PLALETET-RICH PLASMA (PRP) COMPOSITION, SYSTEM AND METHOD OF PREPARING THE COMPOSITION FOR OSTEOARTHRITIS TREATMENT

Field of Invention

[001] The present invention relates to a platelet-rich plasma (PRP) composition, system and method of preparing a platelet-rich plasma (PRP) composition for osteoarthritis treatment.

Background

[002] Osteoarthritis (OA) is a common debilitating disease characterized by chronic pain and symptoms of arthropathies such as articular cartilage damage, synovitis, subchondral bone remodeling and osteophyte formation. The prevalence of OA is increasing steadily due to the aging population and worldwide obesity epidemic. It brings a great social burden and imposes a major challenge on public health. OA could impact not only a patient's quality of life but may cause disability by progression of the disease. In view of this, there is great need to develop disease-modifying osteoarthritic drugs (DMOADs), and also an increase in understanding of the inflammatory pathways leading to the onset and progression of OA thereby enabling development of targeted therapies.

[003] Platelet-rich plasma (PRP) is an autologous whole-blood extract containing high concentrations of platelets and growth factor. There are studies in using injections of a patient's own PRP to promote and accelerate the recovery of injured ligaments, muscles, tendons, and joints. For instance, based on Journal of Clinical Medicine 2022 May; 11(9): 2640, Moretti *et al.,* disclosed a study of evaluating the efficacy and safety of PRP injections in patients affected by knee osteoarthritis (KOA). As PRP uses a patient's own immune system to improve OA, such treatment has few adverse effects and requires only a short hospital stay.

[004] There are existing patent applications or patents related to PRP-derived technologies. For instance, a Chinese Patent Application Publication No. CN 103340904 A disclosed a composition for treating osteoarthritis comprising PRP with certain concentration of leukocytes, components containing fat pluripotent cells, polysaccharides, and an optional pharmaceutically acceptable buffer. Another United States of America Patent Application

Publication No. US 2012171179 A1 disclosed a pharmaceutical composition comprising adipose-derived stem cells, PRP, calcium chloride solution and hyaluronic acid.

[005] Nonetheless, there are still concerns regarding clinical efficacy of using PRP, mainly due to the heterogeneity of preparation methods and resulting products, the scarceness of high quality randomized controlled trials (RCTs), and the contradictory results that have been found so far. Besides the poor standardization, the problem in usage of PRP in treating knee osteoarthritis includes: (1) low platelet counts, or inconsistent platelet counts in every PRP treatment which may lead to poor outcome; (2) the already sub-standard platelet counts release sub-optimal alpha granule releasates, in particular growth factors, which lead to a poor quality PRP; (3) short durability of the growth factors and other active releasates intra-articular after an injection due to quick lymphatic drainage system in knee joint; (4) poor affinity of growth factors to the specific cartilage receptors to enable the significant impact of the treatment of PRP for knee osteoarthritis as disease modifying agent.

[006] In view of the abovementioned drawbacks, the present invention provides a platelet-rich plasma (PRP) composition with consistent and stable quality suitable for osteoarthritis treatment. Further, the present invention provides a system and method of preparing the PRP composition.

Summary

[007] The following presents a simplified summary to provide a basic understanding of the present invention. This summary is not an extensive overview of the present invention, and is not intended to identify key features of the invention. Rather, it is to present some of the inventive concepts of this invention in a generalised form as a prelude to the detailed description that is to follow.

[008] The present invention seeks to provide a platelet-rich plasma (PRP) composition with consistent and stable quality for use in osteoarthritis treatment. More particularly, the composition comprising high amount of platelet counts, i.e., at least 1,000,000/μL of platelet counts, or at least 5 times of platelet counts from the basal level. Further, the composition comprises an optimum amount of bioactive molecules released from the PRP for the benefit of osteoarthritis treatment.

[009] The present invention seeks to provide a method and system of preparing a PRP composition. The method and system provide photo-activation to the PRP thereby stimulating release of bioactive molecules therefrom.

[0010] In one embodiment, the present invention provides a platelet-rich plasma (PRP) composition for treatment of osteoarthritis, comprising: a core comprising PRP and an activator compound comprising calcium chloride, wherein bioactive molecules are released in the core upon activation of alpha granules of platelets in the plasma; and an alginate-based wall surrounding the core to prevent haemolysis.

[0011] Preferably, the PRP is pre-activated by photochromatic light and pulsed electric field.

[0012] In one embodiment, the core comprising PRP and the activator compound in a weight ratio between 80 to 90: 20 to 10.

[0013] In one embodiment, the alginate-based wall is formed from an alginate solution having a volumetric concentration of 2.5 % to 10 % of alginate in water.

[0014] In one embodiment, the present invention provides a method of producing a platelet-rich plasma (PRP) composition for osteoarthritis treatment, the method comprising the steps of: separating a whole blood to obtain a PRP; activating the PRP to stimulate release of bioactive molecules from platelets in the PRP; enriching the activated PRP by adding an activator compound to further stimulate release of bioactive molecules from alpha granules of the platelets; and encapsulating the enriched PRP in alginate nanoparticles.

[0015] Preferably, the activating step is conducted in the presence of photochromatic light.

[0016] Preferably, the activating step is conducted in the presence of photochromatic light and pulsed electric field.

[0017] Advantageously, the activating step is conducted with the photochromatic light at near infrared (NIR) wavelength between 600 nm to 1200 nm.

[0018] Preferably, the activating step is conducted by subjecting the mixture to exposure under the photochromatic light at a predetermined distance and time intervals.

[0019] In another embodiment, the activating step is conducted in the presence of photochromatic light with the pulsed electric fields at sub-microsecond duration low electric field (SMLEF) with bipolar pulses.

[0020] Advantageously, the step of separating the whole blood is conducted by horizontally centrifugating thereof.

[0021] Preferably, the centrifugation is conducted at about 1200 to 1600 rpm.

[0022] In one embodiment, the step of encapsulating the enriched PRP in alginate nanoparticles is conducted by adding alginate nanoparticle to the PRP in the presence of stirring. Accordingly, the enriched PRP concentrate and the alginate solution used is in a volume ratio ranges from 8: 2 to 9: 1, respectively.

[0023] In another embodiment, the present invention provides a system for preparing a platelet-rich plasma (PRP) composition for osteoarthritis treatment, the system comprising: at least a separation tube being configured to extract platelet-rich plasma (PRP) from a whole blood; a centrifugator to provide outward rotation to the separation tube thereby obtaining the PRP; at least a photochromatic light source to subject the separation tube to light at near infrared wavelength; a control panel comprising a processor connected to the centrifugator and the photochromatic light source; and a power connector.

[0024] Preferably, the system further comprises an electric field generator to subject the separation tube to sub-microsecond duration low electric field (SMLEF) with bipolar pulses.

[0025] Advantageously, the centrifugator is located between two photochromatic light sources such that the sample tube is exposed to photochromatic light from more than one direction.

[0026] Preferably, the system further comprises a temperature sensor and/or regulator.

[0027] In the preferred embodiment, the separation tube is located at least 10 cm from the photochromatic light source.

Brief Description of the Drawings

[0028] This invention will be described by way of non-limiting embodiments of the present invention, with reference to the accompanying drawings, in which:

[0029] FIG. 1 illustrates a cross-section of an exemplary system in the present invention.

[0030] FIG. 2 illustrates a cross-section of an exemplary sample tube (before separation of whole blood) used in the system of FIG. 1.

[0031] FIG. 3 illustrates a cross-section of an exemplary sample tube (after separation of whole blood) used in the system of FIG. 1.

Detailed Description

[0032] One or more specific and alternative embodiments of the present invention will now be described with reference to the attached drawings. It shall be apparent to one skilled in the art, however, that this invention may be practised without such specific details. Some of the details may not be described at length so as not to obscure the invention. For ease of reference, common reference numerals or series of numerals will be used throughout the figures when referring to the same or similar features common to the figures.

[0033] The present invention provides an injectable platelet-rich plasma (PRP) composition for treatment of osteoarthritis. Generally, PRP is extracted from whole blood and is injected into a subject to deliver high concentrations of autologous bioactive molecules, including growth factors (GFs) and bioactive proteins, hence it is said to improve tissue healing or regeneration. Among others, the bioactive molecules include basic fibroblast growth factors, platelet-derived growth factors, endothelial-derived growth factor, hepatocyte growth factor, vascular endothelium growth factor, insulin-like growth factor 1, and etcetera. In the present invention, alpha granules of the platelets in PRP are stimulated to improve release yield of the bioactive molecules. Further, proteins involved in membrane trafficking, including vesicle formation, vesicle movement, intracellular targeting are stimulated, thereby

enhancing the release yield from alpha granules of the platelets. Among others, the proteins are vesicle-associated membrane protein 8 (VAMP-8), Syntaxin-2, Rab-GTPases, or the like.

[0034] In the present invention, the whole blood can be collected from one or multiple part of a subject, followed by mixing to obtain a whole blood mixture. In one embodiment, the whole blood mixture is collected from knees and hips of a subject. For instance, the whole blood mixture comprises 10 mL of whole blood drawn from left knee of a subject, 10 mL of whole blood drawn from right knee of a subject, 20 mL of whole blood drawn from left hip of a subject and 20 mL of whole blood drawn from right hip of a subject. The whole blood can be collected by means of a syringe with a large bore needle, such as 21 G type, to ensure efficient collection without causing any possible degradation or activation of platelets in the drawn blood. Anticoagulant, such as acid dextrose citrate or the like, is used during collection.

[0035] The whole blood mixture is subjected to separation and physical activation in a system as shown in FIG. 1. FIG. 1 exemplifies a system comprises a separation tube (10) being configured to extract platelet-rich plasma (PRP) from a whole blood (WB). The separation tube (10) (FIG. 2 and 3) is configured to separate whole blood into fractions including platelet-rich plasma (PRP), platelet-poor plasma (PPP), buffy coat (BC) and red blood cells (RBC). FIG.2 shows a separation tube (10) comprising a body with an inlet (20) configured at the top of the body to receive a whole blood (WB). Further, the tube further comprises two outlets (30, 40) configured in vicinity to the top of the body. The outlets (30, 40) are configured with different extensions into the body to discharge different fraction of a whole blood (WB) upon separation. According to FIG. 2, the outlet (30) has a first channel extended into an upper portion inside the body to discharge the PPP upon separation. Further, the outlet (40) has a second channel extended into a middle portion inside the body to discharge the PRP upon separation. Additionally, the tube comprises a membrane (50) across the body, preferably in about 45 degrees in relative to the tube body, to allow the PRP flow towards the outlet (40) without mixing with the PPP. Accordingly, buffy coat appears below the PRP fraction upon separation. Further, red blood cells appear below the buffy coat fraction. The separation tube (10) used in the present invention is compatible with the system as exemplified in FIG. 1. By which, the separation tube is made of a photochromatic light absorbing material and the material does not cause deflection during photoelectric stimulation. Preferably, it is made of glass. Hence, the separation tube (10) allows further

activation of the PRP fraction immediately, or at a predetermined time, after separation, thereby capturing optimum releasates therefrom. Further, the system comprises a centrifugator to provide outward rotation to the separation tube to improve yield of releasates.

[0036] According to FIG.1, there are two plasma arc lamps (100) to provide photochromatic light at near infrared (NIR) wavelength to the separation tube (10). The separation tube (10) is placed on a tray held by the centrifugator (200) placed between two plasma arc lamps (100). Preferably, separation tube (10) is held at least 10 cm from each of the plasma arc lamps (100). The system further comprises an electric field generator (not shown) to subject the separation tube to sub-microsecond duration low electric field (SMLEF) with bipolar pulses.

[0037] According to FIG. 1, the system further comprises a control panel (300) comprising a processor (not shown) connected to the centrifugator (200), the plasma arc lamps (100), and optionally the electric field generator (not shown). The processor is configured to connect and regulate centrifugation speed, duration of operation (such as duration of photo-activation), and temperature of the system. It shall be understood that the system comprises a power connector (not shown) which can be connected to a local power grid, or can be configured to self-powered by means of battery or a power generator. Additionally, the system comprises a temperature sensor and/or regulator (not shown). Additional features to enhance the system may be included, such as but not limited to, a touch screen monitor, safety fuse, etcetera.

[0038] According to the preceding description, the whole blood mixture is filled into at least one of the separation tubes as shown in FIG. 1. Accordingly, the whole blood mixture, is centrifuged to obtain the PRP. The separation tube (10) is placed in the centrifugator (200) in a manner whereby it can be exposed to photochromatic light from more than one direction as depicted by arrows in FIG. 1. Preferably, the step of separating the blood sample is conducted by horizontally centrifugating the whole blood in the separation tube (10). It was found that horizontal centrifuging yields higher release of platelets from the PRP as compared to vertical centrifuging. Further, the centrifugation is conducted at about 1200 to 1600 rpm, more preferably 1500 rpm.

[0039] In order to obtain effective concentration of bioactive molecules in the PRP, platelets need to be activated. Platelet activation refers to a process whereby the alpha granules of platelets are degranulated to release bioactive molecules, such as growth factors and fibrin, which form clots to stop bleeding and trap the molecules at a site of injury. Platelet activation in the body is instituted by tissue damage and thrombin cascade. However, in clinical applications of PRP, activation is initiated chemically (such as using an activator compound) or physically (such as photo-activation). In the present invention, the composition comprises PRP with an activator compound. In one embodiment, the PRP is first photo-activated by photochromatic light, and further being enriched by addition of an activator compound. In another embodiment, the PRP is first photo-activated by photochromatic light in the presence of pulsed electric field, and further being enriched by addition of an activator compound.

[0040] In one embodiment, the activating step is conducted by subjecting the PRP to exposure under photochromatic light in the near-infrared (NIR) wavelength of 600 nm to 1200 nm, more preferably 600 nm to 800 nm. The activating step is preferably conducted for about 10 to 15 minutes. Further, the activating step is preferably conducted by exposing the PRP to the photochromatic light from a distance of about 10 cm. It is found that such method is able to provide sustained release of bioactive molecules from the PRP.

[0041] In another embodiment, the activating step is conducted by subjecting the PRP to exposure under photochromatic light in the near-infrared (NIR) wavelength and sub-microsecond duration, low electric field (SMLEF) with bipolar pulses. Preferably, parameters involved in the SMLEF includes about 150 ns pulse width, about 650 ns interval between pulses of opposite polarity, and about 4 kV/cm of electric field. Activation of the PRP with SMLEF bipolar pulses is gentler than sub-microsecond, high electric field (SMHEF) pulses, resulting in less platelet microparticles generation but with overall activation levels similar to that obtained with thrombin. Hence, SMLEF provides the means to alter the PRP properties in a controlled fashion, thereby enabling evaluation of their effects on wound healing and clinical outcomes. The activation step triggers lamellipodia extension, numerous filopodia formation, and platelet agglomeration. It was found that PRP activated by the photochromatic light in NIR wavelength has an increased in ATP secretion, calcium release, and P-selectin expression. It was found that the activating step with a combined

photochromatic light and SMLEF is able to increase the platelet count by about 250 times more than basal value, enhance the release of bioactive molecules by about 300 % and activate alpha granular activities by about 55 %.

[0042] Accordingly, the step of enriching the activated PRP is conducted chemically, whereby an activator compound is added to further stimulate release of bioactive molecules from alpha granules of the platelets. The activator compound used is calcium chloride. Further, the enriched PRP is encapsulated in alginate nanoparticles to form the PRP composition in the present invention. In one embodiment, the step of encapsulating the enriched PRP in alginate nanoparticles is conducted by adding the enriched PRP into an alginate solution in the presence of stirring. Accordingly, the enriched PRP and the alginate solution used is in a volume ratio ranges from 8: 2 to 9: 1, respectively.

[0043] In another embodiment, the PRP composition includes a core comprising the activated PRP and an activator compound comprising calcium chloride, wherein bioactive molecules are released in the core upon chemical activation of alpha granules of platelets in the plasma; and an alginate-based wall surrounding the core. It shall be understood that the PRP used in the PRP composition of the present invention includes non-activated PRP and/or physically-activated PRP, as provided in preceding description. According to a preferred embodiment, the PRP composition has a core comprises activated PRP and the activator compound in a weight ratio between 80 to 90: 10 to 20. Further, the alginate-based wall is formed from an alginate solution having a volumetric concentration of 2.5 % to 10 % of alginate in water.

[0044] The PRP composition as described can be administered into a subject by means of injection. The composition may further include other pharmaceutically acceptable carrier and/or additive. The composition can be injected into a subject's knee in a dosage regimen of 10-15 ml solution three times a year or more, if deemed necessary, and depending on severity of the symptoms such as pain score, range of motion, and radiographic severity of the subjects in terms of duration and/or schedule. The PRP composition can be used in association with other intraarticular injection to enhance the efficacy of an osteoarthritis treatment.

[0045] While specific embodiments have been described and illustrated, it is understood that many changes, modifications, variations and combinations of variations disclosed in the text

description and drawings thereof could be made to the present invention without departing from the scope of the present invention.

CLAIMS:

1. A system for activating a platelet-rich plasma (PRP) composition for osteoarthritis treatment, the system comprising:
   a separation tube being configured to extract platelet-rich plasma (PRP) from a whole blood;
   a centrifugator to provide outward rotation to the separation tube thereby obtaining the PRP;
   at least a photochromatic light source to expose the separation tube to photochromatic light at near infrared wavelength;
   an electric field generator to subject the sample tube to sub-microsecond duration low electric field (SMLEF) with bipolar pulses;
   a control panel comprising a processor connected to the centrifugator and the photochromatic light source; and
   a power connector.

2. The system according to claim 1, wherein the sample tube is made with a photochromatic light absorbing material and the material does not cause deflection during photoelectric stimulation.

3. The system according to claim 1 or 2, wherein the centrifugator is located between two photochromatic light sources such that the sample tube is exposed to photochromatic light from more than one direction.

4. The system according to any one of claims 1 to 3, wherein the separation tube is located at least 10 cm from the photochromatic light source.

5. The system according to any one of claims 1 to 4 further comprises a temperature sensor and/or regulator.

6. The system according to any one of claims 1 to 6, wherein the sample tube comprises a membrane slanted in relative to the tube body to allow extracted PRP to flow into a separate compartment without mixing with other components of the whole blood.