1. Pre-clinical (*in vitro* & *in vivo*) studies

1. In vitro comparison of the osteogenic capability of human pulp stem cells on alloplastic, allogeneic, and xenogeneic bone scaffolds

   Heitzer M, Modabber A, Zhang X, Winnand P, Zhao Q, Bläsius FM, Buhl EM, Wolf M, Neuss S, Hölzle F, Hildebrand F, Greven J. BMC Oral Health. 2023 Jan 31;23(1):56. doi: 10.1186/s12903-023-02726-4.

   https://pubmed.ncbi.nlm.nih.gov/36721114/

   Background: A rigorous search for alternatives to autogenous bone grafts to avoid invasiveness at the donor site in the treatment of maxillomandibular bone defects. Researchers have used alloplastic, allogeneic, and xenogeneic bone graft substitutes in clinical studies with varying degrees of success, although their in vitro effects on stem cells remain unclear. Dental pulp stem cells (DPSCs) can potentially enhance the bone regeneration of bone graft substitutes. The present in vitro study investigates the osteogenic capability of DPSCs on alloplastic (biphasic calcium phosphate [BCP]), allogeneic (freeze-dried bone allografts [FDBAs]), and xenogeneic (deproteinized bovine bone mineral [DBBM]) bone grafts. **Methods:** Human DPSCs were seeded on 0.5 mg/ml, 1 mg/ml, and 2 mg/ml of BCP, FDBA, and DBBM to evaluate the optimal cell growth and cytotoxicity. Scaffolds and cell morphologies were analyzed by scanning electron microscopy (SEM). Calcein AM and cytoskeleton staining were performed to determine cell attachment and proliferation. Alkaline phosphatase (ALP) and osteogenesis-related genes expressions was used to investigate initial osteogenic differentiation. **Results:** Cytotoxicity assays showed that most viable DPSCs were present at a scaffold concentration of 0.5 mg/ml. The DPSCs on the DBBM scaffold demonstrated a significantly higher proliferation rate of 214.25 ± 16.17 ($p < 0.001$) cells, enhancing ALP activity level and upregulating of osteogenesis-related genes compared with other two scaffolds. **Conclusion:** DBBP scaffold led to extremely high cell viability, but also promoted proliferation, attachment, and enhanced the osteogenic differentiation capacity of DPSCs, which hold great potential for bone regeneration treatment; however, further studies are necessary.

2. The impact of the size of bone substitute granules on macrophage and osteoblast behaviors *in vitro*

   Fujioka-Kobayashi M, Katagiri H, Kono M, Schaller B, Iizuka T, Safi AF. Clin Oral Investig. 2021 Aug;25(8):4949-4958. doi: 10.1007/s00784-021-03804-z.

   https://pubmed.ncbi.nlm.nih.gov/33538898/

Objective: Bone substitute (BS) size might influence the clinical outcomes of guided bone regeneration (GBR) procedures. The aim of the present study was to investigate the influence of BS size on macrophage (Mϕ) and osteoblast behaviors in vitro. **Materials and methods:** Two different granule sizes (S and M/L) were assessed for four different commercial BSs: deproteinized bovine bone mineral (DBBM), biphasic calcium phosphate type 1 (BCP1), BCP type 2 (BCP2), and carbonate apatite (CO3Ap). The BSs were compared for their impacts on the cell viability and differentiation potential of THP-1-derived Mϕs and human osteoblast-like Saos-2 cells. **Results:** The smaller granules showed higher material volumes and surface areas than the larger granules. Significantly higher viability of Mϕs and Saos-2 cells was observed with the DBBM_L-size granules than with the DBBM_S-size granules. Gene expression experiments in Mϕs revealed few differences between the two sizes of each BS, although higher CD206 mRNA levels were observed in the BCP1_L group and the CO3Ap_M group than in the respective S-size groups on day 1. Only DBBM showed significantly higher mRNA levels of osteogenic markers, including Runx2 and osteocalcin, in Saos-2 cells in the S-size group than in the L-size group. **Conclusions:** The S-size and L-size DBBM granules exhibited clear differences in cell outcomes: cells cultured on the S-size granules exhibited lower cell viability, higher osteopromotive ability, and no noticeable Mϕ polarization changes. **Clinical relevance:** A smaller granule size might be advantageous due to greater bone regeneration potential in the use of DBBM granules to treat defects.

3. Cytocompatibility of Bone Substitute Materials and Membranes

Schafer S, Al-Qaddo H, Gosau M, Smeets R, Hartjen P, Friedrich RE, Nada OA, Vollkommer T, Rashad A. 2021 Jul-Aug;35(4):2035-2040. doi: 10.21873/invivo.12472.

https://pubmed.ncbi.nlm.nih.gov/34182478/

Background/aim: With the demographic change and associated chronic bone loss, the need for cytocompatible bone replacement materials arise in modern medicine. The aim of this *in vitro* study was to investigate the cytocompatibility of eleven different bone substitute materials and membranes. **Materials and methods:** Seven bone substitute materials and four membranes were assessed in vitro. The specimens were tested based on their interaction with MC3T3 pre-osteoblasts, through the utilization of viability, proliferation, and cytotoxicity assays. Cell vitality was evaluated using live-dead staining. **Results:** Although we found minor differences in cytocompatibility among the assessed materials, all tested materials can be considered as cytocompatible with a viability of more than 70% of the negative control, which indicates the non-toxic range as defined in current, international standards (DIN EN ISO 10993-5:2009, German Institute for Standardization, Berlin, Germany). Direct live-dead staining assays confirmed satisfactory cytocompatibility of all tested membranes. **Conclusion:** All examined bone substitute materials and membranes were found to be cytocompatible. In order to assess whether the observed minor differences can impact regenerative processes, further *in vivo* studies need to be conducted.

4. Establishing a new alveolar cleft model in rats to investigate the influence of jaw reconstructions on orthodontic tooth movement

Möhlhenrich SC, Heitzer M, Magnuska Z, Gremse F, Chhatwani S, Danesh G, Hölzle F, Modabber A. Ann Anat. 2021 Jul;236:151713. doi: 10.1016/j.aanat.2021.151713.

https://pubmed.ncbi.nlm.nih.gov/33675947/

Background: The aim of the present investigation was to develop a new cleft model in rats that allows alveolar cleft repair and subsequent tooth movement. **Methods:** A complete continuity-interrupting alveolar cleft was performed on the left-side maxillae of 33 rats through ultrasonic surgery. The clefts were filled with bone wax, and microCT scans were done to analyze the cleft size. After four weeks, the cleft repair was completed using autologous, xenogeneic (human), or synthetic bone substitute. After an additional four weeks, the orthodontic tooth movement was initiated. **Results:** Fourteen rats died during the research, and the study design was constantly adapted accordingly. The main reasons for death included breathing problems during or immediately after the experimental activities (eight animals), followed by two deaths due to circulatory failures. In the remaining 19 animals, the average cleft size was about 2.70 ± 0.46 × 2.01 ± 0.25 × 1.18 ± 0.20 mm, and the mean velocity of orthodontic tooth movement after seven days was between 0.21 ± 0.08 mm in the autologous group and 0.50 ± 0.54 mm in the xenogeneic group. After 56 days, the mean values ranged between 0.67 ± 0.27 mm in the autologous group and 0.82 ± 0.72 mm in the synthetic group. **Conclusions:** Surgical interventions in the oral cavity of rats requires a stronger anesthesia and lead to increased risk of coolant and coagulated blood aspiration. The new alveolar cleft model in rats allows for subsequent orthodontic tooth movement after cleft repair, but only in the mesial root of the first molar.

5. Evaluation of different grafting materials for alveolar cleft repair in the context of orthodontic tooth movement in rats

Möhlhenrich, S.C., Kniha, K., Magnuska, Z. et al. Sci Rep 2021 Jun;11(1):13586. doi: 10.1038/s41598-021-93033-x.

https://pubmed.ncbi.nlm.nih.gov/34193933/

To minimize the postoperative risks posed by grafting autologous transplants for cleft repair, efforts are being made to improve grafting materials for use as potential alternatives. The aim of this study was to compare the bone graft quality of different bone substitutes including the gold standard autografts during the healing processes after cleft repair in the context of orthodontic treatment. In 21 Wistar rats, a complete, continuity-interrupting cleft was created. After 4 weeks, cleft repair was performed using autografts from the hips' ischial tuberosity, human xenografts, or synthetic bone substitutes [beta-tricalcium phosphate (β-TCP)/hydroxyapatite (HA)]. After another 4 weeks, the first molar movement was initiated in the reconstructed jaw for 8 weeks. The bone remodeling was analyzed in vivo using micro-computed tomography (bone mineral density and bone volume fraction) and histology (new bone formation). All the grafting materials were statistically different in bone morphology, which changed during the treatment period. The β-TCP/HA substitute demonstrated less resorption compared to the autologous and xenogeneic/human bone, and the autografts led to a stronger reaction in the surrounding bone. Histologically, the highest level of new bone formation was found in the human xenografts, and the lowest was found in the β-TCP/HA substitute. The differences between the two bone groups and the synthetic materials were statistically significant. Autografts were confirmed to be the gold standard in cleft repair with regard to graft integration. However, parts of the human xenograft seemed comparable to the autografts. Thus, this substitute could perhaps be used as an alternative after additional tissue-engineered modification.

6. Maxillary Sinus Augmentation Using Ceramic Alloplastic Granules or Paste: An Experimental Study in Rabbits

Costa MM, Botticelli D, Moses O, Omori Y, Fujiwara S, Silva ER, Xavier SP. Dent J (Basel). 2021 Jun;9(6):65. doi: 10.3390/dj9060065.

https://pubmed.ncbi.nlm.nih.gov/34205201/

Background: Due to the lack of data comparing the biological behavior of two formulations, granules and paste, of alloplastic graft from microtomographic and histomorphometric points of view, the aim of the present experiment was to compare the histomorphometric and microtomographic healing of two formulations, i.e., granules (MR sites) or paste (MR-inject sites) of an alloplastic graft composed of a combination of beta-tricalcium phosphate and hydroxyapatite used for maxillary sinus lifting. **Methods:** A sinus lifting procedure was carried out bilaterally in 20 rabbits, and the elevated space was filled with either paste or granules of an alloplastic material. A collagen membrane was placed on the antrostomy and the animals were euthanized after 2 or 10 weeks, 10 animals each group. Microtomographic and histological analyses were performed**. Results:** Higher proportions of new bone formation were found at the MR, compared to the MR-inject sites both after 2 weeks (2.65 ± 2.89% vs. 0.08 ± 0.12%; $p < 0.01$) and 10 weeks of healing (34.20 ± 13.86 vs. 23.28 ± 10.35%; $p = 0.022$). **Conclusions:** It was concluded that new bone formation was faster in the MR sites, compared to the MR-inject. However, a longer time of healing should be allowed to make final conclusions about the efficiency in bone formation of the paste formulation of the biomaterial used in the present study.

7. Effects of Different Bone Substitutes on Reactive Oxygen Species Release in Leukocytes *in Vitro*: A Pilot Study

Nomeika D, Jasiunas A, Janužis G, Skrodenienė E, Banienė R, Juodžbalys G. Int J Oral Maxillofac Implants. 2021 May-Jun;36(3):e42-e50. doi: 10.11607/jomi.8592.

https://pubmed.ncbi.nlm.nih.gov/34115064/

Purpose: To evaluate the formation of reactive oxygen species in human leukocytes promoted by bone substitutes that are different in origin and morphology used for jawbone tissue regeneration. **Materials and methods:** This preclinical prospective randomized crossover study involved 10 subjects, from whom venous blood samples were taken. Leukocytes were separated and standardized. Sixty experimental samples consisted of leukocytes incubated with allogeneic, xenogeneic, or alloplastic bone substitutes at different bone weights (12.5 and 25 mg). The control samples consisted only of incubated leukocytes. Reactive oxygen species were quantitatively determined with the fluorimetric method. Statistical analysis was carried out using SPSS 23 software. **Results:** The highest average reactive oxygen species values were obtained in the allogeneic bone substitute group ($P < .05$), while the xenogeneic bone substitute group and control group presented equal reactive oxygen species formation rates ($P > .05$). A proportional difference ($P < .05$) of reactive oxygen species emission was obtained between different masses of bone substitute in the samples. **Conclusion**: Allogeneic and alloplastic bone substitutes affect leukocytes and promote reactive oxygen species emission. Xenogeneic bone substitute presents no leukocyte stimulation and maintains anti-inflammatory conditions. Larger bone substitute mass provokes greater oxidative stress.

8. Possible Implications for Improved Osteogenesis? The Combination of Platelet-Rich Fibrin With Different Bone Substitute Materials

Blatt S, Thiem DGE, Kyyak S, Pabst A, Al-Nawas B, Kämmerer PW. Front Bioeng Biotechnol. 2021 Mar;9:640053. doi: 10.3389/fbioe.2021.640053.

https://pubmed.ncbi.nlm.nih.gov/33816452/

Bone substitute materials (BSM) are widely used in oral regeneration, but sufficient angiogenesis is crucial for osteogenesis. The combination of BSM with autologous thrombocyte concentrations such as platelet-rich fibrin (PRF) may represent a clinical approach to overcome this limitation. This study analyzes the early influence on osteoblast (HOB) *in vitro*. Here, four different BSM (allogeneic, alloplastic, and two of xenogeneic origin) were combined with PRF. After the incubation with osteoblasts for 24 h, cell viability, migration, and proliferation were assessed. Next, marker of proliferation, migration, and differentiation were evaluated on gene and protein levels in comparison to the native BSM and osteoblast alone. Addition of PRF increased viability for both the xenogeneic BSM ($p = 0.0008$, $p = 0.032$, respectively) in comparison to HOB and vs. native BSM ($p = 0.008$), and led to a tendency for increased cell proliferation and migration for all BSM (each $p > 0.05$). On gene basis, allogeneic and alloplastic BSM displayed a significantly increased RUNX2 expression (each $p = 0.050$). Expression of alkaline phosphatase for alloplastic ($p = 0.050$) and collagen-1 for xenogeneic BSM ($p = 0.05$) were significantly increased in combination with PRF. In addition, bone morphogenic protein was expressed significantly higher when xenogeneic material was combined with PRF in comparison to HOB alone (each $p = 0.05$). In summary, the combination of PRF with different BSM increases initial viability and may influence early proliferation and migration potential of osteoblast via RUNX2, alkaline phosphatase, collagen, and BMP2 especially in combination with alloplastic and xenogeneic BSM. Biofunctionalization of BSM using PRF might improve osteogenesis and extend the range of indications.

9. Histological Comparison between Biphasic Calcium Phosphate and Deproteinized Bovine Bone on Critical-Size Bone Defects

Brito MA, Mecca LEA, Sedoski TDS, Mroczek T, Claudino M, Araujo MR. Braz Dent J. 2021 Jan-Feb;32(1):26-33. doi: 10.1590/0103-6440202103583.

https://pubmed.ncbi.nlm.nih.gov/33913998/

The limited options for bone repair have led to an extensive research of the field and the development of alloplastic and xenogeneic grafts. The purpose of this study was to evaluate bone repair with two bone substitutes: deproteinized bovine bone (DBB) and biphasic calcium phosphate ceramic (BCP) in critical-size defect. A total of 8-mm defects were made in the parietal bones of rabbits (n=12). The animals were divided into three experimental groups: sham (defect filled with a blood clot), DBB (defect filled with DBB), and BCP (defect filled with BCP). After the experimental periods of 15 and 45 days, the animals were euthanized and submitted to histomorphometric analysis. The total defect area, mineralized tissue area, biomaterial area, and soft tissue area were evaluated. A greater amount of

immature bone tissue and biomaterial particles were observed in the BCP group compared to DBB and sham at 45 days ($p<0.05$). There was no difference in the qualitative pattern of bone deposition between DBB and BCP. However, the sham group did not show osteoid islands along with the defect, presenting a greater amount of collagen fibers as well in relation to the DBB and BCP groups. There was a greater number of inflammatory cells in the DBB at 45 days compared to BCP and sham groups. In conclusion, BCP and DBB are options for optimizing the use of bone grafts for maxillofacial rehabilitation. Bone defects treated with BCP showed greater deposition of bone tissue at 45 days.

10. Does Platelet-Rich Fibrin Enhance the Early Angiogenetic Potential of Different Bone Substitute Materials? An *In Vitro* and *In Vivo* Analysis.

Blatt, S.; Thiem, D.G.E.; Pabst, A.; Al-Nawas, B.; Kämmerer, P.W. Biomedicines. 2021 Jan;9(1):61. doi: 10.3390/biomedicines9010061.

https://pubmed.ncbi.nlm.nih.gov/33435244/

The impaired angiogenic potential of bone substitute materials (BSMs) may limit regenerative processes. Therefore, changes in the angiogenetic properties of different BSMs in combination with platelet-rich fibrin (PRF) in comparison to PRF alone, as well as to native BSMs, were analyzed *in vitro* and *in vivo* to evaluate possible clinical application. *In vitro*, four BSMs of different origins (allogeneic, alloplastic, and xenogeneic) were biofunctionalized with PRF and compared to PRF in terms of platelet interaction and growth factor release (vascular endothelial growth factor (VEGF), tissue growth factor ß (TGFß) and platelet-derived growth factor (PDGF)) after 15 min. To visualize initial cell–cell interactions, SEM was performed. *In vivo*, all BSMs (_PRF) were analyzed after 24 h for new-formed vessels using a chorioallantoic membrane (CAM) assay. Especially for alloplastic BSMs, the addition of PRF led to a significant consumption of platelets ($p = 0.05$). PDGF expression significantly decreased in comparison to PRF alone (all BSMs: $p < 0.013$). SEM showed the close spatial relation of each BSM and PRF. *In vivo*, PRF had a significant positive pro-angiogenic influence in combination with alloplastic ($p = 0.007$) and xenogeneic materials ($p = 0.015$) in comparison to the native BSMs. For bio-activated xenogeneic BSMs, the branching points were also significantly increased ($p = 0.005$). Finally, vessel formation was increased for BSMs and PRF in comparison to the native control (allogeneic: $p = 0.046$; alloplastic: $p = 0.046$; and xenogeneic: $p = 0.050$). An early enhancement of angiogenetic properties was demonstrated when combining BSMs with PRF in vitro and led to upregulated vessel formation in vivo. Thus, the use of BSMs in combination with PRF may trigger bony regeneration in clinical approaches.

11. Tissue response to biphasic calcium phosphate covalently modified with either heparin or hyaluronic acid in a mouse subcutaneous implantation model

Stojanović S, AlKhoury H, Radenković M, Cvetković V, Jablonska M, Schmelzer CEH, Syrowatka F, Živković JM, Groth T, Najman S. J Biomed Mater Res A. 2021 Aug;109(8):1353-1365. doi: 10.1002/jbm.a.37126.

https://pubmed.ncbi.nlm.nih.gov/33128275/

Biphasic calcium phosphate (BCP) materials are widely employed as bone substitute materials due to their resorption/degradation properties. Inflammation after implantation of such materials represents a prerequisite for bone tissue repair and regeneration but can be also problematic if it is not only transient and if it is followed by fibrosis and scarring. Here, we modified BCP covalently with hyaluronan (HA) and heparin (Hep), glycosaminoglycans that possess anti-inflammatory properties. Beside the characterization of particle surface properties, the focus was on *in vivo* tissue response after subcutaneous implantation in mice. Histological analysis revealed a decrease in signs of inflammatory response to BCP when modified with either HA or Hep. Reduced vascularization after 30 days was noticed when BCP was modified with either HA or Hep with greater cellularity in all examined time points. Compared to plain BCP, expression of endothelial-related genes Flt1 and Vcam1 was higher in BCP-HA and BCP-Hep group at day 30. Expression of osteogenesis-related genes Sp7 and Bglap after 30 days was the highest in BCP group, followed by BCP-Hep, while the lowest expression was in BCP-HA group which correlates with collagen amount. Hence, coating of BCP particles with HA seems to suppress inflammatory response together with formation of new bone-like tissue, while the presence of Hep delays the onset of inflammatory response but permits osteogenesis in this subcutaneous bone-forming model. Transferring the results of this study to other coated materials intended for biomedical application may also pave the way to reduction of inflammation after their implantation.

12. Histological analysis of two biomaterials after socket preservation – Preliminary histological findings

Tomas M, Čandrlić M, Karl M, Juzbašić M, Matijević N, Perić Kačarević Ž, Matijević M. Clinical Oral Implants Research. 2020 Oct; 31(1), 100-100. doi: 10.1111/clr.41_13644.

https://onlinelibrary.wiley.com/doi/10.1111/clr.41_13644

Background: Following tooth extraction, the alveolar ridge undergoes dimensional changes. Various biomaterials are used to reduce alveolar ridge volume loss following tooth extraction by stimulating new bone formation. Maxresorb® inject (Botiss Biomaterials GmbH, Germany) is a biphasic material composed of a water-based gel with 60% HA and 40% β-TCP. BioOss® (Geistlich Pharma AG, Switzerland) is a porous bone mineral matrix which is produced by removing all organic components from bovine bone. **Aim/Hypothesis:** This study aimed to examine the regenerative potential of biphasic calcium phosphate (BCP) (Maxresorb® inject), by assessing histological results of bone biopsy collected from healing sockets and comparing them to inorganic bovine bone (BioOss®), after a healing period of 6 months. **Materials and Methods:** The study is designed as a randomized controlled, two - phase study. In the first phase, 30 patients that qualified for participation in the research were divided randomly into 2 groups of 15 individuals. The bone substitute materials were implanted in extraction sockets after teeth removal. The first group underwent tooth extractions and socket augmentation with Maxresorb® inject. In the other group socket preservation was performed using BioOss®. In both groups, the sockets were covered by native collagen membrane. After 6 months of healing, biopsy was harvested prior to implant placement for histological analysis. Histological sections were examined under the light microscope. **Results:** Preliminary results - The preliminary histological analysis revealed remaining BCP particles in intimate contact with the newly formed bone in the case of Maxresorb® inject. Histological specimens of BioOss® observed under light microscope also showed incorporation of the hydroxyapatite particles into the newly mineralized bone. In both materials, there were no

histological signs of tissue inflammation. Furthermore, both materials exhibited easy handling and patients had no complications during healing period**. Conclusions and Clinical Implications:** Although a small number of patients were treated, along with the preliminary nature of this study, histological findings indicate osteoconductive properties of both materials included in this study. However, additional research is required to obtain histological and clinical features of Maxresorb® inject and BioOss® regarding their osteoconductive properties.

13. Implantation of an Injectable Bone Substitute Material Enables Integration Following the Principles of Guided Bone Regeneration

Barbeck M, Jung O, Smeets R, Gosau M, Schnettler R, Rider P, Houshmand A, Korzinskas T. 2020 Mar-Apr;34(2):557-568. doi: 10.21873/in vivo.11808.

https://pubmed.ncbi.nlm.nih.gov/32111754/

Background/aim: The present study investigates the in vivo tissue reaction and the integration behavior of an injectable bone substitute material (IBS) composed of a water-based gel combined with nano hydroxyapatite particles and biphasic calcium phosphate granules. The results of the IBS were compared to biphasic bone substitute granules (BBSM) of the same chemical composition. **Materials and methods:** The subcutaneous implantation model in 40 female 5-week-old CD-1 mice up to 60 days after implantation was used for conduction of the in vivo experiments. Moreover, established histological, histopathological and histomorphometrical methods were applied. **Results:** The results showed that the IBS was gradually invaded by cells and complex tissue elements. Thus, the implant bed could be distinguished in two areas, i.e. an outer and inner region. While the outer region started to interact with the peri-implant tissue by evoking multinucleated giant cells and at earlier time points by undergoing a continuous high vascularization, the inner part was free of peri-implant cells for at least 30 days, starting to undergo a similar tissue reaction at a later time point. The bone substitute granules allowed for a fast tissue influx between the interspaces of the granules starting at day 10. While the vessel density did not differ in both groups up to the end of the study, the amount of vascularization was significantly higher over the entire observation period in the BBSM group. Moreover, the amount of biomaterial-associated multinucleated giant cells (BMGCs) was significantly higher in the IBS group in the period of between 15 to 30 days after implantation, while comparable BMGC numbers were found in both groups towards the end of the study. **Conclusion:** IBS can build a barrier-like structure that is able to control the soft tissue influx into the central regions of the implantation bed, which could not be observed in other bone substitute granules of the same chemical composition. This directed integration behavior is assumed to be in accordance with the concept of Guided Bone Regeneration (GBR). Furthermore, BMGCs can significantly influence the process of angiogenesis within an implant bed of a biomaterial but not the maturity of blood vessels.

14. A Combination of Biphasic Calcium Phosphate (Maxresorb®) and Hyaluronic Acid Gel (Hyadent®) for Repairing Osseous Defects in a Rat Model

Ahmed AG, Awartani FA, Niazy AA, Jansen JA, Alghamdi HS. Appl Sci. 2020; 10(5):1651. doi: 10.3390/app10051651.

https://www.mdpi.com/2076-3417/10/5/1651

The aim of this in vivo study was to evaluate the efficacy of biphasic calcium phosphate (Maxresorb®, BCP) used in combination with hyaluronic acid (Hyadent®, HyA) gel for regeneration of osseous defects in a rat model. Bilateral femoral condylar bone defects (3 mm diameter and 3 mm depth) were created in 40 male Wistar rats. The defects were grafted as group I (BCP only), group II (BCP + HyA), group III (HyA only), and group IV (empty control). At four weeks and 10 weeks, the bone specimens were evaluated using histological and histomorphometrical analyses to identify the newly formed bone area (NF-BA (%)), as well as the remaining BCP particles (R-BCP (%)). Light microscopic examination indicated the absence of an inflammatory reaction within the bone defects after four weeks or 10 weeks of implantation. Significant new bone regeneration was present in the bone defects grafted with BCP or BCP + HyA biomaterials, as early as four weeks, compared to control groups. The addition of HyA to BCP did not significantly improve bone regeneration at four weeks or 10 weeks. Nevertheless, its role in bone healing and regeneration warrants further investigation.

15. Can the Macrogeometry of Dental Implants Influence Guided Bone Regeneration in Buccal Bone Defects? Histomorphometric and Biomechanical Analysis in Beagle Dogs.

Fernández-Domínguez M, Ortega-Asensio V, Fuentes-Numancia E, Aragoneses JM, Barbu HM5, Ramírez-Fernández MP, Delgado-Ruiz RA, Calvo-Guirado JL, Samet N, Gehrke SA. J Clin Med. 2019 May 7;8(5):618. doi: 10.3390/jcm8050618.

https://pubmed.ncbi.nlm.nih.gov/31067735/

The aim of this experimental animal study was to assess guided bone regeneration (GBR) and implant stability (ISQ) around two dental implants with different macrogeometries. **MATERIAL AND METHODS:** Forty-eight dental implants were placed within six Beagle dogs. GBR was performed to fill buccal defects using maxresorb® bone grafting material and Jason® membrane to cover the graft. The implants were divided into two groups (n = 24 per group): G1 group implants presented semi-conical macrogeometry, a low apical self-tapping portion, and an external hexagonal connection (whereby the cervical portion was bigger than the implant body). G2 group implants presented parallel walls macrogeometry, a strong apical self-tapping portion, and an external hexagonal connection (with the cervical portion parallel to the implant body). Buccal (mouth-related) defects of 2 mm (c2 condition) and 5 mm (c3 condition) were created. For the control condition with no defect (c1), implants were installed at crestal bone level. Eight implants in each group were installed under each condition. The implant stability quotient (ISQ) was measured immediately after implant placement, and on the day of

sacrifice (3 months after the implant placement). Histological and histomorphometric procedures and analysis were performed to assess all samples, measuring crestal bone loss (CBL) and bone-to-implant contact (BIC). **RESULTS:** The data obtained were compared with statistical significance set at $p < 0.05$. The ISQ results showed a similar evolution between the groups at the two evaluation times, although higher values were found in the G1 group under all conditions. Within the limitations of this animal study, it may be concluded that implant macrogeometry is an important factor influencing guided bone regeneration in buccal defects. Group G1 showed better buccal bone regeneration (CBL) and BIC [%] at 3 months follow up, also parallel collar design can stimulate bone regeneration more than divergent collar design implants. **CONCLUSION:** The apical portion of the implant, with a stronger self-tapping feature, may provide better initial stability, even in the presence of a bone defect in the buccal area.

16. Comparison of titanium dioxide scaffold with commercial bone graft materials through micro-finite element modelling in flow perfusion.

Zhang X, Tiainen H, Haugen HJ. Med Biol Eng Comput. 2019 Jan;57(1):311-324. doi: 10.1007/s11517-018-1884-2.

https://pubmed.ncbi.nlm.nih.gov/30117067/

TiO2 scaffolds have previously shown to have promising osteoconductive properties in previous in vivo experiments. Appropriate mechanical stimuli can further promote this osteoconductive behaviour. However, the complex mechanical environment and the mechanical stimuli enhancing bone regeneration for porous bioceramics have not yet been fully elucidated. This paper aims to compare and evaluate mechanical environment of TiO_2 scaffold with three commercial CaP biomaterials, i.e. Bio-Oss, cerabone® and maxresorb® under simulated perfusion culture conditions. **Material and Methods:** The solid phase and fluid phase were modelled as linear elastic material and Newtonian fluid, respectively. The mechanical stimulus was analysed within these porous scaffolds quantitatively. **Results:** The results showed that the TiO_2 had nearly heterogeneous stress distributions, however lower effective Young's modulus than cerabone® and maxresorb®. The permeability and wall shear stress (WSS) for the TiO_2 scaffold was significantly higher than other commercial bone substitute materials. maxresorb® and Bio-Oss showed lowest permeability and local areas of very high WSS. The detailed description of the mechanical performance of these scaffolds could help researchers to predict cell behaviour and to select the most appropriate scaffold for different *in vitro* and *in vivo* performances. Graphical abstract Schematic representation of the establishment procedure. Take the establishment process of cerabone® as an example. Left shows a slice of micro-CT image from cerabone®, and 1.5 mm × 1.5 mm region of interest was shown in the red box. A 1.5-mm3 cube was cut out by Boolean operation in Mimics (Materialise, Belgium), and the cubic model was remeshed in 3-Matic 6.0 (Materialise, Belgium). The cubic model is shown in blue, and the empty space in red.

17. Bone regeneration using composite non-demineralized xenogenic dentin with beta-tricalcium phosphate in experimental alveolar cleft repair in a rabbit model.
Kamal M, Andersson L, Tolba R, Al-Asfour A, Bartella AK, Gremse F, Rosenhain S, Hölzle F, Kessler P, Lethaus B. J Transl Med. 2017 Dec 23;15(1):263. doi: 10.1186/s12967-017-1369-3.

https://pubmed.ncbi.nlm.nih.gov/29274638/

The purpose of this study was to evaluate bone regeneration pattern and quantify bone formation after grafting pre-established experimental alveolar clefts defects model in rabbits using composite xenogenic dentin and β-TCP in comparison to β-TCP alone. **METHODS:** Unilateral alveolar cleft defects were created in 16 New Zealand rabbits according to previously described methodology. Alveolar clefts were allowed 8 weeks healing period. 8 defects were filled with β-TCP, whereas 8 defects filled with composite xenogenic dentin with β-TCP. Bone regeneration of the healed defects was compared at the 8 weeks after intervention. Quantification of bone formation was analyzed using micro-computed tomography (µCT) and histomorphometric analysis. **RESULTS:** µCT and histomorphometric analysis revealed that defects filled with composite dentin/β-TCP showed statistically higher bone volume fraction, bone mineral density and percentage residual graft volume when compared to β-TCP alone. An improved surgical handling of the composite dentin/β-TCP graft was also noted. **CONCLUSIONS:** Composite xenogenic dentin/β-TCP putty expresses enhanced bone regeneration compared to β-TCP alone in the reconstruction of rabbit alveolar clefts defects.

18. In vitro evaluation of an injectable biphasic calcium phosphate (BCP) carrier system combined with recombinant human bone morphogenetic protein (rhBMP)-9.
Fujioka-Kobayashi M, Schaller B, Zhang Y, Pippenger BE and Miron RJ. Biomed Mater Eng. 2017;28(3):293-304. doi: 10.3233/BME-171675.

https://pubmed.ncbi.nlm.nih.gov/28527192/

The aim of the present study was to evaluate the possibility of combining rhBMP9 with an injectable biphasic calcium phosphate (I-BCP, maxresorb inject®), since I-BCP is an easy to handle biomaterial with ideal properties for bone augmentation procedures. The adsorption potential of rhBMP9 as well as the cell behavior of bone stromal ST2 cells were investigated on cell viability, adhesion, proliferation and osteogenic differentiation for I-BCP combined with/without rhBMP9 in vitro. I-BCP demonstrated excellent adsorption/retention potential of rhBMP9 with a slow and steady release over a 10-day period by ELISA. Cell attachment at 8 hours and cell proliferation at one, 3 and 5 days was decreased on I-BCP with/without rhBMP9 when compared to control tissue-culture plastic. While I-BCP had little influence on osteoblast differentiation, its combination with rhBMP9 significantly increased ALP activity at 7 days and mRNA levels of osteoblast differentiation markers including ALP and osteocalcin at 14 days. I-BCP served as an excellent carrier for rhBMP9 clearly demonstrating its osteoinductive potential.

We therefore confirm the great potential of rhBMP9 to serve as a future regenerative growth factor for bone applications.

19. Hydrophilicity, Viscoelastic, and Physicochemical Properties Variations in Dental Bone Grafting Substitutes.

Trajkovski B, Jaunich M, Müller WD, Beuer F, Zafiropoulos GG, Houshmand A. Materials (Basel). 2018 Jan 30;11(2):215. doi: 10.3390/ma11020215

https://pubmed.ncbi.nlm.nih.gov/29385747/

Investigation of the dimensional changes and molecular mobility by Dynamic Mechanical Analysis (DMA) of xenograft (cerabone®), synthetic (maxresorb®), and allograft (maxgraft®, Puros®) blocks in a wet and dry state. While no significant differences could be seen in dry state, cerabone® and maxresorb® blocks showed a slight height decrease in wet state, whereas both maxgraft® and Puros® had an almost identical height increase. In addition, cerabone® and maxresorb® blocks remained highly rigid and their damping behaviour was not influenced by the water. On the other hand, both maxgraft® and Puros® had a strong increase in their molecular mobility with different damping behaviour profiles during the wet state. A high-speed microscopical imaging system was used to analyze the hydrophilicity in several naturally derived (cerabone®, Bio-Oss®, NuOss®, SIC® nature graft) and synthetic DBGS granules (maxresorb®, BoneCeramic®, NanoBone®, Ceros®). The highest level of hydrophilicity was detected in cerabone® and maxresorb®, while Bio-Oss® and BoneCeramic® had the lowest level of hydrophilicity among both naturally derived and synthetic DBGS groups. Deviations among the DBGS were also addressed via physicochemical differences recorded by Micro Computed Tomography, Scanning Electron Microscopy, Fourier Transform Infrared Spectroscopy, X-ray powder Diffractometry, and Thermogravimetric Analysis. Such DBGS variations could influence the volume stability at the grafting site, handling as well as the speed of vascularization and bone regeneration. Therefore, this study initiates a new insight into the DBGS differences and their importance for successful clinical results.

20. Recombinant human bone morphogenetic protein (rhBMP) 9 induces osteoblast differentiation when combined with demineralized freeze-dried bone allografts (DFDBAs) or biphasic calcium phosphate (BCP).

Fujioka-Kobayashi M, Schaller B, Zhang Y, Kandalam U, Hernandez M, Miron RJ. Clin Oral Investig. 2017 Jun;21(5):1883-1893. doi: 10.1007/s00784-016-1983-0.

https://pubmed.ncbi.nlm.nih.gov/27771827/

The aim of the present study was to investigate the effects of rhBMP9 in comparison to the clinically 2 on in vitro cell behavior when combined with two bone graft materials including demineralized freeze-dried bone allografts (DFDBAs) and biphasic calcium phosphate (BCP). **MATERIALS AND METHODS:** The absorption and release kinetics of rhBMPs from DFDBA and BCP were investigated by ELISA. Moreover,

murine bone stromal ST2 cell behavior was investigated on DFDBA or BCP seeded on (1) graft only, (2) rhBMP2 (10 ng/ml), (3) rhBMP2 (100 ng/ml), (4) rhBMP9 (10 ng/ml), and (5) rhBMP9 (100 ng/ml). The effects of rhBMPs on DFDBA and BCP were assessed for cell adhesion, proliferation, and osteoblast differentiation by alkaline phosphatase (ALP) activity, alizarin red staining, and real-time PCR for genes encoding Runx2, ALP, and bone sialoprotein (BSP). **RESULTS:** While both BMPs were gradually released from DFDBA and BCP over time, significantly higher adsorption was observed on BCP when compared to DFDBA. Cell attachment and proliferation was higher on BCP with little influence of either rhBMP2/9. Despite rhBMPs having relatively no effect on cell attachment/proliferation, a pronounced and marked effect was observed on osteoblast differentiation for both rhBMP2/9. Interestingly, it was observed that rhBMP9 induced significantly higher ALP activity, alizarin red staining, and messenger RNA (mRNA) levels of ALP and BSP when compared to rhBMP2. Our results also revealed higher differentiation for rhBMP2/9 with BCP when compared to DFDBA most likely as a result of higher growth factor adsorption. **CONCLUSION**: While both rhBMP2/9 combined with DFDBA or BCP induced osteoblast differentiation, rhBMP9 induced greater osteoblast differentiation when compared to rhBMP2.
CLINICAL RELEVANCE: rhBMP9 may be a recombinant growth factor with higher potential to induce new bone formation when compared to rhBMP2. Further in vivo studies are necessary to characterize its regenerative potential in various animal models.

21. Bone substitute material composition and morphology differentially modulate calcium and phosphate release through osteoclast-like cells.

Konermann A, Staubwasser M, Dirk C, Keilig L, Bourauel C, Götz W, Jäger A, Reichert C. Int J Oral Maxillofac Surg. 2014 Apr;43(4):514-21. doi: 10.1016/j.ijom.2013.10.017.

https://pubmed.ncbi.nlm.nih.gov/24268900/

The aim of this study was to determine the material composition and cell-mediated remodelling of different calcium phosphate-based bone substitutes. Osteoclasts were cultivated on bone substitutes (cerabone®, maxresorb®, and NanoBone) for up to 5 days. Bafilomycin A1 addition served as the control. To determine cellular activity, the supernatant content of calcium and phosphate was measured by inductively coupled plasma optical emission spectrometry. Cells were visualized on the materials by scanning electron microscopy. Material composition and surface characteristics were assessed by energy-dispersive X-ray spectroscopy. Osteoclast-induced calcium and phosphate release was material-specific. maxresorb® exhibited the highest ion release to the medium (P=0.034; calcium 40.25mg/l day 5, phosphate 102.08mg/l day 5) and NanoBone the lowest (P=0.021; calcium 8.43mg/l day 5, phosphate 15.15mg/l day 5); cerabone® was intermediate (P=0.034; calcium 16.34mg/l day 5, phosphate 30.6mg/l day 5). All investigated materials showed unique resorption behaviours. The presented methodology provides a new perspective on the investigation of bone substitute biodegradation, maintaining the material-specific micro- and macrostructure.

22. Donor age-related biological properties of human dental pulp stem cells change in nanostructured scaffolds.*

Bressan E, Ferroni L, Gardin C, Pinton P, Stellini E, Botticelli D, Sivolella S, Zavan B. PLoS One. 2012;7(11):e49146. doi: 10.1371/journal.pone.0049146.

https://pubmed.ncbi.nlm.nih.gov/23209565/

The aim of the present work is to study how biological properties, such as proliferation and commitment ability, of human adult dental pulp stem cells (DPSCs) relate to the age of the donor. Human dental pulps were extracted from molars of healthy adult subjects aged 16 to >66 years. DPSCs were isolated and cultured in the presence of osteogenic, neurogenic, or vasculogenic differentiation medium. Proliferation ability was evaluated by determining doubling time, and commitment ability was evaluated by gene expression and morphological analyses for tissue-specific markers. The results confirm a well-defined proliferative ability for each donor age group at an early in vitro passage (p2). DPSCs from younger donors (up to 35 years) maintain this ability in long-term cultures (p8). Stem cells of all age donor groups maintain their commitment ability during in vitro culture. In vivo tests on the critical size defect repair process confirmed that DPSCs of all donor ages are a potent tool for bone tissue regeneration when mixed with 3D nanostructured scaffolds. CONCLUSIONS: Biphasic calcium phosphate functioned well as a scaffolding material allowing mineralized tissue formation. Furthermore, the addiction of absorbable collagen membranes enhanced bone gain compared with non-membrane-treated sites.

*Study refers to Ossceram (Bredent), which was a private label of maxresorb®.

2. Clinical studies and case series

23. Comparison of Injectable Biphasic Calcium Phosphate and a Bovine Xenograft in Socket Preservation: Qualitative and Quantitative Histologic Study in Humans
Čandrlić M, Tomas M, Karl M, Malešić L, Včev A, Perić Kačarević Ž, Matijević M. Int J Mol Sci. 2022 Feb;23(5):2539. doi: 10.3390/ijms23052539.

https://pubmed.ncbi.nlm.nih.gov/35269686/

This study is the first histologic evaluation of an injectable biphasic calcium phosphate (IBCP) in humans six months after socket preservation according to the principles of guided bone regeneration. After tooth extraction, the alveolar ridge of 21 patients was augmented with IBCP (maxresorb® inject) in the test group, while 20 patients in the control group received a bovine xenograft (BX) (cerabone®). Six months after augmentation, a reentry procedure was performed to collect biopsies of regenerated bone for qualitative and quantitative histologic analysis. A total of 20 biopsies were taken for analysis. Qualitative histologic analysis showed complete integration of the biomaterial and no inflammatory tissue reaction, indicating the biocompatibility of the bone grafts and the surrounding tissue in both groups. Histomorphometric analysis showed comparable results in terms of newly formed bone (IBCP: 26.47 ± 14.71%, BX: 30.47 ± 16.39%) and residual biomaterial (IBCP: 13.1 ± 14.07%, BX: 17.89 ± 11.81%), with no significant difference found across groups (p > 0.05, Mann-Whitney U test). Statistical significance between groups was found in the result of soft tissue percentage (IBCP: 60.43 ± 12.73%, BX: 51.64 ± 14.63%, p = 0.046, Mann-Whitney U test). To conclude, IBCP and BX showed good osteoconductivity and biocompatibility with comparable new bone formation six months after alveolar ridge preservation.

24. Synthetic Injectable Biomaterials for Alveolar Bone Regeneration in Animal and Human Studies
Tomas M, Čandrlić M, Juzbašić M, Ivanišević Z, Matijević N, Včev A, Cvijanović Peloza O, Matijević M, Perić Kačarević Ž. Materials (Basel). 2021 May;14(11):2858. doi: 10.3390/ma14112858.

https://pubmed.ncbi.nlm.nih.gov/34073551/

After tooth extraction, the alveolar ridge undergoes dimensional changes. Different bone regeneration biomaterials are used to reduce bone loss. The aim of this article was to systematically review the literature on the effect of injectable synthetic biomaterials and their advantages and disadvantages for new bone formation in the maxilla and mandible in animals and humans. A literature search was conducted in November 2020 via MEDLINE PubMed, Cochrane, and Embase. Of the 501 records screened, abstract analysis was performed on 49 articles, resulting in 21 studies that met the inclusion criteria. Animal studies have shown heterogeneity in terms of animal models, follow-up time, composition of the injectable biomaterial, and different outcome variables such as bone-implant contact, newly formed bone, and peri-implant bone density. Heterogeneity has also been

demonstrated by human studies. The following outcomes were observed: newly formed bone, connective tissue, residual injectable bone graft substitute, radiographic density, residual bone height, and different follow-up periods. Further studies, especially in humans, based on the histological and biomechanical properties of the injectable delivery form, are needed to draw more concrete conclusions that will contribute to a better understanding of the benefits of this type of biomaterials and their role in bone regeneration.

25. Early implant placement and peri-implant augmentation with a porcine-derived acellular dermal matrix and synthetic bone in the aesthetic area: a 2-year follow-up prospective cohort study

Papi P, Pranno N, Di Murro B, Pompa G. Int J Oral Maxillofac Surg. 2021 Feb;50(2):258-266. doi: 10.1016/j.ijom.2020.07.002.

https://botiss.com/product/early-implant-placement-and-peri-implant-augmentation-with-a-porcine-derived-acellular-dermal-matrix-and-synthetic-bone-in-the-aesthetic-area-a-2-year-follow-up-prospective-cohort-study/

The aim of this study was to evaluate the 2-year follow-up results of early implant placement with simultaneous peri-implant augmentation using an acellular dermal matrix (ADM) and a synthetic bone substitute in the aesthetic zone. Twenty subjects were enrolled in this study, they were either males (eight) or females (12), with a mean age of 47.8 ± 4.45 years and each patient was treated with one implant. Simultaneous contour augmentation with guided bone regeneration was performed using synthetic bone particles (maxresorb®) and an ADM (mucoderm®). Keratinized mucosa width (KMW) and gingival thickness (GT) were assessed at baseline, 1, 3, 6, 12 and 24 months. Marginal bone loss, probing pocket depth, bleeding on probing and plaque index were also recorded. GT and KMW increased between baseline and 1 month, slightly decreased between 1 month and 12 months ($P < 0.001$) and remained stable between 12 and 24 months ($P < 0.001$). After 2 years, mean marginal bone loss level was 0.51 ± 0.63 mm, with no probing pocket depth values >5 mm and no concomitant signs of inflammation registered. Pink aesthetic score was 8.3. Combining an ADM and guided bone regeneration with early implant placement revealed a significant increase of 1.9 mm for GT and 1.6 mm for KMW after 2 years, showing good patient satisfaction regarding the aesthetic outcomes of soft tissues and prosthetic crown.

26. Influence of the type of bone substitute materials on the rates of bone regeneration in sinus lift grafting with lateral approach and delayed implant placement

Papanchev G, Peev S, Georgiev T. Clinical Oral Implants Research. 2020 Oct;31:251-251.

https://onlinelibrary.wiley.com/doi/full/10.1111/clr.189_13644

Background: Dental implantology is the most dynamic area of the dental medicine and today it is an integral part of the daily practice. Often the placement of dental implants must be combined with various augmentation procedures. Such a case is the maxillary sinus floor augmentation, which has

been used for occlusal rehabilitation in the posterior maxilla. Different kinds of bone substitute materials have been used to solve the problems with the shortage and quality of the remaining bone in this region. **Aim/Hypothesis:** The aim of this research is to follow the rates of bone regeneration in sinus lift grafting with synthetic biphasic calcium phosphate-BoneCeramic, xenogeneic bovine hydroxyapatite-Cerabone and biphasic calcium phosphate paste-Maxresorb inj. using instrumental, histomorphometric and imaging methods. **Materials and Methods:** Thirty patients were divided into three equal groups, as each was divided into two subgroups of five people. Each patient underwent a sinus lift with lateral access, and depending on which of the three main groups he fell in, different bone substitute material was used: synthetic biphasic calcium phosphate, xenogeneic bovine hydroxyapatite and biphasic calcium phosphate paste. On the 6th or 9th postoperative month, depending on the subgroup in which each patient falls, a CBCT was performed and the height of the augmented subantral bone was measured. A biopsy was taken using a 4.3 mm trephine bur. The resulting cylINDIAr was fixed in neutral formalin for 24 hours. The sample was stained with a Goldner's Masson trichrome stain, imaged under a scanning microscope and digitized. Staining makes it possible to differentiate mineralized bone by staining it green with NO-mineralized bone, which is stained red. A histomorphometric analysis was performed with the PS CS5 Extended program. **Results:** The obtained results were distributed in tables and statistically processed using descriptive analysis, regression analysis, ANOVA analysis of variance and Student's t-test. They showed that the largest total volume of bone tissue (mineralized and NO-mineralized) in the biopsy sample without residual bone was obtained using xenogeneic bovine hydroxyapatite - 15.7%, followed by biphasic calcium phosphate paste - 14.2% and synthetic biphasic calcium phosphate - 8.2%. The highest values of mineralized bone were obtained with xenogeneic bovine hydroxyapatite, while the NO-mineralized bone was the highest with biphasic calcium phosphate paste. From the analysis of the obtained results it is clear that the sex (P = 0.879) and age (P = 0.143) of the patient do not affect the quantity and quality of the newly formed bone. We did not find a relationship between the volume of bone tissue in the biopsy sample and the time elapsed from sinus lift grafting (P = 0.406). **Conclusions and Clinical Implications:** In conclusion, we can say that sinus lift grafting with lateral approach can be used with equal success in different age groups and always leads to predictable and stable results over time. Xenogeneic bovine hydroxyapatite is a favourite when choosing a bone substitute material. The lack of a statistically significant difference in the volume of the newly formed bone between the 6th and 9th month gives grounds for earlier placement of dental implants reducing treatment time.

27. Evaluation of Efficacy of Surgical Periodontal Therapy with the Use of Bone Graft in the Treatment of Periodontal Intrabony Defects.

Gojkov-Vukelic M, Hadzic S, Pasic E. Med Arch. 2017 Jun;71(3):208-211. doi: 10.5455/medarh.2017.71.208-211.

https://pubmed.ncbi.nlm.nih.gov/28974835/

INTRODUCTION: One of the most important goals of periodontitis therapy is the elimination of deep periodontal pockets. In regenerative periodontal therapy, different types of bone grafts, membranes,

growth factors, etc. are used to improve regeneration of lost periodontal tissue. The aim of this study was to evaluate the effect of surgical therapy supported by the use of bone replacement material in the treatment of deep intrabony pockets, compared to surgical treatment (flap surgery) without the use of bone replacement in advanced periodontitis. **METHODS AND MATERIALS:** The study included 50 patients of both sexes with advanced periodontitis, divided into two groups. After initial periodontal therapy was performed, plaque index (PI), papillary bleeding index (PBI) were verified, and depth of periodontal pockets was measured in both groups. One group (group 1) of the patients underwent surgical therapy, open flap surgery, while the other group (group 2) underwent the same surgical treatment method (open flap surgery), during which bone defects were filled with bone replacement material. **RESULTS:** The results showed that both group 1 and group 2 experienced improvements after periodontal surgical therapy. In group 1, there are no statistically significant changes in all three plaque index measurements (PI), while there has been a significant reduction in PI in group 2 following the surgery. For the PBI index, it was determined that there were statistically significant changes in values in group 1, both after surgical procedures and six months later, as well as in group 2. Statistical analysis of the results of the probing depth of pockets has shown that there are significant changes in the measurement of the depth of periodontal pocket one month after the surgery, as well as six months later, meaning that there has been a significant reduction in the depth of the periodontal pocket one month following the surgery as well as six months later, for both groups. However, we did not determine a statistically significant difference in the probing depth of pockets between these two groups. **CONCLUSION:** Six months after a surgical therapy, clinical parameters showed a reduction of the probing depth of the periodontal pocket in both examined groups. The use of bone replacement did not yield significantly better results in reducing the depth of probing compared to the standard flap surgery. We believe that future research should focus on testing the effectiveness of new regenerative methods and materials (bone replacements with various properties, membranes, and surgical methods) that will result in better treatment results with predictable outcomes.

28. Investigation of peri-implant tissue conditions and peri-implant tissue stability in implants placed with simultaneous augmentation procedure: a 3-year retrospective follow-up analysis of a newly developed bone level implant system.

Lorenz J, Lerner H, Sader RA, Ghanaati S. Int J Implant Dent. 2017 Sep 5;3(1):41. doi: 10.1186/s40729-017-0104-4.

https://pubmed.ncbi.nlm.nih.gov/28875278/

The aim of the present retrospective analysis was to assess peri-implant tissue conditions and document peri-implant tissue stability in C-Tech implants when placed simultaneously with a GBR augmentation procedure. **METHODS:** A total of 47 implants, which were placed simultaneously with a GBR procedure with a synthetic bone substitute material in 20 patients, were investigated clinically and

radiologically at least 3 years after loading. Implant survival, the width and thickness of peri-implant keratinized gingiva, probing depth, bleeding on probing (BOP), the Pink Esthetic Score (PES), peri-implant bone loss, and the presence of peri-implant osteolysis were determined. **RESULTS:** The follow-up investigation revealed a survival rate of 100% and only low median rates for probing depths (2.7 mm) and BOP (30%). The mean PES was 10.1 from the maximum value of 14. No osseous peri-implant defects were obvious, and the mean bone loss was 0.55 mm.
CONCLUSIONS: In conclusion, implants placed in combination with a GBR procedure can achieve long-term stable functionally and esthetically satisfying results for replacing missing teeth in cases of atrophy of the alveolar crest.

29. Monophasic ß-TCP vs. biphasic HA/ß-TCP in two-stage sinus floor augmentation procedures - a prospective randomized clinical trial.

Jelušić D, Zirk ML, Fienitz T, Plancak D, Puhar I, Rothamel D. Clin Oral Implants Res. 2017 Oct;28(10):e175-e183. doi: 10.1111/clr.12983.

https://pubmed.ncbi.nlm.nih.gov/27683073/

The study compares a monophasic (100% ß-TCP) and a biphasic (60% HA and 40% ß-TCP) bone substitute material (BSM) regarding biocompatibility, osteoconductivity and implant stability using histological, radiological and resonance frequency analysis. **MATERIAL AND METHODS:** Sixty-seven sinus floor elevations were performed in 60 patients. One patient group (monophasic bone substitute [MBS], 30 patients, 32 sinuses) was augmented by the use of the monophasic material (Bioresorb®, Sybron Implant Solutions, Bremen, Germany), while the second group (biphasic bone substitute (BBS), 30 patients, 35 sinuses) received a biphasic material (maxresorb®, botiss biomaterials, Berlin, Germany). Cone beam CT images were taken immediately after augmentation and prior to implant placement after 6 months. Trephines were harvested, while the implant bed was prepared. Resonance frequency analysis was performed immediately after implant placement and 6 months later. Descriptive analysis was performed on all augmented sinus (n = 67). For statistical comparison of the groups, one sinus of each bilaterally treated patient was randomly excluded, resulting in 30 sinuses grafted with MBS and 30 sinuses grafted with BBS (n = 60). **RESULTS:** Histomorphometrical analysis of all sinuses displayed comparable results for both groups regarding new bone matrix (MBS 36.16 ± 19.37%, BBS 38.42 ± 12.61%), residual BSM (MBS 30.26 ± 11.7%, BBS 32.66 ± 12.57%) and non-mineralized tissue (MBS 34.29 ± 18.32%, BBS 28.92 ± 15.04) %) ($P > 0.05$, respectively). Radiological volume of BBS was significantly more stable (volume loss of 22.2% for MBS, 6.66% for BBS; $P < 0.001$), and homogeneity of the graft after 6 months was higher for BBS than that for MBS ($P < 0.05$). Resonance frequency analysis endorsed a higher implant stability quotient for BBS after 6 months than that for MBS (MBS 78.31 ± 5.81, BBS 80.42 ± 6.31; $P < 0.05$, Mann-Whitney U-test, respectively). **CONCLUSION:** Both monophasic and biphasic materials show good biocompatibility and osteoconductivity with satisfactory support on implant stability. BBS remains more stable in terms of volume maintenance and radiological graft homogeneity after a healing period of 6 months.

30. Assessment of implant stability following sinus lift procedures with different grafting materials.

Jelušić D, Puhar I, Plančak D. Acta Stomatol Croat. 2014 Mar;48(1):25-32. doi: 10.15644/asc48/1/3.

https://pubmed.ncbi.nlm.nih.gov/27688348/

The objective of this research was to evaluate implant stability following sinus lift with two grafting materials, and to compare it with the results obtained for the implants placed in a pristine posterior maxilla. **MATERIALS AND METHODS:** The study included 44 healthy patients with an existing indication for sinus lift procedure (test group). 46 implants were placed following sinus lift with a pure-phase beta-tricalcium phosphate, while 39 implants were placed following augmentation with 60% hydroxyapatite with 40% beta-tricalcium phosphate material. The control group consisted of 48 healthy patients who were treated with 85 implants but without bone augmentation in posterior maxilla. Astra Tech OsseoSpeed implants were placed in all subjects. Resonance frequency analysis was used in both groups for determining implant stability 4 months after insertion. A mean implant stability quotient (ISQ) was calculated on the basis of 3 measurements. **RESULTS:** No statistical difference was observed in ISQ values of implants placed with and without augmentation procedure (p=0,789). Statistically significant difference was not found when ISQ values of implants placed following particular grafting material were compared with ISQ values of corresponding implants in a pristine bone (p=0.697 and p=0.402). **CONCLUSIONS**: This study demonstrated that the implant stability is comparable among implants placed in the posterior maxilla regardless of sinus lift and grafting procedure. Implants placed in the grafted posterior maxilla can be predictably loaded as the implants placed in a non-grafted, pristine maxilla.

06/2023