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Guided bone regeneration using collagen membranes for sinus augmentation

Xiang Li, Song-ling Chen *, Shuang-xi Zhu, Guo-qing Zha

Department of Oral and Maxillofacial Surgery, The First Affiliated Hospital, Sun Yat-sen University, 58 Zhongshan Road 2, Guangzhou 510080, People's Republic of China

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Abstract

We investigated the effect of guided bony regeneration using collagen membranes for sinus augmentation in the first maxillary molars of 18 adult female beagle dogs. The teeth were extracted bilaterally and the sinus floors were lifted with simultaneous implantation. The grafted material composed of a combination of autografts and Bio-oss™ in a 2:1 ratio. On the experimental side in each dog, collagen membrane was folded at the lateral osteotomy window, at the apex of the implants, and at a certain part of the palatal bone. On the opposite (control) side, the collagen membrane covered the osteotomy window. Six animals were killed at each of 4, 12, and 24 weeks postoperatively. Gross observation, biomechanical testing, and histological examinations were made. On the experimental side, grafted materials showed no obvious resorption or subsidence, and a new bone had formed at the apex of the implants. On the control side, the grafted materials had been shifted and absorbed. Histological examination showed increased formation of a new bone in the experimental group, which matured over time. At 4 weeks, inflammatory cells were present in the control group, which showed less maturation of the new bone. The pull-out force increased with time and, at week 24, there was a significant difference in pull-out force between the two groups ($p < 0.01$). Guided bony regeneration with the enfolded coverage of membrane can effectively reduce resorption of grafted bone on the apical surface of implants and stimulate formation of the new bone in sinus augmentation.

Keywords: Sinus augmentation; Guided bony regeneration; Implant

Introduction

Dental implants have been successfully used in both completely and partially edentulous patients.[1,2] After loss of teeth in the posterior maxilla, insufficient bone height, which is caused by atrophy of bone and pneumatised maxillary sinuses, makes implantation difficult.

Augmentation of the maxillary sinus is one way of attaining sufficient bony height for placement of posterior maxillary implants, and has been highly successful.[3] Since first introduced by Boyne and James,[4] several grafting materials have been used to augment the antral space, including autografts, allogeneic bones, xenografts, or a combination.[5–7] Bio-oss™ is a deproteinised anorganic bovine bone mineral that can be used in sinus augmentation or for widening the alveolar crest, with or without added autografts.[8] When Bio-oss™ is used alone, slow resorption is a shortcoming.[9] Studies have shown that the combination of autografts and non-autografts gave better results in sinus augmentation than the only one kind of material for bone grafts. Some studies, however, have reported problems associated with these materials including absorption of material, no regeneration of bone on top of implants, and migration of grafting materials into the Schneiderian membrane.[10–12] However, no methods have been reported that prevent these materials from being absorbed.

* Corresponding author. Tel.: +86 020 87333122; fax: +86 020 87333122.
E-mail address: chensongling@hotmail.com (S.-l. Chen).

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Guided bony regeneration is well-established in increasing horizontal augmentation, but clinical experience with vertical guided bony regeneration is limited.[13] Traditionally in sinus augmentation a collagen membrane is positioned on the buccal mucosa to cover the antrostomy window to prevent fibrous tissue of the buccal mucosa from growing into bone. In this traditional technique the sinus membrane is in direct contact with grafting material.

To date, to our knowledge, no study has been done to find out if it is necessary to separate the sinus membrane from the grafting material, and whether their separation could reduce the absorption of the grafting material. We have therefore investigated the effect of guided bony regeneration using an absorbable collagen membrane to separate the sinus membrane from the graft material.

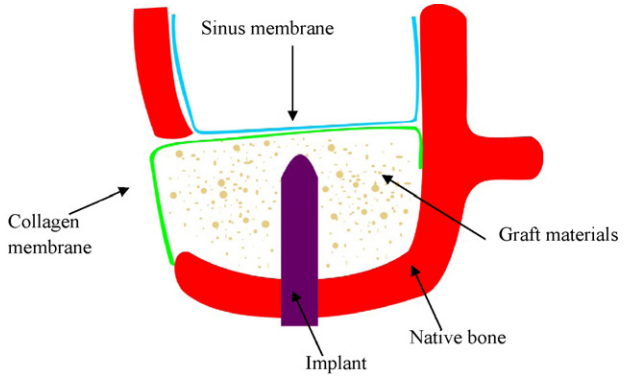

Fig. 1. On the experimental side, collagen membrane is folded at the area of the lateral osteotomy window, the apex of implants, and a certain portion of the palatal bone.

Materials and methods

Experimental model

Eighteen adult female beagle dogs (15–20 kg body weight) were used. The protocol was approved by the Animal Care and Use Committee, Sunyat-Sen University Medical Center, Guangzhou, PRC. All operations were done under general anaesthesia using ketamine 5 mg/kg and xylazine 2 mg/kg given intramuscularly.

After the first maxillary molars of each dog had been extracted bilaterally, and the alveolar ridge had been trimmed to a height of 4–5 mm, the edentulous region was opened by a buccal incision. The mucoperiosteal flap was reflected on the buccal cortical plate from the second maxillary premolar to the second maxillary molar. A round bur was used to remove the lateral bony wall. After the sinus membrane had been raised to 5–6 mm, the implants were placed bilaterally in the maxillary sinus. The cavity was then filled with a combination of autografts and Bio-oss^{TM} in a 2:1 ratio. The right side was used as the control, and was covered in the traditional way in which the Bio-gide^{TM} (Geistlich, Wolhusen, Switzerland) collagen membrane was placed on the lateral walls. In the experimental group, the left side was folded by the collagen membrane (Bio-gide^{TM} collagen membrane was folded at the areas of the lateral osteotomy window, the apex of implants, and a certain portion of the palatal bone) (Fig. 1). Buccal flaps were sutured. The animals were given penicillin 10 μg twice daily for 7 successive days postoperatively.

Six animals each were killed at each of 4, 12, and 24 weeks postoperatively. Six sinuses from each implant were available for the evaluation at each time period and in each treatment group.

Gross observations

The shape of the alveolar ridge, the integrity of the sinus membrane, and the amount of regeneration of new bone at the top of implants were calculated.

Biomechanical testing

Six tissue blocks from each implant were evaluated at 4, 12, and 24 weeks for both experimental and control groups. The block specimens selected for mechanical testing were stored in 10% formalin solution for 24 h. The excess bone was then removed to make the tissue block cylindrical (20 mm × 30 mm). The cover screw was subsequently fastened tightly into the implant. The block specimen was fixed vertically on the base of the testing machine (MTS, 858, US) with the cover screw clamped, ensuring that the direction of pull-out corresponded to the axis of the implant. Measurements of pull-out strength were made using a testing range of 0–1000 N at a speed of 0.4 mm/min until the bone/implant bond failed. The maximum pull-out force for each implant was calculated using stress–strain diagrams.

Histological examination

The specimens of bone were fixed in 10% formalin solution, and decalcified with 10% EDTA for 48 h at 4 °C. They were then dehydrated in increasing concentrations of ethanol, embedded in paraffin, and sliced into sections roughly 4 μm thick. They were stained with haematoxylin and eosin and examined under a light microscope.

Statistical analysis

The significances of differences between the two groups at the specified observation points were measured using Student's *t* test. Probabilities of less than 0.05 were accepted as significant. Analyses were made with the assistance of the Statistical Package for the Social Sciences (SPSS version 10.0, Cary, NC, USA). All data are expressed as mean (SD).

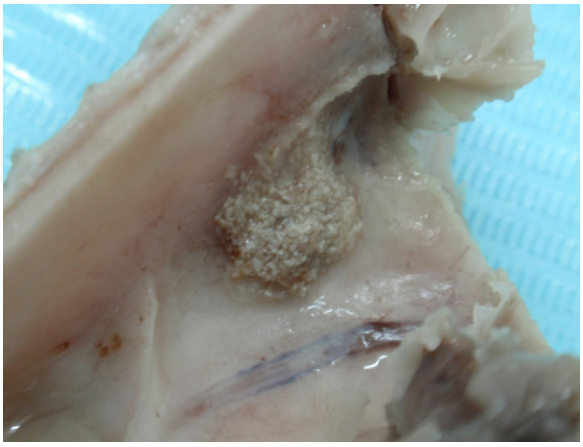

Fig. 2. The grafted material converges on the implant in the experimental group at 4 weeks.

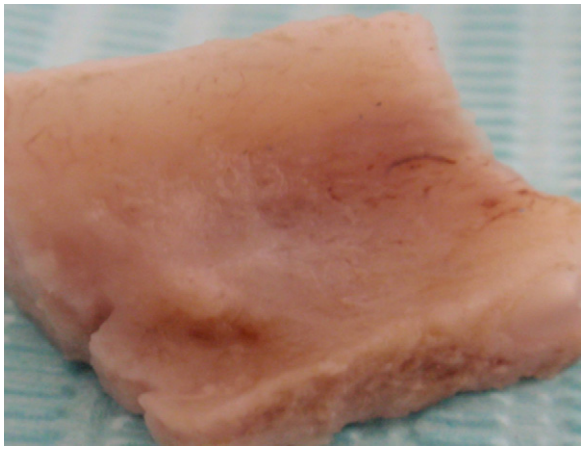

Fig. 4. The implant is covered by thick newly formed bone in the experimental group at 24 weeks.

Results

Postoperative healing was uneventful in all cases, and there were no signs of wound infection in any maxillary sinus cavity before the animals were killed. The alveolar bones looked well-developed, all implants were stable with no crestal resorption of bone, and membranes looked intact.

About 4 weeks after implantation, the grafting material in the experimental group was concentrated at the top of implants with no obvious resorption or subsidence, and with visible particles enfolded by the collagen membrane (Fig. 2). In the control group, on the other hand, there was a clear resorption and subsidence of grafting material, and the tops of the implants could just be seen (Fig. 3).

Twelve weeks after implantation, in the experimental group, grafted bone particles were no longer apparent, and there was a newly formed bone adjacent to the cortical bony wall of the cavity and appearing to surround the top of the implant. In contrast, the newly formed bone in the control group was relatively decentralised, and there was some resorption at the top which was covered by thin bone.

Twenty-four weeks after implantation there was no difference between the newly formed bone and cortical bone in the experimental group. The newly formed bone still surrounded the top, which was covered by thick newly formed bone, and the implant was not exposed (Fig. 4). In contrast, the top of the implant in the control group was exposed with no covering of bone, and the resorption fossa was distinctly visible (Fig. 5).

Biomechanical measurements

There were variable increases in pull-out forces in the two groups over the entire examination period. In both groups, 12-week and 24-week values were significantly greater than those obtained at 4 weeks ($p < 0.01$). At weeks 4 and 12 there were no significant differences in pull-out force between the experimental and control groups. However, at week 24, there

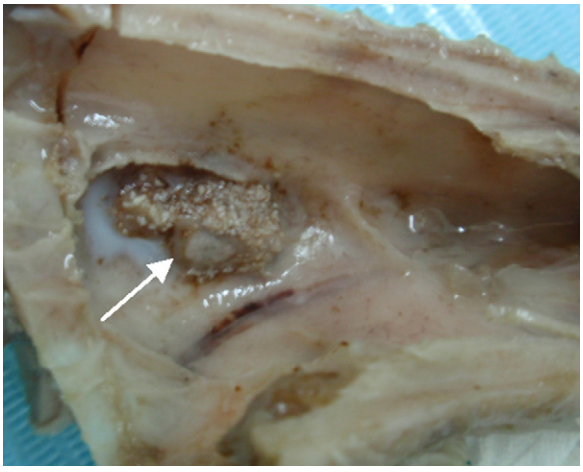

Fig. 3. The grafted material is shifted and absorbed on the control side at 4 weeks.

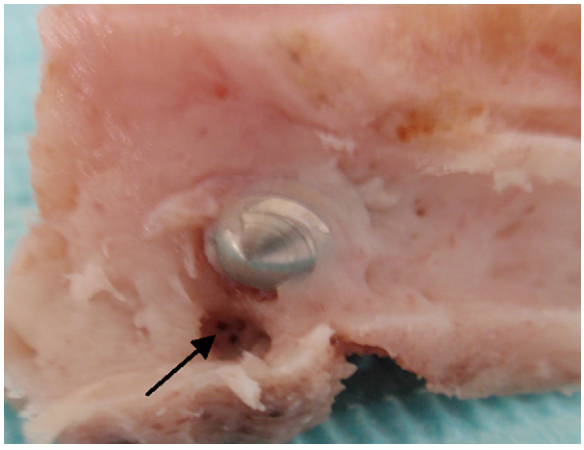

Fig. 5. In the control group, the top of the implant is exposed with no covering of bone and the resorption fossa is distinctly visible at 24 weeks.

Table 1
Mean (SD) pull-out force of implants ($n = 6$).

Time (weeks)	Study groupMean (SD)	Control groupMean (SD)
4	183.7 (28.3)	176.6 (29.4)
12	265.3 (30.6)	253.9 (34.1)
24	558.1 (37.4)*	471.8 (31.5)

* $p < 0.01$ compared with control group at week 24.

was a significant difference between the two groups ($p < 0.01$, Table 1).

Histological examination

Four weeks after implantation in the experimental group, the Bio-oss™ particles were surrounded by numerous fibroblastic cells and fibrous connective tissue. There was a newly formed bone, and numerous osteoblasts lined the Bio-oss™ particles. Bone matrix and capillaries were also present. In one of the control groups, however, many inflammatory cells were observed under the membrane, and some newly formed bones were also found.

Twelve weeks after implantation, a newly formed bone was evident around grafted particles and the number of blood vessels was reduced in the experimental group. The established osseous tissue predominantly surrounded grafted particles of biomaterial and followed their configuration.

Twenty-four weeks after implantation, there were significantly fewer Bio-oss™ particles in the experimental group. The amount of lamellar bone had increased, and fibrous connective tissue was still present. In the control group, however, graft material was more prominent than in the experimental group.

Discussion

Long-term stability of sinus-graft height is important in ensuring the success of the implant.[14] The key to the success of any bone graft is the degree of revascularisation.[15] The sinus membrane cannot directly revascularise the grafting material, but an intact sinus membrane may assist with stability, revascularisation, maturity, and eventual calcification of grafting material.[16]

Although Srouji et al.'s study proved that the sinus membrane had the potential to regenerate bone both in vivo and in vitro,[17] we know of no clinical investigations or animal studies that have shown that the membrane can stimulate bony regeneration. On the contrary, in a study of sinus augmentation in monkeys, Hürzeler et al. reported that new bone mainly existed on the buccal and palatal side of the implants, and no new bone was formed on the apical surface.[18] Hatano et al. also showed gradually increased resorption of grafting material at the top of implants during the observation period.[15] In addition, in an animal study by Miyajima, the volume of the autogenous bone had decreased by about half by 6 months after implantation.[12] Our animal study showed that there was no bone available to cover the top of implants 6 months later in the control group because of obvious resorption of grafting material at the apical surface.

In our experimental group the membrane was insulated from the grafting material. Their resorption was slow, and a new bone developed well on the apical surface of the implants, so we have shown that the sinus membrane cannot effectively stimulate formation of new bone in sinus augmentation.

The reason for resorption of bone at the apical surface of implants is not clear. Hatano et al. held that after implantation the air pressure within the sinus induced collapse of the sinus membrane and bone resorption, which led to loss of vertical bone height.[15] Given that the apical surface of implants has no bone to support because sinus membrane is some distance from the bony wall, grafting material may become dislocated as a result of gravity, air pressure, or movement of the head, which was shown in the present study. Dislocated bone particles may also initiate local inflammation and subsequent resorption of the bone graft.[19] Hürzeler et al. also found that grafting material had a tendency to penetrate the membrane,[18] so the displacement of the grafting material may play a part in resorption of the bone graft at the top of implants after sinus augmentation, although air pressure does seem to be the main factor involved.

In sinus augmentation an absorbable collagen membrane is used on the surface of the buccal mucosa to insulate grafting material from soft tissue. Choi et al. showed that when barrier membrane covered the antrostomy window, the grafted sinus wall was smoother and more new bones were formed. In contrast, without membrane to cover the grafted antrostomy, the residual particles seemed coarser and occasionally protruded out of the sinus wall.[20] The Loma Linda pouch technique, which is used to repair a perforated sinus membrane, completely isolates graft material from the blood supply in the sinus walls.[21] Although it effectively reduces movement or shifting of the membrane, it slows down the remodelling process of graft material.[22]

In the present study membrane was used to cover the buccal mucosa, the newly formed sinus floor, and some portion of the palatal bone. Our results showed that graft material was more concentrated on the apical surface of implants in the experimental group with slower resorption and with no obvious movement. In addition more new bones covered the implant and, after a prolonged healing period, the strength of the bone-implant bond was much stronger and the pull-out force of implants was clearly more than that in the control group. In contrast, in the control, or traditionally covered membrane, group resorption and movement of graft material on the apical surface of the implant were seen with eventual loss of bony coverage.

As the operation in our study immediately followed extraction of teeth, rather than the more usually encountered clinical conditions where a tooth has been missing for a considerable time, the circumstances are not exactly the same as those normally encountered. However, the residual crestal bone

height was trimmed to 5 mm, which has been recommended to achieve sufficient initial implant stability for augmentation of the sinus floor and simultaneous placement of implants.[23] The lifting height, the intactness of the sinus membrane, the position of the window, and the initial stability of the implants were all similar to those found in clinical trials.

References

1. Lindquist LW, Carlsson GE, Jemt T. A prospective 15-year follow-up study of mandibular fixed prosthesis supported by osseointegrated implants. Clinical results and marginal bone loss. *Clin Oral Implants Res* 1996;**7**:329–36.
2. Adell R, Lekholm U, Rockler B, Brånemark PI. A 15-year study of osseointegrated implants in the treatment of the edentulous jaw. *Int J Oral Surg* 1981;**10**:387–416.
3. Chanavaz M. Maxillary sinus: anatomy, physiology, surgery and bone grafting related to implantology—eleven years of surgical experience (1979–1990). *J Oral Implantol* 1990;**16**:199–209.
4. Boyne PJ, James RA. Grafting of the maxillary sinus floor with autogenous marrow and bone. *J Oral Surg* 1980;**38**:613–6.
5. Block MS, Kent JN. Sinus augmentation for dental implants: the use of autogenous bone. *J Oral Maxillofac Surg* 1997;**55**:1281–6.
6. Rabie AB, Lie Ken Jie RK. Integration of endochondral bone grafts in the presence of demineralized bone matrix. *Int J Oral Maxillofac Surg* 1996;**25**:311–8.
7. Zaffe D, Leghissa GC, Pradlliet J, Botticelli AR. Histological study on sinus lift grafting by Fisiograft and Bio-oss. *J Mater Sci Mater Med* 2005;**16**:789–93.
8. Esposito M, Grusovin MG, Coulthard P, Worthington HV. The efficacy of various bone augmentation procedures for dental implants: a Cochrane systematic review of randomized controlled clinical trials. *Int J Oral Maxillofac Implants* 2006;**21**:696–710.
9. Wenz B, Oesch B, Horst M. Analysis of the risk of transmitting bovine spongiform encephalopathy through bone grafts derived from bovine bone. *Biomaterials* 2001;**12**:1599–606.
10. Hürzeler MB, Quìñones CR, Kirsch A, Schüpbach P, Krausse A, Strub JR, et al. Maxillary sinus augmentation using different grafting materials and dental implants in monkeys. Part III. Evaluation of autogenous bone combined with porous hydroxyapatite. *Clin Oral Implants Res* 1997;**8**:401–11.
11. Kahnberg KE, Ekestubbe A, Grondahl K, Nilsson P, Hirsch JM. Sinus lifting procedure. I. One-stage surgery with bone transplant and implants. *Clin Oral Implants Res* 2001;**12**:479–87.
12. Miyajima H. Experimental study on the healing processes after the immediate reconstruction of maxillary bone defect—fresh autogenous iliac bone graft. *Ou Daigaku Shigakushi* 1990;**17**(2):168–82 (in Japanese).
13. Fiorellini JP, Nevins ML. Localized ridge augmentation/preservation. A systematic review. *Ann Periodontol* 2003;**8**:321–7.
14. Peleg M, Garg AK, Mazor Z. Healing in smokers versus nonsmokers: survival rates for sinus floor augmentation with simultaneous implant placement. *Int J Oral Maxillofac Implants* 2006;**21**:551–9.
15. Hatano N, Shimizu Y, Ooya K. A clinical long-term radiographic evaluation of graft height changes after maxillary sinus floor augmentation with a 2:1 autogenous bone/xenograft mixture and simultaneous placement of dental implants. *Clin Oral Implants Res* 2004;**15**:339–45.
16. Artzi Z, Weinreb M, Carmeli G, Lev Dor R, Dard M, Nemcovsky CE. Histomorphometric assessment of bone formation in sinus augmentation utilizing a combination of autogenous and hydroxyapatite/biphasic tricalcium phosphate graft materials: at 6 and 9 months in humans. *Clin Oral Implants Res* 2008;**19**:686–92.
17. Srouji S, Kizhner T, Ben David D, Riminucci M, Bianco P, Livne E. The Schneiderian membrane contains osteoprogenitor cells: in vivo and in vitro study. *Calcif Tissue Int* 2009;**84**:138–45.
18. Hürzeler MB, Quiñones CR, Kirsch A, Gloker C, Schüpbach P, Strub JR, et al. Maxillary sinus augmentation using different grafting materials and dental implants in monkeys. Part I. Evaluation of anorganic bovine-derived bone matrix. *Clin Oral Implants Res* 1997;**8**:476–86.
19. Proussaefs P, Lozada JL, Kim J, Rohrer MD. Repair of the perforated sinus membrane with a resorbable collagen membrane: a human study. *Int J Oral Maxillofac Implants* 2004;**19**:413–20.
20. Choi KS, Kan JY, Goodacre CJ, Lozada JL, Rungcharassaeng K. The effects of resorbable membrane on human maxillary sinus graft: a pilot study. *Int J Oral Maxillofac Implants* 2009;**24**:73–80.
21. Proussaefs P, Lozada JL. The Loma Linda pouch: a technique for repairing the perforated sinus membrane. *Int J Periodontics Restorative Dent* 2003;**23**:593–7.
22. Testori T, Wallace SS, Del Fabbro M, Taschieri S, Trisi P, Capelli M, et al. Repair of large sinus membrane perforations using stabilized collagen barrier membranes: surgical techniques with histologic and radiographic evidence of success. *Int J Periodontics Restorative Dent* 2008;**28**:9–17.
23. Misch CE. Maxillary sinus augmentation for endosteal implants: organized alternative treatment plans. *Int J Oral Implantol* 1987;**4**:49–58.