

Original Article

Evaluation of dental socket healing after using of porous titanium granules: Histologic and histomorphometric assessment in dogs

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ABSTRACT

Background: Different methods have been suggested to preserve bone architecture following traumatic events such as teeth extraction. The purpose of the study was to histologically and histomorphometrically evaluate the dental socket healing after applying porous titanium granules (PTG) in dogs.

Materials and Methods: Four healthy male dogs were involved in the present 6-weeks experimental animal study. Three sockets were surgically created in each side of dog's mandible. One of the sockets in one side was randomly filled by PTG and covered by a resorbable membrane (Tigran + membrane group). Another socket was left unfilled and just covered by the same membrane (membrane group) and the last one was left unfilled and uncovered as the control group. The dogs were killed at two time intervals (2 weeks and 6 weeks, two dogs at each time point). All samples were histologically evaluated under an optical microscope for a new bone formation. Data were analyzed by SPSS ver. 16 and Kruskal–Wallis and Mann–Whitney tests were used to compare data in different groups ($\alpha = 0.05$).

Results: There was a significant difference between the Tigran + membrane and the control group in 2 and 6 weeks in the mean amount of total regenerated bone ($P < 0.05$). The mean amounts of woven, lamellar, and total regenerated bone showed significant differences between 2 weeks and 6 weeks for all three groups ($P < 0.05$).

Conclusions: It can be assumed that the use of Tigran bone substitute with membrane can promote the bone regeneration in bone defects.

Key Words: Bone healing, dental socket, membrane, porous titanium granules

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INTRODUCTION

Tooth extraction will be followed by unavoidable changes in supporting structures and these changes alter the three-dimensional situation of the alveolar process, which can impede restorative fixed treatments.^[1] Resorption processes are accelerated

at the presence of periodontal diseases, and this could make prosthesis treatment plans challenging, so efforts should be made to prevent any changes in the remaining bone structure.^[2] In addition, insufficient amount of the bone in posterior maxilla has been said to hinder the implant placement. This claim can be proved by the high failure rate of short implants.^[3] On the basis of above mentioned statements, the preservation and reconstruction of bone architecture seem mandatory for periodontal and prosthetic treatments.

Different methods have been suggested to preserve and reconstruct adequate volume of bone and to prevent the alveolar ridge resorption following traumatic events such as teeth extraction. The guided

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bone regeneration (GBR) technique is a method that has represented promising results in repairing bone defects. Autogenous bone grafts have been considered the gold standard for these procedures, and their osteoconductive and osteoinductive properties can stimulate bone formation.^[4] Despite their high efficacy in bone reconstruction, the need for the second surgery has been regarded as their main weakness. As a result, so many research studies were conducted to find a suitable alternative for autogenous bone grafts. Bone substitute materials found their niche in the field of dentistry, and they showed promising results in different studies.^[5-8] Hydroxyapatite-based materials are mostly used today. The efficacy of nanocrystal hydroxyapatite paste was evaluated in the Rothamel study,^[9] and it was declared that this material is not effective in ridge preservation as it showed unpredictable resorption pattern. Since nonresorbable materials can withstand external loads and they are resistant to deformation, they can be used with great success in bone defect reconstruction.^[6,7,10-12] Therefore, finding materials that are resistant to resorption and deformity was of great interest in the field of dentistry.

The biocompatibility of titanium (Ti) has been proved in recent years and their use in implants and orthopedic devices is growing widely. This material is highly resistant to corrosion in body fluids and its nonresorbable properties make it potentially an appropriate bone material.^[10,12] Titanium particles can stimulate the activation of complement systems and platelets and can increase the level of platelet-derived growth factor consequently.^[13] This factor has been shown to promote bone growth, and this capability along with large surface area is an advantage for bone reconstruction.^[13] These properties of titanium are incorporated in porous titanium granules (PTG) (Natix™, Tigran Technologies AB, Malmo, Sweden). PTG contain 700–1000 µm diameter granules, and its porous nature makes the bone infiltration through particles possible [Figure 1].

Numerous studies have investigated the efficacy of PTG in treatment of bone defects. Wohlfahrt's study demonstrated that PTG-filled defects showed the higher reconstruction rate compared to same defects after 4 weeks.^[14] In addition, Sabetrasekh *et al.* declared that PTG significantly accelerate the cell proliferation rate compared to deproteinized bovine bone material.^[15] Also in the Wohlfahrt study,^[16] the use of PTG was successful in treatment of degree II

furcation involvement. Contrary to these promising results, Wohlfahrt *et al.* in 2012^[17] declared that no significant improvement was observed in treatment of degree II furcation defects by the use of PTG.

Although PTG was shown to be effective and safe in bone reconstruction, there are conflicting results regarding the efficacy of PTG in different studies. Therefore, this study was designed to histologically and histomorphometrically evaluate the dental socket healing after applying PTG in dogs.

MATERIALS AND METHODS

Study design and sampling

This was a 6-week prospective experimental animal study, which was held with the cooperation of Professors of Torabinejad Research Center. Four healthy male dogs aged 12–15 months and weighing 25–30 kg were included in the study. This study was approved by the animal department of Torabinejad Dental Research Center and local ethical committee of Isfahan University of Medical Science.

Clinical procedure

All dogs were anesthetized using acepromazine 2% (0.02 mL/kg Neurotrano, Alfasan, Woerden, Holland) and ketamine 10% (10 mg/kg ketamine HCl, Alfasan, Woerden, Holland). After atropine (0.02–0.04 mg/kg atropine, Alfasan, Woerden, Holland), injection dogs were intubated and then halothane (Halothane BP, Nicholas Piramal, India) was used to maintain the anesthesia. Periapical radiographs were taken from mandibles' premolar region to diagnose any developmental defect. Lidocaine (Persocaine-E, Lidocaine HCl 2% + Epinephrin 1/80,000, Darou Pakhsh Pharmaceutica Co., Tehran, Iran) infiltration anesthesia was placed in the mucobuccal fold to control the pain and bleeding during the surgical procedure. 0.2% Chlorhexidine was also used around mouth and the skin as prophylaxis.

After sulcular incision from the first mandibular premolar to the first molar, a mucoperiosteal flap was elevated. Second, third, and fourth premolars were hemisected using diamond fissure burs. Mesial root's crown was resected, and the mesial root was reamed and filled with gutta-percha and then it was built up by amalgam (Sina Co., Tehran, Iran). Then, the distal roots were extracted by an elevator without any trauma. This procedure was also performed in the other side. As a result, three sockets were surgically

created in each side and six cavities were created in each dog's mandible [Figure 2].

Sockets were rinsed by normal saline. In each side of the dogs' mandible, sockets were randomly divided to three groups:

1. Tigran + membrane
2. membrane, and
3. control

One of the sockets in one side was randomly filled by NatixPTG (Natix™, Tigran Technologies AB, Malmo, Sweden) and covered by the cytoplast (Osteogenics Biomedical, Inc., USA) resorbable membrane. Another socket was left unfilled and just covered by the same membrane, and the last one was left unfilled and uncovered as a control group [Figure 3]. Finally, eight sockets were filled by Natix PTG and covered by the cytoplast resorbable membrane, eight sockets were just covered by the membrane, and eight sockets were considered control in four dogs.

Surgical flaps were sutured by 3-0 PTFE (Osteogenics Biomedical, Inc., USA). Tramadol 50 mg (5 mg/kg Tehran Chemie Pharmaceutical Co., Tehran, Iran) and ceftriaxone 1 g (Jaber Ebne Hayyan Pharmaceutical Mfg. Co., Tehran, Iran) were injected for 5 days, and dogs were fed on a soft diet for 14 days after surgery. Sutures were removed after 10 days. The dogs were killed at two time intervals (2 and 6 weeks, two dogs at each time point). A lethal injection of 40 mL pentobarbital sodium at 100 mg/mL in 290 g/1000 mL spiritus fortis, 100 mg/kg was given to one of the dogs. Blocks from mandibles' sockets were prepared by a diamond saw (Exacts Apparatebau, Norderstedt, Hamburg, Germany) and then blocks were fixed in 10% formalin solution for 48 h and kept in 70% alcohol. Also, 80 µm slices were prepared by a Buehler IsoMet 5000 high speed precision saw (Buehler; Dusseldorf, Germany) and slices were stained by hematoxylin and eosin (H and E) and investigated under an optical microscope (Nikon E400, Japan) by a pathologist. Histomorphometric analysis with I HMMA_ver. 1 (Sbmu, Iran) software was performed to evaluate the percentage of new regenerated bone including woven, lamellar, and total bone [Figures 4 and 5].

Statistical analysis

Data were analyzed using SPSS software ver. 16 (SPSS Inc., Chicago, IL, USA) and Kruskal–Wallis

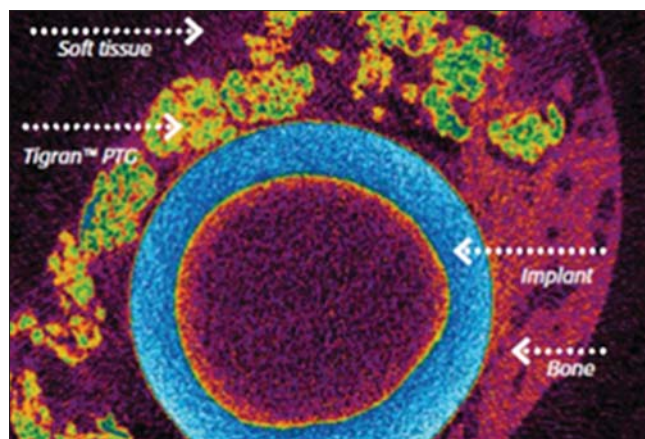


Figure 1: Bone formation in and around titanium granules



Figure 2: Three defects were surgically created in each side of dog's mandible

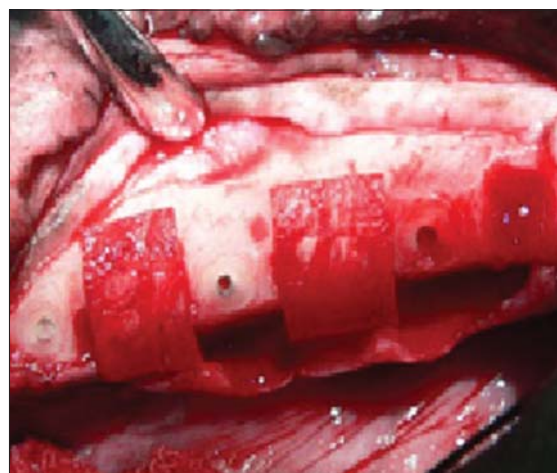


Figure 3: PTG was packed in one of the defects and covered by the membrane, the other defect was left unfilled and just covered by the membrane, and the last one was left unfilled and uncovered as the control group

and Mann–Whitney tests were used to compare data in different groups ($\alpha = 0.05$).

RESULTS

In this study, the minimum and maximum amount of the regenerated woven bone was seen in Tigran + membrane and control groups after 2 weeks, respectively [Table 1, Figure 6]. The mean amount of the regenerated lamellar bone reached its highest

rate in the Tigran + membrane group at 6 weeks [Figures 6 and 8].

The Kruskal–Wallis Test showed that there is a significant difference between different groups at both 2 and 6 weeks in the mean amount of the total regenerated bone. The Mann–Whitney test showed that this significant difference exists

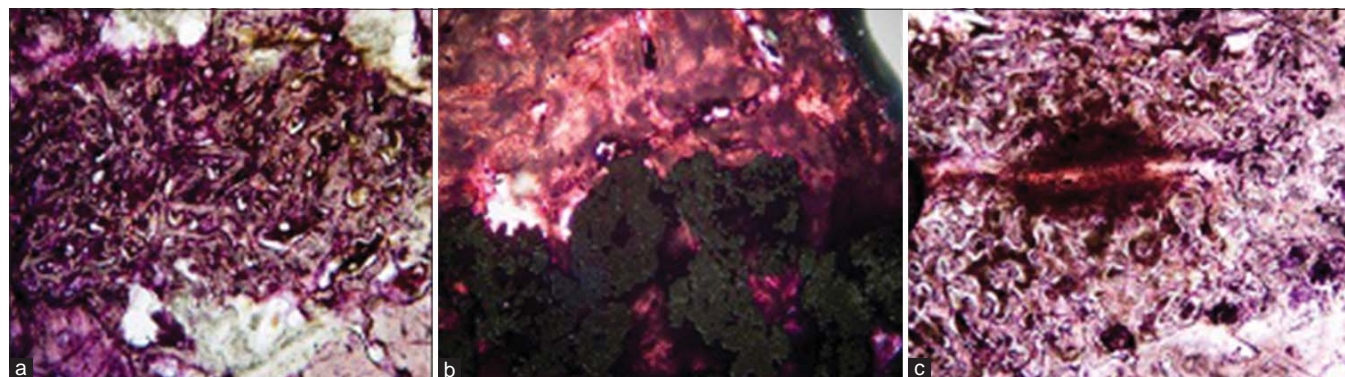


Figure 4: Photomicrographs of bone regeneration at 2 weeks in different groups: (a) control, (b) Tigran + membrane, (c) membrane (H and E, optical microscope; original magnification $\times 100$)

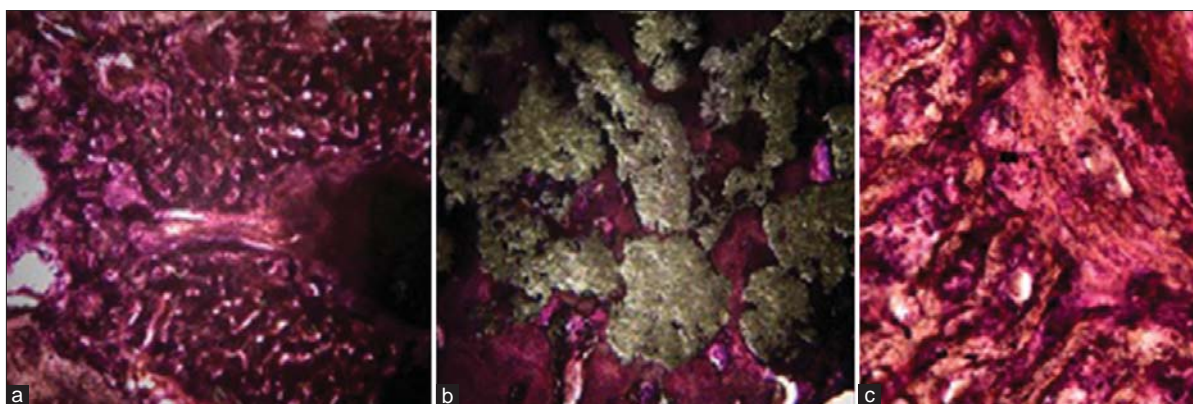


Figure 5: Photomicrographs of bone regeneration at 6 weeks in different groups: (a) control, (b) Tigran + membrane, (c) membrane (H and E, optical microscope; original magnification $\times 100$)

Table 1: The mean amount of the regenerated woven, lamellar, and total bone at 2 and 6 weeks for all three groups

Group	Time point	Woven bone	Lamellar bone	Total regenerated bone
Tigran + membrane	2 weeks	83.00 \pm 9.76	17.00 \pm 9.76	34.75 \pm 5.31
	6 weeks	41.75 \pm 4.64	58.25 \pm 4.64	61.50 \pm 7.00
	Total	62.37 \pm 23.15	37.62 \pm 23.15	48.12 \pm 15.41
Membrane	2 weeks	88.50 \pm 3.10	11.50 \pm 3.10	22.25 \pm 6.02
	6 weeks	48.00 \pm 7.02	52.00 \pm 7.02	53.00 \pm 5.22
	Total	68.25 \pm 22.22	31.75 \pm 22.22	37.62 \pm 17.24
Control	2 weeks	89.25 \pm 2.21	10.75 \pm 2.21	18.50 \pm 4.20
	6 weeks	51.50 \pm 5.44	48.50 \pm 5.44	47.75 \pm 3.30
	Total	70.37 \pm 20.54	29.62 \pm 20.54	33.12 \pm 16.02
Total	2 weeks	86.91 \pm 6.20	13.08 \pm 6.20	25.16 \pm 8.66
	6 weeks	47.08 \pm 6.72	52.91 \pm 6.72	54.08 \pm 7.66
	Total	67.00 \pm 21.30	33.00 \pm 21.30	39.62 \pm 16.79

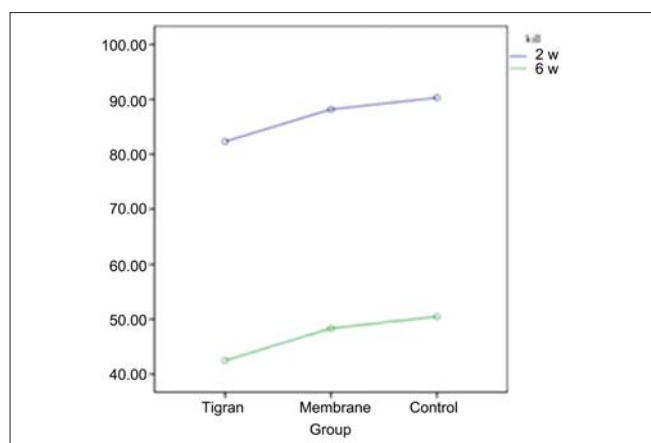


Figure 6: Woven bone regeneration in all groups at two time intervals (tigranmem: Tigran + membrane group, membrane: membrane group, the Y-axis represents the mean amount of the regenerated woven bone)

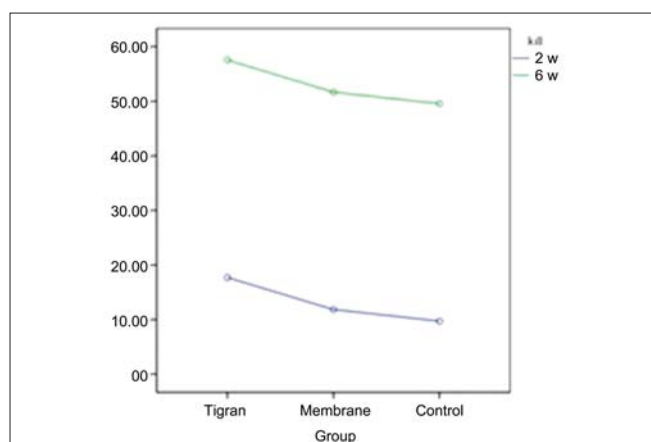


Figure 7: Lamellar bone regeneration in all groups at two time intervals (tigranmem: Tigran + membrane group, membrane: membrane group, the Y-axis represents the mean amount of the regenerated lamellar bone)

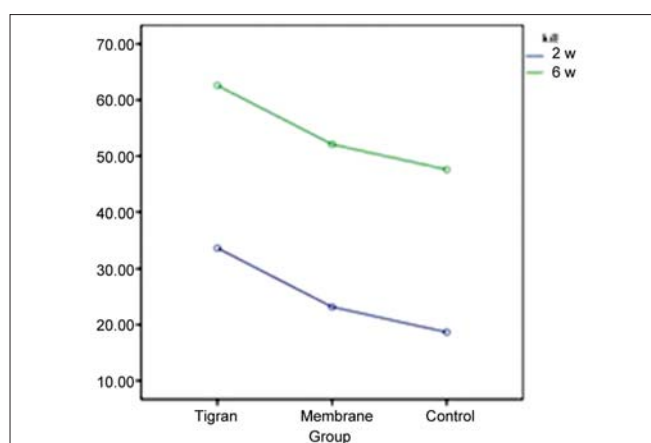


Figure 8: Total bone regeneration in all groups at two time intervals (tigranmem: Tigran + membrane group, membrane: membrane group, the Y-axis represents the mean amount of the total regenerated bone)

between the Tigran + membrane and control groups ($P = 0.026$). In addition, the differences between the Tigran + membrane and membrane groups and between the membrane and control groups were not significant. The mean amount of the regenerated woven and lamellar bone did not show significant difference between groups at 2 and 6 weeks ($P = 0.544$).

The Mann–Whitney test revealed that the mean amount of the regenerated woven, lamellar, and total bone showed significant differences between 2 and 6 weeks for all three groups ($P < 0.05$). In all three groups, the mean amount of the lamellar and woven bone increased and decreased, respectively, as time elapsed from 2 to 6 weeks.

DISCUSSION

Tigran has been recently known as an appropriate bone substitute material in repairing bone defects,^[18,19] but there are no conclusive research studies regarding the efficacy of this material. The present animal study revealed that Tigran can be considered as an appropriate bone substitute material, and it can promote the bone regeneration in bone defects.

Lambert *et al.* showed that Tigran can efficiently act as a bone substitute material in the subnasal region.^[20] Other studies also highlighted Tigran ability in regeneration of the bone defects as a bone substitute material.^[14,16,19,21,22] However, in the Wohlfahrt study^[17] the ability of Tigran was assessed in the treatment of furcation involvement and no significant improvement in clinical endpoints of defect resolution was observed.

The results of this study are in agreement with mentioned research studies that have investigated the PTG efficacy in the treatment of bone defects. In this study, the mean amount of total regenerated bone reached its highest rate in the Tigran + membrane group in both 2 and 6 weeks compared to other groups. This high rate of bone formation can be explained by the osteoconductive property of Tigran, which allows the regenerated bone matrix to act as a scaffold. This ability would enhance the rate of cell penetration into the defect and promote bone regeneration as a result.^[23] Also, Tigran is highly porous and this property increases the surface-to-volume ratio, which is necessary for cell proliferation.^[24]

In addition, the important rule of a membrane should not be neglected in bone defect regeneration.

Osteopromotive materials such as membranes inhibit the fibroblasts proliferation into the defect, and this is a great chance for bone cells to dominate the defect.^[25] In this study, it was shown that membranes can inhibit the epithelial cells proliferation into the defect, and this can promote the bone regeneration process. The results of this study confirm the aforementioned statements as there was no significant difference between sockets covered by the membrane alone and the Tigran + membrane groups regarding the total amount of regenerated bone.

In this study, the minimum and maximum amount of the regenerated woven bone was seen in the Tigran+membrane and control groups, respectively, after 2 weeks. Woven bone is a weak structure and does not have well-organized tissues,^[26] and it is the first bone tissue that is formed in the bone regeneration process.^[27] For the regeneration of the well-structured lamellar bone, hydroxyapatite crystals should be deposited by osteoblast cells. In the second mineralization phase, the mineral contents of the lamellar bone and the size of hydroxyapatite crystals increase and these phenomenon requires time.^[26] On the basis of the required time for bone maturations, it seems rational that the amount of the lamellar bone increased by the time.

On the basis of the aforementioned findings, it can be assumed that a Tigran bone substitute can promote the formation of well-organized mineralized bone as the maximum amount of lamellar bone was regenerated in the Tigran + membrane group after 2 and 6 weeks. Despite the difference between the amount of the regenerated lamellar and woven bone between different groups, the difference is not statistically significant.

In this study, the maximum amount of the total regenerated bone was seen in the Tigran group with membrane coverage; however, the difference in the amount of the total regenerated bone was not significant between the Tigran + membrane and membrane groups. This nonsignificant difference may be attributed to the sample size limitation. Also, it was suggested in the future studies other bone substitute materials to be compared with Tigran for providing the better comparison.

CONCLUSION

On the basis of the mean amount of the total regenerated bone in the Tigran + membrane group,

it can be assumed that the use of a Tigran bone substitute with membrane can promote the bone regeneration in bone defects.

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