Influence of Time and Speed on Platelet Concentration

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Abstract

The use of a product that accelerates healing after surgery or trauma is a very attractive idea. After a tissue destruction, growth factors naturally occur during healing phases or tissue repair or regeneration.
The injection of a platelet concentrate is followed by an increase in growth factor in the surgical site.
This will enable a faster healing. [1-4]

These growth factors are released by platelet release. To concentrate these factors it seems worthwhile to isolate platelets, concentrate and reinjecting them into the surgical site.

Several techniques are used isolate platelets such as PRP, PRF and MPM. All these products are based on platelet concentrates. The result of these products is different in platelets concentration. However, the time and the spin speed will influence on the platelets concentration.

We know that the more the time and the spin speed are shorter, the more the number of isolated platelets is important. We tried in this study to determine the optimal time and spin speed to obtain the higher concentration of platelets.

Keywords

MPM, Platelet, growth factor, centrifugation.

Introduction

The healing process and / or repair is a long and complicated 2process, which involves the growth factors released by platelets. The platelets are the smallest elements in the blood. They were born in the bone marrow, by the fragmentation of megakaryocytes. [5,6]. The platelets are non-nucleated cells, and they contains a large number of vacuoles in the cytoplasm. These vacuoles are extremely interesting because these vacuoles containing PDGF, IGF, or TGF. These factors will act as a messenger between the different cells, activating, preventing their activity or attracting cells on the repairing site by chemotaxis. The platelets are therefore considered as a natural source of growth factor, this is why surgeons are trying to concentrate them and to reinject them at the surgical site.

To isolate and concentrate the platelets, several techniques have been developed, the PRP, PRF, the LPRF the PRGF, or more advanced processes involving a mineral phase as the MPM.

All these techniques are based on centrifuging the patient's venous blood, thus driving the heaviest items toward the bottom of the tube by a gravitational force. Several parameters can influence the centrifugal force. Some parameters are set by the manufacturer, as the distance between the ROTOR and center tubes containing the blood in the centrifuge, or the degree of inclination of the tubes. Others are variable and can be modified by the practitioner as the time and speed of centrifugation.

In this study, we examined the two parameters, which are the only variables under the surgeon's control: time and speed of centrifugation. The aim of this study was to determine the ideal parameters to isolate the best platelet concentrate.

The FRP LPRF, PRGF and the PRP are products that resemble the macroscopic point of view; the MPM is the only one to contain a mineral fraction. The use of PRP being banned in France, we chose to compare the results of the PRF protocol and MPM.

Healing

Following trauma, or injury, the subendothelial structures are exposed. In contact with these structures, and in the presence of calcium, platelets will be activated; they will release their growth factor and undergo structural deformation. The activation and structural deformation platelets allow to fix the fibrinogen on their surface through surface glycoprotein GP IIIa BII [5,6]

The purpose of this activation is to form the platelet plug to stop the bleeding. Using the Willebrand factor, activated platelets will join the breach. The Von Willebrand factor therefore acts as a glue between platelets and subendothelial structures forming the first layer of the platelet plug.

With the accession of fibrinogen on platelet surface glycoproteins, platelets will adhere against each other to form an aggregate: the platelet plug is the one who will stop the bleeding. [5,6] When not activated, fibrinogen is a soluble molecule in the blood. Its activation by thrombin makes it insoluble, and thus form the fibrin or fibrin network. Thrombin is an extremely powerful enzyme. One thrombin molecule can coagulate 1000 times its weight of fibrinogen. [5,6]

The fibrin network once established will strengthen the platelet plug, to make it more stable, more durable. This will constitute the extracellular matrix required for the repair reaction and / or regeneration of damaged tissues.

This extracellular matrix, will serve as a scaffold for cells to migrate by mécanotaxie along the damage d tissue and begin repair. The stability of the fibrin is remarkable, only the intravascular fibrin is capable of fibrinolysis; the fibrin extravascular situation remains stable as we observe on the implant sites.
After complete hemostasis, begins the inflammatory phase, which starts by a site-cleaning phase and then starts the regeneration phase and / or repair. Platelets and other cells such as lymphocytes release their cytokines to attract or activate other cells such as macrophages and fibroblasts, which starts regeneration.

Protocol and Technology

The platelet concentrate is obtained from the centrifugation of the patient's venous blood. By a rotation and at a given speed, a centrifugal force will be applied on the tubes. This force is the strength G. It varies depending on the speed of rotation, the inclination of the tubes into the centrifuge and the distance (D) separating the tubes from the main rotor axis.

The tubes contain blood that contains different elements having different volumes and different masses. During the centrifugation, the G force will be applied to the blood in the tubes. The heavier elements such as red blood cells will migrate faster and further into the tube. While the lighter elements such as platelets will stay in the upper area. (Fig. 2)

However, at a constant force of centrifugation, if the time is extended all the elements even the lightest one, will migrate to the bottom of the tube. The platelets may fracture and release their productsto the bottom of the tube. The upper part meanwhile be an especially platelet-poor plasma (PPP).
The centrifugation time and speed will therefore play an important role in the separation of particles, which influence the wealth platelet concentration.

The purpose of this protocol is to separate a maximum of non-activated platelets to retrieve them and make them and to use them during the surgery

The PRP

Whitman and his team in 1997 described a technique of preparation of platelet concentrates for use in oral and maxillofacial surgery: the PRP. It is to draw blood into tubes containing an anticoagulant to prevent blood clotting during the handling. It also aims to prevent platelet activation and secretion of their growth factors.

Indeed, the growth factors have a very short half-life. For this reason, it is essential to activate platelets at the last moment just prior to injection on the site. Blood will undergo a double centrifuging, platelets are harvested and mixed with bovine thrombin and calcium chloride to activate platelets, and to trigger the coagulation reaction. It should be noted that calcium is an important component for the activation of platelets. [3] The PRP has a large number of growth factors having a major role in tissue regeneration [7]

The PRF

In 2001 Choukroun and his team modified the PRP technique to comply with French law that prohibits the manipulation of the blood. The PRF protocol needs the centrifugation of 10 ml blood for 10 minutes at a speed of 3000 revolution per minute. [1,8] At the end of centrifugation, the result obtained was a separate tube in two parts (Fig 3 A, C.): A red part with red blood cells in the tube bottom (Fig.3 C.), and a yellow part at the top of the tube that is divided itself in é parts (Fig 3 A + B): a liquid party (PPP) (Fig 3 A.), and a gel form. (Fig. 3 B).

The gel is a fibrin, or fibrin network, which must trap all the platelets present in the tube. This fibrin network is formed by the activation of thrombin, which, in turn, activated fibrinogen. (Fibrinogen + thrombin = fibrin).

At this stage of the gel and the platelets are glued and clamped to the fibrin network, and we are unable to count the number Thus to know the number plate, it was counted the number of platelets in the PPP and the red portion of the tube. It has been reported by Dr. Choukroun there was no platelets in the PPP or in the red portion of the tube [1], which suggested that consequently all the platelets were within the gel.

In a 2004 study, which analyzed by ELISA dosage, the presence of platelet's cytokines in the PPP (layer A) and in the PRF. It was able to obtain the result that the PPP contained the same amount of cytokine that exudate FRP. [9,10]
This is explained by the centrifugation speed, centrifugation time and the presence of coagulation activator in the PRF tube.
Indeed, in the PRF tubes, this activation of platelets is due to the siliceous nature of the tube or to the presence of added coagulation factors, so this will activate the platelets that will releases their cytokines. Thus, when the fibrin clot is collected, platelets are already activated and already have released their growth factors. This is why the protocol proposed by Choukroun, does not offer the ability to activate platelets at the moment of injection

the MPM

In 2010, in France, Dr. Perissé and his team developed the protocol of the MPM. (Mineralized Plasmatic Matrix) was born to overcome the shortcomings of the PRF. Indeed the PRF are in gel form, it is not possible to mix the bone substitute or graft particles. It cannot result in a homogenous product and resistant to forces tending to crush the reliefs. However, unarmed mineral and gel state PRF collapses under pressure.
MPM, unlike FRP allows mixing the bone particles and integrating them within the fibrin network into a single product, homogeneous, stable and resistant to forces tending to deform it. The MPM can thus be used for bone graft, with its steady mineral fraction on the site.

The MPM Protocol is centrifuging 4 tubes of 9 ml. of patient's blood for 15 minutes at a speed of 2700 rpm. This operation allows the formation of a red cell concentrate to the bottom of the tube, above of which is observed platelet-rich plasma, fibrinogen, and other cells present in the circulating blood. The part that is directly above the red blood cell layer is called the "buffy coat" and this is the layer that concentrates the maximum platelets. Due to their density, these platelets and white blood cells will be between the top tube and the supernatant of red blood cells are located at the bottom of the tube. [11]

At the end of centrifugation, the tube is divided into two parts. The red lower part (Fig. 4B), containing red cells and yellow top that contain platelets (Fig. 4A). Unlike the PRF, wherein the yellow upper part contains two compartments (a liquid and another form of the gel), the MPM in the upper part is liquid. (Fig. 4 A)

However, this upper part is theoretically divided into two parts, the supernatant which is the PPP (the part that contains only serum) and the lower part of the yellow part containing the platelets. In this MPM protocol, all the yellow part is recovered using a syringe. Therefore, the rich part platelet and poor platelet portion. In this protocol, there is no platelet activating during the centrifugation step as-those are not yet in contact with calcium or with thrombin or silica. The plasma remains in its liquid state. Therefore, non-activated platelets are in greater number and do not release or their granules or their growth factors in the tube.

During centrifugation phase, some platelets may crash against the tube wall and may burst and thus release their granules in the PPP or plasma. However, these factors are not lost. They will be recovered when all plasma will be retrieved by the syringe. The absence of plasma coagulation in gel form in the BPM is due to the properties of the tubes used. For the realization of this protocol, used tubes are tubes containing no anticoagulant such as PRP or clot activator such as FRP.

At the end of centrifugation while the recovered plasma, will be added to the bone substitute or graft. The calcium in the bone substitute or graft will activate platelets and that will release their growth factors in the graft. After a few minutes, the coagulation will occure and a fibrin network will enclose the bone substitute granules, platelets and other cells present in the plasma

Material and Method

Fifteen patients were selected having no health problem or bleeding disorder. Patients signed consent, they were informed about the study, and they agreed to participate. A control sample was taken from patients for platelet count.

Other samples were taken from these patients using empty tubes without anticoagulant or clot activator. The tubes were centrifuged using the same centrifuge. We set the distance between the tubes and the central axis or the rotor 4 mm, and the inclination of the tubes to 30 °. These settings are fixed and invariable for study. Different speeds and times were tested. 15, 12 and 3 min, 2500 rpm and 1200 rpm. At the end of centrifugation, the yellow part of the tube (the upper part that contains platelets) was retrieved using a syringe, placed in another tube, for counting the number of remaining platelets, and to compare compare with the number of platelets in each control tube.

Results

The results confirmed that when the speed and centrifugation time are reduced, the maximum platelet was obtained per µl. The more time or speed increases, fewer platelets are present in the yellow part, and platelets will rush to the tube bottom in the red part with the red blood cells. Speed centrifugation of 1200 rpm for 3 min has doubled the number of platelets per µl compared to its control tube.

Macroscopic comparison between the tubes showed that the yellow part in the tubes centrifuged longer time or at higher speed, was more important than the tubes centrifuged less time or at a reduced speed. (Fig. 6) according to their weight and density, leaving a liquid serum, which may not coagulate.

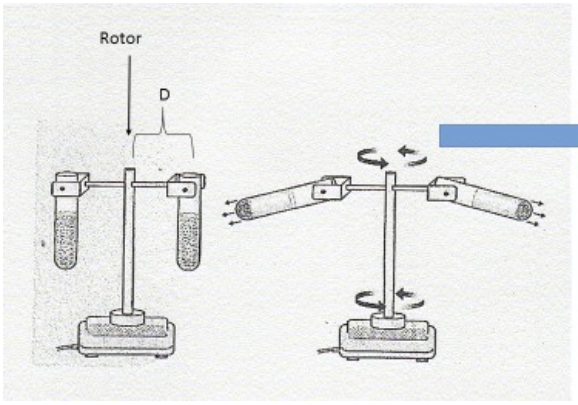
Conclusion

It is important to note that the word platelet concentrate can be misleading. In fact, centrifugation attempts to isolate the maximum amount of platelets that are in the tube without increasing their number. Therefore, the same number of platelets instead of being in 9 ml of blood, it is in a more reduced amount of plasma. Reducing and speed of centrifugation time, increases the number of platelets that are considered as a natural source of growth factor, without affecting the quality of the fibrin network and therefore without affecting its mechanical properties.

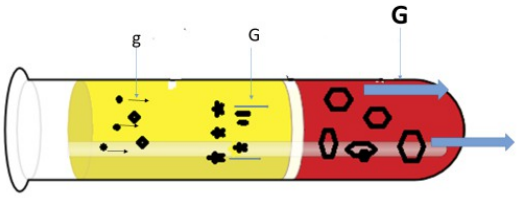
This reduction enables faster completion of the platelet concentrate, 3 min instead of 15 min. In addition, reducing the speed protects the platelets from crashing (crush or less) against the walls of the tubes, and thus allows a late platelet activation.

During our work, we mistakenly left tubes filled with blood in a vertical position. After 15 minutes, we noticed a rush of red blood cells in the bottom of the tube under the influence of "G" Earth's gravity, and the appearance of the desired plasma. (Fig.7)

It will also be noted that the lifetime of the platelets is only 5 to 9 days [12]. So a few days after placing the platelet concentrate in the surgical site, the platelet count will drop very quickly. These plates will certainly be replaced, but the question remains: at what concentration? How many?



Force centrifuge



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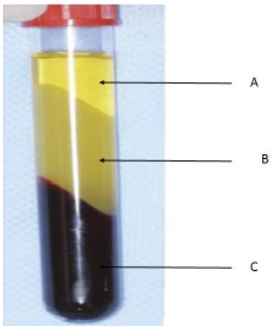

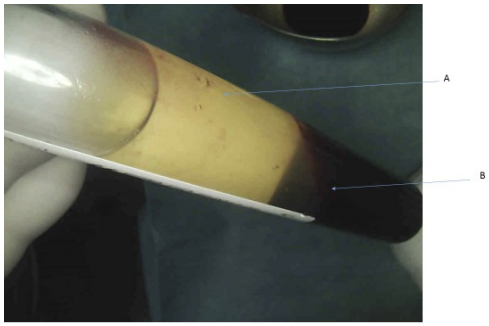

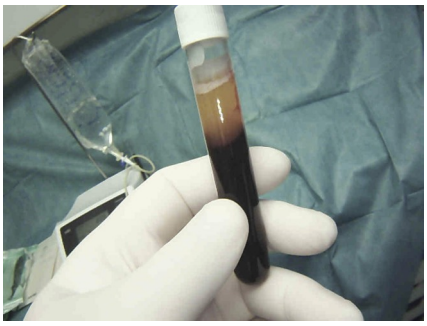

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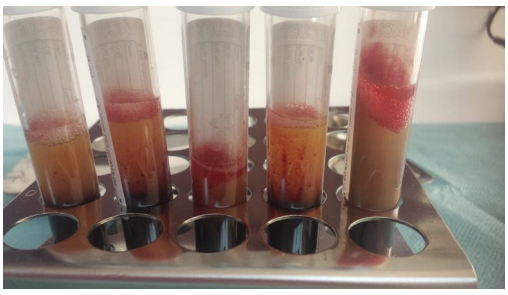

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