Pancreatic cell encapsulation by alginate emulsion and internal gelation

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INTRODUCTION

Diabetes affects ~3% of the worldwide population. In Type 1 diabetes, the insulin-producing β-cells found in cell clusters termed islets of Langerhans are destroyed by the patient's immune system. The current therapy of blood glucose monitoring and insulin injections results in imperfect blood glucose control and long-term complications such as kidney disease and heart failure. Islet transplantation has emerged as an alternative treatment, allowing >80% of patients to become insulin-independent for >1 year (Ryan et al. 2002). The islets used to treat one patient are usually procured from 2 to 3 cadaveric donors. Widespread access to this treatment would require an approximate 100-fold increase in tissue availability (Ricordi 2003). Potential alternatives include the use of insulin-producing cells generated by expanding and differentiating adult pancreatic progenitor cells. Several methods to induce insulin expression in expanded pancreatic tissue involve matrix-immobilized cell culture, for instance in alginate (Tatarkiewicz et al. 2001; Tsang et al. 2007). Alginate encapsulation has also been broadly studied as a method to protect allogeneic and xenogeneic islet grafts from immune rejection (de Vos et al. 2006). Clinical trials of encapsulated islet allotransplants without immune suppression are ongoing (Calafiore et al. 2007).

To meet the demand of ~ 1 million islets to treat one patient, progenitors isolated from one pancreas would need to generate ~ 100 million islet-like cell clusters. The highest throughput reported for a single nozzle encapsulator is 330 mL/h (Schwinger et al. 2002). At this flowrate, > 30 hours would be required to generate the ~ 10 L alginate bead volumes needed per batch. This work describes the adaptation of a highly scaleable emulsion and internal gelation (EM/IG) process to pancreatic cell immobilization, culture and transplantation. EM/IG has been used to encapsulate biological molecules, bacteria, yeast and plant cells (Lencki et al. 1989), but it has never been described for mammalian cell immobilization.

MATERIALS AND METHODS

Alginate and cells: The MIN6 and βTC3 cell lines were maintained in DMEM + 10% fetal bovine serum (medium). The alginate was Sigma A0682 for process optimization, while a higher grade alginate (50/50 mixture of LVM/MVG alginates, Pronova) was used for the transplantation.

EM/IG process: The initial non-optimized EM/IG process was based on the method described by Poncelet et al. (1992) for enzyme immobilization, albeit with reduced processing times and 10 mM HEPES buffering. Briefly, 1 volume cell stock was added to 9 volumes alginate and 0.5 volumes of $CaCO_3$ to obtain a final concentration of 1.5% alginate. Saline (10 mM HEPES, 170 mM NaCl, pH 7.4) was initially used to dissolve the alginate and suspend the $CaCO_3$, but was replaced by 60 mM

MOPS, 127 mM NaCl, pH 7.6 after process optimization. Then, 10.5 mL of the alginate, cell and $CaCO_3$ mixture was added to 20 mL of light mineral oil stirred at 500 rpm. After 12 min (reduced to 3 min after optimization) of emulsification, 10 mL oil + 40 µL of acetic acid was added for 8 min (reduced to 1 min after optimization) to trigger internal Ca^{2+} release. Immediately after gelling, a higher pH was re-established by adding 40 mL of saline containing 10% medium, leading to phase inversion. The agitation was ceased and the entire mixture was centrifuged 3 minutes at 400 x g to accelerate phase separation. The oil and excess saline were removed by aspiration, followed by two washes with medium. For cell growth, half medium changes were performed every second day.

Vibrating nozzle encapsulation or slab formation by external gelation (EG): For the generation of EG alginate beads, the alginate and cell mixture was prepared as above, but process buffer replaced the $CaCO_3$ suspension. Beads were generated by extrusion at 6 mL/h through a 250 µm diameter nozzle using an Inotech encapsulator following the manufacturer's instructions. The beads were agitated 10 minutes in gelling solution (75 mM $CaCl_2$, 10 mM HEPES, 75 mM NaCl, pH 7.4), followed by 5 minutes in saline. The beads were then transferred to a biological safety cabinet and washed twice with medium. For the alginate slabs, the alginate and cell mixture (without $CaCO_3$) was spread at 0.44µL/cm^2 on the bottom of a Petri dish and dropwise addition of gelling solution.

βTC3 transplantation: βTC3 cells were encapsulated at 5e6 cells/mL alginate via the EM/IG or EG processes and kept in culture overnight. The next day, the beads were washed 5 times in saline. The viable cell yield was determined by transferring 1 mL beads into degelling solution. The equivalent of 2.3 e6 cells in beads were transplanted per mouse. The diabetic control mice received 2.3 e6 non-encapsulated βTC3 cells in saline instead. The recipients were male C57BL/6 mice (Jackson) that had been treated with streptozotocin (STZ) 7 days prior to transplantation (Tx). Blood glucose and body weight were monitored 2-3 times per week for 2 weeks before and after the transplantation.

Cell enumeration: To retrieve cells from gelled alginate, 1 volume of gel was added to 4 volumes degelling solution (55 mM citrate, 10 mM HEPES, 90 mM NaCl, pH7.4) and left 15 min on ice on a rotary shaker. Single cells were counted based on trypan blue exclusion by a CEDEX automated cell counter. If cells were in aggregates, the packed cell volume (PCV) was measured instead.

RESULTS AND DISCUSSION

The application of EM/IG to mammalian cell encapsulation has enormous potential due to the scalability and robustness of the process compared to nozzle-based strategies. However, the effect of the required processing steps (contact with $CaCO_3$, emulsification, acidification) on these sensitive cells was unknown. We first sought to determine whether mammalian cells could withstand these conditions and to identify the processing variables that had the most impact on viable cell recovery. The initial process led to less than 40% cell viability following the addition of acid to the emulsion (Figure 1). This was not surprising as the final pH in the alginate beads was ~ 5.0 while the MIN6 viability was shown to be significantly affected after 30 min exposure to pH < 6.5 (data not shown).

Following these observations, the process was improved by 1) adding 10% medium to the citrate degelling solution, 2) minimizing the emulsification and acidification time without affecting the bead size distribution or chelation kinetics and 3) by using a 60 mM MOPS buffer to target a final pH of 6.5 after the addition of the same amount of acid. As a result, the MIN6 cell survival was increased to 90 ± 1%.

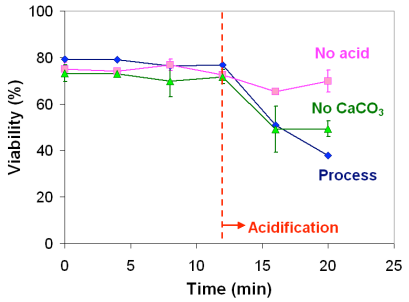

Figure 1 : Sharp decrease in cell viability following acidification of the alginate emulsion

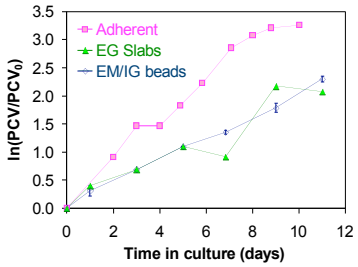

Figure 2: Similar MIN6 cell growth characteristics in the EM/IG beads and in EG alginate slabs

The growth characteristics of MIN6 cells were examined in the EM/IG alginate beads. There was no significant difference between the MIN6 growth rate in the EM/IG beads and externally gelled alginate slabs (Figure 2), indicating that there were no long-term detrimental effects of the EM/IG on MIN6 growth. Interestingly, this was obtained despite structural differences between IG and EG (Quong et al. 1998). However, as expected (Hoesli et al. 2009), the MIN6 growth rate in alginate was significantly reduced compared to adherent culture controls. The growth was also less efficient, with 14.4 ± 0.1% decreased yield of cells per mol glutamine consumed (results not shown). MIN6 expansion was also evident based on visual observation (Figure 3).

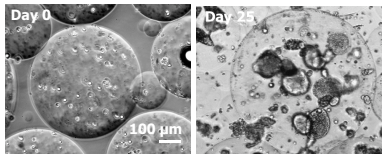

Figure 3 : MIN6 cell growth into islet-equivalent sized spheroids in the emulsion/internal gelation alginate beads

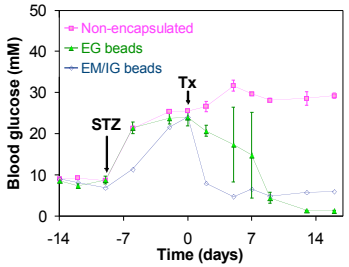

Figure 4: The EM/IG alginate beads protect βTC3 cells from allogeneic rejection in STZ-treated C56BL/6 diabetic mice

Next, the potential of the EM/IG alginate beads to protect grafted cells against allorejection was tested in a pilot study (N=1 for βTC3 cells in EM/IG alginate beads, N = 2 for EG beads and N = 2 for non-encapsulated βTC3 cells) that is currently being revised and replicated. βTC3 cells were

used rather than MIN6 cells because the latter do not express MHC molecules and would not be a suitable allorejection model. As expected, the non-encapsulated βTC3 cells were rejected shortly after transplantation and the animals remained diabetic. On the other hand, the EM/IG and EG encapsulated βTC3 cells normalized the blood glucose levels of mice within 10 days. These results indicate that EM/IG alginate beads could be suitable for immune protection of allografts. Further studies and optimization specifically aimed at improving transplantation performance are required to better assess the potential of the EM/IG alginate beads for immune isolation of islets.

CONCLUSIONS

Alginate immobilization could be a useful tool in many fields of cellular therapy, such as the generation of islets from progenitor cells, chondrocytes from mesenchymal stem cells or hepatocytes and other cell types from embryonic stem cells. For these applications, the generation of large batches of cells in alginate beads would be desirable for validation purposes. This work demonstrates the feasibility of mammalian cell encapsulation using a scaleable EM/IG stirred process with <10% cell losses. This process is suitable for in vitro immobilized mammalian cell culture and potentially even for transplantation purposes.

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