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Biofilm formation and release of fluoride from dental restorative materials in relation to their surface properties

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ABSTRACT

Objectives: To elucidate the impact of surface properties and the release of fluoride from different glass ionomer cements on biofilm formation.
Methods: Standardized specimens manufactured from various classes of glass ionomer cements (GICs), a resin-based composite (RBC), and human enamel were subjected to surface analyses. Subsequent to simulation of salivary pellicle formation, *Streptococcus mutans* biofilm formation was initiated using a drip flow reactor for 48 h and 96 h. Biofilms were characterized by determining viable bacterial biomass and 3D biofilm architecture using SEM and CLSM; the release of fluoride from the specimens was measured using the ion selective micro method in dependence on various experimental conditions (incubation with sterile broth/bacteria/acid).
Results: Surface properties and biofilm formation correlated poorly, while the release of fluoride correlated well with viable streptococcal biomass and SEM/CLSM analyses. For all investigated materials, biofilm formation was lower than on enamel. The release of fluoride showed a significant dependency on the experimental conditions applied; the presence of biofilms reduced fluoride release in comparison to sterile incubation conditions.
Conclusions: Within the limitations of a laboratory study, the results suggest that biofilm formation on GICs cannot be easily predicted as a function of substratum surface parameters. The release of fluoride from glass ionomer cements contributes to control biofilm formation particularly in its early phases.
Clinical significance: Glass ionomer cements can actively control microbial biofilm formation, while biofilms modulate the release of fluoride from GIC materials.

1. Introduction

Glass ionomer cements (GICs) have been introduced in dentistry in the mid-seventies [1], featuring some favourable properties such as a chemical bond to enamel and dentin, a coefficient of thermal expansion almost equal to natural tooth tissues, and the ability to release fluoride over a significant amount of time [2,3]. As a result, GICs are considered as biomaterials that may prevent [3,5] and inactivate [6,7] dental caries and its progression. While early glass ionomer cement formulations featured poor mechanical properties, the recently introduced high-viscosity GICs (HV-GICs) featured significantly improved hardness and stress resistance in comparison to early and conventional GICs. In addition to their application as restorative materials, GICs can successfully be used for cementation of indirect restorations [8].

As secondary caries remains one of the most frequent reasons for failure of dental restorations [9,10], it has often been highlighted that biofilm formation on the surface of dental restorative materials may contribute to the establishment of secondary caries lesions. In GIC restorations, the fluoride released by GICs may serve as a buffer that neutralizes acids secreted by oral bacteria and may inhibit the growth of cariogenic microorganisms [11,12]. Recent studies have highlighted a correlation between the release of fluoride from GICs and the properties of *Streptococcus mutans* biofilms on their surface, suggesting that acidogenicity, dry weight, the amount of extracellular polysaccharides (EPS), as well as biovolumes and EPS thickness are

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significantly reduced in biofilms on the surface of GICs which feature a high release of fluoride [13]. Employing a clinical approach for the analysis of biofilm formation, other researchers pointed out that despite of lacking significant differences in biofilm formation between various materials such as GICs, amalgam, resin-based composite, and ceramic, there was a tendency towards a lower number of viable bacterial cells in biofilms grown on restorations made of GICs and amalgam [14]. Similar results have been published by other groups [15], while some researchers suggested that biofilm formation is not necessarily reduced on fluoride-releasing GICs [16,17]. Thus, the aim of the present laboratory study was to elucidate the impact of surface properties and the release of fluoride from various classes of GICs on *Streptococcus mutans* biofilm formation in dependency on incubation times. The study hypotheses were that (I) *Streptococcus mutans* biofilm formation is not affected by the release of fluoride, (II) the release of fluoride is not influenced by the incubation time, and (III) the release of fluoride is not influenced by exposure of GIC surfaces to biofilms.

2. Materials and method

2.1. Specimen preparation

Standardized specimens were prepared from an experimental light-curing glass ionomer cement (resin-modified GIC; A), a compomer (*Glasiosite*; B), a luting GIC (*Meron*; C), a high viscosity GIC (*Ionostar plus*; D), and from a reference nanohybrid resin-based composite (RBC, *Grandioso*; E, all by VOCO GmbH, Cuxhaven, G). For preparation of a single specimen, a standardized amount of each material was placed into a custom made steel mould with a diameter of 6.0 mm and a height of 2.0 mm, condensed against a glass plate and covered with a cellulose acetate strip (Mylar®) until cured. Compomer, resin-modified GIC, and RBC specimens were light-cured in direct contact for 40 s using a hand-held light curing unit (LCU; SDI Radii plus, SDI, Bayswater, AUS; 1500 mW/cm^2). A total of 83 specimens for each material were produced. The specimens were then subjected to a standardized polishing protocol, including polishing with 1000/4000-grit grinding paper (Buehler, Lake Bluff, IL, USA) using a polishing machine (Motopol 8; Buehler, Düsseldorf, G).

Anterior human teeth extracted for clinical reasons were obtained from the Oral Surgery Unit at the Department of Biomedical, Surgical and Dental Sciences (Milan, Italy). A total of 83 round enamel-dentin slabs with a diameter of 6.0 mm and a thickness of 2.0 mm were cut from the labial surfaces using a water-cooled trephine diamond bur (INDIAM, Carrara, I). Dentin bottoms were removed, and the enamel surfaces (enamel, E) were polished as described for the dental materials above. All specimens were subsequently stored under light-proof conditions in artificial saliva for six days at 37 ± 1 °C prior to the further experiments to allow for maturation of the cement, to eliminate potential impacts of a fluoride burst and, for the resin-modified GIC, the compomer, and RBC, to minimize the impact of residual monomer leakage on cell viability. The artificial saliva used in the present study allows a reproduction of the average electrolytic composition of human whole saliva and was prepared by mixing 100 mL of 150 mM $KHCO_3$, 100 mL of 100 mM NaCl, 100 mL of 25 mM K_2HPO_4, 100 mL of 24 mM Na_2HPO_4, 100 mL of 15 mM $CaCl_2$, 100 mL of 1,5 mM $MgCl_2$, and 6 mL of 25 mM citric acid. The volume was made up to 1 L and the pH was adjusted to 7.0 by pipetting NaOH 4 M or HCl 4 M solutions under vigorous stirring. All specimens undergoing surface analysis were subsequently cleaned using distilled water and applicator brush tips (3 M ESPE, Seefeld, G).

2.2. Surface analysis

2.2.1. Surface roughness

Peak-to-valley surface roughness (R_a) was determined on five randomly selected specimens for each group material using a profilometric contact surface measurement device (Perthen S6P, Feinprüf-Perthen, Göttingen, G). A distance of 1.75 mm was measured in three randomly selected line scans perpendicular to the expected grinding grooves using a standard diamond tip (tip radius 2 μm, tip angle 90°) and a cut off level of 0.25.

2.2.2. Surface free energy

Contact angles between the surface of the various materials and three liquids differing in hydrophobicity (bidistilled water, diiodomethane, ethylene glycol) were determined using the sessile drop method and a computer-aided contact angle measurement device (OCA 15plus, DataPhysics Instruments GmbH, Filderstadt, G). A total of eight drops for each liquid (drop volume 0.2 μL) were analyzed on each of four randomly selected specimens for each material. Left and right contact angles were averaged and the surface free energy was calculated according to the approach introduced by Owens and Wendt [18].

2.2.3. Energy-dispersive X-ray spectroscopy (EDS)

Two randomly selected specimens for each material were subjected to EDS surface analysis. A scanning electron microscope coupled with an EDS probe (EDAX Genesis 2000, Ametek GmbH, Meerbusch, G) was used to acquire full frames of the surfaces of the specimens employing an accelerating voltage of 20 kV, a magnification of 2000× and an acquisition time of 200 s.

2.3. Microbiological procedures

2.3.1. Saliva preparation

Stimulated whole saliva was collected by expectoration from three healthy donors in accordance with the protocol published by Guggenheim et al. [19]. Saliva was collected in chilled tubes, pooled, heated to 60 °C for 30 min to inactivate endogenous enzymes, and was then centrifuged (12.000 × *g*) for 15 min at 4 °C. The supernatant was transferred into sterile tubes, stored at −20 °C, and thawed at 37 °C for 1 h directly prior to the experiments.

2.3.2. Bacteria

Streptococcus mutans ATCC 35668 was cultured according to a previously published protocol [20]. Briefly, Mitis Salivarius Bacitracin agar inoculated plates were incubated for 48 h at 37 °C in a 5% supplemented CO_2 environment. A total of 1% sucrose was added to a pure suspension of the microorganism in Brain Heart Infusion obtained from these plates after an incubation of 12 h at 37 °C in a 5% supplemented CO_2 environment. *S. mutans* cells were harvested by centrifugation (2200 rpm, 19 °C, 5 min), washed twice with phosphate-buffered saline (PBS), and resuspended. The suspension was subsequently subjected to low intensity ultrasonic energy (Sonifier model B-150; Branson, Danbury, CT, USA; operating at 7-W energy output for 30 s) in order to disperse bacterial chains. The suspension was then adjusted to a value of 1.0 on the McFarland scale, corresponding to a microbial concentration of approximately 3.0×10^8 cells/mL.

2.3.3. Biofilm formation

The drip-flow reactor (M-DFR) employed in the present study was a modification of a commercially available Drip Flow Reactor (DFR 110; BioSurface Technologies, Bozeman, MT, USA). The modified design allowed the placement of customized