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Effect of fibrin sealant on early bone healing with tooth ash and plaster of Paris in ovariectomized rats

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난소적출 백서에서 치아 회분말 및 연석고 매식시 조직접합제의 효과

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난소적출 백서에서 치아 회분말 및 연석고 매식시 조직접합제의 효과

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본 연구의 목적은 각종 골 이식재 중 치아 회분말과 치과용 연석고를 혼합한 후 조직접합제를 첨가하여 골다공증이 유발된 흰쥐의 두개골 결손부에서의 초기 치유과정을 통하여 조직접합제의 역할과 골다공증의 경우 치아 회분말 및 연석고 매식시 골치유에 미치는 영향을 알아보는 데 있다.

실험동물은 동일조건 하에서 일정기간 사육한 체중 200mg 이상의 Sprague-Dawley 흰쥐를 사용하였다. 노출시킨 두개골의 정중앙부에 #1/4 round bur를 이용하여 직경 8mm 크기의 원형으로 전층 골결손을 야기시킨 후, 제1군은 아무런 이식을 시행하지 않았고, 제2군은 치아 회분말과 치과용 연석고를 무게비 2:1로 혼합하여 멸균한 이식재를 멸균 식염수와 혼합하여 이식하였고, 제3군은 치아 회분말과 치과용 연석고를 무게비 2:1로 혼합하여 멸균한 이식재를 Tisseel과 혼합하여 이식하였으며, 제4군은 난소적출술을 시행한 후 치아 회분말과 치과용 연석고 (무게비 2:1)를 이식하였으며, 제5군은 난소적출술을 시행한 후 치아 회분말과 치과용 연석고 (무게비 2:1)를 Tisseel과 혼합한 후 이식하였다.

조직 검사를 위해 실험 후 4주와 8주로 나누어 실험군을 희생한 후 매식된 경계부를 포함하여 조직편을 채취한 후, 중성 포르말린 용액에 일정기간 고정하고, 탈회 및 포매과정을 거쳐, Hematoxyline-Eosin으로 이중 염색하여 광학현미경으로 흡수 정도, 신생골의 형성, 염증반응 유무 등의 치유과정을 분석하였다.

각 주에 따른 군별 비교에서 4주의 경우 유의한 신생골 형성의 차이를 보였으며, 1-2군간, 1-3군간, 1-4군간, 2-4군간, 2-5군간, 3-4군간, 4-5군간 비교에서 신생골 형성의 유의한 차이가 있었다. 8주의 경우 유의한($p=0.000$) 신생골 형성의 차이를 보였으며, 1-2군간, 1-3군간, 1-4군간, 1-5군간, 2-4군간, 2-5군간, 3-4군간, 3-5군간, 4-5군간 비교에서 신생골 형성의 유의한 차이가 있었다.

임계한계 이상의 골결손을 수복하기 위해서는 골형성 유도물질의 이식이 필요한데, 치아 회분말, 치아 회분말과 tisseel, 난소절제 후 치아 회분말, 난소절제 후 치아 회분말과 tisseel을 이식한 경우 대조군에 비하여 일부를 제외하고는 통계학적으로 매우 유의한 골형성 증가 소견을 보였다.

난소적출한 군보다는 난소적출을 시행하지 않은 군에서 신생골 형성이 잘 되었으며, 치아 회분말 단독 사용군과 치아 회분말과 tisseel 병용군을 비교하면 치아 회분말 단독 사용군이 더 양호한 골형성을 보였다. 그러나 이는 통계적으로 유의한 차이는 없어 치아 회분말 단독 또는 치아 회분말과 tisseel 병용을 상황에 따라 선택할 수 있으나 난소적출한 군에서는 치아 회분말과 치과용 연석고의 사용을 추천하며, 조직접합제의 사용은 추천하지 않는다.

Introduction

Various types of craniomaxillary and reconstructive surgery are performed in the craniomaxillofacial region for congenital deformities, infections, lesions, and trauma, and many different graft materials have been developed to satisfy this need.[1] The use of autogenous bone harvested from the maxillofacial region results in high success rates of augmentation. However, its application is limited because of several disadvantages, including the need for a donor site as well as pain, swelling, bleeding, and resorption after grafting. Consequently, much effort has been focused on developing an alternative material to autogenous bone for implantation.[2]

Since Dreesman first attempted the use of plaster of Paris (calcium sulfate), tooth ash and plaster of Paris have been studied as bone substitutes in experimental and clinical studies.[3] Particulate dentin (tooth ash and tooth particles) is derived from teeth and is composed mainly of hydroxyapatite (HA).[4] Plaster of Paris is readily available, easily sterilized, inexpensive, completely and rapidly resorbable, biocompatible, and well tolerated by tissues. In addition, plaster of Paris is osteoconductive. It is not osteogenic by itself, but it almost always becomes osteogenic in the presence of bone or periosteum.[2,5-8]

Fibrin sealants of various formulations have been used widely in surgical procedures throughout the body as a means of establishing hemostasis and for tissue approximation. Fibrin sealants have been used at such diverse locations as the heart and pericardial cavity, lung, bowel anastomoses, ovary, nasal sinuses, and skin. Formulations include both autologous preparations, which require peri-operative preparation, and prepackaged commercial kits.[9]

Osteoporosis in humans may be attributable to postmenopausal osteoporosis, secondary osteoporosis related to older age, the administration of corticosteroid hormones, diabetes, pregnancy, lactation, alcohol use, and

tobacco use, among other causes. Diet early in life may also play a role. As postmenopausal osteoporosis progresses immediately after menopause owing to the lack of estrogen produced in the ovaries, the early development of osteoporosis is possible in women with irregular menses or in those who have undergone artificial ovariectomy.[10] The lack of estrogen causes osteoporosis as a result of increased production of bone absorption factors, such as interleukin-1 and -3; decreased production of osteoprotegerin, thus inhibiting bone absorption and osteoclast formation; and decreased sensitivity to corticosteroid hormone-promoting bone formation due to decreased serum calcium concentration. Furthermore, estrogen can stimulate osteoblasts to form bone directly, and this bone formation sometimes decreases when the estrogen stimulus disappears.

This study examined the role of the fibrin sealant during early bone formation with tooth ash and plaster of Paris in ovariectomized rats.

Materials and Methods

Study Animals

This study was approved by the Animal Research Committee of Chosun University. Twelve-week-old Sprague-Dawley rats were selected for the study.

Calvarial critical-size defects (8 mm in diameter) were created in the rats. A critical-size defect is defined as the smallest intraosseous wound in a particular bone and species of animal that will not heal during the lifetime of the animal.

Sixty rats were randomly assigned to five groups, and each group was further divided into two subgroups, which were examined at 4 and 8 weeks after implantation of materials. The defect was filled in different manners: Group 1, no graft; Group 2, tooth ash-plaster graft; Group 3, Tisseel and tooth ash-plaster graft; Group 4, ovariectomy and tooth ash-plaster graft; and Group 5, ovariectomy, Tisseel, and tooth ash-plaster graft. Histologic sections were obtained for histomorphometric analysis of the defects at 4 and 8 weeks after surgery.

Tooth ash and plaster of Paris

Tooth ash was prepared from healthy teeth extracted from humans by washing the teeth in saline solution, ashing them in a furnace at 1200° C, and grinding the product into a powder using 100 mesh (0.149 mm). The high temperature was used to eliminate viruses, bacteria, and fungi. The resulting tooth ash was mixed with plaster of Paris (calcium sulfate hemihydrate, Gypsum Co., USA) at a 2:1 weight ratio. All the materials were sterilized with ethylene oxide before implantation and were mixed with physiological saline solution.

Fibrin sealant

The fibrin sealant, Tisseel Duo Quick (Baxter AG, Vienna, Austria), was used. It consists of deep-frozen Tisseel and thrombin solutions in two disposable syringes. The Tisseel solution contained 100-130 mg total protein, 75-115 mg clottable protein, 70-110 mg fibrinogen, 2-9 mg fibrinonectin, 10-50 IU factor XIII, 40-120 μg plasminogen, 3000 KIU bovine aprotinin, 10-20 mg human albumin, 15-35 mg glycine, 2-4 mg NaCl, 4-8 mg sodium citrate, 0.2-0.4 mg Polysorbate 80, 15 mg creatin monohydrate, and water for a total injection volume of 1 ml. The thrombin solution contained 500 IU thrombin, 50 mg human plasma protein, 5.88 mg $CaCl_2$, 10 mg NaCl, 3 mg glycine, and water for a total injection volume of 1 ml. The Tisseel and thrombin solutions were mixed to form a fibrin clot. The approximate time for resorption was about 2 weeks.

Ovariectomy

General anesthesia was induced in 12-week-old female Sprague-Dawley rats by intraperitoneal injection of ketamine HCl (10 mg/kg). After placing the anesthetized rat in the lateral decubitus position, the ovary was exposed by making a 1-cm incision through the skin, abdominal muscles, and peritoneum in the lateral abdominal area. Then, the ovarian tube was ligated using silk thread, the ovary was excised, and the incision was sutured. An ovariectomy was performed on the other side using the same method.

Implantation

For implantation, each rat was anaesthetized using ether inhalation. The head was shaved and sterilized using a conventional method, and 2% lidocaine HCl containing 1:100,000 epinephrine was injected for hemostatic purposes. An incision was made along the midline of the head to expose the skull. An hole 8 mm in diameter was drilled into the skull, removing the entire layer of the skull, using a 1/4 round bur. An already prepared

mixture of grafting materials was used to close the defect, and the skin was sutured over the skull. An intramuscular injection of 0.05 ml/kg gentamicin (Samwoo Pharmaceuticals, Korea) was administered to prevent infection after surgery. The rats were sacrificed at 4 and 8 weeks after surgery.

Histomorphometric Analysis

After a rat was sacrificed using excess ether inhalation, a bone sample was obtained from the implant site, fixed in 10% neutral formalin for 72 hours, and decalcified in nitric acid for 4 hours. The bone sample was cut into 3-mm-thick sections, which were washed in running water. Each bone sample was treated using an autoprocessing machine (Hypercenter XP, Shandon, UK). After paraffin embedding, each section was cut into 4- to 5-㎛ slices, which were stained with hematoxylin-eosin and Goldner's trichrome and observed under an optical microscope.

Computer-assisted histomorphometry was used to measure the amount of bone formed at the defect site. Images were taken using a Polaroid digital microscope camera (Polaroid, Cambridge, MA, USA) and were analyzed using Image Pro Plus (Media Cybernetics, LP, Silver Spring, MD, USA). Images of each tissue sample were analyzed.

Quantitative Analysis

The Kruskal-Wallis test was used to compare the subgroups and groups overall; the Mann-Whitney U test was used to compare the two subgroups within each group. Values of $p < 0.05$ were considered statistically significant.

Results

Histologic Results

Group 1

Four weeks: There was slight new bone formation, limited to the margin of the bony defect (Fig 1).

Eight weeks: No significant difference was seen in new bone formation compared with that at 4 weeks. Very limited new bone formation was observed at the margin of the bony defect (Fig 2).

In Group 1, the difference in new bone formation between the subgroups was not significant ($p = 0.104$).

Group 2

Four weeks: New bone formation was observed throughout the bony defect. It was woven, anastomosed, and organized. In some areas, it was anastomosed, organized, continuous, and compact/dense (Fig 3).

Eight weeks: Compared with 4 weeks, new bone formation at 8 weeks was more significant not only at the margin but also at the center of the bony defect. The new bone was significantly anastomosed, organized, continuous, and compact/dense (Fig 4).

In Group 2, the degree of new bone formation was not significantly different between 4 and 8 weeks ($p = 0.233$).

Group 3

Four weeks: New bone formation was limited to the margin of the bony defect. The center of the bony defect was filled with inflamed fibrotic tissue and implanted chips. In some cases, continuous new bone formation was observed at the margin of the bony defect (Fig 5).

Eight weeks: Compared with the bone at 4 weeks, the centripetal pattern of new bone formation was increased. The new bone was more organized, continuous, and compact/dense. However, the center of the bony defect

showed no new bone formation and was filled with fibrotic tissue (Fig 6).
In Group 3, the degree of new bone formation did not differ significantly between 4 and 8 weeks (p = 0.276).

Group 4
Four weeks: New bone formation was limited to the margin of the bony defect and lagged behind that in group 3. The center of the bony defect showed no new bone formation and was filled with inflamed fibrotic tissue and implanted chips (Fig 7).
Eight weeks: No significant difference was seen in the degree of new bone formation between 4 and 8 weeks, although the new bone was more organized, continuous, and compact/dense in some areas. However, the center of the bony defect was filled with inflamed fibrotic tissue and implanted chips (Fig 8).
In Group 4, the degree of new bone formation did not differ significantly between 4 and 8 weeks (p = 1.000).

Group 5
Four weeks: New bone formation was limited to the margin of the bony defect. The center of the bony defect was filled with inflamed fibrotic tissue and implanted chips (Fig 9).
Eight weeks: Compared with the bone at 4 weeks, the centripetal pattern of new bone formation had increased slightly. The center of the bony defect was filled with inflamed fibrotic tissue and implanted chips (Fig 10).
In Group 5, the degree of new bone formation did not differ significantly between 4 and 8 weeks (p = 0.345).

Histomorphometric Results

There was a significant difference (p = 0.000) in new bone formation among the 4-week groups. At 4 weeks, there were significant differences between groups 1 and 2 (p = 0.004), 1 and 3 (p = 0.004), 1 and

4 (p = 0.004), 2 and 4 (p = 0.004), 2 and 5 (p = 0.004), 3 and 4 (p = 0.045), and 4 and 5 (p = 0.013). Similarly, there was a significant difference (p = 0.000) in new bone formation at 8 weeks. At 8 weeks, there were significant differences in new bone formation between groups 1 and 2 (p = 0.004), 1 and 3 (p = 0.006), 1 and 4 (p = 0.004), 1 and 5 (p = 0.006), 2 and 4 (p = 0.005), 2 and 5 (p = 0.006), 3 and 4 (p = 0.022), 3 and 5 (p = 0.009), and 4 and 5 (p = 0.022). The new bone formation activities at 4 and 8 weeks are summarized in Table 1.

Table 1. RESULTS FOR NEW BONE FORMATION (unit: ㎟)

	Group 1	Group 2	Group 3	Group 4	Group 5
4 weeks	0.04 ± 0.03	2.28 ± 0.26*	1.85 ± 0.88*	0.86 ± 0.49*,+,$	0.20 ± 0.15+,#
8 weeks	0.07 ± 0.03	2.67 ± 0.60*	2.19 ± 0.99*	0.87 ± 0.54*,+,$	0.29 ± 0.09*,+,$,#

Group 1, no graft; Group 2, tooth ash-plaster graft; Group 3, Tisseel and tooth ash-plaster graft; Group 4, Ovariectomy and tooth ash-plaster graft; Group 5, Ovariectomy, Tisseel, and tooth ash-plaster graft.

* Statistically significant difference relative to Group 1, $p < 0.05$.

+ Statistically significant difference relative to Group 2, $p < 0.05$.

$ Statistically significant difference relative to Group 3, $p < 0.05$.

Statistically significant difference relative to Group 4, $p < 0.05$.

Discussion

Various biomaterials are used in dental surgery, and new biomaterials are continually being developed and introduced. Biomaterials are used for transplantation in oral, maxillofacial, periodontal, and implant surgery to restore hard and soft tissue defects, for guided tissue regeneration, and for esthetic purposes.[2-4,11-23]

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 eliminate additional surgical procedures and risks, bone grafting materials and substitutes are used as alternative grafting materials for ridge augmentation.[24] The ideal grafting materials should (1) be biocompatible, non-carcinogenic, and non-allergenic; (2) show early vascularization; (3) be replaced by new host bone tissue; (4) have strength, resist infection, and be sterile; and (5) undergo surface resorption by the host.[18,19]

Plaster of Paris, which is made of calcium sulfate hemihydrate, is a commonly used grafting material because of its long history of safe use and characteristic complete resorption followed by bone formation.[25] Plaster of Paris is easy to use and disinfect during surgery. It is also inexpensive and can even hinder the fluidity of the implant. Its average solidity enables it to resist rupture after consolidation. Although plaster of Paris cannot guide and induce new bone formation by itself, its resorptive characteristics and biocompatibility assist as a bonding agent when mixed with other materials. In addition, the resorption rate increases and invasion around the tissue is facilitated as the density of the material decreases.[2-4,11,15-23]

Bone-enhancing local factors may have effects secondary to the inevitable surgical trauma or application of peripheral blood, autologous bone marrow,

or the fibrin adhesive system (FAS). Although surgical trauma may trigger a bone response, this does not indicate that further trauma is beneficial for bone healing; the opposite seems to be the case.[26] Albrektsson et al[27] found no positive effects of the FAS. Findings from examinations, including microradiographic and microdensitometric measures, showed that the FAS-treated sites contained less bone than control sites, which had been treated with autologous blood and marrow. These varying opinions indicate that more evidence should be gathered before recommending FAS treatment to accelerate bone regeneration. Kalebo et al[26] inserted bone harvest chamber (BHC) implants in the proximal tibial metaphysis of rabbits to study the effects of autologous bone marrow and the FAS on new bone formation. The specimens were quantified using microradiography-videodensitometry and subjected to further histological examination. The amounts of bone formed in the two chambers treated equally in the same animal were compared. The authors concluded that the conditions for bone regeneration in an osseointegrated titanium implant are excellent and are minimally influenced by locally applied hemostasis, peripheral blood, and autologous bone marrow. By contrast, FAS pretreatment was found to impair bone formation.

In most studies demonstrating a healing-promoting effect of fibrin scaffolds, fibrin clots mixed from the blood of the laboratory animal were used. However, even with these autologous clots, some investigator[28] did not get tissue ingrowth into the clot. Therefore, the theory that the fibrin adhesive acts as a scaffold for tissue ingrowth is apparently not valid in general. In our study, a commercial fibrin gel seemed to function as a hemostatic barrier, reducing the endogenous fibrin response.

Many studies of osteoporosis and intraoral bone loss have reported that bone loss, such as alveolar bone loss, is greater in patients with osteoporosis than in healthy individuals.[29-31] After measuring the trabecular bone matrix density in the mandible, Cao et al[32] reported that the amounts of cortical bone and trabeculae decreased after ovariectomy, with a marked

decrease in the number of trabeculae. Tanaka et al[33] observed that, although no problem existed with the healing itself, the amount of new bone formation was decreased significantly, as the amount of bone absorption exceeded bone production during the process of healing after maxillary molar extraction in ovariectomized rats.

The most commonly used laboratory animal for osteoporosis research is the rat because it is cost effective and convenient to handle. Ovariectomized Sprague-Dawley rats at around 12 weeks of age are frequently used as a model of postmenopausal osteoporosis.[34] However, there are differences between the human and rodent skeletons with respect to bone and mineral metabolism, lamellar bone, and capacity for bone remodeling. Experimental procedures, such as sampling body fluids, removing tissue, and performing surgery, are difficult in the rat because of its small size.[35,36]

Increased resorption is attributable to secondary hyperparathyroidism as a result of the inhibition of intestinal calcium absorption by corticosteroids. The defect in bone formation consists of depressed osteoblast activity with a reduced bone formation rate at the tissue and cell levels. Furthermore, it has been reported that the active formation period was shortened.[37] Vitro studies have confirmed the direct effect of corticosteroids on osteoblasts, and glucocorticoid receptors have been demonstrated in osteoblast-like cells.

Conclusion

To restore a severe bony defect, such as a critical defect, a graft is needed to induce new bone formation. Compared with controls, a significant difference was observed in new bone formation in grafts with tooth ash, tooth ash and Tisseel, tooth ash after ovarian resection, and tooth ash and Tisseel after ovarian resection. Better formation of new bone was observed with tooth ash alone and with tooth ash and Tisseel combined, and the difference between the two was not statistically significant. Therefore, tooth ash alone or tooth ash and Tisseel combined may be used depending on the situation.

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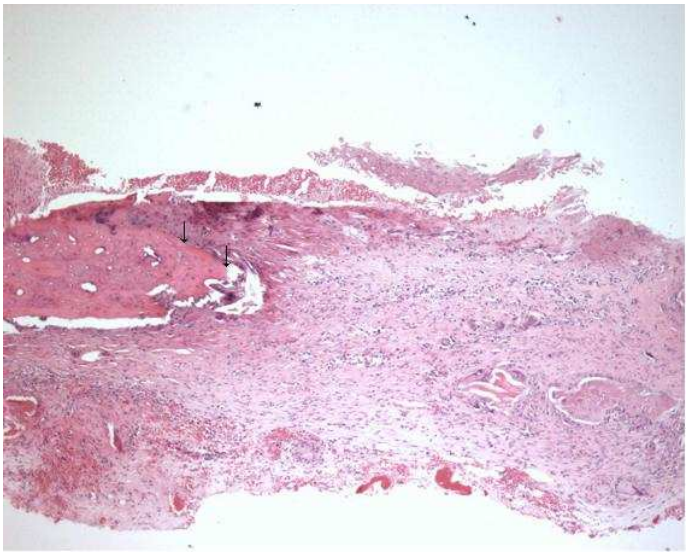

FIGURE 1. Photomicrograph of Group 1 at 4 weeks. Tiny areas of new bone formation (arrows) are seen around the defect margin. The remaining defect is filled with fibrous tissue (hematoxylin and eosin stain, original magnification X40).

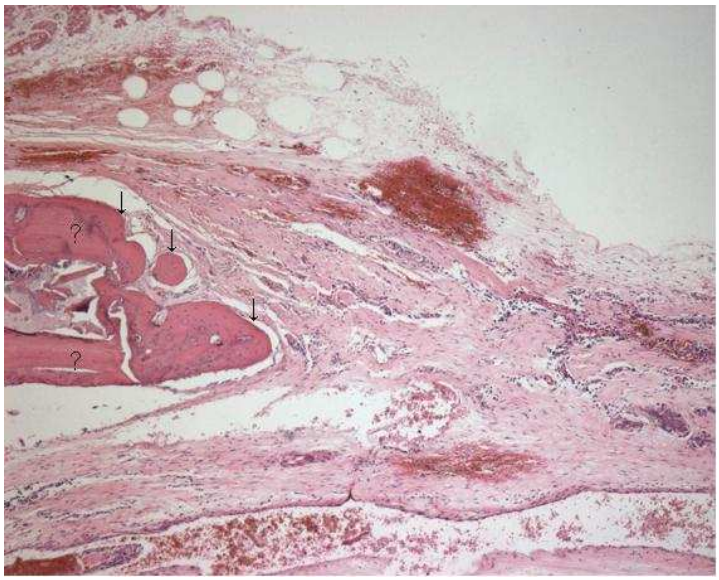

FIGURE 2. Photomicrograph of Group 1 at 8 weeks. Tiny areas of new bone formation (arrows) are seen around the defect margin. The remaining defect is filled with fibrous tissue. Pre-existing bone (asterisks) is present (hematoxylin and eosin stain, original magnification X40).

FIGURE 3. Photomicrograph of Group 2 at 4 weeks. Centripetal, thick, woven bone (arrows) is observed. Pre-existing bone (asterisk) and implant material (thick arrows) are seen (hematoxylin and eosin stain, original magnification X40).

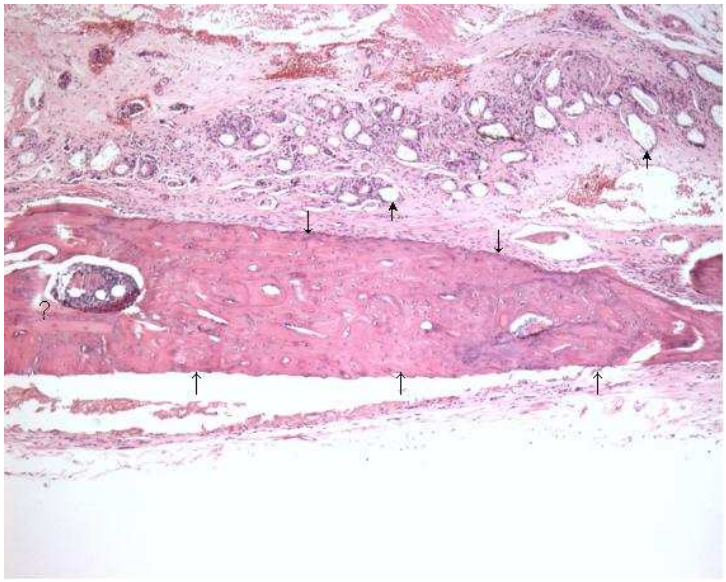

FIGURE 4. Photomicrograph of Group 2 at 8 weeks. The defect is filled with well-formed, continuous, thick, woven bone (arrows). Pre-existing bone (asterisk) and implant materials (thick arrows) are seen (hematoxylin and eosin stain, original magnification X40).

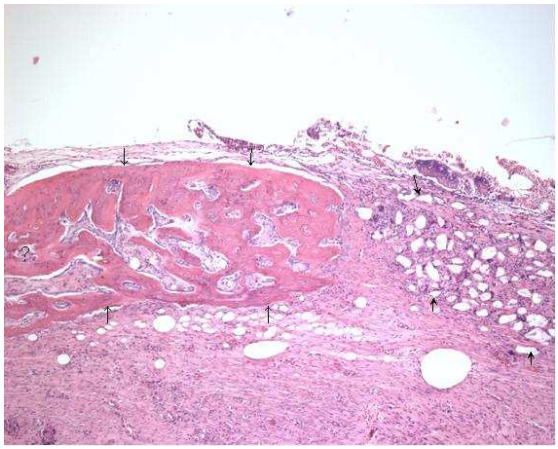

FIGURE 5. Photomicrograph of Group 3 at 4 weeks. Centripetal, anastomosing, thick, woven bone formation (arrows) is seen. Pre-existing bone (asterisk) and implant materials (thick arrows) are present (hematoxylin and eosin stain, original magnification X40).

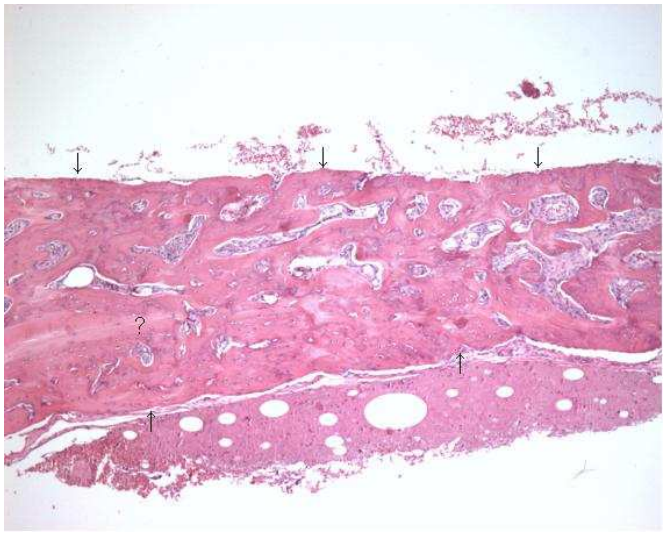

FIGURE 6. Photomicrograph of Group 3 at 8 weeks. Centripetal, thick, woven bone formation (arrows) is noted. Pre-existing bone (asterisk) is seen (hematoxylin and eosin stain, original magnification X40).

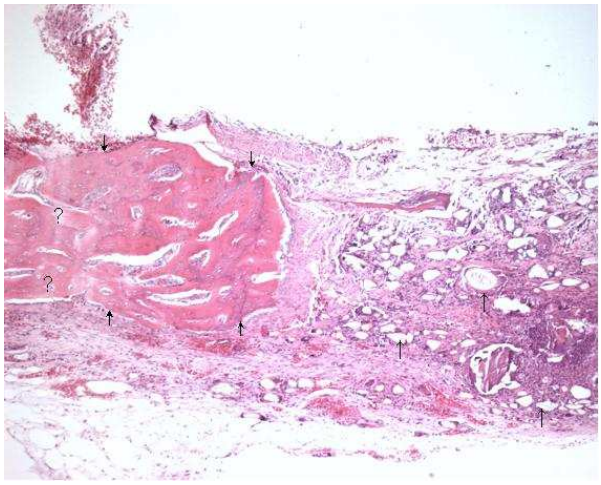

FIGURE 7. Photomicrograph of Group 4 at 4 weeks. New bone (thick arrows) has formed around the defect margin. Pre-existing bone (asterisks) and implanted chips (arrows) are visible in the defect (hematoxylin and eosin stain, original magnification X40).

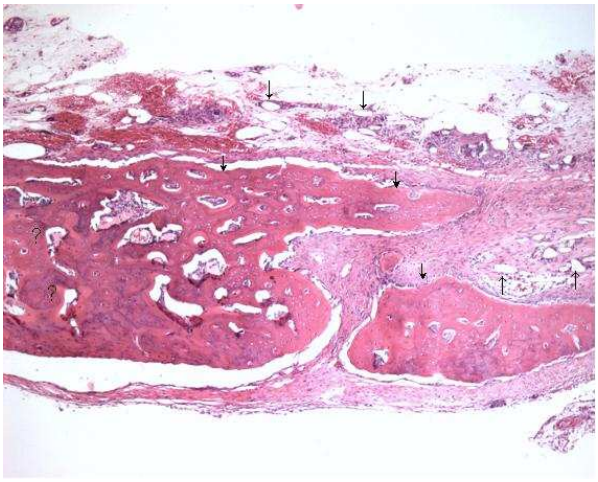

FIGURE 8. Photomicrograph of Group 4 at 8 weeks. Centripetal, anastomosing, thick, woven bone formation (thick arrows) is noted. The center of the bony defect is filled with fibrous tissue and discontinuous new bone. Pre-existing bone (asterisks) and implanted chips (arrows) are seen (hematoxylin and eosin stain, original magnification X40).

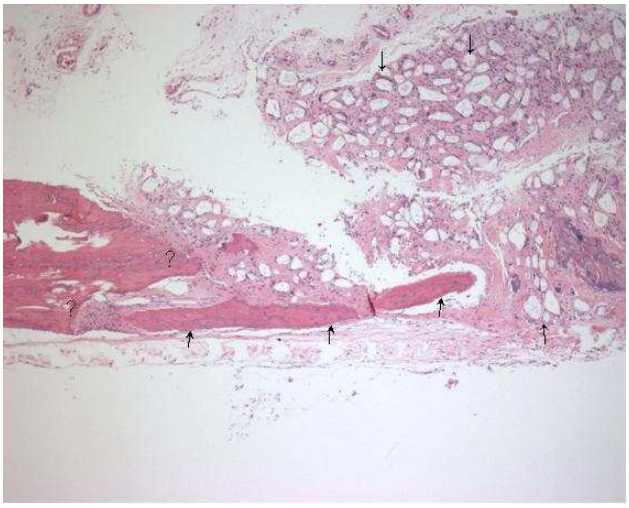

FIGURE 9. Photomicrograph of Group 5 at 4 weeks. Thin, membranous, new bone formation (thick arrows) is seen limited to the defect margin. The center of the bony defect is filled with fibrous tissue and implanted chips (arrows) without new bone. Pre-existing bone (asterisks) is visible (hematoxylin and eosin stain, original magnification X40).

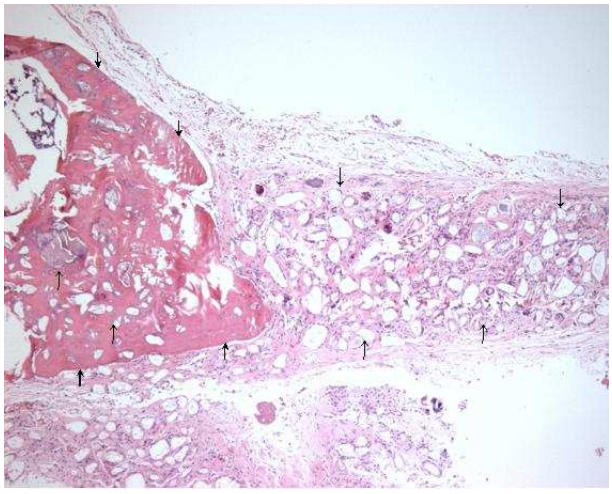

FIGURE 10. Photomicrograph of Group 5 at 8 weeks. Thick, anastomosing, woven bone formation (thick arrows) is seen limited to the defect margin. The center of the bony defect is filled with fibrous tissue and implanted chips (arrows) with no new bone (hematoxylin and eosin stain, original magnification X40).