

## KIT FOR ISOLATION OF PLATELET-RICH PLASMA AND THE METHOD USING THE SAME

### FIELD OF THE INVENTION

5 The present disclosure is related to a kit for isolation of platelet-rich plasma and the method using the same.

### BACKGROUND OF THE INVENTION

Platelet-RICH plasma (PRP) contained buffy coat with rich of platelet and growth factors has introduced to have a better effect on tissue repair, especially in bone, cartilage, skin, dental  
10 alveolar tissue and even as drug/cell carrier for controlled release.

PRP is a blood plasma containing higher-than-baseline levels of platelets. It can be activated by adding thrombin or calcium chloride ( $\text{CaCl}_2$ ) wherein will release the growth factors from alpha granules like fibroblast growth factor-2 (FGF-2), transforming growth factor-beta 1 (TGF- $\beta$ 1), bone morphogenic growth factor-2 (BMP2), insulin-like growth factors 1 (IGF1), platelet-  
15 derived growth factor (PDGF), and the other cytokines. Those are valuable in stimulating, signaling, and promoting cells to involve in tissue repair process. Up to date, the varieties of commercial PRP kits are available on the market where each shows a different predominance in the final products, for instance, the number of harvested platelets, the percentage of leukocytes or erythrocytes, the final volume PRP, price and operation time. Many commercialized PRP  
20 preparation kits on the market followed the protocol as did in fibrin glue preparation; that might be less plasma volume, limited platelet number, too many leucocyte/erythrocytes and inadequate fibrin ultra-structure. Currently, several new PRP devices or kits coming up to the market are generally to use the protocols more readily and user-friendly than before. Even though the first-line clinical people are still not satisfied with most of the PRP kits, no matter in a final volume  
25 of PRP, platelet number, the concentration of growth factors, the number of leukocytes/erythrocytes, or fibrin ultra-structure.

The commercialized PRP kits could be divided into two categories: plasma-based kits and buffy-coat-based kits; that could be briefly described as follows. To collect platelet from whole blood by centrifugation process as the conventional way brings the whole blood into three layers: (1)  
30 plasma layer on the top takes about 55% of whole blood and consists mostly of the least dense component, (2) buffy-coat stays at the middle layer that is less than 1% of whole blood and is

very rich in leucocytes & platelets, (3) the erythrocytes rest at bottom is about 45% of whole blood.<sup>16</sup> The plasma-based kits harvest only the top plasma layer with fewer platelet; on the contrary, the buffy-coat kits harvest both plasma layer and buffy-coat with a higher concentration of platelet as so-called platelet-rich plasma, PRP in short. The platelet could release more growth factors to induce local cells or mesenchymal stem cells to join the repair processes during tissue healing; so that the buffy-coat kits are more welcome to be used in tissue repair or regeneration. The work of the commercial buffy-coat kits available today are commonly based on blood-component density. However, the blood elements are very close in density that makes the separation of blood elements being a big challenge if just by traditional centrifugation. Therefore, the aforementioned commercialized buffy-coat kits are generally contaminated by leucocytes that would induce local inflammation to deteriorate healing process, or filtration by erythrocyte that might lead to local cells necrosis. In addition, the number of granulocytes in PRPs would interfere the formation of fibrin ultra-structure, such as fibrin diameter, pore size and porosity etc., that may be related to the cell migration during the healing process.

In view of this, a need exists for an improved approach which is capable to efficiently separate the platelet from blood plasma, particularly from white blood cells and red blood cells and obtain the platelet-rich plasma.

#### SUMMARY OF THE INVENTION

One aspect provided herein is a kit for isolation of platelet-rich plasma, comprising: a first isolation tube and a second isolation tube. The first isolation tube comprises a first opening and a second opening, and the second isolation tube comprises a first portion, a second portion, and a filter; wherein the first portion has a third opening, the filter is disposed between the first portion and the second portion, the second opening has a diameter in a range of 1 to 5 mm and the filter has a pore size in a range of 1.5 to 4  $\mu\text{m}$ .

Another aspect provided herein is a method of isolation of platelet-rich plasma, comprising: providing the kit of the present disclosure; disposing a blood sample in the first isolation tube through the first opening; centrifuging the first isolation tube to separate a bottom layer, a buffy coat layer, and an upper layer; discarding the bottom layer from the second opening; transferring the buffy coat and the top layer to the second isolation tube through the third opening; centrifuging the second isolation tube to collect the platelet-rich plasma in the second portion.

Other objects, advantages, and features of the disclosure will become more apparent from the following detailed description when taken in conjunction with the accompanying drawings.

#### BRIEF DESCRIPTION OF THE DRAWINGS

5 For the purpose of illustrating the invention, there are shown in the drawings embodiments which are presently preferred. It should be understood, however, that the invention is not limited to the preferred embodiments shown.

In the drawings:

10 Fig. 1 shows the illustrative view of the kit according to the present disclosure; (A) first isolation tube; (B) second isolation tube.

Fig. 2 shows the number of platelets of PRPs isolated by the kit of the present disclosure, commercialized PRP kits, whole blood (WB), and conventional protocol PRP (CP-PRP).

15 Fig. 3 shows the concentration of the growth factor released from activated PRPs of all groups set forth in Table 1: (A) FGF-2, (B) TGF- $\beta$ 1; the same mark (a, b, c, d, or d) means no significant statistical difference, and significance levels were indicated as \* ( $p < 0.05$ ), \*\* ( $p < 0.01$ ), and \*\*\* ( $p < 0.001$ ).

#### DETAILED DESCRIPTION OF THE INVENTION

20 The following embodiments when read with the accompanying drawings are made to clearly exhibit the above-mentioned and other technical contents, features and effects of the present disclosure. Through the exposition by means of the specific embodiments, people would further understand the technical means and effects of the present disclosure adopted to achieve the above-indicated objectives. Moreover, as the contents disclosed herein can be readily understood and implemented by a person skilled in the art, all equivalent changes or modifications which do  
25 not depart from the concept of the present disclosure shall be encompassed by the appended claims.

Furthermore, the ordinals recited in the specification and the claims such as “first”, “second” and so on are intended only to describe the elements claimed and imply or represent neither that the claimed elements have any proceeding ordinals, nor the sequence between one claimed element  
30 and another claimed element or between steps of a manufacturing method. The use of these

ordinals is merely to differentiate one claimed element having a certain designation from another claimed element having the same designation.

Furthermore, the terms recited in the specifications and the claims such as “on” are intended not only directly contact with the other element, but also intended indirectly contact with the other element.

#### Kit For Isolation of Platelet-Rich Plasma (PRP)

Please refer to Fig. 1 and 2. The kit for isolation of PRP comprises a first isolation tube 100 and a second isolation tube 200. The first isolation tube 100 has a first opening 101 and a second opening 102. In a preferred embodiment, the diameter of the first opening 101 is larger than the diameter of the second opening 102. In a more preferred embodiment, the second opening 102 has a diameter in a range of 1 to 5 mm. A first cap 110 is disposed on the first opening 101 and a second cap 120 is disposed on the second opening 102. As shown in Fig. 1, the diameter of the first isolation tube 100 reduces in the portion near the second opening 102 and form a tip-shape accordingly.

The second isolation tube comprises 200 a first portion 210, a second portion 220, and a filter 230. The first portion 210 has a third opening 201. The first portion 210 and the second portion 220 connect to each other and the filter 230 is disposed between the first portion 210 and the second portion 220. The filter 230 has a pore size in a range of 1.5 to 4  $\mu\text{m}$ . A third cap is disposed on the third opening. Hence in the present disclosure, the first, second, and third cap 110, 120, 240 would cover the first, second, and third opening 101, 102, 201 respectively.

In a particular embodiment, the ratio of the first portion 210 and the second portion 220 is between 45:55 to 35:65. In a preferred embodiment, the ratio of the first portion 210 and the second portion 220 is approximately 40:60.

In a particular embodiment, the length of the first isolation tube 100 and the second isolation tube 200 is between 10 to 20 cm.

In a particular embodiment, the first portion 100 and the second portion 200 are dismantlable. However, in another embodiment, the first portion 100 and the second portion 200 are in one piece.

#### Example 1 Preparation of Blood Sample and Isolation of Platelet-Rich Plasma (PRP)

A total volume of 500 ml of whole blood harvested from a swine by an internal jugular venipuncture supplemented with ACD-A anticoagulant (GBiosciences, USA). The blood sample was divided into eight groups to isolate PRP by different protocol, as disclosed in Table 1.

Table 1

| Group                                     | Abbreviation of the isolated product | Description                                                                                                                                                                                                                                                               |
|-------------------------------------------|--------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Whole Blood                               | WB                                   | The whole blood directly harvested from a donor                                                                                                                                                                                                                           |
| Conventional protocol                     | CP-PRP                               | The whole blood was harvested from a donor and go one-round centrifugation by 1600 g force to turn whole blood into three layers. The top two layers were collected and the bottom layer was discarded.                                                                   |
| Tube inserted with 5 $\mu\text{m}$ filter | H5M                                  | Platelet-rich plasma was isolated according to the method disclosed herein, wherein the filter has a cut-off pore size of 5 $\mu\text{m}$ . The second tube is placed in a centrifuge; and then the leukocytes are further filtered out by 2300 g force from the product. |
| Tube inserted with 3 $\mu\text{m}$ filter | H3M                                  | Platelet-rich plasma was isolated according to the method disclosed herein, wherein the filter has a cut-off pore size of 3 $\mu\text{m}$ . The second tube is placed in a centrifuge; and then the leukocytes are further filtered out by 2300 g force from the product. |
| Tube inserted with 2 $\mu\text{m}$ filter | H2M                                  | Platelet-rich plasma was isolated according to the method disclosed herein, wherein the filter has a cut-off pore size of 2 $\mu\text{m}$ . The second tube is placed in a centrifuge; and then the leukocytes are further filtered out by 2300 g force from the product. |
| Commercial kit                            | VB                                   | Commercialized PRP kit                                                                                                                                                                                                                                                    |

PRP is isolated by a two-step process. Firstly, a blood sample is disposed in a first isolation tube through the first opening. The first centrifugation is performed to separate a bottom layer, a buffy coat layer, and an upper layer in the first isolation tube. The bottom layer is discarded from

the second opening and then the buffy coat and the top layers are transferred to the second isolation tube through the third opening, i.e. are disposed in the first portion. The second centrifugation is performed to spin down the plasma in the second isolation tube. As a consequence, the plasma passes through the filter and the PRP is collected in the second portion, whereas the leukocyte is detained in the filter.

#### Example 2 Complete Blood Count

The concentration blood cells and platelets are counted in order to assess the efficiency of isolation. The isolation groups disclosed in Table 1 are respectively counted for the platelet, white blood cells, and red blood cells within by an automated veterinary hematology analyzer (IDEXX Procyte Dx) for complete blood count (CBC). The results are shown in Fig. 2 and Table 2.

Table 3 was to summarize the formulae for the counting and evaluation to all the blood cellular components.

Unless otherwise described, the experiment data in the present disclosure are presented as mean  $\pm$ S.D by using GraphPad Prism software 6. The data were analyzed by one-way ANOVA, followed by Tukey multiple comparison test with significance levels were indicated as \*  $p < 0.05$ , \*\*  $p < 0.01$ , and \*\*\*  $p < 0.001$ .

As shown in Fig.2, the platelet concentration of group CP-PRP and group WB (whole blood) were  $239 \times 10^3/\mu\text{l}$  and  $119 \times 10^3/\mu\text{l}$ , respectively; which was approximately 2-times rich. The platelet number for group H5M, group H3M, group H2M, and group VB are  $8.5 \times 10^3/\mu\text{l}$ ,  $17.0 \times 10^3/\mu\text{l}$ ,  $22.0 \times 10^3/\mu\text{l}$ , and  $13.0 \times 10^3/\mu\text{l}$ , respectively. Among these, the group H2M showed the highest platelet number. Although the platelet concentration of group CP-PRP and group WB was much higher than that of group H2M, however, in view of Table 2, the white blood cells (WBCs) yield in the CP-PRP is much higher than those in H2M, H3M, and H5M and thus the PRP collected in group CP-PRP is probable of causing the intensifying of the local inflammation. WB contained too much both in WBCs and red blood cells (RBCs) that may not only induce local inflammation but also slow down the healing process.

Table 2

| PRP kits       | Initial volume<br>(ml) | Final volume<br>(ml) | Volume Yield<br>(VY %) | Platelet Yield<br>(PY %) | RBC Yield<br>(RY %) | WBC Yield<br>(WY %) |
|----------------|------------------------|----------------------|------------------------|--------------------------|---------------------|---------------------|
| CP-PRP         | 30                     | 15.65±1.32           | 50.24±4.40             | 100.90±8.83              | 0.18±0.03           | 27.83±15.94         |
| H5M            | 4                      | 1.93±0.09            | 48.13±2.29             | 3.44±0.16                | 0.05±0.02           | 0                   |
| H3M            | 4                      | 1.82±0.09            | 45.39±2.37             | 6.48±0.34                | 0.01±0.00           | 0                   |
| H2M            | 4                      | 1.82±0.09            | 45.39±2.37             | 8.39±0.44                | 0                   | 0                   |
| Commercial Kit | 10                     | 3.833±0.29           | 38.33±1.44             | 4.19±0.16                | 0                   | 5.3                 |

Table 3

A.  $Volume\ Yield\ (VY\%) = \frac{volume\ of\ product\ (ml)}{volume\ of\ WB\ processed\ (ml)} \times 100$

B.  $Platelet\ Yield\ (PY\%) = \frac{volume\ of\ product\ (ml) \times Platelet\ concentration\ in\ product\ (x10^9/\mu L)}{volume\ of\ WB\ collected\ (ml) \times Platelet\ concentration\ in\ WB\ (x10^9/\mu L)} \times 100$

C.  $RBC\ Yield\ (RY\%) = \frac{volume\ of\ product\ (ml) \times RBC\ concentration\ in\ product\ (x10^9/L)}{volume\ of\ WB\ collected\ (ml) \times RBC\ concentration\ in\ WB\ (x10^9/L)} \times 100$

D.  $WBC\ Yield\ (WY\%) = \frac{volume\ of\ product\ (ml) \times WBC\ concentration\ in\ product\ (x10^9/L)}{volume\ of\ WB\ collected\ (ml) \times WBC\ concentration\ in\ WB\ (x10^9/L)} \times 100$

Table 2 shows the summary of blood count and the concentration of isolated PRPs yielded. The groups according to the present disclosure, which employ the kit of the present disclosure, including groups H5M, H3M, and H2M collected relatively high final volume and volume yield of PRPs from the whole blood, than those of commercialized kits of group VB

5 The PRP in the groups according to the present disclosure were successfully and efficiently isolated because the filter is permeable only for the platelets and detain other cells.

The groups H2M, H3M, and H5M according to the present disclosure could effectively separate RBCs and WBCs from PRPs, especially for the H2M group. Moreover, the RBCs in groups H5M and H3M are reduced to 0.05% and 0.01%, respectively, which is significantly lower than  
10 the CP-PRP group (conventional protocol).

Accordingly, PRPs isolated by the kit according to the present disclosure (H2M, H3M, and H5M) yield better volume and platelet concentration, which implies that the kit and the method according to the present disclosure efficiently and successfully isolates the PRP from whole blood sample and only extremely low or even no erythrocyte and leukocyte is present in the final  
15 product.

#### Example 3 Growth Factor Quantification

The growth factors quantification of PRPs including fibroblast growth factor-2 (FGF-2) and tumor growth factor-beta-1 (TGF- $\beta$ 1) were quantified by using ELISA kits (Wuhan USCN Business. Co., Ltd., USA) according to the user instructions.

20 The growth factors quantification of the prepared PRPs were shown in Fig. 3. The concentration of growth factors, FGF-2, and TGF $\beta$ -1 in the PRPs prepared from groups H2M, H3M, and H5M according to the present disclosure and CP group were all far above average. The concentrations of growth factors in PRPs prepared from the other commercialized kit were fluctuation very much.

25 As for the concentration of FGF-2 (Fig. 3(A)), the concentration in group H5M, group H3M, group H2M, and group CP were all higher than 750 pg/ml. The concentration in group VB was lower than 500 pg/ml.

As for the concentration of TGF $\beta$ -1 (Fig. 3(B)), the concentration in group H5M, group H3M, group H2M, and group CP were all higher than 1750 pg/ml. The concentration in group VB was  
30 lower than 1500 pg/ml.

The results demonstrates that the concentrations of growth factors in group using the kit according to the present disclosure were relatively stable and far above average.

While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only and can be implemented in combinations. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

What is claimed is:

1. A kit for isolation of platelet-rich plasma, comprising:  
a first isolation tube, comprising a first opening and a second opening, and  
a second isolation tube, comprising a first portion, a second portion, and a filter;  
5        wherein the first portion has a third opening, the filter is disposed between the first portion and  
the second portion, the second opening has a diameter in a range of 1 to 5 mm and the filter has a  
pore size in a range of 1.5 to 4  $\mu\text{m}$ .
2. The kit of claim 1, wherein the ratio of the first portion and the second portion is between  
45:55 to 35:65.
- 10        3. The kit of claim 1, wherein the first portion and the second portion are dismantlable.
4. The kit of claim 1, further comprising a first cap, a second cap, and a third cap to cover the  
first opening, the second opening, and the third opening respectively.
5. A method of isolation of platelet-rich plasma, comprising:  
providing the kit of claim 1;  
15        disposing a blood sample in the first isolation tube through the first opening;  
centrifuging the first isolation tube to separate a bottom layer, a buffy coat layer, and an upper  
layer;  
discarding the bottom layer from the second opening;  
transferring the buffy coat and the top layer to the second isolation tube through the third  
20        opening;  
centrifuging the second isolation tube to collect the platelet-rich plasma in the second portion.
6. The method of claim 5, wherein the centrifugation is performed by 2,200 to 2,400 g.