Microencapsulated Insulin-Producing Cells Accelerate Diabetic Wound Closure

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Statement of Purpose: In the United States chronic wounds affect more than 6 million people, costing $25 billion annually.[1] Chronic wounds are characterized by impaired neovascularization, reduced collagen synthesis, and decreased reepithelialization.[2] Defective insulin signaling has been implicated in impaired wound healing. Insulin stimulates proliferation, secretion, and migration of keratinocytes, endothelial cells and fibroblasts, all essential in wound healing.[3] Topical insulin accelerates wound healing but requires repeated applications.[4] No cell therapy involving insulin has been reported for chronic wounds. Our goal is to use a single application of cells to deliver insulin in a constant or glucose-dependent manner.

Methods: Glucose-responsive insulin secreting GLUT-2 cDNA transfected AtT-20ins-(CGT6) or constant insulin-producing rat islet tumor RIN-m cells were expanded then microencapsulated into polyethylene glycol diacrylate (PEGDA) hydrogels by photopolymerizing a vortex-induced emulsion of cells (1.5×10^4 cells/μL) suspended in a previously described PEGDA precursor solution[5] under white light. Encapsulated cell viability was assessed for 21 days using a calcein AM/ethidium homodimer1 viability kit. Insulin secretion was assessed by ELISA following static stimulation at incremental glucose concentrations. A scratch was made in a confluent layer of HaCaT cells and stimulated with insulin-containing conditioned media from (i) microencapsulated cells at days 1, 7 and 21; (ii) monolayer; or (iii) media from empty microspheres as control.[6] A 1 cm x 1 cm full thickness excisional wound was created on the dorsum of genetically diabetic male mice in accordance with protocols approved by the Rutgers University Institutional Animal Care and Use Committee. Microencapsulated cells or empty microspheres were applied to wounds, and animals were sacrificed on postoperative day (POD) 35 and wound tissue was collected for histological analysis.

Results: Microencapsulated cell viability for RIN-m and AtT-20ins on Day 1 was 66.55 ± 8.76 % and 70.6 ± 8.23 % respectively. Both free and microencapsulated RIN-m cells maintained constant insulin secretion at 0.96 ± 0.06 ng/ml and 0.70 ± 0.33 ng/ml insulin, respectively. Significant increases (3.9 and 3.6 fold greater than control, monolayer and encapsulated, respectively) in insulin release were observed for AtT-20ins cells at incremental glucose concentrations with maximum release at 5.56 mM glucose. Insulin from day 1 RIN-m microspheres caused significant HaCaT cell migration by 24 hours with full closure by 48 hours compared to control (Fig. 1). By 48 hours, significant cell migration was observed for all insulin-conditioned media samples. Microencapsulated RIN-m cells demonstrated significant wound closure at POD 14 (Fig. 2B). At POD 21, wound closures for RIN-m, AtT-20ins and control were 84.3 ± 13.6%, 57.4 ± 36.2% and 73 ± 7% respectively.

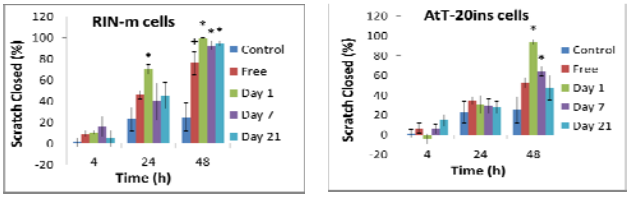

Fig. 1: Keratinocyte scratch assay of cell migration and insulin bioactivity. *, +: Statistical significance between microencapsulated cells vs. control, free cells vs. control, respectively; $p<0.05$. Error bars: standard error of mean.

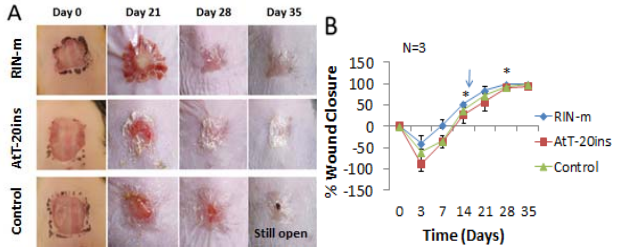

Fig. 2: **A** Gross appearance, **B** % wound closure. Arrow shows microsphere removal on POD 16. *: Statistical significance between microencapsulated cells vs. control; $p<0.05$. Error bars show standard error of mean (SEM).

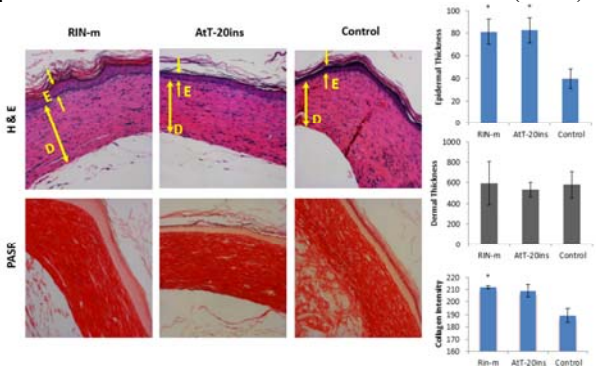

Fig. 3: H&E and PASR stains. Epidermal thickness and collagen fiber density were significantly greater in treated vs control animals. * = significance, error bars = SEM.

Conclusions: Cell microencapsulation within PEGDA hydrogel does not impede cell viability, insulin secretion, or insulin bioactivity, which remained bioactive for at least 21 days as demonstrated by scratch assays. Microencapsulated RIN-m cells demonstrated significant acceleration in wound closure. Future studies will explore dose response and alternate encapsulation geometries. Insulin mediated stimulation of AKT phosphorylation will also be assessed. The wound healing response at earlier time points will be evaluated by immunostaining histological sections for Ki67 and TNF-α.

References:

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