Quantitative evaluation of the bone-forming capacity of biomaterials following external maxillary sinus augmentation and the clinical, histological and microCT results of the concomitant use of a human serum albumin-coated allograft and A-PRF in a similar indication

Ph.D. Thesis

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Budapest
2021

1. Introduction

In recent decades, many biomaterials have been successfully used to substitute autologous bone grafting in cases of an external maxillary sinus augmentation (MSA) with different healing times, yet most authors still consider autologous bone augmentation as the gold standard. If the amount of newly formed bone (NB) in the augmented area is considered to be the primary success factor, it is advisable to quantify the histomorphometric results of studies with different bone substitutes in order to decide whether the use of autologous bone is the most favorable in this respect. Previous systematic reviews on histomorphometric outcomes have tried to synthesize the evidence to identify the most predictable grafting material for MSA, and were performed by pooling the different types of biomaterials into commonly used subgroups (alloplast, xenograft, etc), so that the overall performance could mask the slight differences between the histomorphometric results of these biomaterials.

To rank the available biomaterials for MSA according to their capacity for NB formation on a quantitative basis, a comprehensive literature search for the results of randomized clinical trials (RCTs) is needed. After that, a network meta-analysis (NMA) can be performed, which can handle direct and indirect comparisons simultaneously.

In the field of oral surgery, human serum albumin-coated allografts have been available for several years, and the results of early publications reported that they were used successfully in several indications, and after 6–12 months, graft remodeling capacity was better compared to xenografts.

There are no literature data on the applicability of shorter healing times or the concomitant use of platelet-rich fibrin (PRF) and human serum albumin-coated allografts. Thus, given current implantological trends (use of early loading protocols and composite grafts), further randomized clinical trials are required to determine the appropriate indication area.

2. Objectives

Our objectives included collecting the results of RCTs that reported histomorphometric data based on histological samples taken from the implant preparation sites after two-stage MSA, and evaluating various biomaterials based on the new bone formation capacity in the surgical area using network meta-analysis.

The primary objective of our prospective clinical trial was to use histomorphometry and micromorphometry to examine bone biopsy samples taken after two-stage MSAs, which were augmented with a composite graft of a human serum albumin-coated allograft (BoneAlbumin ™, Orthosera Dental Zrt, Győr, Hungary) and A-PRF. Our secondary goal was to determine the healing times required for implantation and prosthetic loading in these surgical areas by measuring the implant stability quotient (ISQ) values, for which we used a device operating on the principle of resonance frequency analysis (RFA).

In our research, we wanted to answer the following questions:

In the cases of two-stage MSA, in terms of the amount of the newly formed bone in the surgical area, with the use of which biomaterial or combination of biomaterials can we expect the best result? In this respect, is autologous bone transplantation still the gold-standard, or more favorable values can be achieved by using other biomaterials?

Is the microstructure of the augmented area and the pristine bone different when a combination of BoneAlbumin and A-PRF is used for external MSA?

To what extent does the application of different healing protocols affect the histomorphometric and micromorphometric parameters of the augmented areas in the cases of BoneAlbumin and A-PRF composite graft?

Do the stability values of dental implants placed in the augmented area differ 3 and 6 months after external MSA using BoneAlbumin and A-PRF together for grafting material?

What is the ideal healing time for the prosthetic loading of dental implants in the region of the posterior maxilla previously augmented with the combined use of BoneAlbumin and A-PRF?

3. Methods

3.1. Methods of systematic review and meta-analysis

The network meta-analysis was reported in accordance with the PRISMA-NMA Statement. The protocol has been registered in PROSPERO (International Prospective Register of Systematic Reviews) before starting literature research under registration number CRD42019137740.

To search for relevant literature, we reviewed publications published before October 1, 2019 in the electronic databases of the Cochrane Library (CENTRAL), EBSCO, Embase, MEDLINE (via PubMed), and WOS (WOS core Collection).

A systematic search without applied filters or restrictions was performed and the following search key was applied: *("sinus membrane elevation" OR "sinus lift" OR "sinus augmentation" OR "sinus floor augmentation" OR "sinus floor elevation" OR "msfe" OR "sinus graft" OR "maxillary augmentation") AND (graft OR material OR bone)*.

Besides electronic databases, a hand search of cited and citing papers was performed.

All the relevant articles were combined in a reference manager software (EndNote X9; Clarivate Analytics). After removing duplicates, the remaining records were screened in the following three steps: (1) screening by titles, (2) screening by abstracts, (3) screening of the full text. The following information was extracted from all relevant publications: first authors' names, year of publication, study design, number of participants, the average age of the participants, sex distribution, number of surgical sites, number of biopsy samples, applied healing time, the residual ridge height, maxillary sinus width, and the percentage of NB based on histomorphometric records.

Potential sources of bias in the included RCTs were explored using the Cochrane Handbook. Review Manager 5.3 software (Review Manager (RevMan) [Computer program], Version 5.3., Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014.) and the Cochrane Risk of Bias Tool were applied to evaluate seven domains.

To reduce the confounding factors associated with the differences in healing periods applied in the studies, the extracted data were classified into three subgroups: early (bone core biopsy harvesting occurred more than 2 but a maximum of 5 months after MSA), normal (bone core biopsy harvesting occurred more than 5 but a maximum of 8 months after MSA), and late (bone core biopsy harvesting occurred more than 8 months after MSA) healing groups. In the case of the biomaterials used, our goal was to preserve the information of the types and nature of biomaterials by the creating of subgroups. Following the literature review, a total of 42 different subgroups were created based on the different biomaterials and biomaterial combinations used in the studies.

The connections between biomaterial subgroups were examined graphically according to predefined healing categories to identify a network required for NMA. If a connected network was identified, the Bayesian method was used to perform pairwise meta-analyses and NMA.

The Bayesian approach for NMAs describes the range and probability of the parameter of interest (e.g., treatment effect). The posterior distribution produced by this method predicts the new range and probability of plausible values for these parameters with the representation of uncertainty. These properties make the model suitable for drawing direct and indirect probability statements: in our case, to evaluate the relative effect of each biomaterial on new bone formation capability.

All the analyses were carried out under a random effect model. The primary outcome, the NB percentage (continuous), was calculated as mean difference (MD) with 95% credible intervals (95% CrI). Node-splitting analysis was performed for examination of consistency. The model was optimized, and posterior samples were generated using Markov Chain Monte Carlo methods running in four chains. At least 20,000 adaptation iterations were set to determine convergence and 10,000 simulation iterations. The network estimates (pooled estimates of direct and indirect data) of each intervention were presented in comparison with placebo and with each other in a forest plot. The interventions were ranked by their posterior probability by calculating the surface under the cumulative ranking (SUCRA) curve values, and the cumulative probabilities of each treatment were characterized by a single value between 0% and 100%.

To check for publication bias, a visual inspection of funnel plots and Egger's test was performed. All computations were performed using the R (V. 3.5.2) package gemtc (V. 0.8–2) along with the Markov Chain Monte Carlo engine

JAGS (V. 3.4.0), package netmeta (V. 1.1–0), and STATA 16.0 (StataCorp LLC, College Station, Texas, USA).

3.2. Methods of the prospective randomized clinical trial

This clinical trial was conducted in accordance with the Helsinki Declaration and with the Consolidated Standards of Reporting Trials Statement. The study protocol reviewed and approved by the Scientific and Research Ethics Committee of the Health Council of Hungary (31068-7 / 2018 / EÜIG) was registered in the ISRCTN (International Standard Randomized Controlled Trial Number) public database under the registration number ISRCTN10993769 in accordance with international recommendations. In this randomized prospective clinical trial, the following inclusion criteria were applied: systemically healthy adult patients, need for implant-supported fixed restoration in the posterior region of the maxilla, ridge width of at least 7 mm and a residual ridge height of less than 5 mm measured on preoperative cone-beam computed tomography (CBCT).

To calculate the minimum sample size for the trial, the G*Power 3.1 program (v.3.1.9.3, 2017, Institut für Experimentelle Psychologie, Heinrich-Heine-Universität, Düsseldorf, Germany) was used based on data of previous clinical trials. For the expected 1:1 distribution ratio and a 1.05 effect size between the treatments with an alpha level of 0.05 and a power of 80%, 12 cases per group were calculated as the minimum sample size. Taking into account the possible withdrawal of patients, 15-15 MSA per study group were planned to perform.

3.2.1. Surgical procedures

All patients enrolled in the study were treated using the same surgical procedures and biomaterials, the only difference between the two study groups was in terms of the applied healing periods. The surgical sites were randomized into test (3 month healing time) or control (6 month healing time) group after completing MSA, using the tossing coin method performed by a person blinded to the intervention.

At the beginning of the surgery, four tubes, a total of 40 mL of venous blood were drawn from every patient to prepare A-PRF. The tubes were centrifuged for 14 min at 1300 rpm (Duo Quattro Centrifuge, Process for PRF, Nice, France). All surgeries were performed under local anesthesia. The L-shaped mucoperiosteal flap was elevated to access the lateral wall of the maxillary

sinus. A piezo-surgical device and saw-shaped piezo-surgical tips (SmarThor, Megagen Co., Ltd, Daegu, South Korea) were used to carry out the osteotomies required for the lateral access to the maxillary sinus. The bone window was removed and stored in physiological saline solution until the end of the sinus lift. After elevation of the Schneiderian membrane (SM), the composite graft used for augmentation was prepared. In each case, one PRF membrane was shredded for the graft using surgical scissors and mixed with 1.5-2 cm3 of BoneAlbumin and plasma gained from PRF clots during membrane preparation. To protect the mucosa of the maxillary sinus and to cover possible perforations, two PRF membranes were placed on the SM before the graft was inserted, and then the space formed under the mucosa was filled with the composite graft. After insertion of the composite graft, the removed bone window was replaced, and then the osteotomy area was covered with a PRF membrane. The mucoperiosteal flap was closed tension-free with single interrupted non-resorbable sutures in its original position. To control the MSA, a panoramic X-ray was taken immediately after surgery. Antibiotics (1 g amoxicillin-clavulanic acid twice a day for 7 days), anti-inflammatory drugs (275 mg naproxen 3 times a day for 3 days), and chlorhexidine mouthwash (twice a day for 7 days) were prescribed. Sutures were removed 7 days after MSA, when the degree of postoperative pain was rated on a visual analog scale (VAS) for 1 to 10 by the patients. During the healing period, the surgical areas were not loaded with any type of prosthesis.

After 3 (test group) or 6 months (control group) of healing CBCT scans (Planmeca ProMax 3D CBCT, Planmeca Oy, Helsinki, Finland) were performed from all surgical sites prior to implant placement. Implant placement was performed under local anesthesia by raising a full-thickness flap from midcrestal incision. A modular trephine drill (Full-Tech Kft, Szigetszentmiklós, Hungary) designed for the study was used as an initial drill to collect bone core biopsy samples. This trephine (internal diameter of 2 mm and an outer diameter of 2.7 mm) was used to determine the position of the implants, and then the preparation of the implant bed was continued with the drills of the implant system. All patients were treated with Straumann implants (Straumann SP RN implants with Ti-SLA surface, Straumann GmBH, Basel, Switzerland). Directly after implant placement resonance frequency analysis (RFA) was carried out to measure implant stability. A one-stage healing protocol was applied, and interrupted non-resorbable sutures were used to close the flap around the gingiva formers. Antibiotics (1 g amoxicillin-clavulanic acid twice a day for 7 days), anti-inflammatory drugs (275 mg naproxen 3 times a day for 3 days), and chlorhexidine mouthwash (twice a day for 7 days) were prescribed, and the sutures were

removed 7 days after implant placement. Patients were recalled for control at 6, 8, 10 and 12 weeks after implantation, and then prosthetic workflow was started 3 months after surgery. According to the prosthetic situation, screw-retained metal-ceramic crowns or bridges were made as a definitive supply for the implants.

3.2.2. Resonance frequency analysis (RFA)

The stability of the implants was measured with an RFA device immediately after implant placement and postoperatively at 6, 8, 10, and 12 weeks. To determine the implant stability quotient (ISQ) values, SmartPegs (SmartPeg, Osstell AB, Göteborg, Sweden) and an Osstell IDx device (Osstell IDx, Osstell AB, Göteborg, Sweden) were used.

3.2.3. Micromorphometric analysis (microCT)

After biopsy removal, the bone core biopsy samples were placed in a 0.3 mL microcentrifuge tube (Eppendorf tube, Merck KGaA, Darmstadt, Germany) and fixed in 10% formaldehyde, 0.1 M phosphate buffer saline (PBS), pH 7.3 solution. The tubes were code-masked to facilitate blind histomorphometric and micromorphometric analysis. The bone samples were scanned using a microcomputed tomography (μCT) scanner (Bruker 1272 X-ray microtomograph, Bruker μCT, Kontich, Belgium). Scanning was carried out at a resolution of 5.9 μm (60 kV, 166 μA). For image noise reduction, an Al 0.25 mm aluminum filter was used. The average scan duration was 30 min. Raw images were reconstructed by using NRecon software (v.1.7.4.6., Bruker μCT, Kontich, Belgium). The ring artifact correction was 13, and the beam-hardening correction was 25%. CTAn software (v.1.17.7.2, Bruker μCT, Kontich, Belgium) was used to perform 3D morphometric analysis. For each sample, the complex 3D structure was analyzed to identify the pristine bone and the augmented area. In determining the relevant volumes (VOIs) required for quantitative analysis, the transition zone (80–120 segment) was excluded, so the micromorphometric analysis was aimed to compare the pristine and augmented bone regions within the sample in each case. The VOIs differed among the bone core biopsy samples. Therefore, volume-independent metrics were used for the analysis.

3.2.4. Histological and histomorphometric analysis

The bone core biopsy samples were examined by histological and histomorphometric methods following the μCT scanning. The samples were

embedded in paraffin after decalcination and dehydration, and 20 µm sections were prepared. The sections were stained with hematoxylin-eosin stain and digitalized by a slide scanner (Panoramic 1000, 3DHISTECH Ltd., Budapest, Hungary) for histological evaluation. The digital images were transferred to CaseViewer 2.4 (3DHISTECH Ltd., Budapest, Hungary) for histomorphometric analysis. The images were evaluated at 150× magnification to identify the margin between pristine bone and the augmented area to exclude the areas of pristine bone from the analysis. Two representative slides of each histologic sample were selected for the analysis. After staining-based manual segmentation, the percentages of newly formed bone (NB), residual graft particles (RG) and nonmineralized tissue (NMT) were determined with using Adobe PhotoShop (Adobe System Inc., San Jose, CA, USA) and ImageJ for Windows (ImageJ 1.45, 2011, Wayne Rasband, US National Institute of Health, Bethesda, MD, USA) software.

3.2.5. Statistical Analysis

All data were expressed as the mean ± standard deviation. The Shapiro–Wilk test was used to assess the normality of the data distribution. The histomorphometric variables showed normal distribution; therefore, one-way ANOVA was used for statistical analysis. The ISQ values showed a normal distribution at the time of implant placement and were analyzed by one-way ANOVA. Postoperatively, at 6, 8, 10, and 12 weeks of evaluation, the ISQ values showed a non-normal distribution, and these data were analyzed by the independent-samples Mann–Whitney U test. Multiple comparison tests were used to analyze the four datasets of µCT data. The values of bone volume fraction (BV/TV), bone surface/volume ratio (BS/BV), trabecular separation (Tb.Sp), total porosity (Po(tot)) and open porosity (Po(op)) showed normal distributions and were analyzed by one-way ANOVA with Bonferroni and Tukey HSD post hoc tests. The values of the bone surface/volume ratio (BS/TV), trabecular thickness (Tb.Th), Trabecular bone pattern factor (Tb.Th) and connectivity (Conn) showed a non-normal distribution and were analyzed by the independent-samples Kruskal–Wallis test with Bonferroni correction for multiple tests. Statistical analysis was performed using IBM SPSS Statistics 25 software (IBM Corporation, New York, NY, USA), and the differences were considered statistically significant at $p < 0.05$.

4. Results

4.1. Results of the systematic review and meta-analysis

Our systematic literature search resulted in 4055 records after duplicates removal, which were screened for eligibility according to our predefined selection criteria in three steps: screening by titles, screening by abstracts, and finally, screening of the full text. A total of 163 publications were examined in their entirety, 69 of which met all of the selection criteria. A prerequisite for the feasibility of the NMA is that the interventions classified in different subgroups of the NMA are used in the studies and that a network can be created across the different studies between these subgroups. During checking these criteria, an additional 35 publications had to be excluded from the quantitative analysis. Using the dataset from the remaining 34 RCTs, we were able to analyze the histomorphometric results of 28 different biomaterials used for MSA following a healing period of 5-8 months. In our meta-analysis, a total of 378 paired comparisons were performed, of which a significant difference between the effect of the applied biomaterials on inducing new bone formation were identified in only two cases. The use of bovine xenograft + autologous bone marrow concentrate (BMC) composite graft showed significantly better results than using the biomaterials of allograft or biodegradable copolymer subgroups. Based on the NMA, the ranking probability of the 28 biomaterials used for MSA were calculated by estimating the possible rankings associated with each biomaterial. The biomaterials were ranked by summarizing the SUCRA curve as well as the mean ranks (Figure 1.). According to the SUCRA ranking, the most effective biomaterials for the outcome NB% over a healing period of 5 to 8 months after MSA were bovine + BMC (81%), followed by bovine + platelet-rich plasma (PRP) (77%), bioactive glass ceramic + AB 1:1 (70%), nanocrystalline hydroxyapatite in silica gel (70%), and bioactive glass ceramic (70%). AB alone as grafting material took the twelfth position (57%). With its application, there is a 57% chance that the most effective grafting material for two-stage MSA was used in terms of the percentage of newly formed bone in the augmented area after 5-8 months of healing.

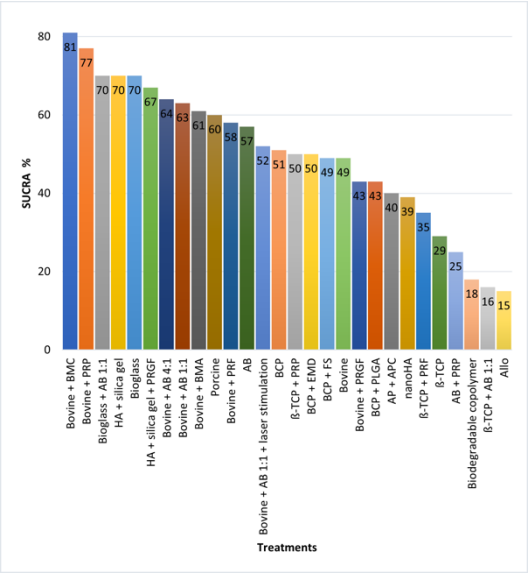

Figure 1.: *The ranking of biomaterials according to their new bone formation efficacy based on the calculation of the surface under the cumulative ranking curves (SUCRA). The figure expresses the cumulative probability for the best rank as a percentage. Abbreviations: autologous bone (AB), allograft (Allo), bovine xenograft (Bovine), porcine xenograft (Porcine), biphasic calcium phosphate (BCP), beta-tricalcium-phosphate (ß-TCP), bioactive glass ceramic (Bioglass), nanocrystalline hydroxyapatite (nanoHA), rigid biodegradable (L-lactic, D-lactic and glycolic acid)copolymer membrane (Biodegradable copolymer), bovine xenograft + autologous bone 1:1 (Bovine + AB 1:1), bovine xenograft + autologous bone 1:1 composite graft followed by laser stimulation (Bovine + AB 1:1 + laser stimulation), bovine xenograft + autologous bone 4:1 (Bovine + AB 4:1), bovine xenograft + platelet-rich plasma (Bovine + PRP), bovine xenograft + platelet-rich fibrin (Bovine + PRF), bovine xenograft + plasma rich in growth factors (Bovine + PRGF), bovine xenograft + bone marrow aspirates (Bovine + BMA), bovine xenograft + bone marrow concentrate (Bovine + BMC), bioactive glass ceramic + autologous bone 1:1 (Bioglass + AB 1:1), beta-tricalcium-phosphate + autologous bone 1:1 (ß-TCP + AB 1:1), beta-tricalcium-phosphate + platelet-rich plasma (ß-TCP + PRP), beta-tricalcium-phosphate + platelet-rich fibrin (ß-TCP + PRF), autologous bone + platelet-rich plasma (AB + PRP), autologous bone + autologous platelet concentrate (AB + APC), biphasic calcium phosphate + fibrin sealant (BCP + FS), poly(lactic-co-glycolic acid)-based polymer (PLGA)-coated biphasic calcium phosphate (BCP + PLGA), biphasic calcium phosphate + enamel matrix proteins (EMD) (BCP + EMD), nanocrystalline hydroxyapatite in silica gel (HA + silica gel), nanocrystalline hydroxyapatite in silica gel + plasma rich in growth factors (HA + silica gel + PRGF).*

4.2. Results of the randomized prospective clinical trial

Twenty-six patients with 30 MSA were enrolled in this study. The mean age was 57.93 ± 7.79 years in the test group and 55.33 ± 8.55 years in the control group. Analyzing preoperative CBCT images, the two groups also showed no significant difference in terms of surgical areas. The residual ridge height was 2.93 ± 1.14 mm in the test group and 3.48 ± 1.04 mm in the control group, and the sinus width, measured in the corresponding area to the previous position of the first molars at the height of the palatonasal recess, was 15.06 ± 0.85 mm in the test group and 14.57 ± 1.41 mm in the control group. In two cases in the test group and in one case in the control group, a small perforation (diameter less than 5 mm) of the SM was observed during the elevation of the sinus membrane, wich was successfully covered by using A-PRF membranes. Except for edema there was no postoperative complication reported in association with sinus augmentation. At suture removal, patients assessed the degree of pain associated with surgery for 3.09 ± 2.05 on a visual analog scale (VAS). After the healing period (3 months in the test group, 6 months in the control group), 26 dental implants were implanted in the test group and 27 in the control group. During implant site preparation, 17–17 bone core biopsy samples, suitable for micromorphometric and histomorphometric examination, were collected from both study groups. At 6 and 8 weeks after implantation 1-1 dental implant was lost due to lack of stability, which was affected the control group. The lost implants were replaced 3 months after their removal, so that in all cases, a screw-retained denture was be made in accordance with the prosthetic plan.

4.2.1. Results of the resonance frequency analysis (RFA)

Regarding the implants' stability, gradually increasing ISQ values were measured during the examined 12-week healing period, with the exception of the two lost implants. In both groups, 8 weeks after implant placement the mean ISQ values exceeded 70 (Table 1).

Table 1.: *The results of implant stability quotient (ISQ) measurement at different time points after implant placement. Statistical significance ($p < 0.05$) is highlighted in bold. Abbreviations: one-way ANOVA (#), independent-samples Mann–Whitney U test (##).*

	Group	N	Mean	Std. Deviation	p-value
ISQ week 0	test	24	68.92	7.56	0.105 #
	control	25	72.20	6.30	
ISQ week 6	test	25	68.52	7.35	**0.003 ##**
	control	24	74.22	4.52	
ISQ week 8	test	26	72.00	7.16	**0.041 ##**
	control	25	75.70	4.76	
ISQ week 10	test	26	74.26	5.79	0.501 ##
	control	24	75.74	4.93	
ISQ week 12	test	26	75.96	4.75	0.345 ##
	control	24	76.96	4.31	

4.2.2. Results of the micromorphometric analysis (microCT)

Each bone core biopsy sample contained both pristine and augmented bone areas. The radiolucency of the allograft particles showed a high degree of similarity to the native bone, so the separation of different bone areas within the samples was performed based on the evaluation of microstructural elements on 3D reconstructions. The pristine bone areas in both groups consisted of an interconnected, thick trabecular network and broad marrow spaces, whereas the augmented bone in both groups was characterized by the convoluted network of thin, immature bone trabeculae, narrow marrow spaces and isolated bone-like formations (partially resorbed RG particles). The transition zone was observed within the samples at a width of 80–120 segment, which was excluded from the selection of VOIs required for quantitative evaluation. Morphometric data of the pristine maxillary bone and the augmented areas of both study groups were compared. The analysis showed no statistically significant difference in comparing the grafted areas of the test and control groups, and no statistically significant difference was observed in either of the morphometric parameters between the pristine bone area of the two groups. The BS/BV, BS/TV and Conn values were significantly higher, and the Tb.Th was significantly thinner in the augmented

areas. Similar values were observed between the native and augmented bone for the BV/TV, Tb.Sp, Tb.Pf, Po(tot), Po(op) micromorphometric parameters (Table 2).

Table 2.: *Results of the micromorphometric analysis. Statistical significance ($p < 0.05$) is highlighted in bold. Abbreviations: bone volume fraction (BV/TV), bone surface/volume ratio (BS/BV), bone surface density (BS/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), trabecular bone pattern factor (Tb.Pf), total porosity (Po(tot)), open porosity (Po(op)), connectivity (Conn), one-way ANOVA with Bonferroni and Tukey HSD post hoc tests (#), independentsamples Kruskal-Wallis test with Bonferroni correction for multiple tests (##).*

	Group	N	Mean	Std. Deviation	p-value
BV/TV	pristine bone-test	17	18.5008	10.1497	0.851 #
	augmented area - test	17	19.1761	8.2003	
	pristine bone - control	17	20.4003	10.5609	
	augmented area - control	17	21.2211	10.0378	
BS/BV	pristine bone-test	17	0.0215	0.0056	**0.000 #**
	augmented area - test	17	0.0297	0.0057	
	pristine bone - control	17	0.0213	0.0063	
	augmented area - control	17	0.0270	0.0061	
BS/TV	pristine bone-test	17	0.0038	0.0021	**0.023 ##**
	augmented area - test	17	0.0056	0.0022	
	pristine bone - control	17	0.0039	0.0016	
	augmented area - control	17	0.0055	0.0024	
Tb.Th	pristine bone-test	17	218.0640	52.7430	**0.017 ##**
	augmented area - test	17	177.0196	28.4101	
	pristine bone - control	17	220.2552	58.7725	
	augmented area - control	17	184.4137	45.6001	
Tb.Sp	pristine bone-test	17	627.6184	238.2173	0.615 #
	augmented area - test	17	569.7924	269.8554	
	pristine bone - control	17	542.3623	234.8811	
	augmented area - control	17	519.8590	242.8923	
Tb.Pf	pristine bone-test	17	0.0043	0.0045	0.072 ##
	augmented area - test	17	0.0078	0.0066	
	pristine bone - control	17	0.0054	0.0046	
	augmented area - control	17	0.0068	0.0050	
Po(tot)	pristine bone-test	17	81.4992	10.1497	0.851 #
	augmented area - test	17	80.8239	8.2003	
	pristine bone - control	17	79.5997	10.5609	
	augmented area - control	17	78.7789	10.0378	

	Group	N	Mean	Std. Deviation	p-value
Po(op)	pristine bone-test	17	81.4899	10.1554	0.851 #
	augmented area - test	17	80.8136	8.2076	
	pristine bone - control	17	79.5864	10.5724	
	augmented area - control	17	78.7685	10.0459	
Conn	pristine bone-test	17	1040.3500	2104.7550	**0.000 ##**
	augmented area - test	17	2197.2400	1911.5760	
	pristine bone - control	17	603.6500	1045.0600	
	augmented area - control	17	1988.9400	2158.4780	

4.2.3. Results of the histological and histomorphometric analysis

A total of 34 bone core biopsy samples were analyzed, and both groups represented 17–17 specimens. Based on histological analysis, signs of gradual graft resorption and remodeling were observed in the augmented areas, which occurred in both groups without foreign body reaction or inflammation. RG particles of the allograft were surrounded by NB and NMT. Based on the histomorphometric evaluation of the sections, NB was 44.89 ± 9.49% and 39.75 ± 8.15%, RG was 12.52 ± 6.25% and 15.67 ± 6.92%, and NMT was 42.59 ± 12.48% and 44.58 ± 13.35% for the test and control groups, respectively. The histomorphometric results of the test and control groups have not shown a significant differences for any of the investigated parameters.

5. Conclusions

5.1. Conclusions based on the results of the meta-analysis

I. The results of the NMA suggest that the use of biomaterials for two-stage MSA does not result in a statistically significant difference in the amount of NB compared to the use of autologous bone, if a healing time of 5-8 months was applied.

II. When various biomaterials are used in combination with autologous bone or autologous cell concentrates (BMC, PRP, PRF, etc.) for MSA with healing times of 5-8 months, the amount of newly formed bone in the surgical area may exceed the values achievable with autologous bone alone as grafting material.

III. For shorter healing protocols applied, faster remodeling ability of autologous bone may still be beneficial. In contrast, the use of biomaterials can significantly reduce the amount of autologous bone required for MSA, resulting in less invasive surgery and shorter surgical time.

IV. The level of evidence for NMA has been reduced due to several factors, so further randomized clinical trials with appropriate information size and unified surgical protocol may be required to support our conclusions based on indirect comparisons.

V. For the healing period of fewer than 5 months, the low number of available randomized trials did not allow to pool a network meta-analysis, which could be a potential area of future research.

5.2. Conclusions based on the results of the clinical trial and resonance frequency analysis (RFA)

I. BoneAlbumin and A-PRF composite graft can be a safely used as a biomaterial for MSA. No postoperative complications other than edema were recorded after the surgeries, and the surgical burden was not significant based on the feedback of the patients.

II. The quality of bone formed in augmented areas is suitable for dental implant placement even after a 3-month healing period.

III. All of the 53 dental implants placed are considered clinically successful, except two that lost implants during the healing process, which resulted in an average 96.2% survival rate 1.5 years after prosthetic loading.

IV. In both healing groups, 8 weeks after implant placement, the ISQ values of the measured implants reached 70, indicating their proper osseointegration. With this two-stage surgical protocol a healing time of 5 months ideally can be sufficient between the external maxillary sinus augmentation and the prosthetic loading of the dental implants.

5.3. Conclusions based on the results of the micromorphometric analysis (microCT)

I. The microstructure of bone augmented with BoneAlbumin and A-PRF is characterized by a network of thin, lamellar, and cylindrical NB trabeculae surrounding the extensive medullary spaces and the residual graft particles, which are showing gradual resorption 3 and 6 months after MSA.

II. The BoneAlbumin and A-PRF composite graft is characterized by extensive remodeling in the first 3 months of healing, which then slows down. Comparing the micromorphometric parameters of the augmented bone samples taken after 3 and 6 months of healing, no diffenence was detected between the two groups.

III. A healing time of more than 6 months may be required for the thickness of newly formed bone trabeculae (Tb.Th) in the augmented areas to reach the values of the native bone.

IV. Higher values of BS/BV, BS/TV, and Conn parameters in the augmented areas can be attributed to the presence of remaining RG in the surgical area.

V. For the BV/TV, Tb.Sp, Tb.Pf, Po(tot) and Po(op) micromorphometric parameters, no difference was detected between the BoneAlbumin and A-PRF composite graft augmented areas and the pristine bone areas respectively.

5.4. Conclusions based on the results of the histological and histomorphometric analysis

I. Based on histological analysis, signs of gradual graft resorption and remodeling were observed in areas augmented with BoneAlbumin and A-PRF, which occurred in both groups without foreign body reaction or inflammation. The remaining particles of the allograft are surrounded by newly formed bone and marrow spaces.

II. Based on the histomorphometric evaluation of the sections, significant new bone formation was observed in the augmented areas after both 3 months (44.89 ± 9.49%) and 6 months (39.75 ± 8.15%) of healing.

III. Comparison of the histomorphometric results of the test and control groups showed no significant difference in any of the examined parameters, based on which can be concluded that a significant part of the remodeling of these composite graft takes place during the first 3 months of the healing.

6. Publications

6.1. Publications related to the thesis

Trimmel B, Gede N, Hegyi P, Szakács Z, Mezey G A, Varga E, Kivovics M, Hanák L, Rumbus Z, Szabó G. (2021) Relative performance of various biomaterials used for maxillary sinus augmentation. A Bayesian network meta-analysis. Clinical Oral Implants Research, 32: 2: 135-153. **IF: 5,977**

Trimmel B, Gyulai-Gaál S, Kivovics M, Jákob N P, Hegedűs C, Szabó B T, Dobó-Nagy C, Szabó G. (2021) Evaluation of the Histomorphometric and Micromorphometric Performance of a Serum Albumin-Coated Bone Allograft Combined with A-PRF for Early and Conventional Healing Protocols after Maxillary Sinus Augmentation: A Randomized Clinical Trial. Materials, 17: 9: 1810. **IF: 3,623**

6.2. Other publications

Minya F, Trimmel B, Simonffy L, Dobó-Nagy C, Gyulai-Gaál S. (2021) Odontoma Removal and Oral Rehabilitation via Insertions of Albumin and Gentamycin Coated Bone Allograft and Dental Implants - A Case Report. Biomedical Journal of Scientific and Technical Research, 33: 5: 26116-26120.

Minya F, Trimmel B, Simonffy L, Gyulai-Gaál S, Lacza Z, Dobó-Nagy C. (2021) Alveolar preservation with albumin and gentamycin-coated allograft after third molar tooth removal. Applied Sciences-Basel, 11: 2: 586. **IF: 2,679**

Trimmel B, Nagy Z, Gyulai-Gaál S. (2021) Új terápiás lehetőségek odontomák kezelésében: Esetismertetés. Fogorvosi Szemle, 114: 1: 26-30.

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Gyulai-Gaál Sz, Minya F, Trimmel B, Simonffy L. (2019) Az implantológia helye a fogcsírahiányos esetek ellátásában. Fogorvosi Szemle, 112: 3: 77-81.

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