Influence of dentinal polyelectrolytes on wet demineralized dentin, a bonding substrate

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Received 19 February 2002; revised 29 August 2002; accepted 6 September 2002

Abstract: The objective of this study was to show the influence of dissolved dentinal polyelectrolytes on the characteristics of dentin (bonding substrate) demineralized by citric acid in the absence or presence of ferric chloride. The demineralizing agent was an aqueous mixture of 0, 1, 3, or 10% ferric chloride in 10% citric acid (10–0, 10–1, 10–3, 10–10, respectively). The hypothesis was that the concentration of dissolved dentinal noncollagenous substances, mainly polyelectrolytes soluble in water, must be decreased by their aggregation with ferric ions, which changes the characteristics of demineralized dentin, the rates of demineralization, and dehydration. Cervical bovine dentin was prepared in 3 × 2 × 2-mm blocks, each weighing 20.0 ± 0.5 mg. The rate of demineralization was investigated by measuring the weight loss resulting from demineralization by immersion in 10 mL of conditioner at 2-h intervals. The dehydration rate of wet demineralized dentin was determined using two methods: (1) weight loss in a desiccator under 263 Pa pressure and (2) differential scanning calorimetry (DSC). Twenty, 12, 8, and 4 h were required to complete demineralization of the blocks with the 10–0, 10–1, 10–3, and 10–10 solutions, respectively. The 10–10 wet demineralized dentin showed the highest rate of dehydration, followed in descending order by the 10–3, 10–1, and 10–0 specimens. Ferric chloride in dentin conditioners provided both a higher rate of dentin demineralization and a higher dehydration rate of wet demineralized dentin. These results suggest that in the presence of ferric chloride, a decreasing amount of dissolved polyelectrolytes aggregated with ferric ions in the substrates may increase the permeability of dentin to water and citric acid. Improvement of monomer permeability is essential to the preparation of good hybridized dentin, providing a more stable and reliable bonding and also protecting the dentin and pulp from infection. A further study of bonding substrates is required in order to understand the role of hybridized dentin in improved dental treatment. © 2003 Wiley Periodicals, Inc. J Biomed Mater Res 66A: 789–794, 2003

Key words: ferric chloride; rate of dentin demineralization; rate of dehydration; citric acid; dentinal polyelectrolytes; bonding to dentin

INTRODUCTION

It is agreed that the adhesion of restorations to tooth substance is desirable in restorative dentistry. Clinical experience suggests that replacement of restorations quite often is required, and research on how to minimize this occurrence has been undertaken for some time now. Improvements in dental materials have not resolved the problem. An intimate attachment of restorations to tooth substrates by adhesion has been the subject of considerable study. Adhesion to enamel has been shown not to be difficult,[1] but adhesion to dentin is complicated. Nakabayashi et al. have proposed that the hybridization of dentin with polymers may make bonding possible and that the impregnation of monomers and their polymerization *in situ* is essential for preparing hybridized dentin.[2] Bonding has been evaluated mainly by measuring the bond strength of adhesives, but it is difficult to explain the bonding mechanism by this means.

Acid and monomer permeability is a characteristic of demineralized dentin, but there are few studies that analyze this permeability. There has been a well accepted consensus that collagen can act as a model of demineralized dentin in dentistry. Higher-ordered structures of collagen, the main component of demineralized dentin, have been studied extensively in an

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Contract grant sponsor: Scientific Research from Ministry of Education, Science, and Sports, Japan; contract grant number: 12470404
Contract grant sponsor: Japan Society for the Promotion of Science (JSPS)
Contract grant sponsor: National Research Council of Thailand (NRCT)

attempt to understand bonding, and it has been shown that the characteristics of demineralized dentin are dependent on the demineralizing agents used.[3]

Some acids induce collagen denaturation but ethylenediaminetetraacetic acid (EDTA), at a pH of 7.4, does not change the structure. Although dentin demineralized with EDTA is not a good substrate with which to prepare hybridized dentin, mixtures of the ferric salt of EDTA and sodium salt are effective in preparing a good substrate for forming hybridized dentin.[4]

However, it is difficult to explain the varieties of demineralized dentin only by the structural changes of collagen caused by different demineralizing agents. Thus solubility parameters of collagen[5,6] and the contact angle of the demineralized dentin surface also have been studied.[7] Collagen is a somewhat hydrophobic polypeptide, but demineralized dentin is hydrophilic. This information suggests that noncollagenous hydrophilic substances may be influential in explaining the characteristics of demineralized dentin bonding substrates. Good wetting agents have shown promising results in promoting good bonding to dentin.[8] On the other hand, a monomer solution of 5% 4-methacryloyloxyethyl trimellitate anhydride (4-META) in methyl methacrylate (MMA) is hydrophobic.[2]

Since 1982, an aqueous mixture of 10% citric acid and 3% ferric chloride (10–3) has been used as a dentin conditioner to remove smear layers, and it has been shown to maintain the high permeability of demineralized dentin.[2] Five-% 4-META in MMA can diffuse and impregnate demineralized dentin, both in dry[2,9] and wet[10] conditions, and it can form a hybrid layer after polymerization initiated by TBB (tri-n-butyl borane). In the absence of ferric chloride, that is, 10% citric acid (10–0) only, well hybridized dentin could not be formed, thus leading to adhesive failure at low tensile bond strengths both in wet and dry conditions.[2,9–11] An aqueous 30% 2-hydroxyethyl methacrylate primer can improve the bonding to dried 10–0 demineralized dentin, but it takes 1 h for priming to occur.[9]

Wet-bonding using phosphoric acid as a dentin conditioner facilitates bonding to the substrates, possibly by preventing the collapse of demineralized dentin. It has been postulated that a higher-ordered structure of collagen is involved in the collapse when it is dried.[12–20] However, the mechanism of collapse has not been well studied.

Hybridized dentin formed using phosphoric acid and 4-META in acetone primer cannot resist the action of strong acid (HCl) and oxidative alkaline (NaOCl) solutions.[21] This chemical challenge may mimic the formation of caries.[9] It has been explained that some demineralized dentin is not converted to hybridized dentin, and therefore demineralized dentin remains between the hybridized and the intact dentin and is susceptible to hydrolysis.[22] Nevertheless, the tensile strength and the durability of wet-bonded specimens using 35% phosphoric acid are poorer than those using 10% phosphoric acid.[23] Therefore the characteristics of wet demineralized dentin are important factors when considering ways to improve wet bonding.

The mechanism whereby ferric chloride maintains higher permeability of demineralized dentin in both dry and wet stages is not well understood. It has been reported that ferric ions affect the diffusion rate of glutaraldehyde into dentin.[4] Examination of the remaining demineralized dentin is possible within a day by using mini-dumbbell-bonded specimens for tensile strength measurement.[24,25] A wet-bonding study of demineralized dentin etched by either 10–3 or 10–0 suggests that the dehydration of demineralized dentin with 4-META in acetone primer is much easier with the 10–3.[10]

These studies postulated a hypothesis that the measurement of the dehydration rate may provide key information in the understanding of the nature of wet demineralized dentin. Dentin is composed of inorganic and organic contents that are mainly hydroxyapatite and collagen fibers, respectively.[26] The noncollagenous macromolecules, proteoglycans (PG), glycosaminoglycans (GAG), and phosphoproteins (PP), etc., have been identified in dentin and predentin.[27–30] Acid conditioning of prepared dentin removes the smear layer and most of the mineral content in the subsurface dentin. The collagen fiber network is exposed and easily collapsed with air-drying[31–33] except for dentin demineralized with a 10–3 solution.[2,9,11] These collagen fibrils are covered with PG, which is composed of a protein core to which one or more GAG chains are attached.[27–30,34,35] Phosphoproteins, PG, and GAG (the dentinal polyelectrolytes) are highly water soluble and polyanionic due to their carboxyl and sulfate groups.[36] Hydrophilic agents and cationic ions, such as ferric ions, easily are attracted by these polyelectrolytes.

It therefore was hypothesized that ferric ions in dentin conditioners may react with anionic sites on PP, PG, and GAG chains and decrease the concentration of dissolved polyelectrolytes in the substrate, which then must affect the permeability of demineralized dentin and increase the rate of demineralization and dehydration of wet demineralized dentin.

The objective of this study was to compare the rate of dentin demineralization and the dehydration rate of wet demineralized dentin conditioned in the absence or presence of ferric chloride in 10% citric acid to determine the influence of dentinal polyelectrolytes on the characteristics of demineralized dentin, the bonding substrate, and the hybridization of polymers with dentin.

MATERIALS AND METHODS

Preparation of bovine dentin blocks

Using a low speed saw (Isomet, Buehler, Lake Bluff, IL), fresh dentin from the cervical third of bovine teeth was prepared as uniform dentin sticks 2 × 2 mm in cross-section. These then were cut into 3 × 2 × 2-mm rectangular blocks with a diamond fissure bur (B11, GC International Co., Tokyo) fitted in a high-speed hand-piece under a copious air-water spray and finished with 180-grit Carbimet paper discs (Buehler, Lake Bluff, IL). The blocks were cleaned with running water, and free water was absorbed on paper (Kimwipes, Kimberly-Clark Co., Tokyo) for 10 s and then weighed before dried in a desiccator with silica gel at 263 Pa for 3 h. Specimens of 20.0 ± 0.5 mg were selected and randomly divided into four groups of 16 dried dentin specimens.

Measurement of observed demineralization rate

Four dried dentin specimens from each group were selected to measure the rate of demineralization with four conditioners: 0, 1, 3, and 10% ferric chloride dissolved in 10% citric acid. They were abbreviated as 10–0, 10–1, 10–3, and 10–10. Each specimen was immersed in 10 mL of each conditioner for 2 h, then soaked in distilled water for 10 s. Excess liquid was absorbed on the paper for 10 s and then weighed before being immersed in the corresponding conditioner again. The weight of demineralized dentin was recorded at 2-h intervals using the previously described method until there was no further decrease. The complete demineralization of all dry demineralized dentin was confirmed using a soft X-ray system (Ohmicron, Shield Cabinet, Ohmic Ltd., Tokyo) operated at 60 kVp and 3 mA for 2 s.

Measurement of dehydration rate

Twelve dried dentin specimens from each group were used to measure the dehydration rate of wet demineralized dentin by two methods: (1) weight loss in a desiccator at 263 Pa, and (2) differential scanning calorimetry (DSC-100, Seiko I, Tokyo). Each dried dentin specimen was demineralized in 10 mL of each conditioner for 20 h. Demineralized specimens were soaked in distilled water for 10 min and dried on paper for 10 s prior to dehydration.

Seven wet demineralized specimens from each groups were dehydrated in a desiccator with silica gel at 263 Pa, the weight of wet demineralized dentin was measured at 0, 15, and 30 min, and 1, 1.5, 2, 4, 8, 20, and 35 h. The percentage of dehydration for each specimen was calculated and recorded at each time interval. The remaining five wet demineralized dentin specimens from each group were dehydrated in the DSC instrument operated between 25° to 150°C at a 3°C/min temperature increase. The temperature of initiation of dehydration in each specimen was recorded.

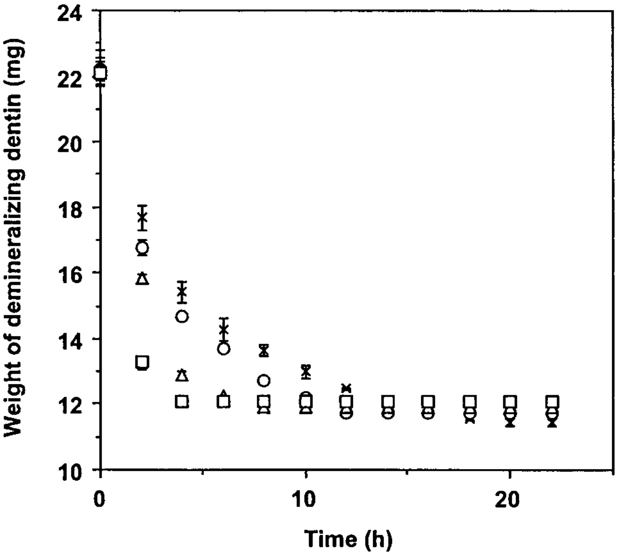

Figure 1. Rate of weight loss of dentin when demineralized with 10–0 (×), 10–1 (○), 10–3 (△), and 10–10 (□) conditioners soaked in distilled water and mopped dry with paper for 10 s at 2 h intervals.

The dehydration data were analyzed using one-way analysis of variance (ANOVA) and the LSD test for significant difference.

RESULTS

The decreasing weight changes resulting from the demineralization of dentin blocks in each group are shown in Figure 1. It took 20, 12, 8, and 4 h, respectively, to complete the demineralization of the blocks using 10–0, 10–1, 10–3, and 10–10 solutions. The percentage of dehydration at the different time intervals during the first 4 h is presented in Figure 2. Significant differences in the amount of dehydration were found among the groups ($p < 0.01$).

No significant differences were revealed between the 10–1 and 10–3 groups at 30 min, 1 h, and 8 h. The levels of dehydration occurring during the first 15 min were as follows: 85% in 10–10, 78% in 10–3, 75% in 10–1, and 66% in 10–0 specimens. It took 4, 8, 20, and 35 h for complete dehydration in the 10–10, 10–3, 10–1, and 10–0 groups, respectively. The initiation temperature for dehydration of the 10–0 specimens was significantly higher than that for the other groups ($p < 0.05$) (Table I). No statistically significant differences were revealed among the 10–1, 10–3, and 10–10 groups.

No significant differences were found in the weight of dry dentin among the groups while a statistically significant lower weight loss was found for dry demineralized dentin for the 10–0 specimens compared with the other three groups ($p < 0.01$) (Table II). The

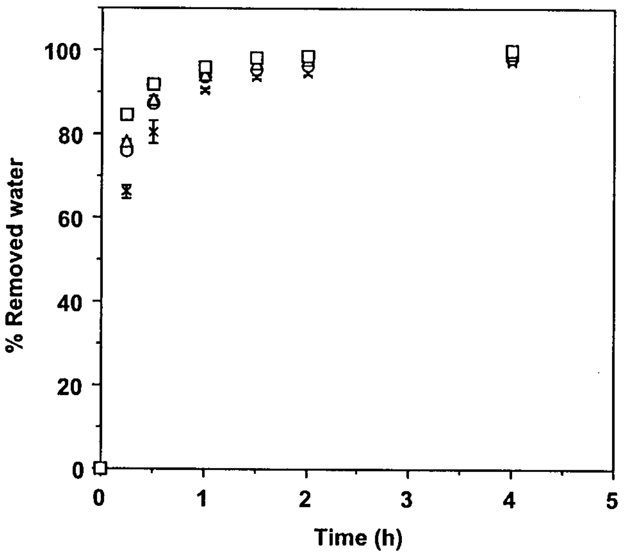

Figure 2. Percentage of water removed from wet dentin demineralized in 10–0 (×), 10–1 (○), 10–3 (△), and 10–10 (□) conditioners and dehydrated in a desiccator.

opacity on the X-ray film of the specimens demineralized with conditioners containing ferric chloride was higher than that for the 10–0 specimens.

DISCUSSION AND CONCLUSIONS

The rate of demineralization increased with the increasing concentration of ferric chloride in the dentin conditioner. Demineralization of dentin conditioned with 10–10 was completed in 4 h while complete demineralization occurred in 8, 12, and 20 h when using 10–3, 10–1, and 10–0, respectively (Fig. 1). The explanation is that the higher concentration of ferric chloride increases the permeability of demineralized dentin, so that the citric acid can diffuse faster and remove a larger amount of the mineral contents. The proteoglycans and polyanionic GAG chains distributed along the collagen fibrils[34,35] easily react with Fe^{3+} due to their higher valence compared with Ca^{2+}. Thus, the ferric ion minimized the amount of dissolved polyelectrolytes.

TABLE I
Mean and Standard Deviation of Initiation Temperature of Dehydration of Wet Demineralized Dentin Conditioned with 10-0, 10-1, 10-3, and 10-10 solutions, as Measured with a Differential Scanning Calorimeter (DSC)

Conditioners	Initiation Temperature of Dehydration (°C)
10-0	73.6 ± 4.5
10-1	68.0 ± 2.9
10-3	67.5 ± 2.9
10-10	66.7 ± 2.3

There is no statistically significant difference among groups connected with the vertical line ($p > 0.05$).

TABLE II
Weight (Mean ± SD, mg) of Dentin Block and Dry Demineralized Dentin, after Drying in a Desiccator for 35 h, of 10-0, 10-1, 10-3, and 10-10 Conditioned Groups

Conditioner	Dentin Block	Demineralized Dentin
10-0	20.27 ± 4.13	4.13 ± 0.12
10-1	20.30 ± 4.46	4.46 ± 0.19
10-3	20.24 ± 4.59	4.59 ± 0.16
10-10	20.18 ± 4.84	4.84 ± 0.28

There is no statistically significant difference between groups connected with the vertical line ($p > 0.05$).

In contrast, in the absence of ferric chloride, citric acid requires at least 8 h longer to complete demineralization of 3 × 2 × 2-mm rectangular blocks of dentin. This supports previous findings that the depth of demineralized dentin increases with increasing concentration of ferric chloride for the same 10-s conditioning periods, that is, 10–0 (2 μm)[9,10], 10–1 (2 μm)[25], 10–3 (4 μm)[2,10], 10–5 (4 μm)[25], and 10–10 (>5 μm).[25]

Increasing concentrations of ferric chloride in 10% citric conditioner increased the dehydration rate of wet demineralized dentin. When dehydrated in a desiccator, 85% of the total water content was removed from 10–10 wet demineralized dentin in the first 15 min. It was significantly faster than for 10–3 (78%), 10–1 (75%), and 10–0 (66%) (Fig.2). Ferric ions aggregated the polyelectrolytes, which made removal of water easier and kept water permeability of demineralized dentin higher; thus the rate of dehydration increased.

The DSC measurements demonstrated a significantly lower initiation temperature for water evaporation among the groups containing ferric chloride than there was for the 10–0 group (Table I). This suggests that concentration of dissolved polyelectrolytes is decreased by ferric ions in the demineralized dentin and that the evaporation temperature of water thus decreases. These results confirmed that ferric chloride makes the dehydration of wet demineralized dentin easier. The initiation temperature of dehydration can be increased with an increase in the fraction of molecules dissolved in the water because of a decrease in the vapor pressure.[37]

The results of this study suggest that ferric chloride in dentin conditioner provides a higher permeability and faster dehydration of demineralized dentin. This is the reason that bonding to dentin using a 10–3 solution in a dry condition works very well[2] while when using phosphoric acid[23,31] and citric acid,[10] the tensile bond strength decreases dramatically. Ferric ions react with polyelectrolytes and make them insoluble. This helps to maintain spaces between collagen fiber networks even after air-drying so that monomers can penetrate and form better hybridized dentin after their polymerization.

In the absence of ferric ions, acids remove the mineral contents and easily allow the exposed collagen to be pasted down by increasing the concentration of dissolved polyelectrolytes with drying, resulting in a loss of diffusion pathways.[27–35] Therefore the monomers cannot diffuse through, resulting in adhesive failure. In a comparison of the effects on wet demineralized dentin of the 10–3 solution with the effects of the 10−0 solution, the ferric ions in 10–3 allow a higher permeability for the 4-META/acetone primer and water, which efficiently dehydrates the wet demineralized dentin. Therefore a better hybridized dentin is produced after monomer impregnation and polymerization.

The 10−0 wet demineralized dentin could not produce reliable bonding, resulting in adhesive failure at lower tensile strengths.[10] This suggests that, just as ferric ions were needed to make the polyelectrolytes insoluble and to maintain the permeability of demineralized dentin, their absence led to the more difficult dehydration. Consequently, the 4-META in acetone primer could not impregnate completely.[10,21]

It has been reported that proteoglycans in cartilage matrix can be condensed by strong dehydrating agents, such as ethanol.[38] Wet bonding using phosphoric acid and an acetone-based primer also results in adhesive failure because of the lower resin content in hybridized dentin,[21,23] primer globules, and resin-water residuals in the dentinal tubules.[18−20] To overcome this problem several applications of primers are needed to dehydrate the wet demineralized dentin.[39]

The higher radiopacity of the specimens conditioned with citric acid and ferric chloride, compared with those conditioned with 10−0, resulted from the presence of ferric ions that reacted with polyelectrolytes distributed along the collagen fibrils. Furthermore, ferric ions can be precipitated to form less soluble Fe_3PO_4 when $FeCl_3$ is present. These also can result in a significantly increased weight of dry demineralized dentin conditioned with citric acid and ferric chloride compared with specimens conditioned with 10−0 (Table II).

The influence of proteoglycans (PGs), glycosaminoglycans (GAGs), phosphoproteins (PPs), etc., on the permeability of demineralized dentin was discussed relative to the preparation of good hybridized dentin. Nakabayashi proposed that hybridized dentin can be considered to be an artificial enamel in the sense of impermeability,[40] which can protect dentin and pulp from stimuli invading them. This is very important in improving dental treatment as it possibly could prevent recurrent caries,[26] something that has not been possible.

In studies designed to show the function of hybridized dentin, it has been recognized that as dentin does not have cells and blood vessels, exposed dentin easily can become infected and cannot heal by itself. This may be the reason why dental materials, as biomaterials, cannot alone provide exposed dentin and pulpal protection during dental treatment; hybridized dentin, however, may be able to induce pseudo-wound healing on injured dentin surfaces.

More needs to be understood about how to prepare good hybridized dentin. The function of dentinal polyelectrolytes may be one of important issues relative to allowing biomaterials to reliably connect with dental tissues.

The authors thank Associate Professor John Harcourt for his editorial assistance in preparing this manuscript.

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