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Microfluidic devices have been fabricated with gelatin and paper clips. Optimization of fabrication and characterization process has been carried out systemically by varying gelatin-to-water ratio, bonding time and connector type. We find that the higher gelatin-to-water ratio and the luer lock syringe tip provide a greater success rate, whereas the influence of bonding time is limited. The Reynolds number was calculated to identify whether the fluid shows laminar or turbulent flow.

Key words: Microfluidics, softlithography, gelatin fluidics, Reynolds number, laminar flow, Do-it-yourself (DIY) and nanofabrication

I. Introduction

Microfluidics is a research and engineering area used to investigate the behavior of fluids through channels at a microscopic level by manipulating small amounts of fluids[1]. This emerging field of technology has applications in several areas of study, including biosensors, pharmaceutical kits and paint mixture kits[2,3]. Especially, one of prominent application is the lab on a chip, which is capable of running and processing several types of medical diagnostic tests[4]. Recent technologies, so-called "organ-on a chip", allows human cells to react with viruses, bacteria and antibodies[5,6].

A typical way to fabricate microfluidic devices requires nanofabrication processes. For example, a negative photoresist is spin-coated onto a silicon wafer to ensure uniform coverage over the entire substrate. A mask with the desired pattern is then placed on the wafer which gets exposed to UV light to transfer the patterns from the mask to the resist. The template is completed after development of photoresist. On this template, there exist patterns for microfluidic devices on silicon wafer. Polydimethylsiloxane (PDMS) is then poured onto the template and cured at high temperature. The PDMS is cut and removed from the template and holes are made in the PDMS using a PDMS puncher. Both PDMS and glass slides undergo oxygen plasma treatment to activate their surface, then activated surfaces are bonded. The device is further cured at high temperature to improve adhesion.

Although the use of microfluidic devices is advantageous and ordinary for many applications, high demand for expensive nanofabrication instruments with high accuracy and precision limits the ability to take full advantage of the opportunities stemming from the concept. Recently, the fabrication protocol of microfluidic devices for Do-it-yourself (DIY) was developed[7]. In this DIY experiment, gelatin and wire were used to replace the PDMS and photoresist, respectively. Diluted dye was injected into the device to understand the influence of channel design and dimension on the behavior of fluids. However, DIY experiments inherently have adhesion issues between gelatin and glass slides as the fabrication process excludes the oxygen plasma bonding process. In this paper, we demonstrated how to improve adhesion between gelatin and glass slide through systematic studies. We propose an optimal process flow and characterization method. We also provide a calculation of Reynolds number to understand the laminar flow of fluids in micro-size channels.

II. Experiment

The fabrication process mainly consists of two sections: fabrication of gelatin cube and pipette injection, followed by data collection and characterization[7]. The fabrication process of the gelatin microfluidics is outlined in Fig. 1. A sheet of plastic film was cut into squares that fit the size of the silicone mold for ice making, then the sheet was placed inside of the mold. This is to make the bottoms of the gelatin flat. Paper clips were unfolded and cut into

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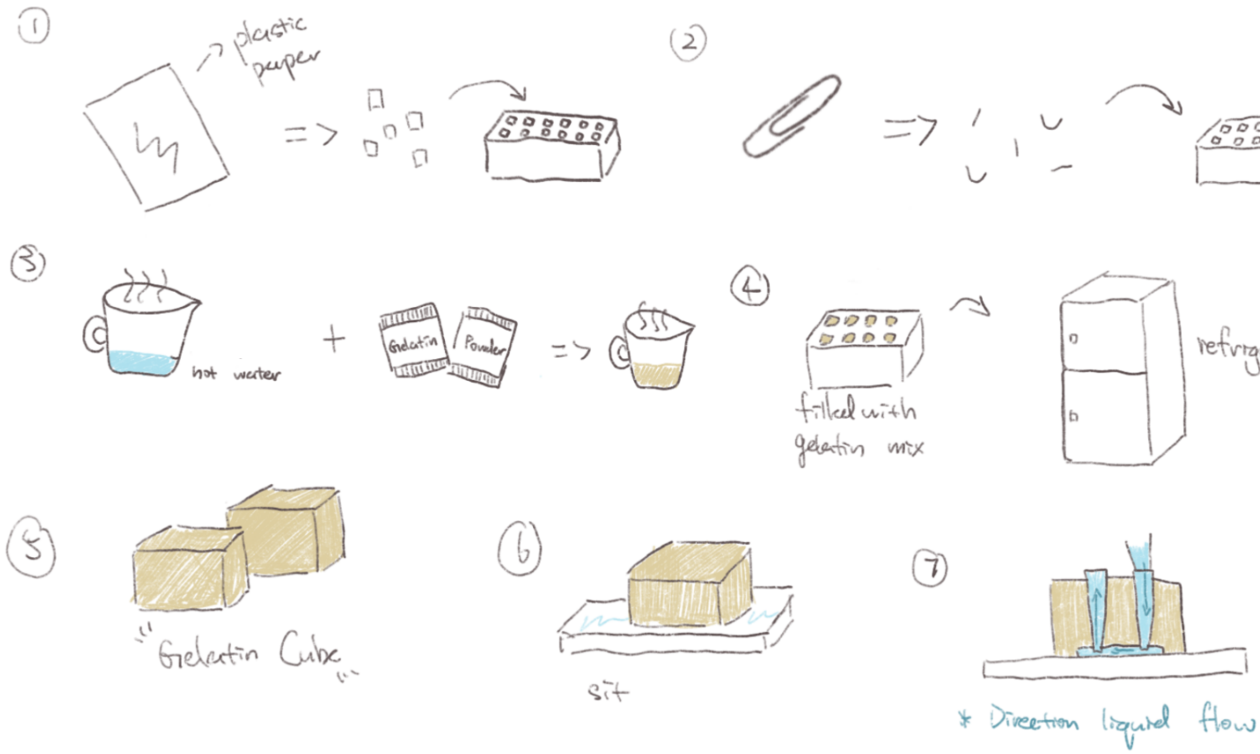

FIG. 1. Process flow to make a microfluidic device with gelatin

short and straight wire pieces. Wire pieces were then put into the middle of each plastic squares inside the silicone mold. The boiled water and gelatin powder (Knox, unflavored) were mixed completely to form gelatin liquid. The liquid was poured into the mold, then cooled in a refrigerator for 20 minutes. The solid gelatin cubes were removed from the mold, and the plastic film and metal pieces were carefully removed. The gelatin cubes were placed on top of separate glass slides and sat until the cubes fully adhered to the glass slides. Connectors, either pipette or luer lock tips, were inserted into the cube, perpendicular to the channel left by the metal piece to make the inlet and outlet. Considering the original connectors might be clogged by gelatin, the old connectors were then replaced with new ones. For the last step, water colored by food coloring was injected from one side of the channel and drained from the other side.

This process, however, has room to improve. One major issue is leakage: injected water occasionally leaked from the crack created by inserting the pipette tips. Another form of leakage is in the interface of gelatin cube and glass slide due to weak bonding, which results in delamination.

For a better DIY laboratory, the influence of gelatin-to-water ratio, bonding time and connector tip types on the device fabrication and characterization were tested systematically. The cooling time in the refrigerator was held constant at 20 minutes. Water mixed with food coloring with the volume ratio of 4 to 1 was used as a fluid flowing through the channel. The leakage was optically observed while flow rates of liquid in the channel were measured in various conditions.

III. Results and Discussion

A. Fabrication of microfluidic device

Figure 2 shows a representative microfluidic device made by a mixture of 10.5 g of gelatin and 118 ml of hot water. The gelatin mold sat on the glass slide for 3 minutes before water injection. The typical fabrication process with PDMS and DIY process with gelatin have similarities and differences. The paper clips in DIY are equivalent to the photoresist in the typical fabrication process. The function of both of them is to create certain patterns on the PDMS or gelatin to serve as a channel for the liquid to flow through. The gelatin in DIY experiment is the same as the PDMS in the typical fabrication. Both are being poured into the tray or Al dish and being solidified in the curing process. Whereas gelatin was cooled down in a refrigerator, PDMS was cured at a high temperature. After the mold was removed, both

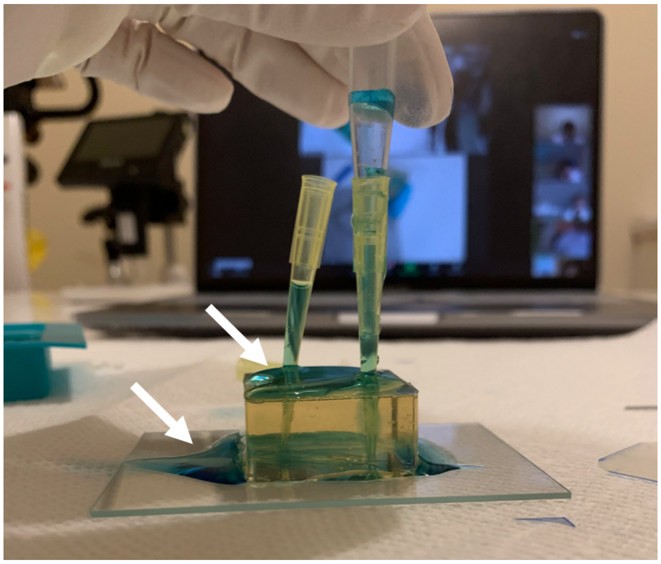

FIG. 2. A microfluidic device made of gelatin. Although colored water flows through the channel, leakages are observed (white arrows). 10.5 g of gelatin were mixed with 118 ml of hot water to make the device, and the bonding time was 3 minutes.

the PDMS chip and gelatin goes through the bonding process to bond them with glass. For the DIY lab, the gelatin is simply put onto a piece of glass for 3 - 30 minutes. The PDMS microfluidic chip, on the other hand, has to go through plasma treatment to make sure it is firmly bonded with the glass. Another difference is the hole punching process to make inlet and outlet. Whereas pipettes are used to punch holes in the gelatin, a hole puncher is used for PDMS.

Although water is flowing through the channel in Figure 2, leakages were observed on the topside of the gelatin and the interface between gelatin and glass slide due to cracks and delamination. This led to inaccurate measurement of the Reynolds number.

B. Optimization of Fabrication and Characterization

Table I shows experimental conditions and results. The success of "N" relates to either leaking or no bond forming. The success of "Y" relates to a secure bond that allows water to flow in the channel consistently. Higher gelatin-to-water ratio did lead to a higher success rate. This trend is observed in sets of samples that used the pipette. When the ratio is 10.5 g of gelatin to 118 ml of water, the success rate is 0%. There is a clear increase in success rate when the concentration of gelatin increases: for both 14 g of gelatin to 118 ml of water samples and 17.5 g of gelatin to 118 ml of water samples, the success rate increases to 50%. Furthermore, the success rate of samples with 21 g of gelatin to 118 ml of water is 100%. The greater gelatin-to-water ratio can make gelatin cubes stiffer and increase the success rate. The stiffer gelatin cubes may have higher resistance to local deformation

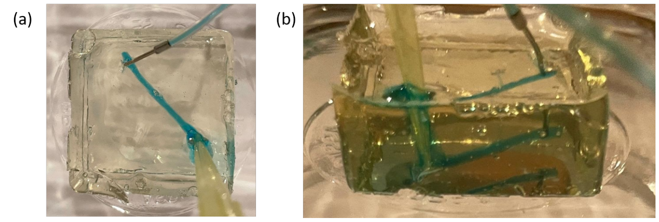

FIG. 3. Gelatin microfluidic devices with optimal conditions (a) plan view (b) tilted view. 21 g of gelatin was mixed with 118 ml of water and the bonding time was 30 minutes. The water passed through successfully.

due to the water injection which leads to the delamination. The stronger chemical bonding may also suppress the probability of cracking to occur.

There is a strong correlation between the use of luer lock tips for syringe and the success rate. 8 out of 16 devices failed when a pipette tip was used for the connector tip, whereas 4 out of 16 devices failed when a luer lock syringe tip was used. Connecting luer lock syringe tip and a syringe also provides and advantage over using pipette tip. One can have more control of the amount of water pushed into the channel.

Longer bonding time between the gelatin cubes and glass slides showed limited dependency on the success rate. The success rate does not increase as the bonding time increases in the 14 g of gelatin to 118 ml of the water sample set that used the pipette. The 17.5 g of gelatin to 118 ml of water set with pipette connector, on the other hand, shows increased success rate as the bonding time increases. Figure 3 shows the microfluidic device made with the mixture of 21 g of gelatin and 118 ml of water. The bonding time was 30 minutes. The water passed through the channel without leakage in both the pipette and luer lock tip.

To create a more durable model, certain steps need to be changed from the original lab. The first is that the powder-to-water ratio of the gelatin should be increased to 3 bags of powder to $\frac{1}{2}$ cup of water. Something to keep in mind while working with stiffer cubes is that making the original hole for the new syringe might be more difficult. Putting the syringe inside the hot water before the start is helpful. This also can help unclog syringes clogged by gelatin. The second change is more optional, but it is helpful to increase the bonding time for glass slides and gelatin cubes. The third change to the original procedure is changing the injection tool from a pipette to a syringe.

C. Calculation of Reynolds number

In characterizing the flow of fluid through a channel, the Reynolds number is often a useful measure to consider. The Reynolds number (Re) is defined as the ratio of inertial forces to viscous forces in the flow of a fluid. If the Reynolds number is low (Re $<$ 2,300), this indicates that the flow is laminar like paint, blood and honey. In other words, the flow is smooth, steady, and non-chaotic.

TABLE I. Every combination of the of the gelatin-to-water ratio, bonding time and connector type tested for the microfluidic devices with gelatin. The success of "N" relates to either no bond forming or leaking. An "Y" correlates to a secure bond that allows water to flow in the channel consistently.

Batch	Gelatin (g)	Water (mL)	Bonding time (min)	Connector type	Success
1	10.5	118	3	Pipette	N
2	10.5	118	10	Pipette	N
3	10.5	118	15	Pipette	N
4	10.5	118	30	Pipette	N
5	10.5	118	3	Luer Lock	N
6	10.5	118	10	Luer Lock	N
7	10.5	118	15	Luer Lock	N
8	10.5	118	30	Luer Lock	N
9	14.0	118	3	Pipette	Y
10	14.0	118	10	Pipette	N
11	14.0	118	15	Pipette	Y
12	14.0	118	30	Pipette	N
13	14.0	118	3	Luer Lock	Y
14	14.0	118	10	Luer Lock	Y
15	14.0	118	15	Luer Lock	Y
16	14.0	118	30	Luer Lock	Y
17	17.5	118	3	Pipette	N
18	17.5	118	10	Pipette	N
19	17.5	118	15	Pipette	Y
20	17.5	118	30	Pipette	Y
21	17.5	118	3	Luer Lock	Y
22	17.5	118	10	Luer Lock	Y
23	17.5	118	15	Luer Lock	Y
24	17.5	118	30	Luer Lock	Y
25	21.0	118	3	Pipette	Y
26	21.0	118	10	Pipette	Y
27	21.0	118	15	Pipette	Y
28	21.0	118	30	Pipette	Y
29	21.0	118	3	Luer Lock	Y
30	21.0	118	10	Luer Lock	Y
31	21.0	118	15	Luer Lock	Y
32	21.0	118	30	Luer Lock	Y

On the other hand, if the Reynolds number is higher (Re > 2,900), the fluid flow through the channel will tend to be turbulent like water. Since the Reynolds number is defined as the ratio of inertial forces to viscous forces, the equation that may calculate the Reynolds number depends upon the cross-sectional geometry of the channel that the fluid is flowing through. In this case, the geometry of the channel was approximated as a simple cylindrical pipe. Equation 1 shows the equation used, as well as the values used (from Table I), to estimate the Reynolds number.

$$\mathrm{Re} = \frac{\mathrm{W\,D}}{\mu\,\mathrm{A}} \tag{1}$$

where Re is Reynolds number, W is the mass flow rate, D is the diameter of channel, μ is viscosity of fluid, and A is the cross-sectional area.

For example, in the case of batch 28 in Table I, it took 3.77 seconds for 2 mL of water to pass through the 16.8 mm of channel. The volumetric flow rate of water, 2/3.77, is 0.53 mL/sec. The diameter of paper clip that determines the diameter of channel is 0.720 mm. The viscosity of water, μ, is 1001.6 Pa $\cdot$ s. Therefore, the Reynolds number of water in the gelatin fluidic device is $9.36 \cdot 10^{-4}$. Since the Reynolds number is many orders of magnitude lower than the typical transition from laminar to turbulent flow, the fluid flow through the channel in this device is considered to be laminar.

IV. Summary

Fabrication and characterization of microfluidic device made of gelatin and paper clip was demonstrated for a DIY experiment. It is found that higher gelatin to water ratio and employing a luer lock connector tip provide a greater success rate. The relationship between glass bonding time and success rate was not obvious. An example of a calculation of the Reynolds number was provided to determine whether the fluid shows laminar flow. These results can be used to improve upon this high school level laboratory as well as give a better introduction to nanotechnology through microfluidics.

V. Acknowledgements

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