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Bacterial adhesion not inhibited by ion-releasing bioactive glass filler

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ABSTRACT

Objective. Bioactive glasses and surface pre-reacted glass-ionomer (sPRG) filler possess cariostatic properties owing to ion release. Many studies investigated potential cariostatic effects; few studies evaluated the surface stability and the structural changes their surfaces undergo in acidic conditions.

Methods. The surface resistance against acid attack and the surface receptiveness for bacterial adhesion and biofilm formation of a sPRG-filled (Beautifil ll, Shofu) and conventional glass-filled (Herculite XRV Ultra, Kerr) resin-based composite (RBC), and a conventional glass-ionomer cement (GIC; Fuji IX GP Extra, GC) were examined. Specimens (n = 3) were immersed in distilled water or lactic acid (pH 4.0) for 3 days. Bacterial growth and biofilm formation were recorded using optical density and SEM.

Results. Upon 3-day immersion in lactic acid, the surface of the sPRG-filled RBC revealed multiple holes, while virtually no change in surface integrity was observed for the conventional RBC and GIC. Bacterial growth measurements revealed that none of the materials inhibited *Streptococcus mutans* ($p < 0.05$). Remarkably, cross-sectional SEM revealed that *S. mutans* had penetrated the etch pits induced by lactic acid in/around the sPRG filler. Ion-release measurements revealed that sPRG-filled RBC released boron and fluoride, while GIC only released fluoride. However, the concentration of ions released by both materials appeared not sufficient to inhibit bacterial growth. Moreover, the structural surface change and resultant increased surface roughness appeared to have promoted biofilm formation.

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Significance. While having bioactive potential through ion release, the stability of surface integrity of bioactive materials is a key-parameter to be assessed with regard to their cariostatic potential.

1. Introduction

The continuous evolvement in dental adhesive technology, such as the development of novel resin-based composites (RBCs) and adhesives, has allowed the size of tooth preparations to be minimized. The dentist can now remove carious tissue selectively and fill the resultant cavity with adhesive materials. Concurrently, dental materials can today be bonded better to tooth tissue. However, secondary caries, which occurs after treatment of a primary caries lesion, could still not be ruled out [1,2]. Several researchers have therefore investigated materials with antibacterial potential to prevent or treat dental caries [3]. Silver compounds, such as silver nitrate, silver fluoride and silver diamine fluoride, have been used in restorative dentistry for many years [4]. Also, silver nanoparticles recently appeared advantageous [5], as they have a large surface area and so exhibit a stronger antibacterial effect than does bulk or ionic silver. In addition, nanoparticles are widely applicable, because they can be mixed with conventional dental materials such as adhesives and RBCs [4]. Other antibacterial substances, such as quaternary ammonium dimethacrylate monomers and 12-methacryloyloxydodecylpyridinium bromide, have been copolymerized in resin-based materials to render them antibacterial [6]. Unfortunately, the actual cariostatic effect of bioactive restorative materials is often found to be limited. Also glass-ionomer cements (GICs) have been attributed antibacterial effects [7]. They can release several types of ions; especially fluoride (F) is detrimental for bacteria [8,9]. A low pH before setting also kills bacteria [9]. Overall, also the ability of GICs to prevent secondary caries remains minor [2].

Few bioactive glasses have been suggested to release antibacterial ions [10,11]. Bioactive glass has an amorphous structure, whereas glass–ceramics are crystallized glasses and composites of a crystalline phase in a residual glassy phase. Bioactive glass consists solely of the elements found in the body material, mainly being silicon (Si), calcium (Ca), sodium (Na), phosphorous (P), and oxygen (O) [12]. Among the diverse kinds of bioactive glass filler, surface pre-reacted glass-ionomer (sPRG) filler has already been used for a relatively long time in some specific commercial RBCs. Most studies investigating this material assortment have focused on ion-release degree and potentially associated antibacterial effects [13–15].

However, only few studies have investigated the surface integrity of ion-releasing bioactive glass filler; the resultant surface receptiveness for bacterial adhesion has also scarcely been studied [11]. Nevertheless, the restoration's surface integrity may be a key factor determining the eventual clinical beneficial effect of the alleged antibacterial properties. Therefore, in order to evaluate the surface integrity of RBCs, containing bioactive glass filler, and its antibacterial efficacy, we compared in this study the bacterial growth and ion-release properties of a sPRG-filled RBC, as compared to that of a contemporary conventional RBC and GIC, this upon prior immersion in distilled water and lactic acid. The null hypotheses tested in this study were that (1) the surface integrity of the sPRG-filled RBC is stable, (2) the sPRG-filled RBC shows a stronger antibacterial effect than the conventional RBC and GIC, and (3) the sPRG-filled RBC releases more ions.

2. Materials and methods

2.1. Material disk preparation (Fig. 1)

The sPRG-filled RBC Beautifil ll (Shofu, Kyoto, Japan), the conventional RBC Herculite XRV Ultra (Kerr, Orange, CA, USA), and the conventional GIC Fuji IX GP EXTRA (GC, Tokyo, Japan) were used in this study. These materials were filled in a 10-mm (diameter) × 2-mm (height) silicone molds (LADD Research, Williston, VT, USA) and covered with a microscope slide-glass plate. In the case of RBC specimens, they were light-cured for 40 s from both sides (for a total curing time of 80 s) using a LED light-curing unit (G-Light Prima II Plus, GC). The GIC specimens were allowed to set for 5 min. The specimens were then polished using a 15-μm diamond lapping film (3 M, St. Paul, MN, USA). A total of 33 disks per material were prepared along with another set of 3 disks for the sPRG-filled RBC (Fig. 1). After being polished, 3 disks were examined by Feg-SEM (see further) without any further treatment (control), while all other disks were either immersed in distilled water (pH 5.8) or in lactic acid (pH 4.0) for 3 days. After storage, the specimens were thoroughly washed with distilled water and air-dried.

2.2. Surface integrity examined by Feg-SEM

Three specimen surfaces per material (n = 3) were coated with a thin layer of osmium (Neo Osmium Coater, Meiwa, Tokyo, Japan), upon which the specimen surfaces were examined using field-emission-gun SEM (Feg-SEM; JSM-6701F, JEOL, Tokyo, Japan) operated at 5 kV and employing an annular semiconductor detector.

2.3. Bacterial strains and culture conditions

Streptococcus mutans (ATCC25175), stored at −80 °C, were subcultured on blood-agar plates (37 °C, 5% CO_2). Colonies from these blood-agar plates were cultivated overnight in brain heart infusion (BHI, Becton Dickinson and Company, Franklin Lakes, NJ, USA) broth; the obtained liquid cultures were used for the experiments.