Validating Hydrogel Microencapsulated Insulin Secreting Cells for Wound Healing

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Introduction:

Previous work demonstrated an accelerated *in vitro* and *in vivo* wound healing response when insulinoma cells were used to deliver insulin to scratches in keratinocyte monolayers and chronic diabetic excise wounds in mice, respectively.[1] When combined with mesenchymal stem cells (MSCs), the response was amplified (healing in 14 vs. 35+ days).[2] To isolate the role of insulin in the response and exclude any potential contribution of an unknown cancer moiety released by the insulinoma cells, the effect on wound healing of multiple lines derived from the same insulinoma was examined.

Methods:

Rat insulinoma (ATCC) RIN-5F, which secretes only insulin and RIN-14B, which secretes only somatostatin were propagated in RPMI-1640 medium (ATCC) supplemented with 10% fetal bovine serum (FBS) and 1% w/v penicillin-streptomycin (pen-strep) in a tissue culture incubator. Cells were encapsulated into polyethylene glycol diacrylate (PEGDA) hydrogels sheets by photopolymerizing cells suspended in a precursor solution formed by combining 0.1 g/mL 10 kDa PEGDA (10% w/v; Laysan Bio, Inc.) with (1.5% v/v) triethanolamine/HEPES buffered saline (pH 7.4), 37mM 1-vinyl-2-pyrrolidinone, 0.1 mM eosin Y. The prepolymer solution was combined with cells (1×10^4 cells/µL), pipetted into 1 cm^2 custom made molds and exposed to white light for 20 seconds to achieve 400 µm thick cell-laden hydrogel sheets containing 2 x 10^6 cells per 400 µm thick hydrogel sheets. Hydrogel sheets were maintained in a 37°C / 5% CO_2 humidified incubator in complete culture media. Conditioned media (CM) containing insulin and/or MSC factors released from the cell-laden hydrogel sheets were collected on days 1, 7 and 21. Scratch assays were conducted with human keratinocytes (HaCaT; Addex Bio, San Diego, CA) as previously described.1 Briefly, HaCaT cells were propagated in DMEM, supplemented with 10% FBS, pen-strep in tissue culture incubators. For scratch assays, 180,000 HaCaT cells/well were seeded onto 24 well plates (Greiner Bio-one; Monroe, NC). Cells were cultured for 48 hours to form a confluent monolayer at which time a single scratch of 415 µm ± 73 µm was produced in the center of the well with a 10 µL pipette tip. The wells were washed twice with Dulbecco's Phosphate Buffered Saline (DPBS) to remove cell debris. HaCaT cells were then stimulated with conditioned media containing insulin. CM was sampled from positive control monolayers on day 1 or cell-laden hydrogels on days 1, 7 and 21. Media from empty hydrogels was used as negative controls. Insulin levels were assessed with ELISA. HaCaT cell migration across the scratch was imaged at 0, 24, 48, 72 and 96 hours using phase contrast microscopy and analyzed on NIH ImageJ software.

Results:

For matched concentrations (high = $2\text{x}10^6$ cells per hydrogel sheet), ELISA results demonstrated decreased insulin release from RIN-14B cells than from the original RIN-m cells at Day 1, greater insulin at Day 7, and equivalent insulin at Day 21 (Figure 1). RIN-5F released the lowest insulin levels at all time points. The original low RIN-m concentration ($0.5\text{x}10^6$ cells per hydrogel sheet) had the highest consistent insulin levels over time. Keratinocytes stimulated with conditioned media from RIN-14B cells migrated faster than those stimulated with RIN-5F cells.

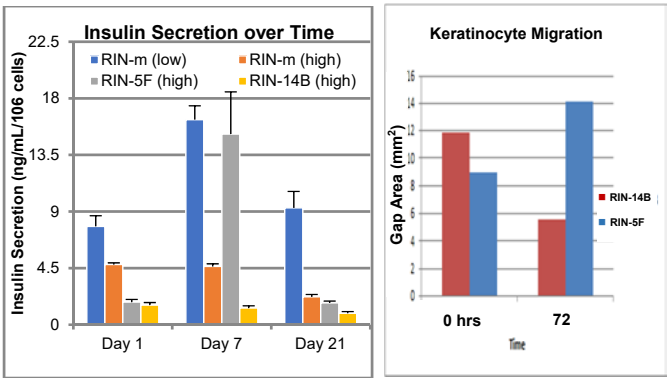

Figure 1. Left: ELISA from original data (RIN-m) compared to RIN-5F and RIN-14B cells. Right: Scratch areas were measured over time to assess keratinocyte migration.

Conclusions:

The decreased insulin levels observed in the RIN-14B cells are consistent with previous data where RIN-m cells encapsulated at higher concentrations produced reduced levels of insulin. These studies will be repeated at low concentrations ($0.5\text{x}10^6$ cells per hydrogel sheet). The accelerated scratch closures observed when keratinocytes were treated with RIN-14B cells, but not with RIN-5F supports our hypothesis that insulin from these cells is responsible for the accelerated response rather than an unknown promotor common to cancer cells.

References:

1. Aijaz A, et al. *Tissue Engineering Part A* 21.21-22 (2015): 2723-732.
2. Aijaz A, et al. "A dual-cell therapy for chronic wounds," *The Society for Biomaterials Annual Meeting*, Minneapolis, MN, Apr 5-8, 2017

Acknowledgements: This work was supported in part by a Rutgers Aresty Undergraduate Research Award and an NSF CAREER Award (CBET-1752079).