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

1. Analyses of the Cellular Interactions between the Ossification of Collagen-Based Barrier Membranes and the Underlying Bone Defects.

   Alkildani S, Ren Y, Liu L, Rimashevskiy D, Schnettler R, Radenković M, Najman S, Stojanović S, Jung O, Barbeck M. Int J Mol Sci. 2023 Apr 6;24(7):6833. doi: 10.3390/ijms24076833.

   https://pubmed.ncbi.nlm.nih.gov/37047808/

   Barrier membranes are an essential tool in guided bone Regeneration (GBR), which have been widely presumed to have a bioactive effect that is beyond their occluding and space maintenance functionalities. A standardized calvaria implantation model was applied for 2, 8, and 16 weeks on Wistar rats to test the interactions between the barrier membrane and the underlying bone defects which were filled with bovine bone substitute materials (BSM). In an effort to understand the barrier membrane's bioactivity, deeper histochemical analyses, as well as the immunohistochemical detection of macrophage subtypes (M1/M2) and vascular endothelial cells, were conducted and combined with histomorphometric and statistical approaches. The native collagen-based membrane was found to have ossified due to its potentially osteoconductive and osteogenic properties, forming a "bony shield" overlying the bone defects. Histomorphometrical evaluation revealed the resorption of the membranes and their substitution with bone matrix. The numbers of both M1- and M2-macrophages were significantly higher within the membrane compartments compared to the underlying bone defects. Thereby, M2-macrophages significantly dominated the tissue reaction within the membrane compartments. Statistically, a correlation between M2-macropahges and bone regeneration was only found at 2 weeks post implantationem, while the pro-inflammatory limb of the immune response correlated with the two processes at 8 weeks. Altogether, this study elaborates on the increasingly described correlations between barrier membranes and the underlying bone regeneration, which sheds a light on the understanding of the immunomodulatory features of biomaterials.

2. Hyaluronic Acid with Bone Substitutes Enhance Angiogenesis In Vivo.

   Kyyak S, Blatt S, Wiesmann N, Smeets R, Kaemmerer PW. Materials (Basel). 2022 May 27;15(11):3839. doi: 10.3390/ma15113839.

   https://botiss.com/product/hyaluronic-acid-with-bone-substitutes-enhance-angiogenesis-in-vivo/

   Introduction: The effective induction of angiogenesis is directly related to the success of bone-substitute materials (BSM) for maxillofacial osseous regeneration. Therefore, the addition of pro-angiogenic properties to a commercially available bovine bone-substitute material in combination with

hyaluronic acid (BSM+) was compared to the same bone-substitute material without hyaluronic acid (BSM) in an in-vivo model. **Materials and Methods:** BSM+ and BSM were incubated for six days on the chorioallantoic membrane (CAM) of fertilized chicken eggs. Microscopically, the number of vessels and branching points, the vessel area and vessel length were evaluated. Subsequently, the total vessel area and brightness integration were assessed after immunohistochemical staining (H&E, alphaSMA). **Results:** In the BSM+ group, a significantly higher number of vessels ($p < 0.001$), branching points ($p = 0.001$), total vessel area ($p < 0.001$) as well as vessel length ($p = 0.001$) were found in comparison to the BSM group without hyaluronic acid. Immunohistochemically, a significantly increased total vessel area ($p < 0.001$ for H&E, $p = 0.037$ for alphaSMA) and brightness integration ($p = 0.047$) for BSM+ in comparison to the native material were seen. **Conclusions:** The combination of a xenogenic bone-substitute material with hyaluronic acid significantly induced angiogenesis in vivo. This might lead to a faster integration and an improved healing in clinical situations.

3. *In Vivo* Analysis of the Biocompatibility and Bone Healing Capacity of a Novel Bone Grafting Material Combined with Hyaluronic Acid

Pröhl A, Batinic M, Alkildani S, Hahn M, Radenkovic M, Najman S, Jung O, Barbeck M. Int J Mol Sci. 2021 May;22(9):4818. doi: 10.3390/ijms22094818.

https://pubmed.ncbi.nlm.nih.gov/34062885/

The present *in vivo* study analyses both the inflammatory tissue reactions and the bone healing capacity of a newly developed bone substitute material (BSM) based on xenogeneic bone substitute granules combined with hyaluronate (HY) as a water-binding molecule. The results of the hyaluronate containing bone substitute material (BSM) were compared to a control xenogeneic BSM of the same chemical composition and a sham operation group up to 16 weeks post implantationem. A major focus of the study was to analyze the residual hyaluronate and its effects on the material-dependent healing behavior and the inflammatory tissue responses. The study included 63 male Wistar rats using the calvaria implantation model for 2, 8, and 16 weeks post implantationem. Established and Good Laboratory Practice (GLP)-conforming histological, histopathological, and histomorphometrical analysis methods were conducted. The results showed that the new hyaluronate containing BSM was gradually integrated within newly formed bone up to the end of the study that ended in a condition of complete bone defect healing. Thereby, no differences to the healing capacity of the control BSM were found. However, the bone formation in both groups was continuously significantly higher compared to the sham operation group. Additionally, no differences in the (inflammatory) tissue response that was analyzed via qualitative and (semi-) quantitative methods were found. Interestingly, no differences were found between the numbers of pro- and anti-inflammatory macrophages between the three study groups over the entire course of the study. No signs of the HY as a water-binding part of the BSM were histologically detectable at any of the study time points, altogether the results of the present study show that HY allows for an optimal material-associated bone tissue healing comparable to the control xenogeneic BSM. The added HY seems to be degraded within a very short time period of less

than 2 weeks so that the remaining BSM granules allow for a gradual osteoconductive bone regeneration. Additionally, no differences between the inflammatory tissue reactions in both material groups and the sham operation group were found. Thus, the new hyaluronate containing xenogeneic BSM and also the control BSM have been shown to be fully biocompatible without any differences regarding bone regeneration.

4. The Influence of Hyaluronic Acid Biofunctionalization of a Bovine Bone Substitute on Osteoblast Activity In Vitro.

Kyyak S, Pabst A, Heimes D, Kämmerer PW. Materials (Basel). 2021 May 27;14(11):2885. doi: 10.3390/ma14112885.

https://pubmed.ncbi.nlm.nih.gov/34072146/

Bovine bone substitute materials (BSMs) are used for oral bone regeneration. The objective was to analyze the influence of BSM biofunctionalization via hyaluronic acid (HA) on human osteoblasts (HOBs). BSMs with ± HA were incubated with HOBs including HOBs alone as a negative control. On days 3, 7 and 10, cell viability, migration and proliferation were analyzed by fluorescence staining, scratch wound assay and MTT assay. On days 3, 7 and 10, an increased cell viability was demonstrated for BSM+ compared with BSM- and the control (each $p \leq 0.05$). The cell migration was enhanced for BSM+ compared with BSM- and the control after day 3 and day 7 (each $p \leq 0.05$). At day 10, an accelerated wound closure was found for the control compared with BSM+/- (each $p < 0.05$). The highest proliferation rate was observed for BSM+ on day 3 ($p \leq 0.05$) followed by BSM- and the control (each $p \leq 0.05$). At day 7, a non-significantly increased proliferation was shown for BSM+ while the control was higher than BSM- (each $p < 0.05$). The least proliferation activity was observed for BSM- ($p < 0.05$) at day 10. HA biofunctionalization of the BSMs caused an increased HOB activity and might represent a promising alternative to BSM- in oral bone regeneration.

2. Clinical studies and case series

5. First Clinical Case Report of a Xenograft–Allograft Combination for Alveolar Ridge Augmentation Using a Bovine Bone Substitute Material with Hyaluronate (Cerabone® Plus) Combined with Allogeneic Bone Granules (Maxgraft®).

   Kloss, F.R.; Kämmerer, P.W.; Kloss-Brandstätter, A. J. Clin. Med. 2023, 12, 6214.

   https://doi.org/10.3390/jcm12196214

   Background: A patient had lost the first left maxillary incisor in the esthetic zone. **Methods:** The defect in the alveolar ridge was reconstructed for an implant-supported restoration using a new xenogeneic bone substitute containing hyaluronate, which was used in combination with allogeneic bone granules. **Results:** After three years of follow-up, the dental implant was stable and showed no signs of infection. **Conclusions:** This is the first case report with a long-term follow-up time of three years of a successful clinical application of a xenograft–allograft combination (cerabone® plus combined with maxgraft®) for alveolar ridge augmentation before dental implantation. Cerabone® plus offers volume stability, provides reliable and efficient structural support of the oral soft tissues in the augmented region(particularly crucial in the aesthetic zone), and preserves the alveolar ridge shape.

6. Immediate implant placement by using natural bovine bone substitute with hyaluronate

   Abillamaa, F., Chemaly , C. ., Maalouf, E. . ., & Trajkovski , B. . (2023). International Journal of Dental Biomaterials Research, 1, 7–12.

   https://doi.org/10.56939/DBR23107a

   Sufficient bone volume is important to allow proper implants osseointegration. The aim of this case report was to observe an immediate implant placement by using xenograft granules with hyaluronate and without any membrane coverage. The augmentation areas were assessed 3 months later during final crown installation and after 1 year and 6 months of implant loading. Satisfactory implant stability, granules osteointegration into newly formed bone, as well as stable soft tissue supported by the granules were observed.

7. Reconstructive Peri-Implantitis Therapy by Using Bovine Bone Substitute with or without Hyaluronic Acid: A Randomized Clinical Controlled Pilot Study

Rakašević D, Šćepanović M, Mijailović I, Mišić T, Janjić B, Soldatović I, Marković A. J Funct Biomater. 2023 Mar 8;14(3):149. doi: 10.3390/jfb14030149.

https://botiss-campus.com/reconstructive-peri-mplantitis-therapy-by-using-bovine-bone-substitute-with-or-without-hyaluronic-acid/

Background: The present pilot study aimed to assess clinical and radiographic efficiencies of bovine bone substitute (BBS) merged with hyaluronic acid (HA) in peri-implantits reconstructive surgery. **Methods:** Peri-implantitis (diagnosed 6.03 ± 1.61 years of implant loading) bone defects were randomly treated either with BBS plus HA (test group) or BBS alone (control group). Clinical parameters including peri-implant probing depth (PPD), bleeding on probing (BOP), implant stability (ISQ), and radiographic changes in vertical and horizontal marginal bone (MB) levels were assessed at six months postoperatively. New temporary and permanent screw-retained crowns were made at two weeks and three months postoperatively. Data were analyzed using parametric and non-parametric tests. **Results:** In both groups, 75% of patients and 83% of implants achieved treatment success after six months (no BOP, PPD <5 mm, and no further MB loss). Clinical outcomes improved over time within groups; however, without significant difference between them. ISQ value obtained significant increases in the test compared to the control group at six months postoperatively ($p < 0.05$). The vertical MB gain was significantly greater in the test group compared to the control ($p < 0.05$). **Conclusions:** Short-term outcomes suggested that BBS merged with HA could improve clinical and radiographic outcomes in peri-implantitis reconstructive therapy.

8. Socket preservation, sinus lift and lateral augmentation by using natural bovine bone substitute with hyaluronate.

Zafiropoulos, G., & Trajkovski, B. (2023). International Journal of Dental Biomaterials Research, 1, 1–6.

https://ijdbr.com/index.php/ijdbr/article/view/12

It is very important to have sufficient bone volume that allows proper implants osseointegration. The aim of this case report was to observe a socket preservation and sinus lift with lateral augmentation by using xenograft granules with hyaluronate and pericardium collagen membrane. The sufficient granules osteointegration into newly formed bone after six months enabled proper implants placement due obvious bone volume increase. The implants were completely integrated into the regenerated bone after four months and then were loaded. The use of xenograft granules with hyaluronate led to successful treatment in combination with pericardium collagen membrane in socket preservation and sinus lift with lateral augmentation.

9. Evaluation between Biodegradable Magnesium Metal GBR Membrane and Bovine Graft with or without Hyaluronate.

Blašković M, Blašković D, Hangyasi DB, Peloza OC, Tomas M, Čandrlić M, Rider P, Mang B, Kačarević ŽP, Trajkovski B. Membranes. 2023; 13(8):691. https://doi.org/10.3390/membranes13080691

https://www.mdpi.com/2077-0375/13/8/691

Bone substitutes and barrier membranes are widely used in dental regeneration procedures. New materials are constantly being developed to provide the most optimal surgical outcomes. One of these developments is the addition of hyaluronate (HA) to the bovine bone graft, which has beneficial wound healing and handling properties. However, an acidic environment that is potentially produced by the HA is known to increase the degradation of magnesium metal. The aim of this study was to evaluate the potential risk for the addition of HA to the bovine bone graft on the degradation rate and hence the efficacy of a new biodegradable magnesium metal GBR membrane. pH and conductivity measurements were made in vitro for samples placed in phosphate-buffered solutions. These in vitro tests showed that the combination of the bovine graft with HA resulted in an alkaline environment for the concentrations that were used. The combination was also tested in a clinical setting. The use of the magnesium metal membrane in combination with the tested grafting materials achieved successful treatment in these patients and no adverse effects were observed in vivo for regenerative treatments with or without HA. Magnesium based biodegradable GBR membranes can be safely used in combination with bovine graft with or without hyaluronate.

10. Full digital workflow for anterior immediate implant placement: implementing guided surgery and one-time abutment concept.

Stavros Pelekanos & Panagiotis Ntovas (2022) LA NOUVELLE REVUE DE PARODONTOLOGIE & D'IMPLANTOLOGIE

https://www.mis-implants.com/upload/pdf/Research/Pelekanos_2022_LA_NOUVELLE_REVUE_DE_PARODONTOLOGIE_DIMPLANTOLOGIE_CONNECT.pdf

Tooth extraction is always followed by loss of vital hard and soft tissues, which can make the forthcoming esthetic rehabilitation a challenge. A biologic orientated treatment strategy has to be followed in order to maintain hard and soft tissue volume. Immediate implant placement, combined with socket seal surgery and socket preservation, can optimize soft tissue conditions and compensate dimensional changes in the alveolar ridge. Furthermore, the placement of a permanent abutment at the time of surgery can significantly reduce both peri-implant bone and soft tissue alterations. Digital

technology can be a valuable tool for planning the treatment, assisting implant placement, providing an accurate soft tissue transfer and fabricating restorations, by mirroring the nearby teeth. The aim of the present article is to introduce a step-by-step protocol, in order to achieve anterior esthetics with immediate implant placement, implementing a full digital workflow.

11. Lateral sinus augmentation by using natural bovine bone substitute with hyaluronate.

Chapanov, K., Deliverska, E., Zafiropoulos, G., & Trajkovski, B. (2022). International Journal of Dental Biomaterials Research, 1, 8–12.
https://doi.org/10.56939/DBR22108ch

https://ijdbr.com/index.php/ijdbr/article/view/12

It is very important to have sufficient bone volume that allows proper implants osseointegration. The aim of this case report was to observe a socket preservation and sinus lift with lateral augmentation by using xenograft granules with hyaluronate and pericardium collagen membrane. The sufficient granules osteointegration into newly formed bone after six months enabled proper implants placement due obvious bone volume increase. The implants were completely integrated into the regenerated bone after four months and then were loaded. The use of xenograft granules with hyaluronate led to successful treatment in combination with pericardium collagen membrane in socket preservation and sinus lift with lateral augmentation.

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