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The role of fibrin rich in platelets and leukocytes in the bone healing of calvary defects: study in rabbits

O papel da fibrina rica em plaquetas e leucócitos na cicatrização óssea de defeitos em calvária: estudo em coelhos

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ABSTRACT

Introduction: L-PRF is a concentrate of platelets and leukocytes in a fibrin network, obtained by autologous centrifugation collected at the time of surgery. It is part of the second generation of platelet concentrates, being low cost, easy to prepare, simple to obtain and has the ability to accelerate the healing of soft and hard tissues.

Objective: To review bone repair of non-critical calvarial defects using L-PRF alone and in association with particulated autogenous bone.

Method: Integrative review of literature taken from the SciELO, Pubmed and Scopus platforms in English and Portuguese using the following descriptors: "L-PRF, bone healing, calvarial defects, rabbit" in AND or OR by the title or summary. After selection, only articles considered relevant were read in full.

Results: 39 articles were included.

Conclusion: L-PRF alone had a positive effect on bone formation over the weeks.

KEYWORDS: L-PRF. Bone healing. Defects in calvaria. Rabbit.

RESUMO

Introdução: O L-PRF é concentrado de plaquetas e leucócitos em rede de fibrina, obtido pela centrifugação autóloga coletada no momento da cirurgia. Faz parte da segunda geração de concentrados plaquetários, sendo de baixo custo, fácil preparo, simples obtenção e tem a capacidade de acelerar a cicatrização de tecidos moles e duros.

Objetivo: Revisar o reparo ósseo de defeitos não críticos em calvária utilizando o L-PRF isoladamente e em associação com osso autógeno particulado.

Método: Revisão integrativa da literatura retirada das plataformas SciELO, Pubmed e Scopus em inglês e português utilizando os seguintes descritores: "L-PRF, cicatrização óssea, defeitos em calvária. Coelhos" em AND ou OR pelo título ou resumo. Após a seleção apenas os artigos cosiderados pertinentes foram lidos na íntegra.

Resultados: Foram incluídos 39 artigos.

Conclusão: O L-PRF isoladamente teve efeito positivo na formação óssea no decorrer das semanas.

PALAVRAS-CHAVE: L-PRF. Cicatrização óssea. Defeitos em calvária de coelhos.

L-PRF after being removed from the glass tube and separated from the clot

Central Message

L-PRF (fibrin rich in platelets and leukocytes) is a concentrate of platelets and leukocytes in a fibrin network, obtained by autologous centrifugation collected at the time of the surgical procedure that offers the advantages of low cost, easy preparation, simple obtainment, and the ability to accelerate the healing of soft and hard tissues. Thus, reviewing its applicability and efficiency in bone repair of non-critical defects is interesting to decide on wound treatment in difficult surgical moments due to the lack of means in ossification.

Perspective

The reconstruction of living tissues is a constant search in tissue bioengineering, both for the replacement of soft and hard tissues. The most diverse health professionals, and especially oral and maxillofacial surgeons, constantly seek materials and technologies that offer the reconstruction of lost bone parts, whether due to tooth extractions, tumors, cysts, traumas, among others. This review provides information on the efficacy of platelet- and leukocytes-rich fibrin (L-PRF) on these needs.

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INTRODUCTION

The reconstruction of living tissues is a constant search in tissue bioengineering, both for the replacement of soft and hard tissues. The most diverse health professionals, and especially oral and maxillofacial surgeons, constantly seek materials and technologies that offer the reconstruction of lost bone parts, whether due to tooth extractions, tumors, cysts, traumas, among others.

The "ideal" graft material should bring together the following skills: a) have unlimited supply without compromising the donor area; b) promote osteogenesis; c) does not present an immune response from the host; d) revascularization quickly; e) stimulate osteoinduction; f) to promote osteoconduction; g) be completely replaced by bone in quantity and quality similar to that of the host.

L-PRF (Platelet and Leukocyte Rich Fibrin) is a concentrate of platelets and leukocytes, obtained by centrifugation of autologous venous blood collected at the time of the operation. It was developed in France in 2001 by Choukroun1, and part of the second generation of platelet concentrates. It offers several advantages, including low cost (autologous blood), ease of preparation (no need for anticoagulants or coagulation activators), ease of obtainment, and high capacity to accelerate the healing of soft and hard tissues. In addition, it incorporates most platelets, leukocytes and growth factors collected from simple blood samples into the fibrin matrix.

Unlike PRP (Platelet Rich Plasma), in L-PRF there is no need to use anticoagulants or coagulation activators, making the procedure less sensitive, easier, cheaper and simpler to obtain.

Several studies have been carried out to evaluate the efficacy of L-PRF, because despite being a promising generation of platelet concentrates, there is suspicion after studies of PRP, since at first it also seemed extremely promising, and then its efficacy was contested.

Current research has shown that the use of L-PRF has contributed to better healing of both soft and hard tissues, mainly because it provides growth factors for longer, 7 to 11 days, unlike PRP in which the factors were released in approximately 8 h. However, there is a need for more in vivo and in vitro researchers to confirm the benefits of L-PRF.

The objective of this study was to review the bone repair of non-critical defects in rabbit calvaria using L-PRF alone and in association with particulate autogenous bone.

METHOD

Integrative review of the literature taken from the SciELO, Pubmed and Scopus platforms in English and Portuguese using the following descriptors: "L-PRF, bone healing, calvarian defects, rabbits" in AND or OR by title or abstract. After selection, only the pertinent articles were read in full. At the end, 39 articles were included.

DISCUSSION

Blood-derived growth factors have been used in medicine for a few decades. However, it is important to note that with the discovery of PRF, the use of platelet concentrates has grown exponentially. Its use in dentistry began in the 90s, with the objective of providing the surgical bed with large amount of platelets to favor growth factors, in order to obtain better tissue healing. However, PRP had number of particularities and difficulties in obtaining, including the need to use anticoagulants and, especially, bovine thrombin for polymerization. Its rapid polymerization made it difficult to incorporate cytokines into the fibrin matrix due to the high rate of bovine thrombin that was required for fibrin polymerization, and as a rigid polymerization effect. As a result, the release of growth factors at the surgical site occurred quickly, instead of slowly.

Research with PRP has shown that the results obtained were not as promising as initially believed.[2-6] With them, it was found that PRP increased the physiological response to trauma and overcame the normal deposition of growth factors; however, although it released large amounts of growth factors, they were only sustained in the initial stage after preparation, for approximately 8 h, that is, a very short period of time.[7]

The evolution to L-PRF

In 2001, Choukroun1 developed a new protocol to concentrate platelets and fibrins in a simple step without blood modification: L-PRF. In it, there is no need for heparin (anticoagulant) or the use of bovine thrombin (coagulation), and according to the authors, this method has a number of advantages over PRP.

L-PRF belongs to the second generation of platelet concentrates, where the fibrin network is much more similar to the natural network. This characteristic leads to more efficient cell migration, and greater cell proliferation, which culminates in better healing. In addition, L-PRF is able to release growth factors slowly and progressively in the fibrin matrix remodeling process, since, in the Choukroun technique, fibrin polymerization occurs in a slow and controlled manner within the centrifuge, producing fibrin matrix of stable three-dimensional architecture, capable of incorporating practically all platelets and most circulating leukocytes, maintaining the release of growth factors at the surgical site for 7-11 days, which has many benefits in tissue healing.[3,4,7-9] It also has the following advantages: a) technical ease of obtainment; b) be obtained from autologous blood; c) requires minimal blood manipulation; d) does not require any type of additive (such as bovine thrombin, calcium chloride or heparin); and e) it is more effective, more efficient and less controversial than the PRP.[5,10,11]

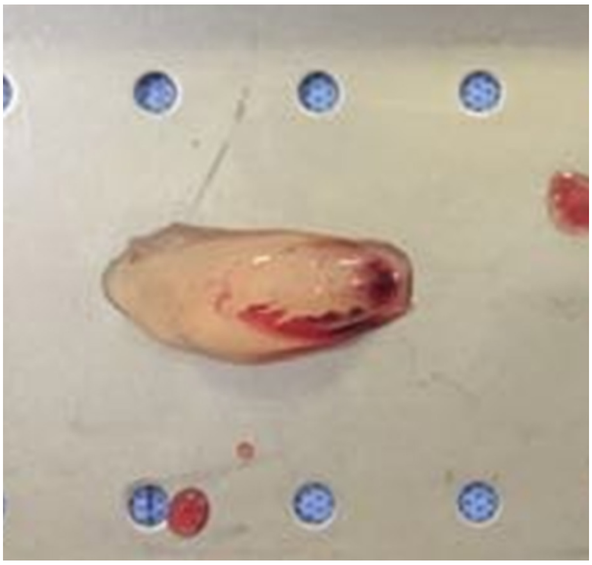


FIGURE 1 — L-PRF after being removed from the glass tube and separated from the clot

The range of use of L-PRF in dentistry and medicine is very wide and is cited to improve alveolar preservation, improve soft tissue healing, improve bone healing, among others.[2,5,6,10,12-18]

Despite having a simple technique, some steps are essential to obtain correct L-PRF, such as great care in blood collection and in the time between collection and the start of centrifugation, which needs to be immediate.[19]

Another very important factor is in relation to the proper positioning of the blood tubes in the centrifuge in order to obtain stability. It is recommended to always place one tube against the other, avoiding instability and vibration. Several authors emphasize that low levels of vibration and thermal variation are essential for adequate PRF obtainment.[19,20-23]

Care must also be taken with the way the membranes are formed and L-PRF is compressed. The specific box for production of L-PRF membranes allows the production of membranes in constant thickness and hydrated, in addition to allowing the collection of serum, which is rich in proteins such as fibroconnectin and vitronectin. This exudate collected from the back can be used to hydrate graft materials, wash the surgical site or store autologous graft, improving the results obtained. The use of gauze to compress the membranes should be avoided as much as possible, since this procedure can also end up removing the growth factors from the membrane, such as PDGF.[3,19,20,24]

The production of L-PRF

The L-PRF production protocols vary according to the centrifugation time, number of revolutions, type of test tube and even the amount of blood to obtain the membranes. The centrifugation time and the number of revolutions depend on the centrifuge, the size of the radius, the angle of the tubes in the centrifuge, among others. Therefore, most centrifuge manufacturers already deliver the speed and time to make each of the desired platelet aggregates, including L-PRF.[19,20,22,23]

There is a line of researchers who claim that the ideal centrifuge for the manufacture of L-PRF would be the Intra-Spin (Intra-Lock International, Boca-Raton, FL, USA). There are studies that show that it is the most stable, with a vibration level between 4.[5] and 6 times lower than that of the other centrifuges, that the temperature of the tubes at the end of the process was lower than in the other commercial brands, that it produces heavier clot and exudate, in addition to showing a thick strongly polymerized fibrin matrix, have living cells with a normal shape, while the other membranes made by the other machines have thin fibrin gel slightly polymerized and most cell bodies appear destroyed. According to several authors, the characteristics of the centrifuge and the centrifugation protocols have a significant impact on the cells, growth factors, and fibrin architecture of L-PRF.[20-22]

The Intra-Spin centrifuge was conceived and created by L-PRF pioneer Joseph Choukroun. However, after a few years, he himself developed another centrifuge, in which the L-PRF would be even more activated (he called it A-PRF), through the use of the A-PRF 12 centrifuge (Advanced PRF, Nice, France) in a different time and speed of revolutions per minute than originally described.

There is, however, a line of researchers who affirm that the use of these specific trademarks is not mandatory, but that there should be a centrifuge that respects the basic requirements necessary for the manufacture of L-PRF. To use other centrifuges, it is necessary to perform calculations to determine the G-force of the centrifuge and adjust the speed and time. Oliveira,23 conducted a study using the Fibrin Fuge 25 centrifuge (Montserrat, Brazil) and concluded that the platelets were intact and adhered to the fibrin network, emitting pseudopods and degranulating, in addition to increased VEGF up to 7 days for all forces (200g, 400g and 800g for 10 min). However, the concentrations were higher when 200g was used.

So, there are many clinicians and researchers who question the extent to which these centrifuges really make a big difference in getting L-PRF and whether this ends up being a commercial appeal for the sale of much higher value centrifuges. They argue that one should not be held hostage to commercial brands, and that the most important thing is to calculate the G-force of the centrifuge that will be used, as well as the centrifugation time. There are centrifuges of the most varied prices on the market, and the Intra Spin centrifuge costs up to 10 times more than others, which often makes it impossible for clinicians to purchase this equipment.

To obtain blood samples from rabbits, a technique was studied that would allow the obtaining of venous blood in sufficient quantity and that would not cause side effects using the auricular vein. The jugular technique of these animals has also been described,25 with more voluminous collection and no adverse events attributed to this procedure.

After centrifugation, the division of the contents of the tubes into 3 parts is visible, with the top layer composed of acellular plasma; the intermediate containing the L-PRF itself; and the base layer showing red blood cells. It is worth mentioning here that the layer of L-PRF that contains the most viable cells is the layer closest to the red blood cells, and therefore, care should be taken in removing the red layer avoiding removing the part of L-PRF that contains most of these viable cells.

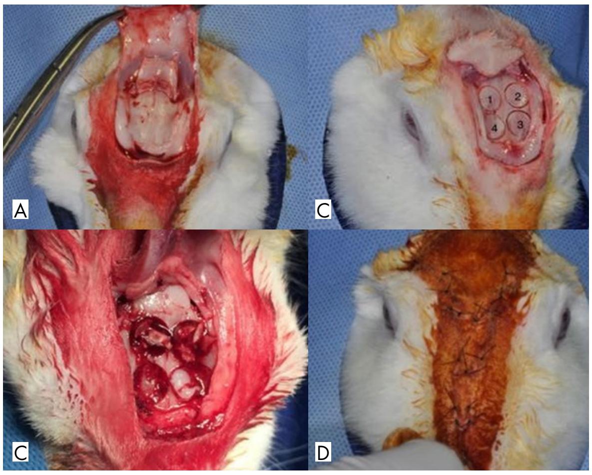
The use of L-PRF and its action on healing

The repair of the different tissues of the human body, including bone, is mediated by different growth factors in a process that begins with the formation of the blood clot and continues with the degranulation of platelets, which release growth factors, such as PDGF, TGF β1 and β2, VEGF and EGF (contained within the granules α platelets). Angiogenesis, which is fundamental in the repair processes, is especially dependent on growth factors from platelet degranulation. In L-PRF, growth factors depend on the fibrin matrix as a support framework and proteolytic protection. They detach from the fibrin mesh slowly and gradually, accompanying fibrinolysis. Platelets act in the process of hemostasis, wound healing and re-epithelialization, release growth factors that stimulate angiogenesis, promote vascular growth and fibroblast proliferation, which provide an increase in collagen synthesis. TGF activates fibroblasts to form protocollagen that results in collagen deposition and wound healing; PDGF increases tissue vascularization, promotes fibroblast proliferation, increases the amount of collagen, stimulates the production of granulation tissue, and improves angiogenesis; VEGF stimulates angiogenesis, mitosis of endothelial cells, and vascular permeability; EGF induces the growth of epithelial tissue and promotes angiogenesis. All this makes healing faster and more efficient, favoring the integration of bone, cutaneous, cartilaginous or fat cell grafts.[23,26,27]

Alloplastic and xenogen materials have a wide indication in bone grafting procedures. However, despite being good osteoconductors, they do not have the property of being osteogenic or osteoinducers. The association of L-PRF and i-PRF with these alloplastic and xenogenous materials can enhance the chances of success, as it contains several growth factors such as PDGF, TGF, IGF, EGF, bFGF.[27-30] In this way, both bone gain can be greater, as well as healing time and lamellar bone quality can also be improved.[9,26,31] L-PRF acts in 4 fundamental phases of the healing process: 1) angiogenesis; 2) immune control; 3) release of growth factors; and 4) recruitment of undifferentiated cells. It also serves as a cover and framework for epithelial cell migration.

The importance of evaluating L-PRF in bone formation is that there are many cases in which there is not enough autogenous bone availability to complete the entire recipient bed. There are cases in which there is not even the possibility of obtaining autogenous bone to place in the recipient bed. In these cases, it is very important to research alternatives to complement the autogenous and/or xenogenous graft, as well as to provide better bone healing, reducing comorbidity in the use of the donor area.

The results of Evans32 showed in an experimental study that there was proportional and significant bone gain between 2 and 6 weeks for the group where L-PRF was placed exclusively (Figure 2). This result is especially important for daily clinical practice, as there are numerous situations in which the use of any type of bone graft (regardless of whether it is autogenous, xenogenous or allogene) is not indicated, and L-PRF alone could be used to avoid infection and maximize the entire tissue response. One of these examples would be the removal of cysts and/or tumors, with or without a safety margin, where the use of grafts is not indicated at the first moment, but for a second with a bone graft. In these cases it can be very interesting to use L-PRF, because in addition to avoiding hemorrhages and bleeding, it would avoid infections and improve the entire tissue response, including angiogenesis and bone neoformation, facilitating a second surgical stage of tissue reconstruction, or even avoiding the need for it.



Source: Evans€

FIGURE 2 — Experimental study in rabbits: A) detachment of the soft tissue and periosteum, leaving the bone tissue of the calvaria exposed; B) definition of each of the groups for osteotomy; C) filling of bone gaps with materials under study; D) final aspect of the experiment.

Many authors have also verified that defects can be successfully repaired with the application of L-PRF alone.[9,14,28,31,33-37] Awadeen[38], evaluated the benefits of L-PRF in critical defects in rats after 1, 2 and 4 weeks and found that those treated with the association of stem cells and L-PRF had better results than with PRF alone. Oliveira[26], conducted a study in which PRF had a positive effect on bone regeneration when associated with Bio-Oss, but not when using L-PRF alone. The association of L-PRF with biomaterials also deserves special attention since it enhances tissue regeneration without the need for a donor bone area. Not all studies are unanimous regarding the advantages of L-PRF, bringing some questions about its stability in osteogenesis.[39,40]

As for the type of centrifuge, many researchers argue that one should not be held hostage to commercial brands, as long as the time and G-force in each centrifuge used are observed.

CONCLUSION

This review shows that there was a statistically significant progressive and proportional increase using L-PRF in non-critical defects in rabbits between 2 and 6 weeks.

Authors' contributions
Conceptualization: Evans Soares de Oliveira, Jurandir Marcondes Ribas-Filho
Methodology: Jurandir Marcondes Ribas-Filho
Project administration: Paulo Afonso Nunes Nassif, Fernando Issamu Tabushi
Writing (original draft): All authors
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