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Preparation of microfluidics device from PMMA for liposome synthesis

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Abstract. Microfluidics has emerged in recent years as a technology that has advantages and is well suited for studying chemistry, biology, and physics at the microscale. A common material which has been widely use to fabricate the microfluidic system is thermoplastic materials. The method of fabricating microfluidic devices has been growing because of advantages such as high-quality feature replication, inexpensiveness, and ease of use. However, the major barrier to the utilization of thermoplastics is the lack of bonding methods for different plastic layers to close the microchannels. Therefore, this study focused on fabricating a microfluidic device on poly(methyl methacrylate) (PMMA) plates by laser engraving. The bonding technique for plastic layers has relied on the application of small amounts of ethanol with conditions of low temperatures (100 ^{o}C), and relatively low pressures (5 tons) for 2 minutes. With this technique, the microfluidic device is created to operate stably, without leakage or cracking even under high pressure. The microfluidic device was applied to synthesize liposomes with a 5:1 ratio of syringe pump velocity between water and lipid solution. The size of liposomes after synthesis is 109.64 ± 4.62 nm (mean ± sd) and the PDI is in accordance with standard conditions (PDI < 0.200).

Keywords: PMMA, microfluidics, liposome, laser engraving.

Classification numbers: 2.4.3, 2.7.1.

1. INTRODUCTION

Microfluidics has been a rising trend among scientists over the last twenty years [1]. With applications in physics and chemistry at the micro-level, microfluidics has shown considerable promise for improving diagnostics and biology studies [2 - 5]. Microfluidic systems are mostly created from poly(dimethylsiloxane) (PDMS), a silicone elastomer that is easy to use, cheap, and can replicate with high precision [6]. However, PDMS has its drawbacks such as incompatibility with high volume fabrication processes. Therefore, the emerging thermoplastic material is a suitable candidate for making micro fluids [7, 8].

Microfluidics on poly(methyl methacrylate) (PMMA) sheets can be produced using a variety of techniques such as thermal embossing injection molding or laser cutting [9]. With its impressive mechanical properties, the microfluidic system made of PMMA can ensure good accuracy compared to the original mold shape. Accordingly, PMMA has great potential of

producing microfluidics with many uses in the near future [10 - 12]. However, one of the major downsides to the application of microfluidic systems is the ability to adhere plastic sheets in the system. Determining how the two base materials quickly join together and ensuring that there is no deformation is a problem. Several methods have been studied and used such as adhesives, heat bonding techniques, and solvent surface treatment techniques, but there still exist problems that need to be solved.

In this research, we investigated the usage of thermal bonding method for PMMA sheets on how to make the bonding fast, efficient, and safe. The optimal thermal bonding method in the presence of absolute ethanol solvents allows for a reasonable cost, non-toxicity, and high speed.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Materials

PMMA FS sheets with a thickness of 3 mm were purchased from Fusheng (Taiwan). Absolute ethanol was from Cemaco Company (Viet Nam). Isopropanol (IPA) solution was bought from Scharlau (Spain). 1,2-dioleoyl-sn-glycerol-3-phosphocholine (DOPC) and cholesterol (Chol) were purchased from Sigma Aldrich (USA). Chloroform solvent was obtained from China.

2.1.2. Devices

A cutting and engraving laser fiber machine with a capacity of 50 W was from Laser Top (Viet Nam). A CO_2 laser cutting machine was purchased from China. A hydraulic heat press machine with a capacity of 15 tons was assembled in Viet Nam. The size of liposome was measured using a Malvern Zetasizer Nano ZS90 from Canada.

2.2. Methods

2.2.1. Creating a microfluidic device

The microsystem design was drawn using CorelDraw 2019 software (Corel Co., Canada) (Figure 1). The design included 2 parts: the laser cutting part and the laser engraving part. The device was composed of 2 PMMA panels with dimensions of 40 × 70 mm, rounded at 4 vertices. The first PMMA sheet had 4 round holes with a diameter of 1.2 mm to create the input and output of the microchannel. One side of the first PMMA plate was engraved with the product information. The second PMMA sheet was a plain sheet of the same size as the first one. The second sheet was engraved with details as shown in the figure with a microchannel dimension of 0.08 mm. After finishing the design, the drawings were fed into the laser cutter to create micro-flow geometry. The PMMA plate laser cutting process was performed on a 50 W CO_2 laser cutter (Viet Nam). The PMMA layers after cutting were cleaned with a clean paper towel and ethanol to remove dust. In this step, the small amount of ethanol applied to the layers via wipes would evaporate within seconds after use and would not affect the bonding thereafter.

Before the two PMMA plates were stacked, absolute ethanol was spread evenly between layers with a pipette just before bonding (20 µL per test sample). This amount of ethanol allowed a partial melting of PMMA particles even when operating under the phase transition temperature of PMMA Tg glass. Two PMMA sheets were placed in the heat press. The heat press system was investigated with pressures of 5 tons, 8 tons, and 12 tons (equivalent to 1.57,

2.51, and 3.76 MPa) at different temperatures (70 ^{o}C, 85 ^{o}C, 100 ^{o}C, and 110 ^{o}C), and within 2-minute and 8-minute intervals.

A B

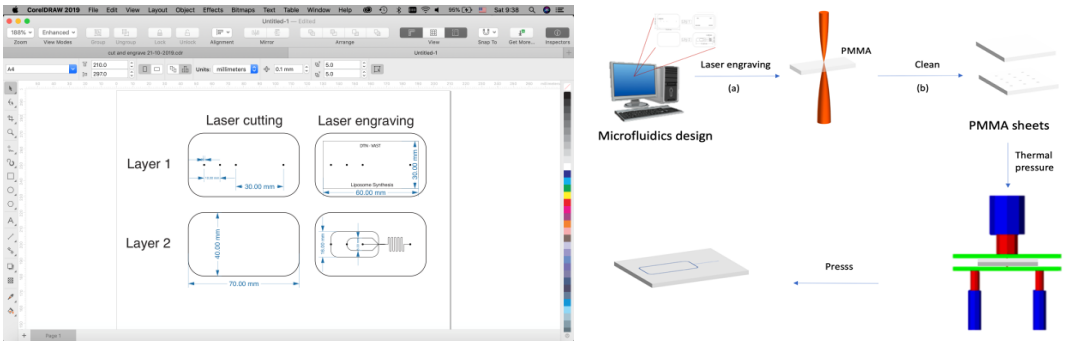

Figure 1. (A) Designing micro-flow system using CorelDRAW 2019 software and (B) process of creating a microfluidic system from PMMA sheet.

2.2.2. Investigation of the bonding tightness between plastic sheets in the microfluidic system

Tests were performed to ensure that no leakage occurred on any part of the microfluidic system surface mounted with UV glue. A red dye solution was pipetted into the microchannel. The microfluidic system was then observed through a microscope.

2.2.3. Surveying the pressure in microfluidic system

The bonding strength between PMMA plates was determined by measuring the maximum pressure of a fluid flow in the microfluidic system.

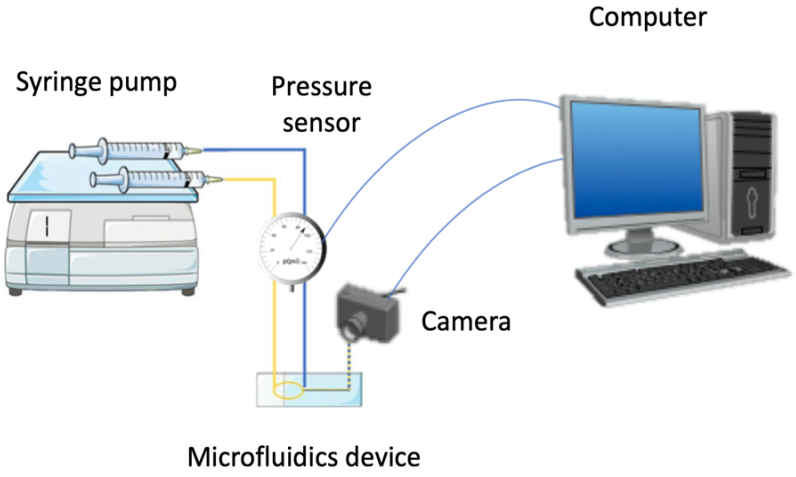

Figure 2. Thermoplastic chip crack pressure detection system.

Figure 2 shows a schematic diagram of the test system which includes a pressure sensor with a working range from 0 to 10 bar and a personal computer recording system synchronized with the image recording system. Pressure was created by pumping water into the microchannel with a needle pump where the output of the microfluidic chip was sealed, so the pressure accumulated inside the microchannel. The use of a three-way connector allowed for the

measurement of the pressure inside the microchannel with a pressure sensor, the results of this pressure measurement were then recorded by a computer. The experiments were performed on a number of microchips, each of which was tested by gradually increasing the pressure from 0 bar to 10 bar, maintaining at 10 bar for 20 seconds, and then gradually decreasing to 0 bar.

2.2.4. Fabrication and investigation of properties of liposomes created from microfluidic device

A mixture of 1,2-dioleoyl-sn-glycerol-3-phosphocholine (DOPC) (7.34 mg) and cholesterol (Chol) (0.77 mg) was dissolved in 10 mL of $CHCl_3$. The DOPC/Chol mixture was transferred from an Eppendorf tube to a 100 mL flask. The solvent solution was separated by a rotary evaporator (Buchi, Switzerland) at a water tank temperature of 30 °C, a tank pressure of 600 mbar, and rotation speed of 60 rpm for the gradual removal of $CHCl_3$. After the evaporation of the solvent, a thin lipid film appeared at the bottom of the flask. Then, 1 mL of methanol was added to the flask to separate the membrane from the bottom of the flask, and the entire solution was transferred to one cylinder (cylinder B) and connected to the liposome microfluidic chip in the number 2 inlet position. The other cylinder (cylinder A) containing distilled water was fed into the microcirculation chip at the digital input position 1. The pump speed in the 2 cylinders was adjusted with the ratio of 3:1, 4:1, and 5:1 ($v_{\text{cylinder A}}/v_{\text{cylinder B}}$) to obtain the outgoing liposome at output 3 on the microcomputer chip. The formed liposome was investigated for its size and dispersion (PDI) by dynamic light diffraction (DLS) method on the Zetasizer Nano ZS90 device in the transparent plastic cuvette.

3. RESULTS AND DISCUSSION

3.1. Manufacturing micro-system

The microfluidic system was fabricated by laser cutting and engraving method. After wiping with ethanol, the PMMA plates were dripped with a small amount of ethanol evenly on the contact surface between the plates and heat-pressed on the hydraulic press. There were three factors affecting the bonding ability between PMMA sheets to be investigated: pressure, temperature, and time. Products after being fabricated were cut and photographed to analyze the bond between PMMA plates.

Three pressure levels, namely 5 tons, 8 tons, and 12 tons, were examined. It could be seen that at low pressure of 5 tons and low temperature, the adhesion between sheets was ineffective, and air bubbles were present between the plastic sheets (Figure.3). When using heat press with a higher pressure (8 tons and 12 tons), some cracks appeared around the wall of the microchannel (red circle, Figure 3). In addition, at these pressures, the internal height of the microchannels was reduced by 1 to 3 % (deflection) while the PMMA sheets were 7 - 8 % higher than those of the microchannels. Therefore, for bonding between PMMA sheets, the pressurization was performed at 5 tons with higher temperatures.

Further tests showed safe bonding after 70 °C and bonding ability remained almost constant from this temperature. The investigation results showed that the binding capacity decreased significantly at 85 °C and then showed a slight increase at 100 and 110 °C due to the boiling point of ethanol at 78 °C under atmospheric pressure. When dripped on the surface of each test sample that was treated at high temperatures, the ethanol is evaporated before applying the plates to the heat press. Solvent evaporation also reduced the crack of the test pieces because the bonding occurred over a much larger area. However, when thermally pressed at 110 °C for 8 minutes, the microchannels completely disappeared and deformed at maximum (nearly 400 % of height), the samples were bound together into a block regardless of the pressure of 8 or 12 tons

(1.57 or 3.67 MPa per specimen). Testing the compression of the microfluidic system at 100 ^{o}C and 5 tons showed that the bond strength was highly dependent on the bonding time for the first 5 minutes. After this time, the ability to form bonds between PMMA sheets was not significantly affected. Although after 5 minutes of bonding, time had no effect on bond strength, the cross-sectional images showed that longer durations resulted in higher distortion, both in terms of channel deflection and growth of the distortion-affected area around the channels (6 ± 6 and 36 ± 4 %, respectively for 8 minutes of bonding, compared to 1 ± 1 and 0 ± 0 %, respectively for 5 minutes of bonding). Therefore, in the experiment, the optimal condition for the bonding between the PMMA plates was the equivalent pressure of 5 tons at 100 ^{o}C for 2 minutes.

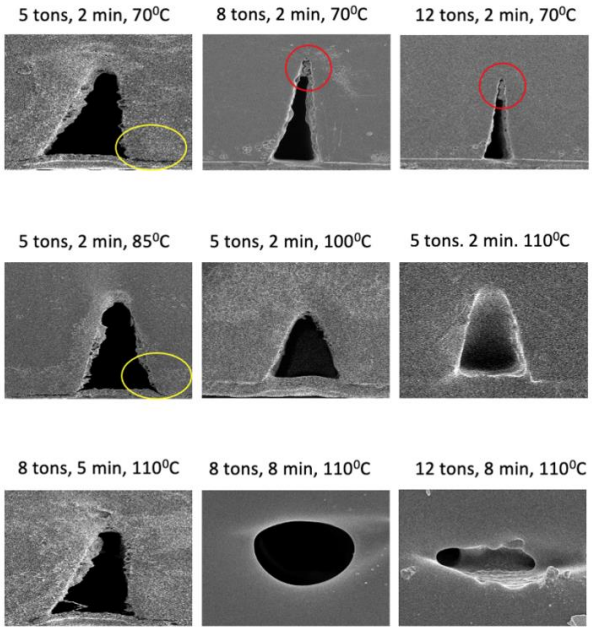

Figure 3. Microchannel characterization according to pressure, time and temperature.

3.2. Testing the microfluidic characteristics

The experiments with a red dye solution showed that no leakage was observed at any point along the microchannel. For the microfluidic pressure test, the pressure resistance capacity of 10 bar was used as a criterion to evaluate the bonding magnitude due to the fact that the microfluidic system needed to meet the pressure capacity of the internal flow systems (in fact most of these systems required less than 2 bar). The results showed that the device with a microchannel could withstand the pressure of 10 bar after bonding.

3.3. Manufacturing liposomes from microfluidic device

The prepared samples were homogeneous white, milky suspensions, without large particles visible to the naked eye. The mean liposome size of 99.84 ± 7.24 nm (mean ± sd), 103.35 ± 6.51 nm (mean ± sd) and 109.64 ± 4.62 nm (mean ± sd), and the PDI dispersion of 0.194, 0.183 and 0.144 were corresponding to a cylinder injection rate (cylinder A / cylinder B) of 3:1, 4:1 and 5:1, respectively. The distribution of liposome particle size is shown in Figure 4. It can be seen that the liposome size was homogeneous with all ratios but the PDI values decreased with increasing velocity ratio. However, liposome concentrations in the final solution would be low at

higher ratios. Therefore, the 5:1 velocity ratio was chosen as the optimal ratio for liposome fabrication.

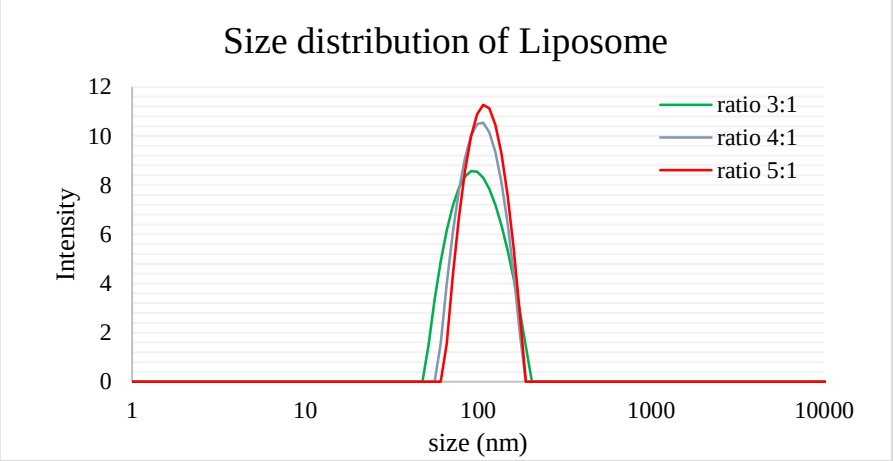

Figure 4. Liposome particle size distribution with syringe pump rate ratio of 3:1, 4:1 and 5:1.

4. CONCLUSIONS

The research has been successful in applying laser engraving technology to create microfluidic systems from PMMA materials. PMMA sheets were bonded together by heat pressing method. The optimal condition of the thermal pressing of PMMA sheets was a pressure of 5 tons, at a temperature of 100 ^{o}C and for a period of 2 minutes. This was a relatively gentle condition to create a bond between PMMA sheets. The created microchips operated stably without leaking or cracking even under a channel flow pressure of 10 bar.

The microfluidic device was applied to prepare liposome particles. The liposome particle size distribution was different depending on the pump velocity ratio of the two solutions. With a 5:1 ratio of syringe pump velocity between water and lipid solution, the most homogeneous liposome particles were created, suitable for further applications in the future.

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Credit authorship contribution statement. Tran Thi Thanh Van: Methodology, Investigation, Funding acquisition. Nguyen Trung Huy, Cao Thi Hong, Vu Quoc Thai, Trinh Quang Dung: Methodology, Experimental process, Formal analysis. Nguyen Thanh Duong: Supervision, Formal analysis, Investigation, Supervision.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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