Chapter 8

Microfluidic Devices Fabrication for Bioelectrokinetic System Applications

Nurul Amziah Md Yunus

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/54390

1. Introduction

The research and development of microfluidic technologies has increased tremendously over the past twenty years. The technology allows designers to create small, portable, robust, low cost and easy-to-use diagnostic instruments that offer high levels of capability and versatility. Microfluidic systems will decrease reagent consumption and reduce cost per analysis. It also reduces analysis time and provides better controllable process parameters in chemical reactions.

Microfluidic is also known as miniaturised total (chemical) analysis system, (μTAS). It is a generic term for a small system or device (microfluidic) designed to perform one or more chemical/biochemical processes. The early stage of microfluidics has been dominated by the development of microflow sensors, micropumps and microvalves in late 1980s and the beginning of 1990s and the concept has been introduced by Manz and co-workers from Imperial College London [1]. Since then, chip-based analytical systems have been rapidly applied to a variety of fields such as separation science, chemical production, DNA analysis, medical diagnostics and environmental analysis [2, 3]. Examples of microfluidic devices are shown in Fig. 1 (a)-(b).

Surprisingly, many fabrication methods used for making a microfluidic device are based on silicon microelectronics fabrication industry, with one of the first devices on silicon by Terry [a gas chromatographic air analyser in the late 70s, but little study was done about producing one again at that time. Interest in this type of technology was revived in the 90s with current microfluidic devices fabricated not only from silicon based but to a range of polymers, glass wafers and foils. This technology is used to build miniature devices as shown in Fig. 1(c) with packaging that could perform as a conventional "big bench-top" such as shown in Fig. 1 (d).

Thus, the challenge has been to create such as medical, chemical, biological devices that are capable of doing a fast and accurate analysis of the reagent and has the capacity to be green technology products in the future. With the recent advances in the synthesis and the characterization of size-selected particles in colloids (submicron and nanometer range) such as blood itself, an investigation on their physical and chemical properties has been made possible [5]. In microfluidic, a fabrication of it devices with integrated microchannels and microelectrodes of dimensions are made comparable to biological cells or particles size. Additionally, if there is a capability of producing small-scale devices, it will allow the development of entirely novel experiments, which currently is rapidly under study.

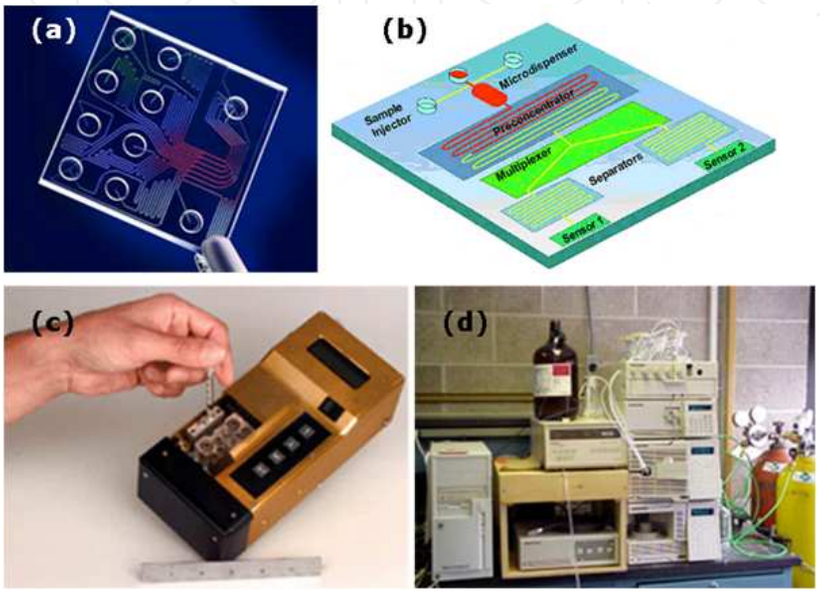

Figure 1. (a) Microfluidics Device –(image is taken from Agilent Technologies website) (b) Possible analysis on the microfluidic device with integrated microfluidic with sample injector, microdispenser, preconcentrator, multiplexor, separator and sensor (c) Handheld microfluidic in the proper packaging (d) Example of a big benchtop system –the conventional version of chemical/biological analytical technique.

Microchannels are very important features for microfluidic systems. There are many ways to fabricate the microchannel, such as by using the silicon based organic polymer, polydimethylsiloxane (PDMS) or the femtosecond laser micromachining and inscription or using deep reactive ion etching (DRIE). The method emphasized in this Chapter will be the fabrication of microfluidic microchannel using the laminate sheet, dry film resist (DFR), for example, the SY series from Elga Europe [7]. This laminate sheet is a biocompatible material and it adheres easily on the co-planar substrate, i.e glass or silicon substrate. It is cheap, simpler and easier to use.

The work is also concerned on the application of electrokinetic technique to bioelectrokinetic monitoring system. Electrokinetic is defined as the study of fluid or particle motion in electric fields. It generally works in direct current, DC and alternating current, AC. Electrokinetics include electrophoresis (EP), dielectrophoresis (DEP), electro-osmosis (EO), electrorotation (ROT) and electroorientation [6]. These electrokinetic processes are able to

manipulate, concentrate, collect, mix and separate different types of bioparticles, biomolecules, organic and inorganic materials.

2. The physics of microfluidic systems

In this section, an overview of the physics of microfluidic will be highlighted because at the microscale, there are many different forces which become significant over forces experienced in everyday life. It is begun with the motion of fluid described by Navier-Stokes equation named after Claude-Louis Navier (1785-1836), a French engineer and physicist and George Gabriel Stokes (1819-1903), a mathematician and also a physicist. The equation was derived from conservation of momentum arguments [8]. This equation arises from applying Newton's second law to fluid motion, together with the assumption that the fluid stress is the sum of a constant viscous term (proportional to the gradient of velocity), plus a pressure term [9]. For an incompressible Newtonian fluid (named after Isaac Newton a mathematician and physicist (1642-1727) [10]) the equation is [11, 12]:

$$\rho_m \frac{\partial \mathbf{u}}{\partial t} + \rho_m \left(\mathbf{u} \bullet \nabla\right)\mathbf{u} = -\nabla p + \eta \nabla^2 \mathbf{u} + \mathbf{f} \tag{1}$$

where ρ is the mass density, $\mathbf{u}$ is the velocity of fluid, p is the pressure, η is the viscosity and $\mathbf{f}$ is the total applied body force. The ratio of the inertial term to the viscous term can be determined from the coefficients in equation which gives a factor referred to as the Reynolds' number (named after Osborne Reynolds (1842–1912)) [13-15] shown in equation (2):

$$\mathrm{Re} = \frac{\rho_m u_o l_o}{\eta} \tag{2}$$

'Re' stands for Reynolds' number, u_o is the magnitude of a typical velocity and l_o is a length scale. It is a dimensionless number and can be estimated as follows: Assuming that typical working fluid is water with typical velocities u_o of 10^{-6} to 10^{-2} m/s. Typical channel radii of 10^{-4} m, $\rho_m = 10^3 kgm^{-3}$ and $\eta = 10^{-3} kgm^{-1}s^{-1}$. For fluids in microfluidic devices, which have small Reynolds number (Re <<1), their behaviour is dominated by viscous forces over inertial forces and the resulting flows are linear and the fluid is considered to move in laminar sheets. If inertial forces are much bigger, Re>>1, turbulent flow will occur. The low values of the Reynolds number in the microfluidic microsystem indicate that, if the fluid is moving under an applied pressure or force, which is suddenly removed, the fluid flow will stop immediately.

2.1. Laminar flow

In microfluidic microsystem, the microchannel is fabricated to guide the fluid flow through the device. This channel can be in any size in the range of less than 5μm to 100s μm and the flow is always laminar. Laminar by definition is a layer. Laminar flow is a condition

whereby the velocity of a particle in a fluid stream is not a random function of time thus the fluid flow follows streamlines [16]. One of the effects of laminar flow is that, two or more streams flowing in contact with each other will not be mixed except by diffusion [17].

The fluid in steady state flow is defined as Poiseuille flow (named after Jean Louis Marie Poiseuille 1797-1869) a French physician and physiologist) [18]. The flow establishes a parabolic flow profile after some distance from the entry point of the fluid into the channel. The velocity is zero at the walls and reaches maximum at the centre of the channel.

2.2. Viscous drag force

When there is particle in fluid, it will experience a viscous drag force due to the action of the fluid on the particles. Viscous drag is the force that resists the movement of a particle through the fluid. The force is proportional to but opposite the relative velocity of the particles. For a sphere particle of radius a in a fluid of viscosity η, viscous drag force is given by Stokes's law [11] in equation (3).

$$\mathbf{F}_{Viscous} = 6\pi\eta a\mathbf{v} \tag{3}$$

$\mathbf{v}$ is the velocity of the particle. For a constant applied force the particle will eventually reach a terminal velocity, which makes it, does not accelerating. If the fluid is at rest, the terminal velocity is simply proportional to the applied force. If the fluid is moving, the terminal velocity experienced by the particle depends on the velocity of the fluid.

For a sphere particle, the friction factor is given by equation (4). The constant *f,* which refers to the friction factor, depends on a range of particle parameter such as size, shape and surface characteristics.

$$f = 6\pi\eta a \tag{4}$$

3. Fabrication of microfluidic process

When the microfluidic is introduced in the early 1990s, a glass substrate is used as a material for the substrate for most microfluidic devices [19-21].The advantages of glass in relation to the other materials are it is chemically inert to most liquid and gases, hydrophilic and optically 100% clear. There are three types of glass that are commonly used in the laboratory. There are consisting of borosilicate, soda lime and fused silica glass. In this work, the borosilicate glass or pyrex glass, which is the type of the microscopic glass slide, is used as the substrate. It can resist strong acids, saline solutions, chlorine, bromine, iodine, and strong oxidizing and corrosive chemicals. This glass can be produced in a mass product since it is not expensive.

The other reason glass slide is used as a substrate, is that glass is hydrophilic, in which it attracts and holds moisture. Most plastics, in comparison, are hydrophobic and need treatment to become hydrophilic. Glass has material properties that are stable in time and it

is thus preferable for applications in which devices are used extensively, such as in high throughput screening. It is a reliable shelf life. It is non-porous, implying that small molecules will not be able to diffuse into it.

3.1. Glass as a substrate

To start utilize glass for microfluidic device, we could cut it into pieces, of dimension for example 2 cm by 1.5 cm, and we call it as fragmented chip glasses. These glasses will go through the cleaning process. They were placed in the glass holder filled with 95% pure water and 5% of soap. Then this holder is placed in the ultrasonic bath (Ultrawave Ltd) for washing away all the fouls with the aid of the beaker filled with DI water for 15 minutes. After cleaning, the glasses are thoroughly rinsed with DI water followed by acetone, methanol and iso-propanol (IPA), the RGB solvents, and blow-dried with nitrogen.

The glasses are placed in the oven for dehydration purposes at the temperature of 200°C for 2 hours minimum after the cleaning process is completed. This step is done to make sure that the glasses used are dried totally so that attachment of laminate sheet mentioned earlier in the introduction on these glasses will be excellent.

3.2. The device

Manz and co-workers introduced photolithographic techniques to microfabricated electrophoretic separation channels [19]. Lithography uses photoresist materials to cover areas on the wafer that will not be subjected to material deposition or removal.

There are two types of photoresist materials, namely, negative and positive photoresists. Negative photoresists are those that will become less soluble in the developer solution when they are exposed to light, forming negative images of the mask patterns on the wafer (the substrate) while positive photoresists are those that will become more soluble in the developer when they are put under the same exposure of light, forming positive images of the mask patterns on the wafer.

Advances in micro and nano fabrication techniques allow small microelectrodes of order of 1μm in size and smaller with selective materials to be manufactured with relative ease. Therefore microfluidic device has been designed and fabricated by taking into consideration the material used for making the microelectrode so that only small amount of voltage needed to gain high current. Thus, this microelectrode structures can provide sufficient force to manipulate particles without requiring high voltage signal generators. This will reduce joule heating in the microfluidic device. Although, it is found that if the microelectrode gap and chamber height is made smaller and lower respectively can impinge on the joule heating problem when higher conductivity medium is employed especially by biologist in biology application, but with selective material for microelectrode i.e biocompatible, it will overcome that problem.

The microelectrodes can be fabricated on approximately 10 cm diameter, (4 inch), 1 mm thick glass wafers or substrates. The total chip that can fit the 4 inch wafer is about 14 chips.

This is depending on the size of the device/chip designed by the engineer for a particular function. The microelectrode will be deposited on the glass wafer by using the electron beam lithography. This technique will ensure the layer of microelectrode is thinner and the electrode width and gap are precisely small.

The microelectrodes are fabricated using gold, Au. The gold layer is essential because of its low resistance and its biocompatible nature, but unfortunately it does not adhere well to the glass surface. Thus titanium, Ti on the other hand, adheres well on the glass substrate and was used to improve gold adhesion. The palladium, Pd layer acts as a diffusion barrier between the titanium, Ti and the gold, Au. The other metal that can promote adhesion to gold, Au and platinum, Pt is chromium, Cr. However Cr is known to diffuse into the overlying gold, Au over time more quickly than titanium, Ti.

Microelectrodes on the glass were patterned using photolithography and ion beam milling over titanium, Ti, seed layer. Pt or Au microelectrodes are chosen because they are highly conductive, chemically unreactive, and able to resist tarnishing and corrosion, which then would allow the maximum current flow through it and hence, greater force or energy could be produced. They are also biocompatible because of its quality of not having toxic or injurious effects on biological systems. Some evaporated metal layers for microelectrode are 10 nm titanium, Ti, 10 nm palladium, Pd, and 100 nm gold, Au. For example, in this work, the width of the wire of the microelectrode is 20 μm and it is made of three layers of metal consisting 30 nm Ti, 100 nm Au and 30 nm Ti. The top layer of Ti is to reduce the effects of corrosion at low frequency and high potentials [22]. The electron-beam lithography technique is used to fabricate smaller size of microelectrode ranging 1-5 μm and gap sizes ranging from 1-10μm and its reproducibly.

3.3. Laminate

The next step is to perform the channel on these glasses. The fresh laminate sheet, dry film resist (DFR) of 50 μm is taken out from the fridge. The purpose of keeping the laminate in cold place is to lengthen the freshness/lifetime/dryness/unwanted crosslink. It is folded in black plastic cover to prevent exposure to or penetration of the UV light. A layer at one side of the laminate is the protective film, the polyethylene (PE) layer, is then peeled off. The photoresist is then applied on the substrate, which is placed on the cardboard as shown in Fig. 1. The substrates are put through the hot-roll laminator with speed 1 m/min and at temperature 100˚C -110˚C. The protective polyester (PET) layer should remain on top of the laminate when the exposure process is performed. This layer is to be removed once the processes of developing and rinsing are followed.

3.4. Adhesive bonding

Our procedure for bonding the fragmented chips uses a technique called adhesive bonding. There are two appliances that can be used for adhesive bonding process, the hot press bonder and the oven. For the hot press bonder, the temperature used must be ramped up by 0.83°C/min, from 150°C to 200°C. The length of time of the first temperature, 150°C is set for

30 minutes while the second temperature 200°C is set for 1 hour. After the course, the temperature is ramped down slowly, with 1°C/min until it settled to the room temperature. This process will take approximately 6 hours to be completed.

For bonding in the oven, the chip will be pressed between several standard size microscope glass slides (rigid plates) with additional big black paper clamps to be used for clamping as shown in Fig. 2. There is other presser that we can use i.e the ceramic type presser. This cheap procedure follows the ideas from Pan et al. [23]. The process is started by setting the temperature ramped up 0.83°C/min to 150°C for 30 minutes, and then it is ramped down 1°C/min to the room temperature. The step is followed by setting up back the temperature 0.83°C/min to 200°C for two hours and then the temperature is ramped down 0.83°C/min to the room temperature. This process will take longer time as compared to the hot press bonder [7].

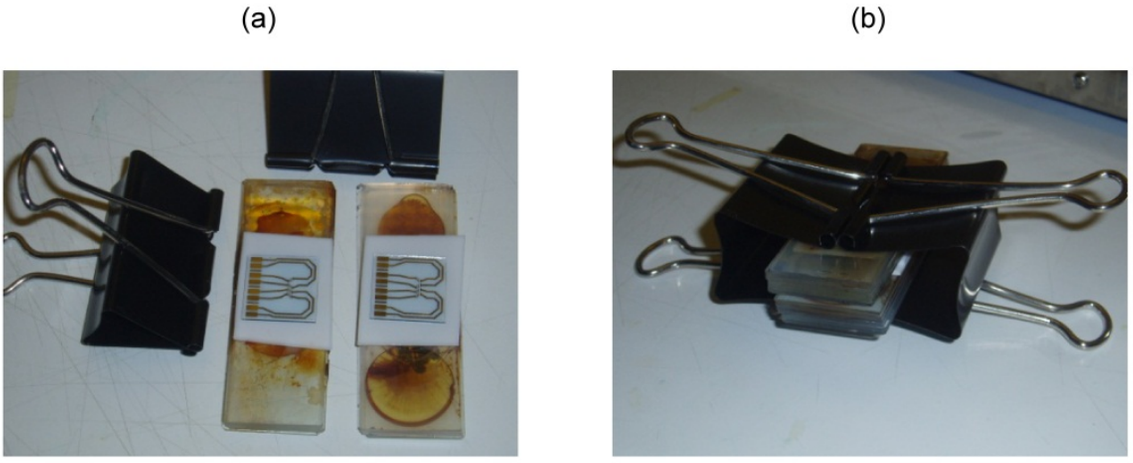

Figure 2. (a) Picture of the substrates that are ready to be placed in between the set of glass slides with Teflon sheets as the absorber/suspension to give room for the expansion and contraction when the temperature rise or fall and (b) they will be clamped with two big black paper clamps which are used for clamping the glasses at its place.

Subsequently, the channel made will be drilled at specific inlets and outlets (marked) to form the holes for the fluid to flow into and out from the channel. The test fluids are flowed through the channel to check for any leakage and clogging before the device get fully utilised [24]. Sometimes the bonded channel are tested using fluorescent dyes to confirm a good seal [25] otherwise the UV glue will be used if the channel has leakage. This UV glue is used to cover the high probability of leak area at the side of the two bonded substrates. The glue will flow by capillary force in between the substrates. And, when the glue has covered the whole area around the channel wall, the chip will then be exposed under the UV light for about 40s. As a result, the close channel will be formed successfully.

3.5. Channel fabrication

The mask design can be created using L-Edit/CleWin or any CAD drawing tools and this design can be transferred in a high-resolution transparency/acetate mask using a high-resolution printer suggested to be from 3386 dpi (dots per inch) to 128000 dpi. Fig. 3 below

shows the ready made acetate mask of the channel. The kind of this mask is preferred and the advantages of it are stated in Table 1.

Illuminated areas will be remained on the glass during development process because we will be using negative photoresist. The exposure time and the developing time are then recorded. This process, of course, requires a very clean substrate (wafer) so that no airborne debris or dust imbedded onto mask when the mask makes contact with the wafer. The imbedded particle could cause permanent damage to the mask and result in defects on the glass wafer with each succeeding exposure.

Parameter	Mask
Pattern	1:1 mask-substrate
Critical dimension	Easy to pattern micron dimensions on mask and substrate
Exposure Field	Entire substrate
Mask technology	Mask has same dimensions as substrate -a rapid prototype
Throughput	Potentially higher
Die (chip/device) alignment and focus	Individual die alignment and focus
Defect density	Defects are not repeated multiple times on a substrate
Surface flatness	For overall global focus and alignment

Table 1. The advantages of using pattern acetate mask-substrate transfer.

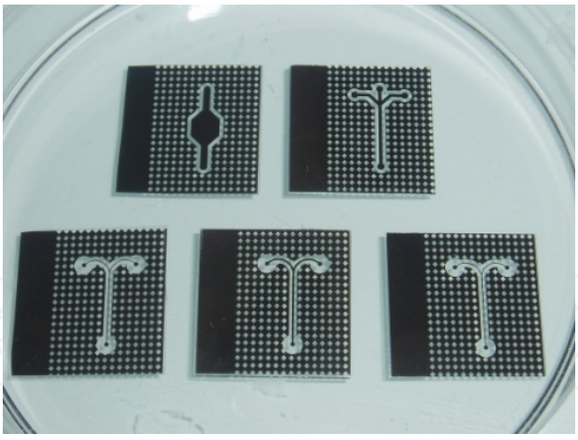

Figure 3. Acetate mask of the channel, with white area is the part where the photoresist is to be remained on the substrate and black area is the part where the photoresist is to be removed after the patterning process.

3.6. Bonding

Prior to bonding, the devices with microfluidic channel are first treated with plasma asher, (Oxford Instruments Plasmalab 80 Plus System) where it is used for ashing, etching and cleaning the surface of polymer, the microchannel. It will remove the surface contamination

and prevent any contamination from interfering the adhesion especially during bonding process. It makes the surface more hydrophilic and thus enhances the adhesive transfer. In other words, plasma treatment will improve polymer analysis, wettability problems, painting/coating of plastics, gluing results and clean surfaces from carbon, grease and oil. The interactions of plasma with a polymer surface can be divided into four general categories as shown in block diagram in Fig. 4.

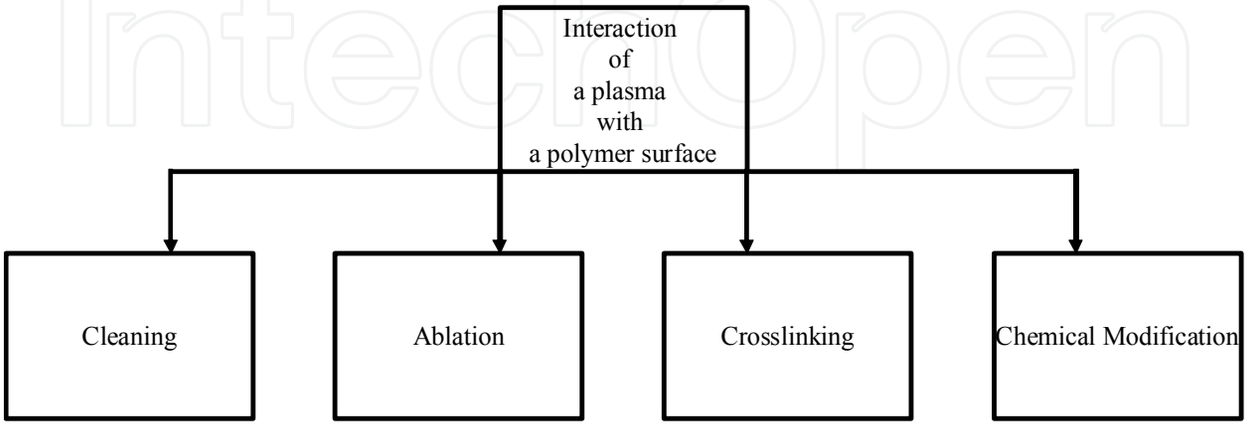

Figure 4. Four main interactions of plasma with a polymer surface. Cleaning, Ablation, Crosslinking and Chemical Modification.

A surface cleaning is the removal of organic contamination. Ablation is to remove material by micro-etching mainly to increase surface area and it is also used to remove a weak boundary layer. A cross-linking or branching is used to strengthen the surface cohesively, and surface chemistry modification is to improve chemical and physical interactions at the bonding inter-phase. The setting of the equipment is shown in Table 2.

Setting	**Condition**
O_2	10 sccm (process gas out)
Pressure	50 (set)
Forward Pressure	100 (RF)
Chiller	20 degree

Table 2. Sample using plasma asher for 2 minutes with its settings and conditions.

These microchannels are sandwiched between two glass substrates to form a closed microchannel for microfluidic systems applications such as a micropump, micromixer and separation systems. For example, the microchannel size is 500 μm wide and the step by step of forming the channel has been discussed in [7]. First, the microchannel is placed at one side of the glass substrate with microelectrodes array on it. The other side of the glass substrate will only with another microchannel on it without microelectrode as shown in Fig. 4. The disadvantage of this configuration with one side of microelectrodes array and two layer of laminates is that the electrokinetic force is weaker due to small gradient of electric field strength generated and it will be difficult to be used to control the bioparticle motion [26].

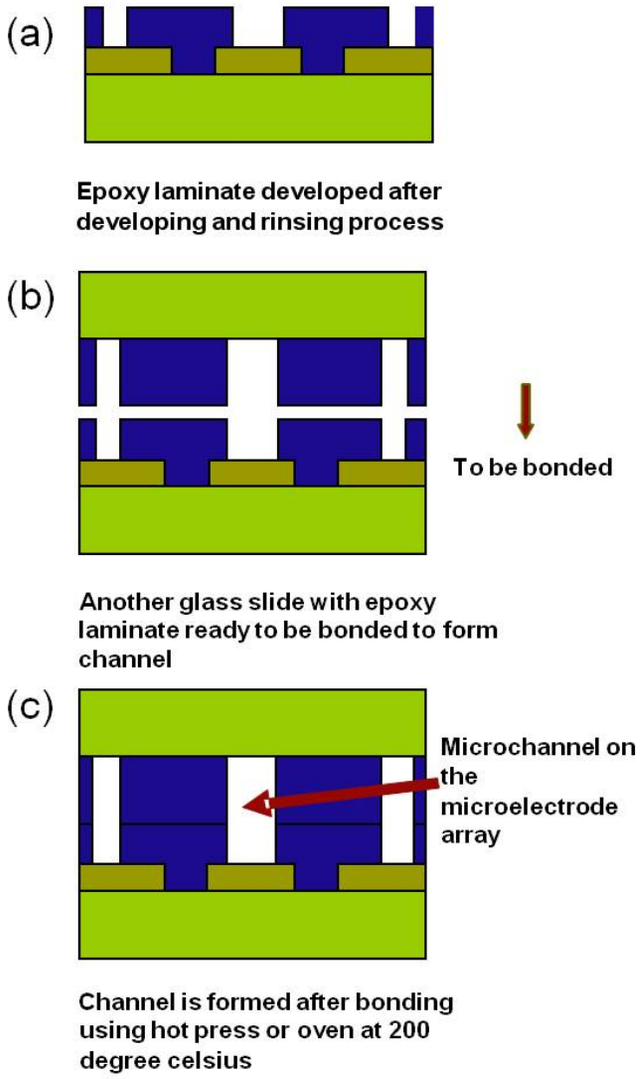

Figure 5. (a) The developing and rinsing process of laminate sheet deposited on the substrate with microelectrodes array (b) The two channels from two glass substrates are aligned and then bonded and (c) hence form the close channel after placing them in the hot press or oven at 200°C.

The electric field strength of the microfluidic device is further increased and this is done by placing the microelectrodes array on the top and the bottom of the glass substrate and with only one layer of laminate as the microchannel. The microfluidic device for bioelectrokinetic working much better with this arrangement, because the gradient of the electric field generated becomes stronger [27]. The thickness or the height of the channel is reduced from 50 μm to approximately 35-37 μm since only one laminate sheet is used [28]. The channel height is reduced compromising the desired electrokinetic operation. For example, we can use it as a separation device to separate a mixture of submicron particles.

Nevertheless, there are times where the bonding process has not been successful. If more than one device is intended to be bonded at the same time using the hot press bonder as shown in Fig. 6, mainly when they are collected to be bonded together, the thickness of the device must really be considered. Otherwise the results shown will be a part of the

microfluidic channel area bonded and a part will not be bonded. Fig. 7 shows the result after using the hot press bonder with collection of devices having different thicknesses to be bonded and assembled in one go.

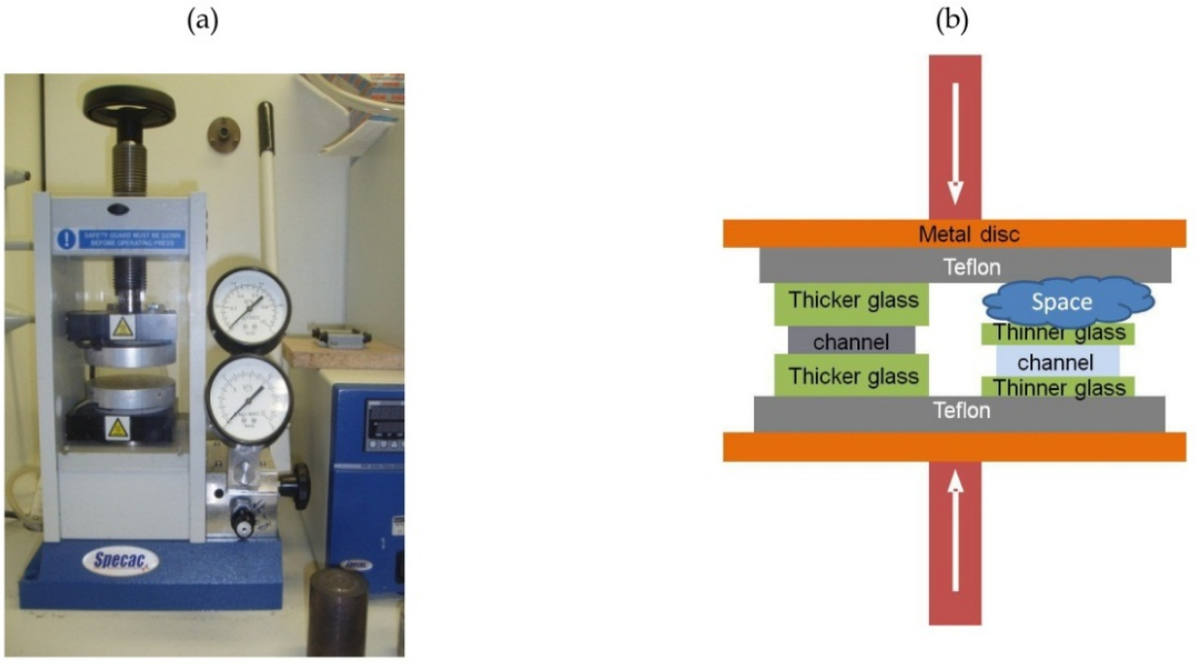

Figure 6. (a) The hot press bonder (b) Illustration of two uneven sized/height devices bonded together in the hot press which will cause the non-bonded area.

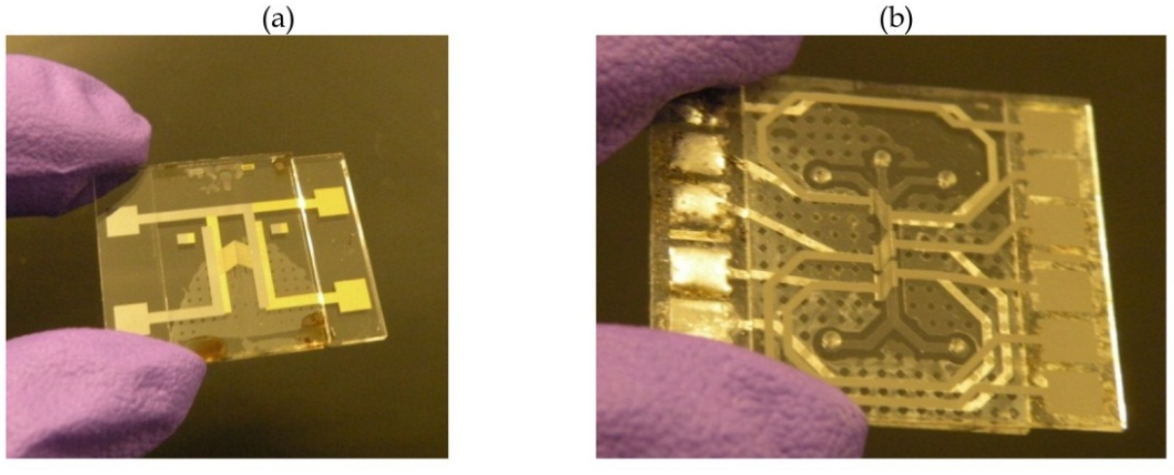

Figure 7. (a) The non-bonded area of the device approximately 2 mm thick was shown at the bottom part of the device with light shaded area (b) the non-bonded area of the device approximately 1.4 mm thick is shown at the right hand top corner near the right most top inlet.

Furthermore, high internal pressures could be developed in the hot press bonder as the fabricated channels were compressed between two contact glasses (wafers), and this might create non-bonding forces as well [29]. These occurrences are not preferred because, the channel will not be formed properly and if the aperture is drilled, there will be a leakage. The optimization of the bonding process for fragmented device is still under investigation.

3.7. Drilling

It is important to exercise a great caution when handling or drilling apertures into the device since the device is small and made from glass. The machine used to drill aperture on the device is from ProxxonGmbH i.e the main adapter NG 5/E with bench drill TBM 220 (220-240V). The drill bit used is a solid carbide drill, DCSPADEG, spade type, 1 mm, 60º point from Drill Service (Horley) Ltd. It is specially made for glass substrate. The sharp point helps to reduce chipping at breakthrough.

The process of drilling will generate heat when the drill bit is spinning and it may break the device. Therefore to reduce heat, the chip is placed in the petri dish filled with water. The other factor to be considered is that the drill bit must be very sharp to avoid fractal crack on the surface of the glass. The speed and the height of the drill bit must also be controlled. Here the speed of drilling is approximately 600rpm.

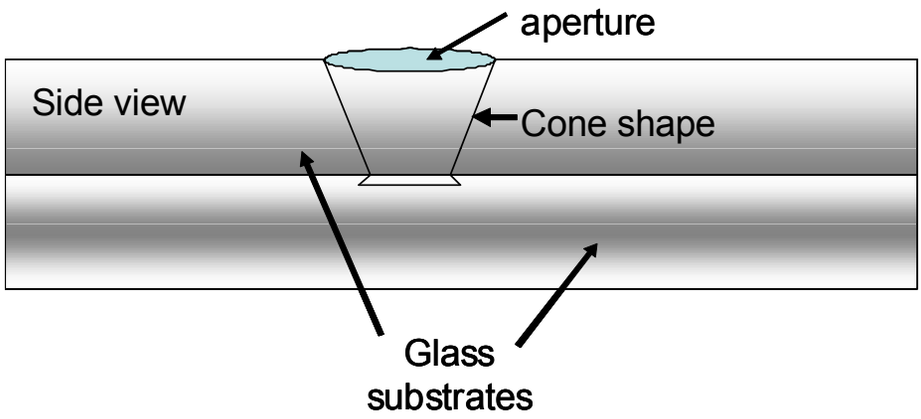

Figure 8. The chip is tilted (side view) to see the shape of the cone has been formed on the both side near the drilled aperture.

The process of drilling starts after the correct place of aperture is marked. In order to make sure that the aperture has already been drilled, the chip is tilted to the side under the light to check whether the shape of the cone has been formed on both glass sides near the drilled aperture as shown in Fig.8. The geometry of the aperture depends on the shape of the drill bit. The device could crack at apertures as shown in Fig. 9(a) and (b). The chip might also break into pieces if the apertures are not drilled properly as shown in Fig. 9(c)-(e) and to overcome this occurence, a temporary tape could be placed on top of the device to prevent leakage as shown in Fig. 9(f).

Since the apertures are drilled using a machine and we are the one who controlled the speed, we might get a different aperture diameter in the end. This will result some differences on the input or output resistance. Once this occurred, there will be a problem if one is working to balance the sheath flow or to sort the particles at the output. In other word, a robotic and controllable driller for microfluidic device should be designed to avoid differences in input and output resistance of the device.

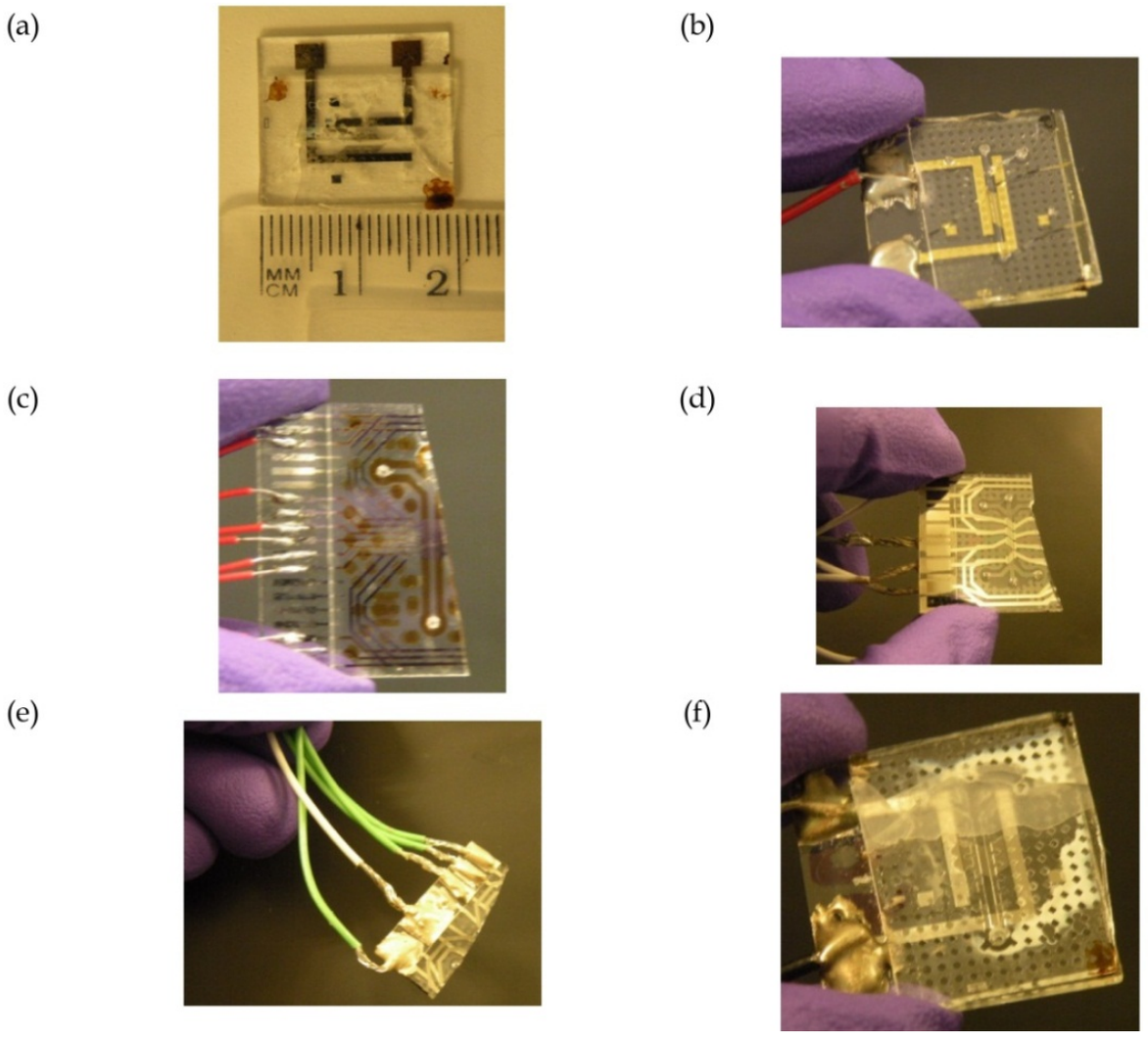

Figure 9. (a) and (b) show the devices crack because of the drilling process. (c), (d) and (e) show the devices break into pieces when there is severe crack occur during drilling process. This breakage will cause trouble during fitting the chip into the holder or handling/preparation of the chip before the experiment. (f) The device is plastered using the tape to cover the other side of the substrate when the drilling process has accidentally passed through the other side of the substrate making the hole throughout the substrates.

3.8. Channel formation

Finally, the channel is produced after laminating and drilling apertures are completed. This close channel is shown in Fig. 10. Fig. 10(a) shows the earlier device tested however has residues in it. Fig. 10(b) shows the successful bonded device under the temperature of 200°C with the length of heating is 2 hours. Fig. 10(c) shows the successful bonded device under temperature 200°C with length of heating of 4 hours. Notice that the image of the device is darker in colour when it is heated longer. Fig. 10(d) shows a device that can be examined by running dyed water into it. This is done to observe if there is any leakage by using the syringe connected with the silicon tube. The bonded device is then tested with chemical resistance by continuously flow the chemical through the channel.

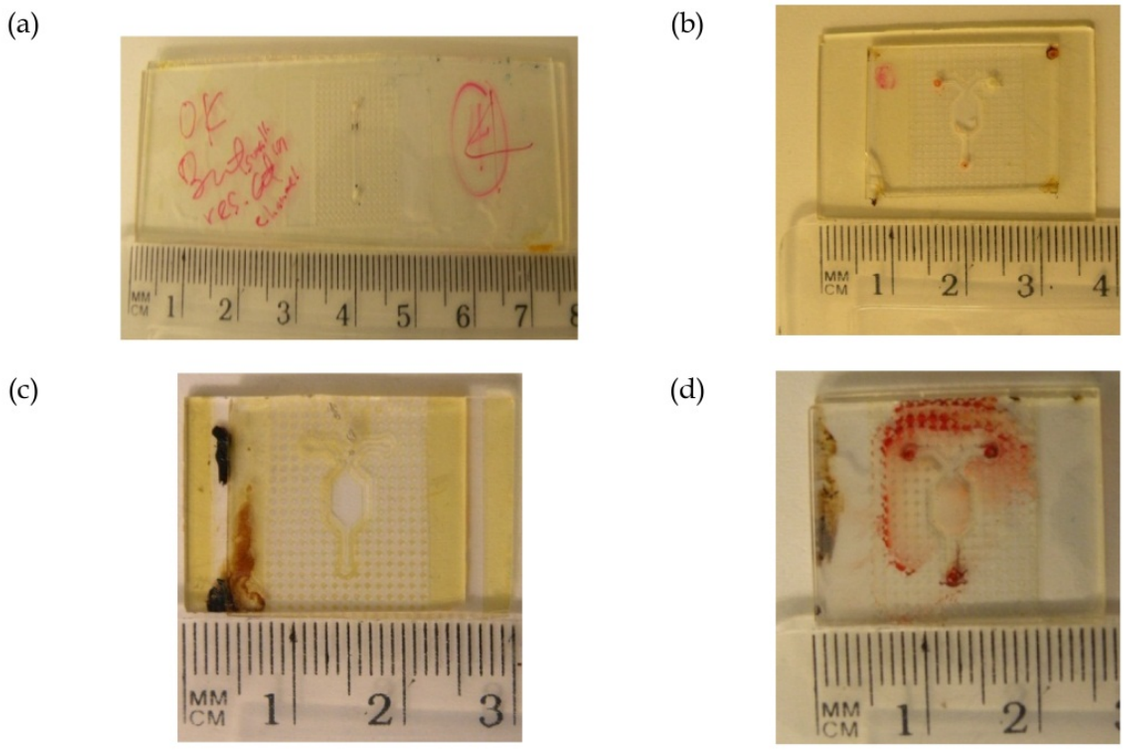

Figure 10. Some tested device using the microscope slide bonded in the oven. (a) a device is successfully bonded but has residues in it and (b) a successful bonded device with in 2 hours. (c) Successful bonded device in the oven 4 hours and (d) bonded device tested using dyes for leakage discovery.

3.9. The device

Fig. 11 shows one of the bonded microelectrodes array device that is ready for the experiment. The connecting wires is used to connect the device to the controller. The gold, Au and chromium, Cr layers are easily leached (dissolved) into the solder therefore soldering iron is not suitable to be used on gold, Au [30]. These wire connections are glued to gold, Au bonding pads, the large connection areas which were included in the microelectrode designs. The glue used is the silver paint or conductive epoxy glue from Chemtronics. It is cured at room temperature for about 4 hours. Table 3 shows the hours in the making of the epoxy conductive glue to cure under the room temperature (RT). This glue is suitable to be used when microelectrodes array is made from gold, Au or platinum, Pt which coated with titanium, Ti.

Hours on Bonding pad	Epoxy Conductive Glue
1 hour	Not cured
2 hour	Not cured
3 hour	Not cured
4 hour	Cured
5 hour	Cured

Table 3. Hours for the epoxy conductive glue to be cured in ambient air/room temperature (RT)

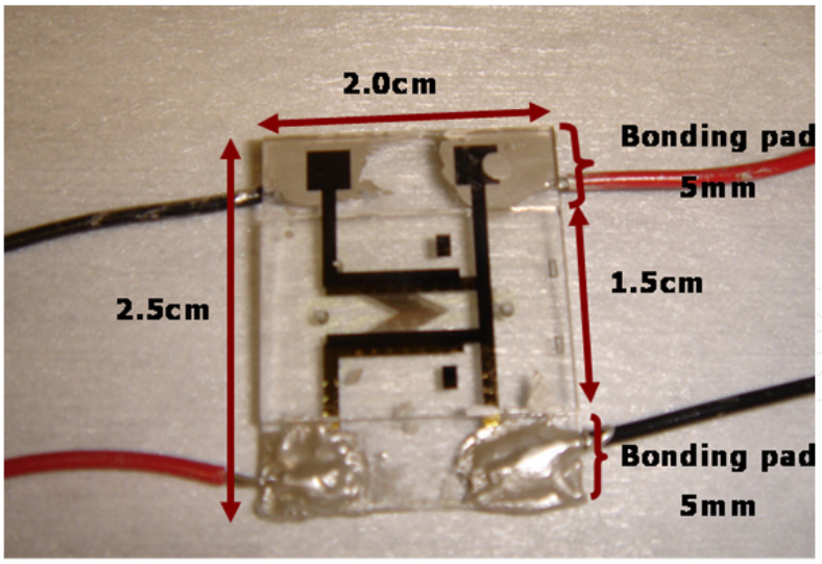

Figure 11. A bonded microelectrodes array with dimensions. The width of the device is 2.0 cm and length is 2.5 cm. The 5 mm length each side of the microfluidic device is made especially for bonding pad of both sided top and bottom microelectrodes [7].

3.10. Device holder

The holder is designed custom made for the device of size 20 mm x 20 mm shown in Fig. 12. The holder base is made from the PEEK material. The lid is made from brass material and it should be firmed enough to hold the device tightly and strongly. The gasket, which is made from polytetrafluoroethylene (PTFE), is a sheet or rubber type ring made of Teflon or a type of sealing material that can fill the space between two objects, the device and the holder, preventing leakage and breaking between the two objects, under compression/pressure.

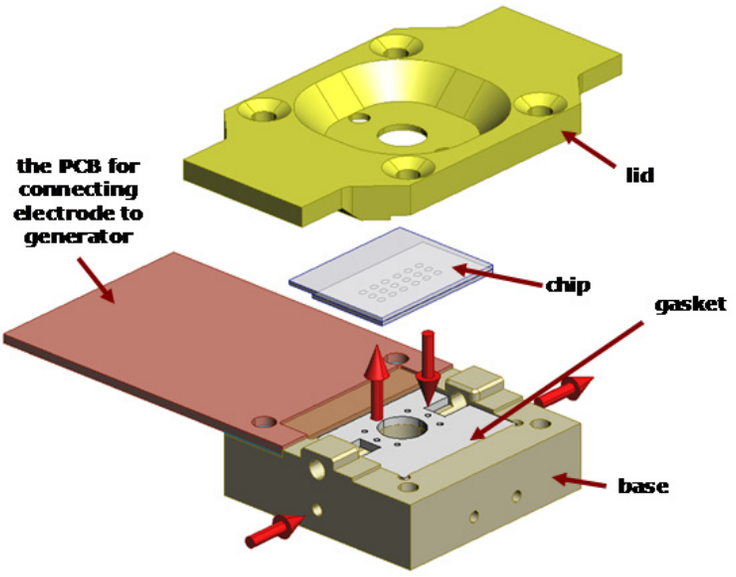

Figure 12. An illustration of a device/chip holder in 3D showing the lid, possible chip, gasket, base and the PCB connector to the electrode on the chip [illustrated by Rupert Thomas].

The device is clamped in the holder tightly as shown in Fig. 13. The holder can clamp the device of at least made of two substrates of each 700 μm thick and above.

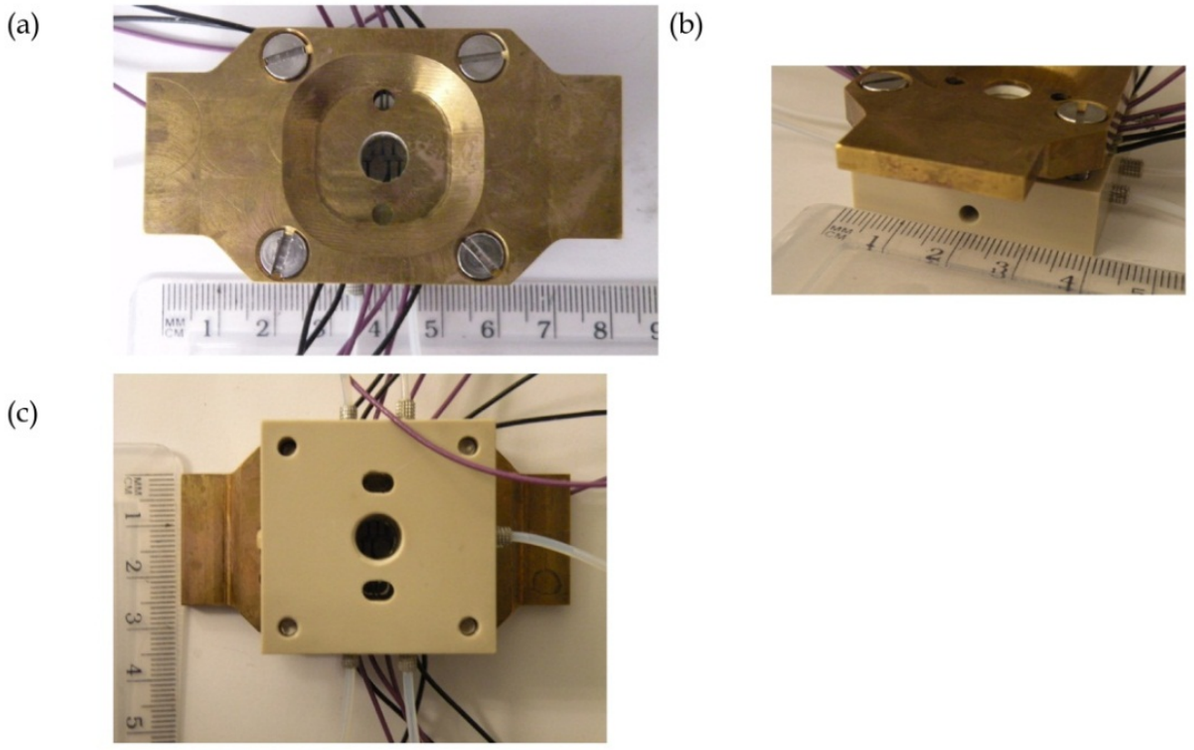

Figure 13. (a) The device in the holder ready to be experimented. (b) The dimension of the base of square shape is 4 mm x 4 mm (c) The top holder is turned upside down, showing the base of the holder.

The holder could be the same but the connectors to the device can be modified depending on the device connectors design. The connectors can be made using the cable of 26 pin and the working pin on the electrode could be selected. Uniquely with this holder, it can be used for viewing the channel of the device on either side of the holder as shown in Fig. 14 (a)-(b) as long as the microscope objective used can meet its working distance. The thickness of the base of the holder is 1 cm and the microscope objective used for this holder is X 10. Fig. 14 (c) and (d) show the dimension of the connectors, made from printed circuit board (PCB).

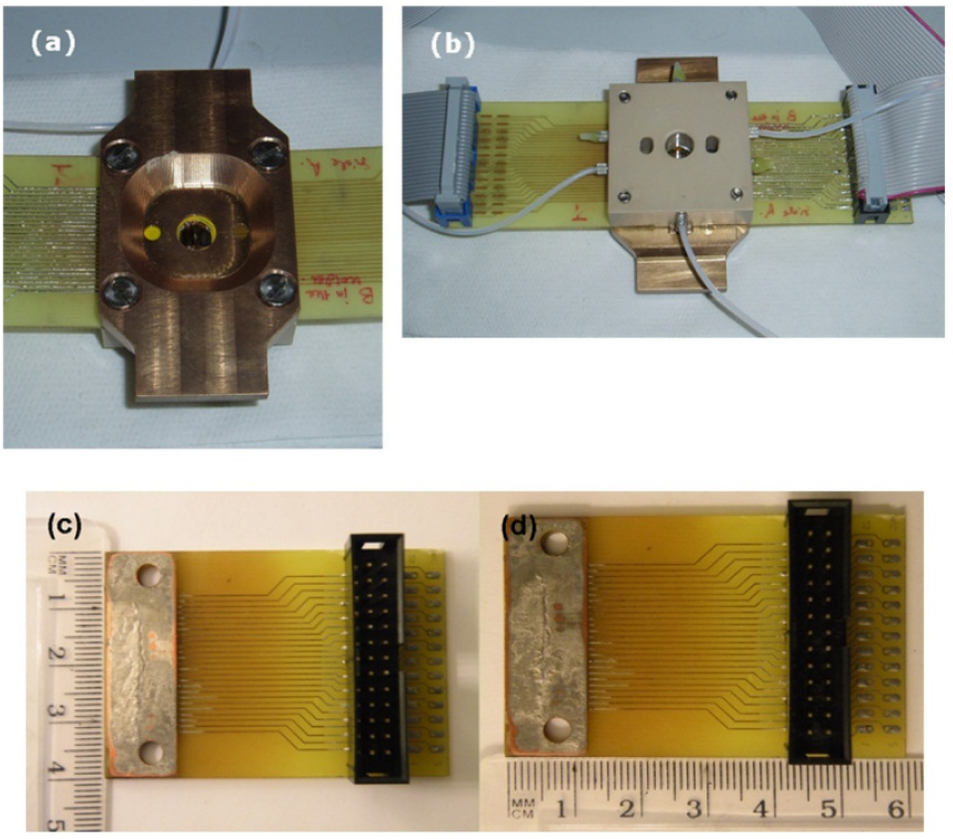

Figure 14. (a) The holder (b) The back of the holder with the connectors (c) and (d) show the dimension of the PCB connectors.

4. Discussion

The microfluidic device (from fragmented chip glasses) is successfully fabricated with an array of microelectrode in it and the microchannel made from the laminate sheet, dry film resists (DFR). The bonding process for the microchannel is seemed excellent to be done in the oven. The thermosetting material for this microchannel makes it can be cured in high temperature and also it cannot be dissolved easily after cured. This is a good indicator for a microfluidic device to be used for longer times in the experiment and during analysis. Hence the complete fabricated microfluidic devices depending on the microelectrode and microchannel designs, they can be used as a particle separator, mixer, focusing device and so on which definitely be functional in the bioelectrokinetic system.

5. Conclusion

In this chapter, the microfluidic device fabrication for bioelectrokinetic system applications is described. The microelectrode is fabricated using a standard microelectronic fabrication technique including the microchannel made of laminate sheet.

In order to make the system efficient, the steps of fabrication and cautions have had to be taken into account. All steps would require thorough precautions and might only need

slight changes in time duration and it will depend on the size of the substrate, the thickness of the microelectrode and microchannel. In fabrication of microfluidic the microelectrode and microchannel dimensions are important for getting a good electrokinetic force to do manipulation of fluid and particle in bioelectrokinetic system [26].

Author details

Nurul Amziah Md Yunus
Universiti Putra Malaysia, Malaysia

Acknowledgement

The author would like to thank Prof. Hywel Morgan and Reader Dr. Nicolas G. Green for ideas, support and laboratory facilities during her research study at Bionanoelectronic Laboratory, Nano Group, School of Electronic and Computer Science (ECS), University of Southampton. A huge appreciation to Dr. Shahanara Banu, Dr. Rupert Thomas, Mr. Andrew Whitton and Ms. Katie Chamberlain for providing valuable discussion and advice to the author in making the microfluidic devices for bioelectrokinetic system applications until successful.

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