Antibacterial Effect of Tannic Acid to Adhesive Resin in Dentine Bonding System

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ABSTRACT

As a preliminary study of antibacterial effect of adhesive resin (bonding agent) in dentine bonding system (DBS), tannic acid solution was applied effectively to give an antibacterial effect to dental DBSs, examining the inhibitory zone size of six types of bacterias. Measuring bound TA concentration in an adhesive resin as a dental DBS which bonded between dentine and resin composite, the results showed that such factors as incubation time and tannic acid concentration in the adhesive resin were important in controlling antibacterial effect to work effectively to bacterial strains.

INTRODUCTION

In dental DBSs, acid solution removed effectively the ground or polished dentine surface by carbide bur prior to dentine adhesion[1,2]. The smeared layer on the dentine was generally removed using a phosphoric acid gel[2], and the acid enlarged the opening of the dentine tubules. An adhesive resin penetrated through the tubles and polymerized there, and the resin composite bonded to the dentine after etching the smeared layer and polymerizing adhesive resin[3-9]. Tannic acid as an an etchant removed the smeared (ground) layer covering the dentine surface without demineralizing underlying dentine, suggesting that tannic acid is effective as an etchant to dentine surface[7,8].

Thus, this study is to examine the antibacterial effect of tannic acid solution to adhesive resin, which includes tannic acid solution, as a preliminary experiment in dental DBS.

MATRIALS AND METHODS

The materials were described as Table 1. The adhesive resin (bonding agent) investigated was a commercial adhesive one (Clearfil Photo Bond, Kuraray Co, Osaka), and an etching solution was tannic acid (TA; Wakou Junyaku Co, Osaka). Three experiments were carried out for the antibacterial effect of tannic acid solution in adhesive resin (bonding agent); 1) the inhibitory zone sizes of six types of bacteria strains were measured for adhesive agent including TA solution, 2) the minimum inhibitory concentration (MIC) of TA solution were also measured, and 3) bound TA concentration of TA-added adhesive resins was measured. The inhibitory zone size of bacteria strains were examined using the same method as the earlier reports[10,11]. They were tested for each of six bacterias; *Streptococus mutans Ingbritt* (IB) 1600, *S. mutans* OMZ 175, *S. sobrinus* OMZ 176, *S. sobrinus* 6715-13, *S. sanguis* ATCC 10556, *S. salivarius* ATCC 9222. All strains were grown in Trypticase Soy Broth (BBL Type; Microbiology Systems, Cockeysville, MD, USA), supplemented with 0.5% yeast extract in an aerobic atmosphere. Bacteria strains were routinedly cultivated in Brain Heart Infusion matrix (Difco Laboratories, Tokyo). Thus, antibacterial effect was evaluated using a disc diffusion method, and disc shaped matrix had 85 mm diameter and 4 mm thick[10,11]. The samples were set on the Brain

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Heart Infusion matrix at 37°C for 24 hours, where the bacteria was cultivated with O.D. 600 (a logarithmic forming period) = 0.7. The MIC of tannic acid was measured for the bonding agent in DBSs, according to an earlier report[10].

Bound TA was determined using four types of bacterial strains; *S. mutans* IB 1600, *S. sobrinus* 6715–13, *S. sanguis* ATCC 10556 and *S. salivarius* ATCC 9222. The resin sample including TA was mixed to contain 1000 μg/ml. A control sample used was adhesive resin without tannic acid. The samples were washed in 0.05 M Tris-HCl buffered saline (pH = 7.2) after centrifuging bacterial strains (O.D. 600 = 0.7) with 10,000 g (15 min at 4°C). They were set in 0.05M Tris-HCl buffer (pH = 7.2) and sonicated for 40 sec (20 KHz) to obtain 3.13×10^{12} cells/ml in the buffer solution. All measurements were carried out for six samples at each experiment.

RESULTS AND DISCUSSION

Table 1 indicates the inhibitory zone size in three kinds of test samples (uncured, immediately after curing and 24 hours after curing of adhesive resin including TA solution or without TA) for six types of bacterial strains tested in this study. The antibacterial effect in TA-added adhesive resin in DBS was observed for all strains, but not-included TA samples showed antibacterial effect except *S. sobrinus* 6715–13 and *S. sanguis* ATCC 10556. Immediately after curing, TA-added samples had a weak antibacterial effect except *S. sobrinus* 6715–13, but not-included TA samples had a weak antibacterial effect for S.sobrinus OMZ 176 and *S. salivarius* ATCC 9222. At 24 hours after curing, TA-added samples showed a weak antibacterial effect for S.mutans IB 1600, *S. mutans* OMZ 175 and *S. sobrinus* OMZ 176, not-included TA samples had no antibacterial effect. Table 2 indicates MIC for bacterial strains tested in this study. Two types of *S. mutans* had higher antibacterial effect than the others, as compared them (300 μg/ml) with 400 μg/ml in the others.

Figures 1a and 1b, respectively, the change of bound TA with incubation time and TA concentration for four types of bacterial strains. To make 40 μg/ml of test samples, TA was added to the samples, and incubated for 15 seconds, and 30, 60 and 120 minutes and centrifuged for 15 min (10,000 g at 4°C). Also, bound TA was measured at 30 minutes as an incubation time when TA con-

Table 1 Antibacterial effect of bonding agent including tannic acid (TA) solution in DBSs. Three samples measured about inhibitory zone size were an uncured bonding agent, a bonding agent obtained immediately after hardening (cure) and a bonding agent obtained at 24 hours after hardening (cure).

	Uncured		Immediately after curing		24 hours after curing	
	TA (+)*	TA (–)**	TA (+)	TA (–)	TA (+)	TA (–)
S. mutans IB 1600	4.20 (2.85)	3.00 (1.38)	0.51 (0.89)	+	+	–
S. mutans OMZ 175	6.83 (2.86)	4.92 (2.56)	0.60 (0.73)	–	0.13 (0.22)	+
S. sobrinus OMZ 176	4.86 (2.34)	4.91 (1.11)	0.99 (0.70)	0.61 (0.66)	+	–
S. sobrinus 6715–13	3.80 (2.24)	–	–	–	–	–
S. sanguis ATCC 10556	0.51 (0.59)	–	0.24 (0.41)	–	–	–
S. salivarius ATCC 9222	4.68 (2.72)	3.14 (2.25)	0.79 (0.32)	0.94 (0.58)	–	–

TA (+)* : Tannic acid included
TA (–)**: not-included tannic acid
n = 6, ( ): SD

Table 2 Minimum inhibitory concentration (MIC) of tannic acid in bonding agent inDBSs when examined by means of six types of bacterial strains.

Strains	MIC (μg/ml)
S. mutans Ingbritt 1600	300
S. mutans OMZ 175	300
S. sobrinus OMZ 176	400
S. sobrinus 6715–13	400
S. sanguis ATCC 10556	400
S. salivarius ATCC 9222	400

centration varied from 40 to 300 μg/ml. TA amount was calculated from calibration curves at 277 nm wavelength with maximum absorption of tannic acid which was dissolved into 0.05 M Tris-HCl buffer. In this study, bound TA concentration in the samples measured, which was defined as the amount bound between TA concentration (sample) and TA solution (added). Bound TA concentration reached to a constant value. Also, bound TA concentration increased until 100 μg/ml, and then reached to constant values for *S. mutans* IB 1600 and *S. sobrinus* 6715–13 in a range of 100 to 300 μg/ml. *S. mutans* IB

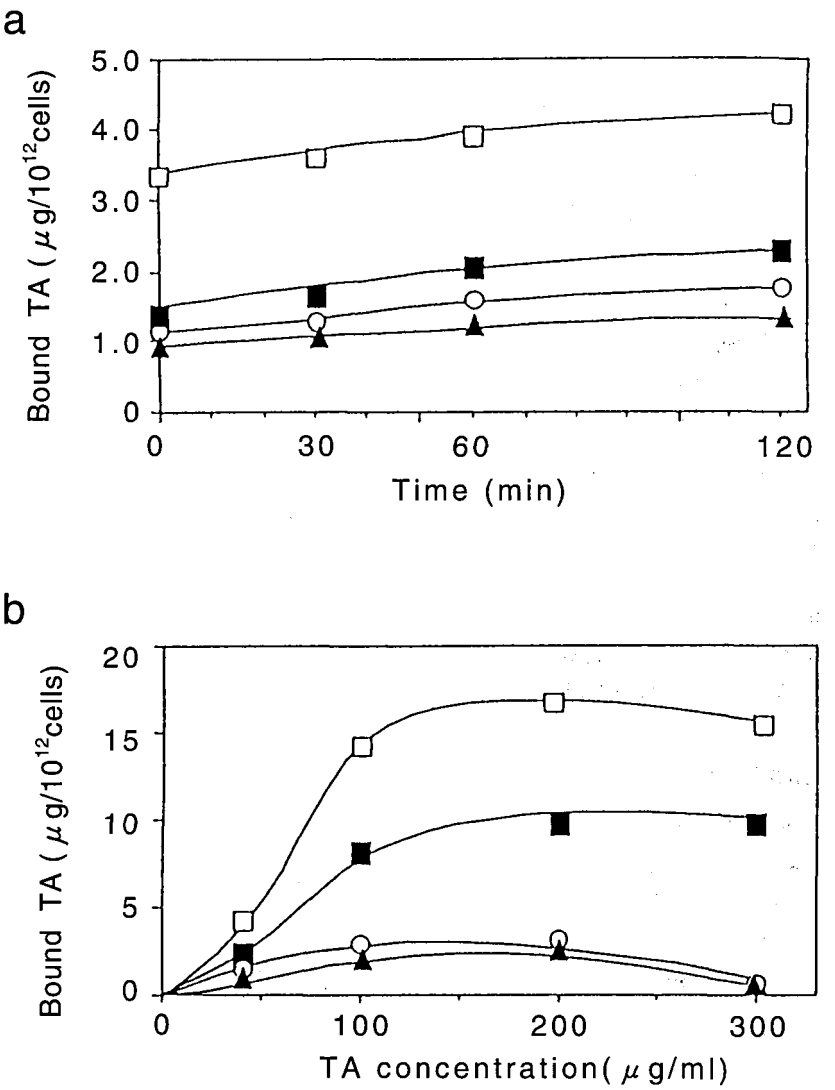

Figure 1 Changes of bound tannic acid with incubation time (a) and tannic acid concentration (b); (a) Tannic acid solution concentration = 40 μg/ml (37°C), and (b) incubation time = 30 min (37°C); □ *S. mutans* Ingbritt 1600, ■ *S. sobrinus* 6715–13, ○ *S. salivarius* ATCC 9222, and ▲ *S. sanguis* ATCC 10556.

1600 had greater bound TA concentration during longer incubation time and also with higher initial TA concentration.

This study showed that an antibacterial effect was found clearly in an adhesive resin with tannic acid in dentine adhesive systems. The antibacterial effect depended on a bond of TA and protein, or a chemical bond between TA and metal ion[12,13]. The result suggests that TA affects bacterial strains associated with the bond. Thus, bound TA concentration was measured, showing that S.mutans IB 1600 had greater bound TA amount than did the other bacterial strains (Figs 1a and 2b). There appeared a relation between antibacterial effect and bound TA. Transmission electron microscopy study showed that there was not a structural change of bacterial strain (*S. mutans*) for TA-treated bacteria strains[13]. The use of adhesive resins including TA was to clarify the antibacterial effect, showing the effect was found clearly by the addition of TA because of gradual TA absorption to bacteria strains. Based on TA effect on human teeth, the bond of TA and protein was formed by OH (TA) and = CO (protein)[13], or a bond of TA and collagen was OH (TA) and NH (collagen)[14]. These results suggest that tannic acid bonds to collagen structure when treated with tannic acid- included bonding agent to etched dentine surface. As a preliminary study of inhibitory zone size, tannic acid solution gave effectively an antibacterial effect (Table 1, and Figs 1a and 1b). Examining bound TA concentration in an adhesive resin as a dentine adhesion system which bonded between dentine and resin composite, their incubation time and tannic acid concentration in the adhesive resin were important factors to control antibacterial effect to work effectively to bacterial strains.

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