DROPLET-BASED MICROFLUIDIC SYSTEM FOR ENCAPSULATION AND CULTURE OF NEURON CELLS IN MICRO-GEL-PARTICLES

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ABSTRACT

We demonstrate integrated microfluidic system performing generation and immobilization of cell-laden micro-hydrogel-particles for real-time, high-throughput neuron culture, analysis of growth and differentiation. Uniform calcium-alginate microcapsules, mimicking the role of extracellular matrix, are formed through three steps: the generation of calcium chloride and cell-laden sodium alginate droplets, fusion of droplets to form micro-gel-particles, and immobilization in chamber for characterization. We observed linear dependence of droplet size on inlet flow rates at T-junctions and defined the optimal channel aspect ratio for enhanced fusion rate. Preliminary viability tests indicate the potential of the device for coupled neuron culture and live imaging of growth.

KEYWORDS: droplet microfluidics, hydrogel, micro-gel-particles, PC12

INTRODUCTION

Droplet microfluidics presents a new paradigm for biological assays [1], where a variety of well-controlled water-in-oil droplets can be formed as isolated microreactors for biomolecule synthesis, drug delivery, and diagnostic testing [2]. The well-defined droplet size facilitates quantitative, high-throughput analysis with reduced reaction times and system complexity. Encapsulation of neuron cells in hydrogel matrices enables novel stem cell-based therapy for diseases such as Parkinson's [3], nerve repair and regeneration [4], and cancer treatment [5]. Microfabrication techniques have been used to droplet generation [6], fusion [7], or effectively create Ca-alginate micro-gel-particles of controlled sizes above 50 μm [8]. In this paper, we demonstrate an integrated solution for stable droplet generation to transport culture media and cells, fusion to form Ca-alginate micro-gel-particles with encapsulated cells, and finally gel-particle immobilization on-chip suitable for real-time, high-throughput live imaging and recording of cell growth.

SYSTEM DESIGN

The multi-phase microfluidic system (Figure 1) consists of functional compartments for aqueous picoliter droplet formation, encapsulation of cells in micro-gel-particles, and culture in an on-chip chamber. Monodisperse aqueous droplets of calcium chloride ($CaCl_2$) and PC12 cell-laden sodium alginate (Na-alginate) are generated in oleic acid carrier medium using perpendicular shear force type T-junctions, with standard droplet size deviation less than 5%. The sizes of $CaCl_2$ and Na-alginate

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droplets can be independently controlled through flow rate regulation to determine relative mixing ratio before fusion. The droplets were fused in 1:1 ratio using an expansion channel forming Ca-alginate micro-gel-particles with embedded PC12 cells. The channel geometry and dimensions are shown in Figure 2. Computational fluid dynamics simulations of droplet fusion in oleic acid were performed to characterize the effect of distance between adjacent droplets (Figure 2a) and channel dimensions (Figure 2b) on fusion efficiency. Lowering channel height also yielded increased fusion efficiency. While maintaining other parameters constant, lowering channel height to 17% of width enables droplet fusion, while fusion did not occur for 33% channel height. This is attributed to increased drag force on larger $CaCl_2$ droplets with lowered channel height. The encapsulating micro-gel-particles are then immobilized in a collection chamber suitable for observation using in-channel pillar structures serving as obstructions for the gel-particles.

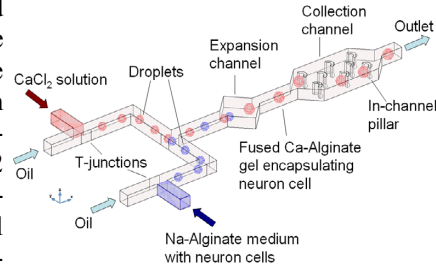

Figure 1: Schematics of the integrated microfluidic system for cell encapsulation in micro-gel-particles.

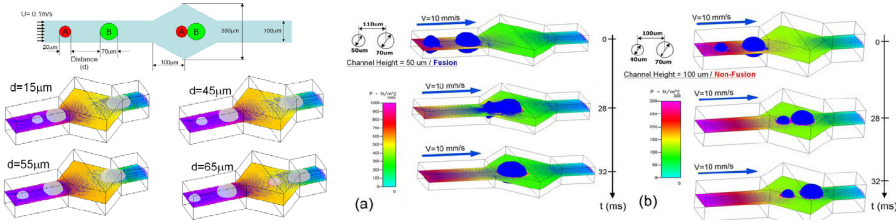

Figure 2: Computational fluid dynamics simulations of droplet fusion efficiency. (a) Effect of droplet separation before expansion, (b) Effect of channel height.

EXPERIMENTAL RESULTS AND DISCUSSION

The microfluidic system was fabricated by soft lithography using polydimethylsiloxane (PDMS) [9]. The channels are of 50µm height for effective fusion. Expansion channels have maximal 200µm width in the middle, and 800µm length. Aqueous droplets of 2% (weight/volume) Na-alginate (Sigma-Aldrich) mixed with PC12 cells and 1% $CaCl_2$ were generated in an oleic acid oil-phase carrier fluid. Precise droplet volume from 60-220pL (Figure 3) were obtained by control of inlet flow rates (10-90µL/hr) while maintaining constant oil flow rate (90µL/hr). Linear dependence of droplet volume on aqueous flow rate with 2pL/(µL/hr) slope was observed.

Generated Na-alginate and $CaCl_2$ droplets were fused in an expansion channel forming Ca-alginate micro-gel-particles with embedded PC12 cells. Oil carrier viscosity and surface tension influence droplet generation and fusion. Oleic acid was found to be more effective than soybean oil for droplet fusion (Figure 4) with over 90% of droplet pairs successfully fused. Half-ring pillar structures (Figure 5) were designed to trap the micro-gel-particles. Culture medium was then infused to replace the oil, once the collection chamber filled with microcapsules. Preliminary viability

tests were carried out through acquiring fluorescent images for stained cells within micro-gel-particles adhered to pillars and trapped within half-rings (Figure 6).

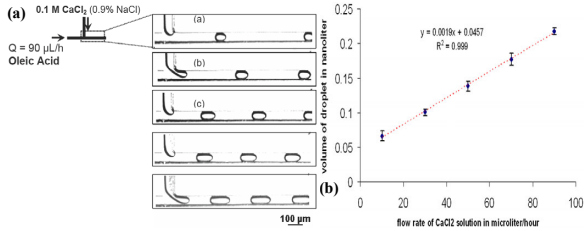

Figure 3: Droplet generation. (a) Photographs of $CaCl_2$ droplets generated at different aqueous flow rates; (b) CaCl2 droplet size vs. infusion rate (Carrier flow: 90µL/hour)

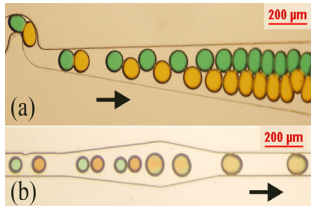

Figure 4:Droplet fusion in (a) Soybean oil (no fusion); (b) Oleic acid, with successful droplet fusion.

Over 50% encapsulated PC12 cells were viable inside micro-gel-particles after trapping. With improved cell densities in micro-gel-particles and collection efficiency in the observation chamber, the device can be further applied to differentiating unique responses of neuronal growth to environmental cues.

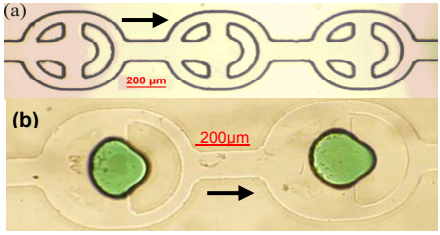

Figure 5: Photomicrographs of (a) in-channel pillar structures and (b) collection of micro-gel particles.

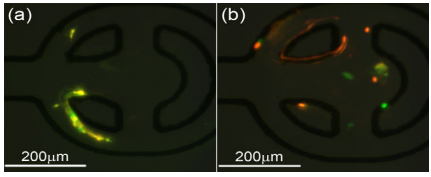

Figure 6: Fluorescent images indicating PC12 viability in the collection chamber (a) Micro-gels adhered to pillar. (b) Trapped in half-rings.

CONCLUSIONS

Droplets of aqueous $CaCl_2$ and Na-alginate were successfully generated, fused to form PC12 cell-laden micro-gel-particles, which were then immobilized for observation in a single-chip microfluidic device. Preliminary viability tests indicate potential of the device for neuron cell culture and analysis of growth and differentiation.

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