Antimicrobial Peptide-Incorporated Polymer Coatings Enhances the Efficacy of Platelet Storage

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Hypothesis

Hydrophilic AMP coupled coatings within PVC bags reduce bacterial load in the storage unit while maintaining biocompatibility with platelets.

Background

Transfusion Units

- Platelets are small, cell fragments activated upon vessel damage and are crucial for hemostasis through aggregation, coagulation, and vessel dilation.
- Platelet concentrate (PLT) transfusions are a lifesaving therapy for preventing or stopping bleeding in patients with low platelet counts or functional platelet disorders.

Current Storage Conditions

- Platelets are stored in plasticized poly(vinyl chloride) (PVC) bags at 22°C under gentle agitation for a maximum of 7 days.

Platelet Storage Lesion

- The hydrophobic PVC surface promotes platelet adhesion and activation.
- Platelet storage lesions (PSLs) lead to a decreased transfusion efficacy[1].

Bacterial Contamination

- Risk of bacterial contamination introduced during collection and exacerbated by storage conditions, which PVC bags cannot effectively prevent.
- Bacterial contamination decreases the safety of transfusion units if administered to patients.

Transfusion Unit Shortage

- 30% of PLT units are wasted in Canada, leading to frequent shortages and loss of collected platelet products[2].

Theory

Develop a hydrophilic, antimicrobial peptide (AMP) coupled coating for platelet storage bags to reduce hydrophobic interactions, decrease platelet adhesion and biofilm formation, preserve platelet viability during storage, and enhance the safety and efficacy of transfusions[3].

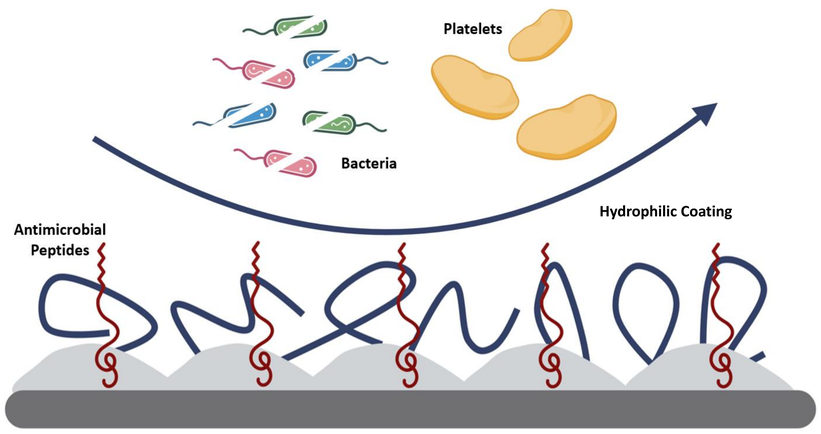


Figure 1. Schematic showing the covalent conjugation of AMPs (red) to the surface of PVC (grey). This design aims to inhibit biofilm formation and eliminate bacteria while preserving platelet health by maintaining platelets in their resting state.

Methods

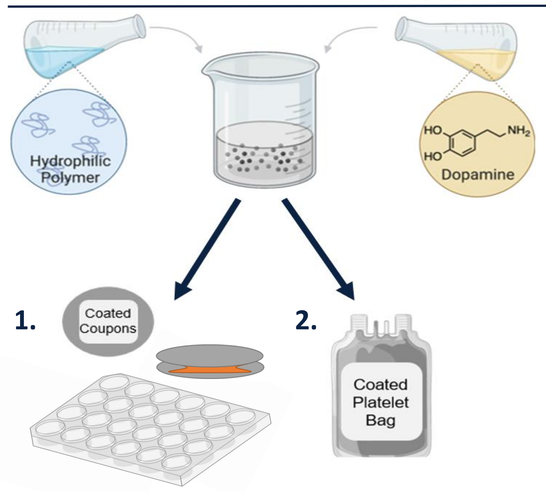


Figure 2. Overview of experimental apparatus where bags are coated via one-step deposition of poly(N,N-dimethylacrylamide) (PDMA) with polydopamine (15:1) to bind the hydrophilic PDMA layer to PVC. AMPs were conjugated using the same process. The protocol was conducted at two scales: **(1)** 1x1 cm PVC coupons placed in 24-well plates & **(2)** 5 mL PVC platelet storage bags, both maintained under standard platelet storage conditions.

Initial Antimicrobial Peptide Screening

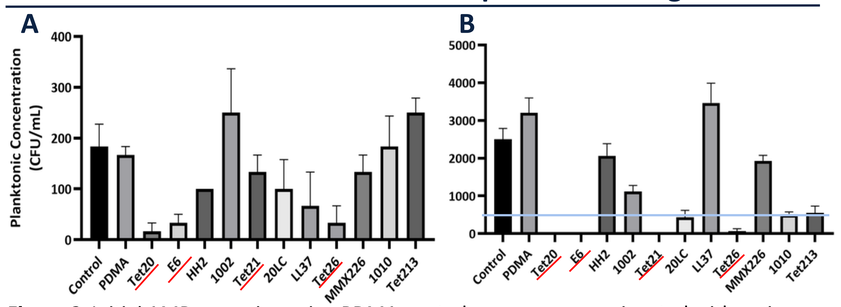
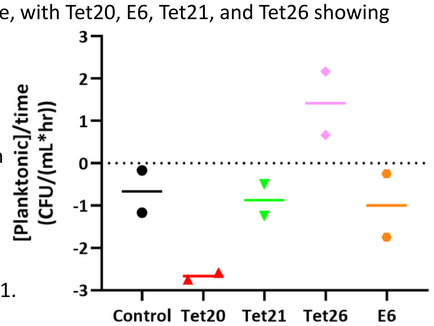


Figure 3: Initial AMP screening using PDMA-coated coupons are conjugated with various AMPs and tested against *Staphylococcus epidermidis* (N=3). Coupons are placed in a 24-well plate filled with Mueller Hinton Broth (MHB) and incubated for **(A)** 4h & **(B)** 24h, after which planktonic bacterial concentrations are measured. AMPs reducing concentrations <500 CFU/mL after 24h are considered effective, with Tet20, E6, Tet21, and Tet26 showing the best performance.

Figure 4: Bactericidal activity of the four most effective AMPs (right) conjugated on PDMA-coated coupons is incubated (24h) in platelet-rich plasma (N=3). Platelet units from Canadian Blood Services (CBS) are pooled from 8 donors to reduce variability. A positive slope for Tet26 indicates no reduction in bacterial concentration, while Tet20 achieves the fastest bacterial load reduction, followed by E6 & Tet21.



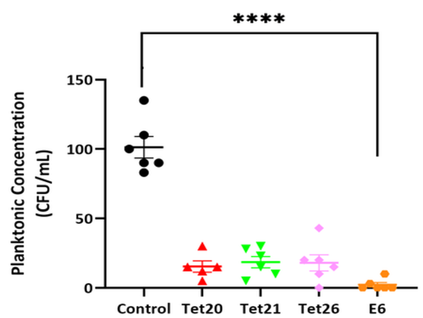


Figure 5: A sandwich apparatus of the PDMA-coated coupons is used for further assessment to better replicate the environment of a platelet storage bag as two coated sides of the bag is always in contact with the stored platelets (N=6). The E6 AMP significantly decreases the planktonic concentration compared to the control when incubated with platelets for 4h (left).

Platelet Quality

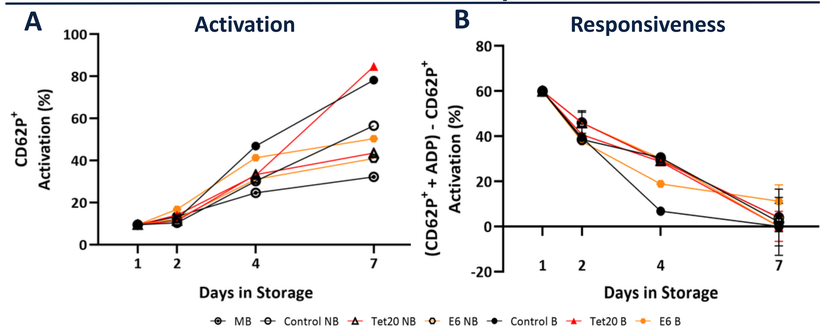


Figure 6: AMPs coupled on PDMA-coated 5 mL platelet bags are used to assess platelet quality on storage days 1, 2, 4, and 7 (N=3). **(A)** Platelet activation is measured by the expression of the surface marker P-selectin. **(B)** Platelet responsiveness is evaluated with adenosine diphosphate (ADP) induced P-selectin expression. E6 demonstrates minimal platelet activation and higher responsiveness throughout the storage period.

Biofilm Formation & Platelet Adhesion

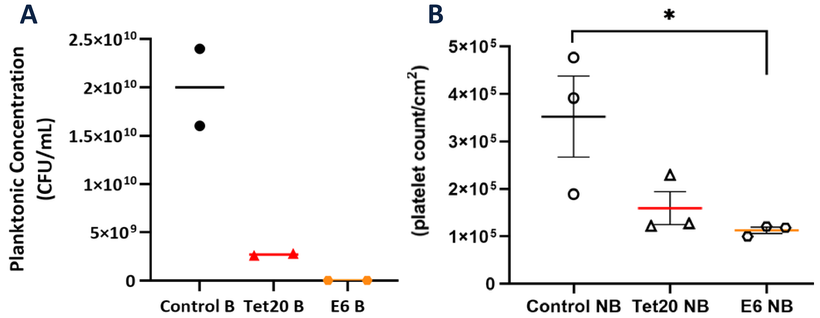


Figure 7: After 7 days of storage, the AMP-coupled polymer-coated surface of the 5 mL bags is used to assess adhesion. **(A)** Biofilm formation (N=2) is evaluated by measuring planktonic concentration, which is minimized by E6. **(B)** Platelet adhesion (N=3), quantified by surface area (cm²), is assessed using an LDH assay. E6 significantly reduces platelet adhesion ($p < 0.05$).

Conclusions & Future Directions

- At a 5 mL scale, hydrophilic E6 AMP-coupled coatings in PVC bags effectively reduce bacterial load while maintaining biocompatibility with platelets.
- Conduct additional iterations at the 5 mL scale using both E6 and Tet20.
- Determine the surface-to-volume ratio for the 5 mL scale.
- Apply findings to inform the design of a 15 mL scale-up.

References

1. Devine DV, Serrano K. The Platelet Storage Lesion. *Clin Lab Med*. 2010;30(2):475-487. doi:10.1016/j.cll.2010.02.002 **2.** Kron A, Vijenthira S, Pendergrast J, et al. Multicenter observational study evaluating the impact of platelet transport bags on product wastage. *Transfusion (Paris)*. 2021;61(5):1383-1388. doi:10.1111/trf.16303 **3.** Zou Y, Lai BFL, Kizhakkedathu JN, Brooks DE. Inhibitory Effect of Hydrophilic Polymer Brushes on Surface-Induced Platelet Activation and Adhesion. Macromol Biosci. 2010;10(12):1432-1443. doi:10.1002/mabi.201000223

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