Influence of wall shear rate on parameters of blood compatibility of intravascular catheters

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Three polymeric materials (silicone, PVC and nylon) were compared in an in vitro perfusion model, whereby 5 ml whole blood were perfused along 1 m lengths of polymeric tubing of 1 mm internal diameter at wall shear rates of up to 1000 s⁻¹. Perfusion took place at 37°C for 30 min. The polymers were investigated for platelet activation, granulocyte secretion, complement activation and contact phase activation. These parameters were also analysed in static contact for comparison. All the parameters measured displayed a dependence on wall shear rate. In all the materials studied, platelet adhesion and platelet activation increased with increasing flow rate. Granulocyte elastase release increased slightly with increasing flow rate up to 300 s⁻¹. Complement activation was greatest for PVC at 1000 s⁻¹, greatest for nylon at 100 s⁻¹, but there was no measurable difference at either rate for silicone. All samples caused an increase in clotting time with increasing wall shear rate. PVC was the most platelet compatible material, nylon the worst. Silicone caused least contact phase activation, PVC and nylon the most. © 1996 Elsevier Science Limited.

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Among the most common types of medical device that comes into contact with blood are intravascular catheters. These are made from one of several varieties of polymer and are used in several different situations of both diagnostic and therapeutic character. It is widely recognized that the placement of such catheters within the vascular system carries the attendant risk of thrombus formation¹⁻³. A considerable amount is known about the mechanisms of the interactions between blood and polymers and a certain amount of information is available concerning variations in blood compatibility between various materials. There is still, however, a considerable uncertainty as to how different materials will perform under given clinical conditions, where variables of the host may be as important as variables of the catheter. Amongst the most important of these variables are the characteristics of blood flow. This should not be too surprising since the interactions between a solid surface and components of a complex fluid flowing over that surface should be dependent upon the characteristics of transport of the critical components in that fluid.

It is well known that the formation of a thrombus is dependent upon either or both the behaviour of platelets at or near the surface and on the protein-based coagulation cascade⁴⁻⁶. The haemodynamic characteristics that control platelet movement and protein transport near the surface are therefore likely to be of considerable significance in controlling events. Naturally it should be expected that there will be considerable differences in behaviour between conditions of turbulent and laminar flow and, even under laminar flow conditions it should be anticipated, and indeed it is observed, that variations in wall shear rates will have an influence on behaviour⁷.

In the experiments reported in this study, a number of parameters of blood compatibility have been assessed, under in vitro conditions, with respect to wall shear rate. A multiparametric approach has been taken and the influence of wall shear rate assessed in each case. The major biochemical and cellular pathways generally regarded as important in material haemocompatibility have been studied, namely platelet adhesion, platelet activation, contact activation, neutrophil activation and complement activation. Platelet adhesion is important as a first stage in the generation of a thrombus and it was this parameter which was previously regarded as the most important indicator of the unwanted reactivity of a material to blood⁸.⁹ Experiments by Feuerstein and Kush¹⁰, however, in which platelets have been observed flowing over a material surface by

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microscopy have shown that many platelets have a short residence time on the surface. Although they can adhere, a proportion become detached, a percentage which is determined to some degree by the surface properties of the material (hydrophobicity, surface roughness, etc.). This brief encounter with the material surface does have a deleterious effect on the platelet, however, and one can easily see the effect of this in their activation state, measured by the appearance of β-thromboglobulin (β-TG) in the surrounding solution.

The adhesion of platelets is, however, an important phenomenon, particularly as they adhere to conformationally altered plasma proteins which have adsorbed to a material surface. The interfacial properties of the surface control the degree and manner of the conformational rearrangements such that large variations in platelet adhesion to materials of apparently similar structure can be observed. Adhesion occurs via a large number of specialized receptors (glycoprotein (GP) IIb/IIIa for collagen, GPIb/IX for vWF). Such specialization allows for the differential degranulation of platelets, even in response to biomaterials, from up to four types of specific storage pool. In these are growth factors, chemotactic factors, acid hydrolases, vasoactive substances and coagulation factors. The surface of an activated platelet is a prerequisite for the formation of enzyme complexes which take part in clotting, whilst the activating stimulus can be transmitted humorally throughout the blood.

This material-dependent activation also occurs with white blood cells. Granulocytes are central to the initiation of an inflammatory response and are active in the propagation of an immune response. Their internal granules contain a large number of highly bioactive compounds which control the functioning of the circulation. This control extends to other cells within the blood. Elastase, for example, reduces the function of platelets by digesting glycoprotein Ib (GPIb) and Granulocytes can be activated either by direct material contact or by virtue of their ability to respond to complement activation via membrane-bound C3b receptors.

Complement activation makes up the humoral arm of the body’s immune defence mechanism and is present within blood. No cellular involvement is required to react with, and destroy, a variety of non-host cells. This protein-based cascade also reacts with artificial surfaces placed within the blood stream, predominantly via the alternate pathway, causing lysis of C3 owing to the assembly of a C3 convertase and resulting in the release of a number of harmful anaphylatoxins (C3a or C5a). This generation of fluid-phase C3a has been utilized in the past as a measure of the complement activating property of a biomaterial, but is now usually replaced by the measurement of iC3b. This is the inactivated cleavage product of C3a generation, C3b.

The other major non-cellular pathway analysed is the coagulation cascade, directly involved in the clotting of blood. Whilst platelets provide a surface for the assembly of proteolytic complexes and the provision of phospholipids and coagulation factors, it is the initiation of the cascade which is responsible for coagulation to occur at all. Whilst there is also an extrinsic pathway leading to coagulation which involves the release of tissue factor from traumatized tissue material-dependent clotting revolves around the activation of a single protein, factor XII (FXII). This process also involves high molecular weight kininogen (HMWK) and prekallikrein (PK) and is known as contact phase activation. The result is the activation of factor XI (FXI), and thereafter the stepwise activation of a series of other factors, including the conversion of fibrinogen to fibrin, the basis of a fibrin clot. The cascade, but not its initiation, also requires a threshold concentration of Ca²⁺ ions, absent from citrated plasma. This property has been used to estimate contact phase activation after biomaterial contact by measuring the time taken for plasma to clot after recalcification, as used in previous studies.

**MATERIALS AND METHODS**

**Materials**

The biomaterials investigated here represent a selection of materials commonly used in surgical practice. Silicone tubing, standard grade from Altec, Alton, Hampshire, UK; transparent vinyl tubing containing octyl phthalate ester plasticizer, standard grade from Portex, Hythe, Kent, UK and autoclavable nylon 6 tubing, standard grade from Jencons, Leighton Buzzard, Bedfordshire, UK, were used. All the tubing were of 1 mm length and 1 mm internal diameter equating to an internal surface area of 38.5 cm². In static contact phase activation experiments, glass was used as a positive control. This was borosilicate glass, Veridia grade and of the same internal dimensions as the test material tubings. It was purchased from Chance Brothers, Malvern Link, Worcestershire, UK.

Flat sheets of PVC, nylon and Dacron were prepared in Trivandrum and used for the assessment of static whole blood-contact response.

**Material characterization**

The three test materials used for dynamic contact experiments were examined for contaminants by X-ray photoelectron spectroscopy (XPS). Atomic composition was quantified at a flood-gun voltage of 1.0 V and sample angle of 30°. Percentages of different carbon environments were calculated by analysis of the C1s peaks by computerized deconvolution.

The transparent vinyl tubings were also tested for plasticizer content by supercritical fluid extraction. In this method, CO₂ at a pressure of >72 bar, 31°C, was incubated with the test material. The high resolving power of the supercritical CO₂ thus formed causes release of the majority of additives and impurities. This extract was analysed for composition by gas chromatography.

**Blood collection and preparation**

Human blood was used in order to eliminate species differences. Selected donors were normal, healthy people who had fasted for more than 8 h and had not received any medication for at least 14 days. Blood samples were collected into 3.8% (w/v) tri-sodium
citrate as anticoagulant at a ratio of 9 parts blood to 1 part citrate. Platelet-rich plasma (PRP) was generated by spinning whole blood at 150 g for 15 min. Platelet-free plasma (PPF) was created by spinning whole blood at 800 g for 15 min to remove most of the blood cells, then spinning the supernatant at 30,000 g in a microcentrifuge for 1 min. This was aliquoted into 250 μl portions and immediately frozen in liquid nitrogen to reduce the differences in contact phase activation between plasma samples.

51Cr-labelled platelets were prepared using a method similar to that of Sakariassen et al.,6 namely by removing the plasma from PRP by successive centrifugation at 500 g for 10 min with Krebs-Ringer solution (4 mM KCl, 107 mM NaCl, 20 mM NaHCO3, 2 mM Na2SO4, 19 mM tri-sodium citrate, 0.5% (w/v) glucose, pH 6.1) and resuspension in fresh solution. Radiolabelling was achieved by incubation of the platelet suspension with 10 μCi ml−1 sodium 51Cr-chromate (Amersham International, Amersham, Buckinghamshire, UK) at room temperature for 20 min. Excess sodium chromate was removed by three successive washings with Krebs-Ringer solution. The radiolabelled platelets were resuspended in native platelet-free plasma, obtained from another portion of blood from the same donor, and the original red blood cells added to give a platelet concentration of 200 × 10^9 ml⁻¹ and a haematocrit of 44%. The pH was adjusted to 7.3.

Blood-material contact

For static contact phase activation experiments, nitrogen-frozen plasma was thawed quickly by placing an aliquot in a 37°C water bath for 60 s, pipetting into the lumen of the sample tubings and incubating statically at 37°C for 10 min. For static whole blood-contacting experiments, flat-sheet versions of the materials were cut into 4 cm² circles and placed onto polystyrene incubation plates. 1 ml blood was placed onto the surface, the lid placed over the plate to reduce evaporation and incubated statically at 37°C for 30 min.

For dynamic experiments, a perfusion system was utilized, similar to that used by Cazenave et al.21 and Poot et al.22 The system consisted of a 1 m length of tubing, held straight and horizontal, and connected at each end to 60 ml syringes via tapered polypropylene ports. Allowing for start and end effects, this arrangement allowed a fully-developed, laminar flow profile along more than 99% of the length of the sample tubes, even at the highest wall shear rate, as defined by Caro et al.23. The syringe containing blood was attached to a Harvard 11 microprocessor-controlled syringe pump (Harvard, South Natick, MA, USA) and 5 ml whole blood perfused along the catheter tubing at varying shear rates at 37°C for 30 min. At higher shear rates blood was constantly re-perfused back and forth through the tube after the initial material contact, allowing a high degree of blood-material contact but with a minimal blood-air interface. Shear rates were calculated using the formula:

\[ \gamma_w = \frac{4Q}{\pi r^2} \]

where \( Q \) is the volume flow rate and \( r \) is the radius of the tubing. Blood was exposed to material surfaces at wall shear rates of 100 s⁻¹, 300 s⁻¹ and 1000 s⁻¹. After dynamic contact, blood was collected and centrifuged at 5000 g for 10 min to separate the plasma, and then at 30000 g for 1 min to obtain platelet-free plasma. This was immediately frozen at −70°C until analysed to prevent post-experimental activation. In the case of plasma for complement activation analysis, specimen stabilizing solution (Quidel, San Diego, CA, USA) was mixed 1:1 with plasma prior to freezing.

Analysis of blood compatibility

For platelet adhesion studies, platelets were labelled with 51Cr and radiolabelled blood was perfused through the tubings. Following perfusion, the tubings were carefully washed with buffered saline to remove non-adherent platelets. It was vitally important that the adhesion data were not influenced by static contact of platelets following perfusion or high shear rates due to washing. To achieve this, at the end of perfusion the inusing syringe was exchanged for one containing saline which was immediately perfused at a wall shear rate of 100 s⁻¹ until all traces of blood had been washed away. The adherent platelets were then counted by placing the tube in a γ-counter. Platelet activation was evaluated by measuring the released amount of β-TG in the perfused blood using an enzyme-linked immunosorbent assay (ELISA) kit from Boehringer Mannheim (Mannheim, Germany).

Contact phase activation was determined by measuring the partial thromboplastin time of blood or plasma contacting the materials. A zero wall shear rate data point was obtained by static incubation using PPF. Dynamic incubation was performed with whole blood to assess the relationship of the whole blood system to contact phase activation. In each case, platelet-free plasma was analysed, by measuring the time for 125 μl plasma to begin clotting after the addition of 125 μl CaCl₂ (25 mM) and 125 μl platelet substitute (Diagen reagent, Diagnostic Reagents Ltd, Thame, Oxfordshire, UK) which had already been prewarmed to 37°C. Clotting was measured by monitoring the change in optical density using a platelet aggregometer (Payton, Toronto, Ontario, Canada).

Neutrophil response was evaluated by quantifying elastase release using an ELISA kit from Merck (Darmstadt, Germany). Total complement activation was measured using an ic3b ELISA kit from Quidel (San Diego, CA, USA). In each case of ELISA analysis, plasma which had been separated and frozen at the end of each experiment was used.

Statistical analysis was performed using a paired t-test. The sample size, \( n \), was 5 in each case.

RESULTS

Material surface characterization

The three test surfaces appeared to be relatively contaminant free, with no evidence of amide, the usual constituent of processing wax. No fluorine was present.
in any sample, but PVC had a small amount of silicone, postulated to be contamination from siloxanes present in storage bags and fixation tape. Broadly, the percentages of each constituent were as expected given the material compositions.

The plasticizer within the vinyl tubing was verified as being an octyl phthalate ether, with a mean content by mass in the range 30–35%.

Platelet adhesion

The response of materials to platelet adhesion in static and flowing blood is shown in Figure 1. The proportion of Cr\(^{51}\) density is given as counts per minute (cpm) of \(\gamma\)-radiation. Since the quantity of chromium in each platelet sample was controlled, this is directly proportional to platelet adhesion. Under dynamic conditions, nylon caused the greatest degree of platelet adhesion at low and high flow rates. There was no significant difference between PVC and silicone, although the mean adhesion for PVC was less in each case than for silicone. It is interesting to note that both at low and high flow rates, the adhesion pattern was the same: PVC < silicone < nylon. The significance values for PVC and silicone compared with nylon were \(p < 0.02\) and \(p < 0.05\), respectively, at 100 s\(^{-1}\), and \(p < 0.1\) for both at 100 s\(^{-1}\).

Also of note was the static response to flat sheet materials. In this case, there was no significant difference between PVC and nylon sheet. The degree of adhesion was of the order of 40 times less than for the tubular materials at 100 s\(^{-1}\). Dacron caused almost three times more platelet adhesion than PVC or nylon, at a significance level of \(p < 0.02\) for both.

Platelet activation

The release of \(\beta\)-TG showed great similarity in comparison with the platelet adhesion response (Figure 2). There was no significant difference between PVC and silicone at low wall shear rate, with nylon activating platelets slightly more. As the shear rates increased, all the materials elicited increased quantities of \(\beta\)-TG in release. In addition, the material dependence was more pronounced at the high wall shear rate. At 1000 s\(^{-1}\), all the values were statistically significant: nylon compared with PVC or silicone (\(p < 0.005\)), silicone compared with PVC (\(p < 0.025\)).

Contact phase activation

One would expect all materials to cause at least some degree of contact phase activation and this was observed (Figure 3). After static contact with plasma, silicone caused the least activation, silicone and nylon producing very similar results. As expected, glass caused a very much greater degree of activation. Again, the flat sheet materials caused a different response compared with their tubular counterparts. Interestingly, the response to nylon and dacron sheet was more severe than that to tubular glass!

After dynamic contact of whole blood to the tubes, the plasma clotting times were all longer than for static contact with plasma (Figure 4). This effect became more pronounced at increasing wall shear rates, such that the time taken for virgin plasma to clot was shorter than for test specimens. Silicone tubing showed the least increase in clotting time, PVC the greatest.

Table 1 Summary of the effect of different materials on the blood response

<table>
<thead>
<tr>
<th>Material dependence with respect to different cellular functions</th>
<th>PVC &lt; silicone &lt; nylon</th>
<th>Silicone ≤ PVC &lt; nylon</th>
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<tbody>
<tr>
<td>Platelet adhesion</td>
<td>PVC &lt; silicone &lt; nylon</td>
<td>Silicone ≤ PVC &lt; nylon</td>
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<tr>
<td>Platelet activation</td>
<td>Silicone &lt; PVC &lt; nylon</td>
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<tr>
<td>Contact phase activation</td>
<td>Silicone &lt; PVC &lt; nylon</td>
<td>Silicone &lt; nylon &lt; PVC</td>
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<td>activation (static)</td>
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<td>inactivation (dynamic)</td>
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<tr>
<td>Neutrophil response</td>
<td>Nylon &lt; silicone &lt; PVC</td>
<td></td>
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<tr>
<td>Complement</td>
<td>Silicone ≤ PVC &lt; nylon</td>
<td>Silicone ≤ nylon ≤ PVC</td>
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<tr>
<td>100 s(^{-1})</td>
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<tr>
<td>1000 s(^{-1})</td>
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Figure 1 Relative platelet adhesion as detected by \(^{51}\)Cr in perfused blood to polymeric tubes and in static blood to polymeric discs.

Figure 2 Platelet activation in resting blood (control) and that perfused through polymeric tubes for 30 min.
Granulocyte activation

There was a significant difference between the neutrophil degranulation into blood after dynamic contact with the tubular biomaterials at all flow rates compared with non-contacted blood (Figure 5), $p < 0.001$ for all materials at $1000 \text{s}^{-1}$; $p < 0.01$ at $300 \text{s}^{-1}$; at $100 \text{s}^{-1}$; $p < 0.01$ for PVC, $p < 0.02$ for silicone, $p < 0.1$ for nylon. In all cases, the degree of elastase release was greater at $300 \text{s}^{-1}$ than at $100 \text{s}^{-1}$ but the same as at $1000 \text{s}^{-1}$. Material dependence was greatest at $100 \text{s}^{-1}$, there being no significant differences between any of the materials at either $300 \text{s}^{-1}$ or $100 \text{s}^{-1}$. PVC elicited the greatest granulocyte response, nylon the least, at $100 \text{s}^{-1}$.

Complement activation

The difference in the degree of complement activation between any of the samples was fairly limited (Figure 6). There was a significant difference between nylon at $100 \text{s}^{-1}$ compared to $1000 \text{s}^{-1}$ with $p < 0.02$, but not PVC at $100 \text{s}^{-1}$ compared with $1000 \text{s}^{-1}$ with $p < 0.2$ and silicone at $100 \text{s}^{-1}$ compared with $1000 \text{s}^{-1}$ with $p > 0.2$. There was also a highly significant difference between nylon at $100 \text{s}^{-1}$ and both PVC and silicone ($p < 0.001$) at that wall shear rate. Interestingly, there was no common trend in change in activation with wall shear rate, as was the case for other parameters. For complement activation owing to perfusion at $100 \text{s}^{-1}$ compared with $1000 \text{s}^{-1}$, there was no difference for silicone, greater activation at $1000 \text{s}^{-1}$ for PVC, but less for nylon.

DISCUSSION

The in vitro study of the haemocompatibility of biomaterials requires the consideration of many parameters: static or dynamic contact, flow rate, wall shear rate, form of biomaterial to be tested, pathway to
consider (platelet adhesion, platelet activation, complement activation, contact phase activation, etc.) and duration of contact. It has previously been demonstrated that haemodynamic circumstances play a significant role in determining localization, growth and fragmentation of thrombi and platelet adhesion in vivo\textsuperscript{20}, and that flow rate controls platelet transport to a surface and their adhesion\textsuperscript{28}. This evidence is supported by observed differences in platelet activity predominance in venous and arterial flow\textsuperscript{27}. Clearly, defining the blood compatibility of a material is a compromise between a number of these factors\textsuperscript{28}.

In this study, contact with blood was performed dynamically so as to achieve a range of wall shear rates consistent with those experienced within the circulation. Static contact was performed in addition for comparison. We have attempted to assess the importance of haemodynamic factors, and possible inter-relations between pathways in some important parameters of blood compatibility. Since flow from the syringes into the sample tubes was via a tapered port of decreasing diameter, flow disturbances would be expected to stabilize. Since the sample tubes were kept straight, one would expect fully-developed, laminar flow to be present along the majority of the tube. Calculations suggest that this would be the case for >99% of the length of the sample. The results should therefore reflect differences obtained by changes in wall shear rate in laminar flow.

It is possible that the perfusion model utilized may unfairly influence the results towards increased activation at higher flow rates since, for a given perfusion time, the blood will contact a given point within the test material a greater number of times. In other perfusion models, for example, that of Cazanave\textit{et al.}\textsuperscript{21}, platelet containing medium is passed only once along the test material. In one set of experiments\textsuperscript{22}, perfusion was allowed to take place over a period of 2–15 min with a range of shear rates spanning 150–1500 s\textsuperscript{-1} and a transit time of 2–21 s. Platelet adhesion was evaluated by analysing the material surface. Platelet adhesion (and aggregation) reactions are, however, extremely time-dependent. Over a range of 30–120 s, platelet aggregate formation was shown to take place in direct proportion with time\textsuperscript{29} at a shear rate of 2000 s\textsuperscript{-1}, whereas platelet α-granule release, as measured by the expression of P-selectin, was shown to be negligible over a period of 30 s, even at a shear rate of 3000 s\textsuperscript{-1}. Our own unpublished data suggest that elevated levels of platelet activation cannot be observed when platelets are subjected to short-term shear rates in excess of 5000 s\textsuperscript{-1}. In our perfusion model, contact of whole blood with a material is allowed to take place over a controlled period of time at a predetermined wall shear rate, allowing fluid-phase phenomena to be observed and analysed. During a one-pass perfusion system, the higher flow rates would have unfairly underestimated fluid-phase time-dependant reactions.

For PVC and silicone there are large differences in adhesion between wall shear rates of 100 s\textsuperscript{-1} and 1000 s\textsuperscript{-1}, with greater adhesion at high flow rate. This is as expected, since platelet transport to a surface is greater at higher flow rates: a greater number of collisions results in a greater degree of adhesion. The result for nylon is slightly anomalous, except that the standard deviation is too large at 100 s\textsuperscript{-1} for discussion.

The smaller degree of platelet adhesion in the static contact experiments again can be accounted for by a lack of transport. This is a particular problem in whole blood where red blood cells tend to sediment under gravity to the bottom of a sample of blood, forcing platelets away from the surface. In addition, a much smaller surface area is presented to the blood in the case of flat sheets (4 cm\textsuperscript{2}) compared with the tubular materials (38.5 cm\textsuperscript{2}). The similarity in response to flat sheet PVC and nylon may be owing to either the difference in contact regime or real differences in surface chemistry and topography between flat sheets and tubes. The massive blood–air interface inherent in a static, flat sheet-contact arrangement appears to have had little adverse effect on the adhesion of platelets. Dacron, a typical vascular graft material, is shown in this model to be a highly platelet incompatible material.

For the same reasons as above, one would have expected β-TG release to increase with increasing wall shear rates, and this is observed. In all three materials, there is approximately three times more platelet secretion at 1000 s\textsuperscript{-1} compared with that at 100 s\textsuperscript{-1}. Nylon, again, is shown to be less compatible with platelets than the other two materials. In this model, using these materials, there is a good correlation between platelet adhesion and activation, a situation which cannot always be assumed.

In the static contact phase activation experiments, silicone is shown to be highly superior to the other materials. This underlines the need for a multiparametric approach to blood compatibility assessment, in that there is a clear divergence in two important and mutually interacting pathways concluding in thrombosis. Interestingly, the flat sheet materials have caused a massive response. In this comparison, almost the same surface area is exposed (4 cm\textsuperscript{2} compared with 5 cm\textsuperscript{2} in the tubes). This huge difference is probably owing to the deleterious effects of a blood/plasma–air interface which will tend to conformationally disturb plasma proteins. Again, Dacron performs extremely badly in this assay. PVC and nylon sheets have completely different responses to contact activation, whereas their tubular counterparts have similar activation properties, strongly suggesting that the tubes and sheets are chemically different.

The rise in partial thromboplastin times after perfusion is interesting and mirrors our own observations (not published) of in vivo cardiopulmonary bypass experiments in sheep. An increase in clotting time can be caused by three factors: a decrease in activation, a reduced concentration of one of the coagulation factors or an increase in serine protease (e.g. factor XIIa) inhibition by some other pathway. The first is unlikely, since the times rise higher than the pre-perfusion plasma aliquots. It is possible that the shearing forces themselves are having an effect on the coagulation pathway. It has been shown that although overall concentrations of adsorbed proteins do not change significantly with increasing wall shear rate, their binding may be changed\textsuperscript{30}. This would not,
in itself, explain the lengthening of the clotting times past the non-activated control, suggesting that some other pathway is involved.

Factor consumption is a possibility. Fibrinogen, owing to its concentration in plasma, easily and quickly deposits onto the surface of a biomaterial in plasma or blood. Gross adsorption could limit the amount available for clotting. However, a very significant reduction in concentration would be needed to lengthen clotting times to that degree. The consumption of other coagulation factors would allow a larger reduction in coagulation rate.

Many inhibitors exist with plasma (C₄-esterase inhibitor, 2α-macroglobulin, 2α-antiproteinase inhibitor, for example). One would expect increasing flow rates to enhance the rates of reaction between inhibitors and serine proteases, especially for those immobilized on a surface. However, the observed time lengthenings are material dependent, not just flow rate dependent. Alternatively, inhibition may come from other pathways. Activation of the fibrinolytic pathway is known to be material dependent to some degree, and is inhibitory to serine proteases in its effects. The degree of inhibition could then be subject to the flow rate, whilst the magnitude of fibrinolytic activation subject to the material in question.

It is possible that other cellular pathways are involved. The activation of platelets, however, would be the basis for the provision of more coagulation factors and might be expected to increase the rate of clotting. Release of elastase is known to be inhibitory to activated clotting factors, but the release of elastase at 300 s⁻¹ appears to be relatively material non-specific, whereas the reduction in coagulation rate is material dependent at that shear rate. It is possible that differential activation of complement is having an effect.

There appears to be a degree, if a somewhat small one, of material dependence to granulocyte activation, which disappears with increasing flow rate. This would suggest that the very action of perfusion, possibly the forced close association with red blood cells or platelets, will cause a degree of activation, as seen at high flow rate. At low wall shear rates, this association would be reduced, causing a reduction in degranulation. In addition, owing to their size (similar to red blood cells), a greater degree of contact with the tube surfaces would be apparent at lower flow rates, accounting for the different degrees of elastase release at 100 s⁻¹.

The response of complement at different wall shear rates is again surprising. None of these materials produced a very large response, and were all similar to each other. However, there was no specific trend of increasing or decreasing activation with increasing wall shear rate. Again one may speculate as to the association of complement activation with other pathways of activation.

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