Intra-Tubular Reactions in Restored Human Teeth: A SEM Study

SUMMARY

The aim of this study was to examine restored human teeth in order to evaluate tissue changes in primary dentin or in primary-secondary dentin continuum. 16 non-carious and restored human molars were used. All teeth were processed for scanning electron microscopic (SEM) analysis. Analysis was focalised at 3 parts of the dentin matrix; at the dentin-restoration interface, at the middle part of the remaining dentinal thickness and at the dentin-pulp interface.

The results showed that in most cases dentinal tubules were wide open, from the dentin-restoration interface to the dentin-pulp interface. No sign of dentinal sclerosis or tubular obturation was found. Consequently, dentinal tubules could provide a pathway for bacteria to penetrate the dentin pulp complex. Nevertheless, dentinal tubules under composite resin restorations seemed to have been sealed by the composite material, at the dentin-restoration interface. Parameters like the age of the patient, the reason of extraction or longevity of restoration did not, indirectly, affect by any way dentin continuum. In general, it can be concluded that unlike previously reported studies, dentinal sclerosis was absent in most of the teeth studied.

Keywords: Dentin Pulp Complex; Pulp Inflammation; Tubule Obturation; Restorative Material

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Introduction

The bulk of the structure of the tooth, both crown and root, is made up of a mineralized tissue known as dentin. The most prominent features of dentin are the dentinal tubules. In the periphery, there are approximately 10,000 to 25,000 tubules per square millimeter with a diameter of 0.5μm. On the contrary, near the pulp there are about 45,000-90,000 tubules per square millimeter, each having a diameter of 2.5 to 3μm. Thus, from a clinical point of view, it should be recognized that dentin beneath a deep cavity preparation is much more permeable than dentin underlying a shallow cavity.

During time, dentinal tubules progressively narrow by the deposition of peritubular dentin in their walls. Additionally, dentinal tubules could be blocked by the precipitation of hydroxyapatite and whitlockite crystals within their structure. These biologic procedures represent dentinal sclerosis. Partial or complete obturation of dentinal tubules may occur as a result of aging, dental caries, or can develop in response to any kind of stimuli, including the affect of some properties of the restorative materials used.

Biologic reactions in dentin pulp complex must be at the centre of attention in restorative dentistry. With all the steps involved in restorative procedure, it is apparent that the biologic effects on dental tissues are multifactorial and the effect from 1 factor alone is impossible to separate from the combined effect of all factors.

Many investigators have studied the effect of dental materials on mineralized dental tissues. Some data are available on the effect of amalgam, resin composite, zinc oxide eugenol cement, and calcium hydroxide on dentin. Besides chemical toxicity, some of the properties of the materials used that are capable of producing injury include acidity, absorption of water, production of heat during setting, or poor marginal adaptation. The aim of this study was to examine non-carious and restored human
teeth in order to evaluate tissue changes in primary dentin or in primary-secondary dentin continuum.

**Materials and Method**

16 volunteers of both sexes, ranging in age from 12 to 72 years, enrolled the study. All had been scheduled to undergo tooth extraction for various therapeutic reasons, including orthodontic therapy, prosthetic rehabilitation or periodontal treatment. In all cases the extracted teeth were molars. Each tooth carried an amalgam or resin composite restoration, had no sign of clinically, or by x-ray, diagnosed secondary caries, and no sign of pulp necrosis, as pulp vitality tests indicated.

Teeth were previously denuded of any soft tissue and stored in 10% formalin. The apical third of the root was removed in order the specimen to consolidate fast. After teeth were vertically sectioned in 2 by a mechanical fracture, they were cleaned in water, immersed in 0.5% sodium hypochlorite solution and gradually dehydrated in alcohol.

**Table 1. Teeth processed for SEM evaluation**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of teeth</th>
<th>Restorative material</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7</td>
<td>5 amalgam, 2 composite</td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>5 amalgam, 1 composite</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>1 amalgam, 2 composite</td>
</tr>
</tbody>
</table>

**Table 2. Specimen details**

<table>
<thead>
<tr>
<th>Teeth</th>
<th>Reason of extraction</th>
<th>Pain symptoms*</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>periodontitis</td>
<td>no</td>
<td>66</td>
</tr>
<tr>
<td>A2</td>
<td>prosthetic</td>
<td>no</td>
<td>52</td>
</tr>
<tr>
<td>A3</td>
<td>periodontitis</td>
<td>no</td>
<td>58</td>
</tr>
<tr>
<td>A4</td>
<td>prosthetic</td>
<td>no</td>
<td>72</td>
</tr>
<tr>
<td>A5</td>
<td>prosthetic</td>
<td>no</td>
<td>52</td>
</tr>
<tr>
<td>A6</td>
<td>prosthetic</td>
<td>no</td>
<td>63</td>
</tr>
<tr>
<td>A7</td>
<td>periodontitis</td>
<td>no</td>
<td>53</td>
</tr>
<tr>
<td>B1</td>
<td>periodontitis</td>
<td>no</td>
<td>38</td>
</tr>
<tr>
<td>B2</td>
<td>unknown</td>
<td>no</td>
<td>34</td>
</tr>
<tr>
<td>B3</td>
<td>prosthetic</td>
<td>no</td>
<td>37</td>
</tr>
<tr>
<td>B4</td>
<td>periodontitis</td>
<td>no</td>
<td>40</td>
</tr>
<tr>
<td>B5</td>
<td>prosthetic</td>
<td>no</td>
<td>35</td>
</tr>
<tr>
<td>B6</td>
<td>prosthetic</td>
<td>no</td>
<td>33</td>
</tr>
<tr>
<td>C1</td>
<td>orthodontic</td>
<td>no</td>
<td>12</td>
</tr>
<tr>
<td>C2</td>
<td>orthodontic</td>
<td>no</td>
<td>15</td>
</tr>
<tr>
<td>C3</td>
<td>orthodontic</td>
<td>no</td>
<td>13</td>
</tr>
</tbody>
</table>

*Vitality tests took place when needed

Teeth were divided into 3 groups according to the patient’s age. In group A patients were at least 40 years old, in group B between 20 and 40 years old, and in group C they were at most 20 years old (Tab. 1). For each the extracted tooth, details concerning the reason of extraction, the presence of symptoms, or longevity of restoration were recorded (Tab. 2).

All teeth were processed for scanning electron microscopic analysis in a JEOL JSM-840 A SEM. When needed, Energy Dispersive (EDS) analysis was performed for qualitative examination of the chemical composition of the material at the tooth-restoration interfaces. In all cases SEM analysis was focused at 3 parts of the dentin matrix; at the dentin-restoration interface, at the middle part of the remaining dentinal thickness and at the dentin-pulp interface (Fig. 1).

![Figure 1. Parts that analysis was focalized at (specimen A1). (a) dentin-restoration interface, (b) middle part of the remaining dentinal thickness, (c) dentin-pulp interface.](image)

**Results**

The results of the SEM analysis showed that dentinal tubules were wide open in most of the cases. No sign of dentinal sclerosis or tubular obturation was found (Tab. 3). Occlusion of dentinal tubules was absent, not only at the dentin-restoration interface, but also at the middle part of dentin and at the dentin-pulp interface. In some cases (specimen B3, A4, B4), it was quite obvious that their structural continuity formed a wide pathway towards the pulp chamber (Fig. 2).

The protective cavity base or liner used under amalgam restoration could not be easily identified using EDS analysis. Nevertheless, no sign of dentinal sclerosis was found under the cavity base application sites.
It has long been recognized that dentinal tubules are the main pathways from any prepared surface to the pulp\(^1\). Consequently, occlusion of dentinal tubules reduces dentin permeability and eliminates pulpal reactions to restorative procedures and materials\(^1,3,8\). As stated before, tissue changes in dentin continuum may occur as an accelerated formation of peri-tubular dentin or by the precipitation of dissolved mineral salts within the tubules\(^3,8\). Additionally, mechanical obturation of dentinal tubules may be achieved by the application of adhesive filling materials\(^8\).

### Discussion

No material that has been developed to date is ideal for all situations, nor is any absolutely perfect for any one situation\(^13\). It has long been recognized that dentinal tubules are the main pathways from any prepared surface to the pulp\(^8\). Consequently, occlusion of dentinal tubules reduces dentin permeability and eliminates pulpal reactions to restorative procedures and materials\(^1,3,8\). As stated before, tissue changes in dentin continuum may occur as an accelerated formation of peri-tubular dentin or by the precipitation of dissolved mineral salts within the tubules\(^3,8\). Additionally, mechanical obturation of dentinal tubules may be achieved by the application of adhesive filling materials\(^8\).
These biological changes in dentin seem to be multifactorial as they depend on many factors, including the type of the material used. Amalgam, still widely used in restorative dentistry, is not considered injurious to the pulp, but requires protection in deep cavities. In spite of its limitations, it is very popular because of its strength, longevity, low cost and relatively ease of handling. One of the most significant disadvantages of amalgam that leads to marginal leakage is lack of adhesion to tooth structure. Amalgam may also discolor dentin because of penetration of mercury. Nevertheless, that is unlikely to initiate any biologic reaction in dentin and is of no importance compared with the amount of mercury released in the oral cavity.

Resin based materials are included among direct adhesive restorative materials. To achieve optimal bonding to dentin, the adhesive must penetrate the de-mineralized dentin, enter the dentinal tubules and their branches and then be polymerized. Beside the variety of the different bonding systems that are commercially available, they all have a unique bonding protocol. In detail, phosphoric acid etching removes the smear layer and de-mineralizes dentin. After conditioning of dentin with acid solution, a collagen fibril network is exposed. Resin monomers are then infiltrated into the collagen network as the primer and resin or a combined primer-resin is applied. However, some concerns remain about pulpal reactions, hypersensitivity and longevity of restoration of this material. Specifically, it has been well documented that using composite resin as a filling material, without a protective base or liner, in deep cavities could impair irreversible pulp damage, due to the diffusion of un-polymerized and toxic components of the material to the pulp through the remaining dentin.

Apart from toxic or allergenic agents in restorative materials, bacteria and their products are also capable of eliciting an inflammatory reaction. The invasion of dentinal tubules by oral bacteria has been clearly demonstrated by many in vivo and in vitro studies. Thus, bacteria present within dentinal tubules may be responsible for pulp infection. In this study, dentinal tubules could provide a pathway for bacteria to penetrate the dentin pulp complex. In detail, the tubule width, as estimated by SEM, was more than 0.5 μm, which is the average diameter of many bacteria species. Unlike previously published data, in this report it is well shown that obturation of dentinal tubules did not occur apart from 5 cases, when resin composite was used as a filling material. In these cases obturation took place only at the dentin-restoration interface. The results found in 5 teeth restored with resin composite indicate a surface seal but not institute anything more.

In this report, parameters like the age of the patient, the reason of the extraction or longevity of restoration did not, indirectly, affect by any way dentin continuum. The most important factor seemed to be the type of the filling material used in each case.

Information about the base cements and liners used in the tested teeth is limited. Generally, it has been documented that zinc oxide-eugenol cements inhibit bacterial growth and promote slight, but statistically significant, increase in hardness on cavity walls. Calcium hydroxide is a substance that promotes limited sclerosis when applied to dentin. Sclerosis is caused by the precipitation of a crystalline material produced by calcium ions within dentinal tubules. Additionally, it has important but time limited antibacterial effect.
attributed to its high pH\textsuperscript{25-27}. On the other hand, glass-ionomer cements have advantages including chemical bonding to mineralized tissues and release of fluorides to the dentin-material interface\textsuperscript{28}.

In this study, EDS analysis showed that most of the intermediate materials used were eugenol based. At all cases, no sign of tubular obturation was found under the cavity base application sites. Additionally, no precipitation of crystalline material occurred when calcium hydroxide was used (Fig. 6). However, it should be well understood that there are other factors determining biological changes at the dentin-base interface, such as the application procedure followed in each case.

Unexceptionally, the procedure followed by each dentist affects the interplay between injury, defence and repair, events of dentin pulp complex\textsuperscript{15,29}. In all cases where amalgam was used, parameters like possible lack of condensation or the low degree of perfection of easily purchasable materials used, contribute to our findings. Nevertheless, it should be well recognised that in order to achieve a surface seal under a restoration, not only materials used should be standardized by international associations, but new materials that provide optimal bonding with dentin should be preferable.

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