Hydrogel Bead Processing for Mammalian Cell Culture

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INTRODUCTION AND OBJECTIVES

Type 1 Diabetes Mellitus is an autoimmune disease that targets pancreatic islet cells that produce insulin. The use of injectable insulin has been an effective means to treat Type 1 Diabetes, though patients still suffer from hypoglycaemic and hyperglycaemic episodes, as well as macro and microvascular complications (The Diabetes Control and Complications Trial Research Group 1993). The transplantation of pancreatic islet cells has been shown to be superior to intensive medical therapy (Thompson 2011) as it targets the underlying cause of the disease (Johnson 2012). The major challenges of transplantation are the lack of high-quality donor tissue and the need for chronic, potent immunosuppression (McCall 2012).

Alginate encapsulation has been extensively studied as a means to protect transplanted cells from immune rejection (deVos 2010). Furthermore, it has been shown that the immobilization of mammalian cells in a three-dimensional hydrogel scaffold such as alginate can induce the differentiation of progenitor cells (Wang 2009). For instance, a 9-fold increase in insulin/DNA content was observed in neonatal porcine islets after 10 days of alginate-immobilized culture (Tatarkiewicz 2001). Alginate encapsulation could therefore be used to generate functional insulin-producing tissue from readily available cell sources such as embryonic stem cells (Rezania 2012). Alginate encapsulation without immunosuppression has also been shown to allow the long-term survival of allogeneic and xenogeneic grafts (Duvivier-Kali 2001). We have previously shown (Hoesli 2010) that pancreatic cells can be encapsulated by a stirred emulsion process (Poncelet 1994). Cell survival during this process was improved by investigating the effect of emulsification time, acidification time, and the buffer used (Hoesli 2010). In this work, we further characterize the combinatorial effects of various process steps on cell survival in order to identify critical process variables that lead to cell losses.

MATERIALS AND METHODS

Emulsification and Internal Gelation Technique

The Poncelet (1994) emulsification and internal gelation technique was adapted to mammalian cells. Alginate beads of 1.5% final concentration were obtained by mixing 10 mL of 1.75 w/v% alginate solution, cells, 0.5 mL of 500 mM $CaCO_3$ and 20 mL mineral oil at 500 rpm for 12 min. To liberate the calcium ions from $CaCO_3$, 20 µL of 16 N acetic acid was then added with 10 mL mineral oil and mixed for another 8 min at 500 rpm. The beads were recovered by adding 40 mL HEPES-buffered saline solution, centrifuging the mixture for 10 min at 1500 rpm, decanting the biphasic mixture, and filtering through a 40 µm nylon strainer. The encapsulated cells were then cultured in 40 mL of culture medium. The viability and cell density were measured using Trypan blue dye exclusion and packed cell volume measurements. All experiments were conducted using the mouse insulinoma MIN6 cell line. Cell survival and growth in the emulsion-generated beads were qualitatively compared to cells encapsulated in alginate beads generated by extrusion and external gelation using a vibrating nozzle cell encapsulator (Inotech).

Factorial Analysis for Characterization

The effects and interactions of the different process steps on cell survival were determined using full factorial design of experiments. The process variables that were investigated included the presence or absence of (1) acetic acid, (2) agitation (shear stress), and (3) mineral oil (emulsification). Cell survival was assessed by measuring the ratio of initial to final cell viability as a function of processing time.

RESULTS AND DISCUSSION

Cell Growth and Viability

The MIN6 cells cultured in the emulsion-generated beads expanded 2-fold over the course of a 10-day culture. At the end of the culture, cell clusters (Figure 1a) with an average cell viability of 63% were recovered. The nozzle extrusion-generated beads are shown in Figure 1b. These beads were less spherical and fewer cell clusters were observed.

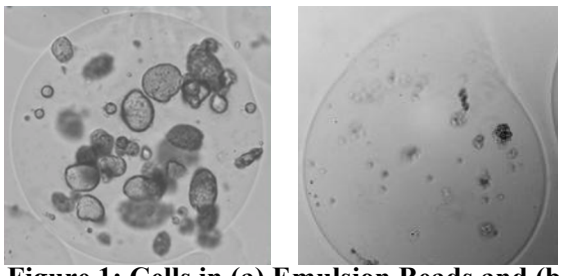

Figure 1: Cells in (a) Emulsion Beads and (b) Extrusion Beads after 10 days of culture

Factorial Analysis

The most significant process variable affecting cell viability was the addition of acetic acid to the emulsion process. Figure 2 shows that removing the acidification step from the process led to a significant increase in the final cell viability, while no significant effect on viability was observed when $CaCO_3$ was removed. Note that acetic acid is added at 12 min into the process time (indicated by arrow).

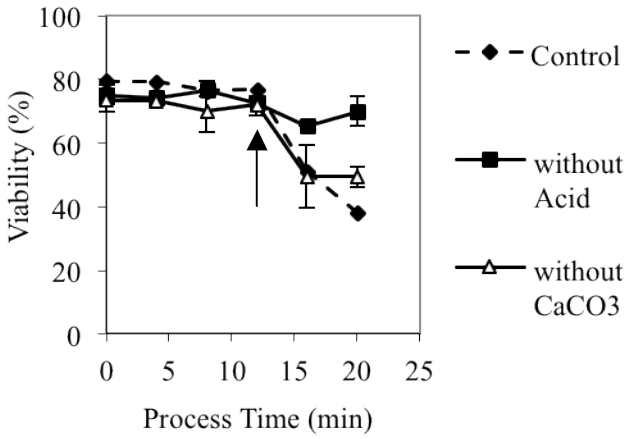

Figure 2: Effect of Acetic Acid on Cell Viability

The ANOVA test for the significant time-dependent variables of the process is shown in Figure 3.

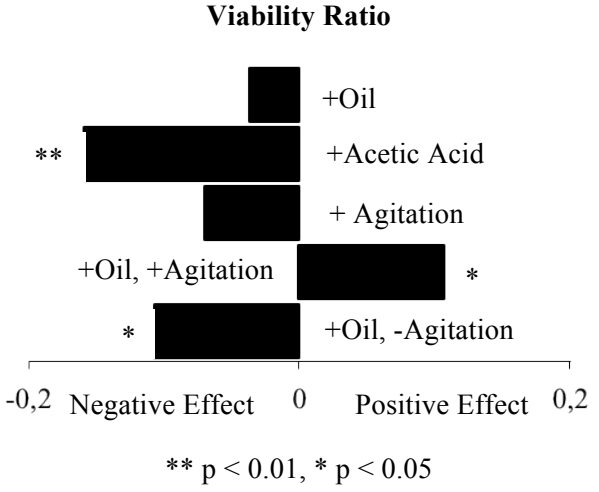

Figure 3: ANOVA Results for Process Variables

These results show that the addition of acetic acid and agitation significantly reduced final cell viability, likely due to the drop in pH and shear forces, respectively. Mineral oil (and emulsification) alone had no significant impact on the viability and actually increased cell survival under agitation, perhaps by reducing the shear forces on the cells.

CONCLUSIONS

Alginate bead generation by emulsification and internal gelation is a promising method to encapsulate large numbers of insulin-producing cells for clinical scale applications. The factorial analysis of the process variables indicated that the addition of acetic acid was the most critical processing step affecting cell survival. Neutralization of the acetic acid was attempted as a solution to this problem. In addition, MIN6 cells were sensitive to agitation (shear stress), an effect that was reduced in the presence of oil. Future work is aimed at characterizing the effect of process variables on bead performance *in vivo* and establishing the efficacy of this method for use in the clinical setting.

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