Lyophilized Human Platelets Interact with Fresh Platelets to Promote Hemostasis Under Shear *In Vitro*

B. Ishler [1], A. Lee [1], S. Torramade-Moix [2], S. Samambar [2], M. Diaz-Ricart [2], G. Escolar [2] and K. Moskowitz [1]

[1] Cellphire Therapeutics, Inc., Rockville, MD

[2] Hematopathology, Pathology Department, CDB, Hospital Clinic Barcelona, IDIBAPS, University of Barcelona, Spain

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INTRODUCTION

Lyophilized human platelets (LHP) are a stabilized platelet derived hemostatic agent under clinical development as Thrombosomes® for treatment of bleeding secondary to thrombocytopenia.

AIMS

To evaluate mechanisms of LHP under shear forces and the potential role of interactions between LHP and fresh platelets during thrombus formation.

METHODS

- Fibrinogen binding to LHP GPIIb/IIIa was assessed by flow cytometry.
- LHP thrombus formation under shear was quantified with the Total Thrombus-formation Analysis System (T-TAS) using microcapillary channels coated with collagen and tissue factor. Thrombus formation was assessed in plasma before and after the addition of LHP (375x10^3 particles/µL).
- LMWH-anticoagulated whole blood at 75x10^3 platelets/µL was perfused over collagen and tissue factor coated microfluidic channels with and without LHP. Triple-color confocal microscopy imaging was applied to differentiate platelets from LHP and assess adherent cells and fibrin interactions.
- Association of LHP and fresh platelets was assessed by aggregometry, and fluorescent microscopy was performed on mixed samples with(out) GPIIb/IIIa antagonism.

RESULTS

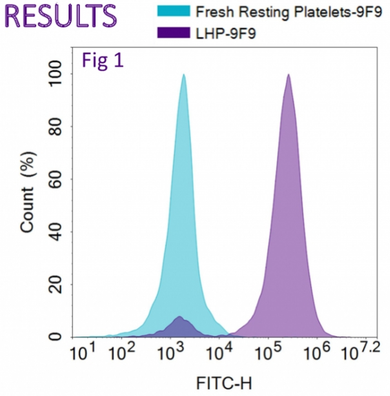

Figure 1: LHP show increased amounts of surface bound fibrinogen relative to fresh platelets after staining with a FITC-conjugated anti-fibrinogen antibody (clone 9F9).

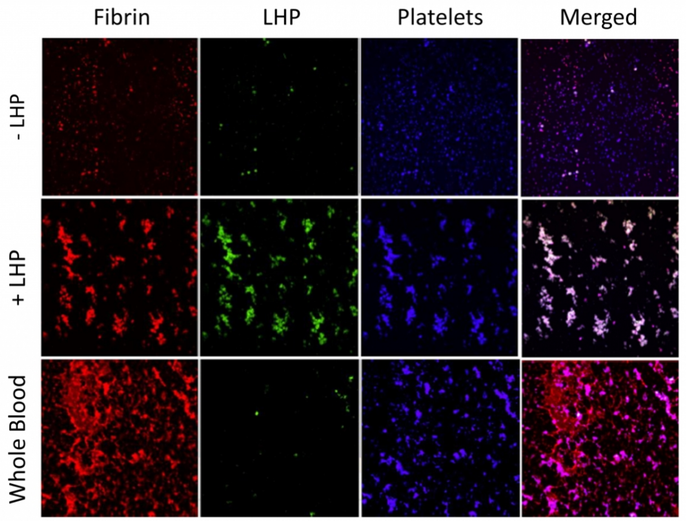

Figure 2: Thrombocytopenic whole blood was perfused over microcapillary channels coated with collagen and tissue factor in the presence and absence of LHP. Triple-color confocal microscopy showed LHP adhering to the capillary and enhancing fibrin and native platelet deposition on the collagen surface.

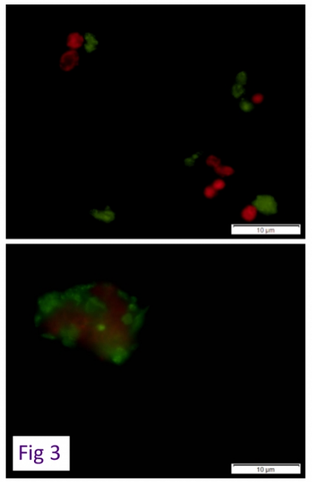

Figure 3: Mixed samples of platelets (red) and LHP (green) were imaged before (top) and after (bottom) stimulation with thrombin. Representative images show the formation of mixed aggregates after platelet stimulation.

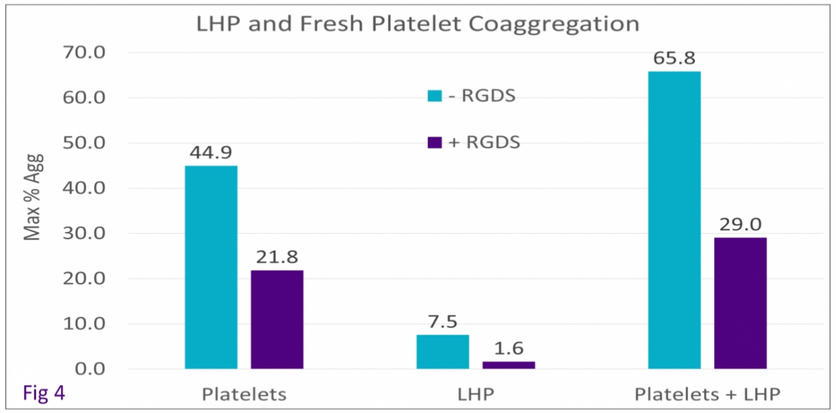

Figure 4: Interactions between fresh platelets and LHP were assessed using light transmittance aggregometry. Samples containing platelets, LHP, or a combination of the two were stimulated with Phorbol myristate acetate. Preventing fresh platelet GPIIb/IIIa activation with RGDS peptide prior to agonist stimulation reduced interactions between fresh platelets and LHP.

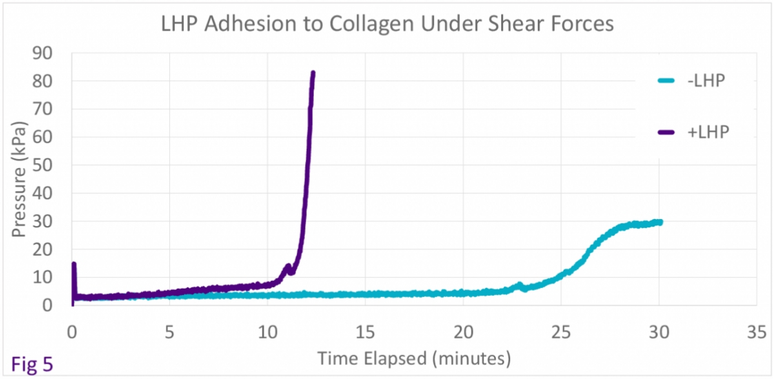

Figure 5: Thrombus formation under shear was measured using the T-TAS in collagen and tissue factor coated microcapillary channels. Adhesion was tracked by monitoring pressure increases over time for plasma samples with and without LHP. Plasma alone did not cause occlusion. LHP in plasma at a concentration of 375x10^3 particles/µL reach total thrombus formation at approximately 12 minutes.

CONCLUSIONS

- LHP adhere to collagen and interact with fresh platelets under shear, likely mediated by LHP surface fibrinogen.
- LHP further enables hemostasis in thrombocytopenic blood.
- LHP may thus function as a hemostatic agent by adhering to sites of injury leading to secondary interactions with circulating platelets and final fibrin formation.

CONFLICTS OF INTEREST

B. Ishler and K. Moskowitz are employees of Cellphire, Inc. holding stock and/or stock options.

CONTACT INFORMATION

Presenting author may be reached at bishler@cellphire.com. Additional information about Cellphire may be found at www.cellphire.com.