Emulsion-based encapsulation of insulin-producing cell clusters

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INTRODUCTION AND OBJECTIVE

Islet transplantation has emerged as a potential long-term treatment for type 1 diabetes. However, the immunosuppressants used to avoid graft rejection can lead to undesirable side effects such as opportunistic infections (Cravedi 2010). Islet encapsulation provides a method to reduce the need for immunosuppression by providing a barrier between the graft and components of the immune system. Diabetic rodents transplanted with allogeneic or xenogeneic islets encapsulated in alginate have remained normoglycemic for up to 1 year without immunosuppression (Duvivier-Kali 2001; Omer 2005; Cui 2009). However, the uncoated alginate beads used in these experiments were permeable to antibodies and likely offered limited protection against the humoral immune response (Omer 2005). To avoid the access of antibodies to the graft, the beads can be coated with cationic polymers. Unfortunately, these polymers lead to complement (Darquy 1994) and macrophage activation (Strand 2001; Juste 2005). Also, positive bead surface charge has been associated with fibrotic overgrowth of the beads (King 2001; de Vos 2007).

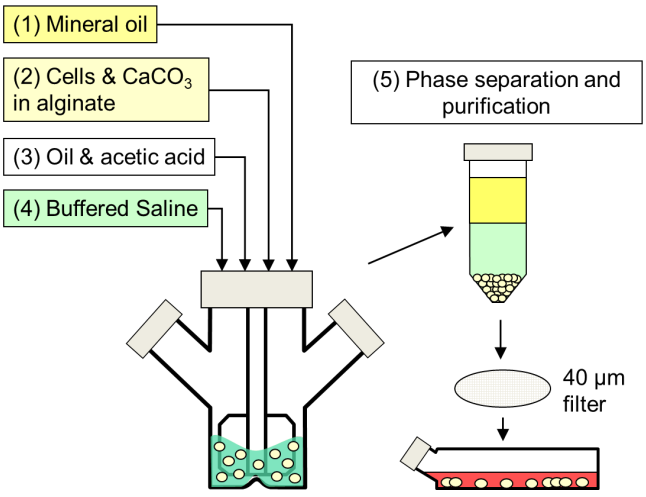

Figure 1: Schematic of the emulsion-based mammalian cell encapsulation process.

We have previously adapted an emulsion-based method (Poncelet 1992) to the encapsulation of insulin-producing beta cells (Hoesli 2009, Figure 1). Contrary to conventional cell encapsulators, the method does not require alginate and cell suspension extrusion through nozzles, and hence allows the generation of higher-concentration 5% alginate beads. These have been shown to exclude antibodies from ~80% of their void volume (Hoesli 2012). Compared to 1.5% extrusion-generated beads, the 5% beads increased the survival of allogeneic beta cells transplanted into mice. The objective of the current work was to further optimize the emulsion process in order to efficiently encapsulate islet-like cell clusters.

MATERIALS AND METHODS

Islet-like cell clusters were generated by culturing mouse insulinoma 6 (MIN6) cells in 1.5% alginate slabs for 4 weeks, such that single cells expanded into cell aggregates. The slabs were generated by pouring a 75 mM $CaCl_2$-containing saline solution over an alginate and cell solution in a Petri dish. The cell clusters were released from the slabs by sodium citrate chelation, followed by washing in medium. The cell clusters were re-suspended in 5% alginate solution containing 95 mM $CaCO_3$. Alginate beads of 947 µm volume-weighted average diameter were generated by emulsion and internal gelation as previously described (Hoesli 2009). As controls, clusters were immobilized in externally gelled 5% alginate slabs or kept in medium and subsequently treated with the same solutions as the beads. The cell clusters were again released from the beads by citrate chelation in order to determine the cell cluster yield and size distribution after cell encapsulation. Cell clusters were dispersed using trypsin and live cells were enumerated after trypan blue staining. Bright field images of the cell clusters were captured and the size distribution of the aggregates was analyzed using the CellProfiler freeware (Carpenter 2006).

RESULTS AND DISCUSSION

Encapsulating cell clusters using the emulsion process presented several challenges compared to encapsulating single cells, such as cell cluster disruption or incomplete encapsulation.

As a model system for encapsulating islets, MIN6 cell clusters were encapsulated in 5% alginate beads. As expected, cell clusters were observed in the beads (Figure 2). Image analysis of the clusters released from alginate revealed no significant difference in cell cluster size between beads, slabs and non-encapsulated controls (106 ± 33, 107± 38 and 114 ± 42 µm diameter, respectively).

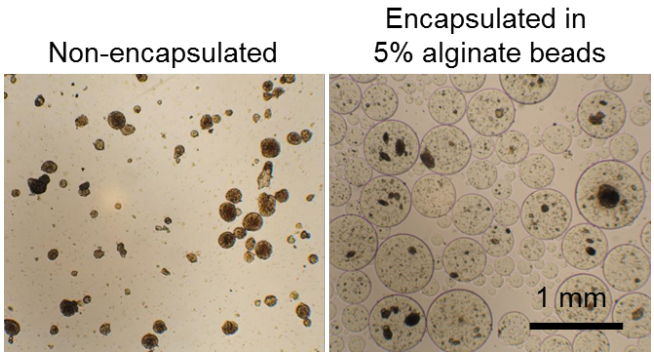

Figure 2: MIN6 cell clusters before and after encapsulation.

The total encapsulated cell yield from the process was ~67%. A similar yield was calculated both from cell cluster counts and from the enumeration of live cells obtained after dispersing the clusters. Potential sources of cell loss include incomplete encapsulation, cell lysis and losses during the bead purification steps. For example, a small fraction of the beads was observed and lost at the oil/water interface due to the presence of encapsulated oil droplets.

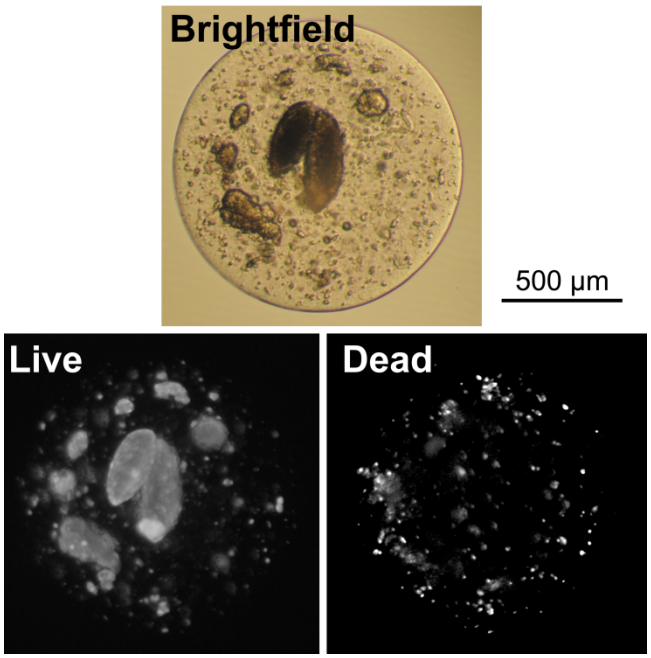

Figure 3. Encapsulated MIN6 cell cluster viability after the emulsion process based on calcein AM (live) and ethidium homodimer (dead) staining.

Finally, live/dead staining (Figure 3) confirmed that the encapsulated cell clusters remained viable, with no significant difference between the encapsulated and the non-encapsulated cell clusters.

CONCLUSIONS

These results demonstrate that viable islet-like cell clusters can be encapsulated by emulsion and internal gelation with >60% encapsulated cell cluster yield. Future work will confirm these results with primary cells and further investigate encapsulated cell function *in vitro* and *in vivo*. The emulsion-based process is a promising scalable technology for cellular therapies requiring immune isolation.

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