

Platelets gliding along surfaces

Using this concept to improve anti-thrombotic treatments

- Platelets are cells involved in thrombosis (blood clotting), an essential process to control bleeding.
- Thrombosis can also occur when it's not needed, potentially causing deadly complications.
- Further understanding of the mechanisms of thrombosis can help prevent these dangerous complications.
- Dr Scott Denardo's research at Duke University Medical Center, USA, has brought to light new evidence on platelet behaviour that could provide the foundation for improving thrombosis-prevention treatments.

Thrombosis, or blood clotting, is an essential biological process that prevents excessive bleeding after a blood vessel gets injured; for example, when you have a cut. Thrombosis is achieved with the help of platelets (small cells that circulate in the blood) and coagulation proteins within the plasma (the liquid part of the blood) as well as on certain surfaces. After an injury, the two components work together to form a thrombus to stop the vessel from bleeding.

Thrombosis can become deadly

Although thrombosis is a mechanism that prevents excessive bleeding, it can also be triggered when no active bleeding is present. This leads to the formation of pathologic thrombus (blood clot) that can cause serious complications, even death. For example, when thrombus blocks

an artery to the heart or the brain, the result can be a heart attack or a stroke, respectively. Moreover, thrombus in major veins is known as deep vein thrombosis. This thrombus can sometimes detach and travel to the lungs where it becomes wedged, preventing adequate blood flow and re-oxygenation – this is known as pulmonary embolism. Thrombosis can also occur on medical devices such as coronary stents, which are used to scaffold open narrowed arteries to the heart. The procedure for placing stents into the arteries to the heart is called percutaneous coronary intervention (PCI). This thrombosis can result in life-threatening complications including heart attack and death during or after PCI.

The risk for thrombosis on equipment within coronary arteries during PCI – and the potential dangerous complications – has led to nearly

50 years of targeted research on the mechanisms of normal and pathologic thrombosis. This research has in turn led to the development of blood-thinning drug treatments to prevent thrombosis during and after PCI. However, the blood thinning ('anti-thrombotic') therapies can also lead to life-threatening excessive bleeding. Research to identify the optimal balance of anti-thrombotic drugs that minimises both pathologic thrombosis and excessive bleeding continues through today.

Dr Scott Denardo at Duke University Medical Center in the USA has modelled the behaviour of platelets inside blood vessels and near medical device surfaces. Some of his observations are just now entering the contemporary understanding of thrombosis. Denardo believes that applying these observations can refine existing anti-thrombotic therapies to improve their safety (less bleeding) while not compromising their effectiveness (preventing thrombosis on PCI equipment, including stents).

A good model with an unexpected observation

To study blood flow behaviour in the vicinity of narrowed (stenosed) arteries and device surfaces, Denardo and his original team at the University of California, Berkeley, created a scaled-up model of a coronary artery. They used an acrylic tube (2.54 cm inside diameter) as

the model artery and a mixture of water and glycerol to simulate blood plasma. The model artery had the capacity for insertion of acrylic and paraffin stenoses, as well as the insertion of a central rod modelling PCI equipment. Rigid microspheres – microscopic beads (3.12 micron diameter) made from polystyrene – were used to facilitate measuring flow velocities using Doppler-based equipment from both inside and outside the model artery. These microspheres were marketed in the late 1980s as precisely following the fluid flow. Also, their diameter and calculated volume are about the same as platelets. Thus, the microspheres were also used to model platelets. For a gold-standard comparison, mathematical models and subsequently early computer-generated simulations were used to independently characterise the fluid flow. Importantly, the models and simulations each applied conventional fluid dynamic theory that assumed no-slip of the fluid (i.e.: zero velocity) at surfaces in the model (Figure 1).

To study platelet behaviour, Denardo and his team created a scaled-up model of a coronary artery and its flow system.

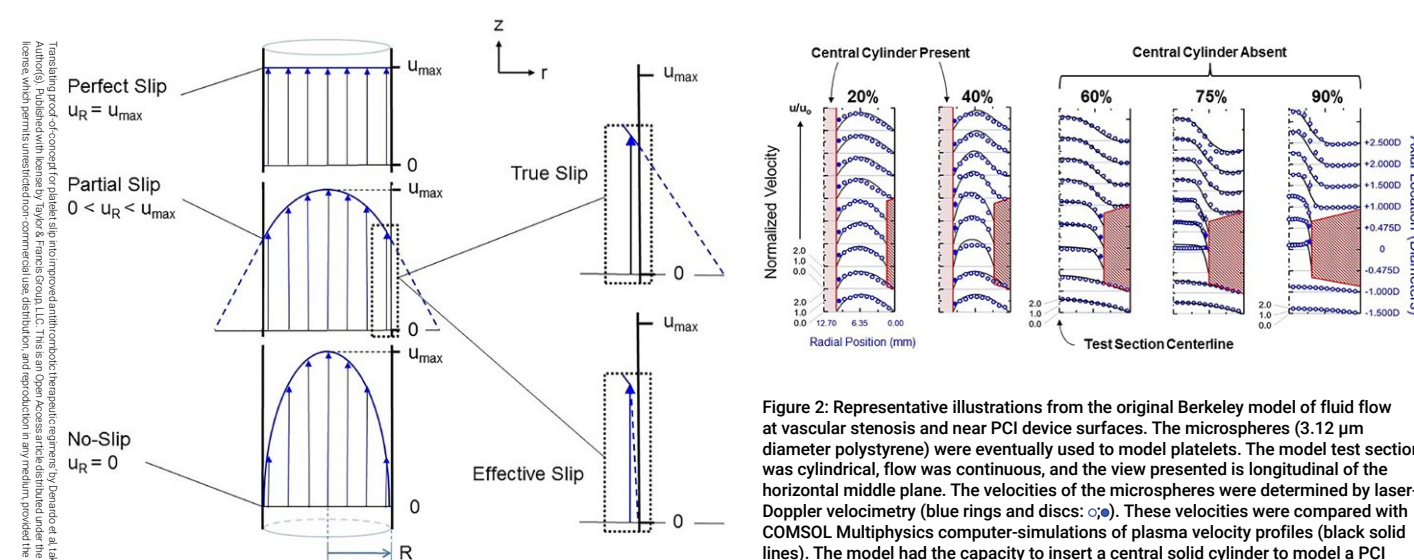


Figure 1: Velocity profile of fluid flow through a cylinder. The length of each arrow is proportional to the velocity. Lower left panel: no-slip boundary condition (i.e.: fluid has zero velocity at the cylinder inside surface). Middle left panel: partial slip. Upper left panel: perfect slip. True slip (upper right panel) indicates the slip layer at the boundary is of molecular dimension, and effective (or apparent) slip (lower right panel) indicates the local velocity varies over a finite, albeit small, mesoscopic distance. Fluid flow is in the z-direction, steady, laminar and incompressible. R=cylinder inside radius; u_R =fluid velocity at cylinder inside surface ("slip velocity"); u_{max} =maximum flow velocity.

Figure 2: Representative illustrations from the original Berkeley model of fluid flow at vascular stenosis and near PCI device surfaces. The microspheres (3.12 μ m diameter polystyrene) were eventually used to model platelets. The model test section was cylindrical, flow was continuous, and the view presented is longitudinal of the horizontal middle plane. The velocities of the microspheres were determined by laser-Doppler velocimetry (blue rings and discs: $\odot, \bullet$). These velocities were compared with COMSOL Multiphysics computer-simulations of plasma velocity profiles (black solid lines). The model had the capacity to insert a central solid cylinder to model a PCI guide wire (a 'cylinder-within-a-cylinder'). The stenosis range was 20–90% by cross sectional area. The Reynolds number of 300 is within the range of normal coronary flow. All velocities were normalised to the mean velocity u_m calculated from direct volumetric measurement of bulk fluid flow. Computer simulations applied the no-slip boundary condition at each surface, noting wall shear stresses in the unobstructed coronary circulation promote minimal plasma slip. The blue discs ($\bullet$) highlight partial or effective slip (i.e.: non-zero velocity) of microspheres in the high shear areas immediately adjacent to the surfaces of the stenoses and central cylinder surface. PCI=percutaneous coronary intervention.

Horizontal View Across Plate Moving Left-to-Right

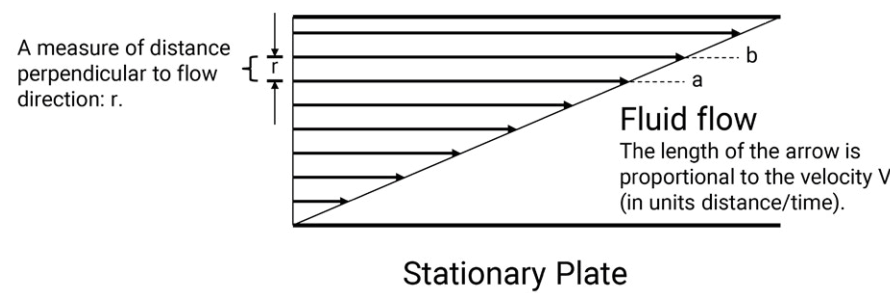


Figure 3: Example of simple shear, for which one plate (upper; viewed from the side) moves parallel to another which is stationary (lower), creating a shearing force on the fluid. The average shear rate in the vicinity of points a and b is: $(V_b - V_a)/r$, where V=velocity. The unit of shear rate is therefore (distance/time)/distance = 1/time.

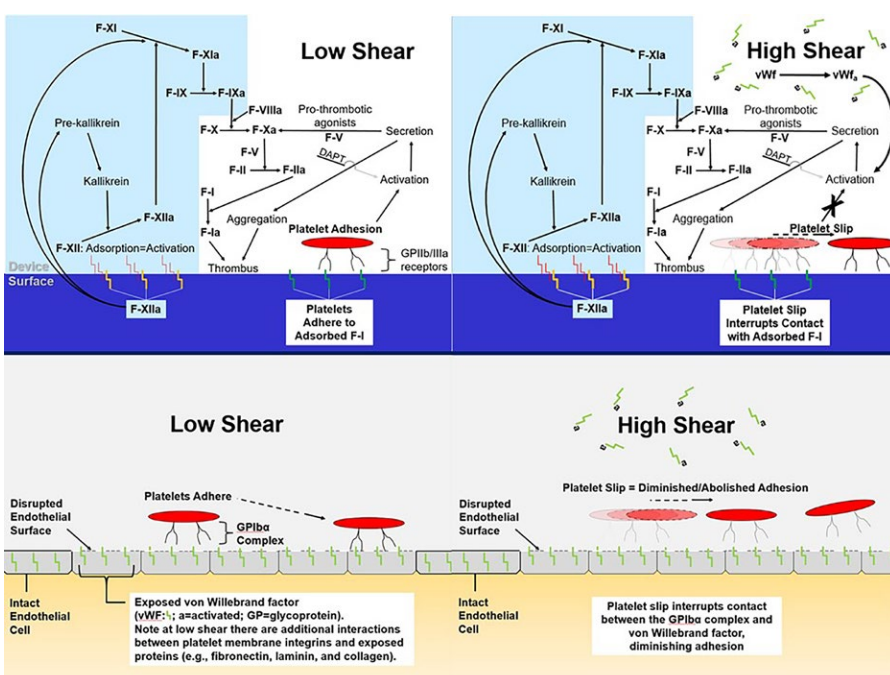


Figure 4: Schematic diagram of the effect of shear rate on platelets at a PCI device surface (upper panel) and at a site of endothelial disruption (lower panel). Left: low shear; right: high shear.

The high-precision external Doppler equipment ('laser-Doppler velocimeter') showed velocities within the more central body of fluid flow that matched mathematical theory and computer simulations (Figure 2). However, near surfaces of higher-grade stenoses and the central rod – where shear rates were high – an unexpected observation was made: the microsphere velocities exceeded the theoretical and simulation velocities; that is, there was apparent slip of microspheres adjacent to these surfaces (Figure 2). These observations were confirmed at the highest shear rates

with the lower-precision internal Doppler measurements (Figure 3 explains shear rate).

This finding defied conventional fluid dynamic theory, led to prolonged confusion in the interpretation of the observations, and met great resistance from the fluid dynamic community.

But something slipped; either the fluid or... the microspheres? The confusion was eventually resolved by accepting the concept that the microspheres did not in fact precisely follow the fluid flow, at least in high

shear conditions. That led to the proof-of-concept for shear-dependent platelet slip.

The gliding platelets

The mechanism of thrombosis and the role of local shear rate continues to evolve. Although the slip of whole blood in microscopic capillary tubes was first inferred nearly 60 years ago, macroscopic slip in arteries cannot be directly visualised nor measured. As a consequence, platelet slip is generally not in the contemporary discussion of thrombosis.

The presence of slip – or functional gliding – of platelets in high shear conditions adjacent to a surface would abbreviate or even extinguish any contact between the platelet and that surface (Figure 4). Importantly, certain antiplatelet medications (eg, Plavix) promote platelet slip. As a consequence, any thrombotic activity of the coagulation system on those surfaces would be diminished or even extinguished. Thus, antiplatelet therapy only, without anticoagulation, would seem sufficient for higher shear conditions.

Put another way, the idea is that if platelets slip at higher shear and don't adhere onto stents, other PCI equipment, or the vessel wall, then there wouldn't be enough contact between them and those surfaces to start interaction with the coagulation proteins to form thrombus. A more practical analogy would involve two people attempting to shake hands, one on a railway platform and the other inside a railway car, with the window open (Figure 5). When the car is motionless or at very low speed, the handshake is successful. However, at higher speed the handshake is impaired or impossible.

Nonetheless, antiplatelet therapy would be necessary, both to promote platelet slip and to prevent their interaction with any activated coagulation proteins in the plasma (e.g., von Willebrand factor; Figure 4). However, anticoagulation therapy – with its exaggerating risk of excess bleeding – would be unnecessary.

Taking advantage of platelet slip

Although the initial interpretation of the fluid dynamic model was clouded by conventional fluid dynamic theory and confusion that the microspheres precisely followed the fluid

flow, the concept was nonetheless laid that something slipped: either the fluid itself or the microspheres. Based on this more general concept and to improve outcomes for his PCI patients, Denardo changed his practice pattern 27 years ago to include antiplatelet therapy only, without anticoagulation, for his non-emergency PCI procedures. His patients did well, and he periodically reported his outcomes in peer-reviewed journals.

His most recent report focused on higher-risk PCI in unstable patients receiving antiplatelet therapy only, without anticoagulation. Among 481 consecutive PCI patients the procedure was successful in 99.2%. The rate of 3-day and 30-day thrombus-associated complications including any detectable heart damage and death were very low: 2.6% (death-0%) and 4.4% (death-0.8%), respectively. Additionally, fatal bleeding events at those times were very low, 0% and 0.2%, respectively. These results all compared favourably with other contemporary PCI reports where a combination of antiplatelet and anticoagulant therapies were used. Denardo and his colleagues concluded that higher-risk PCI can be performed safely and effectively using antiplatelet therapy only, without anticoagulation.

Personal response

What type of studies would be required to further advance the proof-of-concept for platelet slip?

There are two types of studies that would further the proof-of-concept for platelet slip. First, in the basic laboratory, a contemporary validation of the original model of coronary flow and expanded flow cytometry studies. If supportive, then models of flow in other high shear conditions (eg, aortic valve stenosis) may be explored.

Second, additional quantitative clinical research. This clinical research may sequentially involve:

- (1) a propensity score analysis comparing our higher-risk unstable PCI patients receiving antiplatelet therapy only, without anticoagulation, with similar PCI patients who did receive anticoagulation; then, if supportive,
- (2) a registry; and then, if supportive,
- (3) a randomised controlled trial.

How does your model explain the formation of different types of thrombi in arteries and veins?

In broad strokes, thrombi tend to visibly appear either grey or red. Under the microscope, grey thrombi contain primarily fibrin with a variable quantity of platelets,

More questions than answers

But which are the variables that affect the phenomenon of platelet slip? In refining Virchow's famed triad published in 1856 explaining deep vein thrombosis, Denardo postulates three: (1) the local shear rate; (2) the quantity and 'stickiness' of any coagulation proteins in the plasma, on device and vessel walls; and (3) any stickiness of the platelets themselves.

But Denardo has more questions now: What specific physical mechanism accounts for platelet slip at shear rates above a certain threshold? Why is it that, among heart attack patients undergoing visualisation of non-occlusive 'culprit' thrombus, the thrombus tends to appear grey and is platelet rich? Is it that as the 'culprit' plaque narrowing increases, some local shear rates (both upstream and downstream) actually decrease? Or that there is excessive pro-thrombotic proteins exposed upon plaque rupture? And do these concepts apply to other high-shear procedures, such as catheter-based aortic valve replacement?

Denardo and his team are confident that future research with the use of new technologies and perhaps facilitated by AI will answer these questions soon and open the path to more individualised and safer anti-thrombotic treatments.

whereas red thrombi additionally contain red blood cells. Red blood cells are much larger in diameter compared with platelets (about 8 microns vs. 3 microns). Thus, proportionately fewer red blood cells can visually obscure platelets. Finally, grey thrombi tend to form in arteries (higher shear) arteries while red thrombi tend to form in veins (low shear).

In a high-shear arterial environment where platelets slip, the coagulation cascade is less engaged, if at all. Consequently there is less fibrin generated and therefore less of a net to trap red blood cells, hence the grey colour.

However, in the low-shear venous environment where platelets don't slip, the coagulation cascade is engaged, fibrin is generated, and red cells readily trapped. Hence the red colour.

How could this information be used in the future to develop more specialised anti-thrombotic treatments?

This information can be used to focus anti-thrombotic regimens more towards antiplatelet or anticoagulant therapy based upon the shear conditions in the target vessel and/or environment.

Details



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Collaborators

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Bio

Dr Scott Denardo is a Clinical Associate at Duke University Medical Center. His principal career-spanning research has focused on analysis and application of fluid dynamic data he collected in 1989–1990 at the University of California, Berkeley. This research explores microsphere behaviour in different shear conditions with practical applications to biological systems and coronary stent procedures.

Further reading

- Denardo, SJ, et al, (2024) [Translating proof-of-concept for platelet slip into improved anti-thrombotic therapeutic regimens. Platelets](#), 35(1).
- Denardo, SJ, et al, (2021) [Anti-platelet therapy only, without anticoagulation, for percutaneous coronary intervention: A translational application of platelet slip. Journal of Translational Science](#), 7, 1–11.
- Denardo, SJ, et al, (2020) [Validated model of platelet slip at stenosis and device surfaces. Platelets](#), 31(3), 373–382.
- Turitto, VT, Baumgartner, HR, (1979) [Platelet interaction with subendothelium in flowing rabbit blood: effect of blood shear rate. Microvascular Research](#), 17(1), 38–54.
- Virchow, RLK (1856) [Gesammelte Abhandlungen zur Wissenschaftlichen Medizin. Kapitel IV \[Thrombose und Embolie\]. Frankfurt, Germany: Meidinger Sohn & Co.](#)

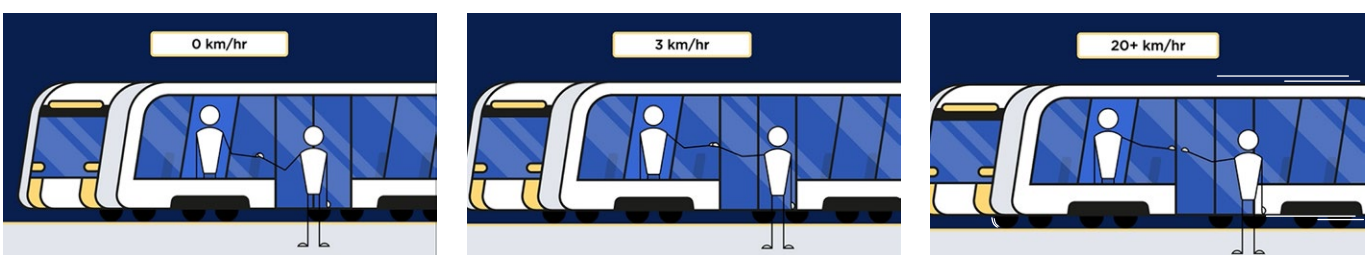


Figure 5: An analogy at the railroad station explaining shear-dependent slip: At zero velocity (stationary) and slow velocity (3 km/hr), the person on the railway car and the person on the platform can easily shake hands through the open window. However, at higher velocity (20 km/hr), the contact time is shortened to the point that the handshake cannot be successfully completed (representing slip).