A Novel Dual-Cell Therapy for Chronic Wounds

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Statement of Purpose: It is recognized that mesenchymal stem cells (MSCs) accelerate healing of chronic wounds, yet MSC therapies are still a translational research focus rather than a clinical reality. Insulin ointments also accelerate wound healing. We investigated a potential synergistic effect of these two differing modes of wound healing. Since topical insulin creams preclude using MSCs, we combined insulin secreting cells (ISCs) and MSCs in a dual-cell therapy approach for wound healing. Our system provides prolonged release of insulin and soluble MSC factors without the need for reapplication. Insulin accelerates wound healing recruiting keratinocytes, endothelial cells and fibroblasts via the PI3-Akt pathway[1], while MSCs improve ISC viability and function[2], regulate inflammation and secrete pro-wound healing factors[3]. Our previous data has shown that ISCs encapsulated within polyethylene glycol diacrylate (PEGDA) hydrogels improved wound healing 1.6 times faster than controls in a diabetic mouse model of chronic wounds.[4] Herein, we demonstrate that ISC and MSC coencapsulation further accelerates wound closure 2.5 times faster.

Methods: RIN-m cells (ISCs) and/or human MSCs were expanded then encapsulated within PEGDA hydrogel sheets by photopolymerizing cells suspended in a precursor solution formed by combining 0.1 g/mL 10 kDa PEGDA (10% w/v; Laysan Bio) with (1.5% v/v) triethanolamine/ HEPES buffered saline, 37mM 1-vinyl-2-pyrrolidinone, 0.1mM eosin-Y, then pipetted into 1 cm^2 custom made molds and exposed to white light for 20 seconds. Encapsulated cell viability was assessed via a calcein AM/ethidium homodimer1 viability kit. Conditioned-media (CM) from hydrogels was collected on days 1, 7 and 21. Concentrations of insulin and MSC factors were measured by ELISA. *In vitro:* Akt phosphorylation and HaCaT migration across monolayer scratch wounds following stimulation with CM were assessed via ELISA and image analysis, respectively. *In vivo:* Cell-laden and empty microsphere (control) hydrogels were applied to 1 cm^2 full-thickness excisional wounds created on the dorsa of genetically diabetic mice. Gross examination was conducted until post-operative day (POD) 28 and wound tissue collected for histology.

Results: Hydrogel sheet thickness was optimized by evaluating ISC viability encapsulated at low (LCD), intermediate (ICD) and high (HCD) cell densities in thin, medium, and thick sheets. Medium thickness hydrogels provided the best average viability at all cell densities (LCD: 82.6 ± 1.3 %, ICD: 85.3 ± 5.2 %, HCD: 81.5 ± 5.9 % on day 1) for at least 21 days and was selected for all subsequent experiments. MSCs improved insulin release from ISCs and secretion levels were 7.8 ± 0.84, 4.7 ± 0.13, 1.3 ± 0.08, 12.9 ± 1.6 ng/mL/10^6cells in LCD, ICD, HCD, dual-cell, ratio 1 (I+M1) sheets, respectively. All single-cell (ISC or MSC) and dual-cell (I+M) hydrogels ranging from high ISC to high MSC ratios maintained bioactivity and factor release until at least 21 days. All low and intermediate ratios of ISC and MSC combinations provided faster scratch closure and increased factor release compared to single-cell type hydrogels, with LCD and I+M1 demonstrating the most significant per cell effects. An increase in TGF-β1 secretion from MSCs was seen in the presence of ISCs (I+M1: 305.2 ± 35.06 pg/mL vs MSC: 80.83 ± 51.17 pg/mL). VEGF release was not detected in MSC monolayers, but was in I+M hydrogels (I+M1: 508.72 ± 61.6 pg/mL); MSCs are known to secrete VEGF to promote islet vascularization. Scratch closures stimulated by CM collected on day 1 from I+M1, ISC and MSC hydrogels were 128.6 ± 10.53, 75.1 ± 6.61, 79.02 ± 8.9 µm/hr/10^6 cells, respectively. Bioactivity of insulin and MSC factors from CM collected on day 1 in stimulating Akt phosphorylation were I+M1: 321.4 ± 23.4, ISC: 243.5 ± 28.8, MSC: 255.1 ± 20.6 % p-Akt/total Akt/10^6 cells. ISC, MSC, and I+M1 hydrogels were applied to excisional wounds in diabetic mice. Animals treated with I+M1 hydrogels healed primarily through reepithelialization without intermediate scab or scar formation as early as postoperative day (POD) 14 compared to ISC and MSC wounds, which healed by contraction and scab formation by POD 21 and controls, which were still open by POD 28. Percent wound closure on POD 18 were 62.9 ± 16.2 %, 65.6 ± 15.2 %, 100 ± 0 % in ISC, MSC and I+M1 treated animals, respectively.

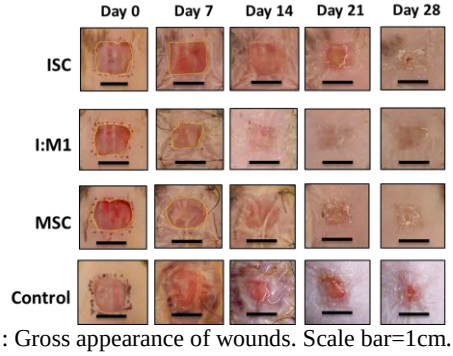

Fig. 1: Gross appearance of wounds. Scale bar=1cm. Scab formation on POD 21 in ISC and MSC, while I:M1 treated wounds have reepithelialized.

Conclusions: Our dual-cell hydrogel dressing has accelerated slow-healing diabetic wounds to heal at a rate faster than normal wounds. Should our treatment move from the benchtop to the clinic, it may transform the treatment of chronic wounds, reducing both costs and duration of treatment. To date, ours is the first cell therapy to combine islets or beta cells with MSCs to improve wound healing. Future work will analyze wound tissue histology and the mechanisms of the synergistic effect of the ISCs and MSCs in accelerating wound healing.

References:

1. Y. Liu, BMC Cell Biol., 10 (2009)
2. Lee EY. Wound Repair Regen. 2009; 17(4):540-7.
3. Kerby A. Cytotherapy. 2013; 15(2):192-200.
4. Aijaz A. Tissue Eng A. 2015; 21(21-22): 2723-32.