



(51) International Patent Classification:

A61K 38/24 (2006.01) A61K 9/48 (2006.01)
A61K 35/14 (2006.01) A61P 17/00 (2006.01)
A61K 9/127 (2006.01)

(21) International Application Number:

PCT/US2013/023490

(22) International Filing Date:

28 January 2013 (28.01.2013)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

13/358,797 26 January 2012 (26.01.2012) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,

HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to the identity of the inventor (Rule 4.17(i))
- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

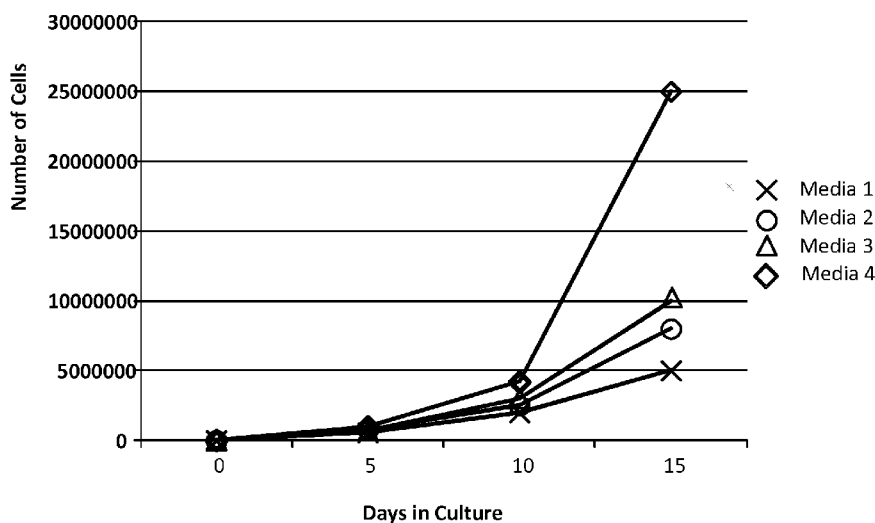
Published:

- with international search report (Art. 21(3))

[Continued on next page]

(54) Title: LYOPHILIZED PLATELET LYSATES

Culture



(57) Abstract: The present disclosure is drawn to compositions and methods of making and using lyophilized platelet lysates. Specifically, a method of preparing a composition suitable for therapeutic use or as a culture medium can comprise steps of concentrating platelets from a platelet source to form a platelet rich portion of the platelet source, and lysing the platelets in the platelet rich portion to form a plurality of lysates. An additional step includes lyophilizing the lysates to form lyophilized platelet lysates in a composition with released concentrations of available growth factors, cytokines, and chemokines. In one example, at 30%, by platelet count, of platelets from a platelet source can be lysed using this process.

WO 2013/113024 A1

- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

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LYOPHILIZED PLATELET LYSATES**BACKGROUND**

Several techniques have been described for the preservation and use of
10 platelets. DMSO and trehalose are two examples of compositions that have
been used for the perseveration of platelets, with or without lyophilization (or
freeze-drying). Cryoprotectant compositions have also been used along with
lyophilization processes to a similar result. Disadvantages with approaches that
15 merely use preservatives and/or lyophilization on intact platelets relate to the fact
that these platelets retain their proteins, receptors, and the like on the surface or
within the platelets. For example, several platelet membrane receptors remain
intact for binding with extracellular factors in response to platelet activation, e.g.,
for platelet adhesion, aggregation, etc. Thus, it would be beneficial to prepare a
more universal preparation of platelets that can be used effectively for many
20 applications, including wound healing, skin treatment, disorders of body tissue
(including body organs) such as lung tissue, or the like.

SUMMARY

25 A method of preparing a composition suitable for therapeutic use or as a
culture medium can comprise steps of concentrating platelets from a platelet
source to form a platelet rich portion of the platelet source, and lysing the
platelets in the platelet rich portion to form a plurality of lysates. An additional
step can include lyophilizing the lysates to form lyophilized platelet lysates in a
30 composition with released concentrations of available growth factors, cytokines,
and chemokines. In one example, at least 30%, by platelet count, of platelets

from a platelet source can be lysed (during the lysing step and often the lyophilizing step as well) using this process.

In another example, a composition suitable for therapeutic use or as a culture medium can comprise lyophilized platelet lysates prepared from source platelets, wherein the lyophilized platelet lysates provide released concentrations of available growth factor, cytokines, and chemokines. In one example, at least 30% of the source platelets that remain in the composition, by platelet count, are lysed to form the lyophilized platelet lysates.

In another embodiment, a method of treating mammalian tissue can comprise applying a composition including lyophilized platelet lysates to a mammalian tissue site for treatment, wherein the lyophilized platelet lysates include released concentrations of available growth factors, cytokines, and chemokines. In one example, the lyophilized platelet lysates are prepared from source platelets, wherein at least 30% of the source platelets that remain in the composition, by platelet count, are lysed to form the lyophilized platelet lysates.

A method of culturing cells or tissue can comprise admixing a media composition including lyophilized platelet lysates with a cell or tissue culture. The media composition can include lyophilized non-lysed platelets as well, and at least 30% of a total platelet count can make up lyophilized platelet lysates.

BRIEF DESCRIPTION OF THE DRAWING

FIG. 1 sets forth a comparison of mesenchymal stem cell cultures using various types of media, one of which includes lyophilized platelet lysates prepared in accordance with examples of the present disclosure.

DETAILED DESCRIPTION

Reference will now be made to the exemplary embodiments and specific language will be used herein to describe the same. It will nevertheless be understood that no limitation of the scope of the invention is thereby intended. Alterations and further modifications of the inventive features illustrated herein,

and additional applications of the principles of the disclosure as illustrated herein, which would occur to one skilled in the relevant art and having possession of this disclosure, are to be considered within the scope of the invention. It is also to be understood that this disclosure is not limited to the particular configurations,
5 process steps and materials disclosed herein, as these may vary to some degree. Further, it is to be understood that the terminology used herein is used for the purpose of describing particular embodiments only, and is not intended to be limiting as the scope of the present disclosure.

It is noted that, as used in this specification and the appended claims,
10 singular forms of "a," "an," and "the" include plural referents unless the content clearly dictates otherwise.

The term "wound" refers to any damage to any tissue of a subject, including damage to the skin as well as damage to deeper tissue, whether the wound is caused accidentally or intentionally, or alternatively by the normal
15 course of a pathological, disease, or degenerative condition. For example, the damage can be as a result of injury or surgery. Non-limiting examples of injuries include ulcers, burns, broken bones, punctures, cuts and scrapes, lacerations, surgical incisions, inflammation, infection, and the like.

As used herein, the term "platelet-containing fluid" refers to any fluid, either
20 biological or artificial, which contains platelets. Non-limiting examples of such fluids include various forms of whole blood, blood plasma, platelet rich plasma, concentrated platelets in any medium, or the like, derived from human and non-human sources.

As used herein, the term "concentrate" or "concentrating" refers to the
25 separation of platelets from the bulk of the plasma, whole blood, or other fluid from which it is present. For example, centrifugation, spectrometry, filtration, decanting, gravity settling, or other methods of concentrating platelets from platelet-containing fluids can be used. When concentrating platelets, it can be desirable to use an anticoagulant (particularly for centrifugation or gravity settling)
30 along with the source of platelets to prevent clotting during the separation of platelets from other components of the blood, plasma, or other fluid.

The term "anticoagulant" refers to compositions that inhibit clotting when concentrating or collecting platelets for use in accordance with examples of the present disclosure. Anticoagulants generally are available as inhibitors of clotting factor synthesis, inhibitors of thrombin, or antiplatelet drugs. Inhibitors of clotting factor synthesis that inhibit the production of certain clotting factors in the liver, include compositions such as warfarin (Coumadin). Inhibitors of thrombin interfere with blood clotting by blocking the activity of thrombin, and include compositions such as heparin and lepirudin (Refludan). Antiplatelet drugs interact with platelets themselves, and include drugs such as aspirin, ticlopidine (Ticlid), clopidogrel (Plavix), tirofiban (Aggrastat), eptifibatide (Integrilin), etc.

The terms "lyophilization," "lyophilize," or the like refer to a freeze-drying or dehydration process that is often used to preserve platelets, but is used somewhat differently in accordance with embodiments of the present disclosure. Specifically, lyophilization is used primarily not just as a preservative process, but rather, to further lyse platelets after initial freeze-thaw or other lysis technique is conducted. In other words, in accordance with examples of the present disclosure, after lysates are formed as described herein, lyophilization provides the added benefit of preserving the growth factors, cytokines, chemokines, and other contents initially enclosed within or bound to the surface the platelets, but which are released when platelets are lysed as described herein, e.g., freeze-thaw lysing. The process typically works by freezing the material and reducing surrounding pressure to allow frozen water in the material to sublime directly from the solid phase to the gas phase.

The term "tissue" includes the full range of small tissue sites to complete organs.

In accordance with the present disclosure, a "lysate" is the composition prepared where platelets are destroyed by disrupting their cell membrane. This can be done chemically, mechanically, by liquid homogenization, or sonication, but in accordance with certain embodiments described herein, the cytolysis is carried out using a freeze-thaw cycle, and to a lesser degree, as part of the lyophilization process. Freeze-thaw lysates can be formed by freezing a platelet suspension and then thawing the material to above room temperature, e.g., 30 °C

to 45 °C, though other freeze-thaw regimens are also included in the scope of the present disclosure, provided they lead to cytolysis of the platelets. With the freeze-thaw technique, this method causes cells to swell and break as ice crystals form, followed by contraction at thawing. Thus, the cyclical swelling and contracting ultimately causes the platelets to break open. Multiple cycles are typically used for more complete lysis, but the “more complete” lysis is not necessarily required in accordance with examples of the present disclosure. Varying degrees of platelet cytolysis can occur, e.g., at least 30%, at least 50%, at least 70%, at least 90%, or up to 100% cytolysis, by platelet count.

The term “lyophilized platelet lysates” or “LPL” are prepared as described herein. However, it is noted that the term lyophilized platelet lysates also includes “lyophilized platelet rich plasma lysates” or “LPRRL” as a specific type of lyophilized platelet lysates. Thus, any discussion of lyophilized platelet lysates (LPL) also includes lyophilized platelet rich plasma lysates (LPRRL), with the understanding that one difference is that platelet rich plasma is used or formed as part of the method or composition. However, in either composition, both include lyophilized platelet lysates.

As used herein, the term “about” is used to provide flexibility to a numerical range endpoint by providing that a given value may be “a little above” or “a little below” the endpoint. The degree of flexibility of this term can be dictated by the particular variable and would be within the knowledge of those skilled in the art to determine based on experience and the associated description herein.

As used herein, a plurality of components may be presented in a common list for convenience. However, these lists should be construed as though each member of the list is individually identified as a separate and unique member. Thus, no individual member of such list should be construed as a *de facto* equivalent of any other member of the same list solely based on their presentation in a common group without indications to the contrary.

Concentrations, amounts, and other numerical data may be expressed or presented herein in a range format. It is to be understood that such a range format is used merely for convenience and brevity and thus should be interpreted flexibly to include not only the numerical values explicitly recited as the limits of

the range, but also to include all the individual numerical values or sub-ranges encompassed within that range as if each numerical value and sub-range is explicitly recited. As an illustration, a numerical range of "about 0.01 to 2.0" should be interpreted to include not only the explicitly recited values of about 0.01 to about 2.0, but also include individual values and sub-ranges within the indicated range. Thus, included in this numerical range are individual values such as 0.5, 0.7, and 1.5, and sub-ranges such as from 0.5 to 1.7, 0.7 to 1.5, and from 1.0 to 1.5, etc. This same principle applies to ranges reciting only one numerical value. Furthermore, such an interpretation should apply regardless of the breadth of the range or the characteristics being described.

With these definitions in mind, it has been recognized that the preparation of lyophilized platelet lysates (LPL) and lyophilized platelet rich plasma lysates (LPRPL) can be highly functional for both research and therapeutic applications, either before or after reconstitution in a liquid carrier. When prepared appropriately, these lyophilized platelet lysates can be stable for extended periods of time when frozen, e.g., -80°C, or even at room temperature in certain embodiments. Though these compositions can be useful in both a research and therapeutic environment without the addition of other ingredients, it is noted that both LPL and LPRPL can include one or more active agent or biologic, such as antifungals, antivirals, antibiotics, growth factors, or chemicals depending on the research or therapeutic application. Furthermore, in certain examples, the LPL or LPRPL can be prepared as part of an encapsulated product, e.g., within liposomes. Liposomes, for example, can be prepared that include multilamellar or unilamellar vesicles. In these examples, LPL or LPRPL can be rehydrated at different concentrations ranging 0.01% to 100%, by volume, and mixed with a liposome solution within its hydrophobic membrane.

In accordance with this, a method of preparing a composition suitable for therapeutic use or as a culture medium can comprise steps of concentrating platelets from a platelet source to form a platelet rich portion of the platelet source, and lysing the platelets in the platelet rich portion to form a plurality of lysates. An additional step can further include lyophilizing the lysates to form

lyophilized platelet lysates in a composition with released concentrations of available growth factors, cytokines, and chemokines.

In another example, a composition suitable for therapeutic use or as a culture medium can comprise lyophilized platelet lysates prepared from source platelets, wherein the lyophilized platelet lysates provide released concentrations of available growth factor, cytokines, and chemokines. In one example, at least 30% of the source platelets that remain in the composition, by platelet count, are lysed to form the lyophilized platelet lysates. Other concentrations of lyophilized platelet lysates prepared from the source platelets can be at least 50%, 70%, 90%, or even up to 100% lyophilized platelet lysates.

In another embodiment, a method of treating mammalian tissue can comprise applying a composition including lyophilized platelet lysates to a mammalian tissue site for treatment, wherein the lyophilized platelet lysates include released concentrations of available growth factors, cytokines, and chemokines. In one example, the lyophilized platelet lysates are prepared from source platelets, wherein at least 30% of the source platelets that remain in the composition, by platelet count, are lysed to form the lyophilized platelet lysates. Other concentrations of lyophilized platelet lysates prepared from the source platelets can be at least 50%, at least 70%, at least 90%, or even up to 100% lyophilized platelet lysates.

A method of culturing cells or tissue can comprise admixing a media composition including lyophilized platelet lysates with a cell or tissue culture. The media composition can include lyophilized non-lysed platelets as well, and at least 30% of a total platelet count can be lyophilized platelet lysates. Other concentrations of lyophilized platelet lysates prepared from the source platelets can be at least 50%, at least 70%, at least 90%, or even up to 100% lyophilized platelet lysates.

In each of the various embodiments herein, whether discussing the compositions or methods, there may be some common features of each of these examples that further characterize options in accordance with principles discussed herein. Thus, any discussions of the compositions or methods alone are also applicable to the other embodiment not specifically mentioned.

In further detail, the present disclosure provides a method for preparing the lyophilized platelet lysates (LPL), and in one example, LPL can be in the form of lyophilized platelet rich plasma lysates (LPRPL). The platelets can be concentrated using any method known in the art, such as centrifugation or
5 commercially available platelet purification devices. After platelet or platelet rich plasma purification or concentration, a resultant concentrated platelet material can be frozen, such as with liquid nitrogen at $-190\text{ }^{\circ}\text{C}$ or to at least $-80\text{ }^{\circ}\text{C}$ using dry ice, ethanol, or other freeze assisting composition, for a period of hours, e.g. 24 hours. After freezing, the concentrated platelet material can then be quickly
10 thawed to above room temperature, e.g., from $30\text{ }^{\circ}\text{C}$ to $45\text{ }^{\circ}\text{C}$, or in one example, at about $37\text{ }^{\circ}\text{C}$. This freeze-thaw cycle can be repeated to increase the concentration of platelet lysates. Generally, the more cycles carried out, the more platelets that will be lysed.

Once the platelet lysates are formed using the freeze-thaw cycling
15 procedure or some other lysing process, LPL (or LPRPL) can then be lyophilized using a commercially available lyophilizer, and then stored for extended periods of times at room temperature or even years at below freezing temperatures, e.g., from $0\text{ }^{\circ}\text{C}$ to $-100\text{ }^{\circ}\text{C}$, or more specifically in one embodiment, at about $-80\text{ }^{\circ}\text{C}$, the product can be stored for about 5 years or more.

20 In another aspect of the present disclosure, LPL can be used for research applications such as cellular or tissue cultures. Specifically, LPL powder or pellets can be reconstituted in a liquid medium (such as DMEM, saline, plasma, DMSO, MEM alpha, RPMI, B-mercaptoethanol, non-essential amino acids, sodium pyruvate, or glutamine, etc.) at appropriate concentrations for a given
25 application. This type of medium, enriched with LPL can be used for culturing mammalian cells, particularly human cells when human platelets are used, for research or therapeutic applications. It should be noted that the use of LPL is useful for growing or deriving various types of cells or tissue, including but not limited to, mesenchymal stem cells from various tissues, human embryonic stem
30 cells, keratinocytes, cardiomyocytes, and many other cell types within the human body, as these lyophilized platelet lysates prepared in accordance with examples of the present disclosure contain growth factors and cytokines that allow growth

of cells and tissues, as well as maintain organs and their functions, etc. The growth of cells in LPL supplemented medium, for example, can be an improvement in many cellular systems compared to existing commercially available medium supplements, such as Fetal Bovine Serum (FBS). In addition, 5 LPL cell culture additive can be kept at room temperature for longer periods of time, which is an advantage over FBS, which expires after less than a week at room temperature.

In another aspect of the present disclosure, LPL can be used for many therapeutic applications, such as a supplement in a medium that will be used to 10 culture cells for therapeutic applications. Thus, LPL can be produced as described herein, but under a cGMP system, which results in LPL produced under minimally manipulated methods and/or cGMP. In addition, it is not required that there be any animal products used to produce LPL of the present disclosure, making the material highly useful for human application.

15 In another aspect of the present disclosure, LPL can be used to treat wounds, ulcers, or burns. As LPL of the present disclosure can be produced under cGMP, topical application to damaged human tissue can be beneficial. As mentioned, LPS can be applied in dry form or rehydrated form prior to application, or as a gel. Alternatively, even if the skin is not damaged by a 20 wound, ulcer, or burn, it can be used for other types of damage that occurs as a result of aging, photodamage, pathological or degenerative disease, or the like. Thus, LPL of the present disclosure can be used at various concentrations and combined with a base cream or other carrier for cosmetic use.

In another aspect, LPL can be rehydrated and nebulized in various 25 concentrations and used to treat lung disorders. Fine powders or rehydrated fine droplets can be inhaled into the lungs for treatment of lung disorders, for example. LPL can also be reconstituted in eye drops as well for treatment of the eyes or surrounding tissue.

In another aspect of the present disclosure, LPL can be rehydrated at any 30 therapeutically effective concentration, or can be kept in powder form, and can be used for orthopedic applications, e.g., bone healing, bone fusion, etc.

In yet another aspect, LPL can be combined with a bandage that can be applied to the wound directly, or rehydrated then applied to the wound. Thus, in part due to the relatively long shelf life at room temperature of LPL of the present disclosure, such a bandage has enhanced wound healing properties and will last longer than many other compositions, giving it a better chance to remain active and effective prior to use. Alternatively, other application approaches can also be used, such as by rehydration and application using a syringe, spray bottle, or other solution dispenser at one of any therapeutically effective concentration when applied to the treatment area. Thus, the administration of the therapeutic compositions of the present disclosure can be done in any acceptable manner known in the medical and pharmaceutical arts.

Specific non-limiting examples of administration methods include the use of fluids, aerosols, sprays, mists, lotions, creams, ointments, gels, gums, lozenges, nebulized droplets or powders, suppositories, drops, washes, dispensing bottles, squeeze tubes, pre-soaked fabric, automatic mixing and/or dispensing devices, syringes, bandages, dermal patches or plasters, etc. In one specific example, LPL can be contained in a kit that can be used in combat or other emergency situations for treating wounds. The kit can store LPL in a more stable form, and then can be reconstituted for immediate use when needed.

With specific reference to the lysing cycle that occurs, and where further lysing occurs during the lyophilization step as described herein, increased amounts of growth factors, cytokines, chemokines, etc., can be present in the resultant material. Examples of these growth factors and other materials that can be present in the resultant material include, without limitation, PDGF, PDAF, VEGF, PDEGF, PF-4, TGF-B, FGF-A, FGF-B, TGF-A, IGF-1, IGF-2, BTG, TSP, vWF, PAI-1, IgG, IgM, IgA, KGF, EGF, FGF, TNF, IL-1, KGF-2, fibropeptide A, fibrinogen, albumin, osteonectin, gro-alpha, vitronectin, fibrin D-dimer, factor V, antithrombin III, a2 macroglobulin, angiogenin, Fg-D, and elastase. In further detail, growth factors, cytokines, or the like that can be present and include, without limitation, LIF, anticancer growth factors such as IGFBP3, eicosanoids such as PGs or leukotrienes, IL-1 TNF alpha, INFs, TNF-a, IL-6, IL-1(a/b), prostanoid metabolites, complement components, reactive oxygen intermediates,

arachidonic acid metabolites, coagulation factors, nitrates, and chemokines. Human derived growth factors, chemokines, cytokines, and hormones can include alpha defensin, alpha synuclein, beta synuclein, 4-1BBL, 6CKine, acidic FGF, activin A, activin R1b, angiopoietin 2, B-DNF, BAFF, BCA-1, BD-1, 5 BMP-2, BMP-4, BMP-7, BMPRA1, BDNF, CNTF, CTGF, CTLA-4Fc, CXCL1, CXCL2, cardiotrophin-1, Cripto, Cystatin C, Dkk-1, EGF AOF, EGF, EMAP II, ENA-78, EPO, Eotaxin, FGF basic AOF, FGF-10, FGF-16, FGF 17, FGF 18, FGF19, FGF4, FGF6, FGF7, FGF8, FGF8b, FGF9, Flt3, G-CSF, GDNF, GMCSF, HGF, HGH, IFN alpha A, IFN alpha A/D, IFN alpha D, IFN alpha a2b, IFN, beta 10 1A, IFN-gamma, IGF1, IGFII, IGFBP-4, IGFBP6, IL1alpha, IL-1Beta, IL10, IL11, IL12, IL13, IL15, IL17, IL17A, IL17F, IL18, IL19, IL2, IL20, IL21, IL23, IL28A, IL28B, IL29, IL3, IL31, IL33, IL4, IL5, IL6, IL7, IL8, IL9, IL10, ITAC, KGF2, Kallikrein11, Kallikrein4, Kallikrein7, LEFTY-A, LIF, Leptin, MCSF AOF, MCSF, MCP-1, MCP2, MCP3, MCP4, MDC, MIG, MIP1alpha, MIP1 beta, MIP3 alpha, 15 MIP3 beta, MIP4, MIP5, midkine, NAP2, NT3, NT4, Neurotactin, neurturin, Oncostatin, osteoprotegerin, PDGF-AA, PDGF-AB, PDGF-BB, PTN, Rank ligand, Rank receptor, RANTES< SCF, SCFAOF, SDF-1 alpha, SDF-1Beta, CD4, CD40L, TNF-RI, TNFRII, TARC, TECK, TGF alpha, TGF1 Beta1, TGF Beta2, TGF Beta3, TNF beta/lymphotoxin, TNF-alpha, TPO, TRAIL, TWEAK, and 20 VEGF. Thus, the compositions prepared in accordance with examples of the present disclosure can be prepared to remove or destroy the cellular information that is commonly found at the cell membrane, and retain many of these growth factors, cytokines, chemokines, etc., for beneficial use as a healant, cell culture medium additive, cosmetic treatment composition, etc.

25

EXAMPLES

The following examples illustrate embodiments of the present disclosure that are presently best known. However, it is to be understood that the following 30 are only exemplary or illustrative of the application of the principles of the present disclosure. Numerous modifications and alternative compositions, methods, and systems may be devised by those skilled in the art without departing from the

spirit and scope of the present disclosure. The appended claims are intended to cover such modifications and arrangements. Thus, while the present disclosure has been described above with particularity, the following examples provide further detail in connection with what are presently deemed to be the most
5 practical embodiments.

Example 1 - Preparation of Lyophilized Platelet Lysates (LPL)

Peripheral blood is collected with an appropriate amount of anticoagulant to prevent clotting of the blood during processing. In this example, 35 mL of
10 blood is collected, which is then centrifuged at 200 x g (1000 rpm) for 10 minutes at 4 °C with the brake set to off mode. After centrifugation, the platelet rich portion is removed and tubes of the platelet rich portion are placed into a freezing range of -190 °C to -80 °C for a period of 24 hours. After 24 hours, the platelets are lysed by thawing quickly at 37 °C. After thawing, the tubes are then returned
15 for another freeze cycle within the same freezing range for a period of 24 hours. The freeze-thaw cycle (one freeze cycle followed by one thaw cycle) is repeated at least once, and more typically, repeated at least twice for a total 3 cycles, e.g., 3 to 6 freeze-thaw cycles. It is notable that sonication, filtration, or the like, can be used to lyse or collect platelets as well, either alternatively or additively with
20 respect to the freeze thaw cycle described herein. After the last thaw cycle, the tubes are refrozen at -80 °C and stored overnight. The following day, the tubes are lyophilized using FreeZone 2.5 Plus (Labconco) under 0.008 mBar at -84 °C for 48 hrs. The lyophilization can further lyse additional platelets as well. Depending on the total volume to be lyophilized, the time may be increased
25 beyond the 48 hours. Likewise, in some embodiments, the time may be decreased to some degree. The LPL, when prepared in this manner, can be stored at room temperature for at least a year, or if at -80 °C, for example, a period of 5 years can be possible. It is noted that the steps of lysing and lyophilizing can result in varying degrees of lysed platelets, e.g., 30%, 50%, 70%,
30 90%, or even up to 100% of the platelets can be lysed, depending varying the number of freeze-thaw cycles, the parameters of the freeze-thaw cycles, the parameters of the lyophilization, etc. Lyophilization alone will not provide the

degree of lysing that can occur when conducting both steps of lysing and lyophilizing in accordance with embodiments of the present disclosure.

Example 2 - Preparation of Lyophilized Platelet Rich Plasma Lysates (LPRPL)

5 The procedure of Example 1 is followed, except for with LPRPL, after the blood is collected as previously described, it is processed using a commercially available PRP preparation device such as the Magellan PRP device by Arteriocyte Medical Systems. The lysing and lyophilization steps of Example 1 can be otherwise the same for the present Example.

10

Example 3 - Use of LPL or LPRPL for Culturing Human Mammalian Cells

 After preparing the LPL of Example 1 or the LPRPL of Example 2, the material can be used to supplement tissue culture media for human cell culture. For example, a concentration of LPL or LPRPL can range from 0.5% to 20% by
15 volume of total lyophilized LPL or LPRPL used for the cell culture. In one specific example, 10 mL of LPL or LPRPL can be used to prepare 100 mL of media.

 In one specific embodiment, cultured human mesenchymal stem cells are isolated from bone marrow and adipose tissue, and a media composition can be prepared as follows:

20

 10mL lyophilized LPL or LPRPL (about 1 gram)
 1mL of NEAA (100X)
 1 mL of Glutamax (100X)
 1 mL of Penn/Strep (100X)
25 97 mL of DMEM LG - without Phenol

In this example, the cells are cultured until about 80% confluency and passaged to generate the desired cell number.

30 Example 4 – Use of LPL or LPRPL for Treating Wounds (Rehydrated Method)

 After preparing the LPL of Example 1 or the LPRPL of Example 2, the LPL or LPRPL can then be rehydrated in a syringe or a spray bottle at a desired

predetermined therapeutic concentration, such as 5%, 10%, 20%, 30%, 40%, 50%, etc., by volume). The concentration can be related to a desired effect, stability, need for specific type of wound to be treated, etc. In any event, the rehydrated LPL or LPRPL is then applied directly onto the site to be treated, and
5 is covered. The application can be a part of one time treatment, or more typically, can be repeated daily, or every three to five days as desired.

Example 5 – *Use of LPL or LPRPL for Treating Wounds (Non-Rehydrated or Powder Method)*

10 After preparing the LPL of Example 1 or the LPRPL of Example 2, the LPL or LPRPL is ground into a fine powder and applied directly onto a wound or site of treatment without first rehydrating the composition. The wound is covered for treatment at the wound or treatment site. The application can be a part of one time treatment, or more typically, can be repeated daily, or every three to five
15 days as desired.

Example 6 - *Use of LPL or LPRPL for Treating Wounds Using a Bandage*

After preparing the LPL of Example 1 or the LPRPL of Example 2, the LPL or LPRPL is ground into a fine powder and placed into a small porous gauze.
20 The gauze is then attached to an adhesive material to form a bandage. In this condition, the LPL or LPRPL bandage is ready to be applied onto various types of wounds directly. Alternatively, the LPL or LPRPL bandage can be rehydrated with saline or water and then placed onto the wound site.

25 Example 7 - *Use of LPL or LPRPL for Treating Lung Disorders*

After preparing the LPL of Example 1 or the LPRPL of Example 2, the LPL or LPRPL is ground into a fine powder and then can be rehydrated at various concentrations determined to have a therapeutic effect for a given treatment regimen. In this example, a concentration of 10% by volume was used. For
30 direct treatment to the lungs, the rehydrated LPL or LPRPL is nebulized and inhaled by the patient for a period of 10 minutes.

Example 8 – *Two-part Rehydration of LPL or LPRPL*

After preparing the LPL of Example 1 or the LPRPL of Example 2, the LPL or LPRPL is ground into a fine powder and placed into one side of a multi-compartment vessel, and on the other side of the vessel, a sterile saline is placed
5 so that the two materials do not contact one another until a seal is broken. Once the seal is broken, the saline mixes with the LPL or LPRPL and they become rehydrated. A spray applicator is then turned to the ON position, and using a pump to press up and down, the reconstituted LPL or LPRPL is applied by spraying directly onto a site to be treated. This is one example of a device that
10 can be used in accordance with examples of the present disclosure. Other devices can also be used for reconstitution and/or application of the LPL or LPRPL described herein.

15 Example 9 – *Use of LPL or LPRPL with an active agent or biologic*

After preparing the LPL of Example 1 or the LPRPL of Example 2, the LPL or LPRPL is ground into a fine powder or can be rehydrated at various concentrations determined to have a therapeutic effect for a given treatment regimen. The LPL or LPRPL is then admixed with an active agent or biologic,
20 such as an antifungal, antiviral, antibiotic, growth factor, or chemical, depending on the therapeutic or research application. The concentration of active agent or biologic can be any concentration that has a therapeutic effect or meets a research goal that is being considered, without limitation.

25 Example 10 – *Comparison of mesenchymal stem cell cultures using various media*

Lyophilized platelet lysates prepared similarly to that described in Example 1 were compared to other media products for culturing mesenchymal stem cells. FIG. 1 graphically shows the results of the study. Specifically, Media 1 included
30 Embryonic Stem Cell (ESC) qualified 10% Fetal Bovine Serum (FBS); Media 2 included 10% Platelet Rich Plasma (PRP); Media 3 included 10% Platelet Lysates (PL); and Media 4 included 5% lyophilized platelet lysates (LPL), each

percentage by volume. As can be seen from FIG. 1, the cells cultured in the media including the lyophilized platelet lysates significantly outperformed the cell cultures that did not include lyophilized platelet lysates.

5 While the invention has been described with reference to certain preferred embodiments, those skilled in the art will appreciate that various modifications, changes, omissions, and substitutions can be made without departing from the spirit of the invention. It is therefore intended that the invention be limited only by
10 the scope of the appended claims.

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CLAIMS

What Is Claimed Is:

- 5 1. A method of preparing a composition suitable for therapeutic use or as
a culture medium, comprising:
 concentrating platelets from a platelet source to form a platelet rich portion
of the platelet source;
 lysing the platelets in the platelet rich portion to form a plurality of lysates;
10 and
 lyophilizing the lysates to form lyophilized platelet lysates in a composition
with released concentrations of available growth factors, cytokines, and
chemokines.
- 15 2. The method of claim 1, wherein the step of concentrating is by
centrifugation.
3. The method of claim 1, wherein the platelet source is whole blood
- 20 4. The method of claim 1, wherein the platelet source is platelet rich
plasma.
5. The method of claim 1, wherein the platelet source is human.
- 25 6. The method of claim 1, wherein the step of concentrating is in the
presence of an anticoagulant.
7. The method of claim 1, wherein after the concentrating step, the
method further comprises the step of processing the platelet rich portion using a
30 platelet rich plasma preparation device to form platelet rich plasma, and wherein
the lyophilized platelet lysates formed are lyophilized platelet rich plasma lysates.

8. The method of claim 1, wherein the lysing step comprises using a freeze-thaw cycle on the platelet rich portion by freezing the platelet rich portion at a range of -190 °C to -80 °C, followed by quickly thawing the platelets at a temperature ranging from 30 °C to 45 °C.

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9. The method of claim 8, further comprising repeating the freeze-thaw cycle until a predetermined degree of cytolysis occurs.

10. The method of claim 9, wherein the predetermined degree of cytolysis is at least 30% cytolysis of the platelets.

11. A composition suitable for therapeutic use or as a culture medium, comprising lyophilized platelet lysates prepared from source platelets, wherein the lyophilized platelet lysates provide released concentrations of available growth factor, cytokines, and chemokines, and wherein at least 30% of the source platelets that remain in the composition, by platelet count, are lysed to form the lyophilized platelet lysates.

12. The composition of claim 11, prepared by the process of concentrating platelets from the source platelets to form a platelet rich portion; lysing the platelets in the platelet rich portion to form a plurality of lysates; and lyophilizing the lysates.

13. The composition of claim 11, reconstituted in a liquid medium.

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14. The composition of claim 11, formulated for application to a tissue site for therapeutic use.

15. The composition of claim 11, formulated into a medium for a cellular culture.

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16. The composition of claim 15, wherein the medium is formulated for *in vitro* use.

17. The composition of claim 15, wherein the medium is formulated for *in vivo* use.

18. The composition of claim 11, wherein the lyophilized platelet lysates are prepared from a mammalian platelet source.

19. The composition of claim 11, wherein the lyophilized platelet lysates are prepared from a human platelet source.

20. The composition of claim 11, wherein at least 50% of the source platelets are lysed to form the lyophilized platelet lysates.

21. The composition of claim 11, wherein at least 70% of the source platelets are lysed to form the lyophilized platelet lysates.

22. The composition of claim 11, wherein at least 90% of the source platelets are lysed to form the lyophilized platelet lysates.

23. The composition of claim 11, formulated into an encapsulant.

24. The composition of claim 23, wherein the encapsulant is a liposome.

25. The composition of claim 11, further comprising a therapeutic agent or drug admixed with the lyophilized platelet lysates.

26. The composition of claim 11, further comprising additional culture media admixed with the lyophilized platelet lysates.

27. A method of treating mammalian tissue, comprising applying a composition including lyophilized platelet lysates to a mammalian tissue site for treatment, wherein the lyophilized platelet lysates include released concentrations of available growth factors, cytokines, and chemokines.

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28. The method of claim 27, wherein the lyophilized platelet lysates are prepared from source platelets, and wherein at least 30% of the source platelets that remain in the composition, by platelet count, are lysed to form the lyophilized platelet lysates.

10

29. The method of claim 27, wherein the lyophilized platelet lysates are from human platelets, and the mammalian tissue site is a human tissue site.

30. The method of claim 29, wherein the tissue site is a skin site.

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31. The method of claim 29, wherein the treatment is cosmetic.

32. The method of claim 29, wherein the treatment is as a wound healant.

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33. The method of claim 27, wherein the tissue site is an internal tissue site.

34. The method of claim 33, wherein the internal tissue site is lung tissue.

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35. The method of claim 33, wherein the internal tissue site is exposed as a result of surgery.

36. The method of claim 27, wherein the lyophilized platelet lysates are applied in the form of a powder.

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37. The method of claim 27, wherein the lyophilized platelet lysates are applied in the form of a reconstituted lysate suspension.

38. The method of claim 37, wherein the reconstituted lysate suspension comprises the lyophilized platelet lysates brought together with saline for application to the tissue site.

5

39. The method of claim 37, wherein the tissue site is the eye.

40. The method of claim 27, wherein the lyophilized platelet lysates are applied in the form of a nebulized inhalant.

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41. The method of claim 27, wherein the lyophilized platelet lysates are applied in the form of an active ingredient on a bandage.

42. The method of claim 27, wherein the lyophilized platelet lysates are in the form of lyophilized platelet rich plasma lysates.

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43. The method of claim 27, wherein an active agent or biologic is applied to the tissue site along with the lyophilized platelet lysates.

44. A method of culturing cells or tissue, comprising admixing a media composition including lyophilized platelet lysates with a cell or tissue culture, wherein the lyophilized platelet composition further includes lyophilized non-lysed platelets, and at least 30% of a total platelet count is lysated.

20

45. The method of claim 44, wherein at least 50% of the total platelet count is lysated.

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46. The method of claim 44, wherein the method is carried out *in vivo*.

47. The method of claim 44, wherein the method is carried out *in vitro*.

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48. The method of claim 44, wherein the lyophilized platelet lysates are lyophilized platelet rich plasma lysates.

49. The method of claim 44, wherein the lyophilized platelet lysates are in powder form when admixed with the cell culture.

50. The method of claim 44, wherein the lyophilized platelet lysates are reconstituted in liquid form when admixed with the cell culture.

51. The method of claim 44, wherein the media composition further comprises one or more of DMEM, DMSO, alpha MEM, RPMI, B-mercaptoethanol, non-essential amino acids, sodium pyruvate, or glutamine.

52. The method of claim 51, wherein the media composition further comprises glutamine.

53. The method of claim 44, wherein the cell or tissue culture is from a mammal.

54. The method of claim 53, wherein the mammal is human.

55. The method of claim 54, wherein the cell culture includes mesenchymal stem cells, human embryonic stem cells, keratinocytes, or cardiomyocytes.

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Culture

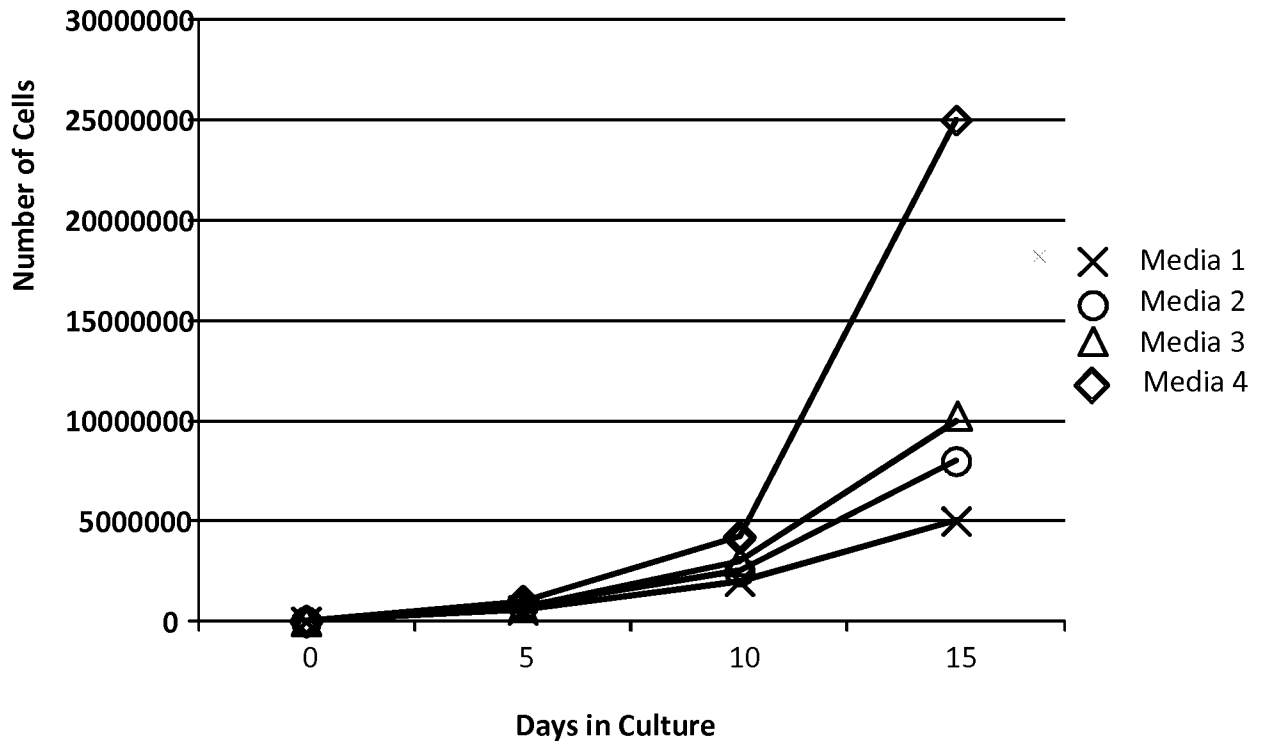


FIG. 1

A. CLASSIFICATION OF SUBJECT MATTER**A61K 38/24(2006.01)i, A61K 35/14(2006.01)i, A61K 9/127(2006.01)i, A61K 9/48(2006.01)i, A61P 17/00(2006.01)i**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K 38/24; A61P 29/00; C12N 5/09; A23L 1/31; C12N 5/071; A61K 9/00; C12N 5/02; A61P 17/00; A61K 35/14

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) & Keywords: lyophilized platelet lysate, concentrating, lysing, lyophilizing, growth factor, cytokine, chemokine

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2011-0123503 A1 (REBULLA et al.) 26 May 2011 See claims 1, 2, 4, paragraphs [0002], [0033]-[0034].	1-26
A		47
A	US 2011-0280952 A1 (CAMELLA et al.) 17 November 2011 See abstract, claims 1, 5, 8, paragraphs [0034]-[0036].	1-26, 47
A	US 2011-0171731 A1 (DIETZ et al.) 14 July 2011 See abstract, paragraphs [0005], [0010].	1-26, 47
A	US 2009-0023211 A1 (PERSSON et al.) 22 January 2009 See claims 1, 3, 4.	1-26, 47
A	US 2011-0200642 A1 (CENTENO) 18 August 2011 See claims 1, 11, paragraphs [0026], [0055].	1-26, 47

 Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family


Date of the actual completion of the international search

21 June 2013 (21.06.2013)

Date of mailing of the international search report

24 June 2013 (24.06.2013)

Name and mailing address of the ISA/KR


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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: 27-46, 48-55
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 27-46, 48-55 pertain to methods for treatment of the human body by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.
2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2013/023490

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