Manufacturability and surface characterisation of polymeric microfluidic devices for biomedical applications

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Abstract
Generally, machining of polymeric microfluidic devices is a one-step manufacturing process. It is economical compared to lithography and can be used for batch production and rapid prototyping. However, surface properties are modified during machining due to the viscoelasticity property of polymers and the mechanical nature of fractures. In this present work, the manufacturing capability of the mechanical micromachining process of polymers has been explored. Surface characteristics like surface roughness, surface energy, and burr formation are investigated. Surface quality is chosen as a contributing factor for defining the manufacturing capability as it is one of the significant factors influencing the physics of fluid flow in micro-channels. In the present work, several manufacturing methods, such as 3D printing, hot embossing, photolithography, and mechanical micromachining, were considered. The surface energy of various surfaces machined using the abovementioned methods is evaluated and compared. It has been observed that mechanical micromachining is the most suitable method as they have less wettability with lower surface energy. Further investigations are carried out by machining microfluidic devices using polymethylmethacrylate (PMMA) and polycarbonate (PC) materials, as they are extensively used in biomedical applications. Surface roughness was measured on the PMMA and PC surfaces after milling. The surface roughness values and surface energies are used for evaluating the suitability of the machining process to fabricate microfluidic devices. Microfluidic devices with serpentine channels were machined on PMMA with a depth of 50 µm and width of 200 µm for evaluating inertial focusing in the channels. These devices were further evaluated for blood cell separation at different dilution rates. It is observed that PMMA is the preferable choice for fabricating microfluidic devices using mechanical micro-milling.

Keywords Mechanical micro-machining · Microfluidics · Polymers · Surface roughness · Surface energy

Abbreviations
PMMA Polymethylmethacrylate
PC Polycarbonate
PDMS Polydimethylsiloxane
PEEK Polyether ether ketone
PI Polyimide
PS Polystyrene
COC Cyclic olefin copolymer
PCE Percentage of cells in the outlet
CE Cell concentration (cells/ml)
Ra Surface roughness parameter

T\text{\textsubscript{g}} Glass transition temperature of the polymers
\sigma\text{\textsubscript{S}} Overall surface energy of the solid (mN/m)
\sigma\text{\textsubscript{L}} Overall surface energy of the liquid
\sigma\text{\textsubscript{SL}} Interfacial tension of solid and liquid
\sigma\text{\textsubscript{L}}\text{\textsubscript{SL}} Energy of adhesion per unit area
\sigma\text{\textsubscript{D}}\text{\textsubscript{L}} Dispersive component of the surface tension of wetting liquid
\sigma\text{\textsubscript{D}}\text{\textsubscript{S}} Dispersive component of the surface tension of solid
\sigma\text{\textsubscript{P}}\text{\textsubscript{L}} Polar component of the surface tension of wetting liquid
\sigma\text{\textsubscript{P}}\text{\textsubscript{S}} Polar component of the surface tension of Solid
\theta Contact angle between the solid and liquid

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1 Introduction

Mechanical micromachining on polymers is widely used in research laboratories for fabricating microfluidic devices, primarily for biomedical applications. Owing to the capability of the applications and commercial advantages, the development of manufacturing for batch and mass production is crucial [1, 2]. Polymers are one of the most widely used biocompatible materials and are suitable for mass production [3]. Currently, polydimethylsiloxane (PDMS) lithography is widely used for fabricating microfluidic devices. Manufacturing methods like injection moulding [4] and hot embossing [5] were successfully used to fabricate microstructures. These methods are more suitable for mass production than at the prototyping stage. Mould masters using mechanical micro-milling were manufactured for the larger-scale fabrication of microfluidic devices, and PDMS was used as a substrate material [6–8]. This process involves significant production times similar to lithography methods and is therefore unsuitable for prototyping. Prototyping requires easy fabrication methods and a low cost of working materials. For this, direct machining techniques like mechanical micromachining will be more suitable. Depending on the machining parameters, mechanical micro-machining introduces surface characteristics like surface roughness and burrs that modify the physics of fluid flow in microchannels and affect biocompatibility [9, 10]. The present work investigates the effects of mechanical micro-milling and surface characteristics like roughness, surface energy, and burr formation on PMMA and PC wafers. The machined surfaces on the wafers are obtained by varying feed rate and depth of cut at a constant speed of 20,000 rpm using a micro-milling tool developed in-house [11]. Surfaces obtained from other fabrication methods like 3D printing, hot embossing, and photolithography are compared with the machined surfaces for surface energy. Furthermore, to evaluate mechanical micromilling, a microfluidic device with serpentine channels is fabricated and investigated for blood cell separation.

1.1 Mechanical micromachining

Mechanical micromachining has primarily been used to machine metallic materials, and theories were developed to achieve better quality [12]. Due to the higher stress variations in micro-tools, the surface quality of the obtained surfaces is affected. The uncut chip thickness in micro-machining is close to the cutting-edge radius, and therefore ploughing action is dominant during material removal. These actions lead to the formation of burrs and side flow of the deformed material, increasing the surface roughness. Surface roughness is the presence of irregular peaks and valleys on the surface inherited by the fabrication process and is measured using surface roughness parameters. Among several, average roughness ($R_a$) is an extensively used parameter to measure surface roughness. It is defined as the arithmetic average of the absolute values of the profile heights over the evaluation length. In the previous studies, the roughness ($R_a$) obtained on PMMA was between 0.13 and 0.3 µm [13], with spindle speed and depth of cut having the most minor and most significant impact on the surface roughness ($R_a$). In a similar work, a single edge diamond tool with a diameter of approximately 50 µm was used to machine PMMA and achieved a surface roughness ($R_a$) < 60 nm [14].

Investigations performed on PMMA, polyether ether ketone (PEEK), and polyimide (PI) materials showed that as the spindle speed and tool diameter increased, the milling temperature was increased [15]. As the temperatures increased, polymers transition into three distinct regimes (glassy, viscoelastic, and rubbery), and the fracture mechanism changes from brittle to ductile. The burr formation of PMMA was relatively less compared to PEEK and PI. Surface quality was affected when the machining took place in the viscoelastic regime due to the sintering of chips to the channels. Burrs are undesirable extended surfaces over the edges of the workpiece, which are caused to the plastic deformation induced by shear strain [16, 17]. This burr formation is unavoidable as it may introduce undesirable forces during flow and affect the bonding of the devices, causing leakages. For an ideal microfluidic device, less surface roughness with no burrs is desirable as they introduce unknown micro-hydrodynamic forces during the fluid flow [18].

1.2 Effect of machining on thermoplastic surfaces

Process parameters like compressive forces, shear forces, and frictional heating affect the surface roughness of polymer machining [19]. If the machining occurs below the glass transition temperature ($T_g$), the material will behave as a solid and either ductile or brittle fracture may be observed. However, as a result of the frictional heating during machining, the viscoelastic property of the polymer comes into effect. This viscoelastic property of polymers creates a machining zone that is neither plastic nor elastic state and is termed as intermittent rubbery area. The arrival of the polymeric material to this rubbery zone depends upon the material’s glass transition temperature. During machining in this rubbery zone, forces from the rotating machining tool act on the polymer chains and cause them to rise along with the rotating tool, as shown in Fig. 1. This rising of polymer chains is called as Weissenberg effect or climbing effect of polymers and depends upon the molecular weight of the polymers. Surface characteristics are modified due to this...
climbing effect of polymers. As PMMA and PC materials have different T_g’s and molecular weights, the surface characteristics of these materials are compared to each other after machining. This comparison will help evaluate the most suitable polymer material among PMMA and PC for mechanical micro-machining within a specified range of parameters.

In a microchannel, the presence of surface roughness causes a pressure drop and affects the surface’s wettability [20–22]. The machined surface could become either hydrophobic or hydrophilic due to the peaks and valleys imparted during machining. These peaks and valleys introduce Wenzel or Cassie-Baxter effects, where the ability of the liquid droplets to penetrate valleys of the surface are altered and affects the surface’s wettability, as shown in Fig. 2. According to Wenzel’s effect, wettability increases as the liquids penetrate the peaks and valleys of a textured surface. According to Cassie-Baxter’s effect, the fluid may sometimes not penetrate the gaps between the textures due to air gaps leading to less wettability. Generally, contact angles and surface energies are measured to characterise these behaviours of the surfaces. Surface energy is a quantitative measurement for evaluating the quality of the surface, where lower surface energy implies higher contact angle and less wetting. It is a relative measurement of the energy that is caused due to surface atoms having an incomplete, unbalanced set of interactions. Therefore, the surface energy of the machined surfaces is compared with surfaces obtained from various fabrication methods.

1.3 Microfluidic devices – biomedical application

Generally, microfluidic devices using the inertial focusing phenomenon [23] for particle separation have channel profiles with different geometries like spiral [24], serpentine [25, 26], and with varying cross-sections [27–29]. Devices with parallel channels were reported for achieving high throughput (240 ml/h) while achieving separation efficiency of 80% of...
pathogenic bacteria from blood flow [30]. The microfluidic channels in inertial focusing devices have dimensions ranging in-between 50 and 650 µm. These dimensions make mechanical micromachining more suitable for fabricating microfluidic devices for inertial focusing and particle separation. Therefore, in the present work, a microfluidic device with serpentine channels appropriate for inertial focusing is fabricated and investigated for blood cell separation. As mechanical micro-milling is the choice for manufacturing microfluidic devices, the design of the microfluidic devices depends on the size of the tools, which is the caveat during machining.

2 Device fabrication – design and force analysis

As shown in Fig. 3, the device has serpentine channels with a width of 200 µm and a depth of 50 µm. The outlets are perpendicular, the middle outlet B has a width of 200 µm, and the extreme outlets A and C have 100 µm channel widths. The cell separation depends on inertial focusing phenomena where randomly dispersed particles in the entrance of a channel migrate laterally to equilibrium positions during the flow in the channels. Different forces act on the cells and position them in the microfluidic channels. The forces acting on particles in inertial focusing is grouped mainly into two categories [31]: (i) primary lift forces [$F_p$] – wall interaction, shear gradient lift, and drag forces; and (ii) secondary forces [$F_s$] – Dean’s flow formation. If $F_s > F_p$, inertial focusing is not adequate, causing mixing of particles. If $F_s < F_p$, inertial forces dominate, resulting in the focusing of the particles. The position of particles in the channel is determined using both primary and secondary forces and varies along the channel cross-section. For a given geometry, the forces can be manipulated by changing flow rates and thus controlling the position of the particles in the liquid flow.

Fig. 3 Design of microfluidic device with serpentine channels and fabrication to approximately 50 µm depth. All dimensions are in mm. (I, inlet; O, outlets)

Fig. 4 Microfluidic channels fabricated using the 3D printing technique. a Schematic of fused deposition modelling. b 3D printed microfluidic device. c Close-up view of serpentine channels in the microfluidic device
Fig. 5 Schematic for development of hot-embossed microfluidic devices

a) SU-8-2005 coated and exposed to UV light

b) SU-8-2025 coated and exposed to UV light with Photo-mask

c) Developed Si-wafer with resist coatings

d) Cr-Ni Seed layer Sputtering

e) Ni-Electroplating

f) Ni-Master

g) Hot-embossing of Ni-master on PMMA

h) Microscopic view of profiles on Ni-Master

i) Hot embossed microfluidic channels on PMMA wafer
3 Materials and methods

3.1 Fabrication methods

In this work, hot embossing, PDMS lithography, 3D printing, and mechanical micro-milling have been used to obtain the surfaces for calculating surface energy. PMMA, PC wafers, and plain glass slides were obtained from Melbourne Centre for Nanofabrication (MCN), Australia. Microfluidic devices fabricated using mechanical micro-machining were further studied for inertial focusing.

3.1.1 3D printing

3D printed microfluidic channels were successfully fabricated with Stratasys F270 and PMMA (VeroClear) material using the fused deposition modelling (FDM) technique. This technique involves extruding polymer into layers using a heated nozzle onto a platform that moves in the Z-direction and is controlled using precision motors. The coordinates for the movement and the layer heights were generated using GrabCAD Print slicing software. Serpentine channels with 1 mm and 0.5 mm channel width and a layer height of 0.127 mm
were fabricated using the FDM technique. As shown in Fig. 4, closed channels were printed using dissolvable support material provided by Stratasys Ltd., and open channels were fabricated without any support material. The design of the printed device consisted of the smallest feature dimension in the channel provided was 0.1 mm. The variations in the dimensions achieved after fabrication showed a deviation of ±0.2 mm.

3.1.2 Hot embossing

A combination of photolithography and electroplating is used to fabricate Ni-master for hot embossing, as shown in Fig. 5. Initially, a silicon wafer is coated with SU-8 2005 and SU-8 2025, at different speeds, exposed to UV light and baked up to 95 °C. The coated wafer is then treated with the SU-8 developer solution. For Ni electroplating, the developed wafer is sputtered with Cr and Ni sputtering and later plunged to galvanic bath for 6 h. After that, the electroplated Ni-master is heated up to 400 °C to remove any residues of SU-8. This Ni-master is used to fabricate hot-embossed microfluidic devices using the hot embosser machine EVG 520 IS at 135 °C and 25 kN force. The detailed procedure for fabricating hot-embossed microfluidic devices is given elsewhere [32].

3.1.3 Photolithography

Silicon wafer coated with AZ40XT (positive resist) was baked, later exposed to UV light with the mask placed on the wafer, and is developed using AZ726MiF solution. The developed wafer is exposed to dry etching gases and coated with Teflon. The PDMS base and the curing agent
(Sylgard 184 from Dow Corning) are mixed at a 10:1 ratio using a centrifugal mixer at 1000 rpm. The mixture is then poured onto the Teflon-coated wafer placed in a Petri dish. This petri dish is placed in a vacuum chamber for 20 min to remove any bubbles formed, later cured in a hot oven at 65°C for 2 hours, and cooled to room temperature. Microfluidic channels formed after curing process, are peeled off later from the wafer, and were used for surface energy measurement. The schematic process for developing the PDMS microfluidic devices is shown in Fig. 6.

### 3.1.4 Device fabrication using CNC micro-milling

The micromachining tool (MMT) [11] setup used for machining, as shown in Fig. 7, has three major systems – mechanical structure, positioning system with its motion driver, controller for the stages, and spindle with its drive system. The high-frequency spindle can reach a maximum speed of 100,000 rpm with a 0.1 µm run-out resolution. The accuracy positioning system, Newport controller (Model ESP 300), with 0.1 µm resolution, was used to control relative motion between the tool and the workpiece. According to the studies performed on the MMT, the vibration amplitude at different speeds varied from 0.2 to 1.4 µm, which shows good rigidity and stability. The limit switches used are optical for the linear stages and have a travel range of 150 mm. The whole setup is mounted on an anti-vibration table with a cast iron base of 20 mm thick to absorb the vibrations.

Grooves with dimensions 8 mm × 5 mm were machined on a 2 mm thick PMMA and PC wafer to measure roughness and surface energy, as shown in Fig. 7c, d. The full-factorial design of experiments was chosen with feed rate (0.5, 1.0, and 1.5 mm/s), depth of cut (5, 10, 15, 20, 25, and 50 µm) as varying parameters, and at a constant speed of 20,000 rpm. Higher feed rates and depth-of-cut values were used to investigate the effect of mechanical micromachining on the surface roughness of machined polymer surfaces with different parameters. Furthermore, increase in the feed and depth of cut has caused frequent breakage of the milling tools. While fabricating serpentine channels, the maximum feed rate of 1.5 mm/s was used only at the first stage to remove more material. In the second stage of machining, a lower feed rate of 0.5 mm/s is used to achieve good surface finish and required depth. A 200 µm two flute end mill used for this experiment was sourced from Reinforced Tooling Solutions, India. Surface roughness and surface energy were measured on the surface of the grooves to determine the most suitable polymer for fabricating microfluidic devices shown in Fig. 3. Initially, grooves of the depth of 100 µm were machined, and later for characterisation, the 100 µm depth grooves initially mentioned were utilised and further machined with the required parameters as mentioned above. For the microfluidic device, the initial depth of cut was 45 µm and followed by 5 µm to achieve 50 µm channel depth. A stepover of 20% is used while machining. Before the surface roughness was inspected, the milled grooves were exposed to compressed air to remove any contaminants attached to the surface.

![Experimental setup for blood flow experiments](image1)

![Microfluidic device mounted under the microscope during blood flow in channel](image2)

**Fig. 8** A photograph of the experimental setup taken during blood flow experiments and the microfluidic device mounted under the microscope and attached to the syringe pump
3.2 Surface characterisation

The roughness measurement on the milled grooves was performed with a Stylus profilometer. As the surfaces are transparent, before viewing the channel using the non-contact profilometer, the surface of the channel was sputtered with gold for 10 min. Veeco non-contact 3D surface profilometer was used to view the surface of the milled microfluidic channel. A stereo microscope (Stemi CS2000) was used to verify burrs present on the side of the wall, and the experiments were repeated three times to validate repeatability. After evaluating the surface characteristics, microfluidic devices with serpentine profiles were machined using suitable parameters.

Fowkes method with Young’s equation (Eq. (1)), Dupre’s equation (Eq. (2)), and Eq. (3) was used to calculate the surface energy by measuring the contact angle from two liquids [33]. Contact angle measurements were carried out using a goniometer. Water ($\sigma_L^P = 46.4 \text{ mN/m}$, $\sigma_H^P = 26.4 \text{ mN/m}$) and diiodomethane ($\sigma_L = \sigma_H^P = 50.8 \text{ mN/m}$) were used as polar and dispersive components for calculating surface energy. The above values were obtained from Kruss’s technical note – TN306e [34].

![Fig. 9 Water and diiodomethane contact angles on polymeric surfaces fabricated using various methods and measured using goniometer for calculating surface energy (showed in the decreasing order of surface energy from top to bottom)](image_url)
Inlet and outlet holes were drilled with a 2 mm drill and connected to the piping using Araldite. Blood samples collected from the volunteers were diluted to 1:40, 1:60, 1:80, and 1:100 dilution rates (1 unit of blood in 40 units of 0.9% NaCl for 1:40 dilution). The diluted blood samples were pumped with a 0.3 ml/min flow rate using a New-Era 300 microfluidic syringe pump. The whole device was mounted under a Nikon Eclipse 80i microscope to view the flow in the channels, as shown in Fig. 8. Blood flow in the experiments was viewed under a microscope at 1:40 dilution, and a further increase in dilution rate will need fluorescent cells for viewing. The blood samples were collected in vacutainers, and the blood cells were counted using ‘BD FACS Canto ii Flow Cytometer’ within 4 h of each flow experiment. The cells were counted for 15 s and 12 µl/min flow rate during flow cytometry. The cell counts were converted into cell concentration (CE) using Eq. (4). After every experiment, the device was washed consecutively with 5%...
hypochlorite, phosphate-buffered saline (PBS), and saline (0.9%NaCl). The percentage of cells collected in the outlets (PCE) was calculated, as shown in Eq. (5).

\[
CE(\text{cells/ml}) = \frac{1000 \times 60 \times \text{Number of cells in the outlet}}{15 \times 12} \quad (4)
\]

\[
PCE(\%) = \frac{\text{Number of cells from the required outlet}}{\text{Total number of cells from all the outlets}} \times 100 \quad (5)
\]

4 Results and discussion

4.1 Surface energy on surfaces fabricated using different techniques

From the contact angle measurements shown in Fig. 9, the PDMS surface is more hydrophobic than other surfaces. The machined PMMA surface is more hydrophobic than other surfaces except PDMS and plain glass slide surfaces. The
surface roughness is in the range of 0.2–0.4 µm for machined PMMA and 0.02–0.03 µm for embossed PMMA surfaces. The contact angles for water on machined and embossed surfaces are 85.58° and 73.64°, respectively, as shown in Fig. 9. These measurements show that machined surfaces have less wettability than embossed surfaces, though machined surfaces have more roughness than embossed surfaces. This deviation indicates that the water did not penetrate the peak and valleys of the machined surface due to air gaps and surfaces following Cassie-Baxter’s effect. Therefore, surface roughness aided in less wettability than embossed and other plane surfaces.

Contact angles were obtained using water and diiodomethane to calculate surface energy on the machined PMMA and PC grooves, as shown in Fig. 7c, d. The contact angles were measured on the inlet of embossed and PDMS devices. Similarly, the contact angles are measured on the plane surfaces of PMMA and PC wafers, 3D printed surfaces, and plain glass slides. As shown in Table 1, the surface energy is measured on the micro-milled grooves of PMMA and PC wafers, plain glass, PDMS, hot-embossed PMMA wafers, and 3D printed PMMA surfaces. The surface of micro-milled PMMA grooves exhibited less surface energy than that of PC grooves, as shown in Fig. 10. Compared to

| S. No. | Peaks and valleys | $R_a$ (µm) | Contact angles | Surface energy (mN/m) | Material (machined Surface) |
|--------|-------------------|-------------|----------------|-----------------------|-----------------------------|
| 1      | Consistent        | 0.3115      | 88.46          | 35.06                 | PMMA                        |
| 2      | Inconsistent      | 0.1676      | 66.95          | 38.05                 | PMMA                        |
| 3      | Consistent        | 0.4766      | 70.65          | 36.14                 | PC                          |
| 4      | Inconsistent      | 0.2640      | 72.23          | 33.52                 | PC                          |
plane surfaces, less surface energy of the machined surfaces was observed and attributed to the Cassie-Baxter’s effect. From the surface-energy point of view, this research shows that PMMA is preferable to PC for fabricating microfluidic devices when using mechanical micro-milling. Comparable surface energy with PDMS or plain glass is desirable as these materials are extensively used in fabricating microfluidic devices.

4.2 Surface roughness measurements

Surface roughness for the CNC machined surfaces were measured on the grooves that are shown in Fig. 7c, d and details of the grooves are given in Sect. 3.1.4. Milling results showed that the least surface roughness obtained is approximately 0.15 µm on PMMA and 0.2 µm on PC surfaces. Lower Ra values were observed at the lower depth of the cut at all feed rates (Tables 2 and 3 for PMMA and PC, respectively), showing the depth of cut is the most significant variable influencing Ra. PMMA and PC materials have different Tgs and are approximately 105 °C and 150 °C, respectively. As the temperature increases, the viscosity of the polymer in the rubbery zone decreases and easing material flow during machining, assuming the temperature during machining is more than Tg. The response of the polymer during machining depends on the rate of disturbance propagation in polymer chains and their respective relaxation times. The disturbance in PMMA polymer chains is early compared to PC due to its less Tg, resulting in a higher volume of material movement on the surface. As the material removal rate changed, this material movement in the rubbery zone resulted in variation of Ra values between PMMA and PC.

Unlike metals, Ra values did not follow any trend, making the surface finish unpredictable (Fig. 11). From surface roughness profiles measured, the peaks and valleys of the surface were consistent at a lower depth of cut and feed rate, as shown in Fig. 12. A lower depth of cut of 5 µm is close to the edge radius in micro-tools, which may have caused ploughing and led to variation in Ra values. Adherence of the polymeric material to the milling tool may have further blunted the milling tool, depriving the machined surface’s quality. This adhesion increases the edge radius of the tool, making ploughing more effective, during which the material is pushed away from the tool path instead of complete removal. Suppose the milling tool stays in the same position during machining, but the temperature during machining is more than Tg. The response of the polymer during machining depends on the rate of disturbance propagation in polymer chains and their respective relaxation times. The disturbance in PMMA polymer chains is early compared to PC due to its less Tg, resulting in a higher volume of material movement on the surface. As the material removal rate changed, this material movement in the rubbery zone resulted in variation of Ra values between PMMA and PC.

Fig. 13  a–b Variations in the channel widths observed on the 100 µm and 200 µm fluidic channel. c Depth of the channels measured using Veeco non-contact 3D profilometer

(a) Dimensions achieved after machining 200µm Channel
(b) Dimensions achieved after machining 100µm channel
(c) Depth of the channel measured using a 3D profilometer
during machining, and rubbing occurs between the milling tool and the workpiece. The viscoelastic polymer chains may climb the rotating tool (Weissenberg effect). Later, the climbed polymer adheres to the tool leading again to shearing action between the tool and workpiece. This cycle of adhering and shearing actions may leave inconsistent peaks on the machined surface, as shown in Fig. 12. The inconsistent peaks on the surface make surface roughness ($R_a$) further unpredictable and affect the wettability of the surface and surface energy on the machined surfaces. Though surface roughness values are high, lower surface energy was observed on the machined surfaces with consistent peaks and valleys, as shown in Table 4. Similarly, higher surface energy values were observed on the surfaces with inconsistent peaks. Thus, the variations in the surface energy and differences between required and dimensions achieved can be attributed to the viscoelastic property of polymers during machining of PMMA and PC surfaces. Microfluidic channels of 100 µm and 200 µm width with a depth of 50 µm were machined on PMMA to evaluate machining process capability. As shown in Fig. 13, an average deviation of 5 µm to 10 µm for 100 µm and 200 µm channels respectively was observed.

**4.3 Formation of burrs**

Burrs are detrimental to the quality of the microfluidic device as it affects the fluid flow. Burr formation is more pronounced in PC than PMMA making PMMA preferable for machining microfluidic devices, as shown in Fig. 14. These burrs on PMMA and PC channel walls are inhibited by the side flow of the polymer chains while machining in a rubbery zone. The interaction stresses between tool and workpiece cause the polymer chains to extrude along the flank face of the tool. PC having higher molecular weight than PMMA needs more significant force to fracture these extruded chains resulting in more burrs. However, burr formation is inevitable and relatively minor burr formation in the PMMA channels can be observed near the edges as shown in the 3D profile in Fig. 15.

**4.4 Blood cell separation using PMMA microfluidic device**

The primary lift forces equilibrate with secondary forces and act on the cells, migrating them across the micro-channels to form cell bands and cell-free regions. Flow experiments
were performed at different dilution rates (1:40, 1:60, 1:80, 1:100) with 0.3 ml/min flow rate in the PMMA microfluidic device fabricated using mechanical micro-milling. Cell bands and cell-free regions consecutively appeared in the serpentine channels due to inertial focusing, as shown in Fig. 16. The inertial focusing phenomenon for blood cell separation was previously used by other researchers with PDMS devices having serpentine channels. In these devices, purity of ~99.95% was achieved, with a flow rate of 0.3 µl/min and at a dilution of 1:20 [35]. In a similar device, purity of 80.3% was achieved using 10 µm polystyrene beads. Separation of white blood cells from whole blood was conducted on the same device and purity of 48% was obtained [26]. In another study, a purity of 91.6% and 99.2% was obtained.

Fig. 16 Experimental photograph of the microfluidic device showing inertial focusing in micro-channel for blood diluted to 1:40 with 0.9% NaCl and with a flow rate of 0.3 ml/min at a magnification of 10 ×. a At the exit of the channel. b Middle of the channel. c–d Shows zoomed view of the section. e Microfluidic device fabricated using mechanical micro-milling on PMMA.

(a) Blood flow near the device outlets showing blood separation

(b) Blood flow near the channel curvature

(c) Magnified view showing separation regions in the channel near the outlets

(d) Magnified view showing separation in the channel curvature

(e) Microfluidic device with serpentine channels fabricated on PMMA using mechanical micro-milling.
during separation of 13 μm and 5 μm polystyrene particles at a flow rate of 0.6 ml/min [25].

In the present work, the occurrence of the inertial focusing phenomenon was successfully observed in the microfluidic channels. Three cell-free regions were observed in the channels at approximately 0–20 μm, 85–115 μm, and 180–200 μm as shown in Fig. 16c. This shows that microfluidic devices fabricated using mechanical micro-machining can be further exploited for blood cell separation. A purity of 81% was achieved from the outlets at 0.3 μl/min and 1:100 dilution. At 1:40 dilution rate, 68% of the cells were removed from one of the outlets. Table 5 gives the cell concentrations (CE) in the collected blood from different device outlets at different dilution rates. As the dilution rates increased, CE in the outlet B increased, as shown in Fig. 17. At higher concentrations (1:40), increased cell interactions might have affected the focusing in the channels. PCE increased from 45 to 62% in the outlet B as the dilution rate increased. This variation of cell concentration in the outlets shows that inertial focusing was more prominent at a 1:100 dilution rate and might be due to the fewer cell interactions. Surface roughness inherited due to machining on PMMA channels might have affected the physics of the microfluidic flows deteriorating the inertial focusing performance. Experiments at 0.3 ml/min and 1:40 were repeated, and similar results were observed.

The advantages of PMMA over PDMS devices is in the ease of manufacturing, low cost of working materials, the ability for batch production, and customised fabrication. For improving the blood plasma separation in PMMA channels, the design of the outlets can be enhanced and further evaluated. These experiments show that inertial focusing can be observed in the microfluidic devices fabricated using mechanical micro-milling on PMMA and can separate particles from the fluid streams.

### 5 Conclusions

In the present work, mechanical micro-milling experiments were carried out on PMMA and PC materials. The surface energy on machined PMMA and PC surfaces was compared with 3D printed, hot-embossed, plain glass slide, PDMS, and other plane surfaces. The surface characteristics of the machined surfaces were comprehensively analysed, including surface roughness, surface energy, and burr formation. PMMA microfluidic devices with serpentine channels fabricated using mechanical micro-milling were examined for blood cell separation. The inertial focusing phenomenon's appearance justified the micro-milling method's applicability to fabricate microfluidic devices for biomedical applications along with the following conclusions:

1. Machined PMMA surfaces are more hydrophobic than embossed, and 3D printed surfaces except for PDMS and plain glass surfaces. Similarly, machined PMMA surfaces showed relatively less surface energy than 3D printed and embossed surfaces. Surface roughness inherited from machining created air gaps in the peaks and valleys. These air gaps might have aided in less wettability and surface energy on the machined surfaces. This shows that machined PMMA is more suitable for fabricating microfluidic devices.

2. A better surface finish with surface roughness (Rₐ) of 0.2 μm was produced on PMMA and PC surfaces using mechanical micro-milling at a lower feed rate (0.5 mm/s), depth of cut (5 μm), and a speed of 20,000 rpm. Viscoelasticity, polymer adhesion, and the sudden appearance of peaks and valleys were noted to be the main factors affecting the predictability of the surface finish.

3. PMMA surfaces have relatively minor burr formation compared to PC surfaces. Microscopic analysis shows that burr formation is inevitable on machined polymeric surfaces.

![Fig. 17 Percentage of cells (PCE) present in different outlets with varying dilutions](image)
4. Inertial focusing was observed on machined microchannels though there were random peaks and valleys, which shows the applicability of the proposed mechanical micro-milling technique in developing microfluidic devices.

5. At 1:100 dilution, 81% of the blood cells were successfully removed from the extreme outlets of the microfluidic device with serpentine channels. As the dilution rate increased from 1:40 to 1:100, the cell removal rate increased from 68 to 81%. It shows that high cell interaction at lower dilution rates affects the performance of inertial focusing. Cell-free zones and cell bands were observed in the microfluidic channels showing inertial focusing in the devices fabricated using mechanical micro-milling.

From the present work, mechanical micromachining proved to be an excellent alternative to MEMS fabrication techniques for the rapid fabrication of microfluidic devices. Multiple microfluidic devices can be fabricated using mechanical micro-milling by avoiding tool breakage with the proposed machining parameters from the present work. The current research can be extended further by adapting advanced techniques like solvent bonding, thermal fusion bonding, and adhesion. Furthermore, including other polymeric materials (COC, PEEK, PS, PP) for developing higher quality microfluidic devices can extend the present research work.

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Author contribution Yugandhar Arcot, G.L. Samuel, and Lingxue Kong designed the research; Yugandhar Arcot conducted the experiments; G.L. Samuel, and Lingxue Kong analysed and interpreted the data; Yugandhar Arcot, G.L. Samuel, and Lingxue Kong wrote the manuscript with inputs from all the authors.

Availability of data and material All the other data required for validation is provided in the manuscript. The data that support the findings of this study are available from the corresponding author upon request. The data provided upon request will include images of surface roughness data, contact angle measurements, and surface energy calculations.

Code availability Not applicable.

Declarations

Ethics approval and consent to participate Approval has been obtained from the Institutional Ethical Committee of the Indian Institute of Technology-Madras to collect blood samples from volunteers, with protocol number: IEC/2018/01/GLS/16. Informed and signed consent was obtained and archived to conduct the experiments and publish results.

Competing interests The authors declare no competing interests.

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