A Confocal Laser Scanning Microscope investigation of different dental adhesives bonded to root canal dentine

K. Bitter[1], S. Paris[1], P. Martus[2], R. Schartner[1] & A. M. Kielbassa[1]

[1]Department of Operative Dentistry and Periodontology, School of Dental Medicine, Campus Benjamin Franklin; and [2]Department of Medical Informatics, Biometry, and Epidemiology, Campus Benjamin Franklin, Charité – Universitätsmedizin Berlin, Berlin, Germany

Abstract

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Aim To evaluate the resin–dentine interface of different adhesive systems and corresponding luting cements proposed for bonding fibre posts to root canal dentine.

Methodology Fifty extracted maxillary canines and central incisors were used. After root canal treatment the teeth were randomly divided into five groups of 10 teeth each. Fibre posts were inserted with five different adhesive systems and corresponding luting cements. Group 1: Clearfil Core/New Bond (Kuraray), group 2: Multilink (Vivadent), group 3: Panavia 21/ED Primer (Kuraray), group 4: PermaFlo DC (Ultradent), and group 5: Variolink II/Excite DSC (Vivadent). The primer was labelled in each case with 0.1% Rhodamine B isothiocyanate (RITC). Each root was sectioned into 2 mm thick slices at 1, 4 and 7 mm below the cementoenamel junction. The resin–dentine interface was evaluated using a Confocal Laser Scanning Microscope; the thickness of the hybrid layer and the number of resin tags were measured. The statistical analysis was performed using nonparametrical tests for comparisons between groups; for overall comparisons the Kruskal–Wallis test was used. Intra-individual analysis within teeth was performed using a linear model.

Results The thickness (μm) of the hybrid layer of group 1 (5.45; SD 1.21), group 4 (3.36; SD 1.59), and group 5 (4.33; SD 1.19) was significantly higher than in the other groups ($P \leq 0.05$). The number of resin tags observed in group 1 was significantly higher than in groups 2–4 ($P < 0.05$), but did not differ from group 5. Each group showed significantly more resin tags in the coronal and in the central part of the root canal than in the apical part ($P < 0.001$).

Conclusion Conditioning of the root canal dentine with phosphoric acid and the use of one- and two-bottle-bonding systems gave a thicker and more uniform hybrid layer with considerably more resin tags than observed after the use of 'self-etching' adhesives. This might provide a more durable bond of the post to root canal dentine.

Keywords: Confocal Laser Scanning Microscopy, dentine bonding agents/dental adhesives, fibre posts, hybrid layer.

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Introduction

Root filled teeth with little coronal tissue are often restored using post and core systems. However, in contrast to the original concepts, recent research has shown that posts do not strengthen the root. Instead, their function is to improve the retention of the core and the coronal restoration (Sorensen & Engelman

Correspondence: Dr Kerstin Bitter, Department of Operative Dentistry and Periodontology, School of Dental Medicine, Campus Benjamin Franklin, Charité – Universitätsmedizin Berlin, Aßmannshauserstr. 4-6, 14197 Berlin, Germany (Tel.: +49 (0)30 8445 6315; fax: +49 (0)30 8445 6204; e-mail: kerstin.bitter@charite.de).

1990). Different studies could show frequent vertical root fractures associated with metallic posts cemented into the root canal (Axelsson *et al.* 1991, Testori *et al.* 1993). In 1990, fibre posts were introduced (Duret *et al.* 1990) and became popular because of their favourable physical properties. Their modulus of elasticity is similar to that of dentine (Asmussen *et al.* 1999), and this seems to reduce stress transmission to the root canal walls by the post, thus avoiding possible root fractures (Isidor *et al.* 1996). The fixation of posts using an adhesive technique allows passive cementation; furthermore, this technique provides increased post-retention in the root compared with conventional methods (Duncan & Pameijer 1998). Moreover, less coronal leakage after adhesive insertion of the posts has been reported (Mannocci *et al.* 2001), which is considered the major issue in successful root canal treatment. The combination of a fibre post and a Bis-GMA-based resin cement has been described as a homogenous structure. Due to this homogeneity fibre, post and resin cement can mechanically replace dentine and contribute to absorb stress (Ferrari & Scotti 2002).

Different bonding systems have been proposed for cementing fibre posts. One- or two-bottle-bonding systems require the demineralization of the root canal dentine with phosphoric acid in a first step. Self-etching primers have been developed for demineralization and bonding in one step. Bonding to root canal dentine requires the consideration of structural features. Ferrari *et al.* (2000) evaluated dentine morphology in root canals in terms of tubule orientation and density. The tubule density was significantly higher in the cervical third than those observed in the central and apical thirds.

The aim of this study was to evaluate the resin–dentine interface of different adhesive systems and corresponding luting cements proposed for bonding fibre posts within root canals using Confocal Laser Scanning Microscopy (CLSM). Moreover, the number of resin tags and the thickness of the hybrid layer was measured. It was hypothesized that there are differences in the penetrating ability of the investigating adhesive systems.

Materials and methods

Fifty maxillary canines and central incisors were used. Teeth with roots having resorption, caries or cracks were excluded. External debris was removed with an ultrasonic scaler and the teeth were stored in 0.1% thymol prior to the study.

After removal of the crown at the cementoenamel junction, the central and the coronal thirds of the canal were enlarged using sizes 1–4 Gates Glidden burs in descending order. The working lengths were visually established by subtracting 1 mm from the lengths of a size 15 file when its tip appeared at the apical foramen. All roots were shaped uniformly at full working lengths to size 50 using reamers with a reaming action and alternating Hedström files with a circumferential filing movement. This was followed by a stepback preparation in steps of 1 mm to size 70. Irrigation was performed with 1 mL of 1% NaOCl solution after every change of file size throughout the cleaning and shaping of the root canal.

After drying with paper points the teeth were filled by means of cold lateral condensation. Size 50 gutta-percha points (VDW, Munich, Germany) served as master cones and size 20 and 25 gutta-percha points were used as accessory points. AH plus (DeTrey Dentsply, Konstanz, Germany) was used as sealer in all cases. Coronal surplus was removed with a heated excavator, and the access cavities and pulp chambers were temporarily filled with Cavit (3M ESPE, Seefeld, Germany) and stored for 24 h at 37 °C in 100% humidity.

The samples were randomly divided into five groups of 10 teeth each. The root canals of each sample were enlarged with a low-speed drill provided by the manufacturer of the post system; the depth of the post space preparation was 9 mm, and the irrigation after the preparation was performed with 0.9% NaCl solution. 'Mirafit White' (Hager & Werken, Duisburg, Germany) fibre posts were tried-in and inserted with different adhesive systems (Table 1).

Group 1

The root canal walls were etched with 35% phosphoric acid for 15 s, washed with water spray and gently air dried. Excess water was removed using paper points. Clearfil New Bond Catalyst and Universal (Kuraray, Osaka, Japan) were mixed and labelled with 0.1% Rhodamine B isothiocyanate (RITC). Bonding was applied using a microbrush tip. Excess primer adhesive solution was removed with a paper point. Clearfil Core Catalyst and Universal Paste (Kuraray) were mixed and applied into the root canal space with a lentulo drill and also onto the post surface. Then, the posts were inserted into the root canal and excess cement was removed.

Table 1 The different adhesive systems used in this investigation

Luting composite	Bonding agent	Manufacturer	Applicable steps	Solvent	Dentine conditioning	Primer ingredients
Clearfil Core	New Bond	Kuraray, Osaka, Japan	2	Ethanol	Phosphoric acid	10-MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, benzoyl peroxide, *N*,*N'*-diethanol *p*-toluidine, sodium benzene sulphinate, ethyl alcohol
Multilink	Multilink Primer A & B	Vivadent, Schaan, Liechtenstein	1	Water	Phosphoric acid	Water, phosphoric acid acrylate, HEMA, polyacrylic acid modified methacrylate resin
Panavia 21	ED Primer	Kuraray, Osaka, Japan	1	Water/ethanol	10 MDP	10-MDP, HEMA, *N*-methacryl 5-aminosalcylic, sodium benzene sulphinate, *N*,*N'*-diethanol *p*-toluidine, water
PermaFlo DC	PermaFlo DC Primers A & B	Ultradent, Salt Lake City, UT, USA	3	Ethanol acetone	Phosphoric acid	Proprietary
Variolink II	Excite DSC	Vivadent, Schaan, Liechtenstein	2	Ethanol	Phosphoric acid	HEMA, Bis-GMA, glycerine dimethacrylate, phosphoric acid acrylate, highly dispersed silica, ethanol

Group 2

Multilink Primer A & B (Vivadent, Schaan, Liechtenstein) were mixed and labelled with 0.1% RITC. The primer was applied for 15 s into the root canal with a Microbrush tip, gently air-dried and excess removed using a paper point. Multilink cement was applied as described above and the posts inserted into the root canal space.

Group 3

Panavia ED Primer Liquid A & B (Kuraray) were mixed, labelled with 0.1% RITC and applied for 15 s onto the root canal walls. The posts were inserted using Panavia 21 cement and excess cement removed.

Group 4

The root canal was etched with 35% phosphoric acid for 15 s, washed out with water and air dried. Excess water was removed using paper points. Perma Flo DC Primer A (Ultradent, Salt Lake City, UT, USA) was labelled with 0.1% RITC and applied onto the root canal walls for 15 s. Subsequently, Primer B was applied; excess primer was removed using a paper point. Perma Flo DC Luting Cement was applied into the root canal space by means of a lentulo and onto the post surface. The fibre posts were then seated and excess cement was removed.

Group 5

The conditioning of the root canal dentine was performed with phosphoric acid as described above. Excite DSC (Vivadent), labelled with 0.1% RITC, was applied into the root canal space and excess cement was removed with a paper point. Variolink II Catalyst and Base were mixed, and the cement was applied into the root canal and the post surface. The post was seated and excess removed.

Crown build up was performed freehand using Clearfil Core in all samples and the teeth were stored in saline solution for 24 h at 37 °C.

All samples were embedded in methacrylate resin (Technovit 4071; Heraeus Kulzer, Wehrheim, Germany) and placed on a slide perpendicular to the long axis of the tooth. Sections of the root were performed with a microtome saw (Exakt Apparatebau, Norderstedt, Germany) at 1, 4 and 7 mm below the cemento-enamel junction. Each section represented the coronal, middle and apical part of the post space preparation. The resulting samples of each tooth were 2 mm thick and placed on a slide for polishing (Exakt

Mikroschleifsystem; Exakt Apparatebau, Norderstedt, Germany); the sectioned surfaces were polished with a series of silicon carbide abrasive papers (1200, 2400, 4000 grit) using running tap water as a lubricant. The samples were kept humid during the whole study.

Confocal Laser Scanning Microscopy was performed with a 'Leica TCS NT' microscope (Leica, Heidelberg, Germany). An Ar/Kr mixed gas laser was used as the light source. Excitation light had a wavelength maximum at 568 nm. The intensity of the excitation light as well as the amplification of the photomultiplier was kept constant during the investigation period. CLSM images were recorded in fluorescent mode. The detected light was conducted through a 590 nm long-pass filter, thus, fluorescent light emitted from the specimen was discriminated from reflected and scattered light. The visualized layer was selected 10 μm below the sample surface and images were recorded with an oil immersion objective (40×, numerical aperture 1.25). The size of the images recorded was 62.5×62.5 μm^2, and the resolution was 512×512 pixel.

Images were recorded at four standardized areas of each sample (Fig. 1). In order to quantify the thickness of the hybrid layer, the measurements were performed at four different locations on each image, and a mean calculated. The number of resin tags represented in the standardized images were counted.

Comparison of the materials was performed by calculating means for the different teeth amongst the sections and the measuring points. Thus, per tooth only one aggregated measurement entered the statistical analysis.

Due to the unequal variances of measurements between different groups, nonparametrical analyses were chosen for comparisons between groups. Overall comparisons were performed using the Kruskal–Wallis test. In case of significance, pairwise comparisons were performed using the Mann–Whitney test. The Bonferroni correction (factor 10 for all pairwise comparisons) was used to correct for multiplicity. Intraindividual comparisons within teeth were performed using a linear model, and adjusting for dependency of data from the same tooth by the use of generalized estimating equations. The level of statistical significance was set at $P = 0.05$ (two-sided). All data of the measurements were subjected to the statistical software package SPSS 11.5 (SPSS Inc., Chicago, IL, USA).

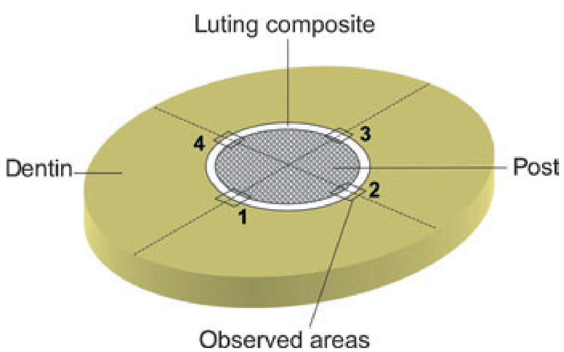

Figure 1 Preparation of specimen. The measurements were taken at point 1–4 of the sample.

Results

Overall significant differences between the materials were observed for the number of resin tags and the mean thickness of the hybrid layer (both $P < 0.005$).

Hybrid layer thickness

No significant differences could be found between Clearfil (New Bond) and Variolink II (Excite DSC) regarding the thickness of the hybrid layer and the number of resin tags. The mean thickness of the hybrid layer, which was measured at 16 points of each sample (4 points per image) was 5.45 μm (SD 1.21 μm) for Clearfil and 4.33 μm (SD 1.19 μm) for Variolink.

Number of resin tags

The mean values for the number of resin tags were 14.51 (SD 2.61) for the material Clearfil and 13.43 (SD 1.28) for Variolink. Clearfil had significantly higher values for the number of resin tags and the mean thickness of the hybrid layer than Multilink ($P < 0.005$), Panavia 21 ($P < 0.005$) and PermaFlo DC ($P = 0.02$ for the number of resin tags and $P = 0.05$ for the mean thickness of the hybrid layer). Multilink showed significantly more resin tags than Panavia 21 ($P = 0.02$), but there was no difference with regard to the thickness of the hybrid layer. No significant difference could be shown for the number of resin tags between Multilink and PermaFlo DC, but the mean thickness of the hybrid layer of PermaFlo DC was significantly greater ($P = 0.02$). The results of both values measured with Panavia 21 were significantly lower compared with PermaFlo DC and Variolink. The P-values were $P < 0.005$ other than $P = 0.02$ for the value 'number of resin tags' of Panavia 21 compared with PermaFlo DC. The number of resin tags of Variolink was significantly higher ($P = 0.02$) than those of PermaFlo DC, but there was no significant difference in the thickness of the hybrid layer ($P = 0.165$). A comparison of all materials used in this study regarding the thickness of the hybrid layer and the number of resin tags is shown in Figs 2 and 3.

Location

The number of tags was significantly higher in the coronal region of the prepared root canal space than in the middle and apical regions for each material ($P < 0.001$; linear model). The thickness of the hybrid layer was not affected by location in the root canal. The evaluation of a correlation between the number of resin tags and the thickness of the hybrid layer in the same tooth was adjusted by generalized estimating equations. Clearfil ($P < 0.001$), Panavia 21 ($P = 0.016$) and PermaFlo DC ($P = 0.016$) had a significant correlation of the two variables; this could not be shown for Multilink ($P = 0.35$) and Variolink ($P = 0.27$).

Discussion

One of the main advantages of using the CLSM for evaluating the adhesive layer of different bonding agents is that samples can be kept humid during the

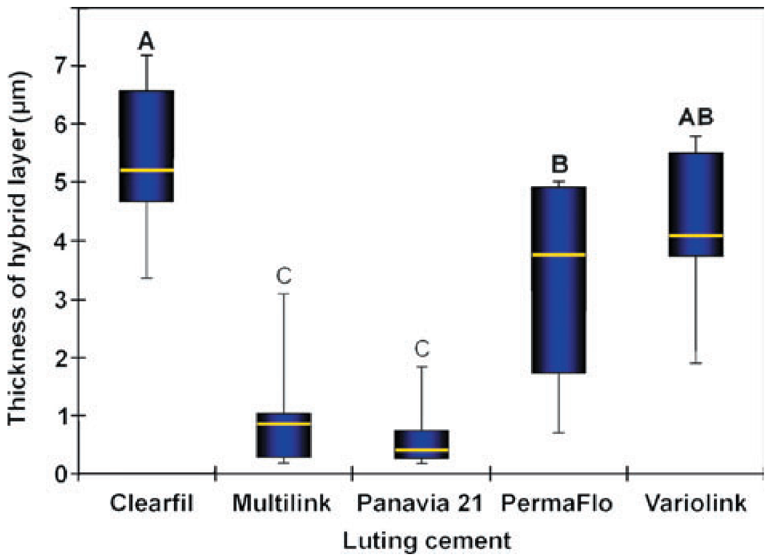

Figure 2 Box-and-whiskers plot showing the thickness of the hybrid layer of the different observed adhesive systems and corresponding luting cements. Different letters indicate significant differences ($P \leq 0.05$).

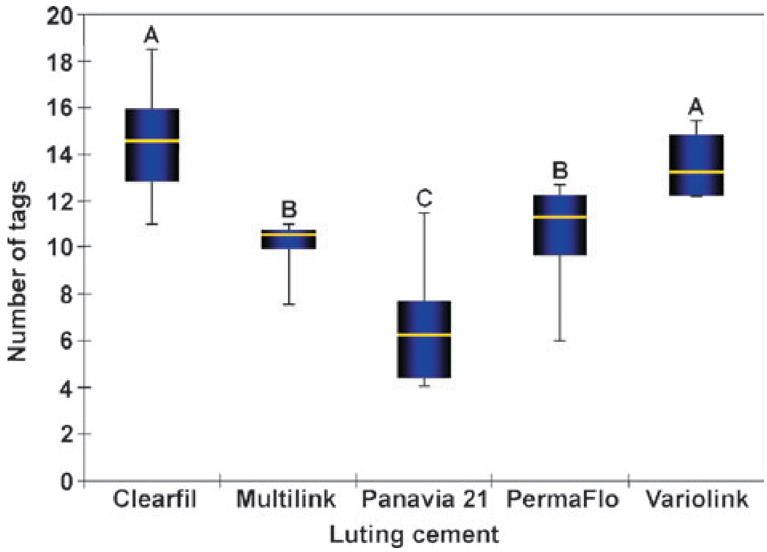

Figure 3 Box-and-whiskers plot giving the number of resin tags of the different adhesive systems and corresponding luting cements. Different letters indicate significant differences ($P < 0.05$).

examination. As drying of the samples is not necessary, this leads to a decreased risk of shrinking artefacts. Furthermore, the subsurface can be analysed without destroying the specimen, thus preparation artefacts can also be excluded.

Studies of the morphology of root canal dentine showed variations in the structure like accessory root canals, areas of resorption, embedded and free pulp stones, and varying amounts of irregular secondary dentine. Some areas were devoid of dentinal tubules (Mjör *et al.* 2001). These irregular features lead to different requirements for bonding to root canal dentine compared with coronal dentine.

The interdiffusion zone of demineralized intertubular and peritubular dentine and polymerized resin was described first by Nakabayashi (Nakabayashi *et al.* 1982) and is essential for an ideal bond to dentine (Nakabayashi *et al.* 1992). Penetration of monomers into dentinal tubules are called 'resin tags'. The penetration of resin to the depths of the demineralized dentinal tubules produced by the etching agent is an important feature in reducing microleakage (Titley *et al.* 1995). The formation of the hybrid layer is dependent on the penetration qualities and surface behaviour of various dentine bonding agents and on the condition and permeability of the dentinal surface (Walshaw & McComb 1994).

Hybrid layer thickness in root canal dentine has not been extensively reported in the dental literature. In the present study a hybrid layer was detectable within all materials tested, even if the thickness of the self-etching adhesives 'Multilink' and 'Panavia, ED Primer' was small. It has been previously described that there is a difference in the degree of substance exchange between 'etch and rinse' and 'self-etch' adhesives. The exchange intensity induced by etch and rinse adhesives exceeds that of self-etch adhesives (Van Meerbeek *et al.* 2003). This could explain the small hybrid layer thickness of the self-etching adhesives observed in the present investigation. Figure 4 shows a representative CLSM image of the self-etch adhesive 'Multilink'.

The mean thickness of hybrid layers for the materials 'PermaFlo DC', 'Variolink/Excite' and 'Clearfil/New Bond' were in a range from 3.78 to 5.2 µm. A CLSM image of the total etch adhesive system 'New Bond/Clearfil' is shown in Fig. 5. These values are in accordance with the observations described by Pioch *et al.* (1996) who found a thickness of the hybrid layer with Optibond between 5 and 8 µm, whilst the material Scotchbond MP had a thickness between 3 and 4 µm. In another investigation the same group observed the thickness of the hybrid layer in correlation with the etching time (Pioch *et al.* 1998). The thickness of the hybrid layer increased with prolonged etching time; for a 15-s etching period the mean thickness of the hybrid layer was 1.89 µm for the adhesive system 'Syntac'

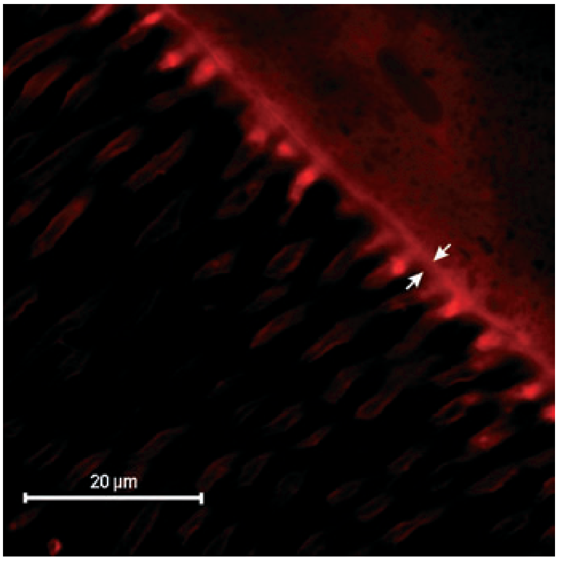

Figure 4 CLSM image of the self-etching adhesive system 'Multilink'. Penetration of the primer into the dentineal tubules (resin tags) and a thin hybrid layer (arrows) are visible in this sample.

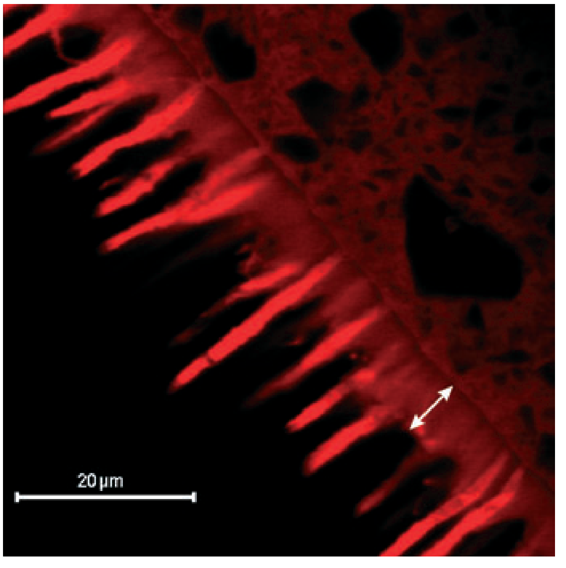

Figure 5 The total etch adhesive system 'New Bond/Clearfil' represented in this CLSM image showed numerous resin tags and a uniform hybrid layer (arrows).

and 2.34 μm for 'Gluma CPS'. These values were smaller than the results of the present study.

Compared with this study similar results could be shown for the bonding agent All Bond 2 in an SEM investigation (Walshaw & McComb 1994). The thickness of the hybrid layer was 5–8 μm when the dentine was conditioned with 10% phosphoric acid. When using the material Scotchbond MP the dentine was conditioned with 10% maleic acid and the thickness of the hybrid layer was 1–2 μm.

In contrast to the findings of the present study Ferrari *et al.* (2000) reported a significant decrease in hybrid layer thickness from coronal to apical. The thickness of the layer of 'All Bond 2 Primer A & B' in the coronal region was 4.2 μm decreasing to 2.5 μm in the middle region and to 1.2 μm in the apical region of the root canal (Ferrari *et al.* 2000). Furthermore, the values of the hybrid layer thickness in that study were smaller than the present findings. In that study, SEM was used which might explain the reduced thickness of hybrid layer, as SEM sample preparation techniques can result in a shrinkage of the bottom half of the hybrid layer, which is often poorly infiltrated. This could lead to a reduced thickness of the hybrid layer in SEM images (Dörfer *et al.* 2000).

The results of the present study showed a significant decrease in the number of resin tags from the coronal to the apical region of the prepared root canal space. This is in accordance with the findings of Ferrari *et al.* (2000), who observed a significantly higher density of dentinal tubules in the coronal third of the root canal than in the middle and apical thirds. Mjör *et al.* (2001) also reported a decreased number of dentineal tubules per mm^2 from about 40 000 in the coronal region of the root canal to 14 400 in the apical region. The authors concluded that the hybrid layer would be more important for adhesion to apical dentine than resin tag formation, because fewer tags are available for resin penetration in this area.

A quality of acetone and ethanol solvents is their action as a hydrophilic carrier and their ability to lower viscosity of bonding agent to enhance penetration into demineralized, collagen-rich dentine. The solvent of the adhesive systems 'New Bond', 'Excite DSC' and 'PermaFlo Primer A & B' used in this investigation was ethanol. 'PermaFlo' also contains acetone. The solvent of the self-etching adhesives 'ED Primer' and 'Multilink Primer' was water which might be a reason for the reduced hybrid layer thickness of these adhesive systems. Another reason might be the more effective demineralization ability of phosphoric acid compared with the self-etching adhesives which allowed a deeper penetration of the adhesive into the dentine. In terms of clinical relevance it has been shown that the thickness of the hybrid layer of mild 'self-etch' adhesives is much smaller than that produced by the strong 'self-etch' or 'etch and rinse' approach, but this has been proven to be of minor importance for effectiveness of bonding (Inoue *et al.* 2001).

Other authors also speculated that as long as the collagen fibrils are optimally infiltrated with resin the hybrid layer does not have to be thick to be strong (Prati *et al.* 1998). Furthermore, other questions have been raised about the importance of the thickness of the hybrid layer. One fact is that prolonged etching times resulted in an increase of the thickness of the hybrid layer but a decrease in bond strengths (Pioch *et al.* 1998, Hashimoto *et al.* 2000). The reason for this might be inadequate hybridization of dentine resulting in a reduction of bond strength; therefore, a complete impregnation of the adhesive resin into the open space of the demineralized dentine is a necessary requirement for high bond strengths.

Another reason for the reduced hybrid layer thickness of the self-etching adhesives may be the inability to penetrate through thick smear layers, which are typical for prepared root canals. Furthermore, it might be speculated that the acidity of the primer could be buffered by the mineral components of the smear layer. This could lead to a reduction of the primer penetration into the underlying dentine (Itou *et al.* 1994).

Tay *et al.* (2000) reported that the thickness of the smear layer has no influence on the adhesive capacity of self-etching agents. Regardless of the thickness of the smear layer the authors observed in all tested groups an authentic hybrid layer of 0.4–0.5 μm. This investigation was performed with an artificial smear layer produced with abrasive papers of different surface roughness which could be of a different kind compared with the smear layer in the root canal. Nevertheless, these data are similar to the present findings, where the mean thickness of the hybrid layer in the Multilink and Panavia groups were 0.41 and 0.85 μm, respectively.

The hypothesis of this study that there would be differences in the penetrating ability of the used adhesive systems was confirmed. The hybrid layer thickness and the number of resin tags of the bonding agents differed significantly amongst the groups. A complete and deep infiltration of the adhesive system into root canal dentine with numerous resin tags, which has been shown for the adhesive systems

'Clearfil New Bond' and 'Excite DSC', is more likely to predict a durable bond of the post to the root canal dentine than the other systems. A previously published study evaluated the microleakage of root filled teeth restored with fibre posts luted with different resin cements (Mannocci *et al.* 2001). Teeth restored with a three-step dental adhesive including conditioning of the root canal dentine with phosphoric acid leaked significantly less than those restored with a self-etching primer. The reason for this might be that the multiple-stage adhesive system was able to produce a more uniform and thick resin–dentine interdiffusion zone than the self-etching primer. This also underlines the importance of a complete and deep infiltration of the adhesive system into the root canal dentine.

Conclusion

Within the limitations of this *in vitro* study a more complete infiltration of adhesive into root canal dentine was achieved after conditioning dentine with phosphoric acid.

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