Streptococcus gordonii Promotes Thrombosis Through Binding Sialoglycans on Platelet Receptor GPIbα in a Microfluidic Device

Ava M. Obenaus[1]*, Lesley Martínez Rodríguez[1], Dang Truong[2], Annie Ke[1], Martha Sim[4], Barbara Bensing[3], Paul Sullam[3], Junmei Chen[4], José A. López[4], Wendy E. Thomas[1], Nathan J. Sniadecki[1]

[1]University of Washington Seattle | [2]University of Washington Bothell | [3]University of California, San Francisco | [4]Bloodworks Northwest Research Institute

I. Introduction

- Infective endocarditis (IE) is initiated when platelets and bacteria form vegetations on the surface of cardiac valves.
- The Siglec-like, serine-rich repeat (SRR) glycoproteins of streptococci selectively bind to sialoglycans on the platelet adhesion receptor GPIbα.
- The pathogenesis of infective endocarditis is thought to be affected by sialoglycan selectivity; however, the specific mechanisms remain unclear.

II. Approach

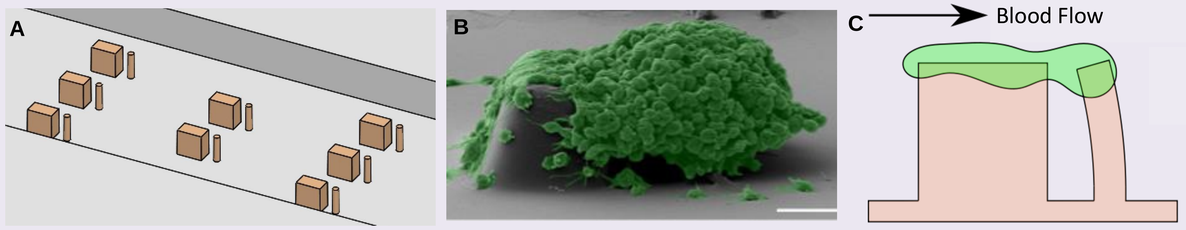


*Figure 1. **(A)** Isometric view of the microfluidic channel with 8 block and post structures. **(B-C)** As blood is perfused through the channel, the block creates a high shear gradient that allows platelets (green) to aggregate [1, 2].*

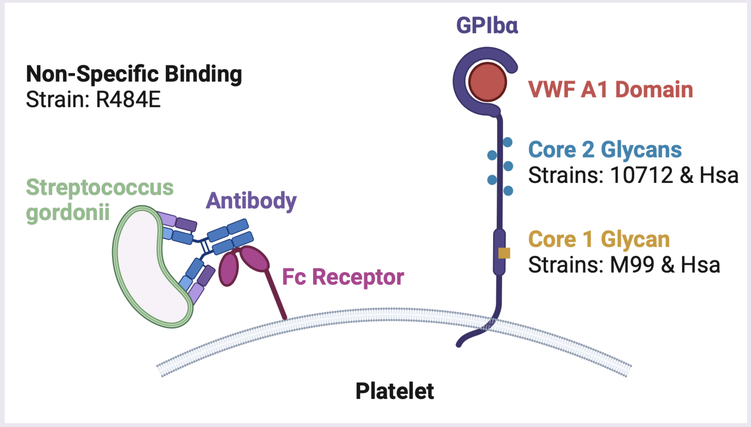


Fig. 2. Sialoglycans on platelet receptor GPIbα and their selectivity for *Streptococci gordonii* variants. Locations of the core 1 glycan and core 2 glycans are shown on GPIbα. *GspB is the wild type form of M99 and binds to Core 1 subtype O-glycans (C1) on GPIbα. The other strains are isogenic variants of GspB, in which the binding region of GspB has been replaced or mutated. 10712 preferentially binds to Core 2 subtype O-glycans (C2), while Hsa binds to both C1 and C2. R484E has a point mutation that disrupts sialoglycan binding to serve as a negative control; however, R484E can still bind non-specifically to platelets through the Fc receptor.*

- Herein, we investigate how *Streptococcus gordonii* affects thrombus formation using a novel **microfluidic device** that forms discrete platelet-aggregates under shear flow (Fig. 1A-C).
- We assessed the **impact of binding selectivity on thrombosis** using four isogenic variants of strain M99: GspB, R484E, Hsa, and 10712 (Fig. 2).
- Sialoglycan selectivity may also determine whether **streptococcal binding triggers GPIbα signaling**. GPIbα can activate platelets via mechanotransduction by unfolding the mechanosensory domain.

III. Results

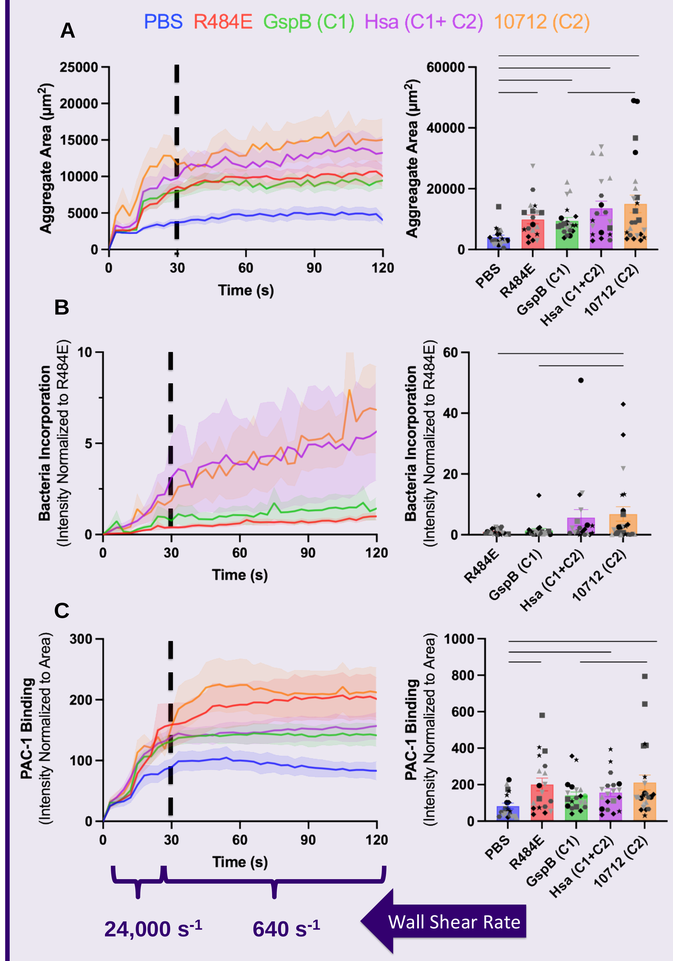
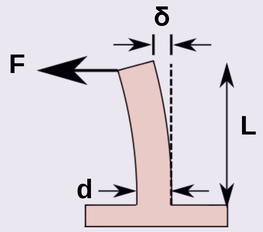


*Figure 3. The **(A)** aggregate area, **(B)** bacteria incorporation, and **(C)** PAC-1 binding are shown over time and 120 seconds after blood enters the channel. **(D)** Thrombi contractile forces are shown over time and at 180 seconds. Platelets encapsulate the top of the block and post. Post deflection (δ) was measured to calculate platelet-plug contractile force (F) using Hooke's Law ($F = k\delta$) where $k = 3\pi Ed^4/64L^3$, E is the modulus of elasticity, d is the diameter of the post, and L is the length of the post. [1,2] Each shape represents a unique donor (n = 7). Line indicates $p < 0.05$ when tested with a linear mixed effects model and pairwise comparisons.*



- All four M99 strains appear **to influence aggregate area** (Fig. 3A).
- 10712 produced thrombi that were significantly larger than GspB (Fig. 3A).
- While all four strains of bacteria were incorporated into the thrombi, **Hsa and 10712 had the highest degree of binding to the platelet-plugs** (Fig. 3B).
- **All four M99 strains increased platelet activation, but 10712 produced more activation than GspB** as seen by PAC-1 binding, an antibody that binds to activated integrin $\alpha_{IIb}\beta_3$ (Fig. 3C).
- All four M99 variants increased thrombi contractile forces. **Hsa produced thrombi that were significantly more contractile than GspB and 10712.**
- Taken together, these results suggest that **bacteria binding to core 2 sialoglycans leads to a higher degree of thrombosis**.

IV. Conclusions & Future Work

- Bacteria **increase platelet activation and aggregation**.
- *Stretococcus gordonii* **binding to core 2 sialoglycans on GPIbα leads to larger amounts of bacteria incorporation and appears to increase thrombosis.**
- Further investigation is needed to determine how thrombosis impacts **virulence** and **the progression of infective endocarditis**.
- We are actively investigating if the **glycoproteins** in the donor plasma impact bacteria binding via western blots.
- Additionally, we are studying **antibodies** in the donor plasma to determine their impact on non-specific bacteria binding via an ELISA assay.

aobenaus@uw.edu

References

[1] Ting et al., *Blood*, 2019

[2] Miles et al., *Blood Advances*, 2021

Funding

Research reported in this publication was supported by the National Heart, Lung, And Blood Institute of the National Institutes of Health under Award Numbers F31HL156697 and AI177473. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.