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The effect of Tissee|™ on bone healing with tooth ash and plaster of Paris mixture in rats

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백서에서 치아 회분말 및 연석고 매식시 티셀이 골치유에 미치는 영향

2006년 2월 일

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백서에서 치아 회분말 및 연석고 매식시 티셀이 골치유에 미치는 영향

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본 연구의 목적은 각종 골 이식재중 치아 회분말과 치과용 연석고를 혼합한 후 골조직의 재생능력을 증가시키는 것으로 알려진 티셀을 첨가하여 흰쥐의 두개골 결손부에 적용하고 초기 치유과정을 관찰, 평가하여 티셀의 역할을 알아보는 데 있다.

실험동물은 동일조건 하에서 일정기간 사육한 체중 200mg 이상의 Sprague-Dawley 흰쥐를 암수 구별없이 사용하였다. 노출시킨 두개골의 정중앙부에 #1/4 round bur를 이용하여 직경 8 mm 크기로 원형의 전층골 결손을 야기시킨 후, 제1군은 아무런 이식을 시행하지 않았고, 제2군은 치아회분말과 치과용 연석고를 무게비 2:1로 혼합하여 멸균한 이식재를 멸균 식염수와 혼합하여 이식하였고, 제3군은 치아회분말과 치과용 연석고를 무게비 2:1로 혼합하여 멸균한 이식재를 티셀과 혼합하여 이식하였으며, 제4군은 티셀만 이식하였다.

조직 검사를 위해 실험 후 4주, 8주로 나누어 실험군을 희생한 후 매식된 경계부를 포함하여 조직편을 채취한 후, 중성 포르말린 용액에 일정 기간 고정하고, 탈회 및 포매과정을 거쳐, Hematoxyline-Eosin으로 이중 염색하여 광학 현미경으로 흡수정도, 신생골의 형성, 염증반응 유무 등의 치유과정을 분석하였다.

시간이 경과함에 따라 2군과 3군에서 골형성이 증가함을 관찰할 수 있었다.

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

임계한계 이상의 골결손을 수복하기 위해서는 골형성 유도물질의 이식이 필요한데, 치아 회분말과 연석고 단독, 티셀 단독은 물론 치아 회분말과 연석고 및 티셀을 혼합하여 이식할 경우 효과적인 신생골 형성을 기대할 수 있을 것으로 판단된다. 이중에서도 특히 치아 회분말 및 연석고 매식후 효과가 가장 탁월한데, 치아 회분말과 연석고 및 티셀의 혼합, 티셀 단독 사용의 순서로 신생골 형성능이 관찰되었다. 그러므로 티셀은 치아 회분말 및 연석고 매식을 병용시 더 좋은 결과를 기대할 수 있을 것으로 사료된다.

Introduction

An autograft is the most suitable option for reconstruction of bony defect. Although an autograft produces good bony regeneration, it has disadvantages, such as limited donor sites, donor site damage as a result of surgery, extended surgery time, and resorption of the graft material. To overcome these limitations, other bone substitutes, including allogeneic bone, xenogeneic bone, and synthetic materials such as ceramics and polyester, have been used in recent years.[1]

In Korea, the bone substitute hydroxyapatite (HA) is expensive because it is imported. Kim et al. used particulate dentin (tooth ash) as an implant material to avoid the high cost of HA. HA is the main ingredient of particulate dentin which can be easily prepared at dental clinics. Particulate dentin has been used as an alternative material for implants with a bioresorbable property. However, the use of particulate dentin presents a problem for the retention of graft materials.[2-14] Calcium sulfate ($CaSO_4 \cdot ½ H_2O$), known as plaster of Paris, has been used as an alloplastic implant in maxillofacial osseous defects and is promising because of its long history of safe use and its characteristically complete resorption followed by new bone formation.[3] Plaster of Paris has been proposed as an effective binding and stabilizing agent for particulate materials.[3]

Studies have suggested that Tisseel™, a fibrin product, may stimulate wound healing by acting as a scaffold for the proliferation of mesenchymal and endothelial cells. This in turn may lead to increased granulation tissue formation and deposition of collagen, thereby enhancing the restoration of a severed tissue compartment.[15]

The purpose of the present study was to evaluate the effect of Tisseel™, used as an adjunct to tooth ash and plaster of Paris mixture, on the early healing of surgically produced bone defects in the skulls of 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. A calvarial critical-size defect (8 mm in diameter) was created in each rat. 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.

Forty-eight rats were randomly assigned to four groups, and each group was further divided into three sub-groups which were examined at 4, and 8 weeks after the defects were filled. The defects were filled in 4 different manners: Group 1, no graft; Group 2, tooth ash and plaster of Paris mixture graft; Group 3, Tisseel™ and tooth ash and plaster of Paris mixture graft; and Group 4, Tisseel™ graft. 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 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 tooth ash was mixed with plaster of Paris (calcium sulfate hemihydrate, Gypsum Co., USA) at a weight ratio of 2:1. All materials were sterilized with ethylene oxide before implantation, and mixed with a physiological saline solution.

Fibrin glue

The fibrin sealant Tisseel Duo Quick™ (Baxter AG, Vienna, Austria) was used. It consists of a deep-frozen Tisseel™ solution and a thrombin solution in two separate 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 aprotinin (bovine), 10-20 mg human albumin, 15-35 mg glycine, 2-4 mg sodium chloride, 4-8 mg sodium citrate, 0.2-0.4 mg Polysorbate 80, 15 mg creatine monohydrate, and water for injection volume of 1 ml. The thrombin solution contained 500 IU thrombin, 50 mg human plasma protein, 5.88 mg calcium chloride, 10 mg sodium chloride, 3 mg glycine, and water for injection volume of 1 ml. The Tisseel™ and thrombin solutions were mixed in the application to form a fibrin clot. The approximate time for resorption was about 2 weeks.

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. A hole 8 mm in diameter was drilled in the skull, removing the entire layer of the skull, by using a 1/4 round bur. An 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 rats were 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 was 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-μm 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). Images of each tissue sample were analyzed.

Quantitative analysis

The Kruskal-Wallis test was used to compare the sub-groups and the groups overall. The Mann-Whitney U test was used to compare the sub-groups within each group, and the Wilcoxon signed-rank test was used to compare the groups at each time period. Values of $p < 0.05$ were considered statistically significant.

Results

Group 1

At 4 weeks: Limited new bone was observed at the margin of the bony defect (Fig 1).
At 8 weeks: No significant difference was seen compared with the 4-week groups. Limited new bone formation was present at the margin of the bony defect (Fig 2).

In Group 1, there were no significant differences in the degree of new bone formation over time.

Group 2

At 4 weeks: A new bone formation was observed (Fig 3).
At 8 weeks: A significant amount of new bone formation was observed compared with that in the 4-week group (Fig 4).

In Group 2, there were no significant differences between 4 and 8 weeks ($p = 0.138$).

Group 3

At 4 weeks: The anastomosing pattern of woven bone seen was intense. In some portions, the bone was organized and became continuous, with some compact and dense bone (Fig 5).
At 8 weeks: The amount of new bone formation was increased significantly compared with that in the 4-week group. The pattern of new bone formation was more organized and continuous, with some compact and dense bone (Fig 6).

In Group 3, there were no significant differences between 4 and 8 weeks ($p = 0.276$).

Group 4

At 4 weeks: New bone formation was limited to the margin of the bony defect. The area not filled with new bone was filled with fibrotic tissues showing mild inflammation (Fig 7).

At 8 weeks: The new bone formation showed a significantly increased centripetal pattern compared with that in the 4-week group. The new bone was relatively organized, continuous, and compact/dense. The center of the bony defect was filled with a non-continuous, thin layer of new bone and fibrotic tissues (Fig 8).

In Group 4, there was significant difference in new bone formation between 4 and 8 weeks ($p = 0.043$).

New bone formation was significantly different overall for the 4-week samples ($p = 0.000$), and 8-week samples ($p = 0.001$). 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 ($p = 0.004$), and 3 and 4 ($p = 0.010$) at 4 weeks. At 8 weeks, there were significant differences between Groups 1 and 2 ($p = 0.004$), 1 and 3 ($p = 0.006$), 1 and 4 ($p = 0.006$), and 2 and 4 ($p = 0.016$).

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
4 weeks	0.067 ± 0.033	2.283 ± 0.256*	1.845 ± 0.878*	0.3483 ± 0.084*,+,$
8 weeks	0.038 ± 0.026	2.667 ± 0.602*	2.190 ± 0.985*	1.3500 ± 1.192*,+

Group 1, non-graft group; group 2, tooth ash and plaster of Paris mixture group; group 3, Tisseel™ and tooth ash and plaster of Paris mixture group; and group 4, Tisseel™ graft group.

* 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, $p < 0.05$.

Discussion

To aid in the stimulation of osteogenesis and the reconstruction of bone defects in cases of injury, disease, and congenital malformation in dentistry, orthopedics, and neurosurgery, various types of bone grafts and biomaterials have been developed.[4] The ideal graft material would have the ability to facilitate osteogenesis, stability when implanted with the graft, low risk of infection, ready availability, low antigenicity, and a high level of reliability.[16]

Research on graft materials has been steadily increasing since HA materials were first developed. HA is the primary inorganic, natural component of bone, is extremely biocompatible, and bonds readily to adjacent hard and soft tissues.[17] However, the high cost of HA and its complicated application during surgery are drawbacks associated with its use. One major challenge is that HA can create significant problems through rejection and the inability of the graft material to develop a stable fusion with the surrounding bone owing to its fluid nature.[3]

The advantages of a mixture of tooth ash and plaster of Paris are the lack of a foreign body reaction, excellent osteoconduction, excellent absorption, possible immediate use, and low cost. The mixture directly binds newly formed bone to increase the stability and firmness of the bone and to prevent granular movement.[1-14] Few report have discussed fibrin sealant as a bone graft material, but there are many studies on hemostasis and the acceleration of wound healing with fibrin sealants.[1]

Fibroblasts play a major role in wound healing. During the healing process, collagen is synthesized to fix and secure surrounding tissues to promote healing. Reticulated fibrin then promotes the growth of fibroblasts. Fibrin sealant maintains a close contact between wound adhesion and the skin graft, inducing the proliferation of capillaries to aid the tissue regeneration.[1] Diamond et al.[18] suggested that, in addition to helping achieve hemostasis, fibrin sealant alters components of the plasminogen

activator system, which may be beneficial in the reduction of postoperative adhesions during the healing process. Fibrin sealant is also effective in the last stage of coagulation. Fibrinogen, through the action of thrombin, is divided into fibrin monomers, which polymerize by hydrogen bonding and interact with factor XIII to form a stable fibrin network with good adhesive qualities and physiological stability. Aprotinin then adds to the fibrin network, preventing the rapid fibrinolysis induced by plasmin.[19] Thus, as a result of its adhesive, hemostatic, and wound healing properties, fibrin sealant has been used successfully in many areas of surgery.[20]

As a potent osteogenic implant, fibrin has a bone induction effect.[21] Bosch et al.[22] reported that fibrin sealant promotes capillary sprouting to enhance connective tissue formation, eventually leading to new bone formation. Furthermore, the use of fibrin sealant at the time of autologous cancellous bone transplantation was found to promote remodeling.[23] Noh et al.[1] reported new bone formation by 28 days after bone chip implantation, but the formation was delayed compared with that in the fibrin sealant-mixed group. In the present study, sites that were grafted with tooth ash and plaster of Paris mixture alone had a higher percentage of surface contact between bone and graft particles than the grafted sites with tooth ash and plaster of Paris mixture and Tisseel™. Carmagnola et al.[24] used a graft containing Bio-Oss™ mixed with Tisseel™ to augment a ridge defect in the dog mandible. This procedure failed to stimulate new bone formation, and a fibrous capsule formed around the graft particles during healing. As the site exposed to treatment was not protected with a barrier membrane during healing, it was hypothesized that the capsule formation resulted from micro-movements that occurred between the graft and the host bone. It was further suggested that the sealing material (i.e., Tisseel™) may have impaired early vascularization of the biomaterial and consequently prevented the growth of host bone into the grafted alveolar ridge defect.

The observations made in the present study are in agreement with findings from previous animal experiments. Albrektsson et al.[25] inserted titanium implants pretreated with a fibrin product (Fibrin Adhesive System; FAS) or with autologous blood and marrow into rabbits. The implants were removed and examined after 4-5 weeks of healing. Findings from the examinations, including microradiographic and microdensitometric measures, showed that the FAS-treated sites contained less bone than control sites that had been treated with autologous blood and marrow. Albrektsson et al. concluded that more evidence should be gathered before recommending FAS treatment to accelerate bone regeneration. Lucht et al.[26] studied bone formation and regional blood flow following the use of fibrin sealant in autologous cancellous bone transplantation for standardized defects in the dog tibia. They concluded that the fibrin sealant did not alter blood flow or new bone formation, but a tendency toward diminished new bone formation was found in some grafts. Kalebo et al.[27] inserted bone harvest chamber (BHC) implants in the proximal tibial metaphysis of rabbits to study the effect of autologous bone marrow and a FAS on new bone formation. The specimens were quantified by microradiography-videodensitometry and subjected to further histological examination. The amounts of bone formed in two equally treated chambers in the same animal were compared. They 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. FAS pretreatment, on the other hand, was found to impair bone formation.

The present study demonstrated that the addition of a fibrin sealant can enhance new bone formation in mechanically produced bone defects. Kania et al.[28] placed two different fibrin products (Autocolle™ and Tissucol™, i.e., Tisseel™; Immuno AG, Vienna, Austria), alone or mixed with a porous, resorbable, ceramic biomaterial, in bone cavities that had been surgically prepared in the tibiae of rabbits. They observed that, after 1 month, the addition of a fibrin sealant (Autocolle™ or Tissucol™) to the ceramic

biomaterial led to a significant increase in bone formation compared with that due to the ceramic biomaterial alone. At 2 months, a significant fibrin sealant-mediated enhancement of bone repair was observed only with Autocolle. At 6 months, the amount of bone formation was similar to that of adult bone in non-operated animals regardless of the initial material. Control cavities, on the other hand, were invaded with only fibrous tissue at each time period.

Conclusion

A graft is needed to induce new bone formation when restoring a critical or severe bony defect. Effective bone formation can be expected using ash, Tisseel™, or an ash-Tisseel™ combination. Ash is especially effective, followed by the ash-Tisseel™ combination and Tisseel™. Thus, Tisseel™ may yield a better result when used in combination with ash.

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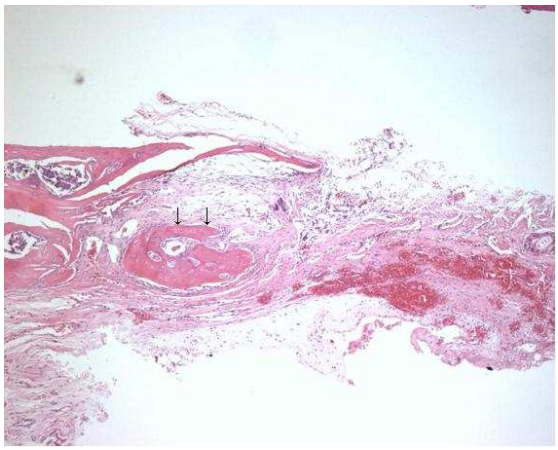

FIGURE 1. Photomicrograph of group 1 at 4 weeks. Minute new bone formation (arrows) is seen around the defect margin (hematoxylin and eosin stain, original magnification X40).

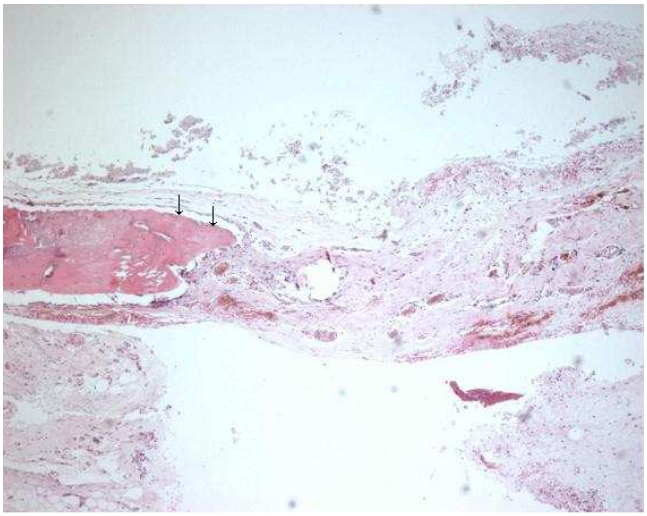

FIGURE 2. Photomicrograph of group 1 at 8 weeks. Minute new bone formation (arrows) is seen around the defect margin (hematoxylin and eosin stain, original magnification X40).

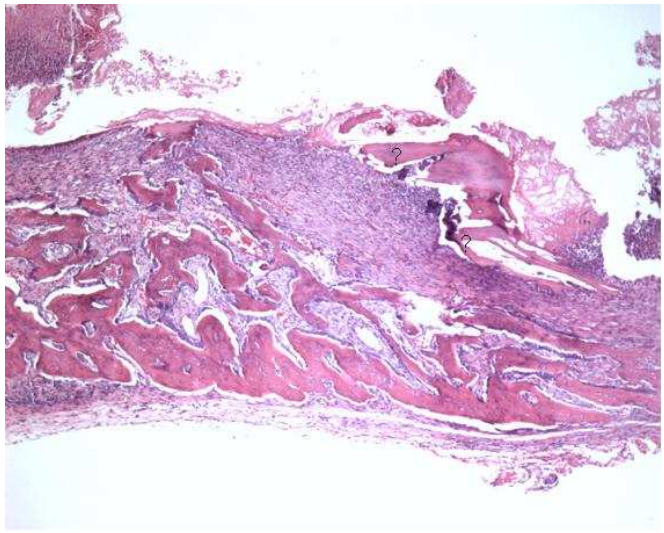

FIGURE 3. Photomicrograph of group 2 at 4 weeks. A centripetal woven bone formation is obvious. Preexisting bone (asterisks) is seen (hematoxylin and eosin stain, original magnification X40).

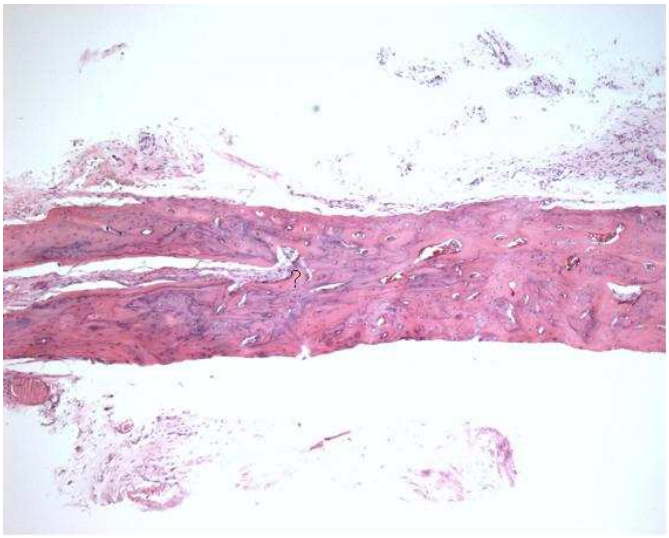

FIGURE 4. Photomicrograph of group 2 at 8 weeks. The defect is filled with well-formed woven bone. Preexisting bone (asterisk) is seen (hematoxylin and eosin stain, original magnification X40).

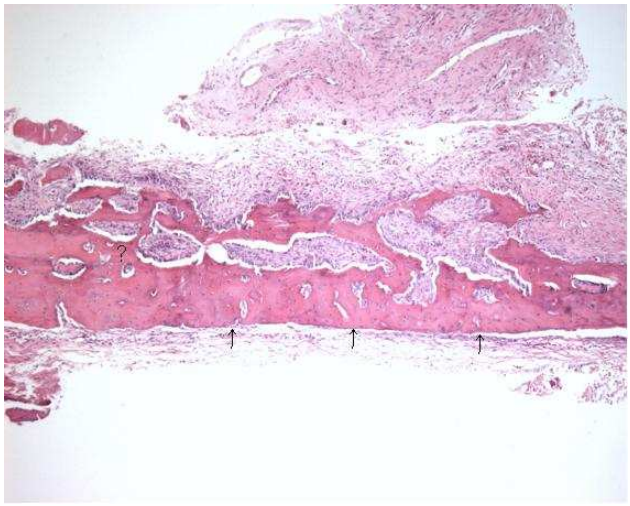

FIGURE 5. Photomicrograph of group 3 at 4 weeks. A centripetal, continuous, thick woven bone formation is noted. Preexisting bone (arrows) is seen (hematoxylin and eosin stain, original magnification X40).

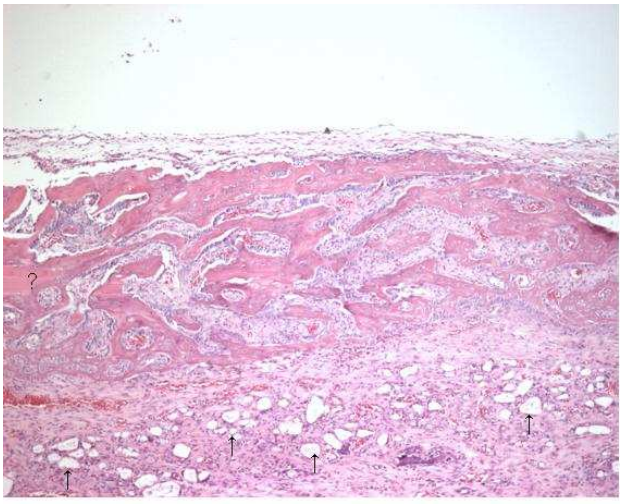

FIGURE 6. Photomicrograph of group 3 at 8 weeks. A centripetal, anastomosing, thick woven bone formation is noted. Preexisting bone (asterisk) and implanted chips (arrows) are seen in the lower portion (hematoxylin and eosin stain, original magnification X40).

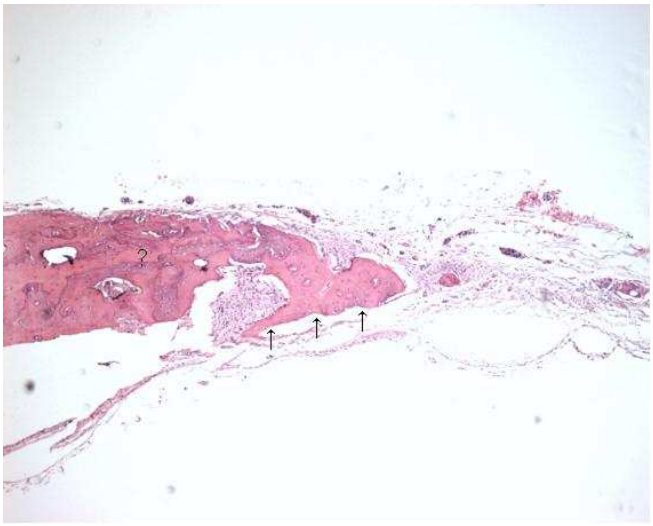

FIGURE 7. Photomicrograph of group 4 at 4 weeks. New bone formation is limited around the defect margin (arrows). Preexisting bone (asterisks) is seen (hematoxylin and eosin stain, original magnification X40).

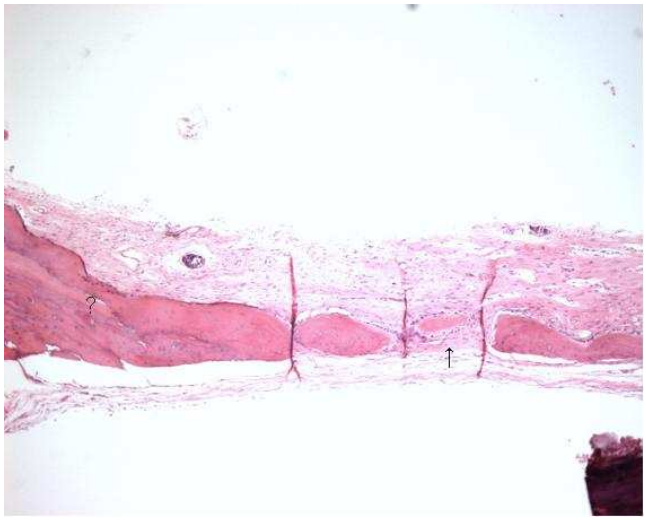

FIGURE 8. Photomicrograph of group 4 at 8 weeks. A centripetal, thick, membranous new bone formation with a central defect (arrow) is seen. Preexisting bone (asterisk) is visible (hematoxylin and eosin stain, original magnification X40).