US011654428B2

(12) **United States Patent**
Batra et al.

(10) **Patent No.: US 11,654,428 B2**
(45) **Date of Patent: May 23, 2023**

(54) **METHODS, SYSTEMS AND APPARATUS FOR SEPARATING COMPONENTS OF A BIOLOGICAL SAMPLE**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **17/721,586**

(22) Filed: **Apr. 15, 2022**

(65) **Prior Publication Data**

US 2022/0258156 A1 Aug. 18, 2022

Related U.S. Application Data

(63) Continuation of application No. 17/424,871, filed as application No. PCT/US2020/014446 on Jan. 21, 2020.

(60) Provisional application No. 62/794,961, filed on Jan. 21, 2019.

(51) **Int. Cl.**

C12N 5/078	(2010.01)
B01L 3/00	(2006.01)
B04B 5/04	(2006.01)
A61K 35/16	(2015.01)

(Continued)

(52) **U.S. Cl.**

CPC ***B01L 3/50215*** (2013.01); ***A61K 35/16*** (2013.01); ***A61K 35/19*** (2013.01); ***B01D 17/0217*** (2013.01); ***B04B 5/0414*** (2013.01); ***C12N 5/0644*** (2013.01); *B01L 2200/026* (2013.01); *B01L 2200/0652* (2013.01); *B01L 2300/042* (2013.01); *B01L 2300/046* (2013.01);

(Continued)

(58) **Field of Classification Search**

CPC B01L 3/50215; B01L 2200/026; B01L 2400/0409; B04B 5/0414; C12N 5/0644; A61K 35/16; A61K 35/19; B01D 17/0217

See application file for complete search history.

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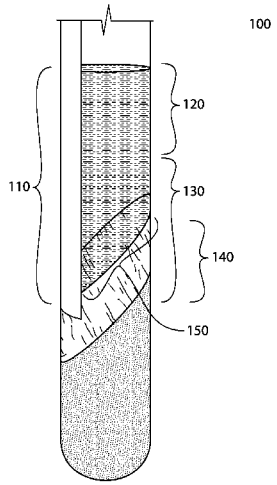
(57) **ABSTRACT**

Described herein are methods, systems and apparatus for separating components of a biological sample; as well as methods of using compositions prepared by same.

25 Claims, 9 Drawing Sheets



(51) **Int. Cl.**
A61K 35/19 (2015.01)
B01D 17/02 (2006.01)

(52) **U.S. Cl.**
CPC *B01L 2300/0832* (2013.01); *B01L 2400/0409* (2013.01)

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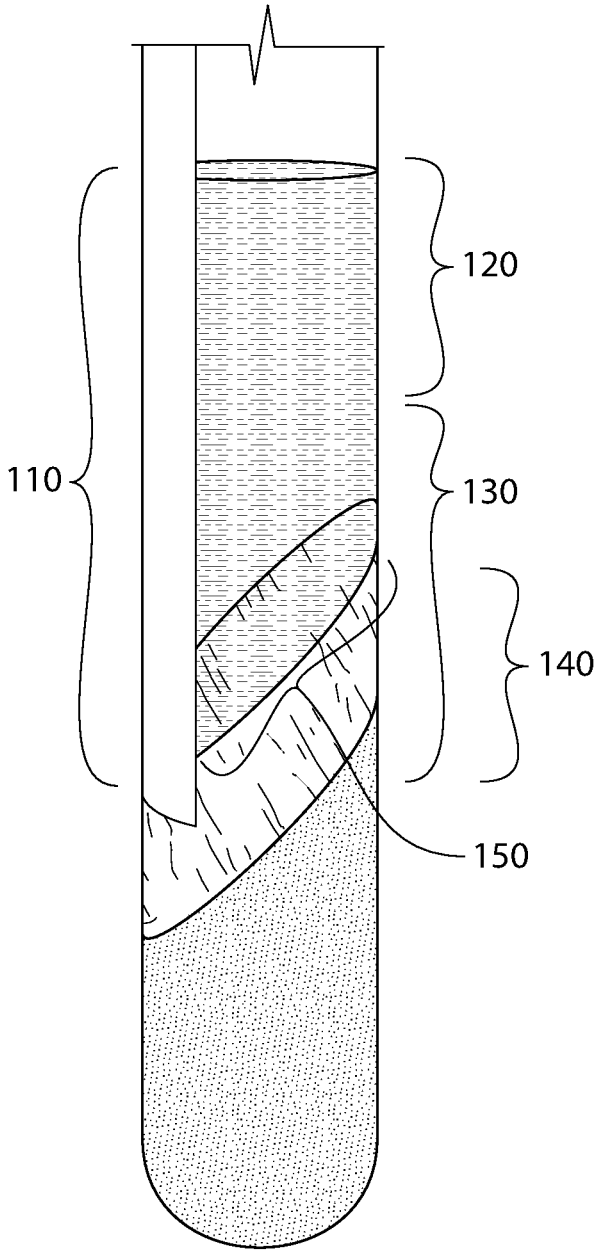

FIG. 1

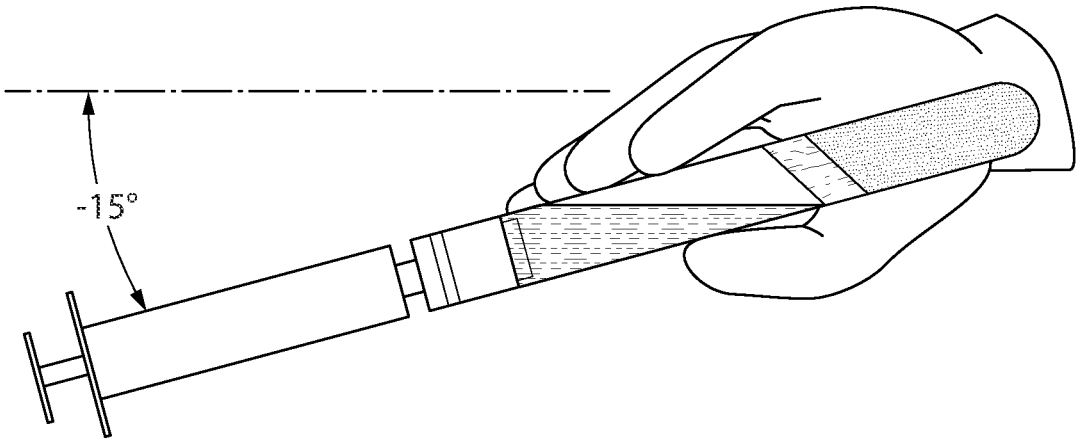

FIG. 2

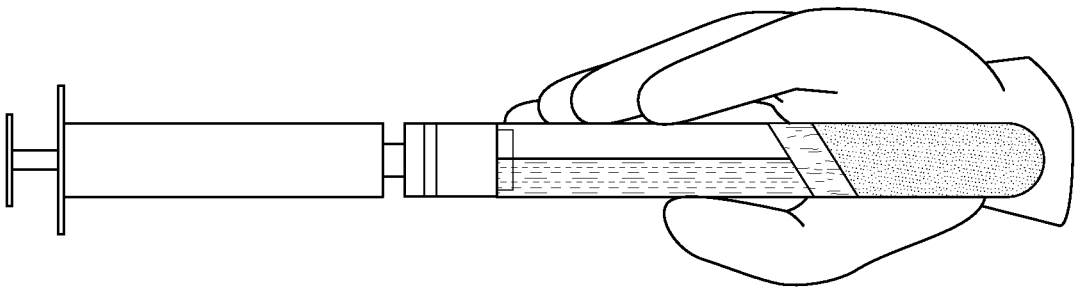

FIG. 3

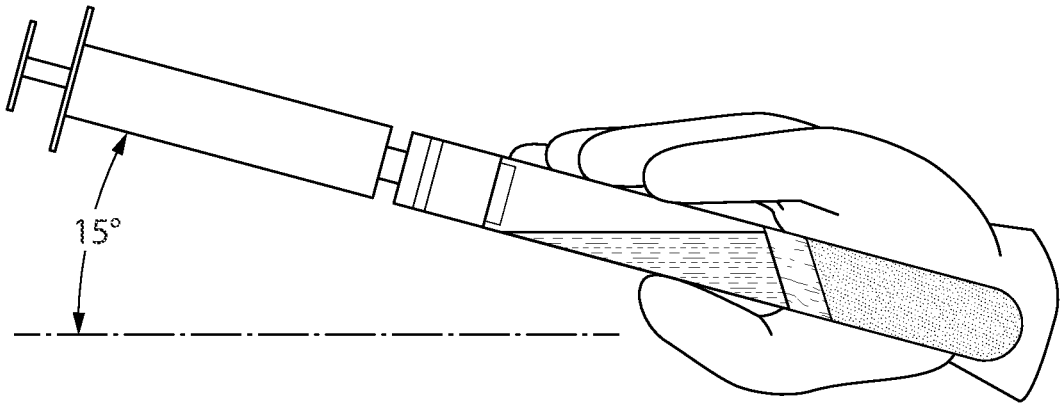

FIG. 4

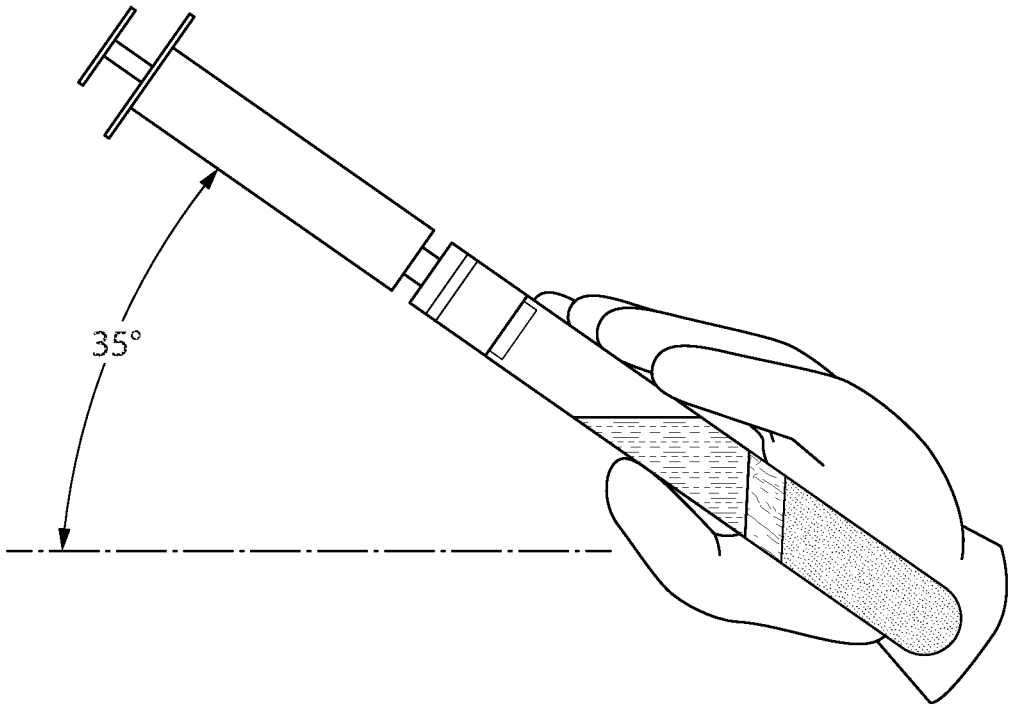

FIG. 5

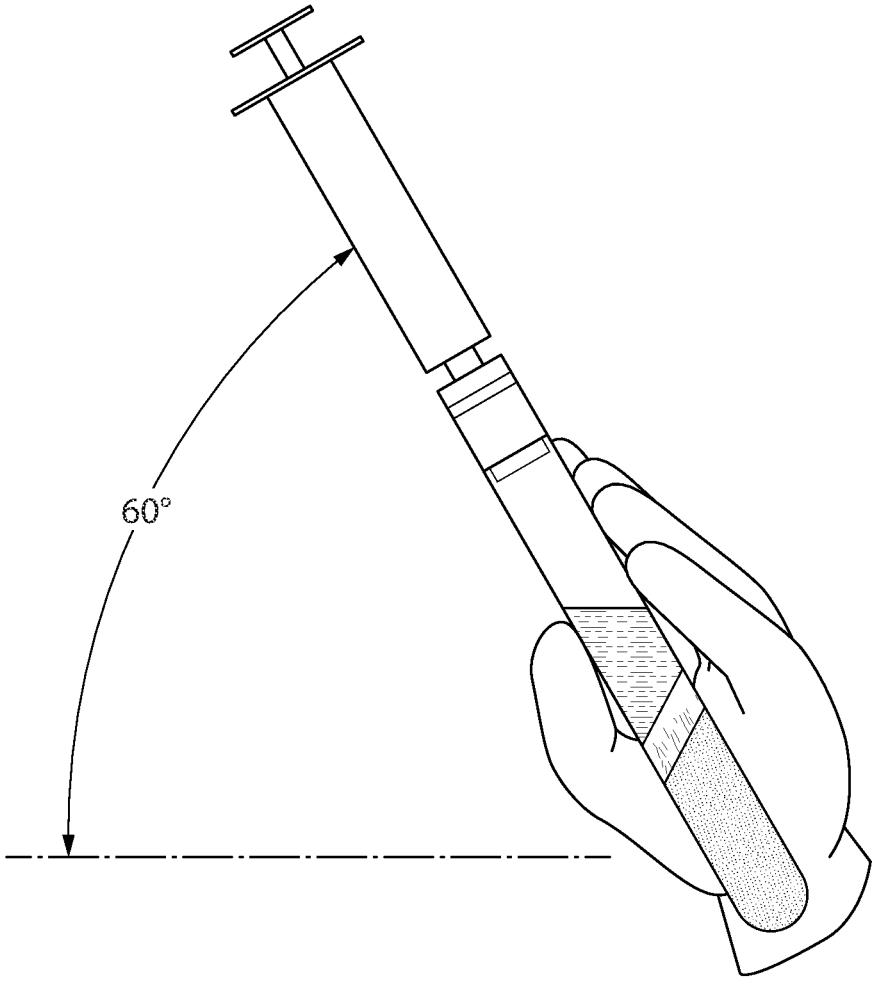

FIG. 6

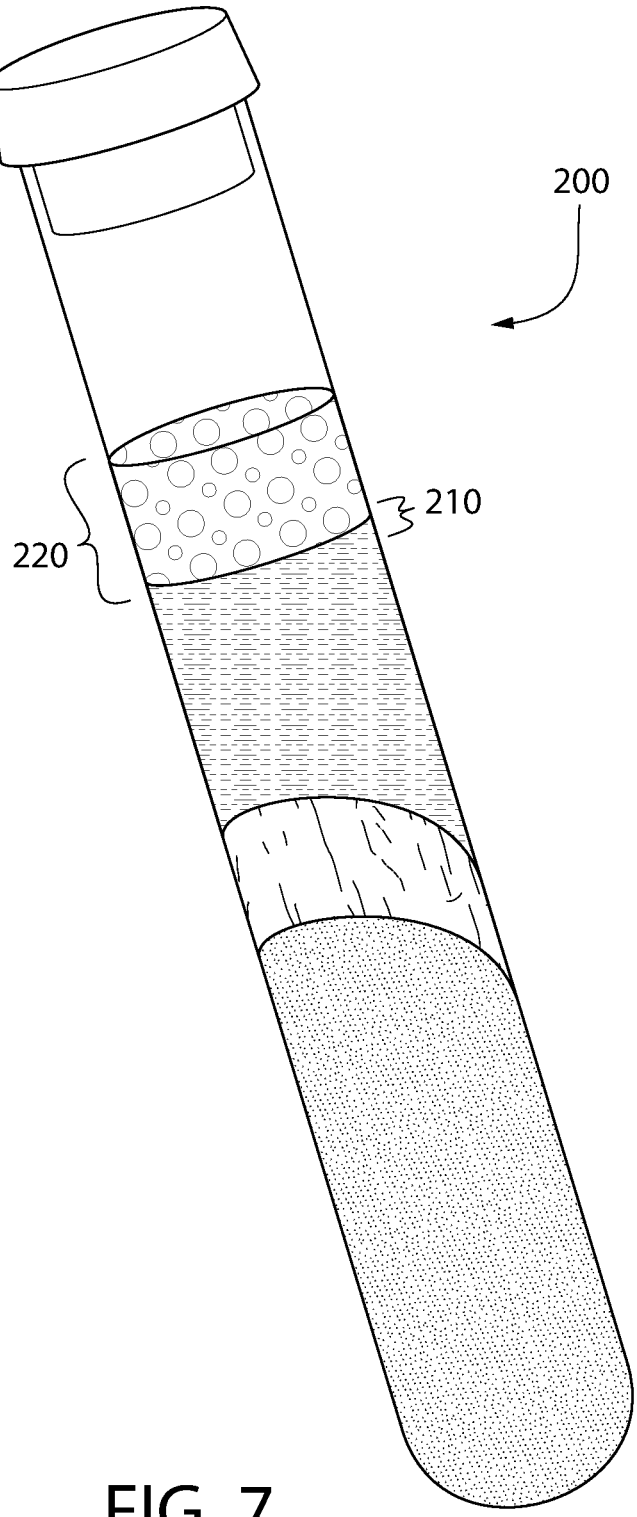

FIG. 7

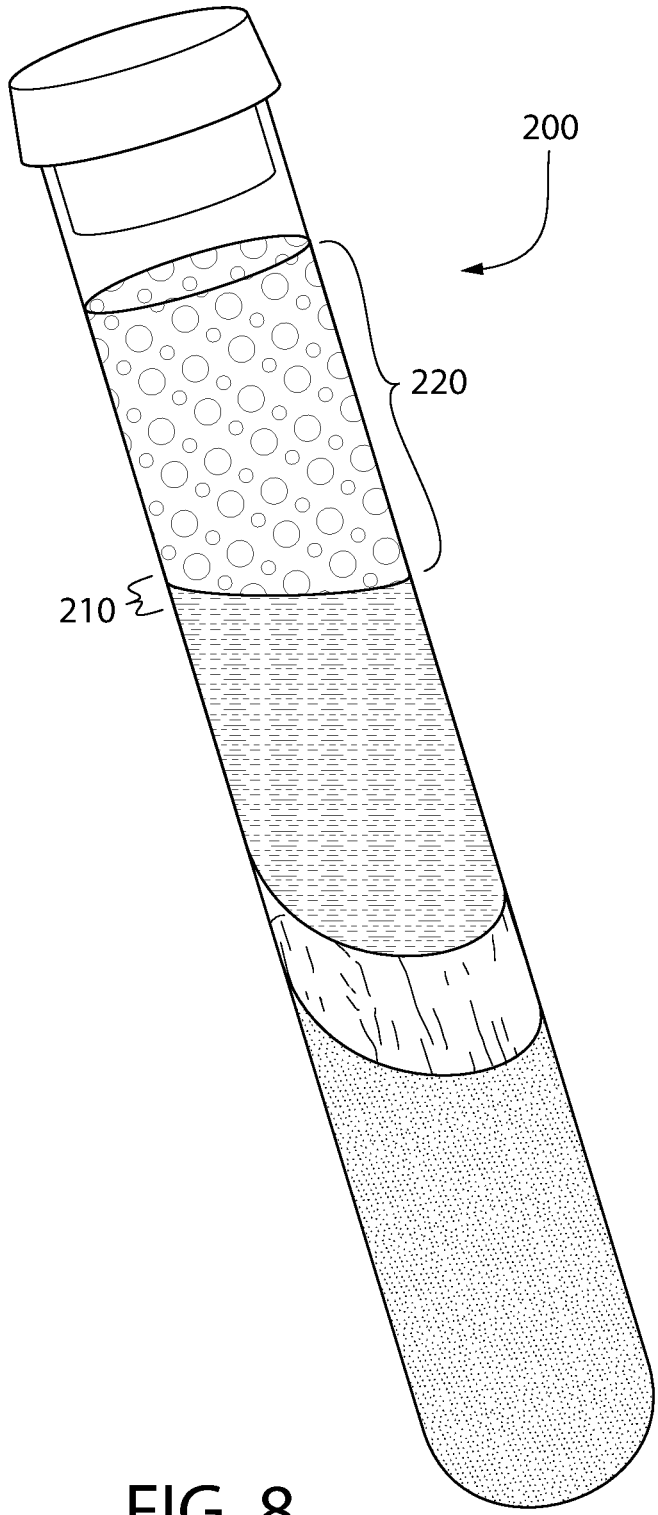

FIG. 8

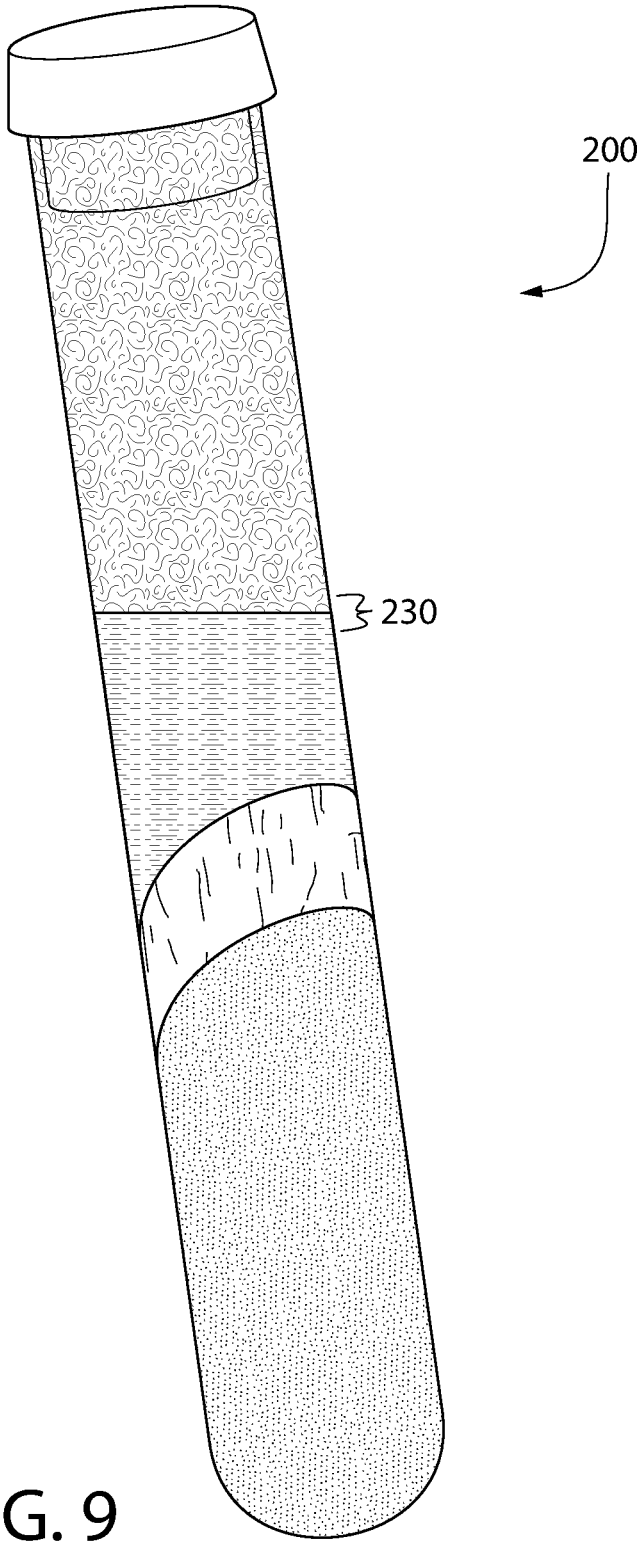

FIG. 9

METHODS, SYSTEMS AND APPARATUS FOR SEPARATING COMPONENTS OF A BIOLOGICAL SAMPLE

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation of U.S. application Ser. No. 17/424,871 filed Jul. 21, 2021, which is a US 371 application from PCT/US2020/014446 filed Jan. 21, 2020, published as WO 2020/154305 on Jul. 30, 2020, which claims the benefit of priority from U.S. Provisional Application No. 62/794,961 filed Jan. 21, 2019; the contents of which are hereby incorporated herein in their entireties.

BACKGROUND

Platelet-rich plasma (PRP) is generally understood to be a concentrate of platelets and plasma, that also contains growth factors, such as Platelet-Derived Growth Factor (PDGF); Transforming Growth Factor group (TGF); Epidermal Growth Factor (EGF); Vascular Endothelial Growth Factor (VEGF); Fibroblast Growth Factor (FGF); and Keratinocyte Growth Factor (KGF), which regulate the healing cascade by signaling surrounding cells to repair damaged tissue and regenerate new tissue.

Various methods and systems for preparing PRP are known; but for a variety of reasons, these methods and systems do not consistently provide efficient platelet capture. For example, devices and systems utilizing a separator gel, tend to have issues with platelets adhering to the separator gel. As a result, the clinician is often left with a less than desirable number of platelets available for administration to a patient.

Thus, there remains a need for simple, cost-effective, reliable and clinically useful methods for overcoming the aforementioned challenges; and that enrich platelet concentrations and increase the number of platelets available for administration to a patient. Embodiments of the present invention are designed to meet these and other ends.

SUMMARY

In some embodiments, the claimed invention is directed to methods for separating components of a biological sample, the method comprising: introducing a biological sample having a plurality of components to a tube comprising: a lumen; a proximal end; a distal end; an interior wall; and an exterior wall; applying a force to said tube for a time sufficient to separate said plurality of components; and agitating said tube at an angle (e.g. from about 5° to about 60°) effective to enrich the concentration of a component of the biological sample (e.g. platelets).

Other embodiments provide compositions comprising a product produced by any one of the methods or systems described herein. While other embodiments provide methods of using a product produced by any one of the methods or systems described herein

Still further embodiments provide system for separating components of a biological sample comprising: a biological sample; a tube; a means for applying a centrifugal force to said tube; and a means for agitating said tube.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** depicts an exemplary tube of the present invention after centrifugation.

FIG. **2** depicts a first comparative agitation angle.

FIG. **3** depicts a second comparative agitation angle.

FIG. **4** depicts a first exemplary agitation angle according to certain embodiments of the present invention.

FIG. **5** depicts a second exemplary agitation angle according to certain embodiments of the present invention.

FIG. **6** depicts a third exemplary agitation angle according to certain embodiments of the present invention.

FIG. **7** illustrates the foam created by an exemplary embodiment of the present invention.

FIG. **8** illustrates the foam created by another exemplary embodiment of the present invention.

FIG. **9** illustrates the absence of foam when a comparative method is performed.

DETAILED DESCRIPTION

In some embodiments, the present invention provides a method for separating components of a biological sample, the method comprising: introducing a biological sample having a plurality of components to a tube comprising: a lumen; a proximal end; a distal end; an interior wall; and an exterior wall; applying a force to said tube for a time sufficient to separate said plurality of components; and agitating said tube at an angle of from about 5° to about 60°. In some embodiments, the force is a centrifugal force.

As part of the Instructions for Use (IFU), PRP systems typically call for a gentle inversion of the collection tube following centrifugation. The inversion allows for resuspension of the platelets in the sample of PRP.

In some embodiments, the present invention provides a method wherein a tube containing a separated biological sample is agitated along its long axis in a rapid manner at a rate of several times per second. In some embodiments, the method may be performed for a few seconds up to one minute. In certain embodiments, the agitation angle may be slightly negative (−15 degrees) to vertical (+90 degrees).

Without being bound by theory, the present inventors believe that the methods of the present invention create a washing (i.e., lavage of the surface of the separation barrier) that helps to release platelets that may be attached to, or adhere to the surface of the separation barrier thereby increasing the number of platelets available for resuspension and administration to a subject.

Some embodiments of the present invention provide a tube comprising a material selected from: glass; modified poly amide (MPA); polyethylene terephthalate (PET) and any other material which is inert to a biological sample. In some embodiments, the tube comprises a laminate structure wherein an exterior wall of the tube is made of a material different than the interior wall.

In some embodiments, the tube further comprises a stopper. In some embodiments, the stopper comprises a material inert to biological samples. In other embodiments, the stopper comprises a material that does not crumble. In certain embodiments, the stopper comprises butyl rubber or its halo derivative formulations. In further embodiments, the stopper has a hardness of from about forty (40) to sixty (60) Shore A. In other embodiments, the stopper has a hardness designed to provide stable vacuum for from about eighteen (18) to about twenty-four (24) months.

In some embodiments, the tube is capable of receiving biological samples of from about four (4) ml to about one hundred (100) ml. In other embodiments, the tube is designed to receive biological samples of from about eight (8) ml to about fifty (50) ml. Still further embodiments provide tubes designed to receive biological samples of from

about ten (10) ml to about thirty (30) ml. Other embodiments provide tubes designed to receive biological samples of from about eleven (11) ml or about twenty-two (22) ml.

In some embodiments, the tube is selected from: a vacuum, tube, a non-vacuum tube, a plastic tube, a glass tube, a rigid tube, a non-rigid tube, a semi rigid tube and any combination thereof. In some embodiments, the terms "tube", "collection tube", "test tube", and the like, may be used interchangeably.

In some embodiments, the tube further comprises a gel. In some embodiments, the gel comprises a thixotropic gel. In further embodiments, the gel comprises a polymer. In certain embodiments, the gel can be a homopolymer or a co-polymer comprising a combination of monomers. In some embodiments, the gel comprises a polyacrylate, polyolefin or polyester.

Still further embodiments provide a gel having a density at 25° C. of from about 1.03 g/cm^3 to about 1.09 g/cm^3. While other embodiments provide a gel having a density at 25° C. of from about 1.04 g/cm^3 to about 1.07 g/cm^3. In some embodiments, the gel has a density at 25° C. of from about 1.05 g/cm^3.

In some embodiments, the gel has a viscosity at 30° C. of from about 1,000 to about 5,000 cps. In other embodiments, the gel has a viscosity at 30° C. of from about 1,000 to about 4,500 cps. In further embodiments, the gel has a viscosity at 30° C. of from about 1,000 to about 4,000 cps. While other embodiments utilize a gel having a viscosity at 30° C. of from about 1,000 to about 3,500 cps. Still further embodiments provide a gel having a viscosity at 30° C. of from about 1,000 to about 3,000 cps. In other embodiments, the gel has a viscosity at 30° C. of from about 1,500 to about 5,00 cps. In further embodiments, the gel has a viscosity at 30° C. of from about 2,000 to about 5,000 cps. While other embodiments utilize a gel having a viscosity at 30° C. of from about 2,500 to about 5,000 cps. Still further embodiments provide a gel having a viscosity at 30° C. of from about 3,000 to about 5,000 cps.

Yet other embodiments provide a separation barrier that does not comprise a gel, e.g. a solid float. In some embodiments, the float can take on a variety of shapes and may be constructed from a variety of materials. In certain embodiments, the float is comprised of a non-porous material and has a substantially smooth surface. In some embodiments, the separation barrier is selected from a gel; a solid float; and a combination thereof.

In some embodiments, the biological sample is autologous. In some embodiments, the biological sample comprises mammalian blood. In some embodiments, the mammalian blood comprises human blood. In some embodiments, the biological sample comprises whole blood.

Still further embodiments provide a biological sample comprising a first component comprising a plasma fraction and a second component comprising lymphocytes, monocytes and erythrocytes. In some embodiments, a centrifugal force is applied for a time sufficient to form a barrier between the first component and the second component. In other embodiments, a centrifugal force is applied for a time sufficient to form a barrier between the plasma fraction and the second component comprising lymphocytes, monocytes and erythrocytes.

In certain embodiments, the plasma fraction comprises platelets. In some embodiments, the plasma fraction comprises platelet rich plasma (PRP) and platelet poor plasma. In some embodiments, the plasma fraction comprises PRP and high-concentrated PRP. In some embodiments, the plasma fraction comprises PRP, high-concentrated PRP and ultra-high concentrated PRP.

Some embodiments further comprise the step of removing at least a portion of the first component. In some embodiments, from about twenty-five percent (25%) to about seventy-five percent (90%) of the first component is removed, optionally about thirty percent (30%) to about seventy percent (85%) of the first component is removed, about thirty-five percent (35%) to about sixty-five percent (80%) of the first component is removed, about forty percent (40%) to about sixty percent (75%) of the first component is removed, about forty-five percent (45%) to about fifty-five percent (70%) of the first component is removed, about forty-five percent (50%) to about fifty-five percent (90%) of the first component is removed, about fifty percent (50%), about sixty percent (60%), about seventy percent (70%), about eighty percent (80%), or about ninety percent (90%), of the first component is removed.

In some embodiments, the tube is agitated for a time sufficient to provide a plasma fraction having a straw color with a pinkish hue. In other embodiments, the tube is agitated for a time sufficient to provide a plasma fraction having a hue angle, h, in the CIELAB system of from 310 to 350 degrees. In further embodiments, the tube is agitated for a time sufficient to provide a plasma fraction having a hue angle, h, in the CIELAB system of from 310 to 345 degrees. In some embodiments, the tube is agitated for a time sufficient to provide a plasma fraction having a hue angle, h, in the CIELAB system of from 310 to 340 degrees. In still further embodiments, the tube is agitated for a time sufficient to provide a plasma fraction having a hue angle, h, in the CIELAB system of from 310 to 335 degrees. While in other embodiments, the tube is agitated for a time sufficient to provide a plasma fraction having a hue angle, h, in the CIELAB system of from 310 to 330 degrees. Still other embodiments provide methods wherein the tube is agitated for a time sufficient to provide a plasma fraction having a hue angle, h, in the CIELAB system of from 310 to 325 degrees. Yet other embodiments provide methods wherein the tube is agitated for a time sufficient to provide a plasma fraction having a hue angle, h, in the CIELAB system of from 310 to 320 degrees.

In some embodiments, the tube is agitated for a time sufficient to create a visually perceivable foam layer. In some embodiments, the foam layer is created on a surface of the plasma fraction. In some embodiments, the appearance of the foam layer correlates with the suspension of a clinically significant number of platelets in the plasma fraction. In other embodiments, the appearance of the foam is a signal that a clinically significant number of platelets are available for extraction and administration to a patient.

In some embodiments, the foam layer has a thickness of from about one (1) millimeter to about five (5) millimeters, optionally from about two (2) millimeters to about five (5) millimeters, or three (3) millimeters to about five (5) millimeters. While in other embodiments, the foam layer has a density of from about 0.01 g/cm^3 to about 0.25 g/cm^3, optionally from about about 0.05 g/cm^3 to about 0.25 g/cm^3, about 0.1 g/cm^3 to about 0.25 g/cm^3, about 0.15 g/cm^3 to about 0.25 g/cm^3, or about 0.2 g/cm^3 to about 0.25 g/cm^3.

In some embodiments, the tube is agitated for from about five (5) seconds to about sixty (60) seconds, optionally from about 5 seconds to about 50 seconds, about 5 seconds to about 45 seconds, about 5 seconds to about 40 seconds, about 5 seconds to about 35 seconds, about 5 seconds to about 30 seconds, about 5 seconds to about 25 seconds,

about 5 seconds to about 20 seconds, about 5 seconds to about 15 seconds, or about 5 seconds to about 10 seconds.

In some embodiments, the agitation is stepwise. In some embodiments, the stepwise agitation comprises a plurality of five second intervals of agitation. In other embodiments, the stepwise agitation further comprises a break between five second intervals. In certain embodiments, the break is from about 0.1 seconds to about 5 seconds.

In some embodiments, the agitation is a rhythmic motion. In some embodiments, the agitation creates a longitudinal or transverse wave-like motion in the biological sample. In some embodiments, the agitation creates a mixed longitudinal and transverse wave-like motion in the biological sample.

In some embodiments, a centrifugal force of from about 500 g to about 5000 g is applied to said tube. In other embodiments, a centrifugal force of from about 750 g to about 5000 g is applied to said tube. While in other embodiments, a centrifugal force of from about 1000 g to about 5000 g is applied to said tube. In yet other embodiments, a centrifugal force of from about 1500 g to about 5000 g is applied to said tube. In some embodiments, a centrifugal force of from about 2000 g to about 5000 g is applied to said tube. In some embodiments, a centrifugal force of from about 2500 g to about 5000 g is applied to said tube. In some embodiments, a centrifugal force of from about 3000 g to about 5000 g is applied to said tube. In other embodiments, a centrifugal force of from about 3000 g to about 4000 g is applied to said tube. While in other embodiments, a centrifugal force of from about 1500 g to about 2500 g is applied to said tube.

In some embodiments, the centrifugal force creates a plasma-gel interface between a surface of the gel and a surface of the plasma fraction. In some embodiments, the plasma-gel interface comprises platelets. In certain embodiments, the platelets in the plasma-gel interface are releasably bound to a surface of the gel. In some embodiments, the agitation releases platelets from the plasma-gel interface. In some embodiments, the platelets released from the plasma-gel interface are suspended in the plasma fraction.

In some embodiments, the tube further comprises (or contains) an anticoagulant. In some embodiments, the anticoagulant is selected from: a citrate salt (e.g. buffered sodium citrate); an EDTA salt (potassium-ethylenediaminetetra-acid); citrate-theophylline-adenosine-dipyridamole (CTAD); hirudin, benzylsulfonyl-d-Arg-Pro-4-amidinobenzylamide (BAPA); citric/citrate dextrose (ACD); heparin; an iodo acetate salt; an oxalate salt; a fluoride salt; and a combination of two or more thereof. Certain embodiments of the present invention do involve the use of a tube comprising an anticoagulant. In such embodiments, the biological sample may have been pre-treated with anticoagulant or the biological sample does not need to be anticoagulated.

Other embodiments provide compositions comprising a product of any one of the methods or systems described herein. Still further embodiments provide for the use of a composition comprising a product of any one of the methods or systems described herein for treating or preventing alopecia, bed sores, wrinkles, pain, tendonitis, arthritis, acne, scarring, crow's feet, ligament sprains and tears, and/or skin lesions.

Still further embodiments provide systems for separating components of a biological sample comprising: a biological sample; a tube; a means for applying a centrifugal force to said tube (e.g. a centrifuge); and a means for agitating said tube. In some embodiments, the systems described herein further comprise a means for measuring color in a biological sample. In some embodiments, the means for measuring color in a biological sample is selected from a spectrophotometer and a densitometer.

In some embodiments, the centrifuge is selected from a fixed angle centrifuge and horizontal spin centrifuge, or a swinging bucket centrifuge.

In some embodiments, the means for agitating the tube is adapted to linearly agitate the tube. In some embodiments, the means for agitating the tube is a tube rocker.

Some embodiments of the present invention provide a system as described herein further comprising a platelet counter. While other embodiments further comprise a processor. In some embodiments, the processor is wirelessly coupled to the means for applying a centrifugal force; the means for agitating the tube; the means for measuring color in a biological sample; and the platelet counter. In some embodiments, the means for applying a centrifugal force; the means for agitating the tube; the means for measuring color in a biological sample; the platelet counter; and the processor are contained in a single apparatus.

As used herein, the term "available platelet count" (or "APC") is intended to refer to the number of platelets that are readily accessible to the clinician for administration to a subject in need thereof.

In some embodiments, the methods and systems described herein increase the available platelet count ("APC") by at least about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 100%, about 105%, about 110%, about 115%, about 120%, about 125%, about 130%, about 135%, about 140%, about 145%, about 150%, about 200%, about 250%, about 300%, about 400%, or about 500%, versus the platelet count provided by a control system. In some embodiments, the control system substantially similar system those encompassed by the present invention, except for the absence of a means for agitating the tube; and/or a substantially similar system wherein the means for agitating the tube is only able to agitate the tube at an angle less than 5°, or at an angle greater than 60°.

In some embodiments, the means for agitating the tube is adapted to agitate the tube at an angle of from about 5° to about 60°, about 5° to about 55°, about 5° to about 50°, about 5° to about 45°, about 5° to about 40°, about 5° to about 35°, about 5° to about 30°, about 5° to about 25°, about 5° to about 20°, about 5°, about 10°, about 15°, about 20°, about 25°, about 30°, about 35°, about 40°, about 45°, about 50°, about 55°, or about 60°. As used herein, "agitation angle" and the like are intended to refer to the angle measured from horizontal.

In some embodiments, the methods and systems described herein provide an available platelet count ("APC") of greater than about 375,000 platelets/microliter, about 400,000 platelets/microliter, about 425,000 platelets/microliter, about 450,000 platelets/microliter, about 475,000 platelets/microliter, about 500,000 platelets/microliter, about 525,000 platelets/microliter, about 550,000 platelets/microliter, about 575,000 platelets/microliter, about 600,000 platelets/microliter, about 625,000 platelets/microliter, about 650,000 platelets/microliter, about 675,000 platelets/microliter, about 700,000 platelets/microliter, about 725,000 platelets/microliter, about 750,000 platelets/microliter, about 775,000 platelets/microliter, about 800,000 platelets/microliter, about 825,000 platelets/microliter, about 850,000 platelets/microliter, about 875,000 platelets/microliter, about

900,000 platelets/microliter, about 925,000 platelets/microliter, about 950,000 platelets/microliter, or about 975,000 platelets/microliter.

Other embodiments provide methods for: suspending platelets in a post-centrifuged biological sample; increasing APC in a biological sample; and/or enriching the platelet count in a biological sample, comprising: centrifuging a collection tube containing a biological sample and a thixotropic gel; and agitating the collection tube at an angle and rate effective to create a layer of foam on top of said biological sample.

For avoidance of doubt, at least a portion of any one of the methods described herein could be suitable for use in any one of the systems described herein.

Referring first to FIG. **1**, an exemplary tube (**100**) containing a biological sample post-centrifugation is depicted. As shown therein, the plasma fraction (**110**) comprises platelet poor plasma (**120**) and platelet rich plasma (**130**), wherein the platelet rich plasma (**130**) has a portion of ultra-high platelet concentration, sometimes referred to as ultra-high platelet rich plasma (**140**). Also depicted in FIG. **1** is the plasma-gel interface (**150**).

FIGS. **2-6** depict three exemplary agitation angles of the present invention and two comparative agitation angles. As discussed herein, the agitation angle is measured from the horizontal plane.

Referring next to FIGS. **7-8**, a tube (**200**) containing a biological sample after centrifugation and agitation in accordance with certain embodiments of the present invention is depicted. FIGS. **7** and **8** also depict the layer of foam (**220**) that appears on the top surface of the plasma fraction (**210**).

In contrast to FIGS. **7** and **8**, tube (**200**) of FIG. **9** does not include a layer of foam on the top surface of the plasma fraction (**230**).

Further areas of applicability of the present invention will become apparent from the detailed description provided hereinafter. It should be understood that the detailed description and specific examples, while describing exemplary embodiments, are intended for purposes of illustration only and are not intended to limit the scope of the present invention.

EXAMPLES

Example 1

A series of experiments were conducted to compare exemplary methods of the present invention to current methods of preparing PRP; and to understand how certain features impact platelet suspension and capture. In particular, agitation angle, agitation time, color of the biological sample and the presence of foam were evaluated. Change in color and the presence of foam were evaluated at various time points during the experiments. As described in Table 1 (below), experiments conducted with exemplary agitation angles and agitation times of the present invention provided surprisingly increased platelet counts with minimal to no infiltration of unwanted cells from the biological sample (e.g. erythrocytes). The results of these experiments are described in Table 1 (below).

TABLE 1

Method	Agitation Angle (°)	Agitation Time (seconds)	Color	Platelet Count (100K/µl)
Whole Blood	n/a	n/a	Deep red	193
Comp. Ex. 1*	n/a	n/a	Straw	355
Comp. Ex. 2	15	60	Deep red	n/a
Comp Ex. 3	45	10	Straw	397
Ex. 1	15	10	Straw w/pink hue	505
Ex. 2	15	30	Straw w/rose hue	436
Ex. 3	45	5	Straw w/pink hue	416
Ex. 4	45	30	Straw w/rose hue	415
Ex. 5	45	60	Light rose	480

Comp. Ex. 1 was a method performed in accordance with techniques known in the industry, wherein the tube was gently inverted horizontally twenty times.

As illustrated by the data described in Table 1 (above) the exemplary methods of the present invention surprisingly increased platelet counts with acceptable levels of infiltrate. A foam layer was also observed with each of the exemplary methods of the present invention. Without being bound by theory, the present inventors believe that agitation angle, agitation time and agitation rate are critical to achieving clinically maximal platelet counts. In addition, the appearance of foam on top of the plasma fraction provides a signal to the clinician that the desired platelet concentration has been achieved, as it correlates with the increased platelet counts.

Example 2

Additional experiments were conducted to further demonstrate the increased platelet counts provided by exemplary methods of the present invention. Five (5) samples from different donors were studied to evaluate the effect of the inventive methods at discrete time intervals ranging from five (5) seconds to one (1) minute. The impact of various agitation angles, ranging from −15° to 90° from horizontal, were also evaluated. The impact of both fixed angle and swing-bucket centrifuges was also evaluated. Platelet counts were performed using an automated Horiba ABX Micros 60 Hematology Analyzer (Horiba Instruments, Inc., Irvine Calif.).

The results of these experiments are described below in Tables 2 to 6. In each experiment 3 mL of platelet poor plasma (PPP) was removed before the platelets were counted in the PRP sample.

WB=Whole Blood

IFU=Instructions for Use

IFU platelet counts refer to platelet counts obtained using techniques known in the industry, wherein the tube was gently inverted horizontally twenty times.

TABLE 2

WB Platelet Count = 202/IFU Platelet Count = 241

Setting			Agitation Time (Seconds)							
Centrifuge	(speed × time)	Angle	5	10	15	20	30	40	50	60
Fixed	3300 × 10	−15	270	391	432	478	469	n/a	—	—
Fixed	3300 × 10	0	281	402	460	572	564	596	577	—
Fixed	3300 × 10	15	300	376	442	520	563	626	607	—
Fixed	3300 × 10	45	266	358	302	371	n/a	—	—	—
Fixed	3300 × 10	90	305	398	n/a	—	—	—	—	—

TABLE 3

WB Patelet Count = 335/IFU Platelet Count = 437

Setting			Agitation Time (Seconds)							
Centrifuge	(speed × time)	Angle	5	10	15	20	30	40	50	60
Fixed	3300 × 10	−15	442	461	438	425	n/a	—	—	—
Fixed	3300 × 10	0	498	529	571	602	639	n/a	—	—
Fixed	3300 × 10	15	501	535	581	672	602	628	638	649
Fixed	3300 × 10	45	471	495	502	462	n/a	—	—	—
Fixed	3300 × 10	90	521	n/a	—	—	—	—	—	—

TABLE 4

WB Platelet Count = 263/IFU Platelet Count = 358

Setting			Agitation Time (Seconds)							
Centrifuge	(speed × time)	Angle	5	10	15	20	30	40	50	60
Fixed	3300 × 10	−15	379	401	n/a	—	—	—	—	—
Fixed	3300 × 10	0	421	482	508	637	605	n/a	—	—
Fixed	3300 × 10	15	439	521	595	639	678	654	638	—
Fixed	3300 × 10	45	444	507	582	604	n/a	—	—	—
Fixed	3300 × 10	90	402	n/a	—	—	—	—	—	—

TABLE 5

WB Platelet Count = 321/IFU Platelet Count = 447

Setting			Agitation Time (Seconds)							
Centrifuge	(speed × time)	Angle	5	10	15	20	30	40	50	60
Swing	3300 × 10	−15	487	521	591	667	582	—	—	—
Swing	3300 × 10	0	598	671	778	864	701	728	—	—
Swing	3300 × 10	15	608	788	901	853	846	—	—	—
Swing	3300 × 10	45	570	605	739	704	698	—	—	—
Swing	3300 × 10	90	683	721	629	—	—	—	—	—

TABLE 6

WB Platelet Count = 172/IFU Platelet Count = 304

Setting			Agitation Time (Seconds)							
Centrifuge	(speed × time)	Angle	5	10	15	20	30	40	50	60
Swing	3300 × 10	−15	387	419	601	662	583	539	—	—
Swing	3300 × 10	0	408	488	573	701	745	705	728	—
Swing	3300 × 10	15	584	707	853	690	707	—	—	—

TABLE 6-continued

WB Platelet Count = 172/IFU Platelet Count = 304										
Setting			Agitation Time (Seconds)							
Centrifuge	(speed × time)	Angle	5	10	15	20	30	40	50	60
Swing	3300 × 10	45	551	674	779	871	720	—	—	—
Swing	3300 × 10	90	402	336	—	—	—	—	—	—

As illustrated by the data described in Tables 2 to 6 (above), exemplary methods of the present invention produce unexpected increases in platelet counts when compared to the platelet counts produced by conventional methods. These differences are not only numerically significant, but they also provide a clinically significant advance to the state of the art. Although the optimal time and angle may vary, the data unequivocally show that the agitation method, across the range of times and angles studied, increased platelet counts, thereby increasing the therapeutic dose of platelets that can be delivered to a subject.

Although several embodiments of the invention have been disclosed in the foregoing specification, it is understood by those skilled in the art that many modifications and other embodiments of the invention will come to mind to which the invention pertains, having the benefit of the teaching presented in the foregoing description and associated drawings. It is thus understood that the invention is not limited to the specific embodiments disclosed hereinabove, and that many modifications and other embodiments are intended to be included within the scope of the appended claims. Moreover, although specific terms are employed herein, as well as in the claims which follow, they are used only in a generic and descriptive sense, and not for the purposes of limiting the described invention, nor the claims which follow.

What is claimed is:

1. A method for:

suspending platelets in a post-centrifuged biological sample;

increasing the number of platelets available for administration to a patient; and/or

enriching the platelet count in a biological sample,

comprising:

centrifuging a collection tube containing a biological sample and a separation barrier;

maintaining the collection tube at an angle of from about −15° to about 90°; and

agitating the collection tube at said angle and at a rate effective to create a thin layer of foam on top of said biological sample.

2. The method according to claim **1**, wherein the collection tube further comprises an anticoagulant.

3. The method according to claim **2**, wherein the anticoagulant is selected from: a citrate salt; an ethylenediaminetetraacetic acid (EDTA) salt; citrate-theophylline-adenosine-dipyridamole (CTAD); hirudin, benzylsulfonyl-d-Arg-Pro-4-amidinobenzylamide (BAPA); citric/citrate dextrose (ACD); heparin; an iodo acetate salt; an oxalate salt; a fluoride salt; and a combination of two or more thereof.

4. The method according to claim **1**, wherein the biological sample comprises a plurality of components.

5. The method according to claim **4**, wherein the centrifugation is performed at a force of from about 500 g up to about 4000 g for a time sufficient to separate the plurality of components in the biological sample.

6. The method according to claim **5**, wherein the separation barrier forms a barrier between the plurality of components of the biological sample.

7. The method according to claim **1**, wherein the biological sample comprises whole blood.

8. The method according to claim **1**, wherein the biological sample comprises a first component comprising a plasma fraction; and a second component comprising lymphocytes, monocytes and erythrocytes.

9. The method according to claim **8**, wherein the plasma fraction comprises platelets.

10. The method according to claim **9**, wherein the plasma fraction comprises platelet rich plasma and platelet poor plasma.

11. The method according to claim **10**, further comprising the step of removing at least a portion of the first component.

12. The method according to claim **8**, wherein the foam layer is created on a surface of the plasma fraction.

13. The method according to claim **8**, wherein the separation barrier comprises a gel.

14. The method according to claim **13**, wherein a centrifugal force creates a plasma-gel interface between a surface of the gel and a surface of the plasma fraction.

15. The method according to claim **14**, wherein the plasma-gel interface comprises platelets.

16. The method according to claim **15**, wherein the platelets in the plasma-gel interface are releasably bound to the gel surface.

17. The method according to claim **16**, wherein the agitation releases platelets from the plasma-gel interface.

18. The method according to claim **17**, wherein the platelets released from the plasma-gel interface are suspended in the plasma fraction.

19. The method according to claim **1**, wherein the collection tube is agitated for from about one (1) second to about sixty (60) seconds, optionally from about four (4) seconds to about forty (40) seconds, about five (5) seconds to about sixty (60) seconds, about 5 seconds to about 50 seconds, about 5 seconds to about 45 seconds, about 5 seconds to about 40 seconds, about 5 seconds to about 35 seconds, about 5 seconds to about 30 seconds, about 10 seconds to 30 seconds, about 10 seconds to about 20 seconds, about 5 seconds to about 25 seconds, about 5 seconds to about 20 seconds, about 5 seconds to about 15 seconds, or about 5 seconds to about 10 seconds.

20. The method according to claim **1**, wherein the collection tube is agitated for a time sufficient to provide a plasma fraction having a hue angle, h, in the CIELAB system of from 310 to 350 degrees.

21. The method according to claim **1**, wherein the method increases the available platelet count (APC) by at least about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 100%, about 105%, about 110%, about 115%, about 120%, about 125%, about

130%, about 135%, about 140%, about 145%, about 150%, about 200%, about 250%, about 300%, about 400%, or about 500%, versus the platelet count provided by a control system.

22. The method according to claim **1**, wherein the foam layer has a density of from about 0.01 g/cm^3 to about 0.25 g/cm^3.

23. The method according to claim **1**, wherein the collection tube is maintained at an angle of from about 0° to about 90°.

24. The method according to claim **1**, wherein the collection tube is maintained at an angle of from about 5° to about 60°.

25. The method according to claim **1**, wherein the collection tube is maintained at an angle of from about 15° to about 45°.

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