Effect of tannic acid solution on collagen structures for dental restoration

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This study examined the effect of tannic acid solution on dissolution of dentine collagen and morphological aspects of tendon collagen. Using root dentine, which was cut off from bovine anterior tooth, dentine powders were obtained by the pulverization and lyophilization. They were subject to an application of 1, 3, 5 or 10% tannic acid (TA) solution for 1, 3, 6, 12 or 24 h. TA-treated dentine powders were treated with 40% phosphoric acid (PA) for 30 s at 20 °C and additionally with trypsin. Released hydroxyproline in Woessner's assay after a hydrolysis in 6 N HCl at 110 °C for 20 h was assumed to be dissolved dentine collagen. Released hydroxyproline in a control sample without acid treatment decreased from 100 to about 60% with increased TA concentration of 1 to 10%, and decreased with increased incubation times of 1 to 24 h when applied by 5% TA solution. Scanning electron microscopy results established the morphological effect of their surface characteristics due to such treatments as 40% PA for 30 s and 5% TA for 6 h, or 40% PA after 5% TA treatment, yielding collagen structures protected by TA to attack from phosphoric acid.

1. Introduction

In vitro study revealed that tannic acid solution removed effectively the smear dentine surface (ground, or polished dentine surface) while leaving the dentinal tubules [1], because the surface became covered by an adherent layer of cutting debris (smear layer in dental field). It lowered dentine permeability and interfered with an attempt to bond restorative resin materials directly to dentine [1, 2]. The use of tannic acid protected the dentine and pulp from complications during the composite restoration procedure. Removing the smear layer by such acids as phosphoric acid, the permeability of dentine may increase and induce structural change of dentine collagen [2]. Clinicians report that etchant is often applied inadvertently to dentine because of difficulty to etch restrictively to enamel. Basic studies clarified the anti-caries activity of tannic acid solution using bovine dentine and human dentine [3–6]. Tannic acid as an etchant removed the smeared layer covering the dentine surface without demineralizing underlying dentine, suggesting that tannic acid is effective as an etchant to dentine surface [7, 8]. The results suggest that tannic acid solution is applicable as an etchant to dentine surfaces. Therefore, we examined the released amount of soluble dentine collagen from dentine powders and observed the exposed tendon collagen surfaces by acid solution, based on our previous plot studies. This study examined the effect of tannic acid on dissolution of dentine collagen from bovine dentine and tendon collagen structures from bovine Achilles' tendon.

2. Materials and methods

Dentine used in this study was covered peripherally by enamel on the crown by soft cementum on the root surfaces. Dentine is the only innervated hard tissue of tooth, but remains relatively insensitive as long as it is covered. The etching solution used was tannic acid (TA; Wakou Junyaku Co, Osaka). First, released amounts of hydroxyproline in dentine powders were determined according to the procedures shown in Table I. The crown was cut off from bovine anterior tooth, and pulp, soft tissue and cementum were removed. Root dentine which was cleaned with distilled water was set in liquid nitrogen for pulverization and followed to lyophilization. The dentine powders (DPs) of 0.42 to 0.84 mm size, which were obtained using sieves (M-2 Type Tsutsui testing sieves; Tsutsui Rikagaku Kikai, Tokyo, Japan), were treated with TA solution, and TA-treated DPs were treated by phosphoric acid (PA; Katayama Kagaku Kougyou, Osaka, Japan) and trypsin (Table II) (Trypsin, Sigma Chemical, St. Louis, MO, USA). The use of TA was detailed as follows. The effect of TA concentration (1, 3, 5, 10%) on dissolved hydroxyproline at incubation time of 6 h was determined. The incubation times were 1, 3, 6, 12 and 24 h. Their TA-treated DPs were treated by PA of 40% for 30 s at 20 °C and followed by cleaning with distilled water and lyophilization. Continuous trypsin treatment was done for the TA-treated DPs at 37 °C for 16 h, or 60 °C for 30 min. The treated samples which contained soluble collagen were prepared after a hydrolysis (6 N HCl at 110 °C for 20 h),

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TABLE I Preparation of dentine powders cut off from bovine anterior teeth, which were obtained according to the experimental schedule. See text

Bovine anterior tooth
↓ Cut off the crown
↓ removal of pulp, soft tissue, cementum
↓ Wash with distilled water
Root dentine
↓ Pulverization in liquid N_2
↓ Wash with distilled water
↓ Lyophilization
Dentine powder (0.42–0.84 mm)

TABLE II The method to determine the amount of hydroxyproline from dentine powders. First, dentine powders were treated with TA (tannic acid), and PA (phosphoric acid) and trypsin treatments were carried out. After hydrolysis, the amount of hydroxyproline was determined. See text for details

Dentine powder	Trypsin treatment
↓ TA treatment	↓ 0.1 mg Trypsin/ml PA buffer
0, 1, 3, 5, 10%	(pH 8.0)
0, 1, 3, 6, 12, 24 h	37 °C, 16 h
↓ Wash with distilled water	60 °C, 30 min
↓ Lyophilization	↓ 12 000 r.p.m. 15 min
TA treated DP	
↓ PA treatment	
40%, 30 s, 20 °C	Spt
↓ Wash with distilled water	↓ Hydrolysis
↓ Lyophilization	6 N HCl 110 °C, 20 h
	Determination of hydroxyproline
	Woessner's assay

TABLE III Scanning electron microscopy (SEM) observation of collagen from bovine Achilles' tendon. Tendon collagen was treated as described by three types of surface treatments

Type I collagen from bovine Achilles' tendon
↓ Treated with
40% PA 30 s
5% TA 6 h
5% TA 6 h + 40% PA 30 s
↓ Wash with distilled water
↓ Lyophilization
↓ Coating
Observation by SEM

whose samples were centrifuged at 12 000 r.p.m. for 15 min. The dissolved hydroxyproline in their DPs in Woessner's assay was determined [9], which corresponded to soluble amounts of collagen. Secondly, collagen from bovine Achilles' tendon (Collagen Insoluble Type I, Sigma Chemical) was used, because tendon collagen is the same collagen structure type as dentine collagen. The tendon collagen samples were treated with 40% PA for 30 s. The samples which were washed with distilled water and lyophilized were prepared for scanning electron microscopy study (SEM; ALPHA 10, Akashi, Tokyo, Japan) at 15 kV and × 10 000 magnification (Table III).

3. Results

Fig. 1 shows released hydroxyproline from dentine powders, representing that it decreased to about 60% with increased concentration of TA from 1 to 10% at incubation time = 6 h. At constant TA concentration of 5%, Fig. 2 shows the released amount of hydroxyproline

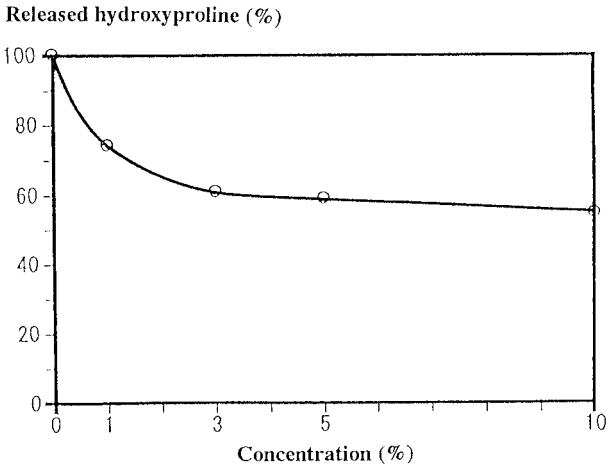

Figure 1 The effect of TA (tannic acid) concentration on released hydroxyproline in dentine powders containing dentine collagen at incubation time = 6 h.

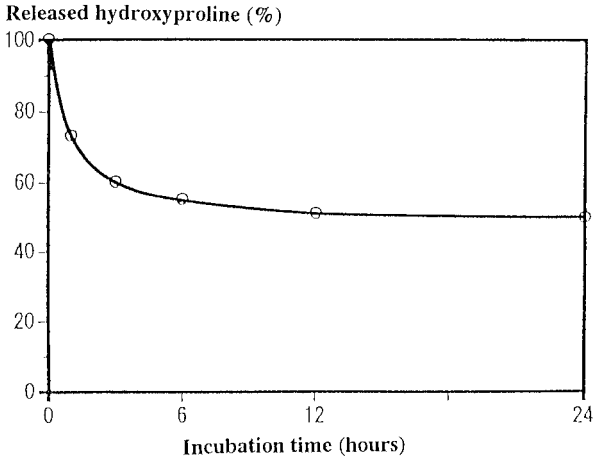

Figure 2 The effect of incubation time on released hydroxyproline at TA concentration = 5%.

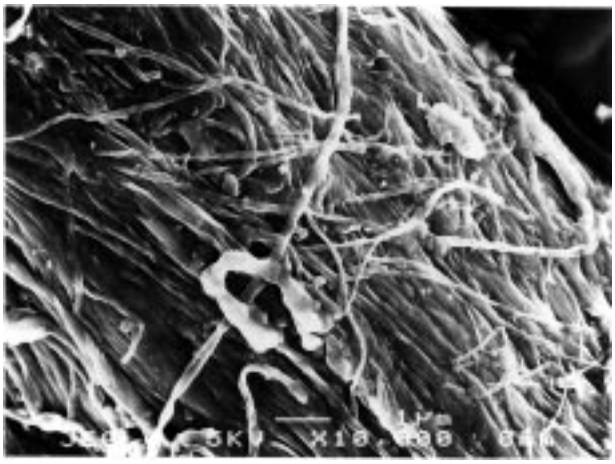

Figure 3 SEM micrograph of tendon collagen surface prepared with untreated samples (control sample). Collagen is seen covering fiber structures.

which decreased to about 50% with increased incubation times 1 to 24 h. At more than 6 h of incubation time with TA treatment, the released amounts of hydroxyproline achieved a constant rate. The results showed that the soluble amount of collagen was less in TA-treated samples than in the untreated sample and TA solution was applied effectively as an acid-etching conditioner.

Fig. 3 shows S an EM micrograph of a control sample without acid solution. Fig. 4 shows micrographs of collagen surface from bovine Achilles' tendon which was treated with 40% PA for 30 s (upper), 5% TA for 6 h (middle) or 40% PA for 30 s after 5% TA for 6 h (lower). A control sample showed fiber structures with transversely striated structures in tendon collagen, whereas the treated collagen structure was protected, leaving fiber structures when treated by TA followed by PA. Collagen surface dissolved with PA treatment without showing fiber structures. If acid-etch technique is used, it is very important in dental dentine bonding systems that acid

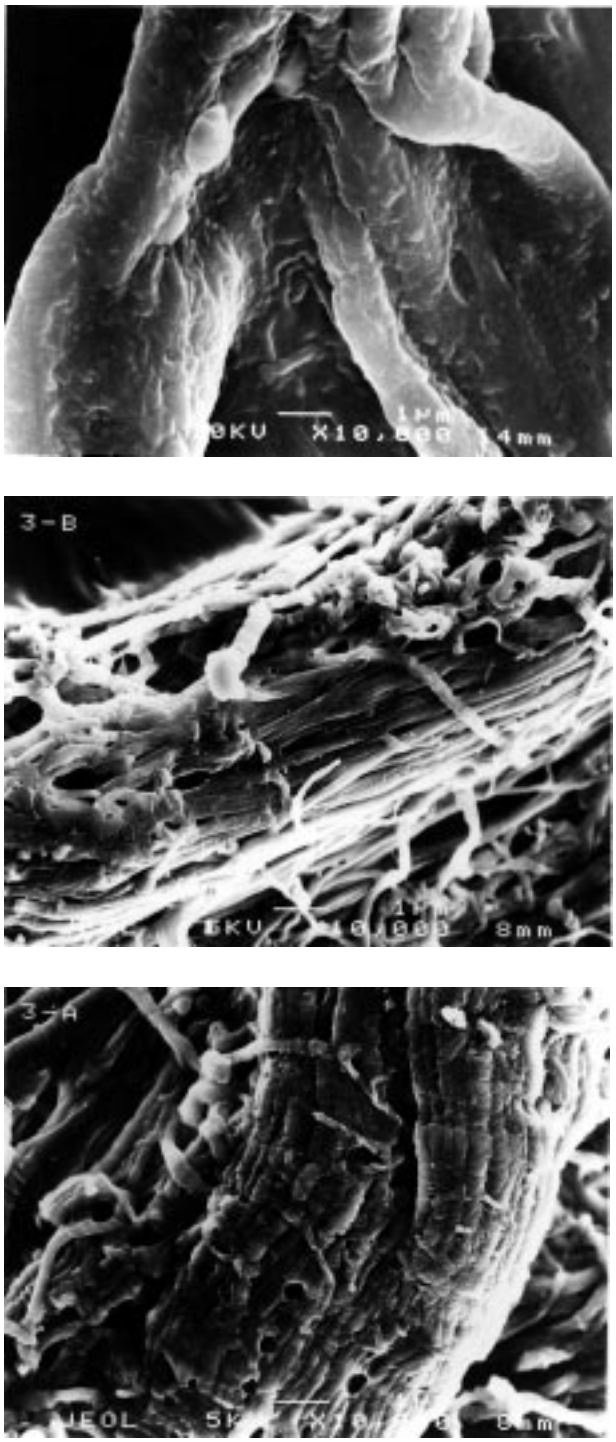

Figure 4 SEM micrographs of collagen surface prepared with the acid treatment in Table III. 40% PA for 30 s (upper), 5% TA for 6 h (middle) and 40% PA for 30 s after 5% TA treatment for 6 h (lower). There were three samples per group.

concentrations should be lowered to the levels which are isotonic with body fluids and the use of TA protects dentine collagen from the strong attack by phosphoric acid (PA).

4. Discussion

There are two points of view regarding smear layer in dental dentine bonding systems [10]. One is that it produces an effective cavity liner to reduce dentine permeability, and the other is that it interferes with adhesion of restorative materials to dentine. Therefore, the use of PA as common acid etchant was effectively applied to remove smear layer (prepared cut, or polished dentine surface), but the effects of removing dentine plugs in dentinal tubule and dissolving the pertubular dentine of the parent tubule have been reported [1, 2, 10, 11]. Another method was to treat the smear layer with 3% oxalic acid, which removed the smear layer and replaced it with an insoluble calcium oxalate [2, 10]. On the contrary, regarding the smear layer when treated by TA, a previous study showed that 5% TA effectively removed the smear layer while leaving the dentinal tubules plugged with cutting debris [8].

This study examined the effect of tannic acid (TA) on collagen structures, because the dentine was demineralized by acid etching of smear dentine surface and then collagen fibers existed. This examination showed that TA solution under certain conditions effectively affected collagen structures. The decreased value of released hydroxyproline at incubation time of 6 h was obtained with increasing TA concentration and an application of 5% TA decreased released hydroxyproline (soluble collagen) with increased incubation time. The smear layer was removed without increasing the size of the tubules by applying 20% or 25% TA solution for 15 to 60 s, but incomplete removal of the smear layer occurred when treated by the TA concentration [12]. The higher TA concentration used in this study lowered the amount of dissolved dentine collagen, showing the fiber structures left on collagen structure when treated with 5% TA. The decreased tendency of released hydroxyproline was also observed with increasing duration of exposure of dentine collagen to TA solution.

This study has shown the clinical significance of dental dentine bonding system as follows. Acid etching of dentine is not harmless, but acids are more irritating than they need to be, because acid concentration and etching times are usually excessive when dental clinicians apply to dentine surface with acid. Acid etching of dentine was proposed as clinical attempts to increase the acid-etched area of dentine for bonding. The acid etching was to demineralize the porosities of the dentine matrix which were created by dissolving away hydroxyapatite minerals from the collagen components of dentine. The etching time was limited to that required to produce optimum dentine bonding [2, 10, 11], because a pulpal reaction to acid etching of dentine occurred with strong solutions of acids such as 60% phosphoric acid or 50% citric acid. For dental dentine bonding, a collagen-mesh network is created on the demineralized dentine, that is, a resin-impregnated hybrid layer as an interdiffusion zone [13, 14]. The demineralization is achieved even

when dentine is etched with dilute acid for a short time [15]. The use of TA solution is given in this study, because most bonding systems use acidic conditioners designed to remove the smear layer (prepared cut or polished dentine surface) and create dentine/resin composite interface. This study has shown an excellent effect of TA on dentine collagen using PA after pretreatment with TA while leaving fiber structures on tendon collagen. TA solution is thus expected to be available as an etchant to collagen surface within a demineralized dentine.

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