Original Article

Novel flow microreactor with blood capillary network-like microchannels

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Abstract

A flow microreactor with microchannels has been widely used for biocatalytic synthesis. For high throughput production, multiple microreactors are assembled into a single module to increase the number of microchannels available for biocatalytic synthesis. However, the assembled microreactors inevitably waste much their own space for the precise assembly (e.g. space for screw holes), indicating difficulty of drastic increase in density of microchannels. Increase in the microchannel density means increase in the performance per unit volume of the microreactors. In this study, we propose a novel microreactor that has a great potential to increase in the microchannel density. Blood capillary network-like microchannels in polydimethylsiloxane (PDMS) matrix were created using sacrificial cotton candy microfibers. The mean residence time of the microreactor prepared using 150 ± 20 mg cotton candy (MR150) was about twice as long as that prepared using 20 ± 5 mg cotton candy (MR20). Moreover, productivity of biodiesel of MR150 was about three times higher than that of MR20. These results show that the density of microchannels and performance per unit volume of our microreactor can be improved only by increasing the amount of cotton candy enclosed in PDMS.

Key words

microreactor, microchannel, capillary network, enzyme, biodiesel

1. Introduction

Flow microreactors have contributed greatly to biotechnology and biomedical research (Bolivar and Nidetzky, 2013). A microchannel reactor is one of flow microreactors and has been widely used for biocatalytic synthesis (Bolivar et al., 2017; Carvalho et al., 2017; Tamborini et al., 2018). The microchannels are created on a flat plate (Song et al., 2012; Stojkovič et al., 2011). For high throughput production, multiple microreactor plates are assembled into a single module to increase the number of microchannels available for biocatalytic synthesis (Thomsen and Nidetzky, 2009; Lawrence et al., 2013). However, the assembled microreactors inevitably waste much their own space for the precise assembly (e.g. space for screw holes), indicating difficulty of drastic increase in density of microchannels. Increase in the microchannel density means increase in the performance per unit volume of the microreactors.

The microchannel network with highest channel density is a blood capillary network (Borenstein et al., 2002). In the human body, the distance between capillary blood vessels of 10-20 μm in diameter is only 100-200 μm to supply sufficient oxygen and nutrients to the cells (Carmeliet and Jain, 2000). Many researchers have attempted to create artificial microchannel network that mimics the blood capillary network (Takei et al., 2012; 2016; Wang et al., 2021). Bellan et al. reported blood capillary network-like microchannels in polydimethylsiloxane (PDMS) matrix created using sacrificial cotton candy (Bellan et al., 2009). This method has a great potential to increase in the microchannel density in PDMS matrix by increasing the amount of cotton candy enclosed in PDMS. To our best knowledge, the dense microchannel network has not been applied to a microreactor. Hence, in this study, we examined a potential of the microchannel network as a microreactor. Specifically, we focused on verifying that the density of microchannels and the performance of the microreactor increased with increase in the amount of cotton candy enclosed in PDMS. Biocatalytic transesterification using lipase for biodiesel production was adopted as the test reaction (Kawakami et al., 2011; Sakai et al., 2010).

2. Materials and methods

2.1 Fabrication of microchannel reactor

Microchannel network were created in PDMS matrix according to the procedure reported by Bellan et al. with minor modification (Bellan et al., 2009). Sucrose cotton candy (melt-spun sugar microfiber) was made using a commercially available cotton candy machine. A wire (1 mm in diameter) was passed into a silicone tube (3 mm in outer diameter, 1 mm in inner diameter) and then wrapped with cotton candy (Figure 1 (a)). Liquid dimethylsiloxane and polymerization initiator (Sylgard 184 silicone elastomer kit, Dow Corning Corp., Midland, MI, USA) were mixed in a 10:1 ratio by weight, and 15 ml of the monomer solution was poured into a plastic petri dish containing cotton candy and left at about 100 Pa for 24 h at room temperature. The polymerization was then completed by leaving at ambient pressure and 75 ℃ for 2 h. For the formation of the microchannels, after removing the wire from the silicone tube to create millimeter-sized channels, the PDMS was immersed in a bath of water at 80 ℃ for 1 h to dissolve the cotton candy. Cotton candy used and cross-sections of PDMS after being immersed in hot water were observed using a scanning electron microscope (SEM, S-3000, Hitachi Ltd., Tokyo, Japan).

2.2 Pulse input test

Distilled water was kept flowing in the microchannel network at 1 μl/min using a syringe pump. Three microliter of an aqueous gardenia dye solution (10 mg/ml) was injected into the inlet of the microreactor. Every 1 min after the dye injection, 1 μl of sample was collected from the outlet. The sample (1 μl) was diluted with distilled water (3 μl) and the absorbance at wavelength 533 nm was measured using a spectrophotometer (NanoDrop-8000, Thermo Fisher Scientific, Waltham, MA, USA).

2.3 Determination of amount of lipase adsorbed to PDMS surface

The sucrose was added to a stainless steel cylindrical container (5 cm in diameter) and left at 200 °C for 1 h to melt. After melting, it was cooled down at room temperature to prepare solid sugar plate with a thickness of 10 mm on the bottom of the cylindrical container. Liquid dimethylsiloxane mixed with polymerization initiator was poured onto the sugar plate, and the monomer solution was solidified according to the above-mentioned procedure. The interfacial area between the PDMS plate and the sucrose plate was 19.7 cm^2. After removing the bottom plate of the cylindrical container, the sugar plate was completely removed by immersing the container in hot water several times. Five milliliter of Ca^{2+}/Mg^{2+}-free phosphate-buffered saline (PBS(–), pH7.4) containing 8 mg/ml lipase (Lipase PS-SD from B. cepacian, Amano Enzyme Inc., Nagoya, Japan) was added on the PDMS surface that had been in contact with the sugar plate, and then gently shaken at 25 °C for 1 h. After removing the aqueous lipase solution, 5 ml of a fresh lipase solution was added and then gently shaken at 25 °C for 1 h. This process was repeated four more times. The lipase concentration of the solution after 1 h of immersion was determined by the Bradford assay. The amount of lipase adsorbed on the PDMS surface after each immersion was calculated from the concentration. The total amount of lipase adsorbed after 6 times of immersion was calculated by adding up the amount at each immersion.

2.4 Productivity of biodiesel

Microreactors with different channel densities were fabricated using 20 ± 5 mg or 150 ± 20 mg of cotton candy. An aqueous lipase solution (8 mg/ml) was kept flowing in the microchannel network at a flow rate of 14 μl/min for 12 h at 25 °C to immobilize lipase on the channel surface by hydrophobic interaction. Ten milliliter of 1-butanol, which is miscible with water and rapeseed oil (Riken, Saka, Japan), was flowed into the channel at a flow rate of 0.1 ml/min so that a mixture of rapeseed oil/1-butanol/water could be flowed. The mixture of rapeseed oil/1-butanol/water with a molar ratio of rapeseed oil to 1-butanol of 1:3 and a water concentration of 1wt% was fed into the microchannel at a flow rate of 5.9 μl/min for 30 min at 25 °C (Kawakami et al., 2011; Sakai et al., 2010). The concentration of C18 butyl ester was measured using a gas chromatograph (Clarus500, PerkinElmer Co., Ltd., Waltham, MA, USA). A calibration curve for n-butyl esters was obtained from butyl oleate.

2.5 Statistical analysis

Data are presented as mean ± deviation. Statistic differences between two groups were determined using a two-tailed Student's *t*-test.

3. Results and discussion

This research used cotton candy microfibers as a template of blood capillary network-like microchannels. The diameter of the microfibers was 33 + 31 μm (n = 100) (Figure 1 (b)). Many branches were observed in the microfibers. This suggests that microchannel network with many branches can be created using the microfibers. The cross-section of PDMS without cotton candy had a smooth surface (Figure 1 (c)). On the other hand, numerous microchannels with a diameter of 26 ± 35 μm were observed in the cross-section of PDMS containing cotton candy after immersion in hot water. The diameter shows that the microchannels were formed by dissolving cotton candy in hot water, as reported by Bellan et al (2009).

Next, two types of microreactors with different microchannel densities were prepared using 20 ± 5 mg (MR20) or 150 ± 20 mg cotton candy (MR150). The theoretical microchannel volume and volume ratio of microchannel to microreactor are shown in Table 1. In order to visualize the microchannels, India ink was flowed from the inlet into the channel at a flow rate of 20 μl/min (Figure 1 (d)). We confirmed that the ink passed through the microchannel and eventually came out through the outlet.

Mean residence time of both microreactors was determined by pulse input test. A small amount of aqueous dye solution was injected into the inlet of the microreactor while keeping water flowing at a flow rate of 1 μl/min. Figure 2 shows the relationship between the absorbance of sample obtained at the outlet and the time after the dye injection. The mean residence time of MR150 determined by integrating the data in Figure 2 was about twice as long as that of MR20 (n = 3, Table 1). These results indicate that the mean residence time can be increased by increasing the amount of cotton candy enclosed in PDMS.

Although the theoretical microchannel volume of MR150 was about seven times larger than that of MR20, the increase in the mean residence time of MR150 over MR20 was only about twofold. As mentioned above, the channel network should have numerous branches. The branches allow water to flow along the shortest route where the pressure drop is the lowest. This should be the reason why the mean residence time was not proportional to the theoretical microchannel volume.

Next, a biocatalytic reaction for biodiesel production was adopted to compare the performance of both microreactors.

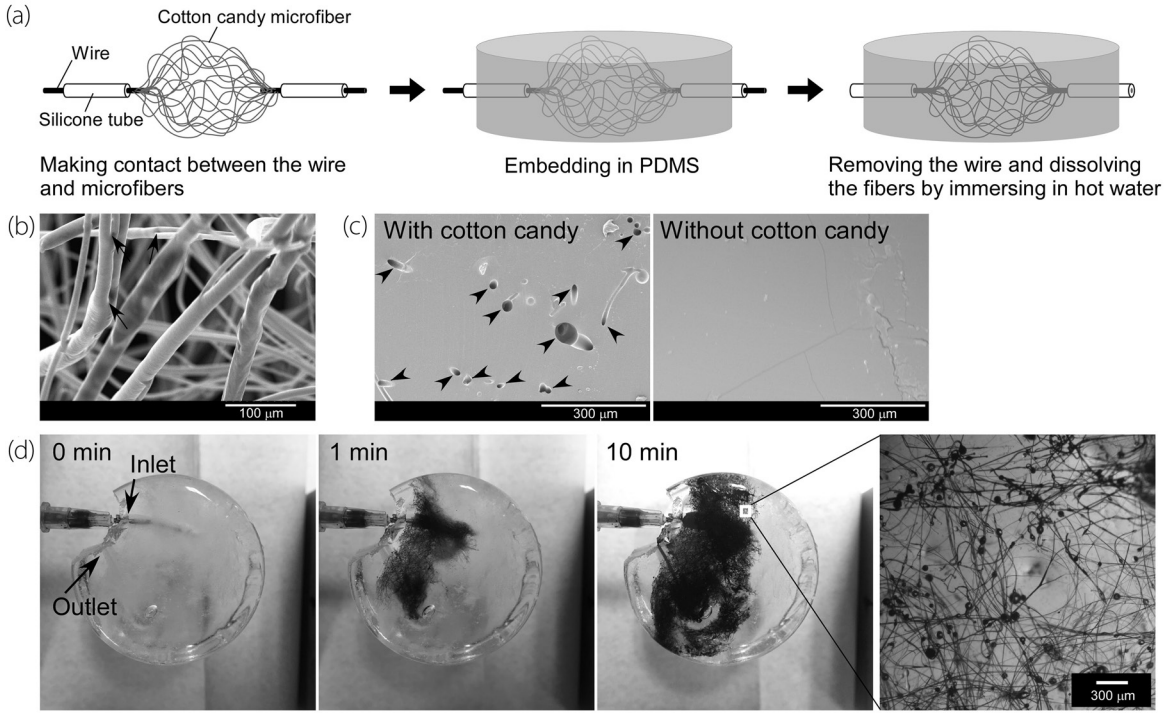

Figure 1: (a) Procedure for creation of blood capillary network-like microchannels in PDMS matrix using cotton candy; (b) SEM image of cotton candy microfibers. Arrows show branch points; (c) SEM image of cross-sections of PDMS without cotton candy and PDMS with cotton candy after immersion in hot water. Arrow heads show cross-sections of microchannels; (d) Progressive perfusion of India ink throughout the microchannel network and magnified image

Table 1: Characteristics of microreactors in this study

	MR20	MR150
Weight of cotton candy used (mg)	20 ± 5	150 ± 20
Theoretical microchannel volume (μl) [a]	9 – 16	81 – 107
Theoretical volume ratio of microchannel to microreactor (%) [b]	0.6 – 1.1	5.4 – 7.1
Mean residence time at a flow rate of 1 μl/min (min)	5.9 ± 1.0 [c]	10.3 ± 0.5

Notes: [a] The volume was calculated based on density of sucrose (1.59 g/cm^3). [b] The volume was calculated by dividing theoretical channel volume by PDMS volume (15 ml) (≈ microreactor volume). [c] $p < 0.01$ v.s. MR150.

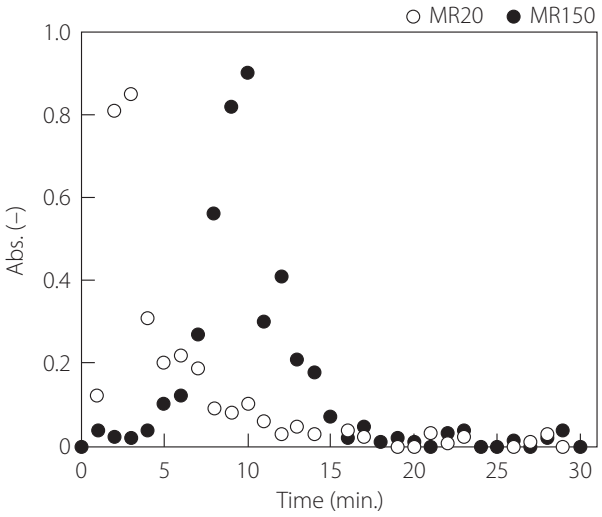

Figure 2: Relationship between the absorbance of sample obtained at the outlets of microreactors and the time after the dye injection into the inlets

Based on the fact that PDMS has hydrophobic nature, lipase was immobilized onto the microchannel surface by physical adsorption through hydrophobic interaction (Kim et al., 2006). Because it was extremely difficult to directly determine the amount of lipase adsorbed onto the microchannel surface, we examined the amount in another approach described in "Determination of amount of lipase adsorbed to PDMS surface" section. The examination shows that the amount reached steady value within 6 times of immersion of PDMS surface in an aqueous lipase solution and the steady amount was 76 ± 10 μg/cm^2-PDMS (n = 3). The microchannel surface would have the same amount of lipase. A mixture of rapeseed oil/1-butanol/water was flowed into the microchannels for biodiesel production. The very low concentration of water (1 wt%) in the mixture indicates the negligible amount of lipase desorbed from the channel surface (Sakai et al., 2010). Figure 3 shows the concentration of butyl oleate (biodiesel) in the sample collected from the outlet after 30 min of perfusion of the mixture (n = 5). The butyl oleate concentration of MR150 was about three times higher than that of MR20. These results show that the performance per unit volume

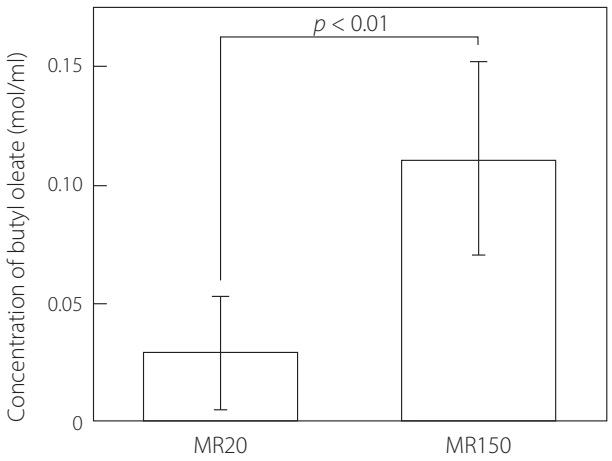

Figure 3: Productivity of biodiesel of microreactors

of our microreactor can be improved only by increasing the amount of cotton candy enclosed in PDMS.

In Figure 2, the mean residence time of MR150 was about two times larger than that of MR20, but the biodiesel production capacity of MR150 was about three times higher than that of MR20. This would be due to the fact that the flow rate was increased from 1 μl/min (for determination of the mean residence time) to 5.9 μl/min (for biodiesel production). The increase in the flow rate (applied pressure) would enable the liquid to flow into the microchannels that had not been perfused when the mean residence time had been determined. This indicates that increased flow rate (applied pressure) would be effective to improve use efficacy of microchannels.

4. Conclusion

We focused on verifying that the density of microchannels and the performance of our microreactor increased with increase in the amount of cotton candy enclosed in PDMS. The mean residence time of MR150 was about twice as long as that of MR20. Moreover, productivity of biodiesel of MR150 was about three times higher than that of MR20. These results suggest that drastic increase in the performance of the microreactor would be achieved by increase in the amount of cotton candy used.

References

Bellan, L. M., Singh, S. P., Henderson, P. W., Porri, T. J., Craighead, H. G., and Spector, J. A. (2009). Fabrication of an artificial 3-dimensional vascular network using sacrificial sugar structures. *Soft Matter*, Vol. 5, 1354-1357.

Bolivar, J. M., Luley-Goedl, C., Leitner, E., Sawangwan, T., and Nidetzky, B. (2017). Production of glucosyl glycerol by immobilized sucrose phosphorylase: Options for enzyme fixation on a solid support and application in microscale flow format. *Journal of Biotechnology*, Vol. 257, 131-138.

Bolivar, J. M. and Nidetzky, B. (2013). Smart enzyme immobilization in microstructured reactors. *Chimica Oggi-Chemistry Today*, Vol. 31, 50-54.

Borenstein, J. T., Terai, H., King, K. R., Weinberg, E. J., Kaazempur-Mofrad, M. R., and Vacanti, J. P. (2002). Microfabrication technology for vascularized tissue engineering. *Biomedical Microdevices*, Vol. 4, 167-175.

Carmeliet, P. and Jain, R. K. (2000). Angiogenesis in cancer and other diseases. *Nature*, Vol. 407, 249-257.

Carvalho, F., Marques, M. P. C., and Fernandes, P. (2017). Sucrose hydrolysis in a bespoke capillary wall-coated microreactor. *Catalysts*, Vol. 7, 42.

Kawakami, K., Oda, Y., and Takahashi, R. (2011). Application of a Burkholderia cepacia lipase-immobilized silica monolith to batch and continuous biodiesel production with a stoichiometric mixture of methanol and crude Jatropha oil. *Biotechnol Biofuels*, Vol. 4, 42.

Kim, J. A., Lee, J. Y., Seong, S., Cha, S. H., Lee, S. H., Kim, J. J., and Park, T. H. (2006). Fabrication and characterization of a PDMS-glass hybrid continuous-flow PCR chip. *Biochemical Engineering Journal*, Vol. 29, 91-97.

Lawrence, J., O'Sullivan, B., Lye, G. J., Wohlgemuth, R., and Szita, N. (2013). Microfluidic multi-input reactor for biocatalytic synthesis using transketolase. *Journal of Molecular Catalysis B: Enzymatic*, Vol. 95, 111-117.

Sakai, S., Liu, Y., Yamaguchi, T., Watanabe, R., Kawabe, M., and Kawakami, K. (2010). Production of butyl-biodiesel using lipase physically-adsorbed onto electrospun polyacrylonitrile fibers. *Bioresource Technology*, Vol. 101, 7344-7349.

Song, Y. S., Shin, H. Y., Lee, J. Y., Park, C., and Kim, S. W. (2012). β-Galactosidase-immobilised microreactor fabricated using a novel technique for enzyme immobilisation and its application for continuous synthesis of lactulose. *Food Chemistry*, Vol. 133, 611-617.

Stojkovič, G., Plazl, I., and Žnidaršič-Plazl, P. (2011). L-Malic acid production within a microreactor with surface immobilised fumarase. *Microfluidics and Nanofluidics*, Vol. 10, 627-635.

Takei, T., Kishihara, N., Ijima, H., and Kawakami, K. (2012). Fabrication of capillary-like network in a matrix of water-soluble polymer using poly(methyl methacrylate) microfibers. *Artificial Cells, Blood Substitutes, and Biotechnology*, Vol. 40, 66-69.

Takei, T., Sakai, S., and Yoshida, M. (2016). In vitro formation of vascular-like networks using hydrogels. *Journal of Bioscience and Bioengineering*, Vol. 122, 519-527.

Tamborini, L., Fernandes, P., Paradisi, F., and Molinari, F. (2018). Flow bioreactors as complementary tools for biocatalytic process intensification. *Trends in Biotechnology*, Vol. 36, 73-88.

Thomsen, M. S. and Nidetzky, B. (2009). Coated-wall microreactor for continuous biocatalytic transformations using immobilized enzymes. *Biotechnology Journal*, Vol. 4, 98-107.

Wang, P., Sun, Y., Shi, X., Shen, H., Ning, H., and Liu, H. (2021). 3D printing of tissue engineering scaffolds: a focus on vascular regeneration. *Bio-Design and Manufacturing*, Vol. 4, 344-378.

(Received: December 7, 2021; Accepted: December 20, 2021)