need of additional platelets or platelet-derived material to provide the clotting function that is missing or inadequate.

[0011] In addition to their use "as is" to supply blood clotting functions to subjects in need, platelets, including platelets, are studied extensively in the laboratory to characterize their properties and understand their precise role in the blood clotting cascade. Research on platelets provides information on blood clotting factors that are supplied by the platelets, factors that interact with the platelets to promote clotting and wound healing, and factors that are necessary to activate platelets or otherwise attract platelets to, and retain them at, a site of injury.

[0012] Both the therapeutic and research uses for platelets require that platelets, or compositions derived from platelets, be available in a form that is biologically active. Currently, platelets for therapeutic uses (e.g., infusion for hemostasis) are typically provided as freshly isolated products, which are less than five days old, and in some cases, preferably no more than three days old. As can be immediately recognized, maintaining an adequate supply of fresh platelets for use in subjects in need is costly and results in loss of a large amount of platelets due to expiration prior to use, particularly in rural settings and combat theaters. Furthermore, because fresh platelets are so important for use in therapy, it can be difficult and expensive to obtain fresh platelets for research purposes. The compositions and methods provided herein, however, can provide alternatives to fresh platelets for therapy and research uses in the medical and veterinary arts.

[0013] In one example, a composition can include cryopreserved platelets, and a cryoprotectant, wherein the composition comprises less than 6% (v/v) of dimethyl sulfoxide (DMSO). In one aspect, the present disclosure refers to a composition comprising: cryopreserved platelets; and a cryoprotectant comprising dimethyl sulfoxide (DMSO) in an amount of about 0.1% (v/v) to about 3% (v/v).

[0014] In some examples, the cryopreserved platelets are cryopreserved human platelets. In some examples, the composition is substantially free of DMSO.

[0015] In some examples, the cryoprotectant is present at about 1 mM to about 1 M. In some examples, the cryoprotectant is present at about 10 mM 10 to about 500 mM. In some examples, the cryoprotectant is present at about 20 mM to about 200 mM. In some examples, the cryoprotectant is present at about 40 mM to about 100 mM.

[0016] In some examples, the cryoprotectant comprises a component selected from the group consisting of a saccharide, a monosaccharide, a disaccharide, and combinations thereof. In some examples, the cryoprotectant comprises a component selected from the group consisting of sucrose, maltose, trehalose, glucose, mannose, xylose, dextrose, and combinations thereof. In some examples, the cryoprotectant comprises a non-reducing disaccharide.

[0017] In some examples, the cryoprotectant comprises a polymer containing one or more monosaccharide units. In some examples, the cryoprotectant comprises a polymer derived from sucrose and epichlorohydrin. In some examples, the polymer is a polysucrose. In some examples, the cryoprotectant is in an amount of about 3% (w/v) to about 10% (w/v). In some examples, the cryoprotectant is in an amount of about 3% (w/v) to about 7% (w/v). In some examples, the cryoprotectant is in an amount of about 5% (w/v) to about 8% (w/v). In some examples, the cryoprotectant comprises a high molecular weight polymer. In some examples, the high molecular weight polymer has an average molecular weight of at least about 70 kilodaltons (kDa).

[0018] In some examples, the cryoprotectant is selected from the group consisting of serum albumin, dextran, polyvinyl pyrolidone (PVP), starch, hydroxyethyl starch (HES), and combinations thereof. In some examples, the cryoprotectant comprises a sugar alcohol. In some examples, the sugar alcohol is selected from the group consisting of mannitol, sorbitol, xylitol, maltitol, maltitol syrup, lactitol, erythritol, isomalt, hydrogenated starch hydrolysates, serum albumin,

dextran, polyvinyl pyrolidone (PVP), starch, hydroxyethyl starch (HES), and combinations thereof.

[0019] In some examples, the cryoprotectant comprises one or more polyols. In some examples, the cryoprotectant is selected from the group consisting of glycerol, ethylene glycol, polyethylene glycol, sorbitol, propylene glycol, pentaerythritol, a saccharide, hydroxypropyl-β-cyclodextrin, a glycerol oligomer, and combinations thereof. In some examples, the cryoprotectant comprises glycerol.

[0020] In some examples, the cryoprotectant is no more than about 10% (v/v). In some examples, the cryoprotectant is no more than about 5% (v/v). In some examples, the cryoprotectant is no more than about 1% (v/v). In some examples, the cryoprotectant is in an amount of at least about 0.5% (v/v). In some examples, the cryoprotectant is in an amount of at least about 1% (w/v). In some examples, the cryoprotectant is in an amount of about 0.1% (v/v) to about 10% (v/v). In some examples, the cryoprotectant is in an amount of about 0.5% (v/v) to about 7% (v/v). In some examples, the cryoprotectant is in an amount of about 1% (v/v) to about 5% (v/v).

[0021] In some examples, the composition also includes a solvent. In some examples, the solvent comprises water. In some examples, the composition further includes a salt. In some examples, the salt is selected from the group consisting of phosphate salts, sodium salts, potassium salts, calcium salts, magnesium salts, and combinations thereof.

[0022] In some examples, the composition also includes a buffer material. In some examples, the buffer is selected from the group consisting of phosphate buffered saline (PBS), bicarbonate/carbonic acid, such as a sodium-bicarbonate buffer, N-2-hydroxyethylpiperazine-N'-2- ethanesulfonic acid (HEPES), a tris-based buffer, a tris-buffered saline (TBS), and combinations thereof.

[0023] In some examples, the cryopreserved platelets comprises platelets derived from a human.

[0024] In some examples, the composition is a hemostatic composition. In some examples, the hemostatic composition is suitable for treating a human. In some examples, the hemostatic composition is suitable for triggering clotting. In some examples, the hemostatic composition is suitable for reducing or preventing blood loss from injured tissue.

[0025] In some examples, the composition is stable for at least about six months. In some examples, the composition is stable for at least about 12 months. In some examples, the composition is stable for at least about 18 months.

[0026] In some examples, the DMSO present in an amount of less than 5% (v/v). In some examples, the DMSO is present in an amount of less than 4% (v/v). In some examples, the DMSO is present in an amount of less than 3% (v/v). In some examples, the DMSO is present in an amount of less than 2% (v/v). In some examples, the DMSO is present in an amount of less than 1% (v/v). In some examples, the composition contains DMSO in an amount of less 0.001% (v/v). In some examples, the DMSO is in the amount of at least about 0.01% (v/v). In some examples, the DMSO is in the amount of at least about 0.1% (v/v). In some examples, the DMSO is in the amount of at least about 0.5% (v/v). In some examples, the DMSO is in the amount of at least about 1% (v/v). In some examples, the DMSO is in the amount of at least about 3% (v/v). In some examples, the DMSO is in the amount of about 0.01% (v/v) to about 5.5% (v/v). In some examples, the DMSO is in the amount of about 0.5% (v/v) to about 1% (v/v). In some examples, the DMSO is in the amount of about 1% (v/v) to about 3% (v/v). In some examples, the DMSO is in the amount of about 3% (v/v) to about 5.5% (v/v). In some examples, the DMSO is in the amount of about 0.5% (v/v) to about 1% (v/v).

[0027] In a second example, a method of making a composition includes:

obtaining platelets contained in a liquid medium;
suspending the platelets in a suspension medium comprising a cryoprotectant, the suspension medium comprising less than 6% (v/v) of dimethyl sulfoxide (DMSO); and
subjecting the suspended platelets to a temperature sufficient to form a cryopreserved platelet composition.

[0028] In some examples, the DMSO is in the amount of about 0.5% (v/v) to about 1% (v/v).

[0028a] In one aspect, the present disclosure refers to a method of making a cryopreserved platelet composition, comprising:

suspending platelets in a suspension medium comprising a cryoprotectant comprising DMSO in an amount of about 0.1% (v/v) to about 3% (v/v) to form suspended platelets;

loading the suspended platelets with the cryoprotectant by subjecting the suspended platelets to a temperature of between 20C and 42C for between 110 and 130 minutes to form a cryoprotectant-loaded platelet suspension; and

subjecting the cryoprotectant-loaded platelet suspension to a temperature of less than -0.1 ºC, for a time sufficient to freeze the cryoprotetant-loaded platelet suspension, thereby forming the cryopreserved platelet composition.

[0029] In some examples, the obtaining comprises isolating the platelets contained in the liquid medium. In some examples, the isolating comprises applying centrifugation filtration. In some examples, the isolating comprises applying tangential flow filtration.

[0030] In some examples, the suspension medium further comprises a salt. In some examples, the salt is present in an amount of about 0.1 mg/ml to about 10 mg/ml. In some examples, the salt is selected from the group consisting of a phosphate salt, a sodium salt, a potassium salt, a calcium salt, a magnesium salt, and combinations thereof. In some examples, the sodium salt is sodium chloride. In some examples, the potassium salt is potassium chloride.

[0031] In some examples, the suspension medium further comprises a buffering component. In some examples, the buffering component comprises a phosphate buffer. In some examples, the phosphate buffer is selected from the group consisting of a phosphate buffered saline (PBS), a bicarbonate/carbonic acid, a sodium-bicarbonate buffer, N-2-hydroxyethylpiperazine-N'-2- ethanesulfonic acid (HEPES), a tris-based buffer, a tris-buffered saline (TBS), and combinations thereof. In some examples, the buffering component comprises sodium bicarbonate (NaHCO3). In some examples, the buffering component comprises a tris-based buffer.

[0032] In some examples, the cryoprotectant comprises a saccharide. In some examples, the saccharide is selected from the group consisting of a monosaccharide, a disaccharide, sucrose, maltose, trehalose, glucose, mannose, dextrose, xylose, and combination thereof.

[0033] In some examples, the cryoprotectant comprises a polymer derived from sucrose and epichlorohydrin. In some examples, the polymer is a polysucrose. In some examples, the polysucrose is present in the suspension medium in an amount equal to about 3% to about 10% (w/v) of the cryopreserved platelet composition.

[0034] In some examples, the cryoprotectant comprises glycerol. In some examples, the cryoprotectant is no more than about 10% (v/v). In some examples, the cryoprotectant is in an amount of at least about 1% (w/v). In some examples, the cryoprotectant is in an amount of about 0.1% (v/v) to about 10% (v/v).

[0035] In some examples, the suspension medium further comprises an organic solvent. In some examples, the organic solvent is present in an amount of about 0.1% (v/v) to about 2% (v/v) of the composition. In some examples, the organic solvent is present in an amount of about 0.1% (v/v) to about 8% (v/v) of the composition. In some examples, the organic solvent comprises an alcohol. In some examples, the alcohol comprises a short-chain alcohol. In some examples, the short-chain alcohol is selected from the group consisting of methanol, ethanol, propanol, 1-propanol, 2-propanol, and combinations thereof.

[0036] In some examples, the DMSO is present in an amount of less than 1% (v/v). In some examples, the DMSO is in the amount of at least about 0.1% (v/v). In some examples, the DMSO is in the amount of about 0.5% (v/v) to about 5% (v/v). In some examples, the DMSO is in the amount of about 1% (v/v) to about 3% (v/v).

[0037] In some examples, the temperature is less than about -1 °C. In some examples, the temperature is less than about -10 °C. In some examples, the temperature is less than about -30 °C. In some examples, the temperature is less than about -70 °C. In some examples, the temperature is about -70°C to about -90 °C. In some examples, the temperature is about -10°C to about -30 °C.

[0038] In some examples, the platelets are human platelets.

[0039] In a third example, a composition is prepared by any one of the methods provided herein. In some examples, the composition contains DMSO in an amount of less 0.001% (v/v).

[0040] In some examples, the cryopreserved platelet composition comprises glycerol.

[0041] In a fourth example, a method of making a composition includes:

obtaining a liquid medium containing platelets;
suspending platelets in a suspension medium, the suspension medium comprising less than about 6% (v/v) of dimethyl sulfoxide (DMSO); and
subjecting the suspended platelets in the suspension medium to a temperature suitable for forming a cryopreserved platelet composition.

[0042] In some examples, the method further includes adding a pH modifier to the liquid medium containing the platelets until the pH is less than about 7. In some examples, the pH is less than than about 6.8. In some examples, the pH is about 4.0 to about 7.0. In some examples, the pH is about 5.0 to about 7.0. In some examples, the pH is about 6.6 to about 6.8.

[0043] In some examples, the method further includes isolating the platelets contained in the liquid medium. In some examples, the isolating comprises applying centrifugation, tangential flow filtration, or both, to the liquid medium. In some examples, the suspending comprises suspending the platelets in the suspension medium containing a concentration of $2x10^9$ platelets per milliliter (ml).

[0044] In some examples, the method further includes incubating the suspended platelets at a temperature of about 37°C for about 3 hours.

[0045] In some examples, the method further includes placing the suspended platelets in the suspension medium in a sealable container. In some examples, the sealable container comprises a fluorinated ethylene propylene (FEP) bag, a polyvinyl chloride (PVC) bag, or a borosilicate serum vial. In some examples, the suspended platelets in the suspension medium are placed in the sealable container in an amount of about $1x10^7$ platelets to about $3x10^{11}$ platelets.

[0046] In some examples, the temperature is less than about -1 °C. In some examples, the temperature is less than about -10 °C. In some examples, the temperature is less than about -30 °C. In some examples, the temperature is less than about -70 °C. In some examples, the temperature is about -70°C to about -90 °C. In some examples, the temperature is about -10°C to about -30 °C.

[0047] In some examples, the method further includes thawing the cryopreserved platelet composition. In some examples, the thawing comprising submersing the cryopreserved platelet composition in a 37°C water bath for about 8 minutes to about 10 minutes.

[0048] In some examples, the composition is substantially free of dimethyl sulfoxide (DMSO). In some examples, the composition contains DMSO in an amount of less 0.001% (v/v).

[0049] In some examples, the method does not comprise a lyophilization step.

[0050] In some examples, the cryopreserved platelet composition comprises glycerol.

[0051] In a fifth example, a cryopreserved platelet composition may be made by a method that includes:

a. mixing platelets with a medium comprising: i) a cryoprotectant, such as a cryoprotectant comprising trehalose, a polysucrose, glycerol, or combinations thereof; ii) dimethyl sulfoxide (DMSO) in an amount of less than 6% (v/v); and iii) ethanol, to form a first mixture;

b. optionally modifying the pH of the first mixture to a desired pH; and

c. subjecting the first mixture, or the optionally pH-modified first mixture, to a temperature of about -1 °C or less for a predetermined time to form the cryopreserved platelet composition,

wherein the cryopreserved platelet composition comprises cryopreserved platelets,

wherein a first amount of trehalose is comprised in the cryopreserved platelets and a second amount of trehalose in the cryopreserved platelet composition is not comprised in the cryopreserved platelets;

wherein the ratio of the first amount to the second amount of trehalose is greater than the ratio of the first amount to the second amount of trehalose when the composition is substantially free of at least one of DMSO, glycerol, or ethanol.

[0052] In a sixth example, a cryopreserved platelet composition may be made by a method that includes:

a. mixing platelets with a medium comprising: i) a cryoprotectant, such as a cryoprotectant comprising trehalose, a polysucrose, glycerol, or combinations thereof; ii) dimethyl sulfoxide (DMSO) in an amount of less than 6% (v/v); and iii) ethanol, to form a first mixture;

b. optionally modifying the pH of the first mixture to a desired pH; and

c. subjecting the first mixture, or the optionally pH-modified first mixture, to a temperature of about -1 °C or less for a predetermined time to form the cryopreserved platelet composition,

wherein the cryopreserved platelet composition comprises cryopreserved platelets, wherein a first amount of trehalose is comprised in the cryopreserved platelets and a second amount of trehalose in the cryopreserved platelet composition is not comprised in the cryopreserved platelets;

wherein the ratio of the first amount to the second amount of trehalose is greater than the ratio of the first amount to the second amount of trehalose in a second platelet composition, wherein the second platelet composition differs from the cryopreserved platelet composition only in that the second platelet composition is made by a method in which the medium with which the platelets are mixed in step a. does not comprise at least one of dimethyl sulfoxide (DMSO), glycerol or ethanol.

[0053] In a seventh example, a cryopreserved platelet composition may be made by a method that includes:

a. mixing platelets with a medium comprising:

i) a cryoprotectant comprising trehalose, a polysucrose, glycerol, dimethyl sulfoxide (DMSO), or combinations thereof; wherein the sum of the amounts of the DMSO and the glycerol is less than 15% (v/v); and

ii) ethanol,

to form a first mixture;

b. optionally modifying the pH of the first mixture to a desired pH; and

c. subjecting the first mixture, or the optionally pH-modified first mixture, to a temperature of about -1 °C or less for a predetermined time to form the cryopreserved platelet composition,

wherein the cryopreserved platelet composition comprises cryopreserved platelets,

wherein a first amount of trehalose is comprised in the cryopreserved platelets and a second amount of trehalose in the cryopreserved platelet composition is not comprised in the cryopreserved platelets;

wherein the ratio of the first amount to the second amount of trehalose is greater than the ratio of the first amount to the second amount of trehalose when the composition is substantially free of at least one of DMSO, glycerol or ethanol.

[0054] In an eighth example, a cryopreserved platelet composition may be made by a method that includes:

a. mixing platelets with a medium comprising:

i) a cryoprotectant comprising trehalose, a polysucrose, glycerol, dimethyl sulfoxide (DMSO), or combinations thereof; wherein the sum of the amounts of the DMSO and the glycerol is less than 15% (v/v); and

ii) ethanol,

to form a first mixture;

b. optionally modifying the pH of the first mixture to a desired pH; and

c. subjecting the first mixture, or the optionally pH-modified first mixture, to a temperature of about -1 °C or less for a predetermined time to form the cryopreserved platelet composition,

wherein the cryopreserved platelet composition comprises cryopreserved platelets,

wherein a first amount of trehalose is comprised in the cryopreserved platelets and a second amount of trehalose in the cryopreserved platelet composition is not comprised in the cryopreserved platelets;

wherein the ratio of the first amount to the second amount of trehalose is greater than the ratio of the first amount to the second amount of trehalose in a second platelet composition, wherein the second platelet composition differs from the cryopreserved platelet composition only in that the second platelet composition

is made by a method in which the medium with which the platelets are mixed in step a. does not comprise at least one of dimethyl sulfoxide (DMSO), glycerol, or ethanol.

[0055] In some examples, the method further comprises modifying the pH of the first mixture. In some examples, the pH is modified to less than 7.

[0056] In some examples, the method does not comprise a lyophilization step.

[0057] In some examples, the cryopreserved platelet composition comprises glycerol.

[0058] In some examples, the amount of ethanol is less than about 8% (v/v) of the composition.

[0059] In a ninth example, a cryopreserved platelet composition can include:

cryopreserved platelets;
a cryoprotectant comprising:
dimethyl sulfoxide (DMSO) in an amount of less than 6% (v/v);
trehalose; and
ethanol;
wherein an amount of intracellular trehalose present in the cryopreserved platelets, after a predetermined time of cooling a mixture comprising platelets and the cryoprotectant, is greater than an amount of intracellular trehalose in the cryopreserved platelets after the same predetermined time of cooling with a cryoprotectant that is substantially free of at least one of DMSO or ethanol.

[0060] In a tenth example, a cryopreserved platelet composition can include:

cryopreserved platelets;
a cryoprotectant comprising:
at least one of dimethyl sulfoxide (DMSO) or glycerol wherein the sum of the amounts of dimethyl sulfoxide (DMSO) and glycerol is less than 15% (v/v);

trehalose; and

ethanol;

wherein an amount of intracellular trehalose present in the cryopreserved platelets, after a predetermined time of cooling a mixture of platelets and the cryoprotectant, is greater than an amount of intracellular trehalose in the cryopreserved platelets after the same predetermined time of cooling with a cryoprotectant that is substantially free of at least one of DMSO, glycerol or ethanol.

[0061] In an eleventh example, a cryopreserved platelet composition can include:

i) cryopreserved platelets comprising a first amount of trehalose ;

ii) dimethyl sulfoxide (DMSO) in an amount of less than 6% (v/v);

iii) ethanol;

and

iv) a second amount of trehalose that is not comprised in the cryopreserved platelets; wherein the ratio of the first amount to the second amount of trehalose is greater than the ratio of the first amount to the second amount of trehalose when the composition is substantially free of at least one of DMSO or ethanol.

[0062] In a twelfth example, a cryopreserved platelet composition can include:

i) cryopreserved platelets comprising a first amount of trehalose;

ii) dimethyl sulfoxide (DMSO) in an amount of less than 6% (v/v);

iii) ethanol; and

iv) a second amount of trehalose that is not comprised in the cryopreserved platelets; wherein the ratio of the first amount to the second amount of trehalose is greater than the ratio of the first amount to the second amount of trehalose in a second platelet composition, wherein the second platelet composition

differs from the cryopreserved platelet composition only in that the second platelet composition does not comprise at least one of DMSO or ethanol.

[0063] In a thirteenth example, a cryopreserved platelet composition can include:

i) cryopreserved platelets comprising a first amount of trehalose;
ii) at least one of dimethyl sulfoxide (DMSO) or glycerol wherein the sum of the amounts of dimethyl sulfoxide (DMSO) and glycerol is less than 15% (v/v);
iii) ethanol; and
iv) a second amount of trehalose that is not comprised in the cryopreserved platelets;
wherein the ratio of the first amount to the second amount of trehalose is greater than the ratio of the first amount to the second amount of trehalose when the composition is substantially free of at least one of DMSO, glycerol or ethanol.

[0064] In a fourteenth example, a cryopreserved platelet composition can include:

i) cryopreserved platelets comprising a first amount of trehalose;

ii) at least one of dimethyl sulfoxide (DMSO) or glycerol wherein the sum of the amounts of dimethyl sulfoxide (DMSO) and glycerol is less than 15% (v/v);

iii) ethanol; and

iv) a second amount of trehalose that is not comprised in the cryopreserved platelets;

[0065] wherein the ratio of the first amount to the second amount of trehalose is greater than the ratio of the first amount to the second amount of trehalose in a second platelet composition, wherein the second platelet composition differs from the cryopreserved platelet composition only in that the second platelet composition does not comprise at least one of DMSO, glycerol or ethanol.In some examples, the ethanol is about 0.01% (v/v) to about 8% (v/v) of the composition. In some examples, the composition comprises about 3 mg/ml to about 300 mg/ml trehalose. In some examples, the DMSO is in the amount of at least about 0.1%

(v/v). In some examples, the DMSO is in the amount of about 0.5% (v/v) to about 5% (v/v). In some examples, the DMSO is in the amount of about 1% (v/v) to about 3% (v/v). In some examples, the DMSO is present in an amount of less than 1% (v/v).

[0066] In some examples, the cryopreserved platelet composition is substantially free of DMSO. In some examples, the composition is substantially free of glycerol. In some examples, the sum of the amounts of dimethyl sulfoxide (DMSO) and glycerol is at least about 1% (v/v). In some examples, the composition further comprises glycerol. In some examples, the glycerol is in an amount of about 0.1% (v/v) to about 10% (v/v). In some examples, the glycerol is in an amount of about 0.1% (v/v) to about 1% (v/v).

[0067] In some examples provided herein is a cryopreserved platelet composition made by a method comprising:

a. mixing platelets with a cryoprotectant, such as a cryoprotectant comprising trehalose, a polysucrose, glycerol, or a combination thereof, to form a first mixture; and

b. subjecting the first mixture to a temperature of about -1 °C or less to form the cryopreserved platelet composition,

wherein the cryopreserved platelet composition comprises less than 6% (v/v) of dimethyl sulfoxide (DMSO). Accordingly, in some examples, the first mixture referred to herein comprises platelets and the suspension medium comprising the cryoprotectant.

[0068] In some examples provided herein is a method for the preparation of a cryopreserved platelet composition comprising less than 6% (v/v) of dimethyl sulfoxide (DMSO), comprising steps a. and b. herein.

[0069] In some examples of the composition or of the method, the method includes optionally modifying the pH of the first mixture to a desired pH, for example, a pH less than 7.

[0070] In some examples of the composition or of the method, the method does not comprise modifying the pH of the first mixture.

[0071] In some examples of the composition or of the method, the method does not comprise a lyophilization step.

[0072] In some examples, platelets can be obtained prior to the treating step by a separation and collection of platelets, sometimes referred to as platelet apheresis (also referred to as "plateletpheresis"), in which the platelets are separated from whole blood and concentrated in a suitable container. Although most of the plasma is removed during apheresis, some plasma may still remain with the separated platelets. Apheresis platelets can be collected from a single donor and can be equivalent to about 4-6 pooled units.

[0073] In some examples provided herein, the platelets are human platelets and the cryopreserved platelet composition is cryopreserved human platelet composition.

[0074] In some examples provided herein, the platelets are not limited to platelets obtained from humans. For example, certain examples provided herein can include platelets obtained from other animal sources, such as canine platelets. More generally, the platelets may be obtained from an FDA-regulated platelet collection center.

[0075] Particular examples of the subject matter described in this document can be implemented to optionally provide one or more of the following advantages. First, some examples herein may provide cryopreserved platelets, or compositions containing thereof, for medical treatment of a human, such as in a blood transfusion to a human. In various examples, the cryopreserved platelets and/or cryopreserved

platelet compositions provided herein can be used for medical treatment of a mammalian subject, e.g., canine, or equine subjects.

[0076] Second, various examples provided herein provide cryopreserved platelets and/or cryopreserved platelet compositions that is free of dimethyl sulfoxide (DMSO). In some examples, the cryopreserved platelets and/or cryopreserved platelet compositions contain a small amount of DMSO. For example, in some examples, the cryopreserved platelets and/or cryopreserved platelet composition contain an amount of DMSO that is less than 2% (v/v) of the cryopreserved platelets and/or composition. Existing cryopreserved platelet formulations are problematic because they require a large amount of DMSO. DMSO is toxic to humans (and other mammalian subjects) in large doses. The cryopreserved platelets and/or cryopreserved platelet compositions provided herein therefore provide a non-toxic, safer alternative to currently available cryopreserved platelet formulations.

[0077] Third, some examples provided herein include a method of cryopreserving platelets and/or platelet compositions without introducing and using dimethyl sulfoxide (DMSO) during cryopreserving. In some examples, provided herein is a method of cryopreserving platelets and/or platelet compositions, wherein the method does not comprise using DMSO. In some examples, a method of making cryopreserved platelets and/or cryopreserved platelet compositions includes using a small amount of DMSO. For example, in some examples, a method of making cryopreserved platelets and/or cryopreserved platelet compositions includes using an amount of DMSO that is less than 2.% (v/v) of the cryopreserved platelets and/or composition. As discussed above, a large amount of DMSO is used for cryopreservation and can be problematic. Because DMSO is toxic to humans (and other mammalian subjects) in large doses, it would need to be removed from the platelets and platelet compositions prior to being used in a patient. Thus, a method of using lower amounts or trace amounts of DMSO to produce cryopreserved platelets and/or cryopreserved platelet compositions advantageously yields a non-toxic, safer

cryopreserved platelets and platelet compositions that does not require the removal of a toxic constituent

[0078] While multiple examples are disclosed, still some other examples of the present disclosure will become apparent to those skilled in the art from the following detailed description, which shows and describes certain examples of the disclosure. Accordingly, the drawings and detailed description are to be regarded as exemplary in nature and not restrictive.

DESCRIPTION OF DRAWINGS

[0079] FIG. 1 is a bar graph of flow cytometry forward scattering data showing a platelet size distribution of: (i) untreated platelets (“hIDSP”); (ii) thawed untreated platelets ("hIDSP Thawed"); (iii) an exemplary cryopreserved platelet composition without DMSO ("Std LB"); and (iv) an exemplary cryopreserved platelet composition with 1 % (v/v) DMSO ("Std LB w/ 1% DMSO").

[0080] FIG. 2 is a graph plot showing size distribution data, obtained using Thrombolux Dynamic Light Scattering, of: (i) the untreated platelets (hIDSP); (ii) the thawed, untreated platelets (hIDSP Thawed); and the thawed exemplary cryopreserved platelet compositions: (iii) the exemplary platelet composition without DMSO (Std LB), and (iv) the exemplary platelet composition with 1% DMSO (Std LB w/ 1% DMSO).

[0081] FIG. 3 is a bar graph showing the aggregation test data for (i) the untreated platelets (hIDSP); (ii) the untreated platelets (hIDSP Thawed); and the thawed exemplary cryopreserved exemplary platelet composition: (iii) the platelet composition without DMSO (Std LB), and (iv) the platelet composition with 1% DMSO (Std LB w/ 1% DMSO).

[0082] FIG. 4 is a bar graph of flow cytometry forward scattering data showing a platelet size distribution of an exemplary DMSO-free platelet composition: (i) prior to freezing (“Pretreat”); and for thawed exemplary DMSO-free platelet compositions that

WHAT IS CLAIMED IS:

We claim:

1. A composition comprising:

cryopreserved platelets; and

a cryoprotectant comprising dimethyl sulfoxide (DMSO) in an amount of about 0.1% (v/v) to about 3% (v/v).

2. The composition of claim 1, wherein the DMSO is in the amount of 0.1% (v/v) to 2% (v/v).

3. The composition of claim 1, wherein the DMSO is in the amount of about 1% (v/v).

4. The composition of claim 2, wherein the cryoprotectant further comprises one or more components selected from the group consisting of a monosaccharide at a concentration of 1 mM to 1 M, a disaccharide at a concentration of 1 mM to 1 M, and a polysaccharide in an amount of about 3% (w/v) to about 10% (w/v), or combinations thereof.

5. The composition of claim 2, wherein the cryoprotectant further comprises a combination of a polysaccharide and a disaccharide, wherein the polysaccharide is a polysucrose polymer derived from sucrose and epichlorohydrin in an amount of about 1% (w/v) to about 10% (w/v), wherein the disaccharide is at a concentration of 1 mM to 1 M and is selected from the group consisting of sucrose, maltose, and trehalose; wherein the composition further comprises a buffering component, and wherein the pH of the composition is between about pH 4 and about pH 7.

6. The composition of claim 5, wherein the polysaccharide is polysucrose and the disaccharide is trehalose.

7. The composition of claim 6, wherein the polysucrose is present at a concentration of 3% to 10% (w/v) and the trehalose is present at a concentration of 1.5% to 5% (w/v).

8. The composition of claim 6, wherein the polysucrose and trehalose are present in the composition at a ratio of 1:1 to 5:1.

9. The composition of claim 6, wherein the buffering component is selected from the group consisting of phosphate buffered saline (PBS), bicarbonate/carbonic acid, such as a sodium-bicarbonate buffer, N-2-hydroxyethylpiperazine-N'-2- ethanesulfonic acid (HEPES), a tris-based buffer, a tris-buffered saline (TBS), and combinations thereof, wherein the composition further comprises a salt selected from the group consisting of a phosphate salt, a sodium salt, a potassium salt, a calcium salt, a magnesium salt, and combinations thereof; and wherein the composition is a hemostatic composition.

10. The composition of claim 6, wherein the cryoprotectant further comprises a monosaccharide selected from glucose, mannose, dextrose, xylose, and combination thereof, and wherein the monosaccharide and disaccharide in the cryoprotectant are present at a concentration of 1 mM to 1 M.

11. The composition of claim 10, further comprising an organic solvent, wherein the organic solvent is present in an amount of about 0.1% to about 8% (v/v) of the composition, and wherein the organic solvent comprises a short-chain alcohol selected from the group consisting of methanol, ethanol, propanol, 1-propanol, 2-propanol, and combinations thereof, and wherein the composition is stable for 6 months at temperatures between -90 ^{o}C and -0.1 ^{o}C.

12. The composition of claim 11, further comprising one or more polyols selected from the group consisting of glycerol, ethylene glycol, polyethylene glycol, sorbitol, propylene glycol, pentaerythritol, hydroxypropyl-β-cyclodextrin, a glycerol oligomer, and combinations thereof, in an amount of 0.1% to 10% (v/v).

13. The composition of claim 12, wherein the DMSO is in the amount of about 1% (v/v).

14. The composition of claim 13, wherein the composition is in a container comprising $1x10^7$ platelets to $3x10^{11}$ platelets.

15. A method of making a cryopreserved platelet composition, comprising:

suspending platelets in a suspension medium comprising a cryoprotectant comprising DMSO in an amount of about 0.1% (v/v) to about 3% (v/v) to form suspended platelets;

loading the suspended platelets with the cryoprotectant by subjecting the suspended platelets to a temperature of between 20C and 42C for between 110 and 130 minutes to form a cryoprotectant-loaded platelet suspension; and

subjecting the cryoprotectant-loaded platelet suspension to a temperature of less than -0.1 ^oC, for a time sufficient to freeze the cryoprotetant-loaded platelet suspension, thereby forming the cryopreserved platelet composition.

16. The method of claim 15, wherein the DMSO is in the amount of 0.1% (v/v) to 2% (v/v), wherein the cryoprotectant further comprises a combination of a polysaccharide and a disaccharide, wherein the polysaccharide is a polysucrose polymer derived from sucrose and epichlorohydrin in an amount of about 1% (w/v) to about 10% (w/v), wherein the disaccharide is at a concentration of 1 mM to 1 M and is selected from the group consisting of sucrose, maltose, and trehalose; wherein the suspension medium further comprises a buffering component, and wherein the pH of the suspension medium is between about pH 4 and about pH 7.

17. The composition of claim 5, wherein the cryoprotectant further comprises a monosaccharide selected from glucose, mannose, dextrose, xylose, and combination thereof, wherein the monosaccharide and the disaccharide in the cryoprotectant are present at a concentration of 1 mM to 1 M, wherein the polysaccharide is polysucrose and the disaccharide is trehalose, wherein the buffering component is selected from the group consisting of phosphate buffered saline (PBS), bicarbonate/carbonic acid, such as a sodium-bicarbonate buffer, N-2-hydroxyethylpiperazine-N'-2- ethanesulfonic acid (HEPES), a tris-based buffer, a tris-buffered saline (TBS), and combinations thereof, wherein the suspension medium further comprises a salt selected from the group consisting of a phosphate salt, a sodium salt, a potassium salt, a calcium salt, a magnesium salt, and combinations thereof, wherein the cryopreserved platelet composition is a hemostatic composition, and wherein the suspension medium further comprises an organic solvent, wherein the organic solvent is present in an amount of about 0.1% to about 8% (v/v) of the suspension medium and wherein the organic solvent comprises a short-chain alcohol selected from the group consisting of methanol, ethanol, propanol, 1-propanol, 2-propanol, and combinations thereof.

18. The method of claim 15, wherein the method does not comprise a lyophilization step

19. The method of claim 15, further comprising isolating the platelets contained in a liquid medium before suspending the platelets, wherein the isolating comprises applying centrifugation, tangential flow filtration, or both, to the liquid medium.

20. The method of claim 15, wherein the suspended platelets in the cryoprotectant-loaded platelet suspension are placed in a sealable container in an amount of about $1x10^7$ platelets to about $3x10^{11}$ platelets.