Transaction: 194

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Combinatorial Methods To Assess Cellular Response To Bis-GMA/TEGDMA Conversion Levels

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Introduction

Composite materials for dental restorations do not attain complete vinyl conversion after curing [1,2]. To study the cellular response to incomplete conversion, techniques were developed to fabricate dental resins with varying conversion levels. For these initial studies, common resin components, 2,2-bis[p-(2′-hydroxy-3′-methacryloxyprop-oxy)phenyl]propane (Bis-GMA) and triethylene glycol dimethacrylate (TEGDMA), were used.

Materials and Methods

A solution of Bis-GMA and TEGDMA at a mass ratio of 1.8:1.0 was prepared with a camphorquinone and ethyl 4-N,N-dimethylaminobenzoate photoinitiator system. Resins were cured (60 s per side) in a Teflon mold (55 mm × 12 mm x 1.5 mm) between two glass slides using a visible light curing unit (Triad 2000, Dentsply International, York, PA). Conversion gradients were prepared using two methods: the centered, overlaid technique and the off-centered technique. For centered, overlaid fabrication, samples were centered on the sample stage, overlaid with a mask (Fig. 1), and cured. Off-centered gradient fabrication involved placing the sample off-centered on the stage. Using near infrared (NIR) spectroscopy, conversion was calculated as the reduction in =C-H absorption (6164 cm^{-1}) normalized to aromatic absorption (4623 cm^{-1}) [3]. To evaluate the cellular response, each resin was sterilized in 70% volume fraction ethanol, pre-incubated in Roswell Park Memorial Institute (RPMI) medium 1640 for 24 h, and seeded with 500,000 Raw 264.7 macrophages (American Type Culture Collection, Manassas, VA) in medium with 10% volume fraction fetal bovine serum. Control cells were seeded on tissue culture polystyrene. At 24 h, cells were incubated with phosphate buffered saline containing 2 µmol/L ethidium homodimer-1 and 2 µmol/L calcein AM for 5 min at room temperature and imaged using fluorescence microscopy to evaluate cell viability.

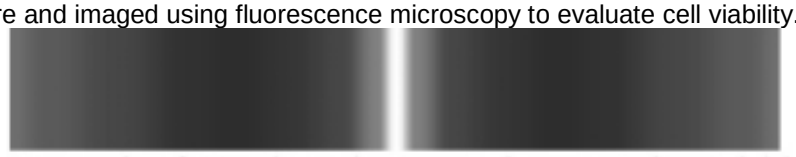

Figure 1. Example of a mask used to create the centered, overlaid conversion gradients. Masks were printed on transparency film using a laser printer.

Results and Discussion

NIR spectroscopy confirmed the presence of conversion gradients for both fabrication methods (Fig. 2). Both methods exploited the non-uniformity of the curing unit. The light intensity was highest in the center of the sample stage and decreased radially. The centered, overlaid technique combined the light source and a mask to create resins with the highest conversion in the center (Fig. 2A). In the off-centered technique, one edge of the sample was aligned with the center of the stage to fabricate a resin with the highest conversion at that edge and the lowest conversion at the opposite edge (Fig. 2B). The higher standard deviations in the centered, overlaid group were likely due to printer inconsistencies. Additional conversion profiles can be

produced by adjusting the mask design, cure time, or sample placement. Macrophages, which play a significant role in inflammation, were selected to evaluate the cellular response. Our preliminary cell studies revealed that cell spreading and attachment were reduced at lower conversions. The viability stain demonstrated that viability decreased as resin conversion decreased. Studies to quantify apoptosis and the macrophage inflammatory response as a function of resin conversion are ongoing.

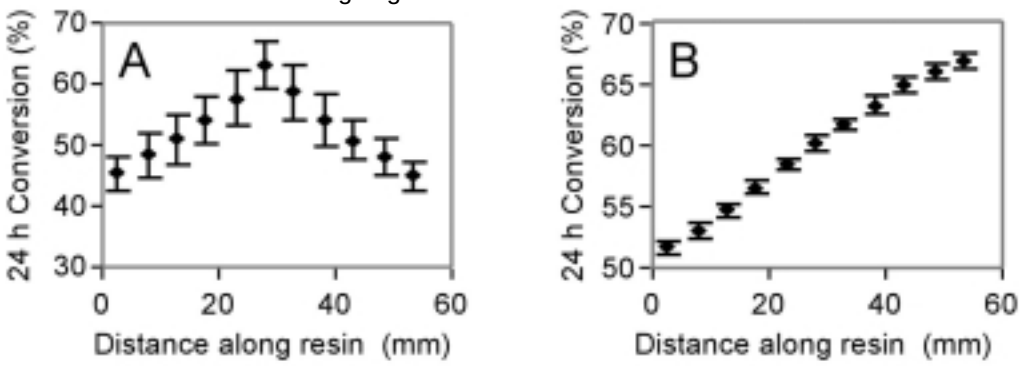

Figure 2. Conversion profiles 24 h after curing (samples stored at room temperature). **A)** Centered, overlaid fabrication ($n = 3$). **B)** Off-centered fabrication ($n = 6$). Error bars represent one standard deviation and are the estimate of standard uncertainty.

Conclusions

Combinatorial approaches are useful for evaluating polymeric dental materials, since a property such as conversion can be thoroughly investigated in a single sample. Our methods for developing conversion gradients could be applied to other dental resins or photopolymerized materials.

References

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Disclaimers