REVIEW

Modified Sealed Restoration and the Development of Dentin Regeneration Therapy

Masahiro YOSHIYAMA¹, Hirotoshi SHIMIZU¹, Yoshihiro NISHITANI¹, Toshiyuki ITOTA¹ and David H.PASHLEY²

¹Department of Operative Dentistry, Okayama University, Graduate School of Medicine and Dentistry, Okayama, Japan
²Department of Oral Biology and Maxillofacial Pathology, School of Dentistry, Medical College of Georgia, Georgia USA
Corresponding author: Prof. Masahiro Yoshiyama, DDS, PhD
  e-mail: yoshiyam@md.okayama-u.ac.jp

SYNOPSIS

The concept of minimal intervention dentistry has evolved as a consequence of our increased understanding of the caries process and the development of adhesive restorative materials. Recently, new concepts of treatments for dentin caries by use of adhesive resins and glass-ionomer cements have been proposed. However, new hard tissue, indicated as the result of applying calcium hydroxide or adhesive resins and/or sterilized by the mixed drugs, formed with a tunnel defect frequency present, running from the medicament interface to the pulp. These reports suggest the urgent necessity for us to the establishment of the biological dentin regeneration therapy like the Modified Sealed Restoration (MSR). In this review, we reported at first about “Resin adhesion to caries-infected dentin”, and then “Dentin regeneration therapy with growth factor (CTGF)” and “In vivo dentin regeneration by adhesive resin containing EVA+C” and finally “Future approaches to establish the dentin regeneration therapy”.

Key words: sealed restoration, resin adhesion, dentin regeneration, EVA+C, CTGF
Introduction

Recently, new concepts of treatments for dentin caries by use of adhesive resins and glass-ionomer cements have been proposed. Table 1 summarizes the new tendency of treatments for dentin caries, and dramatic changes of the roles of adhesive dentistry have occurred from the end of the 20th century. From the mainly restorative dentistry in 20th century, contemporary dentistry shifts towards a minimal intervention (MI) approach, and contemporary operative treatment incorporates the MI philosophy in cavity design\(^1\). Especially, hybridized dentin permits dental treatments that were previously impossible with conventional techniques, opening new previously impossible with conventional technique, opening new frontiers in modern adverted dentistry\(^2\).

Traditional treatment of carious teeth involves removal of all carious tooth structure prior to placement of the restorative materials, often sacrificing more structure than necessary. Handelman et al. proposed using resin sealants to seal carious pits and fissures following acid-etching\(^3\). The careful work of Handelman and his colleagues demonstrated that the residual bacteria became dormant and much less viable\(^4\). This work was followed by a ten year clinical trial by Mertz-Fairhurst et al\(^5\). They radiographically selected lesions that were beyond the EDJ but no more than half-way to the pulp. The enamel was beveled and no carious dentin was removed. After acid-etching, they placed a chemically-cured radiopaque posterior resin composite (Fig.1). They showed no radiographic progression of the lesions and

<table>
<thead>
<tr>
<th>Table 1</th>
<th>New tendency of treatment for dentin caries</th>
</tr>
</thead>
<tbody>
<tr>
<td>ART</td>
<td>Atraumatic restorative technique</td>
</tr>
<tr>
<td>IPC</td>
<td>Indirect pulp capping</td>
</tr>
<tr>
<td>LSTR</td>
<td>Lesion sterilization and tissue regeneration</td>
</tr>
<tr>
<td>MI</td>
<td>Minimal intervention</td>
</tr>
<tr>
<td>MSR</td>
<td>Modified sealed restoration</td>
</tr>
<tr>
<td>DRT</td>
<td>Dentin regeneration therapy</td>
</tr>
</tbody>
</table>

Fig.1 The concept of sealed restoration proposed by Mertz-Fairhurst et al. (1998).
few viable microorganisms when the resin-Sealed lesions were biopsied years after placing resin composites, but further progression of caries in untreated teeth. Their studies were done with relatively old materials rather than with more hydrophilic contemporary adhesive formulations. If residual bacteria in caries-infected dentin can be embedded by adhesive resins, and if these embedded bacteria become dormant, even caries-infected dentin may be conserved without progression of the caries process. We have proposed this concept as "Modified sealed restoration" (MSR) as shown in Figure 2. Moreover, if remineralization of caries-infected demineralized dentin is occurred after MSR with an antibacterial fluoride-releasing adhesive system, we may establish the ultra-conservative therapy of caries-infected dentin (Fig.3).

Recently, exciting biomimetic and tissue engineering approaches to develop the dentin regeneration therapy (DRT). These
might include growth factor, gene or stem cell therapy (Fig. 4). We have focused on the effect of connective tissue growth factor (CTGF) and collagen-immobilized ethylene-co-vinyl alcohol (EVA+C) to the differentiation of pulp cells into odontoblasts.

**Resin adhesion to caries-infected dentin**

However, so as to establish the ultra-conservative therapy of caries-infected dentin, we showed examine the resin adhesion to caries-infected dentin. Recently, a new bond strength testing procedure has been developed which permits the measurement of small (ca. 1mm²) cross-sectional bonded areas. It has been called the microtensile bond strength test (μTBS), and permits the testing of irregular surfaces such as sclerotic and carious dentin. By using μTBS, we were able to evaluate the adhesion of contemporary resin systems to cervical sclerotic dentin⁷,⁸. We have also evaluated the interfacial morphology of two bonding system (Single Bond, 3M and Fluoro Bond, Shofu) to caries-infected dentin, coupled with the measurement of μTBS⁷ and reported that resin bonds made to caries-affected dentin were lower than to normal dentin using either self-etching primer or conventional adhesive systems. Nakajima et al. could demonstrate that very high bond strength could be obtained on caries-affected dentin if the dentin is etched with 35% phosphoric acid and the moist bonding technique is employed⁹.

To establish a new dentin caries treatment involving embedding residual bacteria with adhesive resins, we need to evaluate the adhesive properties of bonding resins to caries-infected dentin. Therefore, we have clarified the adhesive
property of a self etching/self-priming system (Clearfil Liner Bond 2V, Kuraray Medical Inc.) (CV) to normal, caries-affected and caries-infected dentin using μ TBS and transmission electron microscopy (TEM)\textsuperscript{10}. Table 2 shows the composition of the self-etching primer system (CV) employed in our study, and CV contains 10-methacryloyloxy methacrylate (MDP) as an adhesive monomer. The occlusal surface of extracted human third molars with coronal dentin caries were ground perpendicular to the long axis of the tooth to expose a flat surface where the carious lesion was surrounded by normal dentin (Fig.5)
CV to normal dentin was about 44.7±10.1 MPa, while the bond strength of CV to caries-affected dentin was significantly lower (29.9±10.0 MPa) than that to normal dentin (p<0.05). The μ TBS of CV to caries-infected dentin was only 9.8±5.4 MPa which was significantly lower (p<0.05) than CV bonds to caries-affected dentin.

TEM observation of the ultrathin sections of the interface from resin-bonded normal dentin showed a thin hybrid layer (less than 1.0 μ m) was formed by CV in the dentin (Fig.6). Higher magnification TEM revealed the smear layer was completely dissolved. Upwardly-directly banded collagen fibers could be identified along the bonded dentin surface. TEM observation of the interfaces of bonded caries-affected dentin showed a much higher hybrid layer (6-8 μ m) than was seen in normal dentin. A gradient of resin could also be identified from the surface of the hybrid layer downward, with the base of the hybrid layer poorly identified (Fig.7). Bacteria were rarely observed within the dentinal tubules or on dentin surfaces of the bonded car-

The results of the μ TBS of CV to normal, caries-affected and caries-infected dentin are shown in Table2. The μ TBS of
the entrapment of bacteria within some dentinal tubules (Fig.8). Similar to the caries-affected dentin, a gradient of resin could be observed in these thick hybrid layers. Moreover, parts of the dentin surface were not completely wetted by the filled, bonding resin component of the adhesive systems. Other sections revealed thick, erratic hybrid layers that consisted of a superficial layer of completely disorganized and denatured intertubular and peritubular dentin, and an underlying layer of intact hybridized dentin. Bacteria were also trapped within disfigured dentinal tubules.

Dentin regeneration therapy with growth factor (CTGF)

The concept of minimal intervention dentistry has evolved as a consequence of our increased understanding of the caries process and the development of adhesive restorative materials.\textsuperscript{11, 12}

The findings from the recent literature on pulpal cell responses to the application of calcium hydroxide to exposed pulps are described. The initial effect of calcium hydroxide applied to exposed pulp is the development of a superficial three-layer
necrosis. The firm necrosis causes slight irritation and stimulates the pulp to defense and repair. This is followed by the repair process, including migration and proliferation of mesenchymal and endothelial pulp cells and formation of collagen. The mineralization of the collagen starts with dystrophic calcification of both the zone of firm necrosis and the degenerated cells in the adjacent tissue, leading to deposition of mineral in the newly-formed collagen. The presence of calcium ions stimulates precipitation of calcium carbonate in the wound area and thereby contributes to the initiation of mineralization. In the recent report, the success rate in this method was 81.8%, and showed that direct pulp capping using calcium hydroxide was applicable to carious-exposed pulp, and the degree of bleeding is indicative of the prognosis of this treatment.

Some groups investigated nerve regeneration and proliferative activity in amputated pulp tissue after the application of 4-META/MMA-TBB adhesive resins (4-META resin). At 3 days, fibroblast-like cells were positive for proliferating cell nuclear antigen (PCNA) in both 4-META resin- and calcium hydroxide-treated groups and were located mainly within 0.5 mm from the cut surface. These reports showed that although cell differentiation and nerve regeneration were delayed, wound healing occurred even after the application of 4-META resin to exposed pulp surface the same as calcium hydroxide application.

However, new hard tissue, indicated as the result of applying calcium hydroxide or adhesive resins and/or sterilized by the mixed drugs, formed with a tunnel defect frequency present, running from the medicament interface to the pulp.

These reports suggest the urgent necessity for us to the establishment of the biological dentin regeneration therapy like the Modified Sealed Restoration (MSR).

To establish the new method of the biological dentin regeneration therapy, we pay attention to Connective tissue growth factor (CTGF).

CTGF, which is encoded by an immediate early gene and a member of the CCN family, has been shown to be expressed in osteoblasts, fibroblasts and chondrocytes (as endochondral ossification genetic factor, ecogenin). The expression of CTGF mRNA in osteocytes and osteoblasts became more intense around the periodontal ligament, and the intense expression of CTGF extended to osteocytes situated deep in alveolar bone matrix apart from periodontal ligament in both tension and compression sides.

In our research, we immunohistochemically analyzed the expression of type I collagen, alkaline phosphatase and
osteonectin. Type I collagen was well known as one of matrix protein of hard tissue formation. Alkaline phosphatase and osteonectin were indicators of tissue mineralization. All of the pulp cells in this study showed the presence of type I collagen in extracellular matrix. Type I collagen positive cells showed a tendency to increase the expression in 24 hours after the CTGF stimulation. However in 48, 72, 96 hours stimulation could not change the tendency of expression. The expression of alkaline phosphatase was shown in Fig.9. In 24-48 hours stimulation (including non stimulation), the expression of alkaline phosphatase was not detected. In the case of 72 hours stimulation, alkaline phosphatase positive cells showed a
tendency to localize in some area, and marked expression was detected in cells and extra cellular matrix for 96 hours stimulation. The expression of osteonectin was also shown in Fig.10. Osteonectin showed the marked expression in cells and extra cellular matrix for 96 hours stimulation.

These results suggest that CTGF promotes the production of type I collagen immediately, and then, the expression of alkaline phosphatase and osteonectin are affected by CTGF stimulation.

CTGF is expressed in mechanical stimulated osteocytes\textsuperscript{19}, and different from the growth factors like IGF and/or TGF-\textbeta{} which partially promotes the process of endochondral ossification. It plays broadly important roles in some steps of the formation of hard tissue\textsuperscript{12,20}.

It is thought that CTGF play important roles in the wound healing and the formation of second dentin in human dental pulp, and the result of this study suggested that CTGF would be valid for the regeneration of pulp and dentin.

**In vivo dentin regeneration by adhesive resin containing EVA+C**

We have examined the biocompatibility of an experimental adhesive system containing EVA+C (EVA+C bioprimer) to cultured human pulpal cells, and the results suggest that EVA+C bioprimer shows a very good biocompatibility to these cells in vitro. Moreover, we have cut the exposed cavities in the anterior teeth of the monkey under the general anesthesia, and applied the EVA+C bioprimer to the pulp-exposed surface and filled the cavity with a commercial composite resin (Clearfil MegaBond + AP-X, Kuraray Medical, Tokyo, Japan). After 3 months, we have extracted the filled tooth from the monkey, and observed the condition of regenerative dentin at the exposed pulp by a light microscopy after the EDTA demineralization of the teeth. Figure 11 shows the thinner regenerative dentin on the exposed pulp without EVA+C bioprimer as a control, and Figure 12 shows the thicker regenerative dentin formation on the exposed pulp with EVA+C bioprimer (Patent has been applied).

The mean thickness of the regenerative dentin with EVA+C was 618 \textmu{}m, and that without EVA+C was 357 \textmu{}m. There was a significant difference between the thicknesses of both teeth. Pulpal inflammation was observed in the teeth filled with EVA+C bioprimer.

These results were obtained from the limited in vivo animal study, however suggested that EVA+C bioprimer may promote the regeneration of dentin not only in the pulp chamber but also onto the exposed surface. This study is kindly supported by a Grant-in-Aid (B) (2)
Future approaches to establish the dentin regeneration therapy

All of these approaches require careful consideration of the processes taking place if repair and regeneration are to be achieved in a controlled manner. While delivery of these agents provides one hurdle to be overcome, a greater challenge will be the regulation of their effects if total obliteration of the pulp chamber by the repair process is to be avoided\(^{21}\). A number of other considerations will also require attention, including those of ethical and immunogenetical nature. A perfect understanding of dentin regeneration mechanism should be connected to the development of the dentin regeneration therapy.

References

