The use of microfluidics to encapsulate cells

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Introduction

For successful encapsulation of a particular cell-line, there are several essential capsule requirements. These are; efficient diffusion of materials to and from cells, mechanical stability, retrievability (post-implantation), optimized size, simple and non-toxic manufacturing procedures and a biocompatible encapsulation material. The use of microfluidics could overcome some, if not all of these issues.

Microfluidics is the science of handling small volumes of fluid (liquid or gas). The use of microfluidics provides an exciting new droplet generation method. Microfluidic experiments can easily be performed in sealed, sterilisable, purpose-designed micro-reactors. This means that the reactor and reactants can be kept sterile whilst beads or microcapsules are being formed. It is easier to manipulate and precisely control individual droplets and their properties using microfluidic circuits. Reaction times and cell-reagent exposure times can be strictly controlled via the use of microfluidics. Other advantages to using microfluidic methods for droplet generation are; no harmful solvents are used, and uniform, small droplets (150-400μm) can be produced with a very narrow size distribution.

Microfluidics has previously been used to encapsulate cells, with varying success. Sugiura *et al.* (2005) developed a silicon micro-nozzle array based upon microfluidic principles. HEK cells were successfully encapsulated using this method, although it is not reported for how long cells remained viable. He *et al.* (2005) encapsulated cells and single organelles into picolitre- and femtolitre-volume droplets using microfluidic circuits. However, these droplets were not hardened. Tan *et al.* (2006) have also used microfluidics to encapsulate cells and proteins, this time within lipid vesicles. Viability of HeLa cells was shown for over two hours, but no further time points were reported. Vesicles were reported to be stable for more than 26 days.

Q Chip (Cardiff, UK) has developed a highly flexible microfluidic platform that allows for rapid evaluation of microfluidic circuits and junctions. This evaluation platform has previously been used for the generation of cross-linked hydrogel beads, composed of PVA (polyvinyl alcohol), PEGDM (poly(ethylene glycol) dimethacrylate), acrylamide or HEMA (hydroxyethyl methacrylate). To date, all hydrogel beads were formed via a photochemical method.

The microfluidic system developed by Q Chip is used for the rapid evaluation of microfluidic circuits, consisting of microfluidic channels arranged in defined geometries. To achieve this, micro-channels are machined into a PolyTetraFluoroEthylene (PTFE) surface using a Computer Numerical Controlled (CNC) milling machine; to give a microfluidic "chip" (Figure 1). PTFE is a hydrophobic and rigid plastic. To make the square channels closed on four sides, the chip is covered with a hydrophobic gasket (PFA, 250μm thickness) and sandwiched between a borosilicate disc, and a fluid distribution manifold (Figure 2).

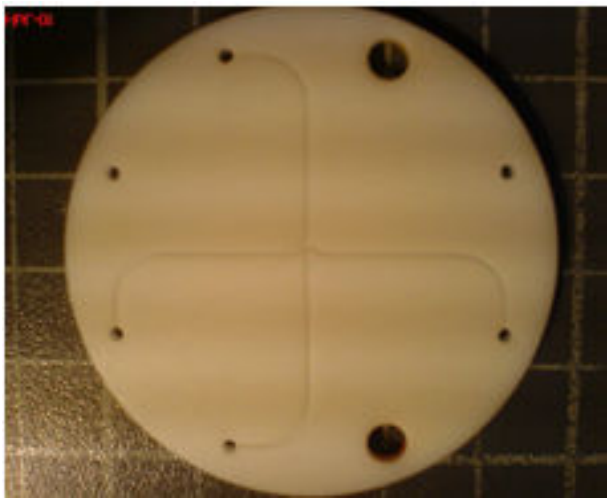

Figure 1: An example of a microfluidic chip

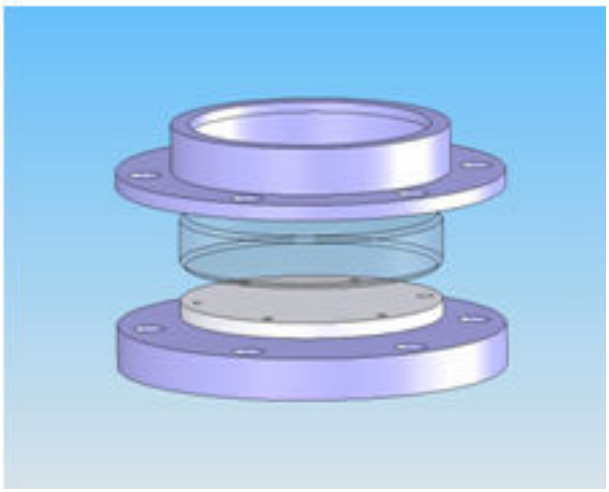

Figure 2: An expanded schematic showing components of Q Chips' microfluidic evaluation rig.

Alginic acid salts, or alginates, have been widely used for polymer encapsulation of cells. It has been shown to be biocompatible and biodegradable (Orive et al., 2004) and can produce ionotropic hydrogels upon exposure to di- and tri-valent cations. Our work continues to make use of alginate to encapsulate cells. Instead of ionically crosslinking alginate via external gelation methods, we have adapted the Poncelet *et al.* (1995) internal gelation method. Internal gelation occurs when Ca^{2+} is liberated, within an alginate droplet, from an insoluble form. The liberation of Ca^{2+} occurs upon exposure to H^{+}, usually in the form of a dilute solution of acetic acid.

Material and methods

Pronova UltraPure, Medium viscosity, High mannuronate (UP MVM) alginate was purchased from NovaMatrix™ (Drammen, Norway). Microcrystalline precipitated calcium carbonate, with an average particle size of 0.07µm was a gift from Speciality Minerals (Birmingham, UK). High oleic sunflower oil was purchased from Statfold (Stafordshire, UK). All cell culture materials were purchased from Invitrogen (Paisley, UK)

In all cell encapsulation experiments, the stainless steel microfluidic rig, glass, gasket, fittings and PTFE chip were autoclaved and then air dried in an oven set to 50˚C. Alginate (2% w/v) and calcium carbonate (0.5% w/v) were suspended in D-MEM/F12 medium without serum at 37ºC. Cells were trypsinised, washed, and counted and then resuspended in alginate-calcium carbonate mixture at a concentration of $1x10^{6}$ cells/ml. The alginate was segmented using sunflower oil as a shielding flow and sunflower oil containing acetic acid (0.5%) as the reactive fluid.

For viability counts single beads were placed on each half of a haemocytometer, medium was removed using tissue. Sodium citrate solution (55mM) was added to each bead followed by trypan blue. A coverslip was placed over the beads and gentle pressure applied to squash the beads. Cell and viability counts were carried out according to literature precedent.

Results and Discussion

Droplets of alginate were formed in a microfluidic circuit. It is obviously important that the crosslinking reaction of the alginate occurs only after droplet generation. However, the reaction

between oil-soluble acetic acid and aqueous-suspended $CaCO_3$ is extremely rapid, and produced solid hydrogel immediately upon the two fluids coming into contact. Hence, it was postulated that the reaction could be delayed via the introduction of a diffusion barrier. A second flow-stream of oil was allowed to flow laminarly alongside the acid-containing oil stream. Hence, protons diffused through the oil flow before reaching the aqueous alginate droplets. In this way solid alginate beads were formed in a single microfluidic circuit. Blank beads were produced via this method. The average diameter of this sample of blank beads was measured to be 277±5µm. The coefficient of variation (CV) was 1.8%. CV was calculated as the standard deviation of the diameter of beads divided by the mean diameter.

HEK293 cells were encapsulated using this novel method. Viability of cells was collected for 90 days (Figure 3). The adapted trypan blue method used was corroborated using FACS analysis. Although there was an initial decrease in cell viability, after roughly 12-14 days viability began to increase and aggregations of cells were observed within the product beads. This strongly suggests the formation of clonal cell colonies.

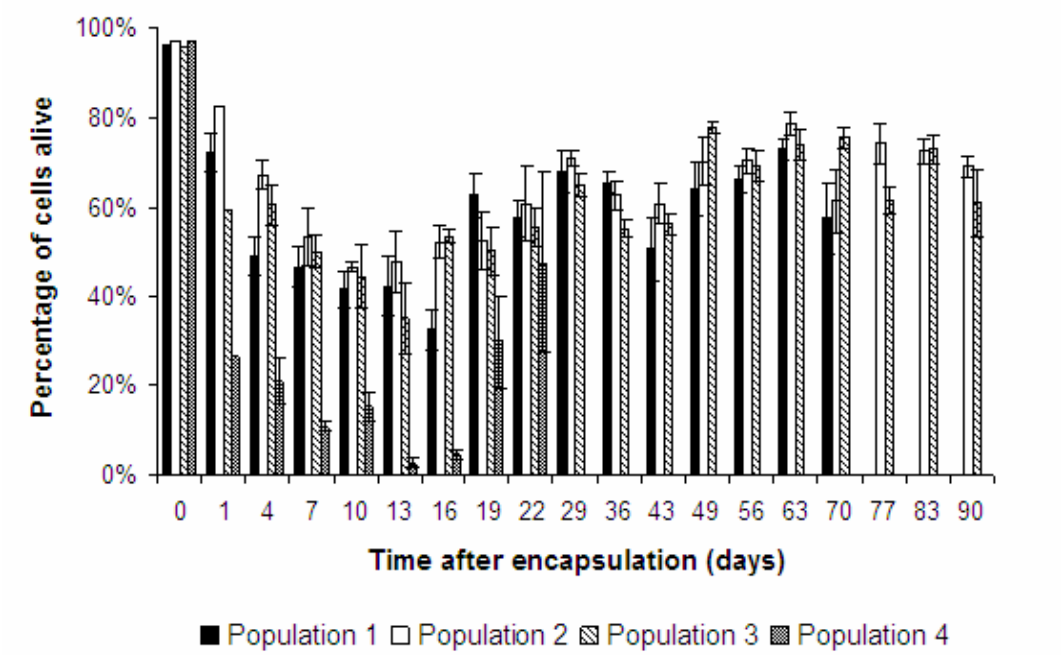

Figure 3: Viability data showing percentage of cells alive. Data collected using an adapted trypan blue method.

A multi-circuit microreactor was designed with 10 microfluidic circuits operating in parallel (Figure 4). As very small volumes of fluid (typically <500nL) are handled in microfluidic circuits, production rates are typically low (in the order of 1000µl/hour per circuit). Consequently, to produce a litre of microcapsules per hour, 1000 parallel channels would be needed. Vertical stacking of many fluidic chips is possible, but gives rise to problems with linking, requiring many tubes, connectors, and pumps (Joanicot and Ajdari, 2005). This can potentially be solved by using a space-saving layout for microfluidic channels, coupled with a single input port for each fluid used with subsequent separation to individual, multiple channels.

Initial experiments making use of the designed multi-circuit microreactor were successful (Figure 5). A production rate of 35ml/hr was achieved. Cells had a viability of 93.5% prior to encapsulation, measured using trypan blue exclusion. After 24 hours, viability was measured to be 51.2%. After 60 days, the viability of the encapsulated cells was seen to be 55.1%.

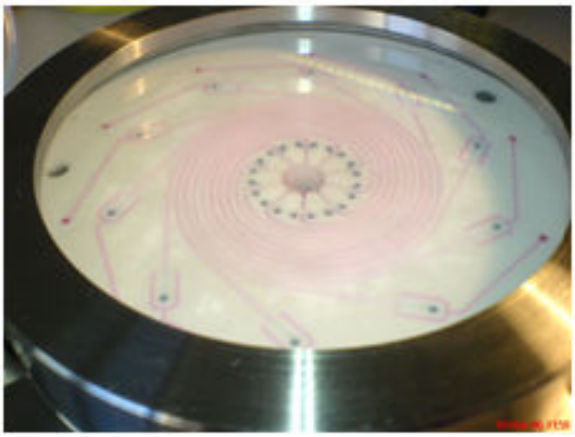

Figure 4: Multi-circuit microreactor in operation

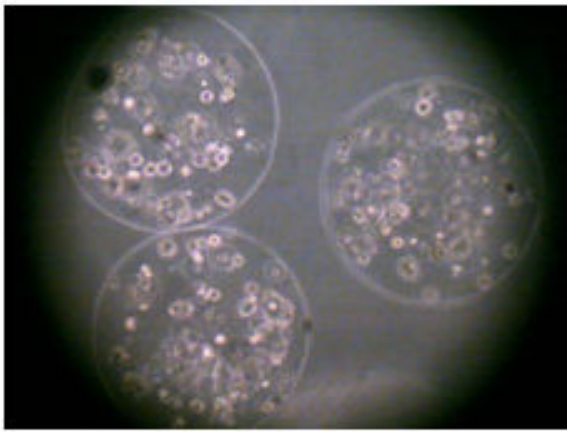

Figure 5: HEK293 cells encapsulated in alginate beads

Conclusions

The work presented here defines a novel method for alginate bead production. This method could be used for encapsulation of DNA, protein or cells. We have successfully encapsulated HEK293 cells.

Future work involves optimization of this device and maximizing cell-viability by modifying the encapsulation methodology. There is also potential for applying poly-electrolyte coatings onto alginate beads. This is a widely used experimental procedure. By further enhancing the microfluidic reactor, it may be possible to create multiple poly-electrolyte layers *on-chip*, allowing complex polymer structures to be synthesized in a single step.

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