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Chitosan microfiber fabrication using microfluidic chips of different sheath channel angles and its application on cell culture

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Abstract

In this study, we successfully produced the chitosan microfibers using the proposed various angles of microfluidic chip, which was also been simulated. By controlling the core and sheath flow rates, we were able to generate laminar flow of different diameters from 15 μm to 40 μm. And the diameter of chitosan microfiber was measured from 20 μm to 50 μm. The microchannel of angle 30° could produce chitosan laminar flow of a smaller diameter than the angle 60° and angle 45° at the fixed flow rates. Finally, the chitosan microfiber was chosen as scaffold and the schwann cell and fibroblast cell with chitosan microfibers were used for cell culture to test effect in tissue engineering application.

Keywords: Chitosan, Microfluidic, Microfibers, Schwann cell, Fibroblast cell

1. Introduction

Chitosan is biodegradable, biocompatible, which makes it a suitable compound for biomedical applications, such as wound management, tissue engineering for nerve regeneration in clinical applications and drug delivery vehicle. Beside, chitosan compound also could application in the environmental protection.

Recently, more and more implantable applications of chitosan have been reported [1]. The bio-properties of chitosan had noted that chitosan fiber has been further fabricated to other medical products. However, the chitosan as the biofiber has visible shortages in its dimension unstably and mechanical properties weakly. Hence, it was observed that using the reagents to cross-link chitosan could improve the tenacity. Application of sodium tripolyphosphate (STPP) for crosslinked chitosan had reported [2]. STPP is a non-toxic polyanion which could take place the cross-linking reaction with chitosan [3, 4].

In tissue engineering application, smaller microfiber has many advantages, such as the bridging of large gaps between the cut ends of the transected tissue or being used as scaffolds for tissue regeneration. For this reason, we will apply to the practiced microfluidic technology [5-8] to achieve this objective. In our study strategy, we discussed the geometric design for influence of the chitosan size and used the devices to generate the chitosan microfibers. Finally, the produced microfibers were used to culture the cells and demonstrate the study could apply to scaffold in tissue engineering.

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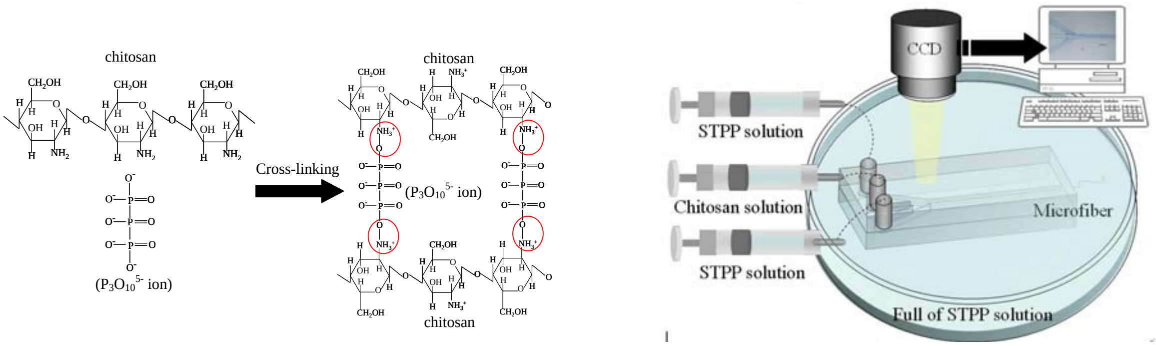
2. Material and method

2.1 Design, Simulation and Fabrication

CFDRC-ACE software is used to simulate the diameter variation of laminar flow in the different angles (including 30°, 45°, 60°). In the fabrication process of microfluidic chip, the various shapes (angle of 30°, 45° and 60°) were designed by using the AutoCAD® 2006, and then SU-8 microchannel mold was fabricated by using MEMS technology. Moreover the width and depth of microchannel are both about 100 µm. The soft lithography was used to form the PDMS microfluidic chip. And the microchannel width was 100 µm measured by optical microscope.

2.2 Experimental Methods

In the experiment, the procedure is as follows. First, the center and side channels are set up with 0.25% chitosan solution (core flow) and 10% STPP solution (sheath flow), respectively. Second, the fluid is injected into the microfluidic chip by syringe pumps (Kdscientific KDS230) programmed by a PC. The hydrodynamic focusing was occurred at a cross-junction position by STPP solution, enabling the construction of chitosan laminar flow along the microchannel. The STPP solution could make the chitosan generate cross-linking reaction and make the chitosan solidify since it contains the $P_3O_{10}^{5-}$ ion. So, the laminar flow of the chitosan could take place the cross-linking reaction by STPP solution to produce the chitosan microfibers, as shown in Fig. 1.



(a) (b)

Fig. 1. (a) The mechanism of chitosan cross-link reaction: the chemical reaction in the reservoir is that the $P_3O_{10}^{5-}$ ions of STPP, indicating the formation of chitosan microfibers. (b) Schematic drawing of the formation of laminar flow in a microfluidic chip. Based on microfluidic to control sheath force, a continuous chitosan laminar flow can be obtained.

3. Results and discussions

3.1 Simulation of the Microfluidic Chip

In the simulation results, we found the laminar flow was generated at cross-junction position by hydrodynamic focusing, as shown in Fig. 2(a)-(c). According to the same density distribute, the different diameter sizes of laminar flow could be observed. Figure 2(d) shows the relationship between the different cross-junction microchannel angle (30°, 45° and 60°) and the diameter size of laminar flow, respectively. Under the core flow at 2.672×10^{-3} m/s, and sheath flow at 2.905×10^{-3} m/s, the chitosan laminar flow diameter decreases as the cross-junction microchannel angle decreases. The results show that the smaller chitosan laminar flow diameter was obtained in the channel of small angle. We found that microfluidic chip with angle 30° could generate laminar flow of smaller diameter size compare to other angles.

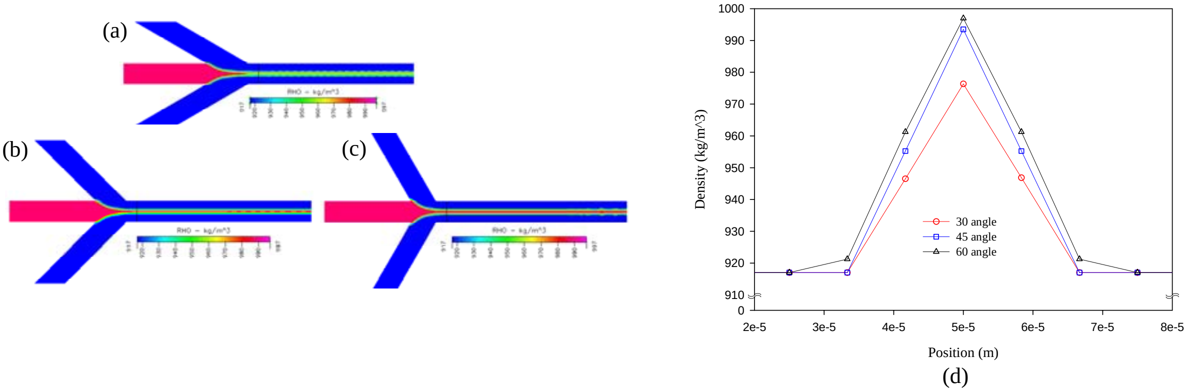


Fig. 2. The simulation results of laminar flow generation (a) 30°, (b) 45°, (c) 60°, (d) the simulation result of relationship between the 30°, 45° and 60° angle and laminar flow.

3.2 Formation of Chitosan Laminar Flow

The laminar flow of chitosan solution was formed by altering chitosan solution and STPP solution flow rates, respectively. By controlling the chitosan and STPP solution flow rate, we could adjust the diameter size of laminar flow, the diameter of chitosan laminar flow was ranged from 15 µm to 40 µm. In devices of different angles, we could observe the same condition, as shown in Fig. 5.

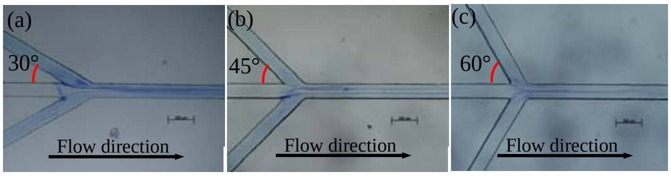


Fig. 3. Chitosan solution was hydrodynamically focused at the non-vertical angle of the channel (a) 30°angle chip, (b) 45° angle chip, (c) 60°angle chip.

3.3 Influence of Channel Angle

Cross-junction microchannel with angle 30°, 45° and 60° were designed for investigating the influence of the generation of laminar flow. By fixing the core flow rate and increase the sheath flow rate, the chitosan laminar flow diameter could decreases in three different angle channel design chip, as shown in Fig. 4(a). By fixing the sheath flow rate and decrease the core flow rate, the chitosan laminar flow diameter could decreases in three different angle channels, as shown in Fig. 4(b). By fixed the core flow and sheath flow rate, we could find the smaller chitosan laminar flow diameter in the angle 30°. So the result shows that microfluidic chip with angle 30° microchannel could generate smaller diameter of laminar flow than other angles.

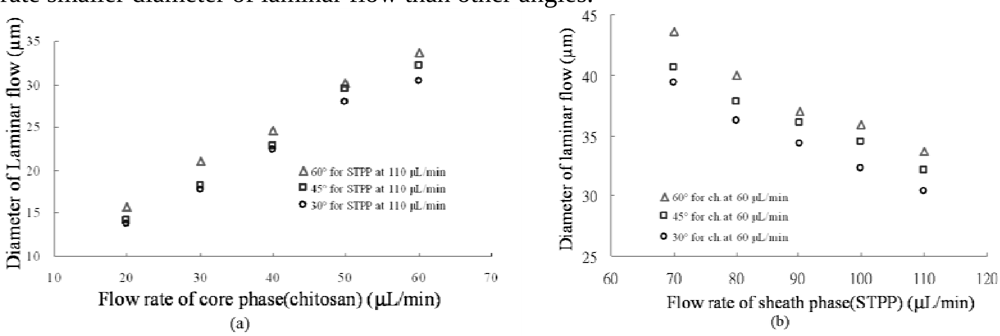


Fig. 4. (a) The relationship between diameter of laminar flow and core and sheath flow rate and (b) the relationship between diameter of laminar flow and different angles.

3.4 Fully Cross-Linked STPP-Chitosan Microfibers and Cell Cultured with Chitosan Microfibers

The chitosan could have cross-link reaction with $P_3O_{10}^{5-}$ of 10% (w/v) STPP solution, and then the chitosan laminar flow was transformed into STPP-chitosan microfibers. The gelled chitosan microfibers were ejected from the outlet of the micro-channel to the reservoir and collected in the STPP solution reservoir. The fully cross-linked microfibers were strong enough and were not aggregated in the 10% STPP solution reservoir. By using SEM for observing the STPP-chitosan microfibers, e the surface of the chitosan microfiber could be observed, as shown in Fig. 5(a)(b). After 24 hours, the schwann cell and fibroblast cell could adhesion on the surface of the chitosan microfibers as shown in Fig. 5(c)(d). But in continuous culture, the Schwann cells have shown the low proliferation. According our observation, the chitosan microfibers were too thin to cause the cell proliferation difficultly. Those results show that the chitosan microfibers could provide a good growth environment for cells, but the size of the chitosan microfibers fabricate by PDMS chip was not fit for cell proliferation.

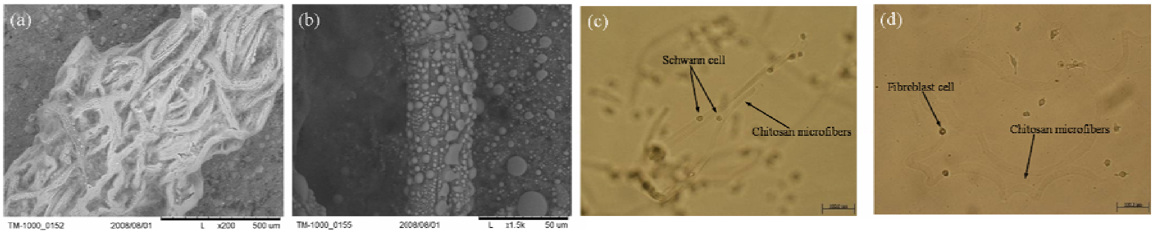


Fig. 5. (a)(b) The SEM morphology of the STPP chitosan microfibers. (c)(d) The schwann and fibroblast cells culture with chitosan microfibers.

4. Conclusions

In this study, we have successfully used the sheath force to generate chitosan laminar flow and chitosan microfibers and demonstrated the influence of angle on the generation. We have found the angle 30° that could generate smaller chitosan laminar at fixed core and sheath flow rate. The chitosan laminar diameter size was ranged form 15 μm to 40 μm. After cross-linking reaction, the chitosan microfiber diameter size was increased from 20 μm to 50 μm. Finally, the chitosan microfibers were applied to culture cells. However, the microfibers are too thin for the cells to culture on chitosan microfibers. In the future, we would use the microfibers to be scaffold, and apply them in the tissue engineering.

References

[1] E. Khor and L. Y. Lim. Implantable applications of chitin and chitosan. *Biomaterials* 2003;**24**:13-22.
[2] S. Shiraishi, T. Imai and M. Otagiri. Controlled release of indomethacin by chitosan-polyelectrolyte complex: optimization and in vivo/evalution. *Journal of controlled release* 1993;**25**:217-225.
[3] F.L. Mi, S.S. Shyu, T.S. Lee and T.B. Wong. Kinetic Study of chitosan-tripolyphosphate complex reaction and acid-resistive properties of the chitosan-tripolyphosphate gel beads prepared by in-liquid curing method. *Journal of Polymer Science: Part B: Polymer Physics* 1999;**37**:1551-1564.
[4] S.T. Leea, F.L. Mia, Y.J. Shena and S.S. Shyub. Equilibrium and kinetic studies of copper (II) ion uptake by chitosan-tripolyphosphate chelating resin. *Polymer* 2002; **42**:1879-1892.
[5] K.S. Huang, T.H. Lai and Y.C. Lin. Manipulating the generation of Ca-alginate microspheres using microfluidic channels as a carrier of gold nanoparticles. *Lab Chip* 2006;**6**:954-957.
[6] K.S. Huang, T.H. Lai, Y.C. Lin. Using a microfluidic chip and internal gelation reaction for monodisperse calcium alginate microparticles generation. *Frontiers in Bioscience* 2006;**12**:3061-3067.
[7] C.H. Yang, K.S. Huang, P.W. Lin and Y.C. Lin. Using a cross-flow microfluidic chip and external crosslinking reaction for monodisperse Tpp-chitosan microparticles. *Sensors and Actuators B: Chemical* 2007;**124**:510-516.
[8] C.H. Yeh, Y.C. Lin, 2008. Using a cross-flow microfluidic chip for monodisperse UV-photopolymerized microparticles. *Microfluidics and Nanofluidics* 2009;2:277-283.