Glass Microchannel Formation by Mycelium

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We propose a new method to fabricate complicated 3-dimensional glass microchannels. We employed mycelium for this purpose. Mycelium possesses a complicated, fine and three-dimensional network structure. We cultivated mycelium in silica compounds, and subsequently silica compounds were heated to be sintered. During this heating process, all the mycelium was burned off and remained a fine network channel structure in a transparent glass chip. We also tried to control the growth of this mycelium. The growth could be changed by growth conditions. In this work, we used cyclic mechanical stimuli for this purpose. We set cyclic tensile strain to the sample under growing mycelium. This cyclic strain caused anisotropic growth of the mycelium in some condition.

Keywords: Microchannel, Glass, Mycelium, Aspergillus oryzae

1. Introduction

In this paper, we employed mycelium to form 3-dimensional microchannel in a glass chip. Microchannels are widely used for lab-on-a-chip including organ-on-a-chip. The popular microchannels are 2-dimensional, and most of them are fabricated by lithography methods [1]. It is more difficult to prepare 3-dimensional channels, some processes have been proposed to fabricate them, such as lamination [2-4], reverse imprinting [5, 6], and the utilization of deformation [7-10] and the 3D printer [11-13]. The blood vessel structure is more complicated than that of microchannels fabricated by these methods.

Authors utilized glass nano powder as a start material of microchannels in this work. We have already developed many kinds of processes to form micropatterned sheets or other micro devices using nano powders [14-25]. Nano powders of ceramics and glasses are popular and already have used in industry fields, so that it is affordable and easy to obtain. We can form nano powder as a powder compact by some ways. Sometimes we compress powder; this is the simplest way to get some form. Also, we can mix powder to polymer binder material. The mixed compound material could show plasticity, and we can form it easier. We have tried nano imprint lithography to fabricate a ceramic or glass sheet with highly fine patterns on it. After forming, powder compacts can be solidified by heating. It is called sintering process.

Instead of forming by machines, we came up with an idea that live organs such as plant root or mycelium could form the nano powder compound. Plants and fungi grow into soil which consists of inorganic particles. Nano particles could also work as soil. We have tried to grow plant seed on nano particles with aqueous binder. The seed grew into particles, and after then, we heated this “soil” with

Fig. 1. Overview of rice-malt, Aspergillus oryzae, employed for this work.
plant to burn off the plant and to sinter the sample. We obtained a ceramic chip with root-shaped microchannels [26]. Silica powder was also used for the same procedure, and a transparent glass chip with microcavity was obtained [27]. In this glass chip, we could fix the structure where plant roots were in symbiosis with arbuscular mycorrhiza.

In this paper, rice-malt, *Aspergillus oryzae*, was employed to form microchannels in glass chips. Figure 1 shows rice-malt we used in this work. Mycelium generated a network structure in the sample, which could be fixed as a cavity in a transparent glass chip after sintering. It is also important to control growth of mycelium. The growth could be changed by growth conditions. We used cyclic mechanical stimuli for this purpose. We set cyclic tensile strain to the sample under growing mycelium.

2. Experimental

2.1. Cultivation and sintering

Aqueous solution of agar and sucrose as cultivation medium. Water was boiled, and 1 mass% agar and 1 mass% sucrose was dissolved. The same mass of silica powder was mixed with the solution to obtain a compound material. We prepared spherical silica powder (SC2500-SQ, Admatechs). The particle size of the prepared silica powder was about 0.5 μm. We dispersed spores into water by dipping edible rice-malt grains. The spores were mixed into the silica compound, and subsequently, mixed sample was poured into a plastic case. After cooling, agar became gelatinous.

The sample was put in an incubator at 35 °C for 2 days. After growth of mycelium, the sample was taken out from the case and was dried in an oven at 50 °C for more than 21.6 ks (6 h). Next, the dried sample was sintered at 1400 °C for 7.2 ks (2 h). The obtained glass samples were observed by an optical microscope.

2.2. Cultivation under cyclic strain.

A flexible container was made using silicone rubber (Ecoflex 00-30, SmoothOn) to be deformed. The container was set to a hand-maid stretching device. The container and the device are shown in Fig. 2. In this experiment, 0.5 mass% agar gel was used as cultivation medium. Sucrose of 1.0 mass% was also added to the gel. Silica powder was not used in this experiment for easy observation.

We prepared 2 kinds of samples following the flows as shown in Fig. 3; one was an agar sample dispersed with spores, and the others was 2 agar sheets which sandwiched spores. Both samples in the silicone container was set in an incubator, and applied cyclic strain of 10 % at 0.5 Hz. Incubation was carried out at 35 °C for 2 days.

3. Results and discussion

3.1. Sintered glass channel

Figure 4 shows photos of a silica sample with mycelium after cultivation (a), after drying (b), and
after sintering (c). Shrinking was observed in each step. After cultivation, a sample gel was distorted a little, and more distortion was observed during the drying stage, but there was less distortion during sintering.

The sintered sample looked clear, but unclear spots were observed inside. Spots were observed by an optical microscope. The image is shown in Fig. 5. The spot was an area where a colony of mycelium grew. From the microscopic image, many pores were also observed. We have not made it clear how these pores generated. It might be possible that bubbles which were generated during mixing the high viscous material had remained in the compact. We should improve the process to remove the bubbles.

3.1. Anisotropic growth under cyclic strain

We cultivated mycelium in 2 kinds of setup. The first one was with a medium in which spores were dispersed (Fig. 3a), and the other one was that spores were sandwiched between agar sheets (Fig. 3b). Figure 6 shows the mycelium in the former setup. There were colonies which were similar to the result in the previous section. The growth was isotropic that means the colony grew radially even under unidirectional cyclic strain.

In the latter case, mycelium grew differently between agar sheets. Figure 7 shows colonies after cultivation. The colony which grew in a static state was isotropic, whereas the mycelium was aligned vertical direction, which was the same direction to the cyclic strain.

The strain caused anisotropic growth of mycelium. This is one possible way to control the growth. We will try to cultivate mycelium in a silica compound to fabricate a glass chip with controlled grown channels. This is our future work.

4. Conclusion

We propose a method to form microchannels in a glass chip by growth of mycelium. We employed silica nano powder as a starting material and fabricated a glass chip with fine channels. In order to control the growth of the mycelium, we cultivated mycelium under uniaxial cyclic strain state. Anisotropic growth was observed in some condition.

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