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Flow-dependent platelet behaviour in blood–material interactions

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Platelet activation and adhesion are important parameters characterizing blood compatibility of biomaterials. A platelet transport theory based on convection diffusion, which describes the influence of wall shear rate, platelet concentration, axial position, hematocrit and red cell size, was originally proposed by Turitto and Baumgartner and later expanded by Aarts. This theory was applied in an *in vitro* perfusion system for three different materials with wall shear rates between 100 s^{-1} and 4300 s^{-1} in order to cover the regions of diffusion controlled, reaction controlled and intermediate platelet adherence. Platelet diffusivity and platelet vessel wall surface reactivity were determined for these cases and the constants m and n were calculated using the relation between platelet diffusivity and shear rate as expressed by the following power law function: $D_w = m^* \gamma_w^n$.

1. Introduction

The importance of rheological conditions on the transport of blood elements to the wall was recognized long ago. In particular, platelet activation and adhesion are considered to be important parameters characterizing the interaction between a biomaterial and blood [1–9]. Similarly, studies on the measurement and prediction of blood flow under various conditions: steady or pulsatile flow, turbulent or laminar flow, flow in tubes with distensible wall, flow in bifurcations and so on, have been performed [10–16]. Unfortunately, complete analysis of mass transport in each case of blood flow is very difficult for a number of reasons: (i) a large number of parameters influence the system; (ii) the values of some of these parameters, such as the membrane properties of blood cells, are not exactly known; or (iii) the exact influence of plasma proteins or released factors on the mass transport of the various elements is also unknown. This has led to investigations using blood analogues or to investigations using many simplifications in order to describe mathematically the real situation.

Turitto and Baumgartner in a series of papers [5–8] tried to apply the normal convective diffusion theory in the case of platelet transport in whole blood flowing in pipes or annuli. Later Aarts [9] expanded this theory by describing the influence of wall shear rate, platelet concentration, axial position, hematocrit and red cell size on platelet diffusivity. The theory was applied in the case of blood flow in annuli in order to calculate platelet diffusivity values for different shear rates and hematocrits.

More recently investigators have recognized the influence of flow conditions in blood–material interactions [17–21]. The necessity to evaluate the performance of new and traditional blood-compatible materials has led researchers to design many different test methods, using static or dynamic conditions, whole blood, plasma or protein solutions, different anticoagulants and so on, in order to have appropriate *in vitro* tests to characterize the blood-compatibility of biomaterials [22–29]. The current opinion [17–21] concerning the design of such a test is that it should take into account the use of the final product since flow conditions (shear rates, turbulence, secondary flows, etc.), duration of contact, size of the contact surface area and the actual placement site are very important parameters to be considered, in addition to the surface finish due to fabrication, and to sterilization effects.

Considering platelet adherence on a biomaterial one can distinguish chemical factors (blood and vessel wall components), at high shear rates, and physical factors (hemodynamics), at low shear rates, influencing the whole process. In the intermediate region, both factors are important. This paper applies the previously mentioned theory to each of these three different flow regimes.

2. Materials and methods

In order to evaluate platelet transport and adhesion kinetics at various flow rates, three different materials, PVC, polypropylene and glass in tube form (i.d. = 0.6 mm, l = 15 cm) were perfused with citrated whole blood with constant hematocrit (0.4) and wall shear rates ranging from 100 s^{-1} to 4300 s^{-1}.

The "capillary perfusion model", introduced by Cazenave and Mulvihill [22] as modified by Poot [23, 24] was used (Fig. 1). A syringe pump (Infu 362, BIOBLOCK) which can be driven by a computer to provide various constant flow rates is connected to three syringes and experiments were carried out at room temperature (20 ± 3 °C).

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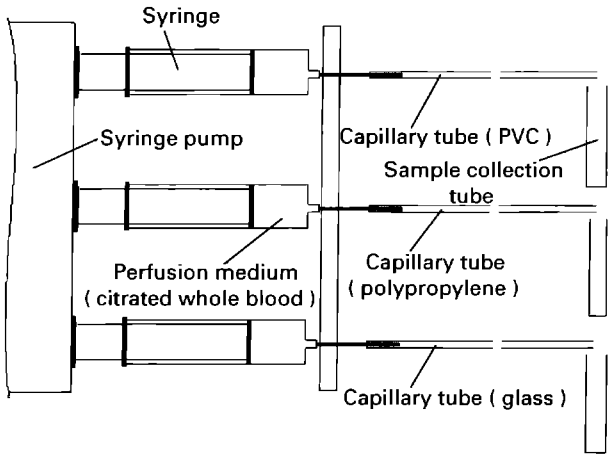

Figure 1 Capillary perfusion model.

The test materials were PVC 7536 and polypropylene with well characterized surface properties and hemocompatibility [22, 23, 25] and glass tubes provided by Omega Dot Tubing, Glass Company of America.

Citrated whole blood was used as perfusate, immediately after venipuncture. Blood was taken from healthy donors and hematocrit values were 0.4 ± 0.2. Perfusion time and collected blood volume were different, depending on the shear rate, but blood volume did not exceed, in any case, 5 ml in order not to have saturation effects.

Determination of platelet adherence was carried out by counting the number of platelets (by means of a Whole Blood Counter (Sysmex)) in influent (control) and effluent blood (after contact with the material).

Blood was treated as a Newtonian fluid (assumption valid for shear rates higher than $100\ s^{-1}$ [30, 31]) and the shear rate was calculated by the following formula (laminar flow in tubes):

$$\gamma = 32Q/\pi d^3$$

where Q is the blood flow rate and d is the internal tube diameter.

After the determination of the number of adhered platelets the theory proposed by Turitto and Baumgartner [6–8] was used in order to calculate platelet diffusivity at the various shear rates and the platelet vessel wall surface reactivity for each material. This last parameter is independent of the shear rate and will be evaluated at the high shear rate regime, where it plays the major role.

Using the following equation for the platelet flux in the case of a tube:

$$J(x) = C_0/(1/K + 1/(\gamma_w D_w^2/6.43x)^{1/3})$$

where J = platelet flux (platelets $cm^{-1}\ s^{-1}$)
C_0 = platelet bulk concentration (platelets cm^{-3})
K = platelet vessel wall surface reactivity ($cm\ s^{-1}$)
γ_w = shear rate at the vessel wall (s^{-1})
D_w = platelet diffusivity at the vessel wall ($cm^2\ s^{-1}$)
x = axial distance from the leading edge of the vessel segment (cm)

Two limiting cases can be distinguished.

Diffusion-controlled platelet adherence
If

$$1/K \ll 1/(\gamma_w D_w^2/6.43x)^{1/3}$$

which reduces the equation to:

$$J(x) = 0.54C_0(\gamma_w D_w^2/x)^{1/3}$$

Reaction-controlled platelet adherence
When

$$1/K \gg 1/(\gamma_w D_w^2/6.43x)^{1/3}$$

which reduces the equation to

$$J(x) = KC_0$$

The first case is approached experimentally with low wall shear rates $100\ s^{-1}$ to $200\ s^{-1}$, the second case for shear rates $>2600\ s^{-1}$, while in the intermediate region the full equation is applied.

3. Results

The equation for reaction-controlled platelet adherence: $J(x) = KC_0$ was used to evaluate platelet vessel wall surface reactivity, K, for the three tested materials after determining platelet adhesion at high shear rates $2600\ s^{-1}$ and $4300\ s^{-1}$ (Table I).

For the diffusion-controlled case, the equation $J(x) = 0.54C_0(\gamma_w D_w^2/x)^{1/3}$ was integrated over the length of the tube, L, to determine the diffusivity coefficient, D_w, at shear rates $100\ s^{-1}$–$200\ s^{-1}$.

At the intermediate wall shear rates ($400\ s^{-1}$–$1600\ s^{-1}$) the main equation for platelet flux was used. First it was analytically integrated over the length of the tube, L, and subsequently, using the previously determined values for platelet vessel wall surface reactivity, it was numerically solved (see Appendix) in order to provide values for platelet diffusivity (Table II).

Platelet diffusivity is found to be shear dependent and increases with increasing shear rate (Fig. 2). This dependency is given by the power law function $D_w = m\gamma_w^n (m = 1.64 \times 10^{-8}, n = 0.7)$.

TABLE I Platelet vessel wall surface reactivity for three tested materials

Material	Platelet vessel wall surface reactivity ($\times 10^{-4}$ cm s^{-1})		
	Shear rate		
	2600	4300	Mean
PVC	6.34 ± 4.8	8.37 ± 3.5	7.35 ± 3.9
Polypropylene	25.8 ± 10	13.1 ± 4.0	19.5 ± 9.7
Glass	21.8 ± 9.4	17.8 ± 1.5	19.9 ± 6.4

TABLE II Platelet diffusivity versus wall shear rate

Wall shear rate (s^{-1})	Platelet diffusivity ($cm^2 s^{-1}$)	Standard deviation ($cm^2 s^{-1}$)
100	4×10^{-7}	0.8×10^{-7}
200	8×10^{-7}	1×10^{-7}
400	1×10^{-6}	1.5×10^{-7}
800	2×10^{-6}	3×10^{-7}
1600	3×10^{-6}	6×10^{-7}
2600	4×10^{-6}	4×10^{-7}
4300	6×10^{-6}	3×10^{-7}

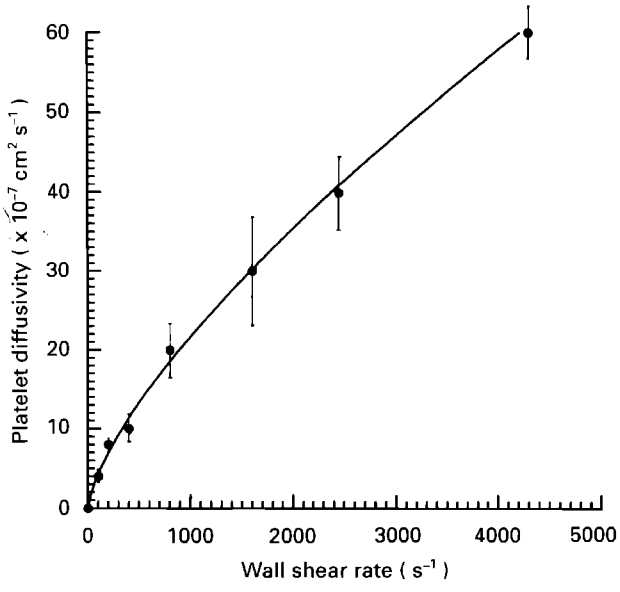

Figure 2 Platelet diffusivity versus wall shear rate.

4. Discussion

Platelet adhesion on biomaterial surfaces is considered to be a very important parameter for the hemocompatibility of the test material. The lack of standardized experiments in this domain is manifested by the existence of a variety of experiments in order to evaluate platelet adhesion on biomaterials. Static or dynamic experiments, different wall shear rates, hematocrits and anticoagulants are some of the parameters which can provide controversial results between different groups.

Our results suggest that the theory proposed by Turitto and Baumgartner [6–8] as expanded by Aarts [9] can adequately describe shear-dependent transport of platelets to the vessel wall in flowing blood. A power law correlation between platelet diffusivity and wall shear rate was determined, $D_w = m\gamma_w^n$, indicating the strong influence of flow on platelet adhesion. The values of platelet diffusivity found in this study range between 4×10^{-7} $cm^2 s^{-1}$ to 6×10^{-6} $cm^2 s^{-1}$, which are higher than the values reported by Aarts *et al.* [9] or Turitto *et al.* [5, 8] (Tables II and III). The value of exponent n is 0.7, which is in accordance with the value reported by Aarts *et al.* [9] at a hematocrit of 0.4, but it is higher than that which can be derived in the paper by Turitto and Baumgartner [8], namely $n = 0.42$. Comparison between our data and those reported by Aarts *et al.* [9] or Turitto *et al.* [5, 8] has an inherent difficulty because Aarts used aspirin-treated radiolabelled human platelets which can give a lower number of adhered platelets, and Turitto *et al.* used citrated rabbit or canine whole blood at lower hematocrit values which can diminish the red cell induced platelet diffusivity. The use of rabbit blood and lower hematocrit values in the study by Turitto and Baumgartner can also explain the difference in the value of n. Moreover the tested surface was arterial segments while in our study it was artificial materials.

Generally platelet diffusivity in the case of whole blood is greatly enhanced by the presence of red cells due to local fluid motion generated by individual red cell rotation, and an effective diffusivity (proposed by Keller [3, 4]) has been defined, as $D_e = D + D_p$ where D is the Brownian molecular diffusion coefficient and D_p is the rotation-induced diffusion coefficient: $D_p = Ca^{2\gamma}$ where C is a constant to be determined and a the red cell radius. For wall shear rates of the order 500 s^{-1}, D_p was estimated to be 10^{-5} $cm^2 s^{-1}$, in the case of normal whole blood.

Considering the hemocompatibility characterization of biomaterials one should note that two parameters, platelet wall surface reactivity and platelet diffusivity, play a very important role at high and low wall shear rates respectively. The influence of chemical factors (plasma and vessel wall components) can be better observed at high wall shear rates (reaction-controlled platelet adherence). In terms of their platelet wall surface reactivity PVC was found to be the best of the three tested materials while there were no significant differences between polypropylene and glass. On the other hand at lower shear rates (diffusion-controlled platelet adherence) platelet adhesion is dependent only on physical factors (hemodynamics) and all three materials presented not significantly different values of platelet adhesion. It should be noted, however, that the method used to determine the number of adhered platelets (using a whole blood counter) is not very accurate and this, along with the nature of such experiments (using whole blood), may be the explanation for the high standard deviations (Fig. 2). The use of more accurate methods (for example radiolabelling of platelets [23, 24] or freeze capture methods [32]) could ameliorate the precision of the experiment, but includes the danger of alteration of the platelet functionality.

These results suggest that characterization of biomaterials in terms of platelet adhesion and activation should include experiments both at high and low shear rates in order to distinguish the influence of chemical and physical factors. The final choice of the material should take into account the final application considering very carefully the flow conditions.

The above observation for platelet transport and adhesion can be generalized also in the case of other blood–material interactions where the Brownian molecular coefficient is very low compared to the value of rotation induced diffusion coefficient (for example in the case of protein adsorption where D is in the order of 10^{-7} $cm^2 s^{-1}$). This could also suggest

TABLE III Platelet diffusivity values in blood flow

Author (year)	Perfusion medium (hematocrit)	Test surface	Wall shear rate (s^{-1})	Platelet diffusivity ($cm^2 s^{-1}$)
Turitto *et al.* (1972)	Canine blood (EDTA) (0–0.5)	Polyethylene tubing (i.d. 0.25–0.5 mm)	40–440	$0.1–2 \times 10^{-7}$
Turitto and Baumgartner (1975)	Citrated rabbit blood (0.3–0.4)	Subenthothelium of arterial segments (l = 0.7–2 cm, i.d. 0.7–15.3 mm)	6.3–832	$1.1–3.4 \times 10^{-7}$
Aarts *et al.* (1986)	Human blood (aspirin-treated) (0–0.6)	Subendothelium of human umbilical arteries (l = 4 cm)	0–1200	$1.05 \times 10^{-9} \gamma_w^{f(h)}$ where h is the hematocrit value $f(h) = 0.297 + 1.29h - 0.9h^2$
Missirlis and Michanetzis (1995)	Human citrated blood (0.4)	PVC, polypropylene and glass tubing (l = 15 cm, i.d. = 0.6 mm)	100–4300	$1.64 \times 10^{-8} \gamma_w^{0.7}$

that other mechanisms influencing platelet adhesion and activation, like diffusion of platelet activating reagents released (ADP, vWF, etc.), can be strongly dependent on flow conditions [33–35]. Thus we have a complicated system which requires much more investigation in order to have a better insight into blood–material interactions

5. Conclusions

These results suggest that convective diffusional mass transport can adequately describe shear-dependent transport of platelets to the vessel wall in flowing blood. They also imply that characterization of blood-compatible materials in terms of platelet adhesion must include experiments both at high and low shear rates in order to distinguish the influence of chemical factors (plasma and vessel wall components) from the influence of physical factors (hemodynamics) involved in the case of blood–biomaterial interactions.

Appendix. Convective diffusional mass transport theory

The steady state equation for mass transfer in a tube is

$$V_x \frac{\delta c}{\delta x} = \frac{\delta}{\delta r}\left(D \frac{\delta c}{\delta r}\right)$$

Assumptions and simplifications

(1) Blood can be treated as a homogeneous, Newtonian fluid (valid for wall shear rates above $100\ s^{-1}$, at normal hematocrits (0.4) and tube radius greater than 100 μm).
(2) Laminar flow (valid for $100\ s^{-1} < \gamma_w < 4300\ s^{-1}$).
(3) The diffusion coefficient (platelet diffusivity D) is assumed to be in the form $D = \alpha\gamma^n$ (where α is a coefficient of proportionality, n a power law constant to be determined and γ the fluid shear rate).
(4) There is no surface saturation by adhering platelets during the experiment.

The general equation providing the platelet flux $J(x)$ is

$$J(x) = \frac{C_0}{\frac{1}{K} + \frac{1}{(\gamma_w D_w^2/6.43\,x)^{1/3}}}$$

Diffusion-controlled platelet adherence

Valid in the case of low shear rates $100\ s^{-1} < \gamma_w < 200\ s^{-1}$:

$$J(x) = 0.54 C_0 (\gamma_w D_w^2/x)^{1/3}$$

Platelet adherence dependent on wall shear rate and independent of tested material.

Reaction-controlled platelet adherence

Valid for high shear rates $\gamma_w > 1300\ s^{-1}$:

$$J(x) = K C_0$$

Platelet adherence dependent on the tested material and independent of the wall shear rate.

Integration formulas

Although platelet flux is dependent on axial position x we can define an effective average flux for a vessel of length L:

$$J = \frac{\int J(x)\,dx}{\int dx}$$

Using the general equation providing the platelet flux $J(x)$, we need to calculate the definite integral (from 0 to L):

$$\int \frac{C_0}{\frac{1}{K} + \frac{1}{(\gamma_w D_w^2/6.43x)^{1/3}}} dx$$

Substituting $(1/KC_0)$ by A and $(1/C_0)(\gamma_w D_w^2/6.43)^{1/3})$ with B we have to calculate the definite integral (from 0 to L):

$$\int \frac{1}{A + Bx^{1/3}} dx$$

which finally gives

$$\int J(x)\mathrm{d}x = \frac{-3A}{B^2}x^{1/3} + \frac{3}{2B}x^{2/3} + \frac{3A^2}{B^3}\ln[A + Bx^{1/3}]$$

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