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Effects of fibrin glue on bone formation in combination with deproteinized bone xenografts in humans

조 선 대 학 교 대 학 원

치 의 학 과

김 문 수

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지도교수 김 수 관

이 논문을 치의학 박사학위신청 논문으로 제출함.

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조 선 대 학 교 대 학 원

치 의 학 과

김 문 수

김문수의 박사학위논문을 인준함

위원장 이화여자대학교 교 수 김 명 래 인

위 원 조선대학교 교 수 김 광 원 인

위 원 조선대학교 교 수 이 상 호 인

위 원 조선대학교 교 수 임 성 철 인

위 원 조선대학교 교 수 김 수 관 인

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조선대학교 대학원

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사람에서 이종골과 조직 접합제의 혼용후 골형성 효과에 관한 연구

김 문수
지도교수 : 김 수관
조선대학교 치의학과
구강악안면외과학 전공

본 연구의 목적은 각종 골 이식재중 이종골과 골조직의 재생능력을 증가시킬 것으로 여겨지는 조직 접합제를 첨가하여 사람의 상악동에 이식한 후 치유과정을 통하여 조직 접합제의 골형성에 관한 효과를 알아보는 데 있다.

제1군은 대조군으로, 총 25명의 환자가 포함되었으며 이종골을 멸균 식염수와 혼합하여 이식하였고, 제2군은 실험군으로, 총 9명의 환자가 포함되었으며 이종골과 조직 접합제를 혼합하여 이식하였다. 그리고 9개월을 기준으로 세분하였다.

조직 검사를 위해 2차수술이나 임프란트 식립시 조직편을 채취한 후, 중성 포르말린 용액에 일정기간 고정하고, 탈회 및 포매과정을 거쳐, Hematoxyline-Eosin으로 이중 염색하여 광학 현미경으로 이식재의 흡수 정도, 신생골의 형성, 이식재 및 신생골의 비율 등의 치유과정을 분석하였으며 조직형태계측학적으로 분석하였다.

1군이 다양한 시기에 생검된 것에 비하여 2군은 대부분 7개월 이후의 장기 관찰된 증례로만 구성되어 이식후 조기 변화는 그룹간 적절히 비교할 수 없었다. 2군은 1군에 비하여 좋은 신생골 형성을 보였으나 통계학적 유의성은 없었다. 2군은 1군에 비하여 단기적으로는 이식재의 흡수가 빠르고 좋았으나, 장기적으로는 1군에서 더 좋은 이식재의 흡수를 관찰할 수 있었다. 그러나, 이들 모두 통계학적인 유의성은 없었다. 1군은 2군에 비하여 조기에 층판골을 형성하지만 통계학적 유의성은 없었다. 그리고 장기적으로는 양 그룹간 층판골 형성능의 차이는 없었다.

본 연구결과로 미루어 보아 이식재 식립후의 신생골 형성에 따른 수술 부위의 안정성은 2군의 술식이 더 유리할 것으로 추정되며, 향후 기간에 따른 체계적인 연구가 필요하리라 사료된다.

Introduction

When considering a lateral approach to the sinus, the major differences between the various surgical methods are the type of graft material used and the choice of immediate or delayed implant placement.[1] In case of severe atrophy of the maxillary alveolar process, sinus floor elevation and implant insertion are usually performed in two stages.[2] When an autogenous bone graft is used, it takes approximately 6 months following augmentation for the transplanted bone to be integrated and substituted by osteoconduction (creeping substitution). Alternatively, autogenous bone transplants can be replaced by bone substitutes, such as Bio-Oss® (Geistlich Söhne AG, Wolhusen, Switzerland), to avoid donor site morbidity.[3]

Bio-Oss®, a frequently used alternative bone substitute, has been evaluated in several animal[4,5] and clinical studies.[6-8] This bone substitute is derived from porous bovine bone material processed to yield natural bone material lacking the organic component; it is reported to have good tissue acceptance and to provide a scaffold for new bone deposition with natural osteotrophic properties.[9]

Histologic data regarding the outcomes of treatments involving sinus grafting in humans are scarce.[10] Even when specimens were obtained at different clinical centers, they were retrieved from patients for whom the surgical procedure was successful.

In implant dentistry, numerous techniques have been studied for promoting and accelerating the osseous healing of dental implants and bone grafts by increasing the bone regenerative potential.[11] These techniques include the application of platelet-rich plasma (PRP),[12-14] bone morphogenetic protein,[15-17] growth factors,[18-20] and fibrin glue.[21-28]

Fibrin glue in various formulations has been used widely in various surgical procedures by means of establishing hemostasis and for tissue approximation. Fibrin sealants have been used in such diverse locations as the heart and pericardial cavity, lung, bowel, ovary, nasal sinuses, and skin. Formulations include both autologous preparations, which require peri-operative preparation, and prepackaged commercial kits.[29]

Fibrin glue is a physiological matrix whose principal component, fibrin, plays fundamental roles in the process of blood clotting and wound healing.[30] It is a potentially suitable biological vehicle for cell transplantation since it has proven biocompatibility, biodegradability, and binding capacity to cells.[31] Fibrin-stabilizing factor XIII, contained in fibrin glue, favors the migration of undifferentiated mesenchymal stem cells (MSCs) on the highly cross-linked structure of the glue, and it enhances the proliferation of these cells.[32]

The use of fibrin glue to improve bone regeneration is well documented,[21-28]

and platelet-rich fibrin (PRF) is an autologous fibrin matrix used to enhance bone generation.[33] Tayapongsak et al[21] found that it facilitates the placement of graft material in the recipient cavity by preventing the displacement of the bone graft particles during placement. It also helps the remodeling process begin earlier by accelerating the migration of fibroblasts and the revascularization process.

In bone reconstruction, the combination of bioceramics and fibrin glues may have synergistic effects. The mechanical strength of the composite is superior to that of the ceramic alone.[34] Furthermore, the bioceramic/fibrin glue composite is stabilized initially through its adaptation and adhesion to the walls of the bone defect. The fibrin might also enhance its biological properties by acting positively on angiogenesis, cell attachment, and proliferation.[35,36]

Few study has examined composites consisting of Bio-Oss® and fibrin glue. Therefore, this histologic study evaluated whether fibrin glue in combination with Bio-Oss® enhances bone regeneration in sinus floor elevation in humans.

Materials and Methods

This study was approved by the Institutional Review Board of Chosun University, Korea. Thirty-six sinus grafts were performed in 34 patients with an alveolar crest bone height in the posterior maxilla of 3 to 5 mm before grafting. The sinuses were grafted using Bio-Oss® alone or mixed with fibrin glue. Informed consent was obtained from all patients.

Group 1 was the control group and included 25 patients who received a xenograft mixed in saline. Group 2 comprised 9 patients who received a xenograft and fibrin glue. The study was further subdivided at the time of 9 months.

Patient selection

Patients were enrolled in this study if they had no systemic or local contraindications: no history of uncontrolled diabetes, no radiation therapy to the head or neck in doses over 5,000 rads, no chemotherapy within the 12 months preceding surgery, no active sinus infection, no uncontrolled periodontal disease, and no psychological problems that would prevent long-term treatment. Smokers were advised to reduce or refrain from smoking.

Surgical technique

Immediately before surgery, the patients rinsed with a 0.2% chlorhexidine digluconate solution for 2 minutes. Local anesthesia was obtained with lidocaine containing epinephrine 1:100,000.

A crestal incision, slightly displaced toward the palate, was made, and a vertical releasing incision was placed in the canine area to facilitate flap elevation. A mucoperiosteal flap was elevated, exposing the lateral wall of the sinus. A bony window, averaging 15 X 10 mm, was outlined using a no. 6 round carbide bur without perforating the sinus membrane. After mobilizing the window, the sinus membrane was elevated starting from the inferior border of the osteotomy site. The lateral window was pushed inward and elevated superiorly, creating a new horizontal ceiling, as the membrane was carefully dissected from the medial and inferior walls of the sinus.

The graft material (Bio-Oss®) was hydrated with saline solution and gently packed into the sinus until it filled the entire cavity. In Group 2, the Bio-Oss® was mixed with fibrin glue. Immediate implant placement was indicated when sufficient native bone was available to achieve primary stability after placement. The procedure was delayed 6 to 18 months after grafting for cases in which it was considered impossible to anchor and stabilize an implant in the subsinus ridge. Screw-type, machined-surface implants were used in the patients.

All the implants were submerged. The abutments were connected during two distinct postoperative periods, at 6 and 18 months post-implant placement. Bone cores from the graft sites were taken for histologic examination. Three to 4 weeks after soft tissue healing, the final abutments were connected, implant stability was tested manually, and prosthetic treatment was carried out.

Histologic examination

Bone cores were harvested from the lateral wall using a 2-mm-diameter trephine bur under sterile saline irrigation. The biopsies were retrieved from areas located between the implants, about 10 mm from the alveolar ridge, at a mean depth of 7 mm.

Microscopic examination and histomorphometric analysis

After fixing the harvested bone in 10% formalin using the conventional method and treating the bone in nitric acid (De-Cal Rapid® Pational Diagnosis, Atlanta, GA) for approximately 4 hours to decalcify, blocks were made to obtain representative sections. After washing and processing the tissues using an autoprocessing machine (Hypercenter XP, Shandon, UK), the blocks were embedded in paraffin. The embedded blocks were cut into 4−μm-thick slices, stained with hematoxylin–eosin (H&E), and observed under an optical microscope. The degree of new bone formation (bone forming activity) and

composition ratio of the tissue sample were determined by measuring and comparing the area of new bone formation using computer-assisted histomorphometry.[3] Images of each tissue sample were taken with a digital camera (Polaroid, Cambridge, MA) and analyzed using Image Pro Plus (Media Cybernetics, Silver Spring, MD).

● Bone forming activity = area of new bone formation / whole surface area of the specimen X 100 (%)
● Composition ratio = areas of woven bone : lamellar bone : residual implant material (%)

Statistical analysis

To confirm the significance after measuring the area of new bone formed, Student's *t*-test was performed. $P < .05$ was considered statistically significant.

Results

Group 1, <9 months

New bone was formed around the implanted Bio-Oss® chips. Under higher magnification, organized trabecular woven bone around the implanted Bio-Oss® chips was noted (Figs. 1, 2). The bone forming activity was 46.3 ± 30.0% (Table 1). The composition ratio of the tissue sample was 47.7 : 14.6 : 37.7 (areas of woven bone : lamellar bone : residual implant material, Table 1).

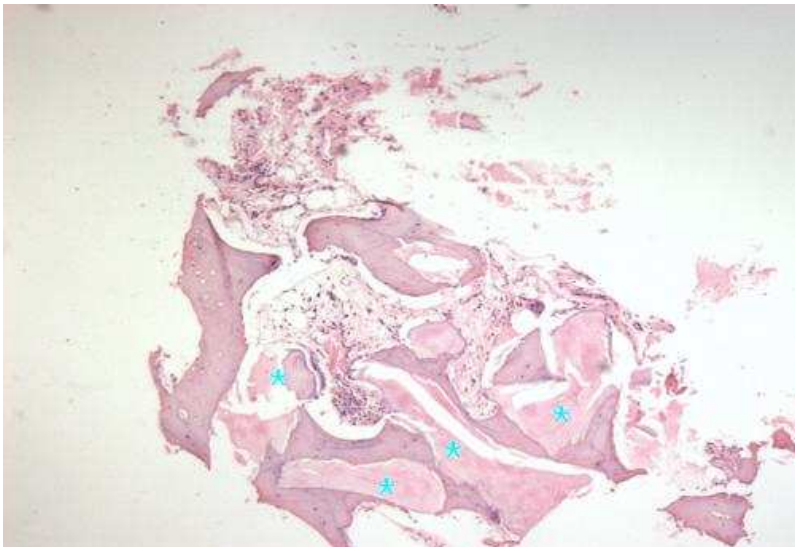

FIGURE 1. New bone was seen around the implanted Bio-Oss® chips (asterisks) (hematoxylin and eosin stain, original magnification X40).

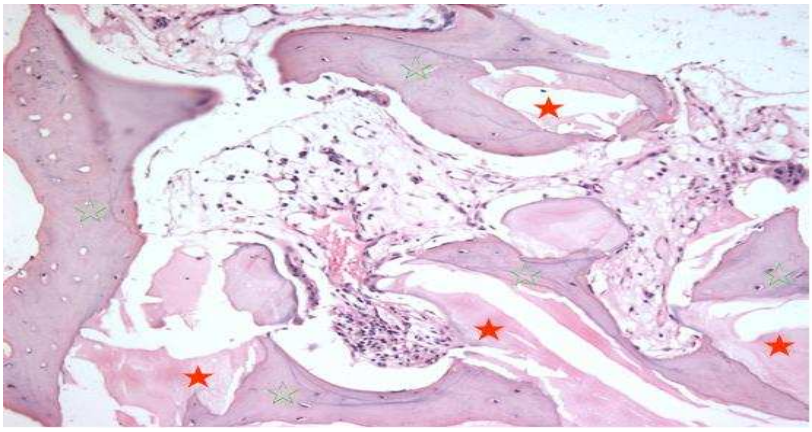

FIGURE 2. Higher magnification of the histopathologic findings. Organized trabecular woven bone (open asterisks) was seen around the implanted Bio-Oss® chips (closed asterisks) (hematoxylin and eosin stain, original magnification X100).

Group 1, ≥9 months

New bone was formed around the implanted Bio-Oss® chips. The new bone forming activity increased slightly (56.5 ± 31.5%) compared to Group 1 at <9 months (Table 1), although the difference was not significant (p=0.21). Under higher magnification, organized trabecular woven bone was seen around the implanted Bio-Oss® chips. Focal lamellar bone formation was also noted (Figs. 3, 4).

The composition ratio of the tissue sample was 63.5 : 13.5 : 23.9 (Table 1). In this group, the proportion of woven bone formation increased compared to Group 1 at <9 months, although the difference was not significant (p=0.07).

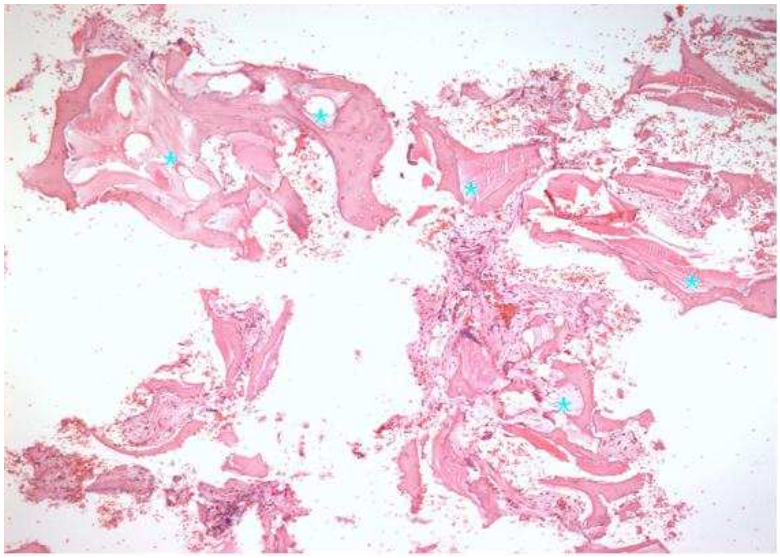

FIGURE 3. New bone was seen around the implanted Bio-Oss® chips (asterisks). The new bone forming activity increased slightly (hematoxylin and eosin stain, original magnification X40).

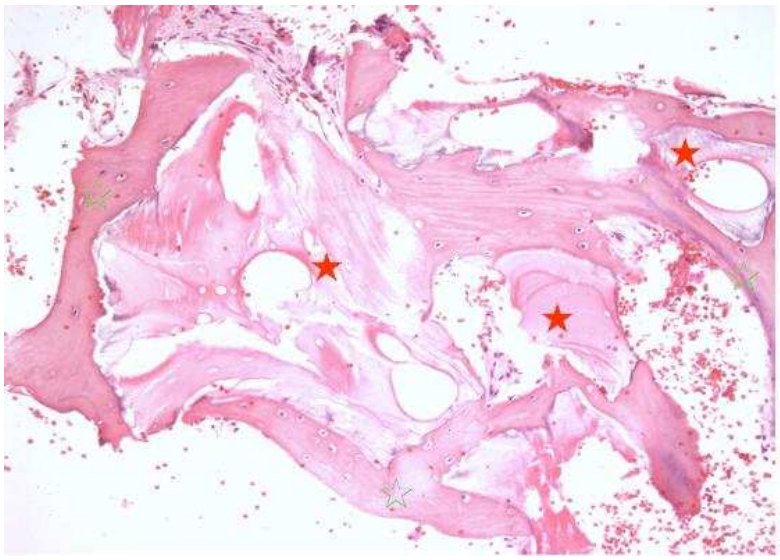

FIGURE 4. Higher magnification of the histopathologic findings. Organized trabecular woven bone (open asterisks) was seen around the implanted Bio-Oss® chips (closed asterisks). Focal lamellar bone formation (arrows) is noted (hematoxylin and eosin stain, original magnification X100).

Group 2, <9 months

New bone was seen around the implanted Bio-Oss® chips. Under higher magnification, organized woven bone formation was seen around the implanted Bio-Oss® chips (Figs. 5, 6). The new bone forming activity increased slightly (57.7 ± 39.3%) compared to Group 1 at <9 months (Table 2), but the difference was not significant ($p=0.277$; Table 3).

The composition ratio of the tissue sample was 80.0 : 3.3 : 16.7 (Table 2). In this group, the proportion of woven bone increased significantly ($p=0.034$) compared to Group 1 at <9 months (Table 4).

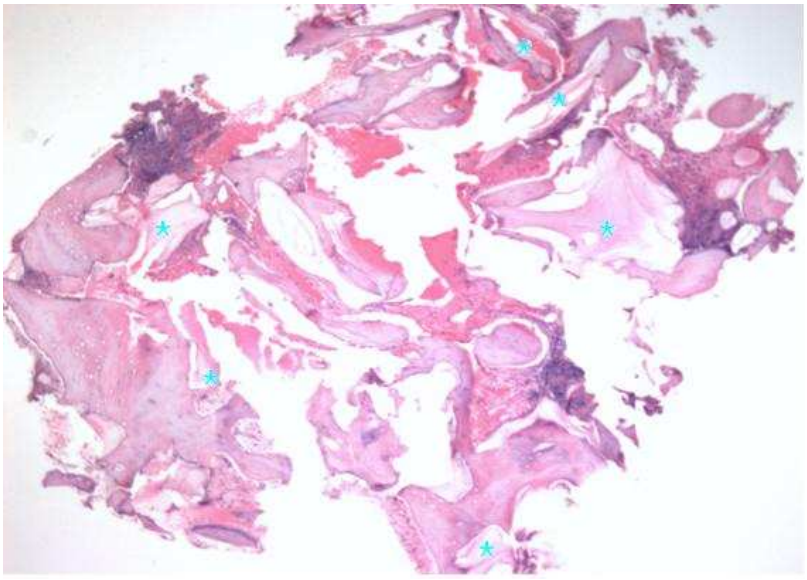

FIGURE 5. New bone was seen around the implanted Bio-Oss® chips (asterisks) (hematoxylin and eosin stain, original magnification X40).

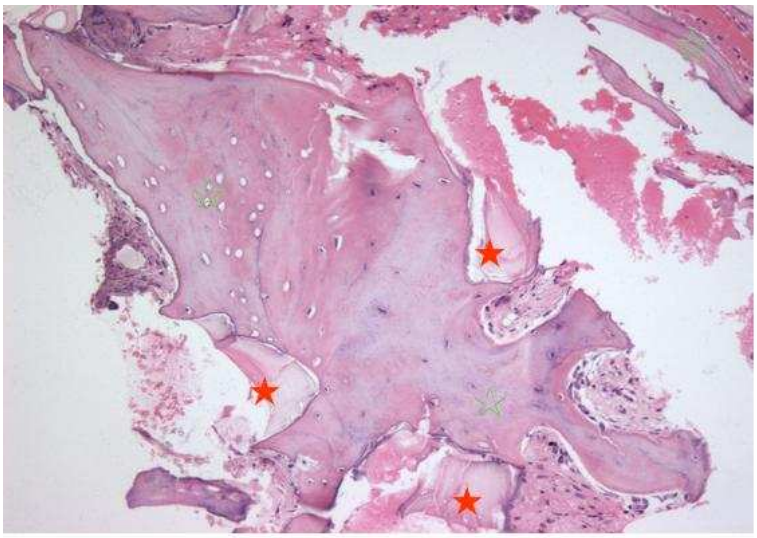

FIGURE 6. Higher magnification of the histopathologic findings. Organized woven bone (open asterisks) was seen around the implanted Bio-Oss® chips (closed asterisks) (hematoxylin and eosin stain, original magnification X100).

Group 2, ≥9 months

Thick woven bone was seen around a few implanted Bio-Oss® chips. Although the new bone forming activity (82.0 ± 18.9) increased (Table 2), it was not significantly different from Group 1 at ≥9 months (p=0.056, Table 3) or from Group 2 at <9 months (*P*=0.13). Under higher magnification, thick woven bone with a few implanted Bio-Oss® chips was seen (Figs. 7, 8).

The composition ratio of the tissue sample was 53.0 : 14.0 : 31.0 (Table 2). The proportion of woven bone decreased significantly (p=0.038) compared to

Group 2 at <9 months, while the proportion of lamellar bone increased significantly (*P*=0.049).

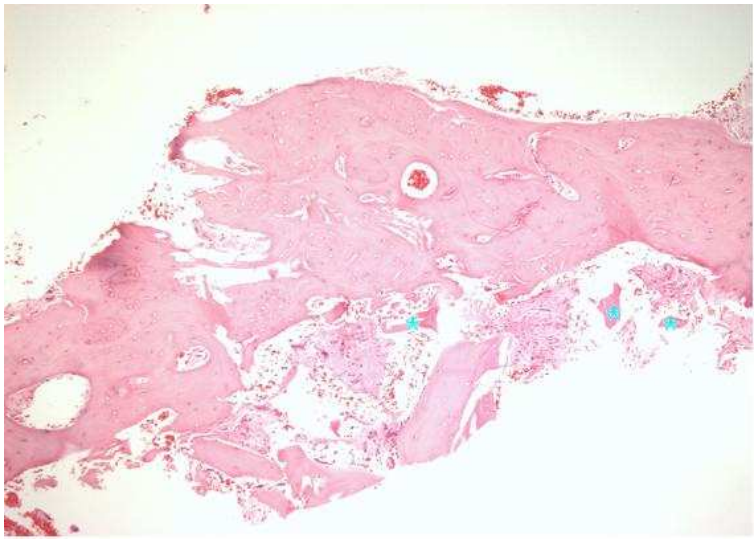

FIGURE 7. Thick woven bone around a few implanted Bio-Oss® chips (asterisks) is seen. The new bone forming activity increased markedly (hematoxylin and eosin stain, original magnification X40).

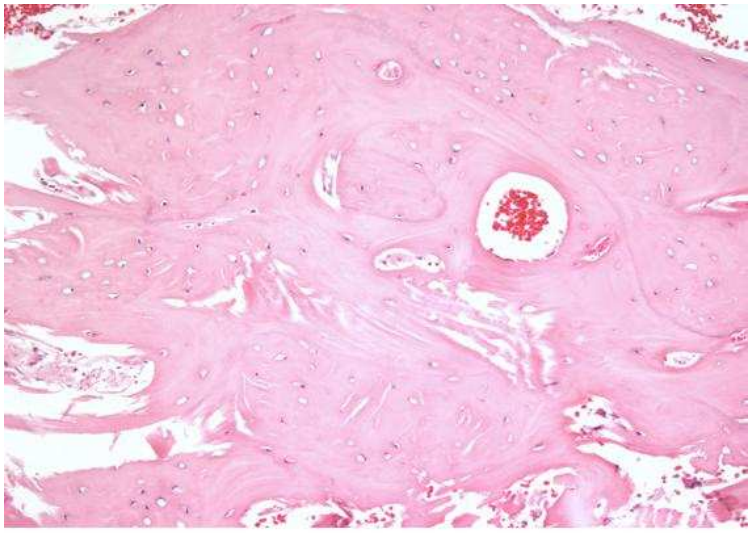

FIGURE 8. Higher magnification of the histopathologic findings. Thick woven bone is seen with a few implanted Bio-Oss® chips. (hematoxylin and eosin

stain, original magnification X100).

Table 1. Bone forming activity and composition ratio after implant surgery in Group 1

Time (months)	n	Bone forming activity (%)*	Composition ratio (%)#		
			Woven bone	Lamellar bone	Bio-Oss®
<9	12	46.25±30.01	47.73±25.63	14.55±12.34	37.73±32.97
≥9	13	56.54±31.45	63.46±24.70	13.46±16.12	23.85±25.91
P value		0.20608	0.0703	0.429	0.130

*Bone forming activity = area of new bone formation/area of total sample X 100 (%)

#Composition ratio = area of woven bone : lamellar bone : residual implant materials (%)

Table 2. Bone forming activity and composition ratio after implant surgery in Group 2

Duration (months)	n	Bone forming activity (%)*	Composition ratio (%)#		
			Woven bone	Lamellar bone	Bio-Oss®
<9	4	57.70±39.26	80.00±20.00	3.33±5.77	16.67±20.82
≥9	5	82.00±18.91	53.00±31.31	14.00±19.88	31.00±8.22
P value		0.1271	0.0377	0.04953	0.1718

*Bone forming activity = area of new bone formation/area of total sample X 100 (%)

#Composition ratio = area of woven bone : lamellar bone : residual implant materials (%)

Table 3. Comparison of the bone forming activity after implant surgery between Groups 1 and 2

Duration (months)	Bone forming activity (%)		P value
	Group 1	Group 2	
<9	46.3±30.0	57.7±39.3	0.277
n	12	4	
≥9	56.5±31.5	82.0±18.9	0.056
n	13	5	

* Bone forming activity = area of new bone formation/area of total sample X 100 (%)

Table 4. Comparison of the composition ratio after implant surgery between Groups 1 and 2

Duration (months)	Composition ratio (%)*		P value
	Group 1	Group 2	
<9			
woven bone	47.7±25.6	80.0±20.0	0.034
lamellar bone	14.6±12.3	3.3±5.8	0.080
Bio-Oss®	37.7±33.0	16.7±20.8	0.161
≥9			
woven bone	63.5±24.7	53.0±31.3¶	0.198
lamellar bone	13.5±16.1	14.0±19.9§	0.472
Bio-Oss®	23.9±25.9	31.0±8.2	0.291

* composition ratio=percentage area of woven bone formation vs. percentage area of

lamellar bone formation vs. percentage area of residual implant materials (%)

¶ The proportion of woven bone was statistically significant decreased compared to Group 2, <9 months, p< .05.

§ The proportion of lamellar bone was statistically significant increased compared to Group 2, <9 months, p< .05.

Discussion

Autografts are the ideal material for reconstructing hard tissue defects. They undergo osteogenesis, osteoinduction, and osteoconduction, do not pose a risk of immune rejection, and require short recovery times. However, only a small amount of tissue can be harvested, and absorption and the secondary defects at the donor site are major drawbacks. To avoid harvesting an autograft and thereby eliminating the additional surgical procedure and associated risks, bone grafting materials and substitutes are used as alternative grafting materials for ridge augmentation.[37] The ideal grafting materials should be biocompatible, noncarcinogenic, and nonallergenic; show early vascularization; be replaced by new host bone tissue; strength, resist infection, and be sterile; and undergo surface resorption by the host.[38,39]

The use of bovine hydroxyapatite (Bio-Oss®) has been suggested for maxillary sinus grafting procedures before or in conjunction with implant placement.[40,41] Bio-Oss® is deproteinized, sterilized bovine bone with 75 to 80% porosity and a crystal size of approximately 10 nm in the form of cortical and cancellous granules and blocks. It has inner macropores of similar size to natural cancellous bone, appears to be replaced by host bone more readily than hydroxyapatite when used in alveolar ridge restoration, and appears to undergo physiological remodeling with incorporation into the host bone. Moreover, Bio-Oss® is highly

biocompatible with oral hard tissues in animals and humans and meets the criteria of an osteoconductive material.[41] The maturation of Bio-Oss® can take up 9 months when used for sinus augmentation.[40] Therefore, the study period was divided at 9 months.

Fibrin tissue adhesive material (FAM) is available as fast (about 5 seconds) and slow (about 1 min) setting types. FAM is supplied in two vials. One contains fibronectin, fibrinogen, factor XIII, and aprotinin (an anti-fibrinolytic agent), while the other contains thrombin and calcium chloride. The components become a gel when mixed. FAM acts as both a tissue adhesive and a hemostatic material. It also enhances wound healing.[42,43]

Fibrinogen is obtained from pooled, single-donor, or autologous blood. Pooled fibrinogen products such as Tisseel, Tissucol, and Fibrin Sealant (Immuno AG, Vienna, Austria) are available commercially and are used widely in Europe and Canada, but not in the United States because of the possible risks of transmitting hepatitis, human immunodeficiency virus, and other diseases. Therefore, US practitioners turn to single-donor fresh-frozen plasma or autologous blood.[21]

Fibrin is believed to stimulate the growth of fibroblasts and osteoblasts.[22] Bosch et al[44] demonstrated that fibrin adhesive improved bone graft incorporation and remodeling by significantly reducing the size of the gaps between bony fragments and accelerating revascularization. Moreover, the multiplication of

bacteria in the fibrin clot is significantly slower than in a comparable blood clot.[21]

In addition to the physical benefit of using fibrin glue in reconstructive bone surgery, the glue also accelerates the bone graft healing process,[21,45] as the fibrin network acts as a scaffold for invading cells[33] and as a carrier for bone induction.[46]

In general, the smaller a bone defect is, the more easily periodontal tissue regeneration is achieved.[42] Ohazama et al[42] studied simple bone defects, involving buccal bone, which extended halfway into the interproximal and inter-radicular regions, whereas Warrer and Karring[47] studied deep, complex bone defects that involved inter-radicular bone only. FAM might be suitable for small bone defects, such as dehiscences and bony clefts. The differences in the experimental jaw could also have contributed to the differences in results. Ohazama et al[42] studied the mandible only, while Warrer and Karring[47] studied both the mandible and maxilla.

Ohazama et al[42] reported that FAM produced significant differences in new bone formation by 8 weeks after surgery compared to the control sites ($P < .05$). This suggests that FAM enhances the osteogenic potential.[43] The results of experiment 2 suggested that FAM indeed enhances bone formation. In our study, the histomorphometric analysis showed that the bone structures in the control (Bio-Oss® alone) and test (Bio-Oss® plus fibrin glue) groups were similar.

FAM might act as a material for creating space. It might also act in conjunction with a growth factor, such as bone morphogenic protein (BMP), insulin-like growth factor, or platelet-derived growth factor.

The histologic analyses highlight other advantages of using Tisseel. Tisseel with Bio-Oss® enhances the graft volume without affecting the maturation of the new bone.

In this study, the histologic similarities observed between the two groups (Bio-Oss® alone, Bio-Oss® plus fibrin glue) should enable sinus floor augmentation surgery with a shorter healing period before implant placement. Furthermore, the quantity of the bone material used to fill the sinus cavity can be reduced safely without negatively affecting the final bone density. Finally, the fibrin glue appears to be suitable for treating sinus membrane perforation, permitting the surgery to be completed. The use of fibrin glue, in addition to a bone graft material, to perform sinus floor augmentation is attractive from a histologic perspective.

Conclusions

Thirty-six sinus grafts were performed in 34 patients with an alveolar crest bone height in the posterior maxilla of 3 to 5 mm before grafting. The sinuses were grafted using Bio-Oss® alone or mixed with fibrin glue. Informed consent was obtained from all patients.

Group 1 was the control group and included 25 patients who received a xenograft mixed in saline. Group 2 comprised 9 patients who received a xenograft and fibrin glue. The study was further subdivided at the time of 9 months.

This histologic study evaluated by hematoxylin–eosin (H&E) and histomorphometric analysis whether fibrin glue in combination with Bio-Oss® enhances bone regeneration in sinus floor elevation in humans. The degree of new bone formation (bone forming activity) and composition ratio of the tissue sample were determined by measuring and comparing the area of new bone formation using computer-assisted histomorphometry.

The following results were obtained;

1. Compared to Group 1, in which biopsies were performed at various times, long-term observations were conducted in Group 2, mainly after 7 months, making it difficult to compare the early changes in the two groups.
2. The new bone formation was better in Group 2 than in Group 1, but the

difference was not significant.

3. The absorption of the graft material was faster in Group 2 than in Group 1, in the short term, but better in Group 1 over the long term, although the difference was not significant.
4. Lamellar bone was formed earlier in Group 1 compared to Group 2, but the difference was not significant. The long-term difference was not evident in lamellar bone formation.
5. Overall, the surgery site stabilized earlier with new bone formation in Group 2 than in Group 1, but the difference was not significant.

New bone was seen in Bio-Oss® and with Bio-Oss® plus Tisseel, although the difference was not significant.

Combining a fibrin sealant and Bio-Oss® could lead to improved scaffolds for bone tissue engineering based on the synergistic effects of the biomaterials. Therefore, Bio-Oss® or Bio-Oss® plus Tisseel may be used depending on the situation.

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