

Change of dentin permeability in different storage media after resin coating

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Purpose: The objective of this study was to evaluate the dentin permeability with/without resin coating in different storage media and periods.

Materials and Methods: Prepared bovine dentin disks were divided into three groups; 1) left uncoated as a control, 2) resin-coated with Clearfil SE Bond only, and 3) coated with the combination of SE and Clearfil Protect Liner F. The hydraulic conductance of each specimen was measured after storage in either deionized water or artificial saliva. The hydraulic conductance value was expressed as the percentage of baseline hydraulic conductance.

Results: For the control group, the mean percentage of hydraulic conductance to baseline hydraulic conductance after 1, 30, and 90 days were 103.7%, 126.0%, and 128.6% in deionized water, and 92.5%, 64.4%, and 62.2% in artificial saliva, respectively. The mean percentage of hydraulic conductance to baseline hydraulic conductance of coated with the combination of SE and Clearfil Protect Liner F after 1 day in artificial saliva yielded the lowest (2.5%) among all the groups.

Conclusion: Application of the resin coating to dentin surface demonstrated remarkable reduction of dentin permeability over time.

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Key Words: artificial saliva, dentin permeability, hydraulic conductance, resin coating, storage media

Introduction

The post-operative sensitivity and pulpal irritation of the indirect restorations are major drawbacks in clinical dentistry. The protection of the dentin and pulp from the mechanical, chemical, and biological stimuli exerts clinical benefits for the patients. According to the widely accepted Brannstrom's hydrodynamic theory, the exposed dentin provokes the movement of intertubular fluid, which activates pulpal nerves and causes pain.¹ Based on the theory, reduction of dentin permeability leads the reduction of post-operative sensitivity and pulpal irritation. Various materials *i.e.* dentin desensitizers, varnishes, topical fluorides, and strontium chloride have been used to mitigate dentin sensitivity.²⁻⁵ Some authors have used dentin bonding systems to reduce the dentin permeability by sealing dentinal tubules.^{6,7}

A resin-coating technique was introduced in the early 1990's in order to overcome the problems of post-operative sensitivity and pulpal irritation in preparations of the inlay/onlay and fullcrown restorations.⁸ In this technique, after cavity preparation for indirect restoration and before taking an impression, the prepared tooth surface is covered by the resin coating material, which is a dentin bonding system or a combination of a dentin bonding system and a flowable resin composite. It was demonstrated that the resin coating technique improved the dentin bonding performance of resin cements,⁹⁻¹³ and prevented marginal leakage beneath the restorations.^{14,15} Nowadays thin-film coating materials have been developed for crown preparations. The resin coating technique is also effective to reduce coronal leakage of the endodontically treated teeth.¹⁶

Several studies showed that the dentin adhesive materials are effective to seal the exposed dentin and

reduce the dentin permeability.^{6,17} On the other hand, it has been reported that adhesive materials absorb water,¹⁸ which may affect dentin permeability after resin coating. Deionized water has been often used as a storage media for measurement of dentin permeability;^{19,20} however, under clinical situation the coating materials are likely to be exposed to saliva with contains a considerable amount of minerals and affect water sorption and permeability in a different manner. Thus, the effects of artificial saliva as a storage media to simulate the oral cavity environment should be investigated. However, there was little information in the literature about the effects of different storage media and coating materials on dentin permeability with the lapse of time.

Thus, the objective of this study was to evaluate the dentin permeability with/without resin coating in different storage media and periods. The null hypothesis tested was that coating material, storage media or storage period did not affect dentin permeability.

Materials and Methods

Specimen preparation

Thirty-six bovine incisors, stored frozen, were used in this study. The teeth were put in room temperature for 1 day before specimen preparation and were kept moist to prevent dehydration. They were hand-scaled to remove soft tissue remnants. The labial surfaces of the teeth were ground to expose the dentin using a model trimmer (Y-230, Yoshida, Tokyo, Japan). Then dentin disks (approximately 10 mm in diameter and 0.75 mm in thickness) were prepared using a slow-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) and a diamond point (F102R, GC, Tokyo, Japan) under running water. Dentin disks were ground flat and a homogeneous smear layer was created using 600-grit silicon carbide paper under running water for 30 s. The specimens were then cleaned in distilled water in an ultrasonic cleaner for 3 min to remove any debris and the smear layer. Subsequently, the permeability of each dentin specimen at baseline was measured according to the procedures as described below. The specimens were randomly allocated to three groups according to the following dentin treatments: 1) dentin surface was left uncoated (control); 2) coated with a self-etching adhesive system, Clearfil SE Bond (SE, Kuraray Noritake Dental, Tokyo, Japan) (SE); and 3) dentin was coated with a combination of SE and a micro-filled low-viscosity resin composite, Clearfil Protect Liner F (PLF, Kuraray Noritake Dental) (SE+PLF).

Table 1. Materials used in this study

Materials	Code	Lot No.	Compositions	Directions
Clearfil SE Bond	SE	011580	Primer: 10-MDP, HEMA, hydrophilic dimethacrylate, photo-initiator, water Bond: 10-MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, photo-initiator, silanated colloidal silica	Apply for 20 s, blow air gently. Apply, blow air mildly, and light cure for 10 s.
Clearfil Protect Liner F	PLF	0073DA	Bis-GMA, TEGDMA, fluoride-methyl methacrylate, camphorquinone, silanated colloidal silica, organic filler	Light cure for 20 s.

All materials are manufactured by Kuraray Noritake Dental, Tokyo, Japan.

Abbreviations: 10-MDP, 10-methacryloyloxydecyl dihydrogen phosphate; HEMA, 2-hydroxyethyl methacrylate; Bis-GMA, bis-phenol A diglycidylmethacrylate; TEGDMA, triethylene glycol dimethacrylate

Compositions and instructions of the materials used in this study were listed in Table 1. In the SE and SE+PLF groups, the SE primer was first applied to the dentin surface for 20 s and dried with gentle air blow. The SE bonding resin was then applied, dried with mild air blow and light-cured for 10 s using a halogen light

curing unit (Optilux 501, Demetron Kerr, Danbury, CT, USA). In the SE+PLF group, PLF was additionally placed on the cured SE adhesive with a micro-brush, and light cured for 20 s. Furthermore, the specimens were divided into two-subgroups according to the storage media ($n=6$). The specimens were stored at 37°C in 50 mL of either deionized water or artificial saliva (1.0 mM CaCl₂, 3.0 mM KH₂PO₄, 3.08 mM NaN₃ and 100 mM NaCl, pH 6.5) for 1, 30, and 90 days, respectively. The storage media for each group was replenished every week.

Measurement of dentin permeability

Dentin permeability was measured using a fluid filtration system working under liquid pressure of 703 cmH₂O.²¹ Each dentin disk was placed in a split-chamber device and between two rubber O-rings, which had 0.196 cm² inner surface area for infiltration of deionized water. Each dentin disk was coated outside of the area of O-ring with two-layers of nail varnish to maintain the exact position of the disk for repeated measurements. The rate of fluid movement across dentin was measured by following the progress of a small bubble within 1.14 mm inner-diameter micro-capillary tube filled with deionized water lying on the top of a millimeter scale. The movement of the air bubble was metered every 5 min for successive 15 min. The average of the results of every 5 min was then calculated into hydraulic conductance (Lp) using the following equation:

$$Lp = \frac{Q}{P \times A}$$

where Lp is hydraulic conductance in $\mu\text{L min}^{-1} \text{cm}^{-2} \text{cmH}_2\text{O}^{-1}$, Q is fluid flow in μL per min, P is hydrostatic pressure across dentin in cmH₂O and A is the dentin surface area in cm².

Scanning electron microscopic (SEM) observations

The dentin disks of the coating groups (SE and SE+PLF) were prepared in the same manner as described in the specimen preparations for the dentin permeability measurement. Unpolymerized oxygen-inhibition layer of the coating layer on the dentin surface was removed with alcohol-soaked cotton pellets. The dentin disks were then embedded in an epoxy resin (Epoxicure, Buehler). After curing the epoxy resin, the specimens were sectioned perpendicularly to the dentin surface using a low-speed diamond saw (Isomet) to obtain approximately 1 mm thick slices. Each slice was subsequently polished with silicone carbide papers followed by diamond pastes (DP-Paste, Struers, Ballerup, Denmark) down to 0.25 μm particle size. The specimens were cleaned ultrasonically in distilled water for 5 min at the end of each step and dried at room temperature for 24 hours. They were then subjected to argon-ion etching (EIS-1E, Elionix, Tokyo, Japan) for 6 min at 0.2 mA and 1 kV in order to disclose the interfacial structure and finally gold sputter-coated (SC-701AT, Elionix) for SEM observation. Thickness of the coating layer for the SE and SE+PLF groups was measured by using an SEM (JSM-5310LV, JEOL, Tokyo, Japan) under $\times 500$ magnification at three points of the coating layer, which were at the center of the coating layer and at a distance of 1 mm from the center on either side. The mean value of the thickness of the coating layers was calculated for each group ($n=6$).

Additionally, dentin disks without resin coating (control) were stored in either deionized water or the artificial saliva for 1, 30, and 90 days. Following the storage procedures, they were removed from the storage media and dried in a desiccator for 1 day. They were then fractured vertically with a wire cutter (YS-602, YDM, Tokyo, Japan) at the center of the disk. After gold-sputter coating, the dentin surface and inside of their dentinal tubules at the fractured dentin disks were obliquely observed under SEM at $\times 2,000$ magnification ($n=3$).

Statistical analysis

The results from dentin permeability measurement were statistically analyzed by the following methods.

The Lp value obtained immediately after ultrasonic cleaning served as the baseline Lp for each specimen. The Lp values of each coating group after 1, 30, and 90 days were expressed in percentage of the baseline Lp ($Lp\%$), respectively. The data were statistically analyzed with three-way ANOVA followed by multiple comparisons using t -test with Bonferroni corrections as *post-hoc*. The factors were coating, storage media and storage period. All the statistical procedures were performed at a 95% confidence level with using Statistics package (ver. 16 for windows SPSS, Chicago, IL, USA).

Results

Measurement of dentin permeability

The mean $Lp\%$ and standard deviation for each group are summarized in Table 2. ANOVA test revealed that $Lp\%$ values were significantly affected by the three factors; coating material, storage media, and storage period ($p < 0.001$ for each). The interaction among these three factors was also significant ($p < 0.001$). There were significant differences between the control and SE groups, between the control and SE+PLF groups, and between the SE and SE+PLF groups, respectively ($p < 0.01$). The $Lp\%$ of the control group stored in deionized water significantly increased up to 30 days ($p < 0.05$) and then plateaued ($p > 0.05$), while the $Lp\%$ of the control specimens stored in artificial saliva significantly decreased up to 30 days ($p < 0.05$) and did not show any change ($p > 0.05$) at 90 days when the measurement was repeated. For the coating groups stored in deionized water, there were significant differences between 1 day and 90 days ($p < 0.05$), while for those stored in artificial saliva there were no significant differences among 1, 30, and 90 days ($p > 0.05$). The lowest $Lp\%$ mean value was obtained in the SE+PLF after 1 day storage in artificial saliva.

Table 2. Hydraulic conductance, expressed as percentages ($Lp\%$)

Coating	Storage media	Storage time		
		1 day	30 days	90 days
Control	Deionized water	103.74 (1.69)	126.00 (12.77) ^a	128.62 (14.35) ^a
	Artificial saliva	92.53 (4.67)	64.35 (5.23) ^b	64.24 (6.91) ^b
SE	Deionized water	12.01 (5.96) ^c	18.87 (5.53) ^{c,d}	23.42 (5.82) ^d
	Artificial saliva	5.09 (2.53) ^e	7.52 (3.77) ^e	9.32 (4.51) ^e
SE+PLF	Deionized water	6.12 (1.84) ^f	10.31 (4.49) ^{f,g}	15.36 (6.70) ^g
	Artificial saliva	2.48 (1.25) ^h	3.69 (0.92) ^h	4.46 (1.51) ^h

All values are mean (SD).

In each row, the values marked by the same superscript lowercase letters are not significantly different ($p > 0.05$).

SEM observations

The SEM images of the coating layers of the SE and SE+PLF groups were shown in Fig. 1. Good adaptation was found at the interface between the coating layer and dentin in both SE and SE+PLF groups. The thickness of SE and SE+PLF was $22.1 \pm 3.7 \mu\text{m}$ and $126.0 \pm 19.5 \mu\text{m}$, respectively.

The SEM images of specimens without resin coating (control) stored in either deionized water or the artificial saliva for 1, 30, and 90 days were shown in Fig. 2. In the deionized water group, dentinal tubule orifices were open and no precipitations were observed either on the dentin surface or inside of the dentinal tubules at any storage period (Figs. 2a-c). On the other hands, some precipitations were observed on the dentin surface in the artificial saliva group, which gradually increased over the storage periods. However, the precipitations were not observed inside the dentinal tubules (Figs. 2d-f).

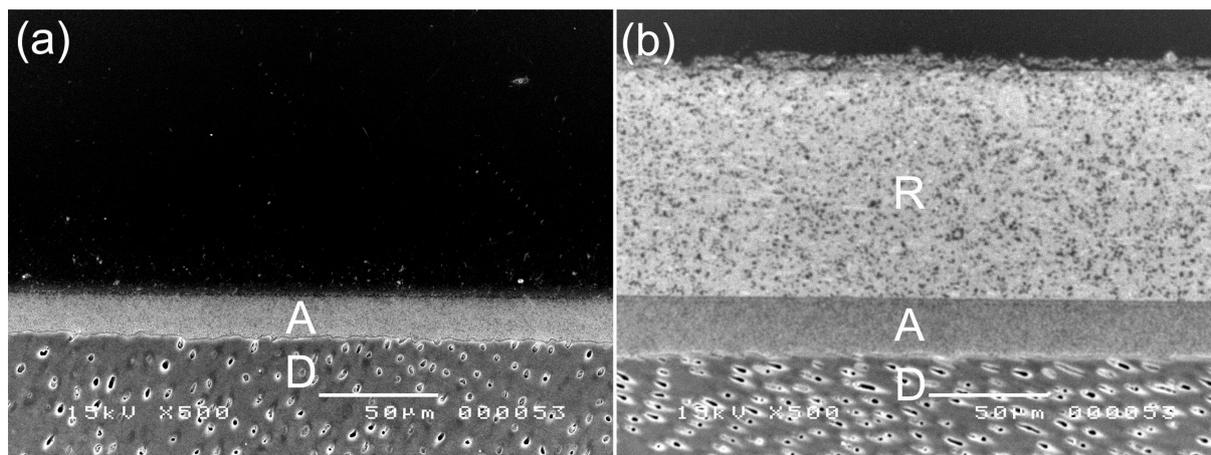


Fig. 1 SEM images of the dentin surface with SE (a) and SE+PLF (b): A, adhesive; D, dentin; R, resin composite

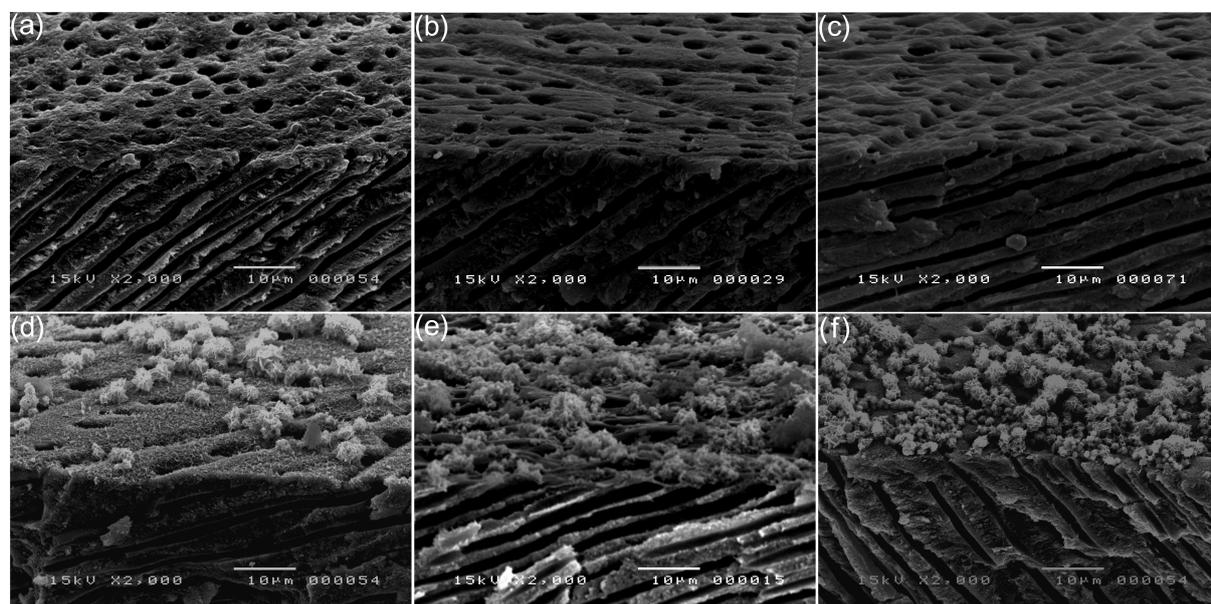


Fig. 2 SEM images of dentin surface and dentinal tubules of the dentin disks stored in deionized water for 1 day (a), 30 days (b), and 90 days (c), and in artificial saliva for 1 day (d), 30 days (e), and 90 days (f). While minimal changes are apparent in deionized water (a-c), deposition of mineral precipitants is evident in the artificial saliva with time (d vs. e and f).

Discussion

Both deionized water and artificial saliva were used as storage media in this *vitro* study. The current results indicated that there was a significant difference in dentin permeability between the two storage media where the $Lp\%$ of control group increased in the deionized water, while in the artificial saliva it decreased. In line with this finding, SEM observation of the dentin surfaces in the control group (Fig. 2) revealed that dentinal tubule orifices widened in deionized water over the storage periods (Figs. 2a-c). On the other hand, in the artificial saliva group, a scattering of small precipitants, probably mineral crystals covering the dentinal tubule orifices were observed (Fig. 2d). Such precipitations were more obviously seen with longer storage periods (Figs. 2e and 2f). It should be noted that the precipitations were not observed inside the dentinal tubules. It is speculated that morphological changes inside the tubules and at peritubular dentin were too small to be detected. In the deionized water group, mineral loss had likely taken place due to dissolution of peritubular dentin

minerals, resulting in increased dentin permeability. It was previously reported that storage of dentin resulted in reduction of its mechanical properties due to demineralization in the unsaturated solution.²²

In a solution saturated with regard to a mineral crystal, such as the artificial saliva used in this study, crystal deposition from the solution may occur on the dentin surface or in the dentinal tubules and peritubular dentin surfaces inside the tubules, resulting in constriction of the tubules and decreased dentin permeability. These hydrodynamic changes may be limited because the dentin permeability reached a plateau after 30 days in both storage media.

A dentin bonding agent has been used as a dentin desensitizer. The adhesive systems contain hydrophilic monomers and/or acidic monomers that in addition to the physical obstruction of tubule through surface coverage and formation of resin tags, have a potential to coagulate with proteins in fluid proteins in the dentinal tubules, resulting in reduction of the dentin permeability.²³ Therefore, application of the resin coating to the exposed dentin is effective in the clinical situation.²⁴

Selection of dentin bonding system for the resin coating is an essential issue to obtain clinical success of the indirect restoration. The two-step self-etch adhesive used in the current study was composed of a self-etching primer (SE primer) and a bonding resin (SE bond). An acidic functional monomer, 10-methacryloxydecyl dihydrogen phosphate (10-MDP) is present in both SE primer and SE bond. The 10-MDP in the primer is considered to play multiple roles; to demineralize the smear layers and the underlying dentin, to promote monomer penetration into dentin, and to react with calcium/hydroxyapatite intensively.²⁵ Nakabayashi *et al.*,²⁶ reported that the hybrid layer formation is essential to obtain good dentin bonding. Recently, the acid-base resistant zone (ABRZ) was found beneath the hybrid layer in the self-etch adhesive systems.²⁷ It was reported that acid resistance at the ABRZ forefront decreased when 10-MDP was excluded from the bonding resin in an experimental two-step adhesive system.²⁸ On the other hand, it has been reported that 10-MDP may act as a hydrophilic component in the polymerized bonding layer and increase water sorption over time.²⁸

The application of PLF on SE reduced dentin permeability. PLF is a low-viscosity microfilled composite mainly formulated using cross-linking dimethacrylates, such as bisphenol A glycidyl methacrylate (Bis-GMA). Therefore, the PLF resin composite is more hydrophobic than the SE adhesive alone. Application of PLF on SE protects the underlying adhesive, and increases thickness of the coating layer and also increases hydrophobicity of the coating layer. The mean thickness of the coating layer of SE+PLF was 126.0 μm , which was approximately five times thicker than that of the SE (22.1 μm). Application of a flowable resin composite on the cured adhesive can eliminate the oxygen inhibited layer of the adhesive, since the uncured resin in the oxygen inhibited layer is subsequently polymerized by diffusion of free radical from the flowable resin composite. It is believed that the adhesive itself is improved in quality (*i.e.* better cross linking, improved conversion ratio and higher mechanical property), contributing to durable bonds at the interface.²⁹⁻³¹

Nevertheless, it should be noted that none of the methods could completely eliminate dentin permeability and reach zero $Lp\%$ in the present study. It is inferred that dentin is inherently a permeable tissue, and a complete elimination of its hydrolytic conductance may be structurally very difficult. On the other hand, it has been reported that dental composites absorb water and release unreacted monomers in aqueous environment.³² The water ingress into dental composites in the oral cavity, with time, leads to deterioration mainly due to a hydrolytic breakdown of the bond between silane and filler particles, filler-matrix debonding or even hydrolytic degradation of the fillers.³³ In addition to the degradation effects, water sorption may alter resins due to

plasticizing effects through expansion, increasing the effective free volume and the ease of movement of chain segments, thus reducing the elastic modulus of the material. These effects have been observed during storage in both deionized water and artificial saliva.³⁴ Similar to the control (uncoated) groups, significant differences were observed between artificial saliva and deionized water in both coated groups. The higher permeability in deionized water was attributed to the effects of water on degradation of both the dentin and the resin-dentin interface.³⁵

Based on the present results, the null hypothesis that coating material, storage media or storage period did not affect dentin permeability was rejected. Further study should be carried out to evaluate the clinical performance of the resin coating for the indirect restorations. Application of the resin coating to dentin surface demonstrated significant reduction of dentin permeability over time. However, reduction of dentin permeability was strongly influenced by coating strategy and storage media. Artificial saliva is more favorable storage media than deionized water for *in vitro* permeability measurement.

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