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

1. Biodegradable magnesium fixation screw for barrier membranes used in guided bone regeneration

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   https://pubmed.ncbi.nlm.nih.gov/35310352/

   An ideal fixation system for guided bone (GBR) regeneration in oral surgery must fulfil several criteria that includes the provision of adequate mechanical fixation, complete resorption when no longer needed, complete replacement by bone, as well as be biocompatible and have a good clinical manageability. For the first time, a biodegradable magnesium fixation screw made of the magnesium alloy WZM211 with a MgF2 coating has been designed and tested to fulfill these criteria. Adequate mechanical fixation was shown for the magnesium fixation screw in several benchtop tests that directly compared the magnesium fixation screw with an equivalent polymeric resorbable device. Results demonstrated slightly superior mechanical properties of the magnesium device in comparison to the polymeric device even after 4 weeks of degradation. Biocompatibility of the magnesium fixation screw was demonstrated in several in vitro and in vivo tests. Degradation of the magnesium screw was investigated in in vitro and in vivo tests, where it was found that the screw is resorbed slowly and completely after 52 weeks, providing adequate fixation in the early critical healing phase. Overall, the magnesium fixation screw demonstrates all of the key properties required for an ideal fixation screw of membranes used in guided bone regeneration (GBR) surgeries.

2. Biodegradable magnesium barrier membrane used for guided bone regeneration in dental surgery

   Patrick Rider, Željka Perić Kačarević, Akiva Elad, Drazen Tadic, Daniel Rothamel, Gerrit Sauer, Fabien Bornert, Peter Windisch, Dávid Botond Hangyási, Balint Molnar, Emely Bortel, Bernhard Hesse, and Frank Witte. 2022. Bioactive Materials 14:152–68. doi: 10.1016/J.BIOACTMAT.2021.11.018.

   https://pubmed.ncbi.nlm.nih.gov/35310351/

   Barrier membranes are commonly used as part of the dental surgical technique guided bone regeneration (GBR) and are often made of resorbable collagen or non-resorbable materials such as PTFE. While collagen membranes do not provide sufficient mechanical protection of the covered bone defect, titanium reinforced membranes and non-resorbable membranes need to be removed in a second surgery. Thus, biodegradable GBR membranes made of pure magnesium might be an alternative. In this study a biodegradable pure magnesium (99.95%) membrane has been proven to have all of the necessary requirements for an optimal regenerative outcome from both a mechanical and biological perspective. After implantation, the magnesium membrane separates the regenerating

bone from the overlying, faster proliferating soft tissue. During the initial healing period, the membrane maintained a barrier function and space provision, whilst retaining the positioning of the bone graft material within the defect space. As the magnesium metal corroded, it formed a salty corrosion layer and local gas cavities, both of which extended the functional lifespan of the membrane barrier capabilities. During the resorption of the magnesium metal and magnesium salts, it was observed that the membrane became surrounded and then replaced by new bone. After the membrane had completely resorbed, only healthy tissue remained. The in vivo performance study demonstrated that the magnesium membrane has a comparable healing response and tissue regeneration to that of a resorbable collagen membrane. Overall, the magnesium membrane demonstrated all of the ideal qualities for a barrier membrane used in GBR treatment.

3. Design of a migration assay for human gingival fibroblasts on biodegradable magnesium surfaces

Amberg, R., A. Elad, D. Rothamel, T. Fienitz, G. Szakacs, S. Heilmann, and F. Witte. 2018. Acta Biomaterialia 79:158–67. doi: 10.1016/j.actbio.2018.08.034.

https://pubmed.ncbi.nlm.nih.gov/30172066/

A novel regenerative approach to Guided Bone Regeneration (GBR) in dental surgery is based on the development of biodegradable and volume stable barrier membranes made of metallic magnesium. Currently used volume stable barrier membranes are made of titanium-reinforced PTFE or titanium-reinforced collagen membranes, both, however, are accompanied by a high incidence of wound dehiscence resulting in membrane exposure, which leads to an increased infection risk. An exposed membrane could also occur directly after insertion due to insufficient soft tissue coverage of the membrane. In both cases, fast wound margin regeneration is required. As a first step of soft-tissue regeneration, gingival fibroblasts need to migrate over the barrier membrane and close the dehiscent wound. Based on this aim, this study investigated the migration behaviour of human gingival fibroblasts on a magnesium surface. Major experimental challenges such as formation of hydrogen bubbles due to initial magnesium corrosion and non-transparent material surfaces have been addressed to allow cell adhesion and to follow cell migration. The designed scratch-based cell migration assay involved vital fluorescent cell staining on a pre-corroded magnesium membrane to simulate in vivo wound dehiscence. The assay has been used to compare cell migration on pre-corroded magnesium to titanium surfaces and tissue culture plastic as control substrates. First results of this assay showed that human gingival fibroblasts migrate slower on pre-corroded magnesium compared to plastic and titanium. However, the scratch was finally closed on all materials. Compared to titanium surfaces and tissue culture plastic, the surface roughness and the surface free energy (SFE) could not explain slower cell migration on magnesium surfaces. Immunohistological investigations of cellular structure revealed, that magnesium ions increased focal adhesion at concentration of additionally 75 mM MgCl2 in cell culture medium.

The use of our designed cell migration assay has shown that ionic medium alterations due to magnesium corrosion has a higher impact on the cell migration rate than surface alterations.

Statement of Significance

The design of a migration assay on non-transparent magnesium surfaces will add the option to study cell response to surface modifications, coatings and the corrosion process itself under life view conditions.

4. Effect of physical cues of altered extract media from biodegradable magnesium implants on human gingival fibroblasts

Amberg, R., A. Elad, F. Beuer, C. Vogt, J. Bode, and F. Witte. 2019. Acta Biomaterialia 98:186–95. doi: 10.1016/j.actbio.2019.07.022.

https://pubmed.ncbi.nlm.nih.gov/31352109/

Volume stable barrier membranes made of magnesium are very promising in Guided Bone Regeneration (GBR) to treat periodontal bone defects in dentistry due to their excellent biocompatibility and biodegradability. During the degradation process the cells are exposed to the alteration of various parameters, so called physical cues, involving surface alterations due to the formed corrosion layer and medium alterations arising from the dissolved corrosion products. Cell migration of human gingival fibroblasts (HGF), as a crucial parameter for optimal healing process in GBR, has been investigated on magnesium membranes and revealed that medium alterations by dissolved corrosion products have a higher impact on cell migration than surface alterations. However, the effect of each altered medium parameter on cell migration has not been adequately studied, but their roles are crucial to explain the slower migration rate on magnesium surfaces compared to titanium and tissue culture plastic surfaces. Our study investigates the single effect of Mg^{2+}, Ca^{2+}, H_2 and increased osmolality as well as the effect of magnesium extracts, which contain a dynamic mixture of previous parameters on cell migration, proliferation and viability of HGF. We showed that at 75 mM Mg^{2+} concentration and at 0 mM Ca^{2+}, respectively, the cell migration rate is greatly reduced. In complex magnesium extract media, we found that a temporarily increased ratio of Mg^{2+} to Ca^{2+} conditioned a slow HGF migration rate. Based on these findings and the characterization of supernatants from HGF migration assays on Mg membranes, we propose, that the slower migration rate of HGF can be explained by the altered ratio of Mg^{2+} to Ca^{2+}, caused by increasing concentrations of Mg^{2+} and decreasing concentrations of Ca^{2+} in the vicinity of the corroding Mg implant, combined with a constantly increased molecular hydrogen concentration in the supernatant. These results are cell type specific and should be checked carefully, if necessary, for Mg implant performance.

Statement of Significance

The study is providing a systematic approach to explain the main effects of extract medium parameters (physical cues) such as magnesium or calcium ion concentration, osmolality and dissolved molecular hydrogen and CO_2 in cell culture media modified by co-incubating with corroding magnesium implants on the migration rate of human gingival fibroblasts (HGF). This study uncovers for the first time the combinatory effect of slightly increased molecular hydrogen and the change in Mg^{2+}/Ca^{2+} ratio on HGF cell migration.

5. Biocompatibility Analyses of HF-Passivated Magnesium Screws for Guided Bone Regeneration (GBR)

Ole Jung, Bernhard Hesse, Sanja Stojanovic, Christian Seim, Timm Weitkamp, Milijana Batinic, Oliver Goerke, Željka Perić Kačarević, Patrick Rider, Stevo Najman, and Mike Barbeck. 2021. International Journal of Molecular Sciences 22(22). doi: 10.3390/ijms222212567.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8624161/

Background: Magnesium (Mg) is one of the most promising materials for human use in surgery due to material characteristics such as its elastic modulus as well as its resorbable and regenerative properties. In this study, HF-coated and uncoated novel bioresorbable magnesium fixation screws for maxillofacial and dental surgical applications were investigated in vitro and in vivo to evaluate the biocompatibility of the HF coating. Methods: Mg alloy screws that had either undergone a surface treatment with hydrofluoric-acid (HF) or left untreated were investigated. In vitro investigation included XTT, BrdU and LDH in accordance with the DIN ISO 10993-5/-12. In vivo, the screws were implanted into the tibia of rabbits. After 3 and 6 weeks, degradation, local tissue reactions and bony integration were analyzed histopathologically and histomorphometrically. Additionally, SEM/EDX analysis and synchrotron phase-contrast microtomography (µCT) measurements were conducted. The in vitro analyses revealed that the Mg screws are cytocompatible, with improved results when the surface had been passivated with HF. In vivo, the HF-treated Mg screws implanted showed a reduction in gas formation, slower biodegradation and a better bony integration in comparison to the untreated Mg screws. Histopathologically, the HF-passivated screws induced a layer of macrophages as part of its biodegradation process, whereas the untreated screws caused a slight fibrous tissue reaction. SEM/EDX analysis showed that both screws formed a similar layer of calcium phosphates on their surfaces and were surrounded by bone. Furthermore, the µCT revealed the presence of a metallic core of the screws, a faster absorbing corrosion front and a slow absorbing region of corroded magnesium. Conclusions: Overall, the HF-passivated Mg fixation screws showed significantly better biocompatibility in vitro and in vivo compared to the untreated screws.

6. Analysis of a Pure Magnesium Membrane Degradation Process and Its Functionality When Used in a Guided Bone Regeneration Model in Beagle Dogs

Patrick Rider, Željka Peri´c, Peri´c Kačarevi´ Kačarevi´c, Akiva Elad, Daniel Rothamel, Gerrit Sauer, Fabien Bornert, Peter Windisch, Dávid Hangyási, Balint Molnar, Bernhard Hesse, and Frank Witte. 2022. Materials 2022, Vol. 15, Page 3106 15(9):3106. doi: 10.3390/MA15093106.

https://pubmed.ncbi.nlm.nih.gov/35591440/

For the surgical technique of guided bone regeneration (GBR), the choice of available barrier membranes has until recently not included an option that is mechanically strong, durable, synthetic and resorbable. The most commonly used resorbable membranes are made from collagen, which are restricted in their mechanical strength. The purpose of this study is to evaluate the degradation and regeneration potential of a magnesium membrane compared to a collagen membrane. In eighteen beagle dogs, experimental bone defects were filled with bovine xenograft and covered with either a magnesium membrane or collagen membrane. The health status of the animals was regularly monitored and recorded. Following sacrifice, the hemimandibles were prepared for micro-CT (µ-CT) analysis. Complications during healing were observed in both groups, but ultimately, the regenerative outcome was similar between groups. The µ-CT parameters showed comparable results in both groups in terms of new bone formation at all four time points. In addition, the µ-CT analysis showed that the greatest degradation of the magnesium membranes occurred between 1 and 8 weeks and continued until week 16. The proportion of new bone within the defect site was similar for both treatment groups, indicating the potential for the magnesium membrane to be used as a viable alternative to collagen membranes. Overall, the new magnesium membrane is a functional and safe membrane for the treatment of defects according to the principles of GBR.

7. Biodegradation of a Magnesium Alloy Fixation Screw Used in a Guided Bone Regeneration Model in Beagle Dogs

Patrick Rider, Željka Perić Kačarević, Akiva Elad, Daniel Rothamel, Gerrit Sauer, Fabien Bornert, Peter Windisch, Dávid Hangyási, Balint Molnar, Bernhard Hesse, Michel Assad, and Frank Witte. 2022. Materials 15(12). doi: 10.3390/ma15124111.

https://pubmed.ncbi.nlm.nih.gov/35744169/

Nowadays, the most commonly used fixation systems are non-resorbable, but new resorbable magnesium alloy fixation screws have been introduced recently. Therefore, the aim of this study was to compare the magnesium fixation screw and the commonly used non-resorbable titanium screw in an animal model. Four 3-wall defect sites were covered with collagen membranes in the mandible of twenty beagle dogs (two sites on the left and two on the right). Each membrane was fixed with either four magnesium screws or four titanium screws. Post-operative follow-up revealed the expected observations such as transient inflammation and pain. Both groups showed a good healing response,

with no differences between groups. Micro-CT analysis showed no significant difference between groups in terms of BV/TV or soft tissue volume. The void volume in the magnesium fixation screw group continued to decrease on average between the different timepoints, but not significantly. Furthermore, a gradual progression of the degradation process of the magnesium screws was observed in the same group. Magnesium screws and titanium screws showed equal performance in tissue regeneration according to GBR principles. An additional advantage of magnesium screws is their resorbable nature, which eliminates the need for a second surgical step to remove the screws.

Clinical studies and case series

1. Application of Biodegradable Magnesium Membrane Shield Technique for Immediate Dentoalveolar Bone Regeneration

   Akiva Elad, Patrick Rider, Svenja Rogge, Frank Witte, Dražen Tadić, Željka Perić Kačarević, and Larissa Steigmann. 2023. Biomedicines 11(3):744. doi: 10.3390/biomedicines11030744.

   https://www.mdpi.com/2169592

   For the first time, the clinical application of the first CE registered magnesium membrane is reported. Due to the material characteristics of magnesium metal, new treatment methodologies become possible. This has led to the development of a new technique: the magnesium membrane shield technique, used to rebuild the buccal or palatal walls of compromised extraction sockets. Four clinical cases are reported, demonstrating the handling options of this new technique for providing a successful regenerative outcome. Using the technique, immediate implant placement is possible with a provisional implant in the aesthetic zone. It can also be used for rebuilding both the buccal and palatal walls simultaneously. For instances where additional mechanical support is required, the membrane can be bent into a double layer, which additionally provides a rounder edge for interfacing with the soft tissue. In all reported clinical cases, there was a good bone tissue regeneration and soft tissue healing. In some instances, the new bone had formed a thick cortical bone visible in cone beam computed tomography (CBCT) radiographs of the regenerated sites, which is known to be remodeled in the post treatment period. Overall, the magnesium membrane shield technique is presented as an alternative treatment option for compromised extraction sockets.

2. Guided Bone Regeneration Using a Novel Magnesium Membrane: A Literature Review and a Report of Two Cases in Humans

   Blašković, M.; Butorac Prpić, I.; Blašković, D.; Rider, P.; Tomas, M.; Čandrlić, S.; Botond Hangyasi, D.; Čandrlić, M.; Perić Kačarević, Ž. Guided Bone Regeneration Using a Novel Magnesium Membrane: A Literature Review and a Report of Two Cases in Humans. J. Funct. Biomater. 2023, 14, 307. https://doi.org/10.3390/jfb14060307.

https://www.mdpi.com/2323800

Guided bone regeneration (GBR) is a common procedure used to rebuild dimensional changes in the alveolar ridge that occur after extraction. In GBR, membranes are used to separate the bone defect from the underlying soft tissue. To overcome the shortcomings of commonly used membranes in GBR, a new resorbable magnesium membrane has been developed. A literature search was performed via MEDLINE, Scopus, Web of Science and PubMed in February 2023 for research on magnesium barrier membranes. Of the 78 records reviewed, 16 studies met the inclusion criteria and were analyzed. In addition, this paper reports two cases where GBR was performed using a magnesium membrane and magnesium fixation system with immediate and delayed implant placement. No adverse reactions to the biomaterials were detected, and the membrane was completely resorbed after healing. The resorbable fixation screws used in both cases held the membranes in place during bone formation and were completely resorbed. Therefore, the pure magnesium membrane and magnesium fixation screws were found to be excellent biomaterials for GBR, which supports the findings of the literature review.

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

Blašković, M.; Blašković, D.; Hangyasi, D.B.; Peloza, O.C.; Tomas, M.; Čandrlić, M.; Rider, P.; Mang, B.; Kačarević, Ž.P.; Trajkovski, B. Evaluation between Biodegradable Magnesium Metal GBR Membrane and Bovine Graft with or without Hyaluronate. Membranes 2023, 13, 691. https://doi.org/10.3390/membranes13080691

https://www.mdpi.com/2403672

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.

4. Possible Applications for a Biodegradable Magnesium Membrane in Alveolar Ridge Augmentation–Retrospective Case Report with Two Years of Follow-Up

Palkovics, D.; Rider, P.; Rogge, S.; Kačarević, Ž.P.; Windisch, P. Possible Applications for a

Biodegradable Magnesium Membrane in Alveolar Ridge Augmentation–Retrospective Case Report with Two Years of Follow-Up. Medicina 2023, 59, 1698. https://doi.org/10.3390/medicina59101698

https://www.mdpi.com/2491342

Background and Objectives: A rigid, resorbable magnesium membrane was recently developed, combining the advantages of resorbable and non-resorbable membranes. Our aim was to describe the application of this membrane for guided bone regeneration (GBR). Materials and Methods: This case report described the treatment and 3D evaluation of two cases utilizing a resorbable magnesium barrier membrane. In Case #1, GBR was performed with a bilayer tunnel flap. The magnesium barrier was placed fixed subperiosteally through remote vertical incisions. In Case #2, GBR was performed using a split-thickness flap design. Volumetric and linear hard tissue alterations were assessed by 3D cone-beam computed tomography subtraction analysis, as well as with conventional intraoral radiography. Results: Case #1 showed a volumetric hard tissue gain of 0.12 cm3, whereas Case #2 presented a 0.36 cm3 hard tissue gain. No marginal peri-implant hard tissue loss could be detected at the two-year follow-up. Conclusions: The application of conventional resorbable collagen membranes would be difficult in either of the cases presented. However, the rigid structure of the magnesium membrane allowed for the limitations of conventional resorbable membranes to be overcome.

5. Horizontal and Vertical Defect Management with a Novel Degradable Pure Magnesium Guided Bone Regeneration (GBR) Membrane—A Clinical Case

Frosecchi, M. Horizontal and Vertical Defect Management with a Novel Degradable Pure Magnesium Guided Bone Regeneration (GBR) Membrane—A Clinical Case. Medicina 2023, 59, 2009. https://doi.org/10.3390/medicina59112009

https://www.mdpi.com/2561930

Background and objectives: In guided bone regeneration (GBR), large defects comprising both horizontal and vertical components usually require additional mechanical support to stabilize the augmentation and preserve the bone volume. This additional support is usually attained by using non-resorbable materials. A recently developed magnesium membrane presents the possibility of providing mechanical support whilst being completely resorbable. The aim of this case report was to describe the application and outcome of the magnesium membrane in combination with a collagen pericardium membrane for GBR. Materials and methods: A 74 year old, in an otherwise good general health condition, was presented with stage 2 grade A periodontitis and an impacted canine. After extraction of the impacted canine, a defect was created with both vertical and horizontal components. The defect was augmented using the magnesium membrane to create a supportive arch to the underlying bone graft and a collagen pericardium membrane was placed on top to aid with the soft tissue closure. Results: Upon reentry at 8 months, complete resorption of the magnesium devices was confirmed as there were no visible remnants remaining. A successful augmentation outcome had been achieved as the magnesium membrane in combination with the collagen membrane had maintained the augmented bone well. Two dental implants could be successfully placed in the healed augmentation. Conclusions: In this case, the magnesium membrane in combination with a collagen pericardium membrane presented a potentially viable alternative treatment to titanium meshes or

titanium-reinforced membranes for the augmentation of a defect with both horizontal and vertical components that is completely resorbable. It was demonstrated that it is possible to attain a good quality and quantity of bone using a resorbable system that has been completely resorbed by the time of reentry.

6. Regeneration of Intrabony Defects Using a Novel Magnesium Membrane

Hangyasi, D.B.; Körtvélyessy, G.; Blašković, M.; Rider, P.; Rogge, S.; Siber, S.; Kačarević, Ž.P.; Čandrlić, M. Regeneration of Intrabony Defects Using a Novel Magnesium Membrane. Medicina 2023, 59, 2018. https://doi.org/10.3390/medicina59112018

https://www.mdpi.com/2563134

Background and Objectives: Due to their specific morphology, the regeneration of intrabony defects (IBDs) represents one of the greatest challenges for clinicians. Based on the specific properties of a magnesium membrane, a new approach for the surgical treatment of IBD was developed. The surgical procedure was described using a series of three cases. Materials and Methods: The patients were healthy individuals suffering from a severe form of periodontitis associated with IBD. Based on radiographic examination, the patients had interproximal bone loss of at least 4 mm. Due to its good mechanical properties, it was easy to cut and shape the magnesium membrane into three different shapes to treat the specific morphology of each IBD. In accordance with the principles of guided bone regeneration, a bovine xenograft was used to fill the IBD in all cases. Results: After a healing period of 4 to 6 months, successful bone regeneration was confirmed using radiological analysis. The periodontal probing depth (PPD) after healing showed a reduction of 1.66 ± 0.29 mm. Conclusions: Overall, the use of the different shapes of the magnesium membrane in the treatment of IBD resulted in a satisfactory functional and esthetic outcome.

7. Resorbable magnesium metal membrane for sinus lift procedures: a case series

Elad, A., Pul, L., Rider, P. et al. Resorbable magnesium metal membrane for sinus lift procedures: a case series. BMC Oral Health 23, 1006 (2023). https://doi.org/10.1186/s12903-023-03695-4

https://pubmed.ncbi.nlm.nih.gov/38097992/

Background: The purpose of this case series was to demonstrate the use of a magnesium membrane for repairing the perforated membrane in both direct and indirect approaches, as well as its application in instances where there has been a tear of the Schneiderian membrane. Case presentation: The case series included four individual cases, each demonstrating the application of a magnesium membrane followed by bone augmentation using a mixture of xenograft and allograft material in the sinus cavity. In the first three cases, rupture of Schneiderian membrane occurred as a result of tooth extraction, positioning of the dental implant, or as a complication during the procedure. In the fourth case, Schneiderian membrane was perforated as a result of the need to aspirate a polyp in the maxillary sinus. In case one, 10 mm of newly formed bone is visible four months after graft placement. Other cases showed between 15 and 20 mm of newly formed alveolar bone. No residual magnesium membrane was seen on clinical inspection. The vertical and horizontal augmentations proved stable and the dental implants were placed in the previously grafted sites. Conclusion: Within the limitations

of this case series, postoperative clinical examination, and panoramic and CBCT images demonstrated that resorbable magnesium membrane is a viable material for sinus lift and Schneiderian membrane repair. The case series showed successful healing and formation of new alveolar bone with separation of the oral cavity and maxillary sinus in four patients.