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Methods for Encapsulating Cells into Hydrogel Sheets for Wound Healing

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Introduction: Hydrogels are cross-linked polymer networks that are widely used in tissue engineering due to their specific characteristics, such as hydrophilicity, biocompatibility, and tunable mechanical properties that are similar to a variety of soft tissues. Alginate is most commonly used to form cell-encapsulating hydrogels. Such hydrogels shield encapsulating cells from immune attack. Alginate can itself be immunogenic,[1] therefore we encapsulated cells within synthetic polymer hydrogels formed using polyethylene glycol diacrylate (PEGDA). Building on prior research whereby RIN-m insulinoma cells were encapsulated into sheets to deliver insulin for wound healing, we encapsulated additional insulinoma cell lines at 2 cell densities to optimize their secretion profiles from encapsulating hydrogels.

Methods: Rat insulinoma RIN-5F, which secretes only insulin and RIN-14B, which secretes only somatostatin were propagated in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% w/v penicillin-streptomycin (pen-strep) in a tissue culture incubator. Cells were encapsulated into PEGDA hydrogel sheets by photopolymerization of precursor solution. Precursor solution was formed by combining 0.1 g/mL 10 kDa PEGDA (10% w/v) with (1.5% v/v) triethanolamine /HEPES buffered saline, 37mM 1-vinyl-2-pyrrolidinone, 0.1 mM eosin Y. The precursor solution was combined with cells at two different cell densities (1×10^4 or 4×10^4 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 $0.5x10^6$ or $2x10^6$ per 400 µm thick hydrogel sheets (Fig 1).

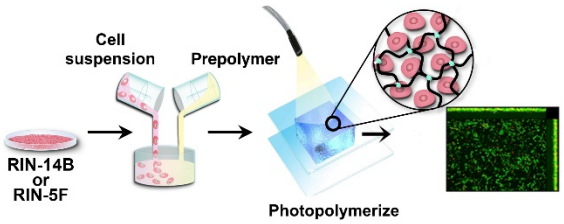

Figure 1. RIN insulinoma cells were expanded, harvested, combined with prepolymer solutions, photo-polymerized in masks, and kept in culture for 21 days.

Hydrogel sheets were maintained in a 37°C / 5% CO2 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. HaCaT cells were propagated in DMEM, supplemented with 10% FBS (v/v) and 1% pen-strep (v/v), seeded onto 24 well plates at $1.8x10^5$ cells/well in tissue culture incubators. Scratch assays were conducted with human keratinocytes as previously described with conditioned media containing insulin.[2] 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: ELISA results showed that the maximum insulin released from RIN-5F hydrogel sheets was 15.2±3.3 ng/mL/10^6 cells at $2x10^6$ cells per sheet, which compared well to RIN-m encapsulated at $0.5x10^6$ cells per sheet, which released 16.3±1.1 ng/mL/10^6 cells. At $2x10^6$ cells per sheet, RIN-m had released only 4.6±0.2 ng/mL/10^6 cells. The maximum insulin released from RIN-14B was 1.6±0.2 ng/mL/10^6 cells. Scratch assays showed scratches closing faster when exposed to conditioned media from RIN-5F cells (Fig 2).

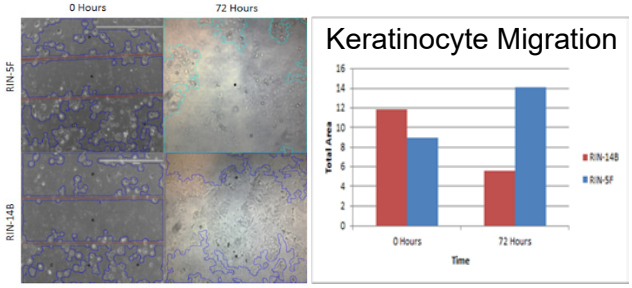

Figure 2. Images: Scratches at 0 and 72 hours for RIN-5F and RIN-14B cells with analyzed area from the ImageJ software. Graph: Keratinocyte migration induced by RIN-14B and RIN-5F cells.

Conclusions: ELISA results demonstrated insulin could exit the hydrogel sheets while scratch assays demonstrated that the insulin exiting was bioactive. The study here assessed cell function rather than viability, as our priority is insulin release. Hydrogel encapsulation did not impede insulin release and function.

References:

1. Ménard M, et al. *JBMR B: Appl Biomat*. 2010 May; 93(2):333-40.
2. Aijaz A, et al. *Tissue Engineering Part A* 21.21-22 (2015): 2723-732.

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