Effects of Channel Surface Finish on Blood Flow in Microfluidic Devices

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Abstract

The behaviour of blood flow in relation to microchannel surface roughness has been investigated. Special attention was focused on the techniques used to fabricate the microchannels and on the apparent viscosity of the blood as it flowed through these microchannels.

For the experimental comparison of smooth and rough surface channels, each channel was designed to be 10mm long and rectangular in cross-section with aspect ratios of ≥100:1 for channel heights of 50 and 100μm.

Polycarbonate was used as the material for the device construction. The shims, which created the heights of the channels, were made of polyethylene terephthalate. Surface roughnesses of the channels were varied from R_z of 60nm to 1.8μm.

Whole horse blood and filtered water were used as the test fluids and differential pressures ranged from 200 to 5000Pa. The defibrinated horse blood was treated further to prevent coagulation.

The results indicate that a surface roughness above an unknown value lowers the apparent viscosity of blood dramatically due to boundary effects.

Furthermore, the roughness seemed to influence both water and whole blood almost equally. A set of design rules for channel fabrication is also presented in accordance with the experiments performed.

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1. INTRODUCTION

Microfluidic devices for the manipulation of complex biological fluids are attracting considerable interest because of their potential to be applied in medical diagnostics (Tüdos et al., 2001) in the form of hand held Point of Care (POC) devices. Blood is a biological fluid of particular interest because it contains a number of different biomarkers that can provide valuable information on the health of the patients. These include for example the cardiac marker for heart diseases B-type natriuretic peptide (BNP) (Gardner et al., 2006) and the protein S100B for the early detection of stroke (Hill 2005).

Polymer microfluidic devices are suitable candidates for POC devices because they can be manufactured in high volume at an affordable cost using microreplication technologies such as hot-embossing or micro-injection moulding (Marson et al., 2007). Both these technologies require a mould (or master) being manufactured onto which the polymer is then replicated. The mould is normally fabricated out of hard metals such as steel by a variety of metal microfabrication technologies. The quality of the manufactured channels in terms of surface finish depends on the choice of the manufacturing technique and on the control of the manufacturing process.

It is well known that blood is a non-Newtonian liquid and when blood is running through tubes and vessels a cell-free layer exists near the walls (Merrill et al. 1963). The thickness of the cell-free layer increases with decreasing diameter for diameters between 50µm and 300µm (Fig. 4). It is also known from a number of previous studies, that the thickness and the viscosity of this layer with very low cell concentration is dependent on the particle size, the channel diameter and the haematocrit value (Sharan and Popel 2001).

For Newtonian liquids, Wang H. and Wang Y. (Wang and Wang 2007) reported that the higher the wall roughness the greater the velocity gradient at the channel wall. At a Reynolds number above 100 and a relative roughness (ε) larger than 10%, flow separation occurs and regular perturbation methods, which are typically used to predict fluid flow in a microchannel, cannot be used any more. The effect of high roughness (R_Z) walls (Hodes et al., 2006) or hydrophobic surfaces (Choi et al., 2002) is better described with the Navier slip boundary model. Thus, the velocity of the bulk liquid flowing in a rough channel rises in comparison to that in a smooth channel. However, it is still unclear what influence a rough wall has on non-Newtonian liquids such as whole blood. This paper focuses on quantifying this influence in view of the choice of a suitable manufacturing process for metal micro-moulds for the manufacture of polymer microfluidic devices for blood manipulation.

2. MATERIAL AND METHODS

The viscosity of blood is variable and it is not possible to measure it within a channel directly. Thus, the viscosity has to be measured by calculating the

volume flow and the differential pressure. To be able to measure these values, they must be kept constant over a certain time at a constant temperature. Therefore, in the experiments to be described, the volume flow was driven with a peristaltic pump (Minipuls3, Model M312 from Gilson Inc.), and the differential pressure was measured with pressure sensors (HCXPM005D6V, HCXM100D6V and HCX005D6V from Sensortechnics). The readout of the sensors was carried out via a LabView 8.5 program connected to an A/D converter (LabJack U3 High Voltage A/D). Using this signal the pump speed has been controlled via a closed loop-control back over the A/D converter.

The volume flow was measured by monitoring the increase in outflow blood weight (Mettler balance AT460) over a period of 10 minutes.

All experiments were performed in a temperature-controlled laboratory at $21\pm1^{\circ}$C and all measurements for water (used as a reference) and whole blood were repeated two or three times to check the repeatability.

Whole horse blood was obtained from TCS Biosciences Ltd. (HB035). It was delivered already defibrinated but, in addition, 6g/l of trisodium citrate was added to prevent its coagulation.

To magnify the effects of the inner walls on the flow, the wall surface was made as large as possible, while keeping the velocity profile along the microchannel constant. Therefore both channels (rough and smooth) have been built as rectangular conduits (Lima, 2008) with a constant width and length of 10mm and with channel heights of 50 and 100µm respectively (Fig. 1). For comparison, at least two different channel heights with two different roughnesses were measured at three differential pressures (200, 300 and 400Pa for the 100µm channel and 1000, 3000, 5000Pa for the 50µm channel). The appropriate pressure range was selected to maintain comparable flow velocities for both the 50 and the 100µm channels.

For additional comparisons, a three-dimensional computational model of the device was created using commercial software (Gambit), and then FLUENT 6.3 was used to undertake the flow calculations. The model was meshed with 700,000 cells and all wall boundaries were treated as solid with a no slip condition. A velocity inflow configuration was chosen for the inlet and an outflow condition was used at the outlet.

A polycarbonate (PC) polymer sheet (Makrolon® GP clear 099) was selected for the test device. The depth of the channel was created by inserting a polyethylene terephthalate (PET) sheet (Polymex®) of the desired thickness between two 10mm thick PC sheets and fixed in position by super glue after clamping.

The device cavities, inlets and outlets were machined on a Kern Evo Micromachining Centre.

The "rough" surface (R_z = 1.8 ±0.6µm) was generated by micromilling using the Kern Evo with a 1.5mm endmill. The spindle speed was 10,000rpm and the feed rate was 750mm/min.

The "smooth" surface was the native PC sheet (Makrolon®, Bayer) as received from the supplier. The measured roughness was R_z = 57 ±2nm.

The total thickness of each device was measured directly by a micrometer with an accuracy of about 1µm. From this measurement it was possible to calculate the actual depth of the microfluidic channels.

The roughness measurements were carried out with a Taylor Hobson Form Talysurf 120L and afterwards calculated by a Scilab program. Three roughness measurements on three different regions were made on the rough

surface and one on the smooth surface for comparison.

3. DESIGN OF EXPERIMENTS

The aim of the work is to measure a change of volume flow (or viscosity) by changing the inner surface roughness of the channel (Fig. 1) while keeping all the other parameters constant. The Hagen-Poiseuille equation (1) describes the change of volume flow (I_V), related to the differential pressure (ΔP), viscosity (η), hydraulic diameter (d_h), cross-sectional area of channel (A), and length (l) (Tipler and Mosca 2004).

$$I_V = \frac{V}{t} = \frac{A \cdot d_h^2 \cdot \Delta P}{32 \cdot \eta \cdot l} \qquad (1)$$

For a rectangular channel with the height (h) << width (w) of the channel and with $l \approx w$, equation 1 can be simplified to equation 2 as follows:

$$\eta \approx \frac{h^3}{8} \frac{\Delta P}{I_V} \; ; \text{ with } d_h = 2\,h \qquad (2)$$

Equation 2 was used in this work to calculate the apparent viscosity (η) by measuring the volume flow through the channel and the differential pressure between the inlet and the outlet at a known channel height. This experiment was repeated with two different wall roughnesses to compare the results with each other and to search for differences.

4. RESULTS

Figure 2 shows a 2D velocity slice for the 10×10mm microchannel. A laminar flow is clearly seen since the velocity vectors are parallel to each other with the same value of 10mm/s across the whole width (equal velocities at the channel exit are depicted in green in Figure 2).

The results from the ANSYS simulation show that the fluid flow conditions along the whole channel are constant; therefore the effects at the walls can be compared. Turbulences have unpredictable influences on the viscosity and therefore must be avoided. Interestingly, the predicted laminar flow characteristics have been illustrated by the presence of a separation of the flow in Figure 3 below, which traces a route across the experimental device aligned with the modelled velocity vectors. It is likely that such an unintentional feature has been caused by agglutination of blood components (cells and/or protein) at the edge of the flow inlet.

The experiments carried out on water showed that the calculated volume flow in the rough channel was significantly higher than for the smooth one. In particular, the apparent viscosity of water in the rough channels dropped to 58.1 ±7.8% and to 79.3 ±7.9% (where 100% represented the viscosity of water in the smooth channel) in the 57µm and 109µm deep channels respectively (Table 1). This suggests a decrease in the flow resistance and a decrease in the apparent viscosity that is in distinct contrast to the predictions made by previous simulations (Kulkarni, 2004).

TABLE 1

COMPARISON OF THE APPARENT VISCOSITY OF WATER AND WHOLE HORSE BLOOD IN A ROUGH (R_z = 1.8µm) VERSUS A SMOOTH (R_z = 60nm) CHANNEL (100%). TWO DIFFERENT CHANNEL HEIGHTS AND TWO DIFFERENT BLOOD HAEMATOCRIT VALUES ARE SHOWN, n = NUMBER OF SAMPLES

Measured channel depth (µm)	Apparent viscosity Water	Apparent viscosity sample 1 (44 % haematocrit)	Apparent viscosity sample 2 (42 % haematocrit)
57±1	58.1 ±7.8%	66.4 ±2.2 % (n=9)	51.6 ±2.7 % (n=10)
109±1	79.3 ±7.9 %	79.7 ±1.9 % (n=12)	77.4 ±3.7 % (n=2)

For horse blood with a haematocrit of 44% (sample 1) the apparent blood viscosity was reduced in the rough channels to 66.4 ±2.2% (where 100% is the viscosity of blood in the smooth channel) in the 57µm deep channel and to 79.7 ±1.9% in the device with a 109µm deep channel. The blood with haematocrit value of 42 % (sample 2) showed a drop in the apparent viscosity of 51.6 ±2.7% in the 57µm channel and of 77.4 ± 3.7% in the 109µm channel (Table 1).

The Reynolds numbers (Re) for the experiments mentioned above were calculated using the equation:

$$\mathrm{Re} = \frac{d_h \cdot \rho \cdot v}{\eta} \tag{3}$$

Where d_h=2h and h is the channel depth, ρ the density of blood (1060kg/m³), v the velocity and η the viscosity. The calculated Re values are reported in Table 2 together with the fluid velocity and the apparent viscosity calculated using equation 2.

It was observed that in all cases the Re for the rough channels was higher than the one for the smooth channels. This result indicates that an increase of surface roughness R_z from 60nm to 1.8µm lowers the apparent viscosity of the blood dramatically, probably due to the presence of boundary effects. Furthermore the roughness seems to influence both water and whole blood almost equally.

TABLE 2

REYNOLDS NUMBER (Re), VELOCITY (v) AND VISCOSITY (η) FOR WHOLE HORSE BLOOD (SAMPLE 1). THE VALUES FOR ROUGH AND SMOOTH CHANNELS ARE PROVIDED FOR TWO DIFFERENT CHANNEL DEPTHS AND THREE DIFFERENT DIFFERENTIAL PRESSURES (Δp). THE ROUGH TO SMOOTH CHANNELS RATIOS ARE ALSO REPORTED FOR COMAPARISON.

Measured channel depth (µm)	ΔP (Pa)	Channel surface R= rough S= smooth	v (mm/s)	R/S v ratio	η (mPa*s)	R/S η ratio	Re	R/S Re ratio
58 ±1	1000	R	6.05 ±1.10		7.1 ±1.4		0.11 ±0.05	
58 ±1	3000	R	19.6 ±2.4		6.5 ±1.0		0.38 ±0.11	
57 ±2	5000	R	31.2 ±7.43		6.8 ±2.2		0.62 ±0.28	
57 ±1	1000	S	3.64 ±0.66	1.66	11.3 ±2.1	0.6	0.04 ±0.01	2.75
57 ±1	3000	S	11.7 ±2.7	1.63	10.7 ±2.5	0.6	0.14 ±0.07	2.71
57 ±1	5000	S	20.0 ±4.4	1.56	10.5 ±2.6	0.6	0.25 ±0.11	2.48
110 ±0	200	R	2.20 ±0.09		13.4 ±0.6		0.038 ±0.003	
110 ±0	300	R	2.87 ±1.01		16.5 ±5.8		0.05 ±0.03	
110 ±0	400	R	4.77 ±0.47		12.5 ±1.2		0.09 ±0.02	
108 ±3	200	S	1.74 ±0.26	1.26	16.6 ±2.0	0.8	0.02 ±0.01	1.90
108 ±3	300	S	2.30 ±0.49	1.25	19.3 ±4.1	0.8	0.03 ±0.01	1.67
108 ±3	400	S	3.47 ±0.92	1.37	17.4 ±4.5	0.7	0.05 ±0.02	1.80

Another experiment was carried out by changing the differential pressure in the device and calculating the corresponding blood velocity. This experiment was repeated in both rough and smooth channel devices with nominal channel depth 50 and 100µm.

It was observed that an increase in the volume flow by changing the differential pressure has a small effect on the apparent viscosity of the blood (Table 2). A roughness effect can only be seen in the channels with nominal depth of 50µm (Table 2).

5. DISCUSSION

The results of the experiments showed a significant change of the apparent viscosity of both water and whole horse blood when comparing rough and smooth surfaces. These effects have already been observed (Hodes et al., 2006, Cohen and Feaster, 2007) but never been compared directly.

A direct comparison was carried out in this work and as a result it can be stated that water and blood flows are very similar with respect to the change in apparent viscosity within microchannels. This can be explained if we assume that the Newtonian cell-free layer which surrounds the central stream

of the blood is the only portion of blood that is affected by the surface roughness. This assumption is supported by the observation that a decrease in haematocrit, which will increase the thickness of the cell-free layer, seems to increase the influence of the wall roughness (Table 1). Furthermore the influence of the channel roughness becomes less important with deeper channels (Table 2) (larger hydraulic diameters). It is known that in deeper channels (above 50µm) the cell-free layer is smaller (Fig. 4) and in the whole channel, the area cross-section for volume flow becomes larger (Sharan and Popel, 2001).

The reason for this effect may be a decrease in the no-slip boundary effect in smaller channels, enabling the plasma at the wall to flow with a velocity significantly higher than zero. Moreover the blood flow velocity is also dependent on the peak-to-valley roughness of the walls. For example Cohen and Feaster (Cohen and Feaster, 2007) predicted a quicker fill time of at least 25% up to more than 50% for microchannels with roughened walls compared to smooth channels. Thus there has to be a limit where the roughness will increase flow resistance and beyond which it will decrease as modeled in the simulations of Wang and Wang (Wang and Wang, 2007).

It is important to note that the plasma layer is highly dependent on the erythrocyte size which is smaller for horses (5-6µm) than for humans (8-10µm) (Jain 1993). The results obtained in this work can therefore be only used to predict qualitatively the behaviour of human blood. As human erythrocytes are bigger than horse erythrocytes, it is expected that the plasma layer will be thicker (Vand, 1948, Baskurt et al. 1997). This could result in a more pronounced effect of surface roughness on human blood flow.

The results obtained are collated in Table 3 in the form of a set of design guidelines for the manufacture of blood microfluidic devices with controlled cell-free layer thickness and blood apparent viscosity.

TABLE 3

DESIGN RULES FOR VARIABLE PARAMETERS THAT INFLUENCE THE APPARENT VISCOSITY AND PLASMA LAYER OF BLOOD

Variable parameters					Parameters influenced	
Haematocrit	Roughness (R_z)	Channel depth	Pressure/ velocity		Apparent viscosity	Plasma layer
42 %	1.8 µm	50 µm	high		low	thick
44 %	60 nm	100 µm	low		high	thin

6. CONCLUSIONS

The aim of this work was to analyse the blood flow behaviour in microchannels with variations in wall surface roughness. Special attention was paid to micromilled surfaces for applications in micromoulding.

The results of the experiments showed a significant drop in the apparent viscosity in the rough-surfaced microchannel compared to the smooth one. This effect seemed to increase with a smaller channel depth and with lower erythrocyte concentration (or haematocrit value). A low haematocrit value corresponds to a thick cell-free layer (Fig. 4); therefore it is possible to state that the effect of the rough surface on the apparent viscosity increases with the cell-free layer thickness.

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Figures captions

Fig. 1 Geometry of the channel within the device; Δp: differential pressure

Fig. 2 Simulation of channel flow from top to bottom in minus y-direction showing velocity vectors and contour velocity values. Fig. 2 a) shows full section and Fig. 2 b) shows the magnified bottom right corner

Fig. 3 Blood flow through the channel of the experimental device from the flow inlet (top left) to the outlet (bottom right); the single arrow (centre) highlights the presence of a separation of the bulk flow, likely caused by blood component agglutination at the flow inlet, which is indicative of laminar flow within the channel

Fig. 4 Variation of plasma layer thickness over channel diameter and for haematocrit values = 60, 45 and 20% (Sharan and Popel, 2001)