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Distribution of inorganic graft material within the PRF block: A integrative review

Diana de Assunção Pereira Ferreira

Dissertação conducente ao Grau de Mestre em Medicina Dentária (Ciclo Integrado)

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Trabalho realizado sob a Orientação do Prof. Doutor Júlio Souza e Co-orientador do Doutor Nuno Sampaio

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Eu, Diana de Assunção Pereira Ferreira, declaro ter atuado com absoluta integridade na elaboração deste trabalho, confirmo que em todo o trabalho conducente à sua elaboração não recorri a qualquer forma de falsificação de resultados ou à prática de plágio (ato pelo qual um indivíduo, mesmo por omissão, assume a autoria do trabalho intelectual pertencente a outrem, na sua totalidade ou em partes dele). Mais declaro que todas as frases que retirei de trabalhos anteriores pertencentes a outros autores foram referenciadas ou redigidas com novas palavras, tendo neste caso colocado a citação da fonte bibliográfica.

DEDICATÓRIA

E assim passaram 4 anos!

Tenho um enorme orgulho em ser enfermeira e essa minha paixão não será esquecida, ela irá sim, complementar esta minha nova paixão pela Medicina dentária. Fazer este percurso foi muito difícil, entre trabalhar por turnos no hospital com horários malucos, e ainda cumprir com todas as tarefas inerentes de todas as unidades curriculares, fez-me muitas vezes pensar "no que me fui meter" … Coragem, Persistência e Determinação são as minhas palavras de ordem, se não fossem elas eu não estaria a redigir este texto em forma de conclusão de um longo ciclo.

Por esse mesmo motivo e primeiramente, dedico a mim mesma a conclusão desta etapa e do quão orgulhosa em mim estou por chegar até aqui.

Ao meu amado filho Pedro, que acompanhou parte deste percurso ainda dentro mim e agora com um aninho vê a mãe alcançar mais uma vitória. Desculpa meu filho, por todo o tempo que não pude partilhar contigo para continuar esta missão, não foi fácil, depois do teu nascimento pensei algumas vezes em desistir por seres tão pequenino, mas a força de vontade foi maior e foi a pensar também no teu futuro que resisti, e no final de cada dia, sentir os teus bracinhos ao redor do meu pescoço, o teu olhar, o teu sorriso, compensavam sempre eram e são o melhor presente do mundo, por muito cansada ou cheia de problemas que esteja, tudo passa quando te tenho no meu colo!

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À minha binómia Sion e à minha trinómia Cleopatra, foi um privilégio conhecer-vos e ter-vos sempre comigo ao longo desta caminhada, levo comigo as melhores recordações graças a vocês, estarão sempre no meu coração.

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RESUMO

O objetivo principal deste estudo, foi realizar uma revisão integrativa sobre a distribuição das partículas do biomaterial de enxerto após mistura com plasma rico em plaquetas (PRF). As seguintes palavras-chave foram aplicadas: (platelet rich fibrin) OR (PRF); AND (block) OR (bone graft); AND (CBCT) OR (micro-CT) OR (microscopy), entre Fevereiro e Maio de 2021. Os critérios de inclusão envolveram, ensaios clínicos randomizados controlados, estudos pré-clínicos randomizados, estudos prospetivos, relatos de caso, um estudo de caso - coorte única, um estudo comparativo e um estudo em animais. Análises obtidas por micro-CT revelaram uma homogeneidade na distribuição de partículas no bloco de L-PRF e revelou um volume médio de 37,7% ± 1,7% para as partículas de DBBM no bloco de L-PRF. A distância média entre as partículas DBBM foi de aproximadamente 60.55 µm (0.0605 mm) na imagem A, e de 226.3 µm (0.23 mm) na imagem B, que foi visualizada nas imagens de SEM (microscopia eletrónica de varredura).

Este estudo é inconclusivo, são necessárias medições e avaliações em meio laboratorial através de microscopia eletrónica, uma vez que as imagens onde se realizaram estas avaliações são insuficientes para validar a veracidade e representatividade destas medidas.

Palavras-chave: platelet rich fibrin, PRF, block, bone graft, CBCT, micro-CT, microscopy.

ABSTRACT

The main aim of this study was to perform an integrative review on the distribution of inorganic graft material particles within the platelet rich fibrin (PRF) block. The following keywords were applied: (platelet rich fibrin) OR (PRF); AND (block) OR (bone graft); AND (CBCT) OR (micro-CT) OR (microscopy), between February and May 2021. Inclusion criteria involved randomized controlled clinical trials, randomized preclinical studies, prospective studies, case reports, a case study - single cohort, a comparative study and a animal study. Micro-CT analyses revealed that the particles were homogenously distributed within the L-PRF block, without contact with each other and revealed a mean volume of 37.7% ± 1.7% for the DBBM particles in the L-PRF block. However, no measurement was performed torecord. The distance between the DBBM particles was approximately 60.55 µm (0.0605 mm) in image A, and 226.3 µm (0.23 mm) in image B, which was visualized in the images by SEM (Scanning electron microscopy).

This study is inconclusive, measurements and evaluations in the laboratory through electron microscopy are necessary, since the images where these evaluations were carried out are insufficient to validate the veracity of these measurements.

Keywords: platelet rich fibrin, PRF, block, bone graft, CBCT, micro-CT, microscopy

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Abreviaturas

A-PRF – Advanced platelet-rich-fibrin

β-TCP – β trícalcium phosphate

BIC – Bone-implant contact

CBCT – Cone-beam computed tomography

CM – Collagen membrane

DBBM – Deproteinized bovine bone mineral

DFDBA – Demineralized freeze-dried bone allograft

ELISA – Enzyme-linked immunosorbent assay

GBR – Guided bone regeneration

HA – Histomorphometric analysis

IGF – Insulin-like growth factor

i-PRF – Injectable platelet rich fibrin

ISQ – Implant stability quotient

L-PRF – Leukocyte and platelet rich fibrin

Micro-CT – Micro Computed Tomography

OD – Optical density (OD)

OFD – Open flap debridement

PGDF – Platelet derived growth factor

PPP – Platelet poor plasma

PRF – Platelet rich fibrin

PRP – Platelet rich plasma

qRT-PRC – Quantitative reverse transcription-polymerase chain reaction

RPM – Revolutions per minute

SEM – Scanning electron microscopy

TGF-β – Beta transforming growth factor

VEGF – Vascular endothelial growth factor

1.Introduction

Various graft materials are used to reconstruct bone defects in the jaws due to tooth loss, trauma, advanced periodontal diseases[1], periapical lesions that require periradicular surgery[2], pathological lesions, and congenital disorders like clefts of the lip, alveolus, and palate[3], that negatively affects the prognosis of dental implants[4], once they need to of adequate bone volume and quality for achieving your osseointegration[5].

To deal with the limitations described above, researchers have explored various bone augmentation strategies and materials, such as harvesting autogenous bone[6], that is the only one that has osteogenic, osteoinductive and osteoconductive qualities, while other materials possess only osteoconductive properties[7], for this is considered the gold standard for grafting, although it has limitations[8], such as donor site morbidity, obtaining limited amounts, high rates of resorption[3], disease transmission and immunogenic response[9]. Because of these disadvantages, other bone graft materials such as allografts, xenografts, and alloplastic bone grafts[3,6] are frequently used for bone augmentation.

Deproteinized bovine bone mineral (DBBM) appeared as one of the most popular employed xenografts, with successful outcomes in histological and clinical perspectives[9].

As stated earlier, the DBBM only has the capacity for osteoconduction but no osteoinduction and osteogenesis, cannot activate new bone regeneration by itself, however is considered as the gold standard xenograft for guided bone regeneration (GBR)[10], however is an material chemically and physically similar to human bone, that act as a scaffold allowing osteogenic cell transportation from the sinus wall to the graft particles increasing the potential of new bone formation[11].

Rather prolonged degradation rate DBBM, usually retards the replacement of new bone formation and prolongs the graft-healing time and fails to synchronize with the osteogenic rate[9]. Hence, combining the graft material with a biologic promoter that contains crucial growth factors may reduce the graft-healing time and enhance the osteoinductive process of bone remodeling[4,9,11].

Platelet rich-fibrin (PRF), an autologous fibrin matrix [1,10], which belongs to the second-generation platelet concentrate, was first developed in France by Choukroun *et al.* [2,3,5,8,12].

The natural fibrin clot in PRF seems to be responsible for the slow release of growth factors[8], such as platelet derived growth factor (PGDF), vascular endothelial growth factor

(VEGF), insulin-like growth factor (IGF) and transforming growth factor (TGF-β)[11], for an extended period.

The injectable platelets rich-fibrin (i-PRF) is a liquid formulation obtained for injectable purpose and bears an extra benefit of aggregating into a fibrin clot shortly after injection. This bio-active material also have growth factors (PDGF, VEGF, TGF-β and so on), that play a vital role in cell migration, proliferation, and vascularization for tissue regeneration[9].

Studies have described that, PRF accelerates early bone regeneration by angiogenesis, chemotaxis, mitosis, and stem cell proliferation in the early phase of bone regeneration[1,9,10]. The combination offers several advantages including healing wound[1,8,11], improved bone maturation and growth, stable grafting, adequate wound sealing and hemostasis, and improved handling of the graft materials[1]. Clinical studies have emphasized that combining growth factor rich PRF and bone grafts could enhance bone quality and quantity[1,3,11].

The Leukocyte and Platelet Rich-Fibrin Block (L-PRF block) has arisen from the combination of a xenograft that is acting as a scaffold with osteoinductive ability and L-PRF membranes that serve as a bioctive nodule with osteoinductive capacity[10], due to its rapid angiogenesis, faster bone remodeling, cost-efficiency and tissue regeneration capacity, thus allowing earlier implant treatment[9]. The combination of activated platelets in the L-PRF membranes and the Liquid Fibrinogen results in a mass production of fibrin, integrating the bone substitute into a strong construct[10].

2.Objective and hypothesis

The main aim of this study was to perform an integrative review on the distribution of inorganic graft material particles within the PRF block.

The distribution of the inorganic graft material within the PRF block is made homogeneously or heterogeneously in space?

3.Method

3.1. Information sources and search strategy

A bibliographic review was performed on PubMed (via National Library of Medicine) considering such database includes the major articles in the field of dentistry.

The following search terms were applied: (platelet rich fibrin) OR (PRF); AND (block) OR (bone graft); AND (CBCT) OR (micro-CT) OR (microscopy). Also, a hand-search was performed on the reference lists of all primary sources and eligible studies of this integrative review for additional relevant publications.

The inclusion criteria included articles published in the English language, until March 1, 2021, reporting surgical approaches using PRF mixed with inorganic graft material, in order to understand its distribution in the block and its influence on the success of bone augmentation. The eligibility inclusion criteria used for article searches also involved randomized controlled clinical trials, randomized preclinical studies, prospective studies, case reports, case study - single cohort, comparative study and an animal study.

Exclusion criteria were as follows: articles in Chinese and Russian; articles that evaluate the effect of PRF membranes and inorganic graft without being mixed in block, or that were mixed and used to fill the void between the bone graft block and the recipient site and on the graft surface.

Studies based on publication date were not restricted during the research process.

3.2. Study selection and data collection process

Studies were primarily scanned for relevance by title, and the abstracts of those non-excluded were assessed. The total of articles was compiled for each combination of key terms and therefore the duplicates were removed using Mendeley citation manager.

The second step comprised the evaluation of the abstracts and non-excluded articles, according to the eligibility criteria on the abstract review. Selected articles were individually read and analyzed concerning the purpose of the present study.

At last, the eligible articles received a study nomenclature label, combining first author names and year of publication. The following variables were collected for this review:

authors' names and publication year, aims/ purpose, study design, PRF protocol, Inorganic graft material, and main outcomes.

4.Results

The literature search on PubMed identified a total of 87 articles although 36 duplicates were removed, as seen in Figure 1. Of the 51 articles, titles and abstracts were read seeking agreement with the inclusion criteria of the presente study and then 13 studies were discarded. After the full reading of the remaining 38 articles, 25 articles were excluded because the application of the PRF and the inorganic graft material were carried out independently, that is, without being mixed in block, and one article in which PRF and inorganic graft material were mixed and used to fill the void between the bone graft block and the recipient site and on the graft surface.

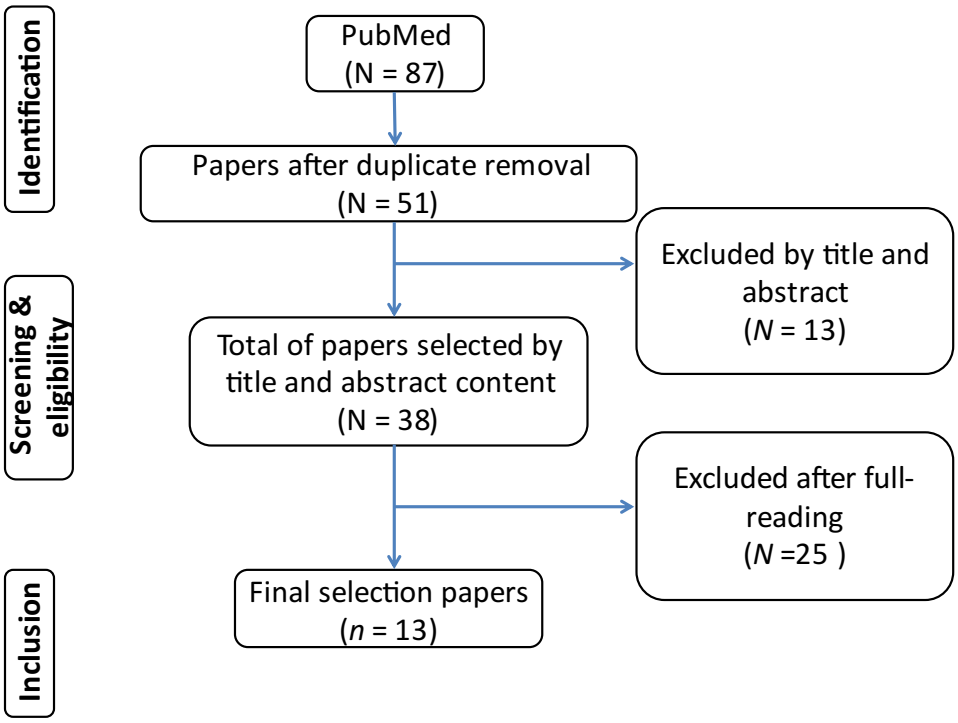

Figure - 1 Flow diagram of the search strategy used in this study

At last, 13 articles were included in the present integrative review. The results of the selection of articles are shown in Table 1.

Table 1- Relevant data gathered from the retrieved studies

Author (year)	Purpose	Study design /analyses	PRF protocol	Inorganic graft material	Main outcomes
Pavani, M.P., Reddy K., Reddy B., *et al.* (2021)	This study assesses bone fill in intrabony defects, following the use of β trícalcium phosphate (TCP) bone graft with and without PRF.	A randomized clinical trial • CBCT	• 6 ml intravenous blood was collected from the antecubital fossa of the patient • The blood was centrifuged using a centrifuge at 3000 rpm for 10 min. • The PRF clot/gel located in the middle of the tube soaked in acellular plasma was removed with tweezers and used as graft material at the experimental site.	β-TCP (SYBOGRAF-T)	Thirty intraosseous defects were treated with: 1. OFD + β TCP + PRF; 2. β TCP alone 3. OFD alone. • All three treatment groups showed a significant reduction in probing poket depth (PD) at 6 months: ○ OFD + β TCP + PRF group 1.8 ± 0.26mm; ○ β TCP group 2.15 ± 0.63 mm; ○ OFD group 2.3 ± 0.48 mm. • Intergroup comparison of plaque index, gingival index and sulcus bleeding index between treatment groups showed no significant difference at different time periods. • The percentage of bone filling: ○ β TCP with PRF group 44.82%, ○ β TCP group 33.22%, ○ OFD group 21.52%.

					• The mean bone filling and the percentage of bone filling were higher in the β TCP + PRF group than in the β TCP and OFD Group.
Al-Mahdi, A. H.; Abdulrahman, M. S.; Al-Jumaily, H. A. H. (2021)	The aim of this prospective study is to investigate and evaluate the quality and quantity of the bone graft that mixed with PRF when used in reconstruction of alveolar cleft in terms of bone density and resorption rate of the bone graft.	A prospective study • CBCT	• 40 ml venous blood sample was collected from each PRF group patients. • Drawn into 4 sterile 10ml evacuated glass tubes without an anticoagulant. • The blood was centrifuged immediately using a Gemmy tabletop centrifuge (plc-5 Gummy Europe) for 10 min at 3000 rpm (approximately 1220 g). After centrifuge, it settles into the following layers: • Red lower fraction containing red blood cells, • Upper straw colored cellular plasma which is platelet poor plasma • Middle fraction containing the fibrin clot (PRF).	Bone graft from the iliac crest.	• 1 month after surgery the mean of bone density of bone grafts: o PRF group (224.25 HU) o Control group (249.5HU), • 6 months after surgery the mean of bone density of bone grafts: o PRF group (411.37HU) o Control group (410 HU). (The bone density was measured and collected in same labeled layer 1 month and 6 months after surgery using CBCT density scale that measured by HU unit that used in CT.) • The bone loss in the width and height according to axial and coronal views in PRF group was significantly lower than that in the control group.

			The upper straw colored layer is then removed and middle PRF is collected.		
Yüceer-Çetiner, E.; Özkan, N.; Önger, M. E. (2021)	The aim of this study is to analyze the effects of autogenous dentin graft and mixture of autogenous dentin graft and platelet-rich fibrin (PRF) applied to the tooth extraction sockets on bone healing process.	A randomized controlled clinical study • Histopathological Evaluation • Immunohistochemical Evaluation • Scanning Electron Microscopy (SEM)	• 10 ml venous blood was obtained in a glass-coated plastic sterile tube (Hema & Lab Medical Products, Ankara, Turkey) without anticoagulant from each patient for the Group DP. • The tubes were centrifuged (EBA 20; Hettich Zentaifugen, Tuttlingen, Germany) at 3000 rpm (approximately 867g) for 10min immediately. After centrifugation, the blood was separated into 3 different layers: • acellular plasma at the top, • erythrocytes at the bottom, • PRF in the middle. The PRF clot was removed using a sterile collet. With a sterile scissors, it was divided into small pieces and mixed with the dentin graft.	Dentin graft	Were evaluated in this study, a total of 57 extraction sockets in 9 patients who were planned to be treated with dental implant after tooth extraction: • Extraction sockets were divided randomly into 3 groups for each patient: ○ 1° group: sockets were filled with autogenous dentin graft (Group D). ○ 2° group: sockets were filled with the mixture of PRF and autogenous dentin graft (Group DP). ○ 3° group: sockets were left empty as the control group (Group C). • After 3 months, histological and immunohistochemical evaluations were performed on the samples taken during the implant surgery. Additionally, samples obtained from each group were examined by scanning electron microscopy. ○ It has been concluded that undemineralized autogenous dentin

					graft has bone formation capacity on early period of bone healing. It can be used as bone graft material in augmentation procedures and its combined use with PRF accelerates new bone formation.
Dhamija, R., Shetty V., Vineeth K., *et al.* (2020)	This in vivo study compared clinical, histological, and radiological differences in bone formation in human extraction sockets grafted with demineralized freeze-dried bone allograft (DFDBA) and platelet-rich fibrin (PRF), with nongrafted	Randomised controlled trial. • CBCT • Histological evaluation • Histomorphological Evaluation	• 5 ml of whole venous blood was collected from each patient at the time of implant placement, in sterile vacutainer tubes of 6-ml capacity without anticoagulant. The vacutainer tubes were then placed in a centrifugal machine REMI R-4C (REMI Laboratory Instruments, Mumbai, India) at 3000 rpm (approximately 1602g) for 10 min at room temperature after which it settled into the following layers: • Red lower fraction containing red blood cells, • Upper straw-colored cellular plasma, • The middle fraction containing the fibrin clot.	Demineralized freeze-dried bone allograft (DFDBA)	The study comprised thirty sockets(having 15 control and 15 test sockets) from patients varying from 25 to 60 years old: ❖ Group I: Control group - no graft was placed and the extraction socket was left to heal normally ❖ Group II: Test group - alveolus was preserved with DFDBA mixed with PRF and placed after extraction. • Lower buccal bone levels were seen in the control group versus test group at all intervals though moderately significant. • Lingual bone levels significantly reduced at all the three intervals for the control group as compared to the test group. • Better bone conversion was noted in the preserved sockets.

	sockets and bone–implant contact (BIC) at 3 and 6 months after implant placement.		The upper straw-colored layer was removed, and the middle fraction which is the PRF was collected.		• The preserved sockets also showed better BIC 3 months after implant placement and loading. • In both groups, ridge width reduced in a time span of 6–7 months but did not show any significant difference between the groups. • In the histological and radiological evaluation, showed insignificant difference in both groups. Indigenously developed DFDBA material shows promising results as an osteoinductive material.
Mu, Z.; He Q.; Xin L.; *et al.* (2020)	This study aims to assess the angiogenic and osteogenic capacity in rabbit sinus model grafted with Deproteinized bovine bone mineral (DBBM) particles soaked	A randomized preclinical study. • SEM • Enzyme-linked immuno sorbent	The development of the low-speed centrifugation concept promoted the creation of a new formulation of platelet-rich fibrin (PRF), namely iPRF: • 1 ml upper layer of transparent pale-yellow sticky liquid in the test tube was collected as iPRF.	Deproteinized bovine bone mineral(DBBM): (Bio-Oss, Geistlich Pharma AG, Wolhusen,	Characterization of DBBM and iPRF+DBBM: ❖ SEM (Stereomicroscope and scanning electron microscop) images showed that DBBM particles are scattered and irregular with porosity. However, after loaded with iPRF, DBBM particles were aggregated and the pores were filled with iPRF, becoming a fibrin clot as a whole. Leukocytes can be observed on the fibrin network of iPRF.

	in injectable Platelet rich fibrin (iPRF), both of which interacted to form an integrated block.	assay (ELISA) • Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) • Micro-CT • Fluorescence assay and VG staining • Histological evaluation		Switzerland)	• Compared to DBBM group, iPRF+DBBM group showed faster bone formation during the early healing period (at 2nd week), while there is no significant difference between two groups in the late period (at 7th week). • Compared to the DBBM group, iPRF+DBBM group showed that new bone formation was mostly detected in the lower part of the SM (Schneiderian membrane) and the upper part of the basal bone. This may be due to the elevated migration of osteoprogenitor cells. • The ratio of new bone formation in iPRF+DBBM group was 1.3 times higher than that of the DBBM group at 4 weeks postoperatively. • Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and vascular tube formation assay were conducted to verify the angiogenic property of iPRF+DBBM in the neovascularization, and results showed that the mRNA levels of VEGF, Col-1 and Ang-1 were significantly higher in the iPRF+DBBM group compared with the DBBM group.

		• Immunofluorescence evaluation • Histomorphometric evaluation			iPRF+DBBM accelerated vascular formation, bone remodeling and substitution of bone graft materials at the early healing period, even though it failed to increase the bone volume in a long-term period. • This integrated grafting biomaterial will have great potential in the application of sinus augmentation, which provides a favorable environment for early implant placement.
Kavitha M., Krishnaveni R., Swathi A.M., et al (2020)	This article describes cases of bone augmentation with a combination of PRP + β -TCP and PRF + β -TCP for treatment of the chronic periapical lesion. The cases were followed for 6	Case Report • CBCT	• Intravenous blood was collected in a 10 ml sterile tube without anticoagulant. • Immediately centrifuged (Remi - India) at 3000 rpm (approximately 1602g) for 10 minutes to obtain PRF gel.	Beta-tricalcium phosphate (β-TCP) (Sybograf-T Eucare Pharmaceuticals (P) Ltda.).	Two cases are presented where periapical surgery was performed and the bone defect was filled with PRP + β -TCP and PRF + β -TCP to promote bone regeneration and healing was evaluated quantitatively using CBCT. • According to table 1 presented in the case report: o In pre-operative: bone density is higher in case 2, o At the end of 6 months there is an insignificant bone density difference in favor of case 1 (PRP + β -TCP),

	months and 1 year and healing was evaluated quantitatively using cone beam computed tomography.				At the end of 1 year this difference inverts in favor of case 2 (PRF + β -TCP).
Castro A.; Cortellini S.; Temmerman A.; *et al.* (2019)	The aim of this study was to characterize the L-PRF block and its components (L-PRF membrane, L-PRF exudate and Liquid Fibrinogen) in terms of release of growth factors, cellular content, and their distribution	*In vitro* study • ELISA • Micro-CT • SEM	• 2 glass-coated plastic tubes of 9ml (red cap, BVBCTP-2, Intra-Spain, Intra-lock, Florida, USA) • 1 plastic tube without coating of 9ml (white cap, WCT, Intra-Spain, Intra-lock, Florida, USA) Centrifuged at 408g force (IntraSpin, Intra-Lock, Florida, USA). • The centrifugation was interrupted after 3 min and the white cap tube was removed from the centrifuge. • The remaining red cap tubes were further centrifuged for 9 min, completing the cycle of 12 min of centrifugation.	Deproteinized bovine bone mineral (DBBM): (Bio-Oss, Geistlich Biomaterials, Wolhusen, Switzerland)	• The L-PRF block consists of deproteinized bovine bone mineral particles surrounded by platelets and leukocytes, embedded in a fibrin network that releases growth factors up to 14 days, with TGF-β1 being the most released growth factor, followed by PDGF-AB, VEGF and BMP-1. • The L-PRF membrane and liquid fibrinogen showed a high concentration of leukocytes and platelets and the microCT and SEM images revealed the bone substitute particles surrounded by platelets and leukocytes, embedded in a fibrin network.

	inside the L-PRF block.		• Immdiatly after removing the white cap tube from the centrifuge, the yellow liquid obtained above the red blood cells was collected whith a sterile (5ml) syringe: liquid is called Liquid Fibrinogen. • When the full centrifugation (12 min) was finalized, the red cap tubes werw removed and two L-PRF membranes were prepared.		
Pichotano E.C.; Molon R.S.; Paula L.G.F.; *et al* (2018)	This case report aimed to describe the effects of leukocyte and platelet-rich fibrin (L- PRF) associated with deproteinized bovine bone mineral (DBBM) and absorbable collagen membrane (CM)	Case report • CBCT • Histological evaluation • Histomorphometric evaluation	• Peripheral blood sample was taken before the surgery, and immediately centrifuged at 3000 RPM (approximately 1038g) for 10min using an appropriate centrifuge (Kasvi K14-0815, Curitiba – Brazil). • After centrifugation, the fibrin clot was removed from the tube and separated. • The L-PRF clot was prepared in the form of a membrane by pressing out the fluids.	Deproteinized bovine bone mineral (DBBM) (Bio-Oss; Geistlich Pharma AG, 88 Wolhusen, Switzerland)	• CBCT evaluation showed an increased bone resorption in the sinus filled with L-PRF and DBBM compared to the left sinus (22.52% and 8.95% respectively). • Implant stability quotient (ISQ) were higher than 68% for all implants tested in all the time points. • Histomorphometric analysis showed higher proportion of newly formed bone in the sinus filled with L-PRF compared to the contralateral side (2118102 and 975535 mm3). • The addition of L-PRF allowed fast healing process evidenced by the higher amount of

	on bone regeneration in maxillary sinus augmentation.				neoformed bone and less fibrous tissue in the sinus, compared to the group without L-PRF. • After 4 months, osseointegration of dental implants installed in the right sinus was successfully achieved. • 6 months after functional loading, stable bone levels were accomplished with the employed protocols.
Cortellini S.; Castro A. B.; Temmerman A. *et al.* (2018)	This proof-of-concept study was to investigate the effects of a new guided bone regeneration technique with a tissue engineering approach.	Case study – Single cohort. • CBCT • Micro-CT	Before starting the surgery, 8 to 16 tubes (9 ml) of venous blood were collected from the patients: • 6 to 14 tubes (red cap, glass coating (BVBCTP-2, IntraSpin, Intra-Lock, Florida, USA) Centrifuge (IntraSpin, Intra-Lock, Florida, USA) at 2700rpm/408g RCF for 12 min, centrifuge rotor radius 5 cm. • 2 tubes (white cap, plastic coating (WCT, IntraSpin, Intra-Lock, Florida, USA))	Deproteinized bovine bone mineral (DBBM) (Bio-Oss Small particles, Geistlich AG, Wolhusen, Switzerland)	• Ten patients (mean age of 50,7 years) representing 15 sites with horizontal alveolar deficiencies were included. • Superimpositio of pre-operative and post-healing CBCT scans showed an average linear horizontal bone gain of 4,6 mm (±2,3), 5,3 mm (±1,2) and 4,4 mm (±2,3), measured at 2, 6 and 10 mm from the alveolar crest, respectively. • The volumetric gain was 1,05 cm3 (±0,7) on average. The resorption rate after 5-8 months was 15,6 % (±6,7) on average. • L-PRF block may be a suitable technique to augment deficient alveolar ridges.

			Centrifuge (IntraSpin, Intra-Lock, Florida, USA) at 2700rpm/408g RCF for 3 min. • The yellow fluid (Liquid Fibrinogen) at the top of the white cap tubes was aspirated with a sterile syringe, without the red part. • After full centrifugation of the red cap tubes, the L-PRF clots were removed from the tubes using surgical tweezers. • The clots were thereafter gently compressed into membranes using a sterile metal box (Xpression, Intra-Lock, Florida, USA).		
Chenchev I.L.; Ivanova V.V.; Neychev D.Z.; *et al.* (2017)	The aim of this case report was to assess the possibility for augmentation of the alveolar ridge in the frontal region of the	Case report • CBCT	A-PRF: • After the venipuncture with a 10 ml vacuum test-tube (Advanced-PRF), 9 ml of blood was taken from the patient. The blood was then immediately put into a PRF DUO (Process for PRF-France)	Bone graft material (do not specify in the article	The control CBCT scan showed good organization of new bone allowing placement of a dental implant. • The good clinical results in this case report show that the addition of PRF and i-PRF to

	upper jaw, utilizing a combination of bone graft material, injectable platelet-rich-fibrin (i-PRF) and advanced platelet-rich-fibrin (A-PRF).		centrifuge for 8min at 1300 rpm (approximately 719g). • The A-PRF membrane, in our methodology, was formed out of two A-PRF clots by putting them on top of one another - the areas bordering with the red part were put at the opposite ends and it was then dried in a metal box - A-PRF Box. i-PRF: • Blood was drawn from the patients in special tubes. The manipulation was performed immediately before using the i-PRF. The tubes were then placed in a centrifugal machine at a 700 rpm (approximately 209g) for 3 min.		bone graft materials improves their properties. • Mixing of bone graft material with pieces of PRF and its infiltration with i-PRF leads to delivery of growth factors inside the wound, which helps the migration of osteopromotor cells and attracts circulating stem cells to the wound (fast angiogenesis). • Adding PRF to the bone graft reduces the required bone graft material volume and improves its manipulative qualities. • The use of PRF accelerates the healing of hard and soft tissues, and the use of PRF membrane instead of another barrier membrane reduces the cost of the procedure.
Moussa M.; El-Dahab O. A.; Nahass	Characterize clinically and radiographically the effect of using platelet-	A controlled trial study. • CBCT	In the test group: • 10 ml of blood per missing tooth space was collected after the local anesthesia administration, and centrifuged (Centrifuge 800, China)	Buccopalatal Bone	• In the 14 sites (7 test group and 7 control group) in 12 patients (7 women and 5 men), all but one autograft (from the control group) integrated successfully after 4 months.

H. E. (2016)	rich fibrin (PRF) autologous graft on the augmentation results of autogenous palatal bone blocks.		at 3500 rpm (approximately 1372g) for 12 to 15 min.		• No statistically significant difference was found between demographic data in the two groups. • There was a statistically significant increase in the buccopalatal bone width in both groups by time as measured by CBCT as well as the manual caliper. • The test group showed statistically significantly lower mean graft resorption than the control group (test, 0.8 ± 0.6 mm; control, 1.6 ± 0.9 mm). • Autogenous palatal bone block surface resorption is significantly decreased by the use of PRF coverage.
Yoon J-S.; Lee S-H.; Yoon H-J. (2014)	The purpose of this study was to investigate the influence of platelet-rich fibrin (PRF) on angiogenesis and osteogenesis in guided bone regeneration	Animal study • Histological evaluation • Immuno histoche	• 3ml blood sample was collected from each rabbit and drawn into 10ml test tubes without an anticoagulant. • The blood was centrifuged immediately using a tabletop centrifuge (406 G, GYROGEN, Daejeon, Republic of Korea) for 10 min at 3000 rpm (approximately 400 g).	Bio-oss (Geistlich-Pharma,Wolhusen, Switzerland)	• In each rabbit, 2 circular bone defects, one on either side of the midline, were prepared using a reamer drill. ○ Each of the experimental sites received bovine bone with PRF, ○ Each of the control sites received bovine bone alone. • At all experimental time points, immunostaining intensity for VEGF was consistently higher in the experimental

	(GBR) using xenogenic bone in rabbit cranial defects.	mical evaluation • Histomorphometric evaluation			group than in the control group. However, the differences between the control group and the experimental group were not statistically significant in the histomorphometrical and immunohistochemical examinations. • The results of this study suggest that PRF may increase the number of marrow cells. However, PRF along with xenogenic bone substitutes does not show a significant effect on bony regeneration. • Further large-scale studies are needed to confirm our results
Pripatnanont, P., Nuntanaranont T.; Vongvatcharanon S.; *et al.* (2013)	This study investigated the effect of platelet-rich fibrin (PRF) on bone regeneration of various grafting materials in	Comparative study • SEM • Radiography • Histological analyses	• 8ml of autologous whole blood collected from the central ear artery of the rabbit before sedation. Blood samples were treated according to the PRF protocol (Dohan et al., 2006a): • The whole blood without an anticoagulant was transferred into a 10 ml glass tube and	Calvarial bone chips and Deproteinized bovine bone (DBB)	• The mean optical density (OD) and histomorphometric analysis (HA) of the percentage of new bone showed that the PRF groups were significantly higher than the non-PRF groups in the autogenous bone graft and the empty defect, but not in the DBB group and the composite group. PRF had a positive effect on bone formation when used alone or combined

	rabbit calvarial defects.	• Histomorphometric analysis	centrifuged at 3000 rpm (approximately 1509g) for 10 min (Labofuge 400R centrifuge, Hereus, Hanau, Germany). A fibrin clot in the middle of the tube was used as an adjunct to grafting material.		with autogenous bone, but not with deproteinized bovine bone.

The main conclusions of the studies are:

- Only one author[10], evaluated the properties of the L-PRF block and its components in terms of release of growth factors, cell content and structure, the remaining 12 authors (92,31%), intend to evaluate angiogenesis and osteogenesis in bone regeneration using the PRF Block with inorganic graft material.
- Study projects include: 3 randomized controlled clinical studys[3,8,13], 1 prospective study[1], 1 randomized preclinical study[9], 1 controlled trial study[6] ,3 cases report[2,7,11], 1 case study-single cohort[4] and 1 comparative study[12].

  Two authors did not indicate the type of study design in their articles, but after carrying out their readings, Castro, A. *et al.*, refer to na *in vitro* study[10], and Yoon, J.S., *et al.*, refer animal studies[5].
- According to the articles studied, these were the types of exams performed: Cone-beam computed tomography (CBCT) (61,54%), Histological evaluation (38,46%), Scanning electron microscopy (SEM) (30,77%), Micro Computed Tomography (Micro-CT) and Histomorphometric evaluation (23,08%), Enzyme-linked immunosorbent assay (ELISA) and Immunohistochemical evaluation (15,38%), Histopatological evaluation, Histomorphological evaluation, Flerescence/Immunoflurescence evaluation and Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) (7,69%).
- Mu, Z. *et al.*, is the only author who does not specify the type of protocol used in the centrifugation of the blood collected for PRF preparation, only mentions the centrifugation time, of 15 min, and removed 1ml of i-PRF[9].
- Regarding blood collections for the PRF preparation protocol, both the quantity and the location of collection differ among authors, and there are also authors who do not mention them.
- As for the centrifugation protocol, the vast majority of authors (61,54%) performed centrifugation with a force of 3,000 RPM in 10 min[1,2,3,5,8,11,12,13]. Castro, A. *et al.*, and Cortellini, S. *et al.*, (15,38%) applied the same centrifugation protocol, using the same centrifuge (IntraSpin, Intra-Lock, Florida, USA) with a force of 408G/2700RPM in 12 minutes[4,10]. Chenchev, I.L., *et al.* performed a protocol for A-PRF (advanced platelet-rich fibrin) by performing a centrifugation at a force of 1300 RPM (approximately 719 G) in 8 minutes and for i-PRF a centrifugation at a force 700 RPM (approximately 209 G) in 3 minutes[7]. Moussa, M. *et al.*, used a centrifugation protocol using a force of 3500 RPM (approximately 1372 G) for 12 to 15 minutes[6]. A conversion of RPM to G force was performed for all types of centrifuges used, and different values were noted for all.

- The most widely used type of PRF is L-PRF (84,62%) (leukocyte and platelet rich fibrin) (REFERENCES), Mu, Z., *et al.*, and Chenchev, I.L., *et al.*, used i-PRF (15,38%) (injectable PRF)[7,9], this last author also used the A-PRF (advanced PRF)[7].
- As for the type of inorganic graft, most used DBBM (46,15%) (demineralized bovine bone mineral), namely BIO-OSS® [4,5,9,10,11,12], Pavani, M.P. *et al.* and Kavitha, M., *et al.* used β-TCP (beta-tricalcium phosphate) (15,38%), namely Sybograft-T® [2,8]. In the remaining studies (38,46%), bone graft from the iliac crest[1], dentin graft[3], DFDBA (demineralized freeze-dried bone allograft)[13], bucopalatal bone[6] and calvarial bone[12] fragments were used.

  Chenchev, I.L. *et al.,* was the only one who did not refer to the type of inorganic graft material, however they state that Bone graft material was used[7].
- Most authors claim that the inorganic graft material with PRF induces greater bone regeneration, bone neoformation and osseointegration [1,2,3,4,6,7,8,9,10,11,12,13] , and one author states that PRF can increase the number of marrow cells, however, together with xenogenic bone substitutes, they have no significant effect on bone regeneration[5].
- Micro-CT analysis revealed that the particles were homogenously distributed within the L-PRF block, without being in close contact with each other. The SEM images from the surface of the L-PRF block showed a dense fibrin mesh covering the whole surface of the block and numerous cells were embedded in this mesh, which seemed to keep the components of the bloock assembled. In the cross-sectional images bundles of fibres could be seen connecting different DBBM particles and these bundles contained clusters of platelets, leukocytes and some red blood cells[10], the images also showed that DBBM particles are scattered and irregular with porosity, however, after loaded with iPRF, DBBM particles were aggregated and the pores were filled, becoming a fibrin clot, as a whole[9].In order to perform some measurements of the distances between DBBM particles, SEM images that presented a measurement scale were selected. The selected images are by the author Castro A., et al. (2018). Figure 2.

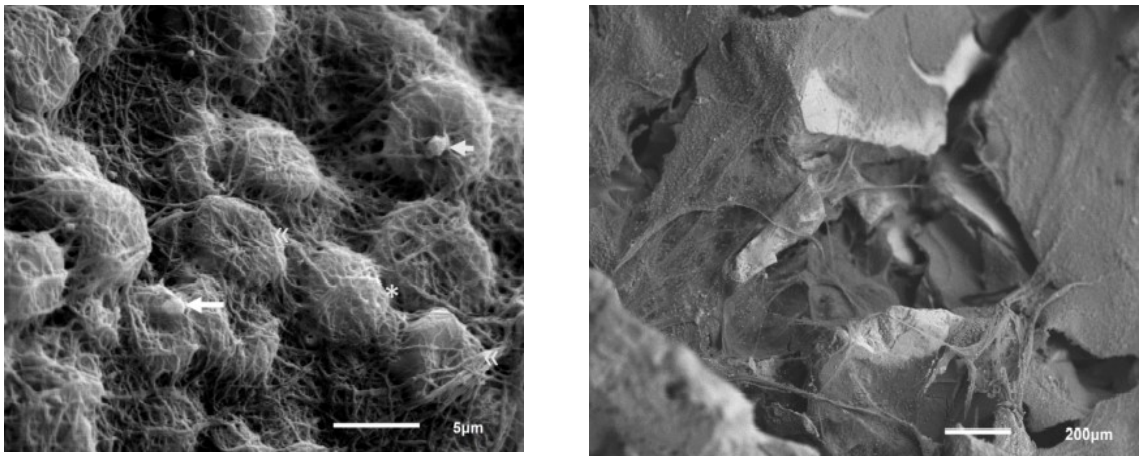

Figure - 2 Image A: L-PRF block surface "cells embedded in the fibrin network" B: LPRF block cross-section "fibres of the liquid fibrinogen connecting the dbbm particles inside the block

Table 2 -Distance between DBBM particles in Image

Distance between DBBM particles	Average distance between particles evaluated in 21 sites.
0	60,55µm ≈ 0,061 mm
0	
0	
0,67 µm	
1 µm	
1,33 µm	
. 1,33 µm	
1,33 µm	
1,67 µm	
1,67 µm	
2,67 µm	
3 µm	
3,67 µm	
4 µm	
4 µm	
5 µm	
6 µm	
6 µm	
7,67 µm	
8,33 µm	

Table 3- Distance between DBBM particles in Image B

Distance between DBBM particles	Average distance between particles evaluated in 9 sites.
0	226,3 µm ≈ 0,23mm
0	
67 µm	
130 µm	
250 µm	
270 µm	
320 µm	
330 µm	
670 µm	

Table 4- Size measurement of DBBM particles in Image

Size measurement of 10 evaluated particles	Average particle size
4 µm	5,15 µm ≈ 0,0052mm
4,2 µm	
4,5 µm	
4,5 µm	
5 µm	
5,3 µm	
5,3 µm	
5,3 µm	
6,7 µm	
6,7 µm	

- On the sample surface (IMAGE A), DBBM particles have a shorter distance average than in the cross section sample (Image B) (0.0605mm <0.23mm) (Table 2 and 3), this difference may be due to greater amplitude of the B image, making the DBBM particles not appear, giving a false feeling of "empty", so the distance between the particles in reality must be smaller than the one measured in the B image.
- Regarding the size of the particles, we have an average diameter of 5.15 µm (0.0052 mm), which is not consistent with what the author Castro A. et al. states that the diameters of mineral particles vary between 65-85 µm (0.065 – 0.085mm)[10], this is due to the fact that DBBM particles vary in size, and may be due to some measurement error (Table4).

5.Discussion

Only one author, evaluated the properties of the L-PRF block and its components in terms of release of growth factors, cell content and structure, and in this evaluation only Micro-CT analysis revealed that the particles were homogenously distributed within the L-PRF block, without being in close contact with each other, but no measurement was performed that could describe the distance between the DBBM particles and the L-PRF. This author also described SEM images from the surface of the L-PRF block showed a dense fibrin mesh covering the whole surface of the block and numerous cells were embedded in this mesh, which seemed to keep the components of the bloock assembled, and in the cross-sectional images bundles of fibres could be seen connecting different DBBM particles and these bundles contained clusters of platelets, leukocytes and some red blood cells[10] (Figure 2).

Most authors (84,62%) affirm that the use of the PRF Block decreases bone loss, increases bone density and neoformation, increases vascularization and improves healing. Pripatnanont, P.*et al.*, it further states that the PRF had a positive effect on bone formation when used alone or combined with autogenous bone, but not with deproteinized bovine bone[12].Yoon, J-S. *et al.*, says that the PRF may increase the number of marrow cells, however, along with xenogenic bone substitutes doot show a significant effect on bony regeneration[5].

All authors cite choukroun's standard protocol, directly or indirectly through other authors who cite it. Choukroun *et al.* (2017), refere in your protocol, that 10 ml of blood was collected and centrifuged for 12 minutes at 2700 rpm (750 g). After centrifuge, it settles into the following layers: Red lower fraction containing red blood cells, upper straw colored cellular plasma which is platelet poor plasma (PPP) and in the middle fraction containing the fibrin clot (PRF). The PRF clot is removed using a sterile collet. The clots were thereafter gently compressed into membranes using a sterile metal box. With a sterile scissors, it´s divided into small pieces and mixed in a 1:1 ratio with inorganic graft material (autologous bone/bone-substitute material)[14] (Figure 3).

Figure - 3 L-PRF block protocol: protocol: 1) collect peripheral blood; 2) centrifuge; 3) Blood tube; 4) centrifugation: A) PPP; B) PRF; C) red blood cells; 5) PRF metal box; 6) Mixed PRF membrane whit DBBM; 7) Sticky bone

Micro-CT analyses revealed that the particles were homogenously distributed within the L-PRF block, without contact with each other and revealed a mean volume of 37.7% ± 1.7% for the DBBM particles in the L-PRF block, however, no measurement was performed. After performing the measurements on the selected images, we can see that the distance between the DBBM particles was approximately 60.55 µm (0.0605 mm) in image A and 226.3 µm (0.23 mm) in image B, and relate this difference with the image magnification, since the elements can be "hidden" by the L-PRF that appears very magnified in image B. Despite this difference between the measurements in the two images, we can conclude that these values demonstrate the proximity between the DBBM particles, corroborating the assertion of the author Castro A. et al., that the particles were homogeneously distributed within the L-PRF block, without being in close contact with each other.

Castro A. et al. states that the diameters of mineral particles range between 65-85 µm (0.065 - 0.085 mm)[10], the measurement I made in image A, shows an average diameter of 5.15 µm (0.0052 mm), which does not match what the author states, this difference may result from the fact that DBBM particles vary in size or may be due to some measurement error.

The evaluation and measurement of the distances performed on images A and B presented great difficulty in relation to the scale presented, the difficulty in distinguishing all the elements of the image, as well as the difficulty in feeling safe in relation to the measurements performed with a ruler and manually, there is always a margin of error that I consider high.

6.Conclusion

The distribution of the inorganic graft material within the L-PRF block is homogeneously or heterogeneously in space? We can conclude that the distribution is homogeneous since the distance between the particles is reduced, keeping the elements of the block together.

This study is inconclusive, measurements and evaluations in the laboratory through electron microscopy are necessary since the images where these evaluations were carried out are insufficient to validate the veracity of these measurements.

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