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Evaluation of bone formation after grafting with Tutoplast™ and BioOss® for the treatment of bone defects around an implant

조선대학교 대학원

치 의 학 과

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지도교수 김 춘 성

이 논문을 치의학 박사학위신청 논문으로 제출함.

2011년 4월

조선대학교 대학원

치 의 학 과

문 경 남

문경남의 박사학위 논문을 인준함.

위원장 서울대학교 교 수 김 영 균 인

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위 원 조선대학교 교 수 김 춘 성 인

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2011년 6월 일

조선대학교 대학원

목 차

표 목 차

도 목 차

국문초록

임플란트 주위 골결손부에 대하여 tutoplast와 bioss를 이용하여 이식후 골형성 평가

문 경 남

지도교수 : 김 춘 성

조선대학교 치의학과 구강악안면외과전공

이 연구의 목적은 쥐의 두개골에 골결손부를 만들고, 임상에서 널리 사용되고있는 Bio-Oss 또는 Tutoplast 를 이식함에 있어서 조직형태학적 평가법을 이용하여 이들 두 골재료의 골전도성을 비교하고 평가하는 것이다.

60마리의 쥐를 대조군 및 실험군(그룹1, 그룹2)으로 구분하였다. : 대조군, 미이식 그룹; 그룹1, Bio-Oss; 그룹2, Tutoplast.

이 쥐들을 6주와 12주 후에 희생시킨 후, 결손부에서 골 표본을 채취하였으며, 이 표본에서 신생골을 조직형태학적으로 분석하였다.

대조군에서는 결손부의 변연에 신생골이 일부 형성되었다. 그러나 중심부에는 골이 형성되지 않았고 섬유성 결합조직이 관찰되었다.

Bio-Oss를 이식한 그룹에서는, 신생골이 인접한 정상골보다 얇았고, Bio-Oss입자가 관찰되었다.

Tutoplast를 이식한 그룹에서는 인접한 골과 점진적으로 융합된 패턴을 보여주었고, Bio-Oss 그룹과 유사하게 일부 부위에서 입자들이 관찰되었다.

12주 후에 채취한 그룹들에서는, 대조군보다 실험군들에서 비율이 상당히 증가하였고, 그룹1 보다 그룹 2에서 더 크게 증가하였다 (p=.001).

Tutoplast 와 Bio-Oss 는 골이식재로서 유용한 것으로 보인다.

Abstract

Purpose: *The purpose of this study was to evaluate the ability of new bone formation of two kinds of bone substitutes (deproteinized bovine bone (Bio-Oss®) or mineralized allogenic bone (Tutoplast™).*

Materials and Methods: *Sixty rats were divided into control and experimental groups (Groups 1, 2): control group, unfilled control; Group 1, Bio-Oss®; Group 2, Tutoplast™, respectively. The animals were sacrificed after 6 and 12 weeks, bone samples were taken from the defect, and newly formed bone was analyzed histomorphometrically.*

Results: *In the control group, some new bone formed in the rim of the defect area, but not in the center and also fibrous connective tissue was observed. In the group 1, newly formed bone was thinner than the adjacent normal bone, and Bio-Oss® particles were observed. In the group 2, showed a pattern of gradual fusion with adjacent bone, as well as particles in some areas, similar to the Bio-Oss®-treated group. In the 12 week groups, the amount of new bone formation was significantly higher in the experimental groups than in the control group, and it was significantly higher in group 2 than in group 1 ($p = .001$).*

Conclusion: *Tutoplast™ and Bio-Oss® graft materials appear to be useful for bone grafts.*

KEYWORDS: *guided bone regeneration, osteoconduction, osteoinduction*

I. Introduction

Autogenous bone is recognized as the gold standard,[1-3] but it has shortcomings due to additional surgeries at the intra- and extraoral donor sites, which consequently results in pain in the donor site, development of complications, prolongation of operation time, and a limitation in the amount of bone that can be harvested.[4,5] To overcome such disadvantages, allobone, xenobone, or alloplastic bone have been developed.[6,7]

Bio-Oss® is a bovine-originated, anorganic, bone graft material prepared from cows by heating at relatively low temperature (300°C) to remove organic substances using alkaline chemicals, and by sterilization with dry heat. Proteins are removed by these processes, pathogens are absent, and this type of bone does not induce an immune reaction, eliminating the possibility of an infection.[6] Additionally, the natural bone structure is maintained, and it is a multiporous structure similar to human bones (75%). Bio-Oss® facilitates the formation of blood vessels and migration of osteoblasts, and new bone is formed by osteoconduction in a bone graft.[6,8-10]

Tutoplast™ is allogenic bone obtained from human cadavers that is manufactured by the following special tutoplast processing technique according to the American Association of Tissue Banks standards:[11] delipidization using acetone and ultrasound, osmotic treatment using distilled water and a saline bath, oxidative treatment using a hydrogen peroxide solution, serial dehydration, and gamma irradiation (17.8 Gy). This is a solvent preserved method, and because it removes water, the mineral matrix is preserved better than freeze-dried allogenic bone. After processing, it is a non-demineralized allogenic graft material that contains all of the minerals and collagen matrix structure of the human skeleton.[11-13]

The purpose of this study was to evaluate the osteoconductivity of two

kinds of bone substitutes (deproteinized bovine bone (Bio-Oss®) or mineralized allogenic bone (Tutoplast™)).

Ⅱ. Materials and Methods

Animals

Experiments were conducted on 60 male 9-week-old Sprague–Dawley rats weighing 200–300 g. Before experimentation, the protocol was evaluated and approved by the Animal Research Committee of Chosun University to ensure that the policies, standards and guidelines for the proper use, care, handling, and treatment of animals were observed. They were maintained under identical conditions, and all animals were healthy.

Materials

Bio-Oss® was purchased from Geistlich Pharma AG (Wolhusen, Switzerland). It is a bovine-originated demineralized omentum graft material, and the 0.25–1-mm particle size was used. Tutoplast™ was obtained from Tutogen Medical GmbH (Neunkirchen, Germany). It is a graft material originating from humans prepared by a solvent preservation method, and the 0.25–1-mm particle size was used.

Experimental groups

Bio-Oss® (group 1) or Tutoplast™ (group 2) was transplanted into a created cranial defect area in 20 rats; ten animals from each group were killed at 6 weeks, and the remaining ten animals at 12 weeks. The control group consisted of animals that did not receive a bone graft. The animals were sacrificed after 6 and 12 weeks

Formation of a cranial defect area and bone grafting

The animals were injected intramuscularly with 2.2 mg of a 2% Rampun® injection solution (Bayer Korea Ltd., Seoul, Korea) and 0.18 mg ketamine chloride injection solution (Yoohan Yanghang, Seoul, Korea). Infiltration anesthesia with lidocaine chloride containing epinephrine diluted to 1:100,000 (Yoohan Yanghang) was applied to the cranial bone defect area to suppress local hemorrhage and pain. The surgical area was sterilized with potadine, and the cranial area was removed. An incision was made along the midline of the head to expose the skull. A hole 8 mm in diameter was then drilled in the skull, removing the entire layer of the skull using a 1/4 round bur. After grafting, the peristeum was sutured, and layer-to-layer suturing was performed with absorbable sutures. After the surgery, 6 mg gentamicin (Joongwei Phamaceutical, Seoul, Korea) was injected intramuscularly to prevent infection.

Sample preparation

Animals were anesthetized as described above and sacrificed in each experimental group. Then, the defect area including the upper periosteum, the scalp, and adjacent healthy bone was resected, fixed in 10% neutral formalin, and decalcified by acid immersion (Calci-Clear Rapid®, National Diagnostics, Atlanta, USA) for approximately 4 hours. In the middle of the bone defect area, three sections of 3-mm thickness were harvested consecutively, washed, and embedded in paraffin using an automatic tissue preparation instrument (Hypercenter XP, Shandon, UK). Paraffin blocks 4–5 μm in thickness were prepared, attached to glass slides, maintained at 68°C on a heat plate for longer than 1 hour, and stained with hematoxylin-eosin or Masson's trichrome stains. The prepared specimens were observed under microscopy and images were captured with the MagnaFire digital camera system (Optronics, Goleta, CA, USA). The region of interest was measured and analyzed for the amount of new bone formation

using the Visus Image Analysis System (Image and Microscope Technology, Daejeon, Korea).

Statistical analysis

The area of bone formed from the edge of defect area to the center was measured (n = 3/sample), and an ANOVA was performed. Scheffe's test was performed to assess significance among the experimental groups. $p < .05$ was considered statistically significant.

Ⅲ. Results

Histological results

A small amount of new bone in the rim of the defect area could be detected in the control group cases, but infiltration by inflammatory cells was limited. However, a new bone formation pattern with fibrous connective tissue, but no bone formation, was revealed in the center of the defect area. More new bone formed in the 12-week group than in the 6-week group, but the bone was thinner than normal bone, with fewer lacunae within the bones (Fig. 1).

In group 1, the Bio-Oss® that filled the defect area was maintained well without invasion of soft tissue. Nonetheless, bone was not formed, a small amount of fibrous connective tissue and Bio-Oss® particles without resorption were observed. The area where new bone formed maintained a thickness that was thinner than adjacent normal bone, and more bone formed in the 12-week group than in the 6-week group. In the 6-week group, an area occurred in which the graft material particles were not fused with adjacent bone and were separated from new bone (Fig. 2). In the 12-week group, new bone could be distinguished from adjacent bone, but areas remained that could not be distinguished. Nevertheless, most particles were not resorbing (Fig. 3).

In the 6-week experimental group transplanted with Tutoplast™, a fusion pattern with adjacent bones having little inflammation was observed. Although more bone formed in the defect area than in the Bio-Oss® graft group, some areas showed a small amount of fibrous connective tissue. Tutoplast™ particles were observed in some areas, but less of a fusion pattern with adjacent bone occurred when compared to the Bio-Oss® graft group (Fig. 4). The 12-week group had more bone formation than the 6-week group, and some areas could not be distinguished from adjacent bone (Fig. 5).

Active osteoblast activity and active formation of new bones were detected around the defect area in all groups, but the amount of new bone formation was greater in the experimental groups than the control group. The new bone was markedly thinner, with abundant fibrous connective tissues. Group 2 had more active formation of new bone compared to group 1.

Histomorphometric analysis

Among the 6-week groups, the amount of new bone formation in groups 1 and 2 were 0.43 ± 0.04㎟ and 0.39 ± 0.03㎟, respectively, which were significantly higher than in the control group (0.22 ± 0.03㎟). In the 12-week groups, the amount of new bone formation in experimental groups 1, 2, and the control were 0.55 ± 0.06㎟, 0.61 ± 0.16㎟, and 0.29 ± 0.02㎟, respectively. The new bone formation was significantly higher in the experimental groups than in the control group, and it was significantly higher in group 2 than in group 1 (p = .001) (Table 1).

Table 1. Mean amounts of new bone formation in Control, Group 1 and 2 at 6 and 12 weeks after placement (㎟)

	Mean ± SD		
Time period (weeks)	Control	Group 1	Group 2
6	0.22 ± 0.03	0.43 ± 0.04*	0.39 ± 0.03*
12	0.29 ± 0.02	0.55 ± 0.06*	0.61 ± 0.16*,†

*Statistically significant difference relative to control (p<0.05).
†Statistically significant difference relative to Group 1 (p<0.05).
SD = standard deviation.

Ⅳ. Discussion

Bone grafting is performed in a bone defect area to reconstruct congenital deformities or to correct injury to the oral and maxillofacial area caused by trauma, facial deformity, tumors, or other diseases, as well as for aesthetic and functional recovery. Bone transplants, autogenous bones, allogenic bones, xenogenic bones, and synthetic bone substitutes have all been used as bone graft materials.[14,15]

Antigens are removed in allogenic bones, and heat-treated, frozen, freeze-dried, or irradiated bones are used for an effective bone graft. However, heat treatment and irradiation impair bone formation after the graft, whereas freezing, freeze-drying, or a demineralized freeze-drying method do not to impede bone formation after grafting.[16] Allogenic bone is supplied as frozen, freeze-dried, or as demineralized freeze-dried bone, and demineralized freeze-dried or non-demineralized freeze-dried bone has been used most widely.

Freeze-dried bones have been used in the orthopedic surgery field since the 1950s, and they have been used in the dental area since the 1970s.[17] Demineralized freeze-dried bone allografts (DFDBAs) mediate the differentiation of host undifferentiated mesenchymal cells to osteoblasts and thus form new bone; DFDBAs were used for the first time in 1975 to treat periodontal defects.[18]

Demineralized bone contains bone morphometric proteins (BMPs), but the bone-forming activity of BMPs is suppressed by inorganic substances within bones; BMPs, however, are not removed during the demineralization process, allowing for excellent osteoinduction of new bone.[19]

Tutoplast™ (TUTOGEN Medical GmbH, Germany) is allobone obtained from the human cadaver. And it is processed through the special process called tutoplast processing technique.[18,20]

It is processed through five steps following delipidization, osmotic treatment,

oxidative treatment, dehydration, and the gamma irradiation (17.8Gy). And the mineralization substrate is well preserved because the moisture is removed by solvent preserved method. It is mineralized allobone has the mineral of the human and collagen substrate.[18]

Bio-Oss® (Geistlich Pharma AG, Switzerland) is a bovine bone derivate that undergoes a low heat (300 ◦C) chemical extraction process by which all organic components are removed, but maintains the natural architecture of bone. The vascularization and migration of osteoblasts occurs readily because it has a multiporous structure similar to human bones. So although osteoinductive ability is absent, it has very high osteoconduction properties.[8] According to a study by Merkx et al.,[21] inflammation was observed after a graft in rats, and after 8 weeks, no infiltration of inflammatory cells occurred. Similarly, we observed no infiltration of inflammatory cells in the group grafted with Bio-Oss®.

Lee et al.[22] compared the osteoconductive effects of deproteinized bovine bone mineral (Bio-Oss®) and solvent-dehydrated allograft (Tutoplast™) in extracted socket of human. They reported the inflammatory cell infiltration was rare in both materials like our study. But, deproteinized bovine bone mineral induced more new bone deposition in the periphery of the native bone particles than solvent-dehydrated allograft. Tudor et al.[23] studied about new bone formation in calvarial defect of pigs using particulated human materials and bovine materials. They reported that the microradiographically measured mineralization rate was 5% to 10% lower than the mineralization rate of autogenous bone grafts, but statistical analysis showed no significant differences after 12 weeks. Our results are slightly different than results of these studies, the new bone formation was significantly higher in Tutoplast™ graft than in Bio-Oss® graft in this study after 12weeks. However, it cannot conclude Tutoplast™ is superior than Bio-Oss® based on this study has limit in which the sample is small.

V. Conclusion

A bone defect area was generated in the rat cranium, and Bio-Oss® and Tutoplast™, which are most widely used in clinics, were transplanted. Using histological and histomorphometric evaluations, the osteoconduction of these two bone substitutes was compared, and the following results were obtained.

1. Some new bone formed in the rim of the defect area in the control group; however, bone did not form in the center and fibrous connective tissue was observed.
2. In the group grafted with Bio-Oss®, newly formed bone was thinner than the adjacent normal bone, and Bio-Oss® particles were observed.
3. The group grafted with Tutoplast™ showed a pattern of gradual fusion with adjacent bone, and particles were observed in some areas, similar to the Bio-Oss®-treated group.

In the 12-week groups, the amount of new bone formation was significantly higher in the experimental groups than in the control group, and it was significantly higher in group 2 than in group 1 (p = .001).

Disclosure

The authors claim to have no financial interest, directly or indirectly, in any entity that is commercially related to the products mentioned in this article.

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Figure Legends

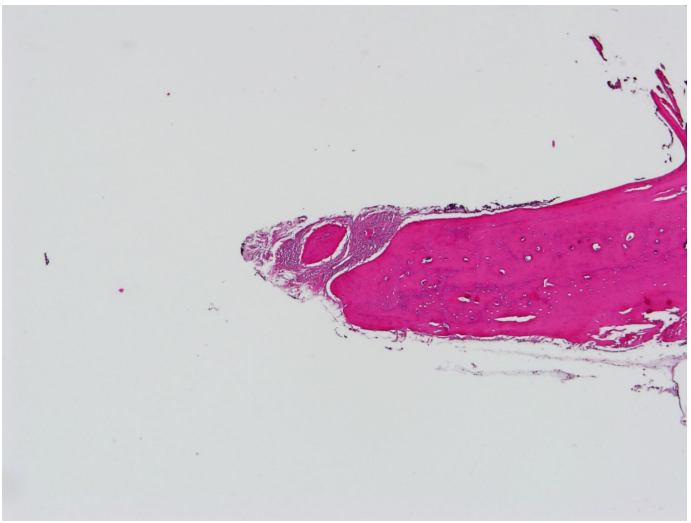

Fig. 1. Histopathological findings of the 12-week control group. The bone was thinner than normal bone, with fewer lacunae within the bones (hematoxylin–eosin stain, original magnification ×50).

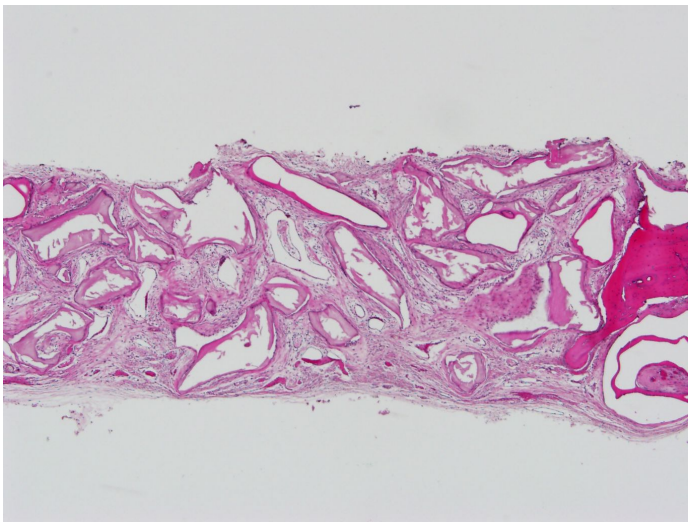

Fig. 2. Histopathological findings of the 6-week group 1. The graft material particles were not fused with adjacent bone and were separated from new bone (hematoxylin–eosin stain, original magnification ×50).

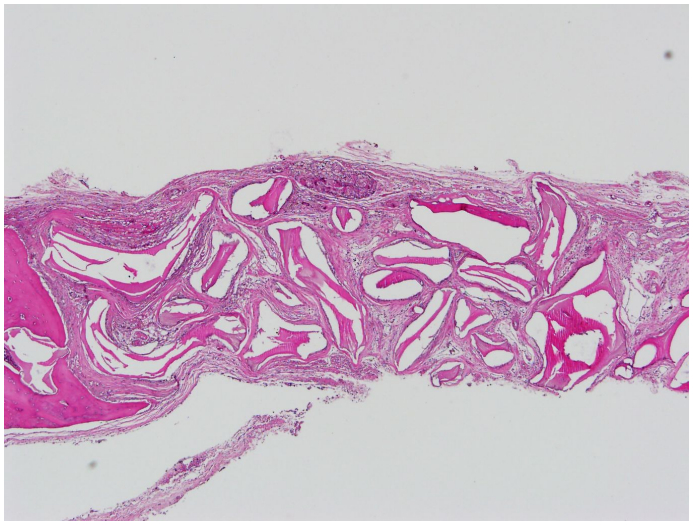

Fig. 3. Histopathological findings of the 12-week group 1. New bone could be distinguished from adjacent bone, but areas remained that could not be distinguished (hematoxylin–eosin stain, original magnification ×50).

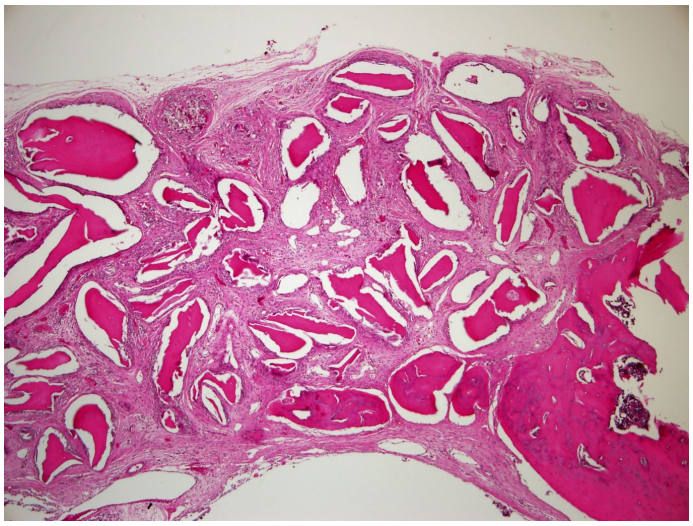

Fig. 4. Histopathological findings of the 6-week group 2. Transplanted with Tutoplast™, a fusion pattern with adjacent bones having little inflammation was observed (hematoxylin–eosin stain, original magnification ×50).

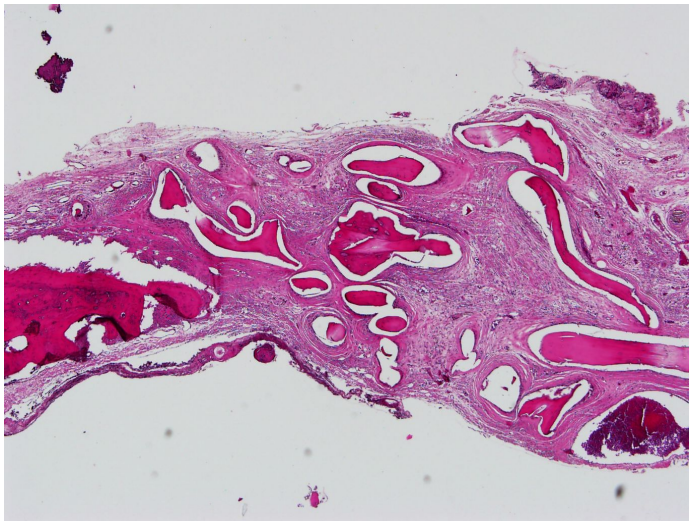

Fig. 5. Histopathological findings of the 12-week group 2. More bone formation than the 6-week experimental group 2 and some areas could not be distinguished from adjacent bone (hematoxylin–eosin stain, original magnification ×50).