Mechanistic study of synthesizing tunable gelatin methacrylate (GelMA) bioinks for rapid and high-resolution stereolithography bioprinting

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Statement of purpose: The performance of the bioprinting process and the properties of the fabricated scaffolds are governed by the bioink properties. Therefore, design and selection of suitable bioink is an indispensable requirement. Gelatin methacryloyl (GelMA) offers excellent biocompatibility, tunable physical properties, and ease of chemical modifications which have made it an attractive choice as bioinks. However, the current preparation methods for GelMA-based bioinks lack the ability to tailor their physical properties for desired bioprinting methods. This work focuses on development of visible light photopolymerizable GelMA bioinks optimized for stereolithography (SLA) bioprinting. We delineate the mechanisms involved in GelMA synthesis by studying 3 crucial parameters. The obtained bioinks maintain low viscosity at room temperature while exhibiting fast photocrosslinking, high strength, low degradation and high biocompatibility.

Methods: GelMA was synthesized by reacting gelatin with glycidyl methacrylate (GMA). Four solvents were used for the reaction - dimethyl sulfoxide (DMSO), reverse osmosis purified water (RO), phosphate buffered saline (PBS) or bi-carbonate buffer (CB). The reaction duration was 3 or 12 hours with initial pH set to high (9) or low (3.5). GelMA was obtained by dialyzing the reaction mixture against RO water followed by lyophilization. The degree of substitution (DS) of GelMA was characterized using ^{1}HNMR spectroscopy. The bioink was prepared with 10% GelMA macromer and Eosin Y photoinitiation system. Hydrogel samples were formed by crosslinking using a DLP-SLA bioprinting system. Compressive modulus was measured using a micromechanical testing machine. The photocrosslinking kinetics were recorded using the micromechanical tester with sinusoidal motion applied to the probe augmented with TMSPMA coated glass applying shear forces to bioink droplet. Bioprinting was performed by projecting the pattern on bioink in a STL bioprinter. NIH 3T3 fibroblast cells and U118 astrocytes were encapsulated in the scaffold. Printed samples were cultured in cell culturing media (90% Dulbecco's Modified Eagle Media, 10% fetal bovine serum, 1% penicillin-streptomycin). The cell morphology was observed on day 5 by staining nuclei with 4′,6-diamidino-2-phenylindole (DAPI) and cytoskeleton with Phalloidin and imaging under fluorescent microscope with EGFP and DAPI channels.

Results: The solvent, initial pH and reaction duration play a significant role in GelMA synthesis and the properties of the resulting hydrogel. Out of GelMA prepared in various conditions, 8 bioinks were found to have gelation point lower than room temperature resulted by combination of high DS and hydrolysis. Presence of an organic base in DMSO catalyzes the nucleophilic substitution reaction and results in high DS while also contributing to high hydrolysis. CB and PBS solvents also exhibit high DS for 12-hour synthesis for all pH conditions (A). However, high pH for 12 hours also extensively hydrolyzes gelatin and a stable hydrogel cannot be formed (PBS-12h-H & CB-12h-H). GelMA synthesized in RO had medium and high DS corresponding to low and high pH reaction conditions. Overall, RO-GelMA had lower degree of hydrolysis. The modulus comparison revealed RO-GelMA to exhibit significantly high strength than other bioinks resulting from a combination of high DS and low hydrolysis (B). Further, RO-GelMA showed faster photocrosslinking and high-resolution printing. High biocompatibility was observed with 3T3 fibroblasts and U118 astrocytes (C(1cm/200µm)-D(1cm/50µm)) [1]. Upon culturing for 30 days, the astrocytes formed an interconnected network and showed an activated state with extended processes (D).

Conslusion: The combination of three key parameters – solvent, pH and reaction duration were shown to tune the properties of the bioink. RO presents a suitable solvent for preparing GelMA with low gelation point and fast photoscrosslinkability, making it an ideal bioink for visible light SLA bioprinting. Growth of encapsulated fibroblasts and astrocytes cells within the hydrogel scaffolds show the suitability of these bioinks for skin and neural tissue engineering. Along with contributing to the bioink synthesis, this investigation also lays the foundation of designing the bioink for targeted tissue types and bioprinting processes.

Reference: [1] Kumar H., Macromol. Biosci., 2020,2000317