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Calibration of a lactic-acid model for simulating biofilm-induced degradation of the dentin-composite interface

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ABSTRACT

Objective. To verify and calibrate a chemical model for simulating the degradation of the dentin-composite interface induced by multi-species oral biofilms in vitro.

Methods. Dentin-composite disks (5-mm dia. × 2-mm thick) were made from bovine incisor roots and filled with either Z100™ (Z100) or Filtek™ LS (LS) composite. The disks, which were covered with nail varnish, but with one of the dentin-composite margins exposed, were immersed in lactic acid solution at pH 4.5 for up to 48 h. Diametral compression was performed to measure the reduction in bond strength of the dentin-composite disks following acid challenge. Scanning electron microscopy (SEM) was used to examine decalcification of dentin and fracture modes of the disks. To better understand the degradation process, micro-computed tomography, in combination with a radiopaque dye ($AgNO_3$), was used to assess interfacial leakage in 3D longitudinally, while SEM was used to determine the path of leakage. One-way analysis of variance (ANOVA) was used to analyze the results, with the level of statistical significance set at $p < 0.05$. The results were compared with those obtained previously using multi-species biofilms for verification and calibration purposes.

Results. After 48 h of acid challenge, the debonding load of both the LS- and Z100-filled disks reduced significantly ($p < 0.05$). In the Z100-filled disks, debonding mostly occurred at the adhesive-dentin interface, while in the LS-filled disks, this happened at the adhesive-composite interface, instead. The degree of dentin demineralization, the reduction in debonding load and the modes of failure observed were very similar to those induced by multi-species oral biofilms found in the previous work. Leakage of $AgNO_3$ occurred mainly along the hybrid layer. The specimens filled with Z100 had a thicker hybrid layer (~6.5 μm), which exhibited more interfacial leakage than those filled with LS.

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Significance. The chemical model with lactic acid used in this study can induce degradation to the dentin-composite interface similar to those produced by multi-species biofilms. With appropriate calibration, this could provide an effective in vitro method for ageing composite restorations in assessing their potential clinical performance.

1. Introduction

In recent years, resin-based composites have become the most widely used restorative material to restore decayed and damaged teeth because of their esthetics and improved clinical performance [1–3]. About 121 million composite restorations were placed in the US by 2006 [4]. However, moderate to large composite restorations have higher failure rates and, as a result, more frequent replacement when compared to amalgam restorations [5]. Composite restorations fail for various reasons, among which secondary caries is the main one [6,7], accounting for up to 55% of the failures reported by dentists [8].

Secondary caries is defined as caries recurring at the interface between tooth and restoration [9], and is the most common reason for the replacement of different kinds of dental restorations [10,11]. Similar to primary caries, secondary caries are caused by substances harmful to the tooth tissues and resins, e.g. acids and enzymes produced by biofilms accumulating at the restoration margins [12]. It has been reported that biofilms accumulate more easily on composite than amalgam restorations due to the former's lack of antibacterial properties [13,14]. Moreover, biofilms have been shown to increase the surface roughness of resin composites [15], which could enhance the adherence of biofilms/plaque on their surfaces. Interfacial defects, which can be inherent of the restorative materials or caused by operator errors, biofilm challenge, occlusal forces or polymerization shrinkage of the resin composite, facilitate the leakage of harmful substances into the interfacial space. The exposed collagens in the hybrid layer are subject to hydrolytic and enzymatic degradation [5], leading to interfacial breakdown, the development of secondary caries and finally failure of the composite restoration. Although an almagam restoration is not bonded to the surrounding tooth tissues, its corrosive products provide an effective seal against the infiltration of the harmful substances. These are the major reasons cited for the higher failure rate seen in composite restorations when compared to amalgam restorations.

A main topic in dental materials research has been the search for a representative laboratory test to simulate the ageing, for example by biofilm challenge, of dental restorations observed clinically. In a previous study, degradation of the dentin-composite interface was effected by using multi-species oral biofilms grown in a CDC reactor [16]. The multi-species biofilms were produced directly from plaque samples collected from donors with a history of early childhood caries [17]. Therefore, they were considered to be more realistic than single-species biofilms. The CDC reactor was set up in such a way that, when pulsed with sucrose solution to simulate food intake, the pH of the medium followed the Stephan Curve as seen in the oral environment. That is, it fell rapidly and remained below the threshold of 5.5 for dentin demineralization during the course of sucrose pulsing, and it returned quickly to a value above 6.0 when sucrose-pulsing was stopped. Significant dentin demineralization was found in dentin-composite disks challenged with the sucrose-pulsed biofilms for 2 days [16]. The bond strength of these disks was also significantly reduced ($p < 0.001$). In contrast, dentin-composite disks subjected to the same biofilms but without sucrose pulsing did not exhibit significant demineralization or loss of bond strength. Since the drop in pH with sucrose-pulsing was the main difference in the challenges experienced by the two groups of specimens, we hypothesize that a low-pH chemical model can simulate the above degrading effects of the multi-species biofilms on the dentin-composite interface.

The inorganic acids produced by biofilms, e.g. lactic, acetic, propionic, succinic and formic acids, are the main cause of caries formation [18]. Among these, lactic acid has long been used to produce artificial carious lesions [19–21]. Fatigue failure of dentin is also accelerated when exposed to lactic acid [22]. Moreover, lactic acid may degrade resin composites, changing their surface hardness [23]. In this study, therefore, a simple chemical model using lactic acid is used to simulate biofilm-induced degradation of the dentin-composite interface. The results are compared to those obtained previously in vitro using multi-species biofilms [16] for verification and calibration purposes.

2. Materials and methods

2.1. *Preparation of dentin-composite disks*

Dentin-composite disks were prepared using bovine incisors and the same restorative materials as reported previously [16]. As shown in Fig. 1, after removing the soft tissues, the crown portion above the cementum-enamel junction (CEJ) and the apical portion of ~3-mm long were cut off from the bovine incisors with a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA). The root canals were enlarged to 2 mm in diameter, and the roots trimmed into cylinders of 5 mm concentric and parallel with the enlarged root canals. After that, the root dentin cylinders were filled incrementally with the composites Z100™ or Filtek™ LS (3M ESPE, St. Paul, MN, USA) using the corresponding adhesives (Single Bond Plus and LS Adhesive System, respectively) and following the manufacturer's protocols. Finally, the filled cylinders were sliced into 2-mm thick disks and stored in de-ionized water at 4 °C before testing.