Disruption of Cell-Cell Contact-mediated Notch Signaling via Hydrogel Encapsulation Reduces Mesenchymal Stem Cell Chondrogenic Potential

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Statement of Purpose: Cell-cell contacts and subsequent Notch signaling are essential for initiation of mesenchymal stem cell (MSC) chondrogenesis. However, subsequent deactivation of Notch is also required to support commitment to the chondrogenic lineage. In development, Notch-mediated chondrogenesis is initiated by MSC condensation and the formation of cell-cell contacts. Recent literature demonstrated that Notch influences Wnt/β-catenin, critical for MSC differentiation, through perturbations in cell-cell contacts[1]. Chondrogenesis is typically studied in vitro through emulation of abundant cell-cell contacts in pellet cultures; however, cells are often delivered within biomaterials-based scaffolds, such as hydrogels, to improve cell localization and therapeutic efficacy in vivo. To explore differences in MSC chondrogenic potential based on these two different culture platforms, this work explored hydrogel-encapsulated and pellet-cultured MSC Notch and Wnt/β-catenin signaling and chondrogenesis in vitro.

Methods: Human MSCs (Lonza) as well as mouse embryonic fibroblasts (C3H10T1/2, ATCC, surrogates for MSCs in signaling work due to superior transfectability[2]) were utilized. Pellets were prepared by centrifuging cells at 1000 RPM for 5 minutes, and hydrogels were prepared by photopolymerizing a mixture of cells, 10 wt% poly(ethylene glycol) dimethacrylate (MW 10kDa), and photoinitiator lithium acylphosphinate (LAP, 0.05wt%) in phosphate-buffered saline ($2.5x10^5$ cells/pellet or hydrogel). Cells were cultured with chondrogenic supplements[3] where indicated. Cell-cell contacts were visualized via immunostaining of N-Cadherin with the Vectastain ABC Kit (Vector Labs) and the LIVE/DEAD kit (Life Technologies). Notch and Wnt/β-catenin activity were quantified with RBPJ-κ and TOP/FOPFlash plasmids (Addgene), respectively, and the Luciferase Kit (Promega), normalized to DNA (Invitrogen). Sox9 gene expression was evaluated through qRT-PCR using the E.Z.N.A. Total RNA Kit (Bio-Tek), iScript™ and SybrGreen (Bio-Rad).

Results: The effect of cell-cell contacts on chondrogenesis was compared between pellets and hydrogels. Compared to pellets cultured in chondrogenic media, hydrogel-encapsulated MSCs exhibited significant 5.8- and 2.9-fold reductions in Sox9 gene expression at days 14 and 21, showing that even with growth factors, hydrogel encapsulation inhibits chondrogenesis. Next, LIVE/DEAD stained cells in pellets and hydrogels were imaged via confocal microscopy, confirming normal morphology and >90% viability. Samples were stained for N-Cadherin; cell peripheries of hydrogels (Fig. 1A) stained less intensely than pellets (Fig. 1B), indicating a relative abundance of cell-cell interactions in pellets. The discrepancy in cell-cell interactions was also illustrated in cell signaling. Hydrogel encapsulation, compared to pellets, resulted in a 7.5-fold decrease in Notch. As previously described, inactive Notch inhibits Wnt through post-translational degradation of β-catenin[1]; consistently, we show a significant 95.1-fold reduction in Wnt signaling in hydrogels treated with a Wnt agonist, 6-bromoindirubin-3'-oxime (BIO), compared to pellets. The use of N-[N-(3,5-Difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT) as a Notch inhibitor in pellets led to similar effects on Notch (4.9-fold reduction) and Wnt with BIO treatment (3.0-fold reduction), confirming the importance of Notch and Wnt signaling in chondrogenesis. Finally, these effects were examined with gene expression profiles (Fig. 1C). Pellets treated with DAPT to mimic temporally relevant inactivation of Notch[4] underwent significant 1.7- and 1.4-fold increases in Sox9 at days 14 and 21 compared to untreated pellets. Moreover, pellets treated with DAPT in the absence of chondrogenic supplements underwent significant 3.7- and 3.4-fold increases in Sox9 at days 14 and 21 compared to hydrogels cultured in chondrogenic media. In contrast, BIO-treated pellets underwent a significant 2.7- and 8.4-fold decrease in Sox9, while BIO- and DAPT-treated pellets underwent significant 2.4- and 9.4-fold reductions compared to pellets, possibly due to Wnt-activated expression of Hoxa11- and Hoxd11, which suppress cartilage development[5].

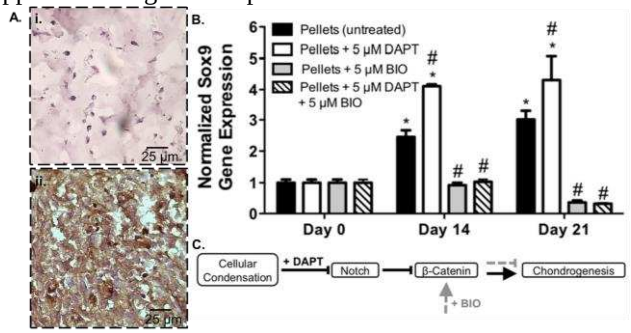

Figure 1. Hydrogels (Ai) and pellets (Aii) were immunostained for N-Cadherin (brown) (bar = 25 μm). MSC pellet Sox9 expression is enhanced with DAPT but reduced with BIO (n=3, avg±SEM, *p<0.05 vs. day 0, #p<0.05 vs. untreated pellets) (B). Cell-cell contacts and transient Notch signaling are necessary for MSC chondrogenesis (C).

Conclusions: Herein we showed that hydrogel encapsulation and failure to form cell-cell interactions and activate Notch signaling significantly reduces MSC chondrogenesis (Fig. 1D). With an improved understanding of cell-cell interactions and cell function, more effective biomaterials-based tissue regenerative strategies can be realized through synergistic application of biophysical and biochemical signals that emulate both the cell microenvironment and cell-cell interactions.

References: 1. Kwon C. Nat Cell Biol. 2011;13:1244-51. 2. Zhao L. Calcif Tissue Int. 2009;84:56-64. 3. Mackay AM. Tissue Eng. 1998;4:415-28. 4. Hardingham TE. J Anat. 2011;13:1244-51. 5. Kuss P. J Clin Invest. 2009;119:146.