Effect of artificial surface electric charge on laminar flow shear-induced single platelet loss

A.T. Shawaqfeh and T.M. Alkhamis
Faculty of Eng., Mutah University, Mutah, P.O. Box 7, Karak 61710, Jordan
e-mail: alkhamis@ahu.edu.jo , talkhamis2000@yahoo.com

This study is designed to investigate the effect of electric charge associated with artificial surface on single platelet loss when known shear stress is applied. A capillary flow system is designed in a form of electric capacitor for the purpose of electric charge separation. Either positive or negative charge is applied to the walls of capillary tubes and the system is pressurized to obtain known wall shear stress. Platelets are then counted in sheared and unsheared samples and single platelet loss is then calculated. Charged surfaces are compared to neutral ones, and the effect of varying the electric voltage is also studied. Results indicate that application of an electric charge to a blood-contacting surface modifies it with respect to single platelet loss. Positive charged surfaces showed the best results compared to negative charged surfaces, which come next, and neutral surfaces, which are the worst. Applying positive charge to copper surface reduced single platelet loss as much as 54% relative to neutral surfaces, whereas negative charge reduced single platelet loss by 23%. It is also concluded that optimum voltage range is up to 22 V after which neutral surfaces become better than charged ones. Moreover, very low voltages are seen to cause more damage than neutral surfaces. Therefore, design of blood-contacting surfaces under shear must take into account the type of the electric charge associated with the surface.

Keywords: Platelet, Shear-induced, Artificial, Surfaces, Laminar

1. Introduction

The need for prosthetic devices is growing very fast. A large number of cardiovascular procedures is conducted all over the world and these involve: Cardiac catheterization, peripheral vascular procedures and open heart surgery [1-2]. Not only these procedures require short blood foreign surface contact, but many other cases such as artificial kidney, artificial lung (blood oxygenators), artificial arteries, and artificial heart, require long term continuous contact with surfaces accompanied with application of huge shear forces. Some of these surfaces show less potential to blood damage than others [3-5]. Blood damage is usually defined as adhesion and aggregation of platelets and is related to the type of surface and bulk flows which lead to thrombus formation that is vital to human life.

Many previous studies have dealt with either surface effect, or bulk flow effect, or a combination of both on Single Platelet Loss (SPL) [6-11]. Shear forces and surface effects were proved to dominate over any other effect [3,12]. All events of reported blood damage were shown to be surface controlled [3,4,13]. Even the initial stages of platelet adhesion, which require adsorption of plasma proteins, were observed to be controlled by the surface [14]. Bulk effects were seen to be important in hemolysis, which is defined as release of hemoglobin and associated matter under shear from Red Blood Cells (RBCs) [3].

Previous surface studies dealt mainly with comparison between the effects of known biomaterials on platelet adhesion [15-18]. Some studies dealt with correlating contact angles with spreading of platelets and consequently their loss from the bulk [19]. However, the effect of surface charge distribution was neither studied nor correlated to platelet loss. Charge distribution is physiologically important since electric charges occur naturally on the lining of endothelial surfaces. Negative charge is a characteristic of all cell membranes and the interior walls of blood conduits. One of the

criteria of red blood cells substitute production is to charge their membranes with negative charge [19-20]. Therefore, any biomaterial or blood-contacting surface must be recognized by its type of charge as one parameter that may be correlated with blood damage as it flows past this surface.

In this study, an experimental system is designed to stimulate electric charges in blood conduits. This system is in a form of an electric capacitor, which separates positive charges from negative charges on its two plates. One of the plates is an aluminum sheet while the other is the capillary tube that contains blood. The system is tested with real blood flow and the effect of positive and negative charges on Single Platelet Loss (SPL) is measured.

2. Methodology

2.1. Experimental system design

2.1.1. Capacitor

The system that is used to simulate blood conduits electric charge can be characterized as a capacitor. Its components are shown in fig. 1, and is composed of an aluminum sheet of 100 cm x 70 cm that is adhered to a wooden board of the same dimensions and contained within a metal frame of 74 cm x 104 cm. The aluminum sheet is then covered by a plastic sheet of 100 cm x 70 cm, which acts as an insulating material between the metal sheets of the capacitor. The other metal sheet of the capacitor is made of copper capillary tubes that are routed along paths and are separated

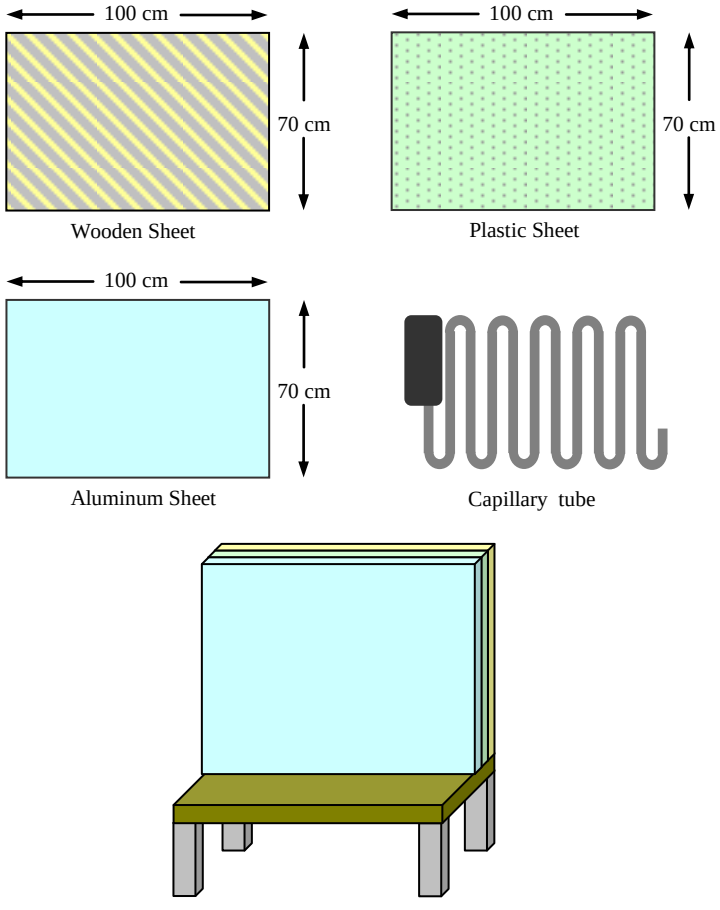


Fig. 1. Parts of the capacitor with their dimensions.

by enough distance, *i.e.*, 5 cm to avoid any possible charge interference between lines of the capillary tubes. A total number of 19 nails at each side were enough to distribute all types of capillary tubes used in the study. Copper is used in this study as a commercial surface, which is expected to have significant effect on SPL for better differentiation between the effects of surface electric charge. During operation, one type of charge will reside in the aluminum sheet; while the other type of charge will reside in the copper tube. Therefore, the inner surface of the tube, which is in contact with the blood, will be charged with a single electric charge.

2.1.2. Experimental setup

The capacitor described above formed the major part of the experimental setup. The complete setup of the experimental system is shown in fig. 2. The entrance of the capillary tube is connected to a small cylinder that is also made of copper and eventually is used as a reservoir to store blood before initiation of shearing experiments. Different sizes of the reservoirs were used in conjunction with different capillary diameters, to make sure that enough blood volume is used for every experiment. The reservoir is then completed to a pressure gage, which regulates flow of nitrogen from a nitrogen cylinder to the blood reservoir at a required pressure, from which wall shear stress can be calculated. Nitrogen is used to apply the required pressure because it is considered an inert gas with respect to blood [21]. A DC power supply (Farnell instrument LTD, Yorkshire, U.K.) is used as a source of the required voltage to the plates of the capacitor. Its negative and positive leads are connected to the plates of the capacitor as required by the procedure (i.e.) if a negative charge is needed on the capillary side then the power supply negative lead is connected to it and vice versa.

2.1.3. Lengths of capillary tubes

To assume fully developed flow of blood and to avoid entrance and exit effects on the flow of blood within the system with respect to the imposed pressure gradient, a large L (length) to D (diameter) ratio is used. The minimum ratio was obtained according to the relation:

$$L/D = 6000\,, \tag{1}$$

which was verified and discussed elsewhere [22]. For the different capillary sizes used, the minimum lengths are shown in table 1.

Ref. [23] described the entrance length L_e necessary for development of centerline velocity to within 99% of its fully developed value in a circular cross section for Reynolds number range of: $Re<1$, according to the equation:

$$L_e/D = 0.059 + 0.056\,Re\,. \tag{2}$$

This experimental study predicted values of L_e/D to be less than 0.6, which makes L_e of negligible values relative to the capillary tubes lengths.

2.1.4. Pressure-wall shear stress-shear rate correlations and mean residence time calculations

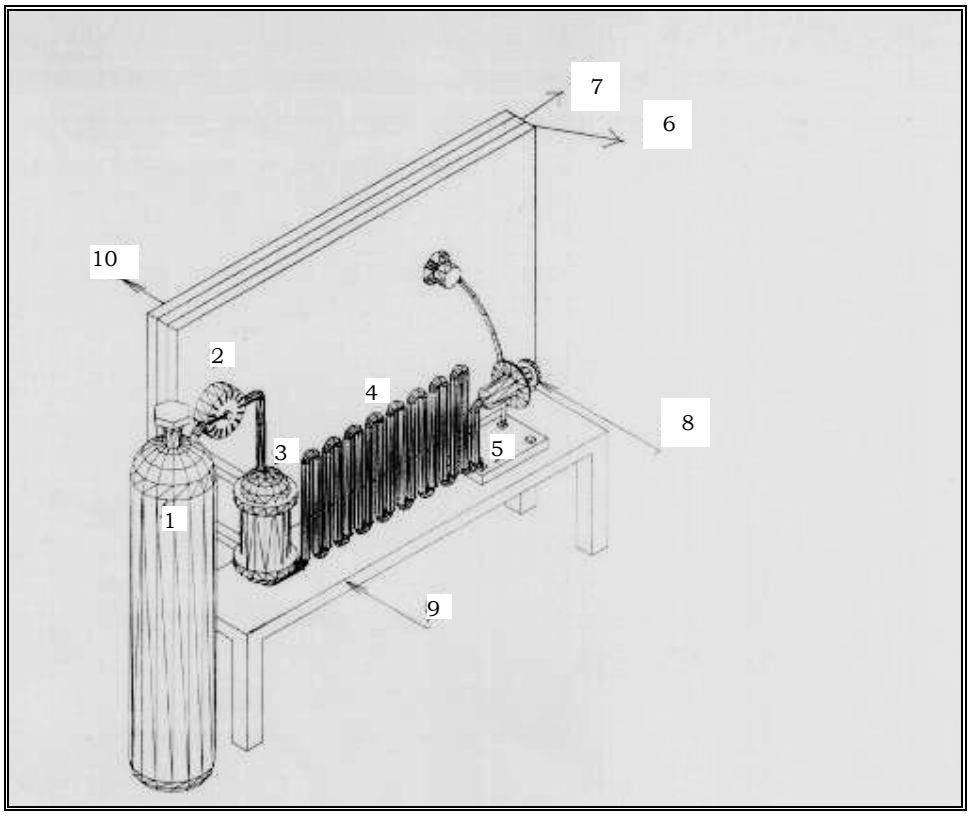
The pressure regulator was connected to the nitrogen line that connects the nitrogen cylinder to the blood reservoir. Wall shear stress (τ_R) for every specified pressure drop (ΔP), radius (R) and length (L) was calculated using the equation:

$$\tau_R = \frac{\Delta P \times R}{2L}\,. \tag{3}$$

The corresponding shear was determined using an asymptotic blood viscosity (μ) value of 3.5 cp. Blood in the range of wall shear stress studied can be considered a Newtonian fluid [3]. Wall shear rate (γ_R), therefore, was obtained using the equation:

$$\gamma_R = \frac{\tau_R}{\mu}\,. \tag{4}$$

Shear rate-shear stress values were obtained using eq. (4) above and the required units. The results are seen in table 2. Residence time ($\bar{t}$), which is defined as the time required to process one volume of the



1. Nitrogen cylinder 2. Pressure regulator
3. Blood reservoir 4. Capillary tubes (the first side of the capacitor)
5. Power supply 6. Plastic sheet (insulator)
7. Aluminum sheet (the second side of the capacitor) 8. Syringe pump system
9. Stand 10. Wood frame

Fig. 2. Experimental setup.

Table 1
Minimum lengths of capillary tubes allowed for minimizing end effects

Tube diameter (mm)	Minimum length (m)
1.00	6.0
1.25	7.5
1.50	9.0

Table 2
Wall shear rate values corresponding to applied wall shear stress

Wall shear stress (Pa)	Wall shear rate (s^{-1})
7.5	2142
10.0	2857
20.0	5714
24.5	7000
30.0	8571
33.3	9514

capillary tube and is obtained by dividing V (volume of the tube) over Q_{av} (average flow rate of blood in the tube). Substitution of the specific expressions for the two terms will result in the following equation:

$$\bar{t} = \frac{32 \times L^2 \times \mu}{\Delta P \times D^2}. \quad (5)$$

2.2. Blood samples

Blood was obtained from healthy donors who fasted over night. Donors were asked if they had taken any medications, such as aspirin (ASA), at least 14 days prior to donation. Only those who took no medications, as mentioned above, were considered for dona-

tion. Donors were told that their blood is being used for medical research. Blood samples were collected in plastic bags containing Citrate Phosphate Dextrose Adenine (CPDA) as an anti-coagulant. Blood samples were transferred within 15 minutes and were tested within two hours of donation.

2.3. Experimental procedure

The experimental system was cleaned by distilled water followed by rinsing with 1% saline solution. The blood sample was charged into the clean blood reservoir by slow gravity flow through blood bag tubing. For each test, a standard sample was withdrawn to be as a reference for comparison with sheared samples. The gas cylinder was then connected to the reservoir and the system was pressurized to the prescribed level. After shearing, few milliliters were discarded, and then 4 milliliters of sheared blood were collected for platelet loss determination. Finally, platelets were counted in sheared and unsheared samples, and then single platelet loss (*SPL*) was determined using the equation:

$$SPL(\%) = \left[\frac{(PCST - PCSH)}{PCST}\right] \times 100, \qquad (6)$$

where *PCST* represents platelet count in standard sample and *PCSH* represents platelet count in sheared sample.

3. Results and discussion

The system that used proved to be adequate for testing the effect of mechanical stresses combined with electric charge on blood damage. Results on blood viscosity as a function of wall shear rate are compared with results from cone-and-plate viscometer and are shown in fig. 3. The flow of blood within the shear rate range studied is apparently Newtonian. Values of asymptotic blood viscosity obtained from the capillary viscometer flow and depicted in the figure agree up to more than 94% with those obtained using cone-and-plate viscometer. The shear rate within the gap of the cone-and-plate device is known to be constant; while it varies along the radius of the capillary and it has its maximum value at the surface. Therefore, the small difference in viscosity values obtained indicates that the system is justified to be used in application of the required mechanical stresses on blood flowing within it.

Fig. 4 shows single platelet loss % (*SPL*), the parameter that was chosen to represent the effect resulting from the application of mechanical stresses or electric charge, versus wall shear stress. It is evident that blood damage, through platelet adhesion at the surface of aggregation in the bulk, occurs mainly at relatively low stresses (i.e.) 7.5 Pa. Asymptotic value is reached at higher shear stresses (20-35 Pa). Therefore, only platelet adhesion seems to be the controlling factor in this range of shear stresses, and no significant blood damage is evident. These results agree very well with the work of others using different devices [3].

Fig. 5 shows the results obtained on *SPL* as a function of wall shear stress when different electric charges are applied to the surface of the capillary tube. Neutral surface showed the highest platelet loss, which indicates that at the voltage used (10 *V*) application of any type of electric charge to the surface improves the quality of the surface with respect to blood compatibility. However, negative surface charge showed higher *SPL* than positive charges. In principle, this may not be possible if the platelets only come into contact with the artificial surface, since negative charges are associated with living cells membranes [24]. Therefore, this may indicate that intermediate material diffuses prior to any platelet contact and changes the electric nature of the synthetic surface. Plasma proteins are known to adhere to non-endothelial surfaces and their diffusion is augmented by the presence of larger particles such as platelets and red blood cells [8,9,14,16]. Moreover, the modified surface with different electric characteristics is now acting as a new substrate for platelet contact. This needs further research to explain the exact mechanism of platelet adhesion to electrically charged synthetic surfaces in the presence of plasma proteins. The figure indicates that the lowest damage is seen to be

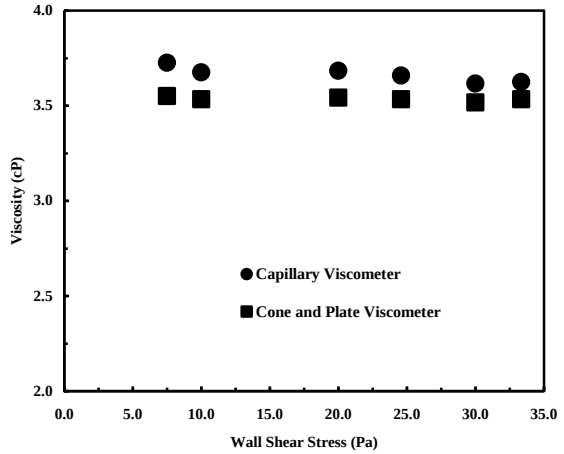


Fig. 3. Viscosity of blood (cP) versus wall shear stress in capillary viscometer and shear stress in cone-and-plate viscometer.

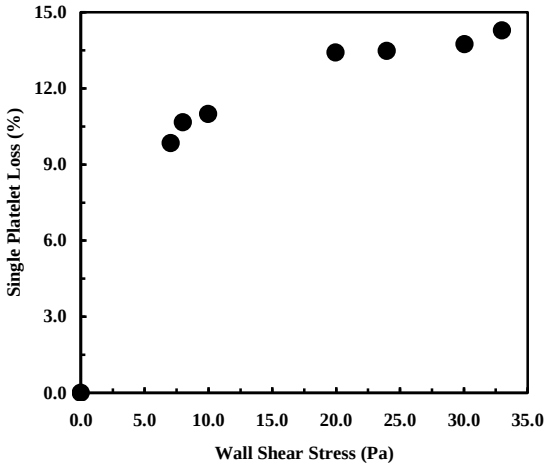


Fig. 4. Effect of wall shear stress (Pa) on single platelet loss (%) in capillary viscometer.

associated with applying positive charge to the surface.

If we introduce a damage index defined as shear-averaged SPL and compare it for the three surface types, we obtain a 13% average for the neutral surface, 10% for the negative, and 6% for the positive surface. This indicates that the extent of blood damage is reduced by about 23% when a negative charge is applied to the neutral non-endothelial surface and by 54% when a positive charge is applied to the surface. This result is considered to be significant in modification of commercial surfaces for applications in blood handling equipment or artificial organs applications.

The effect of increasing the voltage that is applied to the capacitor and hence increasing the electric charges on its plates is shown fig. 6. A similar trend to the one mentioned above is seen up to 20 V. Above that the platelets on charged surfaces seem to be more affected. Results for voltages as low as 75 mV showed high *SPL* up to a level of 41% for negatively charged surface and 32% for positively charged surface [25]. Therefore, application of electric voltage up to 22 *V*, certainly, modifies commercial surfaces with respect to platelet interaction. High voltage values may affect the membranes of platelets and cause immediate rupture that initiates the release reaction and hence substantially increase *SPL* through adhesion and aggregation.

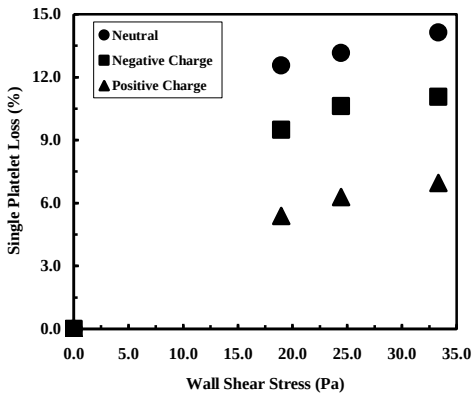


Fig. 5. Effect of electric charge and wall shear stress (Pa) on single platelet loss (%) in capillary viscometer.

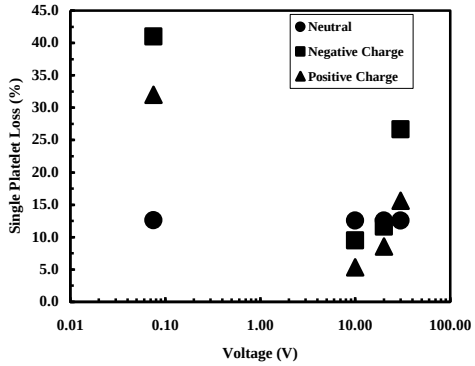


Fig. 6. Effect of increasing voltage on single platelet loss (%) in capillary Viscometer at wall shear stress of 20 Pa.

Table 3
Effect of residence time versus wall shear stress on single platelet loss

Wall shear stress (Pa)	Residence time (s)	Single platelet loss (%)
7.5	22.1	9.83
10.0	16.6	10.97
20.0	8.3	13.46
24.5	9.2	13.50
30.0	9.2	13.77
33.3	5.0	14.34

Table 3 gives residence time associated with presented wall shear stress and the corresponding *SPL*. The data presented shows that even for low residence times the values of *SPL* are high when the corresponding shear stress is high. This indicates that *SPL* is very sensitive to application of shear stress. Therefor it leads us to conclude that surface effects are more dominant that bulk effects and once a platelet gets into contact with the surface it becomes activated and adheres to that surface or to another already adhered platelet. Shear stresses in this case also enhance the interaction by augmentation of their transport to such surfaces especially in the presence of red blood cells.

5. Conclusions

In conclusion, application of an electric charge to a blood-contacting surface modifies it with respect to *SPL*. Positive charged surfaces showed the best results compared to negative charged surfaces, which come next, and neutral surfaces, which are the worst. Applying positive charge to commercial surfaces reduced SPL as much as 54% relative to neutral surfaces, whereas negative charged reduced *SPL* by 23%. It is also concluded that optimum voltage range is up to 20 Volts after which neutral surfaces become better than charged ones. Moreover, very low voltages are seen to cause more damage than moderate voltages. Therefore, design of blood-contacting surfaces under shear must take into account the type of the electric charge and the applied voltage associated with the surface.

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