Ref: Ro J Stomatol. 2024;70(4)
DOI: 10.37897/RJS.2024.4.12

Effectiveness of autologous mesoconcentrate (PRGF) in clinical dentistry: electron microscopic analysis

Anatolii Pavlovych Oshurko[1], Ihor Yuriiovych Oliinyk[2], Nataliia Bohdanivna Kuzniak[1]

[1]Department of Surgical Dentistry and Maxillofacial Surgery of Bukovinian State Medical University, Chernivtsi, Ukraine
[2]Department of Pathological Anatomy of Bukovinian State Medical University, Chernivtsi, Ukraine

Anatolii Pavlovych Oshurko **ORCID ID:** 0000-0002-3838-2206
Ihor Yuriiovych Oliinyk **ORCID ID:** 0000-0002-6221-8078
Nataliia Bohdanivna Kuzniak **ORCID ID:** 0000-0002-4020-7597

ABSTRACT

This paper provides a scientific assessment of a widely implemented technology in regenerative medicine, particularly clinical dentistry, subject to protocol compliance with the method of ordering fibrin filaments, which ensures the formation of a three-dimensional structure that serves as a biological scaffold with multifunctional properties. The preservation of autologous genetic information with full stimulating content, with a spectrum of active trophic and repair factors – growth factors, in particular, of platelet origin, provides a reliable prognosis of "controlled" tissue regeneration.

The aim of the study is to substantiate the effectiveness of the use of autologous plasma products – plasma rich in growth factors (PRGF) in clinical dentistry, and their interfractional differentiation according to the results of electron microscopic analysis.

We have studied the density of formed fibrin fibers in terms of the number (in 10 μm^2) and morphometric value of the cross-section (Ø) in the PRGF - F1 and F2 fractions of mesoconcentrate products, namely, insulating membranes (M) and wrapping blocks (B), using electron transmission microscopy. For evidentiary analytical substantiation, we performed scanning electron microscopy of fibrin strands, which provided high-resolution images of sample surfaces without destroying their architectonics.

The obtained results confirm the working hypothesis, i.e., the acceptable effectiveness of the study based on statistical analysis. This highlights the reliability of the results with the appropriate level of significance in the interfraction difference of PRGF F1-M, B and F2-M, B, with a description of the interrelated diametric differences.

This analysis of the accumulation and quality of fibrin filament formation based on the results of electron microscopic examination, comprehensively confirmed by scanning electron microscopy and in the above fragment of clinical application, gives priority to the PRGF method with targeted fractional use (F1, F2) of mesoconcentrate products for targeted tissue regeneration, in particular in clinical dentistry according to its extensive indications.

Keywords: plasma rich in growth factors, fibrin, atrophy, bone tissue, mandible, human

INTRODUCTION

The use of blood plasma in the medical industry and practice has not only made geographical progress but also significantly expanded the indications for therapeutic and prophylactic treatment [1,2]. Over the past decade, a large number of scientific studies of cell separation have been published [3,4], and the results of clinical trials using cellular technologies [5]. There is a growing demand for clinical selectivity not only in terms of methods and techniques [6-8] but also in terms of their ergonomics and controllability at the stages of patient rehabilitation.

Corresponding author:
Ihor Oliinyk
E-mail: olijnyk1961@gmail.com

Article History:
Received: 27 May 2024
Accepted: 16 December 2024

However, one of the most topical issues in the use of autologous plasma by the Endoret-PRGF method is the guarantee of maintaining the genetic stability of cells even outside the body [9]. That is, obtaining platelet-derived growth factors, which are dimeric glycoproteins, from the patient's venous blood by centrifugation. Moreover, the plasma with platelets is separated from other blood cells with a low leukocyte content, which distinguishes it from the PRF (Platelet Rich Fibrin) technique.

To ensure the predicted results of stem cell transplantation [10], the scientific vector is focused not only on growth factors but also on a fibrin fiber-based matrix with preservation of natural blood clotting processes, which is due to the conversion of soluble fibrinogen into an irreversible fibrin gel, of which platelet-rich plasma is and remains an integral part [11,12].

Such bioconstructions based on fibrin fibers are increasingly used in regenerative medicine as a connective framework for restoring the volume of hard tissue. After all, the fibrin matrix not only has a three-dimensional structure with a spectrum of active trophic and repair factors but also retains the ability to create specific cytoarchitectonics that can be easily modeled in preclinical and clinical positioning, depending on the request of the operating field. Therefore, this study will provide an understanding of the differentiation of mesoconcentrate products by fibrin fiber diameter and density for their clinical use, the formation of a matrix base with the preservation of inter-fibrin spaces for continuous processes of trophic support, and constant physiological remodeling.

AIM

To substantiate the effectiveness of the use of autologous mesoconcentrate products - plasma rich in growth factors (PRGF) in odontological practice in the treatment of bone atrophy caused by the loss of the masticatory group of the dentition, based on the results of electron microscopy.

MATERIALS AND METHODS

The material was collected during planned dental operations using plasma for controlled tissue regeneration. The study subjects were divided into three groups according to the age of the patients, namely: group I - 25-45 years, n = 10; group II - 46-60 years, n = 10; group III - 61-75 years, n = 10.

The preparation of autologous plasma products was based on compliance with the protocol of the Endoret - PRGF BTI technique, Spain, which we describe in our previous research work [13], with a detailed protocol for the collection and formation of autologous cell grafts, and by the terms of the signed international agreement on scientific cooperation under No. 12-05/10 of 17.11.2021, on writing a doctoral dissertation on the topic: "Substantiation of rehabilitation of patients with bone atrophy complicated by topographic and anatomical features of the mandibular canal".

The study of the order of fibrin filaments in 10 μm2 and their transverse size (Ø) on electron micrographs was carried out using the optional morphometry of the transmission electron microscopy method, using the transmission electron microscope PEM-100-01 at magnifications from ×1000 to ×10000, which generated electron beams passing through extremely thin samples of the material under study, which facilitated a preliminary visual assessment of the fractional quality of PRGF - F1 and F2 plasma products - insulating membranes (M) and obturating blocks (B).

Scanning electron microscopy of the fibrin strands was performed, preserving at all times the architecture of the sample and the electrical conductivity.

To obtain micrographs of the fibrin strands under study, the samples were fixed in a 1.5% glutaraldehyde solution and then washed three times with distilled water. After rinsing, the stage of procedural freeze-drying was carried out: the wet samples were frozen and then placed in a vacuum chamber, where moisture was sublimated from the frozen tissue samples. Thanks to this drying process (1.5-2 hours), the tissue did not lose its volume and no strands clumped together. The dried tissue samples were glued to cylindrical copper tables with subsequent metallization of the tissue surfaces by thermal spraying of a thin copper layer (up to 20 nm). Sputtering was carried out in a VUP-5 sputterer.

The experimental part of electron microscopic studies was carried out on a scanning electron microscope JEOL T220A.

A magnification of ×5000-10000 was used to scan the surface of the samples, and the accelerating voltage in all experiments was 20 kV.

In the statistical analysis, first of all, the mean morphometric values (M) and the error of the mean number (±m) of plasma products in both fractions were determined, the values of which are presented in the table below.

Taking into account the fact that the sample differs from the normal distribution, nonparametric methods, in particular, the Mann-Whitney U test, were chosen to compare the quantitative values of interfractional plasma products, with parallel analytical substantiation of the data in the form of the median (Me) and interquartile range (IQR).

Protocol No. 2 of 21.10.2021 of the Commission on Biomedical Ethics of Bukovinian State Medical University (Chernivtsi, Ukraine) stipulated and approved the regulatory bioethical standards of the

planned research work, which was conducted after patients read and signed an informed consent to participate in research in compliance with the main provisions of the GCP (1996), the Council of Europe Convention on Human Rights and Biomedicine (04/04/1997), the World Medical Association Declaration of Helsinki for the Ethical Principles of Scientific Medical Research Involving Human Subjects (1964-2013), Order of the Ministry of Health of Ukraine No. 690 of 23.09.2009 (as amended by Order of the Ministry of Health of Ukraine No. 190 of 31.01.2023).

RESULTS

The proper ordering of fibrin strands serves not only as a biological framework with multifunctional properties but also contains all genetic information (Figure 1) with a full-fledged stimulating content - growth factors, in particular, of platelet origin.

The proven effectiveness of clinical use of mesoconcentrate products, namely isolation membranes, which are widely used in targeted tissue regeneration - closing bone augmentation, burn zones, in particular, maxillofacial area, after surgical treatment of neurovascular structures in areas of their exposure, etc. and obturating blocks - to close oroantral connections, compensation for lost tissue, for its intended purpose, confirms its biological effectiveness according to the results of this work. To scientifically substantiate the results of the study, with their subsequent application in clinical dentistry, we conducted a detailed analysis of the density (accumulation) of fibrin filaments in 10 μm^2 and their diameter (Figure 2), which is ensured by careful adherence to protocols at all clinical and laboratory stages and excludes study subjects who, according to diagnostic screening, are carriers of chronic diseases, including blood diseases.

The results of the statistical analysis presented in (Table 1) cannot confirm patterns of change in quantitative or qualitative characteristics depending on age. The high density of fibrin clot formation, which

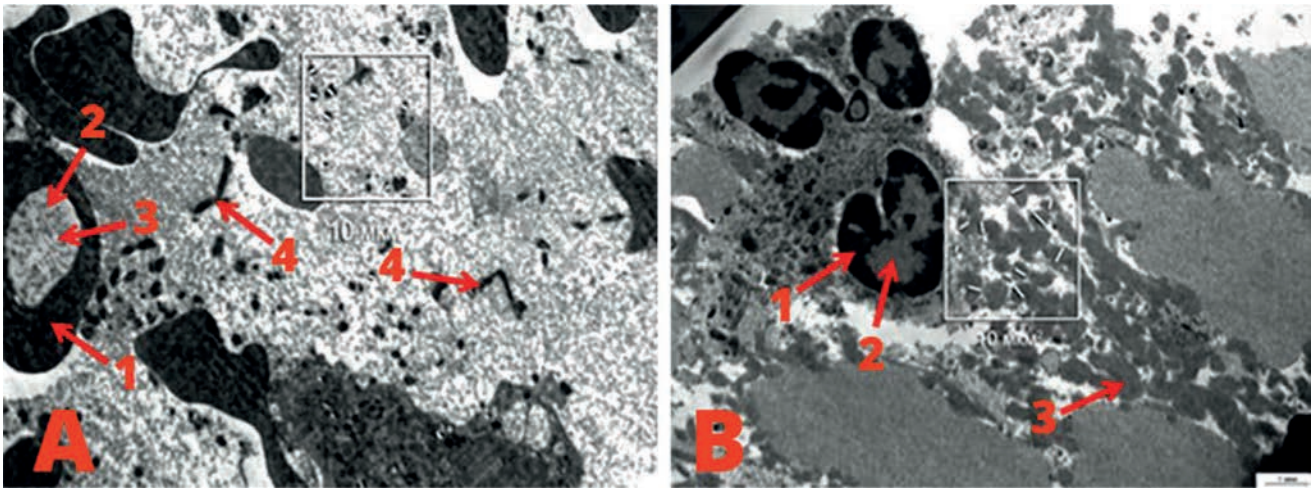

FIGURE 1. Electronogram, ×5000. A) Fibroblasts at different stages of their maturity: 1. Cytoplasm; 2. Nucleus; 3. Nucleolus; 4. Single fibrin strands. B) Fibroblasts at the stage of fibrin fiber formation: 1. Cytoplasm; 2. Nucleus - destruction of the carioma; 3. Formed fibrin fibers

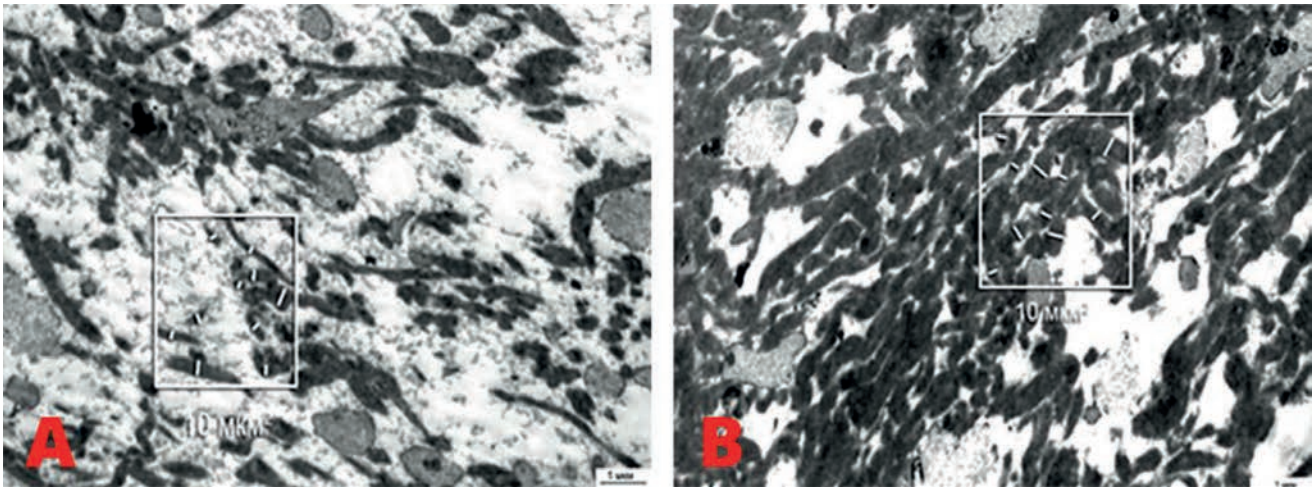

FIGURE 2. Electronogram, ×5000. Investigation of fibrin density and fiber diameter in mesoconcentrate products: A) F2-M; B) F2-B

is most saturated with growth factors, in the first group (25-45 years), with values of 50 units per 10 μm2 in the F2 fraction, was investigated and emphasized by analytical visualization in Figure 2, B. However, the indicated fractional density of fibrin fibers decreases in the third group of the study (≥61 years) to 29 per 10 μm2, outlining the rates of blood aggregation and biological possibilities of separation during centrifugation in these individuals. High orderliness was studied in the second group (individuals aged 46-60 years), where the occurrence of fibrin fibers is 36 per 10 μm2, which characterizes the corresponding reparative and regenerative tissue capabilities of the human body.

Realizing that the mechanisms of fibrin fiber formation occurred outside the body and the phases of formation of its protofibrils into fibrils were accelerated as a result of controlled rotational lateral-horizontal aggregation, but ensuring the completeness of the proper conditions for predicting the preservation of quantitative and qualitative characteristics in their fractional distribution remains an important preclinical stage in controlled tissue regeneration. It is impossible to achieve such results of the formation of a typical fibrin fiber structure when the formation processes occur in the human body in such a way that the phases proceed on the surface or in the middle of the platelet clot, which prevents the association of protofibrils due to their limited mobility. Fibers form chaotically connected thin strands, with rupture and overlap of subordinate fibrillar structures, forming wide interfibrillar spaces, or lack thereof.

Investigating the diameter (Ø) of a fibrin fiber as a morpho-histological structure, the formation of which depends on the multifactorial influence of the biological environment on its tissue reactions, their balance, and variability, we present an analysis with the values of the median number (Me) and interquartile range (IQR), which gives an idea of the variant formation.

In the first group, the Me value of the F1 fraction is 0.19 μm with IQR (0.18, 0.20) - F1-M and IQR (0.17, 0.21) - F2-B. The diameter decreases in the PRGF fraction (F2), where the fibers forming the membrane are Me (IQR) = 0.14 (0.11, 0.18) μm, and the wrapping block is Me (IQR) = 0.16 (0.15, 0.19) μm. However, when testing the hypothesis, the p-value did not confirm the high reliability of the results (see Table 1).

The ordering of fibrin fibers in the fractionally formed mesoconcentrate products is the same in terms of the average diameter and is Me = 0.22 μm with an interquartile range of 0.20-0.25 μm in the second group of the study, except for the diameter in the F2-M fraction, which decreases by 0.4 μm, where Me (IQR) = 0.18 (0.16, 0.18) μm.

The third group of the study (patients aged ≥ 61 years old) is represented by a gradual interfractional difference in the formed plasma products. The PRGF - F1 fraction has a fibrin fiber diameter in the formed membrane Me = 0.20 μm with an interquartile range (0.18, 0.29) μm, however, their volume increases by 0.14 μm in the formed block (clot) and acquires a value of Me (IQR) = 0.34 (0.32, 0.39) μm. In PRGF - F2 fractions, the diameter of fibrin fibers in both mesoconcentrate products decreases and is 0.18 μm, IQR (0.17, 0.21) μm for F2-M = Me and 0.25 μm, IQR (0.20, 0.34) μm for F2-B = Me.

Like every researcher, in this paper, we set out to

TABLE 1. Comparison of the density and diameter of fibrin fibers formed from insulating membranes (M) and wrapping blocks (B) of human mesoconcentrate (blood plasma), n = 30, obtained by the Endoret - PRGF method (Human Technology, BTI, Spain)

Research groups	Research parameters	PRGF (F1-M)	PRGF (F1-B)	PRGF (F2-M)	PRGF (F2-B)
I - the first group (25-45 years old), n=10	Density, number in μm²	24.00	25.00	18.00	50
	Me (IQR)	0.19 (0.18, 0.20)	0.19 (0.17, 0.21)	0.14 (0.11, 0.18)	0.16 (0.15, 0.19)
	M, ±m	0.19±0.02	0.18±0.04	0.14±0.04	0.18±0.05
	p (reliability of the result)	0.791		0.131	
II - the second group (46-60 years old), n=10	Density, number in μm²	16.00	22.00	24.00	36.00
	Me (IQR)	0.22 (0.20, 0.24)	0.22 (0.21, 0.23)	0.18 (0.16, 0.18)	0.22 (0.20, 0.25)
	M, ±m	0.23±0.04	0.23±0.04	0.17±0.04	0.21±0.05
	p (reliability of the result)	0.970		0.035*	
III - the third group ≥ 61 years old, n=10	Density, number in μm²	16.00	29.00	18.00	29.00
	Me (IQR)	0.20 (0.18, 0.29)	0.34 (0.32, 0.39)	0.18 (0.17, 0.21)	0.25 (0.20, 0.34)
	M, ±m	0.23±0.11	0.36±0.10	0.20±0.07	0.27±0.08
	p (reliability of the result)	0, 019*		0,024*	

Note*: significance level $p < 0.05$

confirm the working hypothesis, i.e., the acceptable results of the study. Statistical analysis (see Table) confirmed the high reliability of the results with a significance level of $p < 0.05$ in the third group of the study, where in the fractional comparison between PRGF F1-M and F1-B, $p = 0.019$ with a slightly lower level of $p = 0.024$ in the fractional comparison between PRGF F2-M and F2-B, which describes the significance of the diametric differences that are interconnected (Figure 3, Figure 4).

The results of the study have been clinically tested [9] not only for the preparation and interfractional distribution (Figure 5) but also for the use of mesoconcentrate products in the form of insulating fibrin membranes (F1-M), to cover bone allograft or postimplantation site, a combination of fibrin membranes (F2-M) and fibrin wrapping blocks/coils (F2-B) to fill and isolate sequestral or resection, osteotomy cavities (Figure 6), etc. Below we present a practical application in a clinical setting to emphasize the importance of these principles in promoting tissue integration, healing, and full biological regeneration.

The analysis of the density and diameter of fibrin fibers based on the results of electron microscopic examination, comprehensively confirmed by scanning electron microscopy (see Figure 3, Figure 4) and in the above fragment of clinical application (see Figure 6), gives priority to the PRGF method with targeted fractional use (F1, F2) of mesoconcentrate products for targeted tissue regeneration, in particular in clinical dentistry according to its extensive indications.

DISCUSSIONS

The clinical rationale is indisputable regarding the need for fractional distribution of autologous mesoconcentrate, followed by the formation of fibrin membranes that will primarily perform a barrier function and fibrin clots (blocks) with high platelet concentration, which are used for controlled tissue regeneration.

A randomized and controlled clinical trial of bone healing to preserve the cancellous process using xenografts and allografts compared with the use of plasma-rich growth factors (PRGF), conducted by Stumbras A, et al. [14], emphasizes the greatest formation of new mineralized tissue in the PRGF group according to their histomorphometric analysis.

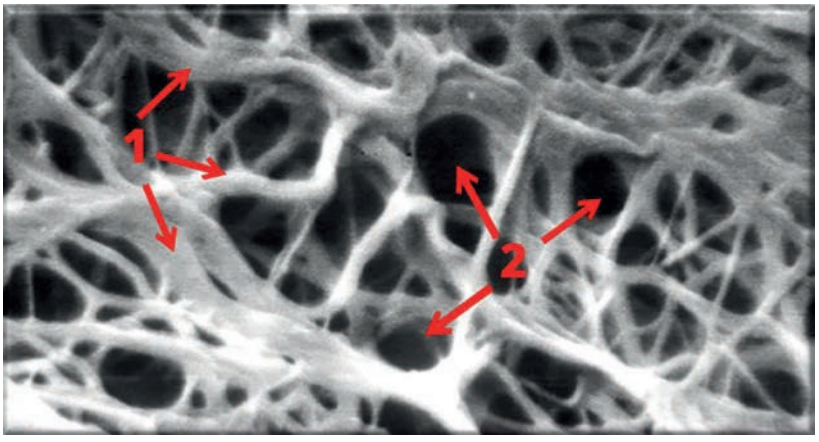

FIGURE 3. Scanning electronogram, ×10000. Study of the autologous fibrin membrane, according to the fractional distribution of F2 Endoret - PRGF (Human Technology, BTI, Spain): 1. Fibrin fibers; 2. Interfibrine space

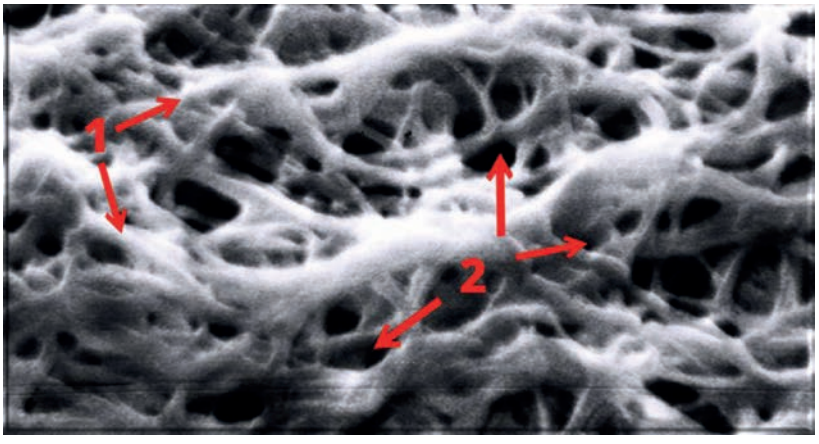

FIGURE 4. Scanning electronogram, ×10000. Investigation of an autologous fibrin clot, according to the fractional distribution of F2 Endoret - PRGF (Human Technology, BTI, Spain): 1. Bonding of fibrin fibers; 2. Interfibrine space

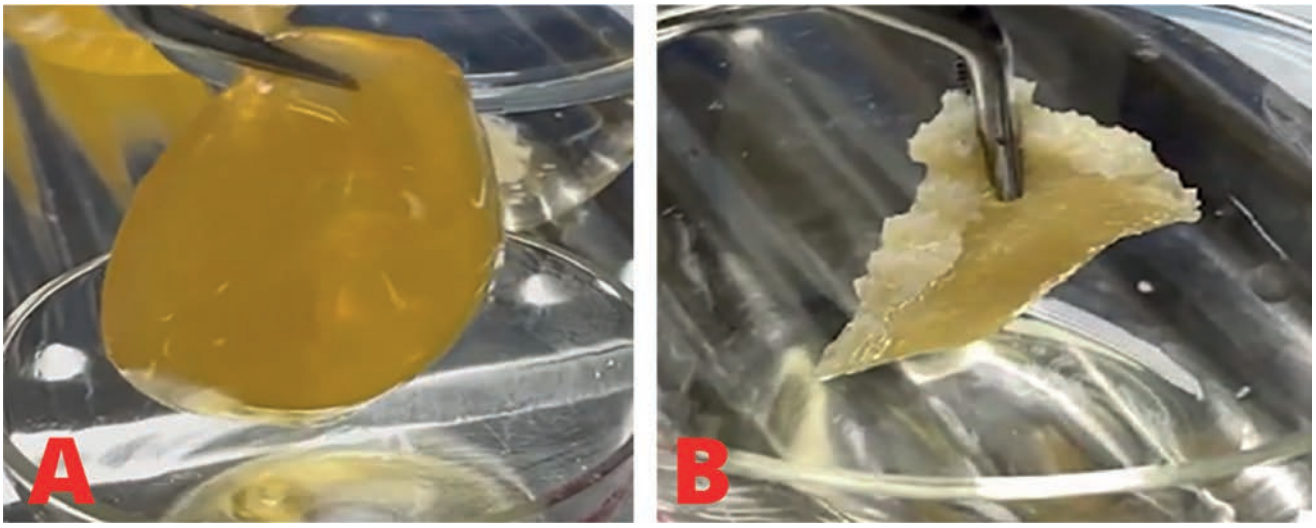

FIGURE 5. A) Fibrin clot formed from fraction F1 Endoret - PRGF for the preparation of an insulating membrane; B) Bone allograft formed using fractional plasma F2 Endoret - PRGF

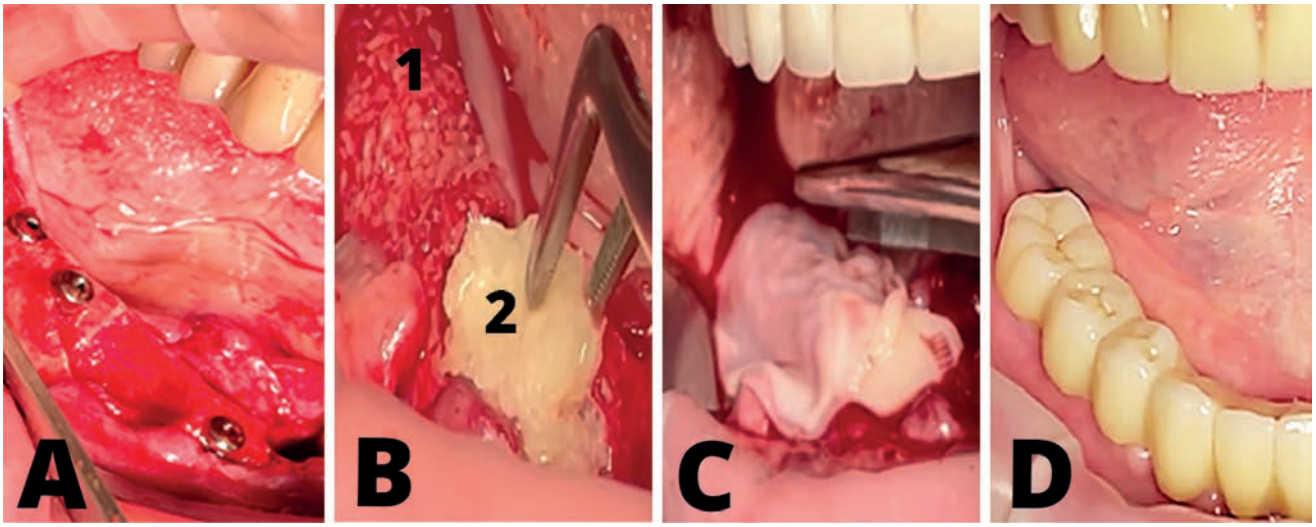

FIGURE 6. Stages of rehabilitation of an edentulous patient with mixed bone atrophy of the mandible complicated by sequestral destructive changes: A) Postimplantation and post-sequestrectomy surgical area on the right side of the mandible; B) 1. Restoration of the volume of the atrophied cellular part and body of the mandible in the vertical and horizontal directions using bone allograft enriched with automeso-concentrate. 2. Closure of the post-extraction cavity using obturating blocks of fractional plasma F2 Endoret - PRGF; C) Application of an insulating membrane formed from fractional autoplasma F1 Endoret - PRGF to block epithelial proliferation, the influence of unprogrammed resorptive processes and preservation of post-extraction bone volume; E) The final result of the patient's comprehensive rehabilitation with the restoration of chewing efficiency and functionality of the mandibular dentition on the right side

A systematic review was conducted to investigate the histomorphometric changes that occur during controlled cellular preservation using autologous plasma of different concentrations, in particular, the ARP technique, performed with fibrin, platelet- and leukocyte-rich fibrin (L-PRF) and pure platelet-rich plasma (P-PRP), the authors Caponio VCA, et al.[15] confirmed their effectiveness in the formation of new bone, however, they did not find significant differences in the fractional use.

Anitua E, et al. [16] argue that such randomized clinical trials aimed at evaluating the use of leukocyte-rich plasma (L-PRP) or pure platelet-rich plasma (P-PRP) for bone regeneration and/or preservation of cancellous bone also do not provide adequate clarification of the effectiveness of these techniques.

Excellent results of clinical treatment are reported in DuVal M, et al. [17], using plasma rich in growth factors according to the PRGF method, with complete regeneration of not only soft tissue but also bone tissue to its pre-pathological state and subsequent successful implant rehabilitation in the same area of the edentulous jaw. PRGF has been proven to be an effective technique in minimizing or eliminating bone deformities that are usually associated with conventional treatment or post-sequestration intervention.

Many claimed techniques implemented in dental practice, which have a single goal - controlled tissue regeneration, are based on the use of autologous platelet plasma, including extracellular vesicle-rich plasma (PVRP), which, according to Troha K, et al. [18], do not have a prediction of a clear tissue organization, which distinguishes them from the technique presented by us.

We are aware of sophisticated cell separation techniques, but, according to Bastian F, et al. [19], they are aimed only at selecting cells of a certain series that have no or limited further clinical and biological application.

In the study, the authors Alavi SE, et al. [20] confirm that all surface changes used to restore lost volume due to atrophic or inflammatory processes of bone tissue are critical to mimic the natural structure of bone, not only to preserve but also to improve cellular responses. In addition, the interaction between biosensitive scaffolds, growth factors, immune cells, and stromal progenitor cells is essential to promote the stability of bone regeneration. After all, diffuse trophic nutrition from the periosteum can be significantly reduced by the use of artificial "semi-deaf" insulating membranes and their fixing elements, which lead to corticalization of the surrounding trabecular tissues.

A significant advantage of mesoconcentrate products is their autologous nature, which excludes a certain range of preclinical tests and immunoconflict issues, and, no less important, the norms of medical law and bioethical regulation.

Since we did not find any similar works in the bibliometric analysis, we believe that the prospects for further research will be aimed at discussing the results of clinical trials using forms (products) of autologous mesoconcentrate in targeted tissue regeneration.

CONCLUSIONS

The ordering of fibrin fibers in the fractionally formed mesoconcentrate products differs according to the results of intergroup analysis and by the average diameter and density.

The high reliability of the results was established in the third group of the study, where in the fractional comparison between PRGF F1-M and F1-B the value of p=0.019, with a slightly lower level of p=0.024 in the fractional comparison between PRGF F2-M and F2-B, describes the statistical significance of the diametric differences that are interrelated.

The presented rationale is indisputable regarding the need for fractional distribution of autologous mesoconcentrate, followed by the formation of fibrin membranes that will primarily perform a barrier function and fibrin clots (blocks) used for controlled tissue regeneration.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflict of interest: The authors declare that they have no conflict of interest.

Financial support: This research received no external funding.

Author's contributions:
Oshurko A.P.: Work concept and design, Data collection and analysis, Critical review, Final approval of the article
Oliinyk I.Y.: Work concept and design, Critical review, Final approval of the article
Kuzniak N.B.: Writing the article, Critical review, Final approval of the article

REFERENCES

1. Raeissadat SA, Ghazi Hosseini P, Bahrami MH, Salman Roghani R, Fathi M, Gharooee Ahangar A, et al. The comparison effects of intra-articular injection of Platelet Rich Plasma (PRP), Plasma Rich in Growth Factor (PRGF), Hyaluronic Acid (HA), and ozone in knee osteoarthritis; a one-year randomized clinical trial. *BMC Musculoskelet Disord.* 2021;22(1):134. https://doi.org/10.1186/s12891-021-04017-x. PMID: 33536010; PMCID: PMC7860007.
2. Latimer JM, Maekawa S, Yao Y, Wu DT, Chen M, Giannobile WV. Regenerative Medicine Technologies to Treat Dental, Oral, and Craniofacial Defects. *Front Bioeng Biotechnol.* 2021;9:704048. https://doi.org/10.3389/fbioe.2021.704048
3. Tripathi H, Peng H, Donahue R, Chelvarajan L, Gottipati A, Levitan B, et al. Isolation Methods for Human CD34 Subsets Using Fluorescent and Magnetic Activated Cell Sorting: An In Vivo Comparative Study. *Stem Cell Rev Rep.* 2020;16:413-23. https://doi.org/10.1007/s12015-019-09939-7. PMID: 31953639 PMCID: PMC7469922.
4. 4. Huang Z, He D, Li HW. A fluorometric assay of thrombin using magnetic nanoparticles and enzyme-free hybridization chain reaction. *Mikrochim Acta.* 2020;187(5):295. https://doi.org/10.1007/s00604-020-04279-7. PMID: 32347383.
5. Wu V, Klein-Nulend J, Bravenboer N, ten Bruggenkate CM, Helder MN, Schulten EAJM. Long-Term Safety of Bone Regeneration Using Autologous Stromal Vascular Fraction and Calcium Phosphate Ceramics: A 10-Year Prospective Cohort Study. *Stem Cells Translational Medicine.* 2023;12(9):617-30. https://doi.org/10.1093/stcltm/szad045
6. Rezazadeh H, Okhovatfard M. Effect of Platelet Rich Plasma in Regenerative Endodontic Treatment: A Review of Clinical Trials. *Research Journal of Pharmacy and Technology.* 2023;16(11):5562-6. https://doi.org/10.52711/0974-360X.2023.00899
7. Sheean AJ, Anz AW, Bradley JP. Platelet-Rich Plasma: Fundamentals and Clinical Applications. *Arthroscopy.* 2021 Sep;37(9):2732-4. https://doi.org/10.1016/j.arthro.2021.07.003. PMID: 34481615.
8. Arai T, Shiga Y, Mukai M, Takayama N, Tashiro S, Tajiri I, et al. Osteogenic effects and safety of human induced pluripotent stem cell-derived megakaryocytes and platelets produced on a clinical scale. *Regen Ther.* 2024;26:850-8. https://doi.org/10.1016/j.reth.2024.09.012. PMID: 39430581; PMCID: PMC11488481.
9. Anitua E, Fuente Mdl, Troya M, Zalduendo M, Alkhraisat MH. Autologous Platelet Rich Plasma (PRGF) Preserves the Genomic Stability of Gingival Fibroblasts and Alveolar Osteoblasts after Long-Term Cell

Culture. *Dentistry Journal*. 2022;10(9):173. https://doi.org/10.3390/dj10090173/.
10. Frenea-Robin M, Marchalot J. Basic Principles and Recent Advances in Magnetic Cell Separation. *Magnetochemistry.* 2022;8(1):11. https://doi.org/10.3390/magnetochemistry80 10011
11. Solakoglu Ö, Heydecke G, Amiri N, Anitua E. The use of plasma rich in growth factors (PRGF) in guided tissue regeneration and guided bone regeneration. A review of histological, immunohistochemical, histomorphometric, radiological, and clinical results in humans. *Ann Anat.* 2020;231:151528. https://doi.org/10.1016/j.aanat.2020.151528. Epub 2020 May 4. PMID: 32376297.
12. Valiño-Cultelli V, Varela-López Ó, González-Cantalapiedra A. Does PRGF Work? A Prospective Clinical Study in Dogs with A Novel Polylactic Acid Scaffold Injected with PRGF Using the Modified Maquet Technique. *Animals (Basel).* 2021;11(8):2404. https://doi.org/10.3390/ani11082404. PMID: 34438861; PMCID: PMC8388684.
13. Oshurko AP, Oliinyk IYu, Kerimova TM, Pompii ES. Clinical protocol for the preparation and electron microscopic analysis of the obtained products of autologous mesoconcentrate – plasma rich in growth factors (PRGF). *Clinical and Preventive Medicine.* 2023;6(28):37-45. https://doi.org/10.31612/2616-4868.6.2023.04
14. Stumbras A, Januzis G, Gervickas A, Kubilius R, Juodzbalys G. Randomized and Controlled Clinical Trial of Bone Healing After Alveolar Ridge Preservation Using Xenografts and Allografts Versus Plasma Rich in Growth Factors. *J Oral Implantol.* 2020;46(5):515-25. https://doi.org/10.1563/aaid-joi-D-19-00179. PMID: 32315435.
15. Caponio VCA, Baca-González L, González-Serrano J, Torres J, López-Pintor RM. Effect of the use of platelet concentrates on new bone formation in alveolar ridge preservation: a systematic review, meta-analysis, and trial sequential analysis. *Clin Oral Investig.* 2023;27(8):4131-46. https://doi.org/10.1007/s00784-023-05126-8. Epub 2023 Jul 13. PMID: 37439800; PMCID: PMC10415431.
16. Anitua E, Allende M, Alkhraisat MH. Unravelling Alveolar Bone Regeneration Ability of Platelet-Rich Plasma: A Systematic Review with Meta-Analysis. *Bioengineering (Basel).* 2022;9(10):506. https://doi.org/10.3390/bioengineering9100506. PMID: 36290474; PMCID: PMC9598440.
17. DuVal M, Alkhraisat MH. Adjunctive Plasma Rich in Growth Factors in the Treatment of Osteomyelitis and Large Odontogenic Cysts Prior to Successful Implant Rehabilitation: Case Report. *Dent J (Basel).* 2023;11(8):184. https://doi.org/10.3390/dj11080184. PMID: 37623280; PMCID: PMC10453440.
18. Troha K, Vozel D, Arko M, Bedina Zavec A, Dolinar D, Hočevar M, et al. Autologous Platelet and Extracellular Vesicle-Rich Plasma as Therapeutic Fluid: A Review. *Int J Mol Sci.* 2023;24(4):3420. https://doi.org/10.3390/ijms24043420. PMID: 36834843; PMCID: PMC9959846.
19. Bastian F, Melayah D, Hugoni M, Dempsey NM, Simonet P, Frenea-Robin M, et al. Eukaryotic Cell Capture by Amplified Magnetic in situ Hybridization Using Yeast as a Model. *Front Microbiol.* 2021;12:759478. https://doi.org/10.3389/fmicb.2021.759478. PMID: 34790184; PMCID: PMC8591292.
20. Alavi SE, Gholami M, Shahmabadi HE, Reher P. Resorbable GBR Scaffolds in Oral and Maxillofacial Tissue Engineering: Design, Fabrication, and Applications. *J Clin Med.* 2023;12(22):6962. https://doi.org/10.3390/jcm12226962. PMID: 38002577; PMCID: PMC10672220.