A Novel Method to Encapsulate Insulin-secreting cells

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Statement of Purpose: Microencapsulating donor cells within an immunoprotective hydrogel that permits both nutrient/waste exchange and the release of therapeutic cell products is essential for long-term functional success of cell-based therapies. The microencapsulation of insulin-secreting cells is heavily researched in order to optimize the process for diabetes applications. The bulk of this research uses alginate microspheres, which are themselves immunogenic.[1] In studies using the non-degradable polymer poly(ethylene glycol) diacrylate (PEGDA), microencapsulation via interfacial polymerization maintained cell viability and returned diabetic rats to normoglycemia without eliciting an immune reaction.[2] We describe a bulk method to rapidly microencapsulate within PEGDA hydrogels AtT-20ins cells, which secrete insulin in response to changing glucose levels.

Methods: Insulin-secreting GLUT-2 cDNA transfected AtT-20ins-(CGT6) were grown in high glucose Dulbecco's modified Eagle's medium, supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin in a humidified incubator at 37°C/5% CO_2. Cell-laden microspheres were formed as previously reported.[3] Briefly, hydrogel 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 (pH 7.4), 37 mM 1-vinyl-2-pyrrolidinone, 0.1 mM eosin-Y, and mixed with AtT-20ins cells for a final concentration of 1.5×10^4 cells/µL. A hydrophobic photoinitiator solution containing 2,2-dimethoxy-2-phenyl acetophenone in 1-vinyl-2-pyrrolidinone at 300 mg/mL was combined in mineral oil 3 µL/mL. The cell-prepolymer suspension was added to the mineral oil solution and microspheres were formed under white light by vortex-induced emulsification. Following microencapsulation, an MTS assay was conducted to determine encapsulation efficiency. Cell viability was assessed using LIVE/DEAD® Viability/Cytotoxicity Kit for mammalian cells (Invitrogen), and imaged under epifluorescent microscope. Insulin secretion from microencapsulated cells was compared against free cells at passage 18 by static and dynamic stimulation at basal (1mM) and higher (5.5mM, 16.7mM and 25mM) glucose concentration. Insulin concentration was determined by Ultra-Sensitive Mouse/Rat Insulin ELISA kit (EMD Millipore).

Results: Encapsulation efficiency as determined by the MTS assay was 37.9±14.6%. The cell cytotoxicity assay demonstrated the effect of microencapsulation on cell viability (Fig. 1). On Day 1 cell viability was 80 %, at Day 11 cell viability was 67.5 %. Insulin release at basal glucose concentration (1 mM) was 1.32 ng/ml and 0.494 ng/ml for free and encapsulated cells, respectively. Cells were exposed to increasing concentrations of static glucose levels until they released 2 ng/ml insulin. Free cells required a stimulation of 25 mM glucose while encapsulated cells only needed 5.5 mM glucose stimulation. This corresponded to maximum fold increases of 1.55 and 4.08 in insulin release for free and encapsulated cells, respectively (Fig. 2).

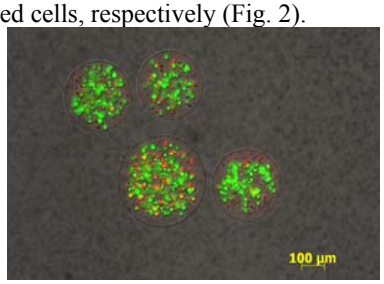

Fig. 1: Viability stain of microencapsulated AtT-20ins cells; (red-dead, green-viable)

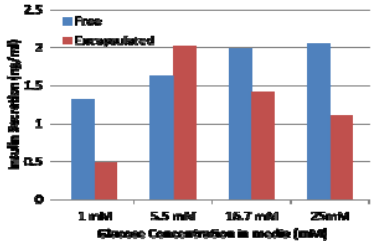

Fig. 2: Insulin secretion under various glucose stimuli

Under dynamic incremental glucose concentration, both free and encapsulated cells show a similar trend with an initial boost in the rate of insulin released over ten minutes (Fig. 3). The highest rate of insulin secretion was observed when cells were stimulated with 16.7 mM glucose for both free and encapsulated cells (0.029 ng/ml/min and 0.045ng/ml/min of insulin, respectively).

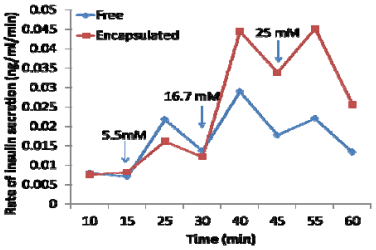

Fig. 3: Rate of insulin secretion under incremental glucose concentration

Conclusions: Initial cytotoxicity and glucose stimulation studies demonstrate that bulk cell microencapsulation within PEGDA does not severely affect cell viability and improves cell function as indicated by diffusional release of insulin through the permeable microspheres. Further studies need to be conducted to assess in vivo performance of microencapsulated cells. Utilizing an insulin-secreting cell line can address the concern of the limited supply of donor beta cells while encapsulation within a bio-inert hydrogel will prevent immune rejection.

References:

[1] Ménard M et al. Biomed Mater Res B Appl Biomater. 2010 93(2):333-340
[2] Cruise GM. Cell Transplant 1999; 8:293-306
[3] Olabisi RM. Tissue Eng. 2010; 16(12): 3727–3736