

An innovative method for three-dimensional bone reconstruction of the anterior mandible with preserved dentition using an allogeneic bone block: A 6-month follow-up

Sylwia Hnitecka^{1,A–F}, Marzena Dominiak^{2,A,E,F}, Cyprian Olchowcy^{3,D–F}, Tomasz Gedrange^{4,5,A,E,F}

¹ Department of Maxillofacial Surgery, Wrocław Medical University, Poland

² Department of Dental Surgery, Wrocław Medical University, Poland

³ Collegium Medicum, Jan Długosz University in Częstochowa, Poland

⁴ Department of Oral Surgery, Wrocław Medical University, Poland

⁵ Department of Orthodontics, Dresden Medical University, Germany

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;

D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online)

Adv Clin Exp Med. 2025;34(5):763–775

Address for correspondence

Sylwia Hnitecka

E-mail: sylwia.hnitecka@gmail.com

Funding sources

None declared

Conflict of interest

None declared

Acknowledgements

The authors want to express their gratitude to Dr. Krzysztof Kujawa from the Centre for Statistical Analysis at Wrocław Medical University for his valuable insight regarding the statistical analyses.

Received on December 11, 2023

Reviewed on January 2, 2024

Accepted on June 10, 2024

Published online on August 9, 2024

Cite as

Hnitecka S, Dominiak M, Olchowcy C, Gedrange T. An innovative method for three-dimensional bone reconstruction of the anterior mandible with preserved dentition using an allogeneic bone block: A 6-month follow-up. *Adv Clin Exp Med.* 2025;34(5):763–775. doi:10.17219/acem/189840

DOI

10.17219/acem/189840

Copyright

Copyright by Author(s)

This is an article distributed under the terms of the Creative Commons Attribution 3.0 Unported (CC BY 3.0) (<https://creativecommons.org/licenses/by/3.0/>)

Abstract

Background. Bone defects around the teeth affect a large portion of the population. Bone regeneration in the area of existing teeth is completely different from that in an edentulous area. To date, no method has been developed for three-dimensional (3D) bone reconstruction in regions with preserved teeth.

Objectives. This study aimed to radiologically evaluate the results of the new method of 3D mandibular bone reconstruction in preserved dentition using a custom-made allogeneic bone block with a 6-month follow-up.

Materials and methods. Alveolar ridge dimensions were radiographically assessed before and 6 months after reconstruction using cone beam computed tomography (CBCT) scans in 32 patients (192 teeth). Reconstruction used a bone block that had been previously planned and prepared using CAD/CAM technology.

Results. The observed changes in alveolar bone dimensions were highly significant in most cases ($p < 0.001$). The closer to the tooth root apex, the lower the bone growth in the sagittal dimension (average of the mean values for each tooth examined in the measured heights): CEJ2: 2.9 mm, ½ CEJ2: 2.7 mm, ¼ CEJ2: 1.9 mm, and API: 1.4 mm. The maximum bone growth in the vertical dimension was observed on tooth 43 (9.9 mm), followed by 32 (9.8 mm), 33 (8.5 mm), 31 (8.4 mm), 42 (8 mm), and 41 (7 mm). The degree of decrease in vestibular dehiscence of the bone was greater the closer the tooth was to the midline (average of –3.8 mm and –3.4 mm for the central incisors; average of –2.8 mm and –2.6 mm for the lateral incisors; average of –2.6 mm and –2.5 mm for the canines).

Conclusions. The results prove that it is possible to prevent bone dehiscence in patients undergoing orthodontic treatment, increasing the ability and effectiveness of covering recessions and improving the morphology of the lower part of the face.

Key words: allograft, mandibular reconstruction, allogeneic bone block, bone reconstruction, orthodontics

Background

A variety of techniques and materials can be found in the available literature, and their continuous improvement is aimed at increasing the effectiveness of alveolar bone regeneration procedures. The methods reported use bone granules, chips, wedges, bone rings, plates, and blocks, including individualized ones, among other things. The effects of using autogenous and allogeneic bone, as well as xenogenous and alloplastic materials, are constantly being compared.^{1–5}

However, these procedures are based on achieving the effect of bone growth in edentulous sections of the alveolar process, mainly as preimplantation preparation. To date, many cases have been evaluated and described that bone regeneration in edentulous sections is currently considered a predictable procedure, provided that certain rules are followed.² The situation of bone regeneration in areas with existing teeth is completely different.

Dental reports on the regeneration of periodontal vertical bone defects (intrabony) confirm the possibility of achieving satisfactory results.^{6–8} Nevertheless, the three-dimensional (3D) regeneration of the alveolar ridge in the dental area poses a problem, especially when it comes to regeneration in the vertical dimension.

It is important to address this issue and look for solutions because the problem of bone defects around the teeth affects a large part of the population.^{9,10} This problem becomes especially important when orthodontic treatment is required or when advanced gingival recessions need to be covered.^{11–13}

It turns out that the procedures for adequate bone regeneration require careful planning and consideration not only of the type of graft material and its ready-to-use form but also of the surrounding anatomy, with special attention to the quality and quantity of the soft tissues and the function of the surrounding muscles.^{14,15}

In this article, we present a novel and innovative method for 3D bone reconstruction of the anterior mandible with preserved dentition using an allogeneic bone block (ABB), focusing on the method of appropriate patient qualification, treatment planning, and the necessary preparatory steps for the basic bone regeneration procedure.

Objectives

This prospective, nonrandomized study aimed to radiologically evaluate the results of a 3D bone reconstruction method for the anterior mandible with preserved dentition with a 6-month follow-up. The main goal was to measure changes in bone dimensions in the anterior mandible based on preoperative cone beam computed tomography (CBCT) analysis and after 6 months.

Materials and methods

Study group

The analysis included data from CBCT scans and the medical records of 32 patients who had undergone anterior mandibular reconstruction surgery using a 3D ABB (as below) and appeared for a 6-month follow-up and CBCT scan. Patients were treated in a private dental practice in Wrocław, Poland, between 2018 and 2021.

Participants were initially referred for surgical consultation for the following reasons:

1. Clinically confirmed gingival recession and consecutive radiographically visible bone defects in the alveolar part of the mandible – both in patients who have never received orthodontic treatment and in those undergoing and following orthodontic treatment.
2. Radiologically identified bone defects without concomitant gingival recession in patients who had a CBCT prior to orthodontic treatment, taking into account the movements of the anterior mandibular teeth.

The analysis included adult (non-growing) patients with preserved dentition, at least in the anterior mandible (teeth 33–43), who had signed an informed consent form for the procedure and participation in the study. This study included patients with bone defects covering the anterior region of the mandible in the area of teeth 33–43 (from the right canine to the left canine) with various configurations and the advancement of dehiscence and/or fenestration.

Patients undergoing orthodontic treatment were also included. In these patients, tooth movements in the analyzed area were suspended for the duration of surgical treatment (passive treatment for 6 months). Smoking patients were advised to give up smoking and were informed about its possible negative effects on healing. Diabetics were also not excluded from the study, provided the disease was stable. There was no upper age limit.

The exclusion criteria included systemic diseases and drug treatments that could affect bone tissue (e.g., Paget's disease, osteoporosis, use of bisphosphonates, or denosumab), previous surgical and periodontal treatments in the anterior mandible, craniofacial anomalies, and previous trauma to the mandible. Patients who did not report for the control CBCT 6 months after reconstruction (4 patients) were also not evaluated.

The primary goal was to quantify bone growth in a population that had a similar procedure performed in a standardized way – values that could be reliably compared.

Research components

The clinical examination mainly included periodontal assessment, identification of possible periodontal pockets (probing depth >4 mm), presence and advancement of the gingival recession, biotype, presence of calculus



Fig. 1. Initial clinical situation of the example patient. Prominent alveolar yokes are visible

and plaque, abnormal frenal attachments (especially on the lower lip), and mentalis muscle tension. It was also determined if the patient had received orthodontic treatment and what type of malocclusion it was, as well as if a gingival graft was required (connective tissue graft (CTG) or free gingival graft (FGG) or both) (Fig. 1).

To adequately prepare and manage the patient, the FLOS concept was developed. This approach consists of physiotherapy, speech therapy, osteopathy, and dental procedures if it's required, due to:

1. a lot of tension of facial muscles and the floor of the mouth from a short lingual frenulum,^{16,17}
2. infantile types of swallowing and other similar,
3. wrong posture,¹⁸ and
4. non-carious lesions such as wedge defects or abfraction defects.

The ABB was prepared and obtained in the patented manner presented in the previous article.¹⁹ Briefly, precise cephalometric measurements were first performed with the calculation of the ANB angle and the determination of the face type, since the value of the ANB angle after reconstruction should not exceed 4–5°. The position of the incisors and mandible and the inclination of the mandibular incisors were assessed. The relationship between the maxillary and mandibular incisors was also considered. The crestal bone level was the decisive parameter. In cases undergoing or planning to undergo orthodontic treatment, bone loss should also be predicted in relation to tooth movement. Therefore, the ALi-CEJ2-B angle was measured. Three points were marked: the deepest concavity on the anterior surface of the mandibular symphysis (point B), the apical point of the anteriormost mandibular central incisor (Iia), and the point 2 mm apical to the cemento-enamel junction (CEJ) of the incisor, which reflects the sulcus depth.

The CBCT scans played a special role in planning the bone block. The area of the bone defect was divided into 4 external regions and 1 internal region, and the reference points were set on the recipient bed. The point is that the bone graft must be very suitable. Therefore, the internal

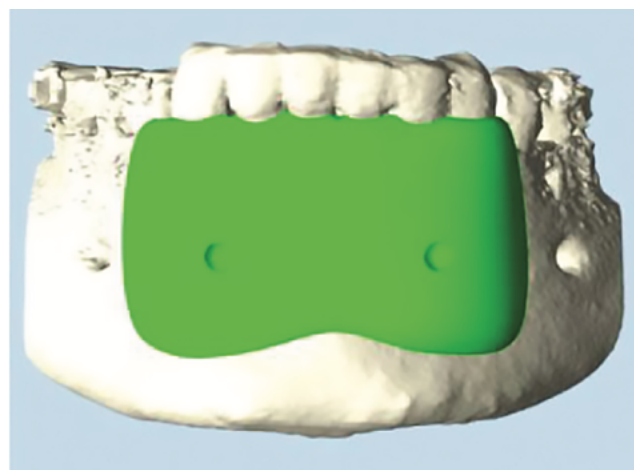


Fig. 2. Bone block planning based on cone beam computed tomography (CBCT). The defect planning is divided into 5 regions (4 external and 1 internal). The position of the screws was taken into account in the planning

surface of the block was assessed to make the contact area with the underlying bone as wide as possible (Fig. 2).

Target values were updated to include actual reference point values, and a new range of target values was added to the diagnostic defect area. The bone block body was connected to the first analyzed plane of the mandibular segments with the bone block used. The shape of the chin and the design of the block were also determined. It was important to keep the thickness of the block similar to the thickness of the possible physiological bone regeneration. In addition, the longitudinal axes of the basal symphysis and alveolar symphysis were positioned as parallel to each other as possible, and the total angle created between them did not exceed 10°. The shape of the chin and the design of the block were also checked.

The size of the ABB depended on the size of the bone defect. The assessment was made by the surgeon based on the analysis of the horizontal cross-section of the alveolar process and objectively by orthodontic analysis. The width was assessed from the right to the left canine to ensure that it matched the shape of the mandible in all dimensions. The incisor crowns were measured, and the value determined the arch shape between the canines. The position of the teeth and the mandibular alveolar region were drawn on CBCT scans, and the desired size of the arch was added.

The height of the bone block was determined using 2 measurements in the direction of the crown and the apex, while the level of the crestal bone was positioned 2–3 mm below the CEJ level, which corresponds to the biological width of teeth with healthy periodontium.

The final step was computer-aided design and fabrication (CAD/CAM). A virtual model of the mandible was created by converting CBCT scans and intraoral scans into digital models. A bone block design was placed on the model, and the actual and nominal points were combined to form

Table 1. Clinical steps for preparing the bone block recipient bed

Protocol for 3D bone reconstruction	
1. With recessions	
a. FLOS technique and botox injection in mental muscle – big tension	12–2 weeks before surgery
b. cutting of high attachment of frenum – lingual, buccal, central	4 weeks before surgery
1.1 free gingival graft – is required when are II–IV Miller class of recessions, shallow vestibule	12 weeks to next ST or bone procedure
1.2. soft tissue augmentation	12 weeks to bone surgery
a. thin biotype – superficial connective tissue graft (CTG)	
b. thick biotype – subepithelial CTG	
1.3. 3D bone block- severe loss of the bone	6 months to ortho treatment
2. Without recessions	
a. FLOS technique and botox injection in mental muscle – big tension	12–2 weeks before surgery
b. cutting of high attachment of frenum – lingual, buccal, central	weeks before surgery
1.1. deepening of vestibule – is required when shallow vestibule is present – FGG	4 weeks to next ST or bone procedure
1.2. soft tissue augmentation	12 weeks to bone surgery
a. thin biotype – superficial CTG	
1.3. 3D bone block – severe loss of the bone	months to ortho treatment

ST – soft tissue; CTG – connective tissue graft.

a single unit. A suitable living donor was selected based on adequate cancellous and/or cortical bone volume with the correct bone density. After milling, the block was cleaned, packaged, sterilized, and sent to the surgeon.

Each patient was operated on in the same way and by the same surgeon. Before bone reconstruction was started, it was determined whether the condition of the soft tissues was sufficient. If the biotype was too thin and gingival recession was advanced, FGG and/or CTG were used first (3 months before bone reconstruction). Excessive tension of the mentalis muscle with a specific “orange peel” sign was reduced with an intramuscular injection of botulinum toxin (Table 1).

Bone reconstruction was performed under local anesthesia and with an antibiotic (0.6 g clindamycin) administered orally 1 h before the procedure. First, an intrasulcular incision was made that was 2 spaces wider mesially and

distally than the planned reconstruction area, and the mucoperiosteal flap was elevated above the mental eminence to create a catch bed without excessive tension. The bone dehiscences and concavities of the mandibular alveolar region were then exposed (Fig. 3). The root surfaces were then mechanically cleaned of debris, and a surgical drill was used to decorticate the compact bone in the interdental spaces to improve vascularization. The advanced platelet-rich fibrin (PRF) membranes (A-PRF)²⁰ were prepared using the patient’s peripheral blood and placed on the surface. Allogeneic bone particles were placed in the deepest bone defects, followed by the insertion of an individualized 3D block of allogeneic bone. The ABB was stabilized in the desired position with 2 titanium screws (Fig. 4), which were removed after 6–8 weeks. Screws approx. 8 mm in length were used. The A-PRF membranes were placed on the outer surface of the block, and sometimes a pure resorbable collagen



Fig. 3. View after mucoperiosteal flap elevation. Advanced bone dehiscences, fenestrations and exposed tooth roots are visible



Fig. 4. View of the block after fixation with titanium screws to the bone base

barrier membrane previously soaked with a liquid fraction of PRF (i-PRF) was placed apically. The final stage was suturing non-resorbable 6-0 sutures using the suspended and mattress suturing technique, which prevents excessive flap tension. An antibiotic, 0.6 g of clindamycin (Clindamycin MIP; MIP Pharma GmbH, Blieskastel, Germany) twice daily and painkillers for 3 days were prescribed. The patient was instructed to perform proper oral hygiene with an antiseptic mouth rinse (Alfa; ATOS, Warsaw, Poland) and to use a soft toothbrush (Elgydium Clinic 7/100; Elgydium Pierre Fabre, Paris, France) for postoperative care. Immediately after the procedure, biostimulation using a Nd:YAG laser (10 Hz, 0.5 W) was performed (TwinLight®; Fotona, Ljubljana, Slovenia). The sutures were removed 2 weeks after the procedure. The whole procedure was performed according to the method patented by Dominiak M. and Gerdrange T. (EP3287097B1, European Patent Office, Munich, Germany, 2016).

All CBCT scans (before and 6 months after surgery) were acquired with the Pax Flex3D Vatech computed tomography system (Vatech Europe, Warsaw, Poland). The mandible center image: field of view (FOV): 80 mm width and 50 mm height. The voxel size was 0.200. All images were analyzed using EzDent-i software (Vatech). Defined parameters were measured in the sagittal plane at the center of the central incisors (teeth 31 and 41), lateral incisors (teeth 32 and 42) and canines (teeth 33 and 43). Only the buccolingual bone dimension was measured in the axial plane. The reference lines were perpendicular to each other, and the vertical line was aligned with the long axis of the tooth. The tooth inclination was not determined.

The width of the alveolar ridge was measured at 4 points on the tooth root: at the CEJ-2 mm level (the crestal bone level of the healthy periodontium), at the root apex, at half-length from CEJ-2 to the apex, and at the quarter-length from CEJ-2 to the apex. It was determined if there was dehiscence (the marginal bone level was below the CEJ-2 mm level). The height was measured from the vestibular (HDV) and/or lingual (HDL) side. It was determined whether vestibular and lingual fenestration were present (FV and FL, respectively). If fenestration was present, its height was measured (HFV and HFL). When the vestibular and lingual bone layers were extremely thin, this was noted separately. The value of this dimension was then determined (HVCL – the height of the vestibular cortical layer and HLCP – the height of the lingual cortical layer). If the level of marginal bone on both sides was below the CEJ-2 (bilateral dehiscence), then the measured width at this level corresponded to the width of the tooth; therefore, the value for the alveolar width was considered to be 0. The buccolingual dimension halfway from CEJ-2 to the apex was measured. This value was determined on the axial section, mesial and distal to the teeth (WAM – width of alveolar bone medial and WAD – width of alveolar bone distal) (Fig. 5). A detailed description of the measurement in such cases has already been described.⁹

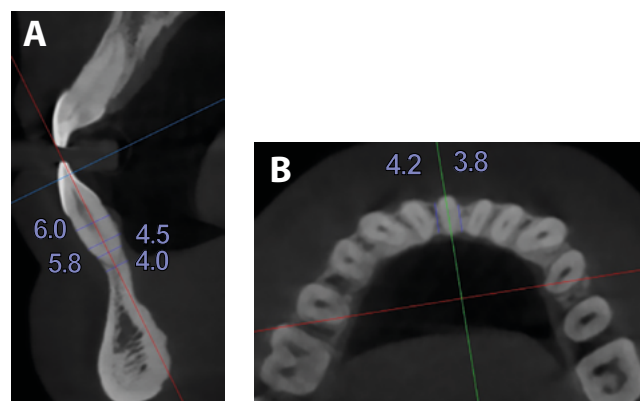


Fig. 5. Scheme of the performance of radiological measurements of the alveolar width – sagittal (A) and axial (B) views

The types and advancement of bone defects were classified according to the classification by Yang et al.²¹ and the classification we made (DM classification) as described in another paper.⁹ (Fig. 6).

In the radiological assessment, existing dehiscences and lingual fenestrations were also noted, although they were not subject to surgical treatment. The lack of changes after reconstruction is related only to the post-surgical observation of the type of changes in a given area (Fig. 7,8).

Statistical analyses

Bone growth was defined as the difference between the measurements taken after 6 months and before treatment in a given patient (repeated measurements). An additional condition of the study was the interdependence of the given variables within the set of all 6 teeth coming from 1 patient. Furthermore, the distribution of differences between the pre- and post-treatment measurements deviated statistically significantly from the normal distribution in 81% of the tests (Supplementary Table 1). Considering the circumstances described above, the pattern of tooth changes was analyzed using a model for all teeth, for 1 of the 12 variables studied (CEJ2, 1/2CEJ2, etc.), using the R package nparLD,²² which is a tool for nonparametric analysis of repeated measures data. We used the command 'nparLD': nparLD(data=dane, CEJ2 ~ Time*Tooth, alpha = 0.05, ...). The effect size in this test is the "relative treatment effect" (RTE). The model evaluated the statistical significance of Treatment, Tooth, and the Treatment × Tooth effects interactions. The results of running this model for the twelve variables included (1) a table with the statistical significance of the effects (listed above), (2) figures visualizing the effects, and (3) a table with the RTE and its 95% confidence intervals (95% CIs). Since the nparLD does not provide a post hoc test for such a model, the last table above served as the basis for detecting statistically significant differences between before and after treatment measurements.

Due to non-normal data distribution in many of the variables, Spearman's rho rank correlation coefficients were used

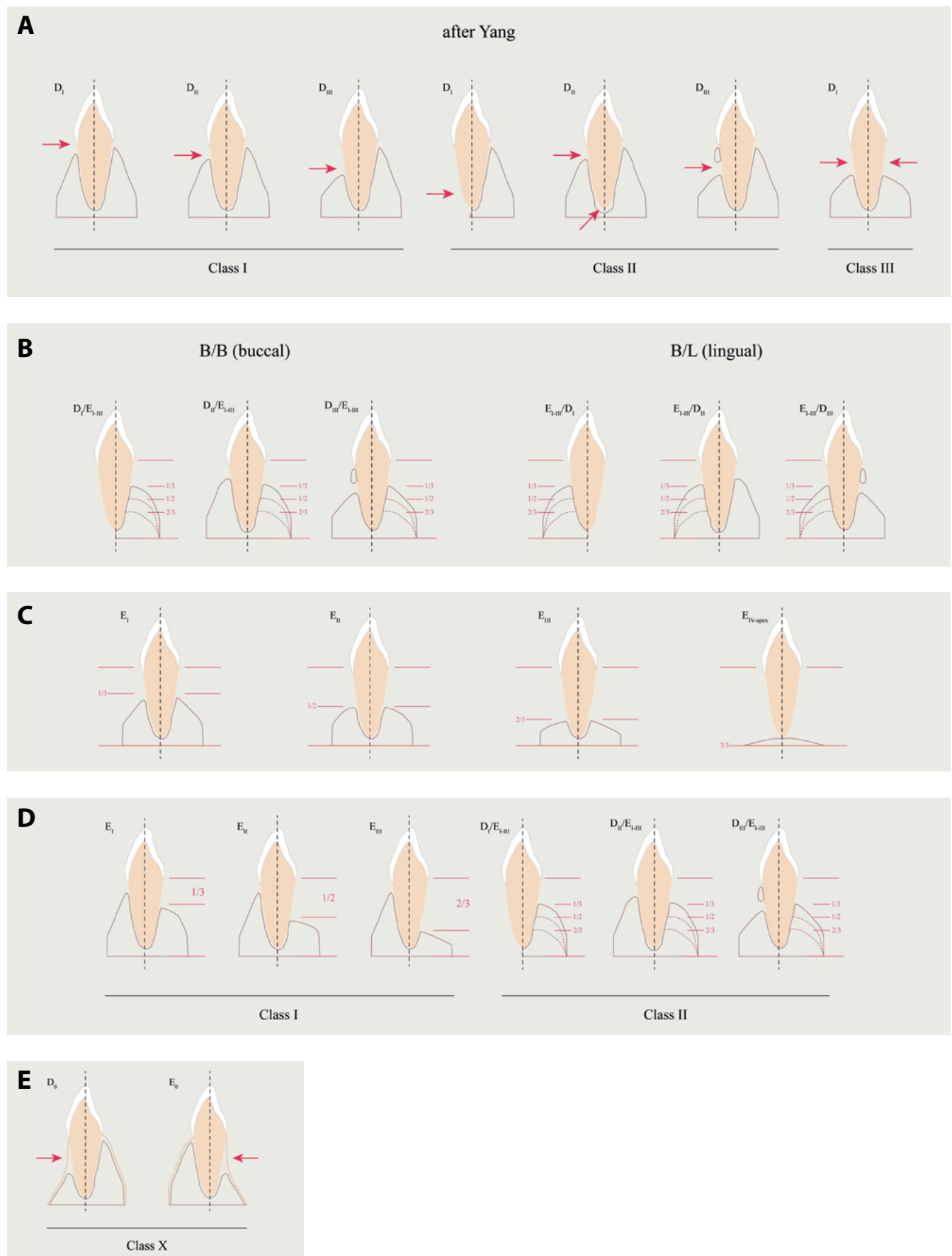


Fig. 6. Yang et al.²¹ (A) and DM⁹ classifications – class I (B), class II (C), class III (D), and class X (E)

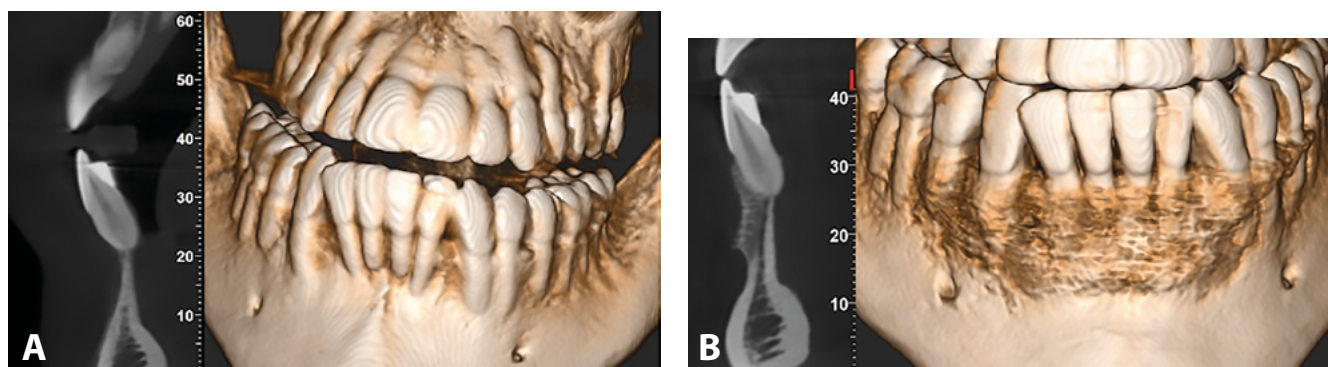


Fig. 7. Cone beam computed tomography (CBCT) scans before (A) and 6 months after bone reconstruction (B) – example patient No. 1

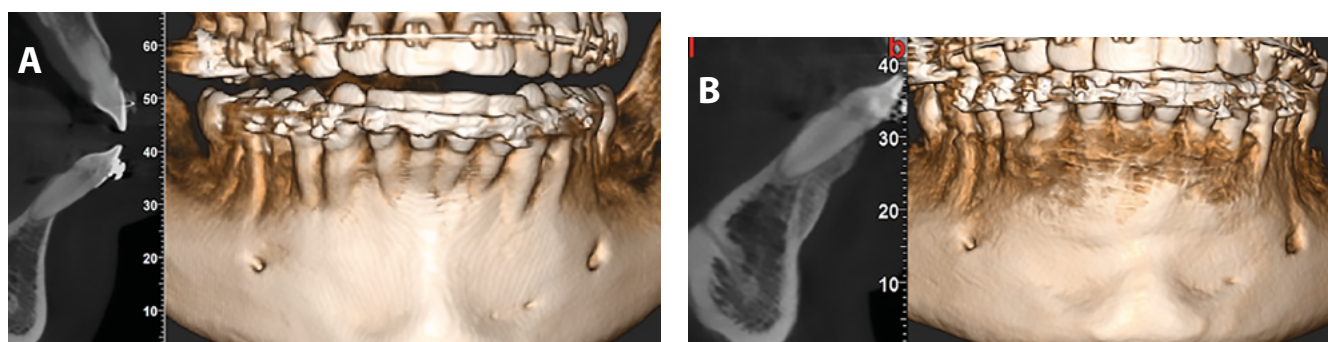


Fig. 8. Cone beam computed tomography (CBCT) scans before (A) and 6 months after bone reconstruction (B) – example patient No. 2

to evaluate the relationship between patient age and changes in bone dimensions. The Shapiro–Wilk test was used to check data distribution normality. Spearman's rho rank correlation coefficients were used to evaluate the monotonic component of the correlation between patient age and changes in bone dimensions. Although the nonparametric test was used in the analysis of the tooth changes, all variables are presented as means and standard deviation (SD), which are more precise than medians and quartiles when tiny changes have occurred. Additionally, the means and median were strongly correlated (Spearman's correlation: $n = 72$, $R = 0.93$, $p < 0.001$), which justifies using the means for presenting bone growth patterns. The statistical description of bone growth parameters is presented in Supplementary Table 2.

The Fisher's exact test was used for the contingency table analysis. As the comparison of bone growth variables between the sexes was considered an exploratory approach in the analysis, no correction for the controlling of Type-I errors was used.

When estimating the required sample size, a postoperative bone growth of at least 1.0 mm, a SD of 1.5 mm and a test power of at least 0.8 were assumed. With these assumptions and an assumed significance level of $\alpha = 0.05$, the minimum sample size was $n = 20$. The selection of patients for the research sample was done successively, and the adequacy of the sample size was continuously checked by estimating the power of the statistical tests used. Patients were included in the research group until the main objective of the study, i.e., bone growth after

surgery measured at CEJ2, 1/2CEJ2 and 1/4CEJ2 levels, was achieved with a test power of $1 - \beta = 0.80$.

The lowest growth was observed in tooth 43. For the measured bone growth at the CEJ2 level (1.6 ± 3.0), the significance of the test with a sample size of $n = 32$ was 0.832 and thus above the assumed minimum value of 0.8. For the same tooth and an increment of 1/4CEJ2 of 1.1 ± 1.3 , the significance of the test was 0.996.

Statistical analysis was performed using the R environment (R Foundation for Statistical Computing, Vienna, Austria, <https://www.r-project.org>) and Statistica v. 13.3 software (TIBCO Software Inc., Palo Alto, USA).

Ethics statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and an approval of the Bioethics Committee of Wroclaw Medical University was obtained (No. KB-284/2023N). The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist was completed.

Results

Overall

The analysis included 32 patients – 25 women (78.1%) and 7 men (21.9%) with a F:M ratio = 1:3.6. Patients' ages

Table 2. The changes in tooth variables as the difference between after and before treatment (means and standard deviation; SD). Statistically significant differences are in bold. Statistical significance was calculated using the Relative Treatment Effect and R-package “nparLD” (see Supplementary Table 3 for details)

Variable	Tooth						Average
	43	42	41	31	32	33	
ΔCEJ2	1.6 ± 3.0	3.3 ± 3.5	3.9 ± 3.2	3.0 ± 3.3	3.2 ± 3.7	2.0 ± 3.5	2.9 ± 2.1
Δ½CEJ2	1.1 ± 1.8	3.3 ± 2.5	3.4 ± 2.4	3.4 ± 2.7	3.2 ± 2.8	2.8 ± 2.0	2.7 ± 1.6
Δ¼CEJ2	1.1 ± 1.3	2.4 ± 1.9	2.4 ± 1.8	2.4 ± 2.1	2.1 ± 1.5	1.2 ± 1.3	1.9 ± 1.3
ΔAPI	0.7 ± 0.9	1.8 ± 1.3	1.8 ± 1.1	1.7 ± 1.1	1.6 ± 1.2	0.8 ± 0.9	1.4 ± 0.8
ΔHD1	-2.6 ± 3.1	-2.6 ± 2.9	-3.4 ± 2.3	-3.8 ± 2.3	-2.8 ± 2.8	-2.5 ± 3.0	-2.9 ± 1.8
ΔHD2	-0.0 ± 0.1	-0.1 ± 0.2	0.1 ± 0.7	-0.1 ± 0.2	-0.0 ± 0.1	0.0 ± 0.1	-0.0 ± 0.1
ΔHBP	-0.1 ± 1.7	-0.4 ± 1.9	-0.5 ± 1.3	-0.3 ± 1.5	-0.8 ± 1.6	-1.0 ± 2.1	-0.5 ± 0.8
ΔHBJ	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	-0.1 ± 0.5	0.0 ± 0.0	-0.2 ± 0.9	-0.0 ± 0.2
ΔHF1	-0.9 ± 2.1	-0.8 ± 1.6	-0.2 ± 1.5	0.0 ± 0.2	-0.8 ± 1.6	-0.4 ± 2.1	-0.5 ± 1.1
ΔHF2	0.0 ± 0.0	-0.0 ± 0.0	-0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	-0.0 ± 0.0
ΔWAM	1.6 ± 1.1	2.4 ± 1.4	2.4 ± 1.3	2.3 ± 1.5	2.2 ± 1.6	1.9 ± 1.4	2.1 ± 1.0
ΔWAD	1.2 ± 1.0	2.1 ± 1.1	2.7 ± 1.6	2.1 ± 1.2	2.1 ± 1.3	1.4 ± 1.1	1.9 ± 0.9

ranged from 18 to 50 years at the time of the procedure; the mean was 32.1 ± 9.2 years.

The largest study group consisted of patients after orthodontic treatment (n = 10; 31.25%; mean age of 35.5 years), followed by study participants before planned orthodontic treatment (n = 9; 28.1%; mean age of 33.5 years) and during orthodontic treatment (n = 9; 28.1%; mean age of 31.6 years). On the other hand, orthodontic treatment was not planned in only 4 study participants (12.5%, mean age of 34 years) who had not been treated previously.

The results of the procedures are shown in Fig. 7,8 using the example of selected patients from the studied group.

The observed changes in alveolar bone dimensions (Table 2) were statistically significant in most variables except for the level of lingual dehiscence (ΔHD2), lingual cortical bone (ΔHBJ) and FL (ΔHF2) (Table 3). The analysis was performed using the R-package nparLD. Among the 9 variables statistically significantly affected by Time (i.e., treatment), no interactions with teeth occurred in 2 variables only (CEJ2 and HBP). In the case of the other variables, such interactions were statistically significant, which means that the changes differed between some teeth (Fig. 9, Supplementary Table 3).

The closer to the tooth root apex, the lower the bone growth in the sagittal dimension. Average of the mean values for each analyzed tooth in the measured heights: CEJ2: 2.9 mm, ½ CEJ2: 2.7 mm, ¼ CEJ2: 1.9 mm, and API: 1.4 mm.

In the sagittal dimension, the level at the:

- CEJ2 had the highest average bone growth at tooth 41 (3.9 mm) and the lowest at 43 (1.6 mm),
- ½ CEJ2 had the highest average bone growth at 31 and 41 (3.4 mm) and the lowest at 43 (1.1 mm),
- ¼ CEJ2 had the highest average bone growth at teeth 31, 41, and 42 (2.4 mm) and the lowest at 43 (1.1 mm), and

– API found the highest average bone growth at 41 and 42 (1.8 mm) and the lowest at 43 (0.7 mm).

Maximum bone growth in the vertical dimension was found at tooth 43 (9.9 mm), followed by 32 (9.8 mm), 33 (8.5 mm), 31 (8.4 mm), 42 (8 mm), and 41 (7 mm).

The degree of decrease in vestibular dehiscence of the bone was greater the closer the tooth was to the mid-line (average -3.8 mm and -3.4 mm for central incisors, 31 and 41, respectively; average -2.8 mm and -2.6 mm for lateral incisors, 32 and 42, respectively; and average -2.6 mm and -2.5 mm for canines 43 and 33, respectively).

The presence of an extremely thin cortical plate before reconstruction was noted in 26 of 192 teeth examined (13.5%) and FV in 28 cases (14.6%). The average height of the vestibular plate was 4.3 mm, while the height of the fenestration plate was 4.1 mm.

Due to the lack of surgical intervention on the lingual side and the elimination of possible orthodontic movements in patients with braces, no differences in the dimensions of bone dehiscences and fenestrations were observed (only values of ±max 0.2 mm, mainly due to measurement errors).

Tangential to the mesial surface of the tooth at the level of ½ CEJ2, the average bone increment in the sagittal dimension (WAM) was 2.1 mm and was greatest at teeth 41 and 42, while distal (WAD) averaged 1.9 mm and was also greatest at tooth 41.

Analysis of the influence of age and sex

There was no statistically significant correlation between patient age and bone growth in any of the variables studied (Supplementary Table 4). There was also no statistically significant difference between men and women in terms of bone growth (Supplementary Table 5).

Table 3. Statistical significance of time (“after” vs “before”), tooth and interaction time × tooth for treatment effects measured with the use of variables CEJ2, 1/2CEJ2, etc. The analysis was performed using the R-package nparLD (see the section “Statistical analyses” for details)

Explained variable	Effect	Statistic	df	p-value
CEJ2	time	62.09	1	<0.001
	tooth	21.49	3.20	<0.001
	time:tooth	1.8	3.98	0.127
1/2CEJ2	time	149.81	1	<0.001
	tooth	27.3	2.69	<0.001
	time:tooth	17.3	3.32	<0.001
1/4CEJ2	time	147.93	1	<0.001
	tooth	20.11	3.48	<0.001
	time:tooth	11.35	3.31	<0.001
API	time	106.77	1	<0.001
	tooth	7.98	3.08	<0.001
	time:tooth	11.63	3.67	<0.001
HD1	time	92.17	1	<0.001
	tooth	3.72	3.32	0.008
	time:tooth	2.41	3.87	0.049
HD2	time	0.45	1	0.504
	tooth	14.66	3.44	<0.001
	time:tooth	0.7	1.69	0.472
HBP	time	12.13	1	<0.001
	tooth	0.36	4.51	0.858
	time:tooth	1.27	4.11	0.280
HBJ	time	1.84	1	0.175
	tooth	0.88	1.89	0.411
	time:tooth	1.65	1.41	0.198
HF1	time	6.81	1	0.009
	tooth	2.78	3.99	0.026
	time:tooth	2.56	3.88	0.038
HF2	time	2.07	1	0.151
	tooth	0.68	1.72	0.484
	time:tooth	0.79	1.90	0.446
WAM	time	160.27	1	<0.001
	tooth	25.2	3.64	<0.001
	time:tooth	5.09	4.27	<0.001
WAD	time	159.63	1	<0.001
	tooth	22.39	3.40	<0.001
	time:tooth	12.72	4.24	<0.001

df – degrees of freedom.

The average bone growth in the group of patients who underwent orthodontic treatment with a passive archwire and in the group of patients without ongoing orthodontic treatment did not differ significantly except for HBJ and HF2, in which small and marginally statistically significant differences occurred (Supplementary Table 6).

Analysis of the impact of adverse features (gingival recessions, thin biotype, excessive function of the mentalis muscle)

Before treatment, the presence of the above factors that might affect the final treatment effect was determined.

In 22 (68.75%) of the cases, the gingival biotype was thin at baseline. The same number of participants ($n = 22$; 66.75%) were diagnosed with significant gingival recession. Almost half of the patients ($n = 14$; 43.75%) showed mentalis muscle hyperactivity.

The chance of achieving an optimal therapeutic effect was 4 times greater in the group of patients without recessions than in the group with recessions ($OR = 4.20$), but the 95% CI was (0.44–39.9) at a $p = 0.38$ using Fisher’s exact test, which should be interpreted as equal chances of an optimal outcome in both groups (not significantly dependent on the presence of recessions). There was no statistically significant difference between patients differing in the occurrence of recessions in relation to bone growth except for marginal significance for HF2 (Supplementary Table 7). A similar lack of dependence was seen in the patient groups with thick and thin biotypes (except for the marginal significance of HBJ) (Supplementary Table 8) and excessive mentalis muscle tension (Supplementary Table 9). Considering the above presentation of the method of preparing the patient for the procedure, the absence of these effects proves the proper implementation of the adopted algorithm – among other recession coverages and elimination of tension.

Predicting optimal treatment effect

The ideal therapeutic effect is evidenced by the ADI class according to DM after treatment. The assessment of treatment outcomes did not take into account the condition of the tissues on the lingual side. This effect was obtained in 24 (75%) patients. In the remaining patients, the ADI class was not definitively achieved, but the therapeutic effect was satisfactory.

The “gold standard” for optimal therapeutic effect was the DM classification, based on which patients were divided into 1 of 2 groups with ideal or satisfactory effects. Continuous variables such as age and changes in bone dimensions after 6 months of treatment were transformed into dichotomous (binary) variables, with cutoff values based on the analysis of receiver operating characteristic (ROC) curves. Patient age is a destimulating (reducing) variable for the chance of achieving an optimal treatment effect. As age increases, the probability of achieving an optimal therapeutic effect decreases. For an age cutoff ≥ 34 years, Sensitivity (Sens.) = 79.2%, Specificity (Spec.) = 75.0%, Accuracy (Acc.) = 78.1%, PPV = 90.5; Likelihood Ratio (+) = 3.17.

The ideal treatment effect was more frequent in the case of an increase in the:

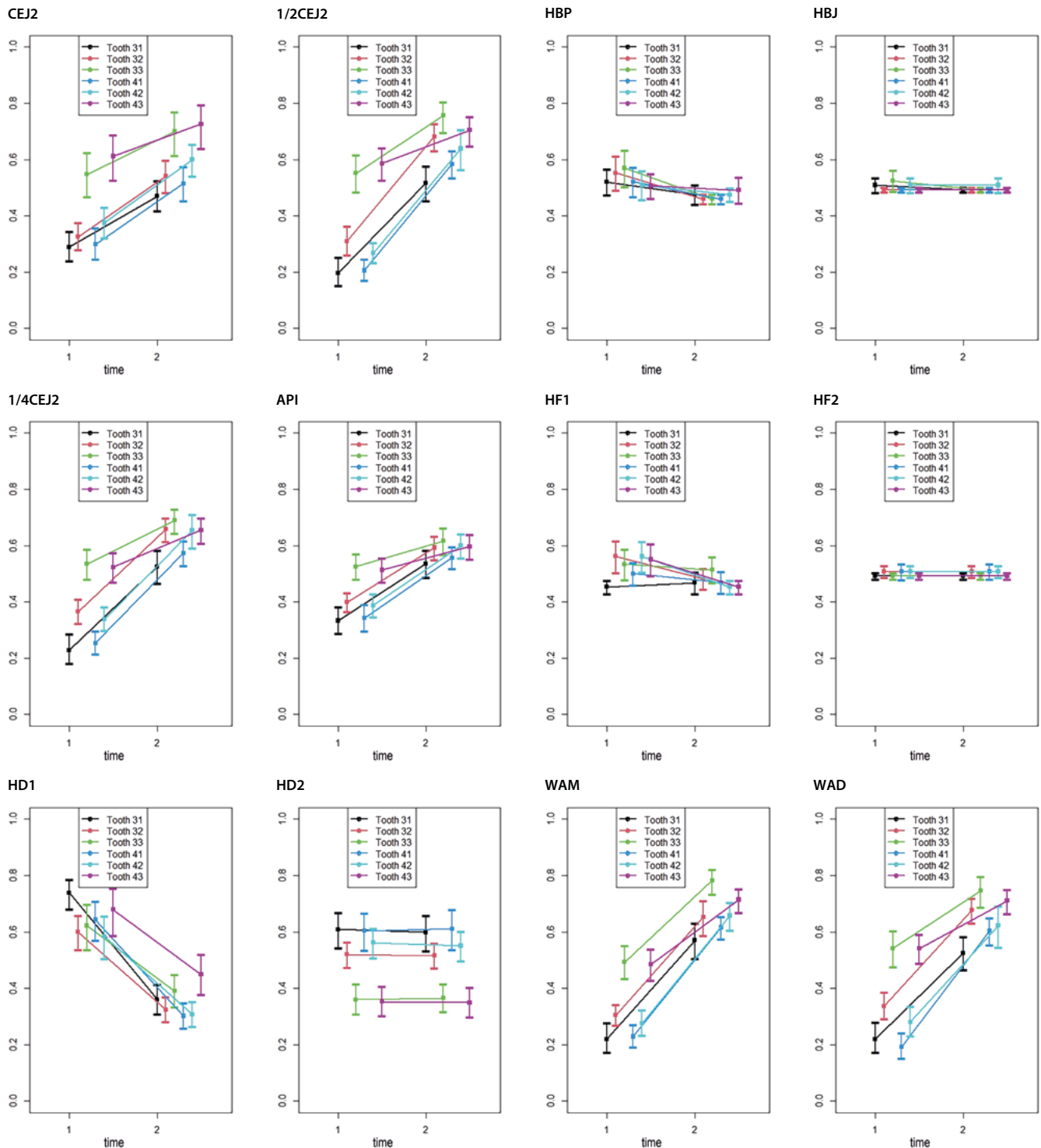


Fig. 9. Relative treatment effect (1 – measurements before treatment; 2 – measurements after treatment) with 95% confidence intervals (95% CIs), calculated with the use of R-package npard (see Supplementary Table 2 for details and statistical significance of the differences)

- Δ CEJ2 of tooth 31 by at least 0.5 mm, tooth 41 by 0.4 mm and tooth 42 by 0.1 mm;
- $\frac{1}{2}$ CEJ2 of tooth 41 by at least 5.9 mm and of tooth 42 by 3.0 mm;
- $\frac{1}{4}$ CEJ2 of tooth 32 by at least 1.6 mm; and
- WAM tooth 33 by at least 2.1 mm.

Discussion

Orthodontic treatment is currently very common in a significant percentage of dental patients, regardless of age. It is well known that proper and effective tooth movement requires the presence of an adequate amount

and quality of bone. Induced tooth movement should only be carried out at the alveolar bone trabeculae.¹² Unfortunately, the occurrence of a gingival recession is still a common side effect. One of the basic assumptions is that the thickness of the anterior part of the alveolar bone should be considered as a limiting factor for orthodontic treatment. Exceeding these anatomical limits is associated with an increased risk of bone loss and the formation of alveolar defects – dehiscences and fenestrations. Anterior teeth in the mandible are found to be most susceptible to these problems, and it has also been observed that the greatest treatment-related bone loss occurred on the side to which a tooth was moved.²³ In addition, pre-existing bone defects often act as “predisposing factors” for gingival recession.¹²

An exposed root surface due to gingival recession is often associated with dentin hypersensitivity, root caries, noncarious cervical lesions, impaired plaque control, and unaesthetic appearances. In addition, untreated gingival recession can lead to further apical displacement over time if the patient does not behave correctly.²⁴ It would be much better to prevent a recession as much as possible.

In the absence of similar methods developed for the purpose described, the analysis of the effectiveness evaluation focuses on the results obtained. First, it is necessary to consider why the ABB and CAD/CAM technology were used and what conditions must be met to achieve a satisfactory effect.

Allografts are a commonly used graft material nowadays. They come from a donor of the same species, which can be fresh frozen, freeze-dried or demineralized freeze-dried bone. This material may not only serve as an osteoconductive scaffold for new bone formation but may also have some osteoinductive potential due to the presence of proteins such as bone morphogenetic proteins.²⁵ No donor site morbidity, less postoperative discomfort, a much larger bone availability, and less bone resorption than autologous bone are leading surgeons to increasingly choose this graft material.²⁶ It is produced and used in various forms, ranging from tiny granules to large 3D blocks.

Brugnami et al. showed that the combination of corticotomy and guided bone regeneration (GBR) in orthodontically treated patients allows for an increase in the dimensions of the “bone envelope” so that the possible deleterious effects of orthodontic movements on periodontal tissues can be overcome, even when the movements are outside the original alveolar anatomy. However, the use of granules with membranes is associated with the movement of the material, the lack of a significantly stable 3D space and a relatively low regeneration potential. Such treatment leads to the formation of a conglomerate of augmentation material so that no new layer of cortical bone is formed with a new point B.²⁷

Knowing the excellent properties of allogeneic bone as a graft material, the search for a better and more

effective method leads to modifications in the shape and structure of the graft.

As early as the end of the 20th century, the idea of using CAD/CAM technology for the fabrication of onlay blocks in augmentation procedures was presented.²⁸ This technology allows for a custom fabrication of allogeneic bone blocks for a variety of alveolar ridge augmentation procedures. Many successful cases have been described, highlighting in particular the accuracy, precision and perfect fit of the bone blocks fabricated using CAD/CAM technology.²⁶

In our cases, the block was placed directly out of the sterile packaging onto the donor bone with a passive fit. Since no shaping or multiple adjustments were required, the open wound time and overall surgical time were significantly reduced.

The ideal therapeutic effect, as defined by the ADI class according to the DM classification after treatment, was achieved in 24 (75%) patients. In others, the ADI class was not fully reached, but the therapeutic effect was satisfactory. Very satisfactory results of maximum bone growth in the vertical dimensions were obtained because, in some cases, even more than 9 mm were reached.

Before performing the basic bone reconstruction procedure, possible complications and their causes should be considered, and an attempt should be made to eliminate them at the preparatory stage.

Common problems with allogeneic bone blocks include wound dehiscence with exposure of the membrane, opening of the incision and exposure of the bone block. These problems are largely due to poor oral hygiene, pre-existing disease, a thin biotype, and thus poor soft tissue management rather than the allogeneic blocks themselves.^{26,29} Therefore, proper soft tissue management should not be a way to treat the above complications but should be an appropriate preparatory phase for advanced bone reconstructions.

Only after the soft tissues (labial side) achieved a stable condition did reconstruction begin. Too thin a biotype, too little keratinized tissue, a shallow vestibule combined with a high and strong frenal attachment, and strong tensions from the mentalis muscle can lead to recession relapse, flap retraction and exposure of the bone block, which could result in a negative outcome, especially during the early phases of healing.¹⁴

In the group of patients we analyzed, there was no statistically significant difference between the patients who differed in the occurrence of recession in relation to bone growth. A similar lack of dependence was seen in the patient groups with thick and thin biotypes and excessive mentalis muscle tension. However, these results were the result of adequate patient preparation. The above factors, which could have a significant negative impact on the final effect of the procedure, were eliminated by gingival augmentation (CTG, FGG or both), ensuring the correct quantity and quality of soft tissue, the depth of the oral

vestibule and the performance of a frenectomy or frenuloplasty, especially in cases of pull syndrome and injections of botulinum toxin into the mentalis muscle. The analysis of the direct influence of the above factors on the effect of reconstruction would have to be based on the division of patients into groups – one group in which the respective factor was eliminated and another in which bone reconstruction was performed with the factor retained. However, this would deliberately expose patients to a worse outcome or to complete failure. The lack of differences is a confirmation of the effectiveness of such preparations of the patient for the procedure.

For the first time, we presented this method as an example of treating a patient with a 6-month follow-up. Finally, the radiographic images revealed the formation of a new layer of cortical bone on the vestibular side and a certain volume of cancellous bone, noting that the block was prepared only from the spongy bone. This is probably related to the way bone blocks are remodeled, which depends on the force and physiological loads acting on them.³⁰ Similar radiological observations were presented by other authors who also showed the formation of a compact bone layer after a 10-month observation.³¹ Hence, the functional adaptation of the bone block to the current morphological and functional conditions is visible. The formation of new cortical bone makes it possible to determine new cephalometric points in this area, especially point D,³² which is important because it determines the directions of possible future orthodontic treatment.

Limitations

A limitation but also further perspectives of this study would include a longer observation period, an analysis of cases in which the methods were applied in other jaw sections, and a separate analysis of changes in bone dimensions during orthodontic treatment, taking into account the inclination of the teeth as we consider different moments of orthodontics treatment (despite suspension of orthodontic movement for the duration of surgical treatment).

Conclusions

This is the first developed and proven method of 3D bone reconstruction in areas with existing teeth. It creates the possibility of safe and predictable reconstruction of vertical and horizontal alveolar bone in the toothed area above the tooth, increases the long-term results of covering gingival recessions through buccal bone reconstruction, enables the prevention of bone dehiscence in orthodontically treated patients, and improves the morphology of the lower part of the face. It can be successfully performed under local anesthesia. A similar method is worth considering for other areas of the oral cavity.

Supplementary files

The Supplementary materials are available at <https://doi.org/10.5281/zenodo.11609828>. The package includes the following files:

Supplementary Table 1. The Shapiro –Wilk's normality test ($n = 32$ for each variable and each tooth) for the differences between before and after the treatment.

Supplementary Table 2. Descriptive statistics of the studied bone growth parameters.

Supplementary Table 3. Relative Treatment Effects for the studied teeth and the statistical significance of the differences between before and after treatment.

Supplementary Table 4. Spearman's correlations of the bone growth parameters (averaged data for all teeth).

Supplementary Table 5. The differences in bone growth parameters (averaged data for all teeth) between sexes.

Supplementary Table 6. The differences in bone growth parameters (averaged data for all teeth) between patients who underwent orthodontic treatment with a passive wire (Wire) and patients without ongoing treatment (Without wire).

Supplementary Table 7. The differences in bone growth parameters (averaged data for all teeth) between patients with recessions (Yes) and patients without recession (No).

Supplementary Table 8. The differences in bone growth parameters (averaged data for all teeth) between patients with thin biotype (Thin) and patients with thick biotype (Thick).

Supplementary Table 9. The differences in bone growth parameters (averaged data for all teeth) between patients with excessive mentalis muscle tension (Yes) and patients without such the feature (No).

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

ORCID iDs

Sylwia Hnitecka  <https://orcid.org/0000-0002-1171-9817>

Marzena Dominiak  <https://orcid.org/0000-0001-8943-0549>

Cyprian Olchowy  <https://orcid.org/0000-0002-6559-3973>

Tomasz Gedrange  <https://orcid.org/0000-0002-3551-6467>

References

1. Jun CM, Yun JH. Three-dimensional bone regeneration of alveolar ridge defects using corticocancellous allogeneic block grafts: Histologic and immunohistochemical analysis. *Int J Periodontics Restorative Dent*. 2016;36(1):75–81. doi:10.11607/prd.1950
2. Sanz M, Vignoletti F. Key aspects on the use of bone substitutes for bone regeneration of edentulous ridges. *Dent Mater*. 2015;31(6):640–647. doi:10.1016/j.dental.2015.03.005

3. Canullo L, Del Fabbro M, Khijmatgar S, et al. Dimensional and histomorphometric evaluation of biomaterials used for alveolar ridge preservation: A systematic review and network meta-analysis. *Clin Oral Invest*. 2022;26(1):141–158. doi:10.1007/s00784-021-04248-1
4. Chavda S, Levin L. Human studies of vertical and horizontal alveolar ridge augmentation comparing different types of bone graft materials: A systematic review. *J Oral Implantol*. 2018;44(1):74–84. doi:10.1563/aaid-joi-D-17-00053
5. Motamedian S, Khojaste M, Khojasteh A. Success rate of implants placed in autogenous bone blocks versus allogenic bone blocks: A systematic literature review. *Ann Maxillofac Surg*. 2016;6(1):78. doi:10.4103/2231-0746.186143
6. Dominiak M, Łysiak-Drwal K, Solski L, Żywicka B, Rybak Z, Gedrange T. Evaluation of healing processes of intraosseous defects with and without guided bone regeneration and platelet rich plasma: An animal study. *Ann Anat*. 2012;194(6):549–555. doi:10.1016/j.aanat.2012.07.007
7. Gupta A, Arora K, Aggarwal P, Kaur K, Mohapatra S, Pareek S. Evaluation of biphasic hydroxapatite and β -tricalcium phosphate as a bone graft material in the treatment of periodontal vertical bony defects: A clinical and digital radiological measurement study. *Indian J Dent Res*. 2022;33(2):152. doi:10.4103/ijdr.IJDR_234_19
8. Kao RT, Nares S, Reynolds MA. Periodontal regeneration – intrabony defects: A systematic review from the AAP Regeneration Workshop. *J Periodontol*. 2015;86(2 Suppl):S77–S104. doi:10.1902/jop.2015.130685
9. Dominiak M, Hnitecka S, Olchowcy C, Olchowcy A, Gedrange T. Analysis of alveolar ridge width in an area of central lower incisor using cone-beam computed tomography in vivo. *Ann Anat*. 2021;236:151699. doi:10.1016/j.aanat.2021.151699
10. Nimigean VR, Nimigean V, Bencze MA, Dimcevici-Poesina N, Cergan R, Moraru S. Alveolar bone dehiscences and fenestrations: An anatomical study and review. *Rom J Morphol Embryol*. 2009;50(3):391–397.
11. Antoun JS, Mei L, Gibbs K, Farella M. Effect of orthodontic treatment on the periodontal tissues. *Periodontol 2000*. 2017;74(1):140–157. doi:10.1111/prd.12194
12. Jati AS, Furquim LZ, Consolaro A. Gingival recession: Its causes and types, and the importance of orthodontic treatment. *Dental Press J Orthod*. 2016;21(3):18–29. doi:10.1590/2177-6709.21.3.018-029.oin
13. Saminsky M, Meir H, Sebaoun A, Tal H. Positive relationship between the dimensions of clinical gingival recessions and associated alveolar bone dehiscences: A biometric study. *Quintessence Int*. 2022;53(4):308–313. doi:10.3290/j.qi.b2644803
14. Stimmelmayer M, Allen EP, Gernet W, et al. Treatment of gingival recession in the anterior mandible using the tunnel technique and a combination epithelialized-subepithelial connective tissue graft: A case series. *Int J Periodontics Restorative Dent*. 2011;31(2):165–173. PMID:21491016.
15. Melo LGN, Almeida ALPF, Lopes JFS, et al. A modified approach for vestibuloplasty in severely resorbed mandible using an implant-retained postoperative stent: A case report. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2008;106(4):e7–e14. doi:10.1016/j.tripleo.2008.05.029
16. Murias I, Grzech-Leśniak K, Murias A, et al. Efficacy of various laser wavelengths in the surgical treatment of ankyloglossia: A systematic review. *Life*. 2022;12(4):558. doi:10.3390/life12040558
17. Olivi G, Signore A, Olivi M, Genovese MD. Lingual frenectomy: Functional evaluation and new therapeutical approach. *Eur J Paediatr Dent*. 2012;13(2):101–106. PMID:22762170.
18. Kalecińska E, Dominiak M, Dominiak P, Anweijer J. Postural disturbances in the presence of the dysfunction of the masticatory system: Case report. *Dent Med Probl*. 2004;41:305–312.
19. Dominiak M, Dominiak S, Targonska S, Gedrange T. Three-dimensional bone block planning for mandibular sagittal bone defect reconstruction. *J Healthcare Eng*. 2020;2020:8829288. doi:10.1155/2020/8829288
20. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part I: Technological concepts and evolution. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2006;101(3):e37–e44. doi:10.1016/j.tripleo.2005.07.008
21. Yang Y, Yang H, Pan H, Xu J, Hu T. Evaluation and new classification of alveolar bone dehiscences using cone-beam computed tomography in vivo. *Int J Morphol*. 2015;33(1):361–368. doi:10.4067/S0717-95022015000100057
22. Noguchi K, Gel YR, Brunner E, Konietzschke F. nparLD: An R software package for the nonparametric analysis of longitudinal data in factorial experiments. *J Stat Soft*. 2012;50(12):1–23. doi:10.18637/jss.v050.i12
23. Jäger F, Mah JK, Bumann A. Peridental bone changes after orthodontic tooth movement with fixed appliances: A cone-beam computed tomographic study. *Angle Orthod*. 2017;87(5):672–680. doi:10.2319/102716-774.1
24. Imber JC, Kasaj A. Treatment of gingival recession: When and how? *Int Dent J*. 2021;71(3):178–187. doi:10.1111/idj.12617
25. Sheikh Z, Hamdan N, Ikeda Y, Grynpsas M, Ganss B, Glogauer M. Natural graft tissues and synthetic biomaterials for periodontal and alveolar bone reconstructive applications: A review. *Biomater Res*. 2017;21(1):9. doi:10.1186/s40824-017-0095-5
26. Pfaffeneder-Mantai F, Meller O, Schneider B, et al. Specially designed and CAD/CAM manufactured allogeneic bone blocks using for augmentation of a highly atrophic maxilla show a stable base for an all-on-six treatment concept: A case report. *Maxillofac Plast Reconstr Surg*. 2022;44(1):21. doi:10.1186/s40902-022-00351-9
27. Brugnami F, Caiazzo A, Mehra P. Can corticotomy (with or without bone grafting) expand the limits of safe orthodontic therapy? *J Oral Biol Craniofac Res*. 2018;8(1):1–6. doi:10.1016/j.jobcr.2017.11.001
28. Eufinger H. Individual augmentation of the atrophic mandible based on CAD/CAM-manipulated computed tomography data: In vitro results. *Int J Oral Maxillofac Surg*. 1994;23(6):399–402. doi:10.1016/S0901-5027(05)80029-3
29. Chaushu G, Mardinger O, Peleg M, Ghelfan O, Nissan J. Analysis of complications following augmentation with cancellous block allografts. *J Periodontol*. 2010;81(12):1759–1764. doi:10.1902/jop.2010.100235
30. Dominiak M, Hnitecka S, Olchowcy C, Dominiak S, Gedrange T. Possible treatment of severe bone dehiscences based on 3D bone reconstruction: A description of treatment methodology. *Appl Sci*. 2021;11(21):10299. doi:10.3390/app112110299
31. Blume O, Donkiewicz P, Back M, Born T. Bilateral maxillary augmentation using CAD/CAM manufactured allogenic bone blocks for restoration of congenitally missing teeth: A case report. *J Esthet Restor Dent*. 2019;31(3):171–178. doi:10.1111/jerd.12454
32. Dominiak M. Własna metoda oceny prognozowania recesji przyzębia [habilitation thesis]. Wrocław, Poland: Wrocław Medical University; 2009.