Cell Encapsulation On Microfluidic Platform

Background

In 1964, T.M.S. Chang began a new area for research for biomedical engineering when he proposed the idea using ultrathin polymer membrane microcapsules for the immunoprotection of transplanted cells, thus defining the concept of bioencapsulation in terms of "artificial cells". [1] Twenty years later, bioencapsulation was successfully implemented to mobilize xenograft islet cells. When implanted in a rat [2], the microencapsulated islet corrected the diabetic state for several weeks. Since then, there has been significant progress towards understanding the biological and technological requirements for successful transplantation of encapsulated cells in vivo.

Application

Bioencapsulation has provided a range of promising therapeutic treatments for diabetes, hemophilia, cancer, and renal failure.

Diabetes:

> Porcine pancreatic islets were microencapsulated in alginate-polylysine-alginate capsules. The capsules was transplanted intraperitoneally into nine spontaneously diabetic monkeys which became insulin independent for periods ranging from 120 to 804 d with fasting blood glucose levels [3]

Hemophilia: (Hemophilia is an inherited bleeding disorder in which blood lacks of many proteins, called clotting factors, which work to stop bleeding.)

> A potentially cost-effective strategy for gene therapy of hemophilia B is to create universal factor IX-secreting cell lines suitable for implantation into different patients. To avoid graft rejection, the implanted cells are enclosed in alginate-polylysine-alginate microcapsules that are permeable to factor IX diffusion. [4]

Cancer:

> Macrophages can kill tumor cells by releasing high levels of nitric oxide (NO) and related reactive nitrogen species such as nitroxyl and peroxynitrite, after up-regulation of expression of the inducible nitric oxide synthase gene (iNOS). These iNOS-expressing cells were trapped within a semipermeable alginate-poly-L-lysine membrane as a means of delivery to tumor sites in a nude mouse model. [5]

Renal failure:

> Polymeric membrane artificial cells (semipermeable microcapsules) containing genetically engineered live cells from the bacteria Escherichia coli DH5 were prepared. The cells remain at all times in the microcapsules and are finally excreted in the stool. During their passage through the intestine, small molecules like urea diffuse rapidly into the microcapsules and are acted on by the genetically engineered cells. This lowers the high plasma urea level to normal in uremic rats with induced kidney failure, and has exciting implications for the use of this and many other types of genetically engineered cells in a number of medical applications. [6]

Methods

In this paper, we will focus on the methods of encapsulating cells into droplets, which has many parameters to be considered: desired mean size, acceptable size dispersion, scale of production and the maximum shear that cells may tolerate.

Current methods

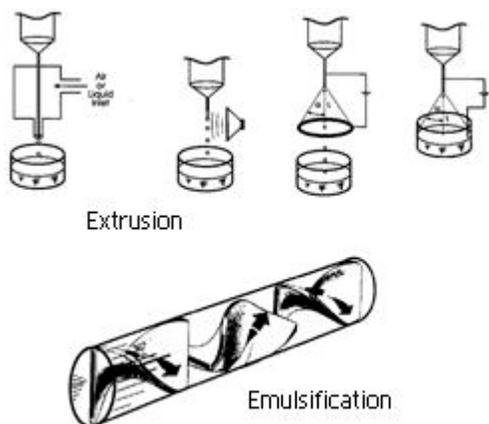Capsules are formulated by droplet extrusion or emulsification. (Figure 1, [9, 10])

Figure1. Current method of encasulation

In the extrusion techniques, often referred to as the drop method, solutions are extruded through a small tube or needle, permitting the formed droplets to freely fall into a gelation bath. The droplets are cross-linked by addition of appropriate reagent to the receiving solution. A typical example is the formation of alginate beads by dropping a sodium alginate solution into a bath containing calcium chloride. In the emulsion technique, solutions are mixed and dispersed in a non-miscible phase often facilitated with a surfactant. When the dispersion reaches the equilibrium, gelating and/or membrane formation is initiated by cooling and/or addition of gelling agent to the emulsion, or by introduction of a cross-linked agent [9]. These methods produce larger droplets. Also, these droplets formed by extrusion method have different membrane thickness and size as they drop in the gelation bath at different time.
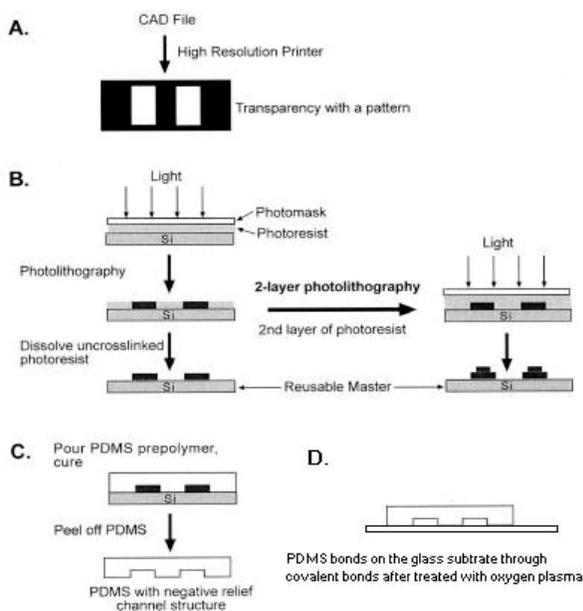
Novel methods:

Figure 2. Schemes for Device Fabrication

Material:
- Poly(dimethylsiloxane) (PDMS) is used as a material for fabricating the microfluidic devices. The PDMS used is supplied in two components, a base and a curing agent. The mixture is introduced on top of a silicon wafer formed from photosensitive polymer with desired channel. (Figure 2, [7])
- Rat neuroblastoma cells (B103) are cultured and used as the main source of cells in the experiment. B103 cells are very strong and can be cultured easily in appropriate media.

Current research and progress [11]:
In our lab, Biomolecular Microsystems and Nanotransducers (BioMINT), we have successfully captured the cells into water droplets in oil medium. The cells have been captured in 100µm diameter droplets in a continuous process that allows for various other on-chip technologies.

The microfluidic design (Figure 3) consists of upstream cell loading and shearing zones and a downstream cell encapsulation zone. The channel depth was 40µm um with width of 30µm and 50µm, for the cell loading and fluid (oil and water) channel, respectively.
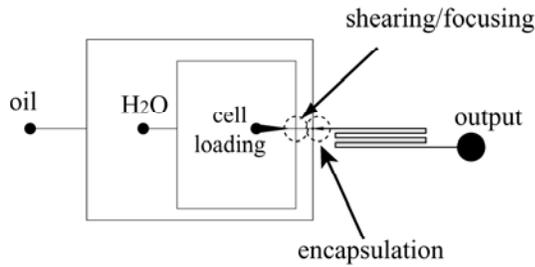
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Figure 3. Device Design

Cell loading consists of two different phases. At first, cells were loading at a densities low enough to reduce cell-to-cell adhesion. The packed cell loading method was used to focus the cells through the loading channel (Figure 4). This focusing region serves two purposes: the first is to shear off the cells into the small groups and send to encapsulation channel and the second is to focus cells media to the center of the channel to avoid the built-up of cell proteins and cell debris on the channel walls.
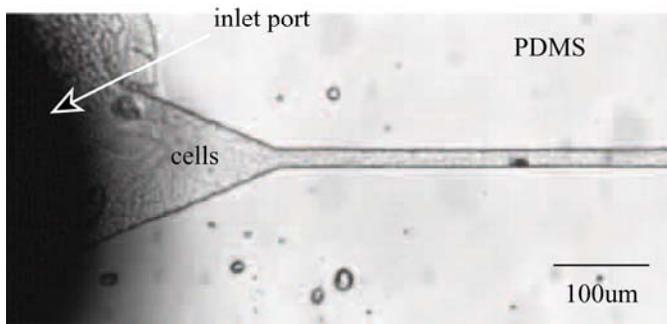

Figure 4. Packed Cell Loading

In the cell encapsulation zone, the cell flow is sheared off into droplets by the flow of two oil streams, oil flows from the upper and lower channels pinching off droplets containing cells and cell media, which are demonstrated in Fig 5a. Fig 5b, c and d are 3 droplets containing a similar number of cells.
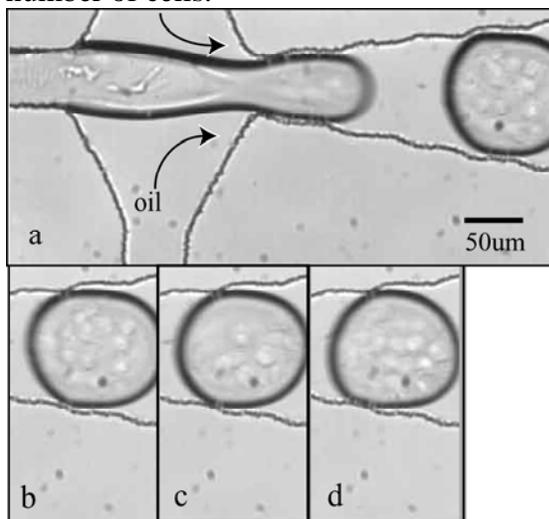

Figure 5. Cell Encapsulation

The droplets size obtained in the previous experiment was 100 µm in diameter, 4 times smaller than the previous reported [8, 10]. The number of cells in each droplet was approximated to be 20-50 cells. However, these results were not stable: some droplets had no cells, and some packed with too many cells. Droplet formation was not stable: it did not consistently produce well-separated droplets as shown in Fig 5. Often cell-to-cell adhesion prevented droplets from pinching off from one another, causing them to fuse.

Goals
- Design channel that produces consistent of droplets formation in both cell number and droplet size.
- Including polymer (alginate) in the water shearing flow for coating the cells.
- Extract the droplets in a continuous process.
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Comparisons between methods

Extrusion/Emulsification	Microchannel
Less control.	Controllable environmental conditions.
Large system.	Small system allowing high throughput and cost effective.
Batch process (extrusion.)	Continuous process.
Inconsistent droplet size and membrane thickness (the droplets are formed randomly through dropping or spinning.)	More control in droplet size and membrane thickness by controlling the channel size and shape.
Separate processing equipment.	Can be incorporated with other microfluidic technologies on the same chip.

Time Line
- The ten-week period will be divided into three phases, approximately three weeks each.
  - *Phase 1*: We will focus on improving consistency of droplet size in the water-oil based media and number of cells in each droplet. This can be achieved by optimizing the cell-shearing region.

  - *Phase 2*: We form droplets with polymer (cross-linked alginate-calcium) coating, no cells involved

  - *Phase 3*: We combine first two phases to produce cells droplets with alginate coating. Based on some current researches in BioMINT Lab, two different techniques of continuous gelation will be tested:

    1. *Droplet fusion*: Cells in alginate droplets and calcium droplets will be prepared in two channels on the same chip. At the junction, the droplets collide to produce cell droplets with polymer coating.
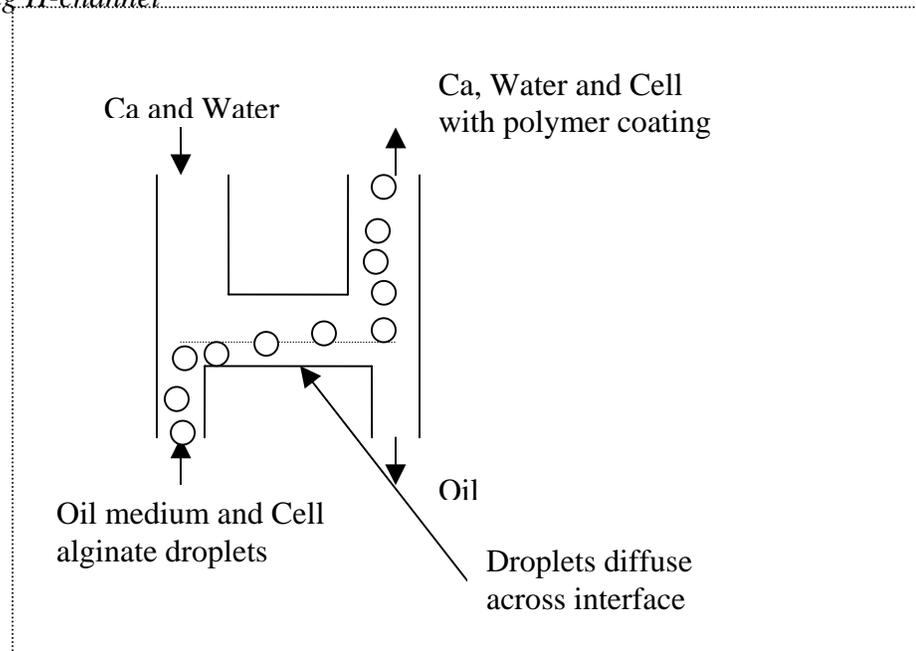
2. Using H-channel

Ca and Water

Ca, Water and Cell
with polymer coating

Oil

Oil medium and Cell
alginate droplets

Droplets diffuse
across interface

Figure 6. H- Filter

- Each phase is comprised of 3 specific tasks in 3 weeks:

Time	Objectives	Student's Tasks	Mentor's Tasks
Week 1	Device Design and Fabrication	-Study L-Edit or CAD software. -Use these layout-editor to establish channel design -Observe the lithography process for making reusable mold master for PDMS devices. -Make PDMS mixture and build the channel device on the glass substrate for testing	-Guide student and source for reference. -Evaluate the design on the scale of feasibility. -Monitor student's progress -Fabricate device wafer in INRF
Week 2	Device Testing	-Cell-culture hood training. -Cell culture: making media, culturing cells, freezing cells -Establish the testing system comprising of syringes and pumps. -Use inverted microscope with built-in high-speed digital camera to observe the flows and cell encapsulations process	-Cell culture training and biohazard safety. -Demonstrate experimental setup and recording methods.
Week 3	Analysis and Optimization	-Will be trained to use different approaches in data and image analysis. -Do math analysis with MathLab or MS Excel -Do image analysis "by hand" and software. -From the collected data, propose a strategy for improving the result in the next cycle of the experiment.	-Explain the importance and utility of collected data. -Introduce data analysis methods and theory.

- The tenth week is used to organize and analyze all the data and synthesize the final results from all of the designs.

Current State and Progress
The research in cell encapsulation in our lab has just successfully tested the microchannel device for capsulating cell-water droplets in oil, marking the advent of new technique create cell capsules. Our goal in this summer is to establish some foundation steps for improving cell encapsulation for future of cell transplantation. The main objective is to combine the microfluidic device with the current polymer material for cell coating in smaller size droplets, with a stable rate in a continuous process.

Cell microcapsulation is a technology with enormous clinical potential for treatment of a wide range of diseases, which have been mentioned earlier. Yet many difficulties remain, some of which certainly challenge our scientific ingenuity. If our technique is tested successfully with B103 cells and alginate coating, we will move one step forward to cells with real applications for clinical trials, which still face lots of obstacles because lacking of standard technology providing steady inputs for all the researches.

The outcome of this research will be evaluated based on two different scales: experimental results and the level of self-improvement for those participating in the research. On a personal perspective, the student will gain knowledge of biomedical engineering in order to progress and become familiar with relevant literature in the field. Furthermore, this will provide an opportunity for the student to work and develop professional skills in research, laboratory techniques, and safety protocols. Finally, the student will experience the lab environment and dynamics of a biomedical engineering collaborative effort, thereby, beginning the path towards a career in research.

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