Solvent-dependent Polycaprolactone/Polyethylene Oxide/Hydroxyapatite Ink for Bone Tissue Engineering

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INTRODUCTION: Critical-sized bone defects resulting from musculoskeletal tumors, infections or trauma pose significant obstacles in orthopedic surgery as they lack the ability to heal naturally. Complete patient recovery is still a challenge despite various treatment options in grafts, reconstructions, and prostheses.[1] Bone tissue engineering (BTE) offers a promising precision solution to address these defects with 3D-printed biocompatible scaffolds. BTE is an area of interest for synthetic scaffold fabrication due to its load-bearing and bioactive requirements. Synthetic polymers provide high mechanical strength and tunable degradation which supersedes that of natural hydrogels. As non-water soluble materials, these polymers require high temperature or incorporation of organic solvents to achieve printability.[2,3] Several studies have successfully utilized synthetic polymers like polycaprolactone (PCL) in BTE scaffolds. PCL is an FDA-approved biomaterial with high biocompatibility, mechanical strength, and accessibility. PCL is soluble in several organic solvents, however solvent selection is rarely examined in ink formulation.[2] Hydroxyapatite has been used as an additive in concentrations from <1% - 85% for improved scaffold bioactivity and mechanical properties.[4,5] Many studies have composed inks from similar biomaterials, but utilized a variety of organic solvents and their composites with differing polarities and stoichiometries. This study aims to optimize a liquid 3D-printable ink composed of PCL, polyethylene oxide (PEO), and hydroxyapatite by varying solvent type during fabrication. Prior research demonstrated successful printing of this composite using the most common solvent, dichloromethane.[5] This study investigates how solvent choice influences ink behavior for extrusion-based printing.

METHODS: Solvents, including dichloromethane (DCM), acetic acid (AA), and chloroform (CH), were tested in a composite ink formulation. Equal amounts of PCL and PEO were dissolved in each solvent along with a surfactant (2-butoxy ethanol) and a plasticizer (dibutyl phthalate) to enhance ink viscosity and homogeneity. Once both polymers were fully dissolved, 65% hydroxyapatite nanoparticles were mixed in until fully combined. The inks were stored in air-tight containers, maintaining stability for over one month. For 3D printing, a TissueScribe extrusion printer was used. A 3mL syringe was loaded with ink topped with a 260μm or 410μm nozzle for extruding lattice scaffold geometries. Printability metrics included flow rate and print speed. Print resolution was assessed by filament width and scaffold dimensions. Ink characterization (n=3 per group) included rheology, Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA, n=2), differential scanning calorimetry (DSC), wettability, and confocal laser scanning microscopy. Printed scaffolds underwent degradation, mechanical, and *in vitro* testing using mesenchymal stem cells and were imaged via scanning electron microscopy.

RESULTS SECTION: Ink composition confirmed no residual solvents were detected in the 3D-printed scaffolds post-drying (**Fig 1A-B**). Similarly, thermal behavior showed no initial solvent evaporation. DSC data showed AA ink had a single endothermic peak where DCM and CH inks had two distinct peaks for each dissolved polymer (**Fig 1C**). Rheological flow behavior between inks differed for all yield and viscoelastic points. DCM as a solvent produced the highest ink viscosity and demonstrated less shear-thinning behavior (**Fig 1D**). Acetic acid resulted in the lowest ink viscosity and highest shear-thinning behavior (**Fig 1D**). Due to a lower viscosity, the AA formula could be printed using a smaller nozzle tip (260 μm vs. 410 μm). Variations in flow rate and print speed influenced filament width and scaffold geometry; higher print speeds produced more uniform filaments, but solvent type affected how flow rate influenced filament resolution. Increased flow rate enlarged resultant filament widths for all solvents, the greatest increase using CH ink and the least in DCM ink. Acetic acid ink demonstrated the greatest variability in print resolution while DCM showed the least. DCM and chloroform-based inks had no significant filament fusion or variation in filament width unlike the AA ink (**Fig 2E-F**). Acetic acid filaments had a significantly rougher surface topography compared to inks using DCM or chloroform (**Fig 2A-D**). DCM and CH inks were statistically more hydrophilic than the AA ink (**Fig 1F**). All scaffolds had similar swelling behavior; however, hydrolytic degradation was immediate in AA scaffolds. CH scaffolds had significantly less mass loss over the 21-day period than DCM scaffolds (**Fig 1E**). Preliminary data from *in vitro* testing using DCM and CH inks suggests scaffolds are not cytotoxic. Further data collection is necessary for formulae comparison on osteoinductive properties.

DISCUSSION: This study demonstrated the impact of solvent choice on the properties and performance of 3D-printable inks for bone scaffolds. One study investigated the effects of methylene chloride and composite variations of this solvent on geometric and mechanical differences between electrospun scaffolds. They saw a similar influential trend between solvent choice and resulting scaffold behavior.[6] Solvent choice depends on the overall research goal and application, but as shown, solvent consideration may lead to improved printability and print resolution. Here, acetic acid improved ink miscibility, but developed scaffolds that underwent rapid degradation, excluding them from cell studies. To influence cell behavior, ink characteristics like surface roughness also varied with solvent type; these micro- or nanoscale surfaces can impact cell adhesion and differentiation.[5] Additionally, increased hydrophilicity in DCM and CH ink demonstrate potential for improved cell attachment and proliferation.[3] These findings reveal solvent selection significantly impacts scaffold resolution, surface roughness, and degradation behavior, all of which can affect their regenerative potential. *In vitro* testing is ongoing and will further illuminate scaffold impact on cell growth and differentiation.

SIGNIFICANCE/CLINICAL RELEVANCE: Investigation of scaffold formulation, specifically how fabrication may influence key properties and performance, may highlight the importance of this decision and provide guidance for future work of scaffold development in bone tissue engineering.

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Figure 1 (left): Material characterization of ink formulae including (A) ink composition using varying solvents: dichloromethane, chloroform, and acetic acid (B) FTIR results compared to pure polymers (C) Shear-thinning behavior (D) contact angle results of scaffold hydrophilicity (E) DSC results of thermal melting behavior (F) Hydrolytic degradation of 3D-printed scaffolds over time. ***p<0.001. **Figure 2 (right):** Scaffold characterization including (A) SEM imaging of DCM (B) chloroform and (C) acetic acid two-layer scaffolds (D) surface roughness (E) filament uniformity (F) filament fusion. ***p<0.001.