Biofilms in restorative dentistry: A clinical report

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One of the main contributing factors to the progression of dental caries and periodontal diseases is the formation and presence of dental bacterial biofilms. Dental biofilms can be found on hard dental structures (enamel and dentin), soft tissues, restorative dental materials, orthodontic appliances, and implants. Biofilm organisms exhibit an altered phenotype with respect to growth rate, gene transcription, and antimicrobial resistance. Biofilms are composed of host constituents, cell-free enzymes, polysaccharides, and bacteria embedded in a matrix of extracellular polymeric substances that they produce to connect and communicate with each other. The process of biofilm development involves several progressive stages. Initially, mostly salivary proteins and cell-free enzymes accumulate on the surface. Specifically, in restorative dentistry, studies have confirmed that formation of biofilm on composite resin restorations degrades and roughens the surface of the restorative material, leading to subsequent colonization of bacteria at the restoration/tooth interface. Invasion of the interface can then begin, causing secondary caries and pulpal pathology. Furthermore, accumulation of biofilm on restorative materials adjacent to the gingival tissue may lead to periodontal diseases.

Biofilms are formed when free-floating microorganisms attach to a surface by van der Waals forces of attraction. This attachment is later strengthened by cell adhesion structures such as pili. The microbes that initially colonize the surface facilitate the development of the biofilm by providing more diverse adhesion sites for other microorganisms. As the microbial population of the incipient biofilm increases, a polymeric matrix develops, which not only serves as a scaffold for the developing biofilm but also protects the microorganisms within it. Biofilms form rapidly in the oral environment, beginning with the formation of the acquired pellicle. During the preparation of indirect restorations, tooth structure is exposed to the oral microbiota, resulting in the adsorption of salivary biopolymers that form the pellicle. During restorative procedures, the initial exposure of the tooth surface to the oral environment may range from a few minutes to a few hours. This, together with leakage under the restorations, may allow the development of the biofilm to a level where it could result in either cementation failure or recurrent caries under the definitive restoration. This fact is of particular importance for interim fixed restorations that are usually fabricated as part of prosthodontic procedures. These interim restorations may leak, leading to further accumulation of biofilms.

This clinical report documents the structure and characteristics of the biofilm formed under a cemented restoration, confirming the need to develop new cementation protocols to disrupt and minimize the formation of biofilm before cementing definitive restorations.
rehabilitation. The treatment plan involved implant-supported fixed restorations. The second right mandibular molar was given a poor prognosis because of severe attachment/bone loss and subsequent extraction was planned.

After 2 weeks, the interim restoration was removed, and the definitive American Dental Association (ADA) type III gold crown (Argdent30; Argen Corp) was evaluated on the tooth, with an assessment of the occlusion and margin fit before cementation. The prepared tooth was cleaned and polished with fine pumice, washed, dried, and isolated with cotton rolls. The crown was airborne-particle abraded, steam cleaned, and loaded with zinc phosphate cement (Dentsply Intl) seated over the tooth with the aid of dynamic seating. Crown margins were burnished, and the cement was allowed to set before removal of excess cement.

The tooth was chosen to investigate the structure and characteristics of the biofilm under a cemented restoration. The patient was notified, and the required consent forms were signed. This study was done with the approval (UP-10-00267) of the University of Southern California IRB.

After months of serving as a vertical stop, the tooth was extracted and evaluated with confocal laser scanning microscopy (CLSM) and scanning electron microscopy.
(SEM). The extracted tooth was placed in 70% ethanol for 48 hours. The tooth was sliced sagittally with a diamond disk (911H Hyperflex disc; Brasseler) under dripping sterile water. For the identification of bacteria with fluorescence in situ hybridization (FISH), the tooth fragments were hybridized for 90 minutes at 46°C with a 50-μL eubacterial probe EUB338 (Cy3) (Integrated DNA Technologies) at a final concentration of 5 ng μL⁻¹ and washed afterward at 46°C for 2×10 minutes with a 2×500 μL washing buffer. For examination with the CLSM (LSM 5 PASCAL inverted; Carl Zeiss Micro-Imaging Inc) using ×10 and ×20 objective lenses, the tooth fragments were positioned face down in sterile water in a slide chamber (Lab-Tek; Electron Microscopy Sciences), and 3 different areas were analyzed.

After CLSM imaging, the tooth fragments were prepared for SEM. The specimens were fixed with 2% glutaraldehyde for 24 hours, dehydrated in a graded ethanol series, mounted with silver adhesive (Electron Microscopy Sciences), sputter coated, and examined with an SEM operating at 5 kV in the secondary electron mode (XL30 SFEG; FEI Co). Three different areas were analyzed.

**DISCUSSION**

The presence and morphologic structure of biofilm underneath the cemented crown 6 months after delivery were analyzed with CLSM and SEM. FISH technique has been successfully used to visually detect and identify bacteria. Here, FISH method was used to detect biofilm bacteria in the interface between the restoration and the tooth structure.

The results of CLSM analysis with immunofluorescence staining confirmed the presence of biofilm not only at the areas close to the margins of the restoration, which is a location with relatively easy access to nutrients and oxygen (Figs. 1, 2), but also at the occlusal interface between the tooth and the restoration, which has less accessibility to nutrients and oxygen (Fig. 3). The results of SEM analysis agreed with CLSM and showed the presence of a biofilm layer at different areas.

![Figure 4](image1.png)

**Figure 4.** Further scanning electron microscopy analysis demonstrating presence of 2 distinct zones within biofilm layer: inner calcified layer; outer less calcified and actively growing layer.

![Figure 5](image2.png)

**Figure 5.** Scanning electron microscopy analysis of biofilm layer, at interface between restoration and tooth structure (lingual margin); showing presence of bacteria with coccus-like morphologic structure at different magnifications (Original magnification ×4, ×20, and ×50).

![Figure 6](image3.png)

**Figure 6.** Scanning electron microscopy analysis of biofilm layer at interface between restoration and tooth structure (occlusal surface); showing presence of bacteria with coccus-like morphologic structure at different magnifications. A, ×4 magnification. B, ×50 magnification. C, ×100 magnification.
at the interface between the tooth and the cemented crown. Interestingly, the SEM images confirmed the presence of 2 distinct strata within the biofilm: the inner layer, which is the calcified part of the biofilm, and the outer layer, which is less calcified and is the growing part of the biofilm (Fig. 4). The existence of biofilms at different areas of the teeth may help explain why restorations fail either due to recurrent decay or failure of cementation, because the biofilms can contaminate cementation protocols.

Streptococci represent the majority of supragingival bacteria in a healthy oral cavity. In the current clinical report, SEM analysis showed the presence of bacteria with coccus-like morphologic structure inside the biofilm layer. Crucially, these bacteria were within the normal size range, indicating that they were not under (permanent) starvation stress (Figs. 5, 6). This observation confirmed that the bacterial biofilm under the crown had sufficient access to the main required nutrients (glucose and oxygen). However, no exuberant densely packed biofilm of the type usually found in patients with advanced periodontitis was observed in the patient. This finding demonstrated certain limitations in either the available space or rich nutrient supply to the biofilm at the interface of the tooth and the restoration.

These findings might not be generalized because, in the current report, the margins were chosen only to compare the biofilm in the marginal area with an area within the confines of the crown. Based on these observations, we suggest the development of new and thorough cementation protocols to disrupt and eliminate biofilms before the cementation of definitive restorations.

CONCLUSIONS

A biofilm layer underneath a cemented gold crown was demonstrated. This biofilm consisted of 2 distinct zones containing bacteria with a coccus-like morphologic structure and normal size. Based on the results of the current report, the existence of biofilms at different areas of the teeth may help clarify why restorations fail due to either recurrent caries or failure of cementation, because biofilms can contaminate cementation protocols.

REFERENCES


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