Professor Susanna Hourani, School of Biomedical and Molecular Sciences, University of Surrey: introduction to platelets, 11 February 2005

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Prof. Hourani gave an introductory session on platelets; she studied platelets for her PhD, but is not actively engaged in platelet research.

Platelets are involved in haemostasis and thrombosis. The latter is a major killer, and a major risk.

Platelets occur within blood vessels. They take part in chemical communication and physical (touch) communication among the various blood-component cells.

Platelets clump (aggregate), but also release things that control the local environment. Red blood cells just carry oxygen. White cells interact with platelets via various compounds. There are also key interactions with endothelium (on the vessel walls) and coagulation cascading, referred to as clotting. Endothelium prevents inappropriate clotting; when endothelial cells are damaged (eg by smoking), there is an increased risk of thrombosis. Smooth muscle (of the vessel wall) doesn't touch platelets unless the endothelium damaged.

Nerves control the diameter of blood vessels, blood pressure etc.

1 Platelet interactions, and platelet endothelium interactions

Three things happen in haemostasis and thrombosis.

1. Stimulated platelets release things and affect local cells in the blood. Platelets are activated when endothelial cells are damaged (etc); when activated, platelets start to stick; they may stick to the blood vessel walls and to each other.

2. Coagulation cascade — clotting — causes liquid blood to become solid. It is triggered primarily (but not exclusively) by the Thrombin enzyme. This takes time to stop bleeding.

3. Local vasoconstriction is also designed to stop loss of blood. It is rapid, and effective in small blood vessels.

There is a fine balance between stimulation and inhibition in platelets. The trigger to aggregation is normally tissue damage, but also undamaged cells triggered in a heart attack or thrombosis — a build up of fat leads to inelastic plaque which splits and reveals the underlying substances (endothelium) that trigger platelet aggregation. Arthroscelosis (hardening of the arteries) also restricts flow.

There is continual interaction among:

- platelets
- coagulation cascade
- endothelial cells
- vascular smooth muscle

1.1 Activated platelets

An activated platelet becomes sticky and changes its shape, from smooth to spiny, increasing its surface area. Essentially, the platelet is held in a disc shape by protein ring. This contracts when the platelet is activated allowing the platelet to change shape. Granules within the platelet are pulled inwards, and as activation proceeds they release their contents.

Activated platelet can change back to passive platelets; platelets that are loosely stuck can unstick, up to a point. The irreversible phases occur when the contents of the granules are released.

Platelet sticks to whatever is exposed. The sub-endothelium contains a collagen protein that is a powerful activator of platelets; — if the endothelium is scraped, platelets stick to it. Then more platelets stick to this, producing a highly-amplified process. Platelets also activate other platelets.

Platelet stickiness is caused by the exposure on the platelet membrane of protein binding sites that bind other proteins. The plasma protein, fibrinogen, is the main (but not only) protein to be attracted and bound. Binding only occurs if a platelet is activated. Fibrinogen is a long, thin protein that lots of other platelets can stick to; it is always present in the blood.

Other things happen in relation to platelet activation.

- The platelet membrane has a lipid bilayer with embedded proteins; these lipids change to release platelet factor 3 ($PF3$), a phosphor lipid that enhances blood clotting.
- Within the platelet, enzymes are switched on to synthesise small chemical mediators (eg. TXA_2,PGD_2, PAF) with a short half life (less than a minute). These chemical mediators thus have only local action, including the recruitment of other platelets. Thromboxin (TXA_2) is the most potent (this is the chemical that is countered by asprin, when taken to reduce risk). PAF is synthesised from white cells.
- Synthesis of NO (nitric oxide) gas triggers other platelets.

The platelets contain two sorts of granules, which release contents on activation:

- dense, delta granules preform as low mole-weight mediators ($5 - HT$, ADP, ATP) affecting local cells;
- alpha granules contain proteins - Fibrinogen and other coagulation etc enhancers; release stimulates cell growth and healing, as well as more platelet activation etc.

When the alpha granules are released, the process leading to platelet aggregation is irreversible — that is, the platelets cannot return to the inactive form.

1.2 Timing

All these processes start in within milliseconds of the triggering damage. The macroscopic effect occurs within seconds, and the whole process is over in minutes — platelets have become stuck, completed their activation process, been released and done. Even though more blood arrives at the wound site, the stimulation stops — inhibitors take the system back into balance.

The platelet response is graded response — platelet shape-change, a bit of clotting stimulation, delta granules, alpha granules, according to level of stimulus. Thus, precise timings are hard to determine.

1.3 Coagulation cascade

Platelets and clotting (the coagulation cascade) are separate but interlinked. Note that a haemophiliac lacks the clotting response, not the platelets or their aggregation.

The coagulation cascade is triggered by an enzyme ($?PF3$). This affects the prothrombin plasma protein, which circulates in blood, stimulating production of thrombin. NB contact with glass also stimulated clotting. Clotting also requires calcium; it can be prevented (eg in vitro) by removing calcium from the blood.

Thrombin triggers Fibrinogen (the active glue between platelets), and forms the Fibrin enzyme. This forms the network that starts clotting, which is then stabilised into a clot.

$PF3$ is the platform for the reactions to occur; activated platelets are necessary and enhance the cascade; the processes occur together — platelet aggregation plus coagulation (positive feedback). Thrombin stimulates platelet aggregation as well as triggering fibrinogen. The platelet alpha granules release (amongst others) PF4, which enhances the coagulation cascade.

2 Platelet activation and inhibition

Platelet *activation* leads to an increase in cytoplasmic calcium. (Ca^{2+}).

Platelet activation is still subject of research; these are some of the known factors only. Essentially,there are two necessary proteins — collagen and thrombin.

Collagen occurs under the endothelium in the blood vessel walls. Platelets stick to it and are activated.

Thrombin is formed in the coagulation cascade, and is a powerful activator of platelets; it can push activation right through to the alpha granule release.

Of the small molecules related to platelet activation, some are released by active platelets to recruit others. (List on 7th slide.):

- ADP/ATP occurs in the dense (delta) granules, but also free in the cytoplasm of all cells — it is the energy currency that makes things happen in cells. Damaged cells release large amounts, which activate platelets — all cells do this. Since platelets also release it during activation, more ADP/ATP arises as aggregation occurs. Platelets use same signal as damaged cells. There is some experimental evidence that endothelial cells release ADP/ATP under hypoxia or fluid stress — since fluid is pumped over them all the time, this happens a lot; Anti-thrombotics sometimes act by blocking ADP on platelets.

- TXA_2, PGH_2 are lipid-derived, synthesised mediators from platelets.

- PAF and $5-HT$ are released by platelets, but are physiologically insignificant — that is, the would not be effective anti-thrombotics.

- Hormonal triggers such as adrenalin, which is stress-released, sensitise the platelets and make it easier to activate. Hence heart-attacks occurring under stress-triggered adrenalin. Short-term exercise raises adrenalin and platelets aggregation — for half-hour exercise, this is about a half-hour recovery as platelets de-aggregate, and this is a high-risk time for heart attacks.

The most important source of platelet *inhibitors* is endogenous anti-thrombotic compounds; endothelium itself is designed to stop aggregation, acting like a Teflon lining for blood vessels.

Prostacyclin is important — the withdrawal of the commercial anti-inflammatory drug, Viox, in December 2004 followed evidence of an increased heart-attack risk; this was probably because Viox blocked synthesis of Prostacyclin, and thus prevented the prevention of aggregation.

Endothelium releases a lot more NO than platelets. NO both promotes and inhibits platelet activation; the more NO the more the inhibition effect.

ADP/ATP can be broken down enzymically to adenosine, which inhibits platelet aggregation. Thus, the immediate stimulus to platelet activation is followed after a delay by inhibition. The cycle can be quite quick experimentally, with the decay occurring over minutes; locally decay may be much faster. Adenosine is also released by all cells if they run out of oxygen, for example because the blood supply to a tissue is cut off. So if a platelet aggregation

blocks a small blood vessel, downstream tissue releases adenosine which starts to break up the aggregation. This is pathologically important.

Some compounds also effect *blood vessels*. As a general rule, platelet inhibition goes with vessel relaxants and v.v. Thus, NO relaxes blood vessels, as does adenosine. Blood vessels are thus also affected by activated platelet releases.

NB the talk slides show what's stored and what's synthesised.

Interaction between *platelet and vessel walls*, specifically the endothelial cells, determine whether a small aggregation disperses or forms a larger aggregation. Healthy endothelium produces substances that prevent aggregation and keep blood vessels from constricting; damage takes away these preventions.

An initial small aggregation could be triggered by small leakages, platelet collision etc; it is always happening, in effect causing minor thromboses. Thrombosis becomes important (recognised) when important blood vessels such as coronary arteries, which take blood somewhere important such as the heart or brain, are blocked. If most endothelium is healthy, the aggregation effect stays local. However, when there is internal damage and the endothelium is damaged, thrombosis can form. The threshold of damage is not clinically quantified. Natural dispersal is on a similar scale to the cycle of scabbing and healing — a few days.

The last two slides summarise these factors, for healthy and damaged endothelial cells respectively. The action of aggregation stimuli on healthy endothelial cells serves to control aggregation (negative feedback). Healthy vessels constantly work to stop aggregation. Sheer stress from moving liquid always happening which stimulates aggregation but also stimulates NO synthesis to prevent aggregation — so if, for instance, blood is not free-flowing, the balance is disrupted.

Damaged endothelium takes out the preventative effects, causing aggregation and vessel constriction. Platelets do not produce enough NO to stop this. But all the reactions are compounds with short half-lifes, so the circulation and propagation of effects does not circulate far — local effects only.

Vessel constriction stops blood loss and reduces oxygen supply — this makes a local aggregation worse.

Because of the short half-lives, active platelets in blood activate, release, then stop; they do not circulate releasing mediators, other than very locally. Experimental work shows that an aggregation only grows at sites where ADP is released; part of an aggregation can break off and move downstream, but this does not cause platelets to activate elsewhere. An aggregation does not spread, because it is very sticky; if platelets are close enough to receive a chemical stimulus, then they are likely to be close enough to stick.

3 Questions answered

A blood vessel might be as small as 100s microns; aggregation clumps, 10s of microns; platelets, 1-2 microns.

A platelet is unstable in that its chemicals break down fast, and it looses its effect fast. This is very hard to examine experimentally.

The speed of blood flow depends on diameter etc. It is a laminar flow, which could theoretically be determined mathematically.

There is little difference across scale of blood vessels — capilliaries have less smooth muscle, but still have collagen proteins.

Normal circulatory systems do not run out of platelets — about 10x per ml.

Platelets don't move independently, they just flow with the blood. The platelet does not become rigid when activated, though a clump becomes relatively rigid. A clot comprises, for example, fibrin and platelets, as well as trapped red blood cells etc.; this forms a scab, and becomes tighter and more rigid, but the individual cells are always flexible.

Trapped substances (eg red blood cells) in clots have been washed in — they are not important in forming the clot. Plasma without red blood cells still clots. Even without platelets, plasma also clots (with right stimuli).

Platelets have no nucleus — can't do things like replicate, or synthesises protein. They are produced in bone marrow, and live about 10 days if not activated. They are barely cells, in the normal sense of the term.

When platelets die, they're broken down (sequestered by spleen).

Asprin is an irreversible inhibitor; it affects platelet function for a week to 10 days, until all the bound platelets die.

However large the hole or cut in a vessel of whatever size, the platelets do same thing. Vasoconstriction helps most in small vessels. The coagulation cascade works with aggregation, but is slower to get going. A completely-cut vessel needs to block so inhibitor loss is desired.

Once a clot (scab) forms, endothelial and other cells reproduce to break up clot and heal wound. Platelets are done with.

Why so many triggers and inhibitors? Natural redundancy.

Our model may need multiple inhibitors and stimulators (clottocytes paper doesn't go much in to this). How much one could get away with is interesting for analysis. Also multiple timescales of trigger and inhibition may be important.

Platelets don't move, just go with the flow — whereas white blood cells can move independently.

Platelets are quite complex, having a complicated control system, even though they have no means to change over time.

Discussion of ways of looking at how successful artificial platelet would be... it could be checked easily, by marking and monitoring ADP production and disaggregation.

Critical timescales are not given in conventional texts, and are very dependent on conditions. A lot of the research is done outside the natural context, where the platelets are already partially activated. Timing data might be found out but cannot necessarily be generalised. Times are not fixed — very high stimulus = instance reaction etc. [This would be fun to play with in simulation.]