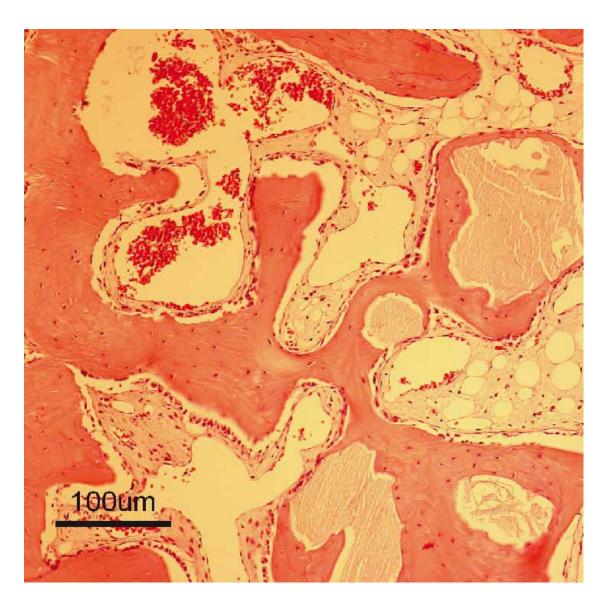
Experimental studies



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REGENERATION SCIENCE





EXPERIMENTAL STUDIES

019

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ORIGINAL ARTICLE Clinical Implant Dentistry and Related Research 2008 Dec;10(4):264-70

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ABSTRACT

Bone substitutes of xenogeneic origin are frequently used as grafting materials for filling bone defects and maxillary sinus floor augmentation procedures. To be effective, bone substitutes must have osteoconductive properties and be completely replaced with new bone with time. In order to improve the clinical handling, it is possible to add collagen gel to prehydrated and collagenated porcine bone (PCPB) particles, with the result of a sticky and moldable material which facilitates its application in the site to be filled.

As the possible influence of the gel on the bone tissue response is not known, the objective of the study was to histologically evaluate the bone tissue responses to PCPB graft with or without collagen gel and to evaluate the resorption/degradation properties of the biomaterials.

For these study, bilateral bone defects (dimensions: 5x8x3 mm) were created in the maxilla of 14 rabbits. The defects were filled with prehydrated and collagenated cortico-cancellous porcine bone (PCPB) particles (OsteoBiol® *Gen-Os*®, Tecnoss®, Giaveno, Italy - granulometry: $250-1000 \,\mu$ m) as control material, or PCPB particles mixed with collagen gel (OsteoBiol® $mp3^{\$}$, Tecnoss®, granulometry: $600-1000 \,\mu$ m) as test material. A collagen membrane (OsteoBiol® *Evolution*, Tecnoss®) was used to cover the defect and to prevent migration of the particles and the wounds were closed with resorbable sutures. Animals were killed after 2 (n=3), 4 (n=3), and 8 weeks (n=8) for histological and morphometrical evaluations.

According to the results of these evaluations, there was no obvious difference between the test and control materials. There were no signs of adverse reactions, and both osteogenesis and angiogenesis followed ordinary time frames. Both materials showed bone formation directly on the particles by typical osteoblastic seams. The bone area increased with time (2-8 weeks) for both sides, from 16,2% (control) and 19,2% (test) to 42,7 and 43,8%, respectively. The PCPB, whether mixed with collagen gel or not, was resorbed by osteoclasts as well as part of remodeling with the formation of osteons within the particles. Morphometry showed a decrease of PCPB area from 19,4% (control) and 23,8% (test) after 2 weeks to 3,7 and 9,3% after 8 weeks, respectively. The histology showed that the membrane had fulfilled its function and was well integrated with the overlaying soft tissues.

CONCLUSIONS

From the findings of this study, it is possible to conclude that mixing collagen gel and PCPB to facilitate the clinical handling does not influence the bone tissue responses to the material, which exhibited osteoconductive properties and was resorbed with time. Both graft materials exhibited osteoconductive properties as bone formation with typical osteoblastic seams observed directly on the surface of the grafted particles. The morphometric measurements showed increased bone area with time in parallel with a decrease of the graft area. The Authors concluded that "collagenated porcine bone exhibits good biocompatibility and osteoconductive properties, whether mixed with collagen gel or not. In this model, the material was resorbed by surface osteoclasts as well as part of remodeling with the formation of osteons".



Experimental evaluation of the effects of ankaferd blood stopper and collagenated heterologous bone graft on bone healing in sinus floor augmentation

ABSTRACT

In case of missing teeth, the use of dental implants is generally more difficult in the edentulous maxilla than in the edentulous mandible because of various complicating factors, as limited bone volume due to maxillary sinus pneumatization and alveolar resorption after tooth loss and poor bone quality. Maxillary sinus augmentation has frequently been proposed as the best option for attaining sufficient bone height and volume for implant placement in the posterior maxilla and for this procedure several grafting materials have been used for augmentation. The aim of this study was to evaluate the effect of collagenated heterologous bone graft (CHBG) and Ankaferd Blood Stopper (ABS), a plant extract, on bone healing after sinus floor augmentation. To the authors' knowledge, this is the first study of the effects of ABS on bone healing in sinus augmentation procedures. In 36 New Zealand rabbits 72 bone defects were created and bilateral sinus augmentation was performed. The maxillary sinuses were grafted with four different biomaterials: blood clot (control group), CHBG (OsteoBiol® Apatos Mix, Tecnoss[®], Giaveno, Italy) (graft group), ABS (ABS group), and ABS + CHBG (ABS + graft group). Material selection was done according to the blocked randomization method. Equal doses of graft materials were used, and mixed homogenously and the bone windows were covered with resorbable collagen membrane (OsteoBiol[®] Evolution, Tecnoss[®]). Twelve rabbits each were sacrificed at 1, 4, and 8 weeks after surgery and on all samples histochemical and immunohistochemical examinations were performed, showing that all the materials used in this study were biocompatible and did not elicit any foreign-body reaction. New bone formation started at the fourth week adjacent to the cortical bone walls, and by the eighth week it was seen in the center of the cavity in all groups. At the fourth week, new bone formation was greater in the ABS and ABS+graft groups than in the other groups. There were osteoclasts around the bone graft materials, but degeneration of the graft was seen only in the ABS+graft group at 4 and 8 weeks.

CONCLUSIONS

In bone regeneration procedure, collagenated heterologous bone graft (CHBG) has been used, thanks to its osteoconductive properties. CHBG proved to integrate well at host sites. In this study, this bone substitute was used alone and in combination with ABS. When used alone, there was no bone formation at the first week, but it increased gradually 1 to 8 weeks. Osteoclast numbers were high at the first week and declined thereafter. When used in combination with ABS, the bone formation rate was similar. Osteoblast density increased 1 to 8 weeks and osteoclast numbers were high in the first week and declined to 8 weeks.

From the results it is evident that in the ABS and the ABS+graft groups, new bone formation was rapid from 1 to 4 weeks, but by the end of the eighth week, new bone formation was similar in all groups. In all groups, new bone formation was increased from 1 to 8 weeks. According to these results, the Authors concluded that ABS may accelerate bone healing.

EXPERIMENTAL STUDIES

094

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ORIGINAL ARTICLE Int J Oral Maxillofacial Implants 2015 Mar-Apr;30(2):279-85

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EXPERIMENTAL STUDIES

098

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ORIGINAL ARTICLE Hindawi Publishing Corporation BioMed Research International 2016-2016-4086870

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Bone regeneration in iliac crestal defects: an experimental study on sheep

ABSTRACT

Successful implant placement requires adequate alveolar ridge dimensions and, if the implant site presents a lack of bone, Guided Bone Regeneration (GBR) is the surgical procedure commonly performed in order to provide an augmentation in terms of volume for the insertion of dental implants. Several types of membranes and biomaterials have been proposed for GBR techniques and the selection of the most appropriate grafting material is one of the key factors in achieving adequate bone formation.

The aim of the present study was to determine the in vivo tissue responses and gap healing patterns around dental implants treated with cortico-cancellous porcine bone blocks, collagenated cortico-cancellous porcine bone versus only membrane in a standardized sheep peri-implant gap-defect model. In the iliac crest of six sheep 4 defects were created for the insertion of an implant and the defects were filled with 1) control, only membrane (OsteoBiol[®] Evolution, Tecnoss[®], Giaveno, Italy); 2) 250–1000 µm cortico-cancellous particulate porcine bone mix (OsteoBiol® Gen-Os®, Tecnoss[®]) + resorbable equine pericardium membrane (OsteoBiol[®] Evolution) (test 1); 3) cancellous equine bone blocks (OsteoBiol® Sp-Block, Tecnoss[®]) + resorbable membrane (OsteoBiol[®] Evolution) (test 2); 4) pre-hydrated collagenated cortico-cancellous porcine bone mix (90% granulated mix, 10% collagen gel) (OsteoBiol[®] mp3[®], Tecnoss[®]) + membrane (OsteoBiol® Evolution) (test 3). The animals were sacrificed after a 4-month healing period and all specimens were processed and analyzed with histomorphometry, with the result that all experimental groups showed an increase of new bone. From the findings it is evident that particles of cortico-cancellous porcine bone 250–1000 μ m particulate mix (CCPB) favour bone formation with a result similar to those obtained with pre-hydrated collagenated cortico-cancellous porcine bone mix (PCCPB). All biomaterials used in the present study were characterized by the presence of bone formation and absence of inflammatory cell infiltrates. However, the defect treated by membrane alone was characterized by the presence of soft tissues and a little immature bone.

CONCLUSIONS

As stated by the Authors, "the function of the graft is not only to improve the space-making capabilities of the membrane, but also to provide additional points on which osteoblasts can start forming new bone. We have shown that CCPB and PCCPB promote bone regeneration in large defects (7 mm wide and 4 mm deep) around dental implants".

In conclusion, this study demonstrates that particulate porcine bone mix and porcine cortico-cancellous collagenated pre-hydrated bone mix, used as scaffolds, induce bone regeneration and these findings suggest that these biomaterials are characterized by a high biocompatibility and can induce a faster and greater bone formation.



Influence of a collagen membrane positioned subjacent the sinus mucosa following the elevation of the maxillary sinus. A histomorphometric study in rabbits

ABSTRACT

In order to allow implant placement in the posterior maxillary regions, it is necessary to increase bone volume by means of sinus floor elevation. This procedure is widely applied and various biomaterials have been recommended to fill the elevated space. In case of a perforation of the sinus mucosa, it has been suggested to apply resorbable collagen membranes to protect the perforation. In order to have further information about the role of a collagen membrane placed subjacent the sinus mucosa, this study aimed to evaluate the healing after elevation of the sinus mucosa when a collagen membrane was placed between the sinus mucosa and a xenoaraft used as filler. In this study, 18 rabbits were used. Sinus mucosa elevation was performed bilaterally. After elevation of the sinus mucosa, a small piece of equine collagen membrane (OsteoBiol[®] Evolution, Tecnoss[®], Giaveno, Italy) was placed subjacent the sinus mucosa at one site (test site), while no membranes were placed within the sinus at the control sites. At both sites, a collagenated cortico-cancellous porcine bone (OsteoBiol[®] Gen-Os[®], Tecnoss[®]) was placed within the elevated space. The subsequent analysis showed that the elevated area was reduced between 2 and 8 weeks of healing by about 25% at the test and 47% at the control sites. After 8 weeks of healing, the mineralized new bone within the elevated space was $18.2\pm5.5\%$ at the test and $26.7\pm7.7\%$ at the control sites. Within the available space at the test site, the percentage was $27.3\pm7.0\%$ after 8 weeks of healing. At 2 and 8 weeks of healing, within the elevated space, the xenograft proportion was $30.9 \pm 4.4\%$ and $6.9 \pm 2.8\%$ at the test, and $35.2\pm7.3\%$ and $9.6\pm4.9\%$ at the control sites, respectively. When the marrow spaces were counted together with the mineralized bone, the total bone formed within the available space after 8 weeks was 46.71% and 55.14% at the test and control sites, respectively.

CONCLUSIONS

From the results of the present study, new bone appeared to form from the native bone of the sinus walls and then propagated toward the middle and the submucosa regions. The collagen membrane contributed to maintain the available area, but the morphometric analyses of the healing in the elevated region after sinus membrane elevation were very similar when an internal collagenous membrane was placed as without the membrane placement. Likewise, the healing process in the elevated region appeared to be largely unaffected by the application of an internal collagenous membrane.



EXPERIMENTAL STUDIES

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ORIGINAL ARTICLE Clinical Oral Implants Research 2017 Dec;28(12):1567-1576

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EXPERIMENTAL STUDIES

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> ORIGINAL ARTICLE Clinical Oral Implants Research 2018 Aug;29(8):821-834

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Reposition of the bone plate over the antrostomy in maxillary sinus augmentation: a histomorphometric study in rabbits

ABSTRACT

After sinus floor elevation, it is common to use membranes in order to cover the lateral access window and this approach showed better results than leaving the antrostomy uncovered. In literature different results have been reported following the two approaches and so the Authors of the present study evidenced the need of further data to describe the influence on healing of the closure of the bone window on the lateral antrostomy and on the integration of the bone window plate to the adjacent bone when ethyl-2-cyanoacrylate is used as fixative. Therefore, the aim of this experimental study was to test if the repositioning of the bony plate secured with a cyanoacrylate (test site) over the antrostomy in maxillary sinus augmentation was superior to the coverage of the antrostomy with a collagen membrane (control site) in terms of bone augmentation area and bone density. Moreover, the Authors assess tissue composition and healing processes 2, 4 and 8 weeks after sinus mucosa elevation within the elevated area and in the antrostomy. Eighteen male New Zealand white rabbits were selected and divided in three groups of different periods of healing, i.e., 2, 4, and 8 weeks, of six animals each. After the exposure of the nasal bone, a rectangular access window was prepared, removing the bony plate. A bilateral sinus mucosa elevation was performed, and the space filled with a collagenated cortico-cancellous porcine bone (OsteoBiol® Gen-Os®; Tecnoss®, Giaveno, Italy). At the test site, the bone plate was repositioned and secured to the walls of the antrostomy with drops of ethyl-2-cyanoacrylate adhesive. At the contra-lateral control sites, an equine collagen membrane (OsteoBiol[®] Evolution, Tecnoss[®]) was used to cover the antrostomy. Per group, 6 animals were sacrificed after 2, 4, and 8 weeks of healing, respectively. The histological evaluation showed that the augmented area after elevation decreased between 2 and 8 weeks from 9.4 ± 1.8 to 4.8 ± 2.8 mm² at the test and from 9.5 ± 2.6 and 5.1 ± 1.6 mm² at the control sites. Small amounts of new bone were seen after 2 weeks in both groups forming from the bony sinus walls and the area of the remaining defects decreased over time at both test and control sites. New bone density increased over time in both groups, with no statistically significant differences. Small residual defects were present both at the test sites in the margin of the bone plate, and at the control sites in the center of the antrostomy.

CONCLUSIONS

The bone healing in the elevated sinus space was similar irrespective of the coverage of the antrostomy. Even if the inference of the results from the present animal study to similar clinical situations in humans has to be considered with care, the Authors concluded that "the protection of the antrostomy by either repositioning the bony plate or covering the window with a collagen membrane resulted in similar outcomes in terms of new bone formation and xenograft resorption inside the available area. After 8 weeks, the bony plate was well incorporated into the subjacent new bone, while at the control sites, the healing was still incomplete. Residual defects were present in both groups".

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Histological and micro-computed tomography evaluations of newly formed bone after maxillary sinus augmentation using a xenograft with similar density and mineral content of bone: an experimental study in rabbits

ABSTRACT

It has been demonstrated that new bone forms after sinus floor elevation, but the tendency of the maxillary sinus to regain the lost space after sinus floor elevation has been documented as well. To counteract the physiological shrinkage of the elevated space, the use of bone fillers has been suggested.

The aim of the present study was to evaluate possible differences in the assessment of bone formation between histological and micro-computed tomography (CT) analyses in maxillary sinuses augmented with a xenograft with similar density and mineral content of bone. Eighteen male New Zealand white rabbits were randomly divided into three groups. After the sinus mucosa elevation, in the test sites an equine collagen membrane (OsteoBiol® Evolution 0.3 mm, Tecnoss®, Giaveno, Italy) was placed subjacent the sinus mucosa and both sinuses were subsequently filled with similar amounts of collagenated cortico-cancellous porcine bone (OsteoBiol[®] Gen-Os[®], Tecnoss[®]; 250–1,000 μm). Six rabbits per group were sacrificed after 2, 4, and 8 weeks of healing. Biopsies were retrieved, scanned in a high-resolution micro-CT, and subsequently subjected to histological assessments. The histological analyses showed that bone increased over time, from 7.5 \pm 2.4% to 27.0 \pm 5.3%, between 2 and 8 weeks of healing. After 2 weeks, higher content of xenograft was found at the histological compared with the micro-CT analyses, especially in the middle regions of the sinus. After 8 weeks of healing, higher percentages of bone were found at the histological compared with the micro-CT analyses, being the differences statistically significant.

CONCLUSIONS

Within the limitation of this study, the Authors concluded that "the outcomes of a micro-CT analysis performed in an early phase of healing may be altered when a resorbable bone substitute with similar density and mineral content of bone is applied".

EXPERIMENTAL STUDIES

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ORIGINAL ARTICLE Clinical and Experimental Dental Research 2018;1–7

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