A Novel Method for the Synthesis and Manufacture of Photocrosslinkable Polycaprolactone-based Biodegradable 3D Scaffolds for Tissue Engineering Applications

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Statement of Purpose: The repair of critical size bone defects due to trauma, disease and tumor resection remains a major clinical problem. The scope of the work presented here aims at addressing this problem thru the synthesis and manufacture of polycaprolactone-based photocrosslinkable scaffolds. Synthesis of poly(caprolactone) fumarate (PCLF) and poly(caprolactone) trifumarate (PCLtF) was conducted by employing a well established poly(propylene fumarate) (PPF) synthesis[1] methodology. The current method employed addresses the shortcomings as reported by Wang[2] with regards to UV crosslinking and overall mechanical properties of PCLF. Enhancement of mechanical properties and bioactivity was studied by the addition of commercially available hydroxyapatite nanoparticles to PCLF and PCLtF scaffolds. X-ray diffraction analysis illustrated adequate nanoparticle dispersion. Successful UV photocrosslinking via stereolithography was obtained with a resolution of 250µm. Preliminary cell adhesion studies show promising results.

Methods: Briefly, PCLF and PCLtF were synthesized via a well established transesterification reaction. Both materials were synthesized from commercially available polycaprolactone derivatives and diethyl fumarate (DEF) under an inert atmosphere in the presence of a zinc chloride catalyst and proton scavenger (hydroquinone). All reagents were used as received (Sigma Aldrich, St. Louis, MO). Synthetic hydroxyapatite nanoparticles with particle size <200nm were used. It should be noted that scanning electron microscopy revealed particles approaching 500nm in diameter. Molecular weight determination was conducted by gel permeation chromatography (GPCMax, Viscotek, Houston, TX). The current method produced a soluble UV penetrable material suitable for manufacture by the rapid manufacturing technology stereolithography. Due to the relative viscosity of the synthesized material an 80% (wt./wt.) mixture (PCLtF or PCLF/DEF) was used for the manufacture of porous scaffolds on a ViperHA stereolithography apparatus (3D Systems, Rock Hill, SC). Hydroxyapatite-containing PCLtF scaffolds (0.5% wt/wt) were examined by x-ray diffraction and scanning electron microscopy. Biocompatibility was studied using rat marrow stromal cells seeded on CAD designed porous disks using Pro/Engineer Wildfire 4.0 (PTC, Needham, MA) and exported as an STL file (Figure 1A). The manufactured disks were cultured in osteogenic media.

Results: Fourier transform infrared spectroscopy revealed successful incorporation of photocrosslinkable alkenes to PCLF and PCLtF. With the current method of fumarate incorporation, PCLF and PCLtF were rendered UV crosslinkable. In addition, the synthesized materials are soluble in DEF which eliminated the need of harsh organic solvents, as well as lowered the viscosity resulting in increased manufacturability. As seen in Figure 1, the pore dimensions of the manufactured porous disk are larger than the model which may be attributed to the cleaning process and resultant swelling of the PCLtF disk. Figure 2 illustrates preliminary results of seeded rat marrow stromal cells on PCLtF porous disks.

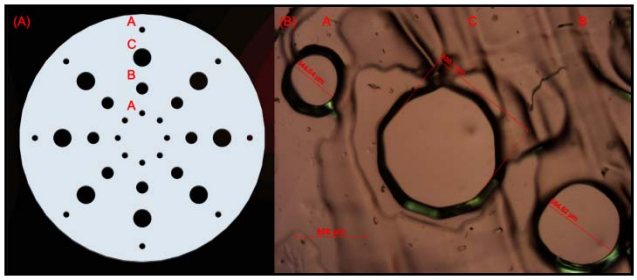

Figure 1. Schematic of CAD designed porous disk (A) with varying pore sizes A) 250µm, B) 500µm, and C) 750µm. Manufactured SL part (B) with measured pore dimensions is shown.

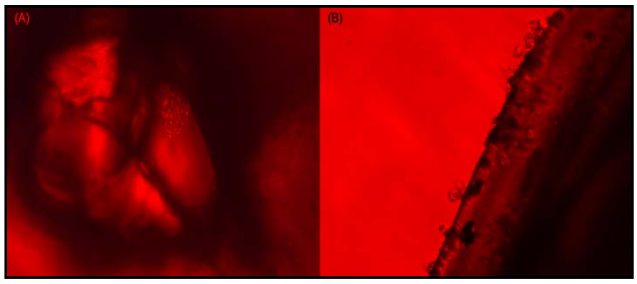

Figure 2. Confocal micrographs of seeded rat marrow stromal cells on manufactured PCLtF porous disks. 1A) Cells attached to inner pore wall. 1B) Cells attached to disk edge.

Conclusions: The present experimental study shows that UV photocrosslinkable polycaprolactone-based scaffolds can be manufactured and subsequent seeding of rat marrow stromal cells attach on porous scaffolds produced by stereolithography. Future work will include the study of living cell cultures via fluorescent dyes as published by Wang, et. al[3] as well as protein and RNA analysis to validate osteoblast phenotypic expression. Extended cell culture studies are needed to determine; rate of proliferation, cell differentiation pathways and rate of extra-cellular matrix deposition.

References:

1. Shung, A.K., Journal of Biomaterials Science-Polymer Edition, 2002. **13**(1): p. 95-108.
2. Wang, S.F., Biomacromolecules, 2008. **9**(4): p. 1229-1241.
3. Wang, Y.H., Biotechnology Progress, 2006. **22**(6): p. 1697-1701.