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The influence of 30-day-old *Streptococcus mutans* biofilm on the surface of esthetic restorative materials—An *in vitro* study

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ABSTRACT

Objective: To evaluate the effects of *Streptococcus mutans* biofilm/restorative materials interaction on surface roughness, hardness and morphology of materials tested.

Methods: Empress 2 (E2), Filtek Supreme (FS), Vitremer (V) and Ketac Molar Easymix (KM) were tested. Twenty-five disks of each material were made and divided into three storage groups: (1) 100% relative humidity ($n = 5$); (2) growth medium (BHI and 1% sucrose) ($n = 5$); (3) *S. mutans* biofilm-growth medium ($n = 15$). Before storage, hardness measurements were immediately obtained from group 1 specimens. After 30 days of storage, the specimens were cleaned in order to obtain the surface roughness and hardness values, besides morphology analysis by scanning electron microscopy.

Results: The surface roughness and hardness values obtained from E2 and FS specimens did not present statistically significant differences among the groups 1, 2 and 3 and between immediate and 30-day-old specimens of each material. However, group 3 specimens of V and KM showed statistically significant higher surface roughness means than other groups. Group 1 specimens of V and KM also showed higher hardness values than the immediate values. Group 3 specimens of V presented decreased hardness values compared with other groups. The scanning electron micrographs showed an increase in surface degradation from group 1 to group 3 for FS, V and KM.

Conclusions: Thirty-day-old biofilm promotes a negative effect on the surface morphology of FS, V and KM, on the surface roughness of V and KM and on the hardness of V.

1. Introduction

The ability of restorative dental materials to withstand the functional force and exposure to various substances in the mouth is an important requirement for their clinical performance for a considerable period of time. Some investigations have demonstrated surface damage to restorative materials caused by the chemical environment of the oral cavity.[1–4] Fundamentally, the factors known to cause these deleterious effects include low pH due to cariogenic biofilm,[5,6] consumption of acidic drinks or foodstuffs,[2–8] and action of enzymes,[9] which can soften the outermost layers and roughen restorative materials.

Dental biofilms harboring cariogenic bacteria (caries-associated microorganisms) are among the virulence factors

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associated with the progression of tooth decay and periodontal diseases. Mutans streptococci are among bacteria proliferating in the dental biofilm. Their virulence is mainly due to their high adhesion capability, acidogenicity and aciduric properties.[10] These *S. mutans* characteristics could be responsible for surface damage to restorations, since this microorganism can be found on any hard surface in the oral cavity, such as enamel, implants, orthodontic appliances or restorative materials.[11,12] Nevertheless, little is actually known about the effects of accumulated dental biofilm on the surface properties and microstructure of restorative materials.

Therefore, the aim of this study was to test the hypothesis that esthetic restorative materials subjected to *S. mutans* biofilm interaction for 30 days differ in surface roughness, hardness and microstructure from those stored in 100% relative humidity or growth medium for the same period.

2. Materials and methods

2.1. Specimen preparation and storage groups

Twenty-five specimens of each aesthetic restorative material described in Table 1 were fabricated using metal rings (10 mm diameter; 2 mm depth) according to the manufacturers' instructions, under aseptic conditions (laminar flow chamber and sterilized instruments). Glass ionomer cements (Vitremer, V and Ketac Molar Easymix, KM) and composite resin (Filtek Supreme, FS) disks were covered with an acetate strip (Probem Ltda, Catanduva, São Paulo, Brazil) and pressed flat with a glass slide to compact the material. FS and V were polymerized with a curing light (Elipar Trilight, 3M ESPE, St. Paul, MN, USA) after the intensity of the light-curing unit to be checked by a curing light meter (Hilux Dental Curing Light Meter, Benliglu Dental Inc., Turkey). KM was allowed to set for 5 min. Following curing, the specimens were not polished to avoid surface contamination. The ceramic disks (IPS Empress 2, E2) were ground flat with up to 1200 μm granulation sandpaper, washed in an ultrasonic bath (Ultrasonic Cleaner, Model USC1400, UNIQUE Ind. e Com. Ltda., São Paulo SP 04709-111, Brazil) and autoglazed. Five specimens from each material were assigned to group 1, five to group 2 and fifteen to group 3. Group 1 specimens were measured for hardness immediately after they were manipulated and then maintained at 100% relative humidity (RH); group 2 were stored in brain heart infusion (BHI) broth (Becton Dickinson and Company, Sparks, MD, USA) supplemented with 1% (w/v) sucrose (Synth, LabSynth, São Paulo, Brazil) without microorganism inoculation; group 3 were stored in this growth medium after early *S. mutans* biofilm adherence on the surfaces of the disks. All these storage conditions were maintained at 37 °C for 30 days in order to analyze changes on the surfaces of the materials afterwards.

The specimens were not sterilized before storage by either physical methods (steam under pressure and gamma rays), chemical methods (solutions) or physico-chemical methods (ethylene oxide and hydrogen peroxide plasma). While these methods may render the specimen sterile, they probably affect the structure and properties of the restorative materials. Pressure, temperature, post-irradiation, chemical components and vacuum can cause degree of polymerization alterations, degradation, cracks formation, among others, modifying the surface of composites and glass ionomers.[13–15]

2.2. Bacteria

Streptococcus mutans UA159 (ATCC 700610) is a proven virulent cariogenic dental pathogen and it was the strain selected for genomic sequencing.[16] To prepare the inoculum, this microorganism was obtained from the culture collection of the Department of Microbiology and Immunology, Dental School of Piracicaba, and grown on *Mitis salivarius* agar plates at 37 °C for 48 h in a 5% supplemented CO_2 environment. Subsequently, single colonies were inoculated into 5 mL of BHI broth supplemented with yeast extract (Himedia Laboratories PVT Ltd., Mumbai, India) and incubated at 37 °C overnight.

Table 1 – Materials used in this study

Materials	Classification	Contents[a]	Batch number
IPS Empress 2 (Ivoclar Vivadent, Schaan, Liechtenstein)	Glass ceramic	Powder: 97% SiO_2, Al_2O_3, P_2O_5, K_2O, Na_2O, CaO, F and 3% TiO_2 and pigments	D59547
		Liquid: water, alcohol, chloride	G13497
Filtek Supreme (3M ESPE, St. Paul, MN, USA)	Resin-based composite	Bis-GMA, Bis-EMA, UDMA, TEGDMA	5AW, 5AT
		Zirconia/silica cluster filler and a non-agglomerated silica filler	
Vitremer (3M ESPE, St. Paul, MN, USA)	Resin-modified glass ionomer	Powder: fluoroaluminosilicate glass; redox system	5JT, 5CK
		Liquid: aqueous solution of a modified polyalkenoic acid, HEMA	5CX, 5EA
Ketac Molar Easymix (3M ESPE, St. Paul, MN, USA)	Conventional glass ionomer	Powder: fluorosilicate glass, strontium and lantanium	193134
		Liquid: polycarbonic and tartaric acids and water	199786

[a] Abbreviation of monomers in alphabetical order: Bis-EMA, ethoxylated bisphenol-A dimethacrylate; Bis-GMA, bisphenol glycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate.