

## The Role of Poly-L-Ornithine, Poly-L-Lysine and Extracellular Matrix Proteins on the proliferation and Function of Pancreatic Insulin-Producing $\beta$ Cells by Complex Alginate Microencapsulation

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**INTRODUCTION:** The properties of alginate bead surface need to be further modified in order to modulate the proliferation or function of encapsulated cells. In this study, we investigated the role of PLL, PLO and extracellular proteins on the surface coating of alginate beads as a microcapsule of insulin-producing  $\beta$  cells from rat insulinoma (Rin-m5F cells).

**METHODS:** Rin-m5F cells were obtained from ATCC. The alginate/cell mixture was extruded as drops through an 18-gauge drawing-up needle into 10 volumes of 1% CaCl<sub>2</sub> or 0.5 % BaCl<sub>2</sub> supplemented with 10mM HEPES buffer, pH 7.4. The alginate beads that entrapped Rin-m5F cells were coated with poly-L-lysine (PLL), poly-L-ornithine (PLO), CN, FN or LN respectively for 15 minutes. They were coated with another layer of 0.04% alginate for 5 min.

Alamar blue assay was used to estimate cell proliferation. Surface analysis was carried out using atomic force microscopy (AFM). The interaction between FITC-labelled CN and alginate beads was uncovered using a Leica confocal microscope. Accumulated insulin secretion in medium was measured using ultrasensitive rat insulin ELISA.

**RESULTS:** Barium alginate beads are more stable and provided similar biocompatibility for Rin-m5F cells compared to calcium alginate beads, which showed progressive rupture after culture for a couple of days. 0.1 % (w/v) of PLL or PLO enhanced the proliferation of encapsulated Rin-m5F cells when barium alginate beads were coated using them (Fig.1). Surface topographic images detected by AFM techniques showed the process of protein covering changed the barium alginate polymeric surface (Fig.2, A&B). Using confocal microscopy, we directly showed that barium alginate beads provided spacious porosity to allow FITC-labelled CN infuse (Fig.2, C). Laminin and collagen type I increased accumulated insulin release, while fibronectin increased cell

proliferation. Indeed, The effect of ECM proteins on cell morphology could be identified up to 5 hours' culture.

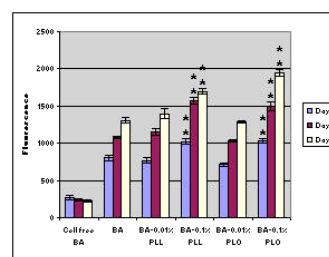


Fig. 1: Effect of PLL and PLO on proliferation of Rin-m5F cells entrapped in alginate microcapsules. \*\*  $P < 0.01$  vs. BA.

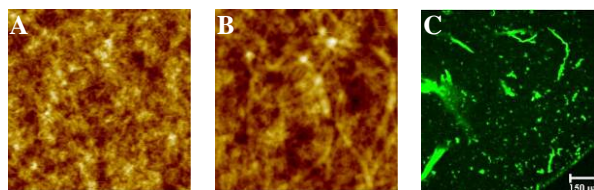


Fig.2: Effect of PLO and CN on physical properties of BA complex. A and B: AFM images. A: BA layer. B: BA layer coated with 0.1  $\mu\text{g}/\mu\text{L}$  Collagen Type I. C: Infusion of FITC-CN into a BA bead.

**CONCLUSIONS:** For Rin-m5F cells in complex 3D alginate beads, 0.1% of PLL and PLO have an effect on proliferation, while CN and LN have an effect on insulin production. These effects might occur through surface modification and cell-surface interaction. These data provide advanced understanding of the role of PLL, PLO and particular ECM proteins in barium alginate microcapsules when insulin-producing cells were encapsulated and cultured.

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