BioSCIENCE Original Article
DOI: https://doi.org/10.55684/2024.82.e00074

Tissue repair with fibrin-rich plasma in soft tissue defects: a study in rabbits

Reparo tecidual com plasma rico em fibrina em defeitos de tecido mole: estudo em coelhos

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ABSTRACT

Introduction: Fibrin-rich plasma (PRF) is an autologous biomaterial that accelerates the healing process of soft and hard tissues. This platelet concentrate also has other advantages such as technical simplicity, rapid production, low morbidity and low cost.

Objective: To evaluate the effects of PRF on soft tissue repair in non-critical defects in the nasal dorsum region of rabbits.

Method: PRF was produced from venous blood collected from 15 rabbits. Two non-critical defects, in a supraperiosteal envelope with approximately 15 mm in diameter, were created on the nasal dorsum of each animal. All were treated differently; the first was filled with PRF membrane (case group) and the second was simply divulsed and sutured edge to edge (control group). The animals were euthanized in the 2nd, 4th and 6th weeks postoperatively. Histological and histomorphometric analyses of the anatomical specimens collected were performed.

Result: In the control group, after 2 weeks, the orthokeratinized stratified epithelium exhibited 8-10 layers of stratification and, in the area corresponding to the surgical bed, the presence of sebaceous glands, sweat glands and hair follicles was not found. The case group presented 6-7 layers of stratification, and it was possible to observe the presence of cutaneous appendages in the area of the operation. After 4 weeks, the control group had stratified epithelium exhibiting 6-7 layers of stratification and discrete neoformation of cutaneous appendages. The PRF group had 5-8 layers of stratification and only 1 small portion of the operated area did not have the presence of appendages. After 6 weeks, the control group presented the skin with a normal appearance, however, it exhibited a smaller amount of cutaneous appendages when compared to the non-operated region. The case group presented 7-9 layers of stratification and an intense amount of cutaneous appendages.

Conclusion: PRF proved to be a promising material for accelerating soft tissue repair

KEYWORDS: Growth factors. Platelet-rich fibrin. Tissue repair.

RESUMO

Introdução: O plasma rico em fibrina (PRF) é biomaterial de origem autóloga que acelera o processo de cicatrização de tecidos moles e duros. Esse concentrado plaquetário, também tem outras vantagens como simplicidade de técnica, rápida obtenção, baixa morbidade e baixo custo.

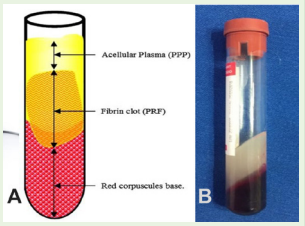
Objetivo: Avaliar os efeitos do PRF no reparo de tecidos moles em defeitos não críticos na região de dorso nasal de coelhos.

Método: PRF foi produzido a partir de sangue venoso coletado de 15 coelhos. Dois defeitos não críticos, em envelope supraperiosteal com cerca de 15mm de diâmetro foram criados no dorso nasal de cada animal. Todos foram tratados de forma diferenciada; o primeiro foi preenchido com membrana de PRF (grupo caso) e o segundo apenas divulsionado e suturado bordo a bordo (grupo controle). Os animais foram submetidos à eutanásia na 2ª, 4ª e 6ª semanas de pós-operatório. Foram realizadas análises histológicas e histomorfométricas das peças anatômicas colhidas.

Resultado: No grupo controle após 2 semanas, o epitélio estratificado ortoqueratinizado exibia entre 8-10 camadas de estratificação e, na área condizente ao leito cirúrgico, não foi encontrada a presença de glândulas sebáceas, sudoríparas e folículos pilosos. O grupo caso apresentou 6-7 camadas de estratificação, sendo possível observar a presença de anexos cutâneos na área da operação. Após 4 semanas o grupo referente ao controle encontrava-se com epitélio estratificado exibindo entre 6-7 camadas de estratificação, e discreta neoformação de anexos cutâneos. O grupo PRF encontrava-se com 5-8 camadas de estratificação e apenas 1 pequena porção da área operada não havia a presença dos anexos. Em 6 semanas o grupo controle apresentava a pele com aspecto usual, entretanto exibia quantidade menor de anexos cutâneos quando comparada à região não operada. O grupo caso apresentou de 7-9 camadas de estratificação e intensa quantidade de anexos cutâneos.

Conclusão: PRF mostrou ser material promissor para a aceleração do reparo de tecidos moles.

PALAVRAS-CHAVE: Fatores de crescimento. Plasma rico em fibrina. Reparo tecidual.



A) Schematic drawing of blood after processing. B) Appearance of blood after centrifugation

Central Message

Therapeutics based on the use of tissue bioengineering or substances that provide tissue growth factors has numerous applicabilities in orthopedic and oro-maxillofacial surgery, whether in the recomposition of hard tissue, or in moles that need filling. It is a fundamental therapeutic approach in areas of craniofacial deformity reconstructions that have a critical size in loci lost due to cysts or tumors.

Prospect

The tissue regeneration capacity provided by fibrin-rich plasma (PRF) mimics the repairing process triggered by platelet-rich plasma (PRP), i.e., the remaining platelet accumulation when activated by the collagen of the grafted area promotes platelet adhesion sites, a condition that favors platelet degranulation. As a consequence of this platelet/collagen interaction, a cascade of events occurs that culminate in the release of cytokines, and growth factors, in the middle of the fibrin clot. In this study, it was possible to show that PRF improves the healing process.

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Conflict of interest: None | Funding: Partly by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) – Funding code 001 | Received: 29/07/2024 | Accepted: 22/10/2024 |
Correspondence: juniordemarco@live.com | Associate Editor: Ronaldo Mafia Cuenca

How to cite:
de Marco J, Reis PGTA, CarmoABC, Lages Neto M, Zeve JLM, Ribas Filho JM. Reparo tecidual com plasma rico em fibrina em defeitos de tecido mole: estudo em coelhos. BioSCIENCE. 2024;82:e074

INTRODUCTION

Therapeutics based on the use of tissue bioengineering or substances that provide tissue growth factors has numerous clinical applicabilities in orthopedic and oro-maxillofacial surgery, whether in the recomposition of hard tissue or in soft tissues that need filling. It is a fundamental therapeutic approach in areas of craniofacial deformity reconstructions that have a critical size in Loci lost by cysts or tumors.[1,2] Optimal tissue reconstruction should combine material with "scaffold" biocompatible with cellular elements, that does not promote graft vs. host reaction and that also provides a tissue environment that mimics the histophysiology of common homeostasis and the morphostasis of the site to be regenerated.[3]

It is assumed that the immediate use of autologous substances that provide synthesis and secretion of growth factors could be considered a plausible technique in the reconstruction of defects. Young and Medawar4 demonstrated success in repairing peripheral nerves in animals sealed with blood plasma.[5] This study, in turn, seems to have pioneered the use of the fibrin sealant technique in general surgery. The product used in the operations provided by Helena Matras consisted of a mixture of fibrinogen and thrombin, forming a local clot that covered the injured area. The natural evolution of this method transformed historical sealants into platelet concentrates when platelets were added to the formula. The first protocols of this platelet-rich plasma combination were called autologous platelet-derived wound healing factors (PDWHF). The platelet content was based on the premise that active platelets are responsible for secreting numerous growth factors, which, hypothetically, can promote cell differentiation, as well as provide adequate levels of extracellular matrix formation. Despite its attractive hypothesis, numerous studies describe that the use of PRP alone may not have the desired effect, sometimes causing a negative impact on the area to be regenerated, such as the myelofibrotic-like effect in the intraosseous area.[6]

These contradictory effects on PRP motivated Choukroun et al.[7] to develop a second generation of platelet concentrates, called Fibrin-Rich Plasma (PRF). In this instance, with the centrifugation of peripheral blood, the formation and physiological fractionation of the clot are now activated without the need for previous use of anticoagulants, which makes PRF a plausible alternative to growth.

Despite the good results demonstrated by Choukroun et al.[7] and other authors,8 additional studies in other research centers are still needed to guide the real effect of PRF on the healing process.

The objective of this research was to analyze the effects of fibrin-rich plasma (FRP) on the repair of non-critical defects performed in the dermis in the nasal dorsum region of rabbits, induced by healing in first intention and to compare these results with the effects of skin healing in first intention, without the use of biomaterials.

METHOD

This work was carried out at Faculdade Evangélica Mackenzie - FEMPAR. It was approved by the Ethics Committee on the Use of Animals (CEUAs/FEMPAR), protocol no. 3211/2017 and followed the ethical principles in animal experimentation, recommended by the National Council for the Control of Animal Experimentation (CONCEA).

We used 15 adult white female rabbits of the New Zealand breed, with no previous diseases, aged between 350-370 days and weighing between 2450-3500 g. All were submitted to the same surgical procedure, with the use of 2 different surgical areas, which received different treatments, one using PRF (n=15) and the other considered control (n=15). In addition, the animals in both groups were allocated into 3 subgroups corresponding to the period of euthanasia (2nd, 4th or 6th postoperative week). It is noteworthy that, for each rabbit, the stipulated euthanasia times were determined by randomization-draw so that there was no bias. The animals were kept in the IES's own vivarium, identified and weighed. They were kept at room temperature, fed with feed of industrial origin suitable for the species (Presence Coelhos) and water ad libitum. A normal day and night cycle was established and fasted for 12 hours prior to anesthesia.

The animals were anesthetized by administering sterile solution of 5% ketamine hydrochloride at the rate of 60 mg/kg and xylazine at the rate of 10 mg/kg. Anesthesia was considered effective when the animal was immobile when handling.

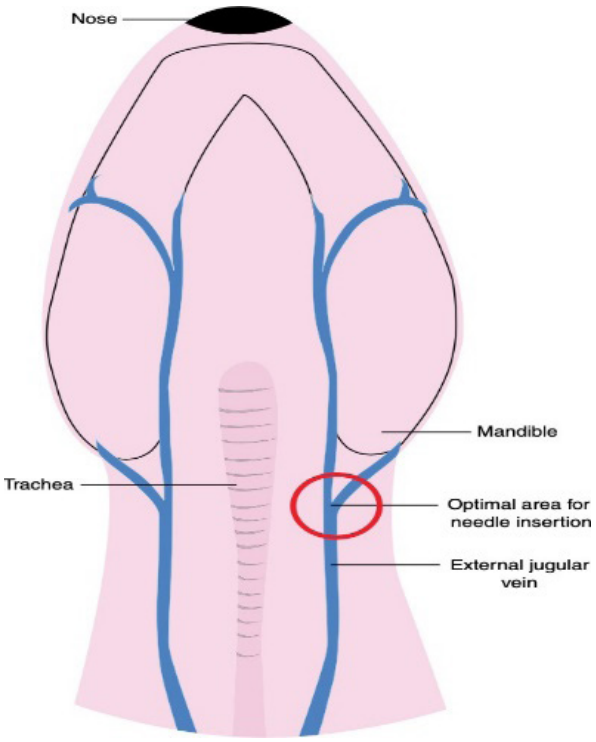
Technique for obtaining peripheral venous blood sample

Neck trichotomy was performed. Since the external jugular vein is located laterally to the midline and superficially to the internal jugular vein and carotid artery, the neck was palpated to locate the trachea. Laterally to it, going towards the mandible, the external jugular vein bifurcates into the maxillary vein and the lingualofacial vein. The external jugular vein is usually located between the trachea and the angle of the mandible (Figure 1). To obtain the blood sample, the external jugular vein was punctured using a scalp size 23.

After the puncture, a 9ml vacuette tube was connected , allowing blood collection. About 6 ml were removed from each rabbit and immediately transferred to the centrifuge.

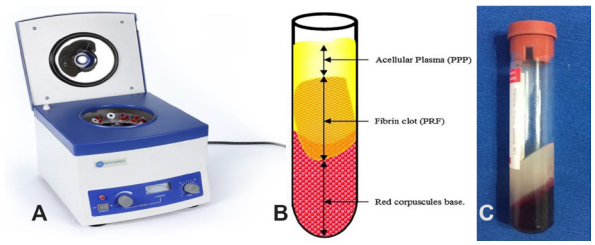
Obtaining the PRF

To obtain fibrin-rich plasma, a simplified method was used. The production was done simultaneously with the surgical procedures. According to the proposed protocol, the PRF was obtained through the centrifugation technique, with the use of a common centrifuge (Figure 2A). This centrifuge allows the placement of up to 12 tubes of 15 ml that can be centrifuged simultaneously.



Source: Nelson et al.9

FIGURE 1 — Schematic drawing illustrating ideal puncture location



Source: Naik et al.[10]

FIGURE 2 — A) Montserrat analog centrifuge model 80-20b; B) schematic drawing of the blood after processing; C) appearance of the blood after centrifugation

The tubes were centrifuged at 1800 rpm for 10 min. After this centrifugation, 3 distinct levels were established (Figure 2B), where the lower part consisted of a layer composed of red blood cells; the intermediate layer by PRF concentrate itself - which resulted in the formation of a three-dimensional fibrin scaffold -, and the upper layer by platelet-poor plasma (Figure 2C).

Surgical procedure

Trichotomy was performed in the skull region extending to the nasal dorsum region of each rabbit (Figure 3A), followed by antisepsis with brushes containing PVPI degerming, followed by washing with 0.9% saline solution, drying, and, finally, new antisepsis with the use of PVPI tincture. Sterile surgical drapes adapted to the region covered the area of the initial procedures.

The surgical area was previously infiltrated by 1 ml of 2% lidocaine with adrenaline 1:100,000. Two envelope-shaped supraperiosteal nasal dorsum incisions were made, exposing the subcutaneous region. After the incision, divulsion was performed, atraumatic separation of the tissues through the natural cleavage planes, where one of the defects received the PRF membrane (Figure 3B), previously removed from the tubes with the aid of anatomical forceps, and after both defects were sutured edge to board with simple stitches (Figure 3C).

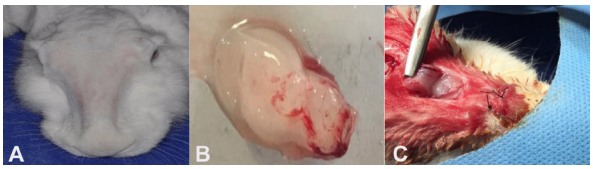


FIGURE 3 — A) Tricotomy of the area to be operated; B) ready-to-use PRF membrane; C) defect receiving the PRF membrane, noting below the case already sutured

Postoperative

Once the anesthetic-surgical procedure was completed, the animals were placed alone in their cages and in a room suitable for anesthetic recovery; were kept in the same place and under the same preoperative conditions, receiving 50 mg of cefazolin sodium for 3 days; the 1 g ampoule was diluted to 20 mL and 0.[1] mg morphine per day for 3 days; The 10 mg ampoule was diluted in 10 ml.

Obtaining the anatomical specimens

The rabbits were euthanized through rapid intravenous administration of 2.5% sodium thiopental at 10 mg/kg. Euthanasia occurred at 2, 4 and 6 weeks postoperatively (5 rabbits in each subgroup). 1 full-thickness flap was performed involving the 2 surgical incisions previously performed to obtain the anatomical specimens.

Preparation of anatomical specimens and histological analysis

The surgical specimens obtained were immersed in 10% formalin solution for 48 h, and each specimen was hemisectioned in order to obtain a cutting plane in the center of the defect so that the entire extension of the area to be studied could be evaluated. Afterwards, the surgical specimens were processed for inclusion and paraffin blocking. Once the blocks were obtained, serial sections with a thickness of 5mm were made, and these were stained with H&E.

Method for histomorphometric analysis

All extensions of the fields referring to the center of the defect were selected. The images were observed under a light microscope coupled to the image capture camera to obtain digital images. Images from the Neubauer camera were obtained in order to establish the grading of the linear measurement for the images of the sample. In the Paint program (Windows, Microsoft) the scales have been established with the Brush icon. Activating the size of the bars, the measurement for each objective was defined from the grid seen in the camera. Based on this principle, a scale of 0.2 mm was established for a 4x objective and 50 µm for a 10X and 40X magnification (Figure 4). A legend with bars and corresponding values was added to the images obtained from the samples, at the

respective magnifications, and the images were analyzed by delimiting the area corresponding to 0.[5] mm2.

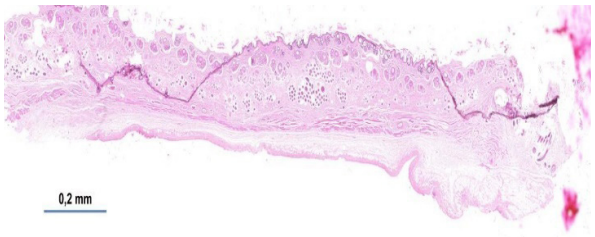


FIGURE 4 — Establishment of the scale with the aid of imaging programs

The histomorphometric parameters were analyzed with the aid of an image analysis program - Image Tool 2.00. Five images containing graft areas were captured, measured in the Paint program and saved in JPEG.

In this program, with the file containing the JPEG image open, the first step was to inform him of the photo's scale pattern. The "settings" icon was selected, followed by "calibrite spatial measurements" (to calibrate the specimen to be analyzed in micrometers). After this selection, the "area" icon was chosen and applied. Through the "area" icon, the perimeter of mature bone, immature bone, fibrosis and medullary area was selected. For each predetermined structure selected, the program provided us, through a spreadsheet, with the respective area in mm2. After selecting the area, the "cont and tag" marker was used to measure the tissue volume until reaching an average of 2 mm2 present in each field. The data of the histological fields analyzed were allocated in a Microsoft Excel spreadsheet, providing final numbers in area, as well as the percentage of each structure analyzed.

Data such as sclerosis, neutrophilic infiltrate, mononuclear infiltrate and fibroblasts were quantified by scoring cells counted in the surgical area determined as follows: Score 0 - from 0 to 1%; Score 1 – between 1 and 25; Score 2 - between 25 and 50%; Score 3 – greater than 50% of cells accounted for in the surgical area. Table 2 in the results section shows the MODA value (highest frequency) determined for each parameter investigated.

Statistical analysis

The variables considered in the histomorphometric analyses of the study were: area of fibrosis, area of thick and fine fibers, area of cutaneous appendages, area of angiogenesis. Mean and standard deviation are shown in Table 1. For comparison of treatments, referring to each parameter studied and within each week of study, the analysis of variance model for repeated measures or the non-parametric Friedman test was considered, followed by the analysis of variance with source of variation or the non-parametric Kruskal-Wallis test. Values of p <0.[05] indicated statistical significance. The data were analyzed with the IBM SPSS Statistics v.20.0 computer program. Armonk, NY: IBM Corp.

RESULT

Qualitative analysis

Control 2 weeks

In the microscopic analysis of the skin fragment referring to the control group, at 2 weeks postoperatively, it was verified that it was a fragment covered by orthokeratinized stratified epithelium, showing between 8-10 layers of stratification. Choron, papillary dermis, and reticular were identified, in addition to areas compatible with delayed granulation tissue, composed of rich vascularization, dense connective tissue, and discrete presence of mononuclear inflammatory cells. However, in the area consistent with the region of the surgical bed, the presence of sebaceous and sweat glands and hair follicles was not identified. A discrete number of mononuclear cells were seen in the cuts. The hypodermis showed no alterations (Figures 5A and 5B)

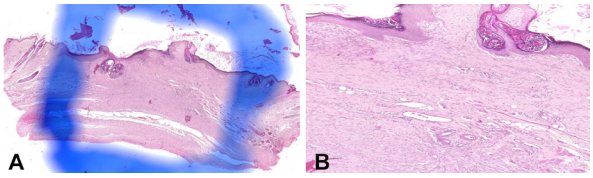


FIGURE 5 — A) Sections at 40x magnification, identifying a fragment of skin without alterations with the demarcated area corresponding to the area compatible with the surgical bed; B) magnification at 100x where epithelium and tissue of late granulation are noted in the area referring to the surgical bed, but there is no cutaneous appendages.

PRF 2 weeks

At 2 weeks postoperatively, specimens that received PRF were found to have a fragment of skin covered by orthokeratinized stratified epithelium, showing between 6-7 layers of stratification. Choryon, papillary dermis and part of the reticular dermis were composed of dense non-modeled connective tissue, permeating fusiform cell content compatible with fibroblasts. In the edges and in the reticular dermis areas of the surgical area, the presence of cutaneous appendages was noted, and in the dermis located peripherally to the surgical bed, the presence of glandular hyperplasia without atypia or dysplastic characteristics was identified. Areas of edema (e) between fibers in the lower portion of the reticular dermis conclude the histological picture ascertained in the specimens (Figure 6).

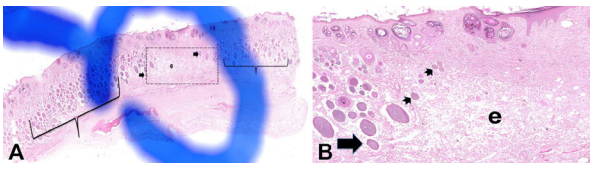


FIGURE 6 — A) 40x magnification, reveals the area of edema (e) demarcated, whose area is demarcated by a dashed rectangle. In the area of the keys, hyperplasia of cutaneous appendages, especially sweat glands, and the presence of glanbular (arrows) in the areas of surgical borders is observed; B) 100x magnification, in addition to the details identified at 40x, areas suggestive of glandular neoformation (arrowhead) are shown, extending to the non-edematous area of the reticular dermis

Control 4 weeks

In the microscopic analysis of the control fragment at 4 weeks postoperatively, little difference was noted between 2-4 weeks. In the current postoperative period, a fragment covered by orthokeratinized stratified epithelium was verified, showing between 6-7 layers of stratification. In the reticular dermis, areas composed of dense connective tissue were identified, with a higher density of collagen fibers (Figures 7A and 7B - demarcated area) through which there was a discrete neoformation of cutaneous appendages (arrows). The hypodermis showed no changes.

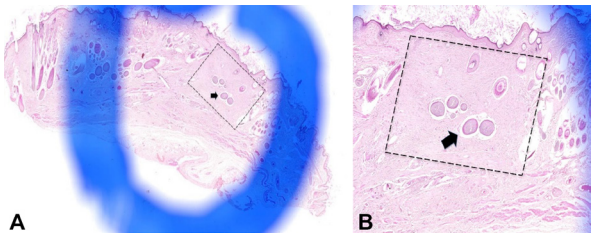


FIGURE 7 — A) 40x magnification shows histological picture in the 4-week control group. In the part demarcated by a dashed line, the area of local fibrosis in the reticular dermis and hypodermis portion is verified. The presence of a glandular opening in the fibrotic area in a slight quantity (arrow) is noted; B) reveals at 100x magnification, details of the fibrotic area and glands without the presence of atypia.

PRF 4 weeks

In the specimens that received the PRF, a fragment of skin covered by orthokeratinized stratified epithelium was verified, showing between 5-8 layers of stratification. Choron, papillary dermis and part of the reticular dermis were composed of dense non-modeled connective tissue, and without characteristics of fibrosis permeating fusiform cell content compatible with fibroblasts. Only a discrete area referring to the surgical bed did not present the presence of cutaneous appendages (Figures 8A and 8B); however, the largest portion referring to the lesioned area the histological picture was compatible with a skin fragment of usual characteristics.

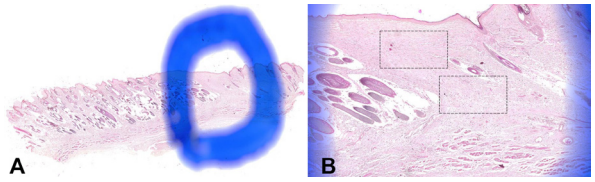


FIGURE 8 — A) Skin fragments in specimens treated with PRF at 4 weeks with usual histological picture, without pathological fibrosis; B) area demarcated in dashed lines identifies area with absence of cutaneous appendages

Control 6 weeks

In the microscopic analysis of the control fragment at 6 weeks postoperatively, a skin fragment with usual aspects is identified. However, in this period, the dermis showed a smaller amount of cutaneous appendages when compared to the areas that were not submitted to surgical conditions. No fibrosis or other conditions were identified that could support pathological conditions in the areas analyzed (Figures 9).

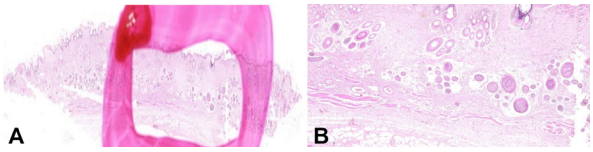


FIGURE 9 — In A=40x and B=100x, there are aspects of dense connective tissue, without pathological alterations in the presence of cutaneous appendages, although smaller when compared to non-surgical areas.

PRF 6 weeks

Specimens that received PRF therapy demonstrated, in the sixth postoperative week, a skin fragment covered by orthokeratinized stratified epithelium exhibiting between 7-9 layers of stratification. Choion, papillary dermis and part of the reticular dermis were composed of dense, non-modeled connective tissue, with a usual appearance permeating an intense amount of cutaneous appendages. The presence of cellular atypia was not found in this period (Figures 10).

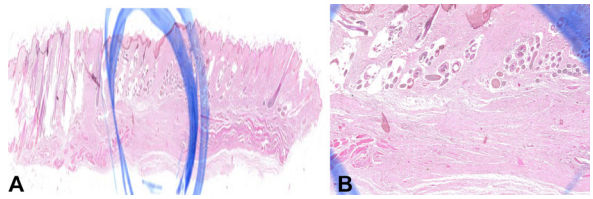


FIGURE 10 — The surgical areas of the control group specimens are shown at A=40 and B=100x, respectively, and aspects of dense connective tissue are verified, with no pathological alterations in the presence of cutaneous appendages.

Qualitative analysis

TABLE 1 – Demonstration of the area of fibrosis, fibers, angiogenesis and cutaneous attachments in the surgical areas

Period analyzed	Parameter	Group		p
		Control	PRF	
2 weeks	Fibrosis area (mm²)	17.16 ± 3.04	11.88 ± 2.35	p= 0.0031
	Fine fiber area	2.93 ± 0.11	3.56 ± 0.89	p=0.0521
	Coarse fiber area	15.36 ± 2.56	8.79 ± 1.99	p=0.0020
	Area of cutaneous appendages (mm²)	-	1.82 ±1.99	p<0.0001
	Angiogenesis/vascular area	3.78 ± 0.68	4.22 ± 0.72	p=0.0632
4 weeks	Fibrosis area (mm²)	9.03 ± 1.84	7.98 ± 1.06	p=0.005
	Fine fiber area	1.02 ± 0.04	1.96 ± 0.01	p=0.0622
	Coarse fiber area	7.90 ± 2.01	6.06 ± 1.83	p= 0.0245
	Area of cutaneous appendages (mm²)	2.56 ± 1.37	2.81 ± 1.85	p=0.0664
	Angiogenesis/vascular area	1.04 ± 0.77	1.36 ± 0.64	p=0.0666
6 weeks	Fibrosis area (mm²)	4.32 ± 0.96	3.39 ± 0.71	p=0.0318
	Fine fiber area	0.88 ± 0.03	0.44 ± 0.08	p=0.0732
	Coarse fiber area	3.68 ± 2.01	2.09 ± 1.83	p=0.0222
	Area of cutaneous appendages (mm²)	3.21 ± 1.87	4.81 ± 1.85	p=0.0332
	Angiogenesis/vascular area	1.04 ± 0.77	1.36 ± 0.64	p=0.0666

TABLE 2 — Mode score for sclerosis, inflammatory infiltrate, and fibroblasts

PeriodAnalyzed	Parameter	Group	
		Control	PRF
2 weeks	Sclerosis	2	2
	Neutrophils	1	0
	Mononuclear	2	1
	Fibroblasts	2	2
4 weeks	Sclerosis	1	0
	Neutrophils	1	0
	Mononuclear	1	0
	Fibroblasts	2	2
6 weeks	Sclerosis	0	0
	Neutrophils	0	0
	Mononuclear	0	0
	Fibroblasts	2	2

DISCUSSION

Described by Choukroun et al.[7] PRF is not - as many authors stipulate - just a "fibrin glue"; much less, too, can it be considered a classic platelet concentrate. It is constituted by an autologous scar matrix, defined as a concentrate of platelets on an intense and important membranous fibrin mesh and, according to some authors, this compound has high regenerative potential.

The tissue regeneration capacity provided by PRF mimics the repairing process triggered by PRP, i.e., the accumulation of remaining platelets, when activated by the collagen of the area to be grafted, promotes platelet adhesion sites, a condition that favors platelet degranulation. As a consequence of this platelet/collagen interaction, a cascade of events occurs that culminate in the release of cytokines, and growth factors, in the middle of the fibrin clot. Overcoming this platelet effect, Ehrenfest, Rasmusson and Albrektsson11 add that the intense density of the fibrin clot acts as a healing biological matrix that serves as a scaffold that sustains cell migration, as well as incites cell proliferation within the fibrin matrix, constituting the first stages of healing.

Within this perspective, Gassling et al.[12] were carried out in a study where they compared the growth of human periosteal cells in PRF membranes when compared to the control using commercial collagen membranes. The study evaluated cell viability and cell proliferation. The results showed that both membranes had viable cells; however, the authors indicated greater cell proliferation in the specimens in which the PRF was inserted.

Also, Gassling et al.[12] proposed the use of fibrin membrane for osteoblast culture. This study compared the compatibility and cell proliferation between fibrin and commercial collagen membranes. Their results showed greater cell proliferation by the PRF membrane, as well as greater cell differentiation in the alkaline phosphatase test, inciting the idea that PRF would be plausible biomaterial for repair, both in growth semantics and in cell differentiation.

In a global histomorphometric analysis of the images of the present study, it was observed that the mean collagen concentration in the wound area showed a statistically significant increase, especially in the analysis focused on coarse fibers as early as 2 weeks, reducing the period of granulation tissue neoformation in specimens that received PRF. Our findings are in accordance with the normal healing process, where the expected - in the wound fibroplasia phase - is an increase in collagen synthesis, and the results demonstrated here corroborate the hypothesis that PRF provides, in fact, rapid regenerative maturation, without the presence of dehiscence or pathological fibrosis.

Although some authors have suggested that PRF heals more quickly than PRP control, no statistically significant differences were found in the comparisons of small fibers and fibroblast score. This bias may reveal a false negative in the present study, since the accounting of fibroblast cells is demonstrated in a score, which stipulates a range of values, minimizing the actual values of cells per mm2.

According to Cieslik-Bielecka et al.[13] An important variable that provides a positive effect of PRF compared to control is the idea that this biomaterial - PRF - produces fibrin matrix architecture. This characteristic structure of the matrix has been considered to be responsible for the therapeutic capacity of PRF, although platelets, leukocytes and growth factors play important roles. In this regard, Choukroun et al.[7] indicated that the fibrin matrix makes open and infected wounds benefit from the use of PRF, as it is able to regulate the process of local immunity, in addition to guiding the migration of the cell epithelium on its surface, stimulating fibroblasts to produce collagen, and promoting angiogenesis. Therefore, PRF membranes protect open wounds, which heal by second intention, such as those in the present study, and accelerate their healing. The fibroblastic role has already been well discussed in previous paragraphs. However, the results described here also corroborate the improvement of immune regulation, demonstrated by the minimization of neutrophilic and mononuclear cell content, and also by epithelial migration.

The context of migration and epithelial proliferation seem to provide an important reflection in this research. In fact, as we did a study with healing in first intention, the analysis of the lining epithelium became a null variable in terms of migration and neoformation; however, the effects of PRF on the area of epithelium that form the cutaneous appendages in the submucosa seem to be intense.

In the present study, it was demonstrated that there is epithelial proliferation of sweat glands and hair follicles peripherally to the area where the defect was produced. The histological results observed in this study suggest that migration and neoformation of new cutaneous appendages intensified at 4 weeks postoperatively, a condition that seems to minimize at 6 weeks, where the quantification of appendages is similar when compared to control. The extrapolation of these results seems to support the aesthetic

hypothesis, since PRF would reduce the effect of local inflammation, create subcutaneous tissue with appendages closer to the area without trauma, minimizing loss of follicles, or reduce the effect of local alopecia production more quickly.

Angiogenesis, immunity and epithelial coverage are, according to Choukroun et al.[7] The 3 important stages of healing and maturation of soft tissues. It was demonstrated here that the PRF membrane was able to support the development of these 3 phenomena simultaneously, although angiogenesis was not statistically different. Similar to what was accounted for for fibroblasts, neoangiogenesis or the presence of vascular conjuncture was performed by score. Here, the percentage range within the same score category is wide, which would minimize the actual angiogenic effect. In addition, only vascular opening and vascular lumen coated with endothelium were considered vascular presence. However, strangulated vessels or microvascularization are not conceivable for analysis by conventional histochemistry. Thus, it is suggestible to complement these results with the use of immunohistochemistry techniques, with the use of anti-CD31, CD34 or even VEFG antibodies so that a more accurate result can be obtained.

Although some particularities have been considered as biases here, it can be suggested that PRF improves the healing process when compared to control.

CONCLUSION

PRF proved to be a promising material for the acceleration of soft tissue (dermis) repair when performed by first intention, having seen that when used there was neoformation of the cutaneous appendages with only 2 weeks postoperatively, while the control group presented slight formation of these structures with only 4 weeks.

Authors' contributions
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