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Studying the results of the application of autogenous, platelet-rich plasma at regenerative therapy of vertical bone defects

ABSTRACT

of dissertation for award of
Educational and Scientific Degree
"DOCTOR"

SCIENTIFIC SPECIALTY:
Therapeutic dentistry

SUPERVISOR:
Prof. Dr. Stefan Vasilev Peev, Ph.D.

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The dissertation contains 230 standard pages and is illustrated with 93 tables, 86 figures, 3 equations and contains 26 appendices. 396 literary sources are cited, of which 6 are in Cyrillic and 390 are in Latin.

The numbers and numbering of the tables and figures in the abstract do not correspond to those in the dissertation.

The dissertation was accepted for public defense by the faculty council of the Department of Periodontology and Dental Implantology on August 28, 2023.

The official defense of the dissertation will be held on 29.11.2023 in Auditorium 103 "Assoc. Dr. Dimitar Klisarov" of the Faculty of Dental Medicine at the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna, in a meeting of the scientific jury.

The defense materials are available in the Scientific Department at the MU-Varna University and are published on the website of the MU-Varna University.

Content

Abbreviations used

BoP – Bleeding on probing
CAL - Clinical attachment level
CBCT – Cone beam computed tomography
DBBM - Deproteinized bovine bone mineral
DFDBA - Demineralized freeze-dried bone allografts
EMD – Enamel matrix derivatives
FDBA - Freeze-dried bone allografts
FFB - Fresh-frozen bone allografts
GBR - Guided bone regeneration
GTR – Guided tissue regeneration
HA – Hydroxyapatite
IL – Interleukin
MMP –Matrix Meratoproteinase
PD – Probing depth
PGE - Prostaglandin
PI – Plaque index
PRF - Platelet-rich fibrin
PRP - Platelet-rich plasma
PTFE – Polytetrafluoroethylene
TCP - Tricalcium phosphate
TNF – Tumor necrosis factor
ECB – Enamel-cementum border
CT - Computed Tomography

1. INTRODUCTION

Periodontitis is the second most common disease in the oral cavity, after carious lesions. It is an infectious disease leading to inflammation and destruction of all periodontal components – gingiva, periodontal ligament, dental root cement and own alveolar bone. Although a number of risk factors can influence the onset, progression and prognosis of periodontitis, the main etiological factor is the specific microorganisms of dental plaque.

The most common clinical symptoms of periodontitis are changes in the color, volume, shape and consistency of the free gingiva, bleeding on probing, loss of clinical attachment level, bone resorption (supra-osseous and infra-osseous defects) and tooth mobility.

The goal of periodontal therapy consists in the elimination of the main etiological factor - the periodontopathogenic microorganisms and regeneration of the destructured periodontal tissues.

Periodontal regenerative therapy is the restoration of the supporting apparatus of the teeth - the periodontium. Guided tissue regeneration is defined as a principle of regeneration in which a barrier membrane is used to eliminate the possibility of the growth of a certain type of unwanted fast-growing tissue (epithelial and connective tissue) and to allow the regeneration of another type of tissue that is slower growing (periodontal ligament, alveolar bone proper and dental cementum).

Over the years, the development of advanced biomaterials has significantly improved the results of the application of various regenerative methods. Different barrier membranes, bone repair materials, different growth factors and combinations of these are used today.

Barrier membranes can be resorbable or non-resorbable. The non-absorbable ones used in practice are polytetrafluoroethylene (PTFE) and titanium-reinforced membranes. The resorbable ones are collagen and synthetic, and the synthetic ones are polylactide, polyglactin (a copolymer of lactic and glycolic acid) and polyethylene glycol.

Bone-restorative materials are applied to preserve or restore the qualities and/or volume of the bone. Depending on their origin, there are several groups of bone-restorative materials: autogenous, allogeneic, xenogenic, alloplastic and composite. Autogenous, allogeneic and xenogeneic transplants are natural, and alloplastic are synthetic materials.

Enamel matrix derivatives (proteins) are pig dental germ extract containing amelogenins (90% of proteins are EMD) and propylene glycol alginate as an alloplastic material. They also contain other proteins, but they are in a significantly smaller concentration. EMDs have been proven effective in stimulating the regeneration of periodontal tissues that are destructured after an inflammatory disease.

Autogenous platelet-rich plasma (PRP) is a platelet-rich substance obtained after specific processing of peripheral blood. Peripheral blood consists of plasma and formed elements - erythrocytes, platelets and leukocytes. Platelets contain three types of granules – α-granules, dense granules and lysosomes. Upon platelet activation and degranulation, α-granules release multiple growth factors. Growth factors have been found to promote cell proliferation, migration and metabolic activity, affect chemotaxis and the production of extracellular matrix proteins. For this reason, bioactive materials such as autogenous platelet-rich plasma (PRP) are increasingly being used in the field of periodontal regenerative

therapy in addition to established biomaterials – barrier membranes and bone repair materials .

To date, however, insufficient evidence is available to lead to a consensus regarding the most appropriate material for use in regenerative therapy of vertical bone defects. There are many controversies arising from the advantages and disadvantages of different biomaterials. On the other hand, autogenous platelet-rich plasma has been the subject of research many times, but always in combination with a bone-restorative material. This led to the need for further investigation and evaluation of the results of the application of autogenous platelet-rich plasma in the regenerative therapy of vertical bone defects both alone and in combination with a barrier membrane alone. For maximum objectivity, the results of the regenerative therapy with the different types of materials used will be evaluated both clinically and paraclinically with the help of cone beam tomography (CBCT).

2. GOAL AND OBJECTIVES

Purpose :

The aim of the dissertation work is to establish the effectiveness of the application of autogenous, platelet-rich plasma in regenerative therapy of vertical bone defects.

To achieve the thus formulated goal, we set ourselves the following

Tasks :

1. Study of the effectiveness of the application of regenerative therapy with autogenous, platelet-rich plasma in vertical defects
2. Investigation of the effectiveness of the application of regenerative therapy with enamel matrix derivatives in vertical bone defects
3. Study of the effectiveness of the application of guided tissue regeneration in vertical bone defects with:
   3.1. Barrier membrane
   3.2. Barrier membrane and autogenous platelet-rich plasma

3. OWN RESEARCH

3.1. MATERIAL AND METHODS

The dissertation research "Investigation of the results of the application of autogenous, platelet-rich plasma in the regenerative therapy of vertical bone defects" was carried out on the basis of the University Medical and Dental Center at the Faculty of Dental Medicine of MU-Varna in the period from August 2022 to July 2023

48 cases out of a total of 30 male and female patients who met all the criteria for participation in the study were included. At the re-evaluation stage, after the systematic and hygienic phase of the treatment sequence, at least one vertical bone defect of the CBCT was found in each participant (Figure 1, Appendix 1).

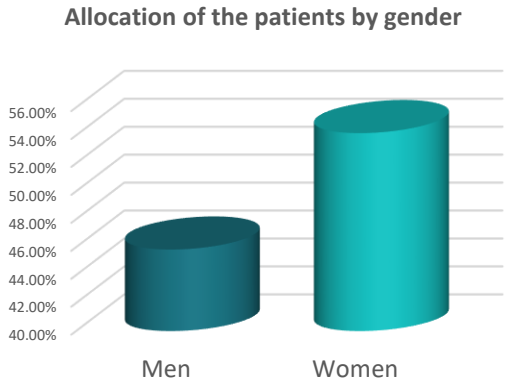

Figure 1

The study included patients aged 31 to 63 years, with a mean age of 45.06 years with a standard deviation of 6.583. The median was 45 years, indicating that ½ of the recipients were under 45 years of age (Figure 2, Appendix 2).

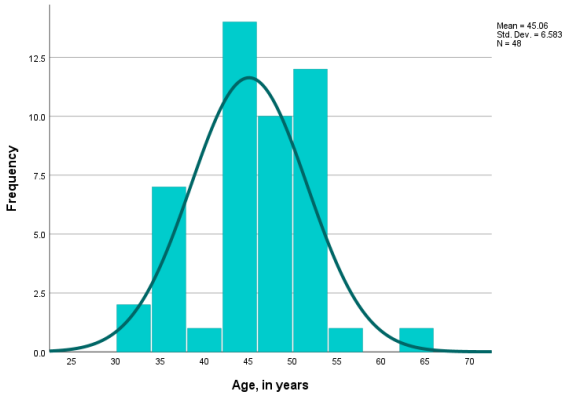

Figure 2

All vertical defects in the present study were randomly assigned to four groups. The first group includes vertical bone defects, where regenerative therapy with autogenous, platelet-rich plasma will be carried out. The second group includes vertical bone defects, where regenerative therapy with enamel matrix derivatives will be carried out. A third group includes vertical bone defects where guided tissue regeneration will be performed with only a barrier membrane. The fourth group includes vertical bone defects, in which guided tissue regeneration with a barrier membrane and autogenous, platelet-rich plasma will take place.

Selection of patients

The study included male and female patients **between the ages of 31 and 63** (fig.2) who met the criteria:

- Signed informed consent;
- Age 18 – 65 years;
- Healthy patients;
- No established systemic diseases;
- Satisfactory personal oral hygiene.

The studied patients have **two-walled, three-walled bone defects or a combination of the listed.**

Each participant in the study was informed about the purpose of the study, the upcoming manipulations, the benefits and risks for the patient (Appendix 3) and signed declarations of informed consent (Appendix 4).

Age, gender and socioeconomic status do not play a role in the allocation of patients to the different groups. The allocation of patients to the different groups occurs randomly.

Criteria for excluding patients from the study:

- Unsatisfactory oral hygiene;
- Untreated inflammatory diseases of the periodontium;
- Horizontal bone loss;
- Single-walled vertical bone defects;
- Conditions of a local and systemic nature that lead to difficulty or obstruction of the recovery process;
- Uncontrolled systemic diseases;
- Malignant diseases;
- Previous radiation therapy in the oral cavity area;
- Taking bisphosphonates;
- Systemic intake of anticoagulants and antiaggregants due to other diseases;
- Smoking;

- Patients who did not meet any of the subjects' inclusion criteria.

Clinical studies

All patients included in the study underwent a systemic and hygienic phase of the treatment sequence in advance. The goal of the systemic phase is to eliminate or reduce the influence of systemic factors on periodontal treatment and to protect the health of the patient and the clinician. For this reason, at the initial examination, each patient fills out a questionnaire about their health status (Appendix 5).

During the hygiene phase, a periodontal card is filled in for each patient included in the study, introduced in the Department of Periodontology and Dental Implantology at the Faculty of Dental Medicine, Varna, by Prof. Dr. Stefan Peev, MD. (Appendix 6). It indicates gingival index (BoP), plaque index (PI), probing depth (PD), margo gingivalis level, furcation involvement, mobility, implant presence and prognosis for each tooth.

An imaging study - **interproximal orthopantomography**, required by every dentist at the start of periodontal treatment to specify the diagnosis and prognosis of each tooth, is mandatory.

Gingival index (bleeding index, BI) according to Animo&Bay, 1975. Bleeding on probing (BoP) is recorded by inserting and gradually moving the periodontal probe into the gingival sulcus or periodontal pocket. After 15 seconds, the result is registered. In the periodontal chart, the presence or absence of bleeding is marked by a "+" or "-" sign in the six areas for each examined tooth (MV, CV, DV, ML, CL, DL). The index is calculated as a percentage ratio between the number of areas with bleeding in relation to all examined.

Plaque control index (PI) according to O'Leary et al., 1972. This index registers the presence or absence of dental plaque on all tooth surfaces. In the periodontal map, the presence or absence of dental plaque is noted with a "+" or "-" sign in the six areas of each examined tooth (MV, CV, DV, ML, CL, DL). The index is calculated as a percentage ratio between the number of areas with dental plaque present in relation to all examined.

After calculating and recording the gingival index (BoP) and plaque index (PI) in the periodontal chart, scaling is carried out. **Scaling** is defined as a procedure in which the tooth surface is instrumented to remove dental plaque, calculus and exogenous tooth stains. The teeth are polished by jet-abrasive method using sodium bicarbonate powder for supragingival application and glycine powder for subgingival application. In addition, a rubber, brush and abrasive paste can be used. The patient is instructed and motivated to observe strict personal oral hygiene.

At the next visit, the periodontal card is completely filled out - depth of probing, level of the margo gingivalis, degree of furcation involvement, tooth mobility, presence of an implant and the individual prognosis for each tooth are recorded.

Figure 3

Probing depth (DS) is defined as the distance measured in millimeters from the gingival margin to the bottom of the sulcus or periodontal pocket. The values were recorded manually with a UNC 15 standardized pressure periodontal probe (Figure 3) in all 6 areas of each tooth (MV, CV, DV, ML, CL, DL). The probe

is inserted parallel to the longitudinal axis of the tooth without losing contact with the tooth surface until resistance is felt from the bottom of the gingival sulcus or periodontal pocket. After its introduction, the probe is moved with a step of 1 mm and an amplitude up-down 1-2 mm around the circumference of the entire tooth, without pulling it out completely. Proximally, the periodontal probe is positioned close to the interdental contact with minimal inclination to detect interdental craters.

Margo gingivalis level is defined as the distance measured in millimeters from the gingival margin to the enamel-cementum boundary (ECM). When the gingival margin is coronal to the ECB, the values are positive, and when it is apical - negative. When the gingival margin is at the level of the ECB, a value of "0" is recorded. In the periodontal map, six values are recorded for each examined tooth for the six specific areas (MV, CV, DV, ML, CL, DL). In the presence of a non-removable prosthetic structure (bridge or crown), the edge of the structure is taken as a reference border when measuring the level of the Margo gingivalis. Registration is done manually with a UNC 15 periodontal probe.

Clinical attachment level (CAL) represents the distance in millimeters from the ECB to the bottom of the periodontal pocket. If in two consecutive visits the CAL value increased there was a loss of the clinical attachment level, if the CAL value decreased there was a gain of the clinical attachment level. This determines CAL as one of the most important indicators that we monitor during the different stages of periodontal treatment.

To determine whether there is **furcation involvement**, probing the furcation areas of teeth with more than one root is performed. This is done using Nabers furcation probes - 1 and 2 (Figure 4). Nabers 1 is used for probing upper first premolars

and upper all molars, and Nabers 2 for lower all molars. The classification of Hamp et al., 1975 applies.

- Class 1 - when Probing horizontally, the probe penetrates up to 3mm
- Class 2 – during horizontal probing, the probe penetrates more than 3 mm, but without completely passing the furcation
- Class 3 – when probing horizontally, the probe passes completely through the furcation.

Each class is divided into three subclasses according to the degree of vertical bone loss in the furcation area (Tarnow&Fletcher, 1984).

- subclass A - vertical bone loss up to 3mm
- subclass B - vertical bone loss from 4mm to 5mm
- subclass C - vertical bone loss over 6mm

The degree of furcation involvement is reflected in the periodontal chart at the designated locations and plotted on the chart with specific designations.

Tooth mobility was determined according to the classification of Miller, 1985.

- 1 – horizontal mobility up to 1mm
- 2 – horizontal mobility over 1mm
- 3 – horizontal and vertical mobility.

Figure 4

The examination is carried out using the handles of two instruments or with the handle of one instrument and one finger.

After completing the periodontal status, the individual **prognosis of each tooth is indicated in the periodontal map**. It can be - certain, doubtful and a tooth irrational to treat. Teeth with a certain prognosis require relatively simple treatment, teeth with a doubtful prognosis usually need additional treatment methods, teeth that are irrational for treatment should be extracted at the stage of the hygiene phase, so that they do not function as plaque-retentive factors.

With a completely completed periodontal map, it is estimated which teeth should be subjected to debridement of the root surface. **Debridement** is a combined concept that includes **Root scaling** - elimination of all deposits of dental plaque, tartar and exogenous staining on the root surface and **Root planing** - elimination of necrotic cementum and endotoxins on the root surface in order to create a biocompatible root surface (smooth, hard and clean). Debridement of the root surfaces is performed with manual Gracey curettes (Figure 5) with vertical, horizontal and oblique movements to tactile sensation for a biocompatible root surface. During the instrumentation, washes are carried out continuously with physiological solution (0.9% NaCl) and infusion solution Metronidazole 500mg/100ml.

Figure 5

After debridement of all periodontal pockets, patients are instructed and motivated to maintain excellent oral hygiene. After waiting for a period of 6 weeks to 6 months, all patients are invited to register **Reassessment of their condition**. In the presence of established periodontal pockets with bleeding that have responded minimally (CAL) or not at all to non-surgical treatment and in the presence of evidence of a bone defect from the OPG, which was appointed before the start of periodontal treatment, sectorial cone-beam computed tomography is appointed tomography (CTS).

Image studies

Using a Planmeca ProMax 3D cone beam tomograph (3D scanner), a three-dimensional X-ray image was taken of each patient who participated in the study after pre-signed informed consent (Appendix 7). Cone-beam computed tomography (CBCT) is performed immediately before regenerative therapy in order to locate and measure the parameters of all vertical bone defects.

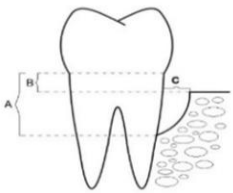

Figure 6

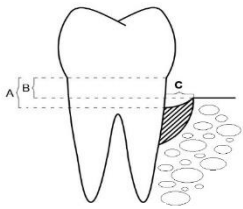

Figure 7

Three measurements were taken on each vertical bone defect (Figure 6):

- A - The distance from the ECB to the base of the bone defect;
- C - The distance from the ECB to the highest bone point from the bone defect;
- C - The width of the defect.

Cone-beam computed tomography (CBCT) was also performed 6 months after the regenerative therapy in order to examine the bone filling of the defect, through new three measurements – A, B and C (Figure 7).

The results of the measurements of the first and second cone-beam computed tomography are compared and it is established how much of the bone defect has been filled after the surgical intervention (Figure 8).

Each cone-beam tomograph (before and after the surgical intervention) has a radiation dose between 600 mGy *cm 3 and 720 mGy *cm 3 and an exposure time of 12.058-12.080 s.

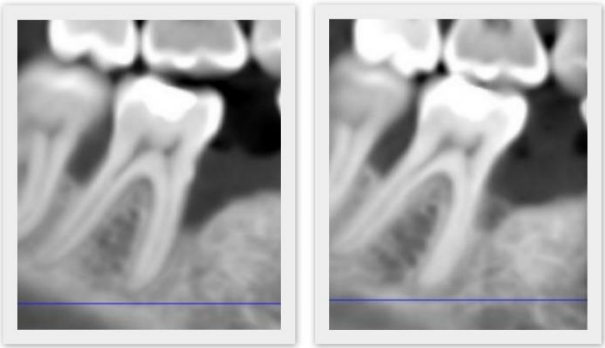

Figure 8

MATERIAL AND METHODS FOR TASK No. 1

Study of the effectiveness of the application of regenerative therapy with autogenous, platelet-rich plasma in vertical defects.

For the implementation of task 1, a comparison was made of the vertical bone defects (clinical and paraclinical) immediately before and 6 months after performing regenerative therapy with autogenous, platelet-rich plasma (PRP). The indicators were clinically compared - Plaque index according to O'Leary et al., 1972, Gingival index according to Animo&Bay, 1975. and in areas with vertical bone defect- Probing Depth, Margo Gingivalis Level and Clinical Attachment Level. Paraclinically, the previously presented A, B and C indicators (Figure 6 and Figure 7) were compared on the CVST.

We studied **12 cases** out of a total of **7 patients both male and female**, systemically healthy, non-smokers with the presence of a vertical bone defect found on CVST (Figure 9). The results are presented in detail in Appendix 8.

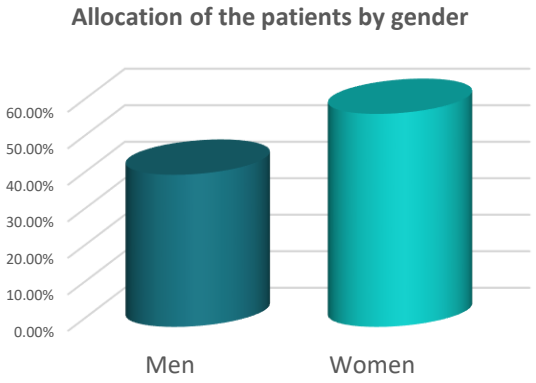

Figure 9

In task 1, patients **between the ages of 36 and 52 participate**, their average age being 45 years with a standard deviation of 5.309. The median was 45 years, indicating that ½ of the recipients were under 45 years of age (Figure 10). The results are presented in detail in Appendix 9.

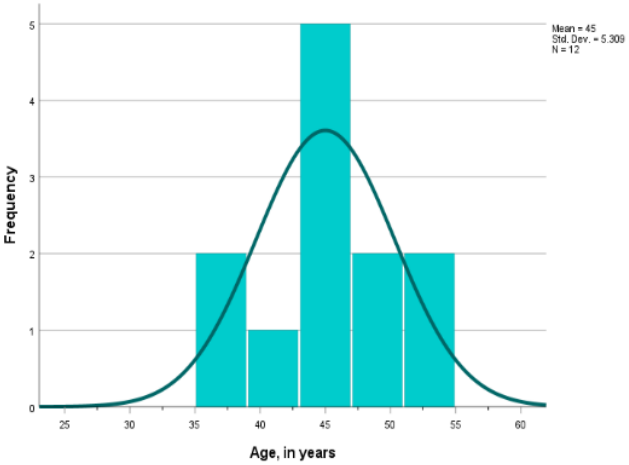

Figure 10

Protocol for obtaining autogenous platelet-rich plasma

The protocol we use to obtain autogenous platelet-rich plasma was published in 2021. by N. Ivanova (1).

Figure 11

As well as the laboratory centrifuge EBA20-HettichLab, Germany (Figure 11), the following laboratory consumables are also needed to obtain PRP - an 8 ml vacutainer with a separating biocompatible inert gel containing a cycloalligate polymer for plasma separation (separating red blood cells) and an anticoagulant 3,2 % trisodium citrate (Figure 12), sterile tubes, sterile syringes of 5 and 10 ml. and 22G sterile needles.

Figure 13

For the final activation of the product, we use calcium gluconate (Calcium gluconicum amp. 10%) (Figure 13).

Figure 12

Venous blood is drawn from the patient, observing all conditions for aseptic and antiseptic. Venipuncture is performed with a 22G needle, the diameter of which prevents pre-activation of platelets. The amount of blood drawn from each patient is 8 ml. The latter is processed within 1 hour (during the operative intervention) of its withdrawal. The drawn blood is transferred from the syringe into the vacutainer. The vacutainer is then shaken to mix the blood with the trisodium citrate.

After the homogenization of the sample, the vacutainer is placed in the laboratory centrifuge EBA20-HettichLab, Germany. A basic principle for working with a laboratory centrifuge is weight calibration, by symmetrically placing the test tubes and the same volume of liquids in them (Figure 14).

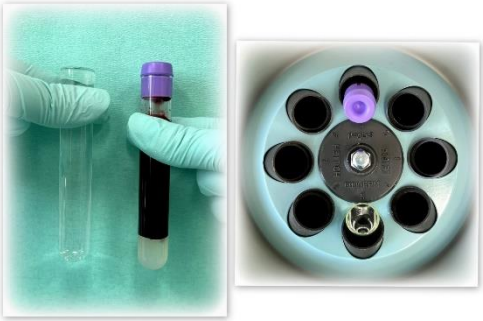

Figure 14

Centrifugation is performed at room temperature 20-22°C in two stages. In the first separation centrifugation, a centrifugal force of 1150xg is used for 10 min., and in the second concentration centrifugation, a centrifugal force of 350xg is used for 5 min. To adjust these values to the centrifuge we are working with, we used information about the technical specifications of the device and the following formulas (Equation 1, Equation 2 and Equation 3).

RPM - Rounds per minute , 6000
RCF - Acceleration , 3461
r - Radius of centrofugation in mm, or the distance from the middle of the axis of rotation to the bottom of the centrofuge's container (for 8ml - 86 mm)

$$RCF = \left(\frac{RPM}{1000}\right) \times r \times 1.118 \Rightarrow RPM = \sqrt{\frac{PCF}{r \times 1.118}} \times 1000$$

Equation 3

First separating centrofugation = 1150xg for 10 mins

$$RPM = \sqrt{\frac{1150}{86 \times 1.118}} \times 1000 = 3458{,}43 \sim 35 \times 100rpm$$

Equation 2

Second concentration centrofugation = 350xg for 5 mins

$$RPM = \sqrt{\frac{350}{86 \times 1.118}} \times 1000 = 1907{,}94 \sim 19 \times 100rpm$$

Equation 1

Through the formulas, we obtained the cycles with which we work in the first and second centrifugation with a laboratory centrifuge EBA20-HettichLab, Germany (Figure 15 and Figure 16).

Figure 15 *Figure 16*

PRP is obtained through the so-called "buffy coat" method, in which the first separating centrifugation takes place at high values of RCF (Relative centrifugal force). As a result, three clearly visible layers are obtained in the vacutainer - the first, the lowest - rich in erythrocytes, the second, rich in platelets and leukocytes - the so-called "buffy coat" and a third, uppermost - platelet-poor layer (platelet poor plasma - PPP). After the end of the first centrifugation, the buffy coat layer and some of the platelet-poor plasma (PPP) were withdrawn using a new 22G needle and a 5ml syringe. and transferred to a new sterile tube (without anticoagulant) for the subsequent second spin. After the second centrifugation, a very small layer of erythrocytes is usually distinguished at the bottom of the tube, a "buffy coat" layer above it, and a layer of PPR again on top. The most superficial portion of the PPP is removed, and the rest together with the "buffy coat" layer (with a total volume of about 3mm) is aspirated using a new 22G needle and a 5ml syringe. and transferred to a new sterile tube, in which this material is subsequently activated calcium gluconate (Calcium gluconicum amp. 10%). The collected plasma (with a volume of 3 ml) is placed in a sterile test tube, to which 1 ml of calcium gluconate

is added. The mixture is homogenized and left to rest at room temperature for 20 minutes (Figure 17).

Figure 17

1. Vacutainer of 8 ml with separating biocompatible inert gel containing cycloalligate polymer for plasma separation and anticoagulant - 3.2% trisodium citrate
2. Vacutainer with 8 ml of venous blood
3. Vacutainer after first separation centrifugation
4. Left - withdrawn buffy coat and platelet-poor plasma (PPP) from vacutainer (right)
5. Tube after second concentration centrifugation
6. The most superficial part of the PPP is removed (right) from the rest together with the "buffy coat" layer (with a total volume of about 3ml) (left)
7. 3 ml including buffy coat and platelet-poor plasma (PPP) (left) and Calcium Gluconate activator (right)
8. 3 ml including buffy coat and platelet-poor plasma (PPP) homogenized with 1 ml of Calcium Gluconate
9. Autogenous platelet-rich plasma (PRP) after 20 min
10. Autogenous platelet-rich plasma (PRP) after 20 min

The resulting gel-like product is used for regenerative therapy of shallow vertical defects or for guided tissue regeneration of deep vertical defects (Figure 18).

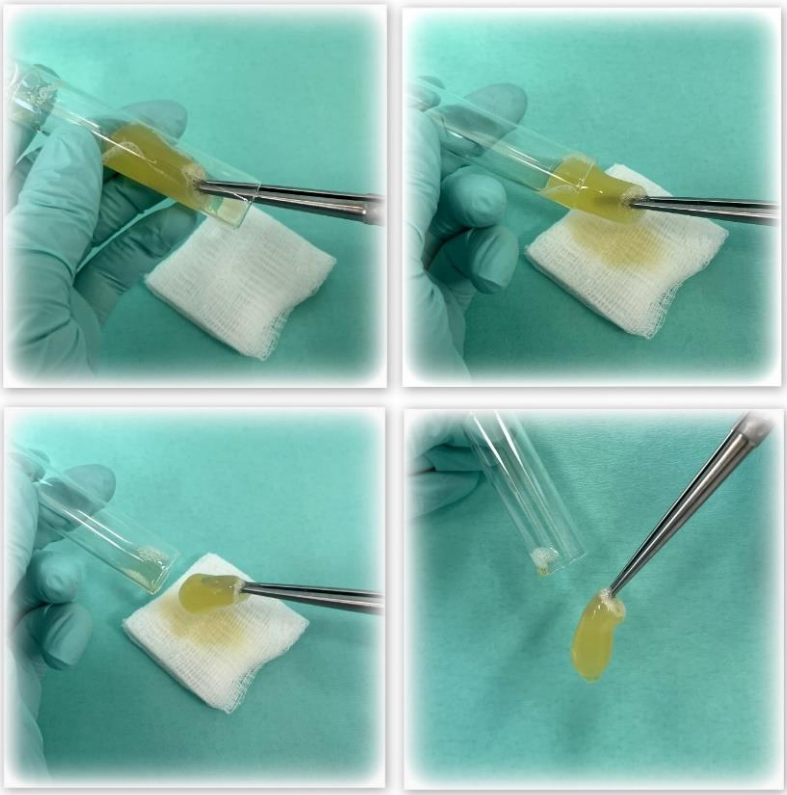

Figure 18

Clinical protocol for regenerative therapy with autogenous platelet-rich plasma in vertical bone defects

The operative field is anesthetized by local anesthesia using 1 carpule of Septanest containing a 4% solution of articaine with adrenaline 1:100000 (Septodont, France). Using a #15 blade scalpel, an intrasulcular incision spanning 1 to 2 teeth medial and distal to the affected area was made. This is followed by preparation of a muco-periosteal flap with a Boozer periosteal elevator. Granulation tissue in the defect is removed by means of SRP with universal curettes Columbia 2L/2R, Columbia 4L/4R and Younger-Good 7/8 (Figure 19) .

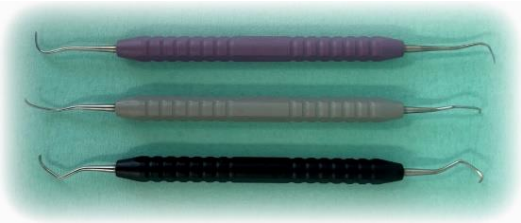

Figure 19

The operative field is washed with physiological solution. The root surface was conditioned for 2 min with 24% EDTA gel (PrefGel, Strauman) (Figure 20 and Figure 21).

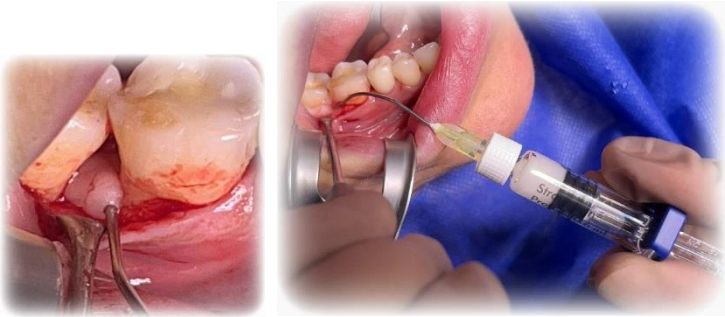

Figure 20

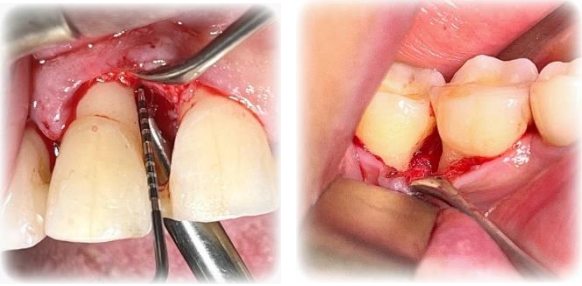

Figure 22

After thorough washing of the EDTA-gel with physiological solution, the previously obtained autogenous, platelet-rich plasma (PRP) is introduced into the vertical bone defect (Figure 22). This is followed by repositioning, adaptation and suturing of the flap using single, interrupted sutures with 5/0 non-absorbable monofilament suture (Dafilon, B.Braun-Melsungen, Germaany).

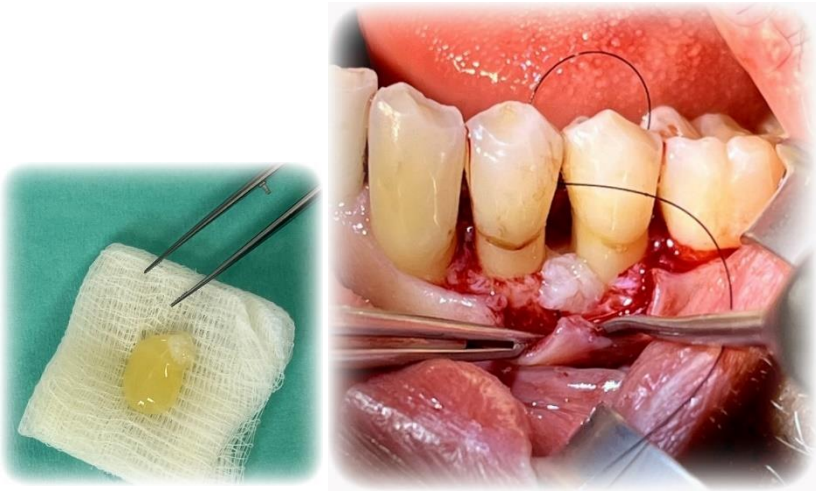

Figure 21

Postoperative care includes an Antibiotic (Amoxicillin, 1000 mg every 8 hours), an NSAID (Aulin, 100 mg every 12 hours) and an oral solution containing chlorhexidine (Eludril Classic).

An appointment is made for a control examination and suture removal between the 10th and 14th day after the surgical intervention.

Six months after the surgical intervention, a visit to the patient is planned to register the current periodontal status and analysis of a new cone-beam computed tomography (CBCT) - necessary for reporting the results.

MATERIAL AND METHODS FOR TASK No. 2

Investigation of the effectiveness of the application of regenerative therapy with enamel matrix derivatives in vertical bone defects.

For the implementation of task 2, a comparison was made of the vertical bone defects (clinical and paraclinical) immediately before and 6 months after performing regenerative therapy with enamel matrix derivatives (EMD). The indicators were clinically compared - Plaque index according to O'Leary et al., 1972, Gingival index according to Animo&Bay, 1975. and in areas with a vertical bone defect - Probing depth, Margo gingivalis level and Clinical attachment level. Paraclinically, the previously presented A, B and C indicators were compared at the CBCT.

We studied **12 cases out of a total of 8 patients both male and female patients**, systemically healthy, non-smokers with the presence of a vertical bone defect established on CVST (Figure 23). The results are presented in detail in Appendix 10.

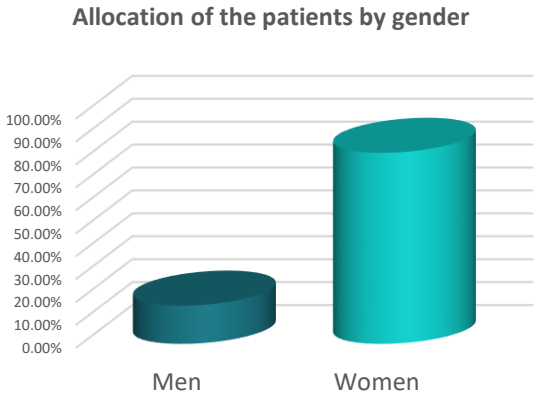

Figure 23

In task 2, patients **between the ages of 31 and 57 participate**, their average age being 46.33 years with a standard deviation of 7.377. The median was 46 years, indicating that ½ of the recipients were under 46 years of age (Figure 24). The results are presented in detail in Appendix 11.

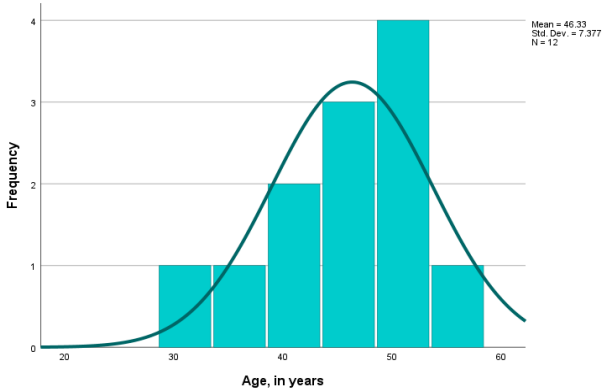

Figure 24

Clinical protocol for regenerative therapy with enamel matrix derivatives in vertical bone defects

The operative field is anesthetized by local anesthesia using 1 carpule of Septanest containing a 4% solution of articaine with adrenaline 1:100000 (Septodont, France). Using a #15 blade scalpel, an intrasulcular incision spanning 1 to 2 teeth medial and distal to the affected area was made. This is followed by preparation of a muco-periosteal flap with a Boozer periosteal elevator. Granulation tissue in the defect is removed by SRP with Columbia 2L/2R, Columbia 4L/4R, and Younger-Good 7/8 universal curettes. The operative field is washed with physiological solution. The root surface was conditioned for 2 min with 24% EDTA gel (PrefGel, Strauman) (Figure 25).

After thorough washing of the EDTA-gel with physiological solution, Emdogain (Straumann, Basel, Switzerland) was applied in the vertical bone defect in contact with the root surface (Figure 26). This is followed by repositioning, adaptation and suturing of the flap using single, interrupted sutures with 5/0 non-absorbable monofilament suture (Dafilon, B.Braun-Melsungen, Germaany).

Figure 26

Figure 25

Postoperative care includes Antibiotic (Amoxicillin, 1000 mg every 8 hours), NSAID (Aulin, 100 mg every 12 hours) and mouth solution containing chlorhexidine (Eludril Classic);

An appointment is made for a control examination and suture removal between the 10th and 14th day after the surgical intervention.

Six months after the surgical intervention, a visit to the patient is planned to register the current periodontal status and analysis of a new cone-beam computed tomography (CBCT) - necessary for reporting the results.

MATERIAL AND METHODS FOR TASK No. 3

Study of the effectiveness of the application of guided tissue regeneration in vertical bone defects with:

- *Barrier membrane;*
- *Barrier membrane and autogenous platelet-rich plasma.*

MATERIAL AND METHODS FOR TASK No. 3.1.

For the implementation of task 3.1. a comparison was made of the vertical bone defects (clinical and paraclinical) immediately before and 6 months after performing guided tissue regeneration with a barrier membrane. The indicators were clinically compared - Plaque index according to O'Leary et al., 1972, Gingival index according to Animo&Bay, 1975. and in areas with a vertical bone defect - Probing depth, Margo gingivalis level and Clinical attachment level. Paraclinically, the previously presented A, B and C indicators were compared at the CBCT.

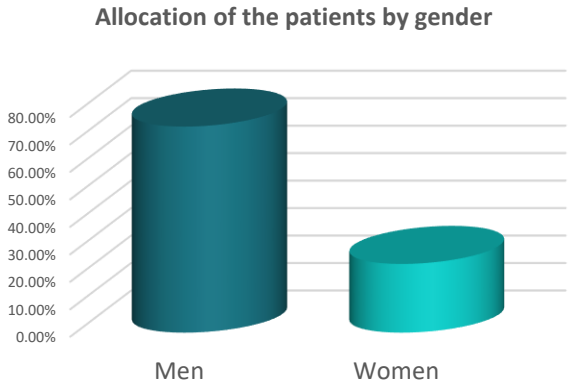

Figure 27

We studied **12 cases out of a total of 9 patients both male and female patients**, systemically healthy, non-smokers with the presence of a vertical bone defect found on CVST (Figure 27). The results are presented in detail in Appendix 12.

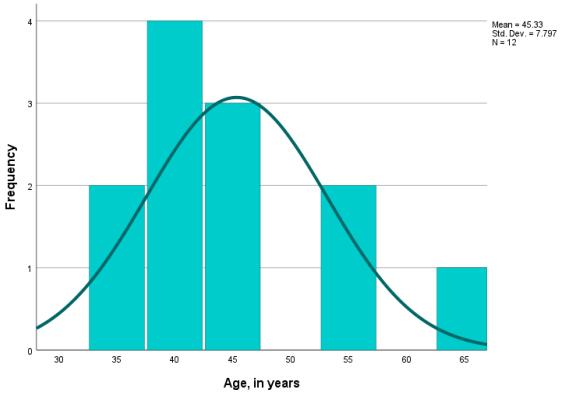

Figure 29

In task 3.1. patients **between the ages of 35 and 53 participated**, their average age being 45.33 years with a standard deviation of 7.797. The median was 45 years, indicating that ½ of the recipients were under 45 years of age (Figure 28). The results are presented in detail in Appendix 13.

A clinical protocol for guided tissue regeneration with a barrier membrane in vertical bone defects

The operative field is anesthetized by local anesthesia using 1 carpule of Septanest containing a 4% solution of articaine with adrenaline 1:100000 (Septodont, France). Using a #15 blade scalpel, an intrasulcular incision spanning 1 to 2 teeth medial and distal to the affected area was made. This is followed by preparation of a muco-periosteal flap with a Boozer periosteal elevator. Granulation tissue in the defect is removed by SRP with Columbia 2L/2R, Columbia 4L/4R, and Younger-Good 7/8

universal curettes. The operative field is washed with physiological solution. The root surface was conditioned for 2 min with 24% EDTA gel (PrefGel, Strauman). After thorough washing of the EDTA gel with physiological solution, the vertical bone defect is covered with a pericardial collagen barrier membrane (Jason Membrane – Botiss, Berlin, Germany) (Figure 29).

Figure 31

The membrane should cover the bone defect a minimum of 2mm beyond its borders and its edges should fit snugly over the surrounding healthy bone and around the tooth necks to prevent epithelial ingrowth. The flap is then repositioned, adapted, and sutured using single, interrupted sutures with nonabsorbable 5/0 monofilament suture (Dafilon, B.Braun-Melsungen, Germaany);

Postoperative care includes Antibiotic (Amoxicillin, 1000 mg every 8 hours), NSAID (Aulin, 100 mg every 12 hours) and mouth solution containing chlorhexidine (Eludril Classic);

An appointment is made for a control examination and suture removal between the 10th and 14th day after the surgical intervention.

Six months after the surgical intervention, a visit to the patient is planned to register the current periodontal status and analysis of the new cone-beam computed tomography (CBCT) - necessary for reporting the results.

MATERIAL AND METHODS FOR TASK No. 3.2.

For the implementation of task 3.2. vertical bone defects (clinical and paraclinical) were compared immediately before and 6 months after guided tissue regeneration with barrier membrane and autogenous platelet-rich plasma. The indicators were clinically compared - Plaque index according to O'Leary et al., 1972, Gingival index according to Animo&Bay, 1975. and in areas with a vertical bone defect - Probing depth, Margo gingivalis level and Clinical attachment level. Paraclinically, the previously presented A, B and C indicators were compared at the SVT.

We studied **12 cases out of a total of 10 patients both male and female patients**, systemically healthy, non-smokers with the presence of a vertical bone defect established on CBCT (Figure 30). The results are presented in detail in Appendix 14.

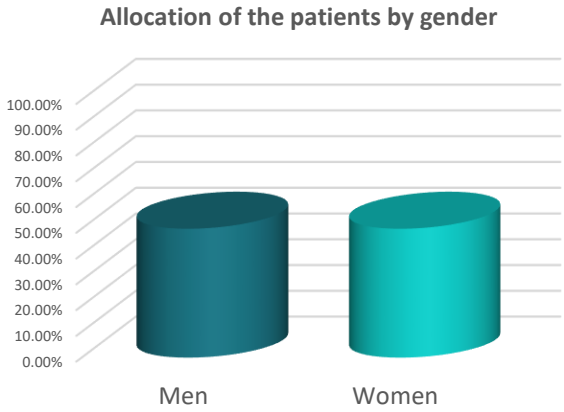

Figure 32

In task 3.2. patients **between the ages of 33 and 52 participated**, their average age being 43.67 years with a standard deviation of 6.228. The median is 44 years, indicating that ½ of the recipients are under 44 years of age (Figure 31). Detailed results are presented in Appendix 15.

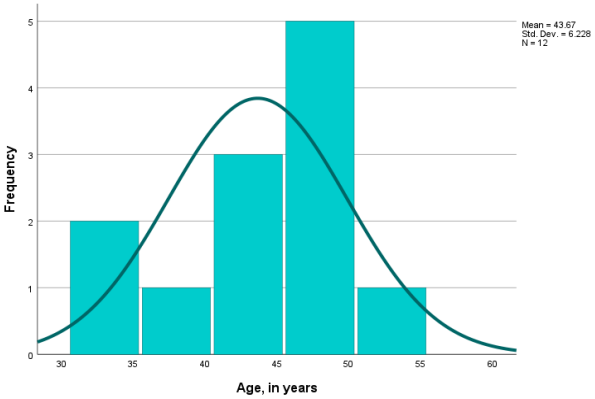

Figure 33

Protocol for obtaining autogenous platelet-rich plasma

The protocol we use to obtain autogenous platelet-rich plasma is described in the Materials and Methods section of Task 1.

Clinical Protocol for Guided Tissue Regeneration with Barrier Membrane and Autogenous Platelet-Rich Plasma in Vertical Bone Defects

The operative field is anesthetized by local anesthesia using 1 carpule of Septanest containing a 4% solution of articaine with adrenaline 1:100000 (Septodont, France). Using a #15 blade scalpel, an intrasulcular incision spanning 1 to 2 teeth medial and distal to the affected area was made. This is followed by

preparation of a muco-periosteal flap with a Boozer periosteal elevator. Granulation tissue in the defect is removed by SRP with Columbia 2L/2R, Columbia 4L/4R, and Younger-Good 7/8 universal curettes. The operative field is washed with physiological solution. The root surface was conditioned for 2 min with 24% EDTA gel (PrefGel, Strauman). After thorough washing of the EDTA-gel with physiological solution – the previously obtained autogenous, platelet-rich plasma (PRP) is introduced into the vertical bone defect. Placement of a pericardial collagen barrier membrane on the autogenous, platelet-rich plasma (Jason Membrane – Botiss, Berlin, Germany). The membrane should cover the bone defect a minimum of 2mm beyond the bone defect and its edges should fit snugly over the surrounding healthy bone and around the tooth necks to prevent epithelial ingrowth. The flap is then repositioned, adapted, and sutured using single, interrupted sutures with nonabsorbable 5/0 monofilament suture (Dafilon, B.Braun-Melsungen, Germaany).

Postoperative care includes Antibiotic (Amoxicillin, 1000 mg every 8 hours), NSAID (Aulin, 100 mg every 12 hours) and mouth solution containing chlorhexidine (Eludril Classic);

An appointment is made for a control examination and suture removal between the 10th and 14th day after the surgical intervention.

Six months after the surgical intervention, a visit to the patient is planned to register the current periodontal status and analysis of a new cone-beam computed tomography (CBCT) - necessary for reporting the results.

3.2. RESULTS AND DISCUSSION

DISCUSSION OF RESULTS

From Figure 32 and Table 1, it can be seen that 50% of teeth with vertical bone defects are molars, followed by premolars with 29.17% and the least in the area of frontal teeth - 20.83%. These results confirm the results of an epidemiological study from 2017. whose aim was to assess the prevalence of infraaxial defects by clinical and radiological studies. It was concluded that vertical defects occur most frequently in the molar region (247). This could be explained by the more difficult access for mechanical cleaning of dental plaque in the distal areas. The results are presented in detail in Appendix 24.

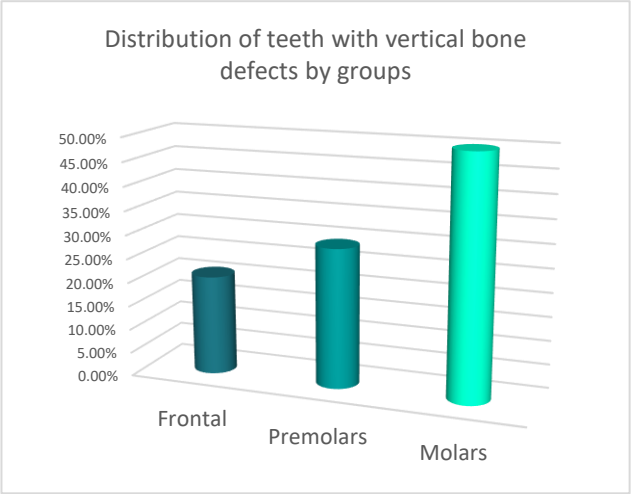

Figure 34

groups of teeth					
		Number	*Relative share (%)*	*Valid rate*	*Cumulative percentage*
Valid	Frontal	10	20.83%	20.83%	20.83%
	Premolars	14	29.17%	29.17%	50.00%
	Molars	24	50.00%	50.00%	100.00%
	Total	**48.00**	**100.00%**	**100.00%**	

Table 1

In discussing our study, it is first of utmost importance to review and compare the plaque and gingival index data obtained from patients in all four groups to determine whether our actual results are mutually exclusive.

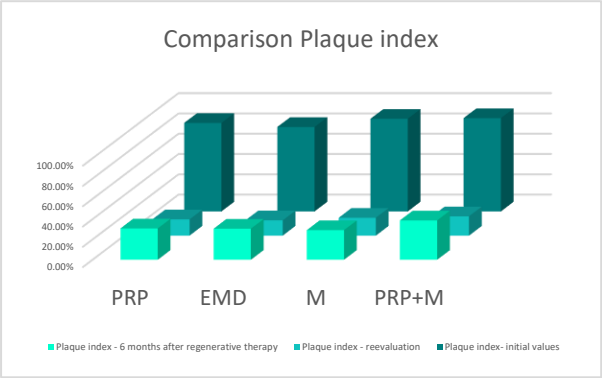

Figure 36

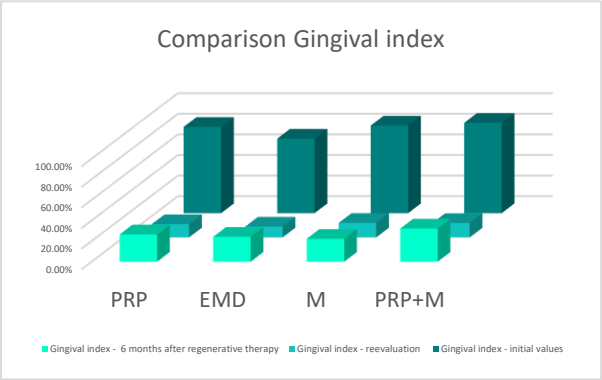

Figure 35

Figure 33 and Figure 34 clearly demonstrate what the mean percentage of plaque index and gingival index was in patients from all four groups in the three reporting periods – 1. Immediately before starting periodontal treatment; 2. Reassessment and 3. Six months after regenerative therapy. There is an undeniable **correlation between plaque and gingival index at the three stages of periodontal treatment in the participants.** The results are presented in detail in Appendix 25.

It is clearly seen that the plaque and gingival index before starting the periodontal treatment in all patients are extremely high, i.e. all study participants started with an unsatisfactory level of personal oral hygiene and a high % of gingival inflammation. At the "Reevaluation" stage, a significant decrease in the values for both indices was observed in all four groups, demonstrating the high cooperativeness of all patients during periodontal treatment. At the "Six months after regenerative therapy" stage, an increase in the results of the plaque index and, accordingly, the gingival index was observed, which can be explained by the deterioration of the patients' personal oral hygiene. A key point is that the Stage Three plaque index values in the four groups were approximately equal (Table 2), allowing us to **rule out plaque and gingival index as modifiers in the results of any of the four groups.**

	Task 1 PRP		Task 2 EMD		Task 3.1. Barrier membrane		Task 3.2. Barrier membrane and PRP	
Indexes	PI	GI	PI	GI	PI	GI	PI	GI
First stage	87.33%	83.75%	83.33%	72.33%	91.42%	85.08%	92.08%	87.75%
Second stage	16.02%	12.83%	15.08%	10.50%	17.58%	14.08%	19.00%	14.00%
Third stage	30.92%	26.50%	30.67%	24.17%	29.25%	22.25%	38.83%	32.17%

Table 2

Probing depth

rom Figure 35, it is clearly seen that when registering the periodontal status during the Hygiene phase, the indicator "Probing depth" has an extremely high average value in all four groups of patients (task 1 - 7.75mm; task 2 - 7.50mm; task 3.1 – 7.58mm and task 3.2 – 8.25mm). On the sixth month after the regenerative therapy (regardless of the applied method), a significant reduction of the probing depth was found compared to the initial values (task 1 – 3.92mm; task 2 – 3.00mm; task 3.1. – 3.42mm and task 3.2. – 4.00mm). Detailed results are presented in Appendix 26. These results are comparable to numerous studies that have demonstrated that regenerative therapy, regardless of the method used, leads to a drastic reduction in probing depth (84, 85, 86, 202, 230, 385).

The ANOVA test proves that the results we obtained for the indicator "Probing depth" at the sixth month after regenerative therapy were not statistically significant between

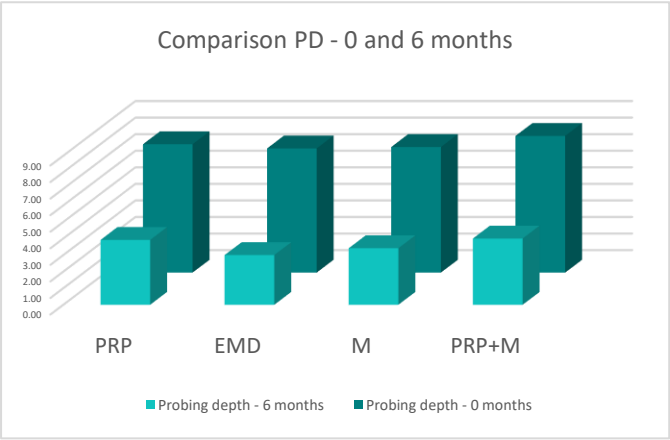

Figure 37

the four groups of patients, F(3,44)=1.228, p=0.311>0.05 (Figure 36 and Table 4).

ANOVA

Probing depth - results

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.729	3	.910	1.228	.311
Within Groups	32.583	44	.741		
Total	35.312	47			

Table 3

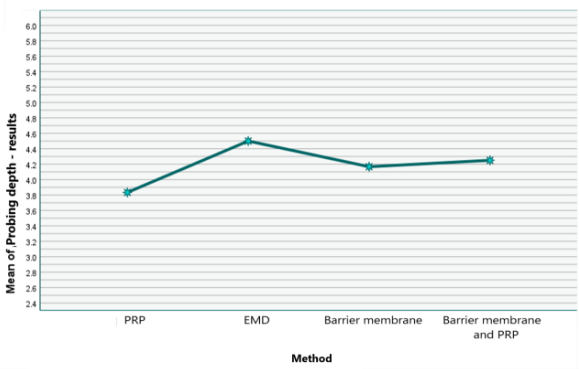

Figure 38

The post-hoc test using Tukey also confirms the above thesis (Table 5).

Multiple Comparisons

Dependent Variable: Probing depth - results

Tukey HSD

(I) Method	(J) Method	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
PRP	EMD	-.667	.351	.244	-1.60	.27
	Barrier membrane	-.333	.351	.779	-1.27	.60
	Barrier membrane and PRP	-.417	.351	.639	-1.35	.52
EMD	PRP	.667	.351	.244	-.27	1.60
	Barrier membrane	.333	.351	.779	-.60	1.27
	Barrier membrane and PRP	.250	.351	.892	-.69	1.19
Barrier membrane	PRP	.333	.351	.779	-.60	1.27
	EMD	-.333	.351	.779	-1.27	.60
	Barrier membrane and PRP	-.083	.351	.995	-1.02	.85
Barrier membrane and PRP	PRP	.417	.351	.639	-.52	1.35
	EMD	-.250	.351	.892	-1.19	.69
	Barrier membrane	.083	.351	.995	-.85	1.02

Table 4

From the descriptive table, it is found that in the patients from Task 1, the probing depth was reduced by 3.83 mm on average. In Task 2 patients, probing depth was reduced by 4.50mm on average. For the patients from Task 3.1. the probing depth is reduced by 4.17mm on average. For the patients from Task 3.2. probing depth was reduced 4.25mm on average (Table 6).

Descriptives

Probing depth - results

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
PRP	12	3.83	.718	.207	3.38	4.29	3	5
EMD	12	4.50	1.000	.289	3.86	5.14	3	6
Barrier membrane	12	4.17	.835	.241	3.64	4.70	3	5
Barrier membrane and PRP	12	4.25	.866	.250	3.70	4.80	3	6
Total	48	4.19	.867	.125	3.94	4.44	3	6

Table 5

Margo gingivalis level

From Figure 37, it is clearly seen that when registering the periodontal status during the Hygiene phase, the indicator "Level of Margo gingivalis" has an average value for task 1 - 0.08mm; task 2 – -0.08mm; task 3.1. – -0.33mm and task 3.2. – -0.58mm. On the sixth month after the regenerative therapy, a relative preservation of the values compared to the initial ones was found (task 1 – 0.17mm; task 2 – -0.58mm; task 3.1. – -0.67mm and task 3.2. – -0.33mm). This means that clinically almost no apical or coronal migration of the gingival margin is observed. The results are presented in detail in Appendix 26.

The ANOVA test proves that the results we obtained for the indicator "Level of Margo gingivalis" at the sixth month after regenerative therapy were not statistically significant between the four groups of patients, $F(3,44)=1.973$, $p=0.132>0.05$ (Figure 78 and Table 78).

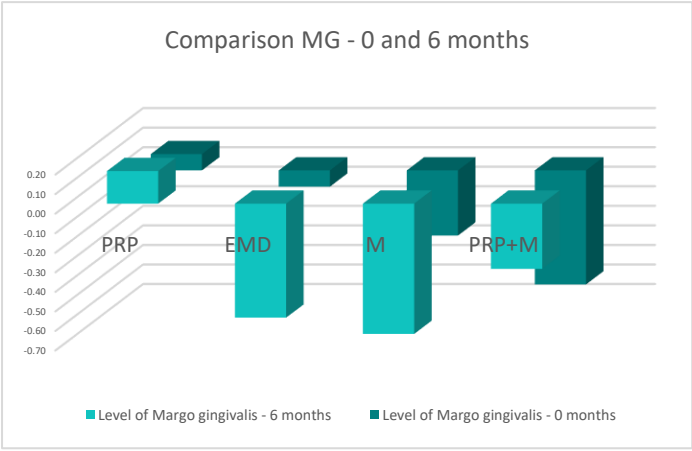

Figure 39

ANOVA

Level of Margo gingivalis - results

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.417	3	1.472	1.973	.132
Within Groups	32.833	44	.746		
Total	37.250	47			

Table 7

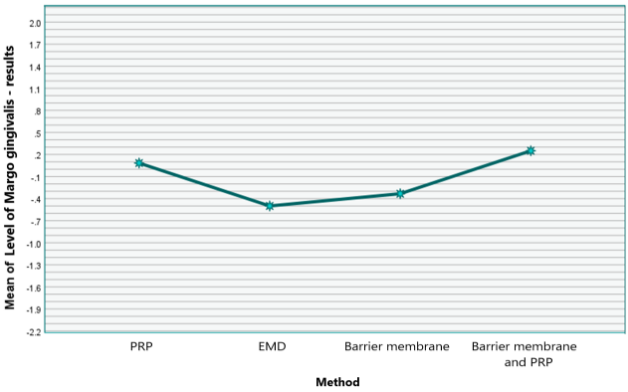

Figure 40

The post-hoc test using Tukey also confirms the above thesis (Table 8).

Multiple Comparisons

Dependent Variable: Level of Margo gingivalis - results

Tukey HSD

(I) Method	(J) Method	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
PRP	EMD	.583	.353	.360	-.36	1.52
	Barrier membrane	.417	.353	.642	-.52	1.36
	Barrier membrane and PRP	-.167	.353	.965	-1.11	.77
EMD	PRP	-.583	.353	.360	-1.52	.36
	Barrier membrane	-.167	.353	.965	-1.11	.77
	Barrier membrane and PRP	-.750	.353	.161	-1.69	.19
Barrier membrane	PRP	-.417	.353	.642	-1.36	.52
	EMD	.167	.353	.965	-.77	1.11
	Barrier membrane and PRP	-.583	.353	.360	-1.52	.36
Barrier membrane and PRP	PRP	.167	.353	.965	-.77	1.11
	EMD	.750	.353	.161	-.19	1.69
	Barrier membrane	.583	.353	.360	-.36	1.52

Table 6

From the descriptive table, it is found that in the patients from Task 1, the gingival margin migrated coronally by 0.08mm on average. In the Task 2 patients, the gingival margin migrated apically by 0.5 mm on average. For the patients from Task 3.1. the gingival margin migrated apically by an average of 0.33 mm. In patients from task 3.2. the gingival margin has migrated coronally by an average of 0.25 mm. (Table 9).

Descriptives

Level of Margo gingivalis - results

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
PRP	12	.08	.996	.288	-.55	.72	-1	2
EMD	12	-.50	.905	.261	-1.07	.07	-2	1
Barrier membrane	12	-.33	.778	.225	-.83	.16	-1	1
Barrier membrane and PRP	12	.25	.754	.218	-.23	.73	-1	2
Total	48	-.12	.890	.128	-.38	.13	-2	2

Table 8

Clinical level of attachment

From Figure 39 and Table 10, it is clearly seen that when registering the periodontal status during the Hygiene phase, the "Clinical level of attachment" indicator for the patients from task 1 is 7.67mm; in the patients from task 2 it is 7.58mm; in patients from task 3.1. is 7.92 mm and the patients from task 3.2. is 8.83mm average. On the sixth month after the regenerative therapy (regardless of the applied method) **, a significant reduction of these values** was found , i.e. acquisition of a clinical level of attachment (task 1 – 3.75mm; task 2 – 3.58mm; task 3.1. – 4 .08mm and task 3.2. – 4.33mm on average). These results are consistent with numerous studies that have demonstrated that regenerative therapy, regardless of the method used, results in a significant gain at the clinical level of attachment (84, 85, 86, 202, 230, 385).

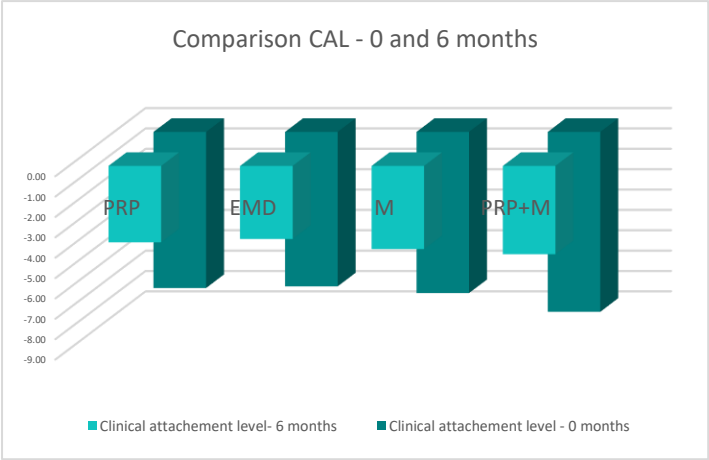

Figure 41

	Clinical level of attachment - medium 0 months	Clinical level of attachment - medium 6 months	Clinical level of attachment - medium Results
Task 1 – PRP (independent)	7.67	3.75	3.92
Task 2 - EMD	7.58	3.58	4.00
Task 3.1. – NTR with Barrier Membrane	7.92	4.08	3.83
Task 3.2. – NTR with PRP and barrier membrane	8.83	4.33	4.50

Table 9

The ANOVA test proves that the results we obtained for the indicator "Clinical attachment level" at the sixth month after regenerative therapy were statistically insignificant between the four groups of patients, F(3,44)=0.795, p=0.503>0.05 (Figure 40 and Table 11).

ANOVA

Clinical attachement level - results

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.229	3	1.076	.795	.503
Within Groups	59.583	44	1.354		
Total	62.813	47			

Table 10

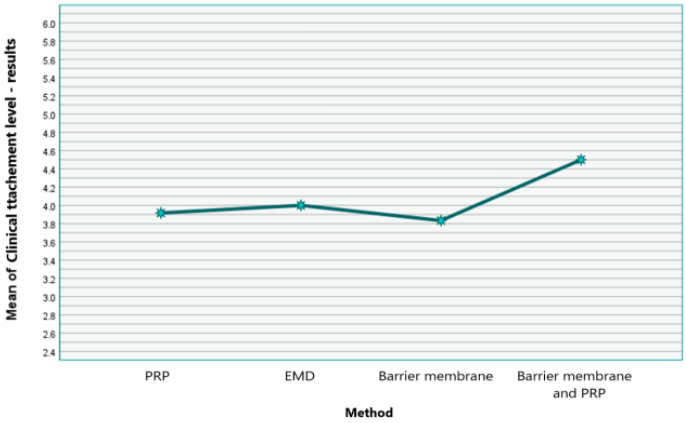

Figure 42

The post-hoc test using Tukey also confirms the above thesis (Table 12).

Multiple Comparisons

Dependent Variable: Clinical attachement level - results

Tukey HSD

(I) Method	(J) Method	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
PRP	EMD	-.083	.475	.998	-1.35	1.19
	Barrier membrane	.083	.475	.998	-1.19	1.35
	Barrier membrane and PRP	-.583	.475	.613	-1.85	.69
EMD	PRP	.083	.475	.998	-1.19	1.35
	Barrier membrane	.167	.475	.985	-1.10	1.44
	Barrier membrane and PRP	-.500	.475	.720	-1.77	.77
Barrier membrane	PRP	-.083	.475	.998	-1.35	1.19
	EMD	-.167	.475	.985	-1.44	1.10
	Barrier membrane and PRP	-.667	.475	.504	-1.94	.60
Barrier membrane and PRP	PRP	.583	.475	.613	-.69	1.85
	EMD	.500	.475	.720	-.77	1.77
	Barrier membrane	.667	.475	.504	-.60	1.94

Table 11

From the descriptive table, it can be seen that Task 1 patients experienced a clinical attachment level gain of 3.92mm on average. In Task 2 patients, a clinical attachment level gain of 4.00mm was observed on average. For the patients from Task 3.1. a clinical attachment level gain of 3.83mm was observed on average. For the patients from Task 3.2. a clinical attachment level gain of 4.50mm on average was observed (Table 13).

Descriptives

Clinical attachement level - results

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
PRP	12	3.92	.996	.288	3.28	4.55	3	6
EMD	12	4.00	1.595	.461	2.99	5.01	1	6
Barrier membrane	12	3.83	.835	.241	3.30	4.36	3	5
Barrier membrane and PRP	12	4.50	1.087	.314	3.81	5.19	3	6
Total	48	4.06	1.156	.167	3.73	4.40	1	6

Table 12

A – The distance from the ECB to the base of the bone defect (of the CBCT)

From Figure 41, it can be clearly seen that in the CBCT analysis of the indicator "A" immediately before the operative intervention, the average value for the patients from Task 1 - 6.37mm, for the patients from Task 2 - 5.85mm, for the patients from Task 3.1. - 7.14 mm and in the patients from Task 3.2. - 7.15mm. On the sixth month after the regenerative therapy (regardless of the applied method), a reduction of indicator "A" was found compared to the initial values (task 1 - 4.68mm; task 2 - 4.34mm; task 3.1. - 5.46mm and task 3.2. – 5.74mm). The results are presented in detail in Appendix 26.

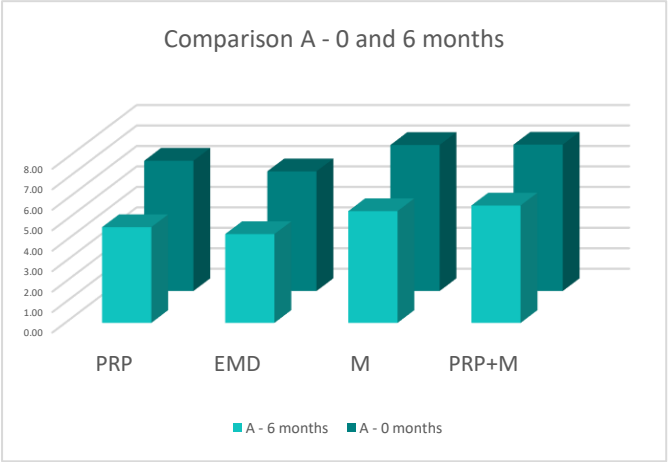

Figure 43

The ANOVA test proves that the results we obtained for the indicator "A" at the sixth month after regenerative therapy were not statistically significant between the four groups of patients, $F(3,44)=0.259$, $p=0.854>0.05$ (Figure 42 and Table 14).

ANOVA

A - results

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.629	3	.210	.259	.854
Within Groups	35.595	44	.809		
Total	36.224	47			

Table 14

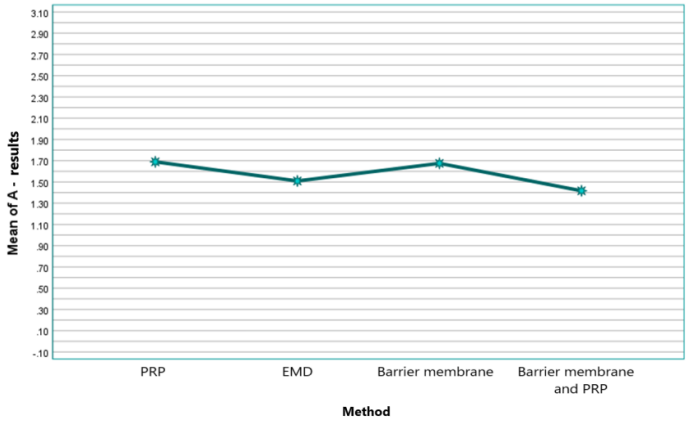

Figure 44

The post-hoc test using Tukey also confirms the above thesis (Table 15).

Multiple Comparisons

Dependent Variable: A - results

Tukey HSD

(I) Method	(J) Method	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
PRP	EMD	.18000	.36719	.961	-.8004	1.1604
	Barrier membrane	.01417	.36719	1.000	-.9662	.9946
	Barrier membrane and PRP	.27250	.36719	.880	-.7079	1.2529
EMD	PRP	-.18000	.36719	.961	-1.1604	.8004
	Barrier membrane	-.16583	.36719	.969	-1.1462	.8146
	Barrier membrane and PRP	.09250	.36719	.994	-.8879	1.0729
Barrier membrane	PRP	-.01417	.36719	1.000	-.9946	.9662
	EMD	.16583	.36719	.969	-.8146	1.1462
	Barrier membrane and PRP	.25833	.36719	.895	-.7221	1.2387
Barrier membrane and PRP	PRP	-.27250	.36719	.880	-1.2529	.7079
	EMD	-.09250	.36719	.994	-1.0729	.8879
	Barrier membrane	-.25833	.36719	.895	-1.2387	.7221

Table 13

From the descriptive table, it is established that in the patients of Task 1, the distance from the ECB to the base of the bone defect was reduced by 1.69 mm on average in six months. In Task 2 patients, the distance from the ECB to the base of the bone defect was reduced by 1.51mm on average over six months. In patients from task 3.1. the distance from the ECB to the base of the bone defect was reduced by 1.68mm on average over six months. In patients from task 3.2. the distance from the ECB to the base of the bone defect was reduced by 1.42mm on average over six months. This means that bone filling was observed in all patients (Table 16).

Descriptives

A - results

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
PRP	12	1.6892	.75883	.21906	1.2070	2.1713	.47	3.07
EMD	12	1.5092	.69571	.20083	1.0671	1.9512	.49	2.95
Barrier membrane	12	1.6750	.91540	.26425	1.0934	2.2566	.41	3.29
Barrier membrane and PRP	12	1.4167	1.15676	.33393	.6817	2.1516	.65	4.76
Total	48	1.5725	.87790	.12671	1.3176	1.8274	.41	4.76

Table 15

B - The distance from the ECB to the highest located bone point from the bone defect (of the CBCT)

From Figure 43, it is clearly seen that in the CBCT analysis of the indicator "B" immediately before the operative intervention, the average value for the patients from Task 1 - 3.19mm, for the patients from Task 2 - 2.64mm, for the patients from Task 3.1. - 3.61mm and in the patients from Task 3.2. - 3.31 mm. On the sixth month after the regenerative therapy (regardless of the applied method), a reduction of the "B" indicator was found compared to the initial values (task 1 - 2.69mm; task 2 - 2.14mm; task 3.1. - 3.12mm and task 3.2. – 2.93mm). The results are presented in detail in Appendix 26.

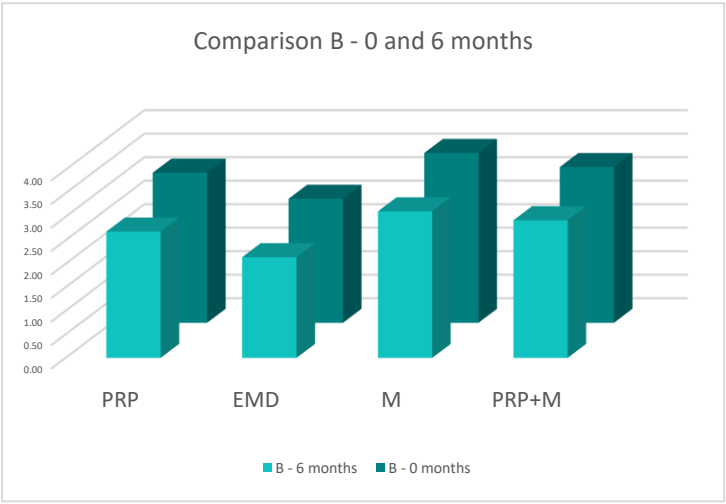

Figure 45

The ANOVA test proves that the results we obtained for the indicator "B" at the sixth month after regenerative therapy were not statistically significant between the four groups of patients, $F(3,44)=0.233$, $p=0.873>0.05$ (Figure 44 and Table 17).

ANOVA

B - results

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.118	3	.039	.233	.873
Within Groups	7.448	44	.169		
Total	7.566	47			

Table 17

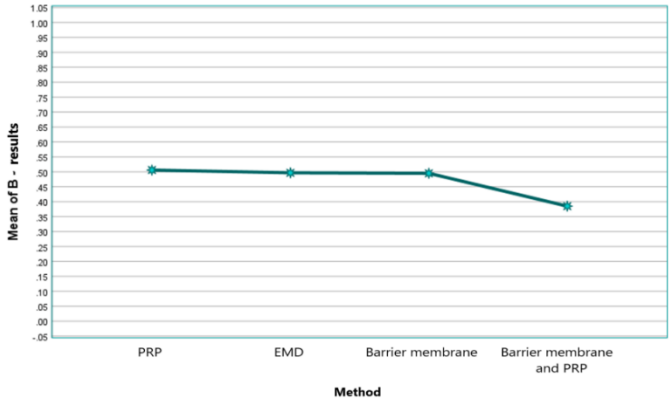

Figure 46

The post-hoc test using Tukey also confirms the above thesis (Table 18).

Multiple Comparisons

Dependent Variable: B - results

Tukey HSD

(I) Method	(J) Method	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
PRP	EMD	.00917	.16796	1.000	-.4393	.4576
	Barrier membrane	.01083	.16796	1.000	-.4376	.4593
	Barrier membrane and PRP	.12083	.16796	.889	-.3276	.5693
EMD	PRP	-.00917	.16796	1.000	-.4576	.4393
	Barrier membrane	.00167	.16796	1.000	-.4468	.4501
	Barrier membrane and PRP	.11167	.16796	.910	-.3368	.5601
Barrier membrane	PRP	-.01083	.16796	1.000	-.4593	.4376
	EMD	-.00167	.16796	1.000	-.4501	.4468
	Barrier membrane and PRP	.11000	.16796	.913	-.3385	.5585
Barrier membrane and PRP	PRP	-.12083	.16796	.889	-.5693	.3276
	EMD	-.11167	.16796	.910	-.5601	.3368
	Barrier membrane	-.11000	.16796	.913	-.5585	.3385

Table 16

From the descriptive table, it is established that in the patients of Task 1, the distance from the ECB to the highest bone point of the bone defect was reduced by 0.51 mm on average in six months. In the Task 2 patients, the distance from the ECB to the highest bone point from the bone defect reduced by 0.50mm on average over six months. In patients from task 3.1. the distance from the ECB to the highest bone point of the bone defect was reduced by 0.50 mm on average in six months. In patients from task 3.2. the distance from the ECB to the highest bone point from the bone defect was reduced by 0.39mm on average over six months. This means that bone filling was observed in all patients (Table 19).

Descriptives

B - results

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
PRP	12	.5058	.48056	.13873	.2005	.8112	.02	1.40
EMD	12	.4967	.19874	.05737	.3704	.6229	.23	.90
Barrier membrane	12	.4950	.35436	.10230	.2698	.7202	.05	1.12
Barrier membrane and PRP	12	.3850	.53015	.15304	.0482	.7218	-.36	1.59
Total	48	.4706	.40122	.05791	.3541	.5871	-.36	1.59

Table 18

C – Width of the bone defect (of the CBCT)

From Figure 45, it is clearly seen that in the CBCT analysis the indicator "C" immediately before the operative intervention has an average value for the patients from Task 1 - 2.37mm, for the patients from Task 2 - 2.07mm, for the patients from Task 3.1. is 1.89mm and in the patients from Task 3.2. is 2.37mm. On the sixth month after the regenerative therapy (regardless of the applied method), a reduction of the "C" indicator was found compared to the initial values (task 1 - 2.01mm; task 2 - 1.84mm; task 3.1. - 1.78mm and task 3.2. – 1.86mm). The results are presented in detail in Appendix 26.

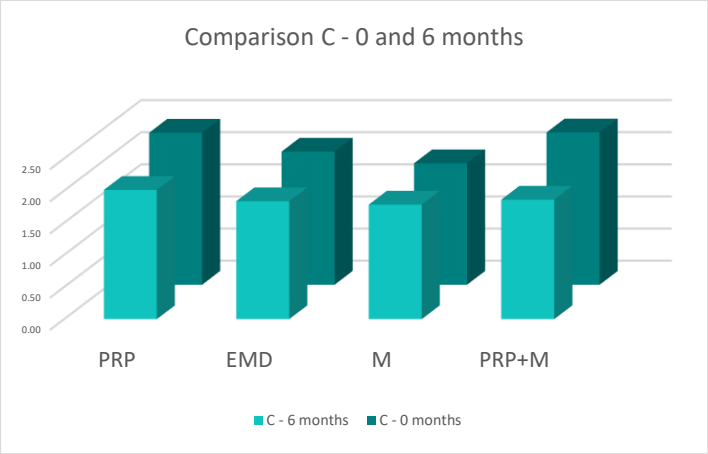

Figure 47

The ANOVA test proves that the results we obtained for the indicator "C" at the sixth month after regenerative therapy were not statistically significant between the four groups of patients, $F(3,44)=0.758$, $p=0.523>0.05$ (Figure 46 and Table 20).

ANOVA

C - results

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.064	3	.355	.758	.523
Within Groups	20.567	44	.467		
Total	21.631	47			

Table 20

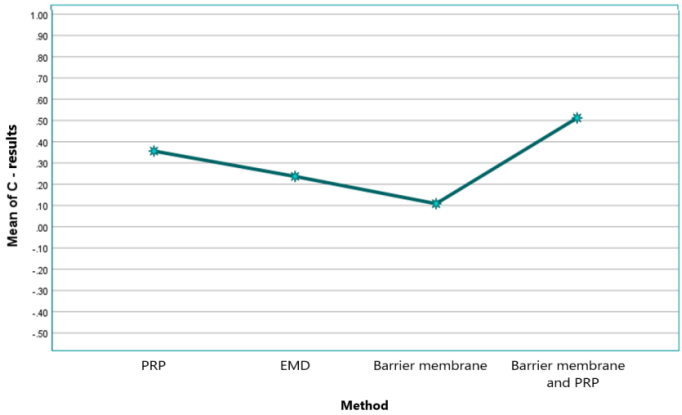

Figure 48

The post-hoc test using Tukey also confirms the above thesis (Table 21).

Multiple Comparisons

Dependent Variable: C - results

Tukey HSD

(I) Method	(J) Method	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
PRP	EMD	.11917	.27912	.974	-.6261	.8644
	Barrier membrane	.24750	.27912	.812	-.4977	.9927
	Barrier membrane and PRP	-.15583	.27912	.944	-.9011	.5894
EMD	PRP	-.11917	.27912	.974	-.8644	.6261
	Barrier membrane	.12833	.27912	.967	-.6169	.8736
	Barrier membrane and PRP	-.27500	.27912	.759	-1.0202	.4702
Barrier membrane	PRP	-.24750	.27912	.812	-.9927	.4977
	EMD	-.12833	.27912	.967	-.8736	.6169
	Barrier membrane and PRP	-.40333	.27912	.479	-1.1486	.3419
Barrier membrane and PRP	PRP	.15583	.27912	.944	-.5894	.9011
	EMD	.27500	.27912	.759	-.4702	1.0202
	Barrier membrane	.40333	.27912	.479	-.3419	1.1486

Table 19

From the descriptive table, it is established that in the patients from Task 1, the width of the bone defect was reduced by 0.36 mm on average in six months. In patients from task 2, the width of the bone defect was reduced by 0.24 mm on average in six months. In patients from task 3.1. the width of the bone defect was reduced by 0.11 mm on average in six months. In patients from task 3.2. the width of the bone defect was reduced by 0.51 mm on average in six months. This means that bone filling was observed in all patients (Table 22).

Descriptives

C - results

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
PRP	12	.3558	.69749	.20135	-.0873	.7990	.00	2.40
EMD	12	.2367	.51215	.14784	-.0887	.5621	.00	1.84
Barrier membrane	12	.1083	.09370	.02705	.0488	.1679	.00	.30
Barrier membrane and PRP	12	.5117	1.05459	.30443	-.1584	1.1817	.00	3.81
Total	48	.3031	.67840	.09792	.1061	.5001	.00	3.81

Table 21

3.2.1. DISCUSSION ON TASK #1

The clinical results obtained at the sixth month after regenerative therapy with autogenous platelet-rich plasma in vertical bone defects reported a decrease in probing depth by 3.83 mm on average, coronal migration of the gingival margin by 0.08 mm on average, acquisition of clinical level of attachment of 3.92mm avg. Bone filling is observed at the CBCT, which is confirmed by the parameters: A – decreases on average by 1.69 mm; B – decreases on average by 0.51mm and C – decreases on average by 0.36mm.

When analyzing the indicators "Clinical level of attachment" at the stage "0 months" and at the stage "6 months after regenerative therapy with autogenous platelet-rich plasma", it is clear that the distance from the gingival margin to the bottom of the pocket decreased on average by 3.917mm, i.e. acquisition of a clinical level of attachment has occurred. At the same time, when analyzing the indicators "A" at the stage "0 months" and at the stage "6 months after the regenerative therapy with autogenous, platelet-rich plasma", it is clear that the distance from the ECB to the base of the bone defect measured on the CBCT was reduced by an average of 1.689mm. This means that **a greater percentage of soft tissue gained than regenerated bone** was observed (3.917-1.689 = 2.228mm).

The analysis of the data from task 1 demonstrates that carrying out regenerative therapy with autogenous, platelet-rich plasma in vertical bone defects is a reliable method, the results of which (clinical and paraclinical) are comparable to those of regenerative therapy with EMD, in guided tissue regeneration with barrier membrane and in guided tissue regeneration with barrier membrane and autogenous platelet-rich plasma. At this

stage, there are still not enough publications regarding the efficacy of PRP as a self-administered material in periodontal therapy, with the exception of a publication from 2018. of Jalaluddin et al. (158) who compared the regenerative efficacy of PRP (alone) and bone repair material in vertical bone defects. The authors concluded that both groups showed promising results after undergoing regenerative therapy.

The results we present demonstrate the potential of autogenous platelet-rich plasma (PRP) as a stand-alone material in periodontal regenerative therapy for vertical bone defects.

3.2.2. DISCUSSION ON TASK #2

The clinical results obtained at the sixth month after regenerative therapy with enamel matrix derivatives in vertical bone defects reported a decrease in probing depth by 4.50 mm on average, apical migration of the gingival margin by 0.5 mm on average, acquisition of a clinical attachment level of 4, 00mm average. Bone filling was observed on the CBCT, which is confirmed by the parameters: A – decreases on average by 1.51 mm; B – decreases on average by 0.50mm and C – decreases on average by 0.24mm.

When analyzing the indicators "Clinical attachment level" at the stage "0 months" and at the stage "6 months" after regenerative therapy with enamel matrix derivatives (EMD), it is clear that the distance from the gingival margin to the bottom of the pocket decreased on average by 4,000mm, i.e. acquisition of a clinical level of attachment has occurred. At the same time, when analyzing the indicators "A" at the stage "0 months" and at the stage "6 months" after the regenerative therapy with enamel matrix derivatives, it is clear that the distance from the ECB to the base of the bone defect, measured on the CBCT, decreased by an average 1.509mm. This means that **a greater percentage of soft tissue gained than regenerated bone** was observed (4,000-1,509 = 2,491mm).

The analysis of the data from task 2 confirms the results obtained from a number of studies related to the regenerative therapy using enamel matrix derivatives in vertical bone defects (132, 351, 355, 356).

The results presented by us confirm the already repeatedly proven biologically active qualities of enamel matrix derivatives, which is why we accept regenerative

therapy with the help of EMD as a standardized method today, used daily in practices.

3.2.3. DISCUSSION OF TASK #3

3.3.3.1. DISCUSSION ON TASK No. 3.1.

The clinical results obtained at the sixth month after guided tissue regeneration with a barrier membrane in vertical bone defects reported a decrease in probing depth by 4.17 mm on average, apical migration of the gingival margin by 0.33 mm on average, acquisition of a clinical attachment level of 3, 83mm average. Bone filling is observed on the CBCT, which is confirmed by the parameters: A – decreases on average by 1.68 mm; B – decreases on average by 0.50mm and C – decreases on average by 0.11mm.

When analyzing the indicators "Clinical attachment level" at the stage "0 months" and at the stage "6 months" after guided tissue regeneration with a barrier membrane, it is clear that the distance from the gingival margin to the bottom of the pocket decreased by an average of 3.833 mm, i.e. acquisition of a clinical level of attachment has occurred. At the same time, when analyzing the indicators "A" at the stage "0 months" and at the stage "6 months" after guided tissue regeneration with a barrier membrane, it is clear that the distance from the ECB to the base of the bone defect measured on the CBCT decreased by an average 1.675mm. This means that **a greater percentage of soft tissue gained than regenerated bone** was observed (3.833-1.675 = 2.158mm).

The analysis of the data from task 3.1. confirms the results obtained from a number of studies aimed at guided tissue regeneration with a barrier membrane in vertical bone defects (68, 69, 371).

The clinical and imaging results we present confirm the effect of barrier membranes described in a number of other studies.

3.3.3.2. DISCUSSION ON TASK No. 3.2.

The clinical results obtained at the sixth month after regenerative therapy with autogenous platelet-rich plasma in vertical bone defects reported a decrease in probing depth by 4.25 mm on average, coronal migration of the gingival margin by 0.25 mm on average, acquisition of a clinical level of attachment of 4.50mm avg. Bone filling was observed on the CBCT, which is confirmed by the parameters: A – decreased by an average of 1.42 mm; B – decreases on average by 0.39mm and C – decreases on average by 0.51mm mm.

In the analysis of the indicators "Clinical attachment level" at the stage "0 months" and at the stage "6 months" after guided tissue regeneration with a barrier membrane and autogenous platelet-rich plasma, it is clear that the distance from the gingival margin to the bottom of the pocket has reduced on average by 4,500mm, i.e. acquisition of a clinical level of attachment has occurred. At the same time, when analyzing the indicators "A" at the stage "0 months" and at the stage "6 months" after guided tissue regeneration with a barrier membrane, it is clear that the distance from the ECB to the base of the bone defect measured on the CBCT decreased by an average 1.417mm. This means that **a greater percentage of soft tissue gained than regenerated bone** was observed (4,500-1,417 = 3,083mm).

The analysis of the data from task 3.2. demonstrated that performing guided tissue regeneration with barrier membrane and autogenous platelet-rich plasma in vertical bone defects is a reliable method, the clinical results of which exceed those of guided tissue regeneration with barrier membrane alone.

At this stage, **no studies were found that tested the potential of the combined administration of autogenous platelet-rich plasma and barrier membrane.** All available studies on the topic investigated the effectiveness of the combined administration of autogenous platelet-rich plasma, barrier membrane and bone repair material (57, 84, 85, 87) or the combined administration of autogenous platelet-rich plasma and bone repair material (180, 363, 385).

The results we present demonstrate the synergistic potential of the undeniable role of the barrier membrane and the qualities of autogenous platelet-rich plasma (PRP).

4. CONCLUSION

Today, the main goal of periodontal treatment consists in the elimination of periodontopathogenic microorganisms and regeneration of lost periodontal structures. Restoring lost tissue as a result of periodontitis is a serious problem that every dental practitioner faces on a daily basis. Vertical bone defects are an inevitable consequence of periodontitis if timely measures are not taken to stop the inflammatory process.

The presence of a vertical bone defect significantly increases the risk of disease progression. This requires a quick and accurate diagnosis. Today, the diagnosis of such defects is carried out clinically and confirmed with the help of cone beam computed tomography (CBCT). CBCT has many advantages over two-dimensional X-ray studies, which is why it is the imaging modality of choice for recording our results.

Over the years, various surgical techniques and materials have been established for the regeneration of periodontal bone defects. Nowadays, the focus is on the development of improved biomaterials to provide even better results in regenerative therapy. Our study confirms the undeniable qualities of some of the biomaterials (enamel matrix derivatives and barrier membranes) that we use continuously in our practice.

Our present study found that there were no statistically significant differences in the clinical and imaging outcomes of the four groups of patients. Thus, we can conclude that autogenous platelet-rich plasma (PRP) and enamel matrix derivatives are materials that demonstrate equally good results as Guided Tissue Regeneration. Here it is necessary to clarify that the use of PRP and EMD in periodontal regenerative therapy has a much

easier surgical protocol compared to GTR and at the same time postoperative complications are less frequent compared to those in GTR (most often bone exfoliation the restorative material and/or exposure of the barrier membrane, which is associated with bacterial contamination). All this gives us reason to conclude that **our preferred methods should be regenerative therapy using PRP or regenerative therapy using EMD over GTR in vertical bone defects.**

The results of the dissertation mainly demonstrate the remarkable potential of autogenous platelet-rich plasma (PRP) as a material for periodontal regenerative therapy. Statistical processing of the data from the study demonstrated the achievement of similar results among all four groups of patients, proving that autogenous platelet-rich plasma (PRP) is a material possessing qualities that can displace previously proven biomaterials used as "gold standard" in periodontal regenerative therapy. However, we must add that the production of the PRP material is more labor-intensive and is associated with possible errors in the methodology. However, the leading advantage of this method is the more affordable price.

The effectiveness of the application of regenerative therapy using enamel matrix derivatives has been repeatedly proven. This surgical method is the easiest to implement, which is why we accept it today as standardized. Its disadvantage compared to alternatively researched material (PRP) is the cost of consumables.

The results of our clinical-statistical study require follow-up over time to be able to evaluate and analyze the data after a longer period of time and in a larger number of patients.

5. CONCLUSIONS

1. Half of the examined patients were under 45 years of age.
2. The distribution of the included participants by gender is as follows - women 56% and men 44%.
3. 50% of teeth with vertical bone defects are molars, followed by premolars with 29.17% and least in the area of frontal teeth – 20.83%.
4. In all studied patients of the four groups, an extremely high percentage of plaque and gingival index was observed immediately before the start of periodontal treatment. At the "Reevaluation" stage, a significant decrease in the values for both indices was observed in all four groups, but at the "Six months after regenerative therapy" stage, a rise in the results of the plaque index and, respectively, the gingival index was observed again.
5. In all studied patients from the four groups, a statistically significant reduction of the "Depth of probing" indicator was found.
6. In all studied patients from the four groups, no statistically significant results were established regarding the indicator "Level of Margo gingivalis".
7. A statistically significant gain in the clinical level of attachment was found in all studied patients from the four groups.
8. In all studied patients from the four groups, a statistically significant reduction of the distance from the ECB to the base of the bone defect was found (CBCT indicator "A").
9. In all studied patients from the four groups, a statistically significant reduction of the distance from the ECB to the highest bone point of the bone defect (CBCT indicator "B") was found.

10. In all studied patients from the four groups regarding the width of the bone defect (CBCT indicator "C") results on the borderline between statistical significance and non-significance are established.
11. In all examined patients - regardless of the applied method of regenerative therapy, an improvement in clinical and imaging indicators was observed. No statistically significant differences were found in the results of the four groups of patients.

CONTRIBUTIONS

Original contributions

1. For the first time, the effectiveness of the application of guided tissue regeneration with a barrier membrane and autogenous platelet-rich plasma in vertical bone defects is investigated.
2. For the first time, clinical and CBCT results of separately use of autogenous platelet-rich plasma (PRP) and enamel matrix derivatives (EMD) in regenerative therapy of vertical bone defects are compared.
3. For the first time, clinical and CBCT outcomes of guided tissue regeneration with barrier membrane alone and with barrier membrane and autogenous platelet-rich plasma (PRP) are compared .

Original contributions to the country

1. For the first time, the effectiveness of the application of autogenous, platelet-rich plasma (on its own) in the regenerative therapy of vertical bone defects was investigated.

Affirmative Contributions

1. We have confirmed the high potential of autogenous platelet-rich plasma (PRP) in regenerative therapy.
2. We have confirmed the proven effectiveness of the application of enamel matrix derivatives (EMD) in the regenerative therapy of vertical bone defects.

3. We confirmed the proven effectiveness of barrier membrane alone in guided tissue regeneration of vertical bone defects.
4. We have confirmed that, regardless of the performed method (of the four studied) of regenerative therapy in vertical bone defects, a significant improvement in clinical and paraclinical indicators is observed.

Publications related to the dissertation work

1. Gerova-Vatsova, T., Peev, S.” Application of autogenous platelet-rich plasma in periodontology.” Scripta Scientifica Medicinae Dentalis [Online], 9.1 (2023): 43-50.

2. Gerova, T., Miteva M. "Barrier membranes used in guided tissue regeneration-advantages and disadvantages." Int J Sci Res 8.10 (2019): 1472-75.

3. Miteva, M., Gerova T. "Bone repair materials used in guided tissue regeneration-advantages and disadvantages." Int J Sci Res 8.10 (2019): 1490-94.

4. Gerova, T., Miteva M. "Application of two-dimensional radiography and CBCT in periodontology." Int J Sci Res 8.11 (2019): 61-5.

5. Gerova, T., Miteva M. "The role of CBCT-imaging technique in periodontology." International J Sci Res 8.11 (2019): 51-4.