Effect of dentinal tubule orientation on the modulus of elasticity of resin-infiltrated demineralized dentin

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The effect of tubule orientation of dentin on the elastic modulus of resin-infiltrated dentin was evaluated. Rectangular cylindrical-shaped dentin specimens with their long axis parallel to and perpendicular to dentinal tubules were prepared from extracted premolars. Twenty-five mineralized specimens of each orientation were evaluated. The remaining specimens were then demineralized. The demineralized specimens and the demineralized following by infiltration with one of these adhesives; Optibond Solo Plus, Single Bond 2 or Prime & Bond NT, from each orientation were evaluated (25 specimens per group). The tubular orientation only affected the elastic modulus of mineralized dentin. The highest elastic modulus was observed for the mineralized dentin when the tensile force was applied parallel to the direction of tubules. The elastic modulus of demineralized dentin was the lowest. The adhesive resins increased the elastic modulus of demineralized dentin, but the differences among the three were insignificant.

Keywords: Mineralised dentin, Demineralised dentin, Resin-infiltrated demineralised dentin, Elastic modulus

INTRODUCTION

Dentin is a major component of teeth. It surrounds the pulp, is covered by enamel, and consists of channels called dentinal tubules. Dentinal tubules are arranged in different directions depending on their positions in the tooth. In coronal dentin, dentinal tubules are curved in the shape of an “s”. In radicular dentin, they are arranged in straight lines. By weight, dentin consists of inorganic material (70%), organic material (20%) and water (10%). The organic material consists of collagen, especially type I (91–92%), and non-collagen proteins (8–9%). The collagen is the primary scaffold for the inorganic components of dentin.

Currently, large numbers of class V cervical lesions are non-carious and have wedge-shaped cavities. These cavities consist of two walls: The occlusal wall includes enamel margin and the dentinal tubules with parallel to the cavity wall. The gingival wall involves dentin margin and the dentinal tubules are perpendicular.

A resin composite with adhesive is always used to restore non-carious cervical lesions. The adhesion between resin and dentin relies on a structure called the hybrid layer. The hybrid layer is demineralized dentin that is infused with resin. To demineralize the dentin in total-etching adhesive systems, phosphoric acid is applied to dissolve the mineral components, exposing a network of collagen fibers. This collagen mesh is then filled with resin, forming the hybrid layer.

The failure of class V restorations with composite resin is still observed. Failures are often caused by leakage at the margins of the restoration. The degree of leakage at the gingival margin is generally greater than at the occlusal margin. Such failure may be caused by the different nature of the margins of a class V cavity, though the different orientation of dentinal tubules may be another factor. A recent study has shown that the orientation of dentinal tubules affects the bond strength of adhesive resin to dentin. The adhesion of resin to parallel-oriented dentinal tubules was stronger than the adhesion to perpendicular-oriented ones, even though some studies have demonstrated that the tubule orientation has no effect to bond strength.

An exhaustive understanding of structural properties related to adhesive restorations and a characterization of the dental hard tissues replaced by adhesive restorations are required to develop new materials.

This study aimed to evaluate the effect of the orientation of dentinal tubules on the elastic modulus of mineralized demineralized specimens reinforced with adhesives. Three types of total-etching adhesives were studied. The hypotheses were as follows: 1) the different orientations of the dentinal tubules would have no effect on the modulus of elasticity of demineralized specimens infiltrated with adhesives, and 2) the different types of adhesives would have no effect on the elastic modulus of demineralized specimens infiltrated with adhesives.
saline solution at 4°C and used within 1 month after extraction.

The specimens were prepared from the cervical part of buccal surface of the teeth. A cylinder was shaped with a rectangular cross-sectional area of approximately 0.5×0.5 mm and a length of about 5 mm using a slow-speed diamond saw under running water (Isomet saw, Buehler Ltd., Lake Bluff, IL, USA). The preparation of specimens from different parts of the teeth is shown in Fig. 1. The area of preparation simulates the cavity wall of a class V lesion. In specimens from the occlusal wall, the dentinal tubules ran parallel to the long axis. In specimens from the gingival wall, the dentinal tubules ran perpendicular to the long axis.

From the 125 premolar teeth, 125 specimens were prepared with dentinal tubules parallel to the long axis and 125 with dentinal tubules perpendicular to the long axis. The orientation of dentinal tubules for each group was confirmed using a microscope (Measuring microscope, Nikon, Tokyo, Japan). The specimens that did not match the criteria were eliminated and replaced with new specimens. Nail varnish was applied to both ends of specimen to limit the testing area to 0.5×0.5×3.0 mm.

Each group of 125 specimens was randomly divided into five subgroups (n=25). The first subgroup was used to test the modulus of elasticity of mineralized dentin. The modulus of elasticity was determined using a universal testing machine (LF Plus Lloyd Instrument, Ametek, Hamspre, UK) with a crosshead speed of 0.2 mm/min.

The remaining 200 specimens (100 with dentinal tubules parallel to the long axis and 100 with dentinal tubules perpendicular to the long axis) were stirred in a demineralizing solution of 0.5 M EDTA (pH 7.4) for 4 days at 4°C. To ensure the complete dissolution of minerals, the demineralised specimens were analysed with radiography. The radiodensity of the specimens were determined by a low-voltage X-ray unit. The modulus of elasticity was then measured for 25 specimens from each group.

The remaining demineralized specimens were immersed in 50% hydroethylmethacrylate (HEMA) in water for 8 h, followed by 100% HEMA for 8 h, and fresh 100% HEMA for another 8 h to remove water from the specimens. This process was performed to ensure that the strongest resin-infiltrated demineralized dentine would be created. The 75 remaining specimens from each group were divided into three subgroups of 25, and each subgroup was immersed in one of the following resin adhesives: Optibond Solo Plus (Kerr, Philadelphia, USA), Prime & Bond NT (Dentsply, Konstant, Germany) or Adper Single Bond 2 (3M ESPE, Minnesota, USA). The selected adhesives normally use HEMA in their formulation, which had the different compositions, solvents and types of filler. The components in the adhesive systems are shown in Table 1. The specimens were totally soaked in adhesive for 12 h and kept in the dark sealed chamber for all immersed time. For the first 8 h, all specimens were soaked in adhesive resin to eliminate the effect of remained HEMA. Then, the

![Fig. 1 The different origins of specimens containing parallel and perpendicular dentinal tubules.](image)

### Table 1 Composition of materials used in this experiment

| Materials                  | Manufacturer             | Components                                                                 |
|----------------------------|--------------------------|-----------------------------------------------------------------------------|
| Optibond Solo Plus (OSP)   | Kerr, Philadelphia, USA  | Bis-GMA, GPDM, HEMA, sodium hexafluorosilicate, barium glass, silica, ethanol |
| Prime & Bond NT (PBN)      | Dentsply, Konstant, Germany | Bis-GMA, HEMA, BPDM, acetone, di-trimethylacrylate resin, PENTA, functionalised amorphous silica, photoinitiators, cetylamine hydrofluoride |
| Adper Single Bond 2 (ASB)  | 3M ESPE, Minnesota, USA  | Bis-GMA, HEMA, ethanol, water, silane treated silica                         |

Abbreviations: Bis-GMA: bisphenol-A-glycidyl methacrylate, GPDM: glycerophosphate dimethacrylate, HEMA: 2-hydroethyl methacrylate
Multiple values with the same superscript indicate no statistically significant difference (\( p > 0.05 \)).

| Orientation of dentinal tubules | Mineralized specimens | Demineralized specimens | Demineralized with adhesive resins infiltrated specimens |
|--------------------------------|-----------------------|-------------------------|--------------------------------------------------------|
|                               |                       |                         | OSP | PBN | ASB |
| 1. Perpendicular              | 1055.51±144.56        | 166.08±80.52            | 521.10±118.29 | 580.56±292.26 | 499.21±273.68 |
| 2. Parallel                   | 2760.51±2073.67       | 163.33±97.26            | 548.11±182.69 | 477.02±195.44 | 617.17±246.15 |

Table 2 Elastic modulus (MPa) measurements of specimens with perpendicular and parallel dentinal tubules

The modulus of elasticity of the specimens was measured by gripping the specimen with a customized testing apparatus 1 mm from each end, leaving 3 mm of specimen for gauge length. The testing apparatus was placed on the testing machine, and the tensile force was applied with the crosshead speed of 0.2 mm/min. The modulus of elasticity was calculated and recorded in megapascal (MPa) by the attached computer with Nexegen software (LF Plus Lloyd Instrument, Ametek, Hampshire, UK). The specimens were kept moist with water during testing.

Data were statistically analyzed by testing the difference in the modulus of elasticity between the groups using analysis of variance (two-way ANOVA) and testing for multiple comparisons with Dunnett T3 with a 95% confidence level.

RESULTS

The statistical analysis revealed that data were normally distributed by using the Kolmogorov-Smirnov Test at a 95% confidence level (\( p > 0.05 \)). There was no equality of variance of the population according to Levene’s test at a 95% confidence level (\( p < 0.05 \)).

By testing the difference of means, the statistical analysis of parametric data using a two-way ANOVA showed that the elastic modulus was affected by both the orientation of dentinal tubules and the types of specimens (mineralized specimens, demineralized specimens, and demineralized specimens infiltrated with adhesive resins) (\( p < 0.01 \)). The effect of the orientation of dentinal tubules and the types of specimens on the elastic modulus was demonstrated (\( p < 0.01 \)). The means of the elastic modulus and the difference in means of elastic modulus between the groups tested by Dunnett T3 multiple comparisons are shown in Table 2.

The maximum elastic modulus tested by tensile force was measured parallel with the orientation of the dentinal tubules in mineralized specimens. This finding was significantly higher than that of specimens tested by tensile force perpendicular to the orientation of dentinal tubules in mineralized specimens.

No statistically significant difference was observed in the elastic modulus among the demineralized specimens infiltrated with different adhesive resins. The orientation of the dentinal tubules also had no statistically significant effect. The elastic moduli of all such groups were significantly less than the elastic modulus of the mineralized specimens.

The elastic modulus of the demineralized specimens was the lowest when compared with other groups. No statistically significant difference was observed in the elastic modulus due to the orientation of the dentinal tubules for these specimens.

DISCUSSION

The results revealed that the elastic modulus of mineralized dentin with perpendicular and parallel dentinal tubules orientation was significantly different (\( p = 0.016 \)) at 1055.51±144.56 and 2760.51±2073.67 MPa, respectively. The results of this study are consistent with experiments by Sano et al.\(^ {11} \). In addition, there was a report that the elastic modulus and ultimate tensile strength were strongly correlated (\( p < 0.001 \))\(^ {12} \). Our results might support the findings of Inoue et al. in 2003\(^ {13} \) and Miguez et al. in 2004\(^ {14} \), which showed a difference in tensile strength between dentin specimens with different dentinal tubules orientation.

The elastic modulus of demineralized dentin with perpendicular and parallel tubular orientation to the tensile force was not significantly different (\( p = 1.00 \)) at 166.08±80.52 and 163.33±97.26 MPa, respectively. These results are consistent with the results of Nishitani et al. in 2005\(^ {15} \) obtained using the dentin of molar teeth. They found that the tensile strength of demineralised dentin with perpendicular and parallel dentinal tubules was not significant different (\( p = 0.252 \)). This result indicated that the directions of the dentinal tubules after demineralization did not affect the strength of the dentin. This finding is most likely due to the
collagen framework of dentin. The collagen structure in dentin is not well organized and is linked together in all directions\textsuperscript{[16,18]}\textsuperscript{.} This finding may explain why the elastic modulus is the same when measured in different directions.

Significant differences were observed when comparing the elastic modulus of mineralized dentin and demineralized dentin for both parallel and perpendicular orientation of dentinal tubules. The mineralized groups demonstrated a higher modulus of elasticity than the demineralized groups. The results were consistent with the results of Sano \textit{et al.}\textsuperscript{[11]}. That study showed that the modulus of elasticity of demineralized dentin was less than that of mineralized dentin.

When the mineral content of dentine is dissolved, the collagen mesh is left. The remaining collagen mesh of demineralized dentin has been shown to be weaker after the minerals of dentin are dissolved\textsuperscript{[18]}. This might reduce the modulus of elasticity of demineralized dentin when compared with perfect teeth containing minerals. This finding implies that the dentin undergoes a dramatic loss of strength when it is demineralized during the acid-conditioning step common in total-etching adhesive systems.

To make the structure of the demineralized dentin stronger, it was reinforced with resin. These resin-infiltrated structures are used for adhesion between the dentin and composite resin\textsuperscript{[4,6]}. Therefore, if the collagen mesh of demineralized dentin is not perfectly infiltrated with adhesive resins, the defect area will be weak and the collagen at the boundary between the composite resin and dentin may be prone to degradation\textsuperscript{[8,10]}.

In demineralized dentin reinforced with resin, the direction of tubules had no effect on the elastic modulus. Therefore, our first null hypothesis was accepted. This finding is probably due to the dissolution of minerals in dentin. The arrangement of collagen fibers to form tubular structure was observed. Collagen fibers are cross-linked together and stretch out of peritubular dentine\textsuperscript{[17]}. Thus, a previous study demonstrated no significant difference in the mechanical properties of demineralized dentin measured in all directions\textsuperscript{[15]}. These results are consistent with our observations of demineralized dentin with adhesive resin reinforcement. Our results show that the elastic modulus did not depend on the direction of the dentinal tubules in resin-infiltrated demineralized dentin.

In addition, the study also found that the types of resin adhesive did not affect the modulus of elasticity of resin-infiltrated demineralized dentin for both tubular orientations. Our second null hypothesis was also accepted. The types of resin adhesives used in this study were Optibond Solo Plus, Prime & Bond NT and Adper Single Bond 2, which had different compositions such as solvents, and types of filler including the quantity and size. However, the main resin monomer in each brand is Bis-GMA, which is a polymerizable polymer chain, affecting the mechanical properties of resin-infiltrated dentin. Presumably, this caused no significant difference in the elastic modulus of resin-infiltrated demineralized dentin among the different adhesive resin.

In our study, the resin-infiltrated demineralized dentin was light irradiated for 1 min, which is longer time than the manufacturers’ instruction and didn’t stimulate the clinical situation. This prolonged irradiation time was performed to eliminate the effect of incomplete adhesive polymerization. Therefore, further study should be established to evaluate the effect of dentinal tubule orientation on the elastic modulus and adhesive polymerization when all adhesive resins were light irradiated as the manufacturers’ instruction or clinical situation and also contained different main resin monomer such as silorane-based composite system.

Moreover, different types of solvents, such as acetone used in Prime & Bond NT and alcohol used in Adper Single Bond 2 and Optibond Solo Plus, did not affect the modulus elasticity of specimens in the study. This result was probably due to the fact that specimens were immersed in the adhesive resins for 4 h, during which the solvent may have evaporated.

In addition, it has been proposed that differences in the mechanical properties of various resin adhesives may be due to different types of resin, quantity and size of filler\textsuperscript{[20]}. Filler content in the adhesive resins Optibond Solo Plus, Single Bond 2 and Prime & Bond NT are 15%, 10% and 10% by volume, respectively\textsuperscript{[21,23]}. Previous research demonstrated that the different amounts of filler corresponded to different mechanical properties of adhesive resins\textsuperscript{[20]}. The size of filler particles in the adhesive resins Optibond Solo Plus, Single Bond 2 and Prime & Bond NT are 400 nm, 70 nm and 50 nm, respectively\textsuperscript{[21,23]}. Those sizes are larger than the 20 nm space between collagen fibers. Filler in adhesive resins should be smaller than 20 nm to be able to penetrate into the dentinal tubules and spaces within networks of collagen after the acid etching\textsuperscript{[20]}. Because the sizes of filler in selected adhesives were larger than the space between collagen fibers, it might be not able to penetrate into the spaces within the collagen mesh. The results of this study were supported by Tay \textit{et al.} in 1999\textsuperscript{[20]}\textsuperscript{.} That study used Prime & Bond NT to penetrate into the demineralized dentin. They found that the filler was larger than the spaces between the collagen and so could not penetrate into the space\textsuperscript{[20]}. There was no relationship between the amount of filler and the strength of resin-infiltrated demineralized dentin in this study. This finding is consistent with the research of Sano \textit{et al.} in 1995\textsuperscript{[15]}. Increasing the strength of the adhesive resin layer can be done by increasing filler content, which does not affect the strength of resin-infiltrated demineralized dentin when the size of the filler is larger than the spaces in the collagen network.

The resin-infiltrated demineralized dentin is comparable to the hybrid layer, which is important in the adhesion between composite resin and dentin. However, the results of this study were obtained under conditions far from those of clinical implication. They should not be extrapolated to values obtained at the interfaces.
between dentin and adhesive resins in typical in vitro dentin-bonding studies.

CONCLUSION
The elastic modulus of dentin was affected by the mineral content and adhesive resin, which penetrated into demineralized dentin. The orientations of dentinal tubules influenced the elastic modulus only for mineralized dentin. The types of adhesive resins used in this study had no effect on the elastic modulus of resin-infiltrated demineralized dentin.

The elastic modulus of resin-infiltrated demineralized resin is higher than that of demineralized dentin. The resin reinforcement of demineralized dentin improves its elastic modulus. Incomplete resin infiltration into demineralised dentin following an acid-etching step creates a weak point at the resin-dentine interface that may be prone to degradation.

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