Creation of a collagen scaffold integrating mesenchymal stem cells and microencapsulated peptides for use in wound healing.

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Statement of Purpose: Current stem cell-based therapies often involve engraftment of dispersed cells into injured tissues with little regard for the status of cells to competently mediate a tissue repair. This practice may be a factor in the inconsistent and disappointing outcomes when stem cells have been tested in clinical studies. Epithelial-mesenchymal transition (EMT) occurs as a prelude to all differentiations undertaken by stem or progenitor cells in the embryo. Taking a lesson from embryonic development, we reasoned that priming stem cells for an EMT prior to engraftment into wounds may promote regenerative healing. To test this novel hypothesis, adult rat bone marrow stromal cells (BMSC's) were seeded onto the top of collagen gels and induced to undergo an EMT in vitro ("Primed"). When gels of EMT-primed BMSCs were sutured into excisional skin wounds (1cm diameter) on adult rats, a significant reduction in scar tissue differentiation ($p < 0.05$) and improved regenerative healing was noted compared to controls. When primed BMSCs were combined with a Cx43 mimetic peptide, αCT1, enhanced healing occurred. Here the αCT1 peptide has been microencapsulated in alginate for controlled delivery. Primed mesenchymal stem cells were added to fabricated 2.5% 0.5mm collagen scaffolds mixed with controlled release microencapsulated αCT1 for use in the wound healing model. The created scaffold has the capability of delivering primed stem cells and prolonged release of peptide into the wound promoting healing. This combination of EMT priming and prolonged peptide release may provide benefits in other wounded tissues, including following myocardial infarction and spinal cord injury.

Methods: Electrospray microencapsulation techniques were used to encapsulate both the αCT1 peptide and a nonsense sequence peptide in alginate. FITC tagging of the peptide was used to characterize the release profile and interaction with cells. Immunohistochemistry and microscopic analysis were used to show priming of rat mesenchymal stem cells on the collagen matrix for induction of EMT.

Results: In multiple studies of the 2.5% collagen physical manipulation occurs when BMSC's are primed in quantities of 75,000 and 125,000 cells per scaffold. Confocal imaging of these samples shows strong occurrences of the EMT markers β Catenin and IST-9 fibronectin. Figure 1 shows an image of these EMT markers.

Microencapsulation of both the αCT1 and nonsense control peptide show stability in vitro at conditions mimicking in vivo temperature and pH. When tested on rabbit lens epithelial cells both encapsulated and unencapsulated αCT1 is shown to have uptake <24 after exposure. The release profile shows our microcapsules containing peptide release in a range of 24-72 hours with degredation at approximately 96 hours when using normal saline solvent.

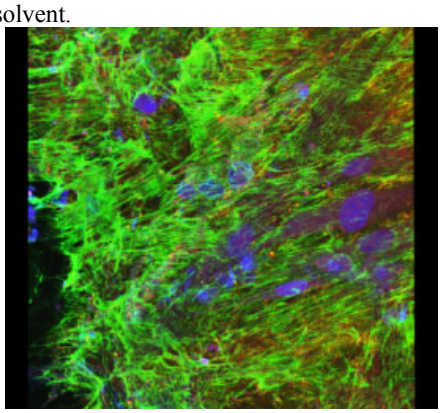

Figure1: Confocal image of 75k seeded collagen. Staining with EMT markers is as follows: Blue= DAPI, Red= β Catenin, Green= IST-9 fibronectin.

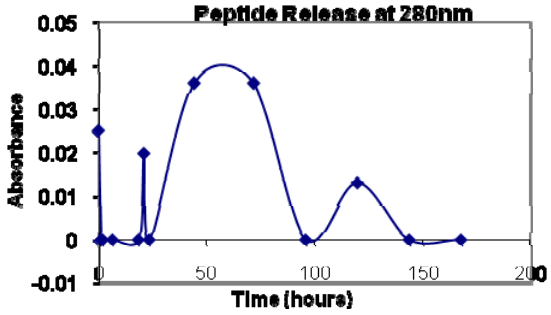

Figure 2: Release profile microcapsules containing αCT1 were placed in 0.9% saline with 37°C incubation for 7 days. Time points were taken after centrifuging at 800 rpm each time. Absorbance readings were taken of the supernatant.

Conclusions: Previous work shows both epithelial cells and BMSCs primed on liquid collagen gels undergo EMT, physically manipulating gels. Here a 2.5% solid matrix is shown to undergo a similar process, indicating BMSCs can be "primed" to interact with each other in a controlled pattern. Microencapsulated αCT1 releases within 24 hours and sustains release for ~72 hours making this ideal for use in wound healing studies where the inflammatory and granulation phases of tissue healing are occurring in this time frame.

References: Ghatnekar, G., M. O'Quinn, et al. (2009). "Connexin43 carboxyl-terminal peptides reduce scar progenitor and promote regenerative healing following skin wounding." Regenerative Medicine **4**(2): 205-223.
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