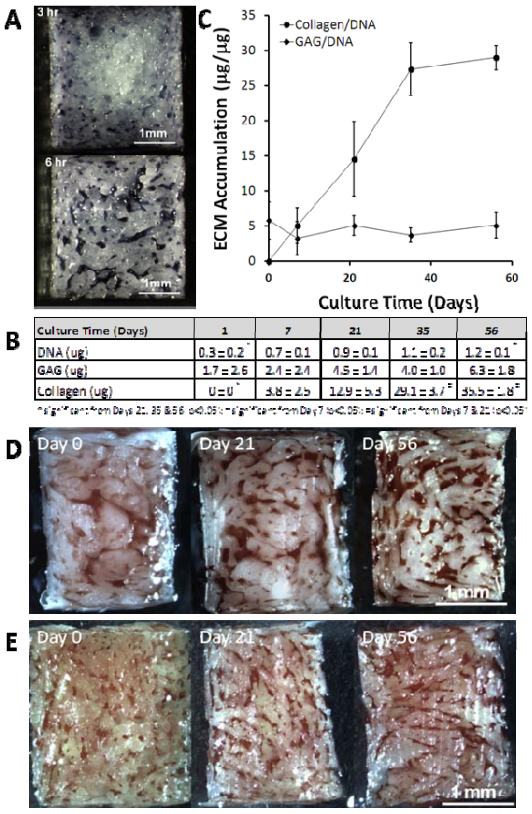
Long-term static culture and fatigue testing of a bi-continuous hydrogel-elastomer scaffold for nucleus pulposus repair

James W.S. Hayami, Stephen D. Waldman, Brian G. Amsden
Queen's University, Kingston, Ontario.

Statement of Purpose: Early stage degenerative disc disease affects the physical properties of the nucleus pulposus and can result in lower back pain due to loss of disc height. The aim of this study was to design and test the long-term capability of a bi-continuous scaffold for the possible use as a nucleus pulposus repair strategy. The bi-continuous scaffold consisted of an *N*-methacrylated glycol chitosan (MGC) hydrogel and an acrylated 30:70 poly(ε-caprolactone-co-trimethylene carbonate) star copolymer (ASCP) elastomer. The formation of the bi-continuous morphology, without any solvents, and the final photocrosslinked scaffold, was accomplished in the presence of cells. The scaffolds were subjected to fatigue testing at physiological strains and characterized for the extracellular matrix accumulation under static culture conditions.

Methods: An 8400 g/mol ASCP and MGC (5% degree of methacylation) were synthesized as described previously [1]. Chondrocytic cells (articular chondrocytes) were isolated from bovine metacarpal-phalangeal joints. Cells were suspended in an 8 w/v% MGC and F12 solution at a concentration of $4x10^7$ cell/mL along with 0.1 w/v% I2959 photoinitiator. 30 wt% of the MGC cell suspension was then mechanically mixed into the ASCP pre-polymer. Aliquots (~30 μL) of the mixture were loaded into cylindrical molds (3.2mm x 3.5mm, d x h). The scaffolds were cross-linked from both sides using long-wave UV for 60 seconds at $50mW/cm^2$. MGC interconnectivity within the scaffolds was measured via extent of penetration of a 1% toluidine blue solution for 0.5, 1, 3 and 6 hours. Scaffolds were cut in half and imaged on a stereomicroscope to determine dye penetration. Fatigue testing at 20 % strain amplitude for up to 10^6 cycles was performed on acellular scaffolds using an Enduratec ELF3200 (Bose) mechanical testing system. Long-term static cultures of cellular and acellular scaffolds were performed in 10% FBS/F12 media for up to 56 days. Equilibrium modulus testing (n=6) was performed on a Mach-1 micromechanical tester (Biomomentum). Biochemistry assays for DNA (Hoechst 33258), GAG (DMMB) and collagen (hydroxyproline) were performed on papain digested samples. In addition, scaffold sections (hand cut) were fixed in 4% paraformaldehyde and stained with either safranin-O or Sirius red.

Results: The interconnectivity of the elastomer phase in the scaffolds resulted in improved mechanical properties (1144±50 kPa) over hydrogel-only scaffolds (101±8 kPa). Interconnectivity of the hydrogel phase was confirmed by the fact that toluidine blue penetrated to the centre of the scaffolds in less than 6h (Fig 1A). Fatigue testing of the acellular scaffolds demonstrated no significant loss in modulus (1106±45kPa) after 10^6 cycles. Long-term static culture of the cellular scaffolds also showed no change in modulus (1115±25 kPa) after 56 days. DNA and collagen accumulation in the cellular scaffolds was significantly higher at the later time points from the initial day 1 values (Fig 1B). Although not significant, a general trend of increasing GAG content over the 56 day culture period was observed (Fig.1B). Normalized to DNA, collagen content increased over time and GAG content showed no change over the 56 day culture period (Fig 1C). These results were confirmed by histology with safranin-O (GAG) and Sirius red (collagen) staining (Fig. 1D and E).



Culture Time (Days)	1	7	21	35	56
DNA (ug)	0.3 ± 0.2	0.7 ± 0.1	0.9 ± 0.1	1.1 ± 0.2	1.2 ± 0.1
GAG (ug)	1.7 ± 2.6	2.4 ± 2.4	4.5 ± 1.4	4.0 ± 1.0	6.3 ± 1.8
Collagen (ug)	0 ± 0	4.8 ± 4.5	14.9 ± 5.3	29.1 ± 4.7	35.5 ± 1.8

Figure 1. A) Toluidine blue dye diffusion test at 3 and 6 h, B) Table of DNA, GAG and collagen values, C) Collagen and GAG normalized to DNA, D) Staining of cellular scaffolds at days 0, 21 and 56 with Safranin-O and E) Sirius red.

Conclusions: The bi-continuous scaffolds were able to withstand fatigue loading up to 10^6 cycles with no significant loss in mechanical properties. Chondrocytic cells cultured within the bi-continuous scaffolds were able to proliferate and accumulate extracellular matrix over the 56 days in culture. Overall the bi-continuous scaffolds show potential for use as a nucleus pulposus replacement for degenerative disc diseases.

References: 1. (Hayami, JWS. Macromol. Biosci. 2011;11:1672-1683.)