Microfluidic Device for the CRISPR Diagnostic of SARS-CoV-2

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ABSTRACT

According to the spread of SARs-CoV-2 in 2019, the current diagnostic methods are Antigen Test Kit (ATK) and Reverse Transcription Polymerase Chain Reaction (RT-PCR), which show the obvious drawback such as low accuracy for ATK and long waiting period for RT-PCR. The purpose of our project is to minimize the diagnostic cost, waiting period, and improve ease of use as well as provide an accurate result of SARS-CoV-2 detection by using a microfluidic device and CRISPR diagnostic assay. The process of diagnosis using CRISPR diagnostic assay includes RNA amplification and CRISPR-Cas detection. We sought to build a microfluidic device that allows the sample of viral RNA to flow continually to the amplification reaction and CRISPR-Cas detection within specified times by using the concept of fluid dynamics.

We explored three fabrication workflows that differed by engraving, cleaning, bonding, and surface treatment techniques. Of which, the most robust fabrication protocol is a three-layer design consisting of a thin polyethylene terephthalate (PET) sheet sandwiched between polymethyl methacrylate (PMMA) layers. The three layers were cut using CO2 laser cutting to create microfluidic channel, inlets, and outlets and bonded by pressure sensitive adhesive (PSA) tape. Flows of fluids with similar properties to CRISPR diagnostic reagents including water and buffer were tested in the device yielding the final design that allows reagents to flow as desired and mix automatically with minimal humans' intervention.

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Firstly, we would like to express our gratitude to the project's advisor Asst. Prof. Pimkhuan Hannanta-anan who greatly supports and conveys the immense knowledge to complete our thesis and research, Microfluidic Device for CRISPR Diagnostic of Sar-Cov2. Without the advisor's involvement, our thesis cannot be perfectly progressed with systematic problem-solving procedure and accomplished with the desired result.

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LIST OF CONTENTS

LIST OF TABLES

LIST OF EQUATION

LIST OF FIGURES

LIST OF SYMBOLS/ABBREVIATIONS

Symbols/Abbreviations	Terms
ACE2	Angiotensin-converting enzyme 2
ATK	Antigen Test Kit
BCoVs	Beta Coronavirus
cDNA	complementary DNA
CAD	Computer-aided Design
CAE	Computer-aided Engineering
CAM	Computer-aided Manufacturing
CNC	Computer Numerical Control
CO_2	Carbon dioxide
Covid-19	Coronavirus 2019
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
DNA	Deoxyribonucleic Acid
DMVs	Double Membrane Vesicles
ER	Endoplasmic Reticulum
EU	European Union
HPMC	Hydroxypropyl methylcellulose
MERS	Middle East Respiratory Syndrome
MERS-CoV	Middle East Respiratory Syndrome Coronavirus
ml	milliliter
mm	millimeter
nsps	Nonstructural Proteins
PCR	Polymerase Chain Reaction
PET	Polyethylene terephthalate
PMMA	Polymethyl methacrylate
PSA	Pressure-sensitive Adhesive
RAT	Rapid Antigen Test
RBD	Receptor Binding Domain

RNA	Ribonucleic Acid
RPA	Recombinase Polymerase Amplification
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
Sar-CoV-1	Severe Acute Respiratory Syndrome Coronavirus 1
Sar-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SARS	Severe Acute Respiratory Syndrome
TMEC	Thai Microelectronics Center
TMPRSS2	Transmembrane Serine Protease 2

CHAPTER 1

INTRODUCTION

1.1 Background

Coronavirus disease is a large strain virus commonly known to cause a wide range of illnesses from common colds to more serious illnesses such as Middle East Respiratory Syndrome (MERS) and severe acute respiratory syndrome (SARS). This disease caused by the SARS-CoV-2 virus. The majority of virus-infected individuals will experience mild to severe respiratory disease and will recover without the need for special care. However, some people will get serious illnesses and need to see a doctor. Serious sickness is more likely to strike older persons and those with underlying medical illnesses including cancer, diabetes, cardiovascular disease, or chronic respiratory diseases. COVID-19 can cause significant illness or death in anyone, at any age. Therefore, it is imperative that there is a rapid and accessible way to detect coronavirus. To reduce the medical costs associated with the treatment and can reduce the mortality rate when we know the results of the covid detection and get treatment quickly.

During the SAR-CoV-2 pandemic, the current diagnostic method with highly accurate result such as Real time polymerase chain reaction (RT-PCR) shows the longer waiting period (1-3 days) compared with Antigen Test Kit (ATK) which gives a result with 15-30 minutes but lower in accuracy. To enhance the diagnostic accuracy and shorten the waiting period, CRISPR diagnostic assay is developed as low-cost and rapid diagnosis with the accuracy close to that of RT-PCR. We want to improve it further by transferring the CRISPR assay to a microfluidic platform which can be made into a test kit that is convenient to use by the preliminary method, just drop the solution into the starting channel, then set the time as set and check the result immediately after the solution flows to the last channel. It is an easy for the public to access, just like testing with Rapid Antigen Test Kit.

Therefore, we observed the advantages in the both ATK and RT-PCR. We will apply the CRISPR assay with the microfluidics. Microfluidic is technology which can flow the fluid inside small channels in microscale. The dimensions of microfluidic is very small. It can contain small amount of substance. This project applies the capillary force formula to figure out the behavior of

the fluid flow inside microfluidic device. The devices are made from acrylic sheet which is a low-cost material when compared to Polydimethylsiloxane PDMS which the scientist normally use.

1.2 Objectives

The overall objective is to fabricate a microfluidic device that can improve ease of use and lower diagnostic costs of the SARS-CoV-2 detection through CRISPR diagnostic assay. The project can be divided into the three following sub aims.

1. To identify the most robust approach to build low-cost microfluidic devices from PMMA.

2. To investigate the fluid behavior inside microchannels.

3. To test the flows of CRISPR assay or fluids with similar properties in microfluidic devices.

1.3 Hypothesis

1.Capillary force formula can be applied in microchannels

2.The microfluidic devices can be used to detect SAR-CoV-2

1.4 Research scope

Biology, programing, and engineering can be applied in this research for building microfluidic devices and observed behavior of the fluid that can flow inside the microchannels. The device is made from PMMA. We expect that the fluid can completely flow in microfluidic device.

1.5 Report outline

The research started from January 2022 to April 2023 for a period of approximately 16 months with a time of 5 days per week.

Table 1 Report outline.

Topics	2022												2023			
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
1.Bernoulli Principle calculation																
2. The first prototype design																
3. Build and test the first prototype																
4.Thermal bonding																
5.Cooperate with NANO																
6.Compare result of the first prototype																
7. Capillary force theory calculation																
8. Sonication of Devices																
9.HMPC Coating																
10. Second prototype																
11. Test water flow on the devices with																

various length																
12. 3 layers bonding with 3M #467MP and normal glue tape																
13. Cooperate with TMEC																
14. Compare the result buffer testing and water testing on different devices																

CHAPTER 2

RELATTED THEORIES

2.1 Microfluidic device

2.1.1 Basic of microfluidic device

Microfluidic devices are widely used in fields such as engineering, medicine, food industrial, chemistry. Microfluidic devices are another diagnostic and experimental option which can vary in complexity depending on the design. The advantages of these devices are that they are inexpensive in their production and diagnostics using reagent volumes lower than the large-scale volume. A microfluidic device can be called miniaturized total analysis or laboratory on a chip. The example of our microfluidic device (Figure 1) is made of PMMA [1].

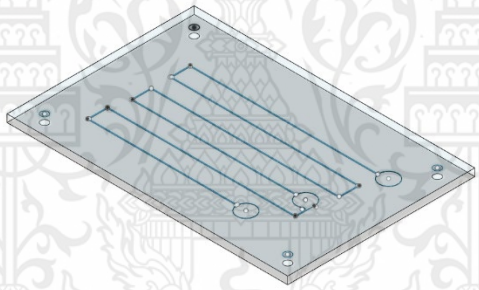


Figure 1 Microfluidic device.

2.1.1.1 Self-propelled microfluidic device

Self-propelled microfluidic device required capillary force to flow the solution without using external force to control.

2.1.2 Usage material for microfluidic device

In the past, microfluidic devices were made of silicon wafers or glass. Silicon is too expensive, opaque, and non-biocompatible. For the glass is transparent in the visible light. It is very hard to etch microchannels on the glass and spend more time in the fabrication methods under the high temperature and pressure. So, there is another option which is thermoplastics. Thermoplastics are transparent, inexpensive, good resistance to chemicals, high strength, and good biocompatibility [1].

2.1.2.1 PMMA

In the research, using a PMMA which is an acrylic-based thermoplastic with transparent, inexpensive, good resistance, good mechanical stability and the PMMA is like glass but inexpensive and lighter than glass [1].

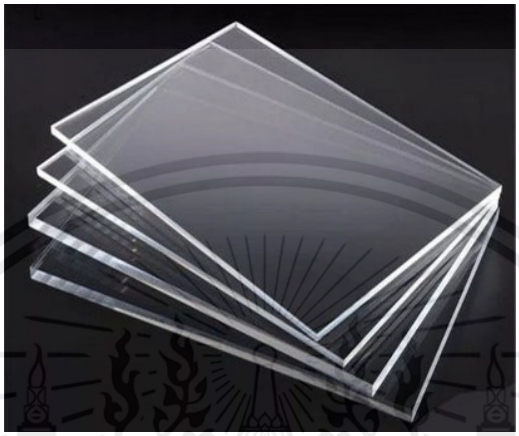


Figure 2 PMMA.

2.1.2.2 PET

In the research, using a PET which is a thermoplastic as the middle layer of microfluidic and since, it can be suitable for cutting with the CO_2 laser machine because it cannot occur toxic gas. Also, it is high transparency, inexpensive, good resistance, good chemical resistance.

Figure 3 PET.

2.1.3 Usage supported material for microfluidic device

In this research, We use 3M Adhesive Transfer Tape 467MP as bonding on the surface between PET and PMMA. It offers exceptional adherence to high surface energy polymers and metal. The adhesive thickness is 0.06 millimeter. There is humidity resistance that has low effect on adhesive performance and also have the temperature resistance when using for short term it can be at 204 degrees Celsius and for long term it can be at 149 degrees Celsius. For the storage and shelf life, it is advised that items be kept in rooms with a temperature of 21 degrees Celsius and a relative humidity of 50%. For 24 months after the date of manufacturing, a product retains its performance and qualities when stored appropriately.

Figure 4 3M Adhesive Transfer Tape 467MP.

2.2 The engraving process of microfluidic device

There are two methods consisting of using CO_2 laser machine and CNC machine

2.2.1 Using CO_2 laser cutter

The process starts with design in drawing software such as Fusion 360 and AutoLaser. After designing, the drawing file will be exported to the CO_2 laser machine and then bringing a PMMA place on the machine under the CO_2 laser beam. The process takes 5 minutes to 1 hour depending on the resolution of the prototype design. The laser device contains a laser source inside the hood of machine, and the laser radiation it emits is directed through mirrors to a movable head that moves in the x-y plane. To direct and focus the laser beam downward onto the stage, the head features a mirror and a focus lens. The important variable is the power and velocity of the beam [2].

2.2.2 Using CNC machine

The process starts with design in drawing software such as Fusion 360 and export file to SOLIDWORKs and then prepare the machine tool to receive the file and send the file using the configured communication settings. The process takes 5 minutes to 10 minutes depending on the resolution of the prototype design, but the disadvantage of this process is when engrave on the PMMA in microscale the result will not accuracy because the surface of PMMA is uneven in microscale that make the direction of milling end is discrepancy.

2.3 Microfluidic device's principle

2.3.1 Bernoulli principle

The Bernoulli principle is based on the assumption of the 2 points on the streamline with the constant density of fluid. The principle focuses on the balance between the pressure, velocity, and elevation that is shown in the equation below [3].

$$\frac{P_1}{W} + Z_1 + \frac{V_1^2}{2g} + h_a = \frac{P_2}{W} + Z_2 + \frac{V_2^2}{2g} + h_f$$

Equation 1 Bernoulli's principle.

where

P = pressure.

W = weight.

Z = elevation.

V = velocity.

g = acceleration of gravity.

h_a = head added by pump between points.

h_f = head loss due to friction between points.

The head loss due to friction between points is when the energy of fluid wants to pass the wall of channel that is shown in the equation below.

$$h_f = f\frac{LV^2}{2gD}$$

Equation 2 Head loss.

Bernoulli's equation describes the velocity of fluid flows inside the channel of a microfluidic device, which is affected by the Head loss or friction that occurs within a pipe and elevation of the microfluidic device. The inequality of the channel between both points can influence the velocity, in which the pressure at both points is equal. The equation is shown below.

$$A_1V_1 = A_2V_2$$

Equation 3 Continuity Equation.

where

A_1 = Area at the point1

V_1 = Velocity at the point 1

A_2 = Area at the point 2

V_2 = Velocity at the point 2

The limitations of Bernoulli's equation must be carefully noted as it is an idealized case. It applies only to steady flow within a streamline and to flow where no energy is lost through turbulence.

2.3.2 Capillary force

Capillary force Equation 4 refers to the flow of a fluid depending on its contact angle, viscosity, and the surface tension. The interaction of the solid surface and the liquid can determine the velocity of the fluid inside the channel [4].

$$Q = \frac{1}{\eta}\frac{\Delta P}{R_F}$$

Equation 4 Capillary force.

where

η = the viscosity of the liquid.

ΔP = the difference in pressure inside and in front of the liquid, which can be taken as the capillary pressure P_C.

R_F = the flow resistance along the flow path.

From the Equation 4, the flow rate is directly proportional to the capillary pressure and indirectly proportional to both flow resistance and the fluid viscosity. Capillary pressure and flow resistance can be calculated by the following (Equation 5, 6).

$$P_C = -\gamma[(\frac{cos\alpha_t + cos\alpha_b}{h}) + (\frac{cos\alpha_l + cos\alpha_r}{w})]$$

Equation 5 Capillary pressure.

where

γ = the surface tension of the liquid.

α = the contact angles.

$$R_F = [\frac{1}{12}(1 + \frac{5h}{6w})\frac{hwR_H^2}{L}]^{-1}$$

Equation 6 Flow resistance.

where

h = the depth of the microchannel.

w = the width of the microchannel.

R_H = the hydraulic radius of the microchannel.

The hydraulic radius R_H can be calculated by.

$$R_H = \frac{hw}{h + w}$$

Equation 7 Hydraulic radius of the microchannel.

From Equation 5, the capillary pressure relies on the contact angle and surface tension accelerating the flow inside of channel. Similarly, the flow resistance is directly proportional to the length of the channel, which means the velocity of the flow will be decreased alongside with the longer distance fluid passes through. To manipulate the flow of fluid, the depth and width of the channel would participate in the equation because the increase or decrease of both values influence the flow rate of the liquid [3].

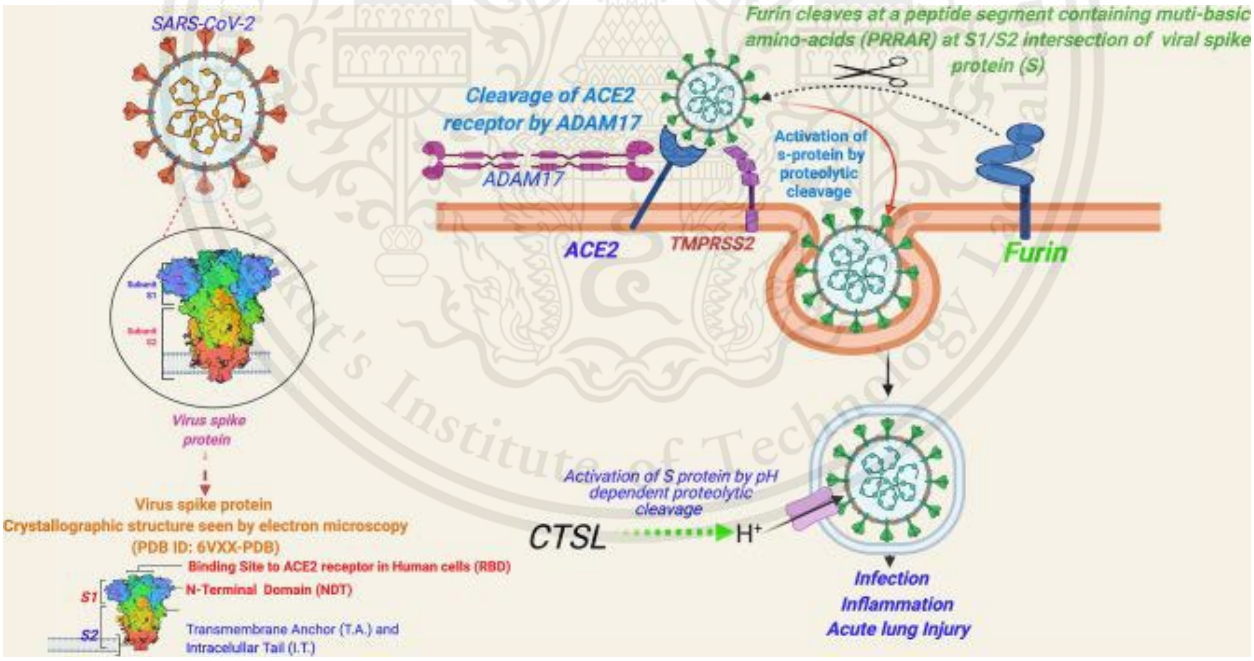
2.3 COVID-19

2.3.1 Fundamental of COVID-19

Covid-19 or coronavirus disease 2019 is described as an infection of severe acute respiratory syndrome coronavirus 2(SAR-CoV-2) leading to the global pandemic. The SAR-CoV-2 belongs the genus of beta coronaviruses (BCoVs) which is similar to SARS-CoV-1 and the Middle East respiratory syndrome coronavirus (MERS-CoV). The disease symptom occurs within the 2 weeks, after exposure, in which the symptoms appear as fever, dry cough, or lose of taste and smell [5].

2.3.2 The entry of virus

The infection begins with the entry of virus into the cells with the cell receptor called angiotensin-converting enzyme 2 (ACE2) which is bound with the receptor binding domain (RBD) of the SAR-Cov-2 virus spike protein. The cleavage of spike protein is responsible by the transmembrane serine protease 2 (TMPRSS2) exposing a part of the viral cell to fuse with the host cell [5] [6].



Inside the cell, the viral genome is translated into the replicase proteins which are later cleaved into the individual nonstructural proteins (nsps). Replication and traslation occur in double membrane vesicles (DMVs) derived from edoplasmid reticulum (ER). The result of RNA translation is then inserted into the ER-Golgi intermediate compartment for assembly of viral proteins. In the final step, the replicated viruses are released out of the cell and invade the other cells [7].

2.4 COVID-19 test

2.4.1 Antigen test for SARS-CoV-2

Antigen tests can detect SARS-CoV-2 antigens before COVID-19 symptoms appear. The antigen, which is a component of the pathogen, will stimulate the immune response. This test looks for antigenicity from surface spike protein. The examination lasts for 15 minutes. Antigen tests can be performed anywhere, without the need for special equipment [8].

2.4.1.1 Rapid Antigen Test

In the COVID-19 situation, RAT is the important method to detect COVID-19 because of the quick examination but this test is less accurate than polymerase chain reaction testing. RAT can be used only in symptomatic individuals and confirmed positive cases at the laboratory [9].

2.4.1.2 Antigen Test Kit

ATK is the device used to detect the SARS-CoV-2 that causes COVID-19. The users who are suspected of being infected with symptoms can swab specimens from the nasal within the first seven days of having symptoms. The results are when the SARS-CoV-2 Nucleocapsid protein Antigens can be detected, two distinct colored lines appear which means it is a positive result assuming that the users are having COVID-19, but the kit show only one colored line appears in the control region and disappear on the test region that means it is a negative result. In addition, the users can repeat the test with the new kit if the users want to make sure the results are correct or not.

Figure 5 Each case of ATK detection.

2.4.1.3 Method of ATK

The example method ALL TEST Brand of using the antigen test kit is first, to insert the soft end of the swab approximately 2cm up the user's nostrils then twist the swab slowly 5-10 times against inside the nasal. Remove the swab from the nose and insert the swab into the tube which contains sodium azide then mix well and rotate for 10-15 seconds with the mucus. Close the cap of the extraction tube and open the small cap of the specimen extraction tube then drop the specimen into the test cassette. Finally, waiting for the result for 15 minutes.

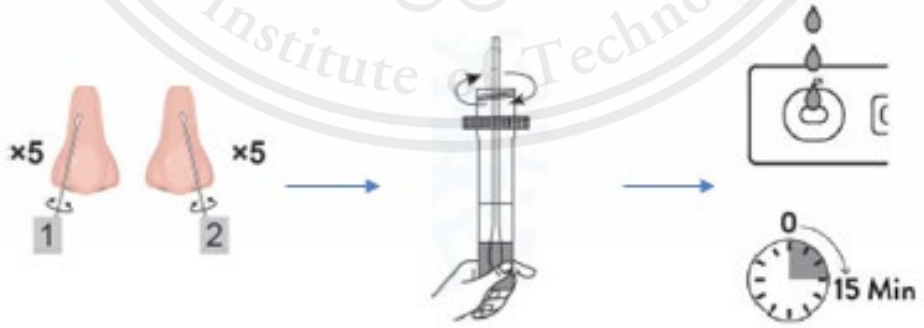


Figure 6 ATK procedure.

2.4.2 Real time Polymerase chain reaction

RT-PCR is the well-known coronavirus 2019 detection method with the high accuracy. This method was derived from polymerase chain method (PCR) using the primer to produce the desired sequence of DNA. To detect viral RNA in the human body, the reverse transcriptase was applied to convert the viral RNA into complementary DNA (cDNA) for the amplification in accordance with the PCR. The RT-PCR can generate a large number of viral DNA sequences within a short amount of time providing the quantitative data with accuracy for viral detection. Therefore, the patients with a small number of viral RNA presence or asymptomatic condition are tested with the positive result [10].Therefore, the patients with a small number of viral RNA presence or asymptomatic condition are tested with the positive result [10].

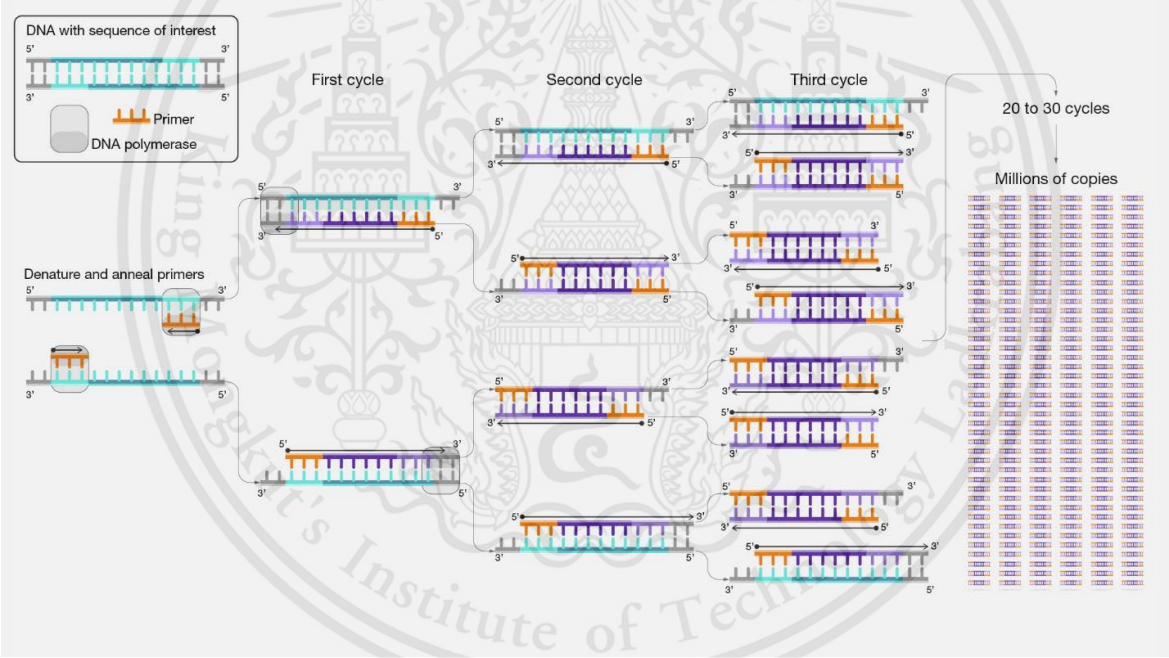


Figure 7 PCR cycle.

The PCR starts with the denatures of DNA into 2 separated strands of DNA and then Taq polymerase will produce the replicated DNA using the original DNA template. The denatures and replication using the Taq polymerase continues in a cycle to amplify the DNA to the enormous number of DNA sequence [11].

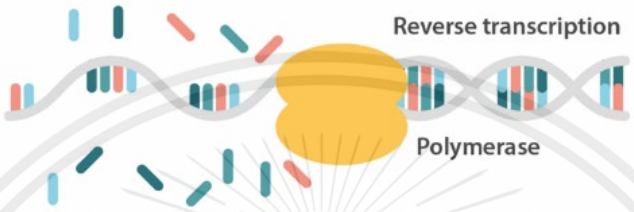


Figure 8 Reverse transcription.

2.5 The developing technology of detection COVID-19

2.5.1 Clustered regularly interspaced palindromic repeat.

CRISPR is a technology which is used to edit genes. CRISPR has been designed and developed. There are two major systems which are Cas endonuclease and guide RNA. Cas endonuclease is the system for denaturing the target of the genome and guide RNA is the system for specifying Cas endonuclease on the target region. There are many Cas endonucleases such as Cas9, Cas12, and Cas13 endonucleases [12].

CRISPR/Cas is the adaptive defense mechanism against viruses. The process starts with The DNA being injected into the bacteria cells by phages then the bacteria's immune system responds to phages by cutting the viral DNA. After that, the bacteria contain bits of viral DNA in a specific site called the CRISPR locus. These snipped bits of viral DNA sequence become protospacers. Then spacers that come from the whilst inserting into the CRISPR locus are interspaced by identical repeats. CRISPR plays a role in the memory of phage infection [13].

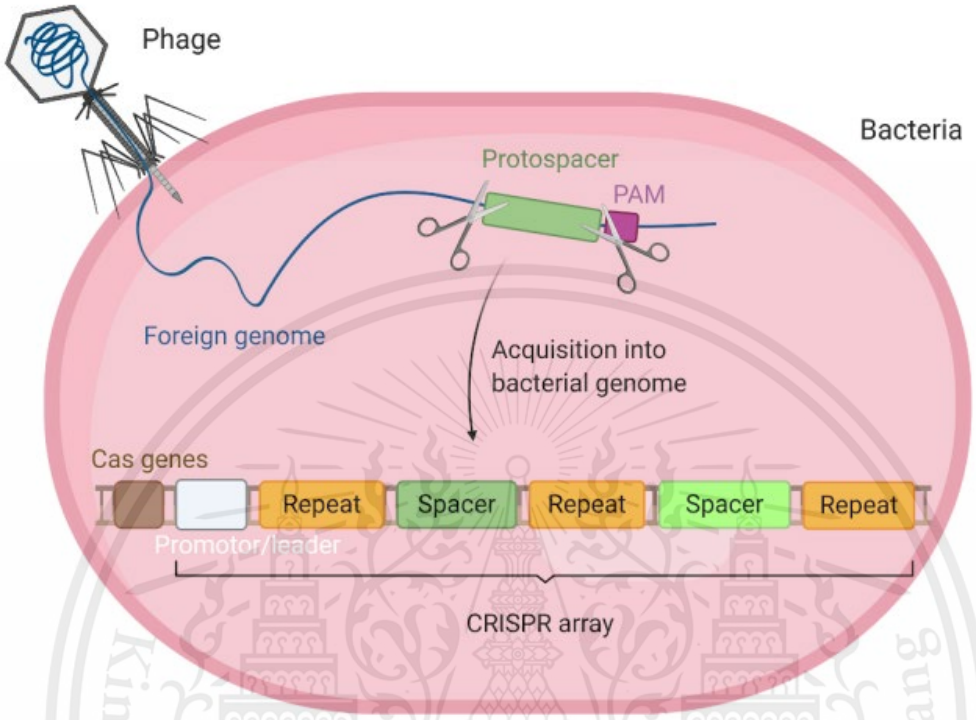


Figure 9 Clustered regularly interspaced palindromic repeat.

The CRISPR diagnostics was derived from the CRISPR/Cas and used as the disease detection method. The components of CRISPR diagnostics include Guide RNA to discover the viral RNA, endonucleases 12a (Cas12a), and quenched fluorescent reporter. The guide RNA will find the target and activate the Cas12a to cleave the quenched fluorescent dye separating quencher and fluorescent dye, which leads to show the fluorescence in case of having the viral RNA.

2.6 Designing Software

2.6.1 Fusion 360

Fusion 360 is the design software application that Autodesk develops. This program combines CAD, CAM, and CAE applications into a single application for response to integration work step by step. The users can start to design, draw, and analyze. There are simulator and animation functions while the user is working. For designing the product or prototype, many tools are accessible, suitable, and convenient also co-working with others on the same project because one can create or join the team. The users can work on both computers and tablets since the data

can be saved in the Autodesk cloud storage but the performance of this program on the tablets is not used at its full potential because some libraries or tools are inaccessible. In this project, working on the computer, the program can be accessed by the student who wants to design because it is free for the students in the institution and the tools are consistent in the engineering field.

The advantages of Fusion 360 are that it is easy for the users to work together through the cloud system and covers the functionality of the operating system on Mac and Windows. The specialties of the program are that it can be designed in a variety of ways so that users can design products according to their preferences and when the users want to simulate whether things can be produced or not. The users can use the simulation, assembly, animation, and rendering functions. After designing, the users can export the files to produce the prototype via a machine such as a CNC machine or 3D printing.

Figure 10 Fusion 360.

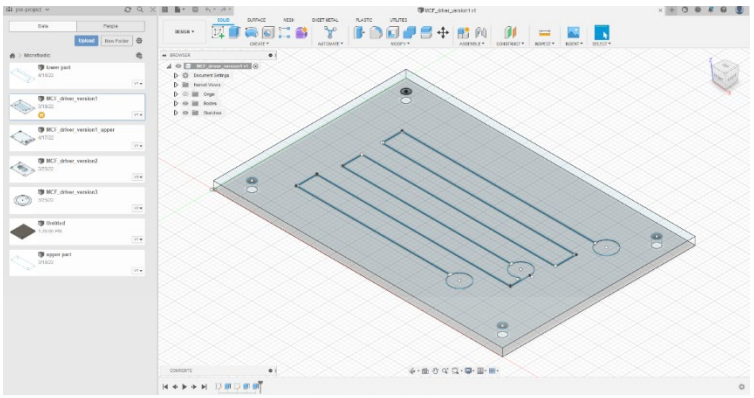


Figure 11 Fusion 360 display screen and example of the designing.

2.6.2 AutoLaser

AutoLaser is the design software application that is used to design the product or prototype to prepare before putting the file into the laser machine for further engraving and cutting. It is suitable for simple designs to make cut or engrave. There is simulation before it is actually cut or engraved.

Figure 12 AutoLaser

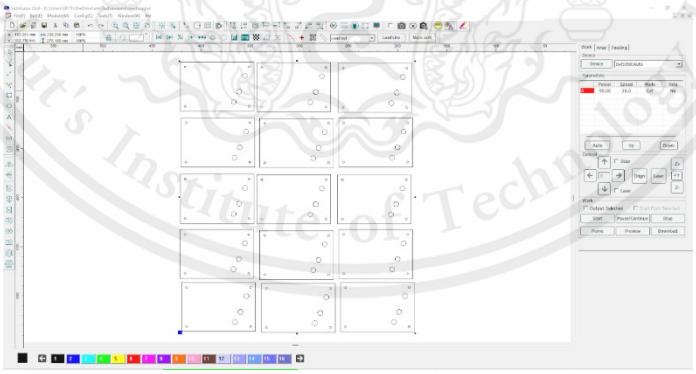


Figure 13 Example of designation.

There are two majors' mode of AutoLaser program that are used in this project. The first mode is cut which is the mode that is designed to cut the material. The material will be cut through or cut off completely. The usage power is dependent on the thickness of material that the users use. The second mode is engraving which is the mode that is designed to carve the material and no need to cut through the surface of material. After designing, there is simulation of the design to preview before exporting the file to the Laser-cut machine. The simulation is on the preview function that is shown in figure 13.

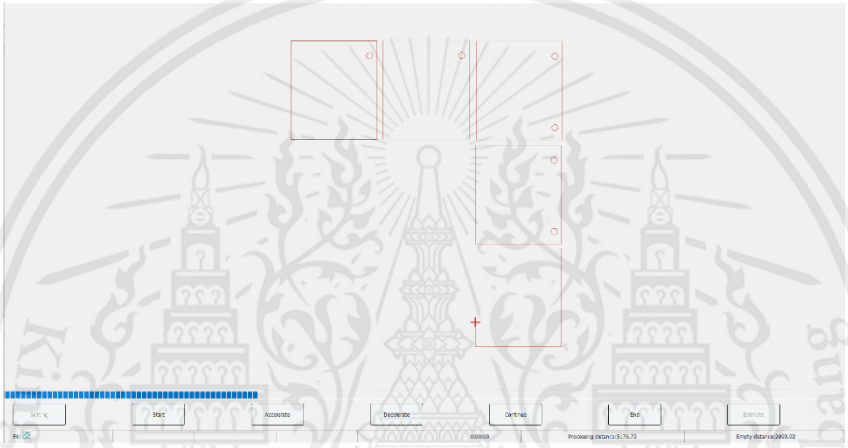


Figure 14 Preview funtion of AutoLaser.

2.7 Laboratory software

2.7.1 ImageJ

ImageJ is the program related to image analysis allowing the users to do the desired measurement and adjust the picture. The program supports a series of images, in which the image multi- reading can be proceeded in short period of time. With the provided function, the program is able to perform an area and pixel calculation on the selected part together with the histogram creation. Image processing such as sharpening, contrast manipulation, smoothing, etc. can be performed from the program.

Figure 15 ImageJ.

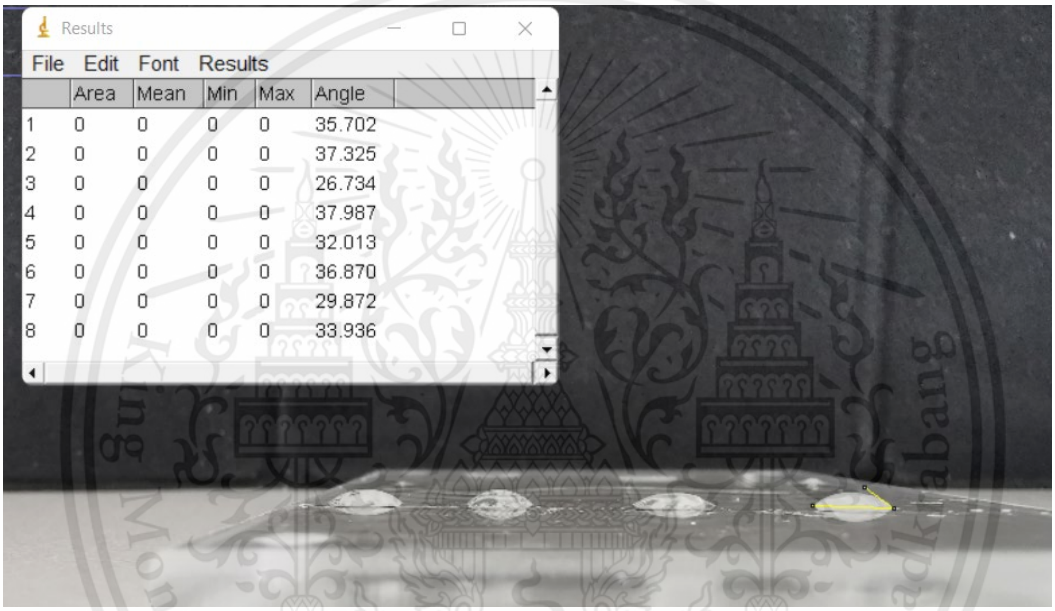


Figure 16 Example of contact angle's measurement

Furthermore, the program can proceed the scaling, rotation, and flipping the image, which is available to the image analysis. The angle and distance are measured through the tools in the program.

2.7.2 Google colab

Google colab is the Google-based website providing collaborative activities on the python coding. The program is suitable for the researcher, students, or data scientist as the data analysis and machine learning can be performed in the provided “Colab notebook”. The code cell on the website can be filled with python coding for the desired purpose.

Figure 17 Google Colab.

2.8 Devices and Machine in project

2.8.1 Micropipette

A micropipe is a device that is used to transfer the amount of less than 0.1 ml of liquid. The micropipette is a necessary skill in biology and chemistry laboratories. The maximum volume that the micropipette can be transferred with that size of micropipette [14].

Figure 18 Micropipette.

2.8.2 Micropipette tips

Micropipette tips are the supporting tools which are used to connect with the micropipette. The micropipette tips can protect the micropipette shafts from contamination and reduce the contamination during suction of solution. The micropipette tips must be fit with the size of micropipette for prevent the leakage of solution.

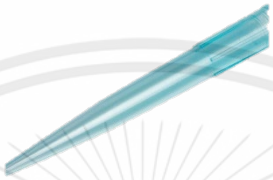


Figure 19 Micropipette tips.

2.8.3 Ultrasonic bath

An ultrasonic bath is the machine used to clean, and sterilization sterilize the surface of the material. The ultrasonic bath will release energy from all areas over a large volume in the bath. This machine can force surface contaminants to release. The users can add the liquid into the bath as a cleaner. The ultrasonic bath might also be useful for liquid degassing, cell separation, or cell lysis [15].

Figure 20 Ultrasonic bath.

The Advantages of this machine are applications requiring less power and diffuse rather than focused energy is better suited for an ultrasonic bath.

The method of ultrasonic bath is ultrasonication that is frequently used to clean and remove rust. This method can be used to clean solid deposits due to cavitation and ultrasonic wave bombardment mechanisms. Cavitation generates heat and energy, which raises the temperature and aids in the dislodging of deposition by loosening it [16].

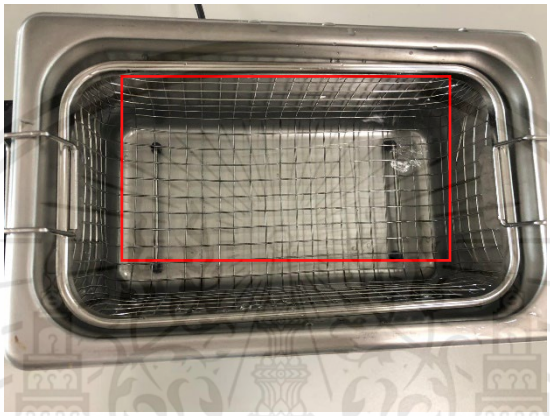


Figure 21 Cleaning the material by ultrasonic bath.

The effect of ultrasonication on PMMA, Crazing phenomenon is fatal affecting the mechanical properties of PMMA because the appearance of crazing on PMMA surface such as creeping behavior, fracture toughness, etc. Due to ultrasonication, the temperature of the solution that used to clean the PMMA surface keep increasing that is the reason why the crazing phenomenon occur. Crazing is a brittle fracture that make PMMA to be deformation and energy absorption which are craze initiation [17].

2.8.4 Hand vacuum pump with manometer

It is the pump that produces the vacuum, not to hold the vacuum. It has a vacuum gauge to measure the vacuum pressure when the pressure is lower than the atmospheric pressure. It can be controlled with one hand, and the vacuum manometer allows to regulate the pressure that is created while manually pumping the fluid.

Figure 22 Hand vacuum pump with manometer.

2.8.5 Hot air oven

It is an electrical device which uses a thermostat to control the temperature inside the device, it can manually adjust the temperature. Inside the device there are aluminum trays to place the object and have vary capacity.

Figure 23 Hot air oven.

2.8.5 The computer numerical control machine

The computer numerical control machine or can be called CNC machine is the machine which creates the products on the materials such as plastics, glass, metals, aluminum, and hard material The process of CNC machine is use of the computer-driven machine tool to produce a part out of solid material in a different shape, depth, wide or many other parameters. The products of CNC are designed by computer design software like Autodesk Fusion 360, SolidWorks Autodesk Inventor, and other software which the CNC can read the language.

The CNC machinist must have skills in both programming and material working to use the full potential of this machine. Most of the software that is used in the CNC is CAD software that is used to create designs. CAD programs include AutoCAD, SolidWorks, Autodesk Fusion 360 and Rhino3D [18].

Figure 24 CNC machine.

2.8.6 The CO_2 laser cutter

An electrically powered gas laser is used in the sheet metal processing method known as CO2 laser cutting. Steel, stainless steel, or aluminum metal sheet material is contour-cut by the laser. With CO2 cutting technology, you have a lot of shaping freedom. Even cutting complicated shapes is made simple by this. In the research, we will use this machine to cut a PMMA which has different in the thickness of sheet. The machine will work along with file that is exported from AutoLaser. Most metals and some non-metals can be cut and engraved using fiber laser technology. These devices can effortlessly cut a variety of metals. However, marking and engraving are the main uses for these lasers. They can engrave a variety of materials such as silver, gold etc. The power in this machine is laser optical/output power. It can cut and engrave materials such as PMMA, PMMA with costing materials etc.

Figure 25 CO_2 laser cutter.

2.8.7 Hydraulic Press

The hydraulic press is the machine which uses the compressive force to press out material on the plate where is placed in the machine. The compressive force is produced by the hydraulic cylinder. The machine works on Pascal's law which is powered by hydraulic pump. A hydraulic system consists of hydraulic motor, piston, pipe. In the machine, there are two cylinder types, the first cylinder is the slave cylinder as a small cylinder. The fluid will be poured into slave cylinder and the fluid in this cylinder is compressed by the piston within, and as a result, it flows via a pipe and into the master cylinder as bigger cylinder and then the fluid is forced back into the original cylinder. The force which is applied on the fluids by the hydraulic motor and the slave cylinder will produce more force than the master cylinder [19].

Figure 26 Hydraulic press.

2.8.8 Vise

Vise is a mechanical tool used to hold an object in place so that work can be done on it. The two parallel jaws of a vise are threaded in and out by a screw and a lever, with one jaw being fixed and the other movable.

Figure 27 Vise.

2.8.9 Compound microscope

The compound microscope uses a compound lens system. There are many lenses on the microscope. For the objectives lens which have different scale consisting of 4x, 10x, 40x and 100x. For the eyepiece lens normally use 10x. In this research, We use Xenon D-117MS compound microscope to look the solution flow inside the microfluidic channel. When placing the object on the microscope, the light will pass through the object. The real image will be obtained by objective lens and then the eyepiece lens will magnify the real image which is the virtual image.

Figure 28 Xenon D-117MS compound microscope.

2.8.10 Stereo scope

The stereo microscope is used to observe low magnification using light reflected from the surface of sample object. There are two lens as same as the compound microscope. This microscope is suitable for looking at large samples or objects. In this research, We used Motic SMZ-171 stereo microscope.

Figure 29 Motic SMZ-171 stereo microscope.

2.9 Related Chemical

2.9.1 Ethanol

Ethanol 99.9% is a pure ethyl alcohol that is prepared in accordance for quality control for pharmaceuticals intended for human and veterinary use.

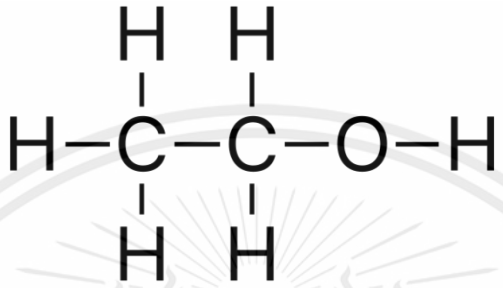


Figure 30 Ethanol formula.

The advantage of Ethanol in this research is that it is used to bond the PMMA in the oven at 60 to 70 degrees Celsius. Ethanol is the alcohol stimulating the PMMA surface which enables the bonding between the PMMA to form under the control temperature.

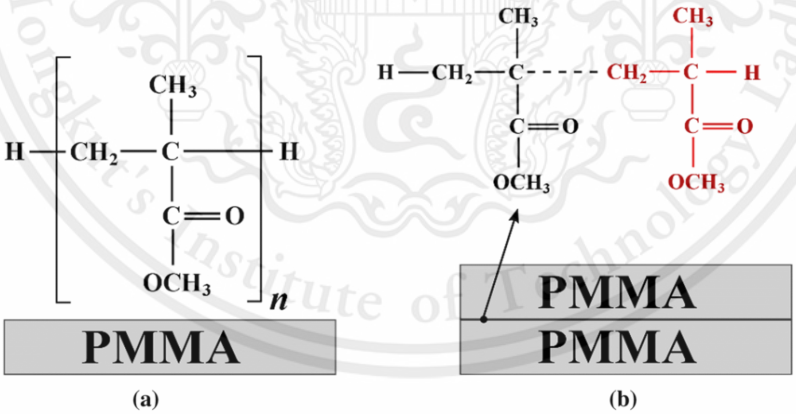


Figure 31 Crosslinking of PMMA.

2.9.2 Propanol

The propanol or isopropyl alcohol is the primary alcohol, which is used as the bonding intermediate for thermal bonding method and as the testing subject in the fluid dynamic.

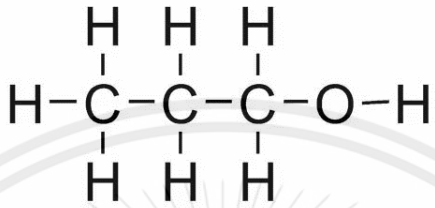


Figure 32 Propanol formula.

2.9.3 Hydroxypropyl methylcellulose

HPMC is a coating agent for the reduction of the water contact angle. With the adjustment of the functional group, HPMC can form the ester bond with the Polymethyl Methacrylate (PMMA) modified to form carboxylic acid group by Nitric acid.

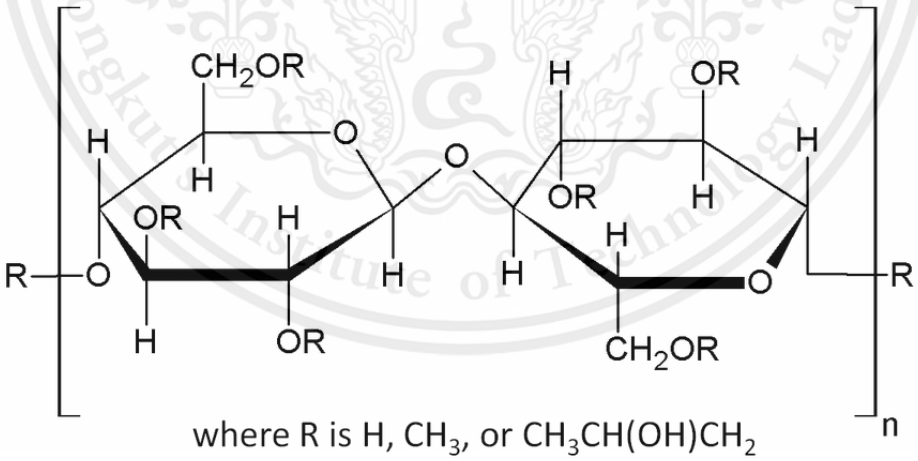


Figure 33 HPMC chemical formular.

2.9.4 Recombinase Polymerase Amplification

Recombinase Polymerase Amplification is the process of using a recombinase to pair the oligonucleotide with the DNA targets for the DNA synthesis.

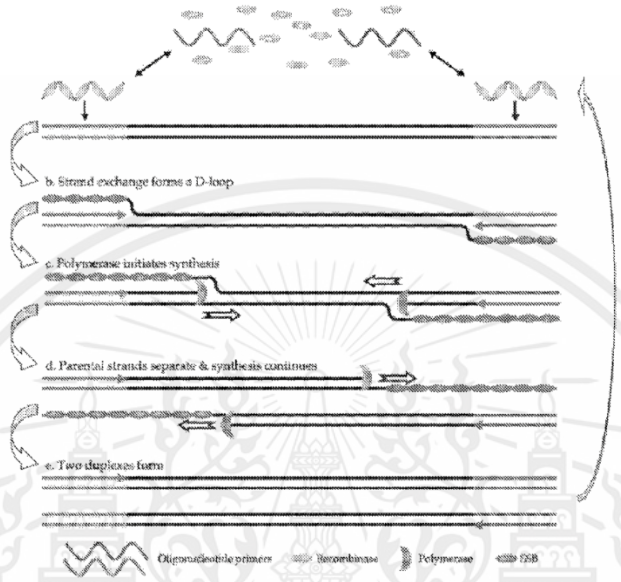


Figure 34 The RPA Cycle.

CHAPTER3

Methodology

The microfluidic research methods are separated into 3 steps including design, build, and test. In this chapter will explain how We build microfluidic device step by step. The PMMA will be the material that research took to do in this research. The design will depend on the capillary force equation.

3.1 Relation material and equipment

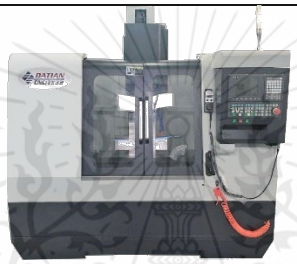
3.1.1 Material

No.	Name	Picture	Function
1	PMMA sheet		The main material to create a microfluidic device. The PMMA sheet will be crafted by CNC or Laser cut to create a channel.
2	PET sheet		The middle layer of 3-layer bonding method.
3	3M #467MP tape		The bonding intermediate for 3 layers bonding method

4	Magic tape		The bonding intermediate for 3-layer bonding method or 2-layer bonding.

Table 2 Material's list.

3.1.2 Equipment

No.	Name	Picture	Function
1	CNC machine		The machine crafts the PMMA sheets to create a water channel.
2	Laser machine		The laser machine cut the PMMA sheet or PET sheet to form a channel.
3	Stereo microscope		The width of the channel can be measured by using the Motic SMZ-171.
4	Compound microscope		The channel can be observed through Xenon D-117MS.

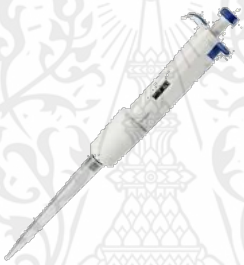
5	Ultrasonication bath		The bath was used to clean the powder inside the device channels.
6	Hot Air Oven		The oven was used to bake the PMMA sheet in the thermal bonding process.
7	Micropipette and tips		The micropipette was used to drop solution into the channel.

Table 3 Equipment's list.

3.1.3 Chemical substance

No.	Name	Picture	Function
1	Absolute Ethanol		The bonding intermediate for thermal bonding method.
2	Propanol		The bonding intermediate for thermal bonding method.
3	HPMC		HPMC was used as the coating substance for increasing the hydrophilicity of the surface.
4	Phosphoric acid		20mM Phosphoric was mixed with HMPC to treat the surface.

5	Nitric acid		The nitric was used to flush the channel prior to coating with HPMC.

Table 4 Chemical substance's list.

3.1.4 Supported equipment

No.	Name	Picture	Function
1	Paper clip		The paper clip was used to hold the PMMA sheets together during the baking process.
2	Magnet bar		The magnet bars were used to hold the PMMA sheets together during the baking process.
3	Air duster		The air duster was used to clear the and dry the channel.

Table 5 Supported equipment's list.

3.2 Diagram of principle

The Bernoulli principle was applied to calculate and cooperate with the computational design, in which the calculation of length and velocity of the device based on this theory was done in Google colab.

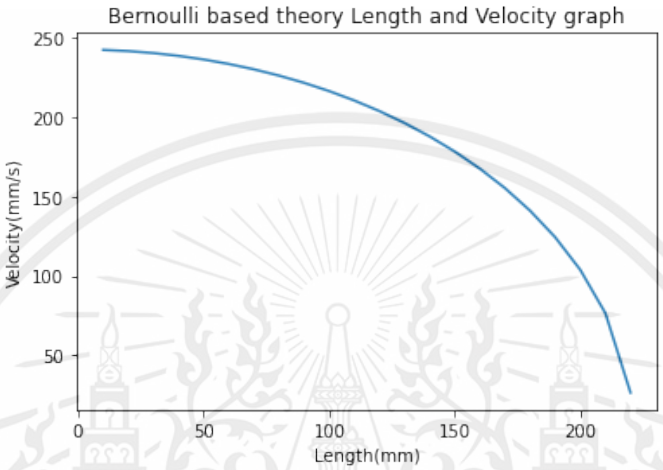


Figure 35 Bernoulli's principle based on length and velocity.

With the small size of the device's channel, the capillary force was cooperated to describe the water flow inside of the fluid, and later incorporated in the computational modeling of the device. The calculation of the velocity, distance, and length was done in Google colab to demonstrate the relationship of the variables.

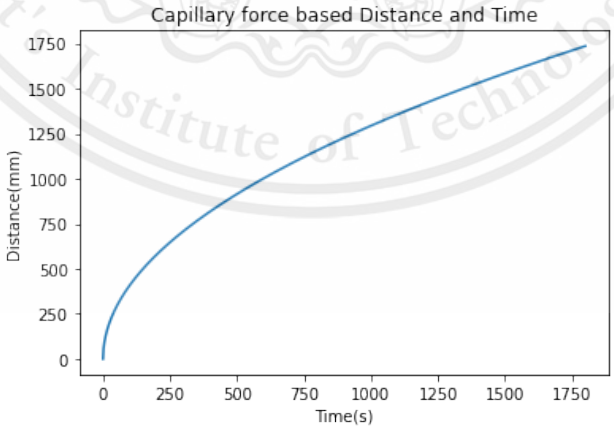


Figure 36 Capillary force based on length and time.

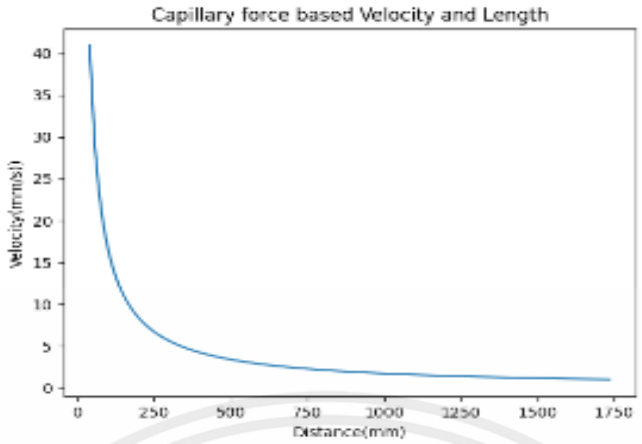


Figure 37 Capillary force based on length and velocity.

3.3 Design the device by Autodesk Fusion 360

The microfluidic device was designed by Autodesk Fusion 360, The microfluidic device was designed for testing the flowing behavior of the liquid inside the channel and designed by referring from capillary force. We would like to check the corresponding between experiment and ideal method.

3.4 Layer type of microfluidic device

In the research, there are two types of microfluidic device's layers.

3.4.1 The 2-layer microfluidic device

This device will have the upper layer and lower layer which are made from a PMMA that is cut by a CNC machine or CO_2 laser cutter.

3.3.1.1 The 2-layer microfluidic device which is came from thermal bonding

We use ethanol to bond the upper layer and lower together with clamps or magnetic bars.

3.3.1.2 The 2-layer microfluidic device which is came from the bonding with tape

We use magic tape or 3M #467MP to bond intermediate between the upper layer and lower layer.

3.4.2 The 3-layer microfluidic device

This device will have an upper layer and lower layer which are the same as the 2-layer. This method is different from the fabrication of the 2-layer device. Due to the middle layer has been added. The middle layer was made from PET. The PET plate was attached with tape on both sides as a bonding intermediate bonding with upper and lower PMMA layers. The 3-layer bonding

technique solves the problem of the inconsistency of the thermal bonding and prevents the leakage inside the channel.

3.5 Microfluidic device fabrication

3.5.1 Building of microfluidic device

These microfluidic devices were divided into three parts which are the upper layer, lower layer, and middle layer by using CO_2 laser cutter and CNC machine to cut or engrave.

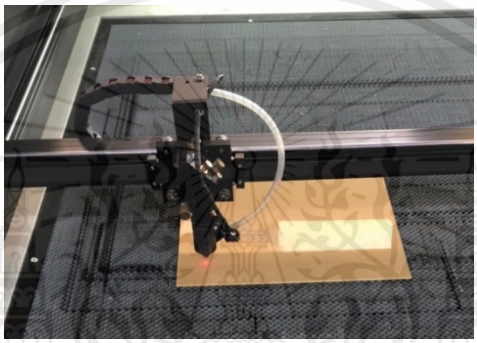


Figure 38 Using CO_2 laser cutter.

Figure 39 Using CNC machine.

3.5.1.1 Using CNC machine to engrave

We only use PMMA to engrave with CNC machine. The micro milling end that we use is 0.1 millimeter. The PMMA samples are cut at 500-700 revolutions per minute.

3.5.1.2 Using CO_2 laser cutter to cut

We use laser cutter to cut PMMA and PET in variety thickness. The power and velocity of laser beam will show in the table below.

PMMA's thickness (mm)	Power of laser beam (%)	Velocity of laser beam(mm/s)
1	80	5
2	80	5
3	85	5
5	90	5

Table 6 Usage of power and velocity of laser beam on PMMA.

PET's thickness (mm)	Power of laser beam (%)	Velocity of laser beam(mm/s)
0.1	20	30

Table 7 Usage of power and velocity of laser beam on PET.

3.5.2 Cleaning strategies

To clean the prototype before bonding to clear the obstacle or scrap. We would like the device clean as possible then there are both basic ways to clean such as using clean with cleaner solution and advance by using ultrasonication.

3.5.2.1 Using cleaner solution

To clean the prototype, this way is easy to clean because cleaner solution such as soap to remove the dirt on the surface of any device's parts before We brings the prototype to the next step. The brush was used to clean the channel first then soap was added to channel. After finishing cleaning the PET or PMMA with channels, the samples were washed with water and left on the tissue paper to dry the samples.

3.5.2.2 Using ultrasonication bath

Prior to the bonding process, the devices from CNC machine and laser cut were placed in the ultrasonication bath with ethanol for clearing the channels obstacles for 5 minutes by pressing sine-wave button. This method, the surface was also treated with Ethanol.

Figure 40 Using ultrasonication bath.

3.5.3 Bonding Strategy

3.5.3.1 Thermal bonding

Ethanol and Propanol were used as the bonding agent between the upper and lower parts of the 2-layer device. Initially, both parts of the device were applied with ethanol or isopropanol then attached to each other by paper clips or clamps. The device was placed in the oven with the temperature of 60-70 degree Celsius to form the bonding for 15 minutes.

3.5.3.2 Bonding with tape.

For 2-layer devices, Magic tape or 3M #467MP was used as bonding between the upper and lower layer of the device and for 3-layer devices, tape was used as bonding among the upper, middle and lower layer then bring to press by using presser such as hydraulic press machine or vise with the force of approximately 200 psi. This method is more convenient than bonding with ethanol or isopropanol because this method just uses tape intermediate the microfluidic device.

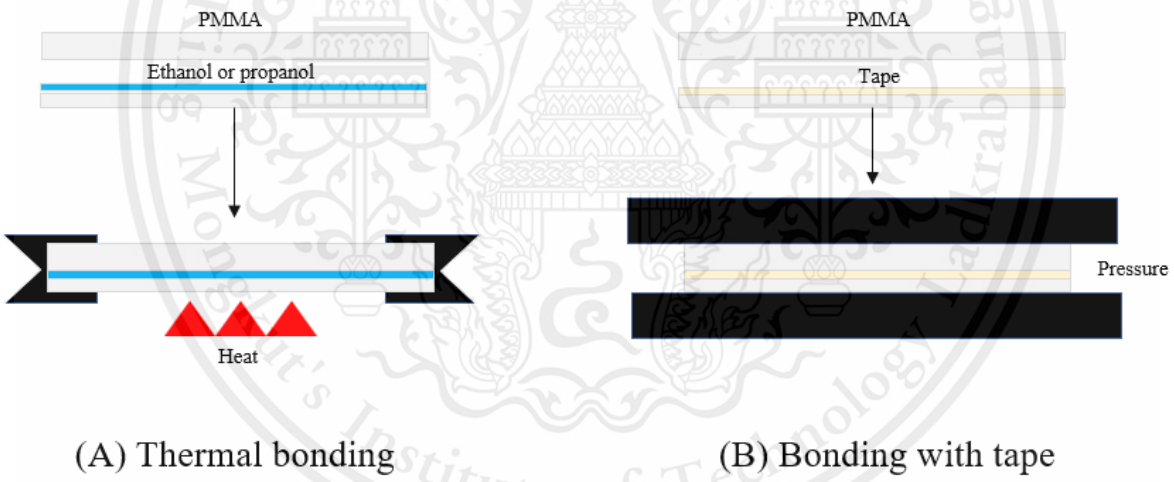


Figure 41 Diagram of thermal bonding and bonding with tape.

3.5.4 Treating with Hydroxy methyl propyl cellulose (HPMC)

To increase the hydrophilicity of the PMMA surface, the 3 mol/L nitric acid was flushed through the devices channel by using hand pump then followed with the flushing of the 0.5 w/v HPMC and 20mM phosphoric acid to form the ester bond between PMMA surface and HPMC. We flushed the nitric acid and HPMC inside the channels by vacuum pump [20].

3.6 Measurement

To measure contact angle when a PMMA was treated by any substance and measure width after cutting.

3.6.1 Contact angle measurement

To measure the contact angle on surface and inside channel.

3.6.1.1 Contact angle on the surface

The water contact angels were measured on the surface of PMMA. The surface will be treated with Ethanol from ultrasonication and HPMC. The contact angle of water plays an important role in the velocity of fluid allowing the fluid to flow inside of the device's channel. The contact angle of water and buffer is measured by taking a photo and measuring in ImageJ program of 10 microliter droplet of water and buffer for ten times.

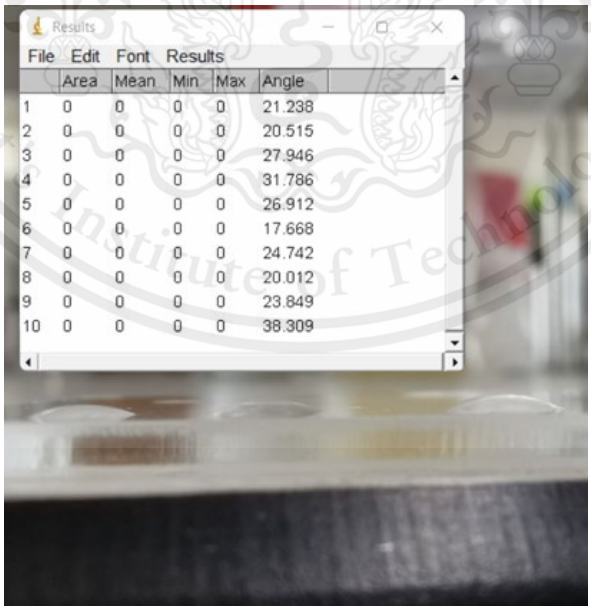


Figure 42 Contact angle measurement on the PMMA's surface.

3.6.1.2 Contact angle inside the channel

The contact angle was measured during the flow of water inside the microchannel by using microscope and ImageJ to measure the angle of the water.

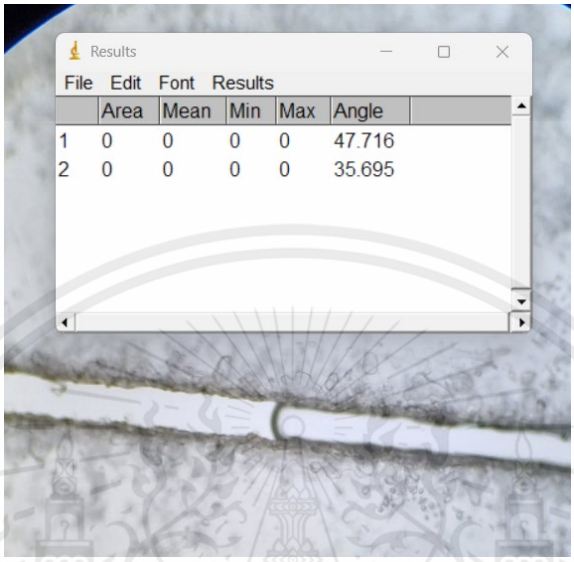


Figure 43 Contact angle measurement inside the channel.

3.6.2 Width measurement

To measure the actual width of channel due to when We were designing the prototype in the drawing software and export file to laser cutter machine, the actual width is different from the width which We set in the drawing software.

3.6.2.1 The measurement through the compound microscope D-117MS

The width of channel was observed under the compound microscope and measured by using the stage micrometer.

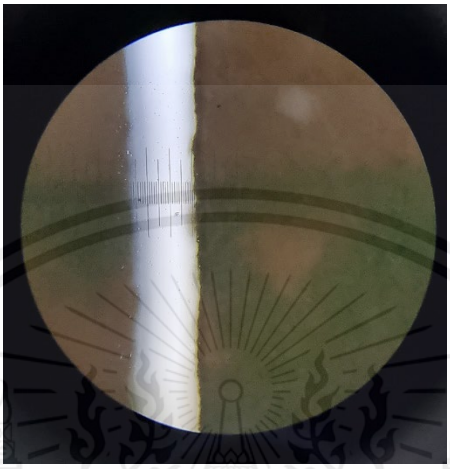


Figure 44 The measurement through compound microscope.

3.6.2 The measurement with the stereo microscope Motic SMZ-171

The width of the channel was observed under the stereo microscope and measured by using the program called Motic Live Imaging Module which can measure the width directly from the camera in the Moticam. We had measured three times each width consisting of 150, 200, 300, 400 and 500 micrometers.

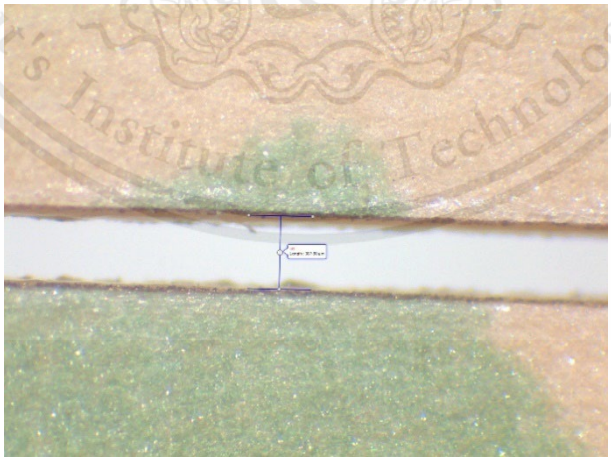


Figure 45 The measurement with the stereo microscope.

3.7 Fluid flow test

The fluid flow was tested with water and rehydration buffer to measure the distance and velocity of the fluid inside channels and to prove the applied formula. The fluid flow was recorded in videos and the distance was measured in the ImageJ program and plot the graph of the fluid.

3.7.1 Flowing into 2-layer devices

There are two cases of flowing into these devices consisting of thermal bonding and bonding with tape.

3.7.1.1 Flowing into devices which are made from thermal bonding method

To flow in the non-treated device and the treated device.

3.7.1.1.1 Flowing into the non-treated device

The water and rehydration buffer will be tested in the non-treated device.

3.7.1.1.2 Flowing into the treated device.

The water will be tested in the treated device because We want to know what is different between flowing of water in the treated and non-treated devices.

3.7.1.2 Flowing into devices which are made from bonding with tape

To flow in the non-treated device and the treated device.

3.7.1.2.1 Flowing into the non-treated device

The water and rehydration buffer will be tested in the non-treated device.

3.7.1.2.2 Flowing into the treated device

The water will be tested in the treated device because We want to know what is different between flowing of water in the treated and non-treated devices.

3.7.2 Flowing into 3-layer devices

The 3-layer devices would be tested the flowing of solution with tape bonding method. Since, they are built from three layers consisting of upper, middle, and lower layer. This experiment will test in the different tape between magic tape and 3M #467MP tape.

3.7.2.1 Flowing in the devices which are made from bonding with magic tape.

There are many bubbles around the channels, and they are difficult to test.

3.7.2.2 Flowing in the devices which are made from bonding with 3M #467MP tape.

This is the best method of this research to test and be able to get varieties result from many devices. Since, We can build more comfortable than other methods.

CHAPTER 4

EXPERIMENTAL RESULT AND DISCUSSION

4.1 Result of fabrication microfluidic device

4.1.1 Fabrication's result of the 2-layer device

Fabrication of microfluidic with 2-layer is dividing the PMMA sheet to 2 pieces for the upper and the lower then they will be bonded by using thermal bonding or pasting the tape method. So, the prototype will be tried to cut and engrave by using a CNC machine and a laser cutter.

4.1.1.1 Cutting result

There are 2 ways to cut or engrave the PMMA sheet. This experiment will focus on the lower layer of the devices.

4.1.1.1.1 Using a CNC machine

4.1.1.1.1.1 layer's surface is not covered with tape

The PMMA sheets were brought to the CNC machine to crave the channels into the lower surface with desired width and depth. After the crafting of channels by CNC machine, the channels were deposited with a powder of the PMMA sheets causing difficulty in clearance of the obstacles inside the channels. To remove the obstacles, We will use the pin to scrape the obstacles but still cannot scrape them all off.

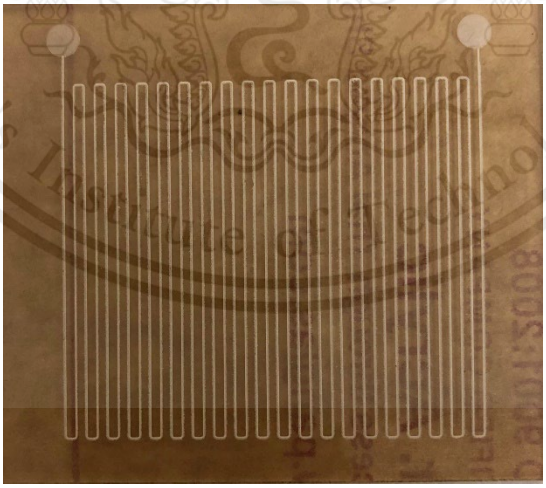


Figure 46 The layer's surface is not covered with tape..

4.1.1.1.1.2 layer's surface is covered with tape

The glue tape was attached to the PMMA surface prior to the crafting of the channels by CNC machine. The result demonstrated that the PMMA sheets were deposited with the glue of the tape causing a blockage of channels and difficulty to remove the glue. This method is not used to test.

Figure 47 The layer's surface is covered with tape.

4.1.1.1.2 Using a CO_2 laser cutter

The PMMA sheets were brought to the laser machine to craft the channels on the lower surface of the PMMA sheets. The width and depth were varied due to the height of the laser and the amount of power of the laser setting causing the burnt of the channels which is difficult to control with accuracy and this method will make the cross-sectional area to a semicircle. The result of engraving shown no different between the layer's surface is covered with tape and not covered with tape.

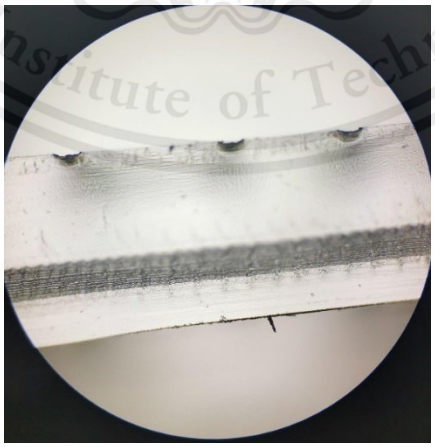


Figure 48 Cross-sectional area of the channels after cutting.

4.1.1.2 Cleaning result

4.1.1.2.1 Using ultrasonication.

After using an ultrasonication bath to sonicate a PMMA sheet, the surface of a PMMA sheet will be destroyed and the crazing phenomenon will affect the solution flow in microfluidics.

Figure 49 Occurring the crazing on the device.

4.1.1.2.2 Using cleaning solution.

This method is enough to clean the layer's surface. The layer's surface is not destroyed.

4.1.1.3 Bonding result

4.1.1.3.1 Thermal bonding result

Bonding the microfluidic device in the oven for 15-20 minutes at 60-80 degree Celsius. Thermal bonding demonstrated the uncertain result of bonding which frequently caused the leakage and blockage inside of the microfluidic devices.

4.1.1.3.1.1 With propanol

Bonding of PMMA sheets with propanol gave the inconsistent result of bonding including the leakage and blockage inside of the devices.

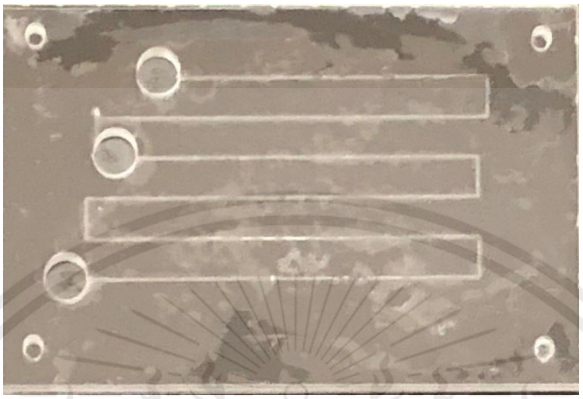


Figure 50 The device after bonding with propanol.

4.1.1.3.1.2 With ethanol

The bonding with ethanol of PMMA sheets caused a similar result of propanol which gave the uncertain result of bonding and caused a leakage and blockage of channels inside device.

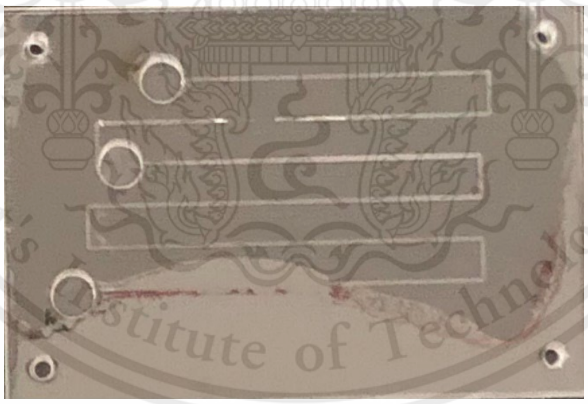


Figure 51 The device after bonding with ethanol.

4.1.1.2 Fabrication's result of the 3-layer device

This method will focus on the tape that we use to bond the layers together. Cutting the middle layer with laser cutter produces a larger width of channel than the design. Nevertheless, the laser can produce the consistent of the width and prevent the deposit of the powder inside the PMMA's channel. All layers can be cut by using a laser cutter. Then clean the layers with cleaner solution and then put all layers together. The important point for this method is the intermediate or middle layer, which was made of PET covered with different tapes.

4.1.1.2.1 Bonding result

4.1.1.2.1.2 The middle layer bonds with magic tape.

The magic tape provided a small amount of errors including a blockage and leakage in some areas of the devices. Although the devices will be pressed by presser, they still have the blockage and leakage inside.

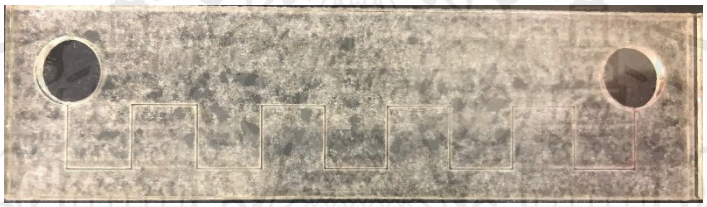


Figure 52 The device was bonded by magic tape.

4.1.1.2.1.2 The middle layer bonds with 3M #467MP tape

The 3M #467MP tape provided tight bonding between the middle layer and both lower and upper layers of the devices. With accurate bonding with other sides, the blockage and leakage rarely occurred and could be prevented and increase the firmness by using presser or vise. The devices look transparent.

Figure 53 The device was bonded by magic tape.

4.2 Measurement result

4.2.1 Result of measurement contact angle

4.2.1.1 On the surface

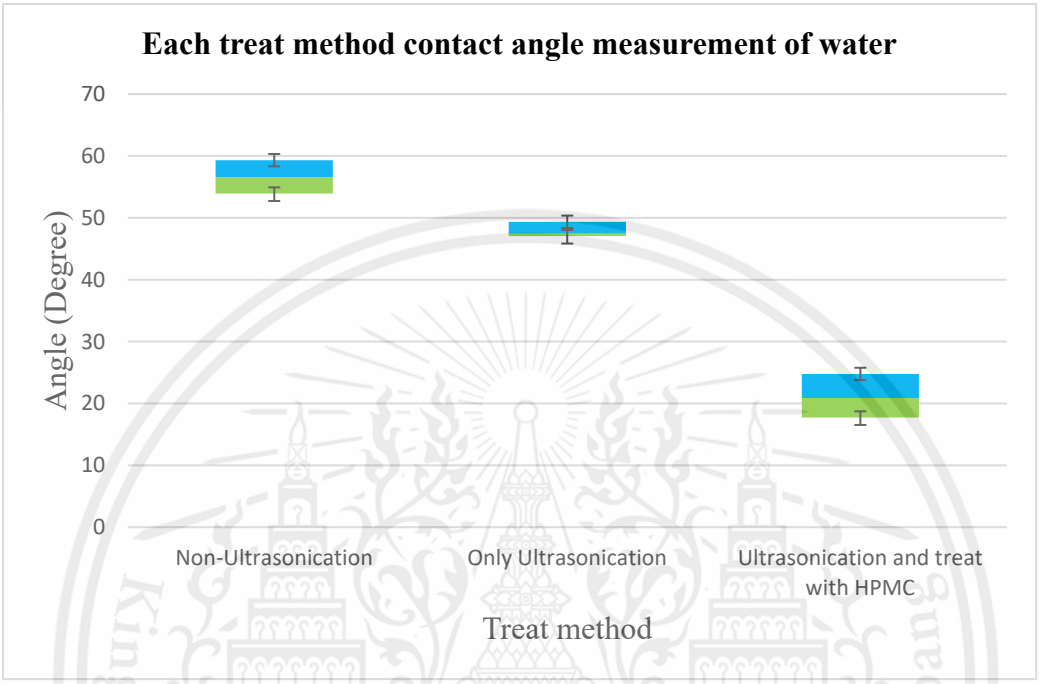


Figure 54 Each treat method contact angle measurement of water.

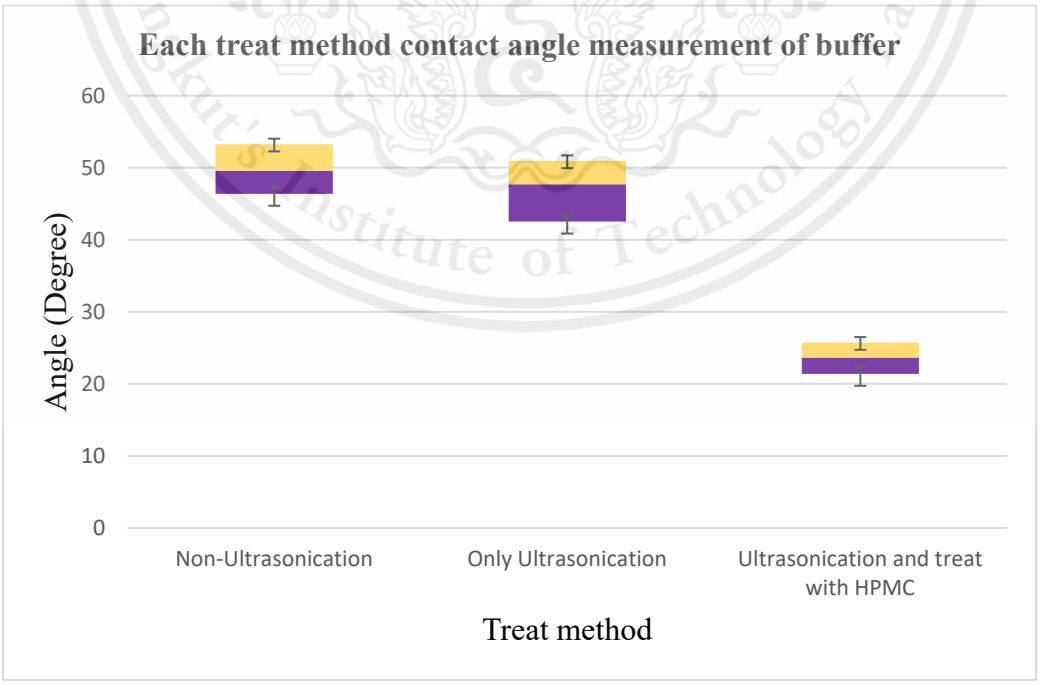


Figure 55 Each treat method contact angle measurement of buffer.

4.2.1.2 Inside the channels

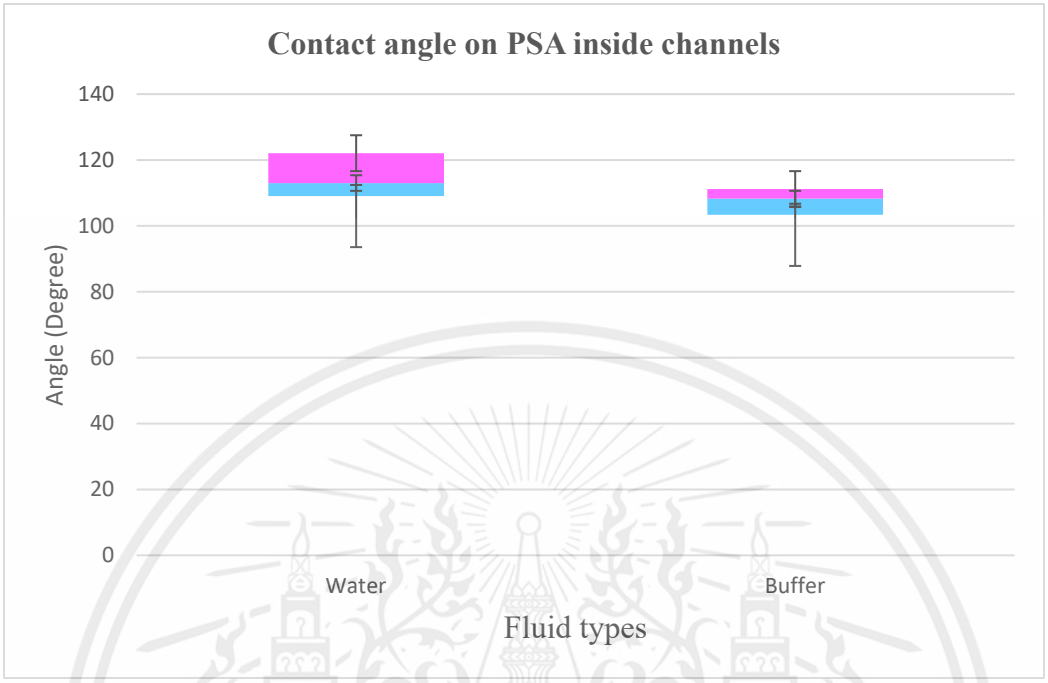


Figure 56 Contact angle on PSA inside channels.

4.2.2 Result of measurement width inside channels

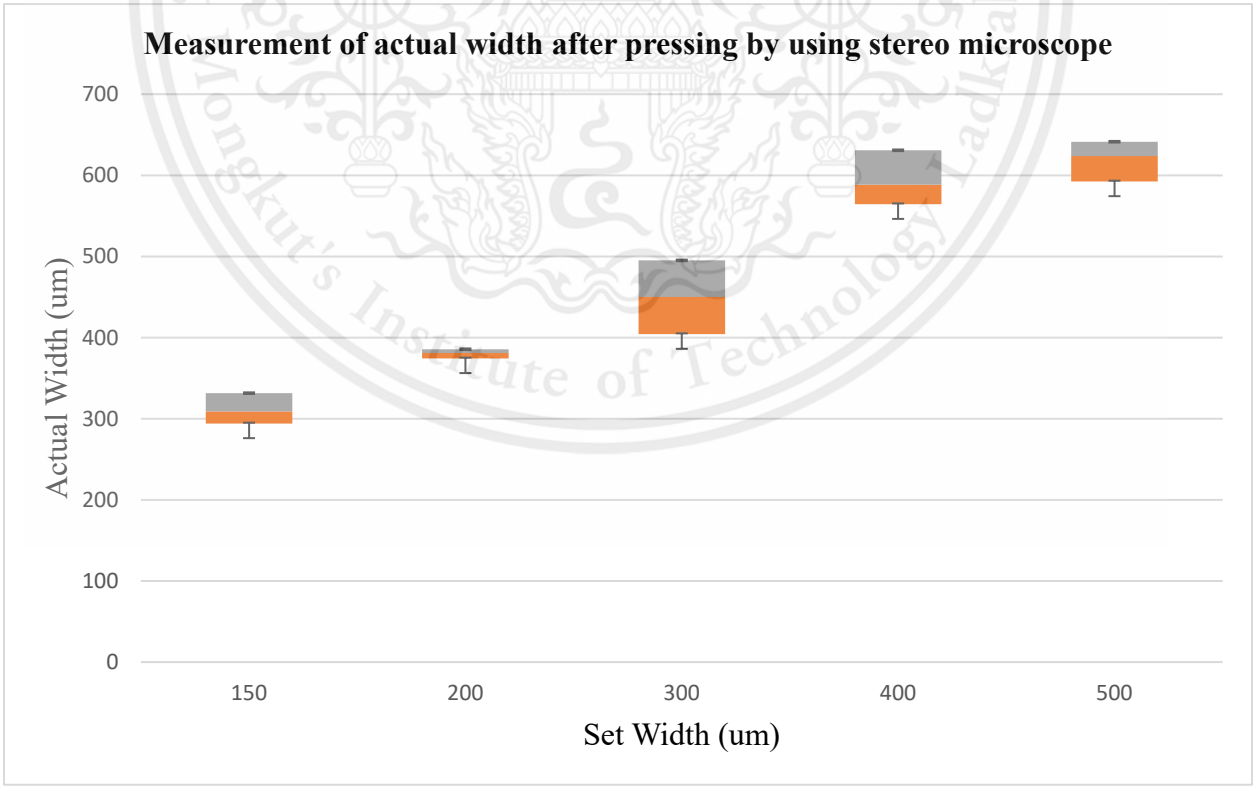


Figure 57 Measurement of actual width after pressing by using stereo microscope.

4.3 Result of fluid dynamic

According to 3.7, We test the flow of fluid in many devices. The result that we receive and can take many samples to comparison are on the 3-layer device because when we consider the various factors, the research observe that the 3-layer devices will give result from many samples and the fluid can flow completely in the system. At the beginning after we changed the method of fabrication from the 2-layer to 3-layer device, the research did not need to use HPMC to treat inside the device's channel because the fluid can flow inside the devices. So, we designed the new devices by starting with the width of channels consisting of 150, 200, 300, 400 and 500 micrometers and the length of channel consisting of 20, 10, 3.5 centimeters and taking to combine to the final device with the total length 8.5 centimeters.

4.3.1 Result of flowing water

4.3.1.1 Inside 20 centimeters length of device

The water was dropped on the devices with depth of 100 micrometers, 20 centimeters length, and various width ranging from 150 micron to 500 micron in accordance with our design. Water can travel the longest distance in devices with 500 microns width for 60 minutes.

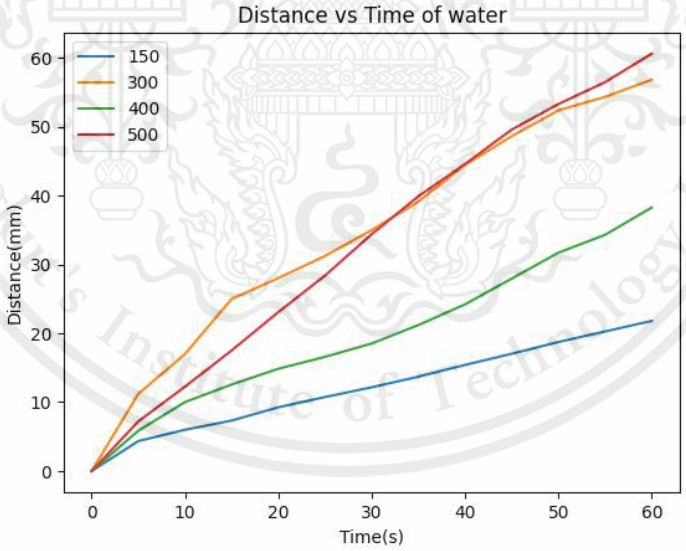


Figure 58 Distance vs Time of water flow in each width.

From the result of different width devices, the approximate distance for 30 minutes which is approximately 35 mm allowing the amplification reaction to be completed.

We observed that, of all the prototypes, the device with 500-um width can be built more robustly than those with smaller width. Then, we chose this 500-um wide channel to experiment with different lengths. The devices with different lengths were tested to show that the water would reach 3.5 cm within 30 minutes. The water in 3.5cm devices showed the average distance approximately 3cm in 30 minutes, while the water on 10cm devices showed the average distance of approximately 35 mm in 30 minutes.

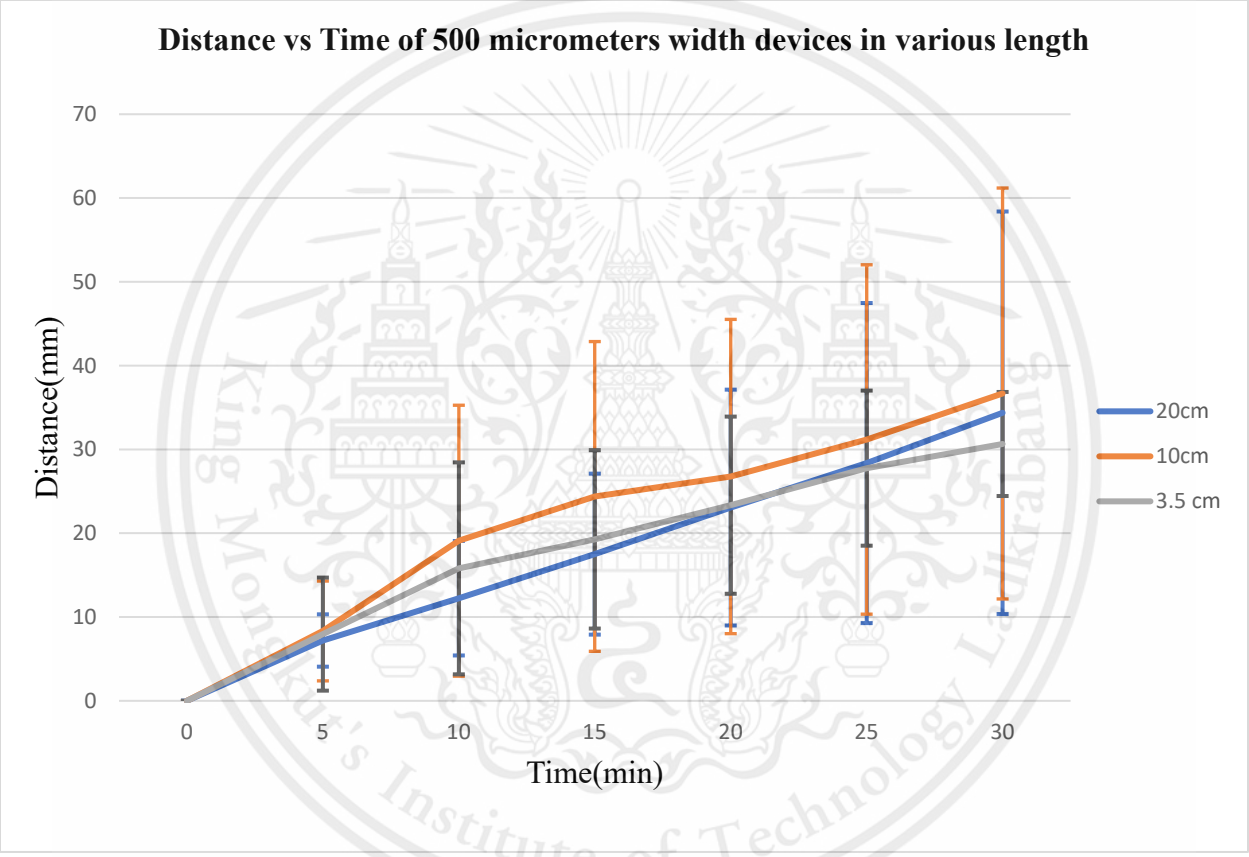


Figure 59 Distance vs Time of 500 micrometers width devices in various length.

4.3.2 Result of flowing buffer

The buffer was tested in 10cm devices as the buffer has a similar fluid property to the actual amplifying reagent. The average distance of buffer at 30 minutes was approximately 50 mm.

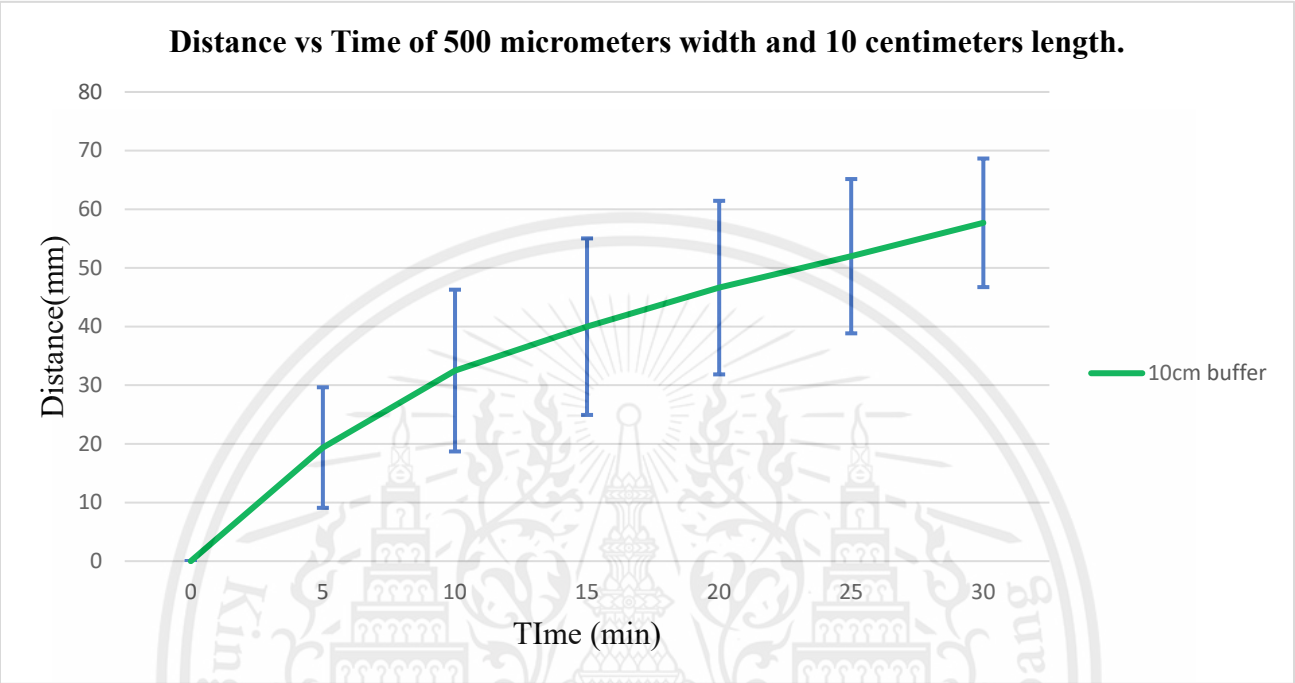


Figure 60 Distance vs Time of 500 micrometers width and 10 centimeters length.

4.4 Comparison between water and buffer

The distance of water and buffer in 10 cm devices were brought to compare.

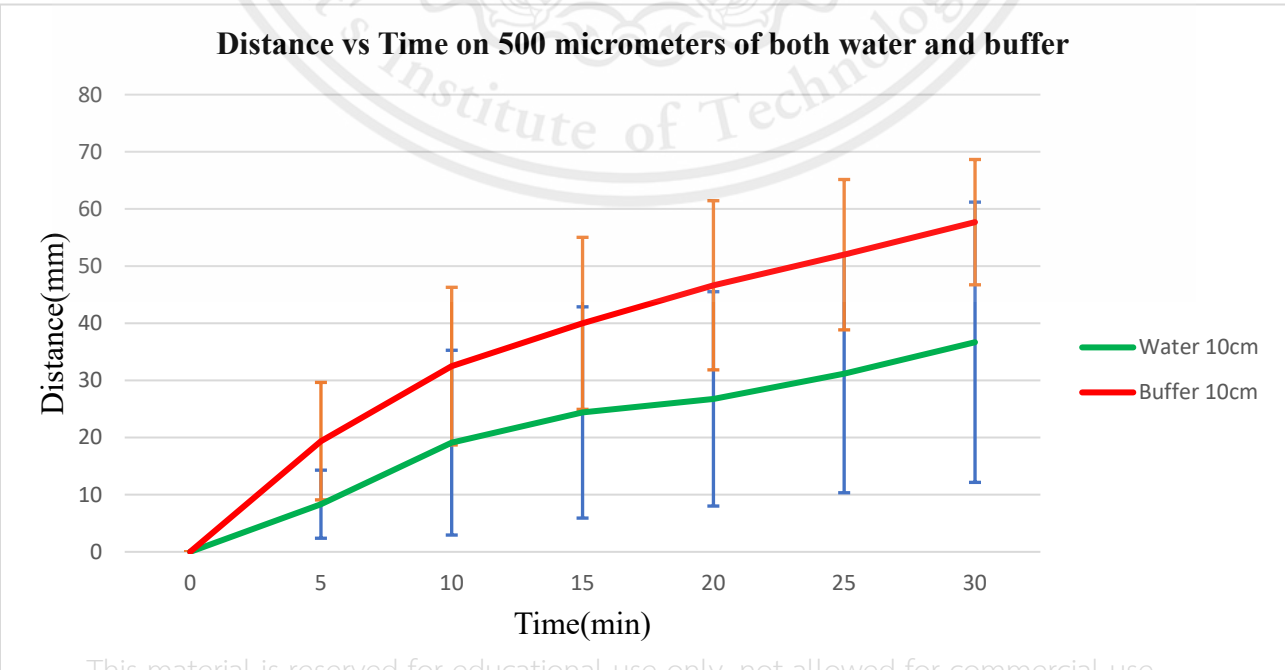


Figure 61 Distance vs Time on 500 micrometers of both water and buffer.

The buffer shows the average distance of 55mm for 30 minutes, which is higher than the water.

4.5 Result of testing on the final prototype.

4.5.1 Result of the first section

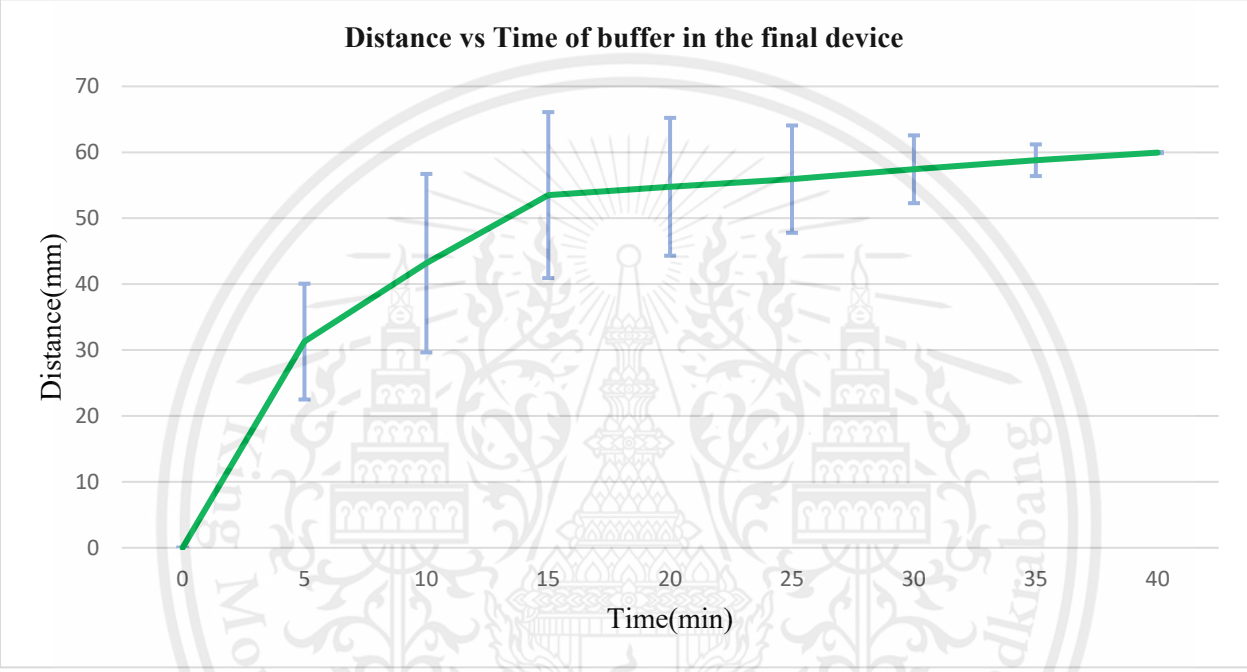


Figure 62 Distance vs Time of buffer in the final device.

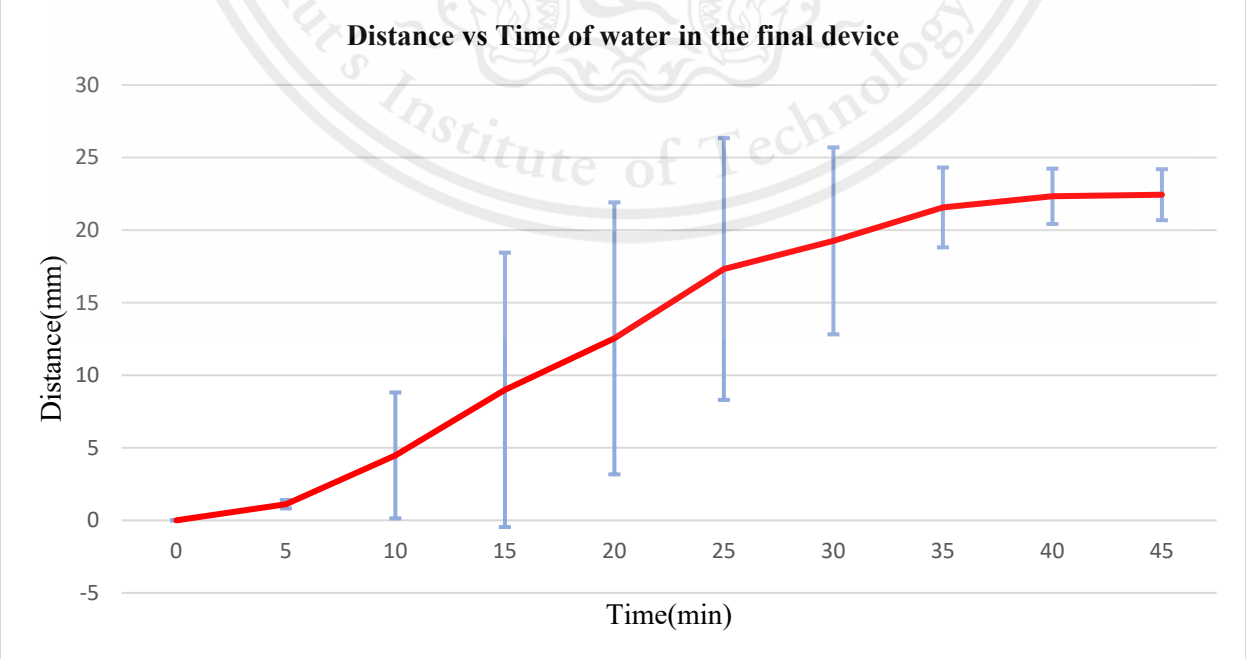


Figure 63 Distance vs Time of water in the final device.

4.6 The prototype discussion

To discuss each design after testing which problem occur or the solution can flow.

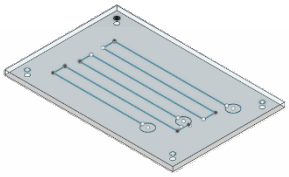
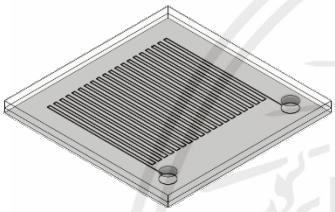
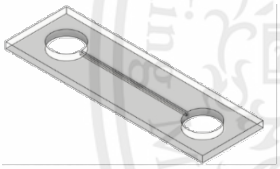
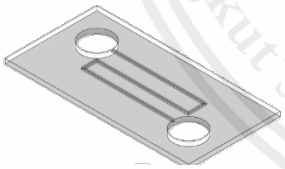
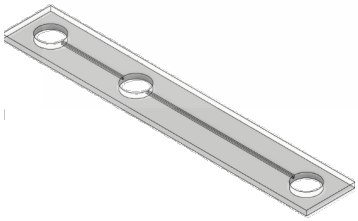
Sketch	Note
	This prototype has a total length of 30 centimeters. There are 3 pits to drop the solution. At the beginning the water cannot flow through to the next pit until being treated with HPMC and then the water can flow. The problem is that there are blockage and leakage inside causing of bonding.
	This prototype has a total length of 200 centimeters. The reason why it is long is because of calculate from capillary force. There are 2 pits because we want to check the actual flow that can flow in this device along with principle. The problem is that there many leakages and make the fluid flow with air bubbles.
 	This prototype has a short length of various such as 3.5, 10, 20 centimeters and width such as 150,200,300,400,500 micrometers because from observation, the water travel too short in 3-layer device. We will decrease the length to test the water flow in various lengths and width.
	This prototype is the final prototype. The first section uses to flow buffer and flow water into the second section. The first is 6 centimeters in length and the second is 2.5 centimeters length.

Table 8 Discussion design.

CHAPTER 5

CONCLUSION

5.1 Conclusion

The purpose of this project is to create a microfluidic device to facilitate the CRISPR technique mechanism including the amplification for 30 minutes and reaction with an CRIISPR-Cas enzyme for 15 minutes.

The calculation for microfluidic devices was involved with Bernoulli's principle to create a design on the first prototype. Nevertheless, the behavior of water in the channels manifested in a different manner to Bernoulli's calculation. The flow of fluid inside the small channels tended to follow the concept of Capillary force referring to the contact angle of the water on an object.

The design was mainly built by using the Fusion 360 to create a 3D structure of the device and the file of design was sent to either industrial engineering department of KMITL to proceed the crafting method using CNC machine or laser machine to cut the channels. The CNC machine frequently caused the deposit of the PMMA powder inside the channels leading to difficulty of removing the obstacles. To avoid the issue, the laser on PET plate attached with 3M #467MP to create a water channel can prevent the cause of the channel's obstacles.

The components of microfluidic devices consist of the PMMA sheets as the upper and lower layers and PET sheets as a middle layer for 3-layer bonding technique.

Sonication was brought to use to clean the channels after the channels were crafted, which was later discovered to cause the crack on the microfluidic surface. The cleaning was adjusted to use the soap and brush to clean the PET plate after the laser cut as the bonding method was changed to 3-layer bonding.

Thermal bonding was applied during the first year of the research by using the 2 layers of PMMA and 99.9% ethanol as a bonding intermediate. The ethanol was painted on both surfaces of upper and lower layer and then the PMMA sheets were held together with paper clip to put into the 65 degrees Celsius for 15 minutes baking process. The result of the thermal method was inconsistency showing the possibility of forming a blockage inside the channels or the leakage. To solve the problem, the method was replaced by using the magic tape as the bonding intermediate at the beginning. At the later period, we used the 3-layer bonding method to minimize the inconsistency of the bonding, leakage, and blockage of the channels. The channels were crafted by either CNC machine or laser machine during the first year to the lower layer surface, which is

then changed to use the laser cut onto the PET plate as the middle layer to fix the value of the channel's depth.

The water represents the fluid property of enzyme and buffer represents the flow property of the amplifier. Therefore, both solutions were brough to test on the designed devices to measure the distance for 30 minutes. The water can travel approximately 30mm on devices with 500 micron of width, 100 micron of depth, and 10 cm of length., while the buffer would reach about 55 mm in 30 minutes.

The contact angle measurement was done by dropping the water onto the PMMA surface and the angle was measured by using ImageJ. Later, the contact angle of water was measured by taking a photo of microscopic view revealing the actual contact angle inside the microfluidic channels.

The treating surface method was initiated by using UV treatment which had no effect on improving the hydrophilicity to the PMMA surface. After the study on the contribution of hydrophilicity using chemical solution, PMMA sheets were flushed with nitric acid and then flushed with the combination of phosphoric acid and HPMC. The chemical method could provide the hydrophilic property to the surface facilitating the flow of water.

The measurement of the channel's width was done by using the microscope and scaling slide. To improve the accuracy of the measurement, Moticam or Motic SMZ-171 was brought to observe the width and measure the width by using the program called Motic Live Imaging Module.

5.2 Suggestion

1. The velocity of the water involves the width, length, depth, and contact angle of the channels.

2. Using 3-layer bonding method can prevent the deposition of the channel's obstacles and the inconsistency of bonding between upper layer and lower layer.

3. HPMC treatment must be initiated with the nitric acid first then flushes with the solution of phosphoric acid and HPMC.

4. The further design may include negative and positive controls to ensure the accuracy of the detection.

REFERENCE

[1] T. N. A. Vo and P. C. Chen, “Maximizing interfacial bonding strength between PDMS and PMMA substrates for manufacturing hybrid microfluidic devices withstanding extremely high flow rate and high operation pressure,” Sens Actuators A Phys, vol. 334, Feb. 2022, doi: 10.1016/j.sna.2021.113330.

[2] M. T. Guler, M. Inal, and I. Bilican, “CO2 laser machining for microfluidics mold fabrication from PMMA with applications on viscoelastic focusing, electrospun nanofiber production, and droplet generation,” Journal of Industrial and Engineering Chemistry, vol. 98, pp. 340–349, Jun. 2021, doi: 10.1016/j.jiec.2021.03.033.

[3] DOE FUNDAMENTALS HANDBOOK THERMODYNAMICS, HEAT TRANSFER, AND FLUID FLOW, “Bernoulli’s Equation,” U.S. Department of Energy Washington, vol.3, Jun. 1992.

[4] C. Liang et al., “One-step selective-wettability modification of PMMA microfluidic devices by using controllable gradient UV irradiation (CGUI),” Sens Actuators B Chem, vol. 273, pp. 1508–1518, Nov. 2018, doi: 10.1016/j.snb.2018.07.034.

[5] A. Kumar et al., “COVID-19 Mechanisms in the Human Body—What We Know So Far,” Frontiers in Immunology, vol. 12. Frontiers Media S.A., Nov. 01, 2021. doi: 10.3389/fimmu.2021.693938.

[6] H. A. S. L. J. B. S. S. A. K. Bohn MK, “Pathophysiology of COVID-19,” Physiology (Bethesda), Sep. 2020. doi: 10.1152/physiol.00019.2020.

[7] A. G. Harrison, T. Lin, and P. Wang, “Mechanisms of SARS-CoV-2 Transmission and Pathogenesis,” Trends in Immunology, vol. 41, no. 12. Elsevier Ltd, pp. 1100–1115, Dec. 01, 2020. doi: 10.1016/j.it.2020.10.004.

[8] G. Popov, O. Nakov, and A. Popova, “Improving the Accuracy of Rapid Antigen Tests for Covid-19 using Data Mining and Blood Test,” in 2022 10th International Scientific Conference on Computer Science, COMSCI 2022 - Proceedings, Institute of Electrical and Electronics Engineers Inc., 2022. doi: 10.1109/COMSCI55378.2022.9912572.

[9] A. Chaudhary, P. Bansal, M. Satija, and V. K. Gupta, “Rapid antigen test for COVID-19: A useful weapon in the arsenal of public health,” Medical Journal of Dr. D.Y. Patil

Vidyapeeth, vol. 15, no. 7. Wolters Kluwer Medknow Publications, pp. S3–S7, Aug. 01, 2022. doi: 10.4103/mjdrdypu.mjdrdypu_951_21.

[10] Juan F. Medrano, “One-Step Versus Two-Step Real-Time PCR,” Department of Animal Science, University of California, vol. 39, July 2005.

[11] E. Green, “Polymerase Chain Reaction (PCR) Fact Sheet,” National Human Genome Research institute Aug. 17, 2020.

[12] H. Rahimi et al., “CRISPR Systems for COVID-19 Diagnosis,” ACS Sens, vol. 6, no. 4, pp. 1430–1445, Apr. 2021, doi: 10.1021/acssensors.0c02312.

[13] S. Berggren, I. Enoksson, C. Stens, “The CRISPR-Cas system.” Department of Physics, Chemistry and Biology Linköping university, June. 2020.

[14] J. Beroz and A. J. Hart, “Universal handheld micropipette,” Review of Scientific Instruments, vol. 87, no. 11, Nov. 2016, doi: 10.1063/1.4966989.

[15] A. O. Driscoll, “Ultrasonic Baths,” Homogenizers, Jun. 27, 2018.

[16] C. N. Cheaburu-Yilmaz, H. Y. Karasulu, and O. Yilmaz, “Nanoscaled dispersed systems used in drug-delivery applications,” in Polymeric Nanomaterials in Nanotherapeutics, Elsevier, 2018, pp. 437–468. doi: 10.1016/B978-0-12-813932-5.00013-3.

[17] Z. M. Xiao, M. K. Lim, and K. M. Liew, “Materials Processing Technology Micromechanics analysis of crazing phenomenon in polymers INDUSTRIAL SUMMARY,” 1995.

[18] Brian Hess, “An overview of CNC machine process,”Astro Machine Work, May 22, 2021.

[19] V. Vaidyanathan, “What is Hydraulic press.”, ScienceABC, Jan 4, 2023.

[20] J. J. Shah, J. Geist, L. E. Locascio, M. Gaitan, M. V Rao, and W. N. Vreeland, “Surface modification of poly(methyl methacrylate) for improved adsorption of wall coating polymers for microchip electrophoresis,” Electrophoresis, vol. 27, no. 19, pp. 3788–3796, May 2006, doi: 10.1002/elps.200600118.