Two-Phase Scaffold For Load Bearing Soft Tissue Repair

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Statement of Purpose: Load bearing soft tissues such as the nucleus pulposus, articular cartilage and meniscus, have limited ability to repair themselves once damaged. Biodegradable scaffolds can provide an immediate solution to repair the damaged tissue and regenerate the native tissue over time. The aim was to combine a cell encapsulating hydrogel with the mechanical properties of an elastomer to produce a bicontinuous cell delivery device for the repair and replacement of load bearing soft tissues. The cell encapsulating hydrogel phase consisted of *N*-methacrylated glycol chitosan (MGC). The elastomer phase of the scaffold was made up of an acrylated 50:50 molar ratio poly(ε-caprolactone-co-D,L-lactide) (ASCP) star copolymer to provide the mechanical properties of the scaffold.

Methods: 50:50 CL:DLLA ASCP [1] and MGC [2] were synthesized as described previously. Micro-CT images of the scaffolds were taken on an Xradia microXCT-400 scanner and reconstructed in ImageVis3D. The density of the MGC regions were artificially increased using 150mg/mL K_2HPO_4, a common CT calibration agent. Primary cells were isolated from articular cartilage of bovine metacarpal-phalangeal joints as described previously [3]. Cells were suspended in a 6 w/v% MGC and F12 solution at a concentration of $4x10^7$ cell/mL. 1 v/v% DMSO was added along with 0.1w/v% I2959 photocrosslinking agent. 30 v/v% of the MGC cell suspension was then mechanically mixed into the 8000 MW CL:DLLA acrylated star copolymer. Aliquots (~50mg) of the mixture were loaded into a cylindrical mold with two adjustable parallel plungers. A UV light source (EXFO) with a 320-390nm filter and 15 mW/cm^2 intensity was used to crosslink the scaffolds, while attached to an in-line rotating device (10 rpm) for 180 seconds. Crosslinked scaffolds were cultured in 5% FBS/F12 media for up to 14 days with media changed every other day. Media was supplemented with 1X antibiotics/antimycotics and 100µg/mL ascorbic acid. Mechanical testing (n=5-6) was performed on a Mach-1 micromechanical tester (Biomomentum) with a 1 kg load cell as described previously [3]. Cells were pre-stained with PKH26 (Sigma) cell tracker prior to seeding within the scaffolds (n=2). Scaffolds were cut and imaged (n=8) along the middle of both planes to determine cell distribution and viability over a 14 day culture period. PKH26 images were converted to binary in ImageJ and counted using the analyze particle feature (2-30 pixel size and 0-1 circularity). Viability within the scaffolds was confirmed with the MTT assay after incubation for 4hrs at 37°C. 600uL of DMSO was added to solubilize the formazan crystals. To facilitate solubilization the scaffolds (n=5-6) were crushed and broken up within microcentrifuge tubes using a metal rod. The suspensions were then centrifuged at 10,000g for 10 minutes. The supernatant was removed and added to a 96 well plate in 200µL aliquots and read at 500nm (max abs.).

Results: 3D reconstruction of the µCT scans (Fig. 1A) revealed a continuous MGC region present throughout the scaffolds (blue). The unlabelled ASCP made up the black void spaces between the MGC regions. PKH26 cell tracking (Fig. 1B) indicated that the cells were well distributed throughout the scaffold and remained intact over a 14 day culture period. Cells counted from the PKH26 images maintained cell numbers from day 3 to day 14 of 235±13 and 278±15, respectively. MTT assay results indicated a relative increase in cell viability over the 3, 7 and 14 day time points as seen in Fig. 1C. Equilibrium modulus values (Fig. 1C) decreased slightly over the 14 day culture period, but were well above the modulus of cell-loaded MGC-only constructs (101±8 kPa). Equilibrium moduli, over the 14 day culture period, were also within the range of values reported for articular cartilage (0.8±0.4 MPa) [4].

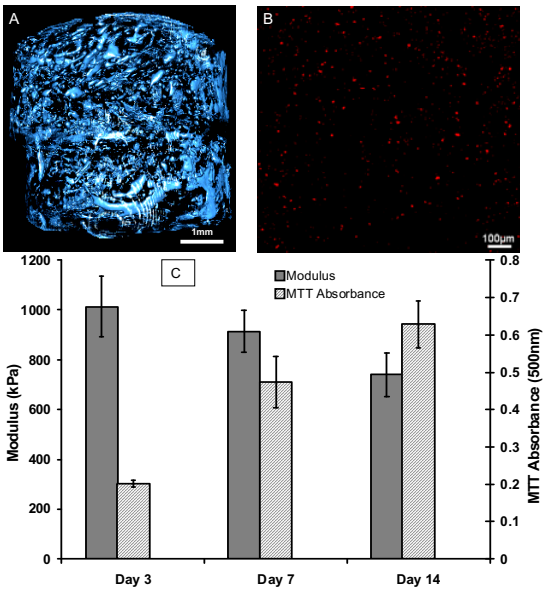

Figure 1. A) 3D reconstruction of µCT scan of 30 v/v% scaffold, B) Confocal image of PKH26 labeled cells within a 30 v/v% scaffold after 14 days, C) Modulus and MTT viability data for 30 v/v% cellular scaffolds after 3, 7 and 14 days in culture.

Conclusions: A cell-loaded bicontinuous two-phase scaffold maintained cell viability over a 14 day culture period. Mechanical properties also remained within the range of load bearing soft tissues after 14 days in culture. Future work will examine cellular activity for a range of cell types over longer culture periods within the scaffolds. Feasibility of this scaffold as an injectable and in situ crosslinkable delivery system will also be investigated.

References:

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