GENERATION OF CHITOSAN MICROFIBER FOR BIO-ARTIFICIAL LIVER MICROCHIP

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ABSTRACT

We demonstrate a method to create microscale chitosan fiber by combining a microfluidic device and *in situ* formation of a 2D shape by winding around the frame. The diameter of these fibers was found to range from a few tenths to hundredths of a micrometer, and the process was optimized to fabricate long (> 1 meter) fibrous chitosan fibers and to wind them. To estimate the capability of these fibers to act as scaffolding for liver tissue formation, HepG2 cells, which are generally used as model for assay of liver function were seeded onto the fibers and cultured.

KEYWORDS: Chitosan microfiber, Bio-artificial liver chip, Liver specific function.

INTRODUCTION

Off-the-shelf artificial bio-organs have been the dream of mankind for the treatment of diseases, regeneration of lost function and prosthesis[1]. In the engineering of tissue and organs, regeneration of liver specific function is the hottest issue[2]. However, the engineering of 3D liver tissues which regulate specific functions still has many challenges such as the inability to properly select scaffold materials that can control the desired shapes. Despite the limits of pure chitosan, we developed a method to synthesize microscale pure chitosan fibers by employing an optimized microfluidic device and controlling the condition and *in situ* formation of a 2D chitosan fiber plane by winding around the frame. Furthermore, we fabricated a bio-artificial liver (BAL) chip by embedding wound fibers in a poly(dimethylsiloxane) (PDMS)-based fluidic channel and analyzed the albumin secretion and urea synthesis.

EXPERIMENTAL

To generate chitosan fibers of various diameters, we designed the co-axial microfluidic chip for the fiber synthesis (Figure 1(a)), with a modification in the dimension of the glass pipette (tip size of pulled pipette ≈ 300 μm). We used 4% (w/v) chitosan solution in 2% (v/v) acetic acid (AA) as the core fluid and 10% (w/v) sodium triphosphate pentabasic (STP) as the sheath fluid to synthesize the chitosan fibers from the interface of the two liquids. By changing the flow rate of the core and sheath fluids, we could change the diameter of the chitosan fibers. The extruded chitosan fibers were wound (fiber length: around 40 cm) onto the windowed polystyrene (PS) frame (Figure 1(b)). Figure 1(c) shows scanning electron microscope (SEM) images of and 3-dimensionally crossed fibers. By adjusting the flow rates of the core and sheath fluids, we could generate fibers of 70 to 150 μm in diameter, and the fibers are continuously extruded without disconnection. Also, we measured the diffusion coefficient of microscale chitosan fiber by fluorescence recovery after photobleaching (FRAP).

Chitosan microfibers were evaluated as scaffolds for liver tissue engineering by culturing HepG2 cells. As a control, cells were seeded at a density of 4.42×10^5 cells/well on cell culture dishes and cultured in 5 mL minimum essential medium. In order to provide the 3-dimensional culturing condition, wound chitosan fibers with cells were also cultured in coverglass-bottom dishes. For further application of our technology, we designed the BAL chip to embed the chitosan fiber wound PDMS frame. After embedding the fiber-wound frame, we seeded the HepG2 cells and perfused the culture medium using a syringe pump. The BAL chip was maintained in an incubator. The fresh media was supplied at a flow rate of 0.1mL/hr, and the albumin secretion and urea synthesis were measured at days 1, 3 and 5 after seeding.

RESULTS AND DISCUSSION

Diameter control of chip-based chitosan fibers was determined by core and sheath flow rates. As the core flow rate increased, at constant sheath flow rate, the diameter was increased. In contrast, as the sheath flow rate increased, at constant core flow rate, the diameter decreased. We utilized core flow rates of 4.2, 4.8 and 5.4 mL hr^{-1} and sheath flow rates of 30, 40, and 50 mL hr^{-1}. The chitosan fibers were divided into 2 groups: those generated at a constant core flow rate of 4.2 mL hr^{-1} (A group) and those generated at a constant sheath flow rate of 30 mL hr^{-1} (B group) as shown in Figure 2. To analyze the diffusion properties within the microfibers, we measured the diffusion coefficient of microscale chitosan fibers by using FRAP technique. Figure 3(c) illustrates the fluorescence recovery curves of fluorescein isothiocyanate (FITC)-dextran (70 kDa) loaded chitosan fibers. The inset of Figure 3(c) shows micrographs before and after laser radiation, and the black point represents the spot on which the laser was focused. Figure 5 shows a micrograph of aggregated cells on the fiber. The cells were stained by a live/dead assay after culturing for 5 days *in vitro*, showing that cell viability was over 95%. Although cells cultured on Petri dishes did not self-aggregate, cells cultured on chitosan mi-

crofibers were spontaneously self-aggregated, forming spheroid-like structures. The hepatic function of HepG2 cells on the chitosan microfiber was evaluated by measuring albumin secretion and urea synthesis over 5 days. We found that HepG2 cells on chitosan fibers showed much higher albumin secretion as compared to the control as shown in Figure 4. It was revealed that albumin secretion on chitosan microfibers was 27.7% than culture dish group. Similarly, the urea synthesis rate of cells on chitosan microfibers was higher than culture dish group (6 % higher). The liver function of hepatocytes could be improved by spheroid formation and glycosaminoglycans.

As a proof-of-the concept, we applied produced chitosan fibers to BAL chip. The poly(dimethylsiloxane) (PDMS) microfluidic platform was fabricated (Figure 6(a)). The chitosan fibers wounded on a frame were embedded in a PDMS platform, and HepG2 cells were seeded and cultured forming clustering around the microfibers. The fresh medium was perfused at a flow rate of 0.1mL hr^{-1} and the albumin secretion and urea synthesis were measured at day 1, 3 and 5 after cell seeding. As shown in Figure 6(b), albumin secretion was increased to 33%, while urea synthesis was increased to 27% after culturing for 5 days *in vitro*. Therefore, self-aggregated spheroids of HepG2 cells adhered on chitosan microfibers could be potentially useful for controlling hepatic function in a well-defined microenvironment.

CONCLUSIONS

We developed pure chitosan-based microfibers in a microfluidic device. In this study, we demonstrated that FRAP-based diffusion properties of pure chitosan microfibers. It was revealed that the diffusion within chitosan microfibers was more active as compared to the calculated diffusion coefficient. HepG2 cells cultured on chitosan-based microfibers were self-aggregated with the spheroid shape, showing higher liver specific function that was confirmed by albumin secretion and urea synthesis. Therefore, our method to create pure chitosan-based microfibers in a microfluidic device could be a potentially useful tool for liver tissue engineering applications.

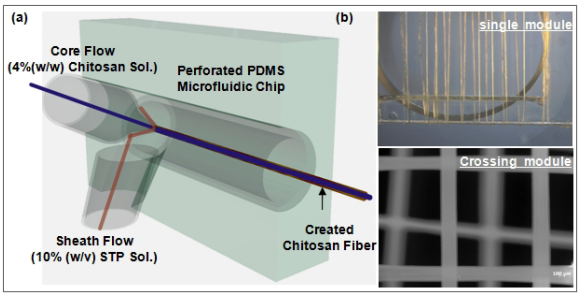


Figure 1. Schematic of the generation of a chitosan microfiber within microfluidic chip which has two stages: generation of the core and sample flow.

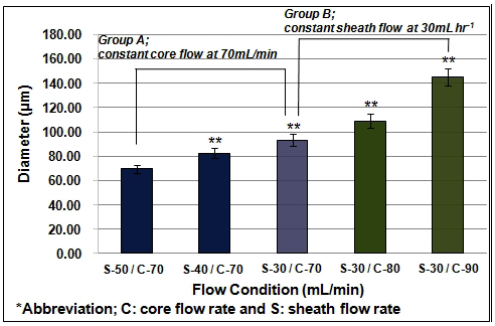


*Figure 2. Variation of controlled fiber diameter as a function of core and sheath flow rates (n = 3, * indicates P < 0.01).*

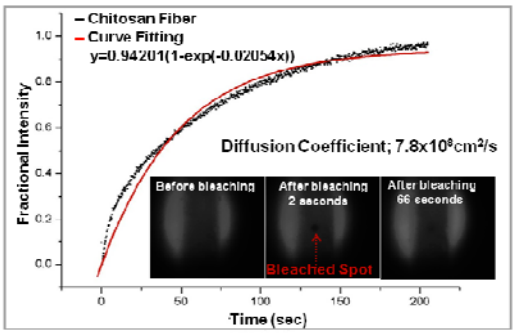


Figure 3. FRAP data for diffusion of 70 kDa FITC-dextran in chitosan fibers; the fluorescence was fully recovered after photobleaching

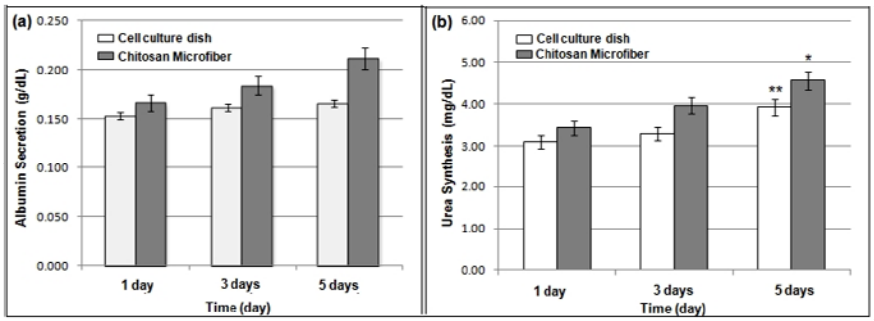


*Figure 4. Albumin secretion and urea synthesis levels synthesized in control HepG2 cells and cells cultured on chitosan fibers. Each point represents the mean of 5 samples (n = 5, * indicates P < 0.05, and ** indicates P < 0.01).*

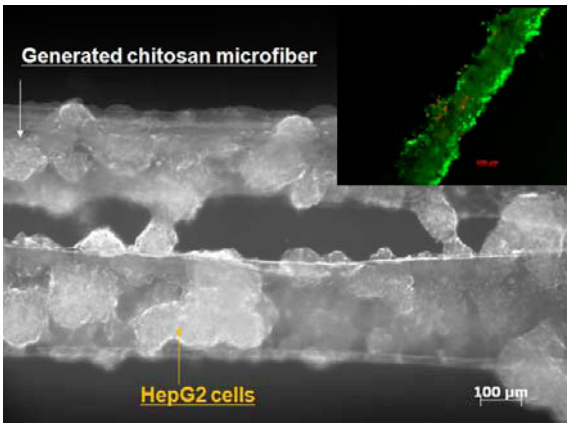
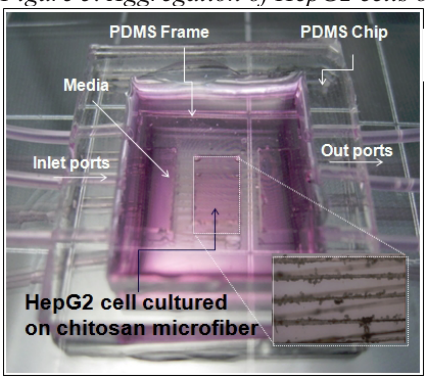


Figure 5. Aggregation of HepG2 cells on chitosan fibers with fluorescent micrograph.

(a)



(b)

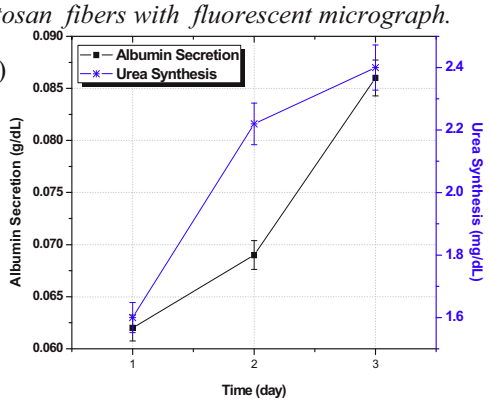


Figure 6. Picture of bio-artificial liver chip and inset shows the HepG2 cells clustered on the chitosan fibers.

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