Addition of Antibacterial Agents to MMA-TBB Dentin Bonding Systems—Influence on Tensile Bond Strength and Antibacterial Effect—

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To produce a bonding system which has both high bond strength and antibacterial properties, an antibacterial agent (vancomycin: VCM or metronidazol: MN) was added to the PMMA powder of 4-META/MMA-TBB resin (CB). The influence of the addition of an antibacterial agent on tensile bond strength to dentin and the antibacterial effect were investigated in this study. Forty-seven freshly extracted bovine first or second incisors were used to measure the tensile bond strength to dentin. The bond strengths to bovine dentin were not significantly decreased by addition of VCM (1%, 2%, 5%), or MN (1%) to CB (p<0.05).

The antibacterial effect of CB containing antibacterial agent on six strains of bacteria was investigated by the agar plate diffusion method, analyzing the appearance of the inhibition zone around a resin disk following anaerobic culturing. The resin disks containing VCM showed antibacterial effects on all of the strains examined; the widths of the inhibition zones were 4-15 mm. The resin disks containing MN showed antibacterial effects on three strains; the widths of the inhibition zones were 0-4 mm.

It was thus possible to produce a bonding system with both antibacterial effect and high tensile bond strength by addition of VCM to PMMA powder.

Key words: 4-META/MMA-TBB resin, Dentin bonding, Antibacterial effect

INTRODUCTION

The bonding ability to the tooth substrate of resinous restorative materials has been improved. Some resinous materials that display excellent bonding to dentin have been developed. Among these materials, 4-META/MMA-TBB resin (CB) has been reported to form a particularly high quality dentin-resin hybrid layer and to achieve effective bonding to dentin1-7. It is reported that excellent histopathological results are obtained with direct pulp capping using CB8.

Kaketa reported that bacteria invaded the cavity floor under the adhesive resin restoration in vivo. Thus we thought that a bonding system which has both high bond strength and antibacterial properties may improve the treatment outcome of resin direct pulp capping therapy.

Several studies have examined the antibacterial properties of dental materials
incorporating antibacterial materials or antibiotics\textsuperscript{9-22}). However, there are few reports of addition of antibacterial properties to resinous dentin bonding materials\textsuperscript{13,14,16,18-22}). It was reported that uncured primer or dental resin containing antibacterial monomer showed an inhibitory effect on the growth of bacteria, both in contact and not in contact with the specimen surface\textsuperscript{20}). After curing, the primer or dental resin containing antibacterial monomer showed an inhibitory effect on the growth of bacteria contacting the specimen surface, but displayed little antibacterial effect on bacteria which did not contact the specimen surface\textsuperscript{18,21,22}).

In this study, to produce a bonding system which has both high bond strength to dentin and antibacterial properties against bacteria which do not contact the bonding material, an antibacterial agent (vancomycin: VCM or metronidazol: MN) was added to the PMMA powder of 4-META/MMA-TBB resin (CB). The influence of the addition of an antibacterial agent on tensile bond strength to dentin and the antibacterial effect were investigated.

MATERIALS AND METHODS

Table 1 shows the materials used in this experiment.

\textit{Preparation of experimental bonding agent}

To prepare the antibacterial bonding agent, an antibacterial agent (VCM or MN) was added to the PMMA powder of CB at 1\% or 2\% or 5\%. This PMMA powder and the mixture of the MMA monomer and TBB catalyst (4 drops: 1 drop) were used by the brush-on technique.

\textit{Specimen preparation for tensile bond strength measurement}

Forty-seven freshly extracted bovine first or second incisors were used to measure the tensile bond strength to dentin without extracting the dental pulp.

The presumed ages of these bovines were all over forty-two months. Teeth which showed severe abrasion were excluded. The teeth were stored in cooled isotonic so-

<table>
<thead>
<tr>
<th>Table 1 Materials used in the experiment</th>
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<tbody>
<tr>
<td>material</td>
</tr>
<tr>
<td>antibacterial agent</td>
</tr>
<tr>
<td>vancomycin</td>
</tr>
<tr>
<td>metronidazol</td>
</tr>
<tr>
<td>bonding system</td>
</tr>
<tr>
<td>Super Bond C &amp; B</td>
</tr>
<tr>
<td>dentin activator</td>
</tr>
<tr>
<td>powder (clear)</td>
</tr>
<tr>
<td>liquid</td>
</tr>
<tr>
<td>catalyst</td>
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<tr>
<td>composite</td>
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</table>
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Dium chloride solution and used within 12 hours after sacrifice. The labial surface was ground flat to expose the dentin using a belt surfacer (Surftmet 1, Buehler, Illinois, USA). The exposed dentin surfaces were polished with wet 1000-grit silicon carbide paper. The surface was rinsed for 30 seconds with a water spray and dried for 30 seconds with an air syringe. The teeth were randomly divided into groups for the tensile bond strength test.

Method of tensile bond strength measurement
The dentin surface was treated for 30 seconds with 10% citric acid and 3% ferric chloride aqueous (Super Bond Dentin Activator, Sun Medical Co., Shiga, Japan). The treated surface was rinsed for 30 seconds with a water spray and dried for 30 seconds with an air syringe. A stainless steel ring (inner diameter=4 mm, height=2.5 mm) was placed on the treated dentin surface. (Fig. 1) The PMMA powder, containing VCM (1%, 2%, 5%) or MN (1%, 2%, 5%), and the mixture of the MMA monomer and TBB catalyst (4 drops: 1 drop) were applied to the treated surface by the brush on technique. Ten minutes after application of the resin mixture, the experimental light-cured composite resin (Metafil 0022, Sun Medical Co., Shiga, Japan) was filled into the ring and cured for 60 seconds with a visible light curing apparatus (Luxor,

Fig. 1 Schematic illustration of the apparatus to measure the tensile bond strength.
I.C.I., Cheshire, England). Twenty minutes after application of the composite, the sample was stored in 37°C deionized water for 24 hours. The tensile bond strength was measured at a cross-head speed of 0.5 mm/min. using a universal testing machine (Instron 1123, Boston, Massachusetts, USA).

The data were statistically analyzed using One Way Analysis of Variance (ANOVA) and Scheffe’s test at $\alpha = 0.05$.

**Measurement of antibacterial effect**

*Streptococcus mutans* (NCTC10449), *Streptococcus sanguis* (ATCC10558), *Streptococcus salivarius* (NCTC8618), *Streptococcus mitis* (NCTC3165), *Actinomyces viscosus* (WVU627), and *Actinomyces naeslundii* (ATCC12104) were used for the antibacterial property test.

The PMMA powder, containing VCM (1%, 2%, 5%) or MN (1%, 2%, 5%), and the mixture of the MMA monomer and TBB catalyst (4 drops: 1 drop) were applied to a polyethylene tube (inner diameter=5 mm, height=2 mm) by the brush-on technique. After 24 hours, the polyethylene tube was removed and the resin disk was used for antibacterial testing. A resin disk containing no antibacterial agent was used as a control. The bacteria were anaerobically (80%N₂+10%CO₂+10%H₂) cultured for 48 hours in brain-heart infusion (Difco, Detroit, MI) broth. The bacterial suspension was spread on blood agar plate (sheep: Denka Seiken Co., Ltd. Tokyo, Japan) to be adjusted to $1 \times 10^4$ CFU/cm²; the resin disks were placed on these plates. After 48 hours’ anaerobic incubation (80%N₂+10%CO₂+10%H₂), the diameter (D) of the inhibition circle was measured with sliding calipers; the width (W) of the inhibition zone was calculated as $W = (D-5)/2$ (mm). Three specimens were tested for each group.

**Measurement of the persistence of antibacterial effect**

*A. naeslundii* was used to measure the persistence of the antibacterial effect. The resin disks were stored in 500 ml of deionized water at 37°C for 7, 30 or 90 days. The deionized water was exchanged every seven days. After storing, the resin disks were used for antibacterial testing for *A. naeslundii* to examine persistence of the antibacterial effect.

The resin disks that came to show no antibacterial effect were bisected along a diameter and placed on the agar plate to contact the cleavage plane to the agar plate. The antibacterial effects for *A. naeslundii* were examined.

**RESULTS**

Table 2 shows the tensile bond strengths of CB containing VCM or MN to bovine dentin. The tensile bond strength of CB containing VCM (1%, 2%, 5%) or MN (1%) indicated no significant difference compared to the control.

Table 3 shows the mean widths of the inhibition zones. No inhibition zone was observed around resin disks containing no antibacterial agent. The resin disks con-
Table 2  Tensile bond strength of CB containing VCM or MN to bovine dentin

<table>
<thead>
<tr>
<th>concentration</th>
<th>VCM</th>
<th>MN</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>24.0 (7.3) 11 (R=4, B=7, I=0)</td>
<td></td>
</tr>
<tr>
<td>1.0%</td>
<td>26.6 (6.0) 6 (R=2, B=4, I=0)</td>
<td>29.1 (8.1) 7 (R=3, B=3, I=1)</td>
</tr>
<tr>
<td>2.0%</td>
<td>22.7 (5.4) 6 (R=4, B=2, I=0)</td>
<td>13.9 (3.4) 6 *(R=0, B=6, I=0)</td>
</tr>
<tr>
<td>5.0%</td>
<td>21.1 (5.2) 6 (R=3, B=3, I=0)</td>
<td>9.5 (4.3) 5 *(R=0, B=5, I=0)</td>
</tr>
</tbody>
</table>

*significantly different from 0% (P<0.05), MPa (S.D.) N (fracture pattern, R: cohesive fracture in composite resin, B: cohesive fracture in bonding resin, I: interface fracture between bonding resin and dentin)

Table 3  Mean width of inhibition zones of the resin disks

<table>
<thead>
<tr>
<th></th>
<th>none</th>
<th>VCM</th>
<th>MN</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0%</td>
<td>1%</td>
<td>2%</td>
</tr>
<tr>
<td>S. mutans</td>
<td>0</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>S. sanguis</td>
<td>0</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>S. salivarius</td>
<td>0</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>S. mitis</td>
<td>0</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>A. viscosus</td>
<td>0</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>A. naeslundii</td>
<td>0</td>
<td>9</td>
<td>13</td>
</tr>
</tbody>
</table>

mm, N=3

Table 4  Mean width of inhibition zones of the stored resin disks and the cleavage plane of bisected resin disk against A. naeslundii

<table>
<thead>
<tr>
<th>storage period</th>
<th>none</th>
<th>VCM</th>
<th>MN</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 day</td>
<td>0</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>7 days</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>30 days</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>90 days</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>cleavage plane</td>
<td>0</td>
<td>6</td>
<td>8</td>
</tr>
</tbody>
</table>

mm, N=3

taining VCM exhibited inhibition zones against all 6 bacteria strains. The resin disks containing MN exhibited inhibition zones against S. mutans, S. mitis, and A. naeslundii, but not against S. sanguis, S. salivarius, or A. viscosus. The widths of the inhibition zones of VCM were greater than those of MN.

Table 4 shows the mean widths of the inhibition zones of the stored resin disks and the cleavage plane of the bisected resin disks along the diameter against A. naeslundii.
DISCUSSION

Selection of bonding system, antibacterial agents, and bacteria strains

The 4-META/MMA-TBB bonding system was used as the dentin bonding system in this study. One of the reasons for choosing this system was that it has excellent bonding properties to dentin\textsuperscript{[1-7]}. The other reasons were that it is suitable for direct pulp capping\textsuperscript{[3]}, and it was easy to add the antibacterial agents because the system is composed of a powder and liquid.

Kaketa\textsuperscript{[23]} reports that bacteria invaded the cavity floor under the adhesive resin restoration \textit{in vivo}, and that these bacteria were almost entirely anaerobic gram-positive rods and cocci. VCM and MN were thus chosen for this study, because VCM shows a high antibacterial effect for gram-positive bacteria, and MN shows a wide spectrum on anaerobes.

Moreover, Kaketa\textsuperscript{[23]} reported that predominant isolates were \textit{Actinomyces} and \textit{Streptococcus}, and facultative anaerobes were more frequently isolated than obligate anaerobes. Thus, gram-positive facultative anaerobes, \textit{Actinomyces} and \textit{Streptococcus}, were used in this experiment.

\textit{A. naeslundii} was used to measure the persistence of the antibacterial effect, because resin disks containing both VCM and MN showed antibacterial effects against \textit{A. naeslundii}.

Tensile bond strength

Needless to say, it is preferable for human teeth immediately after extraction to be used for the adhesion testing of dentin adhesive materials. However, it is extremely difficult to obtain a sufficient number of freshly-extracted human teeth of which the kind and age are uniform. It was reported that the kind, age, and storage conditions of teeth influence the bond strength\textsuperscript{[24-28]}. Sasazaki \textit{et al.}\textsuperscript{[29]} reported that exudation of internal fluid from dentinal tubules was observed within 12 hours after sacrifice on the ground dentin surface of extracted bovine tooth from which the dental pulp had not been extracted, as well as from vital human tooth \textit{in vivo}. Kosuga \textit{et al.}\textsuperscript{[27]} reported there was no significant difference between the tensile bond strengths to first and second lower extracted incisors, from which the dental pulp had not been extracted, of bovines over 3.5-years-old. Therefore, first and second lower extracted teeth, from which dental pulp had not been extracted, of bovines over 3.5-years-old were used in this study.

The addition of VCM (1\%, 2\%, 5\%) and MN (1\%) did not affect the tensile bond strength, but addition of MN (2\%, 5\%) caused a significant decrease. The fracture patterns all indicated cohesive failure in the bonding layer when MN (2\%, 5\%) was added, while the fracture patterns of the controls were cohesive in the composite resin or bonding layer. Therefore, it was suggested that the reduction in tensile bond strength with MN (2\%, 5\%) was caused by a lowering of the mechanical strength of bonding layer due to the addition of MN (2\%, 5\%). On the other hand, the ratio of cohesive failure in the bonding layer of VCM (1\%, 2\%, 5\%) and MN (1
\%\) was similar or decreased compared with the control. Therefore, it was suggested that the tensile bond strength was not decreased by addition of VCM (1 \%, 2 \%, 5 \%) and MN (1 \%), because addition of VCM (1 \%, 2 \%, 5 \%) and MN (1 \%) did not lower the mechanical strength of the bonding layer.

It became clear that addition of antibacterial agents to the 4-META/MMA-TBB bonding system did not decrease the tensile bond strength to dentin when the type and concentration were appropriate.

**Antibacterial effect**

The resin disks containing no antibacterial agent showed no antibacterial effect. Sato et al.\(^{30}\) reported that acrylic resin showed an antibacterial effect, because of the presence of uncured monomer. On the other hand, Yamauchi et al.\(^{31}\) reported that cured 4-META/MMA-TBB resin shows little antibacterial effect. It was considered that the curing reaction of the resin disks used in our study was advanced to some extent, because the resin disks were made 24 hours before the experiment. It was considered that elution of uncured monomer was minimal, and thus the additive-free resin disks showed no antibacterial effect.

The resin disks containing VCM produced inhibition zones against all six bacteria tested; the sizes of inhibition zones were larger than those of MN. It was supposed that diffusion of VCM to the agar plate was more rapid than that of MN, since the solubility in water of VCM is far higher than that of MN. The antibacterial effect was decreased by elongation of the storage period, and eventually disappeared. However, the surface of the cleavage plane of the bisected resin disks along the diameter of resin disks showed an antibacterial effect. It was thought that antibacterial agent was released from resin disk during the prolonged storage periods, but that the antibacterial agent remained in the inner part of the resin disk. The resin disks containing VCM showed an antibacterial effect for longer than with MN, probably because elution of VCM from the resin disk was more rapid than that of MN, since the solubility in water of VCM is far higher than that of MN.

**Antibacterial resinous bonding system**

Yoshikawa\(^{19}\) reported that tannic acid derivatives (TAD) improve the bond strength to bovine dentin, and TAD showed antibacterial effect before polymerization. After polymerization, resin disks containing TAD showed an antibacterial effect on the growth of bacteria in contact with the resin surface\(^{19}\). Imazato et al.\(^{20}\) reported that uncured primer containing methacryloyloxydodecylpyridinium bromide (MDPB) showed an inhibitory effect on the growth of bacteria both in contact and not in contact with the resin surface. After curing, primer containing MDPB showed an inhibitory effect on the growth of bacteria in contact with the resin surface, but displayed little antibacterial effect against bacteria which were not in contact with the resin surface\(^{18,22}\). The present study produced a bonding system which showed both an antibacterial effect against bacteria which were not in contact with the resin surface and a high tensile bond strength to dentin, by addition of VCM (1 \%, 2 \%, 5 \%) to
PMMA powder of CB. Even after polymerization, this bonding system showed an antibacterial effect.

Recently, it was reported that the resinous bonding materials are suitable for direct pulp capping\(^\text{32-36}\). On the other hand, Kaketa\(^\text{23}\) reported that bacteria invaded into the cavity floor under the adhesive resin restoration in vivo, and these bacteria were almost entirely anaerobic gram-positive rods and cocci. The predominant isolates were *Actinomyces* and *Streptococcus*, and facultative anaerobes were more frequently isolated than obligate anaerobes\(^\text{23}\). CB containing VCM (1%, 2%, 5%) in PMMA powder showed an antibacterial effect against gram-positive facultative anaerobes, *Actinomyces* and *Streptococcus*, in this experiment. Thus it was thought that application of this bonding system, which has both high bond strength and antibacterial properties, may improve the treatment outcome of resin direct pulp capping therapy. In clinic, wounds and the dentin are not always germ free; bacteria may be present in the dentin or dental pulp when direct pulp capping is applied. Thus it is thought that this bonding system, which shows an antibacterial effect against bacteria that are not in contact with the resin surface, may improve the treatment outcome of resin direct pulp capping therapy. The effect of application of this antibacterial bonding system to direct pulp capping is the subject of a further study.

Additionally, the dentin treatment solution used in this system was thought to have an antibacterial effect, because this solution was acidic (pH≤1) and bacteria are generally weak to acid. The antibacterial effect of this solution is the subject of a further study.

**CONCLUSION**

The present study produced a bonding system which showed an antibacterial effect and high tensile bond strength to dentin by addition of VCM (1%, 2%, 5%) to PMMA powder of CB. Even after polymerization, this system showed an antibacterial effect against bacteria that did not contact the resin surface.

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