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Cyclic mechanical loading promotes bacterial penetration along composite restoration marginal gaps

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ABSTRACT

Objectives. Secondary caries is the most common reason for composite restoration replacement and usually forms between dentin and the filling. The objective of this study was to investigate the combined effect of cyclic loading and bacterial exposure on bacterial penetration into gaps at the interface between dentin and resin composite restorative material using a novel bioreactor system and test specimen design.

Methods. Human molars were machined into 3 mm thick disks with 2 mm deep × 5 mm diameter cavity preparations into which composite restorations were placed. A ~15–30 μm (small) or ~300 μm wide (large) marginal gap was introduced along half of the interface between the dentin and restoration. *Streptococcus mutans* UA 159 biofilms were grown on each sample prior to testing each in a bioreactor both with and without cyclic loading. Both groups of samples were tested for 2 weeks and post-test biofilm viability was confirmed with a live-dead assay. Samples were fixed, mounted and cross-sectioned to reveal the gaps and observe the depth of bacterial penetration.

Results. It was shown that for large gap samples the bacteria easily penetrated to the full depth of the gap independent of loading or non-loading conditions. The results for all cyclically loaded small gap samples show a consistently deep bacterial penetration down 100% of the gap while the average penetration depth was only 67% for the non-loaded samples with only two of six samples reaching 100%.

Significance. A new bioreactor was developed that allows combining cyclic mechanical loading and bacterial exposure of restored teeth for bacterial biofilm and demineralization studies. Cyclic loading was shown to aid bacterial penetration into narrow marginal gaps, which could ultimately promote secondary caries formation.

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1. Introduction

Key driving forces behind the increased use of dental composite materials are their tooth-like appearance [1] and lack of the potentially toxic element mercury that is present in amalgam [2,3]. Additional clinical advantages include enhanced conservation of tooth structure [4], the ability to be bonded to enamel and dentin surfaces [5], convenient handling, and the ready availability of a wide range of tooth shades. However, annual failure rates up to 15% have been reported for composite restorations, depending on restoration class [6], and a review of the literature has suggested the average lifetime of posterior dental composites is only 6 years [7].

The most common reason for restoration replacement is secondary tooth decay (dental caries) at a dentin-restoration interface [8–12], while the second most observed reason is restoration fracture caused by occlusal loading combined with chemical and physical degradation of the restorative material in the oral environment [13]. Furthermore, it is known that the appearance of secondary caries is associated with bacterial biofilm formation on the dentin-restoration interface. Indeed, the biofilm byproduct lactic acid promotes demineralization of the adjacent dentin which can lead to tooth decay [14,15].

Although bacterial biofilm formation is considered a necessary ingredient, the presence of the biofilm alone does not guarantee secondary tooth decay [16]. Numerous studies have identified the presence of bacteria within marginal gaps between the restoration and the dentin [17–19], and the presence of such gaps is likely an important factor as well. No clinical correlation between marginal gap size and bacterial colonization has been found for resin based composite restorations [20]; however, clinical data is limited and recent in vitro evidence from microbial caries models shows demineralization along the cavity walls of composite restorations increasing in magnitude with larger gap sizes [21,22]. Overall, there is a need for further studies to better understand the factors that control secondary caries formation at the marginal interface between composite restorations and tooth dentin.

Another complication is that teeth are also subjected to cyclic loading during mastication, bruxism (grinding), etc. Cyclic loading may promote marginal gap formation and growth; indeed, some studies have shown the degradation of restoration margins during cyclic mechanical loading [23–27]. Furthermore, it has been demonstrated that the presence of bacteria and a marginal gap does not guarantee further caries formation [15,28,29]. Thus, there may be an additional role of cyclic mechanical loading beyond simply creating a marginal gap or growing it above a critical size to allow bacterial penetration. Based on a survey of the available literature, understanding the mechanism of recurrent decay at the margins of dental composite restorations likely requires the evaluation of the simultaneous effects of bacterial biofilm presence, marginal gaps, and cyclic loading. To date, no such studies have been reported.

Accordingly, the objectives of this paper are the following: (1) to describe a novel bioreactor based test method and test specimen design that has been developed to allow cyclic mechanical loading of simulated tooth restorations within a growing oral biofilm environment, and (2) to study the synergistic effects associated with bacteria and cyclic loading on the marginal penetration of bacteria around dental restorations.

2. Materials and methods

2.1. *Bioreactor fatigue test system*

While bioreactor systems are commonly used for the in vitro study of oral biofilms in controlled laboratory settings [30–35], to date no such systems allow the simultaneous application of cyclic loading to the test sample. The goal was to develop a simulated tooth restoration sample that is practical to reproducibly manufacture and test to study dental restorations in a simulated oral environment with growing biofilms and without external contamination. The sealed bioreactor must provide nutrients to the bacteria in a 5% CO_2 environment at 37 °C, while applying cyclic stress to the restoration.

For the sample design, the physiological size of human teeth dictated the maximum possible dimensions. Based on a survey of typical human teeth it was determined 9 mm diameter disks could be readily machined from the crowns of human molars. Such disks allow the placement of a 5 mm diameter composite restoration on one side (Fig. 1a).

A simple radial symmetric biaxial bending and shear loading geometry was selected to allow even loading on the composite/dentin interface through the backside of bonded restorations with no gaps (Fig. 1a). Loading is applied in the center of the sample by a hemispherical loading rod, while the sample sits on a ring shaped support. The loading rod was guided into the center of the bioreactor using linear bearings that were housed in the bioreactor upper covers (Fig. 1b). To avoid external contamination, the loading rods passed through a flexible rubber barrier where the hole was undersized relative to the rod diameter to ensure a seal was maintained throughout testing. The use of individual bioreactor cells provided a sealed system for individually testing each simulated restoration (Fig. 1b). The bioreactors were fabricated from stainless steel to provide a durable, stiff platform for loading the specimens while also minimizing corrosion and allowing for autoclave sterilization. Bioreactors consisted of five interlocking elements denoted as the upper cover, lower cover, rubber barrier, base and sample stand. The two cover elements and base were manufactured from 316 stainless steel and made up the exterior of the bioreactor. Viton O-rings were used between parts to ensure a sealed system connected with four 316 stainless steel bolts. In order to create an active area for loading, the sample stand (Fig. 1a and b) was designed with a 7.0 mm diameter rounded edge ring that supported the sample. The sample stand was machined from 17-4 stainless steel heat treated at 482 °C for 60 min after machining to achieve peak strength for supporting the specimens. Locating pins were integrated into the lower cover and allowed for the sample to be both located and constrained within the bioreactor and centered over the sample stand for symmetric loading. These locating pins were designed to mesh with the bioreactor base, sample stand and sample itself.

Required nutrients and pH buffering were provided via brain-heart infusion (BHI) liquid media. 5% CO_2 in air was