Dear Sir,

there is increasing evidence that systemic infections play a role in the pathogenesis of cardiovascular or cerebrovascular disorders (1). The incidence of myocardial infarction and stroke increases during acute infections (2), and several viral (3) and bacterial (4) infections are positive risk factors in susceptible individuals. Epidemiological, aetiological and/or laboratory studies have provided support for a link between infectious agents such as Coxsackie B (5), influenza (6), Chlamydia pneumoniae (7), Helicobacter pylori (8) and orally-derived infections (9, 10), and the incidence of coronary artery disease. Streptococcus sanguis is an oral organism which has been isolated from the circulation of individuals following dental extractions (11) and from atherosclerotic plaques (12). Consequently, platelet activation by S. sanguis has been postulated to provide the link between periodontal infections and atherothrombotic conditions (13).

S. sanguis induces platelet aggregation both in vitro and in vivo in a strain- and donor- dependent manner (14). Herzberg et al. have partially characterised the S. sanguis/platelet interaction, using primarily strain 2017–78, and suggest that platelet-activating strains of S. sanguis express a protein, termed PAAP, with homology to collagen (15). Platelet activation by S. sanguis strain 2017–78 is also dependent upon the interaction of IgG with its low affinity platelet receptor, FcγRIIA, in a thromboxane A2-mediated manner (16, 17). There is a concentration-dependent relationship between IgG binding to S. sanguis strain 2017–78 and the induction of aggregation, which in some, but not all, individuals accounts for the responsiveness of their platelets to the bacteria (18). In contrast, polymorphic variation in FcγRIIA does not determine donor response to S. sanguis (18). Other inherent properties of platelets or plasma may contribute to the variability in donor response.

One plausible contributor to donor variability that has not been considered previously is von Willebrand factor (vWF). This large multimeric glycoprotein (GP) promotes platelet adhesion at sites of vascular damage and stabilizes platelet-platelet interactions via interactions with collagen and with both the GP Ib/IX and GP Ibb3 receptors on the platelet surface. Given the structural homology between PAAP and collagen, and the capacity for collagen to bind to vWF, a role for vWF is possible. In the current study a role for vWF in donor/strain-dependent S. sanguis-induced platelet activation was examined.

Platelet aggregation in platelet-rich plasma (PRP) was determined by light transmission in response to three strains of S. sanguis, 2017–78, SK108a and SK112 (platelet:bacteria ratio of 1:2 [17]), in addition to the thrombin receptor activating peptide (TRAP; SFLLRN; 10 µM). The platelets of all donors (n=8) aggregated when challenged with either S. sanguis strain 2017–78 or the soluble agonist TRAP. In contrast, S. sanguis strain SK108a caused the aggregation of platelets in four of eight donors, while S. sanguis strain SK112 failed to produce aggregation in any of the donors (Table 1). These data are consistent with a previous report that strains 2017–78 and SK112 are “aggregating” and “non-aggregating” strains, respectively, whereas strain SK108a produces variable responses (18).

Platelets were suspended in homotypic vWF-depleted plasma (crysosupernatants) prepared by a freeze/thaw technique (19) yielding vWF antigen levels of <0.10 U/ml in seven of eight donors and of 0.35 U/ml in the final donor (Table 1). The levels of IgG binding to S. sanguis strain 2017–78 were measured and aggregation determined, as previously reported (17, 18). The level of IgG binding was similar in PRP (20.3 ± 1.5 µg IgG/mg bacteria; mean ± SEM, n=8) and cryosupernatants (20.6 ± 2.7 µg IgG/mg bacteria; mean ± SEM, n=8), and thus there was no loss of 2017–78-specific IgG during cryoprecipitation. In all individuals, platelet aggregation in response to S. sanguis strain 2017–78 was abolished (Table 1). Similarly, platelet aggregation in cryosupernatants was absent in response to S. sanguis strain SK108a, regardless of whether aggregation had occurred in PRP. No platelet aggregation was observed in response to S. sanguis strain SK112 in cryosupernatants, whereas normal responses to TRAP were retained in all donors (Table 1).

The platelets of a type 3 von Willebrand disease (vWD) patient were examined. This 19-year-old woman of Hispanic origin has a clinical history of recurrent epistaxis, menorrhagia and haemarthrosis. Laboratory findings included FVIII activity of 0.05 U/ml, vWF antigen of 0.02 U/ml and undetectable levels of vWF ristocetin co-factor. The PRP from the vWD patient aggregated in response to TRAP confirming that her platelets were
functionally active, but did not respond to S. sanguis strains 2017–78, SK108a or SK112. When the platelets of a normal donor, which responded to S. sanguis strain 2017–78 and TRAP in PRP, were resuspended in vWD patient plasma they retained responsiveness to TRAP but not to S. sanguis strain 2017–78. Thus the vWD plasma could support platelet aggregation to soluble agonists but not to the S. sanguis, confirming that a plasma component, absent from the vWD plasma, is essential for S. sanguis-induced platelet activation.

Previous studies examining potential roles of vWF and GPIb using other strains of S. sanguis have been inconclusive. For example both Ford et al. and Kerrigan et al., using strains NCTC7863 and 133–79, respectively, demonstrated that anti-GPIb antibodies inhibited platelet aggregation but in a vWF independent manner (20, 21). A role for GPIb was apparently supported in the latter study where platelets from a Bernard-Soulier patient did not respond to strain 133–79. This contradicted a previous study where strains 133–79 and 2017–78 caused the aggregation of Bernard-Soulier platelets (22).

The role of vWF in S. sanguis strain 2017–78-induced platelet activation remains speculative. Several studies have proposed that 2017–78 employs a “collagen-like” mechanism, suggesting that 2017–78 interacts with vWF in a manner similar to collagen inducing a conformational change in vWF, promoting its interaction with GPIb and thereby initiating platelet aggregation. Such a mechanism has been proposed for platelet activation in response to Staphylococcus aureus (23). A second possibility is that IgG, already shown to be important in 2017–78-induced aggregation, activates platelets and causes the conformational change in the α(3)β(3) integrin, allowing vWF binding to the platelet and consolidating aggregation. Although this scenario is possible, it is unclear why S. sanguis would be uniquely dependent on vWF for this function. A third possibility is that vWF serves to facilitate the interaction of S. sanguis with platelets, specifically at the level of the IgG and FcγRIIA. Interestingly, Kerrigan et al. suggested that 133–79-induced platelet activation is FcγRIIA-dependent but occurs in the absence of plasma proteins, including IgG (21).

Regardless of the mechanism, the current study demonstrates that vWF is involved in 2017–78-induced aggregation. Clearly there is considerable inter-strain variability as 2017–78 and 133–79 do not appear to share mechanisms of activation. Furthermore, it is possible that alterations, or polymorphisms, in either vWF or its receptor (GPIb) may account for the observed donor variability and may impact upon the susceptibility of patients with periodontal disease to arterial thrombosis (9, 10).

Acknowledgement
Ms. Eyer was the recipient of a summer studentship from the Network for Oral Research Training and Health.

References
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Table 1: Summary of aggregation responses to S.sanguis strains 2017–78, SK108a and SK112, and to thrombin receptor activating peptide (TRAP) in platelet-rich plasma (PRP: containing vWF) and homotypic cryosupernatants (Cryosup: vWF-depleted).

<table>
<thead>
<tr>
<th>Donor</th>
<th>vWF Ant. (U/ml)</th>
<th>2017–78</th>
<th>SK108a</th>
<th>SK112</th>
<th>TRAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRP</td>
<td>Cryosup PRP</td>
<td>PRP</td>
<td>PRP</td>
<td>PRP</td>
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<td>-</td>
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<td>-</td>
<td>+</td>
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<td>+</td>
</tr>
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<td>-</td>
<td>-</td>
<td>+</td>
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18. Except otherwise noted, all recipient platelets were determined by rocket electrophoresis.


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22. Soberay AH, Herzberg MC, Rudney JD, et al. Responses of platelets to strains of Streptococcus sanguis: findings in healthy subjects, Bernard-Soulier, Glanz-