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Repair techniques for parietal and soft tissue defects using autologous blood-derived preparations

ABSTRACT OF PHD THESIS

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Introduction

The development of this idea of repairing soft tissue defects and integrating prosthetic materials into the repair of parietal defects arose from a presentation at a 2010 congress on the topic of autologous biomaterials, relatively easy to obtain from whole blood, which can provide several functions/roles - either structural support (here, they provide the growth matrix) or immunological support, by locally increasing the amount of growth factors and antimicrobial factors (1). Autologous plasma derivatives are currently used in oral-maxillofacial surgery (BMF) in the coverage of gingival tissue defects after dental implants or in orthopaedic surgery for cartilage, tendon and ligament repair, but also in plastic surgery for soft tissue volumetric augmentation[2,3,4] .

With this study I develop the idea of repairing, by using autologous factors, soft tissue defects and integrating prosthetic materials used in the repair of parietal defects. Today, the use of biomimetic (or biological) matrices has led to a whole range of regenerative therapies, be it collagen gels, collagen sponges or collagen matrices or cell cultures. Autologous blood preparations such as those used in this study are relatively easy to obtain from whole blood.

Biomaterials are defined as substances manufactured to interact with biological systems (in this case, the abdominal wall) for therapeutic purposes (treatment, augmentation, repair or tissue replacement).

Platelet-rich fibrin (PRF) is a solid plasma derivative that plays a structural role by providing fibrin networks on which connective cells migrate, including growth factors and leukocytes for their anti-infective role (2,3). This biomaterial we are trying to adapt to the surface of polypropylene mesh used in the treatment of abdominal hernias and eventrations, by fixing it to their surface. In the case of soft tissue lesions with substance deficiency such as scars, traumatic wounds with substance defect, ulcers of any kind, the PRF will be fixed in the depth of the lesions after the edges have been avulsed, and only after the infectious phenomena have ceased.

Platelet-Rich Plasma (PRP) is a liquid plasma derivative containing a biologically active mixture of growth factors that produce regeneration and healing of damaged tissues (1,2,4). Both plasma derivatives do not elicit an immune response, being autologous material (2).

The method of application of this derivative involves tissue infiltration with PRP into the affected area or the area in which we wish to induce regeneration.

Justification of the topic:

Plasma derivatives such as PRP and PRF represent a modern research direction in biomaterials and regenerative medicine. Although the benefits and mechanisms of action are largely elucidated, and the therapy has existed in the medical field for over 20 years (in dental, orthopaedic and BMF surgery), new applications have been slow to develop. Worldwide, there is still no described procedure using plasma derivatives to integrate prosthetic materials. As for the closure of soft tissue defects, there is evidence of clinical use and it is intensively exploited in Western European clinics. Problems reported in connective tissue defect therapy are the lack of a single protocol and the lack of clear terminology regarding the plasma derivative used (4).

Abdominal parietal pathology is one of the most common pathologies addressed by general surgery (5-7) and, in particular, by clinics where the project is carried out. Soft tissue defects are also a fairly common pathology, requiring a large amount of health resources over a long period of time, and are thus also very costly. The use of plasma derivatives would dramatically decrease the number of hospital days and healing time, which overall would lead to more efficient management with increased cost-effectiveness of therapy. Because an autologous preparation is used, there are no reactions at the injection or fixation site, and it is perfectly compatible with the host organism (2,4,8,9).

The present work brings as a novelty at national level a standardized procedure and a working protocol with plasma derivatives, but also a clarification of the nomenclature of plasma derivatives with specific surgical indications. At the same time, it proposes a personalized, cost-effective, rapid and safe therapy in the treatment of chronic wounds, as well as a new perspective in personalizing and increasing the tissue integration of parietal prostheses. Worldwide there are studies using different substrates to functionalise the plates used in the treatment of parietal defects, but a customised, autologous, *in situ* augmentation technique has not yet been described. Therefore, the process of augmenting and functionalising prostheses with the patient's own plasma derivatives represents a new perspective in the field.

The note of originality comes by describing the first technique to augment the integration process of prosthetic material (polypropylene mesh) used in the alloplastic treatment of abdominal hernias and eventrations using autologous plasma derivatives and by describing a working protocol for the repair of soft tissue defects using plasma derivatives.

It will transfer and extend previously developed knowledge on parietal alloplasty techniques and the use of autologous blood products such as PRP (Platelet Rich Plasma) and PRF (Platelet Rich Fibrin), generating new solutions for rapid integration of polypropylene mesh and repair of soft tissue defects *in vivo*.

Aim of the work: The project aims to increase the performance of the parietal alloplasty technique (for hernia and eventrations) by faster integration and decreased tissue repair time. For soft tissue defects (scabs, ulcers, tissue erosions, old imperfectly closed fistulous tracts, etc.), the study provides a tissue growth matrix using the patient's own blood, thus achieving closure of defects without the need for tissue grafting and with reduced risk of superinfection and rejection.

The use of plasma as a source of concentrating growth factors is not a new concept and has been intensively studied over the last 4 decades. Merging with the field of biomaterials and regenerative medicine instead brings a complex approach to the topic. The benefits of PRP and PRF compounds and their mechanisms of action have been largely elucidated, at least at the theoretical and *in vitro* level, and clinical use has stood the test of time, with new indications developed subsequently. The lack of worldwide processing standardisation to obtain standardised derivatives makes it very difficult to assess actual therapeutic efficacy. At the time of writing this thesis, there is no described process using plasma derivatives for integrating prosthetic materials such as herniated mesh. Repair of soft tissue defects, on the other hand, is the most addressed topic where clinical use data are increasingly available, and is intensively exploited in clinics in Western Europe and beyond. Also in the case of connective tissue defect management there is a lack of standardisation of therapy and a lack of clear terminology regarding the plasma derivative used(4).

Hernias and eventrations are the most common pathology in general surgery(5-7) and even though the recurrence rate has decreased, problems related to tissue integration of prosthetic material have remained relatively constant. This quite frequent pathology requires a large amount of health care resources (related to the increased frequency of admissions for reoperation) over a long period of time and is therefore also very costly.

Therefore, we propose to significantly decrease the number of days of hospitalization and the speed of healing through the use of plasma derivatives, which overall would lead to a cost-effective therapeutic treatment, easy to implement with minimal infrastructure investment compared to bioengineering technologies such as 3D printing or the development of biocompatible functionalized synthetic materials. By using an autologous preparation, we limit the occurrence of immunological reactions at the injection or fixation site as much as possible, thus ensuring repair with a proprietary material with the highest possible degree of compatibility(2,4,8,9).

Considerable progress has been made in the way medicine has developed knowledge of the wound healing process over the past decade, generating large volumes of publications and the development of hundreds of dressing options and therapies. There are numerous studies on chronic wounds, but these largely focus on specific healing products or technologies such as topical therapies, antiseptics, dressings, specific disease states or specific aspects of care such as patient/wound preparation. A simplified overview of evidence-based criteria is needed to facilitate the appropriate management of chronic wounds and early diagnosis of complications in all care settings (hospital, home, nursing home) of such a patient. The aim of this paper is to provide a summary of guidelines for the management of chronic wounds of various aetiologies, using evidence-based medicine, that can be used by wound care clinicians in all theatres of care to assist the entire cycle of care of such a patient (i.e., diagnosis, patient and wound bed preparation, treatment, but also follow-up of progression) for major types of chronic wounds. In addition to this, he has personal experience with the introduction of autologous plasma-derived products into the field of general surgery to accelerate the healing process of tissue-deficient lesions, as well as an application in the field of tissue integration of alloplastic materials, such as parietal prostheses used in the repair of abdominal defects.

This paper is written in the hope that there will be a greater appreciation of the components that make up the entire cycle of wound care, particularly in terms of the dynamic adaptation of therapy according to the evolutionary stage of the wound, but, more importantly, that it will provide the tools necessary to close difficult-to-heal defects and achieve rapid and effective integration of parietal (and other) prostheses.

4. General research methodology

The approval of the Ethics Committee of the Colentina Clinical Hospital was obtained in September 2017 for carrying out the procedures for the collection of plasma derivatives and their use in therapeutic practice, with the registration number and interdisciplinary agreements.

In the first step, eligibility criteria were selected for patients undergoing this type of therapy (with plasma derivatives). This stage is followed by *a period of evaluation of the biophysical behaviour of the plasma derivatives according to the type of protocol used to obtain them and how to improve each type of plasma product so as to have the optimal resource for the planned treatments*.

Based on the literature review and the experience gained from *in vitro* evaluation of plasma derivative samples, we have selected working protocols in order to tailor the therapy to each patient's pathology. This phase is complemented by data obtained during the BioWALL and BioMesh project (4-month phase completed in October 2017).

Phase II involves obtaining blood derivatives and their characterization with the establishment of optimal protocols for obtaining them (phase scheduled to last 3 months and completed in December 2017), followed by the operating protocol and the use of biomaterials generated from plasma (phase that lasted 3 months and was completed in February 2018).

Phase III involves the *in vivo* evaluation of the products with the optimisation of the production processes and the generation of standard protocols, the clinical evaluation of the therapy, biohumoral and imaging (ultrasound) evaluation of the patients until the resolution of the pathology (this phase was planned for 2 years, but for which it was necessary to extend the period to 4 years due to the temporary cessation of surgical activity during the COVID-19 pandemic, the rethinking of the working protocols to be used in the pandemic context and the resumption of the evaluations at the end of the pandemic).

The *in vitro* testing and analysis were carried out in collaboration with the Biophysics Master Department of UMF "Carol Davila", through the kind permission of Prof. Mihaela Moisescu and at the laboratories of the Faculty of Chemical Engineering and Biotechnologies, Department of Biomaterials and Polymer Science of the Polytechnic University of Bucharest, with the help of Prof. dr. eng. Izabela Cristina Stancu, using the

infrastructure provided by these institutions, also with the agreement in the Appendix of this work with no. 4, with the increase of the needs of consumables (vacutainers and collection kits, PRP kits, sterile gloves, etc.), covered by own funds.

The processing and implantation part of PRP and PRF was performed in the operating theatre of the General Surgery Clinic, and the mobile working protocol in the COVID-19 ward. The clinical, photographic and imaging follow-up took place in the Colentina Clinical Hospital, in the premises serving the General Surgery Clinic.

Study 1*: Use of plasma derivatives in chronic wound healing*

The first stage of the study of the biophysical behaviour of the plasma derivatives studied (PRP and PRF) is an observational, *in vitro* study aimed at optimising the protocols for obtaining plasma derivatives and accommodating the working protocols for the hospital surgical theatre. PRF is a natural fibrin-based biomaterial used in tissue healing. It is obtained from patient blood and contains cytokines and growth factors such as transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF) and epidermal growth factor (EGF). The roles of PRF in wound healing are: prolonged release of growth factors at the wound site, proliferation of fibroblasts and osteoblasts, promotion of angiogenesis and induction of collagenogenesis, mechanical adhesion by fibrin and uptake of circulating stem cells.

The aim of this step was to analyse the biophysical characteristics of PRF clots and to investigate the possibility of obtaining a larger PRF clot from the same amount of blood. The final aim of the step is to conclude which protocol is able to cover larger tissue defects and to optimise the safety, efficiency and feasibility of the treatment.

MATERIALS AND METHOD

Venous blood samples were obtained from two healthy donors. Six blood samples were collected from each donor in sterile 6ml anticoagulant-free vacutainers.

The samples were divided into two equal subsets. The first subset was the standard subset which contained only blood (6 ml). The second subset contained 5 ml of blood and 1 ml of calcium gluconate 94 mg/ml . Both subsets were immediately centrifuged by protocol (LW Portafuge E8, 1100 G) for 12 minutes at 23 degrees Celsius. The supernatant obtained after centrifugation is removed from the vacutainer and the yellow part of the fibrin gel is separated from the red part (haema thrombus) using sterile stainless steel scissors. PRF-

the separated PRF is placed on a stainless steel tray and is available for further measurements (weight, size, colour, pH) immediately. They are then placed on a sterile cotton compress and left to dehydrate naturally for 15 minutes. We measured clot sizes and calculated volumes before and after dehydration for both subgroups. Next, samples of PRF clots were prepared and embedded in paraffin and then sectioned at the microtome, followed by staining with Hematoxylin and Eosin and slide fixation for observation under a light microscope (Nikon Eclipse E800).

Macroscopic evaluations coupled with measurements show that the addition of gluconic calcium improves the volume of the PRF clot when added to the blood sample before
centrifugation.

It was observed that the gluconic calcium-treated subgroup had a lower dehydration rate (on average 64.6% of retained volume) at 15 minutes compared to the standard ones (only 24.6% of initial volume), confirming a richer fibrin structure.

The presumption that calcium which plays a role in physiological thrombus formation could promote the formation of a volumetrically richer PRF clot was confirmed by both measurements and microscopic analysis.

Architecturally, the gluconic calcium-treated PRF coagulum is better organised, generating a compact, more robust extracellular matrix with a volume that is maintained at over 60% after the dehydration process.

In the clinical phase, we evaluated which associated pathologies can interfere with the mechanism of action of plasma derivatives, what indications and contraindications these therapies have, what medication interferes with the effects of plasma derivatives. Thus, we established as eligible patients aged between 18 and 90 years, without respiratory or cardiac pathologies that generate hypoxemia, with stable nutritional status (excluding protein-calorie deficiencies of any kind), patients with haematological pathologies affecting thrombocyte status (thrombocytopenia of any kind), with chronic wounds of more than 6 months.

There is also a limit imposed by the ward management for the maximum amount of blood that can be processed (90 ml), from which about 10 PRF clots (2.5/1cm) can be obtained (about 25cm^2). An assessment of the prosthesis coverage strategy performed at

this stage was to use PRF clots spaced at maximum 1cm apart until the mesh is covered, for the PRP injection technique it was determined to inject 0.5ml per $2cm^2$ of tissue under the prosthesis (due to intimate contact with the mesh). The so-called mixed technique (PRF and PRP) attempts to "fill" the gaps between the PRF clots with infiltrated areas with 0.5ml every $2cm^2$ of subprosthetic tissue. Biohumoral status that may interfere with the course of treatment is also analysed, with no additional exclusion criteria identified. The absolute contraindication for the use of plasma derivatives remains also in this study the presence or high suspicion of malignancy, because of the risk of tumour dissemination through concentration of growth factors and circulating tumour cells.

After establishing the protocols for obtaining PRP and PRF, we proceeded to a prospective clinical study on a group of 42 patients from Colentina Clinical Hospital admitted to Surgery I and Surgery II, between 01.03.2018-30.08.2020, diagnosed with chronic surgical wounds of variable etiologies.

Study inclusion criteria:

Patients enrolled were diagnosed with the following pathologies: diabetes mellitus with ulceration, diabetic arteriopathy, non-diabetic arteriopathy, diabetic neuropathy, decubitus lesion.

It can be seen that the shrinkage rate of wounds treated with PRF (87.24%) is higher than the shrinkage rate of standard treated wounds (70.96%). This aspect is makes it necessary to discuss the difference in diameter between PRF-treated and untreated wounds in balance with the contribution of growth factors and extracellular matrix generated by PRF. Wound shrinkage is influenced by several factors, including the size of the wound and underlying tissue, the presence of infection, the immunological response to the wound, the health status of the patient, the type of dressing used and the use of wound shrinkage therapies such as platelet-rich fibrin (PRF) therapy. Given that patient health status, infection status, and dressings used in the study are similar for both the PRF-treated and standard-treated groups, the only variables remain wound size, tissue substrate, and modulation of the immune response. These elements may generate BIAS of the data obtained. According to our data, PRF therapy is effective in accelerating wound healing and contraction, as it triggers activation of cell migration and collagen synthesis and thus creates an environment conducive to lesional contraction. PRF therapy resulted in a 34.2% reduction in lesional surface area, compared to only 16.6% with conventional dressing alone. Other studies have

also shown that PRF therapy can accelerate the rate of wound contraction and provide better recovery outcomes compared with conventional dressings (12,14,19,117).

we examined how a series of chronic wounds measured with IC Measure *software* evolved following treatment with fibrin-rich plasma. This software is a versatile and powerful application for measuring lengths, areas and angles. The simple interface also provides image acquisition and enhancement functionality. Calibration of measurements is performed using a fixed reference mark in the picture, which can be a disadvantage as it is dependent on operator judgement. We measured area and perimeter at different time intervals for each wound and observed a significant reduction in wound area as well as changes in tissue type, indicating healing. The decrease in wound size is an important indicator of healing, although healing rates are not always constant. It could be observed in the wounds described that fibrin-rich plasma helped more in volume coverage, the area contracting to a lesser extent.

In addition, it has been shown that fibrin-richplasma can be successful in treating wounds that have not healed using other treatment modalities. Convenient and inexpensive, *software-based* devices help improve the accuracy of wound measurements and provide clinically relevant information that could demonstrate changes in wound size over time. To more accurately assess the results of fibrin-rich plasma treatment, volumetric assessment *software* would also be useful in the future.

Subsequently, we performed a batch analysis according to wound characteristics: colour, area, perimeter, shrinkage rate.

First, we analysed the evolution of necrotic wounds and those with infection and found that at 48 hours 33.33% of the 15 wounds were epithelialised, while at one week the percentage of epithelialised wounds was 73.33%.

Next, we compared wound progression by colour (initial, 48 hours and 1 week) in patients with a wound area <2500 mm^2 versus patients with an area >2500 mm^2 . We found that in patients with an initial wound area <2500 mm^2 , most wounds were in the inflammation (9 wounds) and granulation (6 wounds) phase and only one patient had a necrotic wound. In patients with an initial area >2500 mm^2 , most were in the inflammation phase (9 wounds) and very few wounds in the granulation phase (3 wounds). Most of the necrotic and infected wounds had an area >2500 mm^2 .

We also analysed the evolution of wound colour according to area at 48 hours and found that many of the wounds with an area <2500 mm^2 had epithelialised (16

wounds), while wounds with an area >2500 mm^2 were in the inflammation (8 wounds) and granulation (6 wounds) phase and only 6 wounds had epithelialised.

At one week, we found that most of the wounds with an area < 2500 mm^2 had epithelialized (23 wounds), only 4 wounds were still in the inflammation phase. In contrast, in patients with an area >2500 mm^2 , only 5 wounds had completely epithelialized, while 4 wounds were in the inflammation phase and the wounds were ischemic.

We then compared the shrinkage of necrotic wounds with the shrinkage of wounds in the granulation phase and observed a higher shrinkage rate in necrotic wounds (79.56%) compared to those in the granulation phase (67.56%). After removal of the scab, if well vascularized and managed, the necrotic wound shrinks faster than a wound that is red and has a more trenant evolution being blocked in the fibrin production stage not going to final epithelialization.

Study 2: ***Enhancing the integration of prostheses used in herniology based on plasma derivatives***

At this stage, we evaluated which associated pathologies may interfere majorly with the mechanism of action of plasma derivatives, what indications and contraindications these therapies have, what medication interferes with the effects of plasma derivatives. Thus, we established as eligible patients aged between 18 and 90 years, without respiratory or cardiac pathologies causing hypoxemia, with stable nutritional status (excluding those with protein-calorie deficiencies of any kind), patients with haematological pathologies affecting thrombocyte status (thrombocytopenia of any kind), with parietal defects at the abdominal wall of maximum 2.5 cm (and an estimated maximum surface area of polypropylene mesh of maximum 51 cm^2). There is also a section management limit on the maximum amount of blood that can be processed (90 ml), from which about 10 PRF clots (2.5/1cm) can be obtained (approximately 25cm^2). An evaluation of the prosthesis coverage strategy performed at this stage was to use PRF clots spaced at maximum 1 cm apart until the mesh was covered. For the PRP injection technique, it was decided to inject 0.5 ml at 2 cm^2 of tissue under the prosthesis (due to intimate contact with the mesh). *The so-called mixed technique (PRF and PRP) attempts to "fill" the gaps between the PRF clots with infiltrated areas with 0.5ml every 2cm^2 of subprosthetic tissue*. Biohumoral status that may interfere with the course of treatment is also analysed, without identifying

additional exclusion criteria. The absolute contraindication for the use of plasma derivatives remains also in this study the presence or high suspicion of malignancy, because of the risk of tumour dissemination through the concentration of growth factors and circulating tumour cells.

The in vitro planning and evaluation phase was carried out during 2017-2018, with a first phase that took place in the laboratories of the Master Biophysics Department of U.M.F. "Carol Davila" Bucharest, where measurements of the physical parameters (weight, volume, cell density, matrix architecture) of plasma derivatives (PRP and PRF) were carried out in relation to the processing protocol (harvesting technique, time from harvest to centrifugation, centrifugation protocol). We used whole venous blood samples collected from healthy volunteers (3 samples of 6 ml from each volunteer) in Vacutest separator gel-free plasma vacutainers (Kima) and processed by Alegra XR 12 centrifugation (Bechman Coulter), following the protocol of L-PRF (centrifugation 12 min at 2700 RPM), I-PRF (5 min, 60G), I-PRF (6 min, 700 RPM (200G)), A- PRF (14 min, 1300 RPM), PRF (15min, 3300 RPM). After separation of PRF clots, weight was determined using a digital analytical balance, volume by compressing the clot into 10 g graduated cylinders and microscopic architecture using the inverted microscope (Axiovision) with 63x magnification.

For PRP evaluation we used samples collected from healthy volunteers - 2 x 4.5 ml vacutainers with 0.109M sodium citrate (BD Vacutainer)/collection which were processed by centrifugation in Alegra XR 12 centrifuge (Bechman Coulter) according to the following protocols: PRP1 (5min at 300G), PRP2 (8 min at 240G), PRP3 (17min at 700G), PRP4 (12 min, 2000G), PRP5 (15min, 250G), PRP6 (7 min at 3300 RPM). After the first centrifugation, transfer with 21G long needle and 5ml syringe the separated plasma obtained into the 6ml Vacutest re-suspension tube (Kima). Re-refuge 5ml PRP samples at 2,500 × g for 7 minutes and separate the concentrated PRP in the 2.7ml 0.109M sodium citrate (BD Vacutainer) tube. The number of platelets in whole blood and PRP samples was determined by automated analyzer. We also evaluated the appearance of PRP-treated PP prostheses on the surface in terms of cellularity at 12 hours. Platelet counts were performed on 1 mm^2 using the same inverted microscope using gentian violet staining.

The second phase involved collaboration with biomaterials and polymers specialists from the Department of Bioresources and Polymer Science of the Faculty of Chemical Engineering and Biotechnologies at Politehnica University of Bucharest, where we

validated the hypothesis that plasma derivatives contribute to faster tissue coverage of polypropylene prostheses through electron microscopy images of PRP-treated mesh samples in murine fibroblast cell cultures.

This analysis in practice supports the theory of integration of prostheses used in herniology based on plasma derivatives - i.e., in the case of PRP for each protocol used, compared to the standard definition of PRP, between 11.24% and 53% more platelets/probe used, and in the case of PRF, PRF, it was found that macroscopically increased fibrin density, i.e., rich cellular component, was observed. Also, at 20x magnification of the PRF clot architecture in hematoxylin-eosin (HE) staining, a richer layer of platelets and a denser fibrillar network (in which leukocytes are also found) is observed at the edge of the clot.

In modern surgical practice it would be useful to apply because the benefits are clearly superior, and the tissue repair, cell matrix support components, as well as the decrease of the inflammatory response generating a tissue incorporation of the prosthesis that can perform the functions in a physiological way, are observed from the first applications/interventions. More interventions are needed in this respect - in order to generate conclusions with high statistical power. Patients who benefit from this therapeutic strategy - complex, personalised and without additional risks, but effective and with visible and rapid results - have a very low risk of adverse effects precisely because of the autologous material.

Both PRP and PRF have proven to be useful and cost-effective for integrating hernia prostheses into tissues. We assume that the positive effect of PRP and PRF on the healing process and the potential to reduce the risk of infection and minimise scarring works similarly for chronic wounds and hernia prostheses. In addition, PRP and PRF can be used to promote angiogenesis, which is the formation of new blood vessels, and can help reduce inflammation.

The *in vivo* study was carried out between 2017-2021 at Colentina Clinical Hospital, General Surgery Clinical Department. From a total of 329 patients with abdominal parietal defects, 270 patients with parietal defects were selected were inguinal, umbilical or small eventrations below 2.5cm of which 42 patients with parietal defects less than 2.5cm (inguinal hernias, umbilical and eventrations) eligible for plasma derivative therapy (26 PRF, 8 PRP, 4 PRP and PRF all with inguinal hernias - 2 PRF therapies for umbilical hernias - 2 PRF therapies for medial trocar eventrations) who also received augmentation

procedures. To simplify the evaluation, we only analysed inguinal parietal defects. Thus, we have a study group of 216 patients with inguinal hernias. Gender distribution male : female →193:23, mean age 61 years. Of these, 191 patients enrolled underwent polypropylene prosthesis fitting surgery in supraaponeurotic (*on-lay*) manner and 25 patients operated laparoscopically in TAPP manner.

Available polypropylene (PP) prostheses (HERNI PRO, type P3,P4 Biosintex, Paha mesh - POLYPROPYLENE MESH and DiproMed model Evolution) anchored with separate polypropylene sutures were used in this study due to its good tensile strength, good tissue integration and low risk of infection [1].

Plasma derivatives were processed intraoperatively from fresh venous blood using Portafuge model E8 centrifuge at 3300rpm and used immediately after obtaining. For PRP we used 4.5 ml citrate vacutainers (BD Vacutainer) and for PRF, 9 ml Clot activator vacutainers (Vacutest Kima). After the mesh was fixed with sutures, we either applied the PRF to the mesh surface using multifilament, synthetic, polyglycolic acid absorbable surgical suture, size 0 (Bicril, BioSintex), or infiltrated the tissues coming in close contact with the mesh with uniformly distributed PRP.

From the description of the 2 groups (standard treatment and PRP), we note that the populations are very similar in terms of mean age (61 years versus 62 years) and gender distribution (11% female versus 7.9%).

Post-surgical healing of inguinal hernia in patients with cardiac pathology may be adversely affected due to the risk of complications associated with the potential for cardiac arrest. Patients with diabetes may require more cautious surgical approaches because of the risk of impaired wound healing and an increased risk of postoperative infection. Patients with visceral adhesions may require more extensive surgery, but depending on the size and severity of the adhesions, hernia surgery may be prolonged by additional time. Obesity is a risk factor for hernia development and may increase the risk of postoperative complications. Chronic viral hepatitis B can interfere with the healing process after hernia surgery, requiring prolonged periods of close monitoring and additional supportive care. The study group shows a similar distribution to the general population so there should be no significant differences between the developmental patterns analysed.

In terms of length of stay, a fairly large change can be observed of about 14% more discharges below average and 17% fewer discharges above average. In order to biostatistically assess whether the pain level on the NRS at 2, 4, 12 weeks after surgery would decrease by compared to standard therapy at 2, 4, 12 weeks, a statistical test should be performed. Specifically, a two-sample t-test could be used to assess whether mean NRS scores at 2, 4, 12 after surgery are significantly different from mean NRS scores observed with standard therapy at 2, 4, 12 weeks. The null hypothesis would be that mean NRS scores with the intervention are not significantly different from NRS scores observed with standard therapy. The alternative hypothesis would be that mean NRS scores with augmentation are significantly lower than NRS scores observed with standard therapy. The p-value from this analysis should be used to determine whether the observed change in NRS scores is statistically significant or not. This assessment on such a small sample cannot generate statistical value so the study will need to be extended to establish the statistical power of the inferred correlations.

The limitations of the present experimental study were the limited amount of whole blood that could be processed and the lack of an anatomopathological evaluation - which for ethical reasons can only be done in patients with complications that require partial or complete prosthesis removal or explantation.

9. Conclusions and personal contributions

As it is not about associations or correlations between variables, but about the evaluation and application of autologous treatments on chronic wounds, this paper focuses on demonstrating a technical principle. I believe that this surgical technique at the borderline of several sciences lends itself to several clinical studies, as well as their introduction into current practice - being the use of materials of autologous nature that have behind them multiple studies attesting to their indisputable role in healing with minimal possibility of developing adverse reactions.

The objectives of the thesis were successfully achieved, so that we were able to safely use plasma derivatives whose preparation we were able to optimize according to therapeutic needs. No adverse reactions of PRP or PRF were encountered throughout the study. If in the field of chronic wound healing PRP and PRF are increasingly making their presence felt in studies, in the field of prosthetic integration in herniology it is a pioneer that attests to the increased integration capacity in patients treated with these derivatives. In particular, PRF is the derivative of choice in both soft tissue defect coverage and herniorrhaphy mesh integration. It, due to the fact that it also comes with the structural support on which to proliferate the neoformation tissue, not just the growth factor amalgam, and significantly accelerates the healing/integration processes.

PRF is a promising treatment option for chronic wound healing with excellent patient outcomes. These plasma derivatives are natural biological healing products obtained by processing whole blood by centrifugation, which have been extensively studied in the medical community and have demonstrated the potential to accelerate healing and tissue regeneration. PRF's clotting properties stop bleeding and enhance healing, while providing a structural component to the wound, and promoting the migration of cells needed for wound repair. In addition, PRF can protect the wound from infection, regenerate tissue and reduce swelling and pain caused by surgery. It has also been shown to reduce the time it takes for wounds to heal, eliminating the need for additional medical care in some cases. On the other hand, PRF has some potential drawbacks: it has risks of exacerbated inflammatory reactions (especially L-PRF, through its leukocyte component), especially if the patient has an exacerbated inflammatory status. These phenomena subside within 24-48 hours of therapy.

The major advantage in both situations is the cost-effectiveness of this therapy. In the case of chronic wounds, plasma derivatives not only save money through rapid healing time compared to dressings, but also compared to the costs of plastic surgery (creating flaps or grafts). While recreating a biological wound barrier through surgical techniques requires at least a few days' hospitalisation and the use of an operating theatre, grafting of PRF clots only requires a small operating theatre or a dressing room, and most of the time the procedure is performed under local anaesthesia. The only device dedicated to this therapy is the centrifuge. Thus, the use of plasma derivatives can be achieved easily, without major technical costs, with a small learning curve that is easy to follow and with results superior to current standard therapies.

The disadvantages of the technique are that it requires a trained person to prepare the plasma derivatives, as the surgery and the preparation of PRP or PRF cannot be done at the same time. Another possible disadvantage is the very short working time of the blood sample and the fact that PRF or PRP samples cannot be stored for later use. In cancer patients with chronic wounds, the use of plasma derivatives is prohibited due to the high risk of tumour dissemination.

It should be investigated in the future whether blood samples taken to treat cancer patients cannot be 'filtered' by circulating tumour cells and whether the local presence of growth factors also causes growth of adjacent tumour tissue, given that healing and oncogenesis share many common steps.

One problem that remains unresolved is how to generate enough to treat larger wounds and parietal defects in a single session. Personally, I am extending research beyond this thesis by attempting to fix autologously derived liquid growth factors onto synthetic extracellular matrix substrates such as collagen haemostatic membranes or collagen sponges used for haemostatic purposes in surgery.

Quantification of healing speed and tissue quality needs to be quantified by further research with greater statistical power. This project started as an experimental study that has been fed by very good results to become a descriptive study of modern techniques with new insights. Complex statistically significant batch studies are needed from which therapeutic efficacy can be calculated. Continued widespread application of PRP and PRF therapy in herniology as a standard procedure could lead to a significant decrease in the failure rate of synthetic prosthesis repairs.

Our own contribution is to find ways to obtain quantitatively and qualitatively better PRF clots than those described in the literature and to use plasma derivatives in the foreign body integration process based on the premises of physiological healing.

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