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Effect of dental monomers and initiators on *Streptococcus mutans* oral biofilms

*Nancy J. Lin**, *Courtney Keeler*[1], *Alison M. Kraigsley*[2], *Jing Ye*[3], *Sheng Lin-Gibson*

Biosystems and Biomaterials Division, National Institute of Standards and Technology, 100 Bureau Drive, MS 8543, Gaithersburg, MD 20878, USA

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ABSTRACT

Objective. Resin-based composites are known to elute leachables that include unincorporated starting materials. The objective of this work was to determine the effect of common dental monomers and initiators on *Streptococcus mutans* biofilm metabolic activity and biomass.

Methods. *S. mutans* biofilms were inoculated in the presence of bisphenol A glycerolate dimethacrylate (BisGMA), triethylene glycol dimethacrylate (TEGDMA), camphorquinone (CQ), and ethyl 4-(dimethylamino)benzoate (4E) at 0.01 μg/mL up to 500 μg/mL, depending on the aqueous solubility of each chemical. Biofilms were evaluated at 4 h and 24 h for pH (n = 3–8), biomass via crystal violet (n = 12), metabolic activity via tetrazolium salt (n = 12), and membrane permeability for selected concentrations via confocal microscopy (n = 6). Parametric and non-parametric statistics were applied.

Results. 500 μg/mL TEGDMA reduced 24 h metabolic activity but not biomass, similar to prior results with leachables from undercured BisGMA-TEGDMA polymers. 50 μg/mL BisGMA reduced biofilm biomass and activity, slightly delayed the pH drop, and decreased the number of cells with intact membranes. 100 μg/mL CQ delayed the pH drop and metabolic activity at 4 h but then significantly increased the 24 h metabolic activity. 4E had no effect up to 10 μg/mL.

Significance. Monomers and initiators that leach from resin composites affect oral bacterial biofilm growth in opposite ways. Leachables, which can be released for extended periods of time, have the potential to alter oral biofilm biomass and activity and should be considered in developing and evaluating new dental materials.

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* *Corresponding author.*
E-mail addresses: nancy.lin@nist.gov (N.J. Lin), campbeco@up.edu (C. Keeler), kraigsleyam@niaid.nih.gov (A.M. Kraigsley), jye@wsgr.com (J. Ye).
[1] Present address: University of Portland, 5000 N. Willamette Blvd., Portland, OR 97203, USA.
[2] Present address: National Institute of Allergy and Infectious Diseases, National Institutes of Health, 5601 Fishers Ln, Rockville, MD 20852, USA.
[3] Present address: Wilson Sonsini Goodrich & Rosati, 650 Page Mill Rd, Palo Alto, CA 94304-1050, USA.
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1. Introduction

Dimethacrylate-based dental resins generally result in incomplete conversion of the monomers to polymers due to vitrification. As a result, measurable levels of leachables can elute from the materials after polymerization [1–3]. Some of the leachable components may exhibit a burst release that declines over time, but in other cases leachables have been shown to elute for extended periods [4], especially if the final degree of conversion (DC) of the polymeric material is low [5]. Leachables are well-established as one of the primary causes for cytotoxicity to mammalian cells associated with dental polymers and composites [6–8]. The biological effects of leachables have also been shown to extend to the other living organisms in the oral environment–the oral microbes [9–11]. For instance, biofilms of *Streptococcus mutans*, an oral pathogen commonly associated with caries formation, have reduced metabolic activity when grown on low DC dental polymers; eluted leachables were identified as the main factor contributing to this effect [12].

Leachables, as a general category, contain many different components, with the constituents depending on material parameters including polymer DC and polymer/composite chemistry. Common monomers and initiators are often found in leachables eluted in aqueous-based solutions. For instance, bisphenol A glycerolate dimethacrylate (BisGMA), triethylene glycol dimethacrylate (TEGDMA), camphorquinone (CQ), and ethyl 4-(dimethylamino)benzoate (4E) have all been identified in leachables from dental materials [2,12–14]. The total quantity, concentration, and composition of the leachables detected depends on the elution conditions, including solvent type, volume of solvent, surface area of the sample, and duration of leaching [1]. Degradation products are also known to elute from dental polymers and composites, and some of these degradation products have been shown to impact bacteria [15,16].

While degradation products have been studied in isolation, the individual effects of monomers and initiators on oral biofilms are not known and are needed to better understand their potential effects on biofilm growth and viability. Leachables are unavoidable with current dimethacrylate-based dental polymers and composites and could potentially be tuned to alter biofilms in a desirable way. Therefore, the objective of this study was to determine the effects of individual dental resin components on *S. mutans* biofilm formation. TEGDMA, BisGMA, CQ, and 4E were evaluated for their effects on biofilm acid production (via pH measurements), metabolic activity, overall biomass, and cell membrane integrity.

2. Materials and methods[4]

2.1. Materials

BisGMA and TEGDMA were obtained from Esstech Inc. (Essington, PA). Bacteria culture reagents were purchased from Becton, Dickinson and Company (Franklin Lakes, NJ), and the BacLight stain was purchased from Invitrogen Corp. (Carlsbad, CA), now part of Thermo Fisher Scientific Corp. All other reagents were purchased from Sigma-Aldrich (St. Louis, MO). All reagents were used as received.

2.2. Monomer/initiator solutions

Stock solutions of 5.0 mg/mL TEGDMA, 0.5 mg/mL BisGMA, 1.0 mg/mL CQ, and 0.1 mg/mL 4E were prepared in 5% dimethyl sulfoxide (DMSO), 95% water (by volume). Chemical structures of the resin components are shown in Fig. 1. The maximum stock concentration of each component depended upon its solubility in 5% DMSO. Dilutions for bacterial experiments were prepared in 5% DMSO, 95% water (by volume) to ensure all samples had the same amount of DMSO.

Laser light scattering (LLS) was performed on each stock solution to test for component aggregation. The stock solutions were diluted 10-fold in phosphate buffered saline (PBS) to mimic the highest final concentration to which biofilms were exposed. A commercial LLS spectrometer (BI-200SM) equipped with a digital time correlator and a compact solid-state diode-pumped Nd:Vanadate (Nd:YVO_4) laser ($\lambda_0 = 532$ nm) was used to perform dynamic light scattering (DLS) at 37 °C with a scattering angle of 90°.

2.3. Bacterial strains and biofilm inoculation

S. mutans Clarke UA159 from the American Type Culture Collection (ATCC 700610) were cultured in Todd Hewitt Broth (THB) overnight at 37 °C with 5% CO_2 (by volume). Cultures were pulse-vortexed and diluted 1:100 in ¼ Todd Hewitt Yeast Extract (THYE) medium (THB + 5 mg/mL yeast extract) supplemented with 30 mmol/L sucrose to prepare the biofilm inoculum. Biofilms were inoculated in 48-well polystyrene plates (Falcon) with 263 μL of inoculum plus 29 μL of resin component solution. This 10-fold dilution for each component resulted in a final concentration of 0.5% DMSO in the biofilm wells. Control wells received either uninoculated growth medium (no growth control) or inoculated growth medium with 29 μL of 5% DMSO (no additive control). Biofilms were cultured at 37 °C, 5% CO_2 (by volume) and analyzed at 4 h and 24 h.

2.4. pH measurements

When biofilms were ready for analysis, growth medium was first collected for pH measurements. Media from triplicate wells were combined to have sufficient volume for the pH electrode. For selected concentrations, kinetics of the pH drop were obtained by measuring medium pH every hour from 0 h to 8 h and from 16 h to 24 h.

[4] Certain commercial materials and equipment are identified in this article to specify the experimental procedure. In no instance does such identification imply recommendation or endorsement by NIST or that the material or equipment identified is necessarily the best available for the purpose.

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