Bacterial invasion of dentinal tubules from the external root surface with and without an intact cemental layer: a confocal laser scanning microscopic study

Abstract

Aim: To evaluate the invasion of two bacterial species; Streptococcus sanguinis (S.s) and Aggregatibacter actinomyctecemitans (A.a) in radicular dentinal tubules with and without an intact cemental layer using confocal laser scanning microscopy.

Methodology: 10 intact freshly extracted human teeth were prepared and divided into 2 groups, Group 1: S.s (1a: S.s without cementum, 1b: S.s with intact cementum) and Group 2: A.a (2a: A.a without cementum, 2b: with intact cementum). The cemental layer on each root was removed from one side and kept intact on the other side. The specimens were incubated in the bacterial suspension for 15 days at 37°C. The specimens were sectioned, mounted, stained with a fluorescent dye and viewed under the Confocal Scanning Electron Microscope at 40X magnification. The penetration of bacteria and presence of live bacteria in dentinal tubules in all the groups was analyzed.

Results: In both the groups there was a significantly higher penetration of bacteria through the exposed dentin as compared to sections with intact cementum. However, there was a significantly higher penetration of Streptococcus sanguinis as compared to Aggregatibacter actinomyctecemitans.

Conclusion: An intact cemental layer protects the dentin from invasion of bacterial species. These bacteria can lead to reinfection of treated periodontal pockets, pulpal inflammation or persistence of periapical infections. Therefore, management of endodontic-periodontic lesions and periodontal therapies should aim at preserving or regenerating a healthy cemental layer.

Keywords: bacterial invasion, confocal scanning electron microscope, cementum, radicular dentin
In this article we evaluated the penetration of two bacterial species, *Streptococcus sanguinis* and *Aggregatibacter actinomycetemcomitans* into the dentinal tubules with and without an intact cemental layer using a confocal laser scanning microscopy. A fluorescent dye was used to stain the nucleic acid and differentiate between live and dead bacteria.

**Materials and methods**

Ten intact freshly extracted single rooted human teeth were used for the study. Teeth were scaled and planed with the help of ultrasonic scaler. The cemental layer was removed from one surface and kept intact on the other surface. The teeth were decoronated with diamond disc at 700rpm and dentin blocks of 5mm length were prepared. The root canal opening was sealed with gutta-percha and dentinal tubule sealant was applied on all the surfaces except the test surface. EDTA was applied on the rest of the surface with the help of microbrush and the teeth were washed with normal saline after 30 seconds. Teeth were then autoclaved in autoclavable pouches to remove any residual bacteria. The teeth were then randomly divided into 2 groups of 5 samples each, Group 1: *Streptococcus sanguinis* (S.s), Sub group a: S.s without cementum, Sub group b:S.s with intact cementum and Group 2: *Aggregatibacter actinomycetemcomitans* (A.a) Sub group a:A.a without cementum, Sub group b: with intact cementum.

Separate inoculums of *Streptococcus sanguinis* and *Aggregatibacter actinomycetemcomitans* were prepared and adjusted to 0.5 McFarland standards. This ensured uniform concentration of both the bacterial species. Teeth were then incubated in the respective bacterial suspensions for 15 days at 37°C. After the incubation time the teeth were then washed in normal saline. The teeth were then split longitudinally and sectioned with the help of diamond disc, chisel and mallet. After the sectioning, the samples were again washed with 100µl PBS using a micropipette to remove any debris.

For the examination of tooth specimens under the confocal laser scanning microscope (CLSM) 100µl of prepared fluorescent stain (LIVE/DEAD Baclight stain, Molecular Probes, Invitrogen Detection Technologies) was applied on the sectioned dentin blocks according to manufacturer’s instruction and the blocks were incubated with the stain at room temperature for 15 minutes in a dark environment to enable bacteria to take up the stain. After 15 minutes the samples were washed with Phosphate Buffer Solution PBS to remove any residual fluorescent stain. Following this an antifade mountant (Dakocytomation) was applied over the sample and the specimen was then mounted on a slide with glycerine and a cover slip was placed. The samples were then subjected to CLSM imaging at 40X Magnification. The confocal images were analysed and number of live bacteria (Green stained) was estimated (Figure 1-4).

![Figure 1](image1.jpg) **Figure 1** Group 1a: *Streptococcus sanguinis* without intact cemental layer.

![Figure 2](image2.jpg) **Figure 2** Group 1b: *Streptococcus sanguinis* with intact cemental layer.

![Figure 3](image3.jpg) **Figure 3** Group 2a: *Aggregatibacter actinomycetemcomitans* without intact cemental layer.

![Figure 4](image4.jpg) **Figure 4** Group 2b: *Aggregatibacter actinomycetemcomitans* with intact cemental layer.

**Statistical analysis**

A statistical analysis was carried out using the SPSS software version 7.1.1. The paired t-test was used to compare the number of live bacteria that penetrated into the radicular dentinal tubules from a surface with and without an intact cemental layer. A p-value of less than or equal to 0.05 was considered statistically significant.

**Results**

In the *Streptococcus sanguinis* group there was significant difference in the number of bacteria that invaded the dentinal tubules without cemental layer (Mean=1072, SD=60.23) and with intact cemental layer (Mean=244.6, SD=7.76); p-value=0.000 (Table 1) (Table 2).

In the *Aggregatibacter actinomycetemcomitans* group, there was...
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Discussion

The results in this study point towards the protective nature of an intact cemental layer. Large numbers of *Streptococcus sanguinis* penetrated into the dentinal tubules when the tooth surface was devoid of cemental layer. This is in accordance with the findings of Meyron et al., where they found rapid penetration of *Streptococcus sanguinis* into exposed dentinal tubules. Fewer number of *A.a* was observed in the dentinal tubules with and without an intact cemental layer. This is in accordance with Giuliana G et al., where they found lower levels of *gram negative* bacteria in periodontal pockets.

Collagen Type I major organic component of dentin, is recognized by oral streptococci and when absorbed into hydroxyapatite surfaces, it serves as an adhesion substrate. The ability of oral streptococci to bind to collagen may facilitate bacterial adhesion to exposed dentin or cementum, and subsequently tissue penetration. The antigen I/II polypeptides, expressed on the surfaces of most indigenous species of oral streptococci, play a major role in mediating Streptococci to collagen.

*A. actinomycetemcomitans* is small, nonmotile, gram negative, saccharolytic, citrophilic, round-ended rod. It binds to collagen I,II,III and V but not IV. It has the ability to invade host cells, penetrate gingival epithelium and survive in eukaryotic cells. These organisms occur in locations like the epithelium wall, epithelial side of basal lamina, connective tissue and alveolar bone.

Few reports have shown the presence of *A. actinomycetemcomitans* in periapical lesions as well. *A. actinomycetemcomitans* is a resident component of the oral microbiota and is commonly found in periodontal infections, but its presence in periapical and root end samples must be considered as one of the causes in persistent endodontic infections.

Although pocket debridement suppresses components of the subgingival microflora associated with periodontitis, periodontal pathogens may return to baseline levels within days or months. The return of pathogens to pre-treatment levels generally occurs in approximately 9 to 11 weeks, but can vary dramatically among patients.

Failure to eliminate residual bacteria in the periodontal pockets, root surfaces and dentinal tubules can cause re-colonization of bacteria and reinfection. Various treatment modalities such as systemic and local antibiotics, laser therapy, photodynamic therapy have been used as adjuncts to non-surgical periodontal therapy to eliminate residual bacteria and have shown promising results in further reducing the bacterial counts.

### Table 1 Tabular form for mean and SD for S.s & A.a

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
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<tbody>
<tr>
<td>1a</td>
<td>1072.4</td>
<td>60.23</td>
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<tr>
<td>1b</td>
<td>244.6</td>
<td>7.76</td>
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<td>2a</td>
<td>172.4</td>
<td>6.54</td>
<td></td>
</tr>
<tr>
<td>2b</td>
<td>13.6</td>
<td>3.04</td>
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1a, S.s without intact cementum; 1b, S.s with intact cementum; 2a, A.a without intact cementum; 2b, A.a with intact cementum

### Table 2 Significant difference in the number of bacteria that invaded the dentinal tubules without cemental layer and with intact cemental layer

<table>
<thead>
<tr>
<th>Groups</th>
<th>95% Confidence interval of the difference</th>
<th>t</th>
<th>df</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Lower</td>
<td>Upper</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td>827.8</td>
<td>61.9</td>
<td>751.8</td>
<td>903.78</td>
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<tr>
<td>1b</td>
<td>158.8</td>
<td>9.49</td>
<td>147.01</td>
<td>170.59</td>
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<tr>
<td>2a</td>
<td>900</td>
<td>61.95</td>
<td>823.07</td>
<td>976.92</td>
</tr>
</tbody>
</table>

1a, S.s without intact cementum; 1b, S.s with intact cementum; 2a, A.a without intact cementum; 2b, A.a with intact cementum
High-resolution confocal microscopic images can be made of either the surface of a sample or beneath the surface. With microscopes running under normal conditions, the optical section thickness is >1μm and the effective penetration into enamel and dentin a maximum of 100μm. Confocal Laser scanning microscopy was used in this study since it allows us to determine the levels of Live (green) and dead (red) bacteria with the help of the fluorescent dye. Also, optical sections of about 1μm can be obtained. Therefore, appropriate images from the inner sections of the specimen can be recorded. Specimen processing techniques can cause disruption of bacterial cells on the surface of the specimen.

The drawback of this study is that it was performed on extracted teeth using single organisms. Future studies can be done using an ex vivo model where freshly extracted teeth can be analyzed immediately after extraction. Also, various treatment modalities can be assessed wherein pre and post treatment levels of live and dead bacteria can be evaluated.

Conclusion

An intact cemental layer is an essential barrier for invasion of bacterial species into the dentinal tubules. Presence of bacterial species in the dentinal tubules can cause re-colonization of bacteria in the periodontal pockets, persistence of periapical infections or cause pulpal inflammation.

Therefore, maintaining the integrity of the cemental layer and attempting to regenerate lost cemental tissues through various regenerative procedures should be of prime importance.

Acknowledgements

None.

Conflict of interest

The author declares no conflict of interest.

References


