

**CRYOPRESERVED PLATELETS, COLLECTIONS THEREOF, AND PROCESSES FOR
PREPARING THE SAME**

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application Serial No. 63/505,219 filed May 31, 2023, and is incorporated herein by reference in its entirety.

STATEMENT OF GOVERNMENT INTEREST

[0002] The invention was made with government support under Contract No. W81XWH20C0030 awarded by the Defense Health Agency (DHA) of the U.S. Department of Defense. The government has certain rights in the invention.

TECHNICAL FIELD

[0003] The present disclosure relates generally to blood products, and, more particularly, to cryopreserved platelet and cryopreserved platelet compositions, including process for preparing same.

BACKGROUND

[0004] Platelets in a liquid stored apheresis form are used to treat blood-related issues, such as hemorrhages and thrombocytopenia. Typically, the platelets used in blood-related treatments have a shelf life of five days. This limits the availability of platelets for blood-related treatments. The short shelf life can also render 10-20% of available platelet collections unusable, causing about 200 million dollars to be lost annually. There are currently no commercially available cryopreserved platelets available for human transfusion.

[0005] Cryopreserved platelets that are described in the field mostly relate to single donor processes, meaning that one apheresis unit is processed into one cryopreserved platelet unit. Apheresis platelet units, and single donor cryopreserved platelet units, have an inherent donor to donor variability since they are both single donor products. This variability includes multiple parameters, not to mention the variation in the percentage of cryoprotectant, such as dimethyl sulfoxide (DMSO), total number of platelets, and platelet concentration in the single donor products. Furthermore, even for CPPs made from pooled platelets, have too much lot-to-lot or batch-to-batch variability in these parameters. Accordingly, there is a long-felt

need in the art to overcome this donor-to-donor and lot-to-lot or batch-to-batch variability in cryopreserved platelets, or cryopreserved platelet compositions.

[0006] It is known that stringent freezing temperature conditions are required for storing cryopreserved platelets, for example, ultra-freezers are required to maintain temperatures at $\leq -65^{\circ}\text{C}$ in order to have a functional platelet product for applications such as in a battlefield, or in a hospital or other patient treatment center. However, depending on geographical locations, such battlefields or hospitals may not be equipped with ultra-freezers due to the size of the freezer unit the cost of one or especially more such freezer units, and/or the lack of an adequate electrical power supply, thereby, making it difficult to store the cryopreserved platelets at such temperatures. Accordingly, there is a long-felt need in the art to have a cryopreserved platelet composition that is effective at controlling bleeding, is readily manufacturable without highly specialized equipment and is capable of being stored in standard -20°C freezers for months or even years.

SUMMARY

[0007] To overcome the above-mentioned and additional problems in the art, the present disclosure provides aspects and embodiments that include frozen platelets, frozen platelet derivatives, cryopreserved platelets, and/or cryopreserved platelet derivatives. In some aspects and embodiments, pooled cryopreserved platelets (CPP) are provided that can be aliquoted into multiple doses of a final product from a starting material that typically includes multiple platelet units. Improved processes of the present disclosure create a final product that exhibits decreased lot to lot variability, when compared to the donor-to-donor variability of current standard of care products (single donor CPP or apheresis platelet units). The novel process aspects and embodiments herein allow for increased in-process controls (IPCs) and reduces variability in the processing compared to single donor CPP processes. The processes provided herein facilitate increased control of the excipients and freezing volume compared to single donor process and prior CPP processes. This increased control reduces variation of the DMSO content, dosage volume, and total cell number that is administered to a patient, which increases the safety profile of a CPP product provided herein. The ability to create multiple doses, such as multiple cryo-vessels of a batch of CPPs from a pool of platelet units allows for quality control testing of the final product for release of lots which is not achievable for single donor CPP.

[0008] Further, in some aspects, provided herein is a process for preparing a cryopreserved platelet composition that can be stored at a temperature higher than -65°C , for example, at a temperature in the range of -10°C to -30°C for a time period of at least 1 month, 3 months, 12 months, or until the

cryopreserved platelets are required for treating a subject in need thereof. Also provided is a frozen platelet composition, which is capable of being stored at a temperature higher than -65°C , for example at a temperature in the range of -10°C to -30°C for a time period of at least 1 month, 3 months, 12 months, or until the cryopreserved platelets are required for treating a subject in need thereof.

[0009] Accordingly, provided herein in one aspect is a process for preparing a cryopreserved platelet composition comprising cryopreserved platelets, said process comprising:

- i) freezing a population of platelets in a cryopreservation medium at a temperature of equal to or less than -50°C to form an initial frozen platelet composition;
- ii) subjecting the initial frozen platelet composition to a temperature of equal to or more than -30°C but less than 0°C , or less than -1°C ; and
- iii) storing the initial frozen platelet composition at the temperature of equal to or more than -30°C but less than 0°C , or less than -1°C , to form the cryopreserved platelet composition comprising the cryopreserved platelets.

[0010] Accordingly, provided herein in one aspect is a process for preparing a cryopreserved platelet composition comprising cryopreserved platelets, said process comprising:

- i) freezing a population of platelets in a cryopreservation medium at a temperature of equal to or less than -50°C to form an initial frozen platelet composition; and
- ii) storing the initial frozen platelet composition at a temperature of equal to or more than -30°C but less than 0°C , or less than -1°C , for at least 1 month to form the cryopreserved platelet composition.

[0011] Accordingly, provided herein in one aspect is a process for preparing a batch of a cryopreserved platelets, comprising:

- a) pooling at least 2 platelet units into one vessel and at least another platelet unit into another vessel, wherein the platelet units are from more than one donor;
- b) centrifuging each vessel to obtain a supernatant comprising plasma, and a pellet comprising platelets;
- c) resuspending the pellet in each vessel to form a resuspension wherein the resuspension has a target weight determined by the number of units pooled or provided in the vessel;
- d) pooling the resuspension from each vessel to form a pooled resuspension in a pooled resuspension vessel;
- e) adding a cryoprotectant to the pooled resuspension vessel having the pooled resuspension to obtain a pooled resuspension having the cryoprotectant;

f) distributing the pooled resuspension having the cryoprotectant from the pooled resuspension vessel among a number of cryo-vessels; and

g) freezing the pooled resuspension having the cryoprotectant in the cryo-vessels, to form the batch of cryopreserved platelets. The target weight in non-limiting examples, can be in the range of 15.0 to 30.0g, 15.0 to 29.0g, 15.0 to 28.5g, or in illustrative embodiments 15.9g to 27.9g times the number of units pooled or provided in the vessel. In illustrative embodiments, the cryoprotectant is or comprises DMSO.

[0012] Accordingly, provided herein in one aspect is a collection of cryo-vessels comprising cryopreserved platelets, wherein the cryopreserved platelets in each cryo-vessel have a biomolecule profile indicative of more than 1 platelet donor, and wherein the concentration of the cryoprotectant, in illustrative embodiments DMSO, in the cryopreserved platelets of a first cryo-vessel is within 15%, 12%, 10%, 9%, 7%, 5%, 3%, 2%, 1%, or 0.5% of the concentration of cryoprotectant, in illustrative embodiments DMSO, in the cryopreserved platelets of a second cryo-vessel. In some embodiments, the collection comprises at least 2, 5, 10, 15, 20, or more cryo-vessels from 1 or in illustrative embodiments, 2, 3, 4, 5 or more batches, wherein a batch of cryo-vessels has an identical set of biomolecule profiles, and wherein each batch of the collection has a different set of biomolecule profiles than any other batch in the collection. In some embodiments, exactly or at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more cryo-vessels are in each batch.

[0013] Accordingly, provided herein in one aspect is a cryopreserved platelet composition comprising cryopreserved platelets, wherein the cryopreserved platelets are stored at about -20 °C to -60°C for a time period of at least 1 month.

[0014] Accordingly, provided herein in one aspect is a composition comprising frozen platelets in a cryopreservation medium in a frozen state, wherein the composition is capable of yielding one or more of the following recited properties after storage for at least 1 month, 2, 3, 4, 5, or 6 months, upon thawing:

- a) is in a liquid state without requiring the addition of a liquid to achieve such liquid state;
- b) exhibits a platelet count of at least $1.0 \times 10^{11}/35$ ml of the composition;
- c) yields a single peak that corresponds to a compromised membrane peak in a membrane integrity assay;
- d) exhibits a CD61-positive-microparticle content of less than 50% of the CD61 positive particles in the composition, and
- e) generates thrombin in an *in vitro* thrombin generation assay.

[0015] Further details regarding aspects and embodiments of the present disclosure are provided throughout this patent application. Sections and section headers are for ease of reading and are not intended

to limit combinations of disclosure, such as methods, compositions, and kits or functional elements therein across sections. Further details regarding aspects and embodiments of the present disclosure are provided throughout this patent application. Sections and section headers are for ease of reading and are not intended to limit combinations of disclosure, such as methods, compositions, or other functional elements therein across sections.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] FIG. 1A and FIG. 1B are non-limiting flowcharts of steps of exemplary processes for preparing a batch or a lot of cryo-vessels comprising cryopreserved platelets.

[0017] FIG. 1C is a schematic of a tubing tree that can be used as per one of the aspects of process herein.

[0018] FIG. 2A shows a non-limiting flow chart of an exemplary process for preparing a cryopreserved platelet composition that is capable of storing at a temperature in the range of -10°C to -30°C.

[0019] FIG. 2B shows a non-limiting flow chart of an exemplary process for preparing a cryopreserved platelet composition that integrates the exemplary process of FIG. 1A and FIG. 2A.

[0020] FIG. 3 displays the target % DMSO that can be achieved using the calculation provided in the single donor process of Vitalant for preparing cryopreserved platelets.

[0021] FIG. 4A shows a freezing volume distribution of 255 units prepared by the single donor process of Vitalant for preparing cryopreserved platelets.

[0022] FIG. 4B shows a freezing volume distribution for units with aggregates observed within 6 hours following thawing and resuspension for 32 units.

[0023] FIG. 5 shows a comparison of the correlation of APC volume to %DMSO of the single donor method for preparing cryopreserved platelets of Vitalant, and CPP pooled process as disclosed herein.

[0024] FIG. 6 shows the correlation between post-expression volume (resuspension volume), and %DMSO for the CPP pooled process as disclosed herein.

[0025] FIG. 7 shows the percentage recovery of platelets for the batches stored at -80°C (single temperature cryopreserved-product) and -20°C (transition temperature cryopreserved-product).

[0026] FIG. 8 shows the percentage aggregation of platelets for the batches stored at -80°C (single temperature cryopreserved-product) and -20°C (transition temperature cryopreserved-product), and comparison with apheresis platelets with arachidonic acid (AA), collagen, and thrombin receptor-activating peptide 6 (TRAP-6).

[0027] FIG. 9 shows the peak distribution of platelets for the batches stored at -80°C (single temperature cryopreserved-product) depicted by “#” and -20°C (transition temperature cryopreserved-product) depicted by “*”, and apheresis platelets depicted by “+”.

DEFINITIONS

[0028] As used herein, “cryopreserved platelets” are frozen platelets that when thawed are in a liquid state regardless of whether any liquid is added to the frozen platelets after thawing. Accordingly, cryopreserved platelets are not fresh platelets and they are not freeze-dried platelet derivatives. During processing cryopreserved platelets are not dried. The term “cryopreserved platelets” does not imply any minimum length of time such platelets are present in a frozen state. However, cryopreserved platelets are typically stable for at least 1, 2, 3, 4, 5, 6, 9, or 12 months, and in illustrative embodiments are stable for at least 18, 24, 36, or 48 hours. Cryopreserved platelets are typically suspended in a cryoprotectant in a frozen state, until thawing before use. In some embodiments herein, cryopreserved platelets are stored for a period of at least 1, 2, 3, 4, 5, 6, 9, or 12 months at a temperature of -20°C.

[0029] As used herein “platelet derivatives” in the context of cryopreserved platelets, or a composition comprising frozen platelets and/or platelet derivatives implies particles that, unlike fresh platelets, do not have intact cell membranes, for example, as demonstrated by a Calcein AM membrane integrity assay (See e.g., Example 8). Accordingly, in some embodiments, a composition provided herein comprises platelet derivatives, and such platelet derivatives are not freeze-dried platelet derivatives.

[0030] Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in Benjamin Lewin, *Genes V*, published by Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew *et al.* (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8). Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. The singular terms “a,” “an,” and “the” include plural referents unless the context clearly indicates otherwise. “Comprising A or B” means including A, or B, or A and B. It is further to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description.

[0031] Further, ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 1 to 49, 1 to 25, 1.7 to 31.9, and so forth (as well as fractions thereof unless the context clearly dictates otherwise). Any concentration range, percentage range, ratio range, or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated. Also, any number range recited herein relating to any physical feature, such as polymer subunits, size or thickness, are to be understood to include any integer within the recited range, unless otherwise indicated. When multiple low and multiple high values for ranges are given that overlap, a skilled artisan will recognize that a selected range will include a low value that is less than the high value.

[0032] As used herein, the symbol "<" means less than or in the context of temperatures, can mean below a recited temperature. As used herein, the symbol "/" in the context of temperatures, can mean to include a range of temperature, for example -20°C +/- 2°C would mean a temperature from -18°C to -22°C. As used herein, "about" or "consisting essentially of" mean $\pm 10\%$ of the indicated range, value, or structure, unless otherwise indicated. As used herein, the terms "include" and "comprise" are open ended and are used synonymously. As used herein, "comprising" is synonymous with "including," "containing," or "characterized by," and is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. As used herein, "consisting of" excludes any element, step, or ingredient not specified in the claim element. As used herein, "consisting essentially of" does not exclude materials or steps that do not materially affect the basic and novel characteristics of the claim. In each instance herein any of the terms "comprising", "consisting essentially of" and "consisting of" may be replaced with either of the other two terms. The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein.

[0033] Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entireties. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

[0034] It is appreciated that certain features of aspects and embodiments herein, which are, for clarity, discussed in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various aspects and embodiments, which are, for brevity, discussed in the context of a single aspect or embodiment, may also be provided separately or in any suitable sub-combination. All combinations of aspects and embodiments are specifically embraced herein and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various aspects and embodiments and elements thereof are also specifically disclosed herein even if each and every such sub-combination is not individually and explicitly disclosed herein.

[0035] While the embodiments of the present disclosure are amenable to various modifications and alternative forms, specific embodiments have been shown by way of example in the drawings and are described in detail below. The intention, however, is not to limit the disclosure to the particular embodiments described. On the contrary, the disclosure is intended to cover all modifications, equivalents, and alternatives falling within the scope of the disclosure as defined by the appended claims.

[0036] It is to be understood that any inventions disclosed or claimed herein encompass all variations, combinations, and permutations of any one or more features described herein. Any one or more features may be explicitly excluded from the claims even if the specific exclusion is not set forth explicitly herein. It should also be understood that disclosure of a reagent for use in a method is intended to be synonymous with (and provide support for) that method involving the use of that reagent, according either to the specific methods disclosed herein, or other methods known in the art unless one of ordinary skill in the art would understand otherwise. In addition, where the specification and/or claims disclose a method, any one or more of the reagents disclosed herein may be used in the method, unless one of ordinary skill in the art would understand otherwise.

DETAILED DESCRIPTION

[0037] The present disclosure addresses many long-felt needs and long-standing problems in the art, such as, but not limited to those mentioned in the Background section herein. To overcome the above-mentioned and additional problems in the art, the present disclosure provides aspects and embodiments that include frozen platelets, frozen platelet derivatives, cryopreserved platelets, and/or cryopreserved platelet derivatives. In some aspects and embodiments, pooled cryopreserved platelets (CPP) that can be aliquoted into multiple doses of a final product from a starting material that typically includes multiple platelet units. The process as per the present disclosure creates a final product that exhibits decreased lot to lot or batch-to-batch variability, when compared to the donor-to-donor variability of current standard of care products

(single donor CPP or apheresis platelet units). Aspects and embodiments herein allow for increased in-process controls (IPCs) and reduces variability in the processing compared to single donor CPP processes. The processes provided herein facilitate increased control of the excipients and freezing volume compared to single donor process and prior CPP processes. This increased control reduces variation of the DMSO content, dosage volume, and total cell number that is administered to a patient, which increases the safety profile of a CPP product provided herein. The ability to create multiple doses, such as multiple cryo-vessels of a batch of CPPs from a pool of platelet units allows for quality control testing of the final product for release of lots which is not achievable for single donor CPP. Additionally, since multiple doses are created from a common pool, the process allows for direct comparisons for stability studies and archive samples can be made for later analysis. Further, illustrative processes herein use approximately 90% less cryoprotectant, such as DMSO to achieve a batch or a lot of cryopreserved platelets as compared to current standard-of-care cryopreserved platelets. And illustrative processes herein provide improved batch-to-batch consistency of cryoprotectant concentrations, such as dimethyl sulfoxide (DMSO) concentrations, total number of platelets, and platelet concentration, than current standard-of-care CPP products.

[0038] For example, the process of preparing a batch of cryopreserved platelets, and a collection of cryo-vessels comprising cryopreserved platelets as disclosed herein provide cryopreserved platelets that are homogenous within a batch and across multiple batches. The homogeneity observed can include but not limited to concentration of DMSO, platelet concentrations, and total number of platelets in cryopreserved platelets. Such homogeneity is generally not observable in cryopreserved platelets produced from a single unit of platelets owing to donor-to-donor variability or prior processes for making CPPs that use a pool of donors.

[0039] Accordingly, as illustrated in FIG. 1A, provided in some aspects, are processes for preparing a batch or a multiple number of batches of cryopreserved platelets. The steps of such aspects are shown in boxes in FIG. 1A, with optional steps in such aspects shown in dashed boxes. Such methods, in non-limiting examples, can include the following steps:

- a) obtaining or providing platelet units from more than one donor (110);
- b) pooling at least 2 platelet units into one vessel and at least another platelet unit into another vessel (120), such that there will be more than one vessel at the end of the step;
- c) centrifuging each vessel to obtain a supernatant comprising plasma, and a pellet comprising platelets (130);

- d) resuspending the pellet in each vessel to form a resuspension wherein the resuspension has a target weight determined by the number of units pooled or provided in the vessel (140);
- e) pooling the resuspension from each vessel to form a pooled resuspension in a pooled resuspension vessel (150);
- f) adding a cryoprotectant to the pooled resuspension vessel having the pooled resuspension to obtain a pooled resuspension having the cryoprotectant (160);
- g) distributing the pooled resuspension having the cryoprotectant from the pooled resuspension vessel among a number of cryo-vessels (170); and
- h) freezing the pooled resuspension having the cryoprotectant in the cryo-vessels, to form the batch of cryopreserved platelets (180).

[0040] Typically, at least 3, 4, or 5 platelet units are provided (110) and available for pooling (120). The platelet units can be from exactly or more than 2, 3, 4, 5 donors. The target weight in non-limiting examples, can be in the range of 15.0 to 30.0g, 15.0 to 29.0g, 15.0 to 28.5g, or in illustrative embodiments 15.9g to 27.9g times the number of units pooled or provided in the vessel. The step preceding the resuspending step (140) can include a step of removing a part of the supernatant for achieving a target weight of the resuspension. Alternatively, the step before the resuspending step can include removing a part of the supernatant and adding a buffer composition before resuspending within the target weight. In illustrative embodiments, the steps of pooling the resuspension (150), and adding the cryoprotectant (160) can be performed using a tubing tree system. In illustrative embodiments, adding a cryoprotectant (160) can include adding dimethyl sulfoxide (DMSO) as the cryoprotectant, and the addition is performed until a target weight of the DMSO that needs to be added is achieved. Typically, the addition of DMSO (160) is performed in the same tubing tree system that is used for pooling the resuspension (150). In illustrative embodiments, distributing the pooled resuspension (170) can be performed using a dosing tree system. In some embodiments, the dosing tree system can be different from the tubing tree system by the design, or can be a different unit or the same unit after cleaning the unit.

[0041] In addition to the above, to address the long-felt need of storing cryopreserved platelets at a temperature higher than -65°C, for example in a -20°C freezer, the present disclosure provides a process to form cryopreserved platelets, which in some illustrative embodiments are cryopreserved platelet derivatives, that includes a transition in freezing temperatures from an initial freezing temperature to a storage freezing temperature. Accordingly, provided herein, in some aspects, is a process comprising a step of initial freezing at a temperature (i.e., initial temperature) less than or equal to -50°C, -60°C, -65°C, -

70°C, -80°C, -85°C, or -90°C, or in the range of -50°C to -85°C, or -60°C to -85°C to form an initial frozen platelet composition, followed by storing the initial frozen platelet composition in a frozen state at a temperature (i.e., storage temperature) equal to or greater than -30°C, but less than 0°C, to form a cryopreserved platelet composition. Surprisingly, it was found that the cryopreserved platelets formed by the process as disclosed herein have the property of being stable and retain hemostatic abilities when stored at higher temperatures as compared to that required for storing conventional cryopreserved platelets. For example, the cryopreserved platelets prepared as per a process disclosed herein can be stored at a temperature of about -30°C, -25°C, -20°C, -15°C, -10°C, or -5°C or at a temperature in the range of -30°C to -5°C, or -30°C to -5°C for at least 1 month up to 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months, or for at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months, or for at least, or up to 1 year, 2, 3, 4, 5, or 6 years, or for between 6 months and 1 year, 2, 3, 4, 5, or 6 years. Accordingly, as illustrated in FIG. 2A, provided in some aspects, are processes for preparing a cryopreserved platelet composition. Exemplary steps of such aspects are shown in boxes in FIG. 2A. Such methods, in non-limiting examples, can include the following steps:

- a) freezing platelets in a cryopreservation medium at a temperature of less than or equal to -50°C (or in some aspects, less than or equal to -55°C, or less than or equal to -60°C) to form an initial frozen platelet composition (280); and
- b) storing the initial frozen platelet composition at a temperature in the range of -30°C to -10°C (in some embodiments in a freezer set at, or at about -20°C) for at least 1 month to form a cryopreserved platelet composition (290). In some embodiments, the cryopreserved platelets formed in such a method are cryopreserved platelet derivatives. Furthermore, in illustrative embodiments, the cryopreserved platelet composition or the cryopreserved platelet derivative composition have one or more recited properties provided herein for frozen platelet and/or frozen platelet derivative compositions.

[0042] In other aspects, as illustrated in FIG. 2B, provided herein are processes for preparing a cryopreserved platelet composition. The steps of such aspects are shown in boxes in FIG. 2B. Such methods, in non-limiting examples, can include the following steps:

- a) obtaining or providing platelet units from more than one donor (210);
- b) pooling at least 2 platelet units into one vessel and at least another platelet unit into another vessel (220), such that there will be more than one vessel at the end of the step;
- c) centrifuging each vessel to obtain a supernatant comprising plasma, and a pellet comprising platelets (230);

d) resuspending the pellet in each vessel to form a resuspension wherein the resuspension has a target weight determined by the number of units pooled or provided in the vessel (240);

e) pooling the resuspension from each vessel to form a pooled resuspension in a pooled resuspension vessel (250);

f) adding a cryoprotectant to the pooled resuspension vessel having the pooled resuspension to obtain a pooled resuspension having the cryoprotectant (260); and

g) distributing the pooled resuspension having the cryoprotectant from the pooled resuspension vessel among a number of cryo-vessels (270). In some embodiments, the pooled resuspension in the cryo-vessels is frozen and stored at a temperature of less than or equal to -50°C .

[0043] Optionally, in some embodiments, a transitional temperature freezing protocol is performed on the pooled resuspension in the cryo-vessels. Accordingly, such embodiments include the following steps: h) freezing the pooled resuspension in the cryo-vessels at a temperature of less than or equal to -50°C to form cryo-vessels each comprising an initial frozen platelet composition (285); and i) storing the cryo-vessels comprising the initial frozen platelet composition at a temperature in the range of -30°C to -10°C for at least 1 month to form cryo-vessels comprising a cryopreserved platelet composition (295).

Preparation of cryopreserved platelets using a transition in freezing temperatures

[0044] Provided herein in one aspect is a process for preparing cryopreserved platelets that include an initial freezing step comprising freezing platelets in a cryopreservation medium, or freezing a pooled resuspension having a cryoprotectant as disclosed herein, at a temperature of less than or equal to -50°C , -55°C , -60°C , in illustrative embodiments, less than or equal to -65°C , -70°C , -75°C , or -80°C , to form an initial frozen platelet composition, and a second step comprising storing the initial frozen platelet composition in a frozen state at a temperature of more than or equal to -40°C , -35°C , -30°C , -25°C , in illustrative embodiments, more than or equal to -20°C , -15°C , or -10°C , but less than 0°C to form cryopreserved platelets, cryopreserved platelet composition, or a batch of cryopreserved platelets. In some embodiments, a composition that includes cryopreserved platelets obtained from a process using a transition in freezing temperatures from an initial temperature equal to or less than some initial target temperature set at a target initial temperature or temperature range that is no warmer than -50°C , and then stored after some period of time, at a temperature that is at a target storage temperature set at a target storage temperature or storage temperature range between about -10°C to about -30°C , can be referred to as a transition temperature cryopreserved-product, or a transition temperature-cryopreserved composition, and such a process can be referred to as a transition temperature cryopreservation process. Also, for

convenience to differentiate a transition temperature cryopreserved product from a cryopreserved product that does not involve such a transition in temperature in its preparation, in some embodiments, a cryopreserved product that is obtained by only freezing and storing at a target temperature at or below -60°C, for example about -80°C, is referred to as a single temperature cryopreserved-product. A skilled artisan will understand that such single-temperature cryopreserved product can in fact, be subjected to a variation in temperatures, but such variation does not include a transition from an initial freezing temperature at or below -50°C to a target storage temperature at -10°C to -30°C. Surprisingly, cryopreserved platelets obtained by a process including a transition in temperature as disclosed herein when stored at a temperature of equal to or higher than -30°C but less than 0°C, or -5°C upon thawing can exhibit hemostatic properties, and in illustrative embodiments are capable of reducing bleeding in a subject, increasing the platelet counts of a subject in need thereof, for example, in a thrombocytopenic subject, or generating thrombin in an in vitro thrombin generation assay, thereby addressing a long-felt need in storage conditions of cryopreserved platelets.

[0045] In some embodiments, an initial frozen platelet composition can be stored at a freezing temperature of more than -40°C, -35°C, -30°C, -25°C, -20°C. In some embodiments, storing of an initial frozen platelet composition can be done for at least 30 minutes, 1 hour, 2, 3, 6, 8, 10, 12, 18, 24 hours, 2 days, 3, 5, 7, 15, 20, 25, days, 1 month, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months, or 1 year, 2, 3, 4, 5, or 6 years. In some embodiments, storing of an initial frozen platelet composition can be done for a time period in the range of 1 month to 10 years, 1 month to 8 years, 1 month to 6 years, 1 month to 5 years, 1 month to 3 years, 1 month to 2 years, 1 month to 1 year, 6 months to 10 years, 1 year to 10 years, 2 years to 10 years, or 3 years to 10 years. In some cases, an initial frozen platelet composition can be stored at a temperature in the range of -40°C to -10°C until the cryopreserved platelets are used for treating a subject in need thereof, in illustrative embodiments, for administering to a subject for reducing bleeding in the subject.

[0046] Typically, in an initial freezing step, a cryopreservation medium having platelets, or a pooled resuspension having a cryoprotectant as disclosed herein, become frozen, and achieve the temperatures as disclosed therein to form an initial frozen platelet composition. For example, freezing a cryopreservation medium having platelets, or a pooled resuspension having a cryoprotectant as disclosed herein at a temperature in the range of -50°C to -85°C comprises subjecting the cryopreservation medium, or the pooled resuspension to the temperature range such that an initial frozen platelet composition is formed at the end of the step, and the temperature of the initial frozen platelet composition is in the range of -50°C to -85°C. An initial freezing step can be performed for a time period until the cryopreservation medium

having the platelets or the pooled resuspension having a cryoprotectant reaches a temperature less than or equal to -50°C , -55°C , -60°C , -65°C , -70°C , -75°C , or -80°C , and the time it takes for the cryopreservation medium or the pooled resuspension to attain the temperature can depend on various factors, not limited to the volume of a cryo-vessel, dimensions of a cryo-vessel, volume of cryopreservation medium having the platelets, concentration of platelets in a cryo-vessel, and composition of a cryopreservation medium in a cryo-vessel. A cryopreservation medium that can be used in a process as disclosed herein can be a cryopreservation medium comprising a cryoprotectant. In illustrative embodiments, the cryoprotectant comprises dimethyl sulfoxide (DMSO). In other embodiments, the cryoprotectant can be any other cryoprotectant apart from DMSO. Other non-limiting examples of suitable cryoprotectants can include saccharides, such as monosaccharides and disaccharides, including sucrose, maltose, trehalose, glucose, mannose, dextrose, xylose, and a combination thereof. In some embodiments, a cryopreservation medium comprising DMSO as a cryoprotectant can have a concentration in the range of 0.001-10%, 0.5-7%, 1-8%, 2-8%, 3-8%, 4-8%, or 5-8%. In some embodiments, an initial freezing step can be done for at least for at least 30 minutes, 1 hour, 2 hours, or 3 hours. For example, an initial freezing step can be done for a time period in the range of 30 minutes to 12 hours, 30 minutes to 10 hours, 30 minutes to 8 hours, 30 minutes to 6 hours, or 30 minutes to 4 hours. In some embodiments, an initial freezing step can be done for more than 12 hours, 2 days, 3 days, 1 week, 1 month, or 6 months. In some embodiments, the temperature during an initial freezing step can be in the range of -50°C to -90°C , -50°C to -85°C , -50°C to -80°C , -50°C to -75°C , -50°C to -70°C , -55°C to -90°C , -60°C to -90°C , -60°C to -85°C , -60°C to -80°C , -60°C to -75°C , or -65°C to -75°C . In illustrative embodiments, the temperature during an initial freezing step can be $-65^{\circ}\text{C} \pm 5^{\circ}\text{C}$, $-65^{\circ}\text{C} \pm 4^{\circ}\text{C}$, $-65^{\circ}\text{C} \pm 3^{\circ}\text{C}$, $-65^{\circ}\text{C} \pm 2^{\circ}\text{C}$, or $-65^{\circ}\text{C} \pm 1^{\circ}\text{C}$. In other illustrative embodiments, the temperature during an initial freezing step can be $-80^{\circ}\text{C} \pm 5^{\circ}\text{C}$, $-80^{\circ}\text{C} \pm 4^{\circ}\text{C}$, $-80^{\circ}\text{C} \pm 3^{\circ}\text{C}$, $-80^{\circ}\text{C} \pm 2^{\circ}\text{C}$, or $-80^{\circ}\text{C} \pm 1^{\circ}\text{C}$. In some embodiments, the time-period during an initial freezing step can depend on the temperature that needs to be achieved. For example, an initial freezing step can comprise a temperature in the range of -60°C to -80°C , for a time period in the range of 1 hour to 7 hours, 1 hour to 6 hours, 1 hour to 5 hours, 1 hour to 4 hours, or 1 hour to 2 hours. In some embodiments, an initial freezing step comprises placing a cryopreservation medium having platelets, or a pooled resuspension having a cryoprotectant in a freezer set at a temperature in the range of -50°C to -90°C , to form an initial frozen platelet composition. [0047] In some embodiments, storing an initial frozen platelet composition comprises subjecting an initial frozen platelet composition to a temperature equal to or more than -30°C , -25°C , -20°C , -15°C , or -10°C but less than -5°C . In illustrative embodiments, an initial frozen platelet composition is subjected to a

temperature of $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$, $-20^{\circ}\text{C} \pm 4^{\circ}\text{C}$, $-20^{\circ}\text{C} \pm 3^{\circ}\text{C}$, $-20^{\circ}\text{C} \pm 2^{\circ}\text{C}$, $-20^{\circ}\text{C} \pm 1^{\circ}\text{C}$, or $-20^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. In some embodiments, storing an initial frozen platelet composition comprises storing in a freezer that is set at a temperature in a range of -10°C to -40°C , -10°C to -30°C , or -15°C to -25°C . Typically, an initial frozen platelet composition when stored at a temperature as disclosed herein or when subjected to a temperature as disclosed herein reaches the intended temperature at the end of the step to form cryopreserved platelets, and after the step, the cryopreserved platelets is stored at the temperature disclosed herein for at least 7, 10, 15, 20, 25 days, 1 month, 2, 3, 4, 6, 8, 10, 12 months, 1 year, 2, 3, 4, 5, 6, 7, 8, 9, or 10 years, or until the cryopreserved platelets are used for administering to or treating a subject in need thereof. In some embodiments, storing an initial frozen platelet composition can comprise storing at a freezing temperature of equal to or higher than -30°C for a time of at least 30 minutes, 45 minutes, 50 minutes, 60 minutes, 70 minutes, 80 minutes, 90 minutes, 2 hours, or 3 hours to form cryopreserved platelets. In some embodiments, a process disclosed herein can comprise subjecting an initial frozen platelet composition to a temperature equal to or higher than -30°C , in illustrative embodiments, in a range of -10°C to -30°C for a time until the temperature of the initial frozen platelet composition reaches the temperature of equal to or higher than -30°C , or in illustrative embodiments, in a range of -10°C to -30°C to form cryopreserved platelets. Typically, once the temperature of the initial frozen platelet composition reaches the temperature in a range of -10°C to -30°C to form a cryopreserved composition, the cryopreserved composition is be stored at a temperature in a range of -10°C to -30°C until the cryopreserved platelets are used for treating a subject in need thereof, or are used for administering to a subject in need thereof.

Composition comprising frozen platelets

[0048] Compositions provided herein in some aspects and embodiments have one or more recited properties (which can also be referred to as recited attributes or recited characteristics). It will be understood that compositions that fall under such aspects or embodiments comprising one or more recited properties exhibit such one or more recited properties, but to fall under such aspects or embodiments that comprise such recited one or more properties does not require that a step is actually performed to demonstrate the one or more recited properties. However, a skilled artisan will understand that such one or more recited properties of a composition can be identified using a method that is set out by a recited property, or by performing a known method, to determine whether a test composition possesses such one or more recited properties. Frozen compositions herein that comprise platelets and/or platelet derivatives, upon thawing exhibit one or more of the following non-limiting recited properties: a) are capable of

exhibiting a platelet count of at least 1.0×10^{11} in 35 ml; b) have about 50% to about 99% of platelets and/or platelet-derived particles in the range of about 1 μm to about 2.5 μm or 5 μm ; c) are in a liquid state without requiring the addition of a liquid to achieve such liquid state; d) yield a single peak that corresponds to a compromised membrane peak in a membrane integrity assay; e) exhibit a CD61-positive-microparticle content of less than 50% of the CD61 positive particles in the composition; f) exhibit an ability to generate thrombin in an *in vitro* thrombin generation assay; g) are capable of inducing aggregation under *in vitro* aggregation conditions comprising an agonist; h) exhibit swirling upon visual observation of the composition; i) exhibit lack of aggregation upon visual observation of the composition; and/or j) exhibit lactadherin positivity in the range of 80-99.5%.

[0049] Provided herein in an aspect is a composition comprising frozen platelets, in an illustrative embodiment, frozen platelet derivatives, in a cryopreservation medium in a frozen state. In some embodiments, a composition comprising frozen platelets in a cryopreservation medium is a composition comprising cryopreserved platelets and/or cryopreserved platelet derivatives. Typically, a composition comprising frozen platelets upon thawing is in a liquid state without the addition of a liquid, such as water or a buffer. Without being bound by any theory, since the process of cryopreservation does not include the step of drying, the platelets in a cryopreservation medium become frozen because the cryopreservation medium is subjected to a freezing temperature, since there is no step of drying, the cryopreservation medium having platelets when thawed is in a liquid state. In some embodiments, a composition herein upon storing for at least 1 month, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months, 1 year, 2, 4, 5, 8, or 10 years at a temperature in the range of -10°C to -40°C is capable of exhibiting a platelet count of at least 1.0×10^{11} , 1.2×10^{11} , 1.4×10^{11} , 1.6×10^{11} , or 1.7×10^{11} /20-35 ml of the composition. For example, a composition herein upon storing for at least 1 month, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months, 1 year, 2, 4, 5, 8, or 10 years at a temperature in the range of -10°C to -40°C is capable of exhibiting a platelet count of at least 1.0×10^{11} in a cryo-vessel, cryo-vial, or a cryo-bag having a volume of 35, 30, 25, or 20 ml, or a volume of around 20-35 ml. Platelet counts can be performed with an automated hematology analyzer, or manually with a hemocytometer. For example, platelet counts of a sample, such as a thawed platelet sample can be determined by using a hematology analyzer, for example, a Beckman Coulter AcT Diff 2 Hematology Particle Analyzer or a Beckman Coulter DxH Hematology Analyzer (Beckman Coulter, beckmancoulter.com). Hematology analyzers are known to be based on the Coulter Principle, which is an electronic method for counting and sizing particles. Although the Coulter Principle can be used to calculate and size many types of particles, the specific application of this principle in hematology is to count and size

white blood cells (WBC), red blood cells (RBC), and platelets (PLT). As a non-limiting method, the platelet count in a composition herein can be derived from an internal continuous PLT/RBC histogram. Particles between 0 and 70 fL are counted and sized as they pass through the RBC aperture. The raw data is evaluated using a proprietary platelet algorithm, such as DxH (available on the Beckman Coulter DxH Hematology Analyzer) to identify the platelet population. The system also performs feature analysis to identify patterns of interference at the low and high ends of the PLT histogram. The algorithm uses both the PLT raw data and the fitted histograms for this process to determine PLT interference patterns, correcting or flagging results, depending on the severity of the interference. The platelet histogram's evaluation improves accuracy by excluding interferences from debris, micro bubbles, red cell fragments or exceptionally small red blood cells. As a non-limiting example of platelet count techniques, Example 5 and Table 6 demonstrate the data for platelet counts per bag by using either the Beckman Coulter AcT Diff 2 Hematology Particle Analyzer or the Beckman Coulter DxH Hematology Analyzer. In some embodiments, platelets can be counted by considering platelets having a diameter in the range of 0.5-5 μ m, 1-4 μ m, 1-3 μ m, 1-2.5 μ m, 1.5-3 μ m, or in illustrative embodiments, 0.5-2.5 μ m, or 2.5-5.0 μ m, typically when measured by flow cytometry or light scattering. In some embodiments, platelets can be counted by considering platelets having a diameter of at least 0.5 μ m, or at least 1 μ m, typically when measured by flow cytometry or light scattering. In some embodiment, particles in a composition that are less than 1 μ m in diameter are microparticles, typically when measured by flow cytometry or light scattering. In some embodiment, particles in a composition that are less than 0.5 μ m in diameter are microparticles, typically when measured by flow cytometry or light scattering. As a non-limiting example of platelet count techniques, flow cytometry can be used for sorting and counting platelets, or platelet derivatives in a composition herein. As is known in the art, different techniques are available for measuring particle sizes of platelets, platelet derivatives, and microparticles, for example platelet derived microparticles. One such technique, in a non-limiting manner, that can be used for measuring particle sizes is flow cytometry. Flow Cytometry is a technique for quantifying characteristics of cells such as cell number, size and complexity, fluorescence, phenotype, and viability. In general, the forward scatter in a flow cytometry is located in line with the laser intercept and is typically considered a measure of the relative cell size. The side scatter is typically located perpendicular to the laser beam intercept and is used to measure the relative complexity of the cell. Commercially available sizing beads can be used to obtain the forward scatter values to calibrate the instrument in order to measure the sizes of the particles. The gates used to measure the size distribution of particles in a composition as disclosed herein are drawn using forward scatter height (FSC-H) signals

generated by latex beads of a known diameter. For example, commercially available sizing beads of 0.5 μ m, and 2.5 μ m can be used to set size gate ranges in a flow cytometry equipment for counting particles that are below 0.5 μ m, such as microparticles or platelet derived microparticles, for counting platelets or platelet derivatives that fall in the range of 0.5 μ m and 2.5 μ m. In some embodiments, a composition comprising frozen platelets or frozen platelet derivatives, or cryopreserved platelets or cryopreserved platelet derivatives in a frozen state, in illustrative embodiments upon storing for at least 1 month, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months, 1 year, 2, 4, 5, 8, or 10 years at a temperature in the range of -10°C to -40°C, upon thawing exhibit a platelet count recovery of at least 65%, 70%, or 75%. For example, a platelet count recovery can be in a range of 60% to 95%, 65% to 95%, 70% to 95%, or 75% to 95%, 70% to 99%, 72% to 99%, or 75% to 99%. A skilled artisan can understand that platelet recovery can be performed by comparing the platelet counts in a composition before freezing and after thawing, to assess the counts after a storage time. In a non-limiting example, percentage platelet recovery can be assessed by using Beckman Coulter AcT Diff 2 Hematology Particle Analyzer or the Beckman Coulter DxH Hematology Analyzer (See Example 7).

[0050] In some embodiments, a composition comprising frozen platelets and/or platelet derivatives herein upon storing for at least 1 month, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months at a temperature in the range of -10°C to -40°C, upon thawing can have a CD61-positive microparticle content of less than 80%, 75%, 70%, 65%, 60%, in illustrative embodiments, less than 50%, 40%, 30%, or 25%. In some embodiments, CD61-positive microparticle content out of all the particles including platelets, platelet derivatives, and microparticles is in the range of 1-30%, 1-25%, 1-20%, 1-15%, 5-30%, 5-25%, or 5-20%. In some embodiments, microparticles, or CD 61-positive microparticles are particles that are less than 0.5 μ m in diameter, typically when measured by flow cytometry or light scattering. In some embodiments, microparticles, or CD 61-positive microparticles are particles that are less than 0.25 μ m in diameter, typically when measured by flow cytometry or light scattering. In some embodiments, microparticles, or CD 61-positive microparticles are particles that are less than 1 μ m in diameter, typically when measured by flow cytometry or light scattering. In some embodiments, at least 70%, 75%, 80% of the particles, typically including platelet, platelet derivatives, and microparticles in the composition are positive for lactadherin. For example, lactadherin positive particles in a composition can be in the range of 70% to 99%, 75% to 99%, or 80% to 99% of the particles in the composition. In some embodiments, lactadherin positive microparticles in a composition can be in the range of 70% to 99%, 75% to 99%, or 80% to 99% of the particles in the composition. Analysing using flow cytometry-based sorting and counting is a non-limiting

technique for calculating the percentage positivity of CD-61 positive microparticles, and lactadherin positive particles. Example 9 demonstrates a technique for calculating the percentage positivity of CD-61 positive microparticles, and lactadherin positive particles in a composition as disclosed herein, and the data is tabulated in Table 8. Various known techniques can be used to determine the sizes of various populations of particles in a composition as disclosed herein. For example, in some embodiments, flow cytometry forward scattering is used for determining the size of the particles. In other embodiments, light scattering, such as Thrombolux Dynamic light scattering is used for determining the size of the particles.

[0051] In some embodiments, a composition comprising frozen platelets, and/or platelet derivatives provided herein, in illustrative embodiments upon thawing, comprises platelets and/or platelet-derived particles, such as platelet derivatives having a particle size (e.g., diameter or max dimension) of at least 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 , or at least about 1.0 μm , , about 0.5 μm to about 5.0 μm . In some embodiments, the cryopreserved platelet composition has 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 platelets and/or platelet-derived particles in the range of about 0.3 μm to about 5.0 μm in diameter, about 0.5 μm to about 5.0 μm , (e.g., from about 0.4 μm to about 4.0 μm in diameter, from about 0.5 μm to about 2.5 μm in diameter, from about 0.6 μm to about 2.0 μm in diameter, about 1 μm to about 5.0 μm in diameter, about 1 μm to about 4.0 μm in diameter, about 1.5 μm to about 4.5 μm in diameter, or about 1 μm to about 3.0 μm in diameter).

[0052] In some embodiments, a composition comprising frozen platelets and/or platelet derivatives, or cryopreserved platelets and/or platelet derivatives herein upon storing for at least 1 month, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months at a temperature in the range of -10°C to -40°C , upon thawing can exhibit an ability to generate thrombin in an in vitro thrombin generation assay. A skilled artisan can use any known test(s) to assess thrombin generation. For example, thrombin generation can be assessed by a thrombin generation assay, and the assay can be performed by semi-automated methods for example using a calibrated automated thrombogram, or using fully automated systems. Thrombin generation assay is a type of coagulation test and is based on the potential of plasma to generate thrombin over time, following addition of activators like phospholipids, tissue factor, and calcium. The results of the assay can typically be calculated as a thrombogram, or thrombin generation curve using computer software after calculation of thrombogram parameters. A non-limiting example of assay conditions of a thrombin generation assay include incubating platelets in the presence of tissue factor, and phospholipids. In some embodiments, an in vitro assay comprises incubating platelets and/or platelet derivatives in the presence of tissue factor and

phospholipids but in the absence of fresh platelets. Thus, in some embodiments, frozen platelets and/or platelet derivatives as disclosed herein can be capable of generating thrombin, for example, when in the presence of a reagent containing tissue factor and phospholipids *in vitro*. For example, in some cases, frozen platelets and/or platelet derivatives, or cryopreserved platelets and/or platelet derivatives (e.g., at a concentration of at least about 10×10^3 particles/ μL , 20×10^3 particles/ μL , 30×10^3 particles/ μL , or 44×10^3 particles/ μL) as described herein can generate a thrombin peak height (TPH) of at least 25 nM (e.g., at least 30 nM, 35 nM, 40 nM, 45 nM, 50 nM, 52 nM, 54 nM, 55 nM, 56 nM, 58 nM, 60 nM, 65 nM, 70 nM, 75 nM, or 80 nM), 50 nM, 75 nM, 100 nM, 150 nM, 175 nM, 200 nM, 250 nM, 275 nM, 300 nM, in illustrative embodiments, when in the presence of a reagent containing tissue factor (e.g., at 0.25 pM, 0.5 pM, 1 pM, 2 pM, 5 pM or 10 pM) and optionally phospholipids. For example, in some cases, frozen platelets and/or platelet derivatives, or cryopreserved platelets and/or platelet derivatives (e.g., at a concentration of at least about 10×10^3 particles/ μL , 20×10^3 particles/ μL , 30×10^3 particles/ μL , or 44×10^3 particles/ μL) as described herein can generate a TPH of about 100 nM to about 350 nM (e.g., about 125 nM to about 350 nM, or about 150 to about 350 nM), in illustrative embodiments when in the presence of a reagent containing tissue factor and (e.g., at 0.25 pM, 0.5 pM, 1 pM, 2 pM, 5 pM or 10 pM) and optionally phospholipids. In some cases, frozen platelets and/or platelet derivatives, or cryopreserved platelets and/or platelet derivatives (e.g., at a concentration of about 4.8×10^3 particles/ μL) as described herein can generate a TPH of at least 25 nM (e.g., at least 30 nM, 35 nM, 40 nM, 45 nM, 50 nM, 52 nM, 54 nM, 55 nM, 56 nM, 58 nM, 60 nM, 65 nM, 70 nM, 75 nM, or 80 nM) when in the presence of PRP Reagent (cat# TS30.00 from Thrombinoscope), for example, using conditions comprising 20 μL of PRP Reagent and 80 μL of a composition comprising about 4.8×10^3 particles/ μL of platelets or platelet derivatives, or cryopreserved platelets and/or platelet derivatives. In some cases, frozen platelets and/or platelet derivatives (e.g., at a concentration of about 4.8×10^3 particles/ μL) as described herein can generate a TPH of about 25 nM to about 100 nM (e.g., about 25 nM to about 50 nM, about 25 to about 75 nM, about 50 to about 100 nM, about 75 to about 100 nM, about 35 nM to about 95 nM, about 45 to about 85 nM, about 55 to about 75 nM, or about 60 to about 70 nM) when in the presence of PRP Reagent (cat# TS30.00 from Thrombinoscope), for example, using conditions comprising 20 μL of PRP Reagent and 80 μL of a composition comprising about 4.8×10^3 particles/ μL of frozen platelets and/or platelet derivatives. In some embodiments, a composition herein can have an IU of at least 0.4, 0.5, $0.7/10^6$ particles. As a non-limiting demonstration of the thrombin generation ability, Example 9 and Table 8 demonstrate the thrombin generation ability of the platelets, or platelet derivatives upon storing. A skilled artisan can use other known

techniques to assess the thrombin generation potential of the platelets, or platelet derivatives as disclosed herein. Accordingly, in some embodiments, a composition herein comprises platelets, or platelet derivatives that retain hemostatic abilities even upon storing at a temperature in the range of -10°C to -30°C for at least 12 months.

[0053] In some embodiments, a composition comprising frozen platelets and/or platelet derivatives, or cryopreserved platelets and/or platelet derivatives herein when stored at a temperature in the range of -10°C to -40°C, in illustrative embodiments, upon storing for at least 1 month, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months, upon thawing can be capable of occluding a collagen-coated microchannel, a tissue factor-coated microchannel, or a collagen- and tissue factor-coated microchannel *in vitro*. For example, such occluding can be determined, for example, by using a total thrombus-formation analysis system (T-TAS®). In some embodiments, a microchannel is collagen-coated microchannel. In some embodiments, a microchannel is tissue factor-coated microchannel, for example, thromboplastin-coated microchannel. In some embodiments, a microchannel is collagen- and tissue factor-coated microchannel. In some cases, frozen or cryopreserved platelets or platelet derivatives as described herein upon thawing, when at a concentration of at least 50×10^3 particles/ μL , 60×10^3 particles/ μL , or 70×10^3 particles/ μL (e.g., at least 73×10^3 , 100×10^3 , 150×10^3 , 173×10^3 , 200×10^3 , 250×10^3 , or 255×10^3 particles/ μL) can result in a T-TAS occlusion time (e.g., time to reach kPa of 60) of less than 30, 25, 20, 15, or 14 minutes, or between 5 on the low end of the range, and 15, 20, or 25 on the high end, or between 10 on the low end of the range, and 15, 20, or 25 on the high end, or between 15 on the low end of the range and 20 or 25 on the high end, for example, in platelet-reduced citrated whole blood. In some cases, frozen or cryopreserved platelets or platelet derivatives as described herein upon thawing, when at a concentration of at least 50×10^3 particles/ μL , 60×10^3 particles/ μL , or 70×10^3 particles/ μL (e.g., at least 73×10^3 , 100×10^3 , 150×10^3 , 173×10^3 , 200×10^3 , 250×10^3 , or 255×10^3 particles/ μL) can result in an area under the curve (AUC) of at least 1300 (e.g., at least 1380, 1400, 1500, 1600, or 1700), for example, in platelet-reduced citrated whole blood. The occlusion time depicts the time it takes the sample to form a thrombus. The lower the time the faster the thrombus formation occurred. The analysis can capture occlusion time (OT) and area under the curve (AUC). OT represents the lag time it takes for the flow pressure to reach a target pressure, such as 60 kPa, 70 kPa, or 80 kPa from the baseline pressure. The AUC is the area under the flow pressure versus time curve which is related to overall thrombus formation. Microchannels or capillaries having different dimensions can be used in a T-TAS system for determining the occlusion times of cryopreserved platelets or cryopreserved platelet derivatives, or frozen platelets or frozen platelet derivatives under different

experimental conditions as provided by numerous commercial suppliers (See e.g., Zacros, Tokyo, JP). For example, a T-TAS PL chip, AR chip, or HD chip can be used for an occlusion (e.g., T-TAS) assay, as are commercially available. Typically, an AR chip for the purposes of T-TAS assay is coated with either collagen, or a tissue-factor, such as thromboplastin, or both. Typically an HD chip for the purposes of T-TAS assay is coated with either collagen, or a tissue-factor, such as thromboplastin, or both. For example, the PL chip can have capillary dimensions of 40 μm X 40 μm ; or an AR chip can have capillary dimensions of 0.3 mm X 80 μm ; or an HD chip can have capillary dimensions of 0.3 mm X 50 μm . Therefore, it is envisioned that a T-TAS assay can be performed to test the ability to occlude a collagen-coated microchannel, utilizing a microchannel or capillary with dimensions in the range of 0.02-0.5, 0.1-0.5, 0.2-0.4, 0.1-0.3, or 0.2-0.3 mm X 25-200, 25-100, 50-100, 40-90, 40-80, or 50-80 μm .

[0054] In some embodiments, a composition comprising frozen platelets and/or platelet derivatives herein, when stored at a temperature in the range of -10°C to -40°C , in illustrative embodiments, upon storing for at least 1 month, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months, upon thawing can exhibit a single peak in a membrane integrity assay typically based upon retention of fluorophore in platelets and/or platelet derivatives. A skilled artisan can contemplate different techniques to study the retention of a fluorophore in particles, such as platelets, and/or platelet derivatives. One such technique is Calcein acetoxymethyl (AM) membrane integrity assay. Calcein AM is a substance that is able to cross the cell membrane and reach the cytosol where Calcein AM gets hydrolyzed by the enzyme esterase to produce fluorescence. Platelets and/or platelet derivatives that are intact are able to retain this fluorescence while non-intact platelets do not. Therefore, based on the fluorescence that is emitted particles can be assessed for their membrane integrity. Accordingly, based on Calcein AM assay, in some embodiments, platelets and/or platelet derivatives in a composition provided herein do not have intact cell membranes, i.e. have compromised membranes. Example 8 is a non-limiting example demonstrating the compromised cell membranes of platelets and/or platelet derivatives in a composition (See FIG. 9). Not to be limited by theory, the observation from FIG. 9 possibly suggests that there are two kinds of population in the single temperature cryopreserved-product (stored at -80°C), a first population that is able to retain less fluorescence of Calcein AM (See first peak from left of “#” in FIG. 9) possibly because of compromised membrane as compared to a second population (See second peak from left of “#” in FIG. 9) showing higher retention of the fluorescence possibly because they have intact membranes. It was further observed that the single peak of the transition temperature cryopreserved-product (stored at -20°C) of all the three batches (See “*” in FIG. 9) corresponded to the first population of the single temperature cryopreserved-product that shows less

retention of Calcein AM. Therefore, it can be inferred that the single population of the transition temperature cryopreserved-product (stored at -20°C) observed in the Calcein AM assay comprise platelets and/or platelet derivatives with compromised membranes. However, surprisingly, in spite of the composition having frozen platelets and/or platelet derivatives, the composition, in illustrative embodiments upon storing at a temperature in a range of -10°C to -40°C, or -20°C +/-2°C for at least 2, 4, 6, or 12 months is able to satisfy the criteria of parameters including platelet count (at least 1×10^{11} platelets per 20-35 ml), typically when counted using hematology analyzer as disclosed herein, pH (more than 6.5), visual observation, such as lack of aggregation and presence of swirling.

[0055] Another non-limiting technique for assessing membrane integrity is by detecting lactate dehydrogenase enzyme (LDH) that is released by the cells having a compromised membrane. LDH is a stable cytoplasmic enzyme that is found in all cells. LDH is rapidly released into the cell culture supernatant when the cell membrane is damaged. According to one of the protocols, LDH activity can be easily quantified by using the nicotinamide adenine dinucleotide (NAD) + hydrogen (NADH) produced during the conversion of lactate to pyruvate to reduce a second compound in a coupled reaction into a product with properties that are easily quantitated. This protocol measures the reduction of a yellow tetrazolium salt, Iodonitrotetrazolium (INT), by NADH into a red, water-soluble formazan-class dye by absorbance at 492 nm. The amount of formazan is directly proportional to the amount of LDH in the supernatant, which is, in turn, directly proportional to the number of cells that have compromised membrane. Accordingly, in some embodiments, frozen platelets or platelet derivatives, or cryopreserved platelets or platelet derivatives in a composition herein have compromised membrane as per LDH assay for assessing membrane integrity.

[0056] In some embodiments, a composition comprising frozen platelets and/or platelet derivatives, or cryopreserved platelets and/or platelet derivatives herein when stored at a temperature in the range of -10°C to -40°C, in illustrative embodiments, upon storing for at least 1 month, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months, upon thawing is capable of showing aggregation under aggregation conditions comprising an agonist, not limited to arachidonic acid, collagen, and TRAP-6. In some embodiments, aggregation conditions comprise an agonist but no fresh or apheresis platelets. In some embodiments, aggregation conditions comprise an agonist but no fresh or apheresis platelets, and no divalent cation. Non-limiting examples of aggregation agonists include, collagen, epinephrine, ristocetin, arachidonic acid, adenosine diphosphate, and thrombin receptor associated protein (TRAP). In some embodiments, frozen platelets and/or platelet derivatives, or cryopreserved platelets and/or platelet derivatives herein upon thawing exhibit

aggregation in the presence of arachidonic acid, in the range of 20-60%, 20-50%, or 30-50%, or aggregation of at least 20%, 30%, 40%, or 50%. In some embodiments, frozen platelets and/or platelet derivatives, or cryopreserved platelets and/or platelet derivatives herein upon thawing exhibit aggregation in the presence of collagen, in the range of 2-50%, 2-40%, or 2-30%. In some embodiments, frozen platelets and/or platelet derivatives, or cryopreserved platelets and/or platelet derivatives herein upon thawing exhibit aggregation in the presence of TRAP-6, in the range of 2-50%, 2-40%, or 2-30%. A non-limiting method to determine aggregation is by using PAP8 Aggregometer (Bio Data Corporation, biodatacorp.com). Example 7 and FIG. 8 demonstrate the aggregation of frozen platelets and/or platelet derivatives, or cryopreserved platelets and/or platelet derivatives herein upon thawing in the presence of collagen, TRAP-6, and arachidonic acid.

Platelet units and Pooling of the units

[0057] Processes provided herein can include platelet units as a starting material or source of platelets. Typically, the platelet units can be apheresis platelet units (APU), however, other sources of platelets can also be included in the process as disclosed herein. The alternative sources of platelets can include whole blood-derived platelets. Platelets can be obtained from whole blood either using the known platelet-rich-plasma (PRP) method or the buffy-coat method. The platelets obtained from the buffy-coat method are known as buffy coat-derived platelet concentrates (BC-PC). A skilled artisan can use platelets from any of the sources available based on the ease of availability and from a commercial standpoint. The platelet units, such as APUs can be accessed from any recognized blood banks or centers that process blood units. The process can be performed at a tertiary care facility that has access to the blood banks. The process can be performed at any facility or a processing center that has access to the blood banks, or the platelet units are supplied to the facility or the processing center. Typically, platelets are available in two forms: pools of whole blood-derived platelet concentrates, and platelets collected via apheresis. Platelet concentrates are prepared from donated whole blood, separated within eight hours of collection, and contain a minimum of 5.5×10^{10} platelets in 1 unit, and individual platelet concentrate units contain about 40 to 50 ml of plasma. Apheresis platelets are collected from a single donor and contain a minimum of 3×10^{11} platelets in 1 unit suspended in 200 to 300 mL of plasma.

[0058] Typically, the platelet units provided herein are from more than 1 donor. For example, the platelet units can be provided from 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more donors. The number of donors can depend on the number of platelet units required for the preparation of cryopreserved platelets. For preparing a batch of cryopreserved platelets comprising more than 10 cryo-vessels, platelet units can be

obtained from more than 8, 9, or 10 donors. For example, for preparing a batch of cryopreserved platelets comprising 12 cryo-vessels, 12 platelet units can be provided. In such cases, each unit can be from a different donor, such that the platelet units are from 12 donors. In other cases, 2 units can be from 1 donor, such that the platelet units are from 6 donors, or 3 units can be from 1 donor such that the platelet units are from 4 donors. A skilled artisan would understand that the number of donors can depend upon the number of platelet units required, and the availability of such units in a blood bank. In some cases, at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 20 or more units can be processed in a single batch. Processes herein can include performing the process as disclosed herein more than once to form more than one batch of the cryopreserved platelets. For example, the process as disclosed herein can be performed multiple numbers of times to form multiple number of batches of cryopreserved platelets. For example, 5 batches of cryopreserved platelets are formed by performing the process 5 times. Similarly, the process can be performed any number of times as per the requirement of the number of batches of the cryopreserved platelets. For example, 2-500, 2-450, 2-400, 2-300, 2-250, 2-200, 2-150, or 2-100 batches can be formed by performing the process as disclosed herein as many numbers of times. In such embodiments, each batch can have 3-50, 3-40, 3-30, 3-25, 3-20, 3-25, 3-12, 4-12, or 5-12 number of cryo-vessels of cryopreserved platelets.

[0059] Process herein includes pooling of platelet units in a vessel, such that a minimum number of units are processed in one vessel until the step of combining the contents of all such vessels for the addition of a cryoprotectant. For example, at least 2 units or 3 units are pooled in a vessel to create a plurality of vessels having pooled platelet units. The pooling of platelet units can be based on the number of platelet units provided, and/or the number of platelet units that can be pooled in one vessel. For example, in case 5 platelet units are provided, 2 units can be pooled in a vessel, and 1 remaining unit can be processed in a separate vessel such that two vessels can have a pooled set of 2 units each and one vessel can have the remaining 1 unit. In case there are 6 platelet units that are provided, then 2 units can be pooled in a vessel such that three vessels can have a pooled set of 2 units each. Pooling of platelet units can be performed in a manner where 3 units or more can be pooled in one vessel. For example, in case 5 platelet units are provided, 3 units can be pooled in a first vessel and 2 units can be pooled in a second vessel such. Alternatively, 3 units can be pooled in a first vessel, and 1 unit each can be processed in separate vessels. The number of units that can be pooled in a vessel can also depend on the type of vessel and the volume that can be processed in the vessel. Typically, a vessel can be apheresis platelet unit (APU) bags. For example, if an APU bag can hold a volume of around 800 to 900 ml for processing, then 2 to 3 units can be

pooled into one vessel, such as an APU bag. For example, APU can hold a volume in the range of 800 to 1600 mL, 800 to 1000 mL, or 1000 mL to 1500 mL. For example, considering APU bags as vessels as per the process disclosed herein, the pooling can be done by using an SCD to weld a plasma transfer set onto an APU bag and then a second APU bag is welded onto the other end of the plasma transfer set. The plasma transfer set is added to extend the working length of the tubing. The two APUs can then be pooled together into a single APU bag. This can be done a multiple number of times to create a plurality of APU bags having pools (2 platelet units) of APU from the initial platelet units. The sterile connecting device used can be a Terumo, TSCD II Sterile Tubing Welder, model number 3me-SC203a (or equivalent). The plasma transfer sets used can be Charter Medical, 24" Tubing, Roller Clamp and Two Piercing Pins, product number 03-220-00 (or equivalent). If there is an odd number of initial platelet units, such as APUs then a plasma transfer set can be welded onto the odd APU and a 600mL transfer bag (Terumo, TeruFlex Transfer Bag, catalog number: 1BB*T060CB71, or equivalent) can be welded onto the other end of the plasma transfer set. The APC of the odd APU remains in the APU bag.

[0060] After pooling the platelet units or separating 1 platelet unit in a separate vessel, the weight of apheresis platelet concentrate (APC) can be determined for each vessel, such as each APU bag. For example, a non-limiting equation for calculating the APC weight of each vessel is:

$$\text{Pooled or single APC Weight} = \text{Pooled or single APU Weight} - \text{Empty Vessel/Bag Weight}$$

[0061] The pooled APU weights can be determined with a scale (Ohaus Adventurer Precision Balance, product number AX8201/E, or equivalent). The empty bag weight is a known value that corresponds to the type of bag that was used for the apheresis platelet collection.

[0062] Process herein includes a step of centrifugation of vessels comprising pooled platelet units, or vessel comprising 1 platelet unit. The centrifugation step is to separate platelets from plasma, and as such can be achieved by centrifuging the vessel at 1000g to 2000g, 1000g to 1500g, or 1100g to 1400g for a time period in the range of 5-30 minutes, 5-25 minutes, or 5-20 minutes. Typically, the vessels can be APU bags and the APU bags can be kept in centrifuge cups that can be centrifuged at 1250g for 10 minutes with maximum acceleration, and with 10 minutes of deceleration.

Plasma expression and resuspension

[0063] Processes herein can include a step of resuspending the pellet that is obtained after the centrifugation step to achieve a target weight of the resuspension within a specific range, for example, 12g

to 32g, 13 g to 30g, 14g to 29g, or 15.9g to 27.9g times the number of platelet units pooled or provided in the vessel that was processed or centrifuged. For example, when 2 units are pooled in a vessel, then the target weight of the resuspension can be two times of 12g to 32g, 13g to 30g, 14g to 29g, or 15.9g to 27.9g, such that the target weight of the resuspension becomes 24g to 64g, 26g to 60g, 28g to 58g, or 31.8g to 55.7g. The target weight of the resuspension can be achieved by removing a part of the supernatant comprising plasma, this step is also known as plasma expression. Plasma expression or removing supernatant can be performed to achieve a target weight of the remainder supernatant and pellet. Alternatively, removing a part of the supernatant can be performed until a target weight of the supernatant that is removed is achieved. The target weight of the supernatant that is to be removed can be determined based on the weight of the remainder supernatant and pellet that is required. For example, when 2 units are pooled in a vessel, then the target weight of the resuspension can be two times of 12g to 32g, 13g to 30g, 14g to 29g, or 15.9g to 27.9g, such that the target weight of the resuspension becomes 24g to 64g, 26g to 60g, 28g to 58g, or 31.8g to 55.7g. Typically, in case when 2 units are provided in a vessel, the target weight of the plasma that needs to be removed can be determined by the weight of the resuspension that is platelet pellet and the remainder plasma in the range of 31.8g to 55.7g, and in one non-limiting example the target weight of the plasma that needs to be removed is determined by the weight of the resuspension that is about 45.6g.

[0064] Typically, the plasma removal target weight for expression can be determined using the below Equation by subtracting 46.5 g from the pooled APC weight. This is done to leave behind approximately 46.5 g of platelet pellet and plasma after the pooled APU is expressed.

[0065] Determination of expression endpoint

$$\text{Plasma Removal Target} = \text{Pooled APC Weight (2 platelet units)} - 46.5 \text{ g}$$

[0066] Each vessel after pooling the platelet units can be taken out of the centrifuge and expressed one-by-one (removing a part of the supernatant). The vessel can be carefully removed from the centrifuge cup, as not to disturb the platelet pellet, and then placed in the plasma expressor (Fenwal Inc., manual plasma extractor, product code 4R4414, or equivalent). The empty APU bag is placed on a scale and tared to weigh the expressed plasma. The pooled APU is then expressed. Once the plasma removal target is reached (± 1.0 g) the expression is stopped. The post-expression pellet weights are determined to ensure that the weight of the platelet pellet and remainder supernatant is within range for further processing (31.8 g – 55.7 g). If the post-expression weight is outside of the range, the supernatant can be added or removed accordingly, until the post-expression weight is within range. Once the post-expression weight is within range, the pellet is

resuspended by gently rocking and massaging the APU bag until the pellet is no longer visible. After the pellet is no longer visible there is a 5-minute ambient temperature resting of the resuspended platelet pellet. The resuspended pellet is then visually inspected for aggregates. If aggregates are observed at this point in the process, and they do not disappear after further resting and agitation, manufacturing management is informed, processing continues, and a 30-minute ambient temperature rest with gentle agitation is added to the process after the addition of 27% DMSO. To accommodate the APU bag having 1 platelet unit, the plasma removal target can be determined by the following equation.

[0067] *Plasma Removal Target (for single Platelet unit) = Odd APC Weight (1 platelet unit) – 23.3g*

[0068] The post-expression pellet weight range (weight of the remainder supernatant and the pellet) is changed to 15.9 g – 27.9 g, to account for the odd APU being a single APU and not a pooled APU.

[0069] Process herein, in some aspects can include resuspending the pellet with a buffer composition, such that the target weight range of 15.9 g – 27.9 g times the number of platelet units is that of the resuspension with the buffer composition. Typically, in such resuspension with the buffer composition, 90%-99.9% of supernatant comprising plasma can be removed and then the pellet can be resuspended with a buffer composition. A buffer composition can comprise a buffering agent, a base, one or more saccharide, optionally a salt, and optionally, an organic solvent. The buffering agent can be any buffer that is non-toxic to the platelets and provides adequate buffering capacity at the temperatures at which the resuspension will be exposed during the process provided herein. Thus, the buffer composition can 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 (tricarballic); benzenepentacarboxylic; maleic; 2,2-dimethylsuccinic; EDTA; 3,3-dimethylglutaric; bis(2-hydroxyethyl)imino-tris(hydroxymethyl)-methane (BIS-TRIS); benzenhexacarboxylic (mellitic); N-(2-acetamido)imino-diacetic acid (ADA); butane-1,2,3,4-tetracarboxylic; pyrophosphoric; 1,1-cyclopentanediacytic (3,3-tetramethylene-glutaric acid); piperazine-1,4-bis-(2-ethanesulfonic acid) (PIPES); N-(2-acetamido)-2-aminoethanesulfonic acid (ACES); 1,1-cyclohexanediacytic; 3,6-endomethylen-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-tricarboxylic (beta-methyltricarballic); 2-(N-

morpholino)propane-sulfonic acid (MOPS); phosphoric; and N-tris(hydroxymethyl)methyl-2-amminoethane sulfonic acid (TES).

[0070] In some embodiments, one or more buffering agents can be present in the resuspension in any suitable amount. In some embodiments, the buffering agent can be present in an amount of 1 mM to 1 M. In some embodiments, one or more buffering agents can be present in about 0.2 to about 20 mg/ml, or about 0.2 to about 2 mg/ml, or about 2 mg/ml to about 20 mg/ml in the resuspension. In some embodiments the buffer composition can comprise one or more salts in about 0.08 to about 8 mg/ml, such as about 0.08 to about 0.8 mg/ml, or about 0.8 mg/ml to about 8 mg/ml in the resuspension.

[0071] The process of preparing the cryopreserved platelets provided herein can also comprise adding to the resuspension one or more salts, such as phosphate salts, sodium salts (e.g., NaCl), potassium salts (e.g., KCl), 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 cryopreserving platelets, or any combination of two or more of these.

[0072] In some embodiments, the salts are present in the resuspension at a concentration of about 1 mM to about 1000 mM, such as about 0.01 M to about 0.2 M. In some embodiments, one or more salts are present in about 0.4 to about 40 mg/ml, or about 0.4 to about 4 mg/ml, or about 4 mg/ml to about 40 mg/ml in the resuspension. In some embodiments, one or more salts are present in about 0.03 to about 3 mg/ml, or about 0.03 to about 0.3 mg/ml, or about 0.3 mg/ml to about 3 mg/ml in the resuspension.

[0073] In some embodiments, these salts are present in the resuspension in an amount that is about the same as is found in whole blood.

[0074] In some embodiments, the process for preparing a cryopreserved platelet composition includes adding to the suspension medium an organic solvent, such as an alcohol, such as ethanol, to the suspension medium. The organic solvent can include one or more alcohols, e.g., short-chain alcohols, such as ethanol. Short-chain alcohols are alcohols having 1 to 6 carbon atoms, in particular 2, 3 or 4 carbon atoms, such as methanol, ethanol, and propanol including 1-propanol and 2-propanol, preferably ethanol. The organic solvent may also be a mixture of different organic solvents. In such a resuspension medium, the solvent can range from 0.1 % to 5.0 % (v/v). In some embodiments, one or more organic solvents are present in about 0.08% (v/v) to about 8% (v/v), or about 0.08% (v/v) to about 0.8% (v/v), or about 0.8% (v/v) to about 8% (v/v) in the resuspension.

[0075] In some embodiments, process herein include resuspending the pellet after the removal of plasma or plasma expression, in a buffer composition. Such a buffer composition can include one or more saccharides. The saccharides can include monosaccharides, disaccharides, or polysaccharides including

sucrose, maltose, trehalose, glucose, mannose, dextrose, xylose, and combinations thereof. In some embodiments, the saccharide for use in the process of preparing cryopreserved platelets provided herein is trehalose. In some embodiments, the polysaccharide is polysucrose, or a combination of any of the above saccharides, in illustrative embodiments trehalose, and polysucrose. Thus, in one embodiment, the first mixture comprises platelets, a cryoprotectant, such as a cryoprotectant comprising trehalose, a polysucrose, or a combination thereof, and a solvent, such as ethanol.

[0076] In some embodiments, a suitable saccharide can include one or more sugar alcohols. Non-limiting examples of sugar alcohols can include mannitol, sorbitol, xylitol, maltitol, maltitol syrup, lactitol, erythritol, and combinations thereof.

[0077] In various embodiments, one or more saccharides can be present in the resuspension in any suitable amount. Such saccharides can be the cryoprotectant or can be one of the cryoprotectants, for examples when one or more saccharides are present in the resuspension along with DMSO. In some embodiments, the saccharide can be present at about 1 mM to about 1 M. In embodiments, the saccharide is present at about 10 mM to about 500 mM. In some embodiments, the saccharide is present at about 20 mM to about 200 mM. In embodiments, the saccharide is present at about 40 mM to about 100 mM. In some embodiments, one or more saccharides are present in about 0.04 mg/ml to about 4 mg/ml, about 0.04 mg/ml to about 0.4 mg/ml, or about 0.4 mg/ml to about 4 mg/ml in the resuspension. In some embodiments, one or more saccharides are present in about 3 mg/ml to about 300 mg/ml, about 3 mg/ml to about 30 mg/ml, or about 30 mg/ml to about 300 mg/ml in the resuspension.

[0078] 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 resuspension, each saccharide can be present in an amount according to the ranges and particular concentrations disclosed herein.

Pooling of the resuspension

[0079] Processes herein, after the step of resuspending the pellet to form a resuspension, can include pooling of the resuspension from each vessels, such as APU bags. Pooling can be done by pooling the resuspension from each vessel, such as APU bags one by one into one pooled resuspension vessel to form a pooled resuspension in a pooled resuspension vessel. Typically, pooling of resuspension from each vessels can be achieved using a pooling tree system. Such a pooled resuspension vessel can be an APU bag that can hold the volumes of the resuspension from all of the vessels. Typically, a pooled resuspension vessel is

a single vessel that can contain the pooled resuspension to which a cryoprotectant is added in a consecutive step. However, in order to accommodate a high number such as 15, 20, 30, 40, 50 or more number of platelet units in a single batch the pooled resuspension from all of the vessels can be pooled and split into two, three, or more number of pooled resuspension vessel.

[0080] A pooling tree system can be designed based on the requirements and platelet units/vessels that are to be processed. An illustrative non-limiting example of a pooling tree system is shown in FIG. 1C.

Referring to FIG. 1C, one non-limiting example of a pooling tree system or a tubing tree system comprises: a first four port cross style manifold (5) connecting a first 14 inch tubing (1), a second 14 inch tubing (1), a third 14 inch tubing (1), and fourth 4 inch tubing (2); a second four port cross style manifold (5) connecting the fourth 4 inch tubing (2), with a fifth 20 inch tubing (3), a sixth 14 inch tubing (1), and a seventh 4 inch tubing (2); a third four port cross style manifold (5) connecting the seventh 4 inch tubing (2) with a eighth 14 inch tubing (1), a ninth 14 inch tubing (1), and a tenth 14 inch tubing (1), and one on/off ratchet clamp (4) on each of the tubing. A non-limiting example of the tubing is TYGON ND 100-65 tubing 3/32" ID X 5/32" OD of different sizes as per the requirement. A further non-limiting description is provided in the table below.

[0081] Table - Tubing Tree

Item#	Description	Quantity
1*	3/32" ID x 5/32" OD Tygon ND 100-65 tubing	14" length (x7)
2	3/32" ID x 5/32" OD Tygon ND 100-65 tubing	4" length (x2)
3*	3/32" ID x 5/32" OD Tygon ND 100-65 tubing	20" length (x1)
4	Pinch Clamp	x10
5**	3/32" ID Hose Barb Cross Connector	x3

* RF Seal on Tygon Tubing Ends.

** Zip ties on each barb and tubing connection (x12).

[0082] For example, in a process in which 12 platelet units are provided, 6 vessels, for example, 6 APU bags can be processed, and after the step of removing of plasma (plasma expression), resuspension from each bag can be pooled in a single bag. Once the 6 pellets are resuspended, the 6 APU bags can be welded onto a sterile tubing tree (Optimum Processing, Inc., part number 02817, or equivalent) to create a "pooling

tree system” and the resuspension from each bag can then be pooled into a single APU bag. It is understood that a skilled artisan can use or custom design any such pooling tree system for the pooling of the resuspension as provided herein.

Addition of cryoprotectant

[0083] Processes herein can include adding a cryoprotectant to a pooled resuspension as disclosed herein. Without being bound by any theory or mechanism, the cryoprotectant stabilizes proteins and other biological substances in the interior of the platelets. The identity of the cryoprotectant is not limited as long as it can enter the platelets and provide a cryoprotectant property. In some embodiments, the cryoprotectant comprises dimethyl sulfoxide (DMSO). In other embodiments, the cryoprotectant is any other cryoprotectant apart from DMSO. Other non-limiting examples of suitable cryoprotectants can include saccharides, such as monosaccharides and disaccharides, including sucrose, maltose, trehalose, glucose, mannose, dextrose, xylose, and combinations thereof. In some embodiments, the saccharide for use in the method of preparing a cryopreserved platelet composition provided herein is trehalose.

[0084] In some embodiments, a cryoprotectant can include one or more sugar alcohols. Non-limiting examples of sugar alcohols can include mannitol, sorbitol, xylitol, maltitol, maltitol syrup, lactitol, erythritol, and combinations thereof.

[0085] In various embodiments, one or more saccharides can be present in the pooled resuspension or in the cryopreserved platelets in any suitable amount. For example, the saccharide can be present at about 1 mM to about 1 M, about 10 mM to about 500 mM, about 20 mM to about 200 mM, or about 40 mM to about 100 mM. As further non-limiting examples, one or more saccharides can be present in about 0.04 mg/ml to about 4 mg/ml, about 0.04 mg/ml to about 0.4 mg/ml, or about 0.4 mg/ml to about 4 mg/ml in the pooled resuspension or the cryopreserved platelets. In some embodiments, one or more saccharides are present in about 3 mg/ml to about 300 mg/ml, about 3 mg/ml to about 30 mg/ml, or about 30 mg/ml to about 300 mg/ml in the pooled resuspension or the cryopreserved platelets.

[0086] In some embodiments, the cryoprotectant can include one or more polyols. For example, suitable cryoprotectants can include glycerol (glycerin), ethylene glycol, polyethylene glycol, sorbitol, propylene glycol, pentaerythritol, a saccharide, hydroxypropyl- β -cyclodextrin, a glycerol oligomer, or combinations thereof. In some embodiments, the cryoprotectant is no more than about 10% (v/v) (e.g., no more than about 9% (v/v), 8% (v/v), 7% (v/v), 6% (v/v), 5% (v/v), 4% (v/v), 3% (v/v), 2% (v/v), 1% (v/v), 0.5% (v/v), or 0.1% (v/v)). In some embodiments, the cryoprotectant is in an amount of at least about 1% (w/v) (e.g., at least about 2% (v/v), 3% (v/v), 4% (v/v), 5% (v/v), 6% (v/v), 7% (v/v), 8% (v/v), 9% (v/v), or 10%

(v/v)). For example, the cryoprotectant is in an amount of about 0.1% (v/v) to about 10% (v/v), about 0.5% (v/v) to about 7% (v/v), about 1% (v/v) to about 5% (v/v), or about 0.1% (v/v) to about 1% (v/v). Glycerol can also be used as a cryoprotectant.

[0087] Typically, process herein includes using DMSO as a cryoprotectant. For example, adding DMSO can be performed until a target weight of DMSO is added to a pooled resuspension as disclosed herein. DMSO can be added to the pooled resuspension until the concentration of DMSO in a pooled resuspension is in the range of 0.001-10% in the pooled resuspension. For example, DMSO concentration can be in the range of 0.005-10%, 0.1-10%, 1-10%, 2-10%, 3-10%, 4-10%, 5-10%, 5.5-10%, 6-10%, 6-9%, 6-8%, 0.001-9%, 0.001-8%, or 0.001-7%. In some embodiments of the process herein, DMSO concentration in the pooled resuspension can be at least 0.001%, 0.005%, 0.05%, 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10%. During performing the process herein, when the suspension from each vessels are pooled to form a pooled resuspension in a pooled resuspension vessel, the amount of a cryoprotectant or DMSO to be added can be calculated based on the weight of the pooled resuspension obtained after pooling the resuspension from each vessel, for example, APU bags. Typically, weight of the pooled resuspension can be determined by adding up weights of all the resuspension from each vessel or APU bag. Pooled APC weight after removal of plasma from all the vessels that initially had 2 platelet units can be added along, if applicable, with the APC weight from the vessel that initially had 1 platelet unit after removal of plasma to arrive at a summation of APC weight, or a total APC weight. The total APC weight at this step can be the post-expression weight and can be arrived at by summing up the resuspension weight of all the vessels/APU bags after removal of plasma.

[0088] *Total Post Expression (after removal of plasma) Weight =
Sumation of resuspension Weights = Total APC Weight*

[0089] An amount of DMSO that needs to be added at this step can vary based on the total APC weight, the stock concentration of DMSO, and the target DMSO concentration that needs to be achieved in the cryopreserved platelets. An illustrative, non-limiting equation for calculating the weight of DMSO that needs to be added to the pooled resuspension is shown below. A stock concentration of 27% DMSO has been considered, and a target DMSO concentration of 6.085% in the cryopreserved platelets in a cryo-vessel has been considered:

[0090] Target weight of 27% DMSO = Total APC Weight * 0.2946

[0091] After the determination of the target weight of DMSO that needs to be added, DMSO can be added to the pooled resuspension by any means that facilitate the addition of DMSO with minimal wastage and leading to uniform mixing of DMSO. The addition of DMSO can be performed directly or indirectly to the pooled resuspension vessel. Typically, a bag or a vessel containing sterile, injectable grade 27% DMSO and 0.66% sodium chloride in water (Bio Life Solutions, BloodStor® 27 NaCl Biopreservation Media, part number 327207, or equivalent) can be welded onto the pooling tree system that was initially used to pool the resuspension from each vessel/APU bags. The 27% DMSO bag can be placed on a scale to weigh how much 27% DMSO is leaving the 27% DMSO bag and entering the pooling tree system. The 27% DMSO bag can be placed higher than the pooling tree system to allow the 27% DMSO to flow gravimetrically. The addition of 27% DMSO Target Weight to the pooling tree system can be performed until $\pm 3.0\text{g}$, $\pm 2.0\text{g}$, or $\pm 1.0\text{g}$ is added to the pooling tree system. The tubing to the 27% DMSO bag can be clamped to prevent additional 27% DMSO from entering the system. The step comprising addition of a cryoprotectant such as DMSO can be performed using a pooling tree system that was initially used to pool the resuspension from each vessel/bag. In such steps, DMSO can be used to rinse the pooling tree system, and the vessels/APU bags that had the resuspension to effectively flush out any platelets that can be stuck to the pooling tree system. Typically, the 27% DMSO that entered the pooling tree system is used to rinse the system to recoup any residual platelet material that remains in the APU bags and in the tubing of the pooling tree system. The 27% DMSO can then be added to the pooled resuspension vessel, for example, an APU bag that contains the pooled resuspension. Tube strippers can be used to strip any 27% DMSO solution that may remain in the pooling tree system, to ensure that all 27% DMSO is added to the platelets.

[0092] It can be understood that a skilled artisan can manipulate the DMSO constant of 0.2946 disclosed in the above equation based on the stock solution of DMSO, and target concentration of DMSO that needs to be achieved in the cryopreserved platelets. For example, the DMSO constant will change if a stock of 10% DMSO is considered rather than 27% DMSO.

Distribution and Freezing

[0093] Processes herein can include distributing a pooled resuspension having a cryoprotectant, such as DMSO to a number of cryo-vessels for a further freezing step. A cryo-vessel can be any appropriate sealable vessel (e.g., a container closure system) in which platelets can be frozen. A cryo-vessel can be an appropriate vial (cryo-vial), ampule, or a bag (e.g., cryo-bag) For example, a cryo-vessel can be a cryo-bag

such as a fluorinated ethylene propylene (FEP) bag or a polyvinyl chloride (PVC) bag. A cryo-vessel can be a borosilicate serum vial. A non-limiting example of a cryo-vessel or a cryobag is 250 mL ethyl vinyl acetate (EVA) thermoplastic container (CryoStore CS250 series, Origen, Austin, TX). The CS250 has a recommended freeze volume of 30-70 mL. The EVA container is also suitable for delivery of the intravenous dosage form being like any other blood container being the appropriate flexibility and transparency. The EVA container is placed in a polyethylene overwrap bag (Helmer, Noblesville, IN) as a secondary container, then placed into a corrugated cardboard box (Mission City Container, San Antonio, TX), internal dimensions 7" x 5 1/4" x 1 5/8", 200 pound test bursting strength, to protect the product from both light and damage.

[0094] In some embodiments of process herein, distributing a pooled resuspension having a cryoprotectant, such as DMSO can be performed by determining a fill-weight or a fill-volume that needs to be distributed to a number of cryo-vessels. For example, a fill-weight can be determined by dividing the weight of pooled resuspension by the number of platelet units provided or processed, such that the pooled resuspension can be distributed to a number of cryo-vessels that is equal to the number of platelet units provided or processed. In some embodiments, a fill-weight can be determined by dividing the weight of pooled resuspension by 1/3, 1/2, 2/3, or 2 times the number of platelet units provided or processed. As a non-limiting example, when 12 platelet units are processed, then the weight of pooled resuspension can be divided by 12 to form a batch comprising 12 cryo-vessels comprising cryopreserved platelets. Alternatively, when 12 platelet units are processed, by dividing the total weight of pooled resuspension having cryoprotectant with appropriate numbers, the pooled resuspension having cryoprotectant can be distributed into 3, 6, 8, 15, 18, or 24 cryo-vessels.

[0095] Distributing herein can be performed using any means that allow maintaining a sterile environment, and a clear passage of the pooled resuspension having cryoprotectant. In illustrative embodiments, distributing can be performed by means of a dosing tree system. A dosing tree system can be same or different in design to the tubing tree system. Typically, in cases where a number of cryo-vessels equal to the number of platelets units are desired, and 12 platelet units are provided for processing then a pooled resuspension vessel, such as an APU bag containing the pooled resuspension (final product) can be welded onto a new tubing tree to fill 12 cryo-bags. This creates a "filling tree system." The weight of the pooled resuspension having the cryoprotectant (final product weight) can then be determined by weighing the pooled resuspension vessel/APU bag and subtracting the empty bag weight. The pooled resuspension (final product) weight can then be divided by 12 to determine the maximum fill weight of the cryo-bags.

The minimum fill weight can be determined by subtracting 2.0 g from the maximum fill weight. This determines the fill weight range of the cryo-bags. Further, 12 cryo-bags (250 mL EVA CryoStore freezing bag, Origen Reference CS250, or equivalent) can then be welded onto a dosing tree system. The filling procedure for a cryo-bag takes place by placing the cryo-bag on the scale, priming the lines of the cryo-bag until just before the pooled resuspension having cryoprotectant enters the cryo-bag, and then taring the cryo-bag. The cryo-bag can then be filled with the pooled resuspension having cryoprotectant until it is within range. The fill weight of each cryo-bag can then be recorded and their volume can be determined by dividing the weight by 1.03 g/mL.

[0096] Process herein after the step of distributing the pooled resuspension having a cryoprotectant includes freezing the pooled resuspension having a cryoprotectant. Freezing can be performed by subjecting the pooled resuspension having a cryoprotectant to low temperatures to about or less than about -1 °C (e.g., about or less than about -5 °C, about -10 °C, about -20 °C, about -30 °C, about -40 °C, about -50 °C, about -60 °C, about -70 °C, about -80 °C, or about of less than about -90 °C). For example, the pooled resuspension having a cryoprotectant in cryo-vessels can be subjected to a temperature of about -70°C to about -90 °, -50°C to about -70 °, -30°C to about -50 °, -10°C to about -30 °, or about -10°C to about -20 °C.). Typically, the cryo-vessels can be subjected to a temperature of about -80°C. Typically, each cryo-bag can be placed into a thawing bag and freezer carton for storage in a -80°C freezer. The units can then be placed in the freezer and the start time of freezing is recorded. The time elapsed from the end of 27% DMSO addition to the start time of freezing, in illustrative embodiments can be equal to or less than 3 hours. The cryopreserved platelets can be thawed for further use by known methods such as exposing the cryopreserved platelets to a non-freezing temperature. For example, the thawing can include submersing the cryopreserved platelets in a 37°C water bath for a suitable amount of time, e.g., about 8 minutes to about 10 minutes.

[0097] Process herein, in some embodiments, after the step of distributing the pooled resuspension having a cryoprotectant includes freezing the pooled resuspension having a cryoprotectant with a transition in freezing temperatures from an initial freezing temperature to a storage freezing temperature. In some embodiments, process herein comprises a step of initial freezing at a temperature (i.e., initial temperature) less than or equal to -50°C, -60°C, -65°C, -70°C, -80°C, -85°C, or -90°C, or in the range of -50°C to -85°C, or -60°C to -85°C to form an initial frozen platelet composition, followed by storing the initial frozen platelet composition in a frozen state at a temperature (i.e., storage temperature) equal to or greater than -

30°C, but less than 0°C, to form cryopreserved platelets, or a cryopreserved platelet composition, thereby forming a batch of cryopreserved platelets.

[0098] Process herein, in some embodiments, can be completed within 4, 3, or 3 hours after addition of cryoprotectant until the freezing step. For example, the process can be completed at a time in the range of 1-3 hours, 1.5-3 hours, 2-3 hours, or 1.5 to 2.5 hours after the addition of cryoprotectant until the freezing step.

[0099] A batch of cryo-vessels comprising cryopreserved platelets obtained from process herein can be assessed for a number of post-manufacturing specifications. A non-limiting set of such specifications can include the following:

[00100] Freeze Volume: 20 mL – 35 mL

[00101] Time from addition of 27% DMSO to freezer: less than or equal to 3 hours

[00102] Frozen by the end of day 2 of platelet age

[00103] Visible aggregates in cryo-bag: none

[00104] %DMSO: 5.65% – 6.52%

[00105] Maximum DMSO in a CPP unit: 2.53 g (the mass corresponding to the maximum freeze volume with the maximum %DMSO).

Variance of cryopreserved platelets within and across batches

[00106] Processes herein, including processes that comprise a transition in freezing temperatures from an initial freezing temperature to a storage freezing temperature can provide cryopreserved platelet composition, cryopreserved platelets, or a batch of cryopreserved platelets having a plurality of cryo-vessels having cryopreserved platelets that can be more homogenous than those of prior methods, and can have a reduced batch-to-batch variability. Such improved homogeneity and reduced batch-to-batch variation, in some embodiments, can be displayed by low coefficient of variance within a batch or across batches of parameters not limited to resuspension weight or volume (post-expression weight or volume) in a vessel, such as APU bag within 15%, 14%, 13%, 12%, 11%, or 10%. For example, resuspension volume in a vessel can have a coefficient of variance in the range of 0.1-15%, 0.1-14%, 0.1-13%, 0.1-12%, 0.1-11%, 0.1-10%, 0.1-9%, 0.1-8%, or 0.1-7% within a batch or across batches. For example, resuspension volume in a vessel can have a coefficient of variance in the range of 0.1-15%, 0.1-14%, 0.1-13%, 0.1-12%, 0.1-11%, 0.1-10%, 0.1-9%, 0.1-8%, or 0.1-7% across at least 2 batches. For example, resuspension volume in a vessel can have a coefficient of variance in the range of 0.1-15%, 0.1-14%, 0.1-13%, 0.1-12%, 0.1-11%, 0.1-10%, 0.1-9%, 0.1-8%, or 0.1-7% across at least 5 batches. For example, resuspension volume in a

vessel can have a coefficient of variance in the range of 0.1-15%, 0.1-14%, 0.1-13%, 0.1-12%, 0.1-11%, 0.1-10%, 0.1-9%, 0.1-8%, or 0.1-7% across at least 15 batches. For example, resuspension volume in a vessel can have a coefficient of variance in the range of 0.1-15%, 0.1-14%, 0.1-13%, 0.1-12%, 0.1-11%, 0.1-10%, 0.1-9%, 0.1-8%, or 0.1-7% across at least 2, 3, 4, 5, or 10 lots, or between 2, 3, 4, or 5 lots on the low end to 100 lots on the high end. For example, resuspending the pellet in each vessel leads to a resuspension in each vessel such that the volume of the resuspension across at least 2, 3, 4, 5, or 10 lots, or between 2, 3, 4, or 5 lots on the low end to 100 lots on the high end has a mean intra-batch coefficient of variance (mean of intra-batch CV) of less than 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, or 7%. In some embodiments, the mean intra-batch coefficient of variance of the resuspension in each vessel across at least 10 batches can be in the range of 1-20%, 1-15%, 1-10%, 1-8%, 2-8%, 3-8%, or 4-8%. For example, resuspending the pellet in each vessel leads to a resuspension in each vessel such that the volume of the resuspension across 2-12 batches has a mean intra-batch coefficient of variance (mean of intra-batch CV) of less than 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, or 7%. In some embodiments, the mean intra-batch coefficient of variance of the resuspension in each vessel across 2-12 batches can be in the range of 1-20%, 1-15%, 1-10%, or 1-8%, 2-8%, 3-8%, or 4-8%. In some embodiments, resuspending the pellet in each vessel leads to a resuspension in each vessel such that the volume of the resuspension in a vessel across at least 5 batches or within a batch varies within 20%, 15%, 12%, 10%, 9%, or 8%. For example, resuspending the pellet in each vessel leads to a resuspension in each vessel such that the volume of the resuspension in a vessel across at least 5 batches or within a batch varies in the range of 3-25%, 7-20%, 7-15%, 7-14%, 7-12%, 7-10%, 3-20%, 3-15%, 3-12%, or 3-10%. In some embodiments, resuspending the pellet in each vessel leads to a resuspension in each vessel such that the volume of the resuspension in a vessel across at least 10 batches or within a batch varies within 20%, 15%, 12%, 10%, 9%, or 8%. For example, resuspending the pellet in each vessel leads to a resuspension in each vessel such that the volume of the resuspension in a vessel across at least 10 batches or within a batch varies in the range of 3-25%, 7-20%, 7-15%, 7-14%, 7-12%, 7-10%, 3-20%, 3-15%, 3-12%, or 3-10%. In some embodiments, resuspending the pellet in each vessel leads to a resuspension in each vessel such that the volume of the resuspension in a vessel across at least 20 batches or within a batch varies within 20%, 15%, 12%, 10%, 9%, or 8%. For example, resuspending the pellet in each vessel leads to a resuspension in each vessel such that the volume of the resuspension in a vessel across at least 5 batches or within a batch varies in the range of 3-25%, 7-20%, 7-15%, 7-14%, 7-12%, 7-10%, 3-20%, 3-15%, 3-12%, or 3-10%. In some embodiments, resuspending the pellet in each vessel leads to a resuspension in each vessel such that the volume of the

resuspension in a vessel across 5-100 batches or within a batch varies within 20%, 15%, 12%, 10%, 9%, or 8%. For example, resuspending the pellet in each vessel leads to a resuspension in each vessel such that the volume of the resuspension in a vessel across 5-100 batches or within a batch varies in the range of 3-25%, 7-20%, 7-15%, 7-14%, 7-12%, 7-10%, 3-20%, 3-15%, 3-12%, or 3-10%.

[00107] Another parameter for assessment of the homogeneity can be the volume or weight of a pooled resuspension having a cryoprotectant, or a cryopreservation medium having platelets, such as DMSO in a cryo-vessel, also known as freeze volume or weight, that has low coefficient of variance within a batch or across batches within 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 3%, 2%, or 1%. For example, the volume or weight of a pooled resuspension having a cryoprotectant in a cryo-vessel, or a cryopreservation medium having platelets can have a coefficient of variance in the range of 0.1-5%, 0.1-4%, 0.1-3%, 0.1-2%, 0.1-1%, 0.1-0.5%, 0.1%-0.4%, 0.1%-0.3%, or 0.1%-0.2% within a batch or across batches. For example, the volume or weight of pooled resuspension having a cryoprotectant in a cryo-vessel, or a cryopreservation medium having platelets can have a coefficient of variance in the range of 0.1-15%, 0.1-14%, 0.1-13%, 0.1-12%, 0.1-11%, 0.1-10%, 0.1-9%, 0.1-8%, 0.1-7%, 0.1-6%, 0.1-5%, 0.1-4%, 0.1-3%, 0.1-2%, 0.1-1%, 0.1-0.5%, 0.1%-0.4%, 0.1%-0.3%, or 0.1%-0.2% across at least 2 batches. For example, the volume or weight of pooled resuspension having a cryoprotectant in a cryo-vessel, or a cryopreservation medium having platelets can have a coefficient of variance in the range of 0.1-15%, 0.1-14%, 0.1-13%, 0.1-12%, 0.1-11%, 0.1-10%, 0.1-9%, 0.1-8%, 0.1-7%, 0.1-6%, 0.1-5%, 0.1-4%, 0.1-3%, 0.1-2%, 0.1-1%, 0.1-0.5%, 0.1%-0.4%, 0.1%-0.3%, or 0.1%-0.2% across at least 5 batches. For example, the volume or weight of pooled resuspension having a cryoprotectant in a cryo-vessel, or a cryopreservation medium having platelets can have a coefficient of variance in the range of 0.1-15%, 0.1-14%, 0.1-13%, 0.1-12%, 0.1-11%, 0.1-10%, 0.1-9%, 0.1-8%, 0.1-7%, 0.1-6%, 0.1-5%, 0.1-4%, 0.1-3%, 0.1-2%, 0.1-1%, 0.1-0.5%, 0.1%-0.4%, 0.1%-0.3%, or 0.1%-0.2% across at least 15 batches. For example, the volume or weight of the pooled resuspension in a cryo-vessel, or a cryopreservation medium having platelets across at least 10 batches has a mean intra-batch coefficient of variance of less than 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, or 2%. For example, the volume or weight of the pooled resuspension having a cryoprotectant in a cryo-vessel, or a cryopreservation medium having

platelets across at least 10 batches has a mean intra-batch coefficient of variance in the range of 0.1-15%, 0.1-14%, 0.1-13%, 0.1-12%, 0.1-11%, 0.1-10%, 0.1-9%, 0.1-8%, 0.1-7%, 0.1-6%, 0.1-5%, 0.1-4%, 0.1-3%, 0.1-2%, 0.1-1%, 0.1-0.5%, 0.1%-0.4%, 0.1%-0.3%, or 0.1%-0.2%. For example, the volume or weight of the pooled resuspension having a cryoprotectant in a cryo-vessel across 2-12 batches, or a cryopreservation medium having platelets has a mean intra-batch coefficient of variance (mean of intra-batch CV) of less than 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, or 2%. In some embodiments, the volume or weight of the pooled resuspension having a cryoprotectant in a cryo-vessel, or a cryopreservation medium having platelets across 2-12 batches can be in the range of 0.05-20%, 0.1-20%, 0.5-20%, 1-20%, 1-15%, 1-10%, or 1-8%, 1-7%, 1-6%, 1-5%, 1-4%, 1-3%, 1-2%, 0.1-2%, 0.1-1%, 0.1-0.5%, 0.1%-0.4%, 0.1%-0.3%, or 0.1%-0.2%. In some embodiments, the volume or weight of the pooled resuspension having a cryoprotectant in a cryo-vessel across at least 5 batches or within a batch varies within 20%, 15%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, or 2%. For example, the volume or weight of the pooled resuspension having a cryoprotectant in a cryo-vessel across, or a cryopreservation medium having platelets at least 5 batches or within a batch varies in the range of 0.05-20%, 0.1-20%, 0.5-20%, 1-20%, 1-15%, 1-10%, or 1-8%, 1-7%, 1-6%, 1-5%, 1-4%, 1-3%, 1-2%, 0.1-2%, 0.1-1%, 0.1-0.5%, 0.1%-0.4%, 0.1%-0.3%, or 0.1%-0.2%. In some embodiments, the volume or weight of the pooled resuspension having a cryoprotectant in a cryo-vessel, or a cryopreservation medium having platelets across at least 10 batches or within a batch varies within 20%, 15%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%. For example, the volume or weight of the pooled resuspension having a cryoprotectant in a cryo-vessel, or a cryopreservation medium having platelets across at least 10 batches or within a batch varies in the range of 0.05-20%, 0.1-20%, 0.5-20%, 1-20%, 1-15%, 1-10%, or 1-8%, 1-7%, 1-6%, 1-5%, 1-4%, 1-3%, 1-2%, 0.1-2%, 0.1-1%, 0.1-0.5%, 0.1%-0.4%, 0.1%-0.3%, or 0.1%-0.2%. In some embodiments, the volume or weight of the pooled resuspension having a cryoprotectant in a cryo-vessel, or a cryopreservation medium having platelets across at least 20 batches or within a batch varies within 20%, 15%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%. For example, the volume or weight of the pooled resuspension having a cryoprotectant in a cryo-vessel, or a cryopreservation medium having platelets across at least 20 batches or within a batch varies in the range of 0.05-20%, 0.1-20%, 0.5-20%, 1-20%, 1-15%, 1-10%, or 1-8%, 1-7%, 1-6%, 1-5%, 1-4%, 1-3%, 1-2%, 0.1-2%, 0.1-1%, 0.1-0.5%, 0.1%-0.4%, 0.1%-0.3%, or 0.1%-0.2%. In some embodiments, the volume or weight of the pooled resuspension having a cryoprotectant in a cryo-vessel, or a cryopreservation medium having platelets across 5-100 batches or within a batch varies within 20%, 15%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%. For example, the volume or weight of

the pooled resuspension having a cryoprotectant in a cryo-vessel, or a cryopreservation medium having platelets across at least 5-100 batches or within a batch varies in the range of 0.05-20%, 0.1-20%, 0.5-20%, 1-20%, 1-15%, 1-10%, or 1-8%, 1-7%, 1-6%, 1-5%, 1-4%, 1-3%, 1-2%, 0.1-2%, 0.1-1%, 0.1-0.5%, 0.1%-0.4%, 0.1%-0.3%, or 0.1%-0.2%.

[00108] In some embodiments of aspects that include process for preparing a batch of cryopreserved platelets, a process for preparing a cryopreserved platelet composition including a transition in freezing temperatures from an initial freezing temperature to a storage freezing temperature, and a collection or a batch comprising cryopreserved platelets, process herein provide an improved homogeneity in terms of concentrations of the cryoprotectant that is present in the cryopreserved platelets in a cryo-vessel of a batch, and across batches such that the mean cryoprotectant concentration, such as DMSO concentration in the cryopreserved platelets, or in a cryopreservation medium having platelets across at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 75, or 100 batches has a coefficient of variance of less than 10%. For example, the DMSO concentration in the cryopreserved platelets across at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 75, or 100 batches has a coefficient of variance of less than 5%. For example, the DMSO concentration in the cryopreserved platelets across at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 75, or 100 batches has a coefficient of variance of less than 3%. For example, the DMSO concentration in the cryopreserved platelets across at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 75, or 100 batches has a coefficient of variance of less than 2%. For example, the DMSO concentration in the cryopreserved platelets across at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 75, or 100 batches has a coefficient of variance of less than 1%. For example, the DMSO concentration in the cryopreserved platelets across at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 75, or 100 batches has a coefficient of variance of less than 0.5%. For example, the DMSO concentration in the cryopreserved platelets across at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 75, or 100 batches has a coefficient of variance of less than 0.4%. Accordingly, in some embodiments of aspects that include a process, or a collection of cryopreserved platelets, DMSO concentration in the cryopreserved platelets across batches or within a batch can be in the range of 0.01-2%, 0.01-1.8%, 0.01-1.6%, 0.01-1.5%, 0.01-1.3%, 0.01-1.1%, 0.01-1%, 0.01-0.8%, 0.01-0.7%, 0.01-0.5%, 0.01-0.4%, 0.02-0.4%, or 0.04-0.4%. For example, the mean cryoprotectant concentration (e.g., DMSO concentration) in the cryopreserved platelets across at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 75, or 100 batches has a coefficient of variance of less than 5%. In some embodiments, the mean cryoprotectant concentration (e.g., DMSO concentration) in the cryopreserved platelets across at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 75, or 100 batches has a coefficient of variance of less than 1%. For example, the mean cryoprotectant

concentration (e.g., DMSO concentration) in the cryopreserved platelets across at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 75, or 100 batches has a coefficient of variance of less than 0.8%, 0.5%, or 0.4%.

Typically, the mean DMSO concentration in the cryopreserved platelets across at least 10 batches can be less than 0.5%. In some embodiments, the cryoprotectant is DMSO, the mean concentration of DMSO in the cryopreserved platelets in a cryo-vessel across at least 10 batches has a coefficient of variance of less than 5%, 4%, 3%, 2%, 1%, or 0.5%. In some embodiments, DMSO concentration in the cryopreserved platelets across at least 10 batches or within a batch can be in the range of 0.01-2%, 0.01-1.8%, 0.01-1.6%, 0.01-1.5%, 0.01-1.3%, 0.01-1.1%, 0.01-1%, 0.01-0.8%, 0.01-0.7%, 0.01-0.5%, 0.01-0.4%, 0.02-0.4%, or 0.04-0.4%. For example, the mean concentration of DMSO in the cryopreserved platelets in a cryo-vessel across at least 20 batches has a coefficient of variance of less than 5%, 4%, 3%, 2%, 1%, or 0.5%. In some embodiments, DMSO concentration in the cryopreserved platelets across at least 20 batches or within a batch can be in the range of 0.01-2%, 0.01-1.8%, 0.01-1.6%, 0.01-1.5%, 0.01-1.3%, 0.01-1.1%, 0.01-1%, 0.01-0.8%, 0.01-0.7%, 0.01-0.5%, 0.01-0.4%, 0.02-0.4%, or 0.04-0.4%. For example, the mean concentration of DMSO in the cryopreserved platelets in a cryo-vessel across at least 50 batches has a coefficient of variance of less than 5%, 4%, 3%, 2%, 1%, or 0.5%. In some embodiments, DMSO concentration in the cryopreserved platelets across 50 batches or within a batch can be in the range of 0.01-2%, 0.01-1.8%, 0.01-1.6%, 0.01-1.5%, 0.01-1.3%, 0.01-1.1%, 0.01-1%, 0.01-0.8%, 0.01-0.7%, 0.01-0.5%, 0.01-0.4%, 0.02-0.4%, or 0.04-0.4%. For example, the mean concentration of DMSO in the cryopreserved platelets in a cryo-vessel across at least 100 batches has a coefficient of variance of less than 5%, 4%, 3%, 2%, 1%, or 0.5%. In some embodiments, DMSO concentration in the cryopreserved platelets across at least 100 batches or within a batch can be in the range of 0.01-2%, 0.01-1.8%, 0.01-1.6%, 0.01-1.5%, 0.01-1.3%, 0.01-1.1%, 0.01-1%, 0.01-0.8%, 0.01-0.7%, 0.01-0.5%, 0.01-0.4%, 0.02-0.4%, or 0.04-0.4%. For example, the mean concentration of DMSO in the cryopreserved platelets in a cryo-vessel across 5-100 batches has a coefficient of variance of less than 1%, or 0.5%. In some embodiments, DMSO concentration in the cryopreserved platelets across 5-100 batches or within a batch can be in the range of 0.01-2%, 0.01-1.8%, 0.01-1.6%, 0.01-1.5%, 0.01-1.3%, 0.01-1.1%, 0.01-1%, 0.01-0.8%, 0.01-0.7%, 0.01-0.5%, 0.01-0.4%, 0.02-0.4%, or 0.04-0.4%. For example, the mean concentration of DMSO in the cryopreserved platelets in a cryo-vessel across 5-500 batches has a coefficient of variance of less than 1%, or 0.5%. In some embodiments, DMSO concentration in the cryopreserved platelets across 5-500 batches can be in the range of 0.01-2%, 0.01-1.8%, 0.01-1.6%, 0.01-1.5%, 0.01-1.3%, 0.01-1.1%, 0.01-1%, 0.01-0.8%, 0.01-0.7%, 0.01-0.5%, 0.01-0.4%, 0.02-0.4%, or 0.04-0.4%. In some embodiments, the concentration of DMSO

in the cryopreserved platelets in a cryo-vessel within a batch has a coefficient of variance of less than 10%, 9%, 8%, 7%, 5%, 3%, 1%, 0.8%, 0.6%, or 0.5%. In some embodiments, the concentration of DMSO in the cryopreserved platelets in a cryo-vessel within a batch or across at least 5 batches varies within 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.4%. In some embodiments, the concentration of DMSO in the cryopreserved platelets in a cryo-vessel within a batch or across at least 10 batches varies within 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.4%. In some embodiments, the concentration of DMSO in the cryopreserved platelets in a cryo-vessel within a batch or across 5-100 batches varies within 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.4%.

[00109] In some embodiments of aspects that include process for preparing a batch of cryopreserved platelets, a process for preparing a cryopreserved platelet composition including a transition in freezing temperatures from an initial freezing temperature to a storage freezing temperature, and a collection or a batch comprising cryopreserved platelets, the concentration of platelets in the cryopreserved platelets in a cryo-vessel within a batch or across batches varies within 20%, 15%, 12%, 10%, or 8%. For example, the concentration of platelets in the cryopreserved platelets in a cryo-vessel within a batch or across batches varies in the range of 2-20%, 2-15%, 2-12%, 2-10%, 5-20%, 7-20%, or 10-20%. For example, the concentration of platelets in the cryopreserved platelets in a cryo-vessel within a batch or across 5-100 batches varies within 20%, 15%, 12%, 10%, or 8%. For example, the concentration of platelets in the cryopreserved platelets in a cryo-vessel within a batch or across 5-100 batches varies in the range of 2-20%, 2-15%, 2-12%, 2-10%, 5-20%, 7-20%, or 10-20%. For example, the concentration of platelets in the cryopreserved platelets in a cryo-vessel across at least 5 batches has a mean intra-batch coefficient of variance of less than 20%, 18%, 15%, 12%, 10%, 8%, 5%, or 4%. In some embodiments, the concentration of platelets in the cryopreserved platelets in a cryo-vessel across at least 5 batches has a mean intra-batch coefficient of variance in the range of 2-20%, 2-15%, 2-12%, 2-10%, 2-8%, 2-6%, or 2-5%. For example, the concentration of platelets in the cryopreserved platelets in a cryo-vessel within a batch has a coefficient of variance of less than 20%, 18%, 15%, 12%, 10%, 8%, 5%, or 4%. In some embodiments, the concentration of platelets in the cryopreserved platelets in a cryo-vessel within a batch has a coefficient of variance in the range of 2-20%, 2-15%, 2-12%, 2-10%, 2-8%, 2-6%, 3-6%, 3-20%, 3-15%, 3-12%, 3-10%, or 3-6%.

[00110] In some embodiments of aspects that include process for preparing a batch of cryopreserved platelets, a process for preparing a cryopreserved platelet composition including a transition in freezing temperatures from an initial freezing temperature to a storage freezing temperature, and a collection or a batch comprising cryopreserved platelets, the total number of platelets in the cryopreserved platelets in a

cryo-vessel within a batch or across batches varies within 20%, 15%, 12%, 10%, or 8%. For example, the total number of platelets in the cryopreserved platelets in a cryo-vessel within a batch or across batches varies in the range of 2-20%, 2-15%, 2-12%, 2-10%, 5-20%, 7-20%, or 10-20%. For example, the total number of platelets in the cryopreserved platelets in a cryo-vessel within a batch or across 5-100 batches varies within 20%, 15%, 12%, 10%, or 8%. For example, the total number of platelets in the cryopreserved platelets in a cryo-vessel within a batch or across 5-100 batches varies in the range of 2-20%, 2-15%, 2-12%, 2-10%, 5-20%, 7-20%, or 10-20%. For example, the total number of platelets in the cryopreserved platelets in a cryo-vessel across at least 5 batches has a mean intra-batch coefficient of variance of less than 20%, 18%, 15%, 12%, 10%, 8%, 5%, or 4%. In some embodiments, the total number of platelets in the cryopreserved platelets in a cryo-vessel across at least 5 batches has a mean intra-batch coefficient of variance in the range of 2-20%, 2-15%, 2-12%, 2-10%, 2-8%, 2-6%, or 2-5%. For example, the total number of platelets in the cryopreserved platelets in a cryo-vessel within a batch has a coefficient of variance of less than 20%, 18%, 15%, 12%, 10%, 8%, or 7%. In some embodiments, the total number of platelets in the cryopreserved platelets in a cryo-vessel within a batch has a coefficient of variance in the range of 2-20%, 2-15%, 2-12%, 2-10%, 2-8%, 2-7%, 3-7%, 3-20%, 3-15%, 3-12%, or 3-10%.

Collection of cryo-vessels comprising cryopreserved platelets

[00111] Provided herein in some aspects, is a collection of cryo-vessels comprising cryopreserved platelets, wherein the cryopreserved platelets in each cryo-vessel have a biomolecule profile indicative of more than 1 platelet donor. The concentration of a cryoprotectant, such as DMSO in the cryopreserved platelets of a first cryo-vessel can be within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% of the concentration of the cryoprotectant, such as DMSO in the cryopreserved platelets of a second cryo-vessel. For example, the concentration of DMSO in the cryopreserved platelets of a first cryo-vessel can be in the range of 0.001-10%, 0.001-8%, 0.001-6%, 0.001-4%, 0.001-2%, or 0.001-1% of the concentration of DMSO in the cryopreserved platelets of a second cryo-vessel. Collection as provided herein can comprise a plurality of cryo-vessels comprising the cryopreserved platelets. Collection can be a collection of cryo-vessels in batches, for example, each batch can have at least 2, 3, 4, 5, 6, 7, 8, 9, 10, or more cryo-vessels, and a collection can have at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 20, 50, or more number of batches. A plurality of cryo-vessels or cryo-containers having the cryopreserved platelets herein, can be referred to as a "batch" or a "lot". Typically, the cryopreserved platelets in each cryo-vessel have a set of biomolecule profiles indicative of more than 1 platelet donor and a batch of cryo-vessels has an identical set of

biomolecule profiles. The set of biomolecule profiles indicative of more than 1 platelet donor in a batch can be different as compared to the set of biomolecule profiles of another batch. Thus, CPPs of a first lot typically have a biomolecule profile of a first set of donors, and CPPs of a second lot typically have a biomolecule profile of a second set of donors, wherein the second set of donors is not identical to the first set of donors. A skilled artisan will understand that there are numerous characteristics that can be used to differentiate CPPs from a first set of donors from CPPs from a second set of donors. Accordingly, a collection of cryo-vessels having CPPs can have a plurality of batches, such that, within one batch the set of biomolecule profiles indicative of more than 1 platelet donor is identical across the cryo-vessels of that batch, and is different from the cryo-vessels of other batches in the collection. Typically, a collection of cryo-vessels as provided herein is homogenous across batches and within batches and has significantly less donor-to-donor variation. Some non-limiting parameters to assess homogeneity can be concentration of cryoprotectant, platelet concentration, total number of platelets, pH, thrombin generation ability (IU), and CD61-positive microparticle concentration. In some embodiments the collections comprise frozen or cryopreserved platelets and/or platelet derivatives having one or more of the recited properties provided herein.

[00112] Collection of cryo-vessels comprising cryopreserved platelets herein, in some embodiments, can include platelet concentrations in manner wherein the concentration of platelets in the cryopreserved platelets in a cryo-vessel within a batch or across batches varies within 30%, 25%, 20%, 15%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, or 4%. For example, the concentration of platelets in the cryopreserved platelets in a cryo-vessel within a batch or across batches varies in the range of 0.5-30%, 1-30%, 2-30%, 3-30%, 0.5-25%, 0.5-20%, 0.5-15%, 0.5-10%, 0.5-7%, or 0.5-5%. For example, the concentration of platelets in the cryopreserved platelets in a cryo-vessel across at least 5 batches varies within 20%, 15%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, or 4%. For example, the concentration of platelets in the cryopreserved platelets in a cryo-vessel across at least 5 batches varies in the range of 0.5-30%, 1-30%, 2-30%, 3-30%, 0.5-25%, 0.5-20%, 0.5-15%, 0.5-10%, 0.5-7%, or 0.5-5%. For example, the concentration of platelets in the cryopreserved platelets in a cryo-vessel across at least 10 batches varies within 20%, 15%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, or 4%. For example, the concentration of platelets in the cryopreserved platelets in a cryo-vessel across at least 10 batches varies in the range of 0.5-30%, 1-30%, 2-30%, 3-30%, 0.5-25%, 0.5-20%, 0.5-15%, 0.5-10%, 0.5-7%, or 0.5-5%. In some embodiments, the concentration of platelets in the cryopreserved platelets in a cryo-vessel across at least 5 batches has a mean intra-batch coefficient of variance within 20%, 15%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, or 4%. For example, the concentration of

platelets in the cryopreserved platelets in a cryo-vessel across at least 5 batches has a mean intra-batch coefficient of variance within 0.5-30%, 1-30%, 2-30%, 3-30%, 0.5-25%, 0.5-20%, 0.5-15%, 0.5-10%, 0.5-7%, or 0.5-5%. In some embodiments, the concentration of platelets in the cryopreserved platelets in a cryo-vessel within a batch has a coefficient of variance within 20%, 15%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, or 4%. For example, the concentration of platelets in the cryopreserved platelets in a cryo-vessel across within a batch has a coefficient of variance within 0.5-30%, 1-30%, 2-30%, 3-30%, 0.5-25%, 0.5-20%, 0.5-15%, 0.5-10%, 0.5-7%, or 0.5-5%.

[00113] Collection of cryo-vessels comprising cryopreserved platelets herein, in some embodiments, can include total number of platelets in a manner wherein the total number of platelets in the cryopreserved platelets in a cryo-vessel within a batch or across batches varies within 30%, 25%, 20%, 15%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, or 4%. For example, the total number of platelets in the cryopreserved platelets in a cryo-vessel within a batch or across batches varies in the range of 0.5-30%, 1-30%, 2-30%, 3-30%, 0.5-25%, 0.5-20%, 0.5-15%, 0.5-10%, 0.5-7%, or 0.5-5%. For example, the total number of platelets in the cryopreserved platelets in a cryo-vessel across at least 5 batches varies within 20%, 15%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, or 4%. For example, the total number of platelets in the cryopreserved platelets in a cryo-vessel across at least 5 batches varies in the range of 0.5-30%, 1-30%, 2-30%, 3-30%, 0.5-25%, 0.5-20%, 0.5-15%, 0.5-10%, 0.5-7%, or 0.5-5%. For example, the concentration of platelets in the cryopreserved platelets in a cryo-vessel across at least 10 batches varies within 20%, 15%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, or 4%. For example, the concentration of platelets in the cryopreserved platelets in a cryo-vessel across at least 10 batches varies in the range of 0.5-30%, 1-30%, 2-30%, 3-30%, 0.5-25%, 0.5-20%, 0.5-15%, 0.5-10%, 0.5-7%, or 0.5-5%. In some embodiments, the concentration of platelets in the cryopreserved platelets in a cryo-vessel across at least 5 batches has a mean intra-batch coefficient of variance within 20%, 15%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, or 4%. For example, the concentration of platelets in the cryopreserved platelets in a cryo-vessel across at least 5 batches has a mean intra-batch coefficient of variance within 0.5-30%, 1-30%, 2-30%, 3-30%, 0.5-25%, 0.5-20%, 0.5-15%, 0.5-10%, 0.5-7%, or 0.5-5%. In some embodiments, the concentration of platelets in the cryopreserved platelets in a cryo-vessel within a batch has a coefficient of variance within 20%, 15%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, or 4%. For example, the concentration of platelets in the cryopreserved platelets in a cryo-vessel across within a batch has a coefficient of variance within 0.5-30%, 1-30%, 2-30%, 3-30%, 0.5-25%, 0.5-20%, 0.5-15%, 0.5-10%, 0.5-7%, or 0.5-5%.

[00114] Collection of cryo-vessels comprising cryopreserved platelets herein, in some embodiments, can include a homogeneity in pH of cryo-vessels in a manner wherein the pH of the cryopreserved platelets in a cryo-vessel within a batch or across batches varies within 5%, 4%, 3%, 2%, 1%, 0.9%, or 0.75%. For example, the pH of the cryopreserved platelets in a cryo-vessel within a batch or across batches varies in the range of 0.001-5%, 0.001-4%, 0.001-3%, 0.001-2%, 0.001-1%, 0.01-1%, 0.05-1%, or 0.5-1%. In some embodiments, the pH of the cryopreserved platelets in a cryo-vessel across at least 5 batches has a mean intra batch coefficient of variance within 10%, 7%, 5%, 4%, 3%, 2%, or 1%. For example, the pH of the cryopreserved platelets in a cryo-vessel across at least 5 batches has a mean intra batch coefficient of variance in the range of 0.001-5%, 0.001-4%, 0.001-3%, 0.001-2%, 0.001-1%, 0.01-1%, 0.05-1%, or 0.5-1%. In some embodiments, the pH of the cryopreserved platelets in a cryo-vessel across at least 5 batches has a coefficient of variance within 10%, 7%, 5%, 4%, 3%, 2%, or 1%. For example, the pH of the cryopreserved platelets in a cryo-vessel across at least 5 batches has a coefficient of variance in the range of 0.001-5%, 0.001-4%, 0.001-3%, 0.001-2%, 0.001-1%, 0.01-1%, 0.05-1%, or 0.5-1%.

[00115] Collection of cryo-vessels comprising cryopreserved platelets herein, in some embodiments, can include a homogeneity in the concentration of CD61-positive microparticles, such that the concentration of CD61-positive microparticles in the cryopreserved platelets in a cryo-vessel within a batch or across batches varies within 20%, 15%, 12%, 10%, 9%, 8%, or 7%. For example, the concentration of CD61-positive microparticles in the cryopreserved platelets in a cryo-vessel within a batch or across batches varies in the range of 1-20%, 1-15%, 1-12%, 1-10%, 3-20%, 3-15%, 3-12%, 3-10%, or 5-10%. In some embodiments, the concentration of CD61-positive microparticles in the cryopreserved platelets in a cryo-vessel across at least 5 batches has a mean intra batch coefficient of variance in the range of 1-20%, 1-15%, 1-12%, 1-10%, 3-20%, 3-15%, 3-12%, 3-10%, or 5-10%. For example, the concentration of CD61-positive microparticles in the cryopreserved platelets in a cryo-vessel across at least 5 batches has a coefficient of variance in the range of 1-20%, 1-15%, 1-12%, 1-10%, 3-20%, 3-15%, 3-12%, 3-10%, or 5-10%. For example, the concentration of CD61-positive microparticles in the cryopreserved platelets in a cryo-vessel across at least 5 batches has a coefficient of variance within 25%, 20%, 15%, 10%, or 8%.

[00116] Collection of cryo-vessels comprising cryopreserved platelets herein, in some embodiments, can include a homogeneity in the thrombin generation ability of the cryopreserved platelets, such that a measure of thrombin generation per 10^6 platelets across batches or within a batch varies within 20%, 15%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, or 2%. A skilled artisan would understand that a measure of thrombin generation can be any appropriate units based on the assay used, for example, thrombin

generation assay can be performed to determine the thrombin generation ability in terms of IU per 10^6 platelets. An illustrative and non-limiting example of a method for assessing thrombin generation is shown in Example 4. For example, a measure of thrombin generation per 10^6 platelets across batches or within a batch varies in the range of 0.5-20%, 0.5-15%, 0.5-12%, 0.5-10%, 0.5-8%, or 0.5-5%. For example, a measure of thrombin generation per 10^6 platelets across batches has a mean intra batch coefficient of variance within 20%, 15%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, or 2%. In some embodiments, a measure of thrombin generation per 10^6 platelets across batches has a mean intra batch coefficient in the range of 0.5-20%, 0.5-15%, 0.5-12%, 0.5-10%, 0.5-8%, or 0.5-5%. For example, a measure of thrombin generation per 10^6 platelets across batches has a coefficient of variance within 20%, 15%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, or 3%. For example, a measure of thrombin generation per 10^6 platelets across batches has a coefficient of variance in the range of 0.5-20%, 0.5-15%, 0.5-12%, 0.5-10%, 0.5-8%, or 0.5-5%.

[00117] Cryo-vessels of CPPs herein are typically prepared using platelets that are pooled from a plurality of donors (e.g. pooled platelets). Thus, cryo-vessels or cryo-vials comprising CPPs herein, and processes for preparing and using the same, typically include a population of CPPs that have a biomolecule profile indicative of more than 1 platelet donor. A skilled artisan will understand that there are various molecular tests that can be used to confirm that CPPs were prepared from a plurality of donors.

[00118] In some embodiments, the biomolecule profile indicative of more than 1 platelet donor is a protein profile. For example, the biomolecule profile can be the amino acid sequence of one or more proteins, for example one or more proteins that are present in or associated with the CPPs in a lot of cryo-vials produced from a pool of donors. Such profile can include, for example, 3 or more amino acid sequences of a target protein from a single gene. These amino acid sequences can be those of polymorphs of the protein. It will be understood that wherein a single donor has one or two amino acid sequences of this protein, for example depending on whether they are homozygous or heterozygous, the presence of 3 or more amino acid sequences for this protein in the CPPs can be indicative of more than 1 donor. Furthermore, if a set of donors whose platelets were pooled to make CPPs of a first lot is not identical to the donors used to make CPPs of a second lot, then the set of amino acid sequence variants/alleles/versions of the target protein(s) in the first lot can be different than the set of amino acid sequence variants/alleles/versions of the target protein(s) in the second lot. For example, if 5 donors are used to make a first pool of platelets used to make a first lot, and 5 different donors are used to make a second pool of platelets for a second lot, for a target protein, there could be up to 10 different alleles/variants/versions in each pool for a protein originally

expressed from a single gene, and at least 1 allele/variant/version of the target protein could be unique to each lot versus the other lot.

[00119] In some embodiments that rely more on quantitative information, the biomolecule profile indicative of more than 1 platelet donor is the presence of two or more alleles/variants/versions/amino acid sequences of at least a first protein from at least a first gene that are significantly different than 50% in frequency within the composition. A 50% frequency would be expected, for example, if such composition was from a single donor that was heterozygous for alleles at the first gene.

[00120] In a similar manner to the discussion above regarding a target protein(s), a biomolecule profile indicative of more than 1 platelet donor can be detected and/or quantified by detecting and/or quantifying nucleic acids that are present in or associated with the CPPs. Such detecting can use techniques such as, but not limiting to, PCR, typically, quantitative reverse transcription polymerase chain reaction (qRT-PCR). qRT-PCR is considered as one of the techniques available for quantifying RNA, such as mRNA in a sample. The RNA to be identified can be RNA specific for an individual donating the platelets, or can be used to identify platelets donated by a single donor. In illustrative embodiments, such an RNA molecule can be detected and/or quantified from a platelet sample for establishing that platelets have been donated by more than 1 individual.

[00121] In some embodiments, a collection of cryo-vessels herein can comprise frozen platelets in a cryopreservation medium in a frozen state, wherein the composition is capable of yielding one or more of the recited properties herein, for example one or more of the following properties after storage for at least 1 month, 2, 3, 4, 5, 6, 8, 10, or 12 months, in an illustrative embodiments at a temperature in a range of -10°C to -30°C, or -20°C +/- 5°C, upon thawing:

- a) is in a liquid state without the addition of a liquid;
- b) exhibits a platelet count of at least $1.0 \times 10^{11}/35$ ml of the composition;
- c) yields a single peak that corresponds to a compromised membrane peak in a membrane integrity assay;
- d) exhibits a CD61-positive-microparticle content of less than 50% of the CD61 positive particles in the composition, and
- e) generates thrombin in an *in vitro* thrombin generation assay. In some embodiments, a composition is capable of yielding two or more, three or more, four, or all of the properties. In illustrative embodiments, a composition is capable of yielding all of the properties. In some embodiments, a

composition is capable of yielding properties a), b), and d). In some embodiments, a composition is capable of yielding properties a), b), d), and e).

Stability of the cryopreserved platelets

[00122] In some embodiments of aspects that include process for preparing a batch of cryopreserved platelets, a process for preparing a cryopreserved platelet composition including a transition in freezing temperatures from an initial freezing temperature to a storage freezing temperature, and a collection or a batch comprising cryopreserved platelets, the cryopreserved platelets herein can be stable when stored frozen at -80°C, -70°C, -60°C, -50°C, -40°C, -30°C, -20°C, or higher. In some embodiments, cryopreserved platelets as disclosed herein, or cryopreserved platelets formed by a process as disclosed herein, in illustrative embodiments, cryopreserved platelets formed by a process that comprises freezing platelets in a cryopreservation medium, or freezing a pooled resuspension having a cryoprotectant as disclosed herein, at a temperature of less than or equal to -50°C, -55°C, -60°C, in illustrative embodiments, less than or equal to -65°C, -70°C, -75°C, or -80°C, to form an initial frozen platelet composition, and a second step comprising storing the initial frozen platelet composition at a temperature of more than or equal to -40°C, -35°C, -30°C, -25°C, in illustrative embodiments, more than or equal to -20°C, -15°C, or -10°C, but less than 0°C, are stable when stored at a temperature of more than or equal to -40°C, -35°C, -30°C, -25°C, -15°C, or -10°C, but less than 0°C. Stability of the cryopreserved platelets present in a cryo-vessel as provided in a collection of cryo-vessels herein, can be assessed by a non-limiting list of parameters including visual inspection of cracks, tears, breaks of the cryo-vessel, such as a cryo-bag, visual inspection of aggregate free swirling of the cryopreserved platelets in the cryo-vessel, such as a cryo-bag, platelet counts per cryo-vessel, and pH of the cryopreserved platelets in the cryo-vessel. For example, in some non-limiting embodiments, cryopreserved platelets herein are stable when stored at a specific temperature, for example, at about -20°C, or higher when the cryopreserved platelets swirl without the presence of any aggregation on a visual inspection. For example, when stored at -20°C +/-10°C, -20°C +/-8°C, -20°C +/-5°C, or -20°C +/-2°C the cryopreserved platelets swirl without the presence of any aggregation on a visual inspection. For example, in some non-limiting embodiments, cryopreserved platelets herein are stable when stored at a specific temperature, for example, at about -20°C, or higher, for example, stored at -20°C +/-5°C the pH of the cryopreserved platelets, typically upon thawing is equal to or more than 6.0, typically, equal to or more than 6.2. For example, the cryopreserved platelets herein upon storing at about -20°C, for example, at -20°C +/-5°C for a period in the range of 1 month-36 months, 1 month-30 months, 1 month-24 months, 1 month-18 months, or 1 month-12 months, typically upon thawing

exhibit a pH higher than 7.0. In some embodiments, the cryopreserved platelets herein upon storing at about -20°C , for example, at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ for a period in the range of 1-12 months, typically upon thawing exhibit a pH higher than 6.2, 6.4, 6.6, 6.8, 7.0, or 7.2. For example, the cryopreserved platelets herein upon storing at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ for a period in the range of 1-12 months, typically upon thawing exhibit a pH in the range of 6.2 to 7.8, 6.4 to 7.8, 6.6 to 7.8, or 7-7.8.

[00123] For example, in some non-limiting embodiments, cryopreserved platelets herein are stable when stored at a specific temperature, for example, at about -20°C , or higher, for example, stored at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ the total number of platelets, typically upon thawing in a cryo-vessel is equal to or more than 1.5×10^{11} , 1.6×10^{11} , or 1.7×10^{11} . In illustrative embodiments, cryopreserved platelets herein, typically when stored at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ for at least 1 month, 2, 3, 4, 6, 8, 10, 12 months, 2, 3, 4, 5, 6, 7, 8, 9, or 10 years, upon thawing have a total number of platelets in a cryo-vessel equal to or more than 1.5×10^{11} , 1.6×10^{11} , or 1.7×10^{11} . In some embodiments, cryopreserved platelets herein, typically upon thawing exhibit a particle size, for example, diameter in the range of $0.5 \mu\text{m}$ to $2.5 \mu\text{m}$, $1 \mu\text{m}$ to $5 \mu\text{m}$, $1 \mu\text{m}$ to $4 \mu\text{m}$, or $0.5 \mu\text{m}$ to $5.0 \mu\text{m}$ such that at least 50% of the platelets after thawing have a diameter in the range of $0.5 \mu\text{m}$ to $2.5 \mu\text{m}$, $1 \mu\text{m}$ to $5 \mu\text{m}$, $1 \mu\text{m}$ to $4 \mu\text{m}$, or $0.5 \mu\text{m}$ to $5.0 \mu\text{m}$. In some embodiments, cryopreserved platelets herein, in illustrative embodiments, upon storing at a temperature in the range of -40°C to -5°C , -30°C to -5°C , or -20°C to -5°C , for at least 1 month, 2, 3, 4, 6, 8, 10, 12 months, 2, 3, 4, 5, 6, 7, 8, 9, or 10 years typically upon thawing exhibit a particle size, for example, diameter in the range of $0.5 \mu\text{m}$ to $2.5 \mu\text{m}$, $1 \mu\text{m}$ to $5 \mu\text{m}$, $1 \mu\text{m}$ to $4 \mu\text{m}$, or $0.5 \mu\text{m}$ to $5.0 \mu\text{m}$ and have a total number of platelets in a cryo-vessel equal to or more than 1.5×10^{11} , 1.6×10^{11} , or 1.7×10^{11} . In some embodiments, at least 50%, 60%, 70%, or 75% of the cryopreserved platelets upon thawing have a diameter in the range of $0.5 \mu\text{m}$ to $2.5 \mu\text{m}$, $1 \mu\text{m}$ to $5 \mu\text{m}$, $1 \mu\text{m}$ to $4 \mu\text{m}$, or $0.5 \mu\text{m}$ to $5.0 \mu\text{m}$. In illustrative embodiments, cryopreserved platelets herein upon thawing have a total number of platelets in a cryo-vessel equal to or more than 1.5×10^{11} , 1.6×10^{11} , or 1.7×10^{11} , and typically, the cryopreserved platelets upon thawing retain hemostatic properties, for example, generating thrombin in an in vitro condition, ability to reduce bleeding in a subject, or ability to increase platelet numbers in a subject in need thereof. In illustrative embodiments, the ability to reduce bleeding in a subject is based on administration of, for example 0.5 to 3 units of frozen platelets, frozen platelet derivatives, cryopreserved platelets, or cryopreserved platelet derivatives. In illustrative embodiments, where 1 unit corresponds to $2.5 \times 10^{11} \pm 4.2 \times 10^{11}$ frozen or cryopreserved platelets and/or platelet derivatives. In some embodiments, methods herein include administering liquid compositions of thawed compositions of frozen platelets, frozen platelet derivatives, cryopreserved platelets, or cryopreserved platelet derivatives provided

herein and/or prepared according to any method provided herein, to a subject to restore hemostasis, reduce bleeding, or stop bleeding in the subject.

[00124] Stability can also be determined by assessing certain parameters after thawing the cryopreserved platelets that are stored at a temperature of more than or equal to -40°C , -35°C , -30°C , -25°C , -15°C , or -10°C , but less than 0°C , in illustrative embodiments, at a temperature in the range of -40°C to -10°C . In some embodiments, thawing of cryopreserved platelets as disclosed herein, or cryopreserved platelets obtained by a process as disclosed herein, can be done by subjecting the cryopreserved platelets to a temperature above the freezing temperature. For example, subjecting the cryopreserved platelets to a temperature above 0°C , for example, at least 5°C , 10°C , 15°C , 20°C , 25°C , 30°C , 35°C . In illustrative embodiments, thawing comprises subjecting the cryopreserved platelets to a temperature in the range of 20°C to 40°C , 22°C to 40°C , 25°C to 40°C , 30°C to 40°C , or 32°C to 40°C . In some embodiments, thawing comprises subjecting the cryopreserved platelets to a temperature of $37^{\circ}\text{C} \pm 5^{\circ}\text{C}$, $37^{\circ}\text{C} \pm 4^{\circ}\text{C}$, $37^{\circ}\text{C} \pm 3^{\circ}\text{C}$, $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$, $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$, or $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. Thawing herein typically comprises subjecting a cryo-vessel or a cryo-vial having cryopreserved platelet as disclosed herein to a water-bath set at a temperature of $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for a time-period until the contents in the cryo-vessel are completely thawed. A skilled artisan can contemplate that the time required for the contents to thaw completely can vary according to the volume of cryopreserved platelets, dimensions of the cryo-vessel and the temperature at which the cryo-vessels were stored before the thawing. Accordingly, thawing can be done by subjecting cryo-vessels to a water-bath set at a temperature of $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for at least 1 minute, 2, 3, 4, 5, 6, 7, 8, 9, or 10 minutes, for example in a range of 2-10, 2-9, 2-8, 2-7, or 2-6 minutes.

Exemplary Embodiments

[00125] Provided in this Exemplary Embodiments section are non-limiting exemplary aspects and embodiments provided herein and further discussed throughout this specification. For the sake of brevity and convenience, all of the aspects and embodiments disclosed herein, and all of the possible combinations of the disclosed aspects and embodiments are not listed in this section. Additional embodiments and aspects are provided in other sections herein. Furthermore, it will be understood that embodiments are provided that are specific embodiments for many aspects and that can be combined with any other embodiment, for example as discussed in this entire disclosure. It is intended in view of the full disclosure herein, that any individual embodiment recited below or in this full disclosure can be combined with any aspect recited below or in this full disclosure where it is an additional element that can be added to an aspect or because it is a narrower element for an element already present in an aspect. Such combinations

are sometimes provided as non-limiting exemplary combinations and/or are discussed more specifically in other sections of this detailed description.

[00126] Provided herein, in one aspect is a process for preparing a cryopreserved platelet composition comprising cryopreserved platelets, or a cryopreserved platelet derivative composition comprising cryopreserved platelet derivatives, said process comprising:

i) freezing a population of platelets in a cryopreservation medium at a temperature of equal to or less than -50°C , in illustrative embodiments for a time until the cryopreservation medium is frozen, to form an initial frozen platelet composition;

ii) transferring the initial frozen platelet composition to a freezer set at a temperature of equal to or more than -30°C , but less than 0°C ; and

iii) storing the initial frozen platelet composition in the freezer for at least 90 minutes, to form the cryopreserved platelet composition comprising the cryopreserved platelets or the cryopreserved platelet derivative composition comprising the cryopreserved platelet derivatives.

[00127] Provided herein, in one aspect is a process for preparing a cryopreserved platelet composition comprising cryopreserved platelets, said process comprising:

i) freezing a population of platelets in a cryopreservation medium at a temperature of equal to or less than -50°C to form an initial frozen platelet composition; and

ii) storing the initial frozen platelet composition at a temperature in the range of -10°C to -30°C for at least 1 month to form the cryopreserved platelet composition.

[00128] Provided herein, in one aspect is a process for preparing a cryopreserved platelet composition comprising cryopreserved platelets, said process comprising:

i) freezing a population of platelets in a cryopreservation medium at a temperature of less than -50°C to form an initial frozen platelet composition; and

ii) storing the initial frozen platelet composition in a freezer set at a temperature of $-20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for at least one month to form the cryopreserved platelet composition.

[00129] Provided herein, in one aspect, is a process for preparing a batch of a cryopreserved platelets, comprising:

a) pooling at least 2 platelet units into one vessel and at least another platelet unit into another vessel, in illustrative embodiments, there are at least 3, 4, or 5 platelet units, and the platelet units are from more than one donor, for example, 2, 3, 4, or more donors;

b) centrifuging each vessel to obtain a supernatant comprising plasma, and a pellet comprising platelets;

c) resuspending the pellet in each vessel to form a resuspension;

d) pooling the resuspension from each vessel to form a pooled resuspension in a pooled resuspension vessel;

e) adding a cryoprotectant to the pooled resuspension vessel having the pooled resuspension to obtain a pooled resuspension having the cryoprotectant;

f) distributing the pooled resuspension having the cryoprotectant from the pooled resuspension vessel among a number of cryo-vessels; and

g) freezing the pooled resuspension having the cryoprotectant in the cryo-vessels, to form the batch of cryopreserved platelets. In illustrative embodiments, the cryoprotectant is dimethyl sulfoxide (DMSO), and the pooled resuspension is with DMSO, to form a pooled resuspension having DMSO. In some embodiments, the resuspension has a target weight that is X times the number of units pooled or provided in the vessel. In some embodiments, the X has a range of 10g to 40g times the number of units pooled or provided in the vessel. Accordingly, the resuspension has a target weight in the range of 10g to 40g times the number of units pooled or provided in the vessel. In illustrative embodiments, the resuspension has a target weight in the range of 15.9g to 27.9g times the number of units pooled or provided in the vessel.

[00130] Provided herein, in one aspect, is a process for preparing a batch of a cryopreserved platelets, comprising:

a) pooling at least 2 platelet units into one vessel and at least another platelet unit into another vessel, in illustrative embodiments, there are at least 3, 4, or 5 platelet units, and the platelet units are from more than one donor, for example, 2, 3, 4, or more donors;

b) centrifuging each vessel to obtain a supernatant comprising plasma, and a pellet comprising platelets;

c) resuspending the pellet in each vessel to form a resuspension;

d) pooling the resuspension from each vessel to form a pooled resuspension in a pooled resuspension vessel;

e) adding a cryoprotectant to the pooled resuspension vessel having the pooled resuspension to obtain a pooled resuspension having the cryoprotectant;

f) distributing the pooled resuspension having the cryoprotectant from the pooled resuspension vessel among a number of cryo-vessels;

g) freezing the pooled resuspension having the cryoprotectant in the cryo-vessels at a temperature of less than or equal to -50°C , to form an initial frozen platelet composition in the cryo-vessels; and

h) storing the initial frozen platelet composition in the cryo-vessels at a temperature of more than or equal to -30°C , but less than 0°C , for example by storing in a -20°C freezer, to form the batch of cryopreserved platelets. In illustrative embodiments, storing is done for at least 7, 10, 15, 20, 25 days, 1 month, 2, 4, 6, 8, 10, 12 months, 2, 4, 6, 8, or 10 years.

[00131] Provided herein, in one aspect, is a collection of cryo-vessels comprising cryopreserved platelets, wherein the cryopreserved platelets in each cryo-vessel have a set of biomolecule profiles indicative of more than 1 platelet donor, in illustrative embodiments, a batch of cryo-vessels has an identical set of biomolecular profiles, and wherein each batch of the collection has a different set of biomolecular profiles than any other batch in the collection,

wherein the collection comprises a plurality of at least 2, 3, 4, or more batches of cryo-vessels,

wherein each batch of cryo-vessels comprises at least 2, 3, 4, or 5 cryo-vessels, and

wherein the coefficient of variance of a mean DMSO concentration in the cryopreserved platelets across the batches is less than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%. In illustrative embodiments, the coefficient of variance of a mean DMSO concentration in the cryopreserved platelets across the batches is in the range of 0.001-10%, 0.001-8%, 0.001-6%, 0.001-5%, 0.001-3%, 0.001-2%, 0.001-1%, 0.001-0.5%, or 0.001-0.1%.

[00132] Provided herein, in one aspect, is a collection of cryo-vessels comprising cryopreserved platelets, wherein the cryopreserved platelets in each cryo-vessel have a biomolecule profile indicative of more than 1 platelet donor, and

wherein the concentration of DMSO in the cryopreserved platelets of a first cryo-vessel is within 25%, 20%, 15%, 10%, 5%, or 1% of the concentration of DMSO in the cryopreserved platelets of a second cryo-vessel.

[00133] Provided herein, in one aspect, is a composition comprising frozen platelets in a cryopreservation medium in a frozen state, wherein the composition is capable of yielding one or more of the following properties after storage for at least 1 month, 2, 3, 4, 5, 6, 8, 10, or 12 months, in an illustrative embodiments at a temperature in a range of -10°C to -30°C , or $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$, upon thawing:

a) is in a liquid state without the addition of a liquid;

b) exhibits a platelet count of at least $1.0 \times 10^{11}/35 \text{ ml}$ of the composition;

c) yields a single peak that corresponds to a compromised membrane peak in a membrane integrity assay;

d) exhibits a CD61-positive-microparticle content of less than 50% of the CD61 positive particles in the composition, and

e) generates thrombin in an *in vitro* thrombin generation assay. In some embodiments, a composition is capable of yielding two or more, three or more, four, or all of the properties. In illustrative embodiments, a composition is capable of yielding all of the properties. In some embodiments, a composition is capable of yielding properties a), b), and d). In some embodiments, a composition is capable of yielding properties a), b), d), and e).

[00134] Provided herein, in one aspect, is a composition comprising frozen platelets in a cryopreservation medium in a frozen state, wherein the composition is capable of yielding one or more of the following properties after storage for at least 1 month, 2, 3, 4, 5, 6, 8, 10, or 12 months, in an illustrative embodiments at a temperature in a range of -10°C to -30°C, or -20°C +/- 5°C, upon thawing:

a) exhibits a platelet count of at least 1.0×10^{11} /35 ml of the composition;

b) yields a single peak that corresponds to a compromised membrane peak in a membrane integrity assay;

c) exhibits a CD61-positive-microparticle content of less than 50% of the CD61 positive particles in the composition, and

d) generates thrombin in an *in vitro* thrombin generation assay, and wherein the composition does not comprise freeze-dried platelet derivatives, or lyophilized platelet derivatives. In some embodiments, a composition is capable of yielding one or more, two or more, three, or all of the properties. In some embodiments, a composition is capable of yielding a), c) and d) of the properties.

[00135] Provided herein, in one aspect, is a cryopreserved platelet composition comprising cryopreserved platelets, wherein the cryopreserved platelets are stored at about -20 °C for a time period of at least 12, 18, or 24 months.

[00136] Provided herein, in one aspect, is a cryopreserved platelet composition comprising cryopreserved platelets, wherein the cryopreserved platelets are stored at about -20 °C for a time period in the range of 1 month to 12, 18, 24, or 36 months.

[00137] Provided herein, in one aspect, is a method of administering cryopreserved platelet composition to a subject, wherein the method includes thawing a cryo-vial of cryopreserved platelets (CPPs) from a collection from any one of the aspects or embodiments herein, or thawing a cryo-vial comprising the

cryopreserved platelet composition of any one of the aspects or embodiments herein, to prepare a liquid comprising CPPs, and administering the liquid comprising CPPs to the subject. In illustrative embodiments, after the administering bleeding of the subject is reduced.

[00138] In some embodiments of any of the aspects or embodiments herein that include a process for preparing a batch of cryopreserved platelets, or for preparing a cryopreserved platelet composition, distributing is performed, in illustrative embodiments distributing in step f) is performed to a number of cryo-vessels that are equivalent to the total number of units provided in a first step, in illustrative embodiments step a). In some embodiments, distributing is performed, in illustrative embodiments distributing in step f) is performed until a target fill weight is achieved in each cryo-vessel, and wherein the target fill weight is determined by the weight of the pooled resuspension having DMSO, in illustrative embodiments the target fill weight is determined by dividing the weight of the pooled resuspension having DMSO by the number of platelet units provided in a first step, in illustrative embodiments, step a). In some embodiments, before resuspending, in illustrative embodiments step c), removing a part of the supernatant comprising plasma is performed until a target weight of the pellet and a remainder plasma is achieved, wherein the target weight is in the range of 15.9g to 27.9g times the number of units pooled or provided in the vessel. In some embodiments, removing the part of the supernatant is performed until a weight of +/-10 g, +/-9 g, +/-8 g, +/-7 g, +/-6 g, +/-5 g, +/-4 g, +/-3 g, +/-2 g, or +/-1 g of the target weight of the pellet and remainder supernatant is achieved. In some embodiments, in step d) pooling the resuspension from each vessel is performed using a tubing tree system, in illustrative embodiments in step e) adding DMSO to the pooled resuspension vessel is performed using the tubing tree system. In some embodiments, in step f), distributing the pooled resuspension having DMSO from the pooled resuspension vessel among a number of cryo-vessels is performed using a dosing tree system. In some embodiments, the process is performed more than once to form more than one batch of the cryopreserved platelets. In some embodiments, the process is performed multiple times to form multiple batches of the cryopreserved platelets.

[00139] In some embodiments of any of the aspects or embodiments herein that include a process for preparing a batch of cryopreserved platelets, or for preparing a cryopreserved platelet composition, in step a) an odd number of platelet units are provided, and wherein one platelet unit is processed in a separate vessel. In some embodiments, in step a) 2 platelet units are pooled in one vessel to form a plurality of vessels. In some embodiments, in step c), the target weight is in the range of 31.8g to 55.7g for the vessels that have 2 platelet units, and in the range of 15.9g to 27.9g for the vessel that has 1 platelet unit. In some embodiments, in step c), the target weight is in the range of 43.5g to 49.5g for the vessels that have 2 units,

and in the range of 20.3g to 26.3g for the vessel that has 1 unit. In some embodiments, in step c), the target weight is in the range of 45.5g to 47.5g for the vessels that have 2 units, and in the range of 22.3g to 24.3g for the vessel that has 1 unit. In some embodiments, in step c), the target weight is about 46.5g for the vessel that had 2 units of the platelets, and is about 23.3g for the vessel that had 1 unit of the platelets. In some embodiments, in step a) 5, 7, 9, or 11 units are provided, and wherein each unit is from a different donor. In some embodiments, in step a) 5 units from 5 donors are provided. In some embodiments, in step a) an even number of units are provided. In some embodiments, in step a) an even number of units are provided, and 2 platelet units are pooled in one vessel, to form a plurality of vessels each having 2 platelet units. In some embodiments, in step a) an even number of units are provided, and the target weight is in the range of 31.8g to 55.7g for each of the vessels. In some embodiments, in step a) an even number of units are provided, and the target weight is in the range of 43.5g to 49.5g for each of the vessels. In some embodiments, in step a) an even number of units are provided, and the target weight is in the range of 45.5g to 47.5g for each of the vessels. In some embodiments, in step a) an even number of units are provided, and in step a) 6, 8, 10, 12, 14, 16, 18, 20, or more units are provided, and wherein each unit is from a different donor. In some embodiments, wherein an even number of units are provided, the pooling is performed for 12 units that are from 12 donors. In some embodiments, at least 2, 3, 4, 5, 6, 7, or 8 units are pooled in a vessel.

[00140] In some embodiments of any of the aspects or embodiments herein that include a process for preparing a batch of cryopreserved platelets, or for preparing a cryopreserved platelet composition, in step e) adding the DMSO is performed to achieve a concentration of DMSO in the range of 0.001-10%, 0.001-9%, 0.001-8%, 0.001-7%, 0.001-6%, 0.001-5%, 0.001-4%, 0.001-3%, 0.001-2%, 0.001-1%, 0.001-0.9%, 0.001-0.8%, 0.001-0.7%, 0.001-0.6%, 0.001-0.5%, 0.001-0.4%, 0.001-0.3%, 0.001-0.2%, 0.001-0.1%, 0.001-0.05%, 0.001-0.01%, 0.01-10%, 0.01-9%, 0.01-5.5%, 0.05-10%, 0.1-10%, 0.5-10%, 1-10%, 1-8%, 1-7%, 1-6%, 1-4%, 2-10%, 2-8%, 2-7%, 3-10%, 3-8%, 3-7%, 4-10%, 4-8%, 4-7%, 5-10%, 5-9%, 5-8%, 5-7%, or 6-10% in the pooled resuspension. In some embodiments, adding the DMSO is performed using a stock solution of 27% DMSO. In some embodiments, the time after the addition of DMSO until the cryopreservation is no more than 1 hour, 2, 3, 4, 5, or 6 hours. In some embodiments, wherein across at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 75, or 100 batches, a) a coefficient of variance of a mean resuspension volume across the batches is less than 10%; and/or b) a coefficient of variance of a mean pooled resuspension volume having DMSO in a cryo-vessel across the batches is less than 5%. In some embodiments, resuspending the pellet in each vessel leads to a resuspension in each vessel such that the

volume of the resuspension across at least 10 batches has a mean intra-batch coefficient of variance (mean of intra-batch CV) of less than 30%, 25%, 20%, 15%, 12%, 10%, 8%, 6%, 5%, 4%, 3%, 2%, or 1%, or can be in the range of 0.1-30%, 0.1-20%, 0.1-15%, 0.1-12%, 0.1-10%, 0.1-8%, 0.1-6%, 0.1-4%, 0.1-2%, 1-10%, 2-10%, 3-10%, 4-10%, 5-10%, or 6-10%. In some embodiments, resuspending the pellet in each vessel leads to a resuspension in each vessel such that the volume of the resuspension across at least 20 batches has a mean intra-batch coefficient of variance (mean of intra-batch CV) of less than 30%, 25%, 20%, 15%, 12%, 10%, 8%, 6%, 5%, 4%, 3%, 2%, or 1%, or can be in the range of 0.1-30%, 0.1-20%, 0.1-15%, 0.1-12%, 0.1-10%, 0.1-8%, 0.1-6%, 0.1-4%, 0.1-2%, 1-10%, 2-10%, 3-10%, 4-10%, 5-10%, or 6-10%. In some embodiments, resuspending the pellet in each vessel leads to a resuspension in each vessel such that the volume of the resuspension across vessels within a batch has a coefficient of variance of less than 30%, 25%, 20%, 15%, 12%, 10%, 8%, 6%, 5%, 4%, 3%, 2%, or 1%, or can be in the range of 0.1-30%, 0.1-20%, 0.1-15%, 0.1-12%, 0.1-10%, 0.1-8%, 0.1-6%, 0.1-4%, 0.1-2%, 1-10%, 2-10%, 3-10%, 4-10%, 5-10%, or 6-10%. In some embodiments, resuspending the pellet in each vessel leads to a resuspension in each vessel such that the volume of the resuspension in a vessel across at least 10 batches or within a batch varies no more than 30%, 25%, 20%, 15%, 12%, 10%, 8%, 6%, 5%, 4%, 3%, 2%, or 1%, or can be in the range of 0.1-30%, 0.1-20%, 0.1-15%, 0.1-12%, 0.1-10%, 0.1-8%, 0.1-6%, 0.1-4%, 0.1-2%, 1-10%, 2-10%, 3-10%, 4-10%, 5-10%, or 6-10%. In some embodiments, the volume of the pooled resuspension having DMSO in a cryo-vessel across at least 10 batches has a mean intra-batch coefficient of variance of less than 30%, 25%, 20%, 15%, 12%, 10%, 8%, 6%, 5%, 4%, 3%, 2%, or 1%, or can be in the range of 0.1-30%, 0.1-20%, 0.1-15%, 0.1-12%, 0.1-10%, 0.1-8%, 0.1-6%, 0.1-4%, 0.1-2%, 1-10%, 2-10%, 3-10%, 4-10%, 5-10%, or 6-10%. In some embodiments, the volume of the pooled resuspension having DMSO in a cryo-vessel across at least 20 batches has a mean intra-batch coefficient of variance of less than 30%, 25%, 20%, 15%, 12%, 10%, 8%, 6%, 5%, 4%, 3%, 2%, or 1%, or can be in the range of 0.1-30%, 0.1-20%, 0.1-15%, 0.1-12%, 0.1-10%, 0.1-8%, 0.1-6%, 0.1-4%, 0.1-2%, 1-10%, 2-10%, 3-10%, 4-10%, 5-10%, or 6-10%. In some embodiments, the volume of the pooled resuspension in a cryo-vessel having DMSO within a batch has a coefficient of variance of less than 30%, 25%, 20%, 15%, 12%, 10%, 8%, 6%, 5%, 4%, 3%, 2%, or 1%, or can be in the range of 0.1-30%, 0.1-20%, 0.1-15%, 0.1-12%, 0.1-10%, 0.1-8%, 0.1-6%, 0.1-4%, 0.1-2%, 1-10%, 2-10%, 3-10%, 4-10%, 5-10%, or 6-10%. In some embodiments, the volume of the pooled resuspension having DMSO in a cryo-vessel across at least 10 batches or within a batch varies no more than 30%, 25%, 20%, 15%, 12%, 10%, 8%, 6%, 5%, 4%, 3%, 2%, or 1%, or can be in the range of 0.1-30%, 0.1-20%, 0.1-15%, 0.1-12%, 0.1-10%, 0.1-8%, 0.1-6%, 0.1-4%, 0.1-2%, 1-10%, 2-

10%, 3-10%, 4-10%, 5-10%, or 6-10%. In some embodiments, in step c) the resuspending is performed using a buffer composition comprising a buffering agent, a base, and one or more of a saccharide. In some embodiments, the saccharide comprises one or more of a monosaccharide, disaccharide, polysaccharide, or a combination thereof. In some embodiments, the saccharide comprises trehalose, and polysucrose. In some embodiments, the buffer further comprises a salt, and an organic solvent. In some embodiments, the vessel in step a) is an apheresis platelet unit (APU) bag. In some embodiments, the APU bag has a volume of at least 600, 700, or 800 mL. In some embodiments, the APU bag has a maximum volume of at least 1000 or at least 1500 mL. In some embodiments, the APU bag has a maximum volume of 1600 mL. In some embodiments, the pooled resuspension vessel is one or more than one APU bag.

[00141] In some embodiments of any of the aspects or embodiments that include a collection of cryo-vessels comprising cryopreserved platelets, a process for preparing a batch of cryopreserved platelets, a process for preparing a cryopreserved platelet composition, or a composition comprising frozen platelets, the collection comprises a plurality of at least 3, 4, 5, 10, 15, 20, 50, 75, or 100 batches of the cryo-vessels. In some embodiments, the biomolecule profile indicative of more than 1 platelet donor, is two amino acid sequences of a first protein from a first gene that are significantly different in frequency within the cryopreserved platelets than 50%, or the presence of more than two amino acid sequences of the first protein. In some embodiments, the set of biomolecule profiles of one batch is different from the set of biomolecule profiles of another batch. In some embodiments, the coefficient of variance of a mean DMSO concentration in the cryopreserved platelets across the batches is less than 15%, 10%, 8%, 6%, 5%, 4%, in illustrative embodiments, less than 3%, 2.5%, 2%, 1.5%, 1%, 0.75%, 0.5%, 0.25%, or 0.1%. For example, the coefficient of variance of a mean DMSO concentration across the batches can be in the range of 15-0.01%, 10-0.01%, 8-0.01%, 6-0.01%, 5-0.01%, 4-0.01%, 3-0.01%, 2-0.01%, or 1-0.01%. In some embodiments, the concentration of DMSO in the cryopreserved platelets of a first cryo-vessel is within 20%, 15%, 12%, 10%, 8%, 6%, in illustrative embodiments within 5%, 3%, 1% of the concentration of DMSO in the cryopreserved platelets of a second cryo-vessel. In some embodiments, the biomolecule profile indicative of more than 1 platelet donor, is the presence of two amino acid sequences of a first protein from a first gene that are significantly different in frequency within the cryopreserved platelets than 50%, or the presence of more than two amino acid sequences of the first protein. In some embodiments, the collection comprises a plurality of at least 2, 3, 4, 5 or more batches of the cryo-vessels wherein the cryo-vessels of each batch have the same biomolecular profile, and the cryo-vessels of each batch have a different biomolecular profile from the cryo-vessels of any other batch in the collection. In some

embodiments, the cryopreserved platelets in a first cryo-vessel when thawed has a volume that varies by no more than 30%, 25%, 20%, 15%, in illustrative embodiments, 10%, 8%, 6%, 5%, 3%, or 1% of the volume in a second cryo-vessel. In some embodiments, the first and the second cryo-vessels are from a different batch of the collection of the cryo-vessels. In some embodiments, the mean DMSO concentration in the cryopreserved platelets across at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 75, or 100 batches has a coefficient of variance of less than 10%, 8%, 5%, 3%, 2%, in illustrative embodiments, less than 1%, 0.9%, 0.8%, 0.75%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, or 0.1%. In some embodiments, the mean DMSO concentration in the cryopreserved platelets across at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 75, or 100 batches has a coefficient of variance in the range of 0.01-5%, 0.01-4%, 0.01-3%, 0.01-2%, 0.01-1%, 0.05-1%, 0.05-0.75%, 0.05-0.50%, or 0.05-0.4%. In some embodiments, the concentration of DMSO in the cryopreserved platelets in a cryo-vessel within a batch or across at least 5 batches varies by no more than 5%, 4%, 3%, 2%, 1%, in illustrative embodiments, no more than 0.5%, 0.4%, 0.3%, 0.2%, 0.1%, or 0.05%. In some embodiments, the cryo-vessels in the batch or the collection comprise the cryopreserved platelets with DMSO in the range of 0.001-10%, 0.01-5.5%, 1-6%, 5-7%, 0.001-8%, 1-10%, 1-8%, 1-7%, 2-10%, 2-8%, 2-7%, 3-10%, 3-8%, 3-7%, 4-10%, 4-9%, 4-8%, or 4-7%.

[00142] In some embodiments of any of the aspects or embodiments herein that include a process for preparing cryopreserved platelets or a cryopreserved platelet composition, for preparing a batch of cryopreserved platelets, a collection of cryo-vessels comprising cryopreserved platelets, or a composition comprising frozen platelets or cryopreserved platelets, the cryopreserved platelets are stable for at least 1 month, 2, 3, 4, 6, 8, 10 months, 1 year, 2, 3, 4, 5, 6, 7, 8, 9, or 10 years at a temperature of -20°C, or -20°C +/-5°C for example, the cryopreserved platelet can be stable for 1 month to 5 years, 1 month to 12 months, 3 months to 5 years, 6 months to 5 years, 1-5 years, or 2-5 years. In some embodiments, the concentration of platelets in the cryopreserved platelets in a cryo-vessel within a batch or across at least 5 batches varies no more than 20%, 15%, in illustrative embodiments, no more than 10%, 8%, 5%, 3%, 2%, or 1%. In some embodiments, the concentration of platelets in the cryopreserved platelets in a cryo-vessel across at least 5 batches has a mean intra-batch coefficient of variance of less than 20%, 15%, in illustrative embodiments, less than 10%, 8%, 6%, 5%, 3%, or 1%. In some embodiments, the concentration of platelets in the cryopreserved platelets in a cryo-vessel within a batch has a coefficient of variance of less than 20%, 15%, in illustrative embodiments, less than 10%, 8%, 6%, 5%, 3%, or 1%. In some embodiments, the total number of platelets in the cryopreserved platelets in a cryo-vessel within a batch or across at least 5 batches varies no more than 20%, 15%, 10%, in illustrative embodiments, less than 8%, 6%, 5%, 3%, or 1%. In

some embodiments, the total number of platelets in the cryopreserved platelets in a cryo-vessel across at least 5 batches has a mean intra-batch coefficient of variance of less than 20%, 15%, 10%, in illustrative embodiments, less than 8%, 6%, 5%, 3%, or 1%. In some embodiments, the total number of platelets in the cryopreserved platelets in a cryo-vessel within a batch has a coefficient of variance of less than 25%, 20%, in illustrative embodiments, less than 15%, 10%, 8%, 6%, 5%, 4%, 3%, 2%, or 1%. In some embodiments, the total number of platelets in the cryopreserved platelets in a cryo-vessel within a batch has a coefficient of variance in the range of 0.01-25%, 0.01-20%, 0.01-10%, 0.01-8%, 0.01-6%, 0.01-5%, 0.01-3%, 0.01-2%, 0.5-10%, 0.5-8%, 0.5-5%, 0.5-3%, 1-10%, 1-8%, 1-6%, or 1-5%. In some embodiments, the cryopreserved platelets comprise plasma in the range of 50-95%, 50-90%, 50-85%, 50-80%, 50-75%, 60-95%, 65-95%, 70-95%, or 70-85% (v/v). In some embodiments, the cryopreserved platelets comprise DMSO in the range of 1-10%, 1-8%, 2-10%, 2-8%, 3-10%, 3-9%, 3-8%, 4-10%, or 4-8% (v/v). In some embodiments, the cryopreserved platelets comprise sodium chloride in the range of 5-30%, 5-25%, 5-20%, 5-15%, or 7-15% (w/v). In some embodiments, the cryopreserved platelets comprise a buffering agent, a salt, one or more saccharide, and at least one organic solvent. In some embodiments, the saccharide comprises trehalose, and polysucrose. In some embodiments, the cryo-vessel is a cryo-bag.

[00143] In some embodiments of any of the aspects or embodiments herein that include a process for preparing cryopreserved platelets or cryopreserved platelet composition that include a transition in freezing and storing temperature, a process for preparing a batch of cryopreserved platelets, or a process for preparing cryopreserved platelets or cryopreserved platelet composition, freezing can comprise freezing a population of platelets in a cryopreservation medium, or freezing a pooled resuspension having a cryoprotectant herein at a temperature of equal to or less than -50°C, -55°C, -60°C, or -65°C, to form an initial frozen platelet composition, and storing can comprise storing the initial frozen platelet composition at a temperature equal to or more than -45°C, -40°C, -35°C, -30°C, -25°C, or -20°C for at least 2, 4, 6, 7, 10, 15, 20, or 25 days, 1 month, 2, 4, 6, 8, 10, or 12 months, 1 year, 2, 4, 6, 8, or 10 years, to form the cryopreserved platelet composition. In illustrative embodiments, storing comprises storing the initial frozen platelet composition in a freezer set at a temperature of -20°C $\pm$ 2°C. In some embodiments, the freezing comprises subjecting the population of platelets in the cryopreservation medium or a pooled resuspension having a cryoprotectant as disclosed herein at the temperature for at least 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 8 hours, 12 hours, 1 day, 2 days, 7 days, 1 week, 2 weeks, 1 month, 2 months, 3 months, or 6 months to form the initial frozen platelet composition. For example, freezing can be done for a time period of 24 hours to 6 months, 24 hours to 5 months, 24 hours to 4 months, 1 day to 6 months, 1 day to 5

months, 1 day to 4 months. In some embodiments, the process further comprises thawing the cryopreserved platelets to form a liquid platelet composition, and administering an effective amount of the liquid platelet composition to a subject in need thereof. In some embodiments, the storing of an initial frozen platelet composition can be done for a time period in the range of 1 month to 10 years, 1 month to 8 years, 1 month to 7 years, 1 month to 5 years, 1 month to 3 years, or 1 month to 1 year. In some embodiments, the cryopreservation medium comprises dimethyl sulfoxide (DMSO) in a concentration in the range of 5% to 8%, for example, DMSO can be 6% $\pm$ 1%, $\pm$ 0.8%, $\pm$ 0.6%, $\pm$ 0.5%, $\pm$ 0.4%, $\pm$ 0.3%, $\pm$ 0.2%, $\pm$ 0.1%. In some embodiments, the cryopreservation medium comprises DMSO in a concentration range of 0.5% to 8%, 2% to 8%, 3% to 8%, or 5% to 8%. In some embodiments, the initial frozen platelet composition is stored at the temperature of in the range of -10°C to -30°C for at least 1 month, 2, 3, 4, 5, or 6 months to form cryopreserved platelets, and the cryopreserved platelets upon thawing exhibits one or more of the following: i) retains the ability to reduce bleeding in a subject in need thereof; ii) exhibit a platelet count recovery of at least 65%, 70%, or 75%; iii) exhibits a pH of equal to or more than 6.2; iv) exhibit an aggregate-free swirling upon a visual inspection; v) exhibits a platelet count of equal to or more than 1.7×10^{11} platelets/bag or platelets/cryo-vessel, or per 20-35 ml. In some embodiments, the mean DMSO concentration in the cryopreserved platelets across at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 75, or 100 batches has a coefficient of variance of less than 10%, 8%, 5%, 3%, 2%, in illustrative embodiments, less than 1%, 0.9%, 0.8%, 0.75%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, or 0.1%. In some embodiments, the mean DMSO concentration in the cryopreserved platelets across at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 75, or 100 batches has a coefficient of variance in the range of 0.01-5%, 0.01-4%, 0.01-3%, 0.01-2%, 0.01-1%, 0.05-1%, 0.05-0.75%, 0.05-0.50%, or 0.05-0.4%. In some embodiments, the concentration of DMSO in the cryopreserved platelets in a cryo-vessel within a batch or across at least 5 batches varies by no more than 5%, 4%, 3%, 2%, 1%, in illustrative embodiments, no more than 0.5%, 0.4%, 0.3%, 0.2%, 0.1%, or 0.05%. In some embodiments, the cryo-vessels in the batch or the collection comprise the cryopreserved platelets with DMSO in the range of 0.001-10%, 0.01-5.5%, 1-6%, 5-7%, 0.001-8%, 1-10%, 1-8%, 1-7%, 2-10%, 2-8%, 2-7%, 3-10%, 3-8%, 3-7%, 4-10%, 4-9%, 4-8%, or 4-7%. In some embodiments, the cryopreserved platelets upon thawing have a diameter in the range of 0.5-5 μ m, 1-4 μ m, 1-3 μ m, or 0.5-2.5 μ m, and wherein the composition upon thawing has a CD61-positive-microparticle content in the range of 10-30%, 10-35%, 10-40%, 10-45%, or 10-50%. In some embodiments, a therapeutically effective amount of cryopreserved platelets or cryopreserved platelet derivatives, frozen platelets or frozen platelet derivatives upon storing at a temperature equal to or more than -45°C, -40°C, -

35°C, -30°C, -25°C, or -20°C for at least 2, 4, 6, 7, 10, 15, 20, or 25 days, 1 month, 2, 4, 6, 8, 10, or 12 months, 1 year, 2, 4, 6, 8, or 10 years, upon thawing retains the ability to reduce bleeding in a subject in need thereof. In some embodiments, a therapeutically effective amount is an amount that is capable of reducing bleeding in a subject as compared to the bleeding before administering the therapeutically effective amount of thawed cryopreserved platelets or cryopreserved platelet derivatives, frozen platelets or frozen platelet derivatives. Accordingly, in some embodiments, a therapeutically effective amount can be at least 0.25 unit, 0.5 unit, 0.75 unit, 1 unit, 2, or 3 units of thawed cryopreserved or frozen platelets. For example, a therapeutically effective amount can be in the range of 0.25 unit to 5 units, in illustrative embodiments, 0.5 unit in a lower end and 3 units, 2 units, or 1 unit in a higher end, or 1 unit in a lower end and 4, 3, or 2 units in a higher end. In some embodiments, 1 unit corresponds to at least 1×10^{11} , 1.5×10^{11} , 2×10^{11} , or 2.5×10^{11} platelets in a volume of at least around 40 ml, 45 ml, 50 ml, 55 ml, or 60 ml. In some embodiments, 1 unit corresponds to platelets in a range of 1×10^{11} to 5×10^{11} , 1×10^{11} to 4×10^{11} , 1×10^{11} to 4×10^{11} , or 2×10^{11} to 3×10^{11} platelets in at least 40 or 50 ml. In illustrative embodiments, 1 unit corresponds to $2.5 \times 10^{11} \pm 4.2 \times 10^{11}$ platelets in 50 ± 4 ml. The unit can refer to a unit of frozen platelets, frozen platelet derivatives, cryopreserved platelets or cryopreserved platelet derivatives, as will be understood depending on the context.

[00144] In some embodiments of any of the aspects or embodiments herein that include a composition comprising frozen platelets or cryopreserved platelets in a cryopreservation medium in a frozen state as disclosed herein, a collection as disclosed herein, or a process for preparing a cryopreserved platelet composition or for preparing a batch of cryopreserved platelets, the composition upon storage for at least 1 day, 2, 4, 6, 7, 10, 15, 20, 25 days, 1 month, 2, 4, 6, 8, 10, or 12 months, 1 year, 2, 4, 5, 6, 8, or 10 years, in illustrative embodiments, at a temperature in a range of -5°C to -30°C, or -20°C $\pm$ 5°C upon thawing exhibits a platelet count of at least $1.0 \times 10^{11}/20$ -35 ml of the composition. For example, exhibits a platelet count of at least $1.0 \times 10^{11}/20$ ml of the composition, $1.0 \times 10^{11}/25$ ml of the composition, $1.0 \times 10^{11}/30$ ml of the composition, $1.0 \times 10^{11}/35$ ml of the composition. In some embodiments, a composition herein upon thawing exhibits a platelet count of at least $1.1 \times 10^{11}/20$ -35 ml of the composition, $1.2 \times 10^{11}/20$ -35 ml of the composition, $1.3 \times 10^{11}/20$ -35 ml of the composition, $1.4 \times 10^{11}/20$ -35 ml of the composition, $1.5 \times 10^{11}/20$ -35 ml of the composition, $1.6 \times 10^{11}/20$ -35 ml of the composition, or $1.7 \times 10^{11}/20$ -35 ml of the composition. In some embodiments, the cryopreserved platelets herein are cryopreserved platelet derivatives. In some embodiments, the frozen platelets herein are frozen platelet derivatives. Typically, a composition as disclosed herein upon thawing is in a liquid state without requiring the addition of a liquid

to achieve such a liquid state. In some embodiments, cryopreserved platelets, or frozen platelets herein do not comprise freeze-dried platelet derivatives or lyophilized platelet derivatives. In some embodiments, a composition upon thawing yields a single peak that corresponds to a compromised membrane peak in a membrane integrity assay. In some embodiments, the membrane integrity assay comprises incubating the composition with calcein acetoxymethyl (AM) to form a treated composition and analyzing the treated composition using flow cytometry. In some embodiments, the membrane integrity assay comprises incubating the composition with calcein acetoxymethyl (AM) for 20 minutes before performing the flow cytometry, and wherein the flow cytometry comprises detecting fluorescence produced by metabolized calcein AM and retained by particles in the treated composition. In some embodiments, the *in vitro* thrombin generation assay comprises generating thrombin in the presence of tissue factor, and phospholipids. In some embodiments, the *in vitro* thrombin generation assay consists of generating thrombin in the presence of tissue factor and phospholipids. In some embodiments, the frozen platelets or cryopreserved platelets herein upon thawing have a diameter in the range of 0.5-5 μ m, 1-4 μ m, 1-3 μ m, or 0.5-2.5 μ m. In some embodiments, a composition comprising frozen platelets herein upon thawing has a CD61-positive-microparticle content in the range of 5-50%, 5-45%, 5-40%, 5-35%, 5-30%, 10-30%, 10-40%, 10-50%, or 10-60%. In some embodiments, a composition as disclosed herein comprise CD61-positive-microparticles having a diameter less than 1 μ m, 0.8 μ m or 0.5 μ m. In some embodiments, a composition herein upon thawing has a pH equal to or more than 6.0, 6.2, or 6.5. In some embodiments, a composition herein upon thawing has a pH in the range of 6.2 to 8.0. In some embodiments, a composition herein have a dispersed property such that after thawing the composition, the frozen platelets therein form no visible aggregates upon a visual inspection. In some embodiments, the composition comprises a dispersed suspension property such that aggregation of platelets in the composition is not observed by visual inspection of the composition after thawing. In some embodiments, the composition has a swirling property, such that swirling of the composition can be observed by visual inspection of the composition after thawing. In some embodiments, a composition comprises a cryopreservation medium comprises dimethyl sulfoxide (DMSO) at a concentration of 0.5% to 10%, 1% to 8%, or 3% to 8%. In some embodiments, in a composition herein, no more than 50% of the frozen platelet derivatives upon thawing are positive for CD62, or Annexin V. For example, in a composition herein, particles positive for CD62 are less than 90%, 85%, 80%, 70%, 65%, 60%, or 50%. For example, in a composition herein, particles positive for Annexin V are less than 90%, 85%, 80%, 70%, 65%, 60%, or 50%. For example, in a composition herein, particles positive for Annexin V are in a range of 1-50%, 1-40%, 1-30%, 1-20%, 1-

10%, 5-50%, 5-40%, 5-30%, or 5-20%. For example, in a composition herein, particles positive for CD62 are in a range of 1-50%, 1-40%, 1-30%, 1-20%, 1-10%, 5-50%, 5-40%, 5-30%, or 5-20%. In some embodiments, frozen platelets or cryopreserved platelets in a composition herein are less activated as compared to freeze-dried platelet derivatives or lyophilized platelet derivatives. In some embodiments, frozen platelets or cryopreserved platelets in a composition are not lyophilized platelet derivatives, or not freeze-dried platelet derivatives. In some embodiments, a composition herein exhibits a platelet count of at least $1.0 \times 10^{11}/20$ ml of the composition. In some embodiments, a composition upon storage for at least 1 day, 2, 4, 6, 7, 10, 15, 20, 25 days, 1 month, 2, 4, 6, 8, 10, or 12 months, 1 year, 2, 4, 5, 6, 8, or 10 years, in illustrative embodiments, at a temperature in a range of -5°C to -30°C , upon thawing exhibits a CD61-positive-microparticle content of less than 50% of the CD61 positive particles in the composition. In some embodiments, a composition upon storage for at least 1 day, 2, 4, 6, 7, 10, 15, 20, 25 days, 1 month, 2, 4, 6, 8, 10, or 12 months, 1 year, 2, 4, 5, 6, 8, or 10 years, in illustrative embodiments, at a temperature in a range of -5°C to -30°C , upon thawing yields a single peak that corresponds to a compromised membrane peak in a membrane integrity assay. In some embodiments, a composition upon storage for at least 1 day, 2, 4, 6, 7, 10, 15, 20, 25 days, 1 month, 2, 4, 6, 8, 10, or 12 months, 1 year, 2, 4, 5, 6, 8, or 10 years, in illustrative embodiments, at a temperature in a range of -5°C to -30°C , upon thawing generates thrombin in an *in vitro* thrombin generation assay. In illustrative embodiments, a composition herein is capable of yielding one or more, two or more, three or more, or all of the properties after storing for 12 months. In some embodiments, a composition comprising frozen platelets in a cryopreservation medium in a frozen state, after storage for at least 1 month, 2, 3, 4, 5, 6, 8, 10, or 12 months, in an illustrative embodiments at a temperature in a range of -10°C to -30°C , or $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$, upon thawing exhibits a liquid state without the addition of a liquid, exhibits a platelet count of at least $1.0 \times 10^{11}/35$ ml of the composition, exhibits a CD61-positive-microparticle content of less than 50% of the CD61 positive particles in the composition, and generates thrombin in an *in vitro* thrombin generation assay.

[00145] Also provided herein are compositions produced by any of the methods described herein. In some embodiments, any of the compositions provided herein can be made by the methods described herein. Specific embodiments disclosed herein may be further limited in the claims using “consisting of” or “consisting essentially of” language.

[00146] The following non-limiting examples are provided purely by way of illustration of exemplary embodiments, and in no way limit the scope and spirit of the present disclosure.

EXAMPLES

[00147] Example 1. Single Donor Method

[00148] A single donor CPP unit is composed of one transfusable dose of apheresis platelets that has been concentrated, by centrifugation and expression, and is cryopreserved in ~6% DMSO at colder than or equal to -65°C (inside a -80°C freezer). The following is a description of the single donor process described in the art, such as in Valeri, C. Robert et al. *Transfusion* 45.12 (2005): 1890-1898, also referred to as the Vitalant method.

[00149] Step 1 - Initial Inspection of APU upon Receipt

[00150] Following receipt of the APU, it is visually inspected for swirling, aggregates, RBC contamination, and intact ports. The unit is checked for an indication that it has been irradiated. If no irradiation indicator is present, then Vitalant irradiates the unit later in the process (step 4). The time elapsed from end of collection to receipt of the APU is confirmed to be less than or equal to 48 hours.

[00151] Step 2 - Determination of Initial APC Volume

[00152] The initial APC volume is determined. Throughout the single donor process, volume of a component is determined using a weight/volume conversion method where the total weight of the component minus the tare weight of the empty bag is divided by the specific gravity of the component. For the purposes of this process the units for various measurements are as follows: weights are in grams (g), volumes are in milliliters (mL), and specific gravities are in grams per milliliter (g/mL). Equation 1 is used to determine APC weight and volume.

[00153] (Equation 1): Determination of APC Weight and Volume

[00154] APC Weight = APU Weight – Empty Bag Weight

[00155] APC Volume = $\frac{\text{APC Weight}}{\text{APC Specific Gravity}}$

[00156] APU weight contains the APC weight and the weight of the empty apheresis collection bag. The terms APC weight and APC volume describe the weight and volume of the apheresis platelet concentrate (APC) that is contained inside of an APU (apheresis platelet unit). APU volume and APC volume can be used interchangeably, as an APU contains the APC volume inside of it. The following steps are used in the manufacturing process to determine APC volume. The APU weight is determined with a scale. The weight of the empty collection bag is subtracted from the APU weight to find the APC weight. Vitalant used the empty collection bag weights that are described in TMRL.801, Common Tare Weights Job Aid. The APC

weight is converted to volume, using a specific gravity of 1.027 g/mL. This specific gravity is from TMRL.301 V.3, “Cryopreservation (Closed System) of Apheresis Platelet Units Procedure” and is considered the known specific gravity of APC, according to literature and standard blood banking practices. This APC specific gravity is used throughout herein.

[00157] Step 3 - APC Volume Reduction (if required)

[00158] If the APC volume is above 375 mL then typically a sample is removed to reduce the APC volume until it is inside of the processing range. A 5 mL transfer set (Charter Medical, 6" Tubing with Piercing Pin and Removeable 5 mL BD™ Syringe, product number 03-220-XL, or equivalent) is connected to the APU using a sterile connecting device (SCD, Terumo TSCD® Sterile Tubing Welder, SC-201A). This 5 mL transfer set is then used to remove the sample. This method of sample removal is used throughout the single donor process to remove samples. After sample removal, the APC volume is redetermined.

[00159] Step 4 - APU Irradiation (if required)

[00160] If the APU was not previously irradiated, then it is irradiated at this point in the process.

[00161] Step 5 - APU Sampling and Confirmation of Pre-Manufacturing Specifications

[00162] The APU is then placed on a platelet incubator/agitator for at least 30 minutes. The APU is inspected for swirling and aggregates again. The APU is then sampled for pH/blood-gas and platelet concentration analysis. A copy of the pH/blood-gas printout is placed in the batch record. After sampling, the APC volume is redetermined. Equation 2 is used to determine the APU total platelet count, using the platelet concentration and APC volume.

[00163] (Equation 2): *Determination of APU Total Platelet Count*

$$\text{Total Platelet Count} = \text{APC Volume} * \text{Platelet Concentration}$$

[00164] The cryopreservation process can continue once it is confirmed that the APU meets all pre-manufacturing specifications. The following excerpt from TMRL.301 V.3 describes the handling of APUs that do not pass the pre-manufacturing specifications, “If the APU does not meet criteria (has a negative swirl, aggregates are present, has a platelet yield $< 3.0 \times 10^{11}$, or a volume < 165 or > 375 mL), place the APU back in the platelet incubator/agitator and discuss with TMRL Director (or designee) how to proceed. Complete a deviation report as necessary per the Occurrence/Deviation Reporting Procedure: TMRL113.”

[00165] Step 6 - 27% DMSO Addition

[00166] Sterile, injectable grade 27% DMSO and 0.66% sodium chloride in water (Bio Life Solutions, BloodStor® 27 NaCl Biopreservation Media, part number 327207, or equivalent) is used to formulate the

APC to a final DMSO percentage (%DMSO) of 5.65% – 6.52%. Throughout this report the APC and DMSO solution is referred to as APC/DMSO. The APU is formulated with 27% DMSO using Table 1:

[00167] Table 1: TMRL.806 V.1, “Weight of 27% DMSO to Add for Freezing Platelet Job Aid”

APC Volume (mL)	165 – 189	190 – 219	220 – 259	260 – 299	300 – 339	340 – 375
27% DMSO Weight to add (g)	50	60	71	81	92	102

[00168] The 27% DMSO bag is welded onto the APU using an SCD. The APU is placed on a scale to measure the weight of the 27% DMSO that is added to the APU. The 27% DMSO bag is suspended above the APU to allow the 27% DMSO to flow gravimetrically for the 27% DMSO addition step. The 27% DMSO is then slowly added to the APU, and the APU is lightly massaged to promote mixing. The 27% DMSO is added at a rate of 10 – 15 g/min until the 27% DMSO target weight is reached. The start and end times of the 27% DMSO addition are recorded. If the rate of 27% DMSO addition falls outside of the desired range of 10 – 15 g/min a deviation report is completed, and manufacturing continues.

[00169] Step 7 - % DMSO Calculation

[00170] The APU is then weighed to find the APC/DMSO Volume and the %DMSO, using Equations 3 and 4.

[00171] (Equation 3): Determination of APC/DMSO Volume

$$\text{APC/DMSO Volume} = \frac{\text{APU/DMSO Weight} - \text{Tare Weight}}{\text{APC/DMSO Specific Gravity}}$$

[00172] Vitalant lists the APC/DMSO specific gravity as 1.03g/mL in TMRL.301 V.3, Cryopreservation (Closed System) of Apheresis Platelet Units Procedure.

[00173] (Equation 4): Determination of %DMSO

[00174] 27% DMSO Dispensed (mL) = APC/DMSO Volume – APC Volume

$$\text{[00175] DMSO\%} = \frac{\text{27\% DMSO Dispensed (mL)}}{\text{APC/DMSO Volume}} * 27\%$$

[00176] The %DMSO is recorded and it is determined if the %DMSO is in range for the product specification (5.65% – 6.52%).

[00177] Step 8 - Sampling for Culture of APU Post-DMSO Addition

[00178] A sample of the APC/DMSO is removed for the purposes of aerobic and anaerobic culturing using BacT/ALERT.

[00179] Step 9 - Platelet Material Transfer

[00180] A tubing extension set (such as a plasma transfer set, Charter Medical, 24" Tubing, Roller Clamp and Two Piercing Pins, product number 03-220-00, or equivalent) is welded between a cryobag (500 mL EVA CryoStore freezing bag, Origen Reference CS500, or equivalent) and the APU to connect the two bags and to extend the working length of the tubing for centrifugation and expression. The APC/DMSO solution is then transferred from the APU bag into the cryo-bag. Any excess air in the cryobag is expressed into the empty APU bag, and the tubing is clamped.

[00181] Step 10 - Centrifugation

[00182] The cryo-bag and empty APU bag then undergo centrifugation at 1250 G for 10 minutes with maximum acceleration, no brake, and slow stop setting set to 3 (Thermo Scientific, Sorvall RC3BP+ centrifuge).

[00183] Step 11 - Expression

[00184] The cryo-bag is carefully removed from the centrifuge cup, as not to disturb the platelet pellet, and is placed in a plasma expressor (such as Fenwal Inc., manual plasma extractor, product code 4R4414, or equivalent). If leaks are noticed, then typically the unit is not used for patient infusion. The plasma expressor is used to remove the supernatant without losing platelets. After expression is complete, the cryobag is weighed. The cryobag should weigh between 50 g – 55 g to meet the volume specification needed and to account for additional volume removed for pre-freezing sampling. If the weight is below 50 g, release the tubing clamp and slowly flow material from the plasma/DMSO waste bag back into cryobag to achieve a weight of 50 g – 55 g. This range is equivalent to 25.5 mL – 30.4 mL. The pre-sampling volume is then determined to ensure that the post-sampling volume will be 20 – 35 mL.

[00185] Step 12 - Platelet Pellet Rest and Resuspension

[00186] A 30-minute ($\pm$ 5 minutes) resting period at 20-24°C is needed prior to resuspension of the platelet pellet. The pellet is resuspended by gentle manual agitation for several minutes. The cryobag is inspected for visible aggregates and it is recorded if aggregates are present.

[00187] Step 13 - Post-Resuspension Sampling

[00188] If the resulting volume after sampling will be less than 20 mL then the sampling is skipped. If the volume is enough to proceed then a pre-freezing sample of ~2 mL is removed. The sample is removed by gently filling a 5 mL syringe (part of the 5 mL transfer set) and expelling back several times to mix the platelet suspension to obtain a well-mixed sample.

[00189] Step 14 - Pre-Freeze Volume Determination

[00190] The pre-freeze volume is determined using a standard empty cryobag tare weight of 22.8 g and a weight/volume conversion that uses the APC/DMSO specific gravity of 1.03 g/mL. This post-freeze volume determination is to ensure that the volume is within range for freezing. The pre-freeze volume range is 20 mL – 35 mL.

[00191] Step 15 - Freezing

[00192] The cryobag is labeled and a final visual inspection of the cryobag is performed to ensure the bag is intact and not leaking. The cryobag's excess tubing is sealed off using a tube sealer (SEBRA® Hand-Held RF Tube Sealing System, model number 2380, or equivalent) and safety seals are added to the remaining tubing. The cryobag is placed into a thawing bag and freezer carton for storage in a -80°C freezer. The unit is placed in the freezer and the start time of freezing is recorded.

[00193] Step 16 - Timing Specifications

[00194] The time elapsed from the end of 27% DMSO addition to the start time of freezing typically be kept less than or equal 2 hours and 45 minutes. Time elapsed from the end of APU collection to placement in the freezer typically be less than or equal to 57 hours. If these time specifications are not met, a deviation report is completed.

[00195] Post Manufacturing Specifications:

TMRL 805 V.2 lists the following post-manufacturing specifications for a CPP unit:

- Freeze Volume: 20 mL – 35 mL
- Time from addition of 27% DMSO to freezer: less than or equal to 2 hours and 45 minutes
- Age at end of processing: less than or equal to 57 hours.
- Visible aggregates: none
- Sterility testing: no growth
- %DMSO: 5.65% – 6.52%
- Maximum DMSO in a CPP unit: 2.53 g (the mass corresponding to the maximum freeze volume with the maximum %DMSO)

Any product not meeting final acceptable criteria (non-conforming products) shall be, at the discretion of the TMRL Director, designated as a miscellaneous product or will be disposed of.

[00196] Analysis of certain important steps in single donor process

[00197] 27% DMSO addition

[00198] DMSO at 5% to 10% is commonly used as a cryoprotectant in biological applications and is added to the APC for its cryoprotective properties.

[00199] Weaknesses in the Previous 27% DMSO Addition Method

[00200] The Vitalant method of adding 27% DMSO based on Table 1 is a very simplistic method of formulating the product, however it has several shortcomings. Dosing the 27% DMSO in this manner creates a process that targets a variable %DMSO. This leads to a product that has an inherently variable %DMSO. Additionally, Table 1 can lead to units that are outside of the %DMSO specification. FIG. 3 illustrates these issues. FIG. 3 displays the target %DMSO that will be achieved using Table 1. Equations 1, 3, and 4 were used to determine the target %DMSO. The variability of %DMSO displayed in FIG. 3 equates to an average percent difference of 10.6%, from the mean of 27% DMSO dosing range. Analysis A explains the math that was used in determining all data points in FIG. 3.

[00201] Analysis A: Analysis of Table 1

[00202] A 189.0 mL APU falls in the 50 g of 27% DMSO dosing range according to Table 1. Using a volume/weight conversion like Equation 1, the APC weight of this APU will be 194.1 g. After the addition of the 50 g of 27% DMSO required by Table 1, the APC/DMSO weight will be 244.1 g, or 237.0 mL. Using Equation 4 to determine the percent DMSO indicates that a 189 mL APU dosed with 50 g of 27% DMSO will be formulated to 5.47% DMSO, which is below the stated specifications.

[00203] Freezing volume

[00204] The fundamental purpose of the centrifugation and expression step is to concentrate the platelets into a lower volume so less DMSO is infused when the CPP transfusion takes place. Centrifugation accomplishes this concentration and reduction in overall volume by pelleting the platelets from the plasma/DMSO during the spin, then excess plasma/DMSO can be aspirated using a plasma expressor. The expression step (Step 11) is the determining factor in the freezing volume. The overall variability that is present in Vitalant's expression step and freezing volume is demonstrated in FIG. 4A (FIG. 2A from Vitalant's Long Term Stability Characteristics of Cryopreserved Platelets at -80°C, Protocol 11-5-J). A large range of volumes is observed, suggesting little control over this processing step. In Vitalant's Long Term Stability Characteristics of Cryopreserved Platelets at -80°C, Protocol 11-5-J, aggregates were observed in CPP units that had low freeze volumes. This demonstrates the importance of controlling the expression and fill volume of the process. FIG. 4B (FIG. 2B from the stability study) gives the distribution of freezing volumes for units in which aggregation was observed after thawing; aggregation was observed only in the lower freeze volumes.

[00205] The previous expression step instructed manufacturing staff to, “remove as much supernatant as possible without losing platelets.” This method of using visual confirmation is uncontrolled and variable, as shown by FIG. 4A.

[00206] **Freezing Protocol**

[00207] The freezing protocol ensures that the freezing is uniform, from unit to unit. All CPP units are placed in a thawing bag and then placed flat in a cardboard box. The flat orientation of the CPP unit ensures uniformity in the heat transfer from the cryobag to the freezer shelf. The same product enclosure system is used for all units, and all units are placed on an empty shelf in a freezer that is at -80°C, to minimize unit-unit variability.

[00208] **Example 2. Exemplary improved process of preparing cryopreserved platelets**

[00209] A non-limiting, exemplary improved processes for preparing cryopreserved platelets using a pool of platelet units as a starting material as disclosed in the Examples, is referred to in these Examples as the pooled CPP process or the exemplary pooled CPP process. The exemplary pooled CPP process accommodates the inclusion and pooling of platelets from 12 apheresis platelet units (APUs), with the goal of improving product control, decreasing variability of the final product, and increasing scalability. The exemplary pooled CPP process incorporates all major process steps that are present in the single donor CPP process of Example 1, however the process has been improved to streamline the process and to create batches of a more homogeneous final product, while limiting batch-to-batch variability of the final product. FIG. 1B is a non-limiting flowchart of the exemplary pooled CPP process. A summary of major steps between the exemplary pooled CPP process and the single donor process is shown below.

[00210] Table 2

Major Step #	Single Donor Process (1 APU)	Exemplary Pooled Process (up to 12 APUs)
1	Formulate APU to ~6% DMSO	Pool 2 APUs Together
2	Centrifuge	Centrifuge
3	Express Plasma/DMSO	Express Plasma
4	Resuspend Platelets	Resuspend Platelets to a target weight

5	Freeze	Pool Resuspended Platelets
6	N/A	Formulate to ~6% DMSO
7	N/A	Fill/Freeze

[00211] The exemplary pooled CPP process creates multiple doses of a homogeneous final product. This allows one to perform representative product characterization testing for all pooled CPP units sent for infusion and allows for a product archive of all batches produced. The single donor process does not allow for the same kind of product quality assurance. Multiple doses from the same final product will also allow one to initiate stability studies that will directly compare the same product at different time points, allowing for a true representation of product stability. This protocol maintains the same product formulation of the cryopreserved platelets (ratios of platelets, plasma, saline, and DMSO) and a similar overall process.

[00212] An illustrative, non-limiting process of preparing the cryopreserved platelets as disclosed herein is shown below.

[00213] The exemplary pooled CPP process has been designed to incorporate up to 12 apheresis platelet units (APUs) from 5 to 10 donors into a single pool to produce up to 12 pooled CPP units. The process can be completed with less than 12 APUs, however a minimum of 5 APUs from 5 donors are needed. The number of initial APUs equals the number of pooled CPP units produced. The pooled CPP product has the same composition as the single donor product (77.5% residual plasma from the APUs, 16.5% of a 0.66% NaCl saline solution, and ~6% DMSO). Like a single donor CPP unit, a pooled CPP unit is composed of one transfusable dose of apheresis platelets that has been concentrated, by centrifugation and plasma expression, and is cryopreserved in ~6% DMSO at colder than or equal to -65°C (inside a -80°C freezer).

[00214] Step 1 - Initial Quality Control (QC) of APUs upon Receipt

[00215] Prior to the APUs being released for manufacturing, the QC department confirms that all APUs meet the following pre-manufacturing specifications, according to QCT-001.

Leukoreduced: Less than 5×10^6 WBCs – responsibility of collecting facility.

Platelet Age: less than or equal to day 2.

Gamma irradiated at 25 Gy, responsibility of the collecting facility.

Ports of APU are intact.

No indication of red blood cell (RBC) contamination.

Aggregate free swirling of APC.

pH: greater than or equal to pH 6.2.

Average APU total platelet count: greater than or equal to 2.5×10^{11} platelets.

[00216] Step 2 - Quality Assurance (QA) Line Clearance

[00217] The QA Department completes line clearance of the processing before manufacturing begins.

[00218] Step 3 - Transfer of APUs to Manufacturing and Manufacturing Inspection of APUs

[00219] Following the QA and QC duties, the APUs are released for manufacturing. The start time of production is recorded when the APUs are removed from the QC platelet incubator and physically given to Manufacturing Staff. Upon receipt of the released APUs, the Manufacturing Staff completes several quality checks to verify the APUs are acceptable. The APUs are visually inspected for swirling, aggregates, red cell contamination, and it is confirmed that the APUs have been irradiated. The Donor ID and expiration date of the APUs are recorded in the batch record for traceability and tracking purposes.

[00220] Step 4 - Creating 2-Unit Pools of APUs

[00221] After all units are determined to be acceptable, an SCD is used to weld a plasma transfer set onto an APU and then a second APU is welded onto the other end of the plasma transfer set. The plasma transfer set is added to extend the working length of the tubing. The two APUs are then pooled together into a single APU bag. This is done 6 times to create 6 pools of 2 APUs from the initial 12 APUs. The sterile connecting device used is a Terumo, TSCD II Sterile Tubing Welder, model number 3me-SC203a (or equivalent). The plasma transfer sets used are Charter Medical, 24" Tubing, Roller Clamp and Two Piercing Pins, product number 03-220-00 (or equivalent).

[00222] Step 5 - Determination of Pooled APC Weight

[00223] The pooled APC weights are determined and recorded for later use in the expression step. For each of the 6 pooled APUs, the pooled APC weight is determined using the following equation:

$$\text{[00224] Pooled APC Weight} = \text{Pooled APU Weight} - \text{Empty Bag Weight}$$

[00225] The pooled APU weights are determined with a scale (Ohaus Adventurer Precision Balance, product number AX8201/E, or equivalent). The empty bag weight is a known value that corresponds to the type of bag that was used for the apheresis platelet collection.

[00226] Step 6 - Centrifugation

[00227] Each pooled APU is placed into a centrifuge cup. The cups are balanced by weight (if needed) and then loaded into the centrifuge. The pooled APUs undergo a 1250 G centrifugation for 10 minutes at maximum acceleration, with a 10-minute deceleration (Beckman-Coulter Avanti J-HC Centrifuge).

[00228] Step 7 - Expression

[00229] The plasma removal target weight for expression is determined using Equation 5 by subtracting 46.5 g from the pooled APC weight. This is done to leave behind approximately 46.5 g of platelet pellet and plasma after the pooled APU is expressed.

[00230] (Equation 5): *Determination of Expression Endpoint*

[00231] *Plasma Removal Target = Pooled APC Weight – 46.5 g*

[00232] Each pooled APU is taken out of the centrifuge and expressed one-by-one. The pooled APU is carefully removed from the centrifuge cup, as not to disturb the platelet pellet, and then placed in the plasma expessor (Fenwal Inc., manual plasma extractor, product code 4R4414, or equivalent). The empty APU bag is placed on a scale and tared to weigh the expressed plasma. The pooled APU is then expressed. Once the plasma removal target is reached (± 1.0 g) the expression is stopped.

[00233] Step 8 - Post-Expression Weight Check

[00234] The post-expression pellet weights are determined to ensure that the weight of the platelet pellet and supernatant is within a target range for further processing (31.8 g – 55.7 g). If the post-expression weight is outside of the range, the supernatant will be added or removed accordingly, until the post-expression weight is within range.

[00235] Step 9 - Resuspension

[00236] Once the post-expression weight is within range, the pellet is resuspended by gently rocking and massaging the APU bag until the pellet is no longer visible. After the pellet is no longer visible there is a 5-minute ambient temperature resting of the resuspended platelet pellet. The resuspended pellet is then visually inspected for aggregates. If aggregates are observed at this point in the process, and they do not disappear after further resting and agitation, manufacturing management is informed, processing continues, and a 30-minute ambient temperature rest with gentle agitation is added to the process after the addition of 27% DMSO.

[00237] Step 10 - Pooling

[00238] Once the 6 pellets are resuspended, the 6 APU bags are welded onto a sterile tubing tree (Optimum Processing, Inc., part number 02817, or equivalent) to create a “pooling tree system” and the resuspended platelet pellet material is pooled into a single APU bag. FIG. 1C is a picture of the tubing tree that was used to create batches or lots of cryo-vessels using the method herein.

[00239] Step 11 - Calculation of 27% DMSO Target Weight

[00240] The sum of the previously measured post-expression weights is used to determine the total post-expression weight, using Equation 6. The total post-expression weight is used in Equation 7 to calculate the 27% DMSO Target Weight needed to formulate the pooled, resuspended platelet material to ~6% DMSO. Equation 7 uses a “DMSO Constant” of 0.2946, derived in the section titled “Development of 27% DMSO Addition Method for Pooled CPP.”

[00241] (Equation 6): *Determination of Total Post-Expression Weight*

[00242] *Total Post Expression Weight = Sumation of Platelet Pellet Weights*

[00243] (Equation 7): *Determination of 27% DMSO Target Weight*

[00244] $27\% \text{ DMSO Target Weight} = \text{APC Weight} * 0.2946$

[00245] Step 12 - Addition of 27% DMSO

[00246] A bag containing sterile, injectable grade 27% DMSO and 0.66% sodium chloride in water (Bio Life Solutions, BloodStor® 27 NaCl Biopreservation Media, part number 327207, or equivalent) is then welded onto the pooling tree system. The 27% DMSO bag is placed on a scale to weigh how much 27% DMSO is leaving the 27% DMSO bag and entering the pooling tree system. The 27% DMSO bag is placed higher than the pooling tree system to allow the 27% DMSO to flow gravimetrically. The 27% DMSO Target Weight ± 1.0 g is added to the pooling tree system. The tubing to the 27% DMSO bag is clamped to prevent additional 27% DMSO from entering the system.

[00247] Step 13 - Rinsing

[00248] The 27% DMSO that entered the pooling tree system is used to rinse the system to recoup any residual platelet material that remains in the APU bags and in the tubing of the pooling tree system. The solution is then added to the APU that contains the pooled, resuspended platelet pellets. Tube strippers are used to strip any solution that may remain in the pooling tree system, to ensure that all 27% DMSO is added to the platelets.

[00249] Step 14 - Fill Volume Determination of Cryobags

[00250] The APU bag containing the final product is welded onto a new tubing tree to fill 12 cryobags. This creates a “filling tree system.” The final product weight is then determined by weighing the APU bag and subtracting the empty bag weight (empty bag weight determination is explained in Step 5 “Determination of Pooled APC Weight”). The final product weight is divided by 12 to determine the maximum fill weight of the cryobags. The minimum fill weight is determined by subtracting 2.0 g from the maximum fill weight. This determines the fill weight range of the cryobags.

[00251] Step 15 - Cryobag Filling Procedure

[00252] The 12 cryobags (250 mL EVA CryoStore freezing bag, Origen Reference CS250, or equivalent) are then welded onto the dosing tree system. The filling procedure for a cryobag takes place by placing the cryobag on the scale, priming the lines of the cryobag until just before the product enters the cryobag, and then taring the cryobag. The cryobag is then filled with the final product until it is within range. The fill weight of each cryobag is recorded and their volume is determined by dividing the weight by 1.03 g/mL.

[00253] Step 16 - Freezing

[00254] Each cryobag is then placed into a thawing bag and freezer carton for storage in a -80°C freezer. The units are then placed in the freezer and the start time of freezing is recorded. Whenever required, after a certain time-period of freezing at -80°C freezer, certain number of cryobags were transferred to a freezer set at a temperature of -20°C, and stored at the temperature to form transition temperature cryopreserved-product. The time elapsed from the end of 27% DMSO addition to the start time of freezing typically be equal to or less than 3 hours.

[00255] Post-Manufacturing Specifications:

[00256] All CPP units produced using the exemplary pooled CPP process, typically, meet the following specifications prior to freezing:

Freeze Volume: 20 mL – 35 mL

Time from addition of 27% DMSO to freezer: less than or equal to 3 hours

Frozen by the end of day 2 of platelet age

Visible aggregates in cryobag: none

%DMSO: 5.65% – 6.52%

Maximum DMSO in a CPP unit: 2.53 g (the mass corresponding to the maximum freeze volume with the maximum %DMSO).

[00257] Summary for Batches with an Odd Number of Initial APUs

[00258] If there is an odd number of initial APUs then the following steps are altered to include additions that will allow for the pooled process to accommodate the single APU. All other steps, calculations, and specifications for the single APU, and the pooled process, remain unchanged.

[00259] Step 4 (Creating 2-Unit Pools of APUs):

A plasma transfer set is welded onto the odd APU and a 600mL transfer bag (Terumo, TeruFlex Transfer Bag, catalog number: 1BB*T060CB71, or equivalent) is welded onto the other end of the plasma transfer set. The APC of the odd APU remains in the APU bag.

[00260] Step 7 (Expression):

Equation 5, which calculates the plasma removal target to determine the expression endpoint, is altered to account for the odd APU being a single APU and not a pooled APU:

$$\text{Plasma Removal Target (for single APU)} = \text{Odd APC Weight} - 23.3g$$

[00261] Step 8 (Post-Expression Weight Check):

[00262] The post-expression pellet target weight range is changed to 15.9 g – 27.9 g, to account for the odd APU being a single APU and not a pooled APU.

[00263] Example 3. Process Development for Pooled CPP as disclosed herein**[00264] Standardization of Weighing Practices:**

[00265] To increase precision and accuracy, a standard orientation of weighing all bags has been implemented for the purposes of weight determination and weight/volume conversion. APU bags are weighed by folding one third of the bag underneath itself and then it is centered and placed to the back edge of the scale, while ensuring all of the bag is on the scale and that no unnecessary tubing is on the scale. Cryobags are placed on the scale in a similar fashion, however their smaller profile does not require the bag to be folded.

[00266] Elimination of Incoming APC Volume Specifications:

[00267] The single donor process had a pre-processing APC volume specification of 165 mL – 375 mL. This volume specification was included to ensure that the APUs were acceptable for their process. APUs outside of this specification would not adhere to the 27% DMSO addition table (Table 1). Due to changes in centrifugation practices and 27% DMSO addition methods this volume specification is no longer relevant for the pooled process. The upper end of this volume specification was included due to a processing constraint stemming from the cryobag size that was previously used and the centrifugation practices of the single donor method. The single donor method requires formulating the APC to ~6%

DMSO prior to centrifugation and transferring the APC/DMSO to a cryobag, then centrifuging the APC/DMSO in the cryobag. An incoming APU with a volume of 375 mL will become 473 mL after the 27% DMSO addition. Since a 500 mL cryobag was used for centrifugation, anything above ~473 mL will either not fit in the cryobag or will result in significant “pillowing” of the cryobag. The pooled process does not add the 27% DMSO prior to centrifugation, so there is no increase in the working volume of the platelet material prior to centrifugation. The pooled process also uses an APU bag for centrifugation rather than a cryobag. An apheresis platelet bag can hold a maximum volume of ~1600 mL and can comfortably hold ~800 mL without pillowing. Therefore, a processing constraint for volume is not necessary with the pooled process. Eliminating this volume specification allows more APUs to be acceptable for processing. Additionally, unnecessary stress on the cryobag has been removed from the process, since the cryobag is not undergoing centrifugation. This practice of centrifuging in the cryobag allows for the possibility of a cryobag bursting in the centrifuge and induces unnecessary stress on the cryobag. This unnecessary stress can cause loss of integrity and increases the chance for potential breakage further downstream in the freezing and thawing steps of product handling.

[00268] Derivation of DMSO Constant:

[00269] The DMSO constant in Equation 7 is derived from the $C_1V_1=C_2V_2$ calculation that is used in Equation 4 to calculate %DMSO. The derivation of the DMSO constant is shown below:

[00270] $C_1 * V_1 = C_2 * V_2$

[00271] $C_1 = 27\% \text{ DMSO}$

[00272] $V_1 = 27\% \text{ DMSO Volume} = X \text{ (the value of interest)}$

[00273] $C_2 = 6.085\% \text{ DMSO (middle of \%DMSO specification)}$

[00274] $V_2 = \text{APC/DMSO Volume} = \text{APC Volume} + 27\% \text{ DMSO Volume} = \text{APC Volume} + X$

[00275] Substitute V_1 and V_2 into the $C_1*V_1 = C_2*V_2$ equation:

[00276] $C_1 * X = C_2 * (\text{APC Volume} + X)$

[00277] Substitute in the known DMSO percentages (C_1 and C_2):

[00278] $27\% * X = 6.085\% * (\text{APC Volume} + X)$

[00279] Rearranging the equation to solve for X gives the following:

[00280] $X = \frac{6.085\%}{27\% - 6.085\%} * \text{APC Volume}$

[00281] $X = 0.2909 * \text{APC Volume} = 27\% \text{ DMSO Volume}$

[00282] This constant of 0.2909 when multiplied by the APC volume will calculate the 27% DMSO volume needed to achieve 6.085 %DMSO. Since the in-process measurements are weights the constant is

then converted to account for this, to eliminate unnecessary weight/volume conversions and to simplify the calculations that the operator must perform. This conversion was done by using the 27% DMSO specific gravity and the APC specific gravity.

$$[00283] \text{ 27\% DMSO Volume} = \frac{27\% \text{ DMSO Weight}}{1.04 \frac{\text{g}}{\text{mL}}}$$

[00284] This specific gravity of 1.04 g/mL is from the excipient SDS.

$$[00285] \text{ APC Volume} = \frac{\text{APC Weight}}{1.027 \frac{\text{g}}{\text{mL}}}$$

[00286] 1.027 g/mL is the known specific gravity of APC.

[00287] Substitute in these formulas for APC volume and 27% DMSO volume into the previous equation that solved for 27% DMSO volume:

$$[00288] \frac{27\% \text{ DMSO Weight}}{1.04} = 0.2909 * \frac{\text{APC Weight}}{1.027}$$

[00289] Rearrange equation to solve for 27% DMSO weight:

$$[00290] \text{ 27\% DMSO Weight} = \left(\frac{0.2909 * 1.04}{1.027} \right) * \text{APC Weight}$$

$$[00291] \text{ DMSO Constant} = \frac{0.2909 * 1.04}{1.027} = 0.2946$$

[00292] This DMSO constant (0.2946) is a unitless value that when multiplied by the APC weight, will determine the weight 27% DMSO needed to formulate the APC/DMSO to 6.085% DMSO (6.09% DMSO when rounded).

[00293] Implementation of 27% DMSO Addition Calculation

[00294] For the exemplary improved pooled process or exemplary pooled CPP process, a method of formulating the CPP product to a fixed %DMSO was developed to eliminate the problems identified in the analysis of the previous 27% DMSO addition method of Example 1 (Table 1 and Analysis A). This exemplary pooled CPP process is intended to reduce batch-to-batch variability of the product formulation and ensure that the APC/DMSO is consistently inside the percent DMSO (%DMSO) specification. Using the DMSO constant produces a method where every or virtually every pooled CPP batch will be formulated to achieve the same target percent DMSO. A tolerance of ± 1.0 g of 27% DMSO has been included to ensure that the percent DMSO is always in-specifications. To demonstrate the theory of this, Analysis B applies the DMSO constant method of formulating a 189 mL APU as performed for the improved exemplary pooled CPP process. A comparison of Analysis B with Analysis A demonstrates how the DMSO constant can eliminate the issues in the prior single-donor method of Example 1.

[00295] (Analysis B): *Case Study of 27% DMSO Addition Methods for Pooled CPP*

[00296] *Weight of 27% DMSO needed = APC Weight * DMSO Constant*

[00297] *Weight of 27% DMSO needed = 194.1 g * 0.2946 = 57.2 g of 27% DMSO*

[00298] Using the same mathematical approach as Analysis A, when a 189.0 mL APU is dosed with 57.2 g of 27% DMSO, the APU in Analysis B is formulated to 6.09% DMSO.

[00299] FIG. 5 is like FIG. 5 and compares the correlation of APC volume to %DMSO of the two 27% DMSO addition methods. FIG. 5 illustrates the increased precision and accuracy of the exemplary pooled CPP process. The old method refers to the use of Table 1 that is used in the single donor CPP process and Analysis A. The exemplary pooled CPP process refers to the use of Equation 7 that is used in the exemplary pooled CPP process and Analysis B. The plot for the exemplary pooled CPP process also displays lines for the tolerances of ± 1 g from the 27% DMSO target weight.

[00300] FIG. 6 illustrates the correlation between post-expression volume and percent DMSO for the exemplary pooled CPP process.

[00301] FIG. 6 encompasses the full range of post-expression volumes that are capable with the pooled process. The lowest achievable post-expression volume is from a 5-unit batch where all APUs are expressed to the lower end of the post-expression range. The highest achievable post-expression volume is from a 12-unit batch where all APUs are expressed to the upper end of the post-expression range. Lines for the ± 1 g tolerances of the 27% DMSO target weight are also included. FIG. 6 demonstrates that percent DMSO of the exemplary pooled CPP process will always be in specification, for all batch sizes (including the minimum batch size of 5 APUs), this eliminates the need for a confirmatory percent DMSO calculation. Table 3 is composed of in-process percent DMSO data from all cGMP-like pooled CPP batches that have been produced (12 batches, encompassing 136 pooled CPP units).

[00302] Table 3

Batch#	28	30	31	35	36	37	38	41	42	44	46	47	Mean	CV
%DMSO	6.14	6.05	6.09	6.06	6.09	6.10	6.09	6.10	6.12	6.09	6.09	6.08	6.09	0.39%

[00303] Table 3 demonstrates the precision (low CV) and accuracy (target %DMSO is 6.09%) that was obtained with the 27% DMSO addition method (a step of exemplary pooled CPP process) embodiment provided in Example 2.

[00304] **Optimization of Excipient Usage:**

[00305] The single donor process of Example 1 requires adding 27% DMSO prior to the platelets being concentrated by centrifugation and plasma expression. Adding 27% DMSO prior to centrifugation

increases the working volume of the solution by ~30%, which limits scalability for the single donor process. An additional hinderance to scalability of the single donor process comes from the fact that this method also requires most of the 27% DMSO to be disposed of as waste, as the majority of the added 27% DMSO is removed during plasma expression. For instance, in Analysis B, a 189 mL APU will have 57.2 g of 27% DMSO added to the whole APU. After centrifugation, if the plasma/DMSO is expressed to retain 28.0 mL of residual plasma/DMSO and platelet pellet, then 216.0 mL of the plasma/DMSO is removed. This equates to 48.7 mL of the 27% DMSO being removed from the CPP unit, while 6.3 mL of the 27% DMSO is retained in the cryobag with the platelets. This is an inefficient use of the product's only excipient as ~90% of the excipient is removed from the final product and disposed of as waste. The pooled process adds the 27% DMSO after centrifugation and plasma removal, therefore the excipient usage is optimized, none is disposed of as waste, and only the amount of 27% DMSO that is required to formulate the product to the target %DMSO is consumed.

[00306] Addition of Plasma Removal Target In-Process Control:

[00307] Equation 5 was created to determine the endpoint of expression to standardize the expression step. This equation uses the pre-centrifugation weight to calculate an endpoint weight for expression. The plasma is weighed as it is expressed to determine when the endpoint is reached. Expression is stopped once the plasma removal target weight (± 1.0 g) is reached. The tolerances on the plasma removal target are included to ensure that the expression step doesn't lead to a post-expression weight that is outside of the range needed to continue processing. These additions to the expression step are included to increase control over the process and decrease batch-to-batch variation. The plasma removal target is calculated using the calculation below.

[00308] (Equation 5): *Determination of Expression Endpoint*

[00309] $\text{Plasma Removal Target} = \text{Pooled APC Weight} - 46.5 \text{ g}$

[00310] This method typically ensures that approximately 46.5 g of platelet pellet and plasma are left behind after expression. After expression, there is typically a weight check to ensure that the weight of the platelet pellet and supernatant is within range for further processing (31.8 g – 55.7 g). If the post-expression weight is outside of the range, the supernatant will be added/removed accordingly until within range.

[00311] Determination of Post-Expression Ranges:

[00312] The single donor plasma expression takes place after the addition of 27% DMSO, and the pooled process centrifugation and plasma expression step take place prior to the addition of the 27% DMSO. This had to be accounted for when determining the post-expression weight ranges. The calculations for determining the post-expression weight ranges are below:

[00313] First, the contribution of the APC in the final APC/DMSO solution typically be determined. The APC volume fraction of the APC/DMSO solution typically be calculated to determine this:

$$[00314] \text{Volume Fraction} = \frac{\text{Solute Volume}}{\text{Solution Volume}}$$

$$[00315] \text{APC Volume Fraction} = \frac{\text{APC Volume}}{\text{APC/DMSO Volume}}$$

$$[00316] \text{APC/DMSO Volume} = \text{APC Volume} + 27\% \text{ DMSO Volume}$$

[00317] Substitute the formula for APC/DMSO Volume into the APC Volume Fraction equation:

$$[00318] \text{APC Volume Fraction} = \frac{\text{APC Volume}}{\text{APC Volume} + 27\% \text{ DMSO Volume}}$$

[00319] As previously discussed in the “Determination of DMSO Constant” section, if formulating the APC/DMSO to 6.09 %DMSO, a formula for 27% DMSO volume is:

$$[00320] 27\% \text{ DMSO Volume} = 0.2909 * \text{APC Volume}$$

[00321] Substitute this equation for 27% DMSO Volume into the APC Volume Fraction formula:

$$[00322] \text{APC Volume Fraction} = \frac{\text{APC Volume}}{\text{APC Volume} + 0.2909 * \text{APC Volume}} = \frac{\text{APC volume}}{(1 + 0.2909) * \text{APC Volume}}$$

[00323] Since APC Volume is in the numerator and denominator of the equation, this value cancels out of the equation and the APC Volume Fraction can be solved:

$$[00324] \text{APC Volume Fraction} = \frac{1}{(1 + 0.2909)} = \frac{1}{1.2909} = 0.775$$

[00325] This means that the APC makes up 77.5% of the CPP product after being formulated to 6.09 %DMSO.

[00326] The single donor freeze volume ranges are for the APC/DMSO product, so to convert them to an equivalent pre-DMSO range, this APU Volume Fraction of 0.775 typically be used:

[00327] **Low End Volume:**

High End Volume:

[00328] $20 \text{ mL} * 0.775 = 15.5 \text{ mL}$

$35 \text{ mL} * 0.775 = 27.1 \text{ mL}$

[00329] The pooled process involves pooling two APUs for centrifugation and expression, so these values are doubled, then they are converted weights for ease of use in the process, using the APC specific gravity of 1.027 g/mL:

[00330] **Low End Weight Specification:**

High End Weight Specification:

[00331] $15.5 \text{ mL} * 2 * 1.027 \frac{\text{g}}{\text{mL}} = 31.8 \text{ g}$

$27.1 \text{ mL} * 2 * 1.027 \frac{\text{g}}{\text{mL}} = 55.7 \text{ g}$

[00332] **Determination of Post-Expression Target Weight of 46.5 g:**

[00333] Vitalant's single donor process had a post-expression volume range of 25.5 mL – 31.3 mL. The middle of this range is 28 mL, or 28.8 g. When rounded to a 1 g interval, this becomes 29 g. This APC/DMSO weight of 29 g was chosen as a starting point for determining the post-expression target weight of the exemplary pooled CPP process. To account for potential dead-space losses introduced by the pooled process, this value of 29 g was increased by 1 g, to become 30 g (or 29.1 mL). This was then converted to an equivalent amount of pre-DMSO product using the same conversions in the previous section:

[00334] $29.1 \text{ mL of APC/DMSO} * \text{APC Volume Fraction} = 29.1 \text{ mL} * 0.775 = 22.6 \text{ mL of APC}$

[00335] 29.1 mL of APC/DMSO is composed of 22.6 mL of APC. The pooled process involves pooling two APUs for centrifugation and expression, so this value of 22.6 mL of APC was then doubled and then converted to a weight for ease of use in the process, using the APC specific gravity of 1.027 g/mL:

[00336] $22.6 \text{ mL} * 2 * 1.027 \frac{\text{g}}{\text{mL}} = 46.4 \text{ g}$

[00337] This value of 46.4 g was then rounded to the nearest half-gram interval to become the post-expression target weight value of 46.5 g.

[00338] **Implementation of New Expression and Filling Methods**

[00339] Table 4 is composed of in-process post-expression and fill/freeze volume data from all cGMP-like pooled batches that have been produced to date using the improved exemplary pooled CPP method of Example 2. These 12 pooled CPP batches encompassed 69 expression steps and 136 manufactured pooled CPP units. The single donor post-expression and freeze volume specification ranges are listed, along with the middle of these ranges. The pooled CPP post-expression volumes have been converted to the equivalent

post-DMSO values, to allow for adequate comparisons, using the previously discussed APC/DMSO volume to APC volume conversion. The minimum and maximum batch-averages from the pooled CPP batches are listed, along with the mean intra-batch CV.

[00340] (Table 4): Summary of New Expression and Filling Methods

Process	Step	Middle/Mean (mL)	Spec. Range (mL)	Batch-Average Range (mL)	Mean Intra-Batch CV
Single Donor	Post-Expression	28.0	25.5 – 31.3	N/A	N/A
	Freeze	27.5	20.0 – 35.0	N/A	N/A
Pool	Post-Expression (n=69)	27.8	20.0 – 35.0	26.5 – 28.8	7.7%
	Freeze (n=136)	26.4		25.0 – 28.1	1.6%

[00341] As discussed in the analysis of the single donor process, the expression step and freeze volume are important for the product, and therefore in illustrative examples, they should be as controlled as possible. The additions to the expression step have created an accurate and precise method, as the pooled CPP post-expression mean is 0.2 mL away from the single donor post-expression volume of 28.0 mL (a 0.7% difference), and the CV is below 10%. Additionally, the pooled process post-expression range is inside of the single donor post-expression range of the process of Example 1. Furthermore, all pooled CPP batches manufactured to-date using the improved exemplary pooled CPP process of Example 2 have been inside the pooled CPP post-expression range and have not required additional handling, manipulation, and weight readjustment. This demonstrates the reliability of the method improvements. The in-process calculations and controls in the expression and filling operations ensure that every cryobag will be filled inside of the 20 mL – 35 mL freeze volume range. The pooled process allows for greater control over the freeze volume as each bag is individually filled from a common pool of product. The freeze volume CV demonstrates that the pooled process has a very controlled filling procedure, which decreases variability in freeze volume. The pooled CPP freeze volume data demonstrates that the improvements in the expression step coupled with the filling procedure will lead to acceptable freeze volumes. As per the improved exemplary processes provided herein in Example 2, 12 batches of CPP as obtained has a mean intra-batch CV of post-expression volume (resuspension volume) of 7.7%, and a mean intra-batch CV of freeze volume (pooled resuspension with cryoprotectant in each cryo-bag) of 1.6%.

[00342] Example 4. Homogeneity of Intra-batch Pooled CPP

[00343] The intra-batch homogeneity was tested across 28 units or cryo-vessels from 6 cGMP-like pooled CPP batches. The pooled CPP batches were manufactured according to the method of Example 2. The platelet count per bag, platelets/ μL , TGA measurements in $\text{IU}/10^6$ platelets, percent positivity of CD61 microparticles, and pH were determined across the 28 units or cryo-vessels from the 6 batches. The platelet count per bag and count/ μL was obtained using either the Beckman Coulter AcT Diff 2 Hematology Particle Analyzer or the Beckman Coulter DxH Hematology Analyzer (Beckman Coulter, beckmancoulter.com).

[00344] Thrombin Generation Assay was performed according to the following steps. Thrombinoscope CAT software was opened, and instrument was set-up according to manufacturer's guideline. PRP reagent containing tissue factor and phospholipids, fluobuffer, and fluo-substrate were prepared according to manufacturer's guidelines (Stago, stago.com). Thawed Octaplas® (Octapharma, octapharmausa.com) thawed TGA dilution buffer were combined to create a buffer containing 30% Octaplas. The combined buffer was used to dilute cephalin 1:50 to be used as a positive control. Octaplas was used to dilute CPP to $1584 \times 10^3/\mu\text{L}$ based on the platelet count data.

[00345] $1584 \times 10^3/\mu\text{L}$ CPP dilution was used to make $325 \times 10^3/\mu\text{L}$, $160 \times 10^3/\mu\text{L}$, and $80 \times 10^3/\mu\text{L}$ serial dilutions of CPP using Octaplas. A multichannel pipette was used to add 20 μL of PRP reagent to each test well and 20 μL of calibrator to each calibration well. Then 15 μL of the $325 \times 10^3/\mu\text{L}$, $160 \times 10^3/\mu\text{L}$, and $80 \times 10^3/\mu\text{L}$ samples were added to each of their test and calibration wells. 65 μL of Octaplas was added to every test and calibration well. 80 μL of 1:50 cephalin was added to the positive control wells. Each plate was inserted into the tray and incubated for 10 minutes at 41°C . After incubations fluo-buffer and fluo-substrate were dispensed into the active wells. The plate was read for 75 minutes at 20 second intervals to capture the full thrombin generation profile. Thrombin generation profile measurements were reported as $\text{IU}/10^6$ particles reading.

[00346] The flow cytometry result for CD61 microparticle positivity was obtained using the Novocyte Flow Cytometry (Agilent, agilent.com) according to the following method. The Novocyte Flow Cytometry was prepared according to manufacturer's guidelines. CPP sample was diluted 100-fold in saline. 10 μL of anti-human CD61-APC (BD Biosciences, bdbiosciences.com) was diluted into 70 μL . A gated isotope control stain mix was created by combining 20 μL of mouse IgG1-APC (BD Biosciences, bdbiosciences.com) with 60 μL of saline. A test stain mix was created by combining 20 μL of the diluted Cd61-APC and 60 μL of saline. The control samples were stained in triplicate by adding 5 μL of 1:100

CPP to 20 μL of the gated isotope control stain mix. The test samples were then stained in triplicate by adding 5 μL of 1:100 CPP to 20 μL of test stain mix. All samples were incubated away from light at room temperature for 20-30 minutes. Once incubation was complete, 400 μL of saline was added to each sample, then 100 μL of each sample was added to each well, beginning with the gating control samples and then the test samples. Well plate was then inserted into tray and plate was run. After sample acquisition was complete, gates were adjusted according to controls and used to determine CD61 positive microparticle count positivity on the test samples. The table below shows the results of the 28 units across the 6 batches.

[00347] Table 5

	Batch#	1 (n=8)	2 (n=2)	3 (n=6)	4 (n=3)	5 (n=3)	6 (n=6)	Mean Intra- Batch CV
Total Plts	Batch Average	2.3E+11	2.3E+11	2.1E+11	2.1E+11	2.0E+11	2.4E+11	4.2%
	Intra-Batch CV	5.1%	4.2%	6.4%	1.6%	2.2%	5.7%	
Plts/ μL	Batch Average	4.7E+06	4.6E+06	4.1E+06	4.1E+06	3.9E+06	4.6E+06	3.7%
	Intra-Batch CV	4.2%	4.8%	5.8%	1.5%	0.4%	5.8%	
IU/ 10^6 Plts	Batch Average	1.6	1.6	1.5	1.5	1.6	1.6	2.0%
	Intra-Batch CV	2.4%	1.5%	0.8%	1.8%	3.0%	2.7%	
CD61 ⁺ MP/ μL	Batch Average	7.4E+06	5.2E+06	3.7E+06	4.4E+06	3.6E+06	6.0E+06	6.9%
	Intra-Batch CV	10.0%	10.6%	7.2%	1.9%	7.3%	4.7%	
pH	Batch Average	6.45	6.29	6.64	6.46	6.47	6.55	0.8%
	Intra-Batch CV	1.1%	1.0%	0.9%	0.4%	0.5%	0.8%	

[00348] The mean intra-batch coefficient of variation are substantially less than 10% demonstrating that the pooled process has very low variability between units with a batch. The low CV's clearly indicate that the pooled process creates batches of homogeneous units. This is not the case with CPP units created from single donor APU that are known to have donor-to-donor variability. The improved exemplary pooled CPP process of Example 2 averages out the donor variability, which decreases batch to batch variability of the product. The mean intra-batch CV achieved using the improved exemplary pooled CPP process of Example 2 was less than 5% (4.2%), that of concentration of platelets (/ul) is less than 4% (3.7%), that of thrombin

generation ability (IU/10⁶ platelets) is less than 3% (2%), that of CD61-positive microparticles is less than 7% (6.9%), and that of pH is less than 1% (0.8%).

[00349] Example 5. One Year Stability Study of Pooled CPP Product Stored at -20°C (transition temperature cryopreserved-product).

[00350] Pooled CPP product was manufactured according to the method of Example 2. The stability of the pooled product was tested at different timepoints for a storage period of 1 year. 7 Apheresis Platelet Units (APU) were pooled resulting in 7 units or cryo-vessels of Pooled CPP product. Initially the 7 units were stored at ≤-65°C freezer for a minimum of 24 hours to form an initial frozen platelet composition. After the initial storage, the cryo-vessels were transferred to a -20°C freezer for storage to form cryopreserved platelets (transition temperature cryopreserved-product). One unit of the Pooled CPP product (transition temperature cryopreserved-product) was removed at different time points and tested according to the criteria below.

[00351] Visual Inspection of cracks, tears, breaks of the unit bag.

[00352] Visual Inspection of aggregate free swirling of the product in the bag.

[00353] Platelet count per bag.

[00354] pH of the product.

[00355] Before initiating each test, the product was removed from the freezer, thawed in a 37°C water bath for 8 minutes, then rehydrated by adding 25mL of 0.9% saline. Visual inspection of breaks, visual inspection of aggregate free swirling, platelet count per bag, and pH of product are indicators for the release criteria of the product. The platelet count per bag was obtained using either the Beckman Coulter AcT Diff 2 Hematology Particle Analyzer or the Beckman Coulter DxH Hematology Analyzer (Beckman Coulter, beckmancoulter.com). The table below details the results of the tests time points.

[00356] Table 6

Timepoint (Months)	Release Criteria			
	Visual Inspection (Pass/Fail)	Aggregate -free Swirling (Pass/Fail)	pH (≥ 6.2)	Platelet Count/Bag (≥1.7X10 ¹¹)
1	Pass	Pass	7.3	2.20E+11
3	Pass	Pass	7.6	2.20E+11

6	Pass	Pass	7.7	2.20E+11
9	Pass	Pass	7.8	2.10E+11
12	Pass	Pass	7.7	2.10E+11

[00357] The results of the stabilization study demonstrate product stability of a transition temperature cryopreserved-product after 12 months of storage at -20°C. The product at all time points passed all release criteria. There were no cracks, breaks, or leaks by visual inspection. Aggregate-free swirl test was confirmed at all time points. The minimum pH and minimum platelet count/bag criteria were achieved at all time points. Although a gradual increase in pH was observed for the 12 months, it did not affect the viability of the product. The platelet count/bag was consistent at the different time points. The findings here confirm the stability of the CPP pooled product (transition temperature cryopreserved-product) at the -20°C storage temperature for 1 year.

[00358] Example 6: Exemplary CPP Manufacturing Method

[00359] The present Example exemplifies one of the methods to produce cryopreserved platelets, which were then used to compare the cryopreserved platelets obtained by a process comprising transition in freezing temperature as exemplified in this method, also referred to as transition temperature cryopreserved-product with the cryopreserved platelets stored only at -80°C, also referred to as single temperature cryopreserved-product.

[00360] Cryopreserved platelets were produced using a single APU per batch of mix ABO and Rh types for all three batches created. The APU was acidified to reach a pH level range of 6.6-6.8 using an Anticoagulant Citrate Dextrose (ACD) solution. The pH level was tested using the Fisher brand Accumet XL 250 pH meter (Oakton Instruments). Once the APU was acidified to a pH of 6.6-6.8 a solution of 27% DMSO was used to achieve a final DMSO concentration of 6%. This was done by first determining the platelet count of the APU and then calculating the volume needed to produce the desired number of aliquots. The volume obtained from the APU was then multiplied by a constant of 0.2909 in order to obtain the correct volume of DMSO solution to add. The AcT diff 2 complete blood count (CBC) analyzer (Beckman Coulter, beckmancoulter.com) was used to determine platelet counts for the purpose of this research. Once a 6% DMSO concentration was obtained, the solution of 6% DMSO and platelet rich plasma (PRP) derived from the APU was centrifuged at 1250g for 20 minutes. Post centrifugation the supernatant plasma/6% DMSO solution was aspirated and reserved for re-suspension. Platelets were then resuspended in approximately half to one mL of plasma/6% DMSO solution, and a concentration test was performed at a 1:20 dilution (50µL of product + 950 µL of PBS) to determine platelet count. The platelet count was then used to

calculate the needed volume of plasma/6% DMSO supernatant that was previously aspirated to achieve a platelet concentration of $8,000 \times 10^3$ platelets/ μL . The count was then confirmed before aliquots of the platelets in 6% DMSO (standard formulation) were made. The final product was aliquoted at a volume of 1.5mL into 5mL cryogenic vials. The aliquots were transferred to the -80°C freezer for 48 hours. After the 48-hour period elapsed half of the aliquots produced were transferred to the -20°C freezer, thereby forming transition temperature cryopreserved-products while the other aliquots remained at -80°C to form single temperature cryopreserved-products.

[00361] Example 7: Comparing transition temperature cryopreserved-product stored at -20°C , and single temperature cryopreserved-product stored at -80°C

[00362] Batch 1, 2, and 3 of each transition temperature cryopreserved-product and single temperature cryopreserved-product prepared by the methods of Example 6 were thawed by transferring the cryotubes to a 37°C water bath for a period of 8-10 minutes. After thawing 1.5mL of saline was added to each cryotube. Products were then allowed to rest for a period of 30 minutes with agitation at room temperature. Once the products were allowed to rest, stability and aggregation testing was performed.

[00363] For stability testing the percent platelet recovery was determined. Percent platelet recovery was done using the AcT diff 2 and determining the percent yield. FIG. 7 shows the % recovery of platelets for the batches stored at -80°C (single temperature cryopreserved-product) and -20°C (transition temperature cryopreserved-product). The average percent recovery amongst the -80°C batches (single temperature cryopreserved-product) was 83% versus 77% when stored at -20°C (transition temperature cryopreserved-product). The goal of the experiment was to obtain a % recovery of greater than 70%, which was obtained by all batches in both storage temperatures. Therefore, the experiment shows similar percent recovery of both storage temperatures, thereby showing that the batches having transition temperature cryopreserved-product (stored at -20°C) are stable even when stored at a temperature higher than the single temperature cryopreserved-product, which is stored at -80°C .

[00364] Aggregation testing was performed using the PAP8 Aggregometer (Bio Data Corporation, biodatacorp.com). Batches 1, 2, and 3 of each product was diluted to 250k/ μL in Hexamethylenetetramine (HMTA) based on the AcT count. In each cuvette 225 μL of diluted sample was added. The cuvettes were then transferred to the incubation wells of the PAP8 Aggregometer. Either 25 μL of collagen (final concentration 10 $\mu\text{g}/\text{mL}$; stock is 100 $\mu\text{g}/\text{mL}$), or 25 μL of TRAP-6 (final concentration 20 μM), or 25 μL of arachidonic acid (AA) (final concentration 500 $\mu\text{g}/\text{mL}$; stock is 5mg/mL) was added and measurements were taken at 2 minutes. FIG. 8 shows the % aggregation of platelets for the batches stored at -80°C (single

temperature cryopreserved-product) and -20°C (transition temperature cryopreserved-product), and comparison with apheresis platelets with AA, collagen, and TRAP-6. Included in the aggregation data collection was an apheresis platelet for comparison. A two-tailed t-test was conducted for comparison between the batches of single temperature cryopreserved-product stored at -80°C against the transition temperature cryopreserved-product stored at -20°C . The AA agonist produced a p value of 0.80, there is no significant difference within the groups. The collagen agonist produced a p value of 0.59, no significant difference. The TRAP-6 produced a p value of 0.45, also with no significant difference. Since there was no statistical difference observed between the batches of both the products, this experiment demonstrated that cryopreserved platelets of transition temperature cryopreserved-product stored at -20°C exhibits stability and hemostatic properties, such as aggregation, similar to the single temperature cryopreserved-product stored at -80°C , thereby addressing a long-felt need of storing cryopreserved platelets at a temperature higher than -65°C .

[00365] Example 8: Calcein Acetoxymethyl (AM) Cell Membrane Testing

[00366] Batch 1, 2, and 3 of each transition temperature cryopreserved-product and single temperature cryopreserved-product prepared by the methods of Example 6 were thawed by transferring the cryotubes to a 37°C water bath for a period of 8-10 minutes. After thawing 1.5mL of saline was added to each cryotube. Products were then allowed to rest for a period of 30 minutes with agitation at room temperature. Once the products were allowed to rest calcein AM assay was performed. Calcein AM is a substance that is able to cross the cell membrane and reach the cytosol where Calcein AM gets hydrolyzed by the enzyme esterase to produce fluorescence. Platelets that are intact are able to retain this fluorescence while non-intact platelets do not. Based off the AcT count a 400 μL sample at a concentration of 1,000,000/ μL was created. 1mM Calcein AM stock solution was diluted in 10 μM in HMTA (1:100). Duplicate samples of 1 μL of 10 μM Calcein and 9 μL diluted cryopreserved platelets of each transition temperature cryopreserved-product and single temperature cryopreserved-product were prepared. All samples were incubated away from open light at room temperature for 20 minutes. 990 μL PBS was added to each sample to dilute. 100 μL of each sample was transferred to an individual well in a 96-well plate. Each sample was acquired on the flow cytometer with the following conditions: parameters: FSC, SSC, FB530 (FITC), stop conditions: 30 μL or 30,000 events, FSC-H threshold larger than 1,000, and flow rate: medium. Included in the assay was an apheresis platelet for comparison. FIG. 9 shows the findings performed for the Calcein AM testing. As expected, the apheresis platelets, indicated by “+” depicts a single peak, and were able to retain more of the Calcein AM as compared to transition temperature cryopreserved-product and single temperature

cryopreserved-product. It was observed that the transition temperature cryopreserved-product stored at -20°C, depicted by “*” in batches 1, 2, and 3 show a single peak depicting a single population of cells based on the membrane integrity while the single temperature cryopreserved-product stored at -80°C, depicted by “#” show two peaks depicting two populations of cells based on the membrane integrity. Furthermore, it was observed that all the three batches of the transition temperature cryopreserved-product stored at -20°C had similar medium sized peaks, possibly demonstrating similar levels of membrane integrity across the batches of the transition temperature cryopreserved-product. The observation possibly suggests that there are two kinds of populations in the single temperature cryopreserved-product, a first population that is not able to retain the fluorescence of Calcein AM (See first peak from left of “#” in FIG. 9) possibly because of compromised membrane and a second population (See second peak from left of “#” in FIG. 9) showing higher retention of the fluorescence possibly because they have intact membranes. It was further observed that the single peak of the transition temperature cryopreserved-product of all the three batches (See “*” in FIG. 9) corresponded to the first population of the single temperature cryopreserved-product that shows less retention of Calcein AM. Therefore, not to be limited by theory, it is believed that the single population of the transition temperature cryopreserved-product observed in the Calcein AM assay has platelets with compromised membranes. However, surprisingly, as shown in Example 7, all the batches 1, 2, and 3 of the transition temperature cryopreserved-product showed a platelet recovery of more than 70%. Similarly, as shown in Example 5, Table 6, transition temperature cryopreserved-products prepared by a process as disclosed in Example 2 after being stored at -20°C even for 12 months were able to pass all the criteria related to aggregate-free swirling, pH, and platelet count.

[00367] Example 9: Characterization of transition temperature cryopreserved-product stored at -20°C for various time points

[00368] Three batches, W4464-22-000010, W4464-22-000013, and W4464-22-000016 of cryopreserved platelets were prepared by the process disclosed in Example 2 with freezing done in a transition manner by freezing the obtained cryo-vessels at a temperature of $\leq -65^{\circ}\text{C}$ for 141 days, 85 days, and 43 days, respectively, to form an initial frozen platelet composition, and storing the initial frozen platelet composition at -20°C for timepoints of 1 month, 3 months, 6 months, and 12 months to form cryopreserved platelets (transition temperature cryopreserved-product). The data included in this Example is only for one of the batches, for the batch W4464-22-000010 until 12 months. Each cryo-vessel consisted of a volume of 20-35 ml in 6% DMSO. Once the time-point was reached, cryopreserved platelets from a cryo-vessel was thawed and tested for the following parameters:

[00369] Visual Inspection: Pass/Fail

[00370] •Aggregate-Free: Pass/Fail

[00371] •pH (≥ 6.2)

[00372] •Platelet Count per Bag ($\geq 1.7E+11$)

[00373] •Thrombin Generation Assay (TGA) (20K/ μ L IU/ 10^6 Particles)

[00374] •CD61 Positive Microparticles $\times 10^6/\mu$ L

[00375] •lactadherin Positive Particles (%)

[00376] Platelet count

[00377] The platelet count per bag was obtained using either the Beckman Coulter AcT Diff 2 Hematology Particle Analyzer or the Beckman Coulter DxH Hematology Analyzer (Beckman Coulter, beckmancoulter.com).

[00378] TGA Protocol

[00379] Fluoroskan Ascent instrument was used to assess the thrombin generation of the products herein. The concentration of cryopreserved platelets, or platelet derivatives from one of the cryo-vessels of a batch of this Example that needs to be tested was determined by using Beckman Coulter® AcT Diff and AcT Diff 2 Hematology Analyzer (EQU-045) or DxH 520 Hematology Analyzer. After the concentration was determined, the platelets were diluted with Octaplas (solvent detergent treated pooled plasma manufactured by Octapharma) to create serial dilution tubes having platelet concentrations of 352×10^3 , 160×10^3 , 80×10^3 platelets/ μ L. For obtaining output for the samples, in-plate dilutions of the platelets of 44×10^3 platelets/ μ L, appropriate volumes of PRP reagent (tissue factor and phospholipids), and Octaplas was added along with the platelets, and a fluorogenic thrombin substrate, FluCa (Stago), into the wells of a 96-well plate. The raw fluorescence intensity of FluCa was measured over time in the Fluoroskan Ascent instrument. The parameters of TGA Count check and TGA particles were generated from the Fluoroskan Ascent instrument. A detailed protocol is provided below.

[00380] Frozen platelets were thawed in a water-bath or plasma thawer set to 37 °C for 8 minutes.

[00381] After thawing, the platelets were diluted by adding 25 mL of 0.9% saline.

[00382] The manufacturer's guidelines were followed in CAT software and the instrument was set up according to manufacturers' guidelines.

[00383] PRP reagent containing tissue factor and phospholipids, calibrator, and fluobuffer and fluo-substrate was prepared according to manufacturer's guidelines.

[00384] Octaplas and TGA dilution buffer were thawed in 37 °C water bath for 10 minutes.

[00385] Thawed Octaplas was added to TGA dilution buffer to create a buffer containing 30% Octaplas.

[00386] The 30% Octaplas solution was used to dilute reconstituted cephalin 1:50 to be used as a positive control.

[00387] Octaplas was used to dilute thawed platelets to $1584 \times 10^3/\mu\text{L}$ based on the platelet count data from the DxH hematology analyzer.

[00388] The $1584 \times 10^3/\mu\text{L}$ dilution of thawed platelets was used to make $325 \times 10^3/\mu\text{L}$, $160 \times 10^3/\mu\text{L}$, and $80 \times 10^3/\mu\text{L}$ serial dilutions of platelets using Octaplas.

[00389] 20 μL of PRP reagent was added to each test well, and 20 μL of calibrator was added to each calibration well.

[00390] 15 μL of the $325 \times 10^3/\mu\text{L}$, $160 \times 10^3/\mu\text{L}$, and $80 \times 10^3/\mu\text{L}$ samples was added to each of their test and calibration wells.

[00391] 65 μL of Octaplas was added to every test and calibration well.

[00392] 80 μL of 1:50 cephalin was added to the positive control wells.

[00393] The plate was inserted into tray and incubated for 10 minutes at 41 °C. After incubation, fluo-buffer was dispensed and fluo-substrate mixture (including a fluorescent-labeled peptide, that when cleaved by thrombin, generates a fluorescent signal) was added into active wells.

[00394] The plate was read for 75 minutes at 20 second intervals to capture full thrombin generation profile.

[00395] Calculation of CD61 positive microparticles and lactadherin positive particles

[00396] Approximately 1 ml of cryopreserved platelets after thawing was diluted with 2-fold saline, and was further diluted 100-fold by serial dilutions.

[00397] CD61, also known as integrin α_3 (GPIIIa) is a glycoprotein subunit of the fibrinogen receptor. GPIIb/IIIa (and other integrin complexes like the vitronectin receptor $\alpha_v\beta_3$) are found on the surface of platelets. They are found in high levels on normal platelets, CD61 is used in this protocol to identify platelet-derived particles. Allophycocyanin (APC) is a fluorochrome with an excitation maximum at 650 nm and an emission maximum at 660 nm. This fluorochrome is detected in channel R660 on the NovoCyte instrument. Appropriate dilutions of CD61-APC were prepared.

[00398] Lactadherin is a milk fat globule-epidermal growth factor-factor 8 protein, binds to phosphatidylserine in a calcium independent manner. Fluorescein isothiocyanate (FITC) is a fluorochrome with an excitation maximum at 495 nm and an emission maximum at 519 nm. This fluorochrome is

detected in channel B530 on the NovoCyte instrument. Appropriate dilutions of lactadherin-FITC were prepared.

[00399] For test staining, the platelets were stained with CD61-APC, and lactadherin-FITC in tubes in a manner where stain mix was added first to the tubes followed by diluted platelets. All the samples were incubated away from light for about 20 minutes. Each sample was acquired on the flow cytometer with the following conditions: parameters: FSC, SSC, FB530 (for FITC), R660 (for APC) stop conditions: 50 μ L or 30,000 events, FSC-H threshold larger than 500, and flow rate: medium (35 μ L/min).

[00400] The "CD61 +" range gate was adjusted on the APC-H histogram such that it includes between 0.8% and 1.0% of the most fluorescent events. The CD61 + gate was copied and pasted from the APC-H histogram of the Gating Control specimen directly to the APC-H histogram of the corresponding Test Sample specimen. Each Test Specimen was examined and replicated to confirm the "CD61+ Microparticles" polygon gate on the APC-H vs FSC-H bivariate plot included the microparticle population, but not the larger platelet population, among CD61 + events for each replicate. The "Lact+" range gate was adjusted on the FITC-H histogram such that it includes between 0.8% and 1.0% of the most fluorescent events. The Lact+ gate was copied and pasted from the FITC-H histogram of the Gating Control specimen directly to the FITC-H histogram of the corresponding Test.

[00401] Results for the specifications relevant for quality criteria are shown in Table 7

[00402] Table 7

Parameter	Specification	Initial	1 Month	3 Month	6 Month	12 Month
Visual Inspection	No Visible Aggregates	PASS	PASS	PASS	PASS	PASS
Swirl Test	Swirling Observed	PASS	PASS	PASS	PASS	PASS
Platelet Count/ Bag	$\geq 1.7E + 11$	2.00E+11	2.30E+11	2.20E+11	2.10E+11	1.90E+11
pH	≥ 6.2	6.8	7.32	7.55	7.6	7.51

[00403] Results for the rest of the specifications are shown in Table 8.

[00404] Table 8

W4464-22-000010	Count/ μ L	TGA Count Check	TGA (20K/ μ L, IU/106 Particles)	CD61 Positive Microparticles/ μ L	Lactadherin Positive Particles (%)	Percentage of CD61 Positive Microparticles
Initial	3.80E+06	557.9	1.5	3.85E+06	81.47%	N/A
1 Month	4.40E+06	465	N/A*	9.10E+05	99.37%	21%
3 Months	4.20E+06	465	N/A*	7.35E+05	99.11%	18%
6 Months	4.00E+06	537.1	0.96	6.62E+05	88.90%	17%
12 Months	3.60E+06	569.7	0.72	4.98E+05	96.10%	14%
Min	3.65E+06	465	0.7	4.98E+05	81.47%	N/A
Max	4.42E+06	569.7	1.5	3.85E+06	99.37%	N/A
Average	4.01E+06	518.94	1.1	1.33E+06	92.99%	N/A
SD	3.14E+05	50.6	0.4	1.42E+06	7.70%	N/A

[00405] Results from Table 7 show that the transition temperature cryopreserved-product, i.e. cryopreserved platelets when stored at a temperature of -20°C for 1 month, 3, 6, and 12 months upon thawing do not show any visible aggregates, and pass the visual inspection of a swirl test, and satisfies the criteria of platelet counts and pH. Therefore, these results demonstrate that the transition temperature cryopreserved platelets herein when stored at a temperature of -20°C for at least 12 months exhibit the properties shown in Table 7.

[00406] Further, results shown in Table 8 demonstrate that in spite of storing at a temperature of -20°C for at least 12 months, cryopreserved platelets upon thawing retain thrombin generation ability. Also, it can be observed that the cryopreserved platelets upon thawing display a CD 61 positive microparticle content in the range of 21% (1 month) to 14% (12 months). Accordingly, it is observed that the percentage of CD 61 positive microparticles gradually decreases over the storage time. Also, cryopreserved platelets herein demonstrate lactadherin positivity in the range of 80-99.5%.

[00407] All references throughout this application, for example patent documents including issued or granted patents or equivalents; patent application publications; and non-patent literature documents or other source material; are hereby incorporated by reference herein in their entireties, as though individually incorporated by reference, to the extent each reference is at least partially not inconsistent with the disclosure in this application (for example, a reference that is partially inconsistent is incorporated by reference except for the partially inconsistent portion of the reference).

[00408] The terms and expressions which have been employed herein are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred aspects, exemplary aspects and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims. The specific aspects provided herein are examples of useful aspects of the present invention and it will be apparent to one skilled in the art that the present invention may be carried out using a large number of variations of the devices, device components, methods steps set forth in the present description. As will be obvious to one of skill in the art, methods and devices useful for the present methods can include a large number of optional composition and processing elements and steps.

[00409] All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. References cited herein are incorporated by reference herein in their entirety to indicate the state of the art as of their publication or filing date and it is intended that this information can be employed herein, if needed, to exclude specific aspects that are in the prior art. For example, when composition of matter are claimed, it should be understood that compounds known and available in the art prior to Applicant's invention, including compounds for which an enabling disclosure is provided in the references cited herein, are not intended to be included in the composition of matter claims herein.

[00410] One of ordinary skill in the art will appreciate that starting materials, biological materials, reagents, synthetic methods, purification methods, analytical methods, assay methods, and biological methods other than those specifically exemplified can be employed in the practice of the invention without resort to undue experimentation. All art-known functional equivalents of any such materials and methods are intended to be included in this invention. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred aspects and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in

the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

[00411] The disclosed embodiments, examples and experiments are not intended to limit the scope of the disclosure or to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. It should be understood that variations in the methods as described may be made without changing the fundamental aspects that the experiments are meant to illustrate.

[00412] Those skilled in the art can devise many modifications and other embodiments within the scope and spirit of the present disclosure. Indeed, variations in the materials, methods, drawings, experiments, examples, and embodiments described may be made by skilled artisans without changing the fundamental aspects of the present disclosure. Any of the disclosed embodiments can be used in combination with any other disclosed embodiment.

[00413] In some instances, some concepts have been described with reference to specific embodiments. However, one of ordinary skill in the art appreciates that various modifications and changes can be made without departing from the scope of the invention as set forth in the claims below. Accordingly, the specification and figures are to be regarded in an illustrative rather than a restrictive sense, and all such modifications are intended to be included within the scope of invention.

What is claimed is:

1. A process for preparing a batch of cryopreserved platelets, comprising:

a) pooling at least 2 platelet units into one vessel and at least another platelet unit into another vessel, wherein there are at least 5 platelet units, and the platelet units are from more than one donor;

b) centrifuging each vessel to obtain a supernatant comprising plasma, and a pellet comprising platelets;

c) resuspending the pellet in each vessel to form a resuspension wherein the resuspension has a target weight based on the number of units pooled or provided in the vessel;

d) pooling the resuspension from each vessel to form a pooled resuspension in a pooled resuspension vessel;

e) adding dimethyl sulfoxide (DMSO) to the pooled resuspension vessel having the pooled resuspension to obtain a pooled resuspension having DMSO;

f) distributing the pooled resuspension having DMSO from the pooled resuspension vessel among a number of cryo-vessels; and

g) freezing the pooled resuspension having DMSO in the cryo-vessels, to form the batch of cryopreserved platelets.

2. The process of claim 1, wherein in step f), distributing is performed to a number of cryo-vessels that are equivalent to the total number of units provided in step a).

3. The process of claim 1, wherein in step f), distributing is performed until a target fill weight is achieved in each cryo-vessel, and wherein the target fill weight is determined by the weight of the pooled resuspension having DMSO.

4. The process of claim 3, wherein the target fill weight is determined by dividing the weight of the pooled resuspension having DMSO by the number of platelet units provided in step a).

5. The process of claim 1, wherein before step c), removing a part of the supernatant comprising plasma is performed until a target weight of the pellet and a remainder plasma is achieved, wherein the target weight is in the range of 15.9g to 27.9g times the number of units pooled or provided in the vessel.
6. The process of the claim 5, wherein removing the part of the supernatant is performed until a weight of ± 1 g of the target weight of the pellet and remainder supernatant is achieved.
7. The process of claim 1, wherein in step d) pooling the resuspension from each vessel is performed using a tubing tree system, and in step e) adding DMSO to the pooled resuspension vessel is performed using the tubing tree system.
8. The process of claim 1, wherein in step f), distributing the pooled resuspension having DMSO from the pooled resuspension vessel among a number of cryo-vessels is performed using a dosing tree system.
9. The process of claim 1, wherein the freezing comprises: freezing the pooled resuspension at a temperature of less than or equal to -50°C to form an initial frozen platelet composition in the cryo-vessels; and storing the initial frozen platelet composition in the cryo-vessels at a temperature from equal to or higher than -30°C to lesser than 0°C or -5°C to form the batch of the cryopreserved platelets.
10. The process of any one of claims 1 to 9, wherein the process is performed more than once to form more than one batch of the cryopreserved platelets.
11. A process for preparing a cryopreserved platelet composition comprising cryopreserved platelets, said process comprising:
- i) freezing a population of platelets in a cryopreservation medium at a temperature of equal to or less than -50°C to form an initial frozen platelet composition; and
 - ii) storing the initial frozen platelet composition at a temperature in the range of -10°C to -30°C for at least 1 month to form the cryopreserved platelet composition.
12. The process of claim 11, wherein the storing comprises storing the initial frozen platelet composition in a freezer set at a temperature of $-20^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

13. The process of claim 11, wherein the freezing comprises subjecting the population of platelets in the cryopreservation medium at the temperature for at least 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 8 hours, 12 hours, 1 day, 2 days, 7 days, 1 week, 2 weeks, 1 month, 2 months, 3 months, or 6 months to form the initial frozen platelet composition.

14. The process of claim 11, wherein the process further comprises thawing the cryopreserved platelets to form a liquid platelet composition, and administering an effective amount of the liquid platelet composition to a subject in need thereof.

15. The process of claim 11, wherein the storing is done for a time period in the range of 1 month to 5 years, 1 month to 3 years, or 1 month to 1 year.

16. The process of claim 11, wherein the cryopreservation medium comprises dimethyl sulfoxide (DMSO) in a concentration in the range of 5% to 8%.

17. A collection of cryo-vessels comprising cryopreserved platelets,

wherein the cryopreserved platelets in each cryo-vessel have a set of biomolecule profiles indicative of more than 1 platelet donor, wherein a batch of cryo-vessels has an identical set of biomolecular profiles, and wherein each batch of the collection has a different set of biomolecular profiles than any other batch in the collection,

wherein, the collection comprises a plurality of at least 2 batches of cryo-vessels,

wherein each batch of cryo-vessels comprises at least 5 cryo-vessels, and

wherein the coefficient of variance of a mean DMSO concentration in the cryopreserved platelets across the batches is less than 1%.

18. The collection of cryo-vessels of claim 17, wherein the collection comprises a plurality of at least 3, 4, 5, 10, 15, 20, 50, 75, or 100 batches of the cryo-vessels.

19. The collection of cryo-vessels of claim 17, wherein the biomolecule profile indicative of more than 1 platelet donor, is two amino acid sequences of a first protein from a first gene that are significantly different

in frequency within the cryopreserved platelets than 50%, or the presence of more than two amino acid sequences of the first protein.

20. The collection of cryo-vessels of claim 17, wherein the set of biomolecule profiles of one batch is different from the set of biomolecule profiles of another batch.

21. The process of any one of claims 1 to 10, wherein the freezing comprises: freezing the pooled resuspension at a temperature of less than or equal to -50°C to form an initial frozen platelet composition in the cryo-vessels; and storing the initial frozen platelet composition in the cryo-vessels at a temperature in the range of -10°C to -30°C , to form the batch of the cryopreserved platelets.

22. The process of claim 21, wherein the freezing comprises subjecting the pooled resuspension at the temperature of less than or equal to -50°C for at least 30 minutes, 45 minutes, 1 hour, 2 hours, or 3 hours.

23. The process of any one of claims 21 or 22, wherein the storing comprises storing the initial frozen platelet composition in the cryo-vessels in a freezer set at a temperature of $-20^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

24. The process of any one of claims 21 or 22, wherein the initial frozen platelet composition in the cryo-vessels is stored at the temperature in the range of -10°C to -30°C for at least 90 minutes, 2 hours, or 3 hours.

25. The process of any one of claims 21 or 22, wherein the initial frozen platelet composition in the cryo-vessels is stored at the temperature in the range of -10°C to -30°C for a time-period in the range of 1 month to 5 years.

26. The process of any one of claims 1 to 9, wherein the freezing comprises freezing the pooled resuspension at a temperature in the range of -50°C to -85°C to form the batch of the cryopreserved platelets.

27. The process of any one of claims 1 to 10, or 21 to 26, wherein in step a) an odd number of platelet units are provided, and wherein one platelet unit is processed in a separate vessel.

28. The process of claim 27, wherein in step a) 2 platelet units are pooled in one vessel to form a plurality of vessels.

29. The process of claim 28, wherein in step c), the target weight is in the range of 31.8g to 55.7g for the vessels that have 2 platelet units, and in the range of 15.9g to 27.9g for the vessel that has 1 platelet unit.
30. The process of claim 27, wherein in step c), the target weight is in the range of 43.5g to 49.5g for the vessels that have 2 units, and in the range of 20.3g to 26.3g for the vessel that has 1 unit.
31. The process of claim 27, wherein in step a) 5, 7, 9, or 11 units are provided, and wherein each unit is from a different donor.
32. The process of any one of claims 1 to 10, or 21 to 26, wherein in step a) an even number of units are provided.
33. The process of claim 32, wherein in step a) 2 platelet units are pooled in one vessel, to form a plurality of vessels each having 2 platelet units.
34. The process of claim 32, wherein the target weight is in the range of 31.8g to 55.7g for each of the vessels.
35. The process of claim 32, wherein in step a) 6, 8, 10, or 12 units are provided, and wherein each unit is from a different donor.
36. The process of any one of claims 1 to 10, or 21 to 35, wherein 2 units are pooled in a vessel.
37. The process of any one of claims 1 to 10, or 21 to 35, wherein at least 3 units are pooled in a vessel.
38. The process of any one of claims 1 to 10, or 21 to 35, wherein in step e) adding the DMSO is performed to achieve a concentration of DMSO in the range of 3-8% in the pooled resuspension.
39. The process of claim 38, wherein the concentration of DMSO is in the range of 5-7% in the pooled resuspension.
40. The process of claim 10, wherein across at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 75, or 100 batches,
- a) a coefficient of variance of a mean resuspension volume across the batches is less than 10%;

and/or

b) a coefficient of variance of a mean pooled resuspension volume having DMSO in a cryo-vessel across the batches is less than 5%.

41. The process of claim 10, wherein resuspending the pellet in each vessel leads to a resuspension in each vessel such that the volume of the resuspension across at least 10 batches has a mean intra-batch coefficient of variance (mean of intra-batch CV) of less than 15%.

42. The process of any one of claims 1 to 10, or 21 to 39, wherein resuspending the pellet in each vessel leads to a resuspension in each vessel such that the volume of the resuspension across vessels within a batch has a coefficient of variance of less than 15%.

43. The process of any one of claims 1 to 10, or 21 to 39, wherein resuspending the pellet in each vessel leads to a resuspension in each vessel such that the volume of the resuspension in a vessel across at least 10 batches or within a batch varies no more than 20%.

44. The process of claim 10, wherein the volume of the pooled resuspension having DMSO in a cryo-vessel across at least 10 batches has a mean intra-batch coefficient of variance of less than 5%.

45. The process of any one of claims 1 to 10, or 21 to 39, wherein the volume of the pooled resuspension in a cryo-vessel having DMSO within a batch has a coefficient of variance of less than 5%.

46. The process of any one of claims 1 to 10, or 21 to 39, wherein the volume of the pooled resuspension having DMSO in a cryo-vessel across at least 10 batches or within a batch varies no more than 10%.

47. The process of any one of claims 1 to 10, or 21 to 46, wherein the vessel in step a) is an apheresis platelet unit (APU) bag.

48. The process of any one of claims 1 to 10, or 21 to 46, wherein the pooled resuspension vessel is one or more than one APU bag.

49. The collection of cryo-vessels or the process of any one of claims 10, or 17 to 20, wherein the coefficient of variance of a mean DMSO concentration in the cryopreserved platelets across the batches is less than 0.5%.

50. The collection of cryo-vessels or the process of any one of claims 10, or 17 to 20, wherein the mean DMSO concentration in the cryopreserved platelets across at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 75, or 100 batches has a coefficient of variance in the range of 0.05-1%.

51. The collection of cryo-vessels or the process of any one of claims 9, 10, or 17 to 20, wherein the concentration of DMSO in the cryopreserved platelets in a cryo-vessel within a batch or across at least 5 batches varies by no more than 0.5%, 0.4%, 0.3%, 0.2%, 0.1%, or 0.05%.

52. The collection of cryo-vessels or the process of any one of claims 9, 10, or 17 to 20, wherein the cryo-vessels in the batch or the collection comprise the cryopreserved platelets with DMSO in the range of 1-10%.

53. The collection of cryo-vessels or the process of claim 52, wherein the DMSO is in the range of 5-8%.

54. The collection of cryo-vessels of the process of claim 53, wherein the DMSO is in the range of 6% +/- 1%, 6% +/-0.75%, 6% +/-0.5%, 6% +/-0.25%, 6% +/-0.1%, or 6% +/-0.05%.

55. The collection of cryo-vessels or the process of any one of claims 9, 10, or 11 to 20, wherein the cryopreserved platelets are stable for at least 1 year at a temperature in the range of -10°C to -30°C.

56. The collection of cryo-vessels or the process of any one of claims 9, 10, or 17 to 20, wherein the concentration of platelets in the cryopreserved platelets in a cryo-vessel within a batch or across at least 5 batches varies no more than 10%.

57. The collection of cryo-vessels or the process of any one of claims 10, or 17 to 20, wherein the total number of platelets in the cryopreserved platelets in a cryo-vessel across at least 5 batches has a mean intra-batch coefficient of variance of less than 6%.

58. The collection of cryo-vessels or the process of any one of claims 10, or 17 to 20, wherein the concentration of platelets (platelets/ μ l) in the cryopreserved platelets in a cryo-vessel across at least 5 batches has a mean intra-batch coefficient of variance of less than 5%.

59. The collection of cryo-vessels or the process of any one of claims 10, or 17 to 20, wherein the thrombin generation ability of the cryopreserved platelets in a cryo-vessel across at least 5 batches has a mean intra-batch coefficient of variance of less than 5%.

60. The collection of cryo-vessels or the process of any one of claims 10, or 17 to 20, wherein the percentage of CD61-positive microparticles in a cryo-vessel containing the cryopreserved platelets across at least 5 batches has a mean intra-batch coefficient of variance of less than 10%.
61. The collection of cryo-vessels or the process of any one of claims 10, or 17 to 20, wherein the pH of the cryopreserved platelets in a cryo-vessel across at least 5 batches has a mean intra-batch coefficient of variance of less than 3%.
62. The process of any one of claims 9, 10, or 11 to 16, wherein the initial frozen platelet composition is stored at the temperature of in the range of -10°C to -30°C for at least 6 months.
63. The process or collection of cryo-vessels of any one of claims 1 to 61, wherein a therapeutically effective amount of the cryopreserved platelets upon thawing retains the ability to reduce bleeding in a subject in need thereof.
64. The process or collection of cryo-vessels of any one of claims 1 to 61, wherein the cryopreserved platelets upon thawing exhibit a platelet count recovery of at least 65%.
65. The process or collection of cryo-vessels of any one of claims 1 to 61, wherein the cryopreserved composition upon thawing exhibits a pH of equal to or more than 6.2.
66. The process or collection of cryo-vessels of any one of claims 1 to 61, wherein the cryopreserved platelets upon thawing exhibit an aggregate-free swirling upon a visual inspection.
67. The process or collection of cryo-vessels of any one of claims 1 to 61, wherein the cryopreserved platelet composition upon thawing exhibits a platelet count of equal to or more than 1.7×10^{11} platelets/bag or platelets/cryo-vessel.
68. The process or collection of cryo-vessels of any one of preceding claims, wherein the cryopreserved platelets comprise plasma in the range of 70-85% (v/v), DMSO in the range of 4-8% (v/v), and sodium chloride in the range of 7-15% (w/v).
69. The process or collection of cryo-vessels of any one of preceding claims, wherein the cryo-vessel is a cryo-bag.

70. A cryopreserved platelet composition or a batch of cryopreserved platelets prepared by the process of any one of claims 1 to 16, 21 to 48, or 62 to 69.

71. A composition comprising frozen platelets in a cryopreservation medium in a frozen state, wherein the composition is capable of yielding the following recited properties after storage for 6 months, upon thawing:

- a) is in a liquid state without requiring the addition of a liquid to achieve such liquid state;
- b) exhibits a platelet count of at least 1.0×10^{11} /35 ml of the composition;
- c) yields a single peak that corresponds to a compromised membrane peak in a membrane integrity assay;
- d) exhibits a CD61-positive-microparticle content of less than 50% of the CD61 positive particles in the composition, and
- e) generates thrombin in an *in vitro* thrombin generation assay.

72. The process, collection, or a composition of any one of claims 1, 11, 17, or 70, wherein the cryopreserved platelets are cryopreserved platelet derivatives, or the frozen platelets are frozen platelet derivatives.

73. The composition of claim 71, wherein the membrane integrity assay comprises incubating the composition with calcein acetoxymethyl (AM) to form a treated composition and analyzing the treated composition by flow cytometry.

74. The composition, process, or collection of any one of claims 71 or 72, wherein the *in vitro* thrombin generation assay comprises generating thrombin in the presence of tissue factor, and phospholipids.

75. The composition, process, or collection of any one of claims 71 or 72, wherein the frozen platelets upon thawing have a diameter in the range of 0.5-5 μ m, 1-4 μ m, 1-3 μ m, or 0.5-2.5 μ m, and wherein the composition upon thawing has a CD61-positive-microparticle content in the range of 10-30%.