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, wherein the cryopreserved platelets are a frozen platelet suspension; and a cryoprotectant comprising dimethyl sulfoxide (DMSO) in an amount of ~~about~~ 0.1% (v/v) to ~~about~~ 3% (v/v) in the composition, wherein the cryoprotectant further comprises a combination of a polysaccharide and a disaccharide, wherein the polysaccharide is a polysucrose polymer, and wherein the disaccharide is selected from the group consisting of sucrose, maltose, trehalose, glucose, mannose, xylose, dextrose, and combinations thereof.

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

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:

obtaining platelets contained in a liquid medium;

suspending the platelets in a suspension medium comprising a cryoprotectant comprising DMSO in an amount of ~~about~~ 0.1% (v/v) to ~~about~~ 3% (v/v) in the composition, wherein the cryoprotectant further comprises a combination of a polysaccharide and a disaccharide, wherein the polysaccharide is a polysucrose polymer, and wherein the disaccharide is selected from the group consisting of sucrose, maltose, trehalose, glucose, mannose, xylose, dextrose, and combinations thereof, to load the suspended platelets with the cryoprotectant; and~~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 cryoprotectant-loaded platelet suspension, thereby forming the cryopreserved platelet composition, wherein the method does not comprise a lyophilization step.

[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.

WHAT IS CLAIMED IS:

We claim:

1. A composition comprising:

cryopreserved platelets, wherein the cryopreserved platelets are a frozen platelet suspension; and

a cryoprotectant comprising dimethyl sulfoxide (DMSO) in an amount of ~~about~~ 0.1% (v/v) to ~~about~~ 3% (v/v) in the composition, wherein the cryoprotectant further comprises a combination of a polysaccharide and a disaccharide, wherein the polysaccharide is a polysucrose polymer, and wherein the disaccharide is selected from the group consisting of sucrose, maltose, trehalose, glucose, mannose, xylose, dextrose, and combinations thereof.

2. The composition of claim 1, wherein the DMSO is present in the amount of 0.5% v/v to 1% v/v or 1% v/v to 3% v/v~~0.1% (v/v) to 2% (v/v)~~.

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

4. The composition of claim ~~2~~1, wherein the polysucrose is present at 3% to 10% w/v and the disaccharide is trehalose present at 1.5% to 5% w/v~~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~~1, 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~~1, wherein the composition is stable for at least 6 months at a temperature of -80 ºC +/- 10 ºC~~the polysaccharide is polysucrose and the disaccharide is trehalose~~.

7. The composition of claim ~~6~~1, wherein the composition further comprises a buffer~~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~~1, 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~~7, 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~~1, 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 1~~0~~, 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 ºC and -0.1 ºC~~.

~~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~~12. The composition of claim ~~12~~11, wherein the DMSO is in the amount of about 1% (v/v).

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

~~15~~14. A method of making a cryopreserved platelet composition, comprising:

obtaining platelets contained in a liquid medium;

suspending the platelets in a suspension medium comprising a cryoprotectant comprising DMSO in an amount of ~~about~~ 0.1% (v/v) to ~~about~~ 3% (v/v) in the composition, wherein the cryoprotectant further comprises a combination of a polysaccharide and a disaccharide, wherein the polysaccharide is a polysucrose polymer, and wherein the disaccharide is selected from the group consisting of sucrose, maltose, trehalose, glucose, mannose, xylose, dextrose, and combinations thereof, to load the suspended platelets with the cryoprotectant; and~~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 cryoprotectant-loaded platelet suspension,

thereby forming the cryopreserved platelet composition, wherein the method does not comprise a lyophilization step.

~~16~~15. The method of claim ~~15~~14, wherein the DMSO is in the amount of less than 1% v/v or from 1% v/v to 3% v/v ~~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~~16. The composition of claim ~~5~~1, 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~~17. The method of claim ~~15~~14, wherein the polysucrose is present at 3% to 10% w/v and the disaccharide is trehalose present at 1.5% to 5% w/v~~method does not comprise a lyophilization step~~.

~~19~~18. The method of claim ~~15~~14, wherein the suspension medium further comprises an organic solvent, wherein the organic solvent is present in an amount of 0.8% to 8% v/v of the suspension medium, 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~~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~~19. The method of claim ~~15~~14, wherein the DMSO is in the amount of less than 3% v/v~~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~~.

20. The method of claim 14, wherein the method further comprises storing the platelets for at least 6 months at a temperature of -80 ºC +/- 10 ºC, wherein after the storing the cryopreserved platelet composition provides adequate hemostatic function and primary coagulopathic function.