RGD Functionalized Polyethylene Glycol Hydrogels Support Proliferation and *In Vitro* Chondrogenesis of Human Periosteum Derived Cells

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Statement of Purpose: The combination of progenitor cells and appropriate scaffolds is a promising area of research in bone and cartilage tissue engineering. For instance, human mesenchymal stem cells (hMSCs), when encapsulated within hydrogels composed of the necessary cues, have demonstrated the potential to differentiate into bone or cartilage.[1,2] Additionally, synthetic hydrogels modified with extracellular matrix (ECM) binding motifs have been shown to stimulate MSCs along the chondrogenic pathway.[3] Further, preconditioning of MSCs on scaffolds in chondrogenic medium has been shown to result in higher chondrogenic gene expression after 28 days *in vitro* and to lead to subsequent *in vivo* bone formation.[4] Here, we utilize human periosteum-derived cells (hPDCs), a progenitor cell population with MSC characteristics[5], paired with protease degradable, functionalized polyethylene glycol (PEG) hydrogels[6] to create tissue-engineered constructs. The objective of this study was to investigate the effects of scaffold composition, specifically exploring the addition of the cell binding motif, Arginine-Glycine-Aspartic Acid (RGD), in combination with various *in vitro* culture conditions, on cell proliferation, chondrogenic differentiation and matrix production of encapsulated hPDCs.

Methods: The hPDCs were encapsulated in degradable 6.5% (w/v) PEG hydrogels crosslinked via a Michael-type addition reaction[6] at cell densities between 1 x 10^6 cells/mL and 10 x 10^6 cells/mL. The encapsulated hPDCs were cultured in growth medium supplemented with FBS to evaluate their proliferation and viability in the hydrogel system and in two different chondrogenic differentiation medium compositions supplemented with either TGF-β1 or BMP-2 to test for *in vitro* chondrogenesis. After culturing for 0 weeks, 1 week and 4 weeks, Live/Dead staining and DNA quantification were performed on the cell-hydrogel constructs to measure cell viability and proliferation respectively. A dimethylmethylene blue (DMMB) assay for glycosaminoglycan (GAG) content and gene expression analysis of *Col IIA*, *Sox-9* and *Aggrecan* were used to quantify chondrogenic differentiation of the hPDCs in the PEG hydrogels. Additionally, histology sections were stained with Safranin-O and Picrosirious Red to observe GAG and collagen content in the newly deposited ECM.

Results: When encapsulated as single cells in functionalized PEG hydrogels, hPDCs maintained a high level of viability, as seen via the Live/Dead staining. The cells encapsulated in the hydrogels with the RGD binding motif displayed a viability of over 90% after 4 weeks, compared to ~30% viability displayed by the cells in the hydrogels without RGD. Similarly, hPDCs in hydrogels that contained RGD exhibited a 5-fold increase in DNA content from 1 week to 4 weeks of culture in growth medium ($p<0.0001$). In contrast, after 4 weeks, the hPDCs encapsulated in hydrogels without RGD displayed a drop in DNA content compared to 1 week of *in vitro* culture in growth medium. Cells encapsulated in the hydrogels and cultured in growth medium did not produce much ECM; however, the DMMB GAG assay demonstrated that the constructs cultured in chondrogenic medium had an increase in GAG production after 4 weeks. In particular, the hPDCs cultured in the hydrogel formulation containing RGD showed a significantly higher amount of GAG accumulation after 4 weeks than the hPDCs encapsulated in the hydrogels without RGD. These results were qualitatively confirmed via histology where the RGD containing samples displayed a higher amount of positive staining by Safranin-O and Picrosirius Red. Quantitative analysis of chondrogenic gene expression also indicated a similar pattern. All the cell-hydrogel constructs cultured in chondrogenic media showed an increase in the expression levels of specific chondrogenic markers from 0 to 4 weeks. In particular, the cells in the constructs functionalized with RGD revealed a higher gene expression profile for chondrogenic markers compared to the hPDCs in constructs without RGD. When looking at the different medium formulations, the hPDCs encapsulated in RGD-functionalized hydrogels showed an increase between 0 and 4 weeks of 100X for *Col IIA*, 2.5X for *Sox-9* and 9X for *Aggrecan* when cultured in the medium supplemented with BMP-2 compared to 15X for *Col IIA*, 3X for *Sox-9* and 3X for *Aggrecan* when supplemented with TGF-β1.

Conclusions: These results demonstrate the potential of RGD-functionalized, enzymatically degradable PEG hydrogels as a 3D culture system for hPDCs to support proliferation and chondrogenic differentiation.The viability and proliferation assays demonstrated that the hydrogels containing the cell adhesive ligand RGD consistently outperformed unfunctionalized hydrogels in terms of the cells' viability and proliferative capabilities within the hydrogels. Furthermore, when cultured in chondrogenic medium, the hPDCs encapsulated in RGD functionalized hydrogels displayed enhanced chondrogenic differentiation when compared to the cells in the hydrogels without RGD. This effect was further augmented when the RGD containing, hPDC-hydrogel system was cultured in the chondrogenic medium supplemented with BMP-2. Overall, these results demonstrate the additive influence that the scaffold and medium composition have on the *in vitro* chondrogenic differentiation of hPDCs.

References:

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