



**PROSPERITAS VESTRA FINIS NOSTRA!**

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**RIDGE PRESERVATION USING GUIDED  
REGENERATION, FREE GINGIVAL GRAFTS, AND  
PLATELET-RICH PLASMA**

**THESIS SUMMARY**

**Research Supervisor:**

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**Varna 2023**

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### **Abbreviations used:**

BGM	bone graft material
CBCT	cone-beam computed tomography
FGG	free gingival graft
Fig.	figure
mGy*cm <sup>3</sup>	milligray cubic centimeter
PPP	platelet-poor plasma
PRP	platelet-rich plasma
PTFE	polytetrafluorethylene
RP	ridge preservation

## **Introduction**

Dental implantology is an innovative specialty that provides full and adequate rehabilitation of the masticatory system and its functions. It is developing at an extremely rapid pace and gives incomparable results in both partial and complete edentulism. Thanks to it, cases that were regarded as insurmountable in the past have found a successful and satisfactory solution.

The establishment of sufficient hard and soft tissue volume is a key prerequisite for successful implant therapy, especially when intraosseous implants are used. There are various reasons for bone and soft-tissue deficiency: anatomical variations; pathological processes of endodontic, periodontal, or mixed nature; traumatic extraction or inability to preserve the bone plates around the dental socket due to an advanced inflammatory process; delayed implant and prosthetic rehabilitation leading to afunctional atrophy of the alveolar ridge.

Numerous techniques are used to address these challenges and create optimal conditions for implantation with sufficient hard and soft tissue volume and, accordingly, functional and aesthetic results.

There are three groups of methods for addressing bone deficiency.

The first group includes measures that minimize bone loss after extraction, such as immediate and early implantation and guided bone regeneration in post-extraction sites or the so-called "ridge preservation" techniques.

The second group includes bone augmentation methods, and the third group includes alternatives to intraosseous implants combined with bone augmentation.

To provide suitable conditions for the subsequent dental restoration, each extraction should be as atraumatic as possible. This aims to preserve the available tissue volume along with the elimination of the pathological process. The next step is guided regeneration in the post-extraction sites, aiming to minimize the expected bone resorption.

Historically, ridge preservation has been described as a method of increasing tissue volume to retain immediate dentures (WD Loo,1968). Today it is mainly applied for the purpose - to limit external resorption of the alveolar ridge and to stimulate bone formation in the socket.

## **Aim and tasks**

### **Aim:**

The present dissertation aims to investigate the influence of platelet-rich plasma, non-porous PTFE barrier membranes, and autogenous free gingival grafts on healing processes in post-extraction sites.

### **Tasks:**

1. To investigate the application of non-porous dense PTFE membranes for guided regeneration in the post-extraction sockets of the posterior teeth (premolars and molars).
  - 1.1. To perform a quantitative evaluation of the bone in the post-extraction sites by measuring the height and width of the alveolar crest immediately after the extraction and the height of the alveolar crest three months after the extraction.
  - 1.2. To analyze the relationship between the quantitative change in the height of the bone plates after three months and some factors (group of teeth, maxillary/mandibular teeth, dental diagnosis, smoking).
  - 1.3. To determine whether the change in height is the same for both bone plates and whether there is a relationship between the width of the bone plates (buccal and palatal/lingual) measured immediately after extraction and the quantitative change in their height after 3 months.
2. To investigate the application of non-porous dense PTFE membranes in combination with platelet-rich plasma for guided regeneration in the post-extraction sockets of the posterior teeth (premolars and molars).
  - 2.1. To perform a quantitative evaluation of the bone in the post-extraction sites by measuring the height and width of the alveolar

- crest immediately after the extraction and the height of the alveolar crest three months after the extraction.
- 2.2. To analyze the relationship between the quantitative change in the height of the bone plates after three months and some factors (group of teeth, maxillary/mandibular teeth, dental diagnosis, smoking).
  - 2.3. To determine whether the change in height is the same for both bone plates and whether there is a relationship between the width of the bone plates (buccal and palatal/lingual) measured immediately after extraction and the quantitative change in their height after 3 months.
  3. To investigate the influence of autogenous free gingival grafts on bone regeneration in the post-extraction sockets of the posterior teeth (premolars and molars).
    - 3.1. To perform a quantitative evaluation of the bone in the post-extraction sites by measuring the height and width of the alveolar crest immediately after the extraction and the height of the alveolar crest three months after the extraction.
    - 3.2. To analyze the relationship between the quantitative change in the height of the bone plates after three months and some factors (group of teeth, maxillary/mandibular teeth, dental diagnosis, smoking).
    - 3.3. To determine whether the change in height is the same for both bone plates and whether there is a relationship between the width of the bone plates (buccal and palatal/lingual) measured immediately after extraction and the quantitative change in their height after 3 months.
  4. To investigate bone regeneration in the post-extraction sockets of the posterior teeth (premolars and molars) without the application of any ridge preservation procedures (control group).

- 4.1. To perform a quantitative evaluation of the bone in the post-extraction sites by measuring the height and width of the alveolar crest immediately after the extraction and the height of the alveolar crest three months after the extraction.
- 4.2. To analyze the relationship between the quantitative change in the height of the bone plates after three months and some factors (group of teeth, maxillary/mandibular teeth, dental diagnosis, smoking).
- 4.3. To determine whether the change in height is the same for both bone plates and whether there is a relationship between the width of the bone plates (buccal and palatal/lingual) measured immediately after extraction and the quantitative change in their height after 3 months.



## **Own Research**

### **Material and methods**

The present study was conducted on the territory of the University Medical and Dental Center and the Faculty of Dental Medicine at the Medical University of Varna "Prof. Dr. Paraskev Stoyanov" for the period June 2022 - April 2023.

The study included 80 patients (fig. 1) aged between 26 and 65 years, divided into 3 age groups according to the WHO classification (fig. 2). A total of 80 teeth were extracted - 34 premolars and 46 molars. The teeth in the present study were divided into 2 groups according to their primary diagnosis: chronic periodontal disease - profunda and periapical lesions (periapical granulomas, periapical cysts, and periapical lesions of diffuse origin). Patients were allocated to four groups by the method of randomization (lottery method). The first group included post-extraction sites where ridge preservation was performed using non-porous dense PTFE membranes while in the second group, we used a combination of these membranes and PRP. In the third group, we performed RP with free gingival grafts. In the fourth group, no RP procedures were applied and it served as a control.

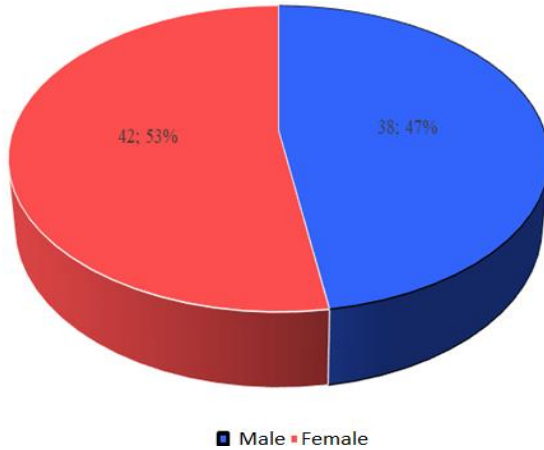


Fig. 1. Gender distribution.

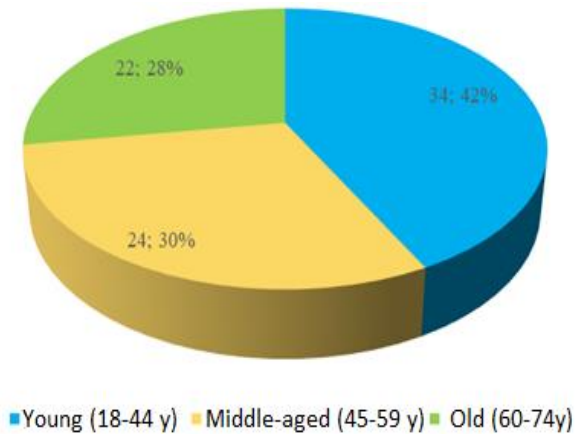


Fig.2 Age distribution.

**Patient Selection:**

The selection was made after careful evaluation of the indications for the treatment and when no general and local contraindications were registered.

**Inclusion criteria:**

1. Patients aged between 18 and 65 years.
2. Patients in good general condition.
3. Patients requiring extraction of premolars or molars.
4. When the post-extraction sites will be restored by a dental implant and/or prosthetic construction.
5. Signed informed consent for participation in the study.

**Exclusion criteria:**

1. Patients with contraindications for general surgical treatment:
  - patients with uncontrolled arterial hypertension;
  - patients with uncontrolled metabolic diseases;
  - patients with AMI in the last 6 months;
  - patients on hemodialysis;
  - patients who underwent chemotherapy and/or radiotherapy in the last year;
  - patients on immunosuppressive therapy;
  - patients on anti-resorptive and anti-angiogenic therapy;
  - patients with concomitant acute diseases at the time of the study;
  - patients with proven psychiatric diseases;
  - pregnancy.
2. Local contraindications:
  - insufficient bone volume after the extraction;
  - acute inflammatory disease or tumor process in the area;
3. Lack of informed consent.
4. Lack of patient motivation to carry out the treatment.

### **Clinical examination:**

After a detailed anamnesis and a thorough clinical examination, we identified the indications and contraindications for inclusion in the study. If the presence of diseases and conditions that pose a risk when performing ambulatory surgery was suspected, consultation with the appropriate medical specialists was required. From the socioeconomic history, special attention was paid to smoking as a bad habit, and the number of cigarettes the patients smoked per day was recorded.

The provisional diagnosis of the tooth was based on the medical history and clinical findings during the examination (which was confirmed or rejected with the subsequent X-ray examination). The indications for the upcoming prosthetic rehabilitation were analyzed, as well as whether there are local contraindications for surgical treatment.

### **Diagnostic imaging:**

Mandatory X-ray examinations include:

- preoperative radiographs - orthopantomography or periapical radiographs, which clarify the diagnosis, anatomical features, and the presence of pathological processes. They give initial information on the existing amount of bone around the tooth before the extraction.

- two postoperative CBCT scans - each with a radiation dose between  $600 \text{ mGy} \cdot \text{cm}^3$  and  $720 \text{ mGy} \cdot \text{cm}^3$  and an exposure time of 12.058-12.080 s. The first is on the day of extraction, and the second – 3 months later. For this purpose, we used the Planmeca ProMax 3D

Max at the Faculty of Dentistry at the Medical University - Varna with Planmeca Romexis image processing software.

On the CBCT scans performed immediately after the manipulation, we made the following measurements:

- We determined the heights of the buccal bone plate ( $A_0$ ) and the lingual/palatal bone plate ( $B_0$ ) on images from paraxial slices in the center of the post-extraction sockets. For this purpose, we drew two straight lines from the highest points of each bone plate to the highest point of the mandibular canal for the teeth of the lower jaw and the lowest point of the maxillary sinus for the maxillary premolars and molars respectively.

- On the same paraxial slice we measured the widths of the buccal bone wall ( $A_w$ ) and the lingual/palatal bone wall ( $B_w$ ). The measurements were taken 3 mm apically from the highest point of the respective bone walls.

- It should be noted that, when the interradicular septum in the molar post-extraction sites was preserved, measurements were performed in both sections of the socket – medial and distal. Thus, the measured areas for the premolars corresponded to their number – 34, while for the molars the measured areas were 62.

As a reference point, we used the abovementioned stable anatomical landmarks instead of the floor of the post-extraction socket. Otherwise, the rapid resorption of the alveolar bone proper (lamina dura) could pose a risk of difficulties and errors in measurement after 3 months.

On the second cone-beam tomography, performed 3 months postoperatively, we measured the height of the bone plates (buccal -  $A_1$  and lingual/palatal -  $B_1$ , respectively) on the same paraxial slice (Fig. 3).

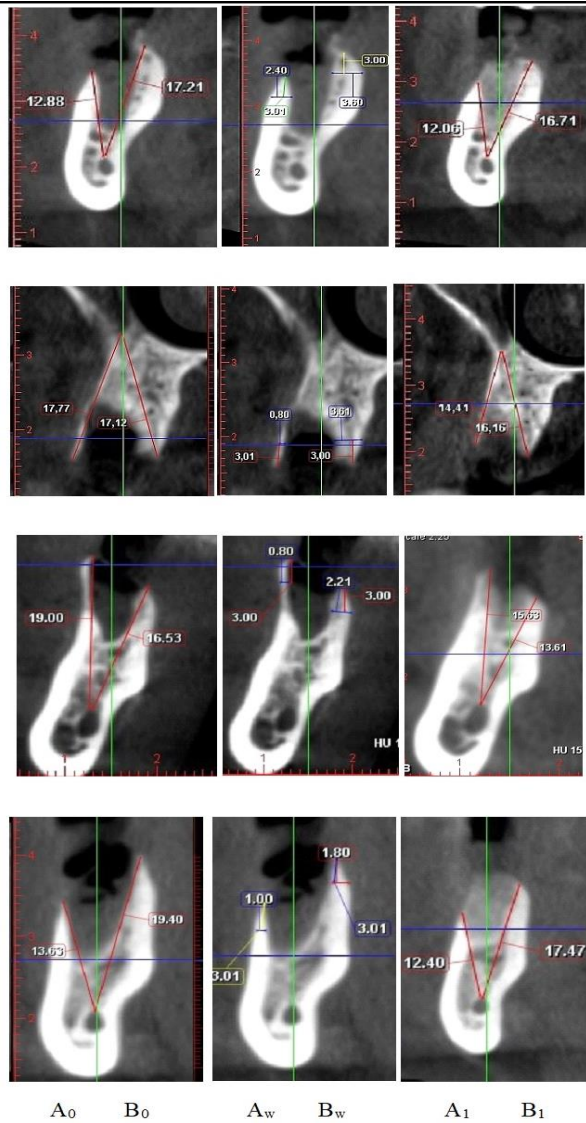


Fig. 3. Measuring the height and width of the bone plates immediately after the extraction and after three months.

### Task 1. Material and Methods.

For task 1, we analyzed the results after the application of the non-porous PTFE membranes as a method of guided regeneration in the post-extraction sockets in 20 teeth of a total of 20 patients aged 26 to 65 years. The average age in the group was  $48.1 \pm 11.34$  years.

In the group 7 (35%) of the patients were male and 13 (65%) were female. There was no statistically significant difference in gender ( $\chi^2 = 1.80$ ,  $P=0.180$ ). Fig.4

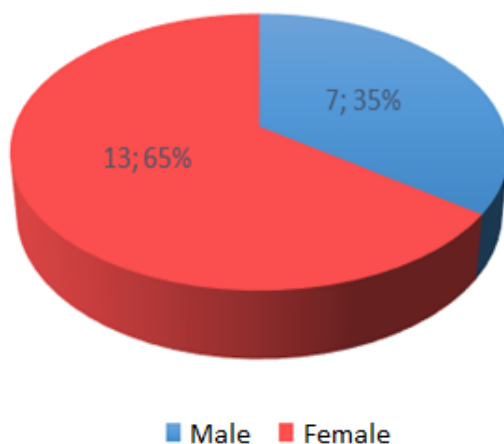


Fig. 4. Gender distribution.

Regarding the smoking status, more patients were smokers than non-smokers but there was no statistically significant difference ( $\chi^2 = 1.80$ ,  $P=0.180$ ). (fig.5)

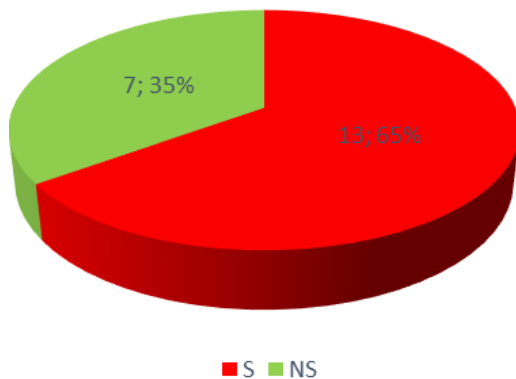


Fig. 5. Distribution of smoking status (S – smokers, NS – non-smokers).

We compared the CBCT images performed on the day of the surgery and 3 months after it. In this experimental group, we observed 20 teeth - 7 premolars and 13 molars. As some molar teeth have preserved interradicular septa, measurements were performed in both medial and distal sections. Thus, this group includes data from 24 post-extraction sites. Diagnosis distribution is presented in Fig. 6.

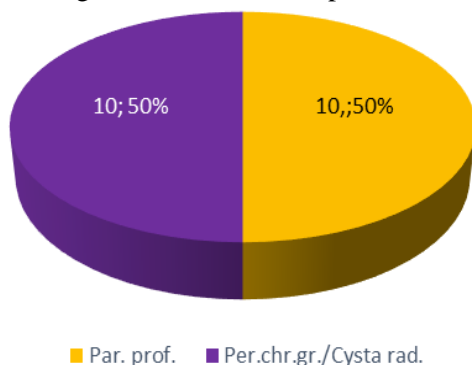


Fig. 6. Diagnosis distribution. Par. prof. – parodontitis profunda; Per. chr. gr.– periodontitis chronica granulomatosa; Cysta rad. – Cysta radicularis.



## Surgical protocol:

Atraumatic extractions, using the necessary surgical instruments (Fig. 8), were performed under local infiltration anesthesia with Dentocaine 40 mg/0.005 mg/ml. When there was a risk of a fracture of the bone walls or the intraradicular septum, multirooted teeth were separated and extracted root by root. Careful curettage of the sockets, smoothing of the bone edges, if necessary, and saline irrigation were performed. Then the soft-tissue edges of the sockets were prepared with a Buser periosteal elevator, forming envelope-shaped mucoperiosteal flaps on both buccal and palatal/lingual sides. The dimensions of the socket orifice were measured, using a periodontal probe, and non-porous PTFE-membranes (permamem®, Botiss biomaterials GmbH, Zossen, Germany) (Fig. 7) were shaped and inserted subperiosteally - 3-5 mm beyond the alveolar walls. The membranes were also tucked under the interdental papillae, but without coming into contact with the adjacent teeth. Crossed mattress sutures were placed over the sockets and, if necessary, some additional interrupted sutures were performed to fix the membranes in place. The suture material we used was 5/0 Polyamide, which is a non-absorbable monofilament. The membranes were left partially exposed.



Fig. 7. Dense PTFE membrane – Permamem®.



Fig. 8. Surgical instruments for tooth extraction and RP using dense PTFE membrane.

### **Postoperative care:**

Patients received postoperative instructions similar to those following a routine dental extraction. Antibiotic prophylaxis was prescribed, usually amoxicillin in combination with clavulanic acid (Augmentin 1000 mg) twice per day for 7 days. When the patient was allergic to penicillins, azithromycin 500 mg for 3 days was prescribed, or clindamycin 300 mg twice per day (for 5-7 days). In addition, non-steroidal anti-inflammatory drugs were prescribed, e.g. Aulin 100 mg every 12 hours and mouthwash with 0.12% chlorhexidine.

Sutures were removed on the 14th postoperative day, while the membrane was removed on the 28th day, using a hemostatic tool

or tweezers. If necessary, the area could be anesthetized beforehand with a contact anesthetic - lidocaine 10% pump spray.

Final epithelization of the tissues after removal of the membrane occurs within 4 weeks.

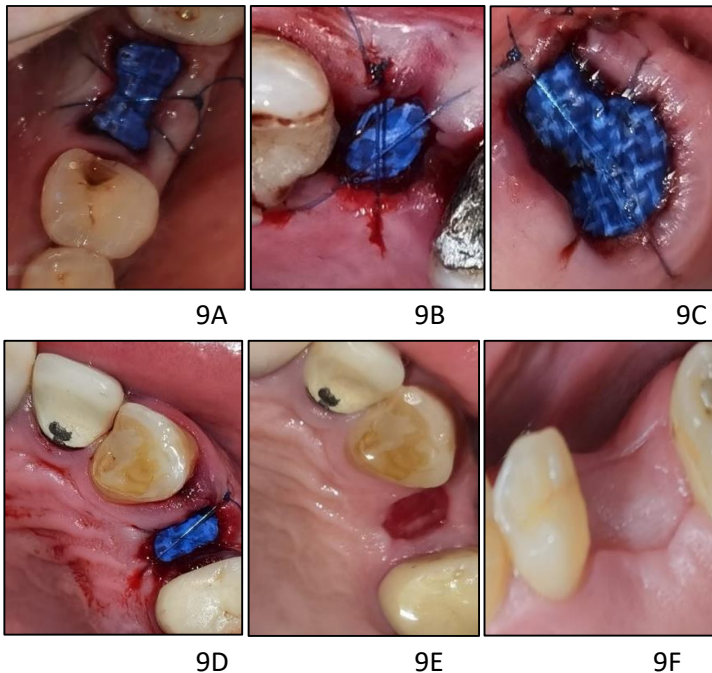


Fig. 9. RP using non-resorbable PTFE membrane. 9A-9D – the day of the procedure; 9E – after removal of the membrane– day 28; 9F – postextraction site covered with epithelium – 3 months after the removal of the membrane.

## Task 2. Material and Methods.

We performed CBCT evaluation of the results after the application of nonporous PTFE membranes combined with PRP as a method for guided regeneration in post-extraction sockets. The experimental group included 20 patients, aged between 29 and 65 years. The average group age was  $48.05 \pm 14.23$  years.

The gender distribution was: 14 males (70%) and 6 females (30%). There was no statistically significant difference in gender ( $\chi^2 = 3.20$ ,  $P=0.074$ ). (Fig. 10)

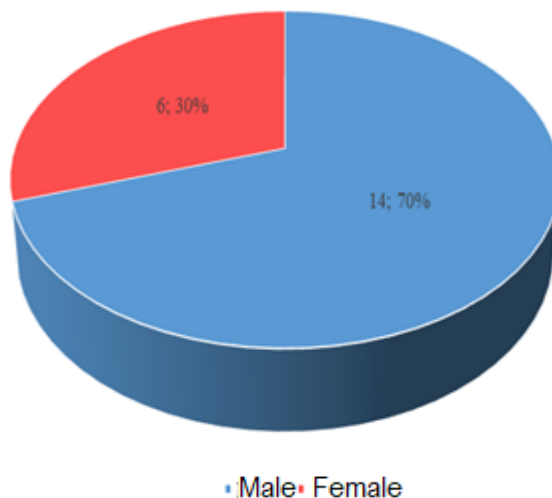


Fig.10. Gender distribution.

Regarding smoking status, the patients in this group are predominantly smokers and there is a statistical significance ( $\chi^2 = 7.20$ ,  $P=0.007$ ). (Fig.11)

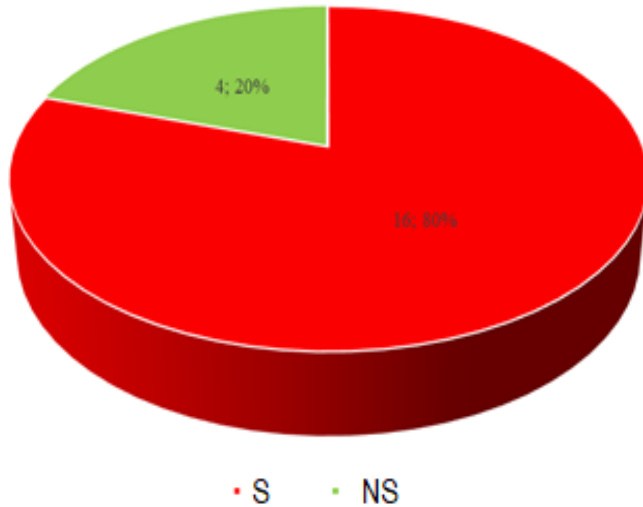
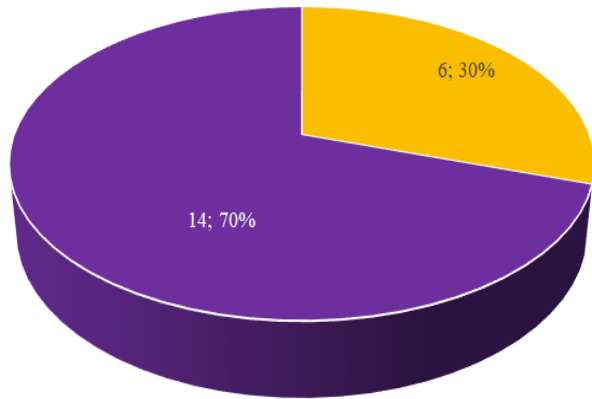


Fig.11. Distribution of smoking status (S – smokers, NS – non-smokers).

The group included 20 teeth – 9 premolars and 11 molars. Since the interradicular septa in some of the molar extraction sites were not damaged by pathological processes, measurements were taken in both medial and distal sections. Therefore, the group included measurements from 27 post-extraction sites. Fig. 12 represents the distribution of teeth according to their diagnoses ( $\chi^2 = 3.20$ ,  $P=0.074$ ).



■ Par. prof. ■ Per. chr. gr./Cysta rad.

Fig. 12. Diagnosis distribution. Par. prof. – parodontitis profunda; Per. chr. gr.– periodontitis chronica granulomatosa; Cysta rad. – Cysta radicularis.

### **Protocol for obtaining platelet-rich plasma:**

For this purpose, we applied the methodology proposed by Ivanova N. (2021) (2). The following devices and consumables were used:

- Laboratory centrifuge EBA20, HettichLab, Germany (fig. 13);
- Vacutainer tubes, containing separating gel (cycloaliphatic polymer gel) and anticoagulant (3.2% trisodium citrate);
- Sterile tubes, syringes, and 22G needles (to avoid premature platelet activation);
- Calcium gluconate solution 8.94 mg/ml, 10 ml.

The venipuncture was performed in the cubital fossa (in an appropriate branch of the median cubital vein or the cephalic vein) by qualified personnel in compliance with all principles of sterility.

The amount of blood drawn from each participant was 8 ml. The blood was then transferred to the vacutainer tube and the sample was inverted 5-10 times to mix with the anticoagulant.

Before the first spin, the rotor was balanced – loaded symmetrically with the sample tube and an opposing tube filled with the same volume of liquid (water). Centrifugation was carried out at room temperature 20-22°C. The first spin (separation) was carried out at 3500 rpm for 10 minutes.

The stages for obtaining PRP belong to the so-called "buffy coat" method in which the first separating spin is performed at high speed. As a result, three visible layers are observed in the vacutainer tube from bottom to top as follows: first layer rich in erythrocytes; second layer, rich in platelets and white blood cells - "buffy coat"; third layer – platelet-poor plasma (PPP).

The middle and most of the PPP layers were aspirated and transferred to a new sterile tube without anticoagulant. The centrifuge was rebalanced and a second centrifugation was performed at 1900 rpm for 5 min. After the second centrifugation (concentration), a small amount of erythrocyte mass was observed at the bottom of the tube, a layer of "buffy coat" above it, and again a layer of PPP on top. Part of the PPP was removed and the remaining amount of plasma and platelet concentrate with a total volume of 3 ml was transferred to a new sterile tube. Then 1 ml of calcium gluconate, which served as an exogenous activator, was added. After careful homogenization, the mixture was left at room temperature for 20 min until the PRP gel formed. The platelet concentrate should be separated from the PPP as soon as possible after centrifugation, as platelets slowly diffuse into the plasma over time.

Blood sampling is performed immediately before the surgical manipulation, and the protocol for obtaining PRP (fig. 14) was performed simultaneously with the extraction.



Fig. 13 - Hettich EBA 20 Centrifuge



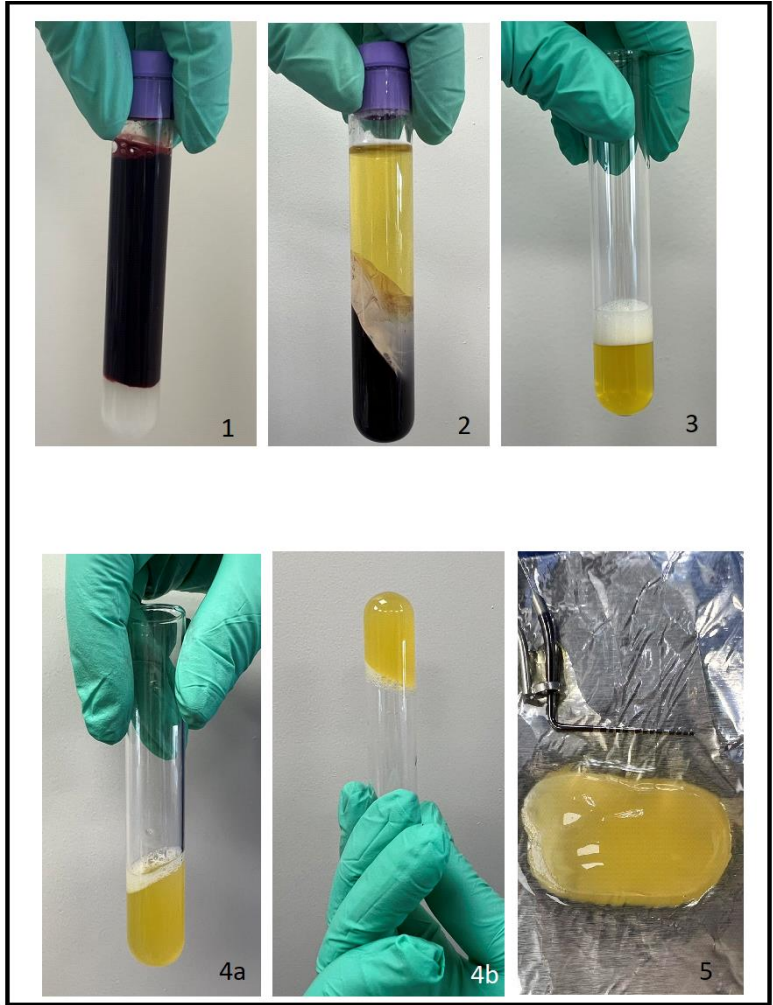


Fig. 14. PRP preparation protocol. 1 – A vacutainer tube with a separating gel, filled with venous blood from the patient; 2 – The vacutainer tube after the first spin (separation); 3 – Test tube after the second spin (concentration); 4,5 – After the activation of the final product.

### **Surgical protocol:**

We applied the same methodology as the one described in task 1 – atraumatic extraction, treatment of the post-extraction wound (curettage, smoothing of bone edges, lavage, hemostasis), and preparation of the buccal and oral soft tissue edges of the socket. The socket was filled with the PRP gel up to the level of the bone edges. A non-porous PTFE membrane was shaped and inserted subperiosteally 3-5 mm from the edges of the socket, as well as under the interdental papillae, without reaching the adjacent teeth. A crossed mattress suture and/or interrupted sutures with 5/0 Polyamide were placed. (Fig.15)

### **Postoperative care:**

Patients received postoperative instructions similar to those following a routine dental extraction. Antibiotic prophylaxis was prescribed, usually amoxicillin in combination with clavulanic acid (Augmentin 1000 mg) twice per day for 7 days. When the patient was allergic to penicillins, azithromycin 500 mg for 3 days was prescribed, or clindamycin 300 mg twice per day (for 5-7 days). In addition, non-steroidal anti-inflammatory drugs were prescribed, e.g. Aulin 100 mg every 12 hours and mouthwash with 0.12% chlorhexidine.

Sutures were removed on the 14th postoperative day, while the membrane was removed on the 28th day, using a hemostatic tool or tweezers. If necessary, the area could be anesthetized beforehand with a contact anesthetic - lidocaine 10% pump spray.

Final epithelization of the tissues after removal of the membrane occurs within 4 weeks.

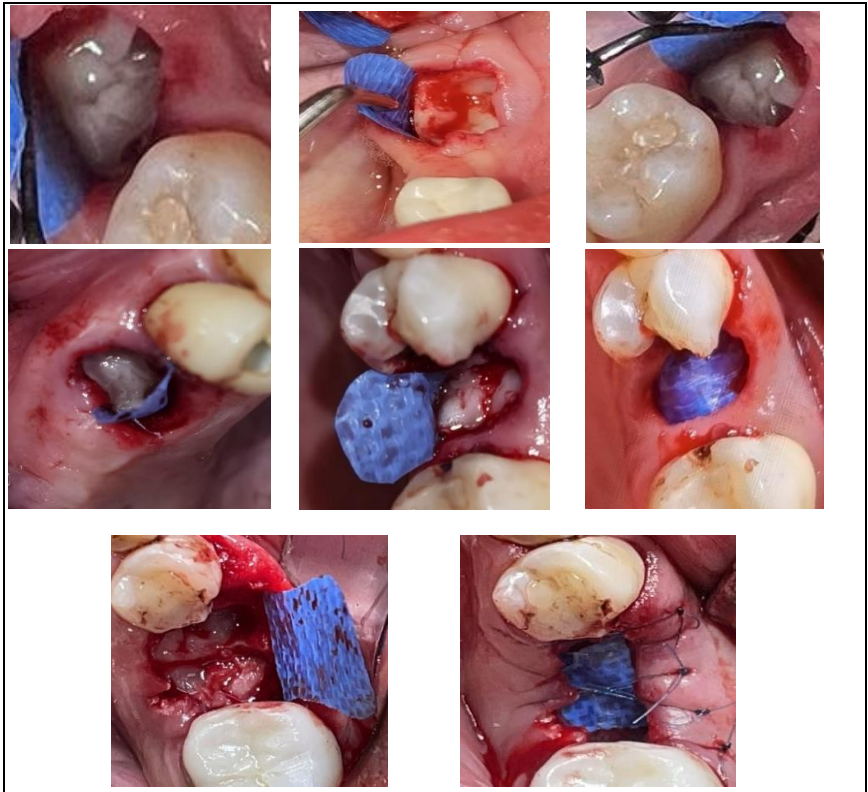


Fig. 15. RP using non-resorbable PTFE membranes in combination with PRP.

### **Task 3. Material and Methods.**

In task 3, we analyzed the results after the application of full-thickness free gingival grafts as a method of RP after tooth extraction. For this purpose, we compared the cone beam tomography performed on the day of the extractions and 3 months after them. A total of 20

post-extraction sockets (in the premolar and molar areas of the dentition) in 20 patients aged 30 to 61 years were included in this group. The average age in the group was  $47.75 \pm 10.97$  years.

In this group 9 (45%) patients were male and 11 (55%) were female, with no statistically significant gender difference ( $\chi^2 = 0.20$ ,  $P=0.655$ ). (Fig.16)

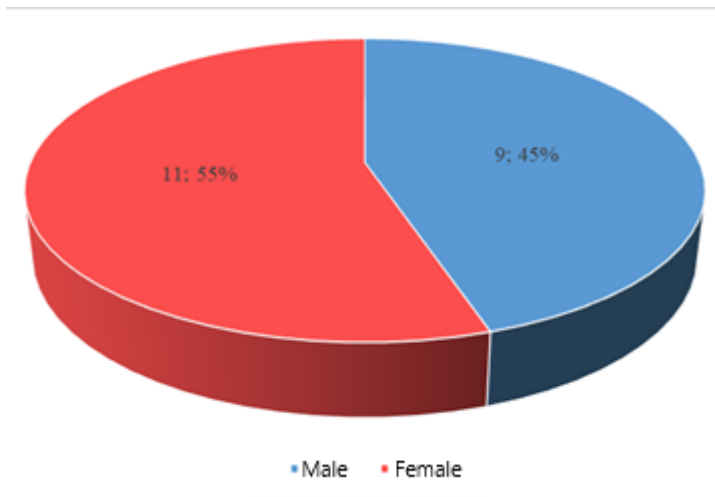


Fig.16. Gender distribution.

Regarding the smoking status, more patients were smokers than non-smokers but there was no statistically significant difference ( $\chi^2 = 0.20$ ,  $P=0.655$ ) (Fig. 17)

Out of all 20 teeth in this group, 10 are premolars and 10 are molars. The total number of examined post-extraction sites is 21. Fig. 18 shows the ratio of teeth according to their diagnoses - no statistically significant difference was found ( $\chi^2 = 1.80$ ,  $P=0.180$ ). (Fig. 18)

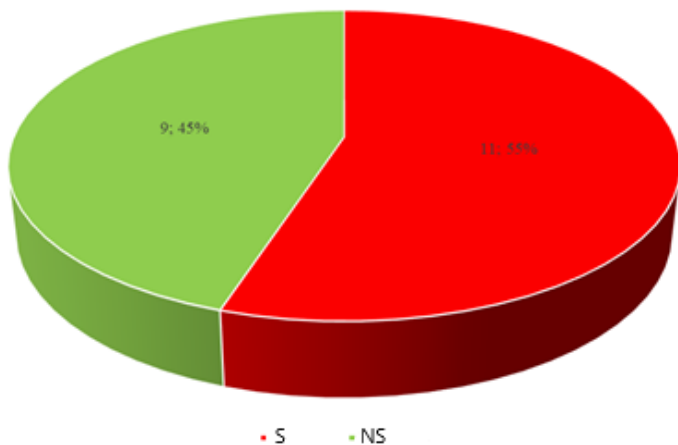


Fig.17. Distribution of smoking status (S – smokers, NS – non-smokers).

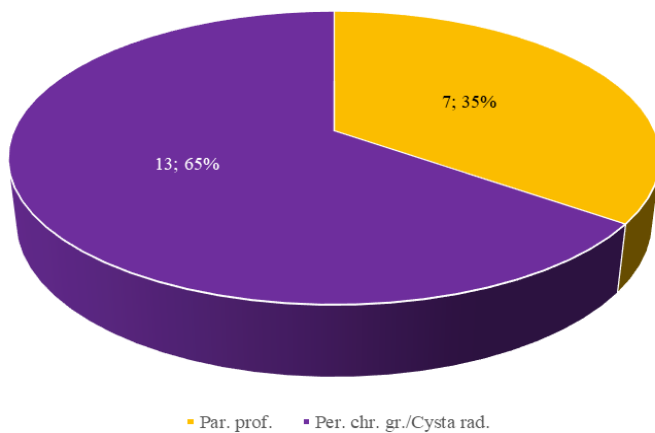


Fig. 18. Diagnosis distribution. Par. prof. – parodontitis profunda; Per. chr. gr.– periodontitis chronica granulomatosa; Cysta rad. – Cysta radicularis.

## **Surgical protocol:**

The first stage was an atraumatic tooth extraction with appropriate surgical instruments and treatment of the post-extraction socket – curettage and smoothing of the bone edges. The next two steps included the preparation of the recipient and the donor sites. (Fig. 19)

### **Preparation of the recipient site:**

It included de-epithelialization of the socket edges with a microblade, compression, and hemostasis. A template, that repeated the shape and exceeded the size of the socket orifice, was cut from sterile foil. This aimed to harvest a graft of slightly larger dimensions than those of the recipient site to compensate for subsequent shrinkage of the graft. It is important to note that the presence of intact interdental papillae in the recipient site was suggested to be critical for optimal graft revascularization.

### **Selection of donor site:**

The selection of the donor site (hard palate or maxillary tuberosity) was based on the anatomical features of the field. The thickness of the soft tissues in the site was measured with a periodontal probe under local infiltration anesthesia. Palatal grafts were harvested from the premolar area of the hard palate, distal to the palatal rugae (plicae palatine transverse) outpassing the distal edge of the first molar or the midpalatal suture and at least 2 mm below the gingival margin of the teeth. The anatomical prerequisites for harvesting from the maxillary tuberosity were: the absence of a wisdom tooth, a well-defined tuber, easy access, and visibility. The shaped foil was applied to the selected area and then a full-thickness gingival graft was formed and harvested. The graft was quickly transferred to the recipient site, adapted, and fixed with several interrupted sutures (or a modified

mattress suture) with 6/0 Polyamide. Hemostasis in both donor and recipient sites was achieved (Fig. 20-22).



Fig.19. Surgical instruments for tooth extraction and RP with FGG.

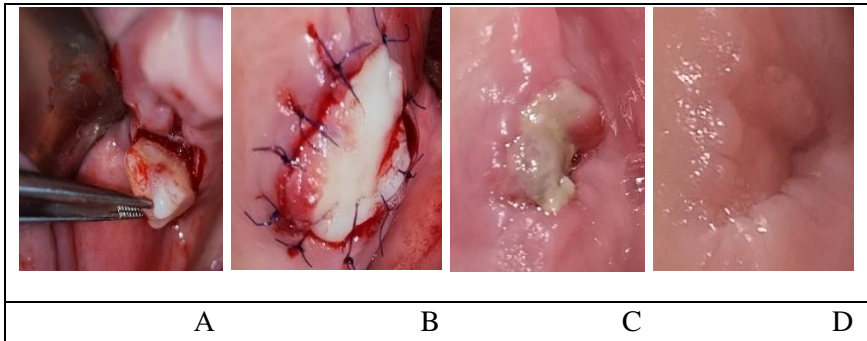


Fig.20. RP with FGG from the maxillary tuberosity – A – harvesting; B – graft adaptation and stabilization; C - day 7 postsurgery – healing process with an area of necrotic tissue; D – recipient site 3 months after the surgery.



Fig. 21. FGG transferred to the recipient site.



Fig. 22. Recipient site - (A) Day 1 postsurgery; (B) Day 12 postsurgery. Donor site – Day 20 postsurgery (C).

### **Postoperative care:**

Patients received postoperative instructions similar to those following a routine tooth extraction. Antibiotic prophylaxis was prescribed, usually Amoxicillin in combination with Clavulanic acid (Augmentin 1000 mg) every 12 hours for 7 days. In addition, non-steroidal anti-inflammatory drugs were prescribed, e.g. Aulin 100 mg every 12 hours and mouthwash with 0.12% chlorhexidine. Sutures were removed in 10-14 days.



It was explained to the patients that there was a greater risk of complications and failure if they smoked during the healing time. Some of them (those who reported that they smoke irregularly or between 2 and 5 cigarettes per day) expressed a desire to be included in the study and a willingness to completely stop smoking while the entire healing period lasts. A few patients (those smoking  $\geq 10$  cigarettes per day) refused to participate in the study.

#### **Task 4. Material and Methods.**

For task 4, we evaluated the bone resorption in 20 post-extraction sockets where no RP procedure was performed. The group involved 20 patients aged between 21 and 65 years. The average age was  $51.10 \pm 11.70$  years.

In the group 12 (60%) patients were male and 8 (40%) were female. There was no statistically significant difference in gender ( $\chi^2 = 0.80$ ,  $P=0.371$ ). (Fig.23)

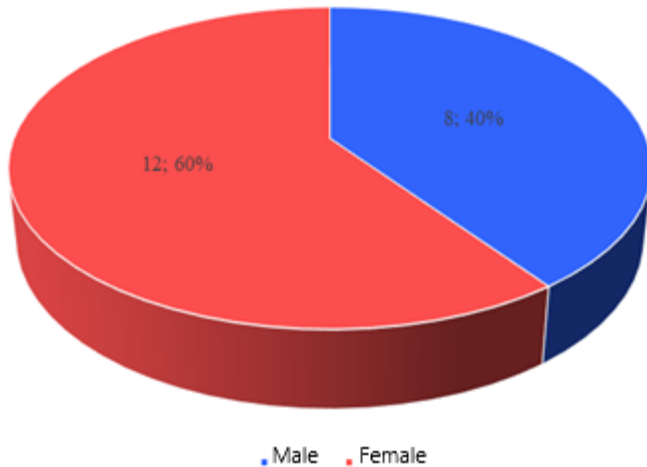


Fig.23. Gender distribution

Regarding the smoking status, more patients were smokers than non-smokers but without any statistically significant difference ( $\chi^2 = 1.80$ ,  $P=0.180$ ) (Fig.24.)

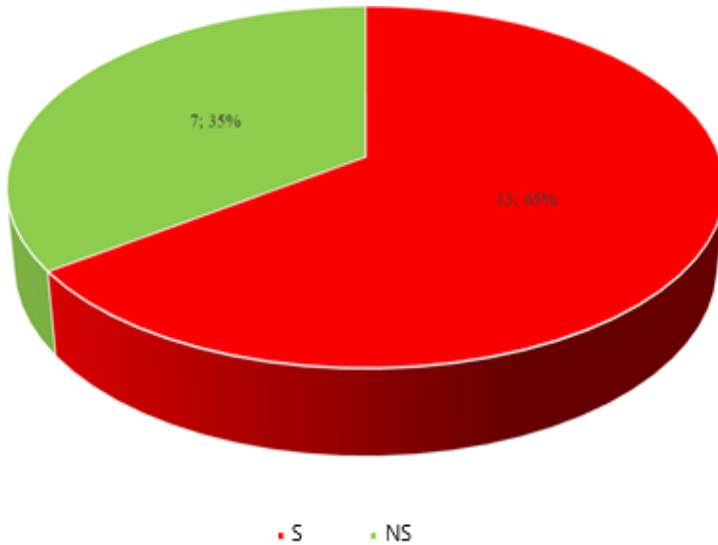


Fig.24. Distribution of smoking status (S – smokers, NS – non-smokers).

Figure 25 demonstrates the ratio of teeth according to their diagnoses - no statistically significant difference was found ( $\chi^2 = 3.20$ ,  $P=0.074$ ).

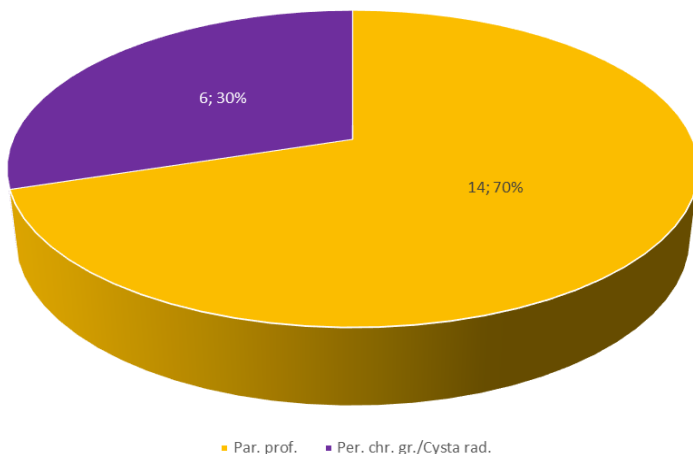


Fig. 25. Diagnosis distribution. Par. prof. – parodontitis profunda; Per. chr. gr.– periodontitis chronica granulomatosa; Cysta rad. – Cysta radicularis.

### **Surgical protocol:**

Atraumatic extraction was performed under local infiltrative anesthesia with Dentocaine 40 mg/0.005 mg/ml. When there was a risk of a fracture of the bone walls or the intraradicular septum, multirooted teeth were separated before the extraction. The next steps include curettage, smoothing of the bone edges, irrigation with saline, and suturing with a crossed mattress suture and/or interrupted sutures. For this purpose, we used 5/0 non-absorbable monofilament material - Polyamide.

### **Postoperative care:**

Patients received postoperative instructions. Antibiotics, non-steroidal anti-inflammatory drugs, and mouthwash, similar to those mentioned above, were prescribed. The sutures were removed in 7-10 days.

## **Task 5. Material and Methods.**

For task 5, we compared the results obtained from the CBCT scans in the four studied groups, which included a total of 80 post-extraction sockets in the posterior areas of the dentition (33 premolars and 47 molars) in 80 patients (38 men and 42 women), aged 26-65 years.

### **Statistical methods**

The following statistical methods were used to process the collected data:

#### **1. Descriptive analysis:**

- 1.1. Alternative analysis – for a description of qualitative variables and grouped data;
- 1.2. Variation analysis – for describing quantitative variables.

Results are presented using measures of central tendency (arithmetic mean, median) and measures of variation (standard deviation, IQR, range) depending on the type of distribution.

#### **2. For hypothesis testing:**

- 2.1. Parametric methods – for normally distributed quantitative variables:
  - Student's t-test when testing hypotheses for the presence of a significant difference between two dependent/independent samples;
  - Pearson's correlation coefficient.
- 2.2. Non-parametric methods - for testing hypotheses for variables deviating from the normal distribution and qualitative quantities:

- Mann-Whitney median test (Mann-Whitney U test) for two independent samples;
- Wilcoxon signed-rank test for related samples;
- H-test of Kruskal-Wallis (Kruskal-Wallis) for three and more independent samples;
- $\chi^2$  – Pearson's chi-squared test;
- Spearman's correlation coefficient;
- Fisher's exact test;
- Cramer's V – a measure of association between two nominal variables;

The level of significance of the null hypothesis is  $\alpha=0.05$ .

### **3. Tabular and graphic methods for visualizing the obtained results.**

Data processing and analysis were performed with the statistical package IBM SPSS version 25.0 (Chicago, IL, USA) and Jamovi Statistical Software, and for graphical analysis – MS Office Excel 2013.

The research was started after receiving permission No. 118/23.06.2022 from the Scientific Research Ethics Committee of the MU "Prof. Dr. P. Stoyanov" - Varna.

## **Results and Discussion**

### **Results and Discussion on Task 1**

Statistically significant ( $p < 0.05$ ) as well as clinically significant differences were taken into account in the analysis of the results. As clinically significant we considered a difference of 1 mm in height/width (Al Hugail et al., 2021).

The average height of the vestibular bone plate in this group was  $10.75 \pm 3.45$  mm and the average width was  $3.25 \pm 1.61$  mm.

Considering the studied factors, the only statistically significant difference was in the width of the vestibular bone according to the jaw (mean difference of 1.36 mm - in the upper jaw  $A_w = 2.40 \pm 0.89$  mm and in the lower jaw  $A_w = 3.76 \pm 1.75$  mm).

Regarding the palatal/lingual plate, the mean height was  $12.69 \pm 3.48$  mm and the mean width was  $3.04 \pm 1.19$  mm. There was no statistically significant difference in the width of the plate according to those factors.

The heights of the vestibular and palatal/lingual plate 3 months after the RP procedure were:  $9.70 \pm 3.29$  mm and  $11.61 \pm 3.52$  mm, respectively. The resulting difference in the vertical size of the plates (bone loss) was of high statistical and clinical significance. For the vestibular wall, it was  $1.05 \pm 0.62$  mm, and for the palatal/lingual wall –  $1.08 \pm 0.59$  mm. These values are close to those reported by Sabe-Alarabab et al. (2019) and Papi et al. (2020) 3-4 months after RP with non-porous PTFE membranes.

No statistically significant difference was reported in the loss of the plates concerning any of the considered factors (jaw, dentition area, diagnosis, smoking). To some extent, these results correspond

with those reported by Hoffman et al. (2008). They performed a retrospective randomized study on the use of non-porous PTFE membranes for RP in 276 post-extraction sockets. The authors examined the influence that the following factors had on the bone level: jaw type, region, socket shape, age, gender, smoking, and bleeding on probing. The results demonstrated that age, gender, smoking, and bleeding on probing do not influence the studied parameters. On the other hand, it turned out that the side of the jaw, the area, and the shape of the socket had influenced the bone level. Almost half of the participants were smokers, but no difference in the treatment outcomes was found between the two groups.

The analysis of the data shows a mean difference of 0.03 mm in the vertical resorption between both plates, which means that they had a similar resorption rate.

Greater resorption of the vestibular/buccal ridge wall compared to the palatal/lingual ridge has been reported in several studies (Araujo et al., 2015; Barone et al., 2008; Fiorellini et al., 2005; Misawa et al., 2016; Schropp et al. 2003). This pattern of resorption can be explained by the concept of the alveolar bone proper (bundle bone) proposed by Araujo and Lindhe (2005). According to their theory, the quantitative ratio of alveolar bone proper to supporting bone is greater in the buccal plate and, as a “tooth-dependent structure,” its loss accordingly leads to greater resorption in the vestibular/buccal plate compared to the palatal/ lingual.

Van der Weijden et al. (2012) also reported that both plates are equally prone to resorption. They found a mean loss in buccal and lingual bone height of 1.67 mm and 2.03 mm, respectively. When considering only studies in which both plates were examined simultaneously (as not all did), then vertical resorption at the buccal plate is on average  $2.59 \pm 1.85$  mm, and for the lingual –  $2.03 \pm 1.78$  mm. Although the buccal bone showed more pronounced resorption

than the lingual bone, the difference between them was only 0.56 mm and did not correspond to that reported by Araujo and Lindhe (41). Some authors (Binkhorst et al., 2023; Horváth et al., 2013) even reported more pronounced vertical resorption in the palatal/lingual plate compared to the buccal plate.

It should be noted that most of these studies concerned post-extraction resorption sockets that healed naturally. In addition, the anterior area of the dentition, where the vestibular wall is usually thinner, was not included in our research. The average width of the bone plates in this experimental group was over 3 mm, while the concept of Araujo and Lindhe is based on the resorption of the thin buccal bone, made up mainly of bundle bone.

We found a weak negative and statistically insignificant correlation between the resorption of both plates, which suggested that they did not affect each other.

A moderate negative, statistically significant correlation was found between the width of the buccal plate measured immediately after extraction and the vertical resorption after 3 months. This demonstrated that as the width of the plate increased, the resorption decreased and vice versa – a stronger vertical loss could be expected with thinner walls. These results correspond to those reported by numerous authors (Abdelhafez et al., 2016; Avila-Ortiz et al., 2020; Couso-Queiruga et al., 2021; Ferrus et al., 2010), but are in contrast to those reported by Mandarino et al. (2018), who found no relationship between the width and the vertical loss of the plates in both experimental and control groups, and Cardaropoli et al. (2014), who found a correlation in the control group, but not when RP was performed.

Regarding the palatal/lingual wall, this correlation is weak negative and statistically insignificant, but it showed a similar tendency with the buccal wall.



Although a statistically significant difference in the width of the vestibular bone according to the jaw was reported (mean difference 1.36 mm), this did not lead to different vertical bone resorption of the plate in both jaws (mean difference – 0.01 mm;  $p = 0.980$ ).

Since bone width has been considered a key factor for vertical bone resorption, these findings suggested that there is probably a critical value in the width.

As the mean widths in both jaws were above 2 mm, this implied that if such a critical threshold existed, it was not above this value.

The existence of such critical value could be the possible reason for the study heterogeneity. This means that below this value resorption is to a greater extent and at a greater speed. According to the literature, this "critical threshold" may be 1 mm or 2 mm. How the results in the present study changed according to these values will be further discussed.

## Results and Discussion on Task 2

Recently, autologous platelet concentrates have gained popularity in the fields of oral surgery, periodontology, and dental implantology not only to promote soft tissue healing but also for accelerated bone regeneration.

Platelet-rich plasma promotes regenerative processes, accelerates their progress, and improves hemostasis. It increases the rate of bone formation from 1.62 to 2.18 times. (Peev, 2023)

The results from the group with combined application of PRP and non-porous PTFE membranes showed an average height of the vestibular bone plate –  $13.92 \pm 4.32$  mm and an average width of the plate –  $2.30 \pm 1.36$  mm. No statistically significant difference was found in the initial measurements on the plate according to the various factors – jaw type, area of dentition, diagnosis, and smoking status.

An average height of  $15.35 \pm 3.45$  mm and an average width of  $3.35 \pm 1.17$  mm were recorded for the palatal/lingual bone. The differences in the height of the bone plate according to the jaw (mean height  $11.93 \pm 4.41$  mm in the upper jaw and  $15.96 \pm 1.83$  mm in the lower jaw), as well as its width (mean width  $2.44 \pm 1.05$  mm in the upper jaw and  $3.88 \pm 0.89$  mm in lower jaw), were statistically significant.

The heights of the buccal and palatal/lingual plates measured after a period of 3 months were:  $12.74 \pm 4.09$  mm and  $14.46 \pm 3.56$  mm, respectively. The change in the vertical dimension (bone loss) that occurred in both plates was of high statistical and clinical significance. At the buccal wall, it was  $1.18 \pm 1.28$  mm, and at the palatal/lingual wall –  $0.88 \pm 0.56$  mm. Therefore, it was more pronounced in the buccal plate, but without significant difference between them.

No statistically significant difference was found in the loss of plates in terms of the considered factors.

The data analysis showed an average difference between the vertical resorption of both plates – 0.3 mm, which implied that they are resorbed evenly.

A weak, statistically insignificant correlation between the vertical resorption of the plates was registered. Therefore, we could conclude that the resorption in each plate proceeded independently and they did not influence each other.

A moderate, negative, statistically significant correlation was found between the width of the buccal plate measured immediately after extraction and the vertical resorption after 3 months.

A strong negative and statistically significant correlation was observed for the palatal/lingual bone wall. This demonstrated that both bone walls were affected by the width as a factor.

In some patients, the vertical resorption of the plates was significantly more pronounced than in others, which could be due to differences in the widths. According to some authors, there is a non-linear relationship between the width of the buccal wall and the resorption of the ridge, and the threshold for this correlation is 2 mm. The correlation was twice as pronounced in areas with a buccal bone width < 2 mm (Qahash et al., 2008).

Although a statistically significant difference of 1.44 mm in the width of the oral wall (Bw) between both jaws was registered, no statistically significant difference in the vertical plate resorption was found (mean difference – 0.1 mm). Given that the width of both plates was over 2 mm, this also suggested that the amount of resorption could increase more significantly below some critical width. Furthermore, it demonstrated that jaw type itself was not a modifying factor for the resorption, and additional area concretization was needed.

Platelet-rich plasma can be used alone but gives better results in combination with bone graft material (BGM). For this purpose, the materials are mixed immediately after the activation of the plasma before the PRP-gel has formed (Peev, 2007).

Most studies on the application of platelet-rich plasma for RP have investigated its utilization in combination with various types of BGM, whereas the present study examined its application only in combination with a barrier membrane. The use of the same surgical protocol and the same type of membranes in the patients of tasks 1 and 2 allowed a comparison between the methods and an analysis of the benefit of the application of PRP without BGM.

### **Results and Discussion on Task 3**

Full-thickness gingival grafts have been used alone or in combination with BGM and/or barrier membranes for RP.

In the present study, we evaluated the application of free gingival grafts as a method for RP to determine their role in the preservation of the vertical crest dimension after tooth extraction.

The results in the group showed an average buccal plate height of  $10.77 \pm 2.91$  mm and an average width of the plate –  $2.37 \pm 1.46$  mm. A statistically significant difference was found only in the height of the buccal plate according to the diagnosis with a mean difference of 2.78 mm.

For the palatal/lingual plate, an average height of  $12.81 \pm 4.02$  mm and an average width of  $2.82 \pm 1.56$  mm were registered. A statistically significant difference was reported regarding the diagnosis.

The heights of the buccal and palatal/lingual plate after 3 months of the extraction were:  $9.14 \pm 2.90$  mm and  $11.55 \pm 4.35$  mm, respectively. The vertical resorption in both plates was of high statistical and clinical significance. For the buccal wall, it was  $1.65 \pm 0.92$  mm, and for the palatal/lingual wall –  $1.27 \pm 0.57$  mm. These results were inconsistent with those reported by Karaca et al. (2015) They evaluated post-extraction volume changes after spontaneous healing and RP with gingival graft, based on CBCT images from the day of extraction and 3 months postoperatively. The authors reported a statistically significant difference in the vertical change of the buccal and palatal bone plates in favor of the use of grafts ( $+0.06$  mm and  $+0.25$  mm vs  $-1.03$  mm and  $-0.56$  mm). In terms of horizontal changes, no statistically significant difference was found between the two groups. In contrast to the results of Karaca et al. are the statements that the height of the socket walls never reaches its original level as that

before the extraction. Ridge preservation could limit but not completely prevent bone resorption (Cardaropoli et al., 2003; De Angelis et al., 2022; Horváth et al. 2013; Morjaria et al., 2014).

Regarding the vertical loss of the palatal/lingual bone plate, we found a statistically significant difference according to the diagnosis ( $1.69 \pm 0.36$  mm in par. prof. and  $1.06 \pm 0.55$  mm in per. chr. gr./ cysta rad.) with a mean difference of 0.63 mm ( $p=0.01$ ). Even if we assume that the reason for this was the originally measured widths, where the difference in values according to the diagnosis was at the border of statistical significance, the correlation analysis between the width of the plate and the loss in its vertical size in all participants of the group did not confirm such a relationship. Therefore, a more thorough analysis of the relationship between these two variables was needed. These results could be related to the periodontal status of the patients. The participants in this group were non-smokers or smoke up to 5 cigarettes per day. Due to the lack of adequate size of the smoker groups, such comparison between them was insufficient.

Data analysis demonstrated a mean difference between the vertical loss of both plates of 0.38 mm, which meant that their resorption was similar.

A weak negative, statistically insignificant correlation was reported between the buccal and palatal/lingual plate resorption.

A strong negative and statistically significant correlation was found between the buccal plate width measured immediately after extraction and its vertical resorption during the first 3 months.

As aforementioned, for the palatal/lingual bone, this correlation was weak, negative, and statistically insignificant, but it showed a similar tendency with the buccal plate.

## Results and Discussion on Task 4

The results in the control group showed an average height of the buccal bone plate –  $13.04 \pm 4.54$  mm and an average width of the plate –  $1.73 \pm 1.19$  mm. Regarding the considered factors, a statistically significant difference was found only in the height of the buccal bone according to the jaw type with an average difference of 6.6 mm (in the upper jaw -  $A0 = 9.83 \pm 3.61$  mm and in the lower jaw  $A0 = 16.43 \pm 2.65$  mm).

The values recorded for the palatal/lingual plate were respectively: mean height –  $13.54 \pm 4.63$  mm and mean width –  $2.99 \pm 1.07$  mm. A statistically significant difference was found in the height of the bone plate according to the jaw with an average difference of 6.69 mm (in the upper jaw –  $B0 = 9.92 \pm 4.18$  mm and in the lower jaw  $B0 = 16.61 \pm 2.04$  mm); as well as concerning the smoking status with a mean difference of 4.03 mm ( $B0 = 12.03 \pm 4.48$  mm in smokers and  $B0 = 16.06 \pm 3.88$  mm in non-smokers).

The heights of the buccal and palatal/lingual plates 3 months after the extraction were:  $9.93 \pm 4.24$  mm and  $11.46 \pm 4.51$  mm, respectively. Vertical bone loss was found in both plates with very high statistical and clinical significance. At the buccal wall, it was  $3.47 \pm 1.96$  mm, and at the palatal/lingual wall –  $2.08 \pm 1.10$  mm.

This difference in the resorption of the plates is consistent with the studies of Araujo and Lindhe (2005). Increased osteoclastic activity during this period leads to resorption of the socket walls, with the vertical bone loss being more pronounced at the buccal plate. The histological results show that the crestal region of the buccal bone plate is composed almost entirely of alveolar bone proper, while in the lingual plate, there is a combination of alveolar bone proper and supporting bone.

A statistically significant difference in the vertical resorption of the buccal plate was found between the different diagnoses. The average bone loss in cases of chronic periodontitis was  $4.02 \pm 2.08$  mm, and in those with chronic periodontitis/radicular cyst, it was  $2.36 \pm 1.12$  mm (mean difference - 1.66 mm).

Zhao et al. (2019) studied alveolar crest volume changes after tooth extraction in the molar regions as well as in teeth with advanced periodontal disease. They found no statistically significant difference in crest height loss in the two jaws over 6 months. However, height resorption is reported to be more pronounced in the buccal wall of the sockets in the lower jaw compared to that in the upper jaw.

Although the difference in the vertical resorption of the buccal bone between both jaws was not statistically significant, the mean resorption in the lower jaw was  $3.93 \pm 2.22$  mm, and in the upper jaw -  $2.92 \pm 2.52$  mm. The mean difference between them was 1 mm, which was of clinical significance and corresponds to the results reported by Zhao et al. and Smukler et al. (2011) who reported that postextraction resorption is more pronounced in the lower jaw than in the upper jaw and at the buccal plate than at the lingual plate.

A statistically significant difference was found between the buccal resorption of both plates (with an average difference of 1.39 mm) in the control group. The resorption was more pronounced at the buccal wall.

A weak, statistically insignificant correlation was found between the resorption of the plates. Therefore, they do not affect each other.

It should be noted that the mean difference between the widths of the buccal and lingual plates, measured immediately after the extraction, was 1.26 mm and that the mean width of the buccal bone was less than 2 mm, while that of the palatal/lingual was more than 2 mm.



According to some authors, when the buccal plate width is < 2 mm, bone resorption is more pronounced (Chen et al., 2007; Qahash et al., 2008; Spray et al., 2000), but the minimum buccal plate width to avoid vertical bone resorption has not yet been established (Huynh-Ba et al., 2010).

Chappuis et al. (2013) reported a mean vertical resorption of 7.5 mm for buccal bone width  $\leq 1$  mm after 8 weeks. In areas with thicker buccal walls ( $>1$  mm), the authors reported a mean vertical loss of 1.1 mm.

The dynamics of bone healing in peri-implant areas in dogs concerning the dimensions of the alveolar crest were evaluated by Qahash et al. (2008). Following fluorescent labeling they found that the resorption of the alveolar ridge depended on the width of the buccal bone. This relationship was nonlinear with a threshold of 2 mm. The correlation was twice as strong for buccal bone < 2 mm, thus, the buccal bone must be at least 2 mm wide to maintain the level of the crest.

At the same time, however, a weak, negative, statistically insignificant correlation was found between the width of the buccal plate, measured immediately after extraction, and the vertical resorption after 3 months.

A weak, negative, statistically insignificant correlation was also reported between the width of the palatal/lingual bone and its loss in height.

Therefore, ridge width was not the only determining factor for the pronounced vertical resorption in this group. It was more pronounced in the buccal wall compared to the palatal/lingual wall, but both losses were of high clinical and statistical significance.

## **Results and Discussion on Task 5**

The present study included 4 research groups - three experimental and one control. In the experimental groups, 3 different methods for RP were applied (application of the non-porous PTFE membranes, a combination of the same type of membranes with PRP, and socket sealing with free gingival grafts), while in the control group, the post-extraction sockets were left on natural healing.

### **Comparative analysis and evaluation of the vertical resorption of the buccal bone plate according to the applied method**

We found a significant difference in the vertical bone loss at the buccal plate between each experimental group and the control one, demonstrating that all RP methods gave satisfactory results in reducing the resorption of the plate.

The buccal bone height loss over 3 months between the three ridge preservation techniques showed no statistically significant difference. When comparing the techniques separately, we found no difference between only the methods with non-porous PTFE membranes (alone or in combination with PRP). These results indicated that the application of PRP did not change the effect of the membrane in terms of vertical bone resorption of the plate. The method with the application of free gingival grafts demonstrated poorer results in preserving the vertical dimension of the wall compared to the other two.

When comparing the loss in buccal bone height over a period of 3 months using the ridge preservation methods on the one hand and the control on the other hand, a statistically and clinically significant

difference was found. These findings demonstrated that the ridge preservation methods achieved better results in preserving the vertical dimension of the buccal wall. It should be noted, however, that the control group presented the smallest plate width initially, which could have contributed to the amount of resorption.

Data analysis showed that the ridge preservation procedures reduced the risk of vertical buccal plate loss of more than 2 mm.

In the present study, the influence of the following factors was considered: jaw type, dentition area, diagnosis, and smoking.

Regarding the vertical bone loss of the buccal plate, a statistically significant difference was reported only in the control group according to the diagnosis (more pronounced in Par. prof. compared to Per. chr.gr./Cysta rad.).

In patients with severe chronic periodontitis, the pattern of ridge resorption is thought to differ from that of extraction due to caries and its complications or tooth fracture (Alexopoulou, 2019). Tooth extractions without any signs of infection were not included in this study, e.g. for orthodontic indications or acute trauma.

We did not find strong evidence for the influence of the studied factors on the loss of plate height.

In all experimental groups, a moderate or strong, negative, statistically significant correlation was found between the width of the buccal plate and its vertical resorption. These results are consistent with the general opinion that the amount of resorption depends on the width of the alveolar walls (Chappuis et al., 2000), but differ from those reported by Cardaropoli et al. (2014). They found no correlation between buccal bone thickness and bone resorption in the RP group, while in the control group, they found a strong negative correlation. The authors concluded that RP could compensate for post-extraction resorption regardless of the buccal bone width. It should be noted that for RP they used bovine xenograft and collagen membrane. In the

control group, a moderate, negative, and statistically insignificant correlation was found between plate width and vertical resorption, which was in contrast to the literature data.

There was marked vertical resorption in the control group, both in thin and thicker socket walls, but since the mean width in this group was  $1.73 \pm 1.19$  mm ( $<2$  mm), we will analyze the width as a factor later in the text.

Data analysis showed that buccal plate width was greater in the lower jaw compared to the upper jaw (in 3 of the groups), as well as at the molar sites compared to the premolar sites (in all groups), but with no statistically significant difference between them. Regarding the vertical resorption of the plate, no statistically significant difference was found concerning the jaw and the area of the dentition. These results correspond to those reported by Moya-Villaescusa and Sánchez-Pérez (2010), who found no statistically significant difference between vertical resorption in single-rooted and multi-rooted teeth. However, it is inconsistent with some other reports, which found a significant difference in resorption between the two jaws and between the premolar and molar regions (Atwood and Coy, 1971; Tallgren, 1972). Temple et al. (2016) performed CBCT analysis on 265 patients and reported bone densification in the distal areas of both jaws. However, generalized conclusions by tooth type cannot be made, as bone density varied between different teeth in the same group, between the different roots of the same teeth, as well as in the coronal-apical direction. This explains why such a division by jaw and teeth is not informative enough.

Although smoking was considered to influence post-extraction resorption and the results of RP procedures, the present study did not confirm these statements. In addition, some other studies have reported that RP produces show similar results in smokers and nonsmokers (Barone et al., 2008; Iasella et al., 2003).

The greater bone loss in some cases could be due to the thin buccal walls of the sockets, consisting mainly of "bundle bone" - a tooth-dependent lamellar bone structure with a thickness of 0.2-0.4 mm (Araujo et al., 2015; Chappuis et al., 2013; Januario et al., 2011).

Some authors indicate a "critical value" in width with a threshold of 2 mm (Araujo and Lindhe, 2005; Qahash et al., 2008; Spray et al., 2000; Vignoletti et al., 2012), while others state that this "threshold" is 1 mm, and for values  $\leq 1$  mm the expected vertical resorption is twice (Cardaropoli et al., 2014, 2015) or three times (Tomasi et al., 2018) more pronounced.

Some authors recommend the application of RP with non-resorbable BGM to compensate for bone resorption in sockets with thin buccal walls ( $\leq 1$  mm) (Ku et al., 2019; Mahesh et al., 2015; Tomasi et al., 2018).

Spray et al. (2000) investigated buccal bone resorption after implant placement in healed areas. They determined the width of the buccal bone plate using a caliper on the day of implantation and after a period of 3-6 months. A significantly greater loss was observed in areas with thinner bone walls. Areas presenting more than 3 mm bone resorption had the smallest mean width of the bone plate (1.3 mm) and vice versa – areas without resorption had a mean width of  $1.8 \pm 1.10$  at the time of implantation. Thus, the authors defined a "critical" thickness of the buccal plate of 2 mm, below which pronounced bone resorption could be expected.

There was statistically and clinically significant vertical wall resorption with a mean value between 1 and 2 mm in all experimental groups. When we evaluated the cases with  $> 2$  mm loss in height, we found that the plate width in 12 out of 13 cases was less than 2 mm. Data analysis demonstrated that buccal bone width less than 2 mm was observed in 46.9% of the cases and above 2 mm - in 53.1% of them, therefore they presented the same frequency.

When comparing the vertical loss at the buccal plate where plate widths were less and more than 2 mm respectively, we found that the loss in the control group was significantly greater in both cases. Therefore, the determining factor for greater resorption is the treatment protocol – with or without RP.

It has been suggested that the width of the buccal wall could serve as a valuable prognostic marker for the expected post-extraction bone resorption (Chappuis et al., 2000; Couso-Queiruga et al., 2021).

Using regression analysis we confirmed that the determining factor for the vertical bone loss was the application/lack of ridge preservation after extraction, while the width had a minor role as a factor and was a weak predictor in naturally healing sockets.

The results demonstrated that the observed methods for RP were effective and significantly compensated for the thin bone walls as a risk factor, but did not completely neutralize it. This finding was in contrast to the statement that in walls >1 mm the regenerative potential is preserved and RP has no particular benefit (Steigmann et al., 2022).

### **Comparative analysis and assessment of vertical palatal/lingual bone plate resorption**

When comparing the loss in palatal/lingual bone height over a period of 3 months, a significant difference, ranging between 0.9 mm and 1.3 mm, was found between each experimental group and the control one. Each of the methods showed better results in preserving the vertical dimensions of the plates than the control group. There was no statistically significant difference in the palatal/lingual vertical bone loss among the studied ridge preservation techniques over 3 months. No significant difference was found between both groups

with non-porous membranes, as well as between the methods with PTFE membranes and free gingival grafts. Between the second and the third group, however, the difference was statistically significant, but without clinical significance.

Data analysis demonstrated that the application of PRP did not improve the results obtained with the PTFE membranes in terms of vertical bone resorption.

A comparison of palatal/lingual bone height loss over a period of 3 months between the ridge preservation methods on one side and the control group on the other showed a significant difference. The resorption was less than that of the vestibular plate which could be related to the initial plate widths in the groups.

Data analysis showed that the application of RP significantly reduced the risk of vertical bone loss greater than 2 mm.

Regarding the factors, expected to influence bone resorption, the only statistically significant difference was in the vertical loss of palatal/lingual height concerning the diagnosis in the group with free gingival grafts ( $1.69 \pm 0.36$  mm in par. prof. and  $1.06 \pm 0.55$  mm in per. chr. gr./ cysta rad.) However, the baseline widths of the plate according to these diagnoses ( $1.90 \pm 0.88$  mm in par. prof. and  $3.28 \pm 1.64$  in per. chr. gr./radicular cyst) was also on the borderline for statistical significance.

Regarding the plate width, a difference was found according to the jaw type (greater for the lower jaw) in three of the four groups (except for the one with gingival grafts, where the two values for the upper and lower jaw were almost the same), as well as according to the region in all four groups (greater in molars). However, these differences were not statistically significant. There was no strong evidence that the considered factors – the type of jaw (upper/lower), area of dentition (premolars/molars), diagnosis (Par. prof. Per. chr. gr./Cysta rad.) and smoking (smokers/non-smokers) influenced the

vertical palatal/lingual bone resorption. This may also be a result of allocating them by jaw type and tooth group rather than more exact area specification. Since all extraction was in infected areas, general conclusions that diagnosis does not influence bone resorption cannot be made.

No statistically significant correlation was found between the width of the plate and its vertical resorption (except in the second group). For this bone wall, a width of less than 2 mm was observed only in 23% of the cases.

A weak, statistically insignificant correlation was reported between the vertical bone loss of both plates in all groups. Furthermore, their resorption proceeded independently, without influencing each other.

It has been suggested that vertical bone loss is more prevalent in the buccal wall than in the lingual one, which could be contributed to the smaller ratio of the alveolar bone propria to the supporting alveolar bone (Schropp et al., 2003; Fiorellini et al., 2005).

Using regression analysis, it was established that the treatment protocol – with or without RP – was the determining factor for the vertical resorption in the palatal bone, as well. When RP was performed, the importance of width as a factor increased.



## Conclusions

1. The application of the considered ridge preservation methods (with non-porous PTFE membranes; with non-porous PTFE membranes and PRP; with free full-layer gingival grafts) reduced the vertical resorption of the buccal and palatal bone plates.

2. The three applied methods gave similar results, but the methods based on guided regeneration (with non-porous PTFE membranes with/without PRP) were superior to the application of free gingival grafts in terms of preserving the vertical dimensions of the walls.

3. Platelet-rich plasma did not improve the results achieved with non-porous PTFE membranes in terms of preserving the vertical dimensions of both bone plates.

4. Ridge preservation procedures could reduce the risk of vertical loss  $> 2$  mm in both bone plates of the sockets.

5. The behavior after extraction – with or without ridge preservation is a determining factor for vertical bone resorption.

6. The width of the buccal bone plate affects the amount of its vertical resorption, but its influence is much less significant than the behavior after extraction (with/without ridge preservation).

7. Ridge preservation methods could significantly compensate for the resorption of thin socket walls, but not completely neutralize the influence of the width as a factor.

8. The width of the buccal plate is greater in the lower jaw compared to the upper jaw, and in the molar region compared to the premolar area, but with no statistically significant difference between them.

9. There is no strong evidence that the considered local and systemic factors (jaw, area of the dentition – premolar/molar, diagnosis, and smoking status) influence the vertical resorption of the alveolar crest over a period of 3 months after extraction.

## **Conclusion**

Implant treatment in areas with reduced bone volume is extremely challenging and requires the application of various methods, especially augmentation procedures, including guided regeneration.

Before tissue augmentation, there is a group of methods aimed at reducing post-extraction resorption and preserving the available tissues. These include immediate and early implantation and loading, as well as ridge preservation techniques.

Prediction of the amount of post-extraction volume changes in each patient is sufficient for making correct clinical decisions and appropriate treatment plans. It is not necessary to perform ridge preservation after each extraction, but it plays an important role in the following situations: thin bone plates (<2 mm); areas with increased aesthetic risk; destruction of the socket walls; multiple extractions; risk of involvement of some anatomical structures and delayed implantation.

In the present dissertation, we examined the application of guided regeneration in post-extraction sockets using non-porous polytetrafluoroethylene membranes alone and in combination with platelet-rich plasma, as well as free full-thickness gingival grafts as a method of socket sealing.

The aim of ridge preservation is not to increase tissue volume at the stage of extraction, but reduce tissue loss between the extraction stage and implantation and eventually eliminate the need for additional augmentation procedures. In other words, ridge preservation aims to create suitable conditions for implantation with minimal invasiveness and lower financial costs.

It is widely believed that as soon as an increase in the available bone volume is required, the application of ridge preservation

procedures is useless. However, some authors demonstrated that the greatest benefits of these methods are found in sockets with thin bone plates (especially the buccal one) and impaired integrity of the bone walls - dehiscences, and fenestrations.

The results of this study showed that the presented methods successfully decreased the amount of vertical post-extraction ridge resorption. They give similar results and no significant superiority of any of them is reported. If their main disadvantages are considered, they would be: the additional materials when using the membranes and PRP and the need for venipuncture in the second method, while for free gingival grafts, the main disadvantage is the second operative field (donor site). Despite the good results these methods show in limiting the amount of bone loss, they cannot fully compensate for the greater resorption in sockets with thin buccal walls. Other authors reported that ridge preservation limited volumetric bone loss regardless of buccal bone width, but they performed guided bone regeneration with membranes and allografts/xenografts.

Post-extraction bone resorption may necessitate additional regenerative procedures during or before implant treatment. Ridge preservation aims to preserve the available tissue volume, avoid the need for guided bone regeneration, and facilitate prosthetic rehabilitation and prosthetic-guided implantation.

## **Contributions**

### Original contributions

1. We proposed our original methodology for measuring the height of the bone plates of the socket relative to the maxillary sinus and the mandibular canal on cone-beam computed tomography.

2. For the first time, the simultaneous use of non-porous PTFE membranes and platelet-rich plasma as a method for guided regeneration in post-extraction sockets was investigated.

3. We proved that the application of PRP did not improve the results of guided regeneration with non-porous PTFE membranes regarding the preservation of the vertical dimensions of both buccal and palatal/lingual socket plates.

4. For the first time, a comparative analysis of the following three ridge preservation methods was performed: with an application of PTFE membranes, with a combination of PTFE membranes and PRP, and with free full-thickness gingival grafts.

### Contributions original for the country

5. For the first time in our country, non-porous PTFE membranes were applied as a method for ridge preservation.

6. For the first time in our country, full-thickness gingival grafts were applied as a method for ridge preservation.

7. For the first time in the country, the influence of ridge preservation methods on the change in the height of the buccal and palatal/lingual bone plate was investigated.

8. For the first time in our country, the influence of the width of the bone plates on the amount of their vertical resorption was investigated.

9. For the first time in the country, the role of the type of jaw, the area of the dentition, the diagnosis, and smoking status on the vertical resorption of the socket walls with/without the application of RP was investigated.

### Confirmatory contributions

1. We confirmed that ridge preservation methods could successfully reduce vertical post-extraction resorption of the socket walls compared to those left on spontaneous healing.

2. We confirmed that the application of non-porous PTFE membranes could reduce the post-extraction vertical resorption of the socket walls.

3. We confirmed that the application of full-thickness gingival grafts could reduce the post-extraction vertical resorption of the socket walls.

4. We confirmed that smoking did not affect the amount of vertical resorption of the socket walls.

## **Publications related to the dissertation**

**1.** Yotsova R, Peev S, Georgiev T. Alveolar ridge preservation using dense polytetrafluoroethylene membranes. A review article. *Scripta Scientifica Medicinae Dentalis*. 2021 Dec 15;7(2).

**2.** Yotsova R, Peev S, Kolarov R. Application of platelet-rich plasma for alveolar ridge preservation. A review article. *Scripta Scientifica Medicinae Dentalis*. 2022 Aug 10;8(1):18-37.

**3.** Yotsova R, Peev S, Kolarov R. Immediate implant placement using customized healing abutments as a method of hard and soft tissue preservation. A review article. *Scripta Scientifica Medicinae Dentalis*. 2022 Aug 11;8(2).