A Micro-fluidic System for the Evaluation of Blood Compatibility of Polymers

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Abstract: We present a new technique for the evaluation of polymer blood compatibility that makes use of a micro-channel array flow analyzer and we describe and characterize the flow dynamics of this instrument. The blood compatibility of four polymers is quantitatively and qualitatively assessed and the results discussed. The blood is allowed to flow through the channels of a polymer coated micro-fluidic chip under adjustable pressure. The chip surface is investigated using optical microscopy during the blood flow and by scanning electron microscopy afterwards. Polymers known for having good blood compatibility exhibited higher flow rate values. Platelets were observed adhering, aggregating and obstructing the channels of the chips coated with polymers known for having poor blood compatibility. This technique has remarkable qualities such as a small blood volume requirement for material tests (100 μL), tuneable flow regimes and the use of human blood.

Key Words: Blood, blood-compatibility, polymer, micro-fluidics, micro-channel array, platelet adhesion.

INTRODUCTION

The direct and indirect costs associated with cardiovascular diseases totaled $431.8 billion in 2007 in the US alone, where more than 6 million cardiovascular operations and procedures were performed during the year 2004 [1]. The selection of materials which exhibit optimal blood-compatibility properties for short and long term applications is one of the major challenges of biomedical engineering, and the demand for cost-effective materials which prevent clot formation when interfacing blood is high.

Polymers are versatile and relatively low cost materials and are commonly employed as biomaterials in biomedical and tissue engineering [2]. Their popularity is attributable to the possibility of synthesizing them in large volumes and of processing and shaping them easily; moreover, several strategies have been established for the functional modification of their surfaces [3]. For example, surface passivation *via* immobilization of biologically inert polymers such as polyethylene oxide [4,5] and polyethylene glycol [6,7] has been shown to minimize the interaction of proteins and cells with materials' surfaces. Alternatively, anticoagulant molecules (e.g. heparin) [8,9] or endothelial cells [10] have been immobilized on polymer surfaces to improve their hemocompatibility. The development of new blood-compatible materials has not, however, been accompanied by the establishment of standard and consistent methods for the assessment and evaluation of materials' hemocompatibility, in spite of guidelines for materials testing provided by the International Standard Organization (ISO) [11]. Nowadays most materials assessment techniques make use of flow chambers [12-15], which often require large volumes of blood samples, with important implications for the cost and feasibility of experiments. Micro-fluidic techniques have recently been shown to have great potential for producing cost effective, rapid and reliable analytical and sensing devices. One of their greatest advantages is their need for only small volumes of biological samples and reagents [16, 17].

In this work we present the use of a micro-fluidic technique to study the interaction of blood with polymers under controlled flow conditions. The surface of a micro-fluidic array chip is coated with the polymer under investigation and the blood is allowed to flow though its channels. Remarkably, the amount of blood required for testing each material is only 100 μL, the blood-material interfacing area being a few mm^2. The blood-compatibility of a polymer is quantified in terms of blood flow rate and duration through the chip.

MATERIALS AND METHODS

The Micro-Channel Array Flow Analyzer

The Micro-Channel Array Flow Analyzer (MC-FAN KH-3; Hitachi Haramachi Electronics Co. Ltd., Japan; Fig. **1**) is a micro-fluidic system devised to study blood-materials interaction under controlled flow conditions [18]. The main part of this system is a disposable micro-channel array chip which is placed inside the flow chamber. In this work we used silicon Bloody 6-7 chips (Hitachi Haramachi Electronics Co. Ltd., Japan) with a 20 nm thick silicon oxide layer at the interface with air. The chips were produced by micro-fabrication technology and their geometry (Fig. **2** and Table **1**) has been studied by the producer company to optimize their flow dynamic properties and therefore their performance.

The micro-fluidic chip is set inside the flow chamber in order to form an array of micro-channels at the boundary with the chamber glass window. The presence of the glass

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window allows observation of the channels during the experiments by means of an optical microscope equipped with a CCD camera (LCL-211H, Watec Co. Ltd., Japan). The flow chamber is held by an XY-stage equipped with micrometric screws to observe different chip areas with the camera. For each measurement, 100 µL of solution is poured into the central hole (reservoir) of the flow chamber. A tube is connected from the central hole of the chip to a volume flow sensor and filled with 0.9% NaCl solution. Another tube connects the flow chamber to a waste bottle and is controlled by a valve (v1 in Fig. **1**). When the valve is opened, the solution flows through the micro-channel array under a pressure determined by the difference in height between the flow sensor and the waste bottle, typically adjusted to 20 cm (1.96 kPa). The flow rate is measured by a stopwatch connected to the flow volume sensor.

The flow chamber is also connected to a 0.9% NaCl solution bottle through a pump. When the pump is activated and the valve v2 opened, the NaCl solution is pumped into the micro-channels of the chip at the pressure of 53 kPa and gathers into the waste bottle. This function is used to rinse the chip.

When blood flows through the channels of the microfluidic chip, the blood components interact with the channel walls' material. Typically some platelets are observed adhering and spreading over the chip surface. If the aggregation of the platelets is large enough to obstruct a channel, the total blood flow rate is reduced and this variation is measured by the volume flow sensor and the stop-watch. The instrument is therefore able to quantify the quality of the interaction of blood with the material's channels.

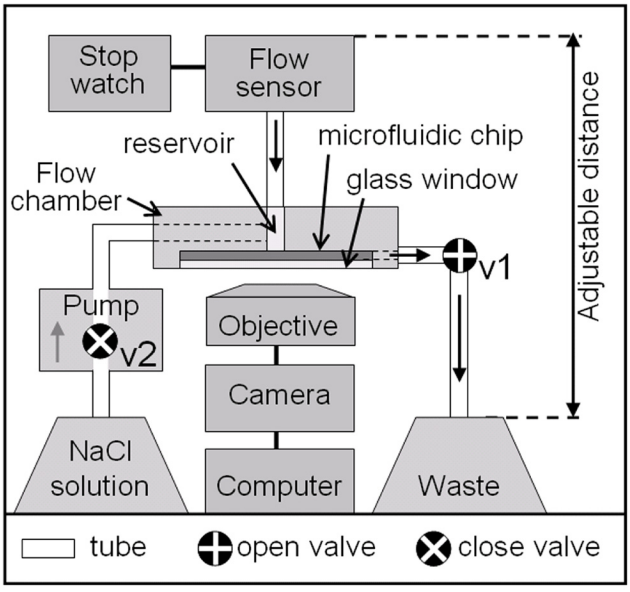

Fig. (1). Schematic of the Micro-Channel Array Flow Analyzer. The position of the valves refers to the configuration used to perform the flow volume measurements. The black arrows refer to the direction of the flow. The grey arrow inside the pump refers to the direction of the flow when the pump is activated and the valve v2 open.

Preparation of the Polymer Coated Micro-Fluidic Chips

The chip surface was cleaned by means of a 20 min bath in piranha solution (1:2 hydrogen peroxide : sulfuric acid by volume) at 120ºC and thoroughly rinsed with MilliQ water.

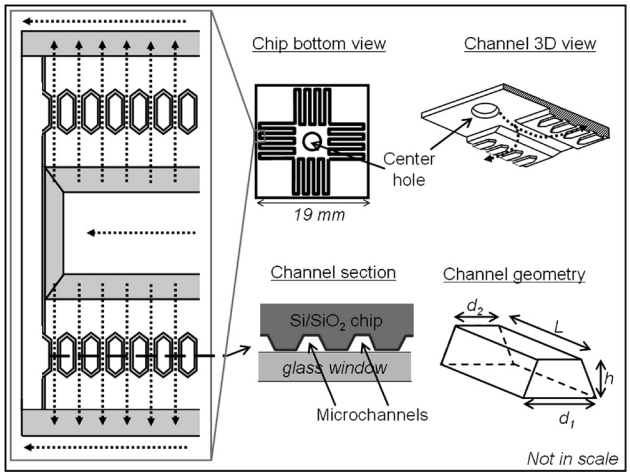

Fig. (2). Schematic of the micro-channel array chip (not in scale). The dotted lines in the inset indicate the flow direction. As shown in the section view, the channels are formed interfacing the chip and the flow chamber glass window. The channel geometry parameters are shown in Table **1**.

Table 1. Micro-Fluidic Chip Geometrical Details, with Reference to Fig. (2)

Chip Dimension	19 mm × 19 mm
Number of channels	8736
Channel length L	30 µm
Channel depth h	4.8 µm
Channel width d_1	10.5 µm
Channel width d_2	4.5 µm

Poly(styrene) (PS), Poly(methylmethacrylate) (PMMA) and Poly(vinylpyridine) (PVP) were purchased from Polymer Source (Canada). 2-methacryloyloxyethyl phosphorylcholine (MPC) was synthesized by the group of Prof. Kazuhiko Ishihara of Tokyo University (Japan). The polymer solutions were prepared by dissolving each polymer in an appropriate solvent (Table **2**), and stoked overnight. The next day, a thin film of the polymer under study was spin coated at 6000 rpm on freshly cleaned chips. The polymer coated chips were stored in MilliQ H_2O overnight and sonicated in a NaCl solution (0.9% NaCl in MilliQ water) for 1 minute before the micro-fluidic experiments.

The polymer film surface was investigated by optical microscopy prior to the measurements to assess the homogeneity of the coating. Identical sample surfaces were investigated by atomic force microscopy (AFM, SPI4000 E-Sweep, Seiko Instruments Inc., Japan); film thickness was evaluated by measuring the AFM section profile of a scratch made in the film utilizing Teflon tweezers.

Characterization of the MC-FAN Flow Dynamic

Clean bare silicon chips were used to characterize the flow behavior of the MC-FAN. 0.5%, 1.0%, 3.0%, 6.0% and 10% (w/v) dextran solutions ($M_w \sim 1.5 \cdot 10^5$, Wako pure

chemical industries Ltd, Japan) and 0.9% (w/v) NaCl solution in MilliQ water were used in these experiments. The viscosity of these solutions was measured at standard conditions by means of a cylinder viscometer (Thermo Haake – Visco Tester 6 L/R, Germany). In a first set of experiments the pressure difference of the MC-FAN was adjusted to 20 cm H_2O (1.96 kPa) and the flow rate of the dextran solutions having different viscosity was measured. A second set of experiments was performed using the 6% dextran solution which was allowed to flow through the chip channels under different pressures.

Table 2. Polymer Film Fabrication Experimental Parameters

Material	Molecular Weight	Solvent	Dilution (%)
SiO_2	-	-	-
MPC	30k	Ethanol	0.3
PVP	138k	Ethanol	0.3
PMMA	190k	Toluene	0.1
PS	220k	Toluene	0.1

Blood Compatibility Evaluation of the Polymer Films

The blood compatibility of the polymer films was evaluated at room temperature by performing micro-fluidic experiments using human whole blood. Blood was collected from a healthy person after informed consent. 1 mL of the collected blood was mixed with 1% (w/v) ethylenediaminetetracetic acid disodium salt (Dojindo Laboratories, Japan) in MilliQ H_2O for blood cell count using a particle counter (PCE-170, Erma Inc., Tokyo, Japan); the average number of platelets in the whole blood measured was $(2.6 \pm 0.3)\cdot 10^5/\mu L$. The blood collected for the micro-fluidic experiments was mixed with heparin solution to be 5 IU/mL. The measurements were performed within 30 minutes of blood collection. The pressure difference between the volume flow sensor and the waste bottle of the MC-FAN was adjusted to 20 cm H_2O (1.96 kPa). For each chip, the flow rate of the NaCl solution was first measured to evaluate the channel volume variation due to the chip fabrication process and the presence of the coated polymer film. Thereafter, the micro-fluidic experiments using human whole blood were performed. The blood flow rate was measured on a bare silicon chip (reference material) before each experiment performed with a polymer coated chip, to monitor the blood coagulation and select consistent results for the data analysis.

After each measurement performed using blood, the chip surface was investigated by optical microscopy and typically some platelets were seen to adhere on the material's surface. The strength of platelet adhesion was qualitatively evaluated by flowing NaCl solution through the micro-channels, under the high pressure of 53 kPa.

Platelet Fixation

After the micro-fluidic measurements with whole blood, the polymer coated chips were removed from the flow chamber and washed three times in 1% Dulbecco's Phosphate Buffered Saline without calcium and magnesium (PBS(-), Nissui Pharmaceutical Co. Ltd., Japan) and shaken, to remove the loosely adhered cells and platelets. The chips were then stored at 4ºC in a 1% gluteraldehyde (Electron Microscopy Science, PA, USA) in H_2O solution for about four hours, rinsed with MilliQ H_2O, dehydrated *via* successive incubations in H_2O/ethanol mixtures with increasing ethanol content (this step was not performed in the case of PVP and MPC polymer coated chips, alternatively a suitable dehydrating agent was used) and stored at 4 ºC. The chips were gold coated before investigation by Scanning Electron Microscopy (SEM, S-4800, Hitachi, Japan).

This study was approved by the Ethics Committee of NIMS.

RESULTS AND DISCUSSION

Characterization of the Fluid Dynamics of the System

The Poiseuille's equation describes the behavior of a Newtonian fluid flowing through a uniform straight pipe having radius R and length L:

$$\dot{Q} = \frac{\Delta V}{\Delta \tau} = \frac{\pi \cdot R^4 \cdot \Delta p}{8 \cdot L \cdot \eta} \qquad (1)$$

where $\dot{Q}$ is the flow rate, ΔV is the volume of fluid that flows in the time $\Delta\tau$, Δp is the pressure difference between the two ends of the pipe and η is the fluid viscosity. The flow is laminar and the velocity profile of the fluid is a parabola (velocity null at the pipe walls). The flow rate $\dot{Q}$ is therefore proportional to the difference of the pressure between the two ends of the pipe and the inverse of the viscosity of the fluid [19].

In analogy with Poiseuille's equation, equation (2) describes the behavior of a fluid that flows through the MC-FAN chip:

$$\dot{Q} = \frac{\Delta V}{\Delta \tau} = \frac{\pi \cdot G \cdot n_c(\tau) \cdot \Delta p}{8 \cdot \eta} \qquad (2)$$

where G is a geometrical factor characteristic of the chip, n_c is the number of open channels of the chip at the time τ *and* Δp is in this case the difference of pressure applied to the system.

The number of channels n_c is normally a constant. When using blood however, the platelets may adhere to the channel walls and obstruct the channels, blocking the blood flow. If these phenomena occur, the number of open channels decreases as the time τ increases. This means that the blood flow rate measured by the MC-FAN depends on the nature of the interaction between the channel walls' material and the blood.

The first phenomenon that occurs when the blood contacts an artificial material is the adsorption of the plasma proteins on its surface and their conformational change. Subsequently, platelets bind the proteins at the surface, eventually adhere and spread over the protein layer, get activated and release coagulation factors which initiate a cascade of reactions leading to the formation of thrombi [2]. Platelet adhesion is therefore able to provide indications of the blood compatibility of materials. The blood flow rate measured by

the MC-FAN depends on platelet adhesion and is therefore used to asses the hemocompatibility of the polymers coating the micro-fluidic chip surface.

The shear rate is an important parameter to take into account when studying blood behavior. It is well known, in fact, that plasma protein behavior and platelet activation is influenced by the shear rate they undergo [20-22]. Typical physiological values of the blood shear rate in the neighborhood of the blood vessel wall are 100 s^{-1} to 2000 s^{-1} in large and small arteries and 20 s^{-1} to 200 s^{-1} in large and small veins. For these high values of shear rate, normal blood can be treated as a Newtonian fluid [19]. In the case of the MC-FAN, the shear rate at the channel walls γ is related to $\dot{Q}$ by the equation:

$$\gamma = \frac{6\dot{Q}}{wh^2 n_c} \qquad (3)$$

where the expression is calculated, for simplicity, for a micro-channel having a rectangular section with height h and width w.

The experiments performed with the dextran solutions aimed to characterize the flow dynamics of the MC-FAN and validate equation (2). Dextran was chosen because it is widely characterized, soluble in water and able to provide solutions with different viscosities by changing its concentration. Moreover, a 6% dextran solution exhibited a viscosity value close to that of human blood (~ 4.4 mPa·s) as shown in Table **3**. Therefore, we considered it a suitable compound to model the flow behavior of blood in the MC-FAN.

The flow rate of the dextran solutions was measured with the MC-FAN under different settings of parameters such as the pressure exerted on the liquid and the solution viscosity. Fig. (**3a**) shows the volume flow rate of dextran solutions having different viscosity η, through the micro-fluidic channels, under a pressure difference of 1.96 kPa (20 cm H_2O).

For simplicity, equation (2) was considered the first order approximation of the flow behavior of the dextran solutions and G was calculated utilizing it. A linear fit of each data set in Fig. (**3a**) was computed and the results are summarized in Table **3**. In Fig. (**3b**) the flow rates resulting from the fits of each data-set in Fig. (**3a**) were plotted against the inverse of the viscosity ($1/\eta$) of the solutions. Using equation (2), the data were linearly fitted and the resulting flow rate was proportional to $1/\eta$ for a factor (14 ± 2) µL·mPa, giving a geometrical factor G=(1.9 ± 0.3) µm^3.

The flow rate does not exhibit purely linear behavior versus 1/η and is therefore not in full agreement with equation (2). Two flow regimes can be identified, one for solutions having viscosity below ~3 mPa·s and one for those above this value, the reasons for which are not fully understood. One possible explanation is that the dextran solutions do not behave like Newtonian fluids for all concentrations. Dextran solutions have been observed to deviate from a Newtonian behavior depending on the dextran molecular weight and concentration, as well as producer company [23].

In a second set of measurements the flow rate of a dextran solution having viscosity η = (4.64 ± 0.02) mPa·s was measured for different pressure settings. The results are shown in Fig. (**4a**), where the dashed lines are linear fits of the data-sets relative to each pressure. The flow rates resulting from the fits of Fig. (**4a**) (see Table **4**) are plotted in Fig. (**4b**) against the pressure used for each experiment. Equation (2) was again considered a first order approximation of the flow behavior of the dextran solution and the data were linearly fitted. The flow rate resulted proportional to Δp for a factor (9.3 ± 0.2)·10^{-4} µL/Pa·s. Substituting this value in equation (2), the resulting geometrical factor was G=(1.62 ± 0.04) µm^3. Table **4** shows also the values of the shear rate computed using equation (3) for the different pressure settings, where the average channel width w has been considered for the calculation (i.e. w=(d_1+d_2)/2=7.5 µm).

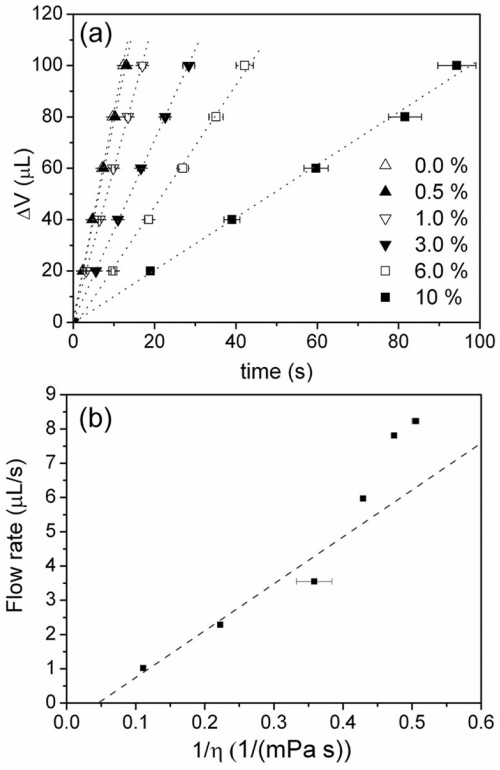

Fig. (3). (**a**) Volume flow rate of dextran solutions having different concentrations and viscosities through the micro-fluidic channels, under a pressure difference of 20 cm H_2O (1.96 kPa) and relative linear fits of the data-sets. (**b**) Flow rates resulting from the fits against the inverse of the viscosity of the dextran solutions and relative linear fit.

Table 3. Viscosity and Flow Rate Measurements of the Dextran Solutions (Pressure: 1.96 kPa)

DextranA (%, m/v)	Viscosity η (mPa·s)	Flow rate $\dot{Q}$ (µL/s)
0	1.94 ± 0.02	8.10 ± 0.11
0.5	2.11 ± 0.01	7.66 ± 0.13
1.0	2.33 ± 0.01	5.87 ± 0.08
3.0	2.8 ± 0.2	3.52 ± 0.03
6.0	4.50 ± 0.01	2.37 ± 0.06
10	9.01 ± 0.01	1.03 ± 0.03

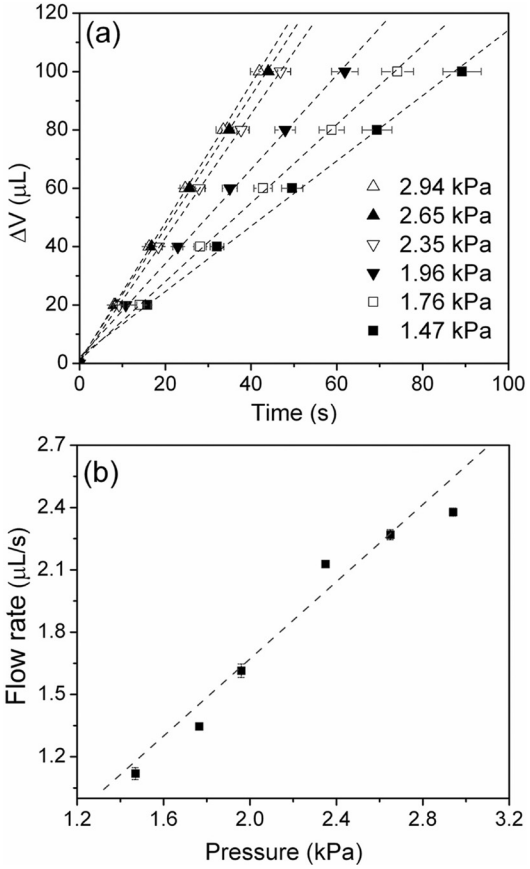

Fig. (4). (**a**) Volume flow rate of a dextran solutions through the micro-fluidic channels under different pressure settings and relative linear fits of the data-sets. (**b**) Flow rate resulting from the fits against the pressure used during the measurement and relative fit.

Table 4. Measurements of the Flow Rate and the Average Shear rate Close to the Channel Walls of a Dextran Solution (Viscosity: (4.64 ± 0.02) mPa·s)

Pressare (H_2Ocm)	Pressare (kPa)	Flow rate $\dot{Q}$ (μL/s)	Shear rate (s^{-1})
30	2.94	2.38 ± 0.02	9460 ± 80
27	2.65	2.27 ± 0.02	9060 ± 80
24	2.35	2.13 ± 0.01	8460 ± 40
20	1.96	1.61 ± 0.03	6400 ± 100
18	1.76	1.35 ± 0.02	5350 ± 70
15	1.47	1.12 ± 0.03	4500 ± 100

The weighted average geometrical factor resulting from the two experiments described in Fig. (**3** and **4**) is G_a= (1.65 ± 0.07) μm^3. Comparing equations (1) and (2), it can be figured that the radius of a straight cylindrical pipe having the same geometrical factor G and length L of the micro-fluidic chip channels is R=(2.65 ± 0.03) μm. The hydraulic radius R_h of a channel is a useful parameter when dealing with channels having a non-circular section and is defined as $R_h=2A/p_w$, where A is the section of the channel and p_w is the wetted perimeter. In the case of a cylindrical pipe, the hydraulic radius coincides with the pipe radius. The hydraulic radius of the wedge-shaped micro-fluidic chip channels is R_h=2.74 μm. This value is compatible with R within a 4% approximation. In analogy to equation (1), equation (2) can be therefore approximated to:

$$\dot{Q} = \frac{\Delta V}{\Delta \tau} = \frac{\pi \cdot R_h^{\,4} \cdot n_c(\tau) \cdot \Delta p}{8 \cdot L \cdot \eta} \qquad (4)$$

where the radius R has been replaced by the hydraulic radius R_h of the channels.

These results suggest that the flow of Newtonian fluids through the MC-FAN can be approximated as laminar. This observation is consistent with the low values of Reynolds numbers *Re* that characterize this system:

$$Re = 4 \cdot \frac{\rho \cdot v \cdot R_h}{n_c \cdot \eta} \qquad (5)$$

where v is the average flow velocity of the solution through the micro-fluidic chip ($v=\dot{Q}/A$) and ρ the solution density. For example, using the values measured for the water solution and shown in Table **3** (0% dextran), $Re\sim 0.14$.

Polymer Film Investigation

The polymer films spin coated on the chip surfaces appeared homogeneous under investigation by optical microscopy. AFM topography measurements were performed on the flat areas of the chips coated with the different polymers and the measured film roughness exhibited typical root mean square (RMS) values below 10 nm. AFM section profile measurements were used to evaluate film thicknesses which exhibited typical values below 50 nm depending on the polymer. The polymer film thickness was also evaluated using the MC-FAN, as described below.

Blood-Compatibility Assay

The MC-FAN was used to characterize the interaction of blood with different kinds of polymers under flow conditions. Using a fluidic technique we obtained an impression of the interaction of blood with materials consistent with the physiological phenomena. Using flow conditions allows, in fact, the simulation of a number of physiological processes important in assessing blood interaction with surfaces. For example, it has been demonstrated that translocating platelets undergo a series of morphological changes in response to increasing fluid shear stress [24], while the activation of plasma proteins such as von Willebrand factors depends on the shear stress they experience [20-22].

A set of micro-fluidic chips coated with 4 different polymers was produced as described previously. The polymers were well known to have a different hemocompatibility (i.e. MPC exhibits good blood compatibility, on the contrary to PS). The silica surface of the chip was used as a reference material.

Before every measurement using blood, changes of the channel geometry due to the presence of the polymer films were tested measuring the flow rate of NaCl solutions. This test was performed also on uncoated chips to evaluate possible defects introduced by the manufacturing process. Fig.

(**5a**) shows the time necessary for 100 μL of NaCl solution to flow through the channels of the chips coated with different polymers ($\Delta\tau_{NaCl}(100\mu L)$). $\Delta\tau_{NaCl}(100\mu L)$ values lower than that of the bare chip relate to the thickness of the polymer films, which depends on the kind of polymer, its molecular weight, solubility in the solvent used to dilute it and experimental parameters. $\Delta\tau_{NaCl}(100\mu L)$ values higher than that of the bare chip might indicate the presence of partial channel obstructions. The error bars represent the statistical variation that was observed repeating the measurements on a number of micro-fluidic chips. The values are consistent within two standard deviations. $\Delta\tau_{NaCl}$ was measured for each individual chip and used to normalize the following measurement performed utilizing blood. The chip was not used for the blood test if its $\Delta\tau_{NaCl}(100\mu L)$ was not consistent within two standard deviations with that measured on a bare silicon chip.

Fig. (**5b**) shows the flow rate of blood flowing through the micro-fluidic chips, which were uncoated and coated with 4 different polymers. Dissimilarly from the measurements performed with dextran solutions, the blood flow rate is not constant during each single measurement, but decreases in time in the case of the chips coated with PVP, PMMA and PS. The data in Fig. (**5b**) were fitted to calculate the blood flow rate; the fit was computed in the time interval $0 < \tau < 40$s for the data relative to PVP, PMMA and PS. The results of the fits are shown in Table **5**, together with the shear rate computed in the same time intervals and the $\Delta\tau(100\mu L)$ values. Each polymer surface exhibits a different blood flow rate and a different $\Delta\tau(100\mu L)$.

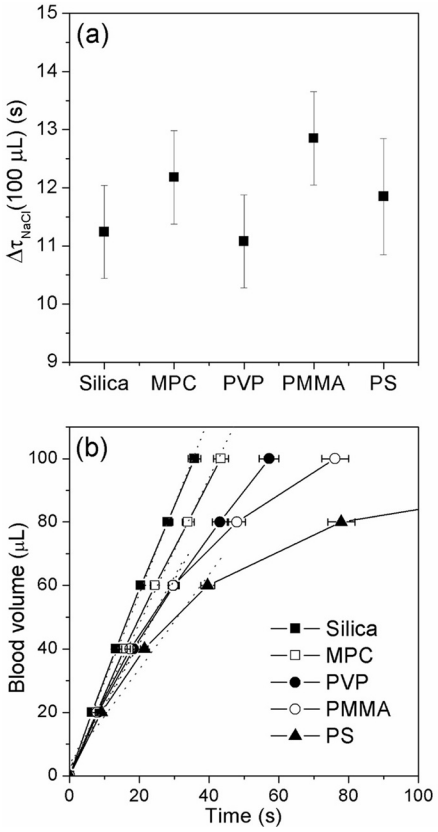

Fig. (5). (**a**) Measurement of $\Delta\tau$ for 100 μL of NaCl solution flowing through the micro-fluidic chips, uncoated (Silica) and coated with different polymers. (**b**) Volume flow rate of blood through the micro-fluidic channels of a MC-FAN (Pressure: 1.96 kPa).

Table 5. Measurements of the Blood Flow Rate and the Average Shear Rate in Proximity of the Channel Walls of the Micro-Fluidic Chips, Uncoated (Silica) and Coated with Different Polymers (Pressure: 1.96 kPa)

Material	$\Delta\tau(100\mu L)$ (s)	Flow Rate $\dot{Q}$ (μL/s)	Shear Rate (s^{-1})
Silica	35 ± 2	2.78 ± 0.05	9900 ± 200
MPC	43 ± 2	2.30 ± 0.05	8200 ± 200
PVP	57 ± 3	1.99 ± 0.11[(*)]	7100 ± 400
PMMA	76 ± 4	2.01 ± 0.15[(*)]	7200 ± 500
PS	190 ± 10	1.49 ± 0.16[(*)]	5300 ± 600

[(*)] The fit was computed for $0 \text{ s} \leq \Delta\tau \leq 40$ s.

The shear rate values shown in Table **5** are higher than those commonly measured in the human body. The analysis of blood-materials interaction performed in this work refers therefore to conditions of high shear rates. However, as seen in the case of Table **4**, our set up allows the control of the pressure applied on the blood and this can be reduced to physiological values. The flexibility of the set up results in the possibility of simulating different physiological states, obtaining an extensive characterization of the materials used to contact blood.

Channel obstruction caused by platelet aggregations was qualitatively investigated by optical microscopy and SEM. For each of the four polymers, Fig. (**6**) shows the optical images of a portion of the coated micro-fluidic chip at three different moments during the blood flow (between 10 and 12 micro-channels are visible in each image). MPC is well known for having high blood-compatibility and is the polymer exhibiting the highest flow rate in Table **5**. During the entire blood flow, no platelet is seen to adhere on its surface (Fig. **6a**). On the contrary, PS is known to have poor blood compatibility. Fig. (**5b**) shows that the blood flow rate through the PS coated micro-fluidic chip is significantly reduced after 40 s. This is due to the formation of platelet aggregations obstructing the micro-fluidic channels, as Fig. (**6b**) shows: the platelets are seen to adhere to the PS film during the blood flow and progressively close a number of channels, the dashed arrows indicating the course of the flow. In some cases, the platelets form large aggregates loosely anchored to the surface. This is what we observe on the PVP surface in Fig. (**6c**): some platelets are seen to adhere to the polymer surface. The chip is rinsed with a high pressure (53 kPa) of NaCl solution at the end of the blood flow to test qualitatively the strength of platelet adhesion to the surface. The platelet aggregations are easily displaced and the surface of the PVP film appears almost completely clean after the rinsing step. This is not the case, for example, with PMMA, as shown in Fig. (**6d**): after rinsing, many platelets still adhere to the surface.

After rinsing, the polymer coated chips can be removed from the MC-FAN and further investigation of the biological samples can be performed. For example, polymer coated chips were investigated using SEM after the blood flow measurements performed with the MC-FAN.

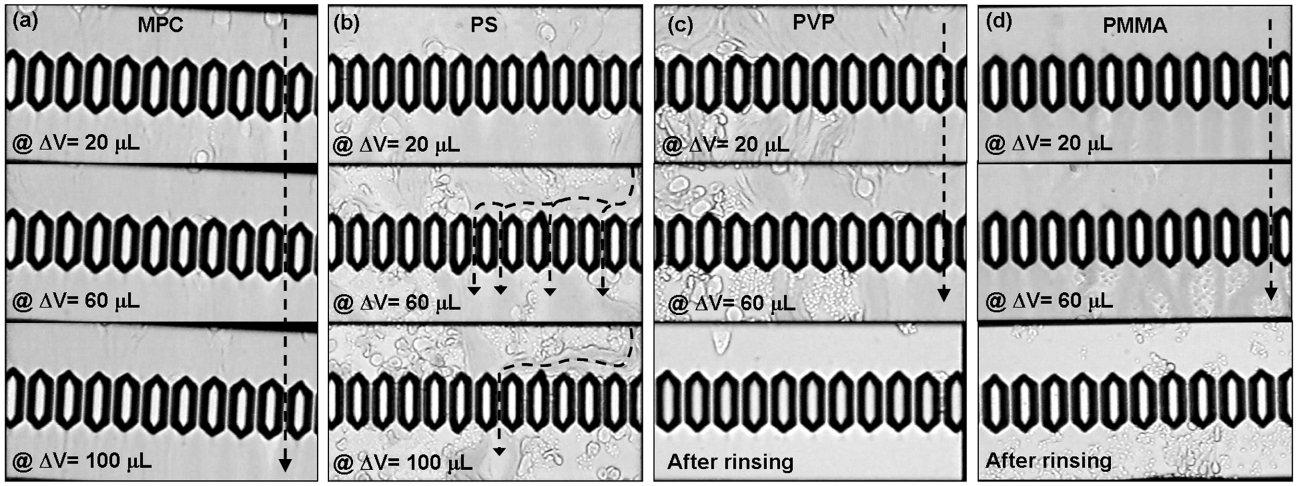

Fig. (6). Optical microscopy images of a portion of the polymer coated micro-fluidic chip surfaces during the blood flow rate measurements with the MC-FAN. Coatings: (**a**) MPC, (**b**) PS, (**c**) PVP, (**d**) PMMA. The dashed arrows indicate the direction of the blood flow. The arrows evidence the closure of three channels in the case of the PS films. Channel length: 30 μm.

Fig. (**7**) shows 3 channels of a PMMA coated chip. An aggregation of platelets obstructing the central channel is clearly visible. Similar aggregations were observed along the channels of the micro-fluidic chips coated with polymers having poor blood compatibility.

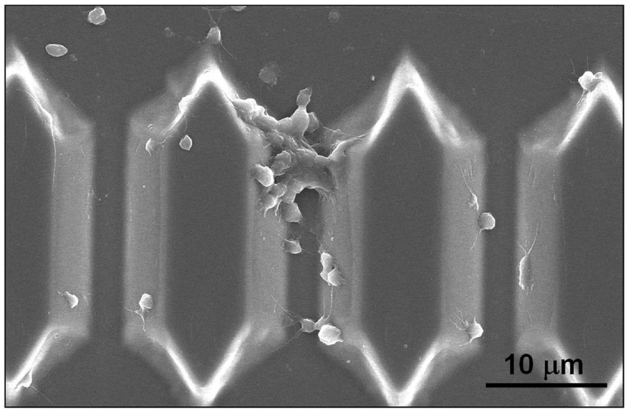

Fig. (7). SEM image of a platelet aggregation which close one micro-channel of a chip coated with PMMA.

CONCLUSIONS

We have presented a micro-fluidic technique for the evaluation of the blood compatibility of materials and we have performed explicative examples using different polymers well known for having different qualities of hemocompatibility.

The blood-materials interaction was qualitatively and quantitatively assessed by means of:

- Measurement of the flow rate of the blood through the micro-fluidic chip. In general, the higher the value, the better the hemocompatibility of the material.
- Measurement of the time required for 100 μL of blood to flow through the micro-fluidic chip ($\Delta\tau(100\mu L)$), preferred when the blood flow rate changes considerably during the flow. In general, the lower the value, the better the hemocompatibility of the material.
- Optical investigation of the chip surface during and after the blood flow. The optical set-up allows observation of the platelets (as well as red and white cells) flowing, adhering to the materials surface and aggregating.
- Qualitative evaluation of platelet adhesion strength to the material's surface.

The MC-FAN possesses significant assets for use in evaluating the blood compatibility of materials:

- Each material test requires a reduce volume of blood, only 100 μL.
- All the measurements are performed under controlled flow conditions.
- Human whole blood can be used instead of animal blood which possibly responds differently to artificial materials because of differences between the species.
- The use of a CCD camera allows visual observation of platelet interaction with the materials and thrombus formation evolution.
- Every measurement is an average of thousands of events, every chip having 8736 channels.

The MC-FAN is therefore an effective *in vitro* technique able to provide rapid first-stage screening of polymer materials to be used in cardiovascular applications and have the potential of reducing the costs, the long times and the sacrifice of animals required by *in vivo* experiments.

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