Novel aqueous two-phase solutions for 3D microfluidic bioprinting applications

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Functionally graded materials (FGMs) are an appealing group of bioactive substances with significant potential for biofabrication. Biphasic emulsion inks have demonstrated utility in structuring cellular configurations and controlled drug release. This study presents the novel application of an aqueous two-phase system (ATP) to manipulate the porosity density of deposited fibers and cellular arrangements. Utilizing a thixotropic agarose fluid-gel, 3D bioprinting of the ATP is performed to capitalize on its unique print-in-place properties for biocompatible ATPs. The continuous phase (Dextran, gelatin, hyaluronic acid methacryloyl (DexMA, GelMA, HAMA)) accommodates the biocompatible dispersed phase within a flow-focusing microfluidic printhead. Fast camera (FAST CAM) analysis examines ATP formation post-fiber extrusion. Materials undergo sterile preparation involving suspension in incremental sodium chloride (NaCl) concentrations. Physico-chemical scrutiny entails scanning electron microscopy (SEM), optical coherence tomography (OCT), and rheological assessments. SEM and OCT imaging characterize ATPs material inks, revealing phase disposition influenced by salt concentration. Rheological measurements calibrate ATP ratio for stable fiber deposition via microfluidic printhead. The dispersed phase's globular diameters correlate with salt concentration. Optically analyzed 3D printed scaffolds quantify dispersed phase density, increased with higher salt concentrations. Larger dispersed phase emulsion diameters result from elevated salt concentration. FGMs are 3D deposited within an agarose fluid-gel as previously described. During 3D deposition, human bone marrow stromal cells (HBMSCs) or myoblast cell line (C2C12) are patterned. Encapsulation of vascular endothelial growth factor (VEGF) and bone morphogenetic protein-2 (BMP-2) occurs within dispersed or continuous phases of the w-w emulsion, followed by 3D printing. Post-3D printing, HBMSCs exhibit comparable viability, spreading, and migration with increased salt concentration. VEGF and BMP-2 in the dispersed phase, patterned in 3D, show enhanced release efficiency dependent on salt concentration, linked to encapsulating droplet attributes. Stimulated by VEGF or BMP-2, implanted HBMSCs demonstrate functional capability, yielding implantable skeletal constructs. C2C12 align along elongated dispersed phases, modulated by temperature and extrusion feed rate ratio. In conclusion, ATP FGM precursors influence dispersion formation attributes and reproducibility, furnishing a functional platform for hierarchical construct 3D printing. This study underscores synergistic interactions between dispersed and continuous biocompatible phases, substantiating the viability of biphasic systems for tissue engineering and regenerative medicine applications.