Biomedical Research 2015; 26 (4): 666-671 *ISSN 0970-938X*
www.biomedres.info

Long-term assessment of bone formation in response to Gen Os and Gel 40 xenografts in an experimental rat model.

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Abstract

The study's aim was to assess long-term bone healing associated with two different xenografts in an experimental model of parietal bone defects in rats. We surgically created two symmetrical, full-thickness, parietal bone defects 5 mm in diameter in each of 12 rats, which were then divided randomly into three groups (eight defects per group): group 1, defects filled were with Gen Os; group 2, defects were filled with Gel 40; and group 3 (control) defects were left empty. There was substantial bone formation in group 1, but no to minimal bone formation was seen in the other groups. Significant differences were observed between groups 1 and 2 and groups 1 and 3 ($p < 0.05$ for both). Gen Os and Gel 40 were both osteoconductive and biocompatible. Based on the long-term outcomes in our study, Gen-Os xenograft is more conducive to bone regeneration, but further studies are required.

Keywords: critical-sized defect, xenograft, bone regeneration, parietal bone

Accepted June 25 2015

Introductıon

Periodontal diseases are destructive by nature, and the basic purpose of periodontal treatment is to regenerate periodontal tissue that has been lost **[1].** This requires new bone formation and cementum, together with newly inserted, functionally oriented fibers at a tooth site previously exposed to the oral environment **[2].** Many graft materials are used therapeutically, including xenografts, which are materials derived from other species with their organic components totally removed. Removal of the organic components prevents immune reactions by the host. The remaining inorganic structure provides a natural matrix and a perfect calcium source [**3].** Xenografts have many advantages **[4];** however, the high price, time-consuming production process, and ethical issues pertaining to animal slaughter are disadvantages **[5].** Thus, production of optimal bone graft material is desirable **[6].**

One potential source of xenograft material is immature calf bone, produced via a process that includes treatment with chemical detergents, freezing, and desiccation. Although the product may be acceptable as graft material to heal minor bone defects, it is not an effective bone substitue [7-8].

A critical-sized bone defect is a defect that will not heal spontaneously without osteopromotive material during the lifetime of the animal [9-10]. It is not entirely understood why a small defect can be repaired, but a large defect cannot [11] , and there is little information regarding how bone formation ceases during the repair of a critical-sized defect. Some studies have investigated healing after application of various biomaterials (e.g., xenografts) in critical-sized defects in animals [12-16].

To the best of our knowledge, Gen Os® and Gel 40® have not been investigated previously as xenografts in rat cranial defects. Therefore, the goal of the present study was to assess the long-term effects of Gen Os and Gel 40 on bone regeneration in experimentally created parietal bone defects in rats.

Materıals and Methods

Animals

Twelve albino Wistar rats weighing 230–300 g were used in the study. Rats were housed in separate cages under standard laboratory conditions and fed a standard food. The investigation was approved by the Animal Ethics Committee of Cumhuriyet University.

Materials

Gen Os (Tecnoss, Giaveno, Italy) is a mixture of cancellous and cortical heterologous porcine bone. The particles range in size from 300 to 1000 µm. Gel 40 (Tecnoss) is a mixture of 60% cortical and cancellous heterologous equine bone (300 µm particle size) and 40% collagens I–III.

Surgical procedure

Rats were anesthetized with a combination of ketamine (Ketalar®; Pfizer, Berlin, Germany) and xylazine HCL (Rompun®; Bayer, Leverkusen, Germany). The dorsal part of the cranium was shaved and then disinfected with povidone-iodine. A 3-cm midline linear skin incision was made on the dorsal part of the cranium. The skin and the periosteum were then dissected gently so that the parietal bones were visible. Two symmetrical, circular, 5-mm diameter, full-thickness bone defects were created with a trephine bur (Meisinger, Düsseldorf, Germany) under saline irrigation. Rats were randomly divided into three groups. In group 1 (n = 8 defects), the defects were filled with Gen Os. In group 2 (n = 8 defects), the defects were filled with Gel 40. In group 3 (n = 8 defects), the defects were left empty as controls. Extreme care was taken to avoid injury to the sinus and dura mater. After application of the products, soft tissues were repositioned and sutured with 3-0 silk suture material. Postoperatively, the appropriate antibiotics and analgesics were administered to the rats for infection and pain control. The sutures were removed 10 days after surgery. Healing was uneventful until the day that the rats were killed and the defects analyzed. No convulsions, inflammation, allergic reactions, or complications around the surgical area were observed.

The rats were killed by intravenous injection of sodium pentobarbital 9 months after the operation. Blocks were taken from the site on the cranium and included the defects and normal bone. Specimens were fixed in 10% buffered neutral formalin for 72 h and decalcified in Shandon TBD-1 rapid decalcifier (Thermo Scientific, West Palm Beach, FL, USA) for 48 h. After rinsing with tap water, specimens were dehydrated in increasing concentrations of ethanol and embedded in paraffin; 7-µm thick sections were made on the transverse plane and stained with Papanicalou's solution 1a (Harris hematoxylin; Merck, Darmstadt, Germany) plus eosin Y 0.5% aqueous solution (Merck) (H&E) and van Gieson's stain (MOS). Histological evaluations of the specimens were performed under a light microscope (Jenamed 2; Carl Zeiss, Jena, Germany), and bone healing was scored. New bone formation was classified according to a previously described semiquantitative classification system [17].

According to this classification system, no or minimal bone healing with fibrous tissue interposition was graded as 0, partial bone healing with occasional fibrous tissue ingrowth was graded as 1, and complete bone healing that bridged the defect was graded as 2.

The nonparametric Kruskal–Wallis and Mann–Whitney U tests were used for statistical analysis of the data. Statistical significance was set to $p < 0.05$. Statistical tests were performed with SPSS version 14.0 (SPSS Inc., Chicago, IL, USA)

Results

There was new bone formation at the center of the defects in group 1 rats. The Gen Os material was surrounded by fibrous connective and bone tissue in some areas (Figures 1, 2). Only minimal amounts of bone formation were detected at the borders of the defects in group 2. In this

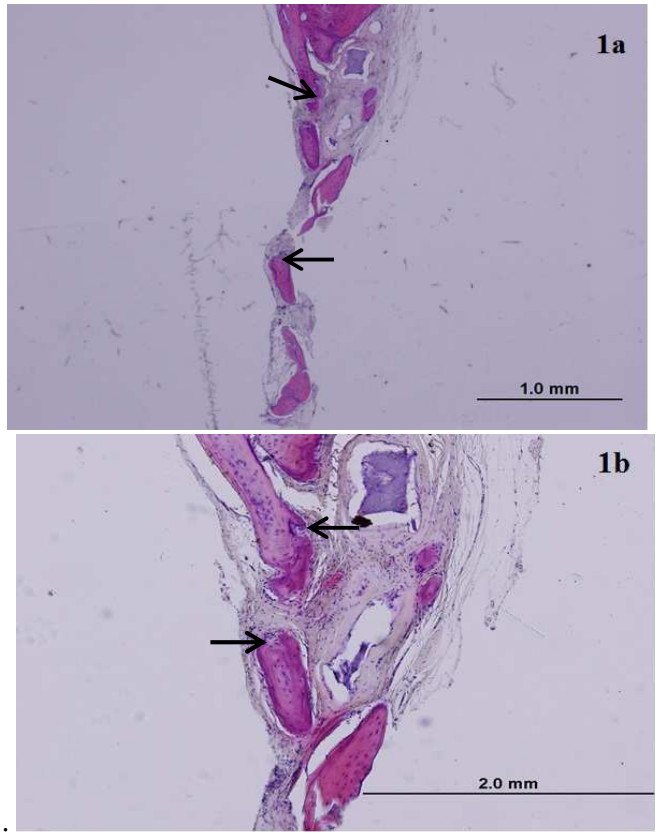

Figure 1. *Gen Os grafts in experimental parietal defects of rats. (a) Gen Os graft 9 months after defect was created. Substantial bone formation(→) has taken place. (b) Islands of new bone formation(→) around the Gen Os graft (20× magnification).*

group, Gel 40 was not resorbed (Figures 3, 4). Many hematopoietic cells and blood vessels were seen in different areas interspersed with Gel 40 (Figure 4b). In the control group, there was no new bone formation in the defect center, although minimal new bone formation was seen at the edges of the residual bone (Figure 5). New bone formation was detected at the defect border in all of the groups. We did not observe any necrosis in the form of cytoplasmic or nuclear abnormalities or tumor formation in the area around the defect. In addition, no inflammation was observed. Differences in the amount of bone healing that had occurred were statistically significant between groups 1 and 3 and between groups 1 and 2 ($p < 0.05$ for both), but not between groups 2 and 3 (Table 1).

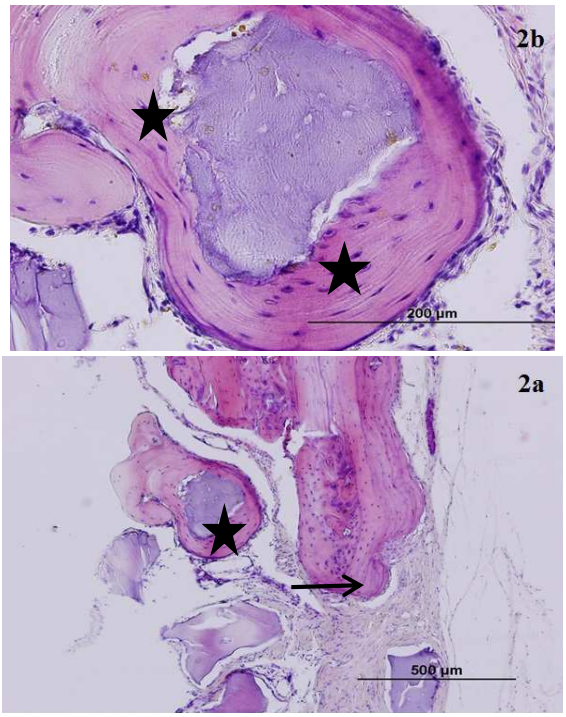

Figure 2. *Gen Os grafts in experimental parietal defects of rats. (a) New bone formation at the edge of the defect(→) and the island of bone formation (*) with encapsulation of bone graft (10× magnification). (b) Islands of new bone formation(*) around the bone graft (40× magnification).*

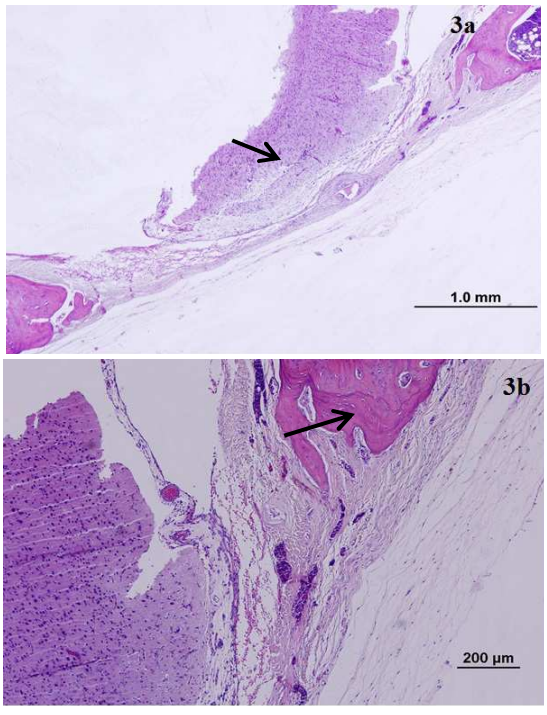

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Figure 3. *Gel 40 grafts in experimental parietal defects of rats. (a) Unresorbed Gel 40(→)in the defect center (4× magnification). (b) Unresorbed Gel 40 and new bone formation(→) at the defect border (10× magnification).*

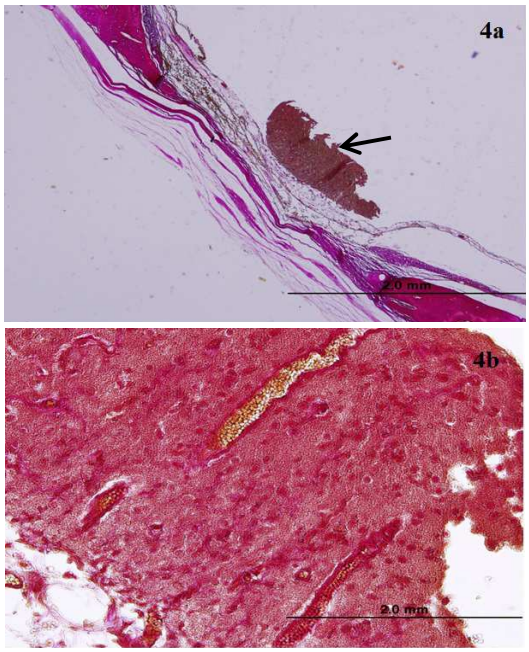

Figure 4. *Gel 40 grafts in experimental parietal defects of rats. (a) Unresorbed Gel 40 in the defect center (→) (4× magnification). (b) Numerous hematopoietic cells and blood vessels interspersed with Gel 40 (40× magnification).*

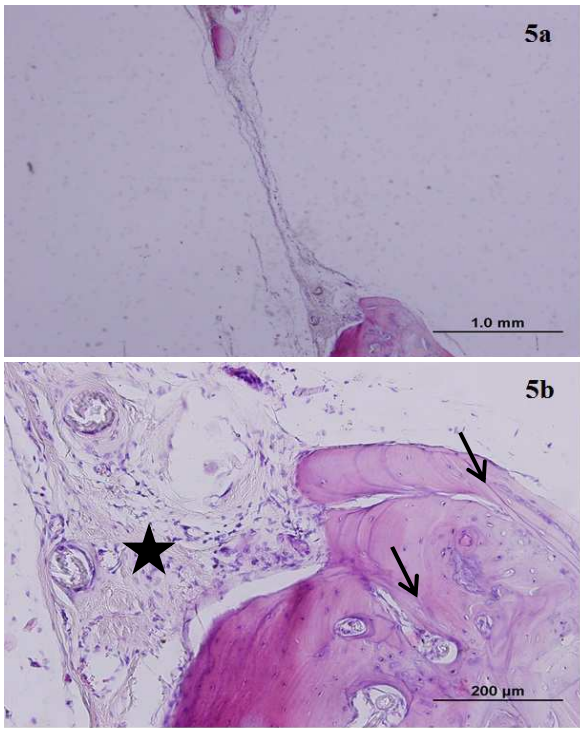

Figure 5*. Untreated experimental cranial defects of rats. (a) Overview of untreated cranial defect (4× magnification). (b) Large amounts of fibrous connective tissue in the defect area(*) and confined bone formation (→) at the defect border (20× magnification).*

Table 1. *Bone healing results in Gen Os, Gel 40, and untreated groups 9 months after creation of parietal defects.*

Groups	Bone-healing scores		
	0	1	2
Gen Os (n = 8)	3	5	0
Gel 40 (n = 8)	8	0	0
Untreated (n = 8)	8	0	0

p < 0.05 for G1 and G2 and for G1 and G3.

Discussıon

In the present study, we evaluated the bone-healing effects of two different types of xenograft, Gen Os and Gel 40, in experimentally created critical-sized defects in the parietal bone of rats. Nine months after the defects were created, we analyzed the results histologically and found that Gen Os, but not Gel 40, appeared to promote bone healing.

In one of the first attempts to investigate healing of the bones of the cranium, little scientific information was yielded, because the defects that were created did not reach the critical size [18]. It is now known that there is a size threshold beyond which a defect cannot heal spontanously without the presence of osteopromotive material. [9-10].The size of critical-sized defects can vary in rats, but in the present study, we selected 5 mm, since this size met our requirements in previous studies [14,19].

To the best of our knowledge, the present study was the first to perform a long-term assessment of Gen Os and Gel 40 xenograft materials in rat critical-sized parietal bone defects. Gen Os and Gel 40 are in the form of dried granules and gel, respectively, which were easy to apply to the defects. Based on our histological analysis, Gen Os promoted new bone growth. In addition, it is likely that the multinucleated giant cells that we observed were reponsible for resorption of the granular material, as previously described [20] . On the other hand, there was minimal to no new bone formation in the Gel 40 group. It is possible that the gel xenograft was still present after 9 months due to the location of the defect and the physical conditions in the adjacent areas. In some of the Gel 40–treated defects, there were cells that likely originated in the bone marrow interspersed among the remaining gel particles. The control defects were filled with fibrovascular tissue and only minimal or no bone formation was observed. Explanations of why critical-sized defects cannot heal spontaneously have been published previously [21,22].

There have been few investigations in which commercially available xenograft materials were tested in experimental models of cranial defects [14-16,23,24]. Generally, these materials are used for clinical purposes [4,25,26] and there have been some experiments in which they were used in experimentally created defects in parts of the body other than the cranium [27-29].

The xenogenic graft material Bio-Oss® has been investigated by many researchers. It is reported to be biocompatible and osteoconductive and is replaced by newly formed bone [4,26]. However, there have been conflicting findings regarding the resorption characteristics of Bio-Oss. Some researchers have shown that it is resorbed [30] , while others have claimed that the resorption rate of Bio-Oss is very slow [31]. In general, good clinical results have been achieved with Bio-Oss for sinus floor augmentation [32] and to fill the sockets of extracted teeth [4,25]. Good results have also been yielded by studies that employed rabbit mandibles [33] and calvaria [34]. Based on our findings, Gen Os is biocompatible and osteoconductive and thus has properties similar to those of Bio-Oss.

In a study similar to the present one, Develioglu et al. [15,16] investigated the effects of Unilab Surgibone® xenografts on bone healing after short- and long-term implantation in rat parietal critical-sized bone defects. According to their results, this material was not resorbed either in the short or long term. Moreover, the xenograft particles were surrounded by a fibrous tissue layer at the implantation site. Osteoclast-like cells were also observed. Unilab Surgibone appeared to be osteoconductive and biocompatible, but it did not have a significant effect on bone regeneration and there was very little resorption. Another experimental study with Unilab Surgibone confirmed that this material does not have cytotoxic effects [34]. By contrast, in the present study, Gen Os was associated with bone healing; thus, this material may significantly promote bone regeneration without adverse effects. In a recent case report in which Unilab Surgibone was used to treat furcation defects in a patient, good results were seen both clinically and radiographically, suggesting that it would be suitable for routine clinical use [35]. By contrast, poor results were reported from a case series [36] in which Unilab Surgibone was used in revision hip surgery. Festa et al [37] used Gen Os to treat extraction sockets in humans and showed that this material, when combined with a membrane, can reduce hard tissue resorption. This is in line with our results for critical-sized defects treated with Gen Os. Moreover, in another study, a mixed bovine bone xenograft was tested in calvarial critical-sized defects in rats. Bone growth was examined 1, 3, 6, and 9 months after the defect was created. According to the results, this xenograft material did not strongly stimulate bone regeneration in the defects [23].

Gel 40 was tested previously in an experimental study of rabbit maxilla defects [38].The authors found that it had good effects on bone regeneration and underwent resorption after 8 wk of observation. This in in contrast with our finding that Gel 40 remained unresorbed. It is possible

that these differing results were due to the different locations of the defects and how the gel was applied. An experimental study of calvarial defects in a rat model with a different gel biomaterial yielded positive findings similar to those of the rabbit maxilla study [39]. Moreover, the different features of maxilla bone also should be considered. Future studies of Gel 40 at different anotomical locations may clarify the resorption process and the effects of the gel on bone healing.

In summary, the present study revealed that Gen Os xenografts promoted bone regeneration compared to Gel 40 and untreated defects. However, further large studies are needed to understand whether either of these materials are suitable for the treatment of periodontal and peri-implant defects.

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