Encapsulation of Nucleus Pulposus Cells in Photocrosslinked Alginate Hydrogels: Cell Viability and Extracellular Matrix Production

Chou AI, Nicoll SB.
University of Pennsylvania, Philadelphia, PA

Statement of Purpose: The intervertebral disc (IVD) is complex fibrocartilaginous tissue divided into 3 distinct regions: the outer and inner annulus fibrosus and the nucleus pulposus (NP). Degeneration of the IVD has become a major health concern in the US, with current surgical treatments resulting in decreased mobility of the spine. A tissue engineering approach may provide an alternative that restores both IVD structure and function. Traditionally, cell encapsulation in ionically crosslinked alginate hydrogels has been used to culture NP cells *in vitro*.[1] However, constructs composed of IVD cells encapsulated in alginate hydrogels exhibit decreased mechanical properties and poor retention of extracellular matrix (ECM) proteins over time.[2] Therefore, the objective of this study was to evaluate cellular viability and protein accumulation of NP cells encapsulated in photocrosslinked methacrylated alginate hydrogels in comparison to ionically crosslinked alginate hydrogels.

Methods: ***Primary Cell Isolation:*** NP cells were isolated from bovine caudal IVDs by collagenase digestion and designated as passage 0. ***Synthesis of Methacrylated Alginate (MA-LVALG):*** Methacrylation modification was based on previous protocols.[3,4] Briefly, methacrylic anhydride at 20X excess was slowly added to a 1% solution of low viscosity alginate (LVALG, Sigma, St. Louis, MO) at 4°C and the pH was periodically adjusted to pH 8 using 5N NaOH for 24hrs. Modified polymer was purified via dialysis for 48hrs and the final product was recovered by lyophilization. Methacrylation was confirmed using ^{1}H-NMR. ***Photocrosslinked Alginate Hydrogels (MA-LVALG):*** $10x10^6$ cells/mL were encapsulated in 2.5 and 3% UV-sterilized MA-LVALG dissolved in 0.05% I2959 (Irgacure 2959, Ciba Specialty Chemicals, Basel, Switzerland) through exposure to longwave UV light for 10min. ***Ionic crosslinked Alginate Hydrogels (LVALG):*** $10x10^6$ cells/mL were encapsulated in $CaCl_2$ crosslinked, 2.5 and 3% UV-sterilized LVALG. ***Cell Culture:*** Passage 2 cells were used.[5] All cultures were incubated at 37°C in DMEM w/ 10% FBS, 50µg/mL L-ascorbic acid and antibiotics. At day 3, constructs were analyzed for viability and ECM production. ***Cell Viability:*** Constructs were evaluated for viability using the MTT Assay Kit (ATCC, Manassas, VA). ***Immunohistochemistry:*** 3-D cultures were fixed in acid formalin and processed for paraffin embedding. Monoclonal antibodies to types I (Sigma) and II (II-II6B3, DSHB) collagen and chondroitin sulfate proteoglycan (CSPG, Sigma) were used with a peroxidase-based detection system (Vector Labs) and DAB as the substrate chromagen. ***Statistical Analysis:*** A two-way ANOVA with a Tukey's post-hoc test was performed to determine the effect of crosslink and w/v%. Significance was set at $p<0.05$. Data represent mean ± standard deviation (n=3).

Results/Discussion: MA-LVALG hydrogels swelled significantly more than LVALG hydrogels after 3 days of culture, with an increase in disc diameter of 30% and 6%, respectively (Data not shown). NP cells encapsulated in MA-LVALG exhibited significantly increased viability compared to LVALG constructs 3 days after encapsulation (Figure 1A). Viable cells were evenly distributed throughout both constructs (Figure 1B,C). NP cells in MA-LVALG hydrogels displayed more pericellular staining of CSPG compared to LVALG hydrogels (Figure 2). Additionally, type I and II collagen staining was similar between the two alginate culture conditions at this early time point (Data not shown).

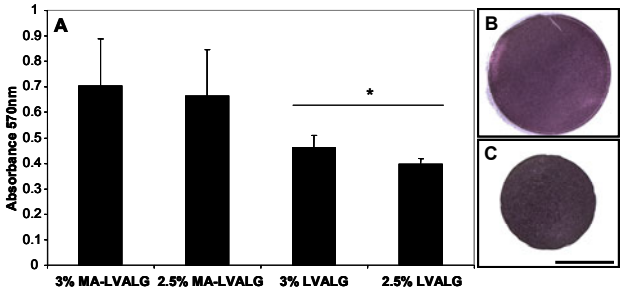

Figure 1: Cell Viability. (A) MTT quantification, Stereomicrographs of (B) 2.5% MA-LVALG and (C) 2.5% LVALG. Scale bar = 5mm. *:significant compared to MA-LVALG

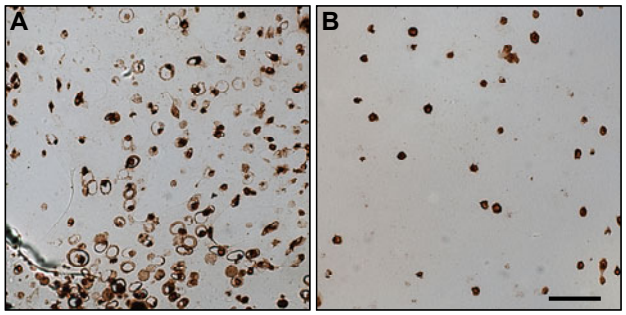

Figure 2: CSPG Staining. (A) 2.5% MA-LVALG and (B) 2.5% LVALG. Scale bar = 50µm.

Conclusions: This is the first study to demonstrate that NP cells can be successfully encapsulated in photocrosslinked alginate hydrogels. Furthermore, viability and proteoglycan protein expression was elevated in MA-LVALG hydrogels compared to LVALG hydrogels and was not affected by the w/v% of the hydrogel. These findings support the use of photocrosslinked alginate hydrogels for cellular encapsulation. Future studies will investigate the long-term viability and protein production of IVD cells encapsulated in photocrosslinked alginate hydrogels as well as the mechanical integrity of these constructs.

References: [1] Maldonado BA. J Orthop Res. 1992;10:677-90 [2] Baer AE. J Orthop Res. 2001;19:2-10 [3] Smeds K. J Biomed Mater Res. 2001;54:115-21 [4] Burdick J. Biomacromolecules. 2005;6:386-91 [5] Chou AI. Spine 2006;31:1875-81