

# The thrombotic potential of oral pathogens

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In recent times the concept of infectious agents playing a role in cardiovascular disease has attracted much attention. Chronic oral disease such as periodontitis, provides a plausible route for entry of bacteria to the circulation. Upon entry to the circulation, the oral bacteria interact with platelets. It has been proposed that their ability to induce platelet aggregation and support platelet adhesion is a critical step in the pathogenesis of the infection process. Many published studies have demonstrated multiple mechanisms through which oral bacteria are able to bind to and activate platelets. This paper will review the various mechanisms oral bacteria use to interact with platelets.

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The primary function of platelets is their adhesion to endothelium or to matrix protein components at sites of the injured vessel wall in the initiation of haemostasis (1). However, platelets are poorly appreciated for their involvement in inflammatory or immune processes in host defence. In this regard, platelets share many similarities with leukocytes well characterised for their role in immuno-protection following invasion by foreign invaders. Just like leukocytes, platelets undergo chemotaxis (2), phagocytose foreign particles (3) and secrete a multitude of products including inflammatory mediators (4), cytokines (5) and antimicrobial peptides (6). Indeed the concept of platelets playing a role in immuno-protection is not a new theory. Studies by Clawson and White in the 1970s demonstrated that platelets were capable of recognising a foreign invader (7–10). In doing so, platelets expressed a number of highly adhesive membrane receptors capable of binding injured or infected tissues. Following this, platelets underwent many cytoskeletal rearrangements in the form of extending pseudopodia that stretched around the foreign invader which promoted engulfment, thus clearing the foreign invader from the bloodstream (3). Therefore, platelets may provide a bridge between innate immunity and host defence.

## The conventional role of platelets in thrombosis

Platelets are small anucleate cell fragments of the larger haematopoietic precursor cell, the megakaryocyte (11).

Being devoid of a nucleus, the platelet has no control of gene expression but has limited capabilities in translational protein synthesis (5). The primary role of platelets in haemostasis is to police the integrity of the endothelium to prevent blood loss (12, 13). Platelets circulate close to the endothelial cell surface as individual entities that ordinarily do not interact with any other cell types and without any stable interaction with endothelial cells as they exist in an anti-adhesive state. Upon trauma or injury to the vascular endothelium, platelets rapidly accumulate at the site of injury. Recruitment is a highly controlled event that is initiated by the adhesive interaction between the exposed extracellular matrix proteins in damaged endothelium and specific membrane receptors on the platelet. Adhesion to the extracellular matrix requires a synergistic function of several membrane receptors which ultimately results in platelet activation and aggregation. There are several different types of matrix proteins exposed upon vessel injury including collagen, vonWillebrand factor (vWf), fibronectin, laminin and thrombospondin. Other adhesive proteins such as fibrinogen/fibrin and vitronectin are not synthesised by endothelial cells, however, do bind to exposed matrix proteins increasing adhesiveness at the damaged site (14, 15).

The platelet expresses a number of membrane proteins with specific binding capabilities for one or more of these adhesive matrix proteins. The initial interaction of platelets with the injured vessel wall occurs between GPIIb and immobilised vWf (16). This interaction initiates the

tethering of circulating platelets to the vessel wall. Platelets typically 'roll' over the vWf in the direction of flow driven by shear forces experienced by the vasculature (1). A loss of interaction between GPIb and vWf on one side of the platelet leads to the formation of another GPIb–vWf interaction on the other side of the platelet which gives rise to a rolling phenomenon. Eventually the platelet will come to a complete stop due to firm adhesion to the injured part of the vessel. This firm adhesion is typically mediated by several membrane receptors, some of which will have become activated as a result of platelet rolling. The adhesion to the extracellular matrix proteins is a result of several platelet receptor interactions; the collagen receptors  $\alpha_2\beta_1$  (17–19) and GPVI (20, 21), the fibronectin receptor  $\alpha_5\beta_1$  (22) and the fibrinogen receptor GPIIb/IIIa (23). Once firmly adhered, the platelets rearrange cytoskeletal components which results in flattening or spreading of the platelet, this is essential in order to withstand the shear forces experienced in the vasculature (1).

At this stage, platelets undergo the release reaction where they release the contents of its stored intracellular granules. These granules contain proteins such as P-selectin which mediates adhesion of platelets to monocytes, neutrophils and lymphocytes, resulting in the formation of platelet leukocyte complexes (24–26). The granules also contain many chemotactic agents which lead to the recruitment of various inflammatory cells; platelet derived growth factor (PDGF) and 12-hydroxyeicosatetraenoic acid (12-HETE) which recruit neutrophils (27, 28); platelet factor 4 and platelet derived histamine releasing factor (PDHRF) which recruit eosinophils in airway disease (29, 30); PDGF and transforming growth factor  $\beta$  (TGF- $\beta$ ) which recruit monocytes and macrophages and TGF- $\beta$  which recruits fibroblasts (31–33). Platelet granules also contain several mediators of tissue damage such as oxygen-free radicals and hydrolytic enzymes. Platelets also release cationic proteins that initiate vascular permeability and mediators that enhance aggregate formation such as adenosine diphosphate (ADP), serotonin (5-HT), Thromboxane A<sub>2</sub> (TxA<sub>2</sub>) and platelet activating factor (PAF; 34,35). More recently it has been shown that platelet granules contain many antimicrobial peptides such as beta-lysin, platelet microbial protein (PMP), neutrophil activating peptide (NAP-2), released upon activation normal T-cell expressed and secreted (RANTES) and fibrinopeptides A and B (36–40).

The flattened part of the platelet forms a new surface for additional platelets to adhere, predominantly through GPIIb/IIIa crosslinking adjacent platelets through a fibrinogen bridge, resulting in aggregate formation. The final step sees an effective plug at the site of injury that is reinforced by the conversion of fibrinogen to fibrin through the coagulation cascade (14).

## Role of platelets in infection

As well as their established role in haemostasis, there is growing evidence for a role for platelets in immunology. In fact it would appear that platelets have an important role to play in the response to infection. Clinical studies would suggest that serious infection is associated with thrombotic events (41, 42). Recent use of antibiotics was shown to be associated with myocardial infarction (43), although treatment of patients with myocardial infarction with antibiotics provided no benefit (44).

The oral bacterial burden has been shown to be associated with the extent of atherosclerosis (45, 46) and cardiovascular disease (47). Periodontal disease is also associated with ischaemic stroke (48, 49) and oral pathogens are also strongly associated with infective endocarditis (50).

## Mechanisms of platelet–bacterial interactions

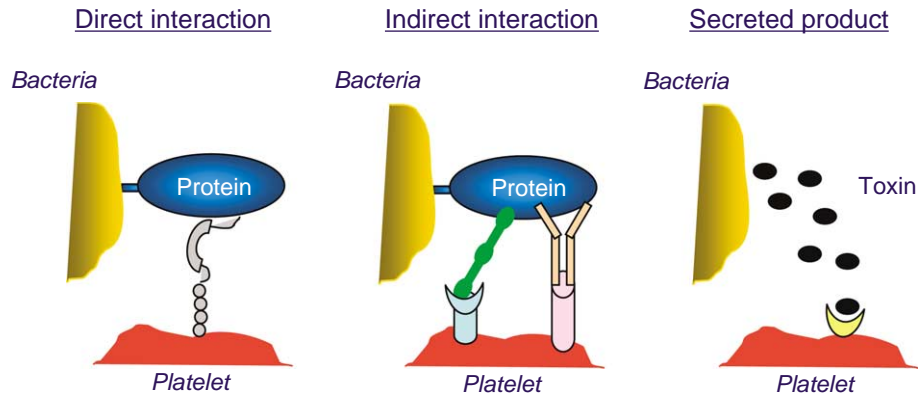
While it is clear that the increased oral bacteria burden is key in the increased risk of thrombotic events, it is not clear if this is due to increased inflammation leading to endothelial dysfunction (51) or is it due directly to the bacteria triggering thrombosis; however, as bacteria can trigger platelet activation, it is likely that this may play a role in triggering the thrombotic events (52).

### Direct interaction

Bacteria can have proteins that can directly interact with a surface receptor on the platelet (Fig. 1). In this case they have ligand-mimetic domains that act as agonists on the platelet receptor. One such direct interaction is that with *Streptococcus sanguinis* which can directly interact with glycoprotein Ib $\alpha$  (GPIb $\alpha$ ), the vWf receptor on the platelet (66). Other potential mediators of platelet activation are lipopolysaccharide (LPS) and lipoteichoic acid (LTA). LPS has been shown to activate platelets in a TLR4-dependent process (53–55), however, some studies have failed to see this platelet activation (56).

### Indirect interaction (bridging protein)

One of the more common mechanisms for interacting with the platelet is the use of a bridging protein (Fig. 1). In this case bacteria bind a plasma protein that is a natural ligand for a platelet receptor e.g. *Staphylococcus aureus*, which has multiple mechanisms for interacting with platelets using a bridging ligand. Clumping factor A and B (57) and fibronectin binding protein (58) on *S. aureus* can both bind fibronectin and/or fibrinogen both of which are ligands for GPIIb/IIIa on the platelet. *Helicobacter pylori* can bind vWf which interacts with GPIb $\alpha$  on the platelet (59). The most common bridging molecule for bacteria to use is IgG. IgG bound to the bacterial surface can interact with the platelet Fc $\gamma$ RIIa receptor and while it appears that this alone cannot stimulate platelet activation it acts in conjunction with other bridging molecules (52, 59, 60). In



**Fig. 1.** Direct interaction: bacteria express proteins that interact directly with a platelet surface receptor. In this case, they have ligand-mimetic domain that act as agonist on the platelet receptor. Indirect interaction: bacteria bind a plasma protein that acts as a bridge and is a natural ligand for a platelet receptor. Secreted products: bacteria also have the potential to secrete products that can in turn directly activate platelets.

the absence of a second bridging, molecule bound antibody can trigger complement formation which can mediate platelet activation via complement receptors in conjunction with the Fc $\gamma$ RIIa receptor (60–62).

#### Indirect interaction (secretion)

Bacteria also have the potential to secrete products that can in turn activate platelets (Fig. 1). *Porphyromonas gingivalis* secretes gingipain, an enzyme that activates the thrombin receptor on platelets which leads to platelet activation (63) and *Escherichia coli* shiga toxin is associated with platelet activation (64) via a novel platelet glycosphingolipid (65).

Bacterial-induced platelet aggregation is different in some respects to that seen with other platelet agonists. Bacterial-induced aggregation is an all-or-nothing response, in that no matter what concentration of bacteria are added to a platelet preparation, the extent of aggregation will always be maximal (often less than that seen with other agonists) or else there is no aggregation. Unlike other agonists there is a lag time to aggregation. Adjusting the concentration of bacteria shortens the lag time to a minimum, but never eliminates it. There appears to be two categories of bacteria: those that have a short lag time of around 2–5 mins, e.g. *S. aureus* and those with a long lag time of 15–20 mins, e.g. *S. sanguinis* or *Streptococcus gordonii*. The short lag time usually indicates the presence of a direct interaction and is dependent on the levels of expression of the interacting protein on the bacterial surface (60). The long lag time usually indicates a complement-dependent aggregation process.

The interaction of platelets with bacteria is not simply one of activation as bacteria are able to either support platelet adhesion or induce platelet aggregation and these are distinct phenomena involving different bacterial proteins. The adhesion process can occur under static

conditions or shear conditions and different proteins are often involved in each process (66).

#### Interaction of oral bacteria with platelets

Oral bacteria differ greatly in their ability to interact with platelets. Kerrigan and colleagues have proposed several different phenotypes for platelet interaction (67, 68). They identified strains of oral bacteria that support platelet adhesion and induce platelet aggregation with a short lag time (*S. sanguinis* strain 133–79) [(67), strains of bacteria that support platelet adhesion and induce platelet aggregation with a long lag time (*S. gordonii* strain DL1), strains that support platelet adhesion but do not induce platelet aggregation (*S. gordonii* strain Blackburn, *S. sanguinis* strain B5.7), strains that cannot support platelet adhesion but can induce platelet aggregation with a short lag time (*S. gordonii* strain Sk12), strains that cannot support platelet adhesion but can induce platelet aggregation with a long lag time (*S. gordonii* strain M99 and *S. sanguinis* strain 7863) and finally strains that cannot support platelet adhesion or induce platelet aggregation (*S. gordonii* strain M5 and *S. sanguinis* strain SK96) (68).

Several studies have now demonstrated that the platelet bacterial interaction may be a lot more complex than this model suggests. It appears that several bacteria have evolved in such a way that many interactions occur at the same time to mediate the same response. For example, it has been shown that *S. gordonii* uses three distinct interactions with the platelet to induce platelet aggregation, deletion of one protein interaction does not affect the aggregation, however, deletion of all three interactive proteins ablates the response. Furthermore, platelets exist in an environment where they are exposed to a range of shear stress. Several studies have characterised the interaction between platelet GPIb $\alpha$  and

vWf under high shear or between platelet GPIIb/IIIa and fibrinogen under low shear.

Other studies suggest that the virulence of several strains of *S. gordonii* in the rat model of infective endocarditis does not correlate with their ability to just interact with platelets. Several strains of *S. gordonii* that were shown to interact with platelets were resistant to polymorphonuclear (PMN) leukocyte-dependent killing (69). These results suggest that the ability of *S. gordonii* to survive in PMN's following phagocytosis may be another important virulence determinant to consider. Therefore, studying the interaction between bacteria, platelets and PMN's is essential to get a more physiological representation of the real picture.

### The interaction between streptococci and platelets

At sites of trauma in the oral cavity, streptococci can gain access to the bloodstream and interact with circulating platelets (70). Septic thrombi (oral streptococci encased in activated platelets) can be found at several sites in the circulatory system, for example, on heart valves, endocardium or indeed in atherosclerotic plaques.

Several approaches have been used to identify the factor responsible for inducing thrombus or aggregate formation (Table 1). Early studies demonstrated that bacteria can either secrete products capable of activating platelets or bind directly to platelets to induce an intracellular signal, either way the outcome is the same – aggregate formation.

*S. sanguinis* was the first oral bacteria shown to have the capability to induce platelet aggregation and to support platelet adhesion (67, 71, 72). *S. sanguinis*-induced platelet aggregation appeared to be dependent on calcium and fibrinogen and in some cases non-specific antibody, whereas platelet adhesion to *S. sanguinis* occurred independently of calcium, fibrinogen or non-specific antibody. This led to the initial classification of the response, a class 1 component-mediated adhesion to the platelet, a class 2 component mediates a calcium-dependent activation and a class 3 component amplifies the response.

Early studies identified components of *S. sanguinis* thought to play a role in binding to and activating platelets. Platelet-associated activating protein (PAAP) was first identified in 1990 (73). PAAP is a 115 kDa glycoprotein, which contains a collagen-like epitope and is constitutively expressed on the surface of *S. sanguinis* (74, 75). PAAP is possibly environmentally regulated during infection in response to high temperature (fever) or exposed collagen (exposed on damaged heart valves) (70). Partial sequence alignment shows homology to the heat shock family of proteins of *Mycobacterium tuberculosis* and *E. coli* (76). PAAP interacts with an unknown platelet receptor which is capable of transducing an intracellular signal leading to platelet activation. Several attempts that have been made to identify the platelet

receptor and potential candidates include the integrin  $\alpha_2\beta_1$  (77) or an integrin-associated protein of 175 kDa (78). As not all donors respond to *S. sanguinis*, it was reported that the ability of PAAP to induce platelet aggregation may be donor specific (79). Platelets release several mediators from stored granules when they become activated of which includes ATP. *S. sanguinis* has been shown to have ectoATPase activity and is capable of hydrolysing ATP to ADP (80, 81). There are several ADP receptors expressed on the platelets, therefore the conversion of ATP to ADP may serve as an amplification step essential for platelet aggregate formation.

Further classification of the streptococcal-induced aggregation response has been described by Kerrigan et al. Type 1 streptococci induce platelet aggregation with a short lag time (<5 mins), type 2 streptococcus induce platelet aggregation with a long lag time (10–15 mins) and finally type 3 streptococci fail to induce platelet aggregation (>20 mins). *S. sanguinis* strain 133–79 is an example of type 1 streptococcus and interacts with platelets via platelet receptor glycoprotein Ib $\alpha$  (the vWf receptor) (67). *S. sanguinis* binds to the N-terminal portion of GPIb $\alpha$  between amino acids 1 and 282. Platelets from patients with Bernard Soulier Syndrome (patients who fail to express GPIb $\alpha$  on the surface of their platelets) fail to interact with *S. sanguinis* (67). *S. sanguinis* lysates were passed through a GPIb $\alpha$  affinity column in order to identify the *S. sanguinis* protein binding to GPIb $\alpha$  (77). This led to the identification of a serine-rich glycoprotein called serine-rich protein A (SrpA). Deletion of SrpA failed to have any effect on the percent platelet aggregation, however, significantly prolonged the lag time. Furthermore, deletion of SrpA from *S. sanguinis* significantly reduced its ability to support platelet adhesion (82). These results highlight the complexity of the interactions between *S. sanguinis* and platelets as multiple proteins must be involved in supporting platelet adhesion and inducing platelet aggregation.

*S. sanguinis* strain NCTC 7863 is an example of a type 2 streptococcus. Ford et al. demonstrated that *S. sanguinis* strain NCTC 7863 induced aggregation of normal platelets suspended in plasma, removal of plasma protein abolished aggregation (62). The long lag time of *S. sanguinis* strain 7863 was progressively shortened by incubating the bacteria in plasma for increasing lengths of time prior to addition to platelets. Since the lag time was due to addition of plasma rather than donor platelets, it was suggested that the rate limiting step for platelet aggregation was assembly or binding of plasma factors on the bacterial cell surface. Subsequent experiments demonstrated that complement assembly on the surface of the bacteria was necessary for aggregation of platelets. Further experiments demonstrated that complement assembly was not enough to trigger platelet aggregation which led to the discovery that IgG and

fibrinogen was necessary to complete the aggregation process (61). More recently, McNicol and colleagues demonstrated that depletion of *S. sanguinis*-specific antibodies from plasma prevented platelet aggregation (83). Addition of antibodies led to rapid phosphorylation of the platelet antibody receptor, Fc $\gamma$ RIIa, occurred following *S. sanguinis* binding (84).

*S. gordonii* is a distinct but close relative of *S. sanguinis* and has been extensively examined for its interactions with platelets. Platelet binding to *S. gordonii* is predominantly mediated by the cell surface glycoprotein, glycosylated streptococcal protein B (GspB). This is a large protein of over 3,000 amino acids and has a predicted molecular weight of 286 kDa. It is heavily glycosylated, primarily with glucose and glucosamine in the cytoplasm and is transported to the cell surface via an accessory system comprising of the SecA2 and SecY2 proteins (85, 86). Sequence alignment demonstrates that GspB is a member of a highly glycosylated serine-rich family of proteins which include *S. gordonii* haemagglutinin streptococcal antigen (Hsa) (87, 88), *S. sanguinis* SrpA (82) and *Streptococcus parasanguinis* fimbria associated protein 1 (FAP1) (89). Hsa is a GspB homologue of *S. gordonii* that was originally characterised as a sialic acid binding haemagglutinin (90). Like GspB, Hsa has also been shown to bind to GPIIb $\alpha$  on human platelets (91). Interestingly, even though both GspB and Hsa bind sialic acid residues on the platelet GPIIb $\alpha$ , there are subtle differences in the binding specificities. For example, GspB specifically binds O-linked sialic residues and the membrane proximal mucin-rich core of GPIIb $\alpha$  (92), whereas Hsa specifically binds N-linked sialic residues on GPIIb $\alpha$  (92) and GPIIb/IIIa (91). Similar to the effect seen using the *S. sanguinis* SrpA mutant, deletion of either GspB or Hsa from *S. gordonii* had no effect on platelet aggregation, however, significantly reduced platelet adhesion (68). Thus, other factors are most likely involved in supporting platelet adhesion and inducing platelet aggregation.

Possibly the best characterised streptococcal adhesins are those of the antigen I/II family of proteins. Initially identified in *Streptococcus mutants*, antigens I/II have now been detected on most oral streptococcal species (93). In *S. gordonii*, the antigen I/II polypeptides have been designated SspA (172 kDa) and SspB (164 kDa). These polypeptides are oligospecific adhesins recognising multiple ligands such as collagen type 1 (94), beta 1 integrins (93), salivary agglutinin glycoprotein (gp-340; 95), as well as several other bacteria including *P. gingivalis*, *Candida albicans* and *Actinomyces naeshlundii* (96–98). Using a proteomic approach to identify differential cell wall protein expression between an aggregating strain of *S. gordonii* (DL1) and a non-aggregating strain of *S. gordonii* (Blackburn), Kerrigan and colleagues identified SspA and SspB as proteins differentially expressed (68). Deletion of SspA and SspB from *S. gordonii* DL1

induced platelet aggregation and supported platelet adhesion as normal. However, deletion of SspA and SspB along with Hsa abolished platelet aggregation and reduced platelet adhesion by 50%, similar to the Hsa mutant alone. Furthermore, overexpression of SspA and SspB in the non-platelet reactive surrogate host *Lactococcus lactis*, induced platelet aggregation but failed to support platelet adhesion (68). These results suggest that *S. gordonii*-induced platelet aggregation is a multifactorial event mediated by several surface proteins.

A major limitation in our current understanding in platelet–bacterial interactions stems from the fact that all of the previous studies have been carried out under static conditions. It has been argued in the literature that data obtained *in vitro* using static binding assays may not be relevant to the fluid dynamic environment encountered in the vasculature (99). There are a growing number of papers in the literature in the last number of years suggesting that the local fluid environment of the circulation critically affects the molecular pathways of cell–cell interactions (100, 101). Platelets perfused over immobilised *S. sanguinis* or *S. gordonii* interacted with a typical rolling behaviour followed by firm adhesion. Deletion of SrpA or Hsa abolished this rolling behaviour, suggesting that these proteins are essential for the initial contact of platelets with the bacteria (68, 82). Platelet rolling with subsequent firm adhesion is characteristic of the platelet interaction with vWf and collagen at the site of injury. Typically, platelets roll along immobilised vWf under high shear conditions. The high shear is necessary to induce a conformational change in vWf thereby making it recognisable by its corresponding receptor on platelets, GPIIb $\alpha$ . As platelets roll along immobilised *S. gordonii* or *S. sanguinis* under low shear conditions, it suggests that the bacterial proteins involved, Hsa or SrpA, are in a conformation already recognisable by platelet GPIIb $\alpha$  and possibly mediate the initial interaction leading to thrombus formation. Furthermore, deletion of Hsa from *S. gordonii* reduced thrombus formation in a catheterised rat model of infective endocarditis (102). The *S. sanguinis* or *S. gordonii* protein that mediates firm adhesion has yet to be identified.

*Streptococcus mitis* is less well characterised in its interaction with platelets. In 2001, Bensing et al. described two surface expressed proteins that were involved in binding platelets namely, PblA (107 kDa) and PblB (121 kDa; 103,104). The genes encoding these proteins reside in the temperate bacteriophage SM1, a member of the siphoviridae family. PblA and PblB are unusual because neither of the protein expresses homology to any other bacterial adhesins described, however, do resemble structural components of bacteriophages (104, 105). Typically, PblA and PblB are released from *S. mitis* are then capable of binding to the bacterial cell wall via the choline

**Table 1.** Identified molecular interactions between oral pathogens and platelets.

Bacterial pathogen	Bacterial factor	Platelet receptor
<i>S. sanguinis</i>	SrpA (82)	GPIb $\alpha$ (67)
	PAAP (73)	Unidentified
	Complement assembly (62)	Fc $\gamma$ RIIIa (83)
<i>S. gordonii</i>	GspB/Hsa (85–88)	GPIb $\alpha$ (92), GPIIb/IIIa (91)
	SspA/B (68)	Unidentified
<i>S. mitis</i>	PblA/B (103,104)	Unidentified
<i>S. mutans</i>	Rhamnose–glucose polymer (106)	Unidentified
<i>S. oralis</i>	Unidentified	Unidentified
<i>S. parasanguinis</i>	FAP (189)	Unidentified
<i>P. gingivalis</i>	Gingipains (110)	PAR1 and PAR4 (63)
	Hgp44 (111)	GPIb $\alpha$ ?

Note: SrpA, serine-rich protein A; PAAP, platelet aggregation-associated protein; GspB/Hsa, glycosylated streptococcal protein B/Haemagglutinin streptococcal antigen; FAP1, fimbria associated protein 1; GPIb $\alpha$ , glycoprotein Ib $\alpha$ ; GPIIb/IIIa, glycoprotein IIb/IIIa; PAR1/4, protease activated receptor 1/4.

residues. Once presented on the cell wall they are capable of mediating platelet binding and also enhance activation of platelet aggregation. Deletion of PblA or PblB reduced thrombus formation in an animal model of infective endocarditis, therefore suggesting that these proteins may be important for platelet deposition onto the infected valve (105).

*S. mutans* has been shown to induce platelet aggregation and support adhesion of human platelets via a soluble serotype-specific rhamnose–glucose polymer (106). This is a direct interaction with the platelets as *S. mutans* induced aggregation in a plasma-free environment in a rapid and saturable manner. Rhamnose polymers are also found in other glycopeptides such as ristocetin. Ristocetin is used to induce agglutination of platelets. The mechanism for ristocetin-induced agglutination involves the binding and bridging of the vWF to platelet GPIb $\alpha$ . Cleavage of the rhamnose tetrasaccharide of ristocetin abolished its ability to induce platelet aggregation in plasma. This suggests that the polymer rhamnose plays an important role in platelet aggregation. Furthermore, depletion of the rhamnose–glucose polymers from the cell wall of *S. mutans*, aggregation was reduced down to 50% of the level of the wild-type strain. These results suggest that the rhamnose backbone play an essential role in inducing platelet aggregation (106).

*Streptococcus oralis* and *S. parasanguinis* do support platelet adhesion, however, not as efficiently as *S. gordonii* or *S. sanguinis* (107, 108). *S. oralis* and *S. parasanguinis* express a protein homologous to the highly glycosylated serine-rich family of proteins, similar to *S. gordonii* Hsa and *S. sanguis* SrpA (109). The lack of efficient adherence to platelets may be due to the absence of the sialic acid-binding moieties necessary to interact with platelet GPIb $\alpha$ . Alternatively the protein may not be readily accessible on the cell surface, for example, if it is blocked

by other surface structures such as a capsule (109). Some strains of *S. oralis* and *S. parasanguinis* did induce platelet aggregation, however, the mechanisms involved have not been elucidated.

### The interaction between other oral bacteria and platelets

*P. gingivalis* contains small vesicles on the outside of their surface that have been shown to interact with many host cells. These vesicles contain factors that have cytotoxic, haemagglutinin, proteolytic and platelet activating activities. Pham et al. identified that gingipain-R was the factor responsible for inducing platelet aggregation (110). Loubakos et al. reported that two forms of purified gingipain-R (50 kDa RgpB and a 95 kDa RgpA) caused aggregation of human platelets with efficiency similar to thrombin through the activation of protease-activated receptors 1 and 4 (PAR1 and PAR4; 63). Naito and colleagues demonstrated that *P. gingivalis*-induced platelet aggregation depends on HagA-encoding genes that intragenically code for adhesins such as Hgp44 (111). Hgp44 adhesin on the bacterial cell surface is processed from haemagglutinin A by the Rgp and Kgp proteinases. Specific antibodies against *P. gingivalis* binds to the platelet antibody receptor Fc $\gamma$ RIIIa is also thought to be an essential step leading to platelet aggregation. Cross-reacting antibodies recognising platelet GPIb $\alpha$  prevented platelet aggregation, suggesting that *P. gingivalis* may be binding to GPIb $\alpha$ . More recent studies investigated the ultrastructural properties of *P. gingivalis*-induced platelet aggregation (112). This study demonstrated that platelets have the ability to engulf or phagocytose *P. gingivalis* during or after platelet activation thereby removing the bacteria from the circulation.

## Clinical implications

It is clear that oral bacteria especially *Streptococci* spp. and *P. gingivalis* are capable of triggering platelet activation. Thrombus formation plays a critical role in infective endocarditis, atherosclerosis, myocardial infarction and stroke, all of which have been associated with periodontal disease. Thus, it is likely that inflammation in the oral cavity allows oral bacteria to access the circulation. Once in the blood, they interact with platelets forming platelet–bacterial aggregates, which can bind to heart valves or interact with sites of atherosclerosis. In the case of a high level of infection, it can lead to septicaemia with systemic activation of platelets, which are cleared from the circulation leading to thrombocytopenia. Reducing bacterial load in the mouth is an important step to preventing thrombus formation, as lesser bacteria will be available to enter the circulation. Several strategies have been employed to address this in the form of oral healthcare programmes. However, in more severe cases root canal or tooth extraction may be more appropriate.

Conventional therapy focuses on the use of antibiotics to treat overt infection and as prophylaxis for patients at high risk, such as patients with damaged or replacement cardiac valves undergoing dental procedures. However, the increase in the incidence of antibiotic resistant strains of bacteria has made it more difficult to treat these infections. The latest guidelines from American Heart Association no longer recommend the prophylactic use of antibiotics except in those patients with very high risk of complications (113).

Targeting the platelet–bacterial interaction may also play an important role in the treatment and prevention of the serious complications of infection. Thus, if the interaction between streptococci and platelets could be prevented, the thrombotic complications of infection could be reduced. While septicaemia may still occur, it would prevent the formation of infected thrombi on the cardiac valve and the formation of septic emboli. It would also prevent the thrombocytopenia associated with sepsis.

There are two approaches to targeting the bacteria–platelet interaction: blocking the bacterial protein or the platelet receptor. The difficulty with blocking the bacterial proteins is that there are so many different proteins involved as each bacterial species uses a different protein and some use three or four different proteins. Targeting the platelet may be a more effective approach as all of the bacteria that activate platelets do so by interacting with three platelet receptors: GPIb, GPIIb/IIIa and FcγRIIIa. The FcγRIIIa receptor is a very promising target as recent reports in the literature suggest that FcγRIIIa acts as a co-signalling receptor for platelet membrane receptors. These reports demonstrate that inhibition of FcγRIIIa blocks downstream signal events upon engagement of either GPIIb/IIIa or GPIbα (114) thus preventing platelet activation. An added advantage is that unlike inhibition

of GPIbα or GPIIb/IIIa, inhibition of FcγRIIIa does not affect normal platelet function. As a result there is a lot of interest in developing small molecule inhibitors of FcγRIIIa. However, as the Fcγ receptor is a crucial player in leukocyte activation in the immune system, small molecule inhibitors may adversely affect the immune function of these cells. *In vivo* experiments will address this caveat.

When oral bacteria gain entry to the circulation as well as coming into contact with platelets, they also come into contact with polymorphonucleocytes such as neutrophils and monocytes. Recent studies have demonstrated that platelets and monocytes from patients with periodontitis are more sensitive to activation with oral bacteria over control patients (115). In addition, platelet complexes with neutrophils and monocytes bound more oral bacteria than uncomplexed neutrophils or monocytes (51). The net result of these platelet leukocyte complexes is cellular priming with increased release of inflammatory mediators and unwanted accelerated thrombosis at sites of vascular injury.

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There is no conflict of interest in the present study for any of the authors.

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