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Experimental simulation of model platelet adhesion to a semi-permeable wall exposed to flow disturbance

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Abstract **A sudden tubular expansion with a semi-permeable wall was constructed from a tubular dialysis membrane to investigate the effects of filtration flow and flow disturbance on particle deposition. The expansion was perfused with a dilute, neutrally buoyant suspension of 1.10 μm diameter polystyrene latex spheres (as models of platelets) in Tris buffer solution containing 10% Dextran T70 and 2% bovine serum albumin. The results showed that adhesion of particles correlated positively with the filtration rate and inversely with the wall shear rate. In the vortex flow region distal to the expansion, particle adhesion was significantly elevated with a maximum at the reattachment point where the wall shear rate was the lowest and particles were constantly carried toward the vessel wall along the curved streamlines. In conclusion, filtration flow has a profound impact on the interaction of blood cells such as platelets with blood vessel walls, and the disturbed flow with a low wall shear rate can enhance the deposits of platelet thrombi to the vessel wall.**

Keywords: platelet adhesion, wall shear rate, filtration rate, flow disturbance

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Adherent platelets are important parts both in thrombus formation and in certain stages of atherogenesis[1]. Both the formation of atherosclerotic lesions and deposition of platelet thrombi develop not randomly and everywhere in the circulation, but localize at certain particular areas in the circulation. In fact, platelet thrombi *in vivo* have been found at sites downstream from bifurcations[2,3], stenoses[4] and venous valves.[5] In these areas, the blood flow is disturbed and the separation of streamlines from the vessel wall and formation of vortices (eddies) are likely to occur. This strongly suggests the involvement of hemodynamic factors in the localization of the vascular diseases.

To understand the role of hemodynamics in the localization of platelet thrombi, Karino et al. have systematically studied the flow patterns in arterial junctions[6,7], stenosis[8] and in the pockets of venous valves[9] by using glass models and transparent natural blood vessels. Detailed information on the behavior of model particles or blood cells such as red blood cells and platelets in these flow areas were obtained. They also investigated the effect of flow pattern and wall shear rate on platelet deposition using a glass sudden tubular expansion coated with collagen[10]. Platelet deposition and aggregation on extracellular matrix (ECM) under defined shear conditions are also quantitatively evaluated using a cone and plate device[11]. Using numerical modeling, David et al. studied the platelet deposition in stagnation point flow[12]. It has been shown that for a constant wall reaction rate, the maximum platelet flux occurs at the stagnation point streamline. This is in direct contrast to that found in experiment where the maximum platelet deposition occurs at some distance downstream of the stagnation point. Unfortunately, these studies did not take the filtration flow across the wall of the blood vessel into consideration.

The blood vessel wall is not impervious but possesses a filtration rate of the order of 10^{-6} cm/s[13—15]. The filtration rate of a blood vessel may be too low to affect the general flow pattern in the blood vessel, but it may probably affect the behavior of blood cells flowing very close to the blood vessel wall, in turn affecting the interaction of the blood cells with the vessel wall. To explore such possibility, an experiment was designed to test the effects of filtration rate and wall shear rate on the adhesion of polystyrene latex microspheres (as models of platelets) to the semi-permeable wall of a sudden tubular expansion. The purpose of this study is to simulate the transport of platelets in the sudden tubular expansion and the interaction of the cells with the vessel wall in the disturbed flow region distal to the sudden tubular expansion. The present paper describes the details of the experimental procedures and the results obtained from this experiment.

1 Methods

A sudden tubular expansion tube geometrically similar to the one in the study of Karino et al.[10] was used in the experiment to study the particle transport behaviour both in the disturbed flow region immediately distal to the expansion and in the fully developed laminar flow region far downstream from the expansion.

(ⅰ) Expansion tube. The sudden tubular expansion (hereafter referred to as an expansion tube) with a permeable wall was constructed from a commercially available cellulose membrane dialysis tube (Canlab, Montreal, Canada) and stainless steel pipes.

Prior to each adhesion experiment, a 7—8-cm-long segment of the dialysis tube having an I. D. of 6.5 mm and a wall thickness of 20 μm was soaked, and washed with deionized water for one day to remove contaminants and glycerol which were adsorbed by the membrane during

the manufacturing process. After thorough washing, the dialysis tube was cannulated at both ends with tightly fitting 2.6 mm I. D., 6.5 mm O. D., thick-walled stainless steel pipes cut at right angles and the ends were firmly tied onto the pipes using surgical sutures. Special care was taken in aligning the axes of the two stainless pipes and the dialysis tube so that they formed a 2.6 mm into 6.5 mm axisymmetric sudden tubular expansion.

The assembled tubular expansion was then mounted onto a solid tube-holder by inserting the two stainless pipes, forming the upstream and downstream sections of the expansion tube, into the concentrically aligned holes drilled through the two parallel walls. These walls were attached to the ends of a base plate and the pipes were held in place by tightening screws in the holder. The expansion tube was then filled with distilled water and was pressurized to check the symmetry of the tube. Only those truly axisymmetric tubes were used for adhesion experiments. The expansion tube and tube-holder were placed at the bottom of a transparent Plexiglas chamber as shown schematically in Fig. 1.

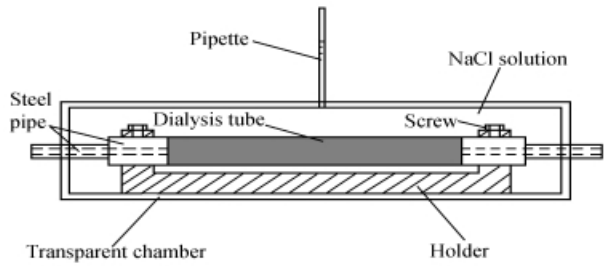

Fig. 1. Schematic drawing of the test section. The test section consists of a tubular expansion with a semi-permeable wall, a tube holder and a transparent plexiglass chamber filled with a saline solution. Filtration flow was created across the semi-permeable vessel wall by means of an osmotic pressure gradient.

(ii) Suspension and fixative solution. A 300 mL neutrally buoyant suspension of particles containing 1.10 μm diameter polystyrene latex microspheres (Duke Scientific Corp., Palo Alto, California) at a particle concentration of 8×10^4 particles/μL, 2% fetal bovine serum albumin (Flow Laboratories, Inc., Mississauga, Ontario), and 10% Dextran T70 (Pharmacia Fine Chemicals, Uppsala, Sweden) in distilled water were prepared for each adhesion experiment. A fixative solution containing 2% glutaraldehyde in distilled water was also prepared. Bovine serum albumin was added to the suspension to coat the surfaces of both the particles and the semi-permeable dialysis tube. The glutaraldehyde solution can react with the serum albumin on the surfaces of the particles and the semi-permeable dialysis tube so that latex particles in contact with the vessel wall could be fixed in place on the semi-permeable membrane by the fixative solution. Dextran T70 was added to the suspension to adjust the density of the suspending phase solution to match that of the latex particles (1.05 g/cm^3), thus preventing the particles from sedimenting. The pH of the suspension was adjusted to 9.2 with Tris buffer and HCl because it has been shown that the aggregation of latex particles is minimal at this pH value[16]. The viscosity of the suspension was 0.08 g/cm • s at room temperature of 23℃± 2℃.

(iii) Flow system. Fig. 2 shows a schematic drawing of the experimental setup used in the present study. As shown in the figure, the expansion tube encased in the Plexiglas chamber (hereafter referred to as a test section) was connected at the upstream port to two head tanks and at the downstream port to a collecting reservoir via flexible plastic tubing. One head tank contained the particle suspension, the other head tank the fixative solution. A three-way stopcock was used to switch the flow from the suspension tank to the fixative solution tank during the course of an experiment. The particle suspension and fixative solution were recirculated between the collecting reservoir and the head tank by using a blood pump. Filtration flow was created across the semi-permeable vessel wall by means of an osmotic pressure gradient. Thus, in order to obtain the desired filtration rates, the transparent chamber encasing the expansion tube was filled with NaCl solutions of various concentrations. The filtration rate across the semi-permeable vessel wall was calculated by measuring the increase in filtrate volume in the transparent chamber with a graduated 20 μL full scale micropipette made from a microhematocrit tube. This micropipette had been installed on the top wall of the chamber by drilling a small hole and gluing the micropipette onto the Plexiglas wall. The change in filtration volume was measured by timing the rise of the meniscus of the filtrate in micropipette. The filtration rate was then calculated based on the measured filtration volume change per second.

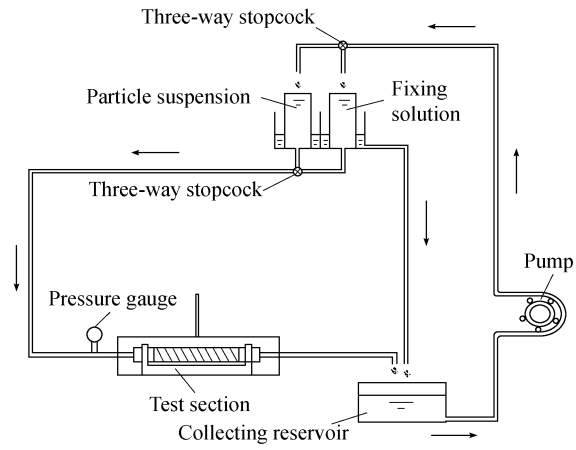

Fig. 2. Schematic drawing of the experimental setup used for particle adhesion experiments. The flow rate in the expansion was controlled by adjusting the height of the overflow head tank or the outflow resistance of the expansion.

(iv) Experimental procedure. In order to coat the inner surface of the dialysis tube evenly with protein, the expansion tube assembly was first dried completely at 50℃, then placed in the transparent filtrate chamber. The cover plate was placed in position and tightened with eight screws positioned symmetrically around the chamber. Silicone grease or a rubber sheet was applied to prevent leakage from the chamber. The semi-permeable membrane was coated with protein by forcing a particle-free 2% bovine serum albumin solution back and forth through the dialysis tube with a syringe. The transparent filtrate chamber was then filled with a NaCl solution of chosen concentration that created the desired filtration rate across the semi-permeable membrane. The test section containing the expansion tube was connected to the head tank and the collecting reservoir. The flow rate in the expansion was controlled by adjusting the height of the particle suspension head tank or the outflow resistance of the expansion. The adjustment was completed in less than 20 s. A steady flow of the particle suspension passed through the dialysis tube for 10 min, when the filtration rate of the suspending phase liquid was repeatedly measured. A high intensity fiberoptic light was used to help observing the flow patterns and measure the length of the annular ring vortex formed distal to the sudden tubular expansion.

After completing a 10 min flow experiment with the particle suspension, the flow was switched to the fixative solution by turning the three-way stopcock. Although glutaraldehyde acts almost instantaneously, the fixative solution passed through the expansion tube for 3 min. During the fixing procedure, special attention was paid to maintain the same Reynolds number (therefore the vortex length) as that set for the flow experiment. After fixing with glutaraldehyde, latex particles were firmly adhered to the surface of the dialysis membrane.

(v) Preparation of samples for analysis. After each experimental run, the dialysis tube forming the outer portion of the tubular expansion was removed from the assembly and cut longitudinally to reveal its inner surface to which the microspheres were adhered. The membrane was then mounted onto a specially designed membrane specimen observing plate with a 12-mm-wide, 50-mm-long window. The specimen was gently washed with the distilled water to remove the loosely attached particles and then dried for 30 min at 50℃.

Once it was dried, the membrane was taut and made a very smooth flat surface. The specimen plate was clamped onto a microscope stage and analyzed under a light microscope at a magnification of 500×. The counting of particles adhered to the surface was carried out using an eyepiece square grid (72 μm×72 μm). All particles appearing within this square were counted while pushing the sample slowly in the lateral direction for each 1 cm so that a total area of 72 μm (axially) × 10 mm (radially) was observed for each subdivision of the membrane. This procedure was carried out at 1.0 mm intervals along the tube axis in the vortex flow region, then changing to 2 or 5 mm intervals far downstream from the vortex. The results are expressed as the number of adhering particles per 10^3 μm^2, i.e. the particle adhesion density. For the distributions of particle adhesion in the vortex flow region, the data are normalized to the average downstream particle adhesion density and are plotted as a function of distance from the origin of the tubular expansion. The average downstream particle adhesion density is the mean value of the particle adhesion densities at the last three locations of the expansion. The Reynolds number *Re* was calculated based on the inlet diameter of the expansion tube.

2 Results

(i) Particle adhesion in the disturbed flow region. Fig. 3 shows a typical photograph of the 1.10 μm diameter polystyrene microspheres adhered to a semi-permeable wall of an expansion tube after a 10-min-flow experiment. Microscopic examinations of the opened dialysis tubes revealed that adhesion of polystyrene microspheres occurred over the entire inner surfaces of the tubes. The distribution of particles was uneven with some scattered small aggregates of particles in most samples. Presumably, this is due to the non-homogeneous nature of the surface structure and porosity of the membrane used in the present investigation. However, even in such samples with no visible tendency in their spatial distribution, it was found that when the adherent particles were counted over the entire area of each subdivision of the membrane assigned to each axial location, there were certain reproducible regularities and tendencies in the normalized particle adhesion density curves as shown in Fig. 4. In the figure, the measured and normalized density of adhering particles was plotted against the axial distance from the origin of the tubular expansion. The mean and standard error (SEM) of mean of the normalized particle adhering

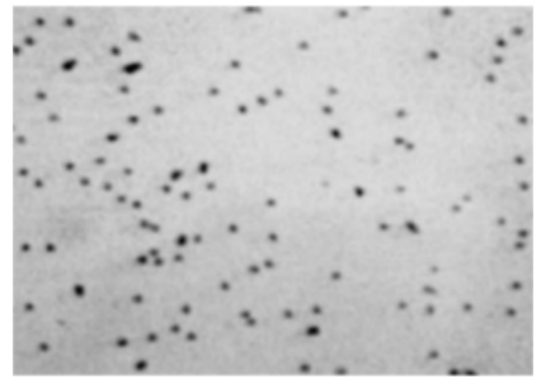

Fig. 3. A typical photograph of the 1.10 μm diameter polystyrene microspheres adhered to the semi-permeable vessel wall of a tubular expansion, showing the distribution of these particles on the transparent dialysis membrane.

density obtained from 8 runs carried out under identical flow conditions are indicated by the thick solid line and the vertical bars, respectively.

At the origin of the expansion, $L = 0$, there was a region where particle number density was unusually high. There, aggregation of particles was so extensive that it was virtually impossible to count. Therefore, this point was excluded and counting was carried out from the axial position $L = 1.0$ mm.

The high particle adhesion density observed at the origin of the expansion was probably due to the presence of a gap between the dialysis tube and the steel pipe where flowing particles, as they approached the corner of the expansion, were caught and formed aggregates and deposited there.

Fig. 4 shows the results obtained at two different Reynolds numbers (*Re*) under the same filtration velocity, $v_w = 2.5 \times 10^{-6}$ cm/s, which is within the range of the measured values for large arteries in man and rabbit[13–15]. The axial lengths of the annular ring vortices measured during flow experiments were about 0.9 cm at *Re* = 75 and 1.4 cm at *Re* = 110. In Fig. 4, the flow reattachment points are indicated by vertical dashed lines at *R*. As evident from the figures, at both *Re*, the particle adhesion density reached a maximum around the reattachment point and was almost constant on both sides of the reattachment point. Although the normalized peak value in particle adhesion density remained the same (1.19 ± 0.06 for *Re*=75 and 1.19 ± 0.05 for *Re*=110), the average downstream adhesion density decreased slightly from 1.94 particles /10^3 μm^2 at *Re* = 75 to 1.88 particles /10^3 μm^2 at *Re* = 110, suggesting a negative effect of high *Re*, hence high flow rate and high wall shear rate on particle adhesion to (interaction with) a semi-permeable vessel wall. To verify this, we carried out another set of experiments to test the effect of wall shear rate on particle wall-adhesion.

(ii) Particle adhesion in regions of fully developed poiseuille flow. In two additional sets of flow experiments, filtration rate and wall shear rate were varied to test their effects on particle wall-adhesion. In these two sets of flow experiments, the number of particles was counted only in regions of fully developed Poiseuille flow.

Fig. 5(a) illustrates the effect of filtration rate on particle-wall adhesion. In this study, experiments were carried out at the same flow rate (*Re* = 75) so that the effect of

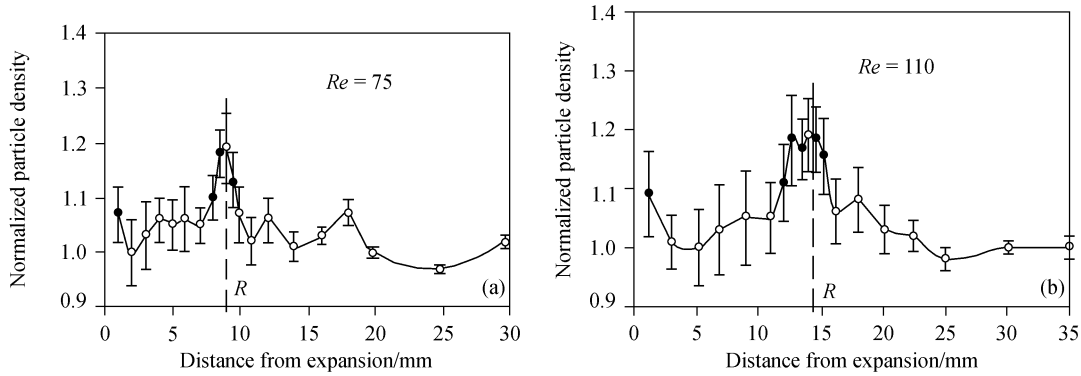

Fig. 4. Plots of the normalized particle adhesion density as a function of the distance from the origin of the expansion, showing the locally enhanced particle adhesion around the reattachment points. The peak value is significantly higher than others ($p < 0.05$) except those indicated by the solid dot. (a) at *Re* = 75; (b) at *Re* = 110.

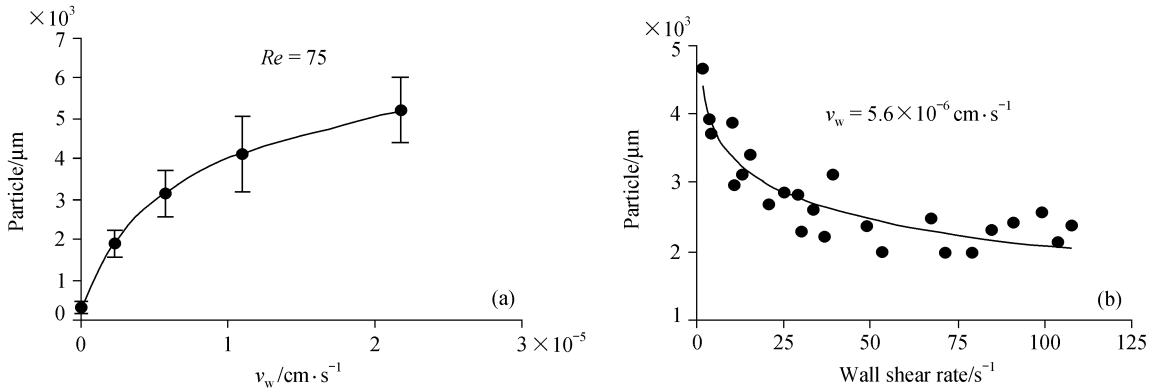

Fig. 5. (a) Plots of the particle adhesion density as a function of the filtration velocity at *Re* = 75. Values are mean ± SEM. Mean particle adhesion densities for a given filtration velocity are significantly different, by *t*-test, from those of all other filtration velocities ($p < 0.05$ in all cases); (b) plot of the number density of adhering particles as functions of the wall shear rate at a filtration velocity, $v_w = 5.6 \times 10^6$ cm/s.

filtration rate could be assessed independently of the contribution on the wall shear rate. As is evident from this figure, particle-wall adhesion was greatly affected by the magnitude of the filtration rate. The particle adhesion density increased nonlinearly with the increasing filtration velocity and tended to approach a constant value as the surface of the membrane was gradually covered by particles.

The effect of wall shear rate on the particle adhesion density was studied by varying the flow rate while maintaining a constant filtration rate. Fig. 5(b) summarizes the results obtained from 24 separate flow experiments carried out at a filtration rate of 5.6×10^{-6} cm/s. As shown in this figure, in the range of low wall shear rate, the adhesion of microspheres to the semi-permeable membrane decreased dramatically with the wall shear rate increasing and gradually approached a constant value when the wall shear rate increased.

3 Discussion

A sudden tubular expansion tube was used to study the adhesion of 1.10 μm microspheres onto the semi-permeable membrane of the expansion tube. The microspheres served as models of platelets that have a size of about 2 μm. The purpose of the present study is to simulate platelet interaction with (adhesion to) blood vessel walls under the influences of filtration flow and flow disturbance. The results of this experimental study have revealed that (1) the adhesion of microspheres onto a semi-permeable vessel wall is affected by two major factors: filtration rate of the vessel wall and wall shear rate; (2) the number density of adhering particles is positively and negatively correlated with the filtration velocity and wall shear rate, respectively; and (3) at a fixed filtration velocity, wall adhesion (prolonged interaction) of particles is greatly enhanced in regions of disturbed flow, resulting in the localized high adhesion of particles around the reattachment (stagnation) point where particles were constantly carried toward the vessel wall along the curved streamlines. This finding differs significantly from that in the experimental study by Karino et al.[10] in a tubular expansion with an impermeable wall, in which the highest platelet adhesion occurred at a position corresponding to the vortex center, where the wall shear rate was the highest theoretically. Such a significant discrepancy between these two experiments is the result of the effect of filtration flow.

As described by Blackshear et al.[17], when a spherical particle is flowing near a filtering membrane, it experiences two types of forces, namely the drag force of filtration, which is directly proportional to the filtration rate and promotes the adhesion of particles onto the filtering membrane, and a migration force of a fluid mechanical origin, which drives the particle away from the vessel wall. Whether the particle adheres onto the wall or not depends entirely upon the relative values of these two forces, or the relative values of the filtration rate v_w and the migration velocity v_p, which is defined as

$$v_p = 0.343\, r_p^2\, G^{3/2} / \upsilon^{1/2}, \quad (1)$$

where r_p is the radius of the spherical particle, G is the wall shear rate, and υ is the kinematic viscosity of the suspending phase liquid. If v_w is greater than v_p, the particle will adhere onto the filtering wall. In the experimental study, the migration velocity of the 1.10 μm diameter polystyrene latex microspheres in the fully developed flow region was on the order of 10^{-6} cm/sec, the same order of magnitude as the filtration rate (based on the fluid parameters: viscosity = 0.08 g/cm • s, density = 1.054 g/cm^3, $G \sim 43$ s^{-1} at $Re = 75$). However, at the reattachment point of the vortex, the wall shear rate was the lowest (theoretically speaking, zero), v_p would therefore be very low at the reattachment point. This may explain why particle adhesion was the highest at the reattachment point when the tubular expansion wall was permeable to water.

In conclusion, the present experimental study has demonstrated that filtration flow across the blood vessel wall does in fact affect the interaction of blood cells with the vessel wall significantly. Further studies on this subject should therefore take the filtration flow of a blood vessel into consideration. The present study was carried out under the steady-state flow conditions, which were very different from the *in vivo* pulsatile flow conditions, therefore the results obtained here should be verified with pulsatile flow in our future work.

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