Growth Factor Release from poly(ethylene glycol) diacrylate Hydrogels as a Treatment for Chronic Wound Healing

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Statement of Purpose: Non-healing, chronic wounds affect 5-7 million people in the United States annually. [1] Because these wounds remain open, there is a high risk of infection, which can lead to amputation in severe cases. Cell therapies have the potential to significantly improve outcomes in these patients by promoting wound healing mechanisms. Mesenchymal stem cells (MSCs) are particularly desirable because they produce factors that reduce inflammation and promote wound healing. The use of MSCs for the treatment of chronic, surgical, and diabetic wounds has demonstrated accelerated healing in clinical trials.[1] A challenge to MSC delivery is that they are subject to immune attack and/or tend to migrate from the site of action. Encapsulating MSCs within hydrogels circumvents these challenges by protecting cells from host immune responders and localizing them to the site of application. Encapsulation within micro-scale hydrogels permits ready waste/nutrient exchange and therapeutic product release. Poly(ethylene glycol) diacrylate (PEGDA) is a synthetic polymer with tunable biomechanical properties that resists protein adsorption and cell adhesion. Fibroblast growth factor 7 (FGF-7) is secreted by MSCs and plays a role in the growth and migration of epithelial cells during wound healing. The objective of this study is to compare viability and growth factor release of MSCs encapsulated in 10 and 20 kDa molecular weight PEGDA microspheres and thin hydrogel sheets.

Methods: Human MSCs (hMSCs) were expanded, then seeded in 6-well plates or encapsulated in 10 or 20 kDa PEGDA microspheres or sheets. Microspheres were prepared by photopolymerizing a vortex-induced water-in-oil emulsion containing PEGDA prepolymer and hMSCs in the aqueous component.[2] Sheets were formed by combining hMSCs and PEGDA prepolymer, then exposing to white light to photopolymerize the solution in a 5mm x 5mm x 0.4mm mold. Viability of cells encapsulated in hydrogel microspheres and sheets was evaluated using a calcein AM/ethidium homodimer assay. Growth factor release from non-encapsulated hMSCs or hMSCs encapsulated in microspheres and hydrogels were determined with ELISA.

Results: Viability of hMSCs encapsulated in 10 and 20 kDa microspheres and sheets exceeded 60% on Day 2 post-encapsulation (Figure 1). Though not statistically significant, cell viability in sheets was approximately 10% higher than in microspheres. Similarly, there was no statistical difference in cell viability between different molecular weight PEGDA microspheres, but 10 kDa microspheres and sheets also showed a 10% higher trend in viability than the 20 kDa group. Concentrations of FGF-7 released from hMSCs encapsulated in microspheres was slightly higher than that detected from non-encapsulated cells in monolayer (Figure 2). Concentrations of FGF-7 released from cells encapsulated in sheets were significantly lower than that released from cells encapsulated in microspheres. There was no significant difference in FGF-7 release detected from 10 and 20 kDa hydrogels, whether sheets or microspheres.

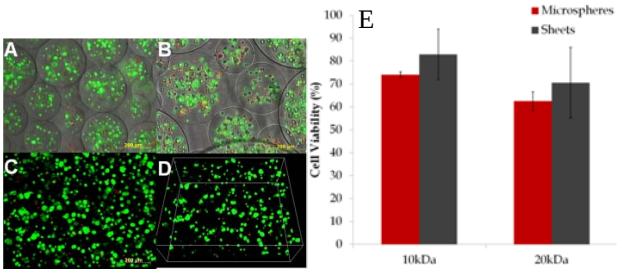

Figure 1. Live/Dead calcein AM/ethidium homodimer 1 stain of hMSCs encapsulated in hydrogel **A,B.** microspheres and **C,D.** sheets (green = live; red = dead). **E.** Quantified hMSC viability when encapsulated in different molecular weight PEGDA (10 or 20 kDa) microspheres and sheets.

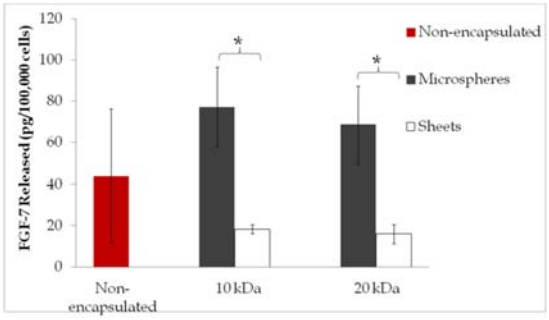

Figure 2. FGF-7 Released from hMSCs in monolayer (Non-encapsulated) and hMSCs encapsulated in PEGDA (10 or 20 kDa) hydrogel microspheres or sheets.

Conclusions: There was no significant difference in cell viability between sheet and microsphere hydrogels; however, the release of FGF-7 from hMSCs encapsulated in sheets was significantly lower. The greater surface area of the microspheres may allow for factors to more easily diffuse from the encapsulated cells to the surrounding media. Alternatively, the relatively violent process of encapsulating cells within microspheres may have stimulated the hMSCs into releasing more FGF-7 than hMSCs encapsulated under the gentle conditions required to form hydrogel sheets. Future work will include microencapsulations for cells that will ultimately be encapsulated in hydrogel sheets. In addition, assays will be performed to detect other crucial growth factors in wound healing, such as vascular endothelial growth factor and epidermal growth factor.

References:

1. Maxson S, et al. Stem Cells Trans Med, 2012; 1:142-149.
2. Olabisi, RM, et al. Tissue Eng Pt A, 2010; 16:3727-3736.