TREHALOSE STABILIZED FREEZE DRIED HUMAN PLATELETS, THROMBOSOMES®, EXPRESS SURFACE MARKERS, THROMBOELASTOGRAM (TEG) VALUES AND SIZE DISTRIBUTION SIMILAR TO TWO TO THREE DAY OLD STORED PLATELETS

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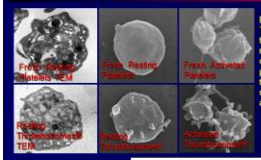
P-0453

ABSTRACT

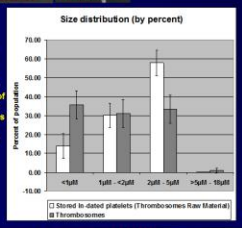
Thrombosomes® is a lyophilized derivative of human platelets developed as a hemostatic product for treatment of civilian as well as combat related trauma and shock conditions associated with life-threatening bleeding. Thrombosomes® is produced by a proprietary process that includes trehalose, other carbohydrates, and a custom lyophilization process. Thrombosomes® preserves several important characteristics including: 1. preservation of morphology and intracellular organelles; 2. particle sizes and distribution similar to in-date stored platelets. 3. expression of adhesion molecules such as GPIb, GPIa, GPIIb/IIIa; and 4. activation potential as evidenced by enhanced Annexin V binding. Thrombosomes® maintains certain platelet functions such as: 1. adhesion to matrix proteins under physiological flow rates and shear, in vitro; 2. generation of thrombin (FIIa) when added to plasma in-vitro; 3. aggregation in response to stimulation by thrombin (FIIa) in an in-vitro buffer system; and 4. enhanced clot formation in thrombocytopenic plasma measured by thromboelastography. In in-vivo models of uncontrolled bleeding, such as external injury produced over a background of busulfan-induced thrombocytopenia (rabbit) and arterial cut (rat), Thrombosomes® has shown efficacy in reducing bleeding and increasing survival time, respectively (P-0452). Thrombosomes® provides a unique hemostatic profile which when combined with its long-shelf life at ambient conditions and durability in extreme conditions, could meet a critical unmet medical need for the treatment of life-threatening bleeding conditions in diverse circumstances and especially where fresh platelets are not available.

MORPHOLOGY and SIZING



Electron micrographs, TEM and SEM, show that platelet morphology and organelles are preserved, as well as the capability to undergo shape change after activation.

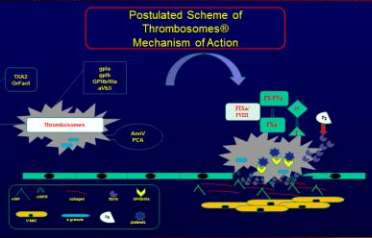
The lyophilization and rehydration process resulted in an increase in the sub-micron sized population of ~20%, a decrease in the 1-5 micron (platelet sized) population of ~22% and a slight increase of particles > than 5 microns in size, <1% when compared to in-date stored platelets.



SURFACE MARKERS

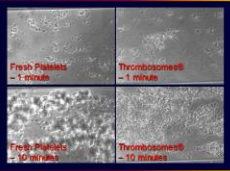
	GPIb	IIbIIIa	Annexin V resting	Annexin V active
Fresh Platelets Percent Positive	98.0	99.6	4.1	48.1
± 1 SD	1.69	0.48	1.17	10.33
Thrombosomes Percent Positive	45.01	98.69	85.01	88.13
± 1 SD	21.24	0.61	10.74	9.88

Flow cytometry analysis reveals that ~45% of GPIb survives the lyophilization and rehydration process, IIbIIIa is completely preserved, and Annexin V binding is 80-90% positive, indicating membrane change and PS exposure.



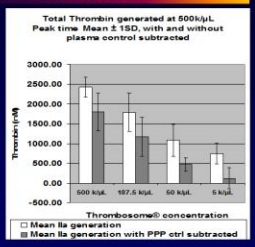
ADHESION

- Adhesion of activated platelets to exposed matrix proteins is the body's first step for initiation of local clot formation.
- Thrombosomes® under physiological shear conditions will adhere to collagen coated glass microchannels.
- Thrombosomes® demonstrated rapid and sustained adhesion and accumulation comparable to fresh platelets.



THROMBIN GENERATION

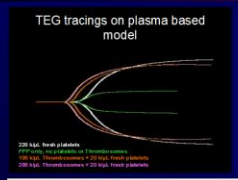
- Once adhered, Thrombosomes® should be able to generate thrombin leading to fibrin deposition at the site of injury
- Thrombin generation by Thrombosomes® has been confirmed using DiaPharma / Technoclone's TGA assay. (in vitro, in plasma)
- Thrombin generation by Thrombosomes® displays a concentration dependant response with high kinetic capacity at equivalent physiological (platelet) levels.
- The addition of Thrombosomes® to PPP resulted in an approximately 300-400% increase in thrombin production.

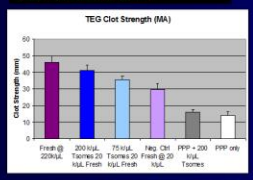


AGGREGATION

- Thrombosomes® is capable of adhering not just to ECM proteins like collagen, but also to each other in response to stimulus from thrombin.
- Helena AggRam optical aggregometer is used to observe an aggregation response of approximately 80% in a buffer based system, comparable with freshly drawn or stored in-dated platelets.

THROMBOELASTOGRAM (TEG)





- TEG measures the kinetics and strength of clot formation throughout the clotting process.
- Thrombosomes® was assayed in a plasma based system using pooled normal plasma.
- Thrombosomes® demonstrated the ability to significantly restore all parameters of clot kinetics in the presence of a priming concentration of platelets (20K/µL) in a concentration dependent manner.
- Thrombosomes® in the absence of a minimal amount of fresh platelets (20K/µL) do not significantly restore TEG parameters.

MATERIALS and METHODS

- Thrombosomes® is produced by a proprietary process involving trehalose and other carbohydrates using a custom lyophilization cycle.
- Sizing characterization is measured by the forward-scatter parameter on FACS, using multicolor-labeled flow cytometry and was validated against a Multisizer III with NIST standard sizing beads.
- Sizing characterization used FITC IbIIIa (IOTest) and PE Annexin V or P-Selectin (BD) to detect cells and screen out non-platelet/Thrombosomes® derived particles, with FITC and PE Isotypes used to standardize settings.
- FITC labeled monoclonal antibodies were used to detect critical platelet surface markers GPIb, (BD) IbIIIa, (IOTest) and P-selectin (BD), FITC labeled Annexin V (BD) was used to detect phosphotidylserine presentation at the lipid membrane, a nidus for procoagulant activity. Percent positive values were calculated against isotype controls.
- In-vitro adhesion studies using the "BioFlux" (Fluxion, San Francisco, CA) were performed with fresh platelets and Thrombosomes at equivalent physiological concentrations in pooled normal plasma at a shear rate of 50 dynes/cm². Flowcells were coated with collagen (Helena) or uncoated as a negative control.
- Thrombin generation was measured in DiaPharma's fluorogenic TGA assay at four concentrations in filtered (0.2µM) pooled normal plasma. Results were read on a Tecan Safirell plate reader and calculated in Excel.
- The modification of the TEG assay using pooled normal plasma in the place of whole blood was developed and validated in order to assay Thrombosomes® in a standard, consistent manner.

CONCLUSIONS

- Thrombosomes®, a lyophilized platelet derived biological product, demonstrates essential functionalities needed to generate blood clots and act as a hemostatic agent. These properties have been shown in various in vitro systems (adhesion, thrombin generation and clot enhancement) and ultimately in experimental models of uncontrolled bleeding. POSTER P-0452
- In addition, Thrombosomes® is safe at efficacious and excess loading doses as shown in GLP safety studies in canine and rabbit models. POSTER P-0454
- We propose that Thrombosomes® could fulfill an important unmet medical need in treating civilian and combat casualties where uncontrolled bleeding leads to life threatening situations and death.