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CENTRIFUGE USE IN DENTAL PRACTICE

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Abstract:

A large amount of different growth factors is concentrated in platelets and leucocytes so idea of getting platelet concentrates due to centrifugation is widely used in medicine and dentistry. Such concentrates are much cheaper and more predictable than use of xenografts and allografts and arises less ethical questions being sometimes indispensable.

Purpose. To describe the main types of platelet concentrates that can be got chairside and used in clinical practice reducing the mess in the terms and systemizing protocols.

Material and Methods. The analysis of the specialized literature in the electronic databases PubMed, Web of Science, Scopus on "PRP", "PRF", "platelet concentrates" and "sticky bone" was done.

Results. The main types of platelet concentrates were described and main principles of the protocols emphasized. History and main principles for counting relative centrifugation forced were also brought to recollection.

Conclusion. PRF is quite a simple low-cost and effective procedure to put into practice. Furthermore, i-PRF or AFG is useful in so-called 'sticky bone' preparation.

Keywords: PRP, PRF, platelet concentrates, sticky bone, AFG

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INTRODUCTION:

The process of healing is quite a relevant problem in modern dentistry and maxillofacial surgery. Today oral surgeons' mind and hands are more occupied with the question of augmentation than extraction. At the same time our patients are not satisfied with revealing pain by deleting rotten teeth but they expect good function and aesthetic rehabilitation and at the same time want it to be cost-effictive.

Lots of methods have been suggested so far: autotransplantation, xeno- and allotransplantation, use of artificial materials and so on- to preserve and gain bone volume. Though if we look precisely at the process of true regeneration beneficial part of it can be given to growth factors big amount of which contains in platelets and leucocytes. Platelets contain high quantities of key growth factors, such as PDGF-AB (platelet-derived growth factor AB), TGFb-1 (transforming growth factor b-1) and VEGF (vascular endothelial growth factor), which are able to stimulate cell proliferation, matrix remodelling and angiogenesis [1]. Meanwhile leucocytes produce large amounts of VEGF, have anti-infectious action and immune regulation [2].

The use of blood-derived products to seal wounds and stimulate healing started with the use of fibrin glues, which were first described 40 years ago and are constituted of concentrated fibrinogen (polymerization induced by thrombin and calcium) [3]. Whitman et al. [4] suggested using platelet concentrates to improve healing and to replace fibrin glues. Consequently the era of platelet concentrates prepared in different ways began.

MATERIAL AND METHODS:

The analysis of the specialized literature in the electronic databases PubMed, Web of Science, Scopus on "PRP", "PRF", "platelet concentrates" and "sticky bone" was done.

RESULTS AND DISCUSSION:

A centrifuge is a device that uses centrifugal force to separate various components of fluid, in our case, blood. So, this item can be used to get either two (heavy red blood cells and plasma with leucocytes and platelets) or three (red blood cells, buffy coat- platelet-rich plasma with leucocytes and platelet-poor plasma) layers. The product of centrifugation depends on conditions: type of the tube used, use of anticoagulants or procoagulants and time and relative centrifugal force (RCF).

The formula to calculate the relative centrifugal force (RCF) can be written as:
RCF (g-Force) = $1.118 \times 10\text{-}5 \times r \times (RPM)^2$,
where r is the radius of the rotor (in centimeters), and RPM is the speed of the rotor in rotation per minute [5]. There are online-calculators to make the process of counting much easier [6].

All parameters are intuitively easy for understanding but RCF is widely misunderstood and improperly used term in articles.

One of the confusions is that various authors have reported centrifugal g-force at the *PRF-clot* (referred to as relative centrifugal force (RCF)-clot – location at which the *PRF-clot* is formed that is not quit representative), whereas others have utilized the international standard method to report g-force calculated at the bottom of centrifugation tubes (*RCF-max*). Furthermore, initial studies reported *RCF-min* values calculated at the upper portion of PRF tubes [7]. Moreover, centrifugation tubes are fabricated from different materials (glass-coated plastic versus glass), centrifugation tubes are of different sizes (9 ml vs. 10 ml), centrifugation tubes are filled with blood to different levels, centrifuges are fabricated using different materials, centrifugation rotors are fabricated with different sizes and rotor radiuses, centrifugation rotors are angled differently [8].

To sum it up, widespread way to describe platelet concentrates by time of centrifugation and RPM is not representative and these parameters should be established for each centrifuge used in clinical practice or science.

***History and types of* platelet concentrates**

Non-self-coagulating concentrates

The original concept leading towards the preparation of platelet concentrates was that concentrated platelets and autologous growth factors could be collected in plasma solutions that could then be utilized in a surgical site to promote local healing [9]. Platelet rich plasma (PRP) is defined as a volume of the plasma fraction of autologous blood with an above baseline platelet concentration [10]. All available PRP techniques have some points in common: blood is collected with anticoagulant and is immediately processed by centrifugation. A first centrifugation step is designed to separate the blood into three layers, red blood cells (RBCs) are at the bottom, acellular plasma (PPP, platelet-poor plasma) is in the supernatant and a 'buffy coat' layer appears in between, in which platelets are concentrated. The next steps vary among the numerous protocols but are an attempt to discard both the RBC layer and the PPP to collect only the 'buffy coat' layer. Finally, the obtained platelet concentrate is applied to the surgical site with a syringe, together with thrombin and/or calcium chloride (or similar factors) to trigger platelet activation and fibrin polymerization. Around the same time period, Anitua et al. [11] formulated a second platelet concentrate also utilizing anticoagulants termed platelet-rich growth factor (PRGF).

Their preparation requires the additional use of bovine thrombin or CaCl2 to coagulate. Furthermore, the preparation must be centrifuged in two separate stages in order to increase platelet concentration without incorporation of leukocytes. To sum it up, we can classify L-PRP, P-PRP and PRGF as non-coagulating concentrates by themselves as containing anticoagulants. Thus, the procedure is quite complicated.

Coagulating platelet concentrates

The Platelet Rich Fibrin (PRF) does not require anticoagulants or bovine thrombin (or any other gelling agent) and can be assumed as a second-generation platelet concentrate [12]. This feature makes this product easily usable, with a low rate of mistakes during the preparation stage [13]. The only things needed are centrifuge and tubes with activator (plastic tubes + SiO2 or glass tubes). A-PRF is an advanced technique preserving more growth factors by reduction RPM (revolutions per minute) of the centrifuge, thus, reducing RCF (relative centrifugal force) [14]. The T-PRF method is based on the hypothesis that titanium may be more effective in activating platelets than the silica activators used with glass tubes in Chouckroun's leukocyte- and platelet-rich fibrin (L-PRF) method. The T-PRF samples seemed to have a highly organized network with continuous integrity compared to the other L-PRF samples [15]. CGF (concentrated growth factors) is a similar protocol as L-PRF but utilizes altered centrifugation speed to produce much larger, denser and richer fibrin matrix containing growth factors [16].

Liquid but coagulating platelet concentrates

Autologous fibrin glue (AFG) which will make sticky bone can be got in a plastic non-coated tube usually described as a white tube [17]. Two layers are formed after centrifugation: the upper layer is AFG layer and the bottom layer is accumulation of red blood cells which will be discarded. Then AFG can be used to mix with xenograft to get so-called *sticky bone* that has quite bigger osteogenic potential than graft mixed with normal saline [18]. As it takes several minutes to coagulate we classify this concentrate as liquid initially though coagulating.

Quite similar initially liquid but coagulating platelet concentrate is *i-PRF*. The main difference with AFG is that i-PRF got at lower RCF [19] and due to Wend et al. [20] decrease in RCF resulted in an increase in cell numbers. Thus the low speed centrifugation concept (LSCC) was introduced as a potential tool to modify PRF scaffolds by fine tuning the centrifugation settings

Varela et al. [21] showed that a higher concentration of platelets and lymphocytes in i-PRF compared to those in peripheral blood took place. Immunohistochemistry staining revealed IL-10, osteocalcin, and TGF-beta factors mainly at the cytoplasm and leukocyte nucleous from the i-PRF for 3 days of incubation. However, the neutrophil-leukocyte proportions were lower for the i-PRF. The difference between neutrophils and lymphocytes can occur due to the difference in their molecular weight during the centrifugation process, thus leading to the highest concentration of lymphocytes in the i-PRF group.

CONCLUSION:

1. All g-forces and RCF-values should be clearly reported at the RCF-max in future studies to avoid future confusion in the field and to improve scientific accuracy in future publications.

2. Decrease in relative centrifugal force results in an increase in cell numbers and growth factors.
3. PRF is quite a simple low-cost and effective procedure to put into practice.

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