Hydrogels that Mimic Developmentally Relevant N-Cadherin Interactions Enhance MSC Chondrogenesis

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Statement of Purpose: Methacrylated hyaluronic acid (MeHA) hydrogels provide a favorable environment that supports the chondrogenesis of MSCs. Many studies have attempted to improve the biological functionality of biomaterial scaffolds by tethering ECM molecules or other bioactive groups [1, 2]. However, little attention has focused on mimicking early cell-cell interactions in tissue engineering scaffolds for stem cell-based cartilage regeneration. N-cadherin is widely considered to be the key factor in directing these cell-cell interactions during mesenchymal condensation [3]. The evolutionarily conserved His-Ala-Val (HAV) motif in the first extracellular domain (ECD1) of N-cadherin is critical to the homotypic protein interaction that mediates the cell to cell adhesion [4]. The aim of this study is to emulate cell to cell surface interactions that are critical to early condensation events by conjugating N-cadherin mimetic peptides onto HA hydrogels.

Methods: Methacrylated HA (MeHA) was synthesized as previously reported[5]. Scrambled peptide (Ac-AGVGDHIGC) and N-Cadherin mimic peptide (Ac-HAVDIGGGC) were obtained from GenScript Inc and the cysteine residue at the C-terminal end permitted reaction to a fraction (10%) of methacrylates via Michael-type addition. Passage 3 human MSCs were photoencapsulated in 1.5 wt% MeHA hydrogel disks (20 million cells/ml) and cultured in chondrogenic media supplemented with TGF-β3 (10 ng/ml) for 28 days (Figure 1). Young's moduli (E_Y) of samples were calculated from static unconfined compression testing. GAG and collagen content was determined via DMMB and hydroxyproline assay, respectively. Real-time PCR was performed using Taqman primers and probes specific for GAPDH (housekeeping gene) and other genes of interest. Samples for histological analysis were embedded in paraffin. Statistical comparisons were performed via ANOVA with Tukey's HSD post hoc with α=0.05.

Results: The coupled N-cadherin peptides significantly increased the expression of chondrogenic markers including type II collagen, aggrecan and Sox 9 on day 1 and 3 of the culture compared to the Scrambled (scramble peptide sequence) and Control (no peptide) groups (Figure 2). No significant differences were observed between the Scrambled and Control groups. This enhanced chondrogenesis was abolished via treatment with N-cadherin specific antibodies, confirming the contribution of these N-cadherin peptides to chondrogenesis (Figure 2). By day 7, the chondrogenic effect of N-cadherin peptides had diminished (Figure 2). After 28 days of culture, hMSC-seeded constructs modified with N-cadherin peptides (Cadherin) possessed higher glycosaminoglycan (GAG) (52% increase), collagen content (83% increase) and mechanical stiffness (137% increase) compared to Scrambled and Control groups. Alcian blue and picrosirius red staining revealed more intense and distributed staining in the pericellular and extracellular space, suggesting superior cartilage matrix elaboration in the Cadherin constructs compared to controls (Figure 3).

Conclusions: The findings from this study indicate that tethering of N-cadherin mimetic peptides enhances early expression of chondrogenic markers and promotes long term cartilage formation. It also demonstrates the promising potential of biofunctionalizing biomaterial scaffolds to emulate the complexity of the natural cell microenvironment during embryogenesis in stem cell-based cartilage regeneration.

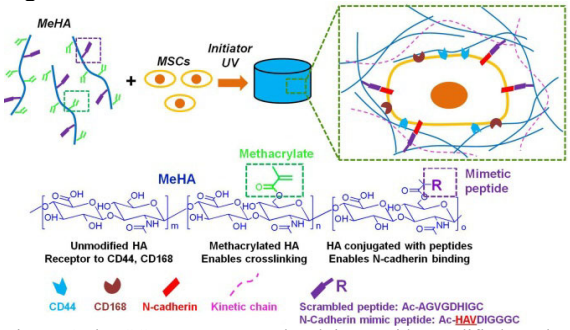

Figure 1. hMSCs were encapsulated in peptide modified methacrylated hyaluronic acid (MeHA) hydrogels prior to in vitro culture.

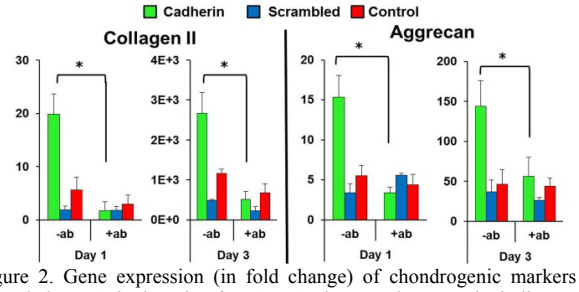

Figure 2. Gene expression (in fold change) of chondrogenic markers in MSC-laden HA hydrogels after 1 or 3 days. '–ab' or '+ab' indicates no treatment or treatment with N-cadherin antibody GC-4 prior to encapsulation. *p<0.05 vs. +ab group of the same scaffold at the same culture time (n=4).

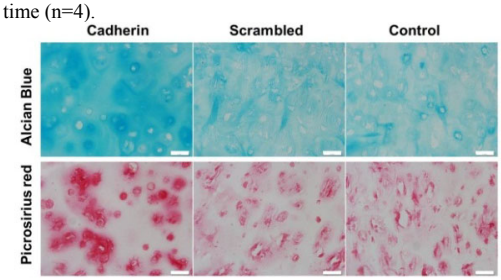

Figure 3. Alcian blue staining (proteoglycans) and picrosirius red staining (collagen) of constructs after 28 days of in vitro culture. scale bar = 50μm.

References: [1]Varghese S, et al. Matrix Biol 2008 Jan;27(1):12-21.[2] Seliktar D, et al. J Biomed Mater Res A 2004 Mar 15;68(4):704-716.[3] Oberlender SA, et al. Cell Adhes Commun 1994 Dec;2(6):521-537.[4] Blaschuk OW, et al. Dev Biol 1990 May;139(1):227-229.[5] Chung C, et al. J Biomed Mater Res A 2006 Jun 1;77(3):518-525.