MICRO-SANDWICH IN MICROFLUIDICS: 3D BIOPOLYMER MEMBRANES FOR CELL ASSEMBLY

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ABSTRACT

We demonstrate the biofabrication of three dimensional (3D) biopolymer membranes and the assembly of two cell lines in a micro-sandwich structure in microfluidics. A freestanding chitosan membrane was first fabricated by using pH gradients generated at the flow interface of two converging flows. The micro-sandwich was then fabricated by cross-linking alginate on both sides of the chitosan membrane with diffusion of calcium ions through the semi-permeable chitosan membrane. Cell assembly was achieved by blending cells into the alginate solution to embed the target cells into the micro-sandwich alginate scaffolds. The cell assembly process is simple, fast and easy to control.

KEYWORDS: Membrane, Cell assembly, three dimensional, biofabrication

INTRODUCTION

3D microenvironments are crucial for *in vitro* study of cell biology, especially for mammalian cells with limited tolerance to hydrodynamic forces of 2D cell culture systems. In 2D cell culture, cells are displaced as a monolayer on a flat substrate. In 3D cell culture, cells are supported in all directions by either neighboring cells or an extracellular matrix (ECM). Moving from 2D to 3D cell culture systems in microfluidics improves the biological relevance [1]. Various natural and synthetic hydrogels have been incorporated into microfluidic cell culture systems to support cells in 3D. However, in many cases ultraviolet photo-polymerization and thermo-initiative gelation are cytotoxic to cells [2-3]. This study demonstrates a simple "biofabrication" of 3D biopolymer membranes from biocompatilbe substrates using stimuli-responsive materials for cell assembly in microfluidic networks.

THEORY AND EXPERIMENTAL

We have recently reported the biofabrication of freestanding, semi-permeable chitosan membranes in microfluidics using the pH gradients generated at the converging interface between a slightly acidic chitosan solution and a basic buffer solution [4], schematically shown in Fig. 1(a). The membrane fabrication process was achieved by enlisting the unique pH-dependent solubility of chitosan, which transits from soluble polycation to gel structure when pH is higher than the

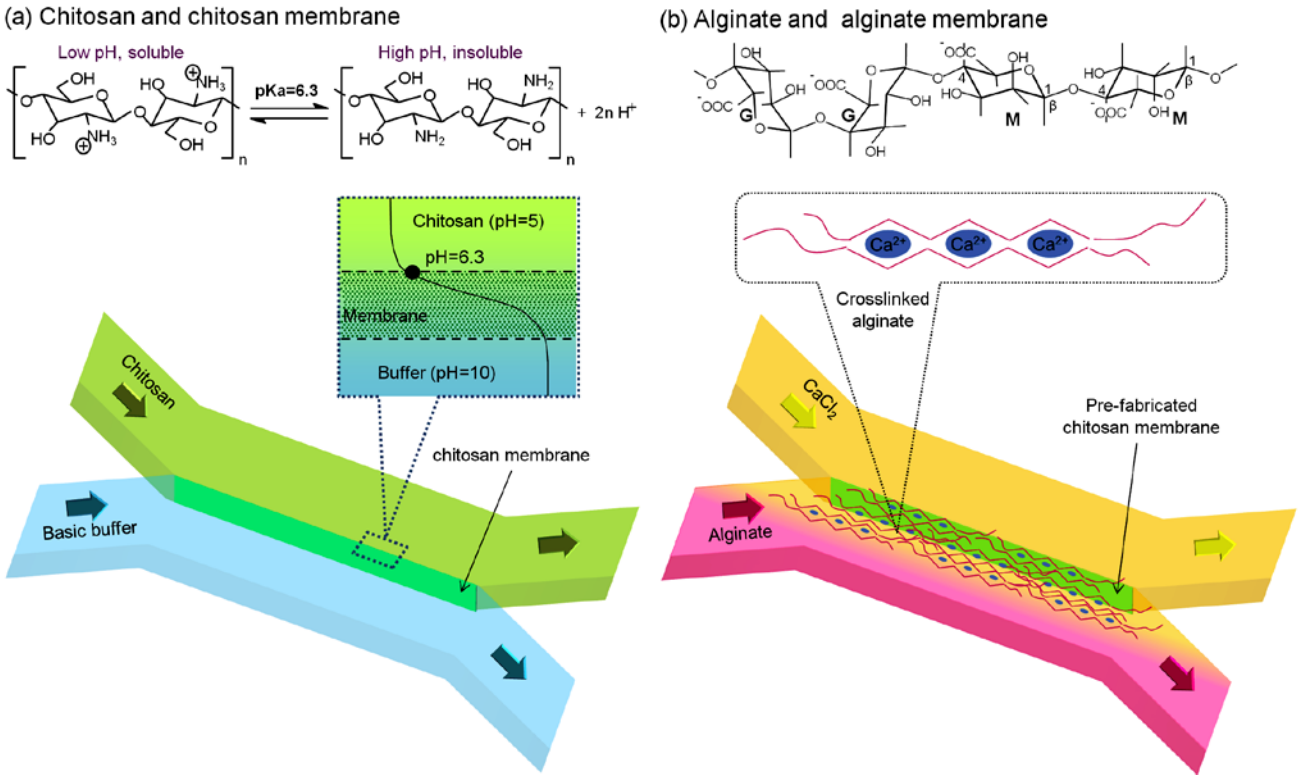

Figure 1: The chemical structures of chitosan and alginate and the schematic biofabrication of biopolymer membranes in microfluidics. (a) A chitosan membrane is first fabricated with local pH gradients generated at the converging flow interface between a slightly acidic chitosan (with a pKa of 6.3) solution and a basic buffer solution. (b) An alginate membrane was fabricated alongside the chitosan membrane with the diffusion of calcium ions through the semi-permeable chitosan membrane.

pKa of 6.3. Here we demonstrate the biofabrication of 3D micro-sandwich scaffolds for cell assembly in polydimethylsiloxane (PDMS) microfluidic devices based on augmenting pre-fabricated semi-permeable chitosan membranes. The microfluidic devices were fabricated with routine soft lithography technique, which has the microchannels of 500-μm wide and 150-μm high. The mechanism of alginate scaffold fabrication is schematically shown in Fig. 1(b), where alginate (1% w/v) is cross-linked into a gel structure along one side of a chitosan membrane by the diffusion of calcium ions (10 mM $CaCl_2$) through the semi-permeable chitosan membrane, which has pore size of a few nanometers [4]. A second alginate scaffold is created on the other side by switching flows, creating a sandwich encompassing the original chitosan membrane. Cells were seeded into both matrices sequentially by blending the target cells with the alginate solutions. Flow rates of the alginate-cell mixtures were below 3 μL/min to ensure successful seeding.

RESULTS AND DISCUSSION

Fig. 2 demonstrates the sequential biofabrication of a micro-sandwich by first fabricating a freestanding chitosan membrane (labeled with TRITC red fluorescence), followed by fabricating two alginate membranes (decorated with 0.2 μm, fluorescence-labeled microspheres) on both sides of the pre-fabricated chitosan membrane. Fig. 3 shows the sequential fluorescent micrographs during the gelation process of alginate membranes on both sides of the chitosan membrane. The thickness of chitosan and alginate membranes is controllable by setting the time period of the gelation process. The alginate gels attach to the chitosan membrane tightly and they form chitosan/alginate complex due to ionic and electrostatic interactions between the polycations (chitosan) and polyanions (alginate).

By using the same mechanism, cell assembly was achieved by blending cells into the alginate solution to embed the target cells in the calcium-crosslinked alginate gel, as commonly used for cell studies in tissue engineering. Fig. 4(a) shows a fabricated chitosan membrane before cell assembly. Fig. 4(b) shows the assembled *E. coli* cells expressing green fluorescent proteins (GFP) (BL21, GFP_{UV}) on one side of the biofabricated chitosan membrane by calcium gelation with

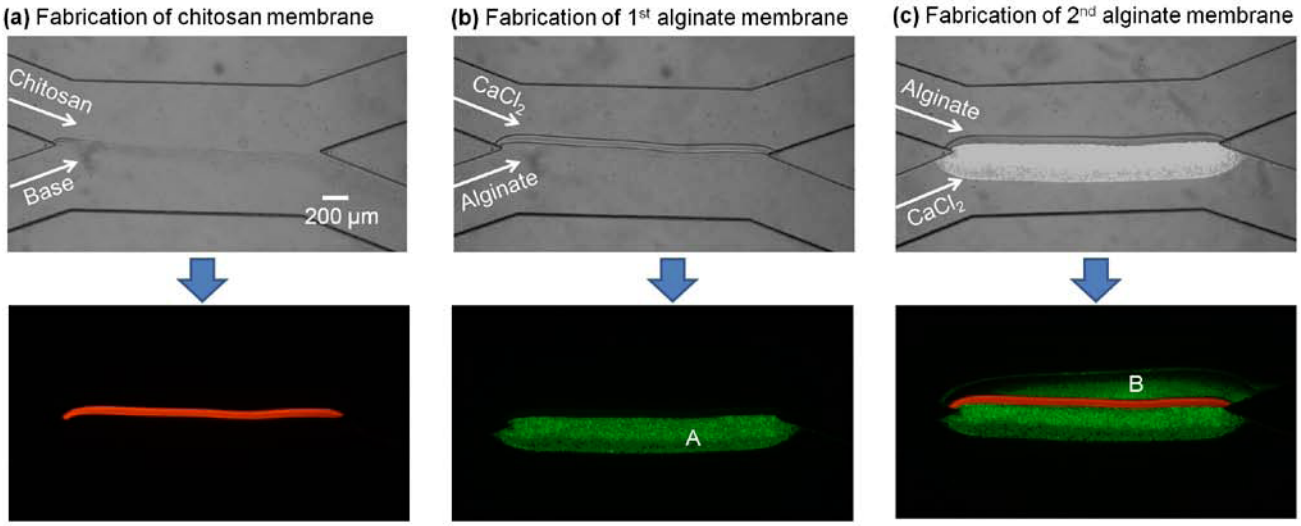

Figure 2: Top view of the sequential biofabrication of a micro-sandwich. (a) Chitosan membrane fabricated as the backbone; (b) Alginate gelation on side A of chitosan membrane by calcium ions diffused through chitosan membrane; (c) Alginate gelation on side B of chitosan membrane by calcium ions diffused through the alginate gel and chitosan membrane.

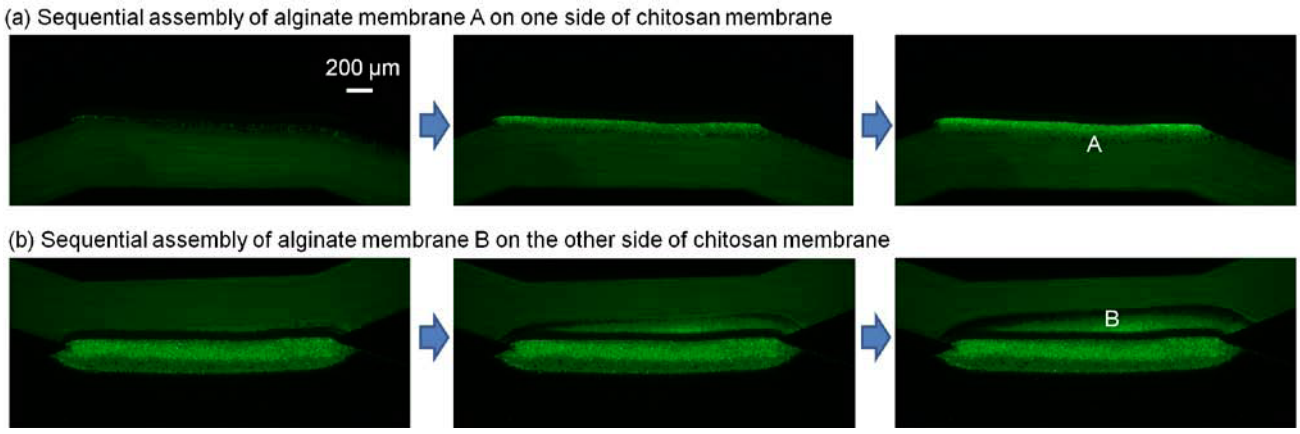

Figure 3: Sequential micrographs showing the gelation process of micro-sandwich membranes. (a) Alginate membrane A on one side of chitosan membrane; (b) Alginate membrane B on the other side.

calcium ion diffused from the other side of the chitosan membrane. By switching the inputs for the alginate/cell mixture and the $CaCl_2$ solution, red *E. coli* cells (BL21, DsRed) were assembled onto the other side of the chitosan membrane (Fig. 4(c)) by calcium gelation as well.

Therefore, the two types of cells were sequentially assembled in the 3D micro-sandwich scaffolds, with a semi-permeable chitosan membrane providing a supporting backbone for the alginate gels (otherwise alginate gels will easily detach from the hydrophobic PDMS microchannel surfaces) and a physical barrier between the two cell types. Nutrients and signal molecules are thereby free to diffuse through the chitosan membrane for cell growth and cell signaling (as demonstrated by the original Ca^{2+} ion diffusion). Importantly, the thickness of the chitosan membrane can be easily tuned with biofabrication time [4], enabling variation in diffusion length between the two alginate membranes. This provides a unique control parameter for cell-to-cell communication studies.

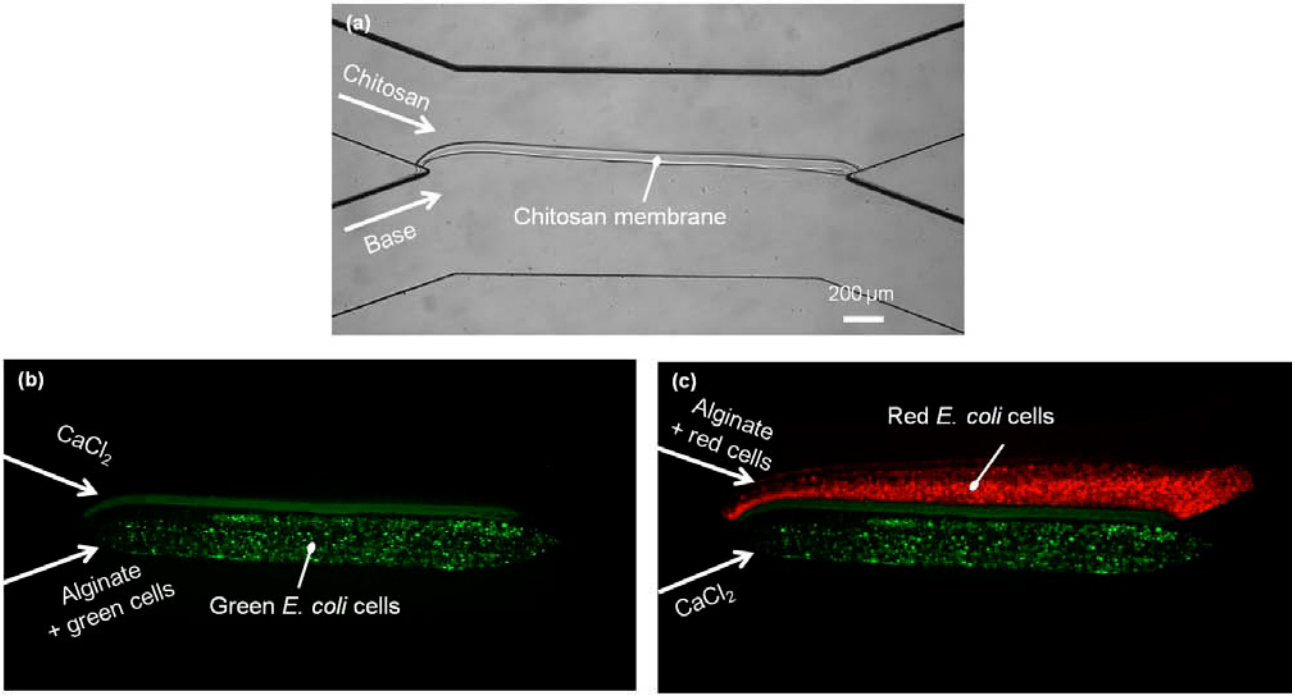

Figure 4: Cell assembly in micro-sandwich membranes. (a) A pre-fabricated chitosan membrane; (b) Assembly of E. coli cells expressing green fluorescent proteins (BL21, GFP_{UV}) in alginate membrane A on one side of the chitosan membrane; (b) Assembly of red E. coli cells (BL21, DsRed) in alginate membrane B on the other side.

CONCLUSION

A novel and simple cell assembly approach was developed for 3D cell cultures within in microfluidic devices. The method incorporates operator-imposed pH gradients at flow interfaces, followed by Ca^{2+} ion gradients enabling diffusion through the pre-fabricated membrane. Cells seeded in such 3D scaffolds are expected to retain biological relevance common among 3D scaffold technologies, but due to the biofabrication method described here, can be placed in configurations that enable detailed study of signaling phenomena to various molecular cues.

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