer of exposed collagen fibres deprived of hydroxyapatite crystals.
At this stage, once the acid solution is washed away, a primer based on amphiphilic monomers may be applied to the dentin surface and it penetrates the collagen meshwork.
The subsequent application of the adhesive resin produces a copolymerisation between the two resin components (primer and adhesive) and an intermingled layer of resin and collagen defined as the "hybrid layer", which was described in Japan in 1982 (Nakabayashi et al. 1982).
The formation of this layer has been confirmed in several studies and it has also been defined as the "resin-reinforced layer" (Nakabayashi 1992) or "resin-dentin interdiffusion zone" (Van Meerbeek et al. 1992b).
The formation of such a layer *in vivo* has also been described (Nakabayashi et al. 1992, 1995, Walshaw & McComb 1994).

It has been suggested that for proper formation of the hybrid layer it is necessary that the collagen does not become denatured during the process of dentin decalcification (Nakabayashi et al '91). For this reason, the use of a 10% citric acid, 3% ferric chloride solution for dentin pretreatment has been recommended (Nakabayashi et al. 1991).
On the other hand, several dentin bonding systems which use strong acid solutions for dentin demineralisation without trying to prevent collagen denaturation also produce a hybrid layer (Van Meerbeek et al. 1992b).

Several investigations have reported that the most important factor for achieving proper diffusion of the primers and adhesive resins into the demineralised collagen meshwork is the maintenance of a wet state of the collagen layer.
Titley and coworkers reported that conventional air-drying of the dentin results in the collapse of the collagen of the demineralised layer and suggested that the penetration of priming agents or resin monomers into this layer may be inhibited (Titley et al. 1994).
Similarly, Gwinnett demonstrated by observations with SEM (Scanning Electron Microscopy) and ESEM (Environmental SEM) that the collagen-rich layer of the demineralised dentin is collapsed and intimately associated with the dentin surface after air-drying, whereas when the dentin remains moist the fibrillar structure of the collagen is maintained (Gwinnett 1994a).
Tay and coworkers confirmed these observations by SEM and TEM (Transmission Electron Microscopy) and demonstrated that hybrid layer formation is strongly reduced in dry dentin compared to wet dentin if water-free primers are used (Tay et al. 1996a).
Several other investigators concluded that the dentin adhesive bond strength to dentin is higher when dentin is maintained blot-wet compared to air-dried (Kanca 1992a, Gwinnett 1992, Gwinnett & Kanca 1992a).
Although there are different ways of keeping the exposed collagen layer uncollapsed, there is agreement that for good primer infiltration it is necessary that the collagen layer maintains or regains its original dimension.
The concept of the collagen layer could be visualised as a sponge that can absorb liquid in the normal state but is almost impermeable when compressed or shrunken.

After acidic pretreatment, the dentin has hydrophobic characteristics (Attal et al. 1994), due to the high protein content (Van Meerbeek et al. 1992a) and its surface energy (or critical surface tension) drops (Erickson 1992).
In order to infiltrate and support the exposed collagen, different amphyphilic monomers are used. These monomers contain two functional groups: one is hydrophilic to interact with the moist dentin substrate, while the other is hydrophobic to interact with the adhesive resin. Such molecules are, for instance, 2-hydroxyethyl methacrylate (HEMA), 4-methacryloxyethyl trimellitate anhydride (4-META) and biphenyl dimethacrylate (BPDM) (Swift et al. 1995).These resin materials are often referred to as primers or adhesion promoters and improve the wettability of the dentin surface by increasing its surface energy (Erickson 1992) or by matching the surface tension of the adhesive resin to the surface tension of the dentin (Pashley & Carvalho 1997). These monomers are used in solutions of acetone, alcohol or water. Depending on the type of solvent, the primers may require different degrees of dentin wetness and may permeate the exposed collagen layer completely or only partially.
It has been reported that primers having acetone as a solvent need a wet dentin surface in order to have the acetone molecules chase the water molecules in the collagen layer, replace them and, by quickly evaporating, leave the space for the HEMA or BPDM molecules to take the places once occupied by water (Kanca 1992b, Jacobsen & Söderholm 1995, Nakabayashi 1996).
A dry dentin surface would not allow acetone penetration and the collagen infiltration by resin would therefore be very limited.
On the other hand, excess of water would also reduce the resin penetration because the acetone would compete with an overwhelming amount of water molecules to infiltrate the demineralised collagen layer.
Thus, the use of acetone as a solvent creates the need for a dentin surface not "too wet" and not "too dry" (Tay et al. 1996b, Gwinnett et al. 1996).
Other investigations demonstrated that primers with increasing amount of water as solvent increase the bond strength to dentin by restoring the shrunken demineralised collagen layer to its original structure and thus allowing for diffusion of the bonding agent (Nishiyama et al. 1995).
It has been suggested that because of this action of the water in the primer, the wet status of the dentin surface becomes less important for proper bonding and the use of bonding systems less technique-sensitive (Van Meerbeek et al. 1998).
However, it has also been reported that primers having water as a solvent give lower bond strength values than primers containing acetone as a solvent (Jacobsen & Söderholm 1995).
The complete diffusion of the primer/bonding resins into the demineralised collagen layer appears to be important since a discrepancy between the depth of decalcification and the depth of the resin infiltration would leave collagen fibres exposed to possible future degradation, hydrolysis and bonding failure (Pashley et al. 1992, Sano et al. 1994).

Porous, mineralised or partially demineralised dentin surface.
Although hybridisation of dentin is nowadays considered to be a major factor in dentin adhesion, some authors emphasise instead the importance of an irregular surface topography under the collagen-rich zone.
Gwinnett reported that only around one third of the shear bond strength to dentin is attributable to resin infiltration of the intertubular dentin matrix and resin tags in the dentinal tubules and demonstrated that elimination of the collagen layer does not affect the bond strength. According to his investigation, collagen should be kept wet or eliminated to facilitate diffusion of the primer along the solid dentin area, which would give two thirds of the bond strength (Gwinnett 1993).
It has also been reported that higher tensile and shear adhesive strength to dentin can be obtained by elimination of the collagen layer with NaOCl (Wakabayashy et al. 1994, Gwinnett 1994b).
The partially demineralised dentin layer underneath the exposed collagen fibres has been reported to differ significantly from dentin surfaces covered by a smear layer with regard to surface roughness parameters. The increase in surface area in the partially demineralised dentin layer, similar to the values registered for air-abraded dentin surfaces, has been suggested to be a factor contributing to the high shear bond strength reported (Gwinnett 1994c). According to Kinloch (1987), there may be distinct advantages in bonding to a rough surface, not necessarily

because of any mechanical interlocking mechanism but due to an improved degree of interfacial contact and more extensive energy dissipative deformations being initiated in the adhesive. Thus, chemical or mechanical dentin pretreatment that increases the roughness of the dentin surface could perhaps affect the adhesion strength of a bonding agent.

The elastic buffer function of the hybrid layer.

It has been suggested that even if the hybrid layer has no effect on shear bond strength, it is important for achieving good marginal adaptation of the restorations since it could act as an elastic buffer, counteracting the effect of composite resin polymerisation shrinkage (Van Meerbeek et al. 1993a, Pashley DH et al. 1993).

An increasing modulus of elasticity from the hybrid layer to the composite resin has been detected, suggesting some elastic properties of the hybrid layer (Van Meerbeek et al. 1993b).

Furthermore, better marginal adaptation of the composite resin to the cavity walls has been reported when a hybrid layer was created compared to restorations performed without hybrid layer formation (Uno & Finger 1995).

The role of the hybrid layer in dentin bonding.

The identification of the role of the hybrid layer in the marginal adaptation of composite resin to dentin is of clinical relevance.

The hybrid layer could represent only an obstacle to resin diffusion into the irregularities of the mineralised dentin surface and a weak point in the bonding formation because incomplete resin infiltration within the demineralised intertubular matrix results in a weak collagen-rich zone, susceptible to hydrolysis and consequent microleakage (Pashley et al. 1992, Sano et al. 1994). In fact, in vivo SEM studies revealed restoration detachment areas due to incomplete resin penetration of exposed collagen (Walshaw & McComb 1994, 1995).

In such a case, a dentin pretreatment technique which would prevent the exposure of a collagen layer or eliminate it should be used for proper dentin bonding.

Resin tags into the dentinal tubules.

The effect of a hybrid layer on dentin bonding has almost never been separated from a possible effect of micromechanical retentions produced by resin tags formed into the open dentinal tubules.

Resin tags have been detected in SEM and TEM studies, in vitro and in vivo, showing many lateral branches with hydrophilic systems which could be important for retention of the composite resin during polymerisation (Van Meerbeek et al. 1992b, Gwinnett and Kanca 1992b, Walshaw & McComb 1994, Gwinnett 1994b, Kanca & Gwinnett 1994, Chappell et al. 1994, Ferrari et al. 1994, Blair et al. 1995).

Several authors pointed out that resin tags were already detected with previous hydrophobic systems proved to be unsuccessful and that bond strength is lower in deep dentin, which presents more tubules and therefore a greater possibility of resin tag formation (Swift et al. 1995, Nakabayashi et al. 1991, Brännström & Nordenvall 1977, Tagami et al. 1990, Pashley EL et al. 1993, Olsson et al. 1993).

These observations are mostly valid for hydrophobic systems in which the tags are not intimately associated with the tubule walls and for which the lower bond strengths in deep dentin were disclosed.

Pashley et al. (1995) elaborated a model for bond strength versus dentin structure considering the relative contribution of resin tags, hybrid layer and surface adhesion to total bond strength to dentin. They suggested that in superficial dentin resin tags would contribute very little to bond strength whereas in deep dentin their contribution would be decisive. The opposite would be true for the contribution of a hybrid layer.

The contraction gap.

The above-discussed polymerisation shrinkage of the composite resin may result in detachment of the restorative material from the cavity walls of the preparation.

The consequent gap between the restoration and the tooth walls can easily become colonised by bacteria. which may induce pulpal inflammation and necrosis.

Growth of bacteria in gaps under composite resin as well as under silicate restorations has been demonstrated (Brännström & Nyborg 1971) and a clear correlation between presence of microorganisms and/or their products in the gaps and pulp reactions found (Brännström & Nyborg 1973, Qvist 1975, Bergenholtz 1977, Brännström et al. 1979).
The presence of a marginal gap may facilitate microleakage, that is, clinically undetectable infiltration of microorganisms, fluids, molecules and ions between the restoration and the tooth walls (Cox 1991).
Investigations have demonstrated that when bacterial microleakage is successfully avoided by a hermetic seal at the outer margins of a restoration, even in case of pulp exposure, the healing of the pulp is ensured (Bergenholtz et al. 1982, Cox et al. 1987, Cox 1991).
Presence of bacteria in the gap may also result in recurrent caries, which has been found to be the main reason for replacement of composite resin restorations (Mjör 1985, Van Dijken 1986).
The presence of gaps at the restoration margins is also said to be the reason for postoperative hypersensitivity. The marginal gap rapidly becomes filled with fluid either from the dentinal tubules or from the oral cavity. Rapid fluid movements into the dentinal tubules induced by cold, osmotic stimulation or by chewing produce activations of the Aδ nerve fibres and consequent sharp-pain sensation (Närhi 1985, Brännström 1986, 1992).

<u>The cervical wall of the cavity.</u>
Due to the mechanical retention achieved by the acid etching technique, the composite resin shrinkage occurring during the polymerisation often does not lead to the detachment of the restorative material from the cavity walls surrounded by enamel.
If a cavity wall is not surrounded by enamel, like class II and class V cavities with a cervical wall in dentin/cementum, the situation is different. In this region, a gap between the restorative material and the dentin may occur due to the polymerisation contraction. The occurrence of marginal gaps in the cervical area has been demonstrated in several studies in vitro and in vivo (Crim et al. 1985, Gross et al. 1985, Davila et al. 1988, Torstenson 1988).
A statistically significant correlation between the marginal adaptation at the cervical margin and the position of the margin has also been reported. When the cervical margin was placed one millimetre coronally to the cementum-enamel junction (CEJ), the restoration seal was perfect, whereas it deteriorated significantly when the cervical margin was placed 0.5 mm coronally to the CEJ, at the CEJ and 0.5 mm apically to the CEJ (Schuckar & Geurtsen 1997).
Thus, the cervical wall of class II and V cavities in dentin appears to be a weak point in the restoration.

Reduction of marginal gaps and microleakage towards the pulp.

<u>Improvements in the marginal adaptation.</u>
Several techniques have been suggested in order to improve the quality of the margins between the composite resin and the cavity walls.

The design of the cavity
Partial compensation for the stress at the bonding areas during the composite resin shrinkage due to the polymerisation process is given by the composite resin plastic deformation, or flow, which takes place at the same time (Davidson & De Gee 1984). The degree of plastic deformation is determined by the material supplied from the free, unbonded, outer surfaces of the restoration (Davidson 1986) and it has been demonstrated that the configuration of the cavity affects the setting stress. The lower the ratio of bonded to free composite resin surfaces, the more flow may compensate for such stress (Feilzer et al. 1987). Thus, it has been suggested that flatter and wedge-shaped cavities would be preferable to butt-joint cavities for improvement of marginal adaptation (Davidson et al. 1984, Asmussen & Munksgaard 1988).

Cervical grooves
It has been suggested that retention grooves at the cervical wall of class II composite restorations may reduce the cervical gap and leakage (Ben-Amar at al. 1988). Such grooves could perhaps also reduce the risk of fracture of the filling and increase the cervical surface,

minimising the effect of stress (thermal and mechanical trauma), which may result in creep and flow. In addition, such grooves in combination with lateral grooves and etched, bevelled enamel would offer more acceptable retention of the composite (Brännström et al. 1991).

The use of low/high viscosity composite
As previously discussed, the plastic deformation (flow) of the composite resin during setting reduces the shrinkage stress. This occurs mainly in composite resins with a low modulus of elasticity like the ones with low filler content. This type of composite shrinks more but on the other hand flow relieves the major part of the polymerisation stress. High-filled composites, with a higher elasticity modulus, compensate the contraction stress much less due to a reduced flow. Furthermore, it has been speculated that once the restoration is in function, the high-modulus, stiff composites cannot flex adequately when subjected to load and may transfer stress to the bonding interface much more than composite resins that have a lower modulus and are more easily deformated. Thus, it has been suggested that the lower the Young's modulus of a restorative resin, the better is the marginal integrity of the restoration (Van Meerbeek et al. 1992a).

Light-cured or self (chemically) cured
Whereas the use of light-cured composite resins has been suggested for the possible control of the setting direction toward the light sources (Lutz et al. 1986), there may be some advantages in using self-curing composite resins. The flow of the composites during setting is very much reduced for light-cured materials compared to chemically-cured ones since the curing rate of the latter is much lower. In other words, the light-cured materials reach their final stiffness much more rapidly than self-cured ones and therefore undergo much less plastic deformation and consequently do not compensate the stress generated by setting shrinkage at the bonding interfaces (Braem et al. 1987). Another advantage of the self-cured composite resins is their lower Young's modulus of elasticity compared to light-cured resins (Van Meerbeek et al. 1993b). This might be due to the fact that with self-cured materials, during mixing of the catalyst and the base pastes, oxygen becomes entrapped in the resin mixture, thereby inducing polymerisation inhibition and a lower degree of cure (Rueggeberg & Margeson 1990). It has been demonstrated that such voids in the self-cured materials not only reduce contraction stress because of polymerisation inhibition by oxygen, but also because they contribute to the amount of free surface and hence to the flow capacity of the material (Alster et al. 1992).
Another disadvantage of light-cured composites is that the polymerisation process of these materials is initiated at the restoration surface. Thus, this surface is eliminated as a source of flow for stress relief (Swift et al. 1995).

Application of the composite resin
The technique of application of the composite resin in the prepared cavity is considered to be very important. Application in bulk theoretically induces a higher stress in the bonded surfaces than application of the composite resin in several layers. This is due to the fact that the volume of polymerisation shrinkage is higher in the case of bulk application. In case of several layers, only the volumetric shrinkage of the last layer would negatively affect the marginal seal of the restoration (Kemp-Scholte & Davidson 1988). The results of several studies are contradictory, however. Some investigations comparing bulk and incremental applications of the composite resin report that reduced marginal gaps were obtained by a 2-step incremental filling technique (Hansen 1986, Crim 1991, Torstenson & Odèn 1989). Other studies have found no significant differences between the marginal adaptation achieved with the two techniques, or for several-layer application (Crim & Chapman 1986, Zidan et al. 1987, Torstenson & Odèn 1989, Eakle & Ito 1990).

Direction of the light application
The self-curing composite resins tend to shrink toward the centre of the mass, whereas light-cured ones shrink toward the light source. For this reason, it has been suggested that the direction of the composite resin setting contraction may be partly controlled by positioning of the light source (Lutz et al. 1986). A 3-step technique has been described for the restoration of class II cavities. The first layer should be applied at the bottom-third of the cervical wall and light-cured with the help of a light-wedge placed in the interproximal area, apically to the

restoration margins. Thus, the polymerisation shrinkage would be directed toward the cervical wall and not toward the occlusal region (Lutz et al. 1986).

Sandwich technique (self/light-cured)
To minimise contraction of composite restorations, it has been suggested that the use of a self-cured composite as the first increment could improve marginal adaptation (Bertolotti 1991, Fusayama 1992). According to this concept, chemically-cured composite will initially cure in the deepest area at the restoration/tooth interface. In the case of a dual cure adhesive, the chemical initiator will trigger and accelerate polymerisation of the chemically cured composite in contact with the adhesive. The composite curing will be directed toward the cavity wall and potentially be strong enough to counteract the tendency of the composite resin to shrink toward the centre of the mass. This curing "toward the tooth" would be enhanced by the tendency of chemically curing composites to start polymerising in the warmest area of the preparation, namely the tooth/restoration interface. According to Fusayama (1992), a deep cavity would be better restored with self-cured composite resin in the deeper portion to ensure adhesion to the cavity wall. The outer portion is then restored with light cured composite resin, for colour match and stability and to avoid air bubbles in the superficial layers of the restoration.

Resin inpregnation of the marginal gap
The routine practice of resin impregnation of potential gaps ("rebonding") has been recommended (Torstenson et al. 1985, Garcia-Godoy & Malone 1987). This technique entails the application of a self-cured bonding agent along the restoration margins once the composite material has set and the excess composite has been removed. In this way, any gap between the restoration and the tooth tissues could be filled with the resin penetrating by capillarity. This technique has been reported to result in gap-free restorations in most of the treated cases (Torstenson et al. 1985, Garcia-Godoy & Malone 1987).

Glass ceramic inserts
The weakest component of a composite resin is the resin that is used as a surrounding matrix to contain the filler particles. If the proportion of resin monomers could be reduced, the volumetric polymerisation shrinkage of the composites and hence the marginal failure of the restorations would be reduced (Jensen & Chan 1985, Freedman 1993). One possible solution would be to displace the composite material from the cavity by insertion of a large piece of inorganic reinforcement material (Bowen et al. 1993). Glass ceramic inserts of a material similar to composite resin fillers have been developed. By bulk replacement of the composite resin, the glass-ceramic inserts increase the filler-to-resin ratio of the restorative material (Freedman 1993). As these inserts also have superior physical properties to composite resins, the resultant restoration should be stronger and subject to less polymerisation shrinkage. In theory, the inserts should reduce the risk of marginal seal failure and also improve the durability of occlusal and interproximal composite resin restorations (Freedman 1993, Bowen & Setz 1986). Experimental evidence on the use of glass ceramic inserts for preventing cervical gap formation and microleakage is somewhat contradictory. Bowen (1987) found that glass inserts reduced microleakage in *in vitro* composite resin restorations. Donly et al. (1989) reported that glass inserts significantly reduced the amount of polymerisation shrinkage in MOD restorations *in vitro*. On the other hand, Gesi & Ferrari (1992), in an *in vitro* study, found no difference in microleakage between restorations with and without glass ceramic inserts. Tani et al (1993) reported that beta quartz ceramic inserts markedly reduced the polymerisation shrinkage of the composite resin but failed to reduce the stress generated by the polymerisation contraction forces. Degrange et al (1993) found less microleakage around composite resin restorations with glass-ceramic inserts than those without, but somewhat larger marginal gaps were detected.

Intermediate resins
It has been claimed that low-viscosity, low-modulus intermediate resins should be applied between the bonding agent and the restorative resin to act as an "elastic buffer" or "stress breaker" relieving contraction stresses. These stresses were found to be reduced by 20-50% by the application of such intermediate resins (Kemp-Scholte & Davidson 1990). Van Meerbeek et al. (1993b) reported a gradient of elasticity from the rather stiff dentin over a more elastic resin-dentin interdiffusion zone and adhesive resin layer to the restorative composite. The gradient

was more substantial in those systems that produced relatively thick adhesive resin layers or provided a filled low-viscosity resin as an intermediate layer between the adhesive resin and the bulk restorative composite.
Staninec & Kawakami (1993) reported higher shear bond strength and less microleakage for restorations performed with a low viscosity resin layer placed between the adhesive and the composite. Similarly, Fortin et al. (1994) found a higher bond strength for bonding systems using partially filled liners (such as Clearfil Liner Bond) as "elastic buffers" underneath the restorative resin.

Dentinal lining
An alternative solution to bonding to dentin has been the use of a liner between the composite resin and the cavity walls. In such a case, the liner should predictably seal the dentinal tubules to prevent infiltration of microorganisms or their products toward the pulp. The liner should not be expected to prevent marginal gap formation. In fact, the ideal liner should bind strongly to the dentin surface and weakly to the composite resin, so that once a marginal gap appears, this is located between the composite resin and the liner instead of between the liner and the dentinal surface (Brännström et al. 1989).
Glass Ionomer Cements (GICs) have been claimed to adhere to dentin due to their acidity, which etches the dentin, thereby producing mechanical retention as well as reacting chemically with hydroxyapatite and collagen (McLean et al. 1985). They shrink by about 4% of the volume and the contraction force is only 40% of that developed by composite resins (Feilzer et al. 1986). However, several investigators have detected microleakage between the dentin and various GICs under composite resin restorations (Shortall et al. 1988, Prati & Montanari 1989, Peutzfeldt & Asmussen 1989, Guelman et al. 1989). It is possible that the composite resin binds to the GIC and that, when it shrinks, it pulls off the GIC from the dentin.
Synthetic varnishes are also produced for lining purposes. Resins based on polytrifluorethylene, polyamides, calcium hydroxyde and polystyrene are available on the market. There is a possibility that varnishes bind strongly to the composite resins and are pulled off from the dentin during the polymerisation shrinkage, creating a marginal gap exposed to microorganism invasion. Brännström et al. (1983) found bacterial growth and pulp inflammation in 20 out of 24 composite resin fillings lined with Copalite resin, whereas in the same experiment, only one cavity lined with Tubulitec demonstrated pulp inflammation (Brännström et al. 1983).

Methods of studying dentin bonding effectiveness.

In vitro methods

Bond strength tests
A common way of evaluating adhesion to dentin by bonding materials is the use of adhesive tests. Often, tension or shear forces are applied until failures in the bonding occur. The amount of force needed is considered to be indicative of the bond strength.
Several different adhesion test apparatuses have been constructed both for tensile bond and for shear bond (Øilo & Olsson 1990, Fowler et al. 1992, Watanabe & Nakabayashi 1994). As a consequence, differences are found in the values obtained for the bond strength depending on different test apparatus designs (Fowler et al. 1992, Øilo 1993). Furthermore, Watanabe & Nakabayashi (1994) suggested that in tensile tests the production of tensile load perpendicular to dentin is often difficult and that in the case of shear tests the break-point does not always correspond to the weakest point.
Another method is the fracture toughness test, which has been suggested to provide appropriate assessment of the fracture resistance of the dentin-composite interface (Tam & Pilliar 1993).
Prati et al. (1992) pointed out that bond strength studies often use flat dentin surfaces as representative of the tooth surface to be bonded and therefore exclude the effects of interfacial stress generated by polymerisation contraction forces as well as the dentin structure with parallel, oblique or cross-cut tubules. In fact, these kinds of studies do not simulate clinical condititons (Retief 1991). Some investigators have also measured dentin bonding strength in three-dimensional cavities prepared in dentin (Stewart et al. 1990).

Studies based on adhesion tests can be indicative of the bonding strength ability of the studied materials and further examinations by light or scanning electron microscopy can reveal the mode and kind of fracture as well as features of the bonding-tooth interface (Øilo 1993, Øilo & Austrheim 1993, Tam & Pilliar 1994). However, their importance in predicting marginal adaptation of restorations or microleakage prevention is questioned.
Prati et al. (1992) studied the relationship between bond strength tests and microleakage measured in the same Class I restorations. The results demonstrated that there was an inverse relationship between dentin bond strength and microleakage for some bonding agents. Furthermore, the bond strength values obtained from flat dentin surfaces were much higher than the values obtained for three-dimensional cavities. On the other hand, Staninec & Kawakami (1993) reported a good correlation between early shear bond strength and microleakage.

Examination of the marginal seal.
The marginal seal of restorations can be studied by different techniques. The most common are the direct measurements of any gap between the restorative material and the tooth structure or the use of microleakage methods.
Cavity margins have been evaluated by means of light or scanning electron microscopy (Taylor & Lynch 1993). The use of SEM requires drying of the specimens. Therefore, the risk of artefactual gaps is high and the use of replicas is suggested (Davila et al. 1988).
Although this technique has some disadvantages, such as being time-consuming, SEM is widely used for marginal gap detection (Gwinnett & Kanca 1992b, Krejci et al. 1993, Abdalla & Davidson 1993). For longitudinal studies, epoxy replicas of the cavity margins may be taken and analysed for detection of marginal gaps (Blunck & Roulet 1989, Roulet et al. 1991). However by this technique only the cavosurface margins can be evaluated and no information about the internal adaptation of the restoration is obtained.
Brännström et al. (1984) and Torstenson & Brännström (1988) suggested the use of another technique for the evaluation of marginal adaptation. After the setting of the composite resin and the removal of the excess composite, a few drops of a self-curing bonding agent to which a fluorescent dye has been added are applied along the cavity margins. This allows penetration of the fluorescent resin (FEB) into any gap by capillarity. After the setting of the disclosing resin, it is possible to section the restorations without risk of alterations in the gap location and dimensions. Measurements of the microphotographs obtained on UV light microscopy allow the evaluation of the exact width and length of the marginal failures.
Marginal adaptation can also be studied by a microleakage test. This is a common technique by which the restored teeth are immersed in an infiltrative agent and later sectioned through the restoration and analysed for dye penetration.
Several dye solutions have been used in the literature, the most common being methylene blue solution 1% (al-Ajam & McGregor 1993), 2% (Mandras et al. 1993) or 5% (Sparks et al.1992), basic fuchsin solution (Retief et al. 1992), 5% eosin solution (Youngson 1992) and silver nitrate solution (Sorensen et al. 1991). Radioisotope diffusion has also been used (Shahani & Menezes 1992). Most frequently, the dye penetration is used as an indicator of presence of gaps between the restoration/tooth interface and a linear assessment of the penetration extension is performed. However, the use of a dye solution can also allow the evaluation of the dentin permeability if the dye penetration into the tubules is scored.
The penetration into marginal gaps is dependent on the molecular size of the disclosing solution, the molecular polarity, the interaction between the dye and the restorative material, capillarity and time (Roulet 1994).
Criticism has been raised against this method due to the fact that acidic dyes such as eosin should be buffered in order to avoid the tooth structure becoming demineralised during the immersion period, allowing increased dye uptake (Youngson 1992). Furthermore, it has been suggested that retention of this dye could be related to the concentration of protein within the dentin rather than to the microleakage potential of the restorative material (Youngson 1992). The same has been suggested for methylene blue, which will stain glycosaminoglycans in the dentin (Farndale et al. 1986).
Some authors have assumed that gap size is positively correlated to microleakage values (Irie et al. 1990). On the other hand, it has recently been shown that even restorations in which no marginal gaps could be detected by SEM can present some leakage. This leakage has been

defined as a nanoleakage since the ions of the disclosing solution penetrated through the demineralised collagen fibres not infiltrated by the adhesive resins (Sano et al. 1994, 1995).
The interpretation of the microleakage data may be difficult. The smallest dye particles measure around 120 nm and the smaller isotope molecules 40 nm, while bacteria are of a size of 0.5-1 µm or larger (Taylor & Lynch 1992). Thus, it might be argued that the use of dye solution or isotope for the evaluation of the restoration margins is too severe since bacteria would not be able to penetrate gaps disclosed by such techniques. However, bacterial products could possibly leak through such spaces and induce pulp inflammation.
The significance of this kind of correlation is considered to be uncertain since there has been no in vivo validation of the in vitro investigations (Retief 1994), although there are reports of similar results obtained in vitro and in vivo with regards to marginal gap detection (Gwinnett & Kanca 1992b, Kanca & Gwinnett 1994) or bond strength (Nakabayashi et al. 1995).

Surface analysis techniques

Instruments for the analysis of surfaces are usually used for homogeneous solids that are not altered by high vacuum. This is not the case with a heterogeneous material such as dentin. The instruments for surface analysis are generally based on an electron or photon beam excitation of the studied surface. The electrons and/or ions which are released from the collision of the beam with the surface are detected and images of the surfaces can be reproduced or the chemical composition of the surface obtained. Auger spectroscopy (Eick et al. 1996), scanning electron microscopy (Van Meerbeek et al. 1993a), transmission electron microscopy (Van Meerbeek et al. 1993a), Fourier transform-infrared spectroscopy (Spencer et al. 1992), Raman spectroscopy (Suzuki et al. 1991), X-ray photoelectron spectroscopy (XPS, chemical analysis)(Eliades & Palaghias 1992) and environmental SEM (Gwinnett 1994a) have been used.

Surface roughness analysis (quantitative data)

Contact profilometry.

The principle for contact stylus instruments is that a pick-up with a stylus traverses over the surface at a constant velocity. A load applied to the stylus keeps the stylus tip in contact with the surface while the vertical movements of the tip are converted to electrical signals and later into digital information or profile lines on chart records (Wennerberg 1996).

Non-contact, optical profilometry.

Several optical profilometers are available today. The principle is that a light beam is used as an optical stylus which is moved over the surface. The light reflected from the surface is collected by a photodetector. Compared to contact methods, the optical profilometers are non-destructive, do not have problems such as wear of the stylus tip, are able to detect smaller structures and are faster. Among these methods, there are interferometry systems, autofocus detection systems and confocal laser scanning microscopy systems. The latter allow high resolution and accuracy also in the vertical plane, giving the possibility of 3D visualisation of biological specimens. This is due to the presence of two blocking filters (pinholes) which remove the light not reflected from the focal plane since the reflected light passes through the pinhole only when the surface is in focus and in this way the out-of-focus contributions to the image are suppressed (Wennerberg 1996). A possible disadvantage of the application of optical profilometry to the analysis of dentin is that the surface analysis must be performed in air with consequent drying of the specimens.

Contact, non-contact electron microscopy.

Instead of mechanical styli or light waves, electrons can be used to produce images of the surfaces. The electron microscopic methods previously mentioned in connection with the surface analysis technique may be used for qualitative description of the surface roughness. For a qualitative as well quantitative description, Atomic Force Microscopy may be used. This instrument measures repulsive forces between a flexible cantilever with a silicon nitride tip and the studied surface. The cantilever deflection produced by the interatomic forces between the tip and the surface is monitored by a laser beam that sends a signal detected by a photodiode (Wennerberg 1996). Atomic Force Microscopy can be used in both contact and non-contact mode and makes it possible to analyse the dentin under moist conditions. This technique has not been widely used yet for dentin examination and only a few studies have been published with AFM observations (Igarashi & Nakabayashi 1996, Kinney et al. 1996, Silikas et al. 1999).

In vivo methods

Considering the limitations associated with in vitro methods, it could be better to perform clinical investigations. Clinical investigations, however, present diagnostic problems. The evaluation of presence/absence of marginal gaps and overhangs by an explorer varies markedly among examiners (Dedmon 1982) and the same disagreement occurs in the evaluation of needs for restoration replacement due to secondary caries (Merret & Elderton 1984). Leinfelder et al. (1982) demonstrated that the smallest marginal ledge that can be clinically detected by a probe is about 0.1 mm. Removal of restorations after diagnosis also reveals the poor correlation between the actual findings and the established diagnoses (Söderholm et al. 1989). Furthermore, clinical signs like pain and percussion and thermal tests are quite insensitive to small differences between techniques and materials. Thus, even though in vitro data are uncertain, in vitro investigations are important for the development of the materials since general clinical use of dental materials is unacceptable as a testing tool (Roulet 1994).

GENERAL AIMS.

The main objective of the present work was to verify *in vitro* the hypothesis that some of the direct techniques available today can give optimal marginal adaptation and prevent microleakage in class II composite resin restorations with cervical margins below the cementum-enamel junction (I-V, VII) .

Other objectives were to test the hypothesis that dentin surface roughness is dependent on dentin pretreatments and correlates to shear bond strength (VI) and to microleakage (VII). Further, to study the influence of a hybrid layer, resin tags and interfacial contacts between the adhesive resins and the dentin surface on shear bond strength (VI) and on microleakage (VII).

MATERIAL AND METHODS

Cavity preparation.

For the investigations, intact human premolars extracted for orthodontic reasons and kept frozen (-8 °C) (I-V) or in deionised water at 6 °C (VII) for not longer than 6 months were used. Intact human third molars stored in deionised water at 6 °C for not longer than 6 months were also used (VI).

For the marginal adaptation/microleakage studies (I-V, VII), class II cavities were prepared on the approximal surfaces of the teeth. Each approximal box was 3-4 mm wide and 1.5-2 mm deep. The cervical wall was located just below the cemento-enamel junction. The cavity was prepared with a fissure bur at high speed, under water cooling. A small round bur at low speed was used to cut retention grooves at the axioproximal line angles. The enamel on the lateral and occlusal walls was bevelled with a diamond point used at low speed (I, VII). At the cervical wall, no grooves (I, VII), or a single groove made with a round bur at low speed (I), or double retention grooves made with a notched chisel (I-V) (Dental Therapeutics, Nacka, Sweden) were made.

Cavity restoration.

The dentin bonding agents, the liners and the composite resins used thoughout the studies are listed in Table 1.

The composite resins were applied in bulk (I-V) or in two increments (III-V, VII).

Once the composite resin had set, the excess restorative material was removed with hand instruments.

Marginal adaptation and microleakage analysis.

In order to investigate the quality of marginal adaptation and the dentin protection achieved, methods allowing direct evaluation of the gap size and methods detecting patency of dentinal tubules were used: FEB (I-III) and toluidine blue solution (II-V, VII) penetration extent was analysed by light microscopy; restoration replicas were analysed by SEM (IV).

FEB

After the setting of the composite resin and the removal of the excess composite, a few drops of a self-curing bonding agent (Enamel Bond, 3M) to which a fluorescent dye (Zyglo Penetrant ZL-22A Magnuflux Corp, Chicago, IL, USA) had been added were applied along the cavity margins. This allows penetration of the fluorescent resin (FEB) into any gap by capillarity. The teeth were later ground longitudinally from lingual to buccal, perpendicular to the restorations and the surface polished under water cooling. Measurements of the microphotographs obtained at UV light microscopy (Ortoplan Leitz, Wetzlar, Germany) allowed the evaluation of the exact width and length of the marginal failures.

Toluidine blue solution

After the restorations were completed, the apical part of the root was removed with a diamond disc and a cavity was prepared on the root canal using a round bur at low speed. The deepest part of the cavity was filled with Coltosol temporary cement (Coltene, Alstatten, Switzerland), over which the bonding and the composite resins were applied and cured. Finally, a layer of nail varnish was brushed onto the entire tooth surface, except for a one millimetre area nearest the composite resin filling. The teeth were then immersed in a solution of 0.5% toluidine blue for 20-22 hours at 37 °C. The teeth were later ground longitudinally from lingual to buccal, perpendicular to the restorations (II, III) or sectioned mesio-distally using an Exakt Cutting Grinding System (Exakt Apparatebau, Norderstedt, Germany) (IV, V, VII). Three or four sections were obtained from each tooth. The sections were manually polished using waterproof papers (grain size 1000), mounted on a glass plate, examined and photographed under UV (Ortoplan) or a light microscope (StereoZoom, Bausch & Lomb, New York, USA). Of the 3-4 sections, the deepest penetration observed was scored to represent the result for the restoration. Different microleakage scores were used in the investigations.
Presence or absence of dye solution in the dentinal tubules was also recorded.

Exclusion criteria

Restorations were excluded if excess composite resin material was seen at the margins, possibly preventing penetration of the FEB (or in some cases of the dye).

Replica analysis by SEM

The specimens were treated for 30 s with 3% EDTA (Tubulicid Plus, Dental Therapeutics) and rinsed with 1% NaOCl for few seconds to remove the smear layer from the ground surface. The specimens were dried for 10 s by an air-blast, a surface-tension-reducing agent was applied to the specimens and an impression in light body addition silicone impression material was taken. The impression was poured in epoxy resin. The replica was removed from the impression, washed with distilled water, dried and vacuum coated with a 15 nm-thick gold layer. The replica was later processed by SEM (Autoscan, Siemens, Erlanger, Germany).

<u>Shear bond strength test.</u>

For the shear bond strength study (VI), tooth slices from different tooth areas were obtained from third molars and embedded in epoxy resin. After different dentin pretreatments, the surface area to be bonded was delimited by a paraffin paper fitted to a teflon mould with a 2 mm diameter and 3 mm thickness. The specimen preparation followed the recommendation of the ISO/TR 11405 (1994) for the application of the bonding agent and for the composite resin material to fit the split mould. The bonding system was applied according to the manufacturer's instructions and the composite resin was applied in bulk to fit the mould and light-cured. Directly after curing, the specimens were removed from the apparatus and immersed in water at 37°C for 24 h. After this, the specimens were positioned in the loading rig of a Lloyd LRX (Lloyd Instruments, Fareham, UK) and sheared until failure with a cross-head speed of 0.7 mm/minute.

<u>Analysis of the dentin surfaces.</u>

In study VI the pretreated dentin surfaces were analysed by SEM, Topscan 3D and AFM.

Qualitative assessment
The tooth slices were impregnated with osmium tetroxide according to the OTOTO technique; dehydrated in progressively increasing concentrations of ethanol, embedded in T-butanol and dried in a vacuum chamber. The specimens were later bonded to SEM stubs with conductive silver, coated with a 15 nm-thick gold layer using an Emitech K550 apparatus (Emitech, Ashford, England) and processed by SEM (DSM 982 Gemini; Zeiss, Berlin, Germany).

Surface roughness.
To investigate the dentin surfaces at different resolution levels, two instruments were used.
TopScan 3D (Heidelberg Instruments, Heidelberg, Germany): an optical profilometer using a small laser spot as an optical stylus. The system employs the method of confocal laser scanning microscopy, which results in a high resolution in the optical Z direction (height). The laser scanning is achieved by moving the laser spot along three axes. The 3D image of the surface is reassembled from the data by a computer (Wennerberg et al. 1992).
For numerical description, four different roughness parameters were used:

- S_a: the arithmetic mean of the departures of the roughness profile from the mean plane, measured in µm. This parameter gives a good general description of height variations but it is insensitive to wavelength and occasional high peaks and low troughs.
- S_{sk}: a measure of the symmetry of the profile about the mean plane. It describes the shape of the height distribution. Equal numbers of peaks and troughs have zero skewness. Profiles with more peaks than troughs have a positive skewness; profiles with peaks removed or deep scratches have a negative skewness.
- S_{cx} represents the average mean spacing of profile peaks at the mean plane in the X direction. It is a space descriptive parameter.
- S_{dr} represents the developed surface area ratio. This is the ratio of the developed surface area to the sampling area. This parameter includes information from height as well as space.

The size of the measured area was for all measurements 250 X 250 µm. The resolution in depth was 20 nm. The grid size in the X direction was 1 µm while that in the Y direction was 3 µm.

Atomic Force Microscope (AFM) (Dimension 3000 SPM, Digital Instruments, Santa Barbara, California, USA): a profilometer which measures the interaction between a sample and a tip attached to a flexible cantilever. The interatomic forces between the tip and the surface deflect the cantilever. The deflection is monitored by a laser beam that sends a signal detected by a photodiode as the cantilever is moved (Wiesendanger 1994). Under optimal measuring conditions, the AFM has a possible horizontal resolution of about 10 pm and a vertical resolution of 1 pm. Only the roughness parameter S_a was used. Measurements were made in a 10x10 µm area and in a 1x1 µm area of the dentin surfaces. The measurements were performed in a tapping mode in saline solution. All measurements used 256x256 pixels, that is a distance of 0.039 µm between two scans in the case of a 10x10 µm measuring area, or a distance of 0.0039 µm between two scans when the measuring area was 1x1 µm.

Statistical Analyses
For the analysis of the results, the statistical methods used were: the ANOVA test (I, VI, VII) the Chi-square test (I), the Fisher's exact test (IV, V) and the Student-Newman-Keuls test (VI). The results in Studies II and III were not statistically analysed at the time of publication. However, the statistical analysis (Chi-Square and Student-Newman-Keuls) for Studies II and III is presented in the results section.

STUDY I

The specific aim of the study was to test the hypothesis that the use of cervical retention grooves can reduce the extent and width of cervical gaps in Class II composite resin restorations.

Materials and Methods: 30 human intact premolars teeth stored frozen until use, in which 60 class II cavities were prepared. In 20 cavities two cervical grooves were made with a special notched chisel (Dental Therapeutics), in 20 only one cervical groove was made with a round bur at low speed and in 20 cavities no grooves were made. Scotchbond 2 and P50 (3M Dental Products) were used as restorative materials. The presence of gaps was disclosed by FEB.

STUDY II

The specific aim was to test the hypothesis that the use of a bonding agent, Scotchbond 2, in combination with one of five different thin liners prevents microleakage toward the pulp. Another aim was to test the hypothesis that the technique of "resin infiltration of the gap" (or "rebonding") results in microleakage prevention. In another series of experiments, the hypothesis tested was that the removal of the superficial smear layer only with an EDTA-containing detergent, Tubulicid Red Label (Dental Therapeutics), does not negatively affect the marginal adaptation obtained with a glass-ionomer liner (Vitrebond). Another part of the study tested the hypothesis that limiting application of the bonding agent to the bevelled and etched enamel only, in cavities treated with liners, does not affect the microleakage results compared to application of the bonding to the entire cavity.

Material and Methods: 203 class II restorations were made in intact premolar teeth, stored frozen, using either a glass-ionomer cement (Vitrebond) or four other liners (Thermoline, Hydroxyline, Barrier Dentin Sealant, Tubulitec). Scotchbond 2 was applied to the whole cavity or only to the bevelled and etched enamel. P-50 was applied in bulk.

To study not only the number of gaps, but also their width, the fluorescent resin-penetrating technique (FEB) was applied. In order to evaluate if dentin impermeability was achieved by the different liners, some of the teeth were immersed for 20-24 hours in a 0.5% toluidine blue solution.

STUDY III

The specific aim was to test the hypothesis that contraction gaps and microleakage can be prevented or reduced by the use of one of the bonding systems All Bond, Superbond D Liner, Superbond C&B and Tokuso Light Bond, which use different dentin pretreatments. Another aim was to test the hypothesis that the application of a composite resin in two increments with a metal matrix band produces similar marginal adaptation to bulk application of the composite with a plastic matrix strip.

Materials and Methods: 80 human intact premolar teeth were stored frozen until use. One hundred and sixty restorations were made in class II cavities. The four different bonding agents were applied in 40 cavities each. Half of the restorations were performed by bulk application of the composite resin using a plastic matrix strip and half by two horizontal increments using a metal matrix band. Presence of marginal contraction gaps, their width and extent was disclosed by the FEB technique, whereas the patency of tubules was disclosed by the 0.5% toluidine solution technique.

STUDY IV

The specific aim was to test the hypothesis that prevention of marginal gap formation can be achieved by the use of a self-cured composite as a first increment followed by the application of a light-cured composite as second increment. Another aim was to test the hypothesis that the amount of gaps along the restoration margins may be reduced by hygroscopic expansion of the restorative material.

Materials and methods: 20 human intact premolar teeth, stored frozen, were employed and 40 class II restorations were produced.

Two different bonding agents (All Bond 2 and Superbond D Liner) were compared in combination with two self-cured composites (Bisfil 2B and Palfique Self-Cured) applied as first increments to the cavities. As the second increment for all the restorations, the same light-cured composite (Palfique Estelite) was used.

The dye penetration technique was used for 24 hours directly after the placement of the filling, and SEM was used to study the condition of the interface and the location of any gap in the same specimen after storage in water for 1-4 months, considering the possibility of hygroscopic expansion.

STUDY V

The Specific aim of this study was to test the hypothesis that inclusion of glass ceramic inserts (Beta Quartz Glass-Ceramic inserts, Lee Pharmaceuticals, South El Monte, CA, USA) in the composite resin applied in class II cavities can improve the marginal seal of restorations with cervical margins in dentin.

Materials and Methods: 40 intact premolar teeth were stored frozen until use. Eighty class II cavities were prepared with both approximal surfaces of each tooth being restored. Restorations with and without Megafill inserts in combination with either All Bond 2 or Tokuso Light Bond bonding agents and the light-cured composite Palfique Estelite were made. The teeth were divided into four groups, each group comprising twenty cavities (All Bond 2/Palfique Estelite; All Bond 2/Palfique Estelite/Glass Ceramic inserts; Tokuso Light Bond/Palfique Estelite; Tokuso Light Bond/Palfique Estelite/Glass Ceramic Insert).

The teeth were immersed in a solution of 0.5% toluidine blue for 24 hours at 37 °C and then analysed for presence of gaps and penetration of dye into dentinal tubules.

STUDY VI

The specific aim was to define the morphology and roughness of dentin from different tooth areas after various pretreatments to test the hypothesis that dentin surface roughness is dependent on dentin pretreatments and correlates to shear bond strength as well as to study the influence of a hybrid layer, resin tags and interfacial contacts between the adhesive resins and the dentin surface on shear bond strength.

Material and Methods: 38 extracted third molars, stored in deionised water, were used, each providing two sections of cervical (c) and lateral (l) dentin. Five pretreatments were performed: A) application of a 0.2% EDTA solution, B) abrasion with Al_2O_3 particles followed by 0.2% EDTA , C) 10% H_3PO_4, D) 10% H_3PO_4 and immersion in a collagenase solution for 1 h, E) control: no treatment. Z100 composite resin cylinders were bonded to the specimens with All Bond 2 bonding resin and tested for shear bond strength. Twelve other specimens from each group were analysed for surface roughness with an optical profilometer (3D Topscan) at the µm level and an Atomic Force Microscope at the nm level. Four of these specimens were further examined by SEM.

STUDY VII

The specific aim was to test the hypothesis that a dentin pretreatment resulting in exposure of a demineralised collagen layer reduces microleakage compared to pretreatments which either eliminate the demineralised collagen layer or do not induce dentin demineralisation, or increase the roughness of the dentin surface.

Material and Methods: 30 premolar teeth stored in deionised water until use in which 60 class II cavities were made with the cervical wall just below the cemento-enamel junction were used. The cavities were restored with All Bond 2 and Spectrum composite resin. The same 5 dentin pretreatments used in Study VI were applied. Directly after restoration, the teeth were immersed in 0.5% toluidine blue solution for 24 h. The extent of dye penetration along the tooth/restoration interface was measured under a light microscope.

RESULTS

Due to the different extent of penetration of the FEB or the toluidine blue solution in the different studies, different scores were used. For the sake of simplicity, in the general review of the results, the scores of each study are transformed to a common score scale for all the investigations: 0= no penetration, 1= penetration of the FEB or the dye not further than the middle of the cervical wall, 2= penetration until the corner between the cervical and the axial wall, 3= penetration along the whole cervical wall and extending along the axial wall (Fig 1).

Effect of retention grooves on marginal gaps (I).
The use of one or two retention grooves did not reduce the amount of restorations with marginal gaps compared to restorations performed without retention grooves (score 0, Fig. 2). Neither did it affect the width of marginal gaps, since no difference between the groups was detected by ANOVA: the mean marginal gap width for restorations performed without grooves was 7.3 µm with a range 0-14; for the cavities with one groove, the mean was 8.4, range 0-14; for the two-grooves cavities the mean was 5.8, range 0-18.
The use of retention grooves, however, clearly reduced the extent of the marginal gaps. The Chi-square test indicated a significant difference between the no-retention-grooves and 1 or 2 retention-groove groups as well as between the 1 and 2 retention-groove groups for the penetration scores.

Effect of dentinal liners on microleakage towards the pulp (II)
The formation of marginal gaps along the margins of the restorations was disclosed by the toluidine blue solution in most of the restorations performed without the "resin infiltration" (rebonding) technique. Thermoline, Hydroxyline and Barrier Dentin Sealant failed in 100% of the cases to prevent microleakage into the dentinal tubules, which occurred in 60% of the restorations treated with Vitrebond. The penetration into the dentinal tubules of the dye solution was limited to 11% of the restorations lined with Tubulitec (Fig. 3). No penetration into the tubules was detected for the restorations treated with the "rebonding" technique, which clearly prevented microleakage along the margins of the restorations in 90% of the cases studied with the dye solution. The Chi-Square test revealed a significant difference in the overall comparison of the treatment groups for microleakage towards the pulp ($p<0.001$).
No difference could be detected regarding dye penetration along the margins of the restorations or into the dentinal tubules whether Scotchbond 2 was applied only to the etched enamel or to the entire cavity for the groups treated with Thermoline and Tubulitec.

Effect of Scotchbond 2, Vitrebond and the "rebonding" technique on marginal gaps (II)
Again, in 90% of the cases treated with the "rebonding" or "resin infiltration" technique the formation of a marginal gap was avoided, as detected by the FEB technique (Fig. 4). In almost 80% of the restorations treated with Scotchbond 2 alone, marginal gaps were instead detected. The restorations lined with Vitrebond did not show any difference depending on the dentinal pretreatment with water or with an EDTA solution, since the rate of restorations with marginal gaps was very similar for the Vitrebond groups, being 40-45%. Thus, the removal of the superficial smear layer by use of an EDTA-solution did not affect the marginal adaptation obtained with Vitrebond.
The Chi-Square test revealed a significant difference for the overall comparison of the restorative groups for dye penetration ($p=0.008$).

Effect of All Bond, Superbond D-Liner, Superbond C&B and Tokuso Light Bond on marginal adaptation and microleakage (III)
Almost 90% of the cavities restored with Superbond D-Liner and 70% of those restored with All Bond did not show any FEB or toluidine blue penetration (Fig. 5). The corresponding figures for Superbond C&B and Tokuso Light Bond were approximately 40% and 10%. Only Tokuso Light Bond showed a high number of restorations (about 50%) with penetration along the whole cervical and axial wall (score 3). The Chi-Square test revealed a statistically significant difference ($p=0.0001$) for the overall comparison of the bonding agents. This difference was further analysed with the Student-Newman-Keuls test, which demonstrated a significant difference between bonding agents with the following ranking: SDL, ALB>SCB>TLB.
The width of the marginal gaps disclosed by FEB is presented in Table 2. Apparently, once a marginal gap occurred, its width did not differ much between the treatment groups. However, no statistical analysis of the data was performed, due to the low number of observations in some groups.
The dye penetration test revealed that in almost 90% of the restorations performed with Tokuso Light Bond, the dentinal tubules were patent, suggesting poor adhesion of the bonding system

to the dentin (Fig. 6), since the marginal gaps were located between the dentin and the adhesive. This was also the case for 40% of the restorations performed with Superbond C&B, 20% and 5% respectively for All Bond and Superbond D-Liner restorations. The Chi-Square test revealed a significant difference ($p<0.001$) for the overall comparison of the bonding agents for presence of dye into the dentinal tubules.

Effect of composite resin application technique (bulk or increments) (III)
No differences in marginal adaptation scores were detected by the Chi-Square test ($p=0.8278$) between restorations performed with application of the composite resin in bulk with help of a metal matrix band and in two increments using a plastic matrix strip (Fig. 7). This was true for all the four bonding agents tested.

Effect of a "sandwich" technique (self+light-cured increments)(IV)
All the restorations performed with the combination All Bond/Bisfil 2B showed a good marginal adaptation (Fig. 8). Marginal gaps were also avoided in 70% of the restorations performed with the All Bond/Palfique-self-cured combination. No significant difference between the two All Bond-treated groups was detected. Only 20% of the restorations performed with the combination Superbond D-Liner/Palfique-self-cured were gap-free, while marginal gaps were detected in all the Superbond D-Liner/Bisfil 2B restorations. No statistically significant difference was found between the two Superbond D-Liner-treated groups, whereas Fisher's exact test demonstrated a significant difference between the All Bond and the Superbond D-Liner-treated groups ($p<0.001$). An open communication from the cavosurface margins to the pulp, through patent dentinal tubules, was detected only for Superbond D-Liner/Bisfil 2B-restorations in 40% of the cases.

Effect of hygroscopic expansion (IV)
The SEM evaluation of the restorations after 1-4 months' storage in water revealed that only a few cavities (12%) still showed a marginal gap at the cavosurface margins (Fig. 9). Six more restorations (15%) showed marginal gaps located internally to the cavosurface margins. No statistically significant difference could be detected between the four treatment groups. Thus, the hygroscopic expansion of the composite resin apparently resulted in an improvement of the marginal adaptation and the initial differences between treatment groups disappeared with time.

Effect of Glass Ceramic Inserts (Megafill) (V)
All the restorations performed with All Bond 2/Palfique Estelite showed good marginal adaptation (Fig. 10). The same result was achieved in 65% of the cavities restored with the combination All Bond 2/Palfique Estelite/Glass Ceramic insert. Only 50% of the restorations were gap-free in the Tokuso Light Bond/ Palfique Estelite-restored group, whereas in the Tokuso Light Bond/Palfique Estelite/Glass Ceramic Insert group only 35% of the restorations showed good marginal adaptation.
With respect to dye penetration along the tooth-restoration interface, a significant difference was found between ALB2 and ALB2/GCI ($p=0.0083$), ALB2 and TLB ($p=0.0004$) and ALB2 and TLB/GCI ($p< 0.0001$).
The intergroup differences in dye penetration into the dentinal tubules were not statistically significant.

Effect of dentin pretreatments on the dentinal surface appearance (VI)
The SEM observation showed that the untreated surfaces (Group E) presented a smear layer covering the dentin in both cervical and lateral dentin sections. All the other groups with cervical dentin presented mainly cross-cut dentinal tubules, almost completely occluded by a smear layer in groups A and B, completely open and widened in Group D, and widened and slightly occluded by collagen fibres in group C (Fig. 11). The dentin surfaces of group A and B appeared to be free from smear layer in the intertubular areas. The specimens in group C showed the presence of demineralised collagen fibres, making the dentin markedly microporous. The dentin from group D was completely free from smear layer and collagen fibres. All the lateral dentin specimens presented dentinal tubules mainly longitudinally cut. The solid dentin appearance in the different pretreatment groups was similar to that described for the cervical intertubular dentin (Fig. 12).

Effect of dentin pretreatments on surface roughness (VI)
The data obtained by 3D Topscan measurements demonstrated a statistically significant difference for the height descriptive parameter S_a and the surface developed area ratio parameter S_{dr} in the cervical wall, since the collagenase-treated group (D) showed a significant increase compared to the H_3PO_4-treated group (C), the EDTA-treated group (A) and the untreated group (E) (Tables 3). In the lateral wall, the only significant increase of S_a was detected in group D compared to A and E and an increase in S_{dr} was detected for D compared to E. The other roughness parameters did not show any significant differences and are therefore not presented.

The data obtained by the AFM measurements, when the examined area was 1x1 µm, revealed a significant difference between the mean S_a value of group C (5.28 ± 2.00 nm) and those of groups E and D (11.56 ± 5.24 nm and 11.03 ± 2.41 nm respectively). When the examined area was 10X10 µm no significant differences were detected.

Effect of dentin pretreatments on shear bond strength (VI)
The shear bond values are presented in Fig 13.
The Student-Newman-Keuls test demonstrated a statistically significant difference between groups with the following ranking: D>A,B,C>E.
No statistically significant difference was found for different dentin pretreatments in lateral dentin (longitudinally cut tubule surfaces). In cervical dentin (cross-cut tubule surfaces), the ANOVA test showed that group D was different from group A ($p=0.009$), B ($p<0.001$), C ($p<0.001$) and E ($p<0.001$). Group A differed from group E ($p=0.001$) and group C differed from group E ($p=0.008$). Comparisons between cervical and lateral wall dentin within the same treatment failed to demonstrate any statistically significant difference except for group D ($p=0.006$).
Thus, no difference in shear bond strength was found between the pretreatment which favoured the formation of a hybrid layer by demineralising the dentin surface (C) and those that simply removed the superficial smear layer, allowing for interfacial contacts between dentin and adhesive resin (A, B). The pretreatment which eliminated the collagen fibres (D), favouring the formation of resin tags into the dentinal tubules as well as the interfacial contact between the mineralised dentin and the adhesive resin, showed an increased bond strength.

Effect of dentin pretreatments on microleakage (VII)
The extent of the penetration from the cervical wall toward the axial wall and the pulp was similar for all the groups (Fig. 14). A large amount of restorations from each pretreatment group allowed penetration of the disclosing solution along the whole cervical and part of the axial walls (score 3).
The statistical analysis did not show any difference between pretreatment groups with regard to the extent of dye penetration along the restoration margins and into the dentinal tubules.
Thus, no difference was detected in marginal gap formation between the restorations on which hybrid layer was formed compared to restorations on which only resin tags and/or interfacial contacts between the mineralised dentin and the adhesive resin could occur.

DISCUSSION

The use of one or two cervical retention grooves did not prevent the formation of marginal gaps and did not affect the width of the gaps. However, two retention grooves at the cervical wall of the cavity reduced the extent of marginal gaps. This has been tested in a controlled study only in cavities restored with an adhesive belonging to a superseded generation of bonding agents (Scotchbond 2) and it would be interesting to repeat the investigation with bonding agents of more recent production. Furthermore, the effect of retention grooves under mechanical stresses remains to be evaluated.
Scotchbond 2 has shown clear limitations regarding the production of a good marginal seal (I). Similarly, its use in combination with a glass-ionomer liner (Vitrebond) only partially reduced

the formation of marginal gaps, compared to the use of Scotchbond 2 alone, the rate of failure being still too high for it to be a recommendable restorative technique. In contrast, its use with the "resin impregnation" or "rebonding" technique resulted in a good marginal seal with high predictability (II).

The hypothesis that prevention of contraction gaps and microleakage could be achieved by use of a dentin bonding agent could only partially be verified. This hypothesis had to be rejected for Tokuso Light Bond and Superbond C&B bonding agents (III). Poor marginal adaptation for restorations treated with Tokuso Light Bond was detected throughout the investigations. A possible explanation for the poor results obtained with Tokuso Light Bond could be that the bonding system does not require smear layer removal. Thus, the formation of a possible hybrid layer, interfacial contacts with the mineralised dentin and resin tags might be strongly reduced with consequent reduction of dentin adhesion. The same bonding mechanisms probably did not occur in the case of the highly viscous adhesive of the Superbond C&B system, which may have failed to infiltrate the exposed collagen layer and into the dentinal tubules.

The good marginal adaptation obtained by the use of a "sandwich" technique with the application of a self-cured composite resin as a first increment and of a light-cured one as a second increment may be explained in different ways (IV). According to Bertolotti (1991) and Fusayama (1992), the reason would be the direction of polymerisation shrinkage of the self-cured composite resin. This direction would be towards the cavity walls due to the contact with the dentin adhesive chemical initiator. Another possible explanation would be that the presence of a self-cured restorative material allows increased stress relief for the bonding agent due to retarded curing of the composite resin as well as to an increased free surface due to the material porosity (Braem et al. 1987, Rueggeberg & Margeson 1990, Alster et al. 1992, Van Meerbeek et al. 1992, 1993b). The second explanation is better supported by the results. The chemically-cured bonding agent (Superbond D-Liner) did not produce good marginal adaptation compared to the light-cured one (All Bond), whereas, according to Bertolotti (1991) and Fusayama (1992), the chemical initiator of the adhesive should have better "attracted" the composite polymerisation direction.
It should be noted that the "sandwich" technique, although showing good marginal adaptation for All Bond, did not really improve the quality of the margins of the All Bond-restorations in comparison with the results from Studies III and V, where All Bond and the more recent version, All Bond 2, were used in combination with a light-cured composite resin alone.

The SEM evaluation of the restorations after storage in water suggested a potentially important role of hygroscopic expansion in reducing the presence of gaps along the margins of composite restorations. However, hygroscopic expansion requires time to seal a marginal gap. During this time, in the clinical situation, bacteria could invade the gap and the possibly patent dentinal tubules.
It should be noted that it has been assumed that the difference in gap detection between the first (dye) and the second part (SEM) of the study was due to hygroscopic expansion. Another possibility could be that microleakage occurred in restoration areas that were not revealed as a marginal gap by SEM.
Interestingly, the SEM examination revealed areas of good bonding between restorative material and tooth tissue interspersed with areas of bond failure. Areas of adhesive or cohesive failures internal to the cavosurface margins cannot be colonised by bacteria unless they are already present in the hard tissues or the cavosurface seal later fails. However, the presence of such a gap could result in hypersensitivity since only tubule communication from the fluid gap to the pulp is needed (Brännström 1986).

The poor marginal adaptation obtained by the use of Superbond D-Liner in combination with a "sandwich" technique is in contrast with the very good marginal seal obtained by its use in combination with Palfique light-cured alone. An obvious theoretical explanation for these results is lacking. Incompatibility between products from different manufacturers could be an explanation. Alternatively, performance variability among different batches of the same product could also explain the different results of Studies III and IV for this bonding agent.

The hypothesis that the application of composite resin in bulk would give marginal adaptation and microleakage results similar to those obtained by two-step incremental application was confirmed (III). The findings support previous results (Crim & Chapman 1986, Zidan et al. 1987, Torstenson & Odèn 1989, Eakle & Ito 1990) and are in contrast with others (Hansen 1986, Crim 1991). Theoretically, application in bulk should induce higher stress in the bonded interfaces than application of the composite resin in several layers, since the volume of the polymerisation shrinkage is higher in the case of bulk application (Kemp-Scholte & Davidson 1988). Possibly, in the composite resin applied in bulk, a reduced amount of monomers underwent curing compared to the incrementally placed material, thus reducing the volume of the expected polymerisation shrinkage. Furthermore, it has previously been reported that, in the presence of cervical retention grooves, bulk and incremental application of the composite resins gave similar microleakage scores (Ben Amar et al. 1988). This could be partly due to the mechanical retention offered by the grooves when the light-curing is started at the interproximal area, at the level of the cervical wall.

The hypothesis that restorations reinforced by glass ceramic inserts may show a better marginal seal than restorations without inserts was not supported by the results (V). When All Bond 2 and Palfique were used without glass ceramic inserts, no microleakage was observed. This result could not be repeated with the combination All Bond 2/Palfique and glass ceramic inserts. Moreover, use of a glass ceramic insert did not significantly improve the marginal seal of the cavities restored with Tokuso Light Bond and Palfique.
The results suggested that the main factor influencing the outcome of the marginal seal in these particular restorations was the choice of the bonding agent and/or pretreatment procedures rather than the use of the glass ceramic insert. However, the size of marginal gaps was not analysed and the possibility that the glass ceramic inserts had an effect in reducing the size of the marginal gaps therefore cannot be excluded. It also remains to be investigated if the inserts can increase the resistance of the restorations to chewing forces. In theory, the inserts should have reduced the risk of marginal seal failure since they reduce the amount of composite resin material needed for restoration. Tani et al. (1993) quantified *in vitro* the contraction force and polymerisation shrinkage of composite resins with and without Beta Quartz glass ceramic inserts, using 6 different brands of composite resin. The glass inserts markedly reduced the linear polymerisation shrinkage, but this reduction often did not correspond to a reduction in contraction forces during polymerisation. These observations could explain the lack of reduction of microleakage with the glass ceramic inserts in our investigation.

The hypothesis that dentin roughness relates to dentin pretreatment was confirmed by the 3D Topscan examination. These data support a previous investigation showing that the partially demineralised dentin layer obtained after acid etching and removal of the exposed collagen fibres was significantly different from dentin surfaces covered by a smear layer with regard to surface roughness (Gwinnett 1994c). A correlation between surface roughness and shear bond strength was partly detected, since the collagenase-treated group (D), which showed an increased roughness (S_a) and surface area (S_{dr}), also gave the highest shear bond strength in cervical dentin. On the other hand, such a correlation was limited to these data since the ranking of the pretreatment groups for µm roughness and shear bond strength did not always correspond. It might be speculated that there is a certain level of surface roughness over which an increase in surface area positively affects the bond strength. Such roughness would depend on the size, shape and density of the peaks and the pits in the bonded surfaces (Hansson 1997). Such an increase in surface roughness would have occurred only in group D, cervical dentin. Other factors may have a stronger influence on the adhesion than surface roughness. One of these factors may be the chemical composition of the dentin surface, which, together with roughness and physical parameters (e.g. capillarity), may determine the dentin surface energy and, consequently, the diffusion of the adhesive onto the dentinal surface (Attal et al. 1994).

The absence of a hybrid layer did not affect the bond strength and, in fact, the elimination of the exposed collagen layer resulted in a higher shear bond strength in cervical dentin. These data support previous reports (Gwinnett 1994b, 1994c, Wakabayashy et al. 1994). From the differences in the collagenase-treated group (D), where dentin surfaces with longitudinally cut

tubules showed a reduced shear bond strength compared to the surfaces with cross-cut tubules, it appeared that the formation of several resin tags perpendicularly oriented in relation to the shearing force increased the strength of the adhesion between the dentin and the bonding agent. The fact that EDTA-treated groups (A, B) and the H_3PO_4-treated group (C) had similar shear bond strength values suggested that the formation of interfacial contact between the adhesive resin and the mineralised or partially mineralised dentin surface is the basic bonding mechanism. The result supports previous findings (Gwinnett 1993, 1994b). Whether such a mechanism is based on electrochemical bonding or micromechanical interlocking with the partially mineralised dentin remains to be clarified.
The presence of a collagen layer to produce a hybrid layer did not result in an improved marginal adaptation compared to dentin surfaces deprived of a resin-impregnated collagen layer either. Thus, the elastic buffer effect of a hybrid layer apparently did not occur or was not enough to counteract the effect of curing shrinkage of the composite resins and to improve the seal of the restorations. These results are in contrast with the reports of Uno & Finger 1995 regarding the effect of a hybrid layer on marginal adaptation.
It should be noted that the fracture areas in Study VI were analysed under a light microscope. In order to make a clear distinction between adhesive and cohesive failures (either in dentin or in the adhesive), the use of SEM would have been more suitable. It is possible that failures which have been considered to be adhesive failures between the dentin and the bonding agent may in fact have been cohesive failures within the adhesive resin or within the dentin. A cohesive failure, i.e., a fracture in one of the materials to the side of the interface, indicates that the physical properties of the material have limited the bond strength of the assembly (Øilo 1993). Thus, a failure close to the composite resin, between the composite and the adhesive, would not represent a proper measure of the bond strength produced by the pretreatment. A fracture at the top of the hybrid layer could mean a cohesive failure of the adhesive resin rather than a failure in the adhesive/dentin interface.
The shear bond strength values obtained for the different pretreatment groups were quite low compared with the values obtained for All Bond 2 in previous reports (Kanca 1992a, Gwinnett 1993, 1994b, 1994c). Similarly, in Study VII, the number of restorations showing penetration of the dye solution along the margins and into the dentinal tubules was high. These results with the use of All Bond 2 were in contrast to those obtained in Studies III, IV and V.
A possible reason for this difference could be the method of storage of the specimens before use. In Studies VI and VII, teeth stored in water were used, according to the ISO standard (1994), whereas in the other investigations teeth stored frozen were used. The lack of retention grooves at the cervical wall in the restorations from Study VII could partly explain the results. A third possible reason could be that the restorative combinations used, All Bond2/ Z100 in Study VI and All bond 2/Spectrum in Study VII, result in low bond strength values and in an inferior marginal adaptation compared to the other studied restorative combinations with All Bond. In a recent investigation, lower values of bond strength were detected for the combination All Bond 2/Z100 compared to other bonding agent/composite resin combinations (Leirskar et al. 1998). A possible variability between batches of the adhesive cannot be excluded either.

The use of liners to protect the dentin and the pulp from penetration of the disclosing agent was clearly unsuccessfull with the exception of the use of Tubulitec (II). When this liner was used, the dye penetration was limited to the gap between the composite resin/adhesive and Tubulitec, without extension into the dentinal tubules. For this reason, Tubulitec was used as a liner when the "resin impregnation" technique was tested. Possibly, the impregnation of the gap after the setting of the composite resin could be a useful technique even when the most recently introduced bonding agents are used in order to increase the predictability of good marginal adaptation.
The use of liners in composite resin restorations is nowadays very rare. This is due to several reasons. Etching of the dentin with acidic solutions has become an accepted procedure since it has been known for a long time that acid application does not produce pulpal damage (Brännström & Nordenvall 1978, Nordenvall et al. 1979, Torstenson et al. 1982). Another reason is the progress in dentin adhesion achieved in recent years, which has induced some professionals to believe that the predictability of gap-free restorations is very high. As discussed, few techniques and materials studied showed predictable results. The pretreament of

a cavity with acidic solutions resulted in open and widened dentinal tubules. In case of failure of the marginal seal of the restorations, in several cavities the disclosing solution was detected all the way through the dentinal tubules to the pulp. In other restorations, a marginal gap was detected without penetration of the dye into the dentinal tubules. In such cases, the presence of a liner or of a bonding agent nearer the dentin protected the patent dentinal tubules and the pulp. In the clinical situation, the liner or the adhesive could be degraded by an acidic pH in the long run (Freund & Munksgaard 1990) and the dentinal seal disappear. Thus, the enlarged tubules could offer a route to the pulp for bacteria and toxins. Fortunately, the incidence of secondary caries and pulp inflammation and necrosis in the clinical situation is not as high as the rate of detection of marginal gaps and patency of tubules in *in vitro* studies. This could be due to different testing conditions for the materials in vivo and in vitro. However, another possible explanation could be that, in vivo, the defence mechanisms of the pulp such as production of atubular, reparative dentin reduce the effect of the marginal gaps. Furthermore, the hygroscopic expansion could contribute to later sealing of the restoration margins. Irreversible pulpal damage would therefore occur only in those cases in which some of the reparative mechanisms do not occur.

Due to the impossibility of predicting the effect of the compensatory mechanisms in case of marginal gap formation, a cautious attitude toward the use of composite resins should be adopted in view of the high variations in the marginal adaptation results within the same product.

MAIN FINDINGS

Within the limitations of this thesis, the findings demonstrated that:

- The presence of two retention grooves at the cervical wall reduced the effect of the composite resin polymerisation contraction by reducing the extent of the marginal gaps formed (I).
- The use of Scotchbond 2 (I, II), Tokuso Light Bond (III, V) and Superbond C&B (III) as dentin bonding agents did not prevent marginal gap formation in a predictable way.
- The use of a glass-ionomer cement (Vitrebond) improved marginal adaptation but microleakage towards the pulp still occurred with a high frequency (II).
- The use of liners did not result in reduced microleakage towards the pulp with one exception (II).
- Resin infiltration of the marginal gaps once the restorative material had set ("rebonding") resulted in good marginal adaptation (II).
- The application of a composite resin in bulk, with a metal matrix strip, did not affect the quality of the marginal adaptation compared to the application of the composite resin in two increments with a plastic matrix band (III).
- The use of a self-cured composite resin as a first increment followed by the application of a light-cured one resulted in good marginal adaptation for All Bond restorations and in poorer quality of the margins for Superbond D-Liner restorations (IV). This technique did not result in better marginal adaptation than that obtained by the use of All Bond or Superbond D-Liner in combination with a light-cured composite resin only (III, V).
- The inclusion of glass ceramic inserts did not improve the restoration marginal adaptation compared to the results obtained by the use of the bonding agents in combination with composite resins only (V).
- Hygroscopic expansion resulted in a significant improvement of the marginal seal (IV).
- All Bond and the more recent version All Bond 2 produced a good marginal adaptation in a quite predictable way when teeth stored frozed were used (III, IV, V), whereas a poor marginal adaptation was noted for All Bond 2 in teeth stored in deionised water (VII).
- Superbond D-Liner produced a good marginal adaptation when used in combination with a light-cured composite alone (III) but the performance was poorer in combination with a sandwich technique (IV).

- The roughness of the dentin, at the µm level, was found to partly depend on the surface pretreatment and to show some correlations to shear bond strength (VI).
- The shear bond strength to dentin was independent of the formation of a hybrid layer, whereas the formation of interfacial contacts between adhesive resin and the dentin beneath or deprived of an exposed collagen layer seemed to be the bonding mechanism most affecting the shear bond strength (VI).
- The formation of resin tags in the open dentinal tubules increased the bond resistance to shearing forces (VI).
- Dentin pretreatments resulting in exposure of a demineralised collagen layer did not reduce the microleakage along the restoration margins compared to pretreatments which eliminate the demineralised collagen layer, or do not induce demineralisation of the dentin or increase the dentin surface roughness (VII).

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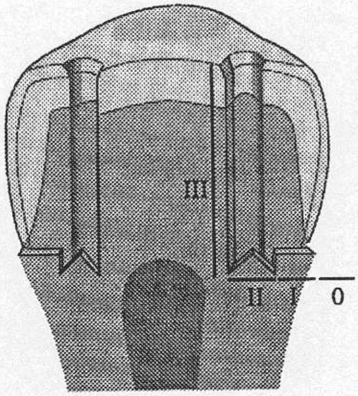

Fig. 1. Schematic drawing of a mesio-distal section of a class II cavity. For a general review of the results, the scores of each study are transformed to a common score scale for all the investigations: 0 = no penetration, 1 = penetration of the FEB or the dye not further than the middle of the cervical wall, 2 = penetration until the corner between the cervical and the axial wall, 3 = penetration along the whole cervical wall and extending along the axial wall

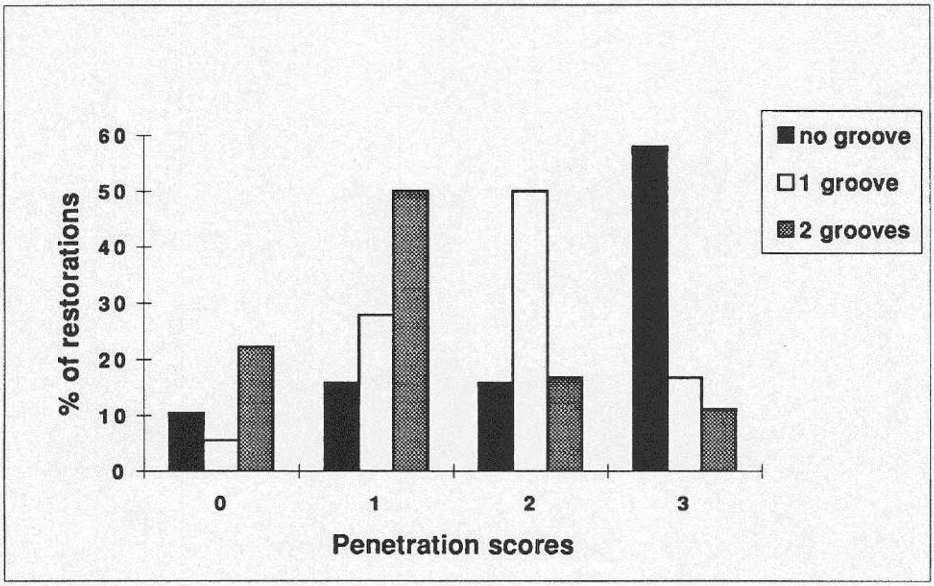

Fig. 2. FEB penetration extent scores for restorations performed without, with one or with two retention grooves.

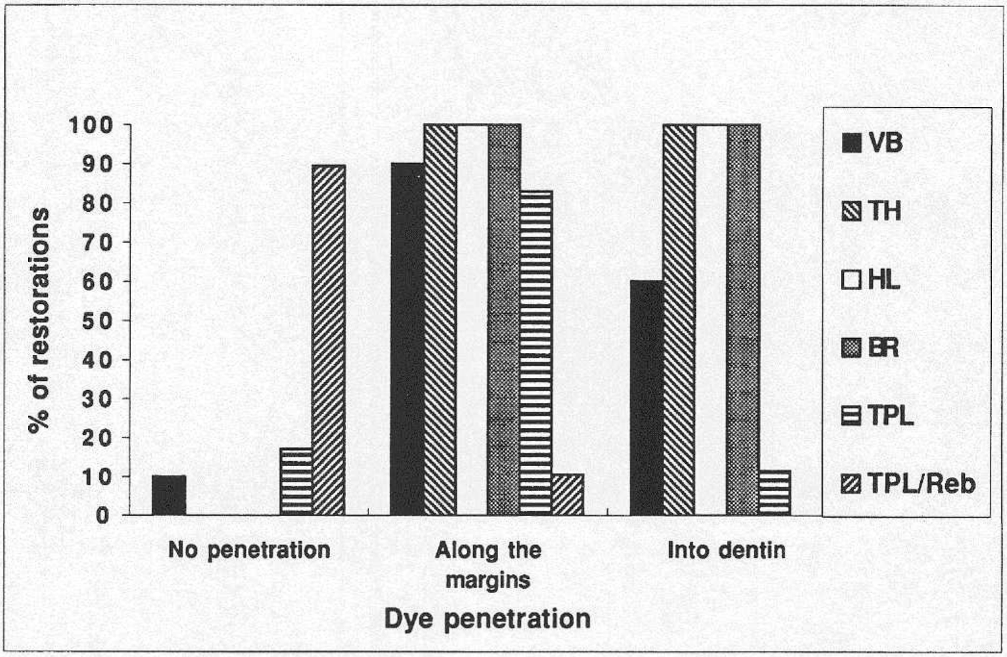

Fig. 3. Distribution of restorations performed with different dentinal liners with regards to dye penetration. TPL/Reb: Cavity lined with Tubulitec and treated with the "rebonding" technique.

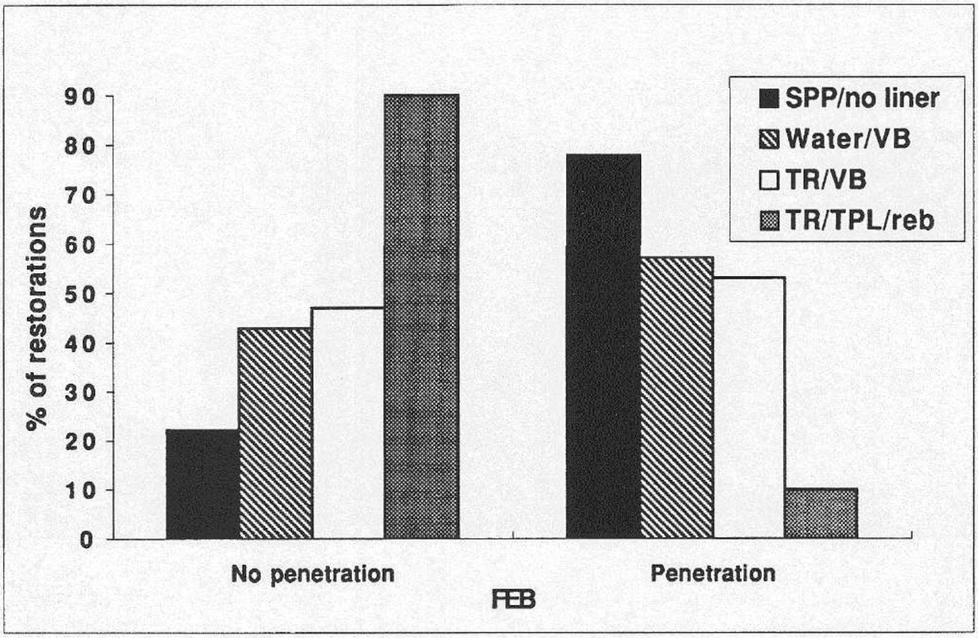

Fig. 4. Distribution of restorations performed with Scotchbond 2 alone or in combination with Vitrebond or with the "rebonding" technique.
SPP/no liner: cavity pretreated with Scotchprep Dentin Primer.
Water/VB: cavity washed with water and lined with Vitrebond.
TR/VB: cavity pretreated with Tubulicid Red and lined with Vitrebond.
TR/TPL/reb: cavity pretreated with Tubulicid red, lined with Tubulitec and restored according to the "rebonding technique".

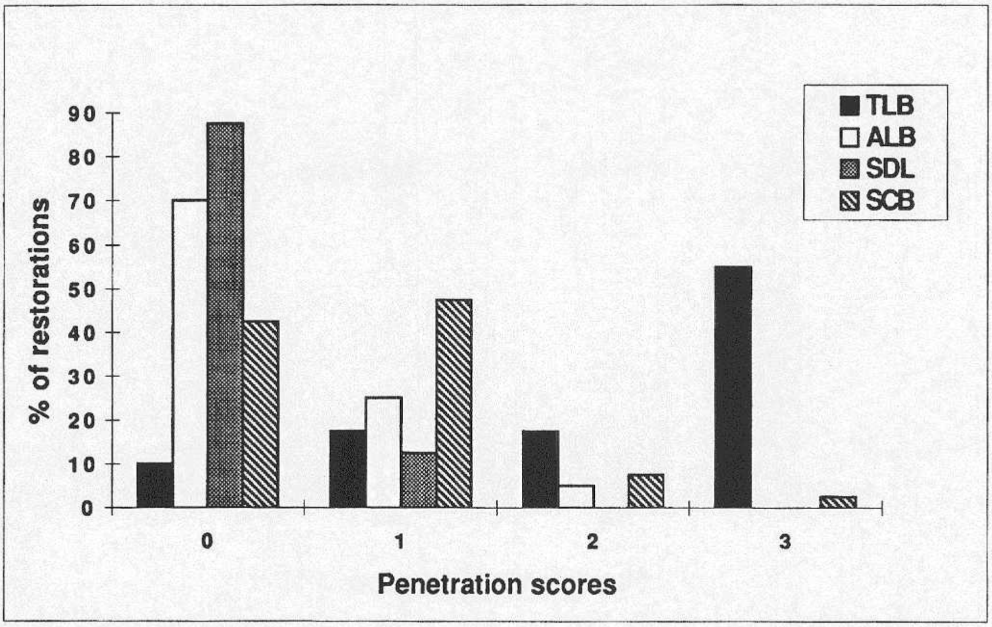

Fig. 5. FEB and toluidine blue penetration extent scores in restorations performed with Tokuso Light Bond (TLB), All Bond (ALB), Superbond D-Liner (SDL) and Superbond C&B (SCB).

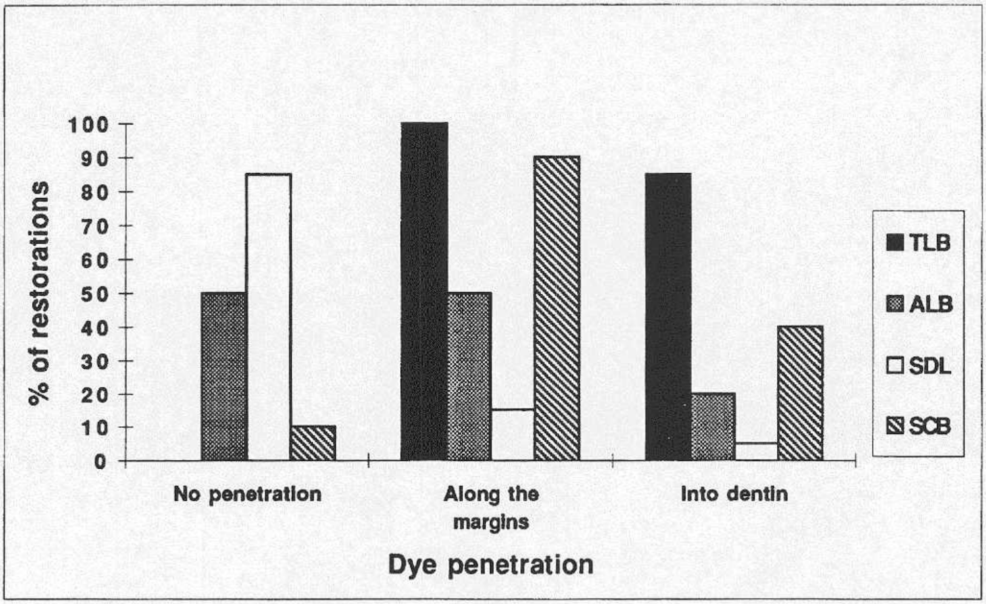

Fig. 6. Distribution of restorations performed with Tokuso Light Bond (TLB), All Bond (ALB), Superbond D-Liner (SDL) and Superbond C&B (SCB) with regards to penetration of the toluidine blue solution.

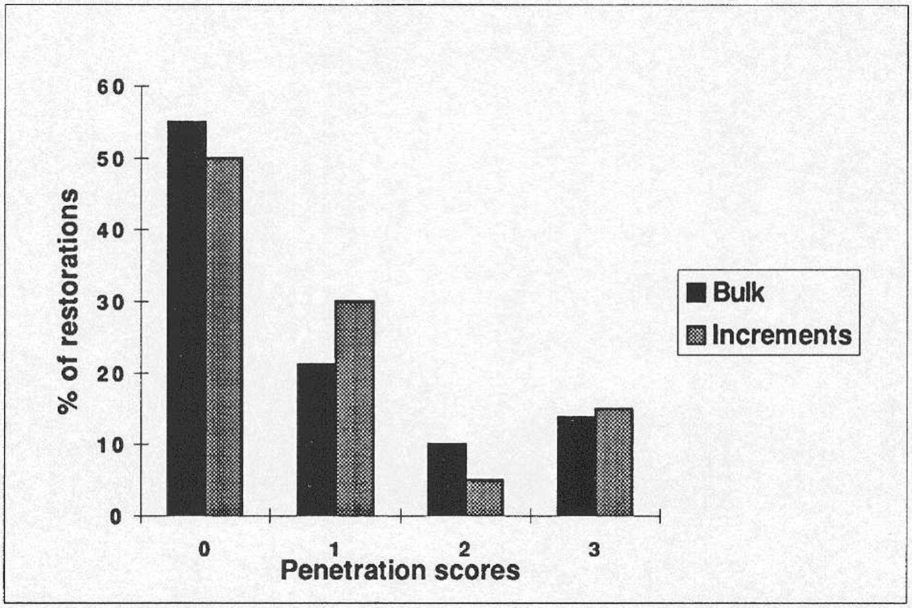

Fig. 7. FEB and toluidine blue penetration extent scores in restorations performed with All Bond, Superbond D-Liner, Superbond C&B and Tokuso Light Bond either with bulk or with incremental applications of the composite resin.

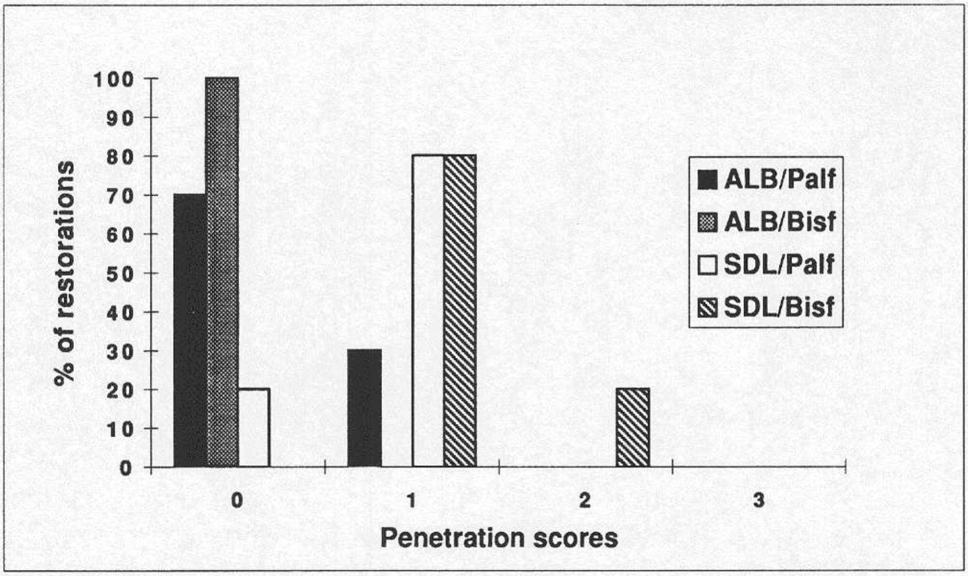

Fig. 8. Toluidine blue penetration extent scores in restorations performed with the combinations All Bond/Palfique-self-cured, All Bond/Bisfil 2B, Superbond D-Liner/Palfique-self-cured, Superbond D-Liner/Bisfil 2B.

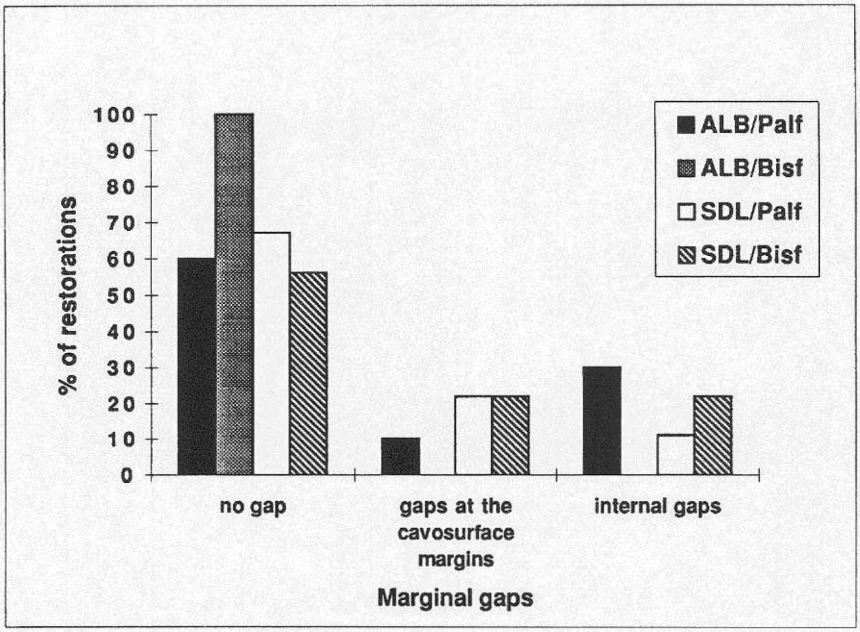

Fig. 9. Marginal gap location detected by SEM in restorations performed with All Bond/Palfique-self-cured, All Bond/Bisfil 2B, Superbond D-Liner/Palfique-self-cured, Superbond D-Liner/Bisfil 2B after water storage.

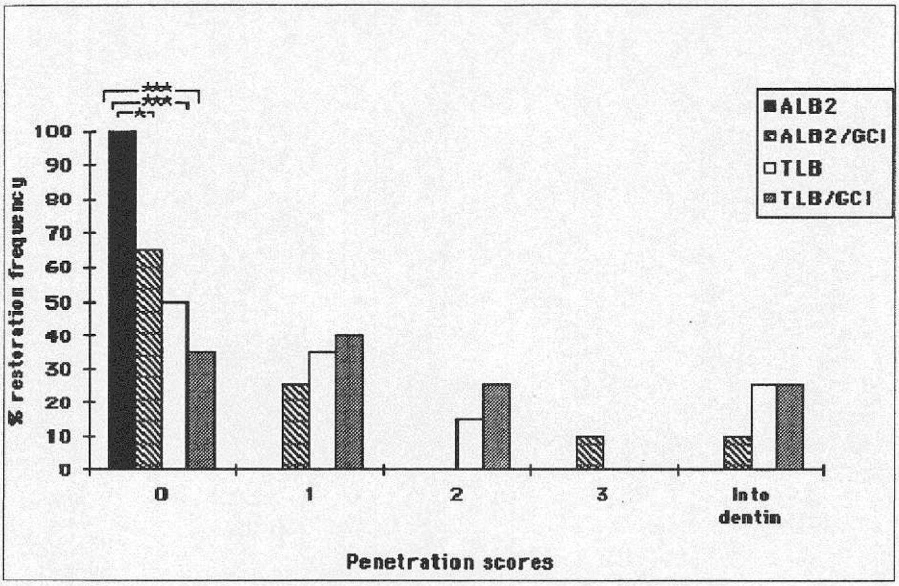

Fig. 10. Toluidine blue penetration extent scores in restorations performed with the combinations All Bond 2/Palfique Estelite (ALB2), All Bond 2/Palfique Estelite/Glass Ceramic insert (ALB2/GCI), Tokuso Light Bond/ Palfique Estelite (TLB), Tokuso Light Bond/Palfique Estelite/Glass Ceramic Insert (TLB/GCI). Statistically significant differences:

*: $p<0.05$;

***: $p<0.001$

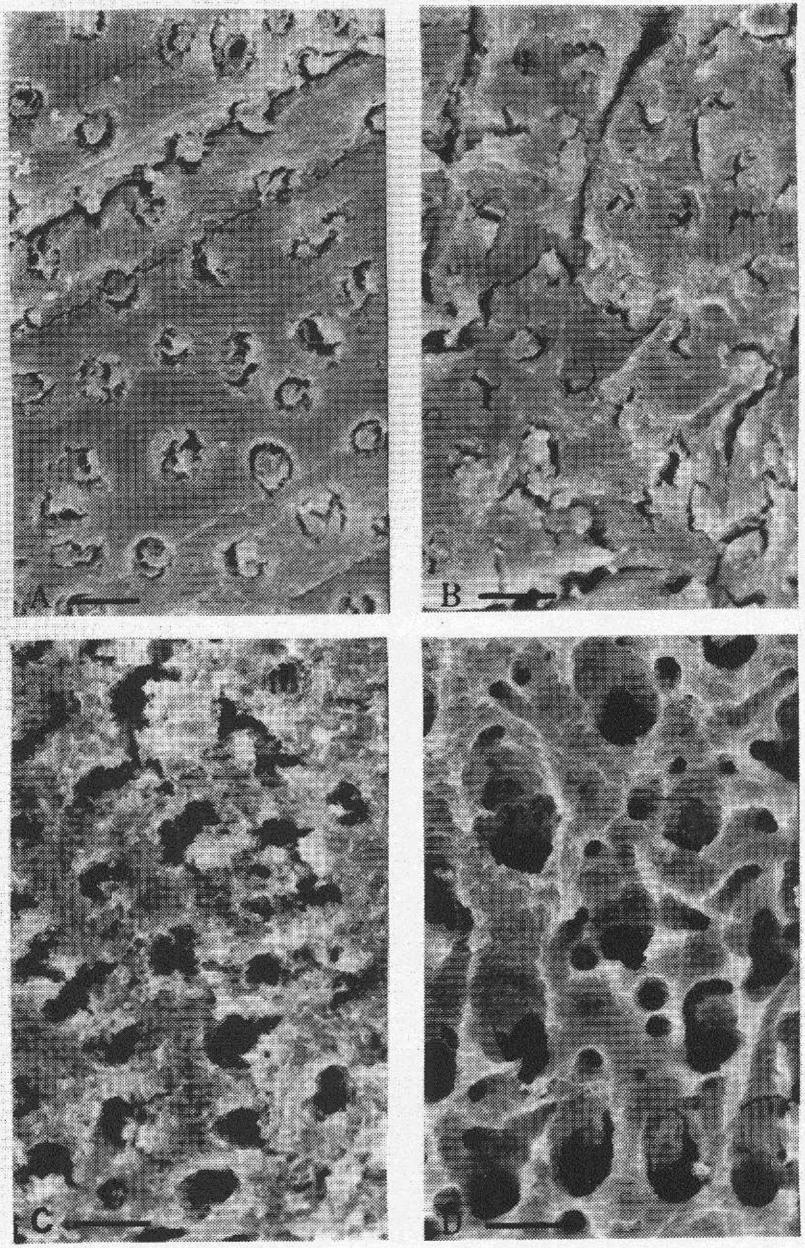

Fig. 11. Cervical dentin sections. Magnification: x2500. (A) EDTA-treated group. The superficial smear layer is removed while smear plugs are occluding the majority of the dentinal tubules. (B) Al_2O_3/EDTA-treated group. The dentin surface is characterised by surface irregularities. The superficial smear layer is removed but smear plugs are present. (C) H_3PO_4-treated group. The intertubular dentin surface is clean from smear layer and covered by a collagen layer. The dentin tubules are free from smear layer and partly covered by collagen fibres. (D) H_3PO_4/collagenase-treated group. The surface is cleaned from smear layer and collagen and the dentinal tubules are widened and open. Note the presence of several secondary canals and anastomoses. The bar represents 5 μm.

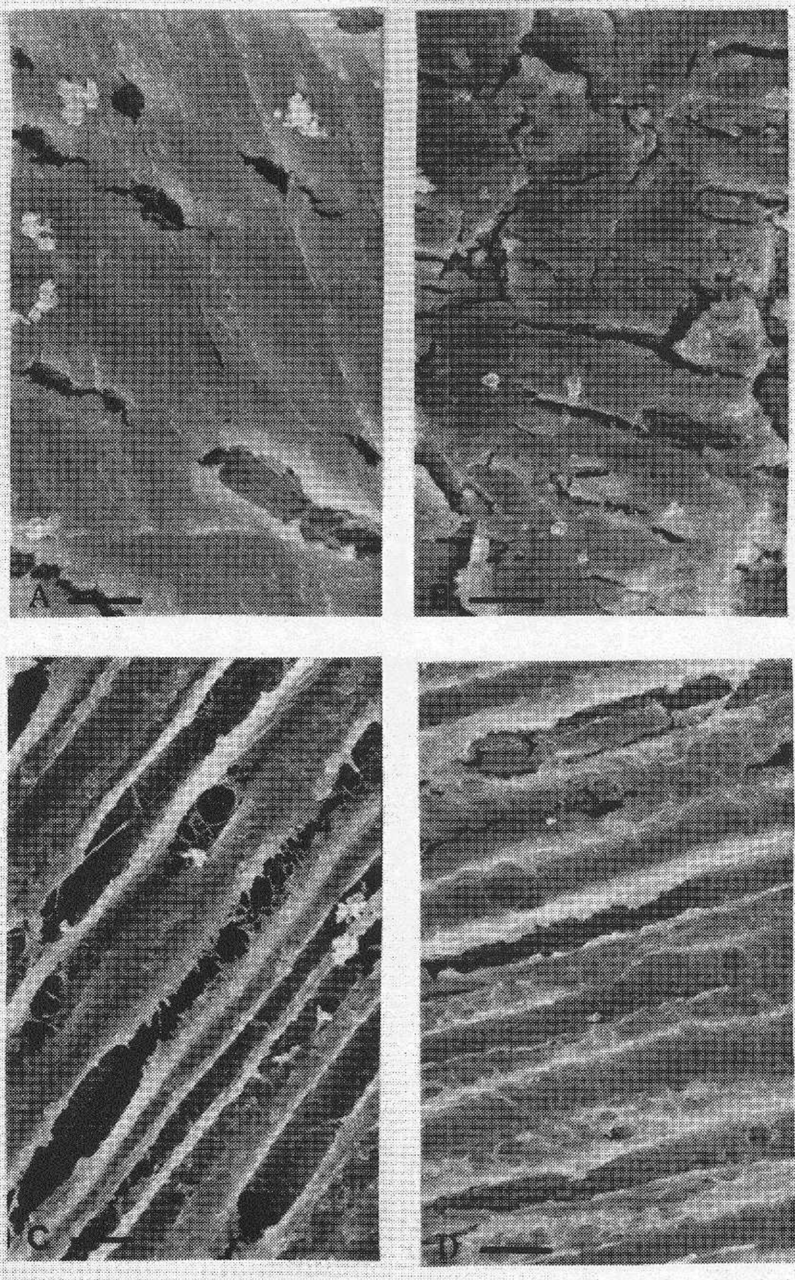

Fig. 12. Lateral dentin sections. (A) EDTA-treated group. The intertubular smear layer is removed while smear layer is still covering the majority of the longitudinally cut dentinal tubules. Magnification: x2500. (B) Al_2O_3/EDTA-treated group. The dentin surface is characterised by surface irregularities. Only the intertubular smear layer is removed. Magnification: x2500. (C) H_3PO_4-treated group. The dentin surface is clean from smear layer and covered by a collagen layer. The dentinal tubules are longitudinally cut. Magnification: x2000. (D) H_3PO_4/collagenase-treated group. The surface is free from smear layer and collagen fibres. Magnification: x2500. The bar represents 5 μm.

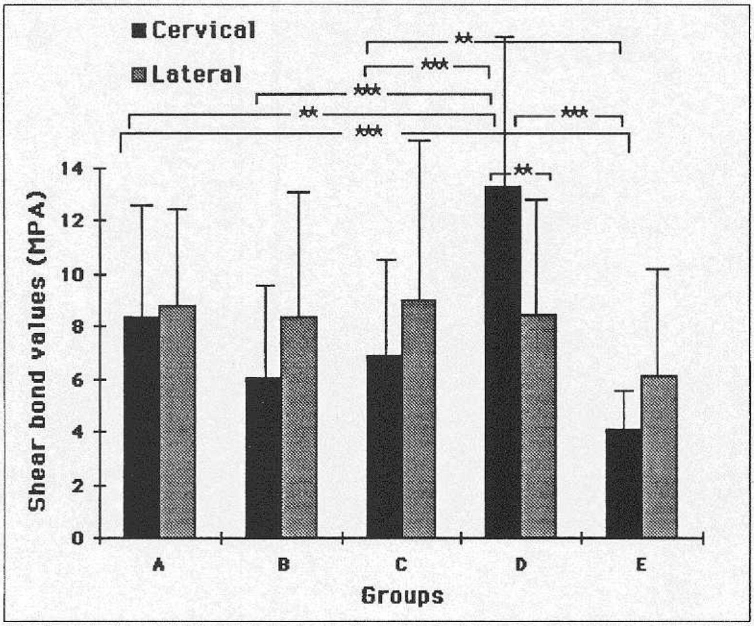

Fig. 13. Shear bond strength mean values (Mpa) and standard deviation (bars).
Statistically significant differences:
**: $p<0.01$
***: $p<0.001$

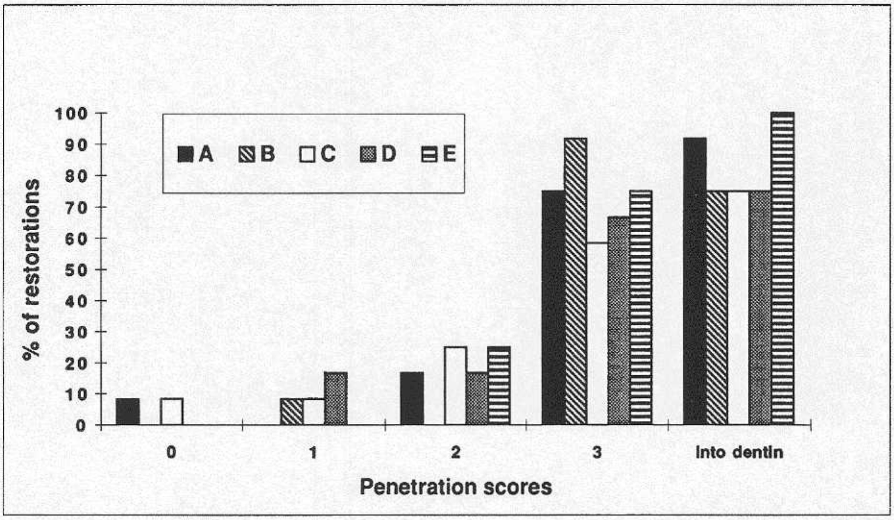

Fig. 14. Toluidine blue penetration extent scores in restorations performed after different dentin pretreatments. A: EDTA; B: aluminium oxide/EDTA; C: phosphoric acid; D: phosphoric acid/collagenase; E: untreated control.

Table 1. Dental materials used in the investigations.

Abbreviation	Name	Manufacturer	Study
Dentin bondings			
SCT2	Scotchbond 2	3M Dental Products, St Paul, MN, USA	I, II
ALB	All Bond	Bisco, Itasca, IL, USA	III, IV
SDL	Superbond D-Liner	Sun Medical, Kyoto, Japan/ Parkell, Farmingdale, NY, USA	III, IV
SCB	Superbond C&B	Sun Medical, Kyoto, Japan/ Parkell, Farmingdale, NY, USA	III
TLB	Tokuso Light Bond	Tokuyama Soda, Tokyo, Japan	III, V
ALB2	All Bond 2	Bisco, Itasca, IL, USA	V-VII
Dentinal liners			
VB	Vitrebond	3M Dental Products, St Paul, MN, USA	II
TH	Thermoline	Voco Chemie, Cuxhaven, Germany	II
BR	Barrier Dentin Sealant	Teledyne Water Pik, Fort Collins, CO, USA	II
HL	Hydroxyline	Taub Product, Jersey City, NJ, USA	II
TP/L	Tubulitec	Dental Therapeutics, Nacka , Sweden	II
Composite resins			
P-50	light-cured	3M Dental Products, St Paul, MN, USA	I, II
Palfique Estelite	light-cured	Tokuyama Soda, Tokyo, Japan	III-V
Z100	light-cured	3M Dental Products, St Paul, MN, USA	VI
Spectrum	light-cured	Dentsply, DeTrey, Konstanz, Germany	VII
Palfique	self-cured	Tokuyama Soda, Tokyo, Japan	IV
Bisfil 2B	self-cured	Bisco, Itasca, IL, USA	IV

Table 2. Effect of different bonding agents on marginal gap width.

	n° of cavities with gaps		mean (μm)		range (μm)		Total n° of restorations	
	B	I	B	I	B	I	B	I
TLB	9	7	5.5	7.7	2-12	4-16	10	10
ALB	1	1	1.5	3.5	1-2	3-4	10	10
SDL	1	1	3.0	1.5	2-4	1-2	10	10
SCB	1	4	5.0	2.3	4-6	2-6	10	10

B: composite resin applied in bulk
I: composite resin applied in two increments

Table 3. 3D Topscan measurements. Mean values and differences between treatments (Student-Newman-Keuls test).

Site	*Cervical*		*Lateral*	
Parameters	mean Sa (µm)	mean Sdr	mean Sa (µm)	mean Sdr
	0.94 **D**	1.33 **D**	0.91 **D**	1.31 **D**
	0.84 **B**	1.25 **B**	0.80 **C**	1.28 **C**
	0.68 **C**	1.22 **C**	0.74 **B**	1.24 **B**
	0.57 **A**	1.15 **A**	0.60 **A**	1.18 **A**
	0.56 **E**	1.16 **E**	0.55 **E**	1.14 **E**

Groups connected by a bar are not significantly different.

Sa: is the arithmetic mean of the departures of the roughness profile from the mean plane.

Sdr: represents the developed surface area ratio.

A: EDTA pretreatment

B: Aluminium Oxide/EDTA pretreatment

C: Phosphoric acid pretreatment

D: Phosphoric acid/collagenase pretreatment

E: Untreated control

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