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Effect of high local concentrations of antibiotics on early bone formation of tooth ash and plaster of Paris in ovariectomized rat

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난소적출 백서에서 치아 회분말 및 연석고 매식시 고농도의
항생제 국소 적용후 골형성에 관한 효과

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난소적출 백서에서 치아 회분말 및 연석고 매식시 고농도의 항생제 국소 적용후 골형성에 관한 효과

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본 연구에서는 난소적출 백서에서 치아 회분말 및 연석고를 이용한 골다공증 환자 골결손 부위 재건시 항생제 사용에 따른 임상적 유용성을 알아보기 위하여, 난소적출술(ovariectomy)을 시행한 백서의 골결손 부위에 치아 회분말 및 연석고를 매식한 후 항생제를 국소적으로 적용한 후 시간에 따른 골형성 정도를 비교 분석하고자 한다.

실험동물은 동일조건 하에서 일정기간 사육한 체중 200mg 이상의 Sprague-Dawley 흰쥐 48마리를 사용하였다. 노출시킨 두개골의 정중앙부에 #1/4 round bur를 이용하여 직경 1cm 크기의 원형으로 전층골 결손을 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 연석고 (무게비 2:1)를 Gentamicin과 혼합하여 이식하였으며, 제4군은 난소적출술을 시행한 후 치아 회분말과 치과용 연석고 (무게비 2:1)를 Gentamicin과 혼합하여 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 흡수정도, 신생골의 형성, 염증반응 유무 등의 치유과정을 분석하였다.

통계학적인 분석은 SPSS (SPSS for Window version 7.5, Korea)를 이용한 nonparametric Wilcoxon rank test를 사용하였다. P값이 0.05보다 작은 경우에 통계학적으로 유의성이 있는 것으로 간주하였다.

각 주에 따른 군별 비교에서 4주의 경우 역시 유의한 신생골 형성의 차이를 보였으며, 1-2군간, 1-3군간, 1-4군간, 2-3군간, 2-4군간, 3-4군간 비교에서 신생골 형성의 유의한 차이가 있었다. 8주의 경우 역시 유의한 신생골 형성의 차이를 보였으며, 1-2군간, 1-3군간, 1-4군간, 2-3군간, 2-4군간 비교에서 신생골 형성의 유의한 차이가 있었다.

임계한계 이상의 골결손을 수복하기 위해서는 골형성 유도물질의 이식이 필요한데, 치아 회분말과 식염수, 치아 회분말과 gentamycin, 난소절제 후 치아 회분말과 gentamycin을 이식한 경우 대조군에 비하여 매우 유의한 골형성 증가 소견을 보였다. 그러나 치아 회분말과 식염수를 사용한 경우 가장 좋은 결과를 보였고, 치아 회분말과 gentamycin 사용시 치아 회분말과 식염수를 사용한 경우보다 신생골 형성 정도가 약간 저조하였다. 한편 난소절제 시행여부에 따라 치아 회분말과 gentamycin을 평가하였을 때 비록 8주군의 경우 통계학적 유의성은 없었으나 4주군의 경우에는 난소절제에 의하여 통계학적으로 유의한 골형성 억제가 초래되었다.

Introduction

Osteoporosis produces bone fragility, a loss of structure, and increased sensitivity to fractures as the disease progresses because of decreased bone density and weakening of bone microstructure.[1] In recent years, extended life expectancy has resulted in a dramatic increase in the incidence of osteoporosis, resulting in increased long bone and spinal pathology. Furthermore, many researchers have reported that bone loss due to osteoporosis is also affecting the jawbones.[2,3]

Autogenous bone may be used for augmentation rather than allogeneic or alloplastic materials or guided bone regeneration (GBR) techniques.[4-6] A variety of grafting techniques as well as bone graft donor sites are available.[4]

Among the grafting materials, autogenous corticocancellous bone has been termed the gold standard. Osteoinductive factors transferred within the autograft are thought to favor osteogenesis at the augmented area to promote enhanced bone formation and graft stabilization.[7] However, the additional surgical procedure required for autograft harvesting is associated with donor site morbidity. To overcome this problem, substitute materials for autogenous bone, such as demineralized freeze-dried bone, calcium sulfates, calcium phosphates of different origin, and xenografts, have been introduced and used for bone augmentation.[7-14] These alternative materials are readily available, easy to handle, disposable, and do not demand additional surgical procedures.[14]

A method of preventing particle scatter and the loss of graft material is needed. Plaster of Paris has been used as a binder to carry and retain graft particles in periodontal osseous defects. The material is biocompatible and completely resorbed at a fairly rapid rate. An additional benefit is that the plaster can serve as a vehicle for delivery of an antibiotic-graft combination.[15,16-28]

The use of plaster of Paris as a binder may have facilitated the response to grafting by preventing particle scatter and facilitating graft retention and by permitting more precise graft placement and adaptation.[15] Nilveus et al.[29] suggested that the loss of graft material during the healing phase is one factor that can contribute to the failure of bone filling in furcation areas. The material chosen to bind graft particles should at least have a neutral effect and not interfere with the healing response. In addition to its biocompatibility and resorption properties, plaster of Paris has the ability to carry a chemical that can facilitate the regenerative process, such as doxycycline.

Osteoconduction is characterized as bone growth by apposition from the surrounding bone and provides a physical matrix or scaffolding suitable for the deposition of new bone. The most common osteoconductive bone graft materials are alloplasts and xenografts. Bioactive ceramics are the largest family of alloplasts and include calcium phosphates such as hydroxyapatite (HA), which has experienced wide clinical use as a bone substitute in particulate and block forms. HA has also been frequently used as a representative osteoconductive material.[17]

The successful management of open fractures or chronic osteomyelitis often requires serial debridement of the wound and administration of antibiotics. Currently, several techniques for adequate debridement have proven to be effective. Antibiotics, an adjunct to thorough debridement, are usually administered intravenously, but this maneuver may be ineffective as well as potentially toxic. Theoretically, the blood supply of infected or traumatized bone is compromised, and this state prevents adequate local tissue levels of the antibiotic.[30]

Bone grafting plays an important role in reconstructing infected tibial nonunions.[31] Local therapy of antibiotic beads and impregnating antibiotics in the bone graft must be conducted according to the microbiological sensitivity tests.[31]

Several factors have been shown experimentally to affect both the amount and duration of the elution of antibiotic from materials. These include the type of combination of antibiotics, concentration of the antibiotics, surface area available for diffusion, properties of the implanted material, and method of elution.[30]

The present study examined the role of the concentration of antibiotics during early bone formation of 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, Gwangju, Korea. Twelve-week-old Sprague-Dawley rats were selected for the study.

An 8-mm-diameter calvarial, critical-size defect was created in each rat. Forty-eight rats were randomly assigned to four groups, and each group was further divided into two subgroups based on their evaluation at either 4 weeks or 8 weeks after implantation. The defect was filled in different manners: Group 1, non-ovariectomy and non-graft group; Group 2, non-ovariectomy and saline-soaked tooth ash-plaster graft group; Group 3, non-ovariectomy and gentamicin-soaked ash-plaster graft group (gentamicin, 15 mg/rat; Daesung Microbiological Labs Co., Euiwang, Korea); Group 4, ovariectomy and gentamicin-soaked ash-plaster graft group (gentamicin, 15 mg/rat). A defect in the skull measuring 8 mm in diameter requires 0.25 g of tooth ash. Histological sections were obtained for histomorphometric analysis of the defects at 4 and 8 weeks after surgery.

Study Materials

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

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 at the lateral abdominal area. The ovarian tube was then ligated using silk thread. The ovary was excised, and the incision was closed by suturing. Ovariectomy was also performed at the other side using the same method.

Implantation

Each rat was anaesthetized using ether inhalation. The head was shaved and sterilized using the 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 8-mm-diameter hole was drilled in the skull, removing the entire layer of the skull, by using a 1/4 round bur. An already prepared mixture of implantation materials was used to close the defect. The skin was then sutured over the skull. An intramuscular injection of 0.05 ml/kg gentamicin (Samwoo Pharmaceuticals, Daejun, 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 around 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, Waterford, UK). After paraffin embedding, each section was cut into 4 to 5 μm slices, which were stained with hematoxylin-eosin and Goldner's

trichrome and were 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 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 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 at 4 weeks

New bone formation was limited to the margin of the bony defect (Fig 1).

Group 1 at 8 weeks

No significant difference was seen compared to the 4-week group. New bone formation was limited to the margin of the bony defect (Fig 2).

In Group 1, no significant difference was seen in the degree of new bone formation ($p = 0.104$).

Group 2 at 4 weeks

A centripetal pattern was observed in the new bone formation. An anastomosing pattern of woven new bone was seen. In some areas, bone formation was more organized, continuous, and compact/dense (Fig 3).

Group 2 at 8 weeks

Compared to the 4-week group, the degree of new bone formation was increased significantly. The anastomosing pattern of new bone became more intense with the bone becoming organized, continuous, and compact/dense (Fig 4).

In Group 2, the degree of new bone formation showed no significant difference between 4 and 8 weeks ($p = 0.138$).

Group 3 at 4 weeks

Bone formation was limited to the margin of the bony defect. Fibrotic tissues showing chronic inflammation filled the center of the bony defect. In some cases, new bone formation was continuous in the margin of the bony defect (Fig 5).

Group 3 at 8 weeks

Compared to the 4-week group, the centripetal pattern of new bone formation increased further. New bone formation was relatively organized, continuous, and compact/dense. The center of the bony defect was filled with fibrotic tissues showing no new bone formation (Fig 6).

In Group 3, no significant difference was present between 4 and 8 weeks ($p = 0.416$).

Group 4 at 4 weeks

New bone formation was limited to the margin of the bony defect. The center of the bony defect showed no new bone formation and was filled with implanted chips and fibrotic tissues showing chronic inflammation (Fig 7).

Group 4 at 8 weeks

Compared to the 4-week group, a small centripetal pattern of new bone formation was observed. The center of the bony defect was filled with implanted chips and fibrotic tissues showing chronic inflammation (Fig 8).

In Group 4, the degree of new bone formation was statistically significant between 4 and 8 weeks ($p = 0.046$).

Histomorphometric Results

No specific difference was observed when the degree of inflammation was compared between the gentamicin and physiological-saline groups. However, the ash and saline group showed significant new bone formation with less fibrotic tissues accompanying inflammatory cells compared to the ash and gentamicin group. The ash and gentamicin showed less new bone formation and many unabsorbed implanted chips, indicating chronic inflammation. However, acute inflammation and pyogenic inflammation coming from other sources of infection were not observed.

When each week was compared in each group, new bone formation showed a significant difference (p = 0.000) in the 4-week group. A significant difference was seen between groups 1 and 2 (p = 0.004), 1 and 3 (p = 0.004), 1 and 4 (p = 0.004), 2 and 3 (p = 0.004), 2 and 4 (p = 0.004), and 3 and 4 (p = 0.010). Also in the case of the 8-week group. A significant difference was seen in new bone formation between groups 1 and 2 (p = 0.004), 1 and 3 (p = 0.006), 1 and 4 (p = 0.004), 2 and 3 (p = 0.006), and 2 and 4 (p = 0.004).

The new bone formation activities at 2 and 4 weeks are summarized in Table 1.

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

	Group 1	Group 2	Group 3	Group 4
4 wks	0.067 ± 0.033	2.283 ± 0.256*	0.405 ± 0.148*,+	0.235 ± 0.029*,+
8 wks	0.038 ± 0.026	2.667 ± 0.602*	0.600 ± 0.236*,+	0.378 ± 0.136*,+

Group 1, non-ovariectomy and non-graft group; Group 2, non-ovariectomy and saline-soaked tooth ash and plaster graft group; Group 3, non-ovariectomy and gentamicin-soaked ash and plaster graft group (Gentamicin®, 15 mg/rat; Daesung Microbiological Labs); Group 4, ovariectomy and gentamicin-soaked ash and plaster graft group (Gentamicin®, 15 mg/rat; Daesung).

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

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

Discussion

Tooth ash, which consists mainly of HA, is easily obtained from extracted teeth. Plaster of Paris is easy to obtain and sterilize, inexpensive, and completely absorbed. Experimental studies have shown that a mixture of tooth ash and plaster of Paris promotes bone healing while increasing the stability of the tooth ash and that the mixture can be used to restore hard tissue defects, for guided bone regeneration, in oral and maxillofacial surgery, and as a barrier membrane.[16-28]

The greatest advantages of plaster of Paris are its capacity to bond firmly with adjacent bone and its ability to guide new bone formation, which occurs in association with the resorption of the plaster.[32] Plaster of Paris has been used in the treatment of bone defects in the fields of orthopedics, otorhinolaryngology, and oral and maxillofacial surgery. De Leonardis and Pecora (1999)[33] reported that the overall success rate for 130 placed implants at one year postimplantation was 98.5%. In that study, a sinus augmentation procedure was performed using calcium sulfate as the sinus grafting material, indicating calcium sulfate's suitability.

The administration of antibiotics through a variety of routes is a widely accepted method of managing or avoiding wound infection. When bacterial contamination is likely, antibiotics can also be added to the alloplastic material or the autograft. The use of gentamicin-loaded bone cement decreased reinfection in revision arthroplasty and lowered the infection rates in primary arthroplasty. The management of open fractures or osteomyelitis requires serial wound debridement and intravenous antibiotics, such as aminoglycosides. These antibiotics, however, are nephrotoxic and ototoxic at high serum concentrations. For this reason, we have been using the antibiotics locally to minimize systemic adverse effects while eliminating infection. Whether the locally applied antibiotics are inhibitory on the bone regeneration is not clear.[34]

Chan et al.[31] discussed the effects of antibiotic-impregnated bone grafting in infection elimination and bone incorporation. They reported that the antibiotic-impregnated autogenous cancellous bone grafting compared with the pure cancellous bone grafting for the reconstruction of small (2-4 cm) infected tibial defects. Bony union was achieved within 3 to 7 months in the antibiotic-impregnated bone grafting group, and the union rate was 100%. The pure cancellous bone grafting group had a 98% union rate. There was no statistical difference between the union rates of the two groups. The impregnating antibiotics did not appear to have any adverse effects on bone graft incorporation.

Although the local administration of aminoglycosides appears promising clinically, the toxic effects of high local aminoglycoside concentrations on bone and bone healing have not been determined.[35] In an effort to elucidate the effects of high concentrations of aminoglycoside antibiotics on the local incorporation of bone graft, Lindsey et al. studied the incorporation of tobramycin-impregnated bone grafts.[36] The incorporation of tobramycin-impregnated cancellous bone graft (30 mg/g of bone graft) appeared similar histologically, radiographically, and biomechanically to that of defects grafted with cancellous bone alone.

The use of locally applied aminoglycosides has generated increased bone, particularly for the treatment of open fractures and osteomyelitis.[35] This technique delivers high local concentrations of aminoglycosides, frequently >400 g/L, while avoiding systemic toxicity.[19] However, Miclau et al.[35] suggested that it may be beneficial to maintain local tobramycin levels at 200 g/L to avoid levels toxic to bone.

Petri[37] histologically demonstrated the uneventful healing of allografts combined with cephalothin and tobramycin. He suggested the combination could be used in contaminated oral maxillofacial fractures. McLaren and Miniaci[38] reported an in vivo study that explored the effectiveness of antibiotic delivery by using morselized cancellous bone graft as a vehicle for powdered tobramycin. They concluded that local treatment with antibiotic beads could

be achieved for up to 3 weeks without toxic serum concentrations. McLaren[39] again found low concentrations of antibiotic in serum and urine, despite high local tissue levels. This finding prompted McLaren to recommend tobramycin-impregnated cancellous bone graft as an effective method of acutely reconstructing bone loss in compound fractures. Lindsey et al.[40] evaluated the effects of antibiotics on bone graft healing in vivo. They concluded that antibiotic-impregnated bone graft for the treatment of skeletal defects does not roentgenographically, microradiographically, histologically, or biomechanically alter the healing process that occurs with bone graft alone.

Pedersen and Lund[41] indicated that gentamicin has a depressive effect on bone turnover because it reduced ^{45}Ca and titrated proline, which represent mineral and matrix resorption and alkaline phosphatase activity, respectively, and are assumed to be markers of osteoblast activity (i.e., bone formation).[42]

Locally applied aminoglycosides are reported to result in local levels that are >600 times higher than minimal toxic serum levels without producing deleterious systemic effects.[35] The most relevant study of aminoglycoside toxicity to the musculoskeletal system is that on fibroblasts.[35]

Aminoglycoside-containing materials, including plaster of Paris and cancellous bone graft, have been used to deliver the antibiotic locally. In vitro, plaster of Paris is at least as effective as polymethylmethacrylate (PMMA) in the local delivery of aminoglycoside.[35]

Kim et al.[34] suggested that a saline-mixed bone graft is an effective material for the regeneration of osseous defects. However, we believe that when infection is present or the gingiva is also affected, the use of antibiotics would not interfere with bone formation because healing is promoted by the prevention of infection.

In this study, progressive regeneration of new bone was observed at each stage according to the lapse of time.

From the results of this study, impregnating antibiotics did not seem to have any adverse effects on bone graft incorporation in ovariectomized cases, whereas antibiotic-impregnated bone grafting resulted in eliminating infection. We concluded that this treatment protocol provides rapid recovery from osteomyelitis and is an effective and safe method for the management of small infected defects.

Future studies in this area are needed to determine an optimum time for the reentry procedure, indicate the ideal concentration of gentamicin, and identify binders that have a positive rather than a neutral or negative influence on the regenerative healing response.

Conclusion

A graft is needed to induce new bone formation to restore a critical or more severe bony defect. Compared to controls, a significant increase in new bone formation was seen with ash and saline, ash and gentamicin, and ash and gentamicin after ovariectomy. The best result was seen with ash and saline, whereas the effect was slightly less with ash and gentamicin compared to ash and saline. However, the effect of ash and gentamicin was evaluated according to ovariectomy. The results showed that ovariectomy significantly inhibited new bone formation in the 4-week group but not in the 8-week group.

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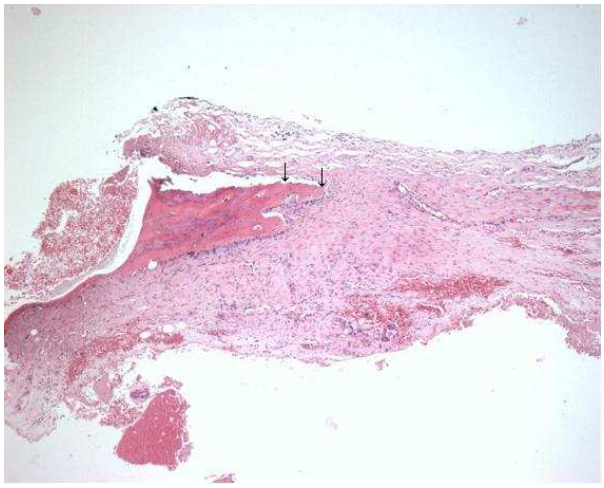

FIGURE 1. Photomicrograph of Group 1 at 4 weeks. Tiny new-bone formation (arrows) around the defect margin was observed. The remaining defect area was filled with fibrous tissue (hematoxylin and eosin stain, original magnification X40).

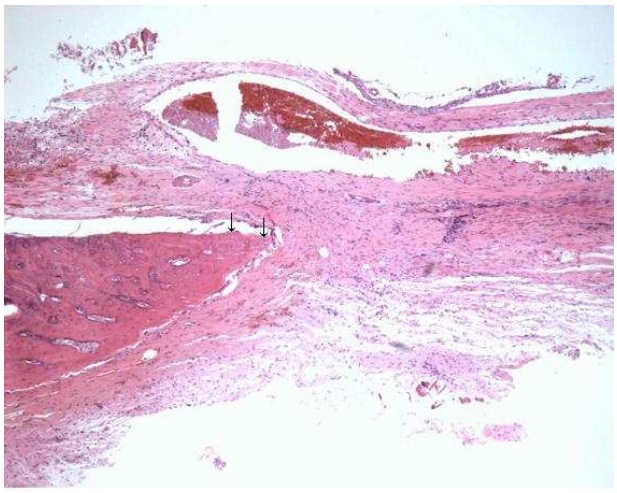

FIGURE 2. Photomicrograph of Group 1 at 8 weeks. Tiny new-bone formation (arrows) around the defect margin was observed. The remaining defect area was filled with fibrous tissue (hematoxylin and eosin stain, original magnification X40).

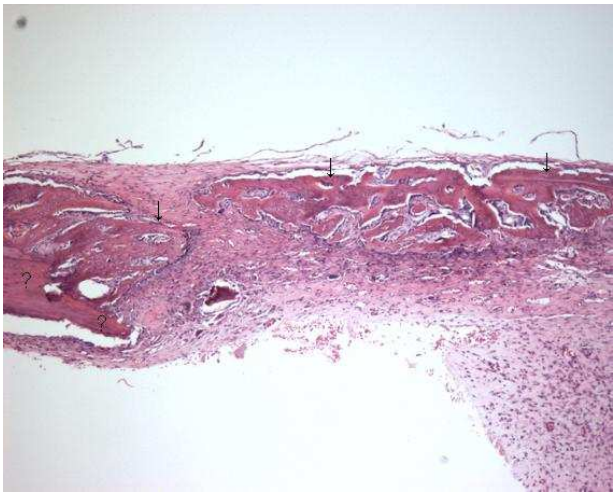

FIGURE 3. Photomicrograph of Group 2 at 4 weeks. Centripetal anastomosing woven bone (arrows) was identified. Preexisting bone (asterisks) was observed (hematoxylin and eosin stain, original magnification X40).

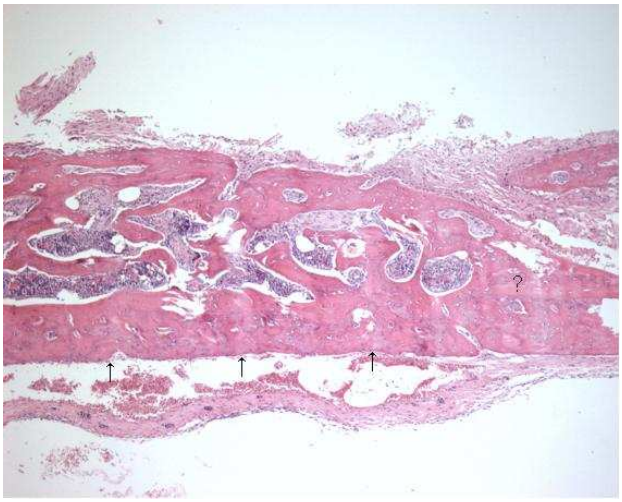

FIGURE 4. Photomicrograph of Group 2 at 8 weeks. The defect was filled with well-formed continuous woven bone (arrows). Preexisting bone (asterisk) was observed (hematoxylin and eosin stain, original magnification X40).

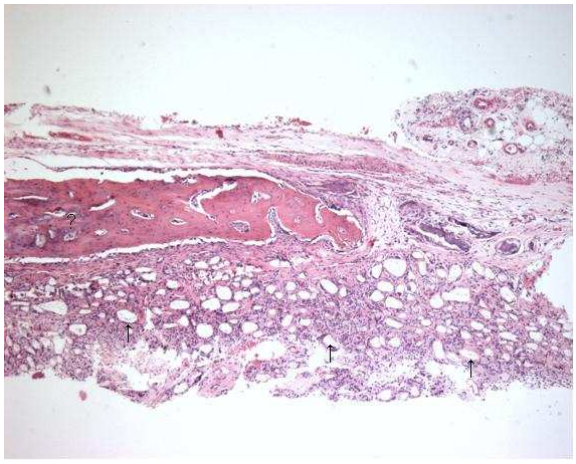

FIGURE 5. Photomicrograph of Group 3 at 4 weeks. Centripetal continuous thick woven-bone formation was noted. The central area of the bony defect was filled with fibrous tissue without new bone. Preexisting bone (asterisk) and implanted chips (arrows) in the lower portion were observed (hematoxylin and eosin stain, original magnification X40).

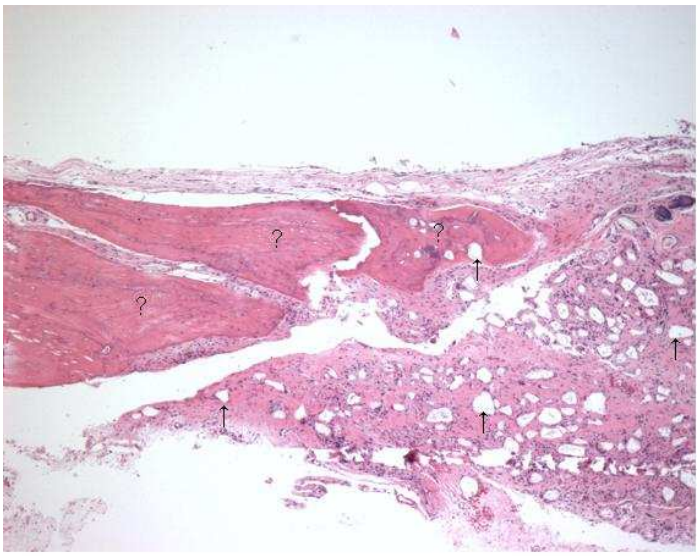

FIGURE 6. Photomicrograph of Group 3 at 8 weeks. Centripetal thick continuous woven-bone formation (asterisks) was noted. Implanted chips (arrows) were found in the lower portion and in the middle of newly formed bone (hematoxylin and eosin stain, original magnification X40).

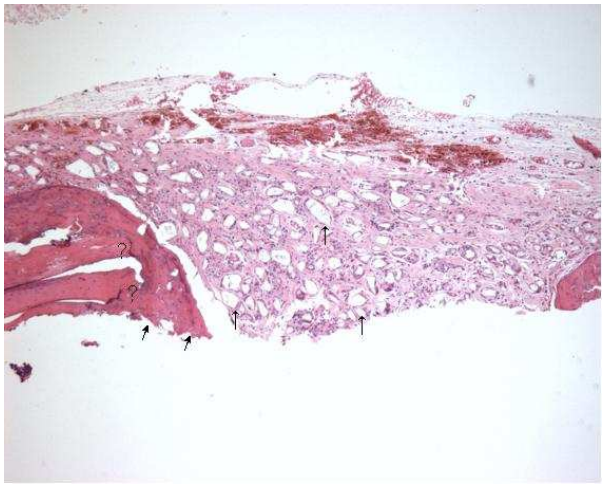

FIGURE 7. Photomicrograph of Group 4 at 4 weeks. Limited new-bone formation (thick arrows) was found around the defect margin. Preexisting bone (asterisks) and implanted chips (arrows) in the defect area were observed (hematoxylin and eosin stain, original magnification X40).

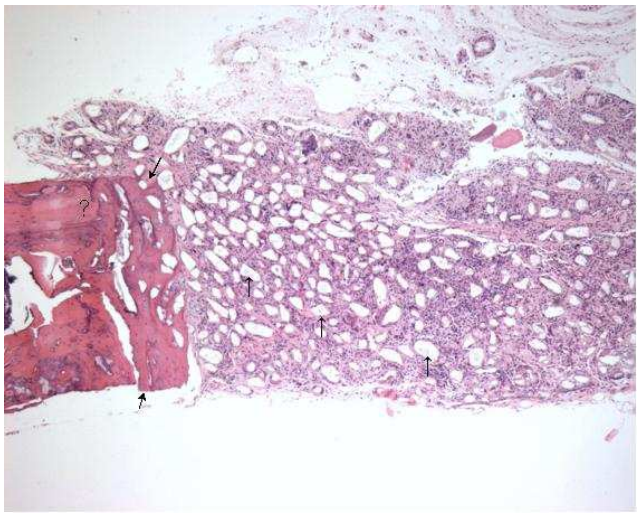

FIGURE 8. Photomicrograph of Group 4 at 8 weeks. New bone formation (thick arrows) around the defect margin was observed. Preexisting bone (asterisk) and implanted chips (arrows) in the defect area were found (hematoxylin and eosin stain, original magnification X40).