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(71) Demandeur/Applicant:
CELLPHIRE, INC., US

(72) Inventeurs/Inventors:
MOSKOWITZ, KEITH ANDREW, US;
ISHLER, BRADEN CARL, US;
DICKERSON, WILLIAM MATTHEW, US;
TANDON, NARENDRA NATH, US;
LEE, AMBER NICOLE, US

(74) Agent: SMART & BIGGAR LLP

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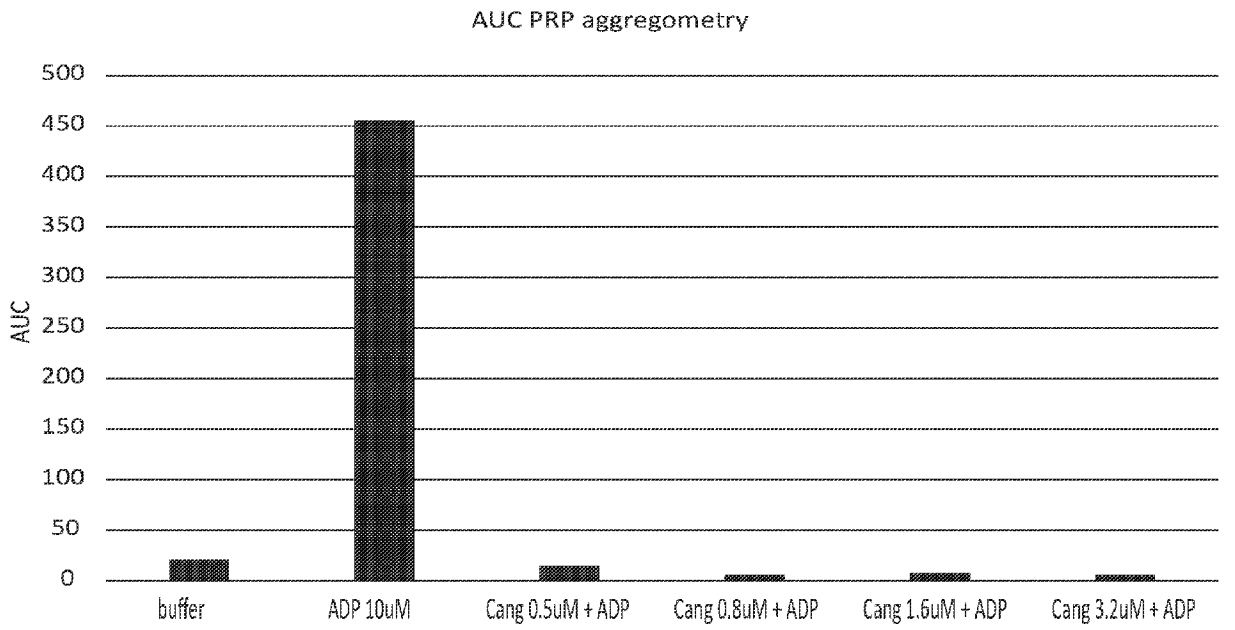

Figure 1

(57) Abrégé/Abstract:
In some embodiments provided herein is a method of treating a coagulopathy in a subject, the method including administering to the subject in need thereof an effective amount of a composition including platelets or platelet derivatives and an incubating agent including one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent, wherein the subject has been treated or is being treated with an antiplatelet agent.

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(71) Applicant: **CELLPHIRE, INC.** [US/US]; 9430 Key West Avenue, Rockville, Maryland 20850 (US).

(72) Inventors; and
(71) Applicants: **MOSKOWITZ, Keith Andrew** [US/US]; 14974 Dawnhaven Drive, Westfield, Indiana 46074 (US). **ISHLER, Braden Carl** [US/US]; 410 Blue Silk Lane, Apt H, Gaithersburg, Maryland 20879 (US). **DICKERSON, William Matthew** [US/US]; 1401 Church St. NW #311, Washington, District of Columbia 20005 (US). **TANDON, Narendra Nath** [US/US]; 7102 Strators Lane, Gaithersburg, Maryland 20189 (US). **LEE, Amber Nicole** [US/US]; 1747 Yale Pl., Rockville, Maryland 20850 (US).

(74) Agent: **MAIZE, Kimberly M.** et al.; Fish & Richardson P.C., P.O. Box 1022, Minneapolis, Minnesota 55440-1022 (US).

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(54) **Title:** THROMBOSOMES AS AN ANTIPLATELET AGENT REVERSAL AGENT

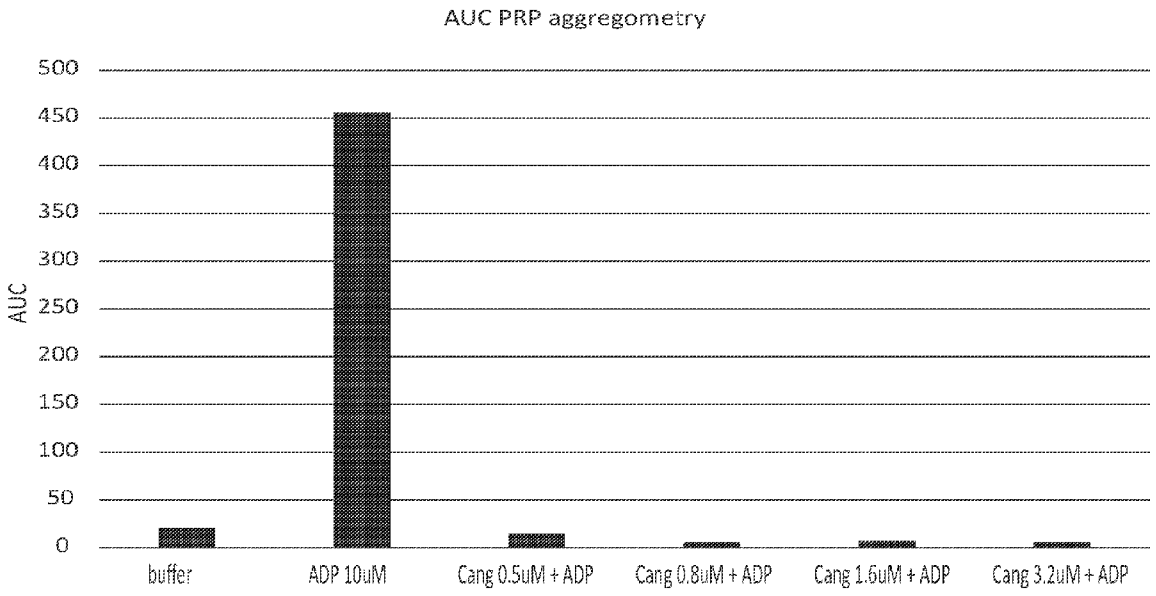

Figure 1

(57) **Abstract:** In some embodiments provided herein is a method of treating a coagulopathy in a subject, the method including administering to the subject in need thereof an effective amount of a composition including platelets or platelet derivatives and an incubating agent including one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent, wherein the subject has been treated or is being treated with an antiplatelet agent.

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THROMBOSOMES AS AN ANTIPLATELET AGENT REVERSAL AGENT

CROSS-REFERENCE TO RELATED APPLICATIONS

[001] This application claims priority to U.S. Provisional Application Serial No. 62/887,923, filed on August 16, 2019 and U.S. Provisional Application Serial No. 63/065,337, filed on August 13, 2020, each of which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[002] This disclosure serves to describe the use of thrombosomes as a treatment for drug-induced coagulopathy. The use of antiplatelet drugs such as aspirin or clopidogrel can result in increased bleeding potential. Here we demonstrate that thrombosomes can circumvent or overcome this inhibition to restore hemostasis.

BACKGROUND

[003] Antiplatelet drugs (also herein called antiplatelet agents) are common in the U.S. adult population and employ multiple mechanisms of inhibiting platelet action. Antiplatelet drugs are used to treat and/or prevent a number of cerebrovascular and cardiovascular diseases

[004] Antiplatelet drugs, however, are responsible for many adverse drug-related events (ADEs). Overdose and adverse events related to these drugs carry the risk of serious bleeding and related complications in the patient population. In addition, subjects treated with antiplatelet drugs face additional complications for surgery, as a subject may need to be tapered off the drugs before surgery, though cessation of therapy could put the subject at an increased risk for heart attack, stroke, or death.

[005] There is therefore a need in the art for the treatment of coagulopathy, such as antiplatelet agent-induced coagulopathy, as well as a need for a solution for preparing subjects taking an anti-platelet drug for surgery.

SUMMARY OF THE INVENTION

[006] Provided herein in some embodiments is a method of treating a coagulopathy in a subject, the method including administering to the subject in need thereof an effective amount of a composition including platelets or platelet derivatives and an incubating agent including one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent.

[007] In some embodiments, provided herein is a method of treating a coagulopathy in a subject, the method including administering to the subject in need thereof an effective amount of a composition prepared by a process including incubating platelets with an incubating agent including one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent, to form the composition.

[008] In some embodiments, provided herein is a method of restoring normal hemostasis in a subject, the method including administering to the subject in need thereof an effective amount of a composition including platelets or platelet derivatives and an incubating agent including one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent.

[009] In some embodiments, provided herein is a method of restoring normal hemostasis in a subject, the method including administering to the subject in need thereof an effective amount of a composition prepared by a process including incubating platelets with an incubating agent including one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent, to form the composition.

[0010] In some embodiments, provided herein is a method of preparing a subject for surgery, the method including administering to the subject in need thereof an effective amount of a composition including platelets or platelet derivatives and an incubating agent including one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent. Implementations can include one or more of the following features. The surgery can be an emergency surgery. The surgery can be a scheduled surgery.

[0011] In some embodiments, provided herein is a method of preparing a subject for surgery, the method including administering to the subject in need thereof an effective amount of a composition prepared by a process including incubating platelets with an incubating agent including one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic

solvent, to form the composition. Implementations can include one or more of the following features. The surgery can be an emergency surgery. The surgery can be a scheduled surgery.

[0012] In some embodiments of the above methods, the subject has been treated or is being treated with an antiplatelet agent. In some embodiments, treatment with the antiplatelet agent can be stopped. In some embodiments, treatment with the antiplatelet agent can be continued.

[0013] In some embodiments, provided herein is a method of ameliorating the effects of an antiplatelet agent in a subject, the method including administering to the subject in need thereof an effective amount of a composition including platelets or platelet derivatives and an incubating agent including one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent.

[0014] In some embodiments, provided herein is a method of ameliorating the effects of an antiplatelet agent in a subject, the method including administering to the subject in need thereof an effective amount of a composition prepared by a process including incubating platelets with an incubating agent including one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent, to form the composition.

[0015] In some embodiments, the effects of the antiplatelet agent can be the result of an overdose of the antiplatelet agent.

[0016] In some embodiments, the antiplatelet agent can be selected from the group consisting of aspirin, cangrelor, ticagrelor, clopidogrel, prasugrel, eptifibatide, tirofiban, abciximab, and a supplement.

[0017] Some embodiments of any of the methods herein can include one or more of the following features. Administering can include administering topically. Administering can include administering parenterally. Administering can include administering intravenously. Administering can include administering intramuscularly. Administering can include administering intrathecally. Administering can include administering subcutaneously. Administering can include administering intraperitoneally. The composition can be dried prior to the administration step. The composition can be rehydrated following the drying step. The composition can be freeze-dried prior to the administration step. The composition can be rehydrated following the freeze-drying step. The incubating agent can include one or more salts selected from phosphate salts, sodium salts, potassium salts, calcium salts, magnesium salts, and

a combination of two or more thereof. The incubating agent can include a carrier protein. The buffer can include HEPES, sodium bicarbonate ($NaHCO_3$), or a combination thereof. The composition can include one or more saccharides. The one or more saccharides can include trehalose. The one or more saccharides can include polysucrose. The one or more saccharides can include dextrose. The composition can include an organic solvent. The platelets or platelet derivatives can include thrombosomes.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] **Figure 1** shows transmission light aggregometry of cangrelor ("Cang") in platelet rich plasma, expressed as the integrated aggregation curve, induced by 10 μM adenosine diphosphate (ADP) activation with and without increasing concentrations of cangrelor.

[0019] **Figure 2** shows the effect of cangrelor, ADP, or a combination thereof on platelet occlusion using T-TAS® technology.

[0020] **Figure 3** is a bar plot of the area under the curve (AUC) for data sets from Figure 2. Replicate data sets from Figure 2 are presented as averages.

[0021] **Figure 4** is a bar plot of the occlusion time for data sets from Figure 2. Replicate data sets from Figure 2 are presented as averages.

[0022] **Figure 5** shows the effect of thrombosomes ("thromb"; 300,000/μL) supplemented to platelet rich plasma in the presence and absence of ADP and cangrelor, at 60, 90, or 115 minutes post-rehydration on platelet occlusion using T-TAS® technology.

[0023] **Figure 6** is a bar plot of the AUC for data sets from Figure 5. Replicate data sets from Figure 5 are shown as averages.

[0024] **Figure 7** is a bar plot of the occlusion time for data sets from Figure 5. Replicate data sets from Figure 5 are shown as averages.

[0025] **Figure 8** is a bar plot of the AUC from aggregation experiments for platelets (at a concentration of 250,000 platelets per μL) treated with collagen (10 μg/mL) and various concentrations of eptifibatide ("Epti").

[0026] **Figure 9** shows the effect of eptifibatide at various concentrations on whole blood using T-TAS® technology.

[0027] **Figure 10** shows the effect of thrombosome ("Tsomes") supplementation (approximately 200,000/μL) on whole blood with and without various concentrations of eptifibatide using T-TAS® technology.

[0028] **Figure 11** is a bar plot of the occlusion time for the data sets from Figure 10.

[0029] **Figure 12** is a bar plot of the AUC for the data sets from Figure 10.

[0030] **Figure 13** shows that thrombosomes (various lots) occlude in the presence of eptifibatide in platelet-poor plasma (PPP).

[0031] **Figure 14** is a bar plot of the AUC for data sets from Figure 13. Replicate data sets from Figure 13 are shown as averages.

[0032] **Figure 15** is a bar plot of the occlusion time for the data sets from Figure 13. Replicate data sets from Figure 13 are shown as averages.

[0033] **Figure 16** is a bar plot of the AUC from aggregation experiments for platelets treated with collagen (10 μg/mL) or arachidonic acid ("AA"; 500 μg/mL) with and without various concentrations of aspirin ("ASA").

[0034] **Figure 17** is a bar plot of the occlusion time for whole blood, whole blood treated with various concentrations of aspirin (ASA), and whole blood treated with various concentrations of aspirin and supplemented with thrombosomes (approximately 200,000-400,000/μL) as measured by response to collagen coated plastic under shear using T-TAS® technology.

[0035] **Figure 18** shows the recovery of thrombus formation promoted by thrombosomes in whole blood in the presence of ASA (200 micromolar), cangrelor (1 micromolar), AP2 6F1 (40 micrograms), as measured by occlusion time on the T-TAS AR chip coated with thromboplastin and collagen.

[0036] **Figure 19** shows the recovery of thrombus formation promoted by thrombosomes in whole blood in the presence of ASA (200 micromolar), cangrelor (1 micromolar) and 6F1 (40 micrograms/mL), as measured by occlusion (pressure) over time.

[0037] **Figure 20** shows the effect of thrombosomes supplementation to aspirin-(ASA-)inhibited whole blood (500 micromolar) on the interaction with plastic immobilized porcine collagen under high shear, as measured by AUC.

[0038] **Figure 21** shows the effect of thrombosomes supplementation to aspirin-(ASA-)inhibited whole blood (500 micromolar) on the interaction with plastic immobilized porcine collage under high shear, as measured by occlusion (pressure) over time.

[0039] **Figure 22** shows the effect of thrombosomes supplementation to aspirin-(ASA-)inhibited whole blood (100 micromolar) on the interaction with plastic immobilized porcine collage under high shear, as measured by AUC.

[0040] **Figure 23** shows the effect of thrombosomes supplementation to aspirin-(ASA-)inhibited whole blood (100 micromolar) on the interaction with plastic immobilized porcine collage under high shear, as measured by occlusion (pressure) over time.

[0041] **Figure 24** shows the effect on peak thrombin of thrombosome supplementation to normal and aspirin-inhibited plasma.

[0042] **Figure 25A** shows the effect of cangrelor alone or cangrelor plus thrombosomes on platelet occlusion using T-TAS® technology.

[0043] **Figure 25B** is a bar plot of the occlusion time for data sets from Figure 25A.

[0044] **Figure 26A** shows the effect of tirofiban alone, or with random donor platelets (RDP) or thrombosomes on platelet occlusion using T-TAS® technology.

[0045] **Figure 26B** is a bar plot of the occlusion time for data sets from Figure 26A.

[0046] **Figure 27A** shows the effect of eptifibatide alone, or with RDP or thrombosomes on platelet occlusion using T-TAS® technology.

[0047] **Figure 27B** is a bar plot of the occlusion time for data sets from Figure 27A.

[0048] **Figure 28A** shows the effect of AP2 alone, or with RDP or thrombosomes on platelet occlusion using T-TAS® technology.

[0049] **Figure 28B** is a bar plot of the occlusion time for data sets from Figure 28A.

[0050] **Figure 29A** shows the effect of thrombosomes on PRP taken from a subject on aspirin therapy using T-TAS® technology.

[0051] **Figure 29B** is a bar plot of the occlusion time for data sets from Figure 29A.

[0052] **Figure 30A** shows the effect of thrombosomes on PRP taken from a subject on aspirin therapy on thrombin generation.

[0053] **Figure 30B** is a bar plot of thrombin generation parameters for PRP taken from a subject on aspirin therapy, with or without added thrombosomes.

[0054] **Figure 31A** shows aggregometry of PRP taken from a subject on ibuprofen therapy, with added buffer, arachidonic acid, or collagen.

[0055] **Figure 31B** shows the effect of ADP on PRP taken from a subject on ibuprofen therapy, with or without thrombosomes.

[0056] **Figure 32** shows the effect of dosing thrombosomes on the bleeding time of mice treated with a superpharmacologic dose of clopidogrel.

DETAILED DESCRIPTION

[0057] Before embodiments of the present invention are described in detail, it is to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the term belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. The present disclosure is controlling to the extent it conflicts with any incorporated publication.

[0058] As used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a saccharide" includes reference to one or more saccharides, and equivalents thereof known to those skilled in the art. Furthermore, the use of terms that can be described using equivalent terms include the use of those equivalent terms. Thus, for example, the use of the term "subject" is to be understood to include the terms "patient", "person", "animal", "human", and other terms used in the art to indicate one who is subject to a medical treatment. The use of multiple terms to encompass a single concept is not to be construed as limiting the concept to only those terms used.

[0059] It is to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting. Further, where a range of values is disclosed, the skilled artisan will understand that all other specific values

within the disclosed range are inherently disclosed by these values and the ranges they represent without the need to disclose each specific value or range herein. For example, a disclosed range of 1-10 includes 1-9,1-5, 2-10, 3.1-6, 1, 2, 3, 4, 5, and so forth. In addition, each disclosed range includes up to 5% lower for the lower value of the range and up to 5% higher for the higher value of the range. For example, a disclosed range of 4 - 10 includes 3.8 - 10.5. This concept is captured in this document by the term "about".

[0060] As used herein and in the appended claims, the term “platelet” can include whole platelets, fragmented platelets, platelet derivatives, or thrombosomes. “Platelets” within the above definition may include, for example, platelets in whole blood, platelets in plasma, platelets in buffer optionally supplemented with select plasma proteins, cold stored platelets, dried platelets, cryopreserved platelets, thawed cryopreserved platelets, rehydrated dried platelets, rehydrated cryopreserved platelets, lyopreserved platelets, thawed lyopreserved platelets, or rehydrated lyopreserved platelets. “Platelets” may be “platelets” of mammals, such as of humans, or such as of non-human mammals.

[0061] As used herein, “thrombosomes” (sometimes also herein called “Tsomes” or “Ts”, particularly in the Examples and Figures) are platelet derivatives that have been treated with an incubating agent (e.g., any of the incubating agents described herein) and lyopreserved (such as freeze-dried). In some cases, thrombosomes can be prepared from pooled platelets. Thrombosomes can have a shelf life of 2-3 years in dry form at ambient temperature and can be rehydrated with sterile water within minutes for immediate infusion. One example of thrombosomes are THROMBOSOMES®, which are in clinical trials for the treatment of acute hemorrhage in thrombocytopenic patients. Typically, agents that inhibit Factor IIa, VIIa, IX, Xa, XI, Tissue Factor, or vitamin K-dependent synthesis of clotting factors (e.g., Factor II, VII, IX, or X) or that activate antithrombin (e.g., antithrombin III) are considered to be anticoagulants. Other mechanisms of anticoagulants are known. Anticoagulants include dabigatran, argatroban, hirudin, rivaroxaban, apixaban, edoxaban, fondaparinux, warfarin, heparin, and low molecular weight heparins.

[0062] As used herein, an “antiplatelet agent” is an antithrombotic and does not include anticoagulants. Examples of antiplatelet agents include aspirin (also called acetylsalicylic acid or ASA), cangrelor (e.g., KENGREAL®), ticagrelor (e.g., BRILINTA®), clopidogrel (e.g., PLAVIX®), prasugrel (e.g., EFFIENT®), eptifibatide (e.g., INTEGRILIN®), tirofiban (e.g.,

AGGRASTAT®), and abciximab (e.g., REOPRO®). For the purpose of this disclosure, antiplatelet agents include agents that inhibit P2Y receptors (e.g., $P2Y_{12}$), glycoprotein IIb/IIIa, or that antagonize thromboxane synthase or thromboxane receptors. Non-limiting examples of thromboxane A_2 antagonists are aspirin, terutroban, and picotamide. Non-limiting examples of P2Y receptor antagonists include cangrelor, ticagrelor, elinogrel, clopidogrel, prasugrel, and ticlopidine. Non-limiting examples of glycoprotein IIb/IIIa include abciximab, eptifibatide, and tirofiban. NSAIDS (e.g., ibuprofen) are also considered to be antiplatelet agents for the purposes of this disclosure. Other mechanisms of anti-platelet agents are known. Antiplatelet agents also include PAR1 antagonists, PAR4 antagonists GPVI antagonists and alpha2beta1 collagen receptor antagonists. Non-limiting examples of PAR-1 antagonists include vorapaxar and atopaxar. As used herein, aspirin is considered to be an antiplatelet agent but not an anticoagulant. Additional non-limiting examples of antiplatelet agents include cilostazol, prostaglandin E1, epoprostenol, dipyridamole, treprostinil sodium, and sarpogrelate.

[0063] In some embodiments, an antiplatelet agent can be selected from the group consisting of aspirin, cangrelor, ticagrelor, clopidogrel, prasugrel, eptifibatide, tirofiban, abciximab, and combinations thereof. In some embodiments, an antiplatelet agent can be selected from the group consisting of aspirin, cangrelor, ticagrelor, clopidogrel, prasugrel, eptifibatide, tirofiban, abciximab, terutroban, picotamide, elinogrel, ticlopidine, ibuprofen, vorapaxar, atopaxar, and combinations thereof. In some embodiments, an antiplatelet agent can be selected from the group consisting of aspirin, cangrelor, ticagrelor, clopidogrel, prasugrel, eptifibatide, tirofiban, abciximab, terutroban, picotamide, elinogrel, ticlopidine, ibuprofen, vorapaxar, atopaxar, cilostazol, prostaglandin E1, epoprostenol, dipyridamole, treprostinil sodium, sarpogrelate and combinations thereof. In some embodiments, the antiplatelet agent can include multiple antiplatelet agents, such as 2 (or more) of any of the antiplatelet agents described herein. In some embodiments, the antiplatelet agent can be aspirin and clopidogrel.

[0064] Cangrelor like clopidogrel, ticagrelor, and prasugrel, blocks the $P2Y_{12}$ (ADP) receptor on platelets. Cangrelor can in some cases be used as a representative of this class of drug. Cangrelor, unlike clopidogrel and prasugrel, does not need hepatic metabolism to become biologically active.

[0065] Eptifibatide is a peptide therapeutic that blocks the fibrin binding role of GPIIb-IIIa receptor on platelets. The drug is typically administered via IV as a 180 μg/kg bolus

followed by 2 μg/kg/min continuous infusion. The blood concentration of eptifibatide is typically about 1-2 μM. Bleeding times generally return to normal within about 1 hour of drug stoppage.

[0066] Aspirin is an irreversible cylcooxygenase (COX) inhibitor. The COX enzyme in platelets is responsible for synthesis of thromboxane A2, prostaglandin E2 and prostacyclin (PGI2). Aspirin permanently inactivates the COX enzyme within platelets, and since platelets do not have the nuclear material to synthesize new enzyme, new platelets must be produced to overcome the aspirin effect. Without thromboxane A2, prostaglandin E2, and prostacyclin (PGI2) platelets are limited in their pro-aggregation activity. Many people are maintained on a low dose of aspirin to prevent unwanted clotting events. Aspirin bioavailability largely varies with administration route, with a single 500 mg dose IV at peaks of 500 μM and the same dose orally at 44 μM.

[0067] The antiplatelet class of drugs is widely used to prevent unwanted clotting episodes that lead to heart failure, stroke, and the like. In many cases, an antiplatelet drug may need to be reversed or stopped. In the case of advanced notice, as in a pre-planned surgery situation, the antiplatelet drug dose can sometimes be stopped before the surgery, preventing unwanted bleeding during surgery. In the case where an antiplatelet agent needs reversing quickly, reversal agents are typically not readily available, are expensive, or carry significant risk to the patient. In the case of need for rapid antiplatelet reversal, a platelet transfusion is typically administered, though the response to this is often only partial reversal. The caveat of this course of reversal is that the newly-infused platelets themselves are susceptible to circulating drug antiplatelet activity whereas, in some embodiments, compositions as described herein (e.g., including thrombosomes) are not. In some embodiments, compositions as described herein (e.g., including thrombosomes) are an active reversal agent. In some embodiments, the hemostatic activity of compositions as described herein (e.g., including thrombosomes) does not succumb to antiplatelet drugs.

[0068] Some exemplary antiplatelet agents and potential methods of reversal are described below.

[0069] Acetylsalicylic acid (ASA; aspirin) - aspirin acts as a COX-1 blocker in platelets, which renders the platelet inactive by irreversibly inhibiting platelet-derived thromboxane formation. Clinically, aspirin is sometimes reversed by a platelet transfusion in emergency situations or by stopping treatment where surgery is scheduled in the future.

[0070] Clopidogrel (e.g., PLAVIX®) - clopidogrel acts as to prevent ADP from binding to its receptor on platelets. ADP binding leads to platelet shape change and aggregation. Clopidogrel is non-reversible. Clinically, clopidogrel is sometimes reversed by a platelet transfusion in emergency situations or by stopping treatment where surgery is scheduled in the future.

[0071] Cangrelor (e.g., KENGREAL®) - cangrelor acts to prevent ADP from binding to its receptor on platelets. ADP binding leads to platelet shape change and aggregation. Clopidogrel is reversible and platelet function is returned approximately 1 hour after stopping infusion. Clinically it is generally preferred when reversal is needed after a procedure.

[0072] Ticagrelor (e.g., BRILINTA®) - ticagrelor acts to prevent ADP from binding to its receptor and acts as an inverse agonist. Ticagrelor is reversible and platelet function can return after approximately 72 hours of the last dosage. Reversal of action of ticagrelor can be affected by the time after the last dose. If the last dose was longer than 24 hours previous, then platelet transfusion can sometimes be therapeutic to reverse the results.

[0073] Effient (e.g., PRASUGREL®) - Effient acts to prevent ADP from binding to its receptor and acts as a non-reversable antagonist. It being a non-reversible antagonist, new platelets must be formed to overcoming its effect. Clinically Effient is reversed by a platelet transfusion in emergency situations or by stopping treatment where surgery is scheduled in the future.

[0074] Eptifibatide (Integrilin) -Eptifibatide acts to block the GpIIb/IIIa and acts as a reversible antagonist. Clinically, Integrilin is reversed by a platelet transfusion in emergency situations or by stopping treatment where surgery is scheduled in the future.

[0075] Platelets infusions are currently used as a treatment method for antiplatelet drugs, but platelet transfusions only act to dilute out the effect of these drugs. In some embodiments, thrombosomes are not reactive to these drugs and maintain their ability to aid in clotting. This makes treatment via thrombosomes entirely unique and introduces a new application for the product.

[0076] Platelet-derived products are not currently used as a treatment method for anticoagulant/antiplatelet drugs, and there are no currently approved reversal agents for antiplatelet agents. As such, emergency treatments (pre-op, trauma, and the like) are typically

blanket precautions to avoid or mitigate hemorrhage. Non-limiting examples include infusion of plasma, red blood cells, and anti-fibrinolytics. Platelet derivatives (e.g., lyopreserved platelets (e.g., thrombosomes)) may be an effective alternative or supplement to these general treatments.

[0077] Without being bound by any particular theory, it is believed that thrombosomes can work at least in part by providing a procoagulant negatively charged surface to augment thrombin generation above and beyond that suppressed by the anti-coagulants. Similarly, without being bound by any particular theory, it is believed that thrombosomes can work at least in part by binding to and co-aggregating with circulating platelets.

[0078] Products and methods are described herein for controlling bleeding and improving healing. The products and methods described herein can also be used to counteract the activity of an antiplatelet agent (e.g., aspirin (also called acetylsalicylic acid or ASA), cangrelor (e.g., KENGREAL®), ticagrelor (e.g., BRILINTA®), clopidogrel (e.g., PLAVIX®), prasugrel (e.g., EFFIENT®), eptifibatide (e.g., INTEGRILIN®), tirofiban (e.g., AGGRASTAT®), or abciximab (e.g., REOPRO®)). The products and methods described herein are directed toward embodiments that can aid in the closure and healing of wounds.

[0079] In certain embodiments, a composition comprising platelets such as lyophilized platelets or platelet derivatives may be delivered to a wound on the surface of or in the interior of a patient. In various embodiments, a composition comprising platelets such as lyophilized platelets or platelet derivatives can be applied in selected forms including, but not limited to, adhesive bandages, compression bandages, liquid solutions, aerosols, matrix compositions, and coated sutures or other medical closures. In embodiments, a composition comprising platelets such as lyophilized platelets or platelet derivatives may be administered to all or only a portion of an affected area on the surface of a patient. In other embodiments, a composition comprising platelets such as lyophilized platelets or platelet derivatives may be administered systemically, for example via the blood stream. In embodiments, an application of the platelet derivative can produce hemostatic effects for 2 or 3 days, preferably 5 to 10 days, or most preferably for up to 14 days.

[0080] Some embodiments provide a method of treating a coagulopathy in a subject, the method comprising administering to the subject in need thereof an effective amount of a composition comprising platelets such as lyophilized platelets or platelet derivatives and an

incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant (also called a lyophilizing agent), and optionally an organic solvent.

[0081] Some embodiments provide a method of treating a coagulopathy in a subject, the method comprising administering to the subject in need thereof an effective amount of a composition prepared by a process comprising incubating platelets with an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent, to form the composition.

[0082] In some embodiments of any of the methods described herein, the coagulopathy is the result of an antiplatelet agent.

[0083] Some embodiments provide a method of treating coagulopathy in a subject, wherein the subject has been treated or is being treated with an antiplatelet agent, the method comprising administering to the subject in need thereof an effective amount of a composition comprising platelets such as lyophilized platelets or platelet derivatives and an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent.

[0084] Some embodiments provide a method of treating coagulopathy in a subject, wherein the subject has been treated or is being treated with an antiplatelet agent, the method comprising administering to the subject in need thereof an effective amount of a composition prepared by a process comprising incubating platelets with an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent, to form the composition.

[0085] Some embodiments provide a method of restoring normal hemostasis in a subject, the method comprising administering to the subject in need thereof an effective amount of a composition comprising platelets such as lyophilized platelets or platelet derivatives and an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent.

[0086] Some embodiments provide a method of restoring normal hemostasis in a subject, the method comprising administering to the subject in need thereof an effective amount of a composition prepared by a process comprising incubating platelets with an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent, to form the composition.

[0087] Some embodiments provide a method of restoring normal hemostasis in a subject, wherein the subject has been treated or is being treated with an antiplatelet agent, the method comprising administering to the subject in need thereof an effective amount of a composition comprising platelets such as lyophilized platelets or platelet derivatives and an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent.

[0088] Some embodiments provide a method of restoring normal hemostasis in a subject, wherein the subject has been treated or is being treated with an antiplatelet agent, the method comprising administering to the subject in need thereof an effective amount of a composition prepared by a process comprising incubating platelets with an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent, to form the composition.

[0089] Compositions as described herein can also be administered to prepare a subject for surgery, in some cases. For some patients taking an antiplatelet agent, it may be difficult or impossible to reduce the dosage of the antiplatelet agent before surgery (e.g., in the case of trauma or other emergency surgery). For some patients taking an antiplatelet agent, it may be inadvisable to reduce the dosage of the antiplatelet agent before surgery (e.g., if the patient would be at risk of a thrombotic event (e.g., deep vein thrombosis, pulmonary embolism, or stroke) if the dosage of the antiplatelet agent were reduced over time.

[0090] Accordingly, some embodiments provide a method of preparing a subject for surgery, the method comprising administering to the subject in need thereof an effective amount of a composition comprising platelets such as lyophilized platelets or platelet derivatives and an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent.

[0091] Some embodiments provide a method of preparing a subject for surgery, the method comprising administering to the subject in need thereof an effective amount of a composition prepared by a process comprising incubating platelets with an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent, to form the composition.

[0092] Some embodiments provide a method of preparing a subject for surgery, wherein the subject has been treated or is being treated with an antiplatelet agent, the method comprising

administering to the subject in need thereof an effective amount of a composition comprising platelets such as lyophilized platelets or platelet derivatives and an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent.

[0093] Some embodiments provide a method of preparing a subject for surgery, wherein the subject has been treated or is being treated with an antiplatelet agent, the method comprising administering to the subject in need thereof an effective amount of a composition prepared by a process comprising incubating platelets with an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent, to form the composition.

[0094] In some embodiments, a surgery can be an emergency surgery (e.g., in the case of trauma) or a scheduled surgery.

[0095] In some embodiments, treatment with an anticoagulant can be stopped (e.g., in preparation for surgery). In some embodiments, treatment with an anticoagulant can continue.

[0096] Some embodiments provide a method of ameliorating the effects of an antiplatelet agent in a subject, the method comprising administering to the subject in need thereof an effective amount of a composition comprising platelets such as lyophilized platelets or platelet derivatives and an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent.

[0097] Some embodiments provide a method of ameliorating the effects of an antiplatelet agent in a subject, the method comprising administering to the subject in need thereof an effective amount of a composition prepared by a process comprising incubating platelets with an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent, to form the composition.

[0098] In some cases, the effects of an antiplatelet agent may need to be ameliorated due to an incorrect dosage of an antiplatelet agent. For example, in some embodiments, the effects of an antiplatelet agent can be ameliorated following an overdose of the antiplatelet agent. In some cases, the effects of an antiplatelet agent may need to be ameliorated due to a potential for interaction with another drug (e.g., a second antiplatelet agent). For example, in some embodiments, the effects of an antiplatelet agent can be ameliorated following an erroneous dosing of two or more drugs, at least one of which is an antiplatelet agent.

[0099] In some embodiments of any of the methods described herein, the composition can further comprise an active agent, such as an anti-fibrinolytic agent. Non-limiting examples of

anti-fibrinolytic agents include ε-aminocaproic acid (EACA), tranexamic acid, aprotinin, aminomethylbenzoic acid, and fibrinogen. In some embodiments, platelets or platelet derivatives can be loaded with an active agent, such as an anti-fibrinolytic agent.

[00100] Clotting parameters of blood (e.g., the subject's blood) can be assessed at any appropriate time during the methods described herein. For example, one or more clotting parameters of blood can be assessed before administration of a composition comprising platelets such as lyophilized platelets or platelet derivatives as described herein, e.g., in order to determine the need for administration of a composition comprising platelets or platelet derivatives as described herein. As another example, one or more clotting parameters of blood can be assessed after administration of a composition comprising platelets or platelet derivatives as described herein, e.g., in order to determine the effectiveness of the administered composition, to determine whether additional administration of the composition is warranted, or to determine whether it is safe to perform a surgical procedure.

[00101] Accordingly, any of the methods described herein can include steps of assessing one or more clotting parameters of blood before administration of a composition comprising platelets or platelet derivatives as described herein, assessing one or more clotting parameters of blood after administration of a composition comprising platelets or platelet derivatives as described herein, or both.

[00102] Any appropriate method can be used to assess clotting parameters of blood. Non-limiting examples of methods include the prothrombin time assay, international normalized ratio (INR), thrombin generation (TGA; which can be used to generate parameters such as, e.g., peak thrombin, endogenous thrombin potential (ETP), and lag time), thromboeleastography (TEG), activated clotting time (ACT), and partial thromboplastin time (PTT or aPTT).

[00103] **Thrombin generation**

[00104] The thrombin generation assay measured the production of thrombin after sample activation via a pro-coagulation agent resulting of thrombin enzymatic cleavage of a fluorescent peptide and release of fluorescent molecule. The peak thrombin is a measure of the maximum thrombin produced, lag time the time to start of thrombin production and ETP as the total thrombin potentially produced.

[00105] In some embodiments, a patient can have a peak thrombin of about 60 nM to about 170 nM, such as about 65 nM to about 170 nM, such as about 65 nM to about 120 nM,

such as about 80 nM, before administration of a composition comprising platelets or platelet derivatives as described herein.

[00106] TEG assesses intrinsic hemostasis via plots of clot strength over time. Calcium chloride ($CaCl_2$) is typically used as the initiating reagent. A TEG waveform (see, e.g., Figure 16) has multiple parameters that can provide information about clotting.

R-time = reaction time (s) - time of latency from start of test to initial fibrin formation.

K = kinetics (s) – speed of initial fibrin formation, time taken to achieve a certain level of clot strength (e.g., an amplitude of 20 mm)

alpha angle = slope of line between R and K - measures the rate of clot formation.

MA = maximum amplitude (mm) - represents the ultimate strength of the fibrin clot.

$\mathbf{A_{30}}$ = amplitude 30 minutes after maximum amplitude is reached- represents rate of lysis phase.

[00107] In hypocoagulable blood states, R-time increases and MA decreases. R-time typically provides a broader response range than MA.

[00108] In the Total Thrombus-formation Analysis System (T-TAS®, FUJIMORI KOGYO CO., LTD), the sample is forced through collagen-coated microchannels using mineral oil. Changes in pressure are used to assess thrombus formation. The Occlusion Start Time is time it takes to reach 10 kPa, and the Occlusion Time = time it takes to each Δ80 kPa using an AR chip (e.g., Zacros Item No, TC0101). According to the manufacturer, an AR chip can be used for analyzing the formation of a mixed white thrombus consisting chiefly of fibrin and activated platelets. It has a flow path (300 μm wide by 50 μm high) coated with collagen and tissue factors and can be used to analyze the clotting function and platelet function. In comparison, a PL chip can be used for analyzing the formation of a platelet thrombus consisting chiefly of activated platelets. A PL chip has a flow path coated with collagen only and can be used to analyze the platelet function.

[00109] The ACT assay is the most basic, but possibly most reliable, way to measure clotting time (t_{ACT}), determined by a magnet's resistance to gravity as a clot forms around it. Typical donor blood has a t_{ACT} ~ 200-300s using only $CaCl_2$.

[00110] Some embodiments provide a method of increasing thrombin generation in a subject, the method comprising administering to the subject in need thereof an effective amount of a composition comprising platelets such as lyophilized platelets or platelet derivatives and an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent.

[00111] Some embodiments, provide a method of increasing thrombin generation in a subject, the method comprising administering to the subject in need thereof an effective amount of a composition prepared by a process comprising incubating platelets with an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent, to form the composition.

[00112] Some embodiments provide a method of increasing peak thrombin in a subject, the method comprising administering to the subject in need thereof an effective amount of a composition comprising platelets such as lyophilized platelets or platelet derivatives and an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent.

[00113] Some embodiments provide a method of increasing peak thrombin in a subject, the method comprising administering to the subject in need thereof an effective amount of a composition prepared by a process comprising incubating platelets with an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent, to form the composition.

[00114] In some embodiments, prior to the administering, the peak thrombin of the subject was below 66 nM (e.g., below 64 nM, 62 nM, 60 nM, 55 nM, 50 nM, 45 nM, 40 nM, 35 nM, 30 nM, 25 nM, 20 nM, 15 nM, 10 nM, or 5 nM). In some embodiments, after the administering, the peak thrombin of the subject is above 66 nM (e.g., above 68 nM, 70 nM, 75 nM, 80 nM, 85 nM, 90 nM, 95 nM, 100 nM, 110 nM, 120 nM, 130 nM, 140 nM, or 150 nM). In some embodiments, after the administering, the peak thrombin of the subject is between 66 and 166 nM. Peak thrombin can be measured by any appropriate method.

[00115] An "effective amount" as used herein is an amount of the composition that comprises an amount of platelets such as lyophilized platelets or platelet derivatives (e.g., thrombosomes) effective in treating the subject. Such an amount of platelets or platelet derivatives (e.g., thrombosomes) includes any appropriate dosage of a composition comprising

platelets or platelet derivatives as described herein that can be administered to the subject. For example, in some embodiments, a dose of a composition comprising platelets or platelet derivatives (e.g., thrombosomes) can include about 1.0×10^7 particles to about 1.0×10^{10} particles, such as about 1.6×10^7 particles (e.g., thrombosomes)/kg to about 1.0×10^{10} particles/kg (e.g., about 1.6×10^7 to about 5.1×10^9 particles/kg, about 1.6×10^7 to about 3.0×10^9 particles/kg, about 1.6×10^7 to about 1.0×10^9 particles/kg, about 1.6×10^7 to about 5.0×10^8 particles/kg, about 1.6×10^7 to about 1.0×10^8 particles/kg, about 1.6×10^7 to about 5.0×10^7 particles/kg, about 5.0×10^7 to about 1.0×10^8 particles/kg, about 1.0×10^8 to about 5.0×10^8 particles/kg, about 5.0×10^8 to about 1.0×10^9 particles/kg, about 1.0×10^9 to about 5.0×10^9 particles/kg, or about 5.0×10^9 to about 1.0×10^{10} particles/kg).

[00116] In some embodiments of the methods herein, the composition is administered topically. In some embodiments, topical administration can include administration via a solution, cream, gel, suspension, putty, particulates, or powder. In some embodiments, topical administration can include administration via a bandage (e.g. an adhesive bandage or a compression bandage) or medical closure (e.g., sutures, staples)); for example the platelet derivatives (e.g., lyopreserved platelets (e.g., thrombosomes)) can be embedded therein or coated thereupon), as described in PCT Publication No. WO2017/040238 (e.g., paragraphs [013]-[069]), corresponding to U.S. Patent Application Serial number 15/776,255, the entirety of which is herein incorporated by reference.

[00117] In some embodiments of the methods herein, the composition is administered parenterally.

[00118] In some embodiments of the methods herein, the composition is administered intravenously.

[00119] In some embodiments of the methods herein, the composition is administered intramuscularly.

[00120] In some embodiments of the methods herein, the composition is administered intrathecally.

[00121] In some embodiments of the methods herein, the composition is administered subcutaneously.

[00122] In some embodiments of the methods herein, the composition is administered intraperitoneally.

[00123] In some embodiments of the methods herein, the composition is dried prior to the administration step. In some embodiments of the method, the composition is freeze-dried prior to the administration step. In some embodiments of the method, the composition is rehydrated following the drying or freeze-drying step.

[00124] In some embodiments, the antiplatelet agent is selected from the group consisting of aspirin (also called acetylsalicylic acid or ASA); a P2Y12 inhibitor such as cangrelor (e.g., KENGREAL®), ticagrelor (e.g., BRILINTA®), clopidogrel (e.g., PLAVIX®), or prasugrel (e.g., EFFIENT®); a glycoprotein IIb/IIIa inhibitor such as eptifibatide (e.g., INTEGRILIN®), tirofiban (e.g., AGGRASTAT®), or abciximab (e.g., REOPRO®)); supplements such as herbal supplements; or a combination of any thereof. Examples of supplements include ginger, ginseng, ginkgo, green tea, kava, saw palmetto, boldo (*Peumus boldus*), Danshen (*Salvia miltiorrhiza*), Dong quai (*Angelica sinensis*) papaya (*Carica papaya*), fish oil, and vitamin E. Examples of herbal supplements include ginger, ginseng, and ginkgo.

[00125] In some embodiments, the antiplatelet agent is aspirin.

[00126] In some embodiments, the antiplatelet agent is cangrelor (e.g., KENGREAL®).

[00127] In some embodiments, the antiplatelet agent is ticagrelor (e.g., BRILINTA®).

[00128] In some embodiments, the antiplatelet agent is clopidogrel (e.g., PLAVIX®).

[00129] In some embodiments, the antiplatelet agent is prasugrel (e.g., EFFIENT®).

[00130] In some embodiments, the antiplatelet agent is eptifibatide (e.g., INTEGRILIN®).

[00131] In some embodiments, the antiplatelet agent is tirofiban (e.g., AGGRASTAT®).

[00132] In some embodiments, the antiplatelet agent is abciximab (e.g., REOPRO®).

[00133] In some embodiments, the antiplatelet agent is terutroban.

[00134] In some embodiments, the antiplatelet agent is picotamide.

[00135] In some embodiments, the antiplatelet agent is elinogrel.

[00136] In some embodiments, the antiplatelet agent is ticlopidine.

[00137] In some embodiments, the antiplatelet agent is ibuprofen.

[00138] In some embodiments, the antiplatelet agent is vorapaxar.

[00139] In some embodiments, the antiplatelet agent is atopaxar.

[00140] In some embodiments, the antiplatelet agent is cilostazol.

[00141] In some embodiments, the antiplatelet agent is prostaglandin E1.

[00142] In some embodiments, the antiplatelet agent is epoprostenol.

[00143] In some embodiments, the antiplatelet agent is dipyridamole.

[00144] In some embodiments, the antiplatelet agent is treprostinil sodium.

[00145] In some embodiments, the antiplatelet agent is sarpogrelate.

[00146] In some embodiments, the antiplatelet agent is a supplement.

[00147] In some embodiments, the antiplatelet agent is an herbal supplement.

[00148] In some embodiments, rehydrating the composition comprising platelets or platelet derivatives comprises adding to the platelets an aqueous liquid. In some embodiments, the aqueous liquid is water. In some embodiments, the aqueous liquid is an aqueous solution (e.g., a buffer). In some embodiments, the aqueous liquid is a saline solution. In some embodiments, the aqueous liquid is a suspension.

[00149] In some embodiments, the rehydrated platelets or platelet derivatives (e.g., thrombosomes) have coagulation factor levels showing all individual factors (e.g., Factors VII, VIII and IX) associated with blood clotting at 40 international units (IU) or greater.

[00150] In some embodiments, the platelets such as lyophilized platelets or platelet derivatives (e.g., thrombosomes) have less than about 10%, such as less than about 8%, such as less than about 6%, such as less than about 4%, such as less than about 2%, such as less than about 0.5% crosslinking of platelet membranes via proteins and/or lipids present on the membranes. In some embodiments, the rehydrated platelets or platelet derivatives (e.g., thrombosomes), have less than about 10%, such as less than about 8%, such as less than about 6%, such as less than about 4%, such as less than about 2%, such as less than about 0.5% crosslinking of platelet membranes via proteins and/or lipids present on the membranes.

[00151] In some embodiments, the platelets such as lyophilized platelets or platelet derivatives (e.g., thrombosomes) have a particle size (e.g., diameter, max dimension) of at least about 0.2 μm (e.g., at least about 0.3 μm, at least about 0.4 μm, at least about 0.5 μm, at least about 0.6 μm, at least about 0.7 μm, at least about 0.8 μm, at least about 0.9 μm, at least about 1.0 μm, at least about 1.2 μm, at least about 1.5 μm, at least about 2.0 μm, at least about 2.5 μm, or at least about 5.0 μm). In some embodiments, the particle size is less than about 5.0 μm (e.g., less than about 2.5 μm, less than about 2.0 μm, less than about 1.5 μm, less than about 1.0 μm, less than about 0.9 μm, less than about 0.8 μm, less than about 0.7 μm, less than about 0.6 μm, less than about 0.5 μm, less than about 0.4 μm, or less than about 0.3 μm). In some embodiments, the particle size is from about 0.3 μm to about 5.0 μm (e.g., from about 0.4 μm to

about 4.0 μm, from about 0.5 μm to about 2.5 μm, from about 0.6 μm to about 2.0 μm, from about 0.7 μm to about 1.0 μm, from about 0.5 μm to about 0.9 μm, or from about 0.6 μm to about 0.8 μm).

[00152] In some embodiments, at least 50% (e.g., at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99%) of platelets such as lyophilized platelets or platelet derivatives (e.g., thrombosomes), have a particle size in the range of about 0.3 μm to about 5.0 μm (e.g., from about 0.4 μm to about 4.0 μm, from about 0.5 μm to about 2.5 μm, from about 0.6 μm to about 2.0 μm, from about 0.7 μm to about 1.0 μm, from about 0.5 μm to about 0.9 μm, or from about 0.6 μm to about 0.8 μm). In some embodiments, at most 99% (e.g., at most about 95%, at most about 80%, at most about 75%, at most about 70%, at most about 65%, at most about 60%, at most about 55%, or at most about 50%) of the platelets such as lyophilized platelets or platelet derivatives (e.g., thrombosomes), are in the range of about 0.3 μm to about 5.0 μm (e.g., from about 0.4 μm to about 4.0 μm, from about 0.5 μm to about 2.5 μm, from about 0.6 μm to about 2.0 μm, from about 0.7 μm to about 1.0 μm, from about 0.5 μm to about 0.9 μm, or from about 0.6 μm to about 0.8 μm). In some embodiments, about 50% to about 99% (e.g., about 55% to about 95%, about 60% to about 90%, about 65% to about 85, about 70% to about 80%) of the platelets or platelet derivatives (e.g., thrombosomes) are in the range of about 0.3 μm to about 5.0 μm (e.g., from about 0.4 μm to about 4.0 μm, from about 0.5 μm to about 2.5 μm, from about 0.6 μm to about 2.0 μm, from about 0.7 μm to about 1.0 μm, from about 0.5 μm to about 0.9 μm, or from about 0.6 μm to about 0.8 μm).

[00153] In some embodiments, platelets are isolated, for example in a liquid medium, prior to treating a subject.

[00154] In some embodiments, platelets are donor-derived platelets. In some embodiments, platelets are obtained by a process that comprises an apheresis step. In some embodiments, platelets are pooled platelets.

[00155] In some embodiments, platelets are pooled from a plurality of donors. Such platelets pooled from a plurality of donors may be also referred herein to as pooled platelets. In some embodiments, the donors are more than 5, such as more than 10, such as more than 20, such as more than 50, such as up to about 100 donors. In some embodiments, the donors are from about 5 to about 100, such as from about 10 to about 50, such as from about 20 to about 40,

such as from about 25 to about 35. Pooled platelets can be used to make any of the compositions described herein.

[00156] In some embodiments, platelets are derived *in vitro*. In some embodiments, platelets are derived or prepared in a culture. In some embodiments, preparing the platelets comprises deriving or growing the platelets from a culture of megakaryocytes. In some embodiments, preparing the platelets comprises deriving or growing the platelets (or megakaryocytes) from a culture of human pluripotent stem cells (PCSs), including embryonic stem cells (ESCs) and/or induced pluripotent stem cells (iPSCs).

[00157] Accordingly, in some embodiments, platelets are prepared prior to treating a subject as described herein. In some embodiments, the platelets are lyophilized. In some embodiments, the platelets are cryopreserved.

[00158] In some embodiments, the platelets or pooled platelets may be acidified to a pH of about 6.0 to about 7.4 prior to the incubation with the incubating agent. In some embodiments, the method comprises acidifying the platelets to a pH of about 6.5 to about 6.9. In some embodiments, the method comprises acidifying the platelets to a pH of about 6.6 to about 6.8. In some embodiments, the acidifying comprises adding to the pooled platelets a solution comprising Acid Citrate Dextrose (ACD).

[00159] In some embodiments, the platelets are isolated prior to the incubation with the incubating agent. In some embodiments, the method further comprises isolating platelets by using centrifugation. In some embodiments, the centrifugation occurs at a relative centrifugal force (RCF) of about 1000 x *g* to about 2000 x *g*. In some embodiments, the centrifugation occurs at relative centrifugal force (RCF) of about 1300 x *g* to about 1800 x *g*. In some embodiments, the centrifugation occurs at relative centrifugal force (RCF) of about 1500 x *g*. In some embodiments, the centrifugation occurs for about 1 minute to about 60 minutes. In some embodiments, the centrifugation occurs for about 10 minutes to about 30 minutes. In some embodiments, the centrifugation occurs for about 30 minutes.

[00160] An incubating agent can include any appropriate components. In some embodiments, the incubating agent may comprise a liquid medium. In some embodiments the incubating agent may comprise one or more salts selected from phosphate salts, sodium salts, potassium salts, calcium salts, magnesium salts, and any other salt that can be found

in blood or blood products, or that is known to be useful in drying platelets, or any combination of two or more of these.

[00161] In some embodiments, the incubating agent comprises one or more salts, such as phosphate salts, sodium salts, potassium salts, calcium salts, magnesium salts, and any other salt that can be found in blood or blood products. Exemplary salts include sodium chloride (NaCl), potassium chloride (KCl), and combinations thereof. In some embodiments, the incubating agent includes from about 0.5 mM to about 100 mM of the one or more salts. In some embodiments, the incubating agent includes from about 0.5 mM to about 100 mM (e.g., about 0.5 to about 2 mM, about 2 mM to about 90 mM, about 2 mM to about 6 mM, about 50 mM to about 100 mM, about 60 mM to about 90 mM, about 70 to about 85 mM) about of the one or more salts. In some embodiments, the incubating agent includes about 5 mM, about 60 mM, about 65 mM, about 70 mM, about 75 mM, or about 80 mM of the one or more salts. In some embodiments, the incubating agent comprises one or more salts selected from calcium salts, magnesium slats, and a combination of the two, in a concentration of about 0.5 mM to about 2 mM.

[00162] Preferably, these salts are present in the composition comprising platelets or platelet derivatives, such as freeze-dried platelets, at an amount that is about the same as is found in whole blood.

[00163] In some embodiments, the incubating agent further comprises a carrier protein. In some embodiments, the carrier protein comprises human serum albumin, bovine serum albumin, or a combination thereof. In some embodiments, the carrier protein is present in an amount of about 0.05% to about 1.0% (w/v).

[00164] The incubating agent may be any buffer that is non-toxic to the platelets and provides adequate buffering capacity to the solution at the temperatures at which the solution will be exposed during the process provided herein. Thus, the buffer may comprise any of the known biologically compatible buffers available commercially, such as phosphate buffers, such as phosphate buffered saline (PBS), bicarbonate/carbonic acid, such as sodium-bicarbonate buffer, N-2-hydroxyethylpiperazine-N'-2- ethanesulfonic acid (HEPES), and tris-based buffers, such as tris-buffered saline (TBS). Likewise, it may comprise one or more of the following buffers: propane- 1,2,3-tricarboxylic (tricarballylic); benzenepentacarboxylic; maleic; 2,2- dimethylsuccinic; EDTA; 3,3-dimethylglutaric; bis(2-hydroxyethyl)imino- tris(hydroxymethyl)-methane (BIS-TRIS); benzenehexacarboxylic

(mellitic); N-(2- acetamido)imino-diacetic acid (ADA); butane-1,2,3,4-tetracarboxylic; pyrophosphoric; 1,1-cyclopentanediacetic (3,3 tetramethylene-glutaric acid); piperazine-1,4-bis-(2-ethanesulfonic acid) (PIPES); N-(2-acetamido)-2- amnoethanesulfonic acid (ACES); 1,1-cyclohexanediacetic; 3,6-endomethylene- 1,2,3,6-tetrahydrophthalic acid (EMTA; ENDCA); imidazole;; 2- (aminoethyl)trimethylammonium chloride (CHOLAMINE); N,N-bis(2- hydroxyethyl)-2-aminoethanesulfonic acid (BES); 2-methylpropane-1,2,3-triscarboxylic (beta-methyltricarballylic); 2-(N-morpholino)propane-sulfonic acid (MOPS); phosphoric; and N-tris(hydroxymethyl)methyl-2-amminoethane sulfonic acid (TES). In some embodiments, the incubating agent includes one or more buffers, e.g., N-2-hydroxyethylpiperazine-N'-2- ethanesulfonic acid (HEPES), or sodium-bicarbonate ($NaHCO_3$). In some embodiments, the incubating agent includes from about 5 to about 100 mM of the one or more buffers. In some embodiments, the incubating agent includes from about 5 to about 50 mM (e.g., from about 5 mM to about 40 mM, from about 8 mM to about 30 mM, about 10 mM to about 25 mM) about of the one or more buffers. In some embodiments, the incubating agent includes about 10 mM, about 20 mM, about 25 mM, or about 30 mM of the one or more buffers.

[00165] In some embodiments, the incubating agent includes one or more saccharides, such as monosaccharides and disaccharides, including sucrose, maltose, trehalose, glucose, mannose, dextrose, and xylose. In some embodiments, the saccharide is a monosaccharide. In some embodiments, the saccharide is a disaccharide. In some embodiments, the saccharide comprises a monosaccharide, a disaccharide, or a combination thereof. In some embodiments, the saccharide is a non-reducing disaccharide. In some embodiments, the saccharide comprises sucrose, maltose, trehalose, glucose (e.g., dextrose), mannose, or xylose. In some embodiments, the saccharide comprises trehalose. In some embodiments, the incubating agent comprises a starch. In some embodiments, the incubating agent includes polysucrose, a polymer of sucrose and epichlorohydrin. In some embodiments, the incubating agent includes from about 10 mM to about 1,000 mM of the one or more saccharides. In some embodiments, the incubating agent includes from about 50 to about 500 mM of the one or more saccharides. In embodiments, one or more saccharides is present in an amount of from 10 mM 10 to 500 mM. In some embodiments, one or more saccharides is present in an amount of from 50 mM to 200 mM. In embodiments, one or more saccharides is present in an amount from 100 mM to 150 mM. In some

embodiments, the one or more saccharides is the lyophilizing agent; for example, in some embodiments, the lyophilizing agent comprises trehalose, polysucrose, or a combination thereof.

[00166] In some embodiments the composition comprising platelets or platelet derivatives, (e.g., thrombosomes), may comprise one or more of water or a saline solution. In some embodiments the composition comprising platelets or platelet derivatives, such as freeze-dried platelets, may comprise DMSO.

[00167] In some embodiments, the incubating agent comprises an organic solvent, such as an alcohol (e.g., ethanol). In such an incubating agent, the amount of solvent can range from 0.1 % to 5.0 % (v/v). In some embodiments, the organic solvent can range from about 0.1 % (v/v) to about 5.0 % (v/v), such as from about 0.3 % (v/v) to about 3.0 % (v/v), or from about 0.5 % (v/v) to about 2 % (v/v).

[00168] In some embodiments, suitable organic solvents include, but are not limited to alcohols, esters, ketones, ethers, halogenated solvents, hydrocarbons, nitriles, glycols, alkyl nitrates, water or mixtures thereof. In some embodiments, suitable organic solvents includes, but are not limited to methanol, ethanol, n-propanol, isopropanol, acetic acid, acetone, methyl ethyl ketone, methyl isobutyl ketone, methyl acetate, ethyl acetate, isopropyl acetate, tetrahydrofuran, isopropyl ether (IPE), tert-butyl methyl ether, dioxane (e.g., 1,4-dioxane), acetonitrile, propionitrile, methylene chloride, chloroform, toluene, anisole, cyclohexane, hexane, heptane, ethylene glycol, nitromethane, dimethylformamide, dimethyl sulfoxide, N-methyl pyrrolidone, dimethylacetamide, and combinations thereof. In some embodiments the organic solvent is selected from the group consisting of ethanol, acetic acid, acetone, acetonitrile, dimethylformamide, dimethyl sulfoxide (DMSO), dioxane, methanol, n-propanol, isopropanol, tetrahydrofuran (THF), N-methyl pyrrolidone, dimethylacetamide (DMAC), or combinations thereof. In some embodiments, the organic solvent comprises ethanol, DMSO, or a combination thereof. The presence of organic solvents, such as ethanol, can be beneficial in the processing of platelets, platelet derivatives, or thrombosomes (e.g., freeze-dried platelet derivatives).

[00169] In some embodiments the incubating agent is incubated into the platelets in the presence of an aqueous medium. In some embodiments the incubating agent is incubated in the presence of a medium comprising DMSO.

[00170] In some embodiments, one or more other components may be incubated in the platelets. Exemplary components may include Prostaglandin E1 or Prostacyclin and or EDTA/EGTA to prevent platelet aggregation and activation during the incubating process.

[00171] Non-limiting examples of incubating agent compositions that may be used are shown in Tables 1-5.

Table 1

Buffer	
Component	**Concentration (mM unless otherwise specified)**
NaCl	75.0
KCl	4.8
HEPES	9.5
$NaHCO_3$	12.0
Dextrose	3
Trehalose	100
Ethanol (optional)	1% (v/v)

Table 2

Buffer A	
Component	**Concentration (mM unless specified otherwise)**
$CaCl_2$	1.8
$MgCl_2$	1.1
KCl	2.7
NaCl	137
NaH_2PO_4	0.4
HEPES	10
D-glucose	5.6
pH	6.5

Table 3

Buffer B	
Component	**Concentration (mM unless otherwise specified)**
Buffer and Salts	Table 4 (below)
BSA	0.35%
Dextrose	5
pH	7.4

Table 3. Buffer B can used when incubating platelets, e.g., for flow cytometry. Such an incubation can be done at room temperature in the dark. Albumin is an optional component of Buffer B.

Table 4

Concentration of HEPES and of Salts in Buffer B	
Component	**Concentration (mM unless otherwise specified)**
HEPES	25
NaCl	119
KCl	5
$CaCl_2$	2
$MgCl_2$	2
glucose	6 g/l

[00172] Table 4 is another exemplary incubating agent. The pH can be adjusted to 7.4 with NaOH. Albumin is an optional component of Buffer B.

[00173] Table 5.

Tyrode's HEPES Buffer (plus PGE1)

Component	**Concentration** (mM)
$CaCl_2$	1.8
$MgCl_2$	1.1
KCl	2.7
NaCl	137
NaH_2PO_4	0.4
HEPES	10
D-glucose	5.6
pH	6.5
Prostagalandin E1 (PGE1)	1 μg/ml

[00174] Table 5 is another exemplary incubating agent.

[00175] In some embodiments, platelets (e.g., apheresis platelets, platelets isolated from whole blood, pooled platelets, or a combination thereof) are incubated with the incubating agent for different durations at or at different temperatures from 15-45 °C, or about 37°C.

[00176] In some embodiments, platelets (e.g., apheresis platelets, platelets isolated from whole blood, pooled platelets, or a combination thereof) form a suspension in an incubating agent comprising a liquid medium at a concentration from 10,000 platelets/μL to 10,000,000 platelets/μL, such as 50,000 platelets/μL to 2,000,000 platelets/μL, such as 100,000 platelets/μL to 500,000 platelets/μL, such as 150,000 platelets/μL to 300,000 platelets/μL, such as 200,000 platelets/μL.

[00177] The platelets (e.g., apheresis platelets, platelets isolated from whole blood, pooled platelets, or a combination thereof) may be incubated with the incubating agent for different durations, such as, for example, for at least about 5 minutes (mins) (e.g., at least about 20 mins, about 30 mins, about 1 hour (hr), about 2 hrs, about 3 hrs, about 4 hrs, about 5 hrs, about 6 hrs, about 7 hrs, about 8 hrs, about 9 hrs, about 10 hrs, about 12 hrs, about 16 hrs, about 20 hrs, about 24 hrs, about 30 hrs, about 36 hrs, about 42 hrs, about 48 hrs, or at least about 48 hrs. In some embodiments, the platelets may be incubated with the incubating agent for no more than about

48 hrs (e.g., no more than about 20 mins, about 30 mins, about 1 hour (hr), about 2 hrs, about 3 hrs, about 4 hrs, about 5 hrs, about 6 hrs, about 7 hrs, about 8 hrs, about 9 hrs, about 10 hrs, about 12 hrs, about 16 hrs, about 20 hrs, about 24 hrs, about 30 hrs, about 36 hrs, or no more than about 42 hrs). In some embodiments, the platelets may be incubated with the incubating agent for from about 10 mins to about 48 hours (e.g., from about 20 mins to about 36 hrs, from about 30 mins to about 24 hrs, from about 1 hr to about 20 hrs, from about 2 hrs to about 16 hours, from about 10 mins to about 24 hours, from about 20 mins to about 12 hours, from about 30 mins to about 10 hrs, or from about 1 hr to about 6 hrs. In some embodiments, the platelets, the platelet derivatives, or the thrombosomes are incubated with the incubating agent for a period of time of 5 minutes to 48 hours, such as 10 minutes to 24 hours, such as 20 minutes to 12 hours, such as 30 minutes to 6 hours, such as 1 hour minutes to 3 hours, such as about 2 hours.

[00178] In some embodiments, the platelets (e.g., apheresis platelets, platelets isolated from whole blood, pooled platelets, or a combination thereof) are incubated with the incubating agents at different temperatures. In embodiments, incubation is conducted at 37°C. In certain embodiments, incubation is performed at 4 °C to 45°C, such as 15 °C to 42°C. For example, in embodiments, incubation is performed at 35°C to 40°C (e.g., 37°C) for 110 to 130 (e.g., 120) minutes and for as long as 24-48 hours. In some embodiments, the platelets are incubated with the incubating agent for different durations as disclosed herein, and at temperatures from 15-45 °C, or about 37°C.

[00179] In some embodiments, platelets (e.g., apheresis platelets, platelets isolated from whole blood, pooled platelets, or a combination thereof) are loaded with one or more active agents. In some embodiments, the platelets can be loaded with an anti-fibrinolytic agent. Non-limiting examples of anti-fibrinolytic agents include ε-aminocaproic acid (EACA), tranexamic acid, aprotinin, aminomethylbenzoic acid, and fibrinogen.

[00180] Loading platelets (e.g., apheresis platelets, platelets isolated from whole blood, pooled platelets, or a combination thereof) with an active agent (e.g., an anti-fibrinolytic agent) can be performed by any appropriate method. See, for example, PCT Publication Nos. WO2020113090A1, WO2020113101A1, WO2020113035A1, and WO2020112963A1. Generally, the loading includes contacting the platelets with the anti-fibrinolytic agent. In some embodiments, the loading can be performed by combining the active agent with the incubating agent. In some embodiments, the loading can be performed in a separate step from the incubating

step. For example, the loading can be performed in a step prior to the incubation step. In some such embodiments, the active agent can be supplied to the platelets as a solution or suspension in any of the incubation agents described herein, which may or may not be the same as the incubating agent used in the incubating step. In some embodiments, the loading step can be performed during the incubation step. In some such embodiments, the active agent can be added to the incubation agent (e.g., as a solid or in a solution or suspension) during the incubation). In some embodiments, the loading step can be performed in a step following the incubation step. In some such embodiments, be supplied to the platelets as a solution or suspension in any of the incubation agents described herein, which may or may not be the same as the incubating agent used in the incubating step.

[00181] An active agent can be applied to the platelets in any appropriate concentration. In some embodiments, an active agent can be applied to the platelets (e.g., as part of the incubating agent or another solution or suspension) in a concentration of about 1 μM to about 100 mM (e.g., about 1 μM to about 10 μm, about 1 μM to about 50 μM, about 1 μM to about 100 μM, about 1 μM to about 500 μM, about 1 μM to about 1 mM, about 1 μM to about 10 mM, about 1 μM to about 25 mM, about 1 μM to about 50 mM, about 1 μM to about 75 mM, about 10 μM to about 100 mM, about 50 μM to about 100 mM, about 100 μM to about 100 mM, about 500 μM to about 100 mM, about 1 mM to about 100 mM, about 10 mM to about 100 mM, about 25 mM to about 100 mM, about 50 mM to about 100 mM, about 75 mM to about 100 mM, about 10μM to about 100 mM, about 200 μM to about 1 mM, about 800 μM to about 900 μM, about 400 μM to about 800 μM, about 500 μM to about 700 μM, about 600 μM, about 5 mM to about 85 mM, about 20 mM to about 90 mM, about 25 mM to about 75 mM, about 30 mM to about 90 mM, about 35 mM to about 65 mM, about 40 mM to about 60 mM, about 50 mM to about 60 mM, about 40 mM to about 70 mM, about 45 mM to about 55 mM, or about 50 mM).

[00182] In some embodiments, the method further comprises drying the platelets. In some embodiments, the drying step comprises lyophilizing the platelets. In some embodiments, the drying step comprises freeze-drying the platelets. In some embodiments, the method further comprises rehydrating the platelets obtained from the drying step.

[00183] In some embodiments, the platelets are cold stored, cryopreserved, or lyophilized (e.g., to produce thrombosomes) prior to use in therapy or in functional assays.

[00184] Any known technique for drying platelets can be used in accordance with the present disclosure, as long as the technique can achieve a final residual moisture content of less than 5%. Preferably, the technique achieves a final residual moisture content of less than 2%, such as 1%, 0.5%, or 0.1%. Non-limiting examples of suitable techniques are freeze-drying (lyophilization) and spray-drying. A suitable lyophilization method is presented in **Table A.** Additional exemplary lyophilization methods can be found in U.S. Patent No. 7,811,558, U.S. Patent No. 8,486,617, and U.S. Patent No. 8,097,403. An exemplary spray-drying method includes: combining nitrogen, as a drying gas, with a incubating agent according to the present disclosure, then introducing the mixture into GEA Mobile Minor spray dryer from GEA Processing Engineering, Inc. (Columbia MD, USA), which has a Two-Fluid Nozzle configuration, spray drying the mixture at an inlet temperature in the range of 150°C to 190°C, an outlet temperature in the range of 65°C to 100°C, an atomic rate in the range of 0.5 to 2.0 bars, an atomic rate in the range of 5 to 13 kg/hr, a nitrogen use in the range of 60 to 100 kg/hr, and a run time of 10 to 35 minutes. The final step in spray drying is preferentially collecting the dried mixture. The dried composition in some embodiments is stable for at least six months at temperatures that range from -20°C or lower to 90°C or higher.

[00185] **Table A: Exemplary Lyophilization Protocol**

	Step	Temp. Set	Type	Duration	Pressure Set
Freezing Step	F1	-50°C	Ramp	Var	N/A
	F2	-50°C	Hold	3 Hrs	N/A
Vacuum Pulldown	F3	-50°	Hold	Var	N/A
Primary Dry	P1	-40°	Hold	1.5 Hrs	0 mT
	P2	-35°	Ramp	2 Hrs	0 mT
	P3	-25°	Ramp	2 Hrs	0 mT
	P4	-17°C	Ramp	2 Hrs	0 mT
	P5	0°C	Ramp	1.5 Hrs	0 mT
	P6	27°C	Ramp	1.5 Hrs	0 mT
	P7	27°C	Hold	16 Hrs	0 mT

Secondary Dry	S1	27°C	Hold	>8Hrs	0 mT

[00186] In some embodiments, the step of drying the platelets that are obtained as disclosed herein, such as the step of freeze-drying the platelets that are obtained as disclosed herein, comprises incubating the platelets with a lyophilizing agent (e.g., a non-reducing disaccharide). Accordingly, in some embodiments, the methods for preparing platelets further comprise incubating the platelets with a lyophilizing agent. In some embodiments the lyophilizing agent is a saccharide. In some embodiments the saccharide is a disaccharide, such as a non-reducing disaccharide.

[00187] In some embodiments, the platelets are incubated with a lyophilizing agent for a sufficient amount of time and at a suitable temperature to incubate the platelets with the lyophilizing agent. Non-limiting examples of suitable lyophilizing agents are saccharides, such as monosaccharides and disaccharides, including sucrose, maltose, trehalose, glucose (e.g., dextrose), mannose, and xylose. In some embodiments, non-limiting examples of lyophilizing agent include serum albumin, dextran, polyvinyl pyrrolidone (PVP), starch, and hydroxyethyl starch (HES). In some embodiments, exemplary lyophilizing agents can include a high molecular weight polymer. By "high molecular weight" it is meant a polymer having an average molecular weight of about or above 70 kDa and up to 1,000,000 kDa. Non-limiting examples are polymers of sucrose and epichlorohydrin (e.g., polysucrose). In some embodiments, the lyophilizing agent is polysucrose. Although any amount of high molecular weight polymer can be used as a lyophilizing agent, it is preferred that an amount be used that achieves a final concentration of about 3% to 10% (w/v), such as 3% to 7%, for example 6%.

[00188] An exemplary saccharide for use in the compositions disclosed herein is trehalose. Regardless of the identity of the saccharide, it can be present in the composition in any suitable amount. For example, it can be present in an amount of 1 mM to 1 M. In embodiments, it is present in an amount of from 10 mM 10 to 500 mM. In some embodiments, it is present in an amount of from 20 mM to 200 mM. In embodiments, it is present in an amount from 40 mM to 100 mM. In various embodiments, the saccharide is present in different specific concentrations within the ranges recited above, and one of skill in the art can immediately understand the

various concentrations without the need to specifically recite each herein. Where more than one saccharide is present in the composition, each saccharide can be present in an amount according to the ranges and particular concentrations recited above.

[00189] Within the process provided herein for making the compositions provided herein, addition of the lyophilizing agent can be the last step prior to drying. However, in some embodiments, the lyophilizing agent is added at the same time or before other components of the composition, such as a salt, a buffer, optionally a cryoprotectant, or other components. In some embodiments, the lyophilizing agent is added to the incubating agent, thoroughly mixed to form a drying solution, dispensed into a drying vessel (e.g., a glass or plastic serum vial, a lyophilization bag), and subjected to conditions that allow for drying of the solution to form a dried composition.

[00190] The step of incubating the platelets with a cryoprotectant can include incubating the platelets for a time suitable for loading, as long as the time, taken in conjunction with the temperature, is sufficient for the cryoprotectant to come into contact with the platelets and, preferably, be incorporated, at least to some extent, into the platelets. In embodiments, incubation is carried out for about 1 minute to about 180 minutes or longer.

[00191] The step of incubating the platelets with a cryoprotectant can include incubating the platelets and the cryoprotectant at a temperature that, when selected in conjunction with the amount of time allotted, is suitable for incubating. In general, the composition is incubated at a temperature above freezing for at least a sufficient time for the cryoprotectant to come into contact with the platelets. In embodiments, incubation is conducted at 37°C. In certain embodiments, incubation is performed at 20°C to 42°C. For example, in embodiments, incubation is performed at 35°C to 40°C (e.g., 37°C) for 110 to 130 (e.g., 120) minutes.

[00192] In various embodiments, the lyophilization bag is a gas-permeable bag configured to allow gases to pass through at least a portion or all portions of the bag during the processing. The gas-permeable bag can allow for the exchange of gas within the interior of the bag with atmospheric gas present in the surrounding environment. The gas-permeable bag can be permeable to gases, such as oxygen, nitrogen, water, air, hydrogen, and carbon dioxide, allowing gas exchange to occur in the compositions provided herein. In some embodiments, the gas-permeable bag allows for the removal of some of the carbon dioxide present within an interior of the bag by allowing the carbon dioxide to permeate through its

wall. In some embodiments, the release of carbon dioxide from the bag can be advantageous to maintaining a desired pH level of the composition contained within the bag.

[00193] In some embodiments, the container of the process herein is a gas-permeable container that is closed or sealed. In some embodiments, the container is a container that is closed or sealed and a portion of which is gas-permeable. In some embodiments, the surface area of a gas-permeable portion of a closed or sealed container (e.g., bag) relative to the volume of the product being contained in the container (hereinafter referred to as the "SA/V ratio") can be adjusted to improve pH maintenance of the compositions provided herein. For example, in some embodiments, the SA/V ratio of the container can be at least about 2.0 cm^2/mL (e.g., at least about 2.1 cm^2/mL, at least about 2.2 cm^2/mL, at least about 2.3 cm^2/mL, at least about 2.4 cm^2/mL, at least about 2.5 cm^2/mL, at least about 2.6 cm^2/mL, at least about 2.7 cm^2/mL, at least about 2.8 cm^2/mL, at least about 2.9 cm^2/mL, at least about 3.0 cm^2/mL, at least about 3.1 cm^2/mL, at least about 3.2 cm^2/mL, at least about 3.3 cm^2/mL, at least about 3.4 cm^2/mL, at least about 3.5 cm^2/mL, at least about 3.6 cm^2/mL, at least about 3.7 cm^2/mL, at least about 3.8 cm^2/mL, at least about 3.9 cm^2/mL, at least about 4.0 cm^2/mL, at least about 4.1 cm^2/mL, at least about 4.2 cm^2/mL, at least about 4.3 cm^2/mL, at least about 4.4 cm^2/mL, at least about 4.5 cm^2/mL, at least about 4.6 cm^2/mL, at least about 4.7 cm^2/mL, at least about 4.8 cm^2/mL, at least about 4.9 cm^2/mL, or at least about 5.0 cm^2/mL . In some embodiments, the SA/V ratio of the container can be at most about 10.0 cm^2/mL (e.g., at most about 9.9 cm^2/mL, at most about 9.8 cm^2/mL, at most about 9.7 cm^2/mL, at most about 9.6 cm^2/mL, at most about 9.5 cm^2/mL, at most about 9.4 cm^2/mL, at most about 9.3 cm^2/mL, at most about 9.2 cm^2/mL, at most about 9.1 cm^2/mL, at most about 9.0 cm^2/mL, at most about 8.9 cm^2/mL, at most about 8.8 cm^2/mL, at most about 8.7 cm^2/mL, at most about 8.6, cm^2/mL at most about 8.5 cm^2/mL, at most about 8.4 cm^2/mL, at most about 8.3 cm^2/mL, at most about 8.2 cm^2/mL, at most about 8.1 cm^2/mL, at most about 8.0 cm^2/mL, at most about 7.9 cm^2/mL, at most about 7.8 cm^2/mL, at most about 7.7 cm^2/mL, at most about 7.6 cm^2/mL, at most about 7.5 cm^2/mL, at most about 7.4 cm^2/mL, at most about 7.3 cm^2/mL, at most about 7.2 cm^2/mL, at most about 7.1 cm^2/mL, at most about 6.9 cm^2/mL, at most about 6.8 cm^2/mL, at most about 6.7 cm^2/mL, at most about 6.6 cm^2/mL, at most about 6.5 cm^2/mL, at most about 6.4 cm^2/mL, at most about 6.3 cm^2/mL, at most about 6.2 cm^2/mL, at most about 6.1 cm^2/mL, at most about 6.0 cm^2/mL, at most about 5.9 cm^2/mL, at most about 5.8 cm^2/mL, at most about

5.7 cm^2/mL, at most about 5.6 cm^2/mL, at most about 5.5 cm^2/mL, at most about 5.4 cm^2/mL, at most about 5.3 cm^2/mL, at most about 5.2 cm^2/mL, at most about 5.1 cm^2/mL, at most about 5.0 cm^2/mL, at most about 4.9 cm^2/mL, at most about 4.8 cm^2/mL, at most about 4.7 cm^2/mL, at most about 4.6 cm^2/mL, at most about 4.5 cm^2/mL, at most about 4.4 cm^2/mL, at most about 4.3 cm^2/mL, at most about 4.2 cm^2/mL, at most about 4.1 cm^2/mL, or at most about 4.0 cm^2/mL . In some embodiments, the SA/V ratio of the container can range from about 2.0 to about 10.0 cm^2/mL (e.g., from about 2.1 cm^2/mL to about 9.9 cm^2/mL, from about 2.2 cm^2/mL to about 9.8 cm^2/mL, from about 2.3 cm^2/mL to about 9.7 cm^2/mL, from about 2.4 cm^2/mL to about 9.6 cm^2/mL, from about 2.5 cm^2/mL to about 9.5 cm^2/mL, from about 2.6 cm^2/mL to about 9.4 cm^2/mL, from about 2.7 cm^2/mL to about 9.3 cm^2/mL, from about 2.8 cm^2/mL to about 9.2 cm^2/mL, from about 2.9 cm^2/mL to about 9.1 cm^2/mL, from about 3.0 cm^2/mL to about 9.0 cm^2/mL, from about 3.1 cm^2/mL to about 8.9 cm^2/mL, from about 3.2 cm^2/mL to about 8.8 cm^2/mL, from about 3.3 cm^2/mL to about 8.7 cm^2/mL, from about 3.4 cm^2/mL to about 8.6 cm^2/mL, from about 3.5 cm^2/mL to about 8.5 cm^2/mL, from about 3.6 cm^2/mL to about 8.4 cm^2/mL, from about 3.7 cm^2/mL to about 8.3 cm^2/mL, from about 3.8 cm^2/mL to about 8.2 cm^2/mL, from about 3.9 cm^2/mL to about 8.1 cm^2/mL, from about 4.0 cm^2/mL to about 8.0 cm^2/mL, from about 4.1 cm^2/mL to about 7.9 cm^2/mL, from about 4.2 cm^2/mL to about 7.8 cm^2/mL, from about 4.3 cm^2/mL to about 7.7 cm^2/mL, from about 4.4 cm^2/mL to about 7.6 cm^2/mL, from about 4.5 cm^2/mL to about 7.5 cm^2/mL, from about 4.6 cm^2/mL to about 7.4 cm^2/mL, from about 4.7 cm^2/mL to about 7.3 cm^2/mL, from about 4.8 cm^2/mL to about 7.2 cm^2/mL, from about 4.9 cm^2/mL to about 7.1 cm^2/mL, from about 5.0 cm^2/mL to about 6.9 cm^2/mL, from about 5.1 cm^2/mL to about 6.8 cm^2/mL, from about 5.2 cm^2/mL to about 6.7 cm^2/mL, from about 5.3 cm^2/mL to about 6.6 cm^2/mL, from about 5.4 cm^2/mL to about 6.5 cm^2/mL, from about 5.5 cm^2/mL to about 6.4 cm^2/mL, from about 5.6 cm^2/mL to about 6.3 cm^2/mL, from about 5.7 cm^2/mL to about 6.2 cm^2/mL, or from about 5.8 cm^2/mL to about 6.1 cm^2/mL.

[00194] Gas-permeable closed containers (e.g., bags) or portions thereof can be made of one or more various gas-permeable materials. In some embodiments, the gas-permeable bag can be made of one or more polymers including fluoropolymers (such as polytetrafluoroethylene (PTFE) and perfluoroalkoxy (PFA) polymers), polyolefins (such as low-density polyethylene (LDPE), high-density polyethylene (HDPE)), fluorinated ethylene

propylene (FEP), polystyrene, polyvinylchloride (PVC), silicone, and any combinations thereof.

[00195] In some embodiments, dried platelets or platelet derivatives (e.g., thrombosomes) can undergo heat treatment. Heating can be performed at a temperature above about 25°C (e.g., greater than about 40°C, 50°C, 60°C, 70°C, 80°C or higher). In some embodiments, heating is conducted between about 70°C and about 85°C (e.g., between about 75°C and about 85°C, or at about 75°C or 80 °C). The temperature for heating can be selected in conjunction with the length of time that heating is to be performed. Although any suitable time can be used, typically, the lyophilized platelets are heated for at least 1 hour, but not more than 36 hours. Thus, in embodiments, heating is performed for at least 2 hours, at least 6 hours, at least 12 hours, at least 18 hours, at least 20 hours, at least 24 hours, or at least 30 hours. For example, the lyophilized platelets can be heated for 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 24 hours, 25 hours, 26 hours, 27 hours, 28 hours, 29 hours, or 30 hours. Non-limiting exemplary combinations include: heating the dried platelets or platelet derivatives (e.g., thrombosomes) for at least 30 minutes at a temperature higher than 30°C; heating the dried platelets or platelet derivatives (e.g., thrombosomes) for at least 10 hours at a temperature higher than 50°C; heating the dried platelets or platelet derivatives (e.g., thrombosomes) for at least 18 hours at a temperature higher than 75°C; and heating the dried platelets or platelet derivatives (e.g., thrombosomes) for 24 hours at 80°C. In some embodiments, heating can be performed in sealed container, such as a capped vial. In some embodiments, a sealed container be subjected to a vacuum prior to heating. The heat treatment step, particularly in the presence of a cryoprotectant such as albumin or polysucrose, has been found to improve the stability and shelf-life of the freeze-dried platelets. Indeed, advantageous results have been obtained with the particular combination of serum albumin or polysucrose and a post-lyophilization heat treatment step, as compared to those cryoprotectants without a heat treatment step. A cryoprotectant (e.g., sucrose) can be present in any appropriate amount (e.g. about 3% to about 10% by mass or by volume of the platelets or platelet derivatives (e.g., thrombosomes).

[00196] In some embodiments, the platelets or platelet derivatives (e.g., thrombosomes) prepared as disclosed herein by a process comprising incubation with an incubating agent have a storage stability that is at least about equal to that of the platelets prior to the incubation.

[00197] In some embodiments, the method further comprises cryopreserving the platelets or platelet derivatives prior to administering the platelets or platelet derivatives (e.g., with an incubating agent, e.g., an incubating agent described herein).

[00198] In some embodiments, the method further comprises drying a composition comprising platelets or platelet derivatives, (e.g., with an incubating agent e.g., an incubating agent described herein) prior to administering the platelets or platelet derivatives (e.g., thrombosomes). In some embodiments, the method may further comprise heating the composition following the drying step. In some embodiments, the method may further comprise rehydrating the composition following the freeze-drying step or the heating step.

[00199] In some embodiments, the method further comprises freeze-drying a composition comprising platelets or platelet derivatives (e.g., with an incubating agent e.g., an incubating agent described herein) prior to administering the platelets or platelet derivatives (e.g., thrombosomes) In some embodiments, the method may further comprise heating the composition following the freeze-drying step. In some embodiments, the method may further comprise rehydrating the composition following the freeze-drying step or the heating step.

[00200] In some embodiments, the method further comprises cold storing the platelets, platelet derivatives, or the thrombosomes prior to administering the platelets, platelet derivatives, or thrombosomes (e.g., with an incubating agent, e.g., an incubating agent described herein).

[00201] Storing conditions include, for example, standard room temperature storing (e.g., storing at a temperature ranging from about 20 to about 30 °C) or cold storing (e.g., storing at a temperature ranging from about 1 to about 10°C). In some embodiments, the method further comprises cryopreserving, freeze-drying, thawing, rehydrating, and combinations thereof, a composition comprising platelets or platelet derivatives (e.g., thrombosomes) (e.g., with an incubating agent e.g., an incubating agent described herein) prior to administering the platelets or platelet derivatives (e.g., thrombosomes). For example, in some embodiments, the method further comprises drying (e.g., freeze-drying) a composition comprising platelets or platelet derivatives (e.g., with an incubating agent e.g., an incubating agent described herein) (e.g., to form thrombosomes) prior to administering the platelets or platelet derivatives (e.g.,

thrombosomes). In some embodiments, the method may further comprise rehydrating the composition obtained from the drying step.

[00202] In some embodiments, provided herein is composition comprising platelets or platelet derivatives (e.g., thrombosomes), polysucrose and trehalose made by the process of obtaining fresh platelets, optionally incubating the platelets in DMSO, isolating the platelets by centrifugation, resuspending the platelets in an incubating agent which comprises trehalose and ethanol thereby forming a first mixture, incubating the first mixture, mixing polysucrose with the first mixture, thereby forming a second mixture, and lyophilizing the second mixture to form a freeze dried composition comprising platelets or platelet derivatives (e.g., thrombosomes), polysucrose and trehalose.

[00203] In some embodiments, provided herein is a method of making a freeze-dried platelet composition comprising platelets or platelet derivatives (e.g., thrombosomes), polysucrose and trehalose comprising obtaining fresh platelets, optionally incubating the platelets in DMSO, isolating the platelets by centrifugation, resuspending the platelets in a incubating agent which comprises trehalose and ethanol thereby forming a first mixture, incubating the first mixture, mixing polysucrose with the first mixture, thereby forming a second mixture, and lyophilizing the second mixture to form a freeze-dried composition comprising platelets or platelet derivatives (e.g., thrombosomes), polysucrose and trehalose.

[00204] In some embodiments, provided herein is a process for making freeze-dried platelets, the process comprising incubating isolated platelets in the presence of at least one saccharide under the following conditions: a temperature of from 20° C. to 42° C for about 10 minutes to about 180 minutes, adding to the platelets at least one cryoprotectant, and lyophilizing the platelets, wherein the process optionally does not include isolating the platelets between the incubating and adding steps, and optionally wherein the process does not include exposing the platelets to a platelet activation inhibitor. The cryoprotectant can be a polysugar (e.g., polysucrose). The process can further include heating the lyophilized platelets at a temperature of 70° C to 80° C for 8 to 24 hours. The step of adding to the platelets at least one cryoprotectant can further include exposing the platelets to ethanol. The step of incubating isolated platelets in the presence of at least one saccharide can include incubating in the presence of at least one saccharide. The step of incubating isolated platelets in the presence of at least one saccharide can include incubating in the presence of at least one saccharide. The conditions for incubating can

include incubating for about 100 minutes to about 150 minutes. The conditions for incubating can include incubating for about 110 minutes to about 130 minutes. The conditions for incubating can include incubating for about 120 minutes. The conditions for incubating can include incubating at 35° C to 40° C. The conditions for incubating can include incubating at 37° C. The conditions for incubating can include incubating at 35° C to 40° C. for 110 minutes to 130 minutes. The conditions for incubating can include incubating at 37° C for 120 minutes. The at least one saccharide can be trehalose, sucrose, or both trehalose and sucrose. The at least one saccharide can be trehalose. The at least one saccharide can be sucrose.

[00205] In some embodiments, provided herein is a method of preparing freeze-dried platelets, the method including providing platelets, suspending the platelets in a salt buffer that includes about 100 mM trehalose and about 1% (v/v) ethanol to make a first composition, incubating the first composition at about 37° C. for about 2 hours, adding polysucrose (e.g., polysucrose 400) to a final concentration of about 6% (w/v) to make a second composition, lyophilizing the second composition to make freeze-dried platelets, and heating the freeze-dried platelets at 80° C for 24 hours.

[00206] Specific embodiments disclosed herein may be further limited in the claims using "consisting of" or "consisting essentially of" language.

[00207] **Exemplary Embodiments**

[00208] Embodiment 1 is a method of treating a coagulopathy in a subject, the method comprising administering to the subject in need thereof an effective amount of a composition comprising platelets or platelet derivatives and an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent.

[00209] Embodiment 2 is a method of treating a coagulopathy in a subject, the method comprising administering to the subject in need thereof an effective amount of a composition prepared by a process comprising incubating platelets with an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent, to form the composition.

[00210] Embodiment 3 is a method of restoring normal hemostasis in a subject, the method comprising administering to the subject in need thereof an effective amount of a

composition comprising platelets or platelet derivatives and an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent.

[00211] Embodiment 4 is a method of restoring normal hemostasis in a subject, the method comprising administering to the subject in need thereof an effective amount of a composition prepared by a process comprising incubating platelets with an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent, to form the composition.

[00212] Embodiment 5 is a method of preparing a subject for surgery, the method comprising administering to the subject in need thereof an effective amount of a composition comprising platelets or platelet derivatives and an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent.

[00213] Embodiment 6 is a method of preparing a subject for surgery, the method comprising administering to the subject in need thereof an effective amount of a composition prepared by a process comprising incubating platelets with an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent, to form the composition.

[00214] Embodiment 7 is the method of any one of embodiments 5-6, wherein the surgery is an emergency surgery.

[00215] Embodiment 8 is the method of any one of embodiments 5-6, wherein the surgery is a scheduled surgery.

[00216] Embodiment 9 is the method of any one of embodiments 1-8, wherein the subject has been treated or is being treated with an antiplatelet agent.

[00217] Embodiment 10 is the method of embodiment 9, wherein treatment with the antiplatelet agent is stopped.

[00218] Embodiment 11 is the method of embodiment 9, wherein treatment with the antiplatelet agent is continued.

[00219] Embodiment 12 is a method of ameliorating the effects of an antiplatelet agent in a subject, the method comprising administering to the subject in need thereof an effective amount of a composition comprising platelets or platelet derivatives and an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent.

[00220] Embodiment 13 is a method of ameliorating the effects of an antiplatelet agent in a subject, the method comprising administering to the subject in need thereof an effective amount of a composition prepared by a process comprising incubating platelets with an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent, to form the composition.

[00221] Embodiment 14 is the method of embodiment 12 or embodiment 13, wherein the effects of the antiplatelet agent are the result of an overdose of the antiplatelet agent.

[00222] Embodiment 15 is the method of any one of embodiments 1-14, wherein the composition further comprises an anti-fibrinolytic agent.

[00223] Embodiment 16 is the method of embodiment 15, wherein the anti-fibrinolytic agent is selected from the group consisting of ε-aminocaproic acid (EACA), tranexamic acid, aprotinin, aminomethylbenzoic acid, fibrinogen, and a combination thereof.

[00224] Embodiment 17 is the method of embodiment 15 or embodiment 16, wherein the platelets or platelet derivatives are loaded with the anti-fibrinolytic agent.

[00225] Embodiment 18 is the method of any one of embodiments 9-16, wherein the antiplatelet agent is selected from the group consisting of aspirin, cangrelor, ticagrelor, clopidogrel, prasugrel, eptifibatide, tirofiban, abciximab, a supplement, and a combination thereof.

[00226] Embodiment 19 is the method of any one of embodiments 9-16, wherein the antiplatelet agent is selected from the group consisting of aspirin, cangrelor, ticagrelor, clopidogrel, prasugrel, eptifibatide, tirofiban, abciximab, terutroban, picotamide, elinogrel, ticlopidine, ibuprofen, vorapaxar, atopaxar, and a combination thereof.

[00227] Embodiment 20 is the method of any one of embodiments 9-16, wherein the antiplatelet agent is selected from the group consisting of aspirin, cangrelor, ticagrelor, clopidogrel, prasugrel, eptifibatide, tirofiban, abciximab, terutroban, picotamide, elinogrel, ticlopidine, ibuprofen, vorapaxar, atopaxar, cilostazol, prostaglandin E1, epoprostenol, dipyridamole, treprostinil sodium, sarpogrelate, and a combination thereof.

[00228] Embodiment 21 is the method of any one of embodiments 1-20, wherein administering comprises administering topically.

[00229] Embodiment 22 is the method of any one of embodiments 1-20, wherein administering comprises administering parenterally.

[00230] Embodiment 23 is the method of any one of embodiments 1-20, wherein administering comprises administering intravenously.

[00231] Embodiment 24 is the method of any one of embodiments 1-20, wherein administering comprises administering intramuscularly.

[00232] Embodiment 25 is the method of any one of embodiments 1-20, wherein administering comprises administering intrathecally.

[00233] Embodiment 26 is the method of any one of embodiments 1-20, wherein administering comprises administering subcutaneously.

[00234] Embodiment 27 is the method of any one of embodiments 1-20, wherein administering comprises administering intraperitoneally.

[00235] Embodiment 28 is the method of any one of embodiments 1-27, wherein the composition is dried prior to the administration step.

[00236] Embodiment 29 is the method of embodiment 28, wherein the composition is rehydrated following the drying step.

[00237] Embodiment 30 is the method of any one of embodiments 1-28, wherein the composition is freeze-dried prior to the administration step.

[00238] Embodiment 31 is the method of embodiment 30, wherein the composition is rehydrated following the freeze-drying step.

[00239] Embodiment 32 is the method of any one of embodiments 1-31, wherein the incubating agent comprises one or more salts selected from phosphate salts, sodium salts, potassium salts, calcium salts, magnesium salts, and a combination of two or more thereof.

[00240] Embodiment 33 is the method of any one of embodiments 1-32, wherein the incubating agent comprises a carrier protein.

[00241] Embodiment 34 is the method of any one of embodiments 1-33, wherein the buffer comprises HEPES, sodium bicarbonate ($NaHCO_3$), or a combination thereof.

[00242] Embodiment 35 is the method of any one of embodiments 1-34, wherein the composition comprises one or more saccharides.

[00243] Embodiment 36 is the method of embodiment 35, wherein the one or more saccharides comprise trehalose.

[00244] Embodiment 37 is the method of embodiment 35 or embodiment 36, wherein the one or more saccharides comprise polysucrose.

[00245] Embodiment 38 is the method of any one of embodiments 35-37, wherein the one or more saccharides comprise dextrose.

[00246] Embodiment 39 is the method of any one of embodiments 1-38, wherein the composition comprises an organic solvent.

[00247] Embodiment 40 is the method of any of embodiments 1-39, wherein the platelets or platelet derivatives comprise thrombosomes.

EXAMPLES

[00248] The results that follow demonstrate the impact of the thrombosomes product in an *in vitro* model of a patient taking antiplatelet drugs. Thrombosomes and other lyophilized platelet products are designed for infusion into a patient's bloodstream following diagnosis of trauma or hemostatic failure. These drugs utilize multiple forms of platelet inhibition mechanisms which inhibit platelet response to adenosine diphosphate (ADP), arachidonic acid, fibrinogen and von Willebrand factor binding to name a few. These include drugs like aspirin, clopidogrel, ticagrelor, effient, cangrelor and eptifibatide.

[00249] **Example 1 – $P2Y_{12}$ inhibitors**

[00250] Cangrelor, like clopidogrel, ticagrelor, and prasugurel, blocks the $P2Y_{12}$ (ADP) receptor on platelets. Cangrelor is used here as a representative of this class of drug.

[00251] Thrombosomes were prepared consistent with the procedure in Example 4. Transmission light aggregometry and T-TAS® experiments were carried out according to Example 4.

[00252] The effect of cangrelor on the aggregation of platelets in platelet-rich plasma (PRP; taken from humans as whole blood and processed to isolate platelets in plasma without white blood cells (WBC) or red blood cells (rbc) was evaluated by transmission light aggregrometry. Aggregation of platelets (platelet rich plasma) in response to agonist-induced activation showed complete inhibition of 10 μM adenosine diphosphate (ADP)-induced aggregation by cangrelor at therapeutic concentration of 0.5 μM-3.5 μM (Figure 1). All doses of cangrelor investigated completely eliminated ADP-induced platelet aggregation in PRP.

[00253] The effect of cangrelor on platelet occlusion under shear was evaluated by T-TAS®. Fresh platelet rich plasma (platelet concentration 278,000/μL; PRP generally has a platelet concentration of about 200,000/μL to about 300,000/μL) stimulated *in vitro* with 10 μM

ADP occluded earlier under high shear than unstimulated platelets (PRP) as determined by AR chip (collagen and tissue thromboplastin) using T-TAS® technology (Figure 2). Cangrelor alone (1 μM) did not exhibit inhibition on occlusion, but when combined with ADP (10 μM), platelet adhesion and occlusion was essentially eliminated. These results are further illustrated in Figures 3 and 4. Without being bound by any particular theory, it is believed that this pattern is observed because platelets have other ADP receptors not blocked by cangrelor that respond to ADP and cause shape change and aggregation where the ADP receptor P2Y12 blocking inhibits collagen binding, and, accordingly, the platelets may bind each other due to ADP stimulation but may be prevented from binding collagen on the coated chip.

[00254] In Figure 3, the area under the curve (AUC) values (derived from data in Figure 2; replicates are averaged and plotted once) are indicative of a combined value of how quickly the thrombus happened in time and how substantial the thrombus is when it does happen. PRP AUC was increased with ADP stimulation. Cangrelor had little effect on AUC value, but when combined with ADP stimulation, the AUC dropped close to zero.

[00255] In Figure 4, the time to occlusion of the AR T-TAS® chip with drug treatment was evaluated. PRP occluded the chip channel at approximately 20 minutes, and stimulation of platelets with ADP decreased that time. Cangrelor had little effect on occlusion times, but addition of ADP stimulation to PRP sample inhibited occlusion essentially completely.

[00256] In the presence of cangrelor with ADP stimulation at the concentrations shown to be inhibitory of platelets, thrombosomes ("thromb" in Figures 5-7) were not inhibited, indicating that thrombosomes can aid in a clot formation even in the presence of cangrelor at therapeutic levels.

[00257] The effect of cangrelor on thrombosomes under shear was evaluated by T-TAS®. Figure 5 shows that thrombosomes (after 60, 90, or 115 minutes of rehydration, as indicated) retain hemostatic function in the absence or presence of cangrelor (1 uM), with ADP (10 uM) present. Unlike platelets, thrombosome occlusion of the T-TAS® AR Chip is unaffected by the antiplatelet effect of cangrelor + ADP. This suggests thrombosomes will maintain expected function when infused into patients receiving cangrelor and similar agents. These results are further illustrated in Figures 6 and 7.

[00258] In Figure 6, the AUC values (derived from the data in Figure 5) are indicative of thrombus formation. There was no effect of cangrelor + ADP on thrombosome adhesion and

occlusion of the T-TAS® AR Chip in plasma; thrombosomes caused a thrombus formation regardless of cangrelor and ADP. The same dose of cangrelor and ADP completely inhibited freshly harvested platelets.

[00259] In Figure 7, the time to occlusion (derived from the data in Figure 5) of the thrombosomes on AR T-TAS® chip with drug treatment was evaluated. There was no effect from cangrelor + ADP on thrombosome time to occlusion using the T-TAS® AR Chip in plasma. The same dose of cangrelor and ADP completely inhibited freshly harvested platelets.

[00260] **Example 2. GPIIb-IIIa inhibitors.**

[00261] The results that follow demonstrate the impact of thrombosomes in an in vitro model of a patient taking a GPIIb-IIIa inhibitor. Eptifibatide, a common antiplatelet drug, competitively inhibits the GPIIb-IIIa receptor on platelets which interact with fibrinogen and von Willebrand factor.

[00262] Eptifibatide is a peptide therapeutic that blocks the fibrin binding role of GPIIb-IIIa receptor on platelets. The drug is typically administered via IV as a 180 μg/kg bolus followed by 2 μg/kg/min continuous infusion. The blood concentration of eptifibatide is typically about 1-2 μM. Bleeding time generally returns to normal within about 1 hour of drug stoppage.

[00263] Thrombosomes were prepared consistent with the procedure in Example 4. Transmission light aggregometry and T-TAS® experiments were carried out according to Example 4.

[00264] The aggregation of platelets (in platelet rich plasma) was evaluated using transmission light aggregrometry. Eptifibatide completely inhibited collagen-induced (10 μg/mL) platelet aggregation in PRP at all concentrations tested, as detected by light transmission aggregometry in PRP. (Figure 8).

[00265] The effect of thrombosomes on shortening clotting times while in the presence of eptifibatide was also studied. The ability of thrombosomes to recover occlusion times was studied on the T-TAS® system. The T-TAS® system measures occlusion time under shear forces with collagen and thromboplastin stimulation. The whole blood profile of occlusion and AUC on the AR T-TAS® chip lengthened and decreased, respectively, with eptifibatide. Eptifibatide extended the occlusion time of whole blood on the T-TAS® AR Chip in a dose-dependent manner. In this experiment, whole blood occluded at 8 minutes, and the occlusion time was extended to 16 minutes with 6 μM eptifibatide (Figure 9). Thrombosomes reversed the

inhibitory effect of eptitibatide on thrombus formation. Eptifibatide inhibition of whole blood occlusion on the T-TAS® AR Chip was reversed by the addition of thrombosomes at approximately 200,000/μL (N=3). When thrombosomes (approximately 200k/μL) were added to the sample of whole blood inhibited with eptifibatide, the time to occlusion decreased to 'normal' at 9 minutes (Figure 10).

[00266] The area under the curve values with thrombosome treatment also increased with thrombosomes compared to that of normal whole blood samples. Figure 11 demonstrates the time to of occlusion of the thrombosomes on AR T-TAS® chip with drug treatment; eptifibatide inhibition of T-TAS® AR Chip occlusion was nearly entirely reversed by the addition of thrombosomes (200,000/μL; N=3). In Figure 12, the area under the curve values were indicative of thrombus formation, where thrombosomes returned inhibition by eptifibatide to normal levels; eptifibatide inhibition of platelet adhesion to and occlusion of the T-TAS® AR Chip is overcome by addition of thrombosomes (200,000/μL; N=3).

[00267] Thrombosomes, unlike platelets, are not inhibited in their ability to occlude under shear in the presence of eptifibatide (Figure 13). Figure 13 shows profiles of thrombus formation of various lots of thrombsomes on AR T-TAS® system were unchanged with eptifibatide treatment. Thrombosomes in platelet poor plasma (PPP) were flowed through the T-TAS® AR Chip with and without 6 uM eptifibatide. There was no effect of eptifibatide on thrombosome adhesion and occlusion. All thrombosome concentrations were approximately 300,000/μL.

[00268] The AUC and occlusion values by T-TAS for thrombosomes (approximately 300,000/μL) in plasma was the same with and without eptifibatide (Figure 14-15). Figure 14 shows the area under the curve values were indicative of thrombus formation, and no changes were observed with eptifibatide in platelet-poor plasma. There was no effect of 6 uM eptifibatide on AUC of Thrombosomes T-TAS® AR Chip occlusion. Figure 15 shows the time to occlusion of the thrombosomes on AR T-TAS® chip was unchanged with eptifibatide. There was no significant influence from 6 μM eptifibatide on thrombosomes occlusion time of the T-TAS® AR Chip in platelet-poor plasma.

[00269] **Example 3. COX inhibitors.**

[00270] The results that follow demonstrate the impact of thrombosomes in an *in vitro* model of a patient taking a COX inhibitor. Aspirin, a common antiplatelet drug, blocks the

COX1 enzyme in platelets. COX1 is responsible for converting arachidonic acid to prostaglandin.

[00271] Aspirin is an irreversible cyclooxygenase (COX) inhibitor. The COX enzyme in platelets is responsible for synthesis of thromboxane A2, prostaglandin E2, and prostacyclin (PGI2). Aspirin permanently inactivates the COX enzyme within platelets, and since platelets do not have the nuclear material to synthesize new enzyme, new platelets must be produced to overcome the aspirin effect. Without thromboxane A2, prostaglandin E2 and prostacyclin (PGI2) platelets are limited in their pro-aggregation activity. Many people are maintained on a low dose of aspirin to prevent unwanted clotting events. Aspirin bioavailability largely varies with administration route, with a single 500 mg dose IV at peaks of 500 μM and the same dose orally at 44 μM.

[00272] Thrombosomes were prepared consistent with the procedure in Example 4. Transmission light aggregometry and T-TAS® experiments were carried out according to Example 4.

[00273] Platelets will aggregate with collagen and arachidonic acid stimulation. Stimulation by arachidonic acid can be completely inhibited whereas collagen stimulation aggregation can only be partially inhibited at concentrations of 100-400 μM aspirin (Figure 16). Figure 16 shows light transmission aggregometry in PRP with collagen (10 ug/mL) and arachidonic acid (AA; 500 ug/mL), which induced platelet aggregation, and that aggregation was inhibited by all doses of aspirin (ASA) tested. Aspirin eliminated arachidonic acid induced platelet aggregation entirely. The PL chip system on the T-TAS® was used to emulate *in vitro* platelet binding and aggregation due to the exposure of collagen in the vasculature under shear conditions. This action of platelets was largely limited in the presence of 100 and 500 μM of aspirin but can be at least partially returned in the presence of thrombosomes (approximately 200,000 to 400,000/μL; Figure 17). Figure 17 shows via area under the curve measurement of whole blood that thrombus formation on the PL T-TAS® chip was inhibited by aspirin with partial return of thrombus formation with thrombosomes.

[00274] **Example 4. Protocols**

[00275] **Generation of Thrombosomes.** Thrombosomes were prepared consistent with the procedures described in U.S. Patent Nos. 8,486,617 (such as, e.g., Examples 1-5) and 8,097,403 (such as, e.g., Examples 1-3), incorporated herein by reference in their entirety.

[00276] **Transmission Light Aggregometry**

[00277] Plasma samples with platelet or thrombosomes or combination of both are loaded into cuvettes and placed into the aggregometry chambers. The chambers warm the sample and provide constant stirring. The initiation of aggregation can be done by multiple types of inhibitor agents not limited to thrombin, ADP, collagen and any agent know to stimulate platelet aggregation. The samples can also have been taken as ex-vivo, or in-vitro supplemented with inhibitors. The instrument begins the assay by first recording the light transmission previous to stimulation for 2 minutes. The stimulant of interest is then introduced by the technician and the change in light transmission is recorded overtime. The increase in light transmission corresponds to increase in platelet aggregation.

[00278] **Evaluation by T-TAS® using an AR chip**. AR chips are characterized by a single channel containing collagen and tissue factor; they can be used to analyze clotting and platelet function.

[00279] The T-TAS® instrument was prepared for use according to the manufacturer's instructions. AR Chips (Diapharma Cat. # TC0101) and AR Chip Calcium Corn Trypsin Inhibitor (CaCTI; Diapharma Cat. # TR0101) were warmed to room temperature. 300 uL of rehydrated thrombosomes were transferred to a 1.7 mL microcentrifuge tube and centrifuged at 3900 *g* x 10 minutes to pellet. The thrombosomes pellet was resuspended in George King (GK) pooled normal human plasma or autologous plasma with or without autologous platelets to a concentration of approximately 100,000- 450,000/uL, as determined by AcT counts (Beckman Coulter AcT Diff 2 Cell Counter). 20 uL of CaCTI with 480 uL of thrombosomes sample in GK plasma were mixed with gentle pipetting. The sample was loaded and run on the T-TAS® according to the manufacturer's instructions.

[00280] **Evaluation by T-TAS® using a PL chip**

[00281] PL chips are run similarly to AR chips but this chip is only coated with collagen alone.

[00282] **Thrombin Generation**

[00283] *Reagent Preparation.* For thrombin generation, the following materials were used from manufacturers, as follows: FluCa Kit (Diagnostica Stago, Cat. No. 86197), Thrombin calibrator (Diagnostica Stago, Cat. No. 86197), PRP Reagent (Diagnostica Stago, Cat. No. 86196), OCTOPLAS®, a solvent detergent treated human pooled plasma (Octapharma, Cat. No.

8-68209-952-04). All frozen reagents were thawed in a 37 °C water bath before use. All reagents were rehydrated with sterile water using the volume printed on the reagent labels. Approximately 2 min after rehydration, the reagents were mixed by inverting vials 5 times, so no chunks or powder left; vortexing was not used. This procedure was repeated approximately 10 minutes after rehydration. All reagents were incubate at room temperature for another approximately 10 minutes (total of approximately 20 min after rehydration). A 30% solution of OCTOPLAS® was prepared by mixing 4.66ml of thrombosomes control buffer (Table B) with 2ml of OCTOPLAS®.

[00284] Table B. Thrombosomes Control Buffer

Component	**Concentration** (mg/mL, except where otherwise indicated)
NaCl	6.08
KCl	0.28
HEPES	2.47
$NaHCO_3$	0.77
Dextrose	0.41
Trehalose	28.83
Ethanol	0.76% (v/v)

[00285] *Sample Analysis - Plate preparation and testing.* For experiments containing thrombosomes, a thrombosomes dilution series was generated (dilutions of 194.4K, 64.8K, 21.6K, and 7.2K per µL were typically used; cell counts are determined by flow cytometry) for each the experimental thrombosomes and the reference thrombosomes. Thrombosomes were rehydrated unless indicated otherwise. The highest-concentration dilution (e.g., 194.4k thrombosomes) was prepared by combining thrombosomes, OCTAPLAS®, and thrombosomes Control Buffer. The rest of the dilution series was prepared by serial 1:3 dilutions in OCTAPLAS®. For each test sample, 20 uL of PRP reagent was added to each sample well (of Immulon 2HB Clear, round-bottom 96-well plate (VWR, Cat. No. 62402-954)) and 20 uL of Thrombin Calibrator was added to each calibrator well. To each sample well and calibrator well, 80 uL of the each of the thrombosomes dilution series was added. Continue until the last dilution. The plate was then incubated in the Fluoroskan Ascent 96 well fluorescent plate reader

(Thrombinoscope) (ThermoFisher Scientific) for 10 minutes. During this incubation phase, the FluCa solution was prepared by adding 40µL of FluCa substrate to the 1.6ml of thawed Fluo-Buffer, vortexing, and returning the solution to the water bath. When incubation was complete, the FluCa solution was added to the Fluroskan instrument according to the manufacturer's instructions. The plate fluorescence was monitored for 75 minutes at an interval of 20 seconds and a temperature of 40-41°C.

[00286] Example 5.

[00287] Additional experiments were carried out with cangrelor and aspirin. Thrombosomes were prepared consistent with the procedure in Example 4. Transmission light aggregometry, T-TAS®, and thrombin generation experiments were carried out according to Example 4.

[00288] The effect of thrombosomes on the recovery of thrombus formation was evaluated using T-TAS® technology and an AR chip. Figure 18 shows the occlusion time of whole blood treated with various combinations of thrombosomes (at a concentration of 250,000 thrombosomes per µL), aspirin (200 µM), cangrelor (1 µM), anti-Integrin alpha-2 (CD49B) antibody 6F1 (40 µg; see dshb.biology.uiowa.edu/integrin-alpha-2-alpha2beta1?sc=7&category=-107 for product/manufacturer information), and anti-GPIIb/IIIa receptor antibody AP2 (20 ug/mL; see kerafast.com/product/2010/anti-glycoprotein-gpiiiagpiib-complex-ap-2-antibody for product/manufacturer information). Figure 19 shows the occlusion over time of untreated whole blood and whole blood treated with thrombosomes (at a concentration of 250,000 thrombosomes per µL), a mixture containing 6F1 (40 ug/mL; anti-CD49b), ASA (aspirin; 200 uM), and cangrelor (1 uM); or a combination thereof.

[00289] The effect of thrombosomes on the recovery of thrombus formation was also evaluated using T-TAS® technology and a PL chip. Figure 20 shows the occlusion time of whole blood treated only with buffer, aspirin (500 µM), or aspirin (500 µM) and thrombosomes (at a concentration of 250,000 thrombosomes per µL). Figure 21 shows the occlusion over time of whole blood, whole blood treated with aspirin (500 µM), or aspirin (500 µM) and thrombosomes (250,000/µL). Figures 22 and 23 show similar experimental data using 100 µM aspirin instead of 500 µM aspirin.

[00290] The effect of aspirin treatment (concentration) on thrombin generation was measured. Thrombosomes were evaluated at concentrations 1450, 1150, 850, 650, 450, 150, 50, and 0 k/uL in PPP from patients taking baby aspirin daily and standard plasma (INR = 1). Figure 24 shows that the peak thrombin value of the aspirin plasma in absence of thrombosomes was below the normal range (about 45 nM; normal range is about 66-166 nM), but with thrombosomes addition, it came back to being within the normal range at even the lowest thrombosomes concentration used (50 k/μL). The values again were saturated at about 800 k thrombosomes and went up to 220 nM – 5 times the value of this plasma in absence of thrombosomes (increase from 45 to 220 nM).

[00291] Example 6. Thrombosomes Reversed Prolonged PRP Occlusion Times Induced by Cangrelor

[00292] Additional experiments were carried out with cangrelor. Thrombosomes were prepared consistent with the procedure in Example 4. T-TAS® was carried out according to Example 4.

[00293] Figures 25A and 25B show that platelet rich plasma treated with 100ng/mL cangrelor and ADP extended occlusion times from 19 to 26 minutes on the T-TAS® flow system (collagen and tissue factor coated channel). The addition of 150k/μL thrombosomes decreased the time back to 15.3 minutes.

[00294] Example 7. Thrombosomes but not Random Donor Platelets (RDP) Reversed Extended Occlusion Times Induced by tirofiban in PRP

[00295] Additional experiments were carried out with tirofiban. Thrombosomes were prepared consistent with the procedure in Example 4. T-TAS® was carried out according to Example 4. Random donor platelets were prepared from whole blood.

[00296] Figures 26A and 26B show that platelet rich plasma treated with 100ng/mL tirofiban extended occlusion times from 18.43 to no occlusion on the T-TAS® flow system (collagen and tissue factor coated channel). The addition of 150k/μL of thrombosomes decreased the time back to 12.94 minutes but RDP only partially recovered at the same count.

[00297] Example 8. Thrombosomes but not Random Donor Platelets Reversed Extended Occlusion Times Induced by Eptifibatide in PRP

[00298] Additional experiments were carried out with eptifibatide. Thrombosomes were prepared consistent with the procedure in Example 4. T-TAS® was carried out according to Example 4. Random donor platelets were prepared from whole blood.

[00299] Figures 27A and 27B show that platelet rich plasma treated with 9μM eptifibatide extended occlusion times from 18.43to over 30 minutes on the T-TAS® flow system (collagen and tissue factor coated channel). The addition of 150k/μL of thrombosomes decreased the time back to 11.56 minutes but not occlusion seen with same number of RDP.

[00300] Example 9. Thrombosomes Reversed Extended Occlusion Times Induced by AP2 (anti-GpIIb/IIIa) in PRP

[00301] Additional experiments were carried out with AP2. Thrombosomes were prepared consistent with the procedure in Example 4. T-TAS® was carried out according to Example 4. Random donor platelets were prepared from whole blood.

[00302] Figures 28A and 28B show that platelet rich plasma treated with 10μg/mL AP-2 extended occlusion times from 18.43 to over 30 minutes on the T-TAS® flow system (collagen and tissue factor coated channel). The addition of 150k/μL of thrombosomes decreased the time back to 13.14 minutes and occlusion was seen at 17.43 minutes same number of RDP.

[00303] Example 10. Thrombosomes Reversed Prolonged Occlusion in PRP from Subjects on Aspirin Therapy

[00304] Additional experiments were carried out with aspirin. Thrombosomes were prepared consistent with the procedure in Example 4. T-TAS® was carried out according to Example 4. Random donor platelets were prepared from whole blood. The subject was on a standard dose of 81 mg/day of aspirin.

[00305] Figures 29A and 29B show that platelet rich plasma taken from an aspirin patient failed to occlude on the T-TAS® flow system (collagen and tissue factor coated channel). The addition of 200k/μL of thrombosomes returned to normal occlusion time to 16 minutes.

[00306] Example 11. Thrombosomes Restore Thrombin Generation in Ex-vivo Aspirin Platelet Rich Plasma

[00307] Additional experiments were carried out with aspirin. Thrombosomes were prepared consistent with the procedure in Example 4. Thrombin generation was carried out according to Example 4.

[00308] Figure 30A shows Thrombin generation of platelet rich plasma from aspirin patient verses normal stimulated with PRP reagent was reversed with 50k/μL of thrombosomes. Figure 30B shows the change from and return to normal thrombin production, time to peak production, and lag time in three repeat aspirin ex-vivo samplings with thrombosomes (50k/μL). (n=3 thrombosome lots, n=2 individuals).

[00309] Example 12. Thrombosomes Restore Hemostasis in PRP from Subject on NSAID Ibuprofen Therapy.

[00310] Additional experiments were carried out with ibuprofen, an NSAID. Thrombosomes were prepared consistent with the procedure in Example 4. Aggregometry and T-TAS® were carried out according to Example 4.

[00311] Platelet rich plasma was taken from subject on 800 mg ibuprofen. Figure 31A shows that a lack of aggregation in response to arachidonic acid confirms NSAID presence in the PRP. Figure 31B shows occlusion on the T-TAS® flow system (collagen and tissue factor coated channel); PRP from the ibuprofen patient demonstrated occlusion, while addition of ADP abolished occlusion. The addition of 150k/μL thrombosome restored occlusion.

[00312] Example 13. Thrombosomes® Restore Bleeding Time in NOD-SCID Mice Treated with Supra-pharmacologic Clopidogrel

[00313] Additional experiments were carried out with clopidogrel. Thrombosomes were prepared consistent with the procedure in Example 4.

[00314] The mouse was treated with clopidogrel for 5 days. The mouse was anesthetized, the tail end was snipped off followed by thrombosomes being immediately administered. The time from tail snip to tail stop bleeding was recorded by visual inspection.

[00315] NOD/SCID mice were treated with ~ 3 times the clinical dose of clopidogrel for 5 days then assessed in the tail-snip bleed model. The bleed time (min) was extended to 17.8 minutes with clopidogrel treatment verses untreated at 9 minutes (data not shown). Treatment

with 8μL/gram of thrombosomes (1.8 x 10^9 particles/mL at 200 μL) decreased bleeding to 12.31 minutes (Figure 32).

[00316] Although the foregoing description is directed to the preferred embodiments of the invention, it is noted that other variations and modifications will be apparent to those skilled in the art, and may be made without departing from the spirit or scope of the invention. Moreover, features described in connection with one embodiment of the invention may be used in conjunction with other embodiments, even if not explicitly stated above. Furthermore, one having ordinary skill in the art will readily understand that the invention as discussed above may be practiced with steps in a different order, and/or with hardware elements in configurations which are different than those which are disclosed. Therefore, although the invention has been described based upon these preferred embodiments, it would be apparent to those of skill in the art that certain modifications, variations, and alternative constructions would be apparent, while remaining within the spirit and scope of the invention. Embodiments of the invention so claimed are inherently or expressly described and enabled herein. In order to determine the metes and bounds of the invention, therefore, reference should be made to the appended claims.

What is claimed is:

1. A method of restoring normal hemostasis in a subject, the method comprising administering to the subject in need thereof an effective amount of a composition comprising platelets or platelet derivatives and an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent, wherein the subject has been treated or is being treated with an antiplatelet agent.

2. A method of restoring normal hemostasis in a subject, the method comprising administering to the subject in need thereof an effective amount of a composition prepared by a process comprising incubating platelets with an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent, to form the composition, wherein the subject has been treated or is being treated with an antiplatelet agent.

3. A method of treating a coagulopathy in a subject, the method comprising administering to the subject in need thereof an effective amount of a composition comprising platelets or platelet derivatives and an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent, wherein the subject has been treated or is being treated with an antiplatelet agent.

4. A method of treating a coagulopathy in a subject, the method comprising administering to the subject in need thereof an effective amount of a composition prepared by a process comprising incubating platelets with an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent, to form the composition, wherein the subject has been treated or is being treated with an antiplatelet agent.

5. A method of preparing a subject for surgery, the method comprising administering to the subject in need thereof an effective amount of a composition comprising platelets or platelet derivatives and an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent, wherein the subject has been treated or is being treated with an antiplatelet agent.

6. A method of preparing a subject for surgery, the method comprising administering to the subject in need thereof an effective amount of a composition prepared by a process comprising incubating platelets with an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent, to form the composition, wherein the subject has been treated or is being treated with an antiplatelet agent.

7. The method of any one of claims 5-6, wherein the surgery is an emergency surgery.

8. The method of any one of claims 5-6, wherein the surgery is a scheduled surgery.

9. The method of any one of claims 1-8, wherein the subject is being treated with an antiplatelet agent.

10. The method of claim 9, wherein treatment with the antiplatelet agent is stopped.

11. The method of claim 9, wherein treatment with the antiplatelet agent is continued.

12. A method of ameliorating the effects of an antiplatelet agent in a subject, the method comprising administering to the subject in need thereof an effective amount of a composition comprising platelets or platelet derivatives and an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent.

13. A method of ameliorating the effects of an antiplatelet agent in a subject, the method comprising administering to the subject in need thereof an effective amount of a composition prepared by a process comprising incubating platelets with an incubating agent comprising one or more salts, a buffer, optionally a cryoprotectant, and optionally an organic solvent, to form the composition.

14. The method of claim 12 or claim 13, wherein the effects of the antiplatelet agent are the result of an overdose of the antiplatelet agent.

15. The method of any one of claims 1-14, wherein the composition further comprises an anti-fibrinolytic agent.

16. The method of claim 15, wherein the anti-fibrinolytic agent is selected from the group consisting of ε-aminocaproic acid (EACA), tranexamic acid, aprotinin, aminomethylbenzoic acid, fibrinogen, and a combination thereof.

17. The method of claim 15 or claim 16, wherein the platelets or platelet derivatives are loaded with the anti-fibrinolytic agent.

18. The method of any one of claims 1-17, wherein the antiplatelet agent is selected from the group consisting of aspirin, cangrelor, ticagrelor, clopidogrel, prasugrel, eptifibatide, tirofiban, abciximab, a supplement, and a combination thereof.

19. The method of any one of claims 1-17, wherein the antiplatelet agent is selected from the group consisting of aspirin, cangrelor, ticagrelor, clopidogrel, prasugrel, eptifibatide, tirofiban, abciximab, terutroban, picotamide, elinogrel, ticlopidine, ibuprofen, vorapaxar, atopaxar, and a combination thereof.

20. The method of any one of claims 1-17, wherein the antiplatelet agent is selected from the group consisting of aspirin, cangrelor, ticagrelor, clopidogrel, prasugrel, eptifibatide, tirofiban, abciximab, terutroban, picotamide, elinogrel, ticlopidine, ibuprofen, vorapaxar, atopaxar, cilostazol, prostaglandin E1, epoprostenol, dipyridamole, treprostinil sodium, sarpogrelate, and a combination thereof.

21. The method of any one of claims 1-20, wherein administering comprises administering topically.

22. The method of any one of claims 1-20, wherein administering comprises administering parenterally.

23. The method of any one of claims 1-20, wherein administering comprises administering intravenously.

24. The method of any one of claims 1-20, wherein administering comprises administering intramuscularly.

25. The method of any one of claims 1-20, wherein administering comprises administering intrathecally.

26. The method of any one of claims 1-20, wherein administering comprises administering subcutaneously.

27. The method of any one of claims 1-20, wherein administering comprises administering intraperitoneally.

28. The method of any one of claims 1-27, wherein the composition is dried prior to the administration step.

29. The method of claim 28, wherein the composition is rehydrated following the drying step.

30. The method of any one of claims 1-28, wherein the composition is freeze-dried prior to the administration step.

31. The method of claim 30, wherein the composition is rehydrated following the freeze-drying step.

32. The method of any one of claims 1-31, wherein the incubating agent comprises one or more salts selected from phosphate salts, sodium salts, potassium salts, calcium salts, magnesium salts, and a combination of two or more thereof.

33. The method of any one of claims 1-32, wherein the incubating agent comprises a carrier protein.

34. The method of any one of claims 1-33, wherein the buffer comprises HEPES, sodium bicarbonate ($NaHCO_3$), or a combination thereof.

35. The method of any one of claims 1-34, wherein the composition comprises one or more saccharides.

36. The method of claim 35, wherein the one or more saccharides comprise trehalose.

37. The method of claim 35 or claim 36, wherein the one or more saccharides comprise polysucrose.

38. The method of any one of claims 35-37, wherein the one or more saccharides comprise dextrose.

39. The method of any one of claims 1-38, wherein the composition comprises an organic solvent.

40. The method of any of claims 1-39, wherein the platelets or platelet derivatives comprise thrombosomes.

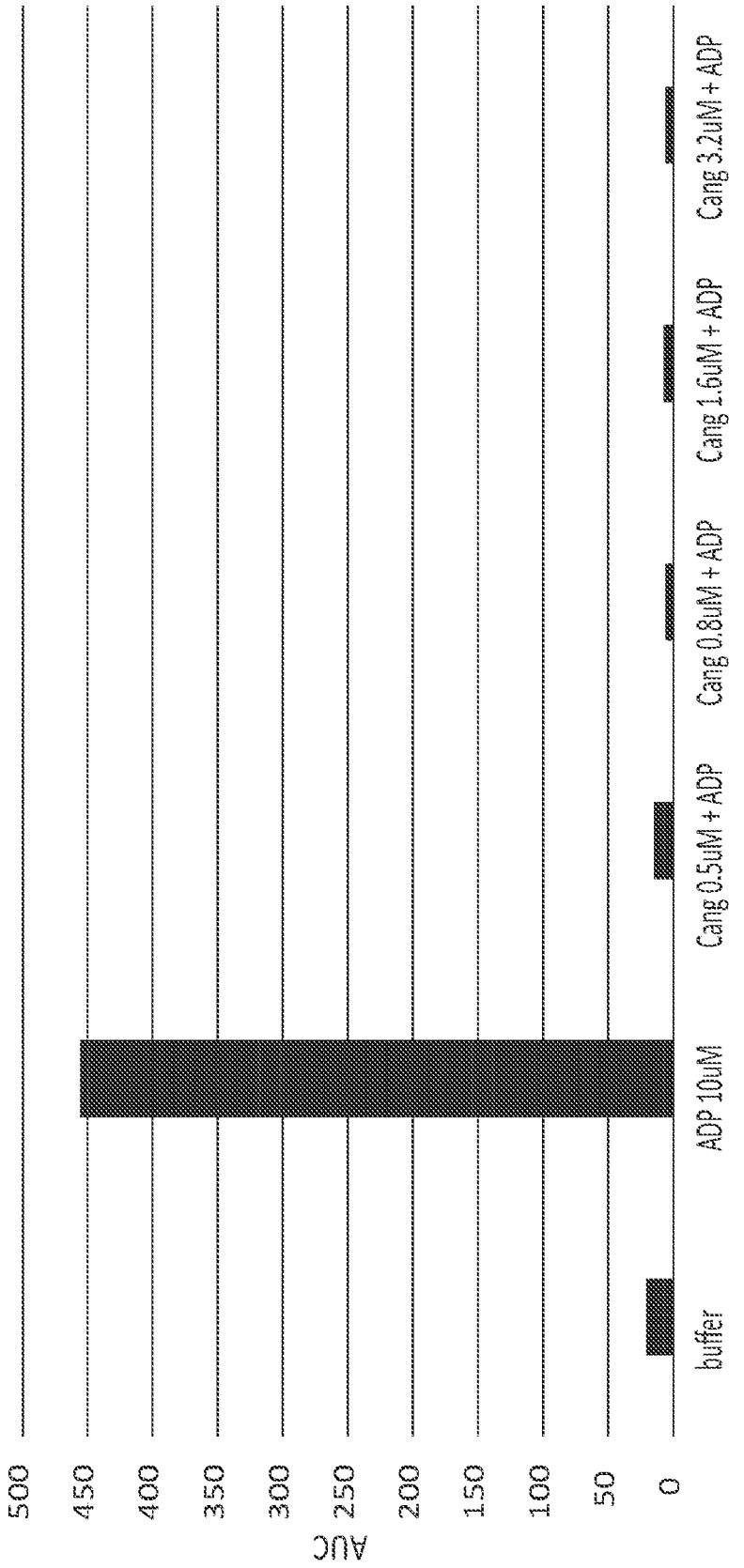
AUC PRP aggregometry
AUC
500
450
400
350
300
250
200
150
100
50
0
buffer
ADP 10uM
Cang 0.5uM + ADP
Cang 0.8uM + ADP
Cang 1.6uM + ADP
Cang 3.2uM + ADP

Figure 1

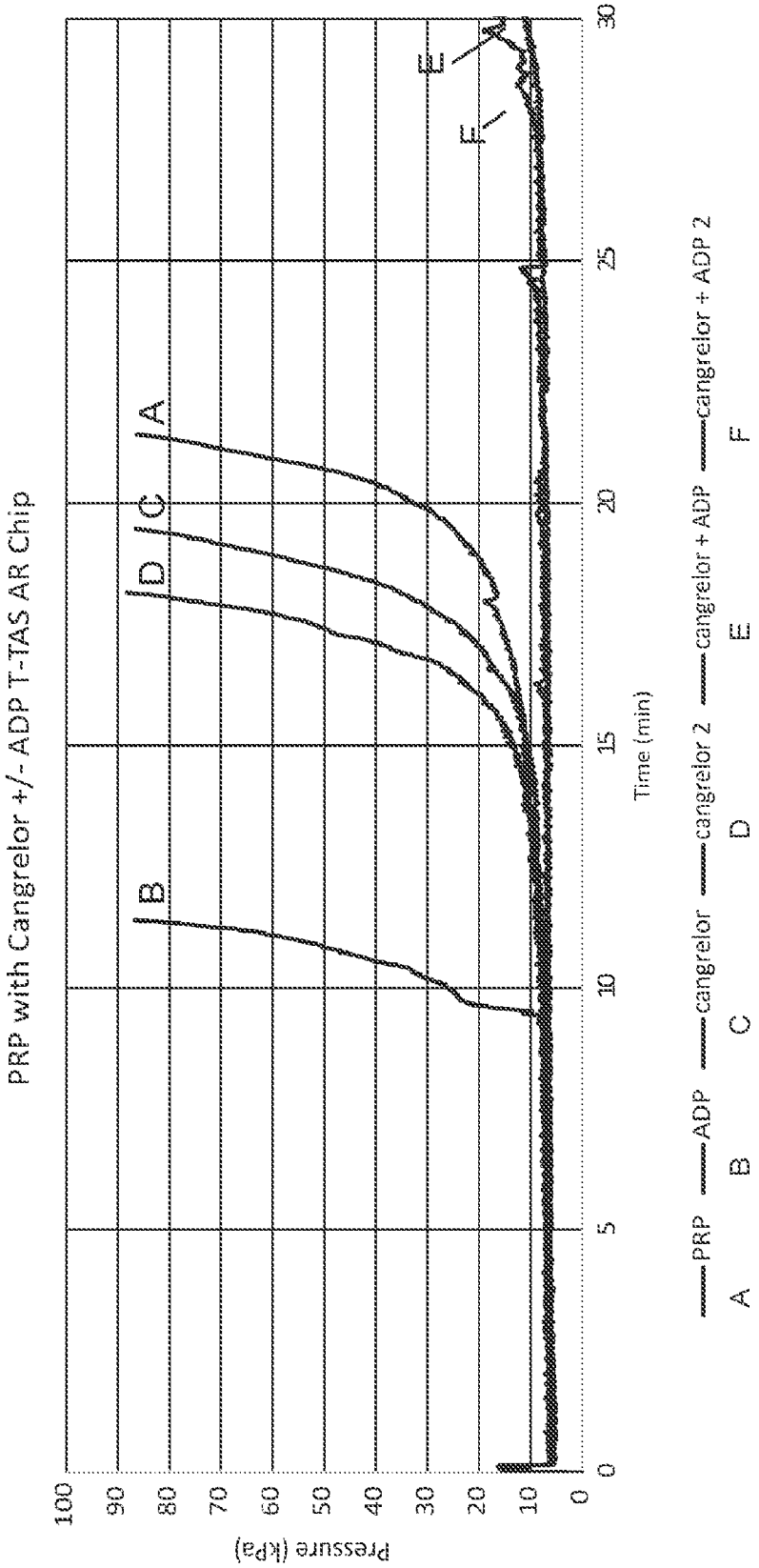
PRP with Cangrelor +/- ADP T-TAS AR Chip
Pressure (kPa)
Time (min)
A
B
C
D
E
F
PRP
ADP
cangrelor
cangrelor 2
cangrelor + ADP
cangrelor + ADP 2

Figure 2

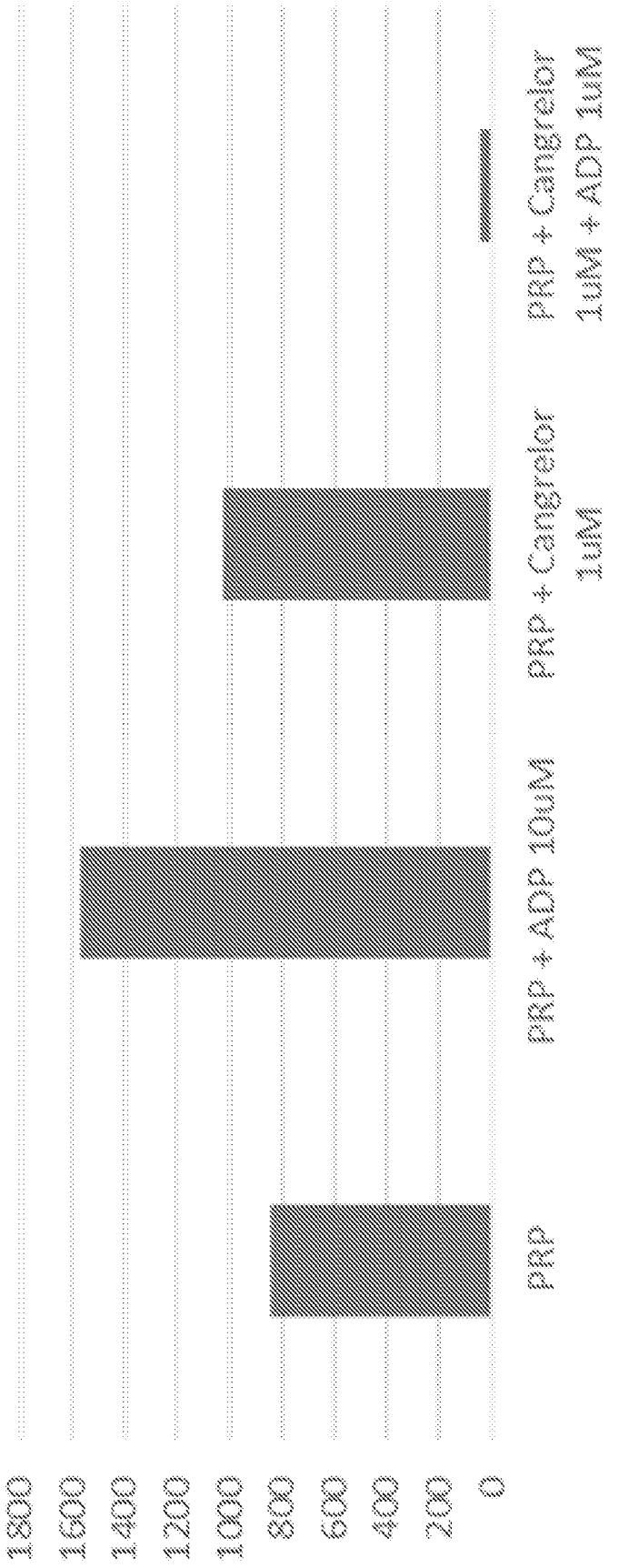
AUC
1800
1600
1400
1200
1000
800
600
400
200
0
PRP
PRP + ADP 10uM
PRP + Cangrelor 1uM
PRP + Cangrelor 1uM + ADP 1uM

Figure 3

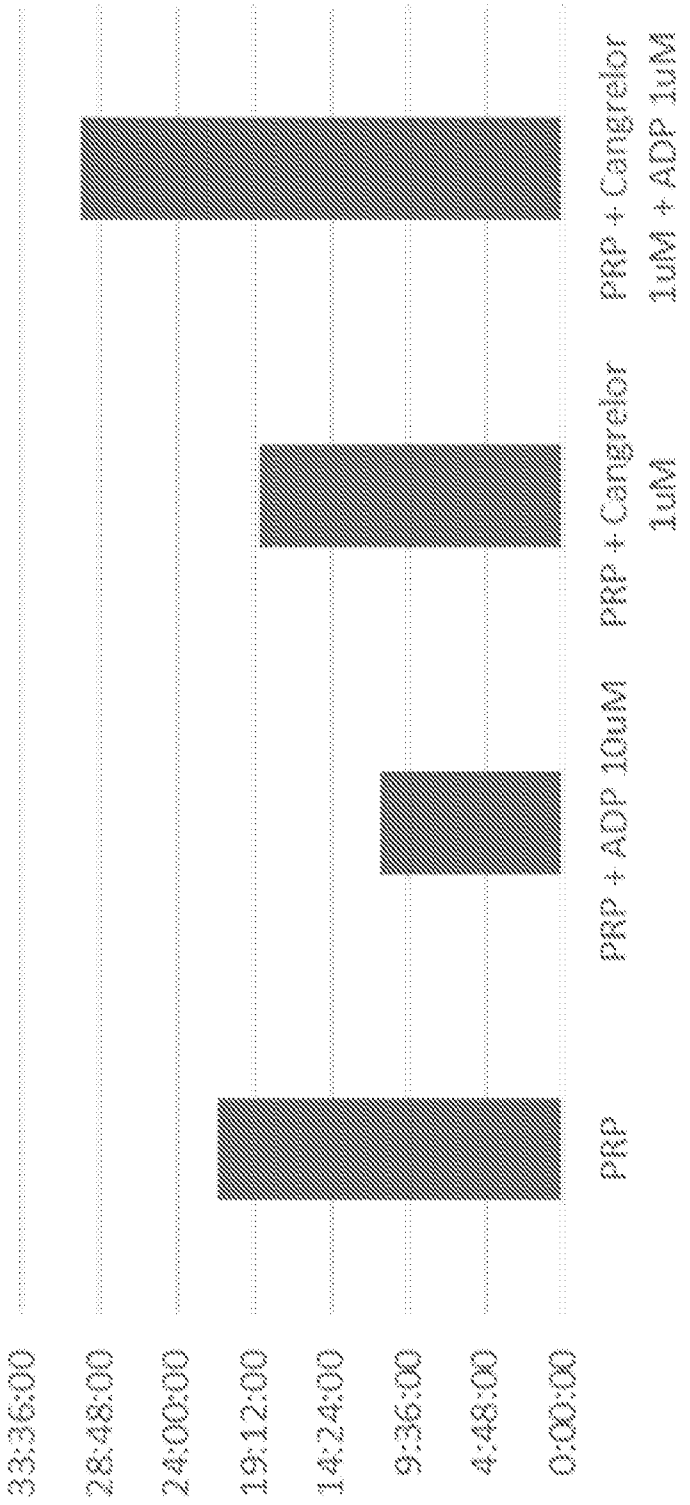
Occulsion
33:36:00
28:48:00
24:00:00
19:12:00
14:24:00
9:36:00
4:48:00
0:00:00
PRP
PRP + AOP 10uM
PRP + Cangrelor 1uM
PRP + Cangrelor 1uM + AOP 1uM

Figure 4

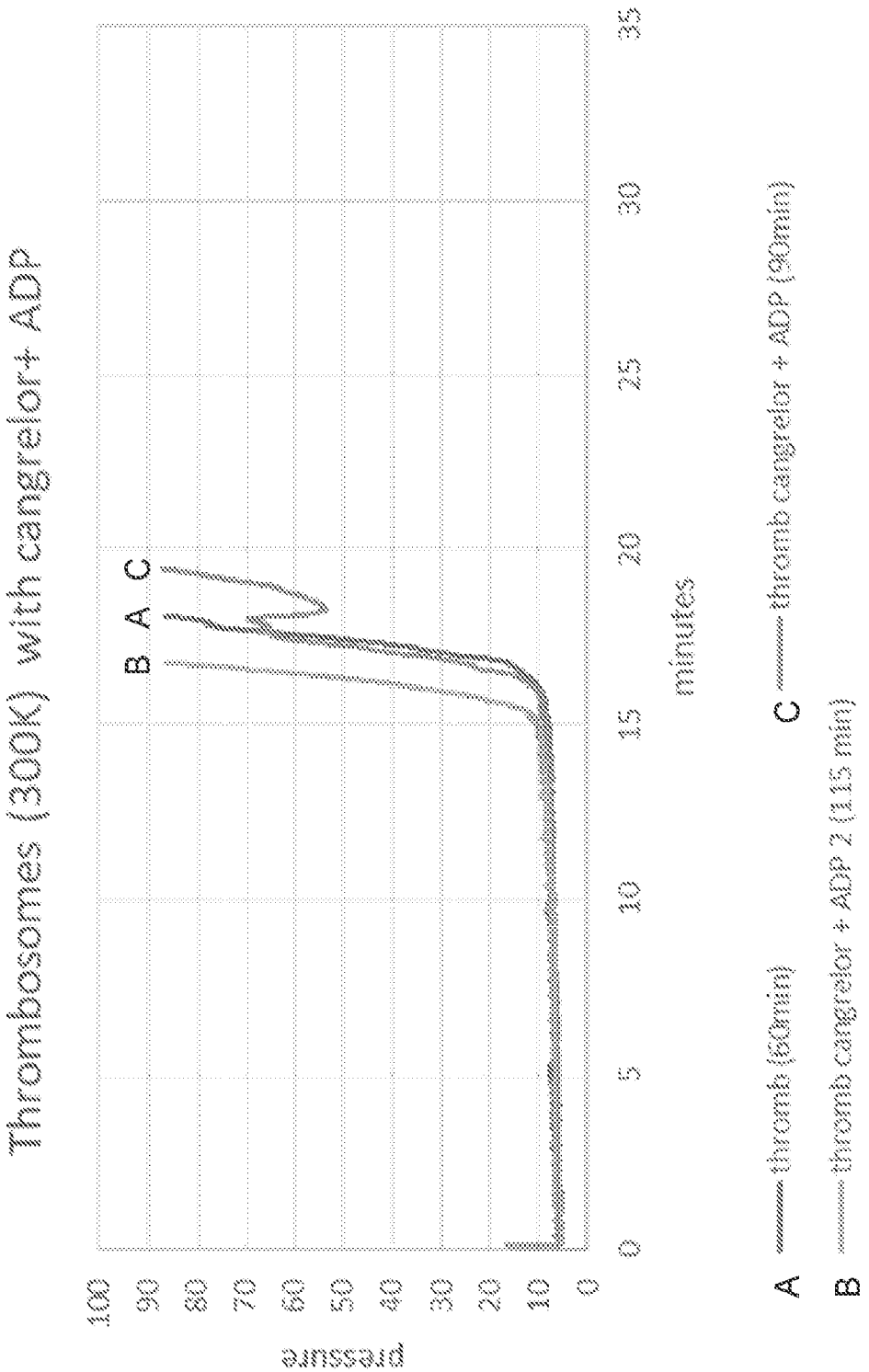
Thrombosomes (300K) with cangrelor+ ADP
pressure
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
minutes
B
A
C
A thromb (60min)
B thromb cangrelor + ADP 2 (115 min)
C thromb cangrelor + ADP (90min)

Figure 5

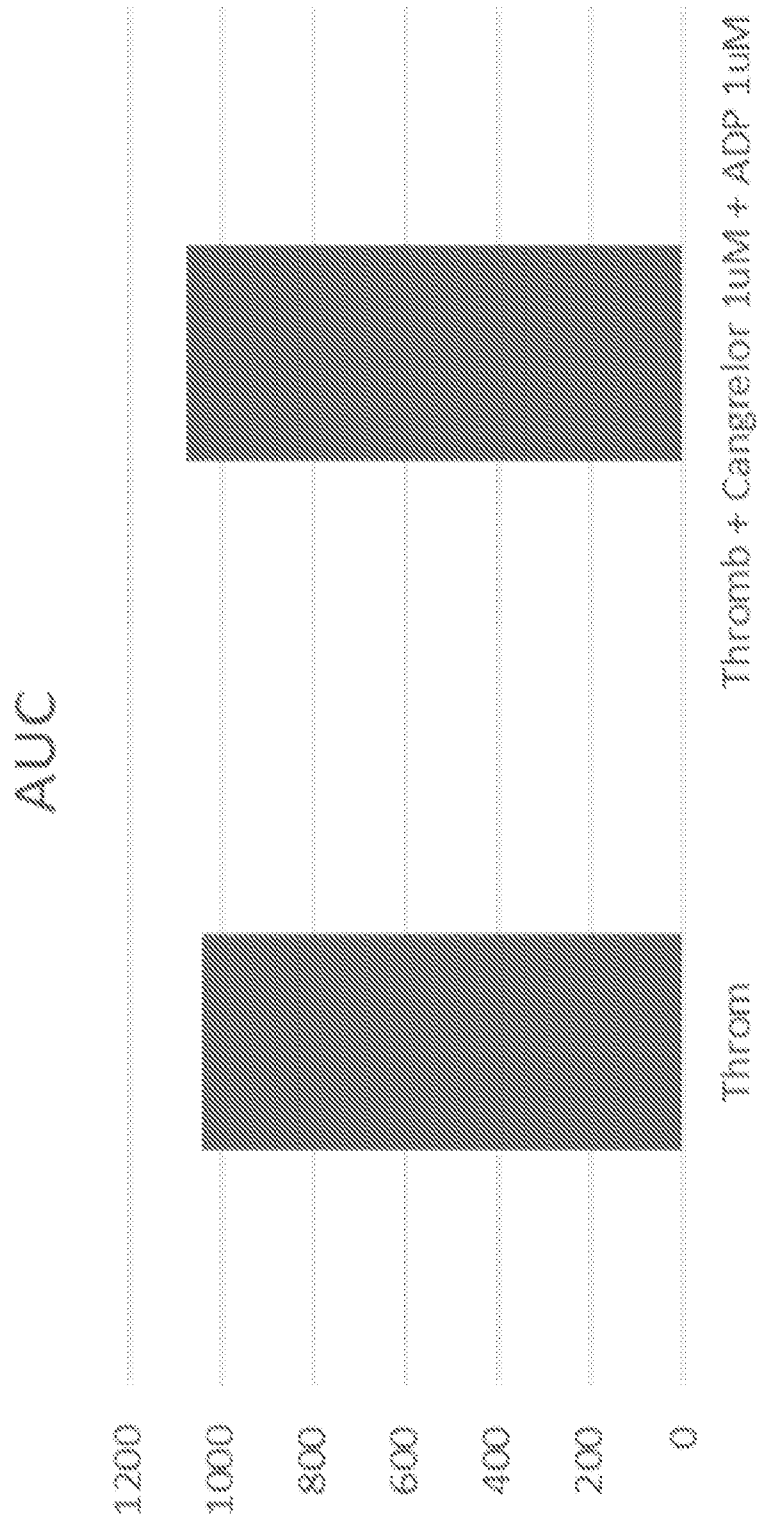
AUC
1200
1000
800
600
400
200
0
Throm
Throm + Cangrelor 1uM + ADP 1uM

Figure 6

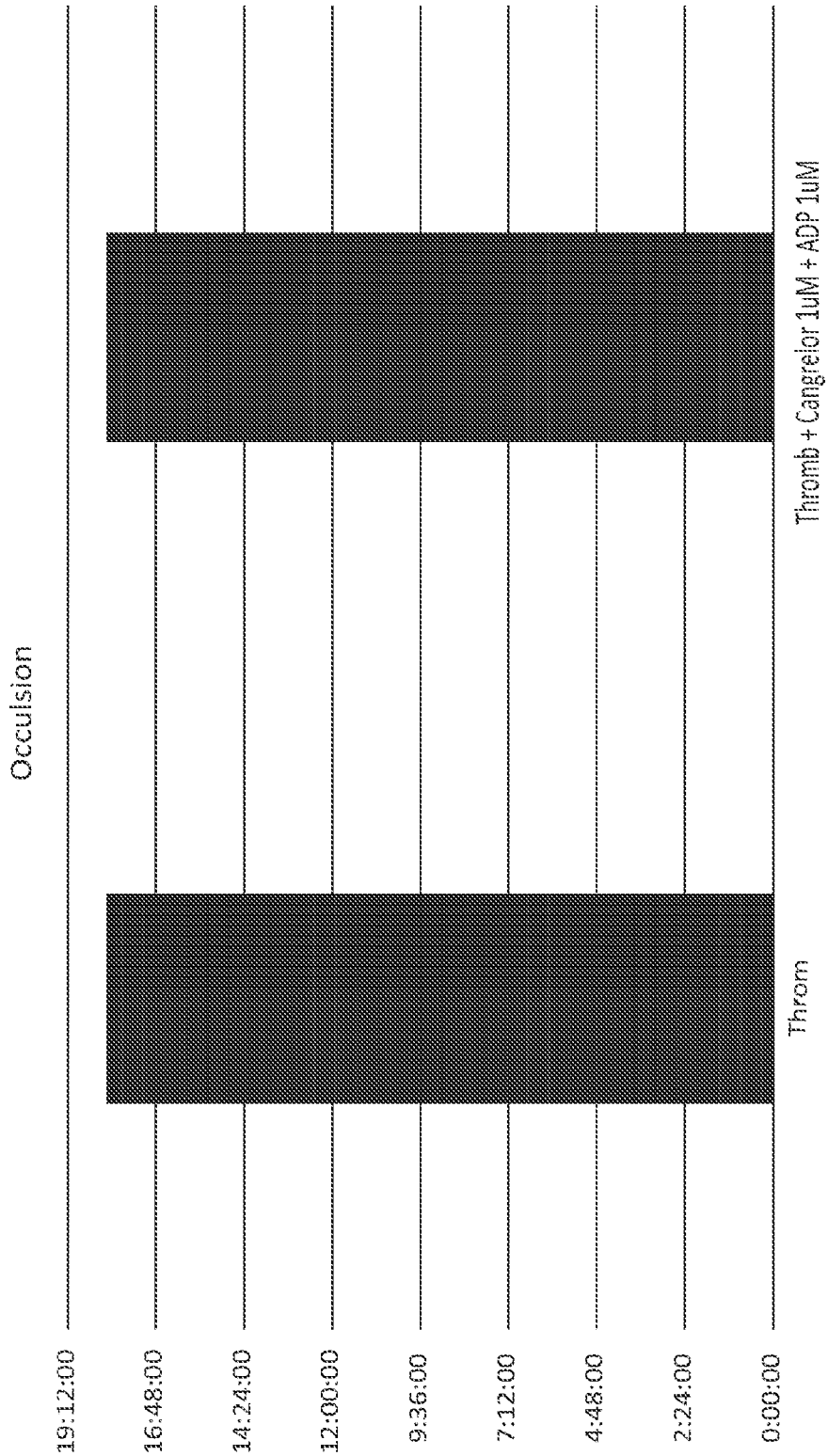
Occulsion
19:12:00
16:48:00
14:24:00
12:00:00
9:36:00
7:12:00
4:48:00
2:24:00
0:00:00
Thromb + Cangreior 1uM + ADP 1uM
Throm

Figure 7

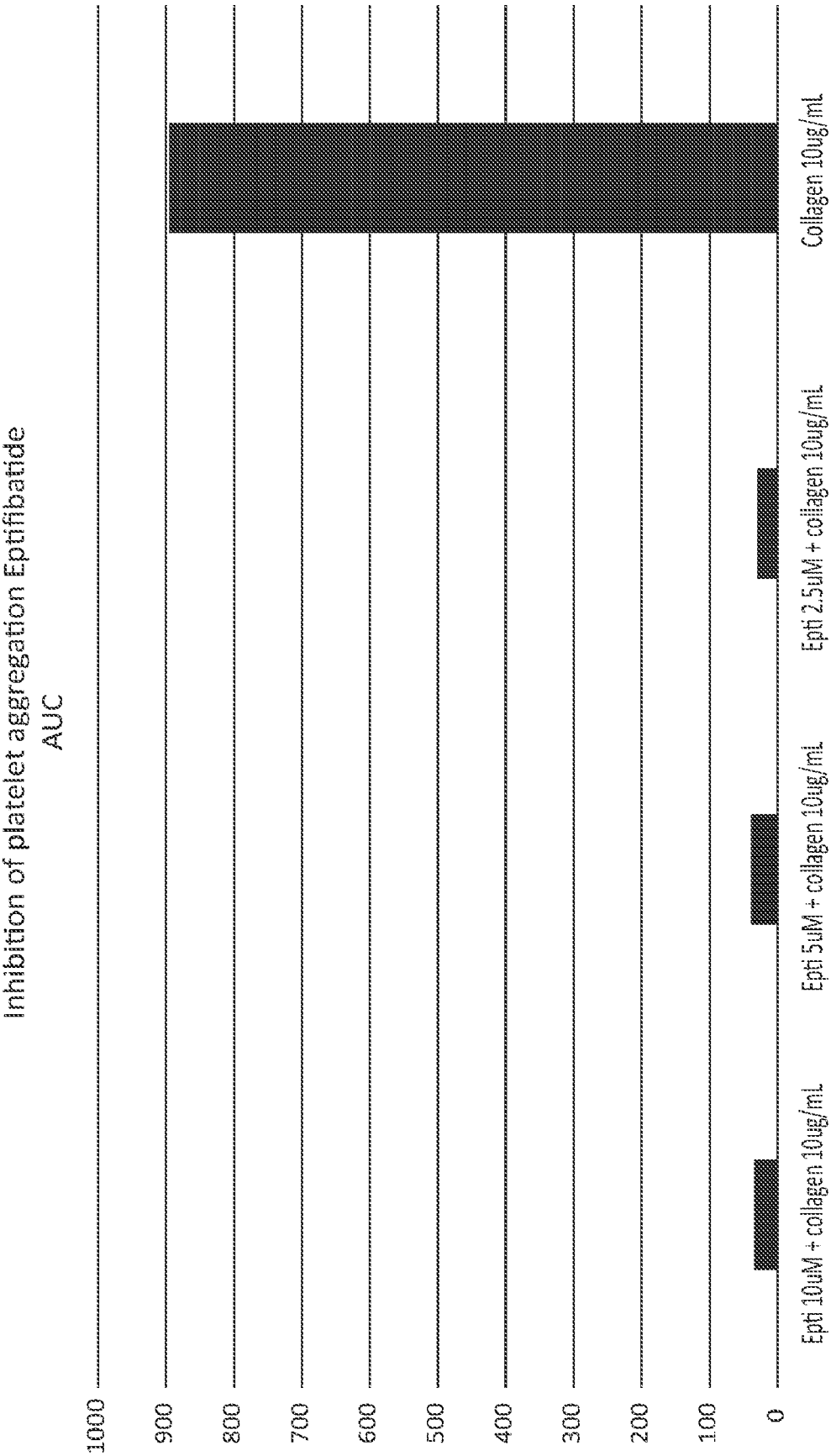
Inhibition of platelet aggregation Eptifibatide
AUC
1000
900
800
700
600
500
400
300
200
100
0
Epti 10uM + collagen 10ug/mL
Epti 5uM + collagen 10ug/mL
Epti 2.5uM + collagen 10ug/mL
Collagen 10ug/mL

Figure 8

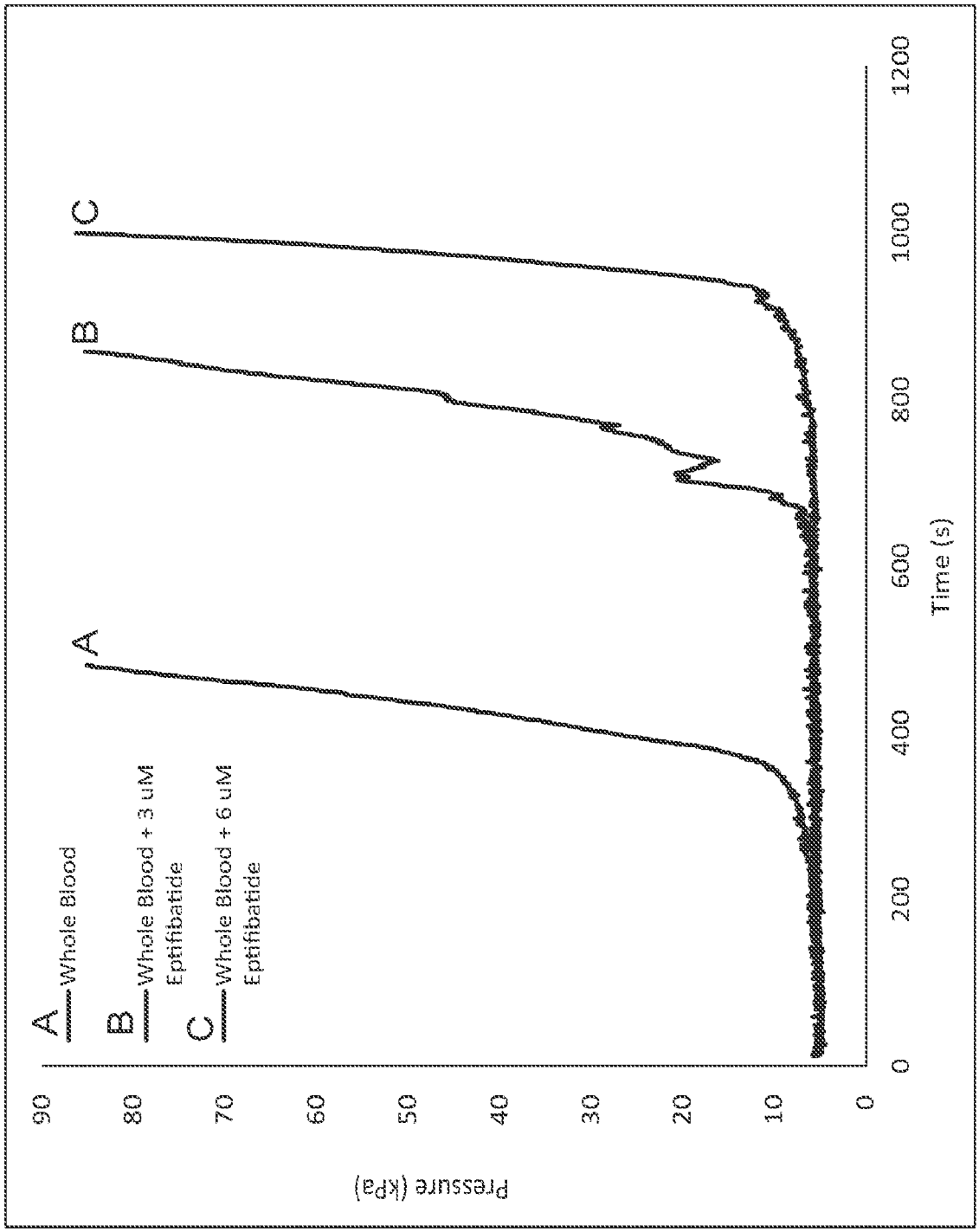
A Whole Blood
B Whole Blood + 3 uM Eptifibatide
C Whole Blood + 6 uM Eptifibatide
A
B
C
Pressure (kPa)
Time (s)
0
10
20
30
40
50
60
70
80
90
0
200
400
600
800
1000
1200

Figure 9

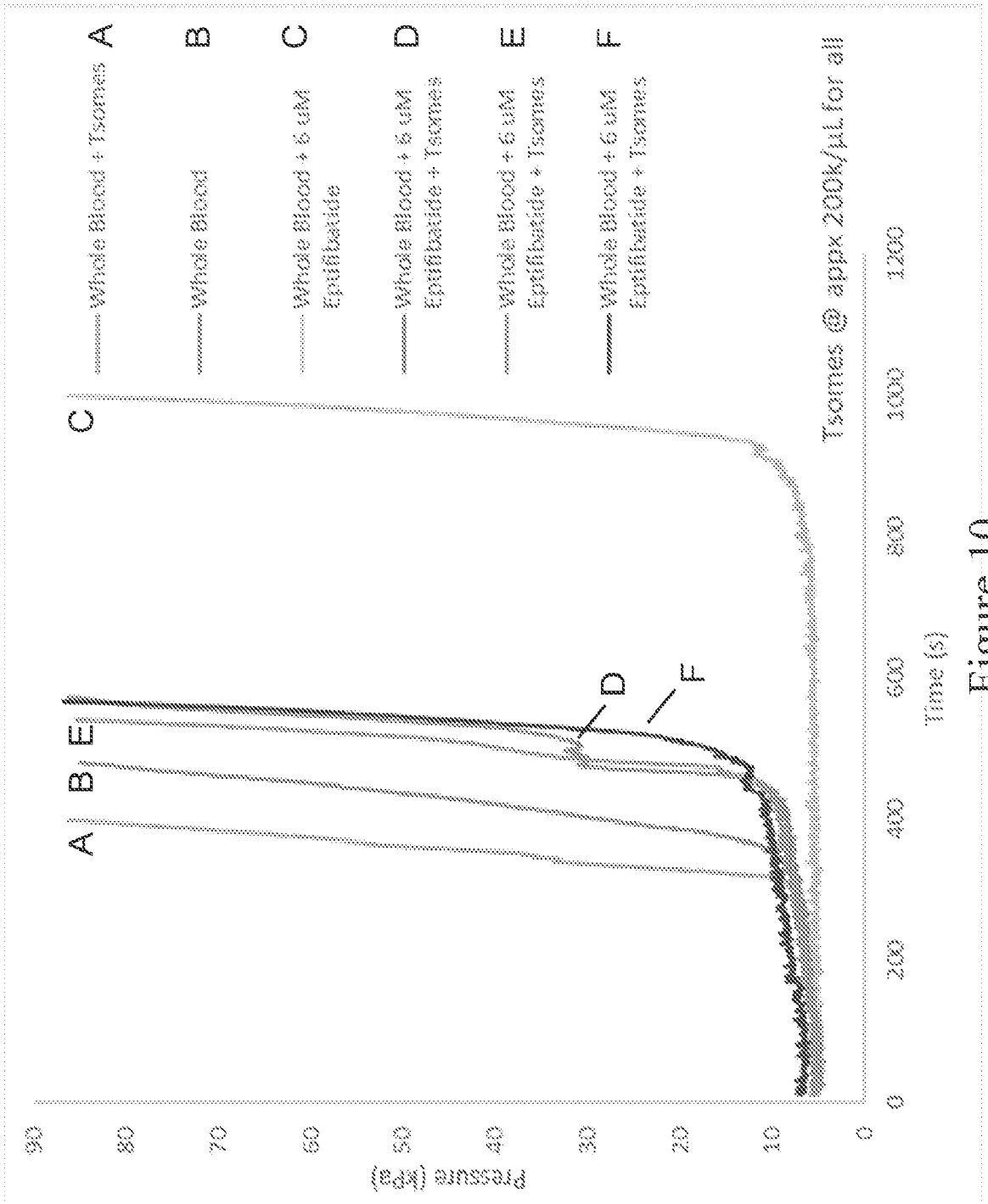
A
Whole Blood + Tsomes
B
Whole Blood
C
Whole Blood + 6 uM Eptifibatide
D
Whole Blood + 6 uM Eptifibatide + Tsomes
E
Whole Blood + 6 uM Eptifibatide + Tsomes
F
Whole Blood + 6 uM Eptifibatide + Tsomes
Tsomes @ appx 200k/uL for all
A
B
E
C
D
F
Pressure (kPa)
Time (s)
0
10
20
30
40
50
60
70
80
90
0
200
400
600
800
1000
1200

Figure 10

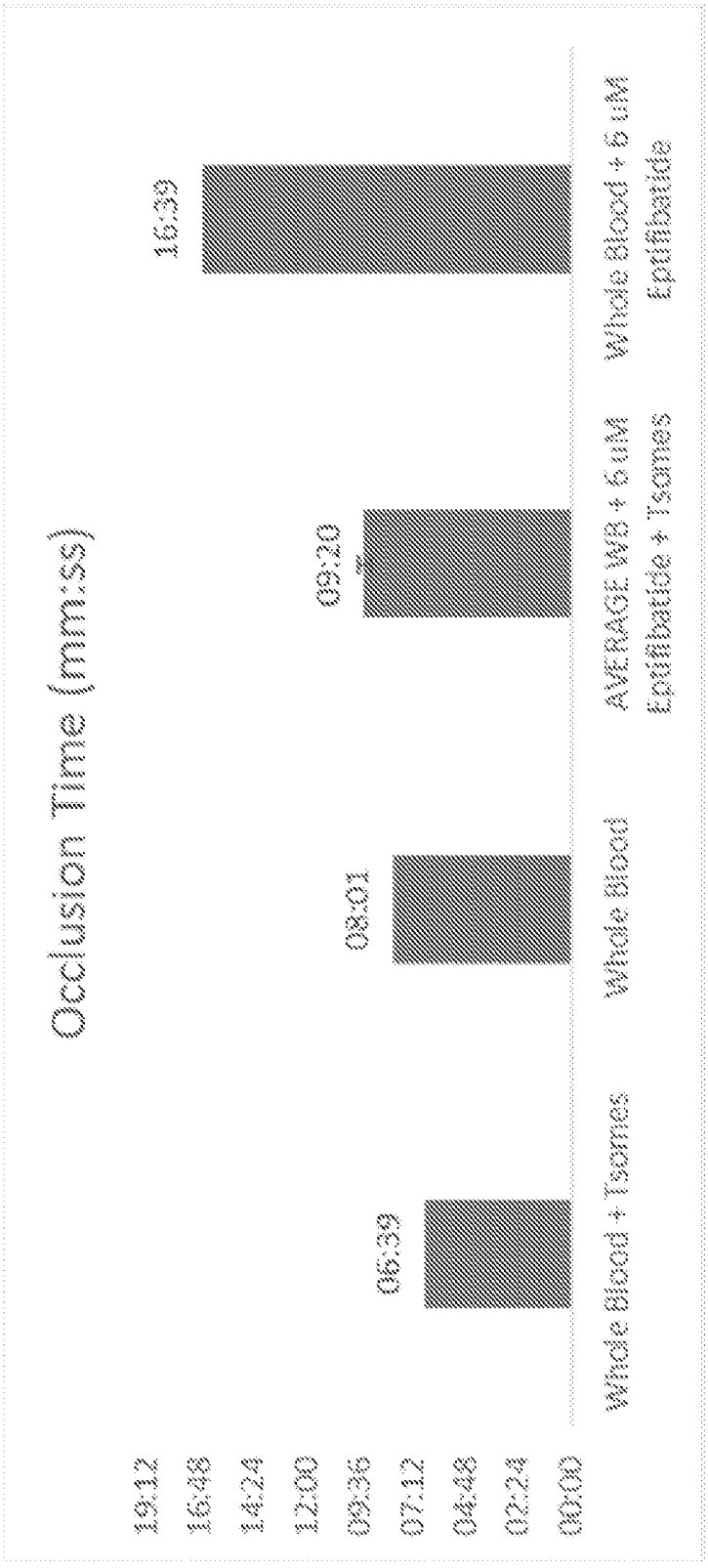
Occlusion Time (mm:ss)
19:12
16:48
14:24
12:00
09:36
07:12
04:48
02:24
00:00
06:39
08:01
09:20
16:39
Whole Blood + Tsomes
Whole Blood
AVERAGE WB + 6 uM Eptifibatide + Tsomes
Whole Blood + 6 uM Eptifibatide

Figure 11

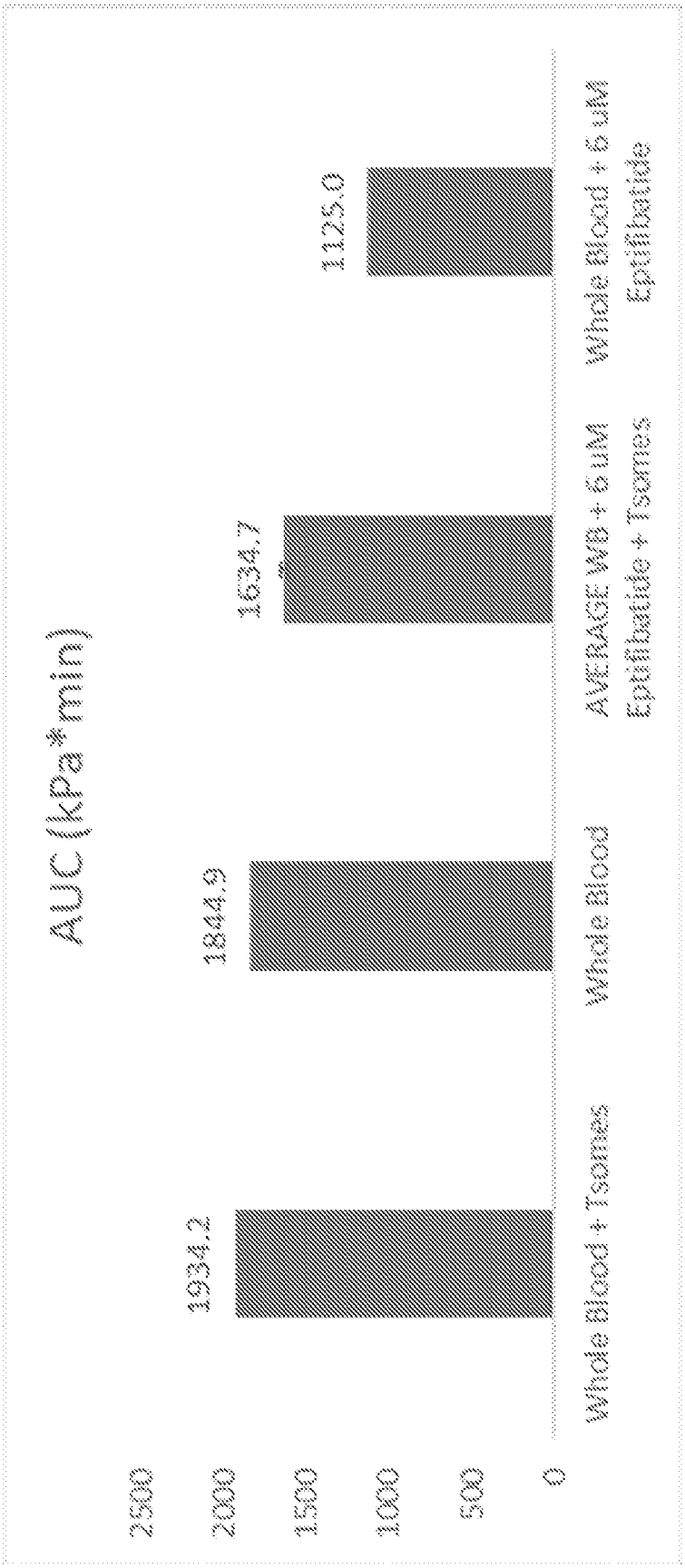
AUC (kPa*min)
2500
2000
1500
1000
500
0
1934.2
1844.9
1634.7
1123.0
Whole Blood + Tsomes
Whole Blood
AVERAGE WB + 6 uM Eptifibatide + Tsomes
Whole Blood + 6 uM Eptifibatide

Figure 12

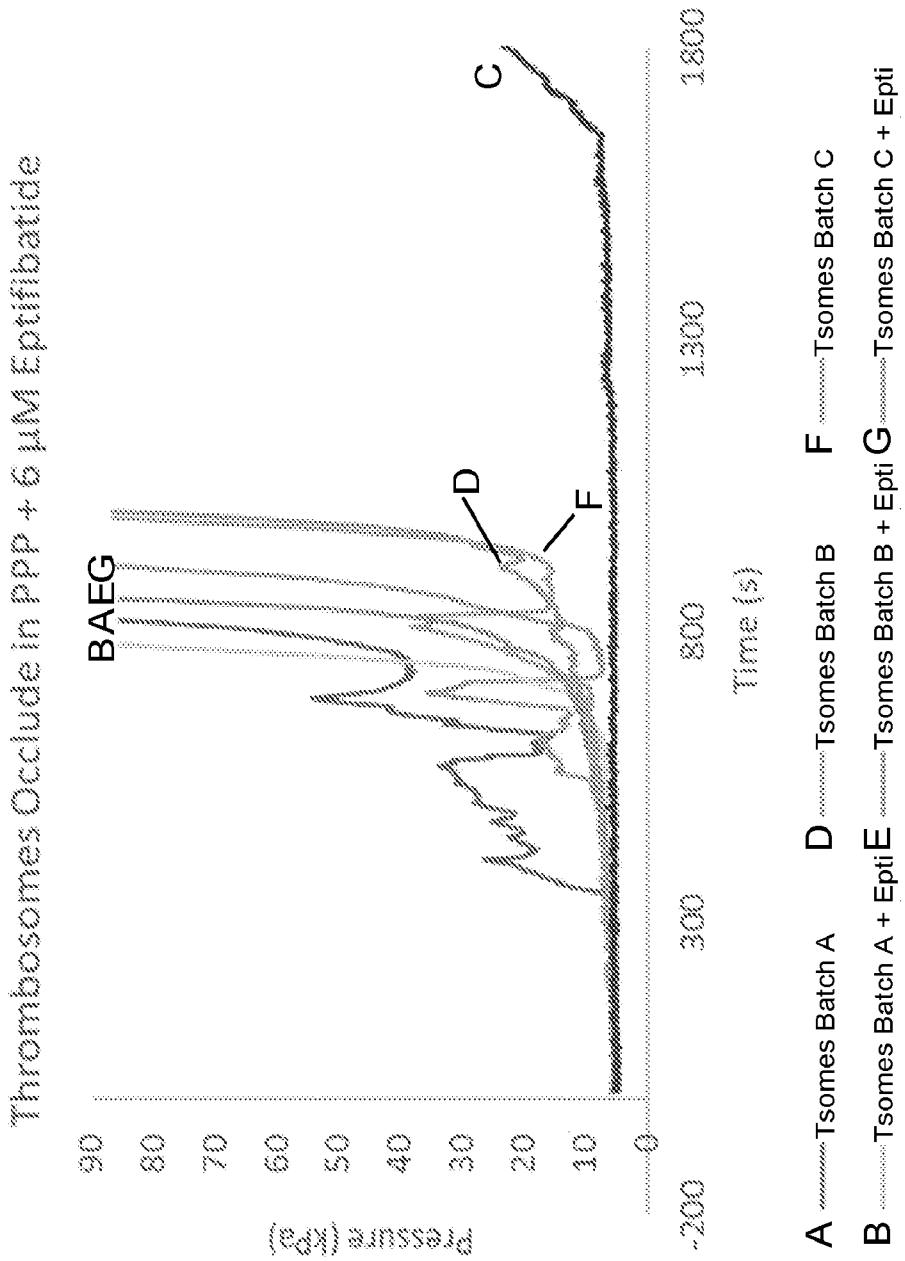
Thrombosomes Occlude in PPP + 6 µM Eptifibatide
Pressure (kPa)
90
80
70
60
50
40
30
20
10
0
-200
300
800
1300
1800
Time (s)
BAEG
D
F
C
A Tsomes Batch A
B Tsomes Batch A + Epti
C PPP Alone
D Tsomes Batch B
E Tsomes Batch B + Epti
F Tsomes Batch C
G Tsomes Batch C + Epti

Figure 13

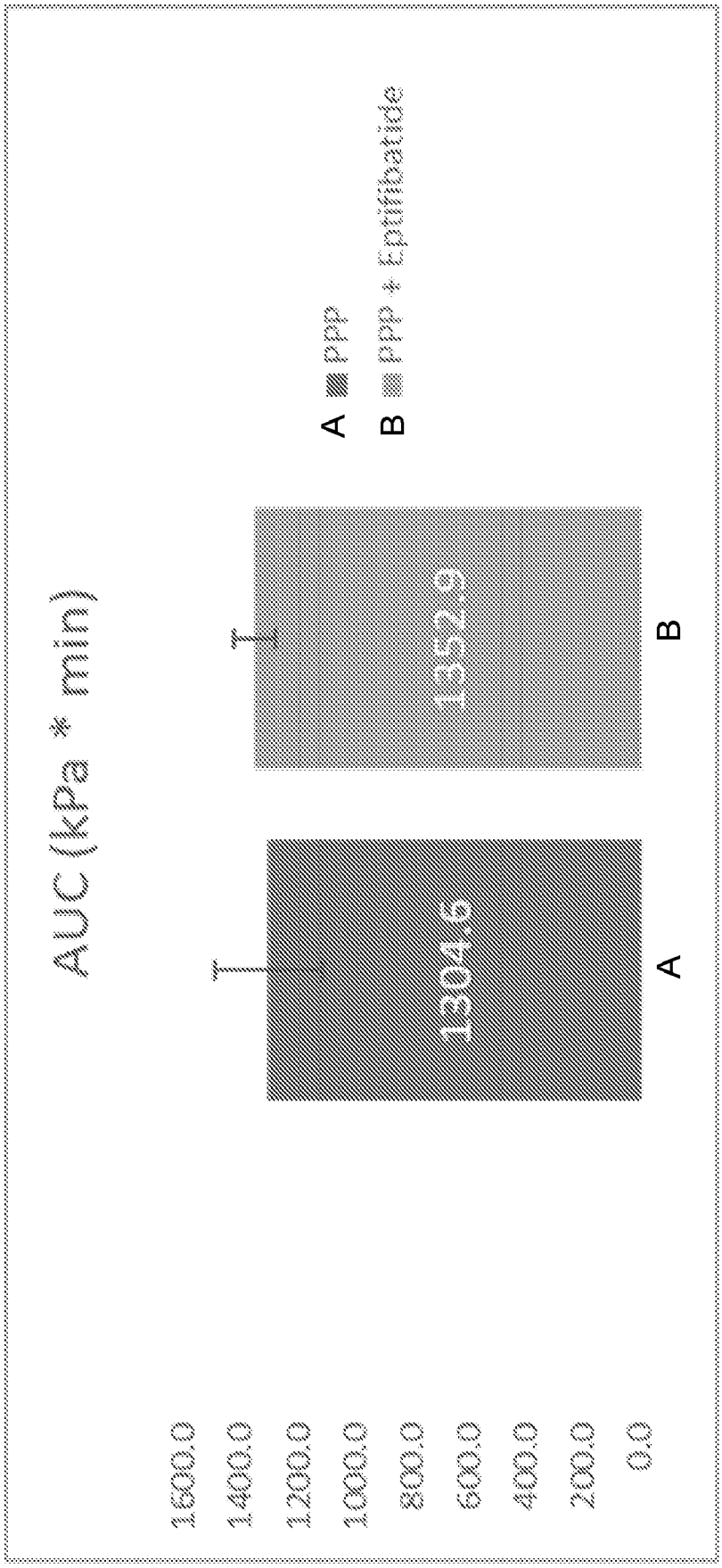
AUC (kPa * min)
1600.0
1400.0
1200.0
1000.0
800.0
600.0
400.0
200.0
0.0
1304.6
1352.9
A
B
A PPP
B PPP + Eptifibatide

Figure 14

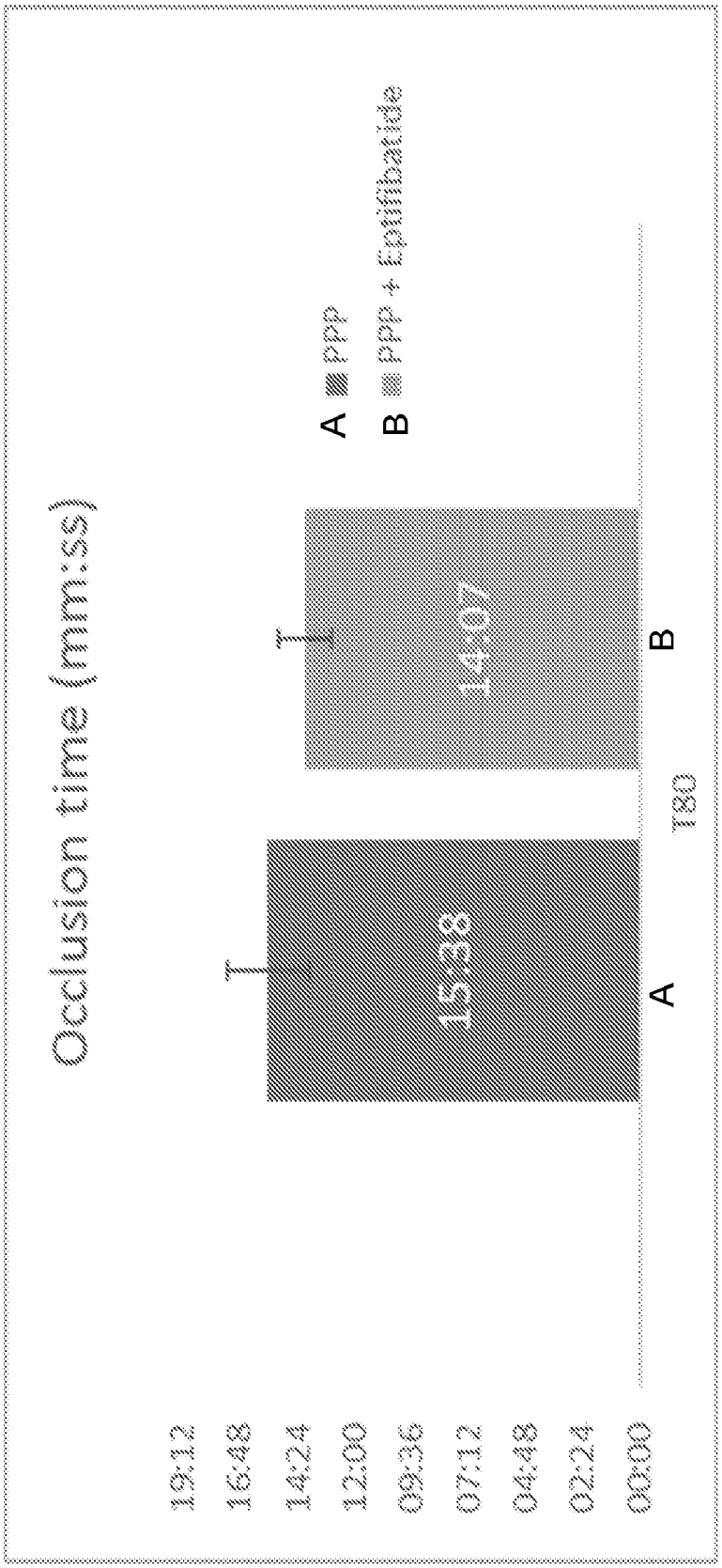
Occlusion time (mm:ss)
19:12
16:48
14:24
12:00
09:36
07:12
04:48
02:24
00:00
A PRP
B PRP + Eptifibatide
15:36
14:07
A
B
T80

Figure 15

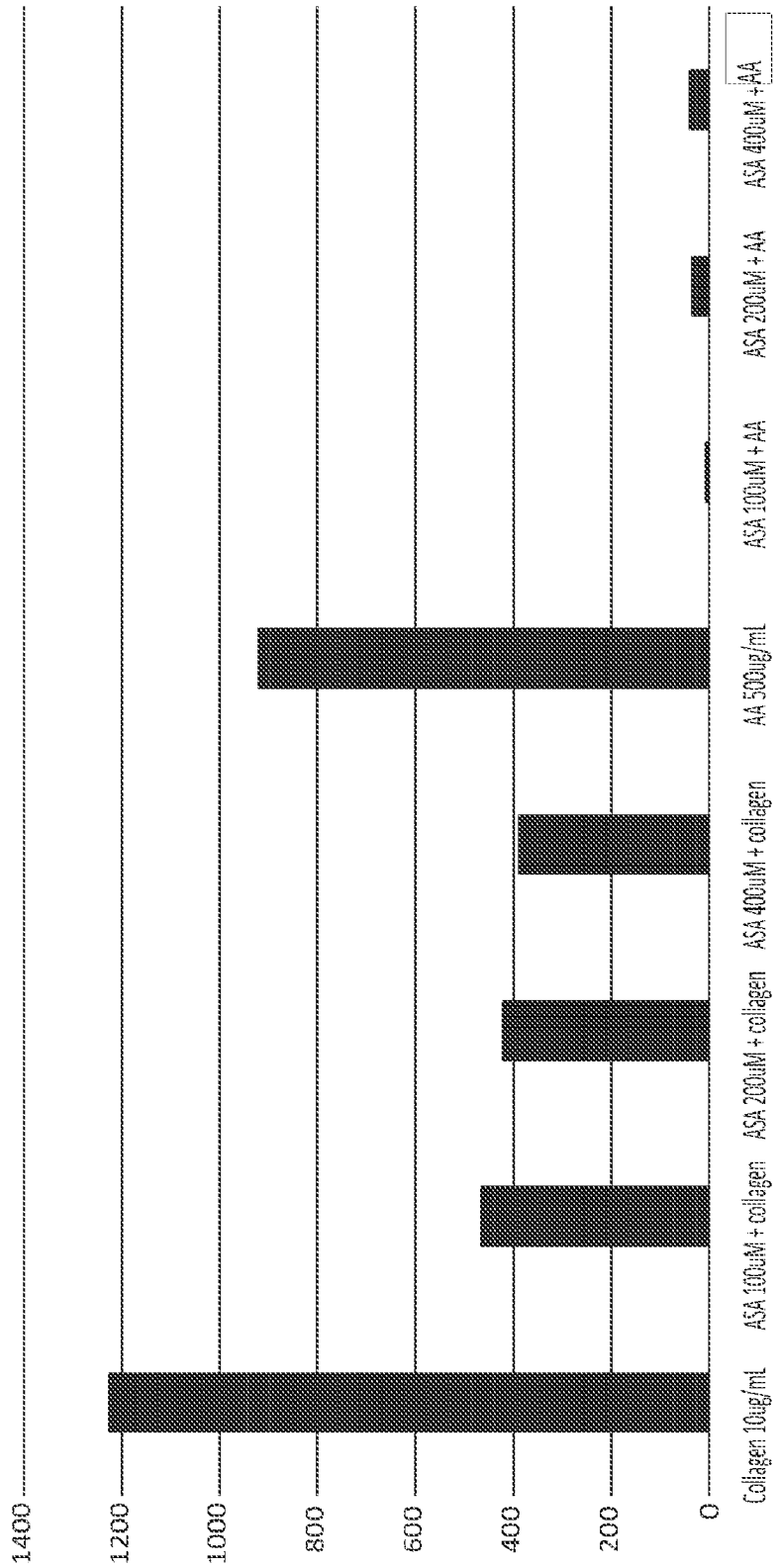
Inhibition of platelet aggregation Aspirin
AUC
1400
1200
1000
800
600
400
200
0
Collagen 10ug/mL
ASA 100uM + collagen 10ug/mL
ASA 200uM + collagen 10ug/mL
ASA 400uM + collagen 10ug/mL
AA 500ug/mL
ASA 100uM + AA 500ug/mL
ASA 200uM + AA 500ug/mL
ASA 400uM +AA 10ug/mL

Figure 16

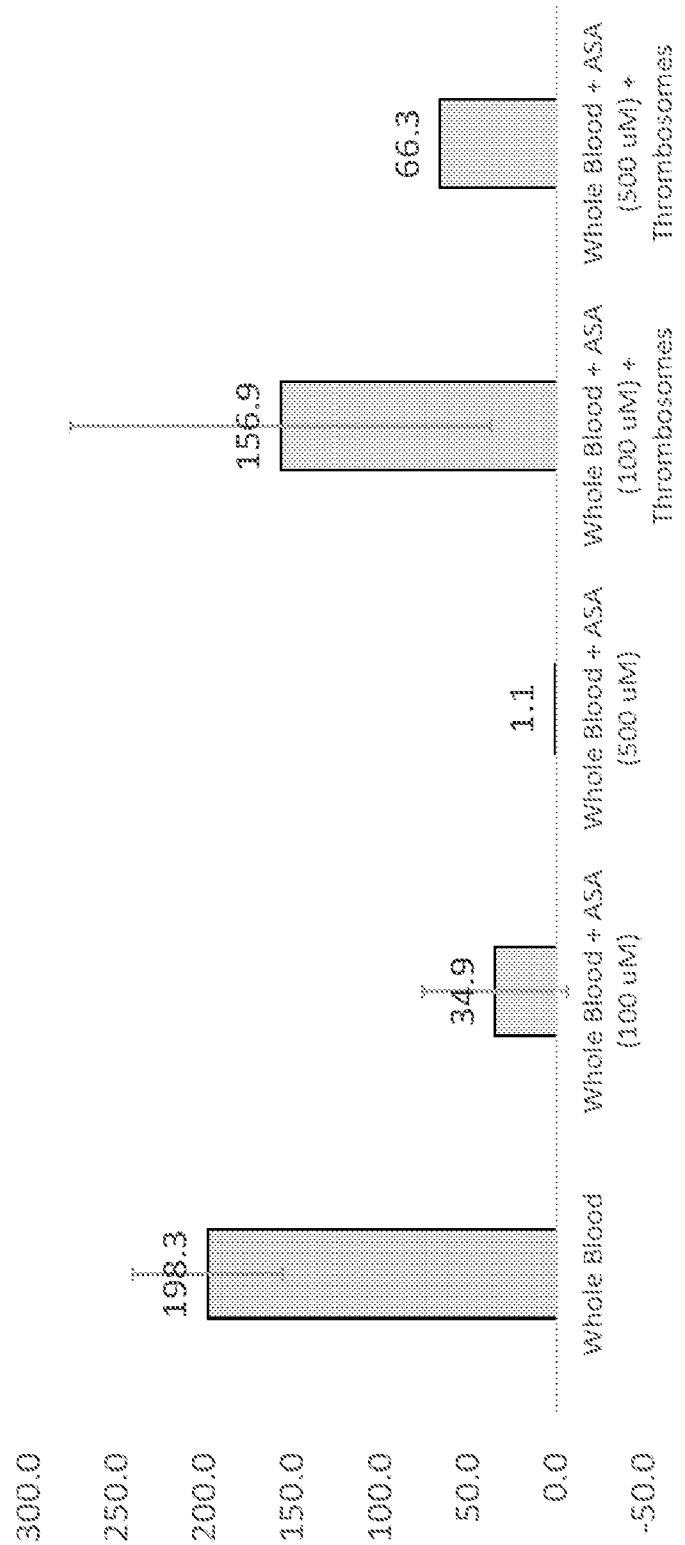
PL Chip AUC
300.0
250.0
200.0
150.0
100.0
50.0
0.0
-50.0
198.3
34.9
1.1
156.9
66.3
Whole Blood
Whole Blood + ASA (100 uM)
Whole Blood + ASA (500 uM)
Whole Blood + ASA (100 uM) + Thrombosomes
Whole Blood + ASA (500 uM) + Thrombosomes

Figure 17

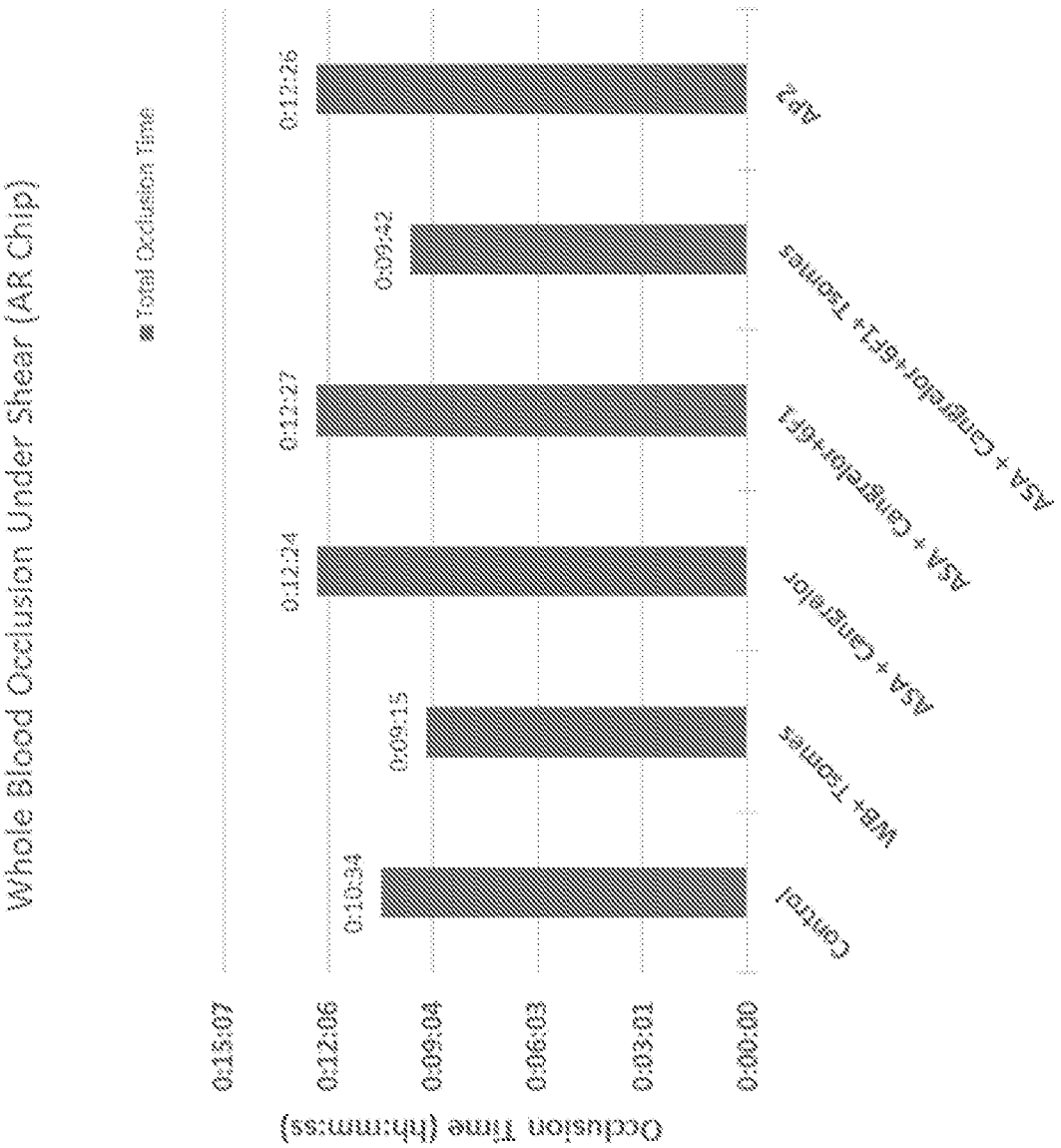
Whole Blood Occlusion Under Shear (AR Chip)
Total Occlusion Time
Occlusion Time (hh:mm:ss)
0:15:07
0:12:06
0:09:04
0:06:03
0:03:01
0:00:00
0:10:34
0:09:15
0:12:24
0:12:27
0:09:42
0:12:26
Control
WB+ Tsomes
ASA + Cangrelor
ASA + Cangrelor+6F1
ASA + Cangrelor+6F1+ Tsomes
AP2

Figure 18

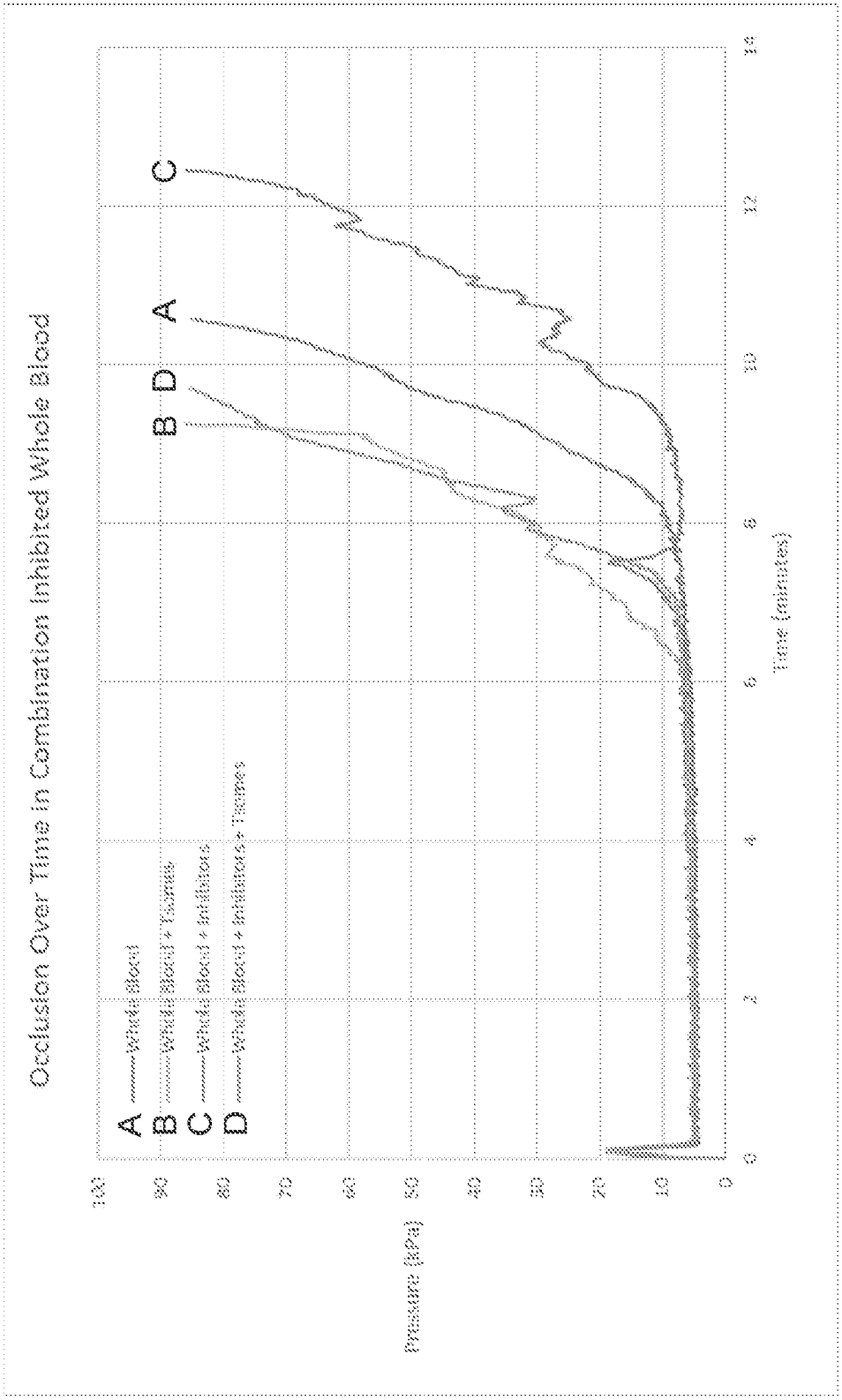
Occlusion Over Time in Combination Inhibited Whole Blood
A —— Whole Blood
B —— Whole Blood + Tecnis
C —— Whole Blood + Inhibitors
D —— Whole Blood + Inhibitors + Tecnis
Pressure (kPa)
Time (minutes)
A
B D
C

Figure 19

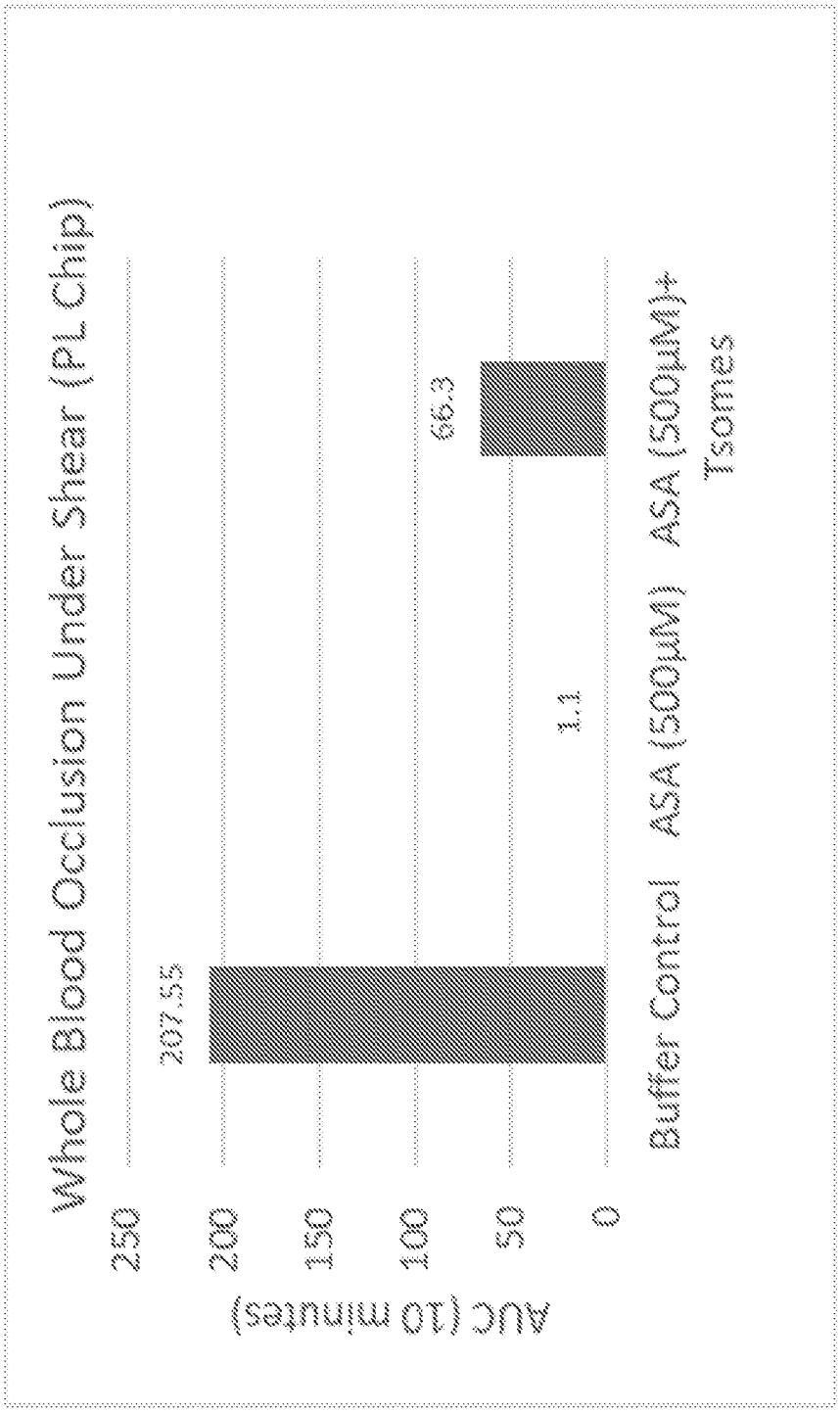
Whole Blood Occlusion Under Shear (PL Chip)
AUC (10 minutes)
250
200
150
100
50
0
207.55
1.1
66.3
Buffer Control
ASA (500µM)
ASA (500µM)+ Tsomes

Figure 20

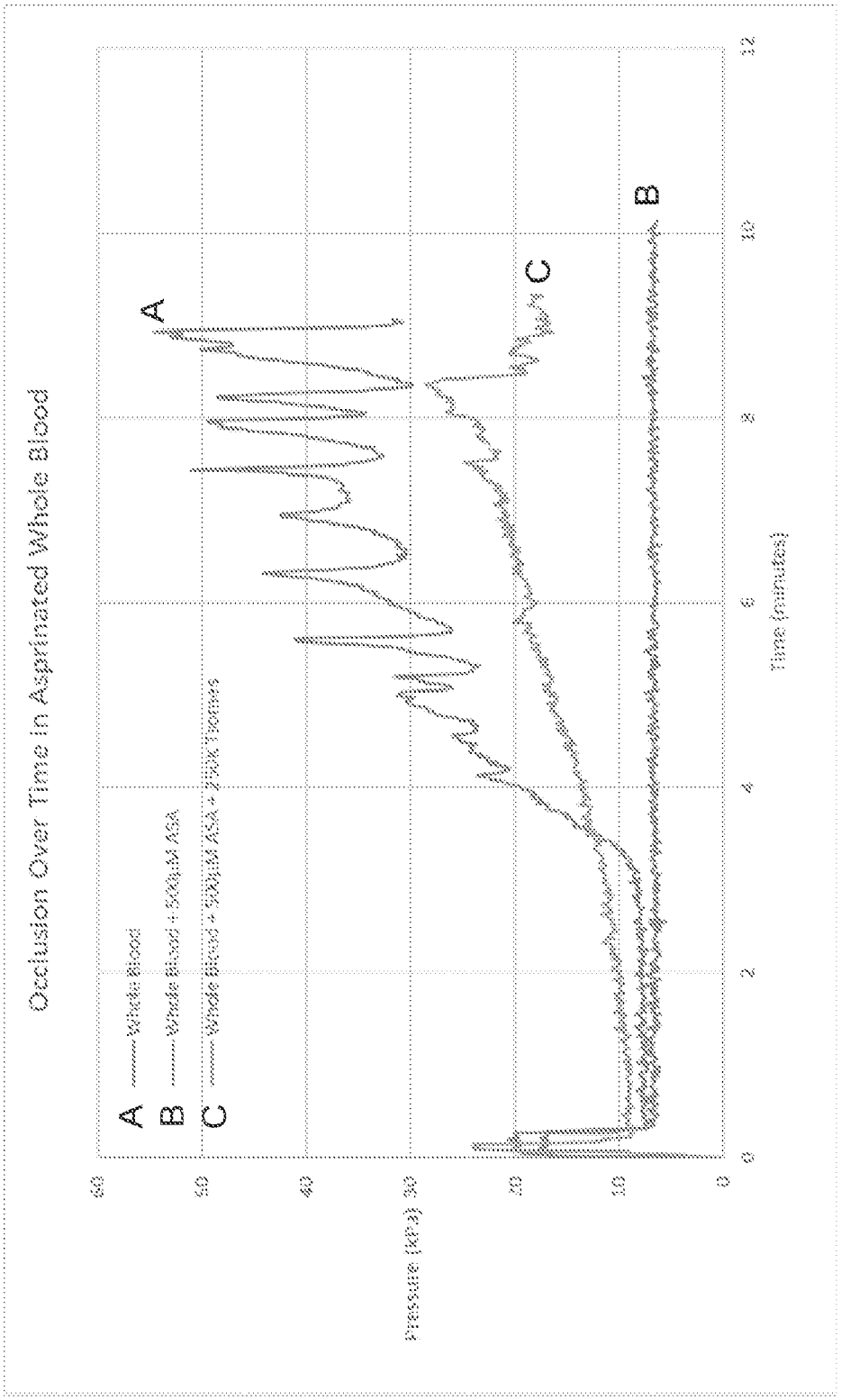
Occlusion Over Time in Aspirated Whole Blood
A ——Whole Blood
B ——Whole Blood + 500µM ASA
C ——Whole Blood + 500µM ASA + TRAP Receptors
A
B
C
Pressure (kPa) aspirated
Time (minutes)
0
10
20
30
40
50
60
0
2
4
6
8
10
12

Figure 21

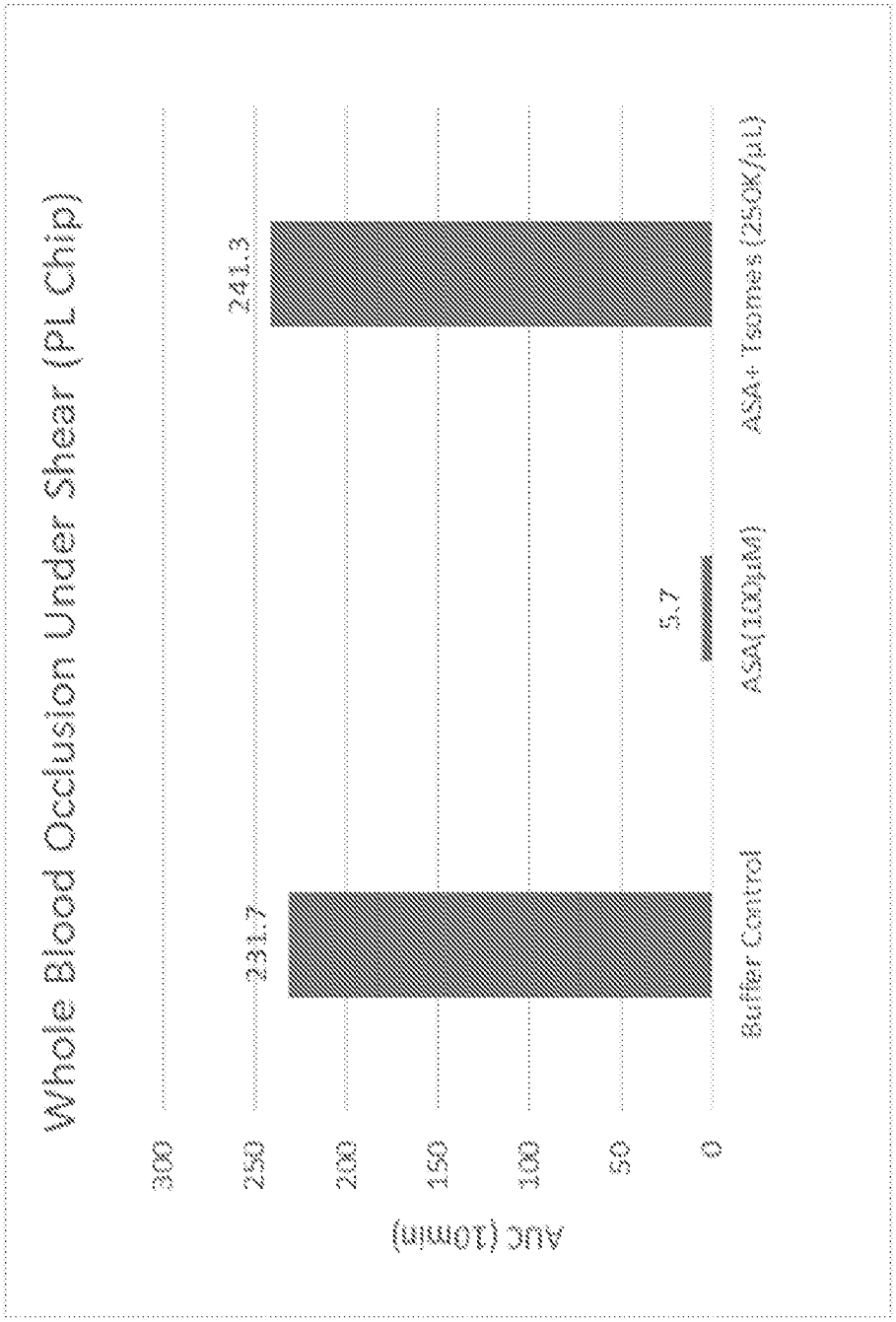
Whole Blood Occlusion Under Shear (PL Chip)
AUC (10min)
300
250
200
150
100
50
0
231.7
5.7
241.3
Buffer Control
ASA(100μM)
ASA+ Tsomes (250K/μL)

Figure 22

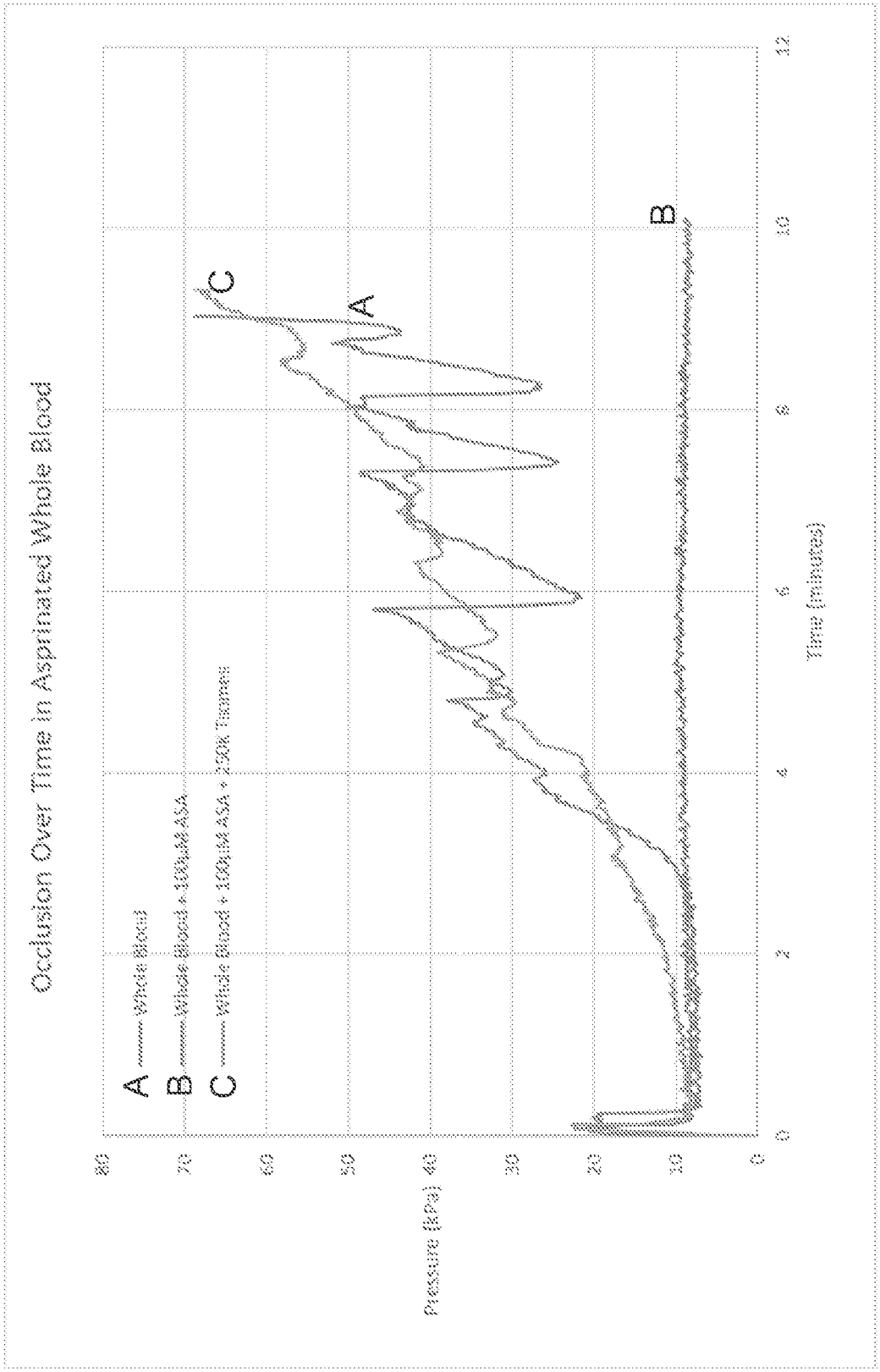
Occlusion Over Time in Aspirated Whole Blood
A —— Whole Blood
B —— Whole Blood + 100µM ASA
C —— Whole Blood + 100µM ASA + 2.50% Tannic
Pressure (kPa)
Time (minutes)
A
B
C

Figure 23

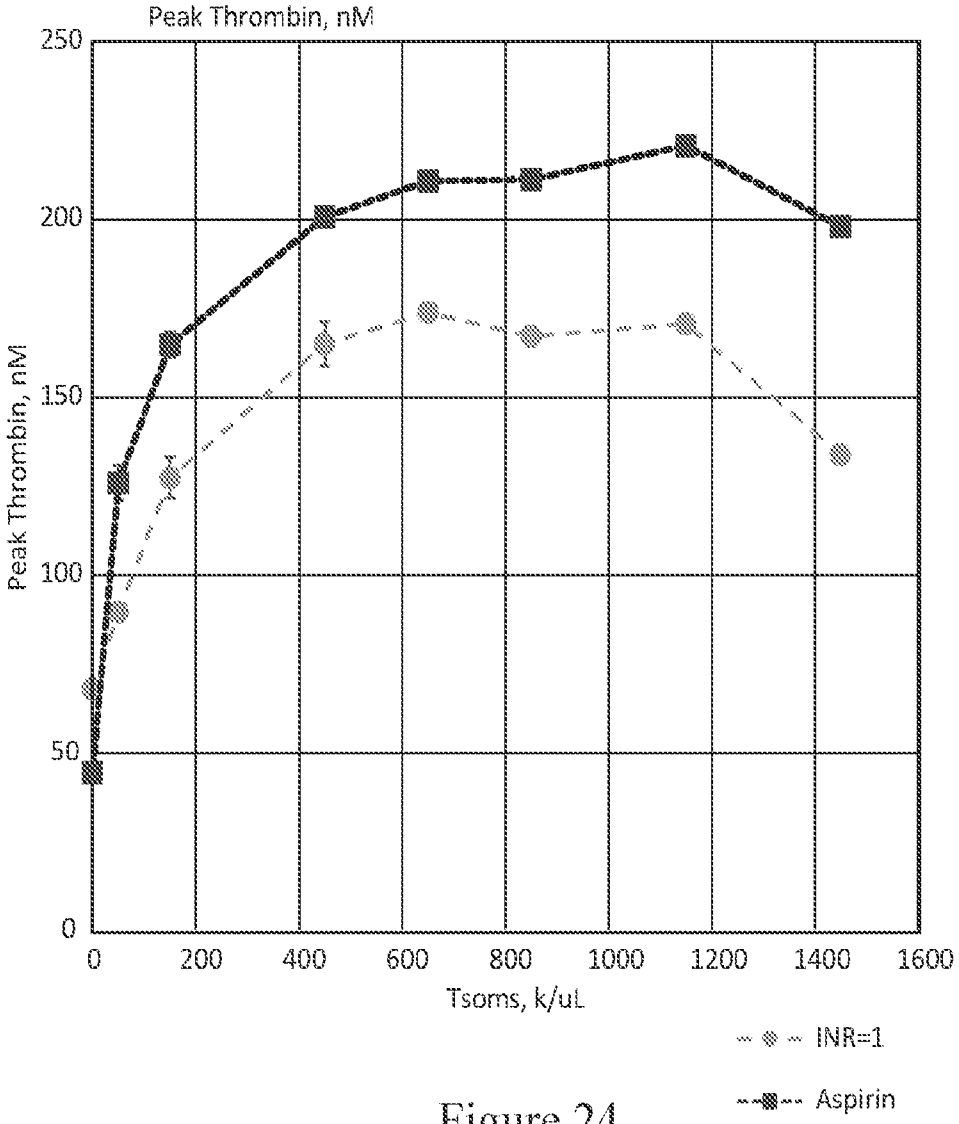
Peak Thrombin, nM
250
200
150
100
50
0
Peak Thrombin, nM
0
200
400
600
800
1000
1200
1400
1600
Tsoms, k/uL
INR=1
Aspirin

Figure 24

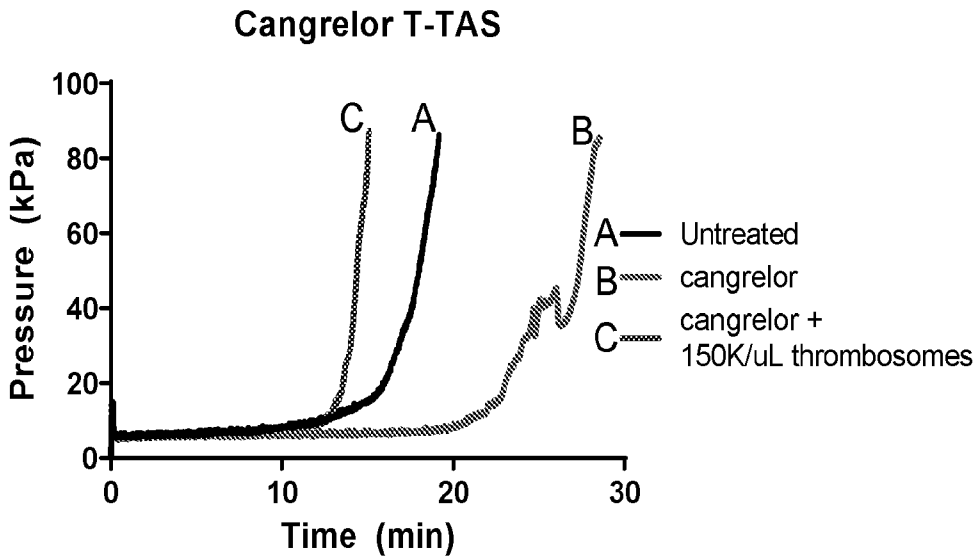

Figure 25A

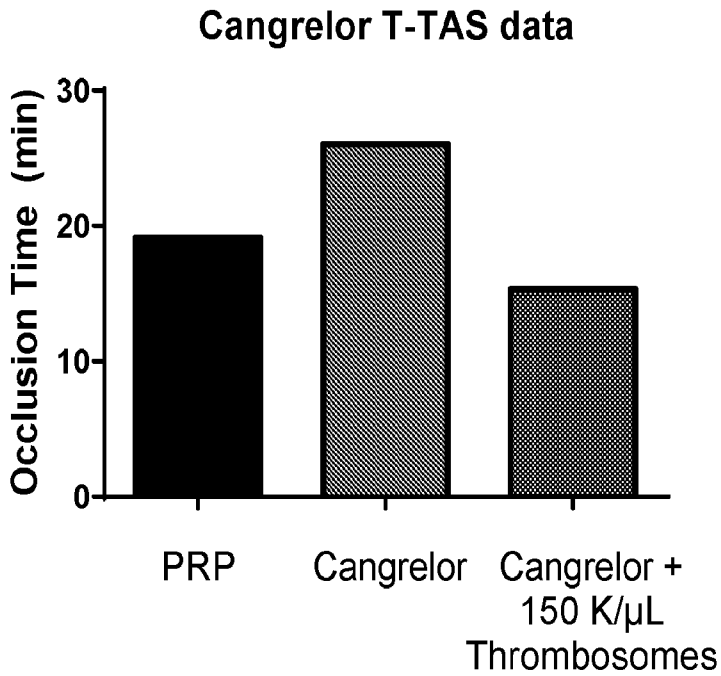

Figure 25B

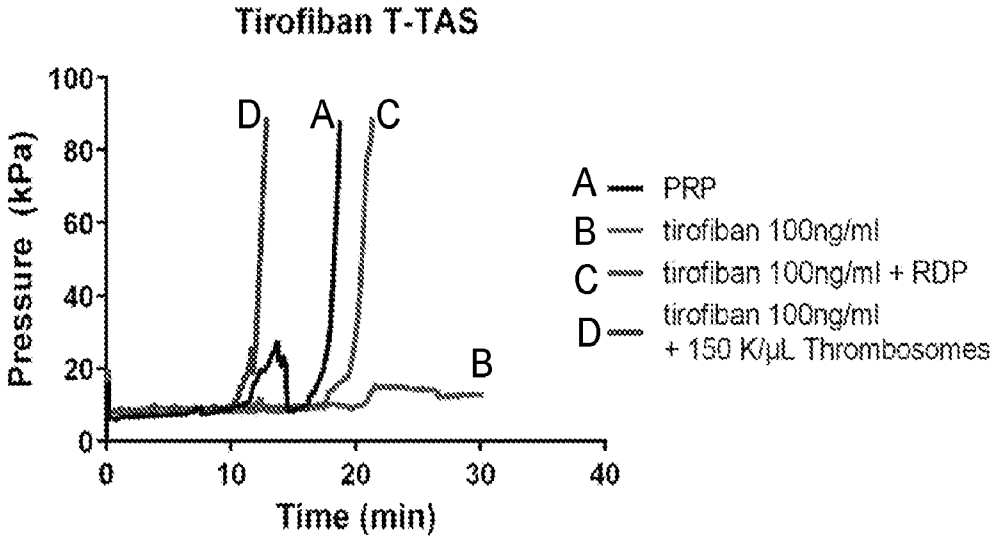

Figure 26A

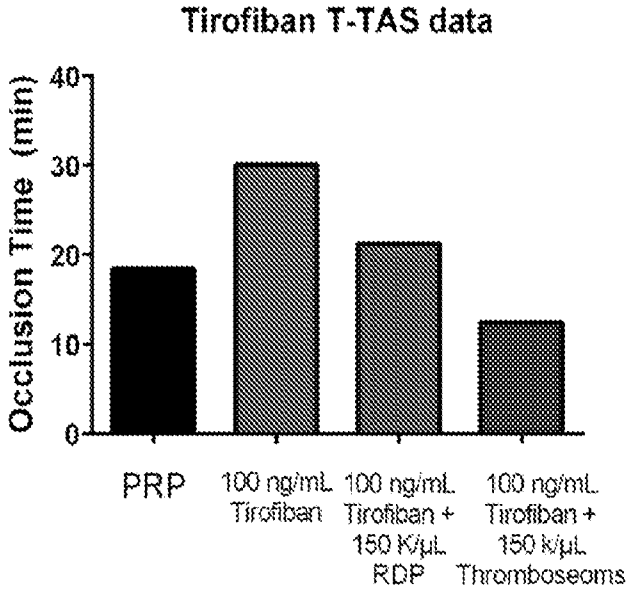

Figure 26B

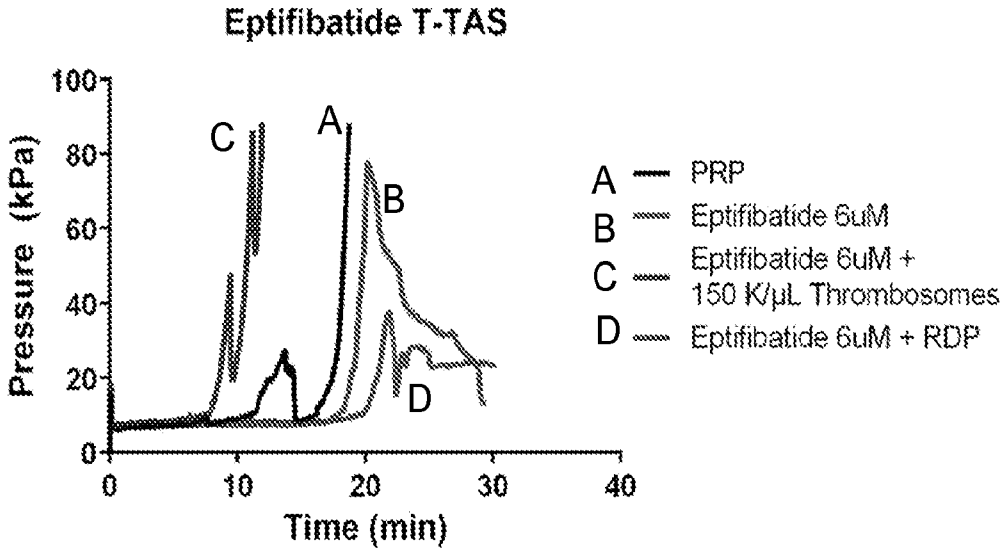

Figure 27A

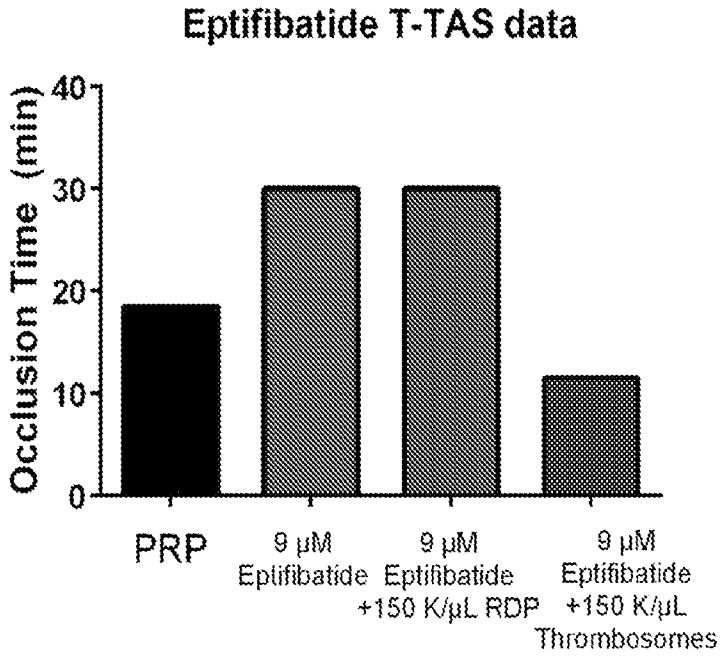

Figure 27B

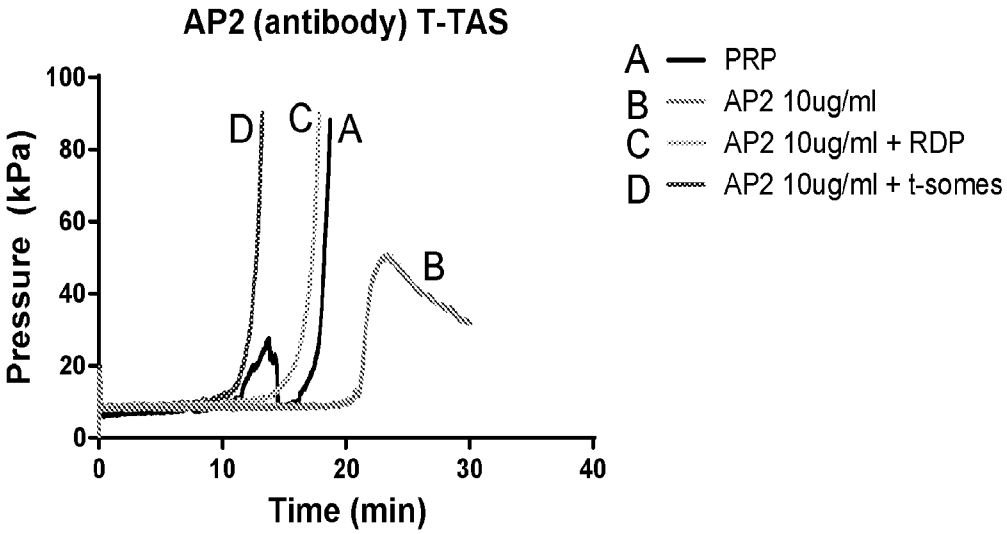

Figure 28A

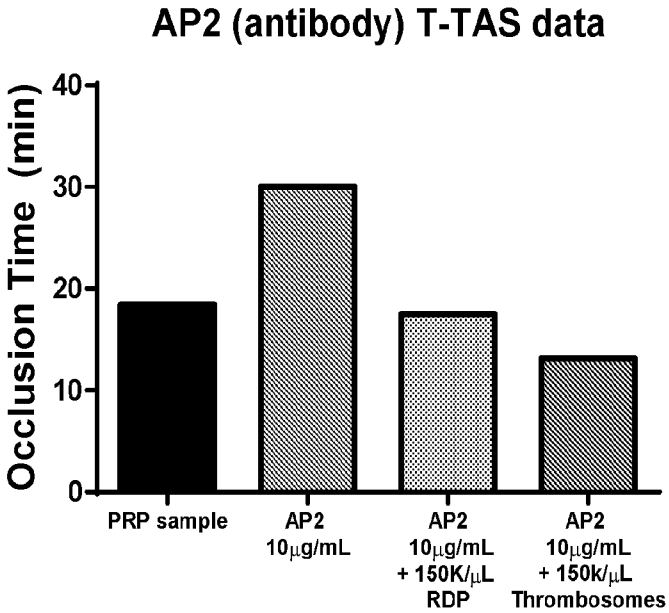

Figure 28B

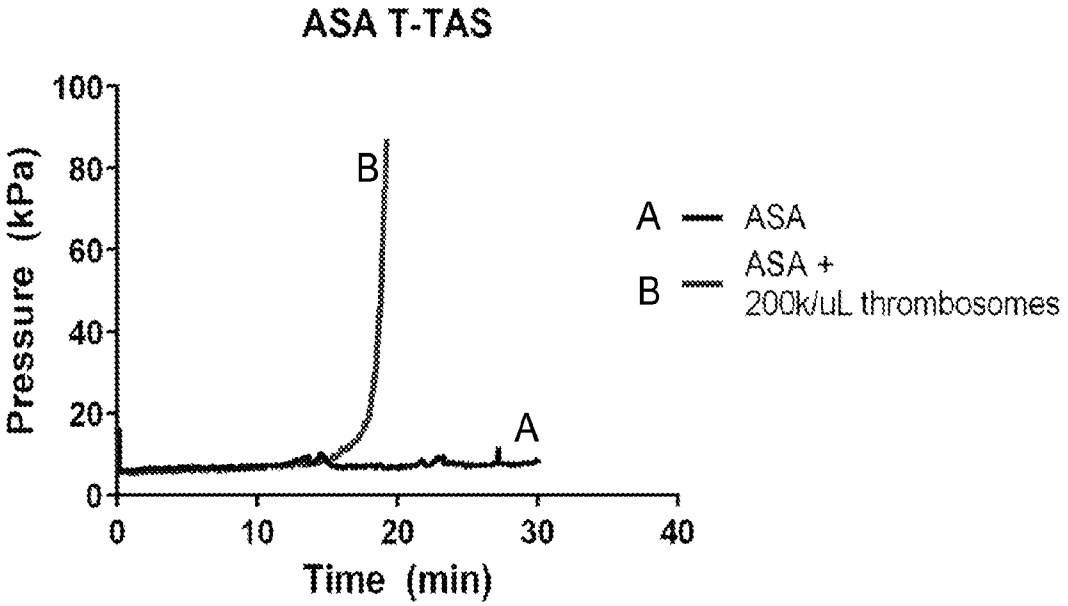

Figure 29A

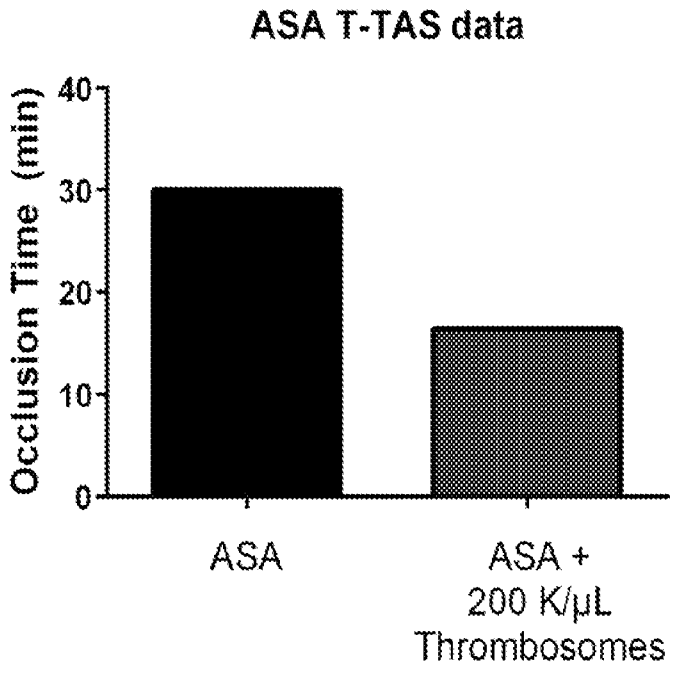

Figure 29B

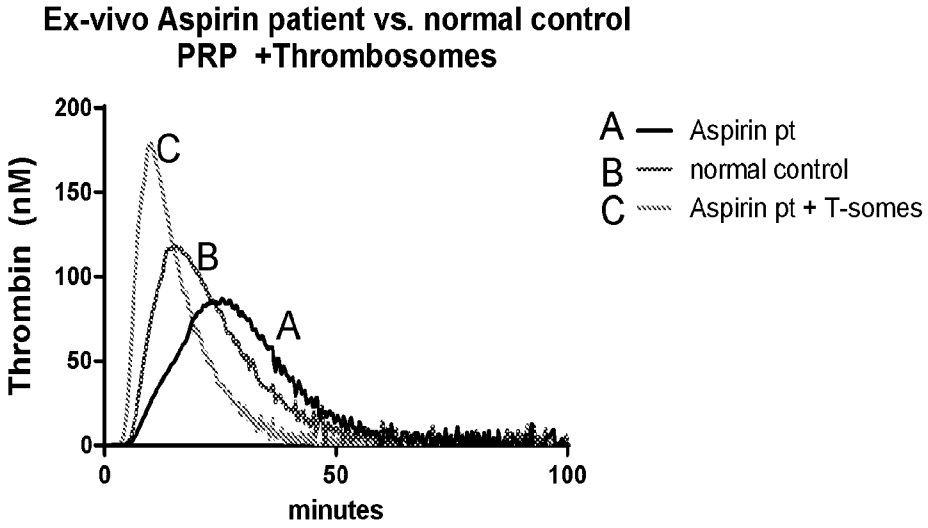

Figure 30A

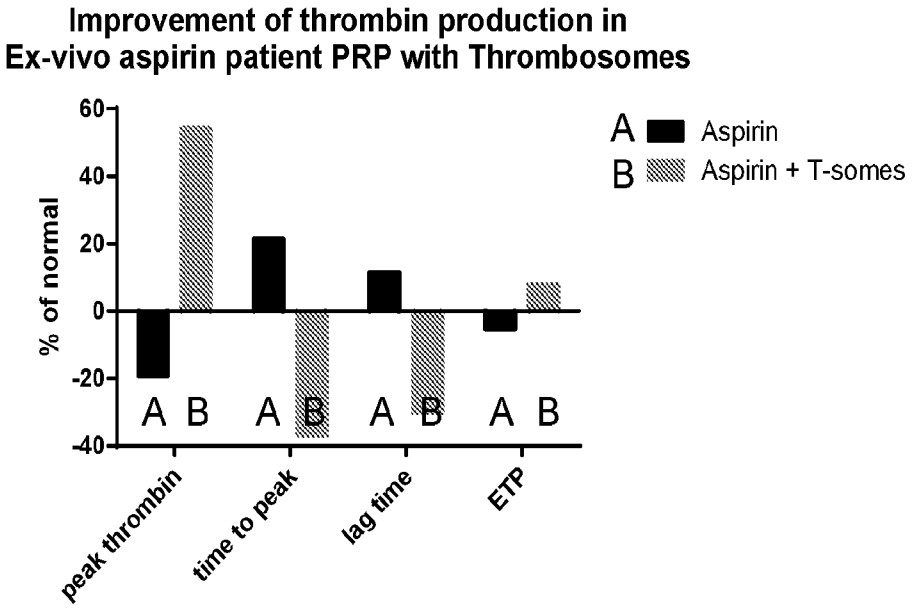

Figure 30B

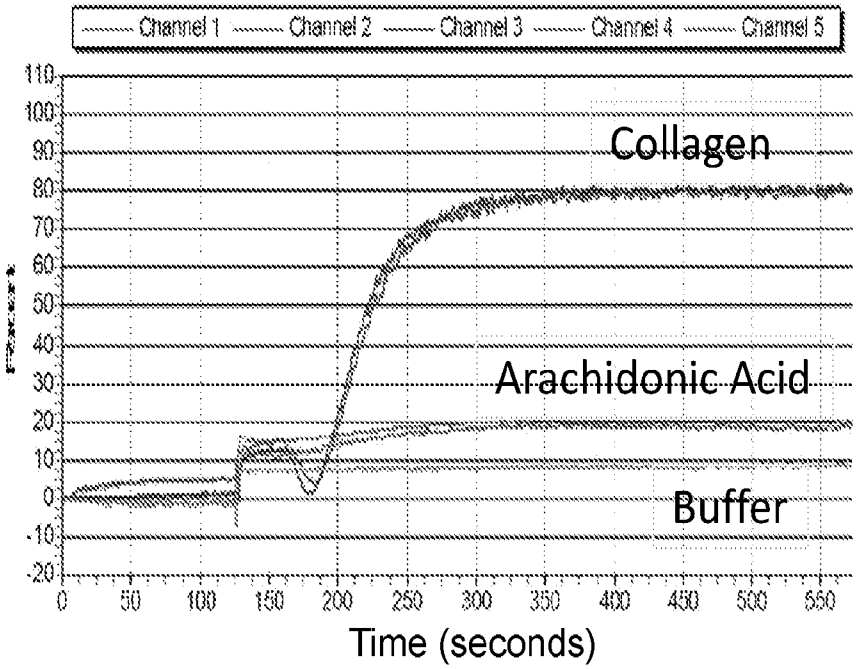

Figure 31A

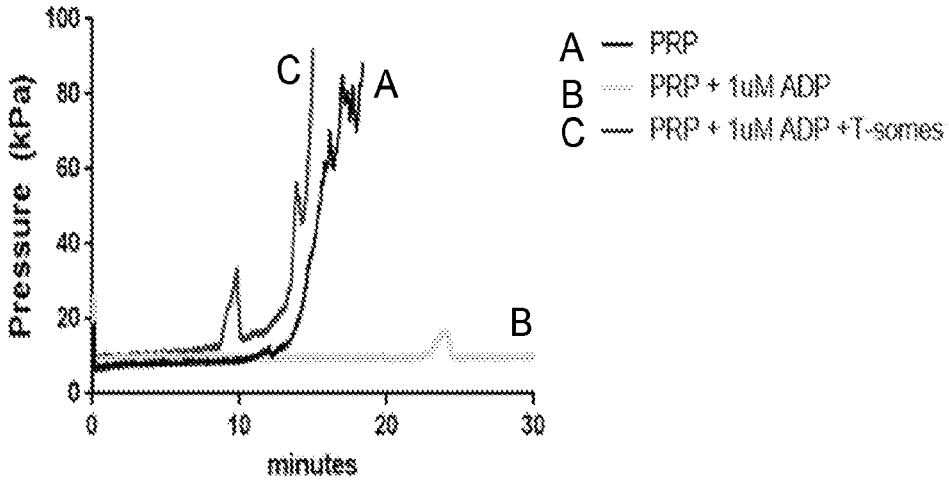

Figure 31B

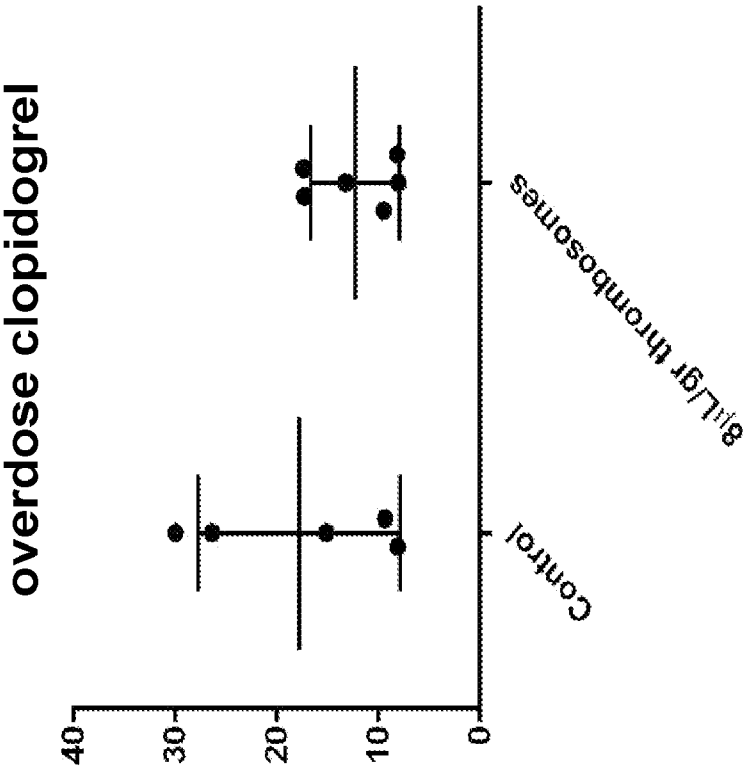
overdose clopidogrel
40
30
20
10
0
Control
8μL/gr thromboxomes

Figure 32

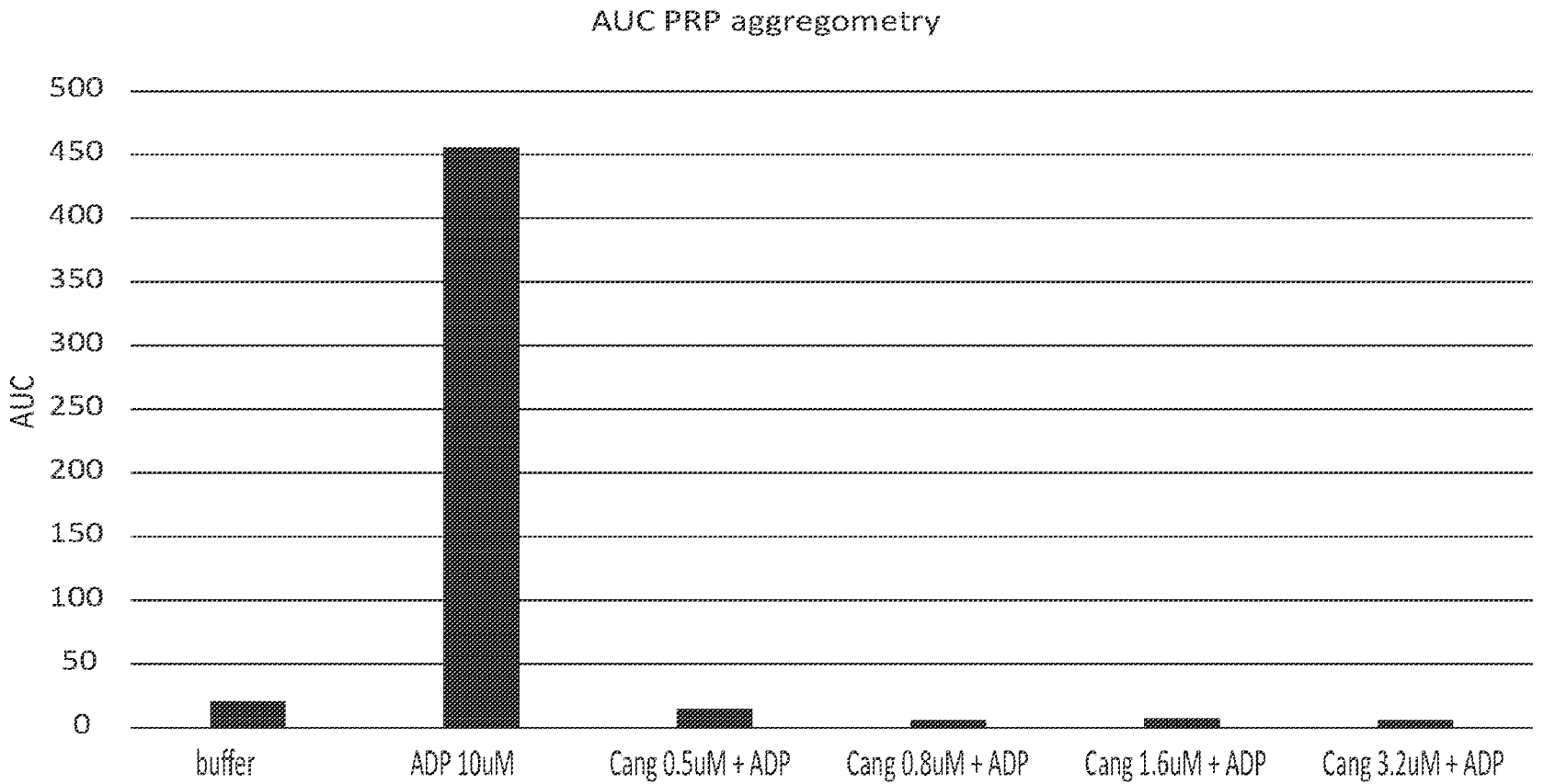
AUC PRP aggregometry
500
450
400
350
300
250
200
150
100
50
0
AUC
buffer
ADP 10uM
Cang 0.5uM + ADP
Cang 0.8uM + ADP
Cang 1.6uM + ADP
Cang 3.2uM + ADP

Figure 1