Rapid Assessment of Dental Polymers Using Gradient Samples

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Statement of Purpose: Dental composites are widely used as restorative materials yet still face challenges including incomplete conversion, shrinkage, residual stresses, and leakage. High-throughput techniques to assess several material properties in a combinatorial manner would prove useful in selecting and optimizing dental polymers and composites. The purpose of this study was to fabricate well-controlled polymer specimens with a range of monomer compositions and material properties and to develop high-throughput techniques to measure the properties. Samples were prepared with variation in monomer composition and irradiation intensity, and the resulting chemical, mechanical, and biological properties were evaluated.

Methods: Commonly used dental monomers, 2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy)phenyl] propane (BisGMA) and triethylene glycol dimethacrylate (TEGDMA), were mixed at various mass ratios (80:20, 70:30, 60:40, 50:50, 40:60, 30:70, and 20:80) and photo-activated with camphorquinone and ethyl 4-*N*,*N*-dimethylaminobenzoate. Specimens were prepared and cured such that they contained discrete changes in composition and continuous changes in irradiation intensity (Fig. 1). Degree of conversion (DC) was measured using near infrared spectroscopy (standard uncertainty of 5 %), and mechanical properties were quantified using nanoindentation (standard uncertainty of 3 %). Specimens were then sterilized and aged in phosphate buffered saline (37 °C, 7 d). RAW 264.7 macrophage-like cells were seeded onto the polymer specimens, cultured for 24 h, and stained with standard live/dead stains, calcein acetoxymethyl ester (calcein AM) to stain live cells and ethidium homodimer-1 (EthD-1) to stain dead cells. A third dye, Hoechst 33342 (H33342), was included to stain cell nuclei and aid in quantifying viability and cell density. Cell images were analyzed using customized image analysis macros. Nuclei stained with EthD-1 represented dead cells, and nuclei stained with H33342 and not costained with EthD-1 represented live cells. Analysis of variance and Fisher's least significant difference test were used to analyze the cell results (95 % confidence interval). Standard uncertainties associated with the viability and density measurements are 15 % and 30 %, respectively.

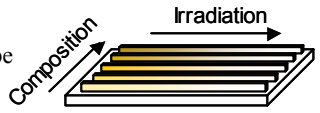

Figure 1. Diagram of gradient sample. Each composition stripe is 60 mm x 3 mm x 1 mm.

Results/Discussion: Continuous gradients in DC as a function of position were confirmed for each composition (Fig. 2a). Elastic modulus also depended on position and composition (Fig. 2b). Cell viability and density varied with DC but not composition, so data from all compositions were pooled. A DC of at least 52 % was required for normal cell viability (Fig. 3), whereas at least

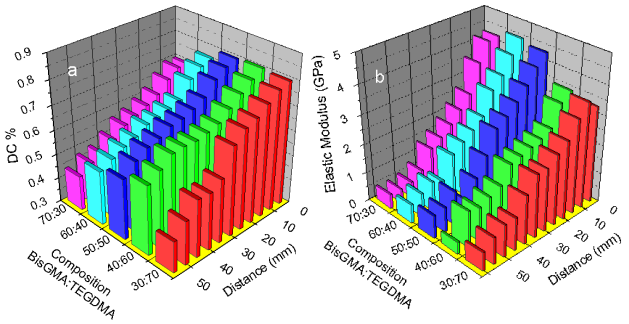

Figure 2. DC **(a)** and elastic modulus **(b)** as a function of composition and distance on the 2D gradient specimens.

60 % DC was required for a regular cell density. This method of quantifying viability and cell density using a slight adaptation of the common live/dead fluorescent staining assay was straightforward, and automated analysis allowed for rapid quantification of the images. Evaluating chemical, mechanical, and biological properties on the same samples thoroughly investigated the effects of composition and irradiation. These results demonstrated that a single composition/irradiation combination to optimize all material properties does not exist. For instance, higher BisGMA content increased the elastic modulus but decreased the maximum DC attained. These results demonstrate the importance of testing multiple properties to assess material performance.

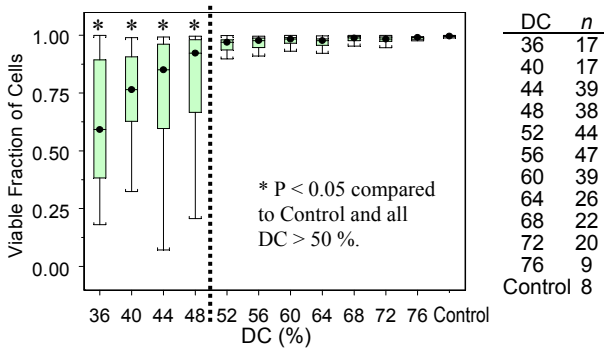

Figure 3. Box plot of cell viability as a function of DC for all compositions combined. Sample size (*n*) is listed on the right.

Conclusions: The use of gradient approaches to screen the effects of monomer composition and irradiation allowed for rapid material assessment. These techniques could easily be applied to novel monomers and composites to enable the selection of compositions and processing protocols that optimize the properties of dental composites and thus accelerate improvements in clinical products.

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