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Original Article

The effects of type-I collagen membrane, hydroxyapatite, and platelet-rich plasma on bone regeneration: An experimental study

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Abstract

Aim: Various methods have been developed to support bone reconstruction, including the use of autogenous, allogenic, and synthetic bone graft substitutes, membranes, and concentrated supplements. This study aims to compare the effects of collagen membrane, hydroxyapatite, and platelet-rich plasma on the bone regeneration process in a rat model.
Methodology: The experiment was conducted with 60 female Wistar albino rats. Subjects were divided randomly into three groups (n: 20). In the first group, the right femurs' defects were left empty (group I), and type-I collagen membrane was applied to the left femurs (group II). Type-I collagen membrane + hydroxyapatite (group III) was applied to the second group, and type-I collagen membrane + hydroxyapatite + platelet-rich plasma (PRP) (group IV) was applied to the third group. Thus, a total of 80 femurs were included in the study, and defects were evaluated histologically on the 10th, 21st, 45th, and 90th days (n: 5).
Results: Groups II and III had better osteogenesis scores than group I, whereas group IV had better results than all other groups.
Conclusion: In the PRP group, the osteogenesis scores were significantly better than the other groups. However, the healing was almost excellent in all three study groups (groups II, III, and IV) at the end of the experiment.

Keywords: hydroxyapatite, type-I collagen membrane, platelet-rich plasma, bone regeneration, osteogenesis

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Introduction

In the oral and maxillofacial region, significant bone defects result primarily from infection, trauma, pathologies, developmental deformities, congenital anomalies, metabolic diseases, and the use of some drugs (1). In bone-tissue reconstruction, the diversity of anatomical regions, the dimensions of the defect, mechanical stresses, and the soft tissue presence bring along clinical difficulties (2). Many techniques have been applied over the years to solve various issues, including bone grafting, which plays a critical role in repairing critical bone defects. Providing a scaffold accelerates the self-repair cycle of the defect area in critical bone injuries. Autografts, synthetic grafts, membranes, platelet concentrations, and cellular treatments are among the preferred methods for bone defects that are difficult to repair, though they have some limitations (3-5).

Guided bone regeneration (GBR) is commonly used to enhance bone growth to restore the lost tissue. By placing barrier membranes on the defect, cells with regeneration potential are allowed to proliferate. Another critical role of the membrane is to create a

barrier over the defect to protect the underlying clot (6,7). Clinically resorbable and non-resorbable membranes are available for use. Since non-resorbable membranes carry a higher risk of exposure and infection after exposure and require primary fixation and a second operation, absorbable membranes are generally preferred (8). Collagen is the most widely used resorbable membrane clinically (9). Collagen membrane is preferred for a number of reasons, including decreased patient morbidity, ease of surgical application, lower incidence of exposure, and the lack of a need for a second operation. On the other hand, an uncontrolled duration of barrier function and the potential collapse of the membrane into the defect area are significant problems with collagen membranes. Often block or granular form autogenous, allogenic, and xenogenic graft materials are used to overcome this problem (10,11).

Hydroxyapatite (HA) granules are highly biocompatible calcium phosphates of heterologous origin with similar properties to mineralized human bone that, due to their similarity to the mineral structure of bone, are readily accepted by the body (12,13). Hence, HA is the most common and preferred graft material.(17)

In 1998, Marx *et al*. showed the effect of PRP on bone defects (16). Platelets play an essential role in wound healing. During and after clot formation, platelets initiate and support wound healing by increasing collagen production, migration of other cells to the damaged area, and initiation of vascular growth; they also contain essential growth factors that provide cell differentiation (17–19). Clinical and animal studies have shown that platelet-rich plasma (PRP) enhances bone formation by increasing the density of growth factor in the bone graft (20). It has been reported that PRP has been used clinically in cartilage, bone, muscle, tendon, and ligament regeneration and frequently in dermatology, head and neck surgery, otolaryngology, cardiovascular surgery, oral and maxillofacial surgery, and periodontology due to the effects of growth factors that increase wound healing and regeneration (21,22). This study aimed to compare the effects of collagen membrane, hydroxyapatite, and platelet-rich plasma on bone regeneration. Early (10th and 21st days) and late (45th and 90th days) new bone formation were investigated. Furthermore, it aimed to compare foreign body reaction, infection, fibrotic encapsulation, physical attachment, immune reaction, resorption of the bone grafts, and biocompatibility.

Materials and Methods

Materials

Animals were anesthetized with ketamine hydrochloride (Ketalar®, Eczacıbaşı, Türkiye) and xylazine hydrochloride (Rompun®, Bayer, Türkiye), sacrificed with sodium thiopental (Thiopental®), and disinfected with povidone-iodine (Betadine®, Kansuk, Türkiye). Hydroxyapatite (Apatos®) and type-I collagen membrane (Denticol®), 3.0 polyglactin suture (Vicryl®, Ethicon Limited, Belgium), 3.0 silk suture (Doğsan®), and antibiotics (Gentamycin®, 0.05 ml/kg) were used.

Experimental Design

The study was conducted on 65 female Wistar albino rats weighing between 200 and 240 g. Five of them were used in PRP preparation, and the remaining 60 were randomly divided into three groups. The study was planned according to the ARRIVE guideline checklist (23) and approved by the Committee of Animal Experiments at Dicle University, Türkiye. (DEHEK:2006/43). The surgical procedures were performed at Dicle University Health Sciences Research and Application Center.

PRP Preparation and Surgical Technique

PRP was obtained as described with some modifications (24). Briefly, five rats were sacrificed to obtain PRP. The animals were anesthetized with 0.2 ml ketamine and 0.1 ml xylazine hydrochloride. The blood was collected into 4.5 ml sodium citrate tubes and centrifuged at 1200 rpm for 10 min. The platelet-rich plasma (PRP) and the platelet-poor plasma (PPP) were then separated from the supernatant. The PRP and PPP were centrifuged again at 7835 rpm for 15 min, and the PPP supernatant was removed. The PRP cell count was determined as 1,600,000 mm3. The entire procedure was conducted at the Biochemistry Department Laboratories, Dicle University Medical School (CS-15 Centrifuge, Serial no: 96 E 6773, 4800 rpm, Beckman, S4180).

Following anesthesia, the right legs of the rats were shaved and then disinfected using povidone-iodine. A skin incision was made over the femur, the area was dissected, and a 10 × 3 × 2 mm bone defect was created on the femur (Fig. 1). In the first group, both femurs of the rats were used. A total of 80 femurs were operated upon during the experiment.

Group I: *The defect is left empty (Group I, n = 20).

Group II: *The defect is covered with type-I collagen membrane (Group II, n = 20).

Group III: Hydroxyapatite and type-I collagen membrane were applied to the defect (Group III, n = 20).

Group IV: PRP combined with hydroxyapatite and type-I collagen membrane was applied to the membrane (Group IV, n = 20).

The collagen membrane was stabilized to the femur with a 3.0 polyglactin suture. The periosteum and subcutaneous tissues were stitched primarily with 3.0 polyglactin sutures and the skin with 3.0 silk sutures. All the animals were injected with antibiotics intramuscularly and closely monitored in separate cages throughout the experiment. On Day 10, Day 21, Day 45, and Day 90, five rats from each group were sacrificed via intraperitoneal injection of thiopentone at lethal doses (28.410 µg/kg). The femurs were extracted and fixed in 10% neutralized formalin. The samples were then decalcified in 5% formic acid, dehydrated in an ethanol series, and embedded in

paraffin blocks. Subsequently, sections of the samples were stained with hematoxylin and eosin.

Osteoblastic activity, foreign body reaction, infection, osteogenesis, fibrous tissue development, physical attachment, biocompatibility, and graft resorption were all assessed under a light microscope. New bone formation was graded as follows: 1 (No Osteogenesis); 2 (Mild Osteogenesis); 3 (Moderate Osteogenesis); 4 (Good Osteogenesis); and 5 (Excellent Osteogenesis).

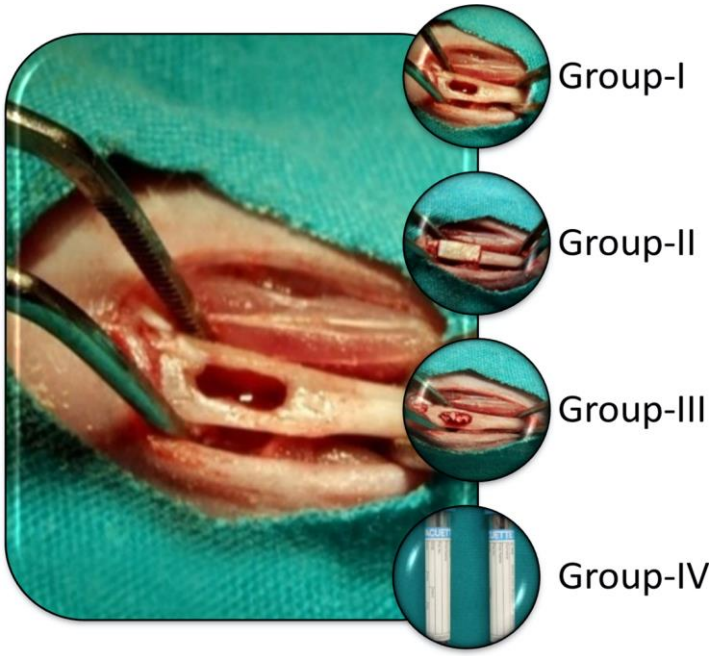

Figure 1. Defect area and biomaterial application to the groups

Statistical analysis

The statistical analyses were performed using SPSS software version 19.0 (IBM Corp., Armonk, NY, USA). Descriptive statistical value is given as the median (max-min). Normality was tested using the Kolmogorov-Simirnov test. Comparison of the groups was performed using the Kruskal-Wallis test, with paired comparisons performed using the Dunn-Bonferroni test. Statistical significance was set at $p < 0.05$ (Table 1).

Table 1. Statistically comparison of the osteogenesis.

	10th day	21st day	45th day	90th day	
Groups	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	
Group-I	1 (1-2)^{aA}	2 (1-2)^{A}	2 (2-3)^{aAB}	3 (3-4)^{aB}	p=0.003
Group-II	3 (2-3)^{cAB}	2 (1-2)^{A}	3 (3)^{bAB}	4 (3-4)^{abB}	p=0.002
Group-III	2 (1-2)^{bA}	2 (1-2)^{A}	3 (3-4)^{bAB}	4 (4)^{bB}	p=0.001
Group-IV	2 (2)^{bA}	2 (2)^{A}	4 (4-5)^{cAB}	5 (4-5)^{cB}	p=0.001
	p=0.037	p=0.426	p=0.002	p=0.004	

Data presented as median (minimum to maximum). Different superscript letters (A, B) indicate statistically significant differences in row. Different lowerscript letters (a, b, c) indicate statistically significant differences in column.

Results

Histological Evaluation

Day 10

There was no new bone formation in the control group, but fibrous tissue formation was observed.

Connective tissue formation and a thin layer of spongious bone formation were observed in Group II, Group III, and Group IV. Although there was an inflammatory response, foreign body reaction and resorption were not observed in any of the experimental groups (Fig. 2).

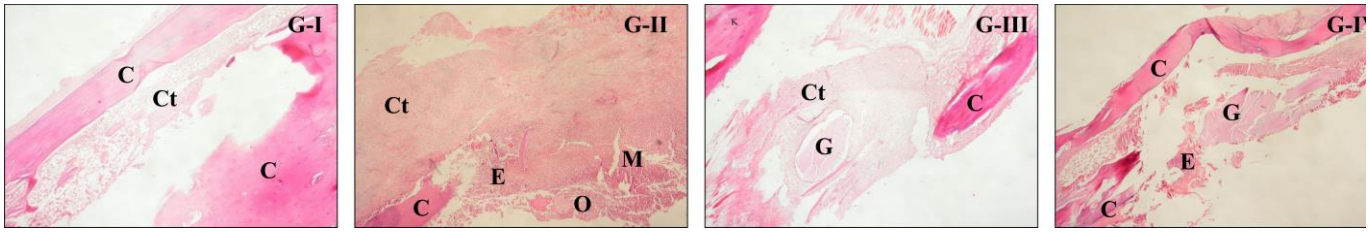

Figure 2. 10th-day histologic sections (HEX40) (C:cortex, O:osteogenesis, Ct:connective tissue, M:membrane, E:enflamation, G:graft),

Day 21

Trabeculae and new bone formation were observed in the control group. Osteogenesis was present at the borders of the defect in Group II, Group III, and Group IV. Heightened connective and inflammatory response were present in all the experimental groups. No foreign body reaction or resorption was observed in any of the groups. Osteogenesis had progressed further in Group IV than in Group III (Fig. 3).

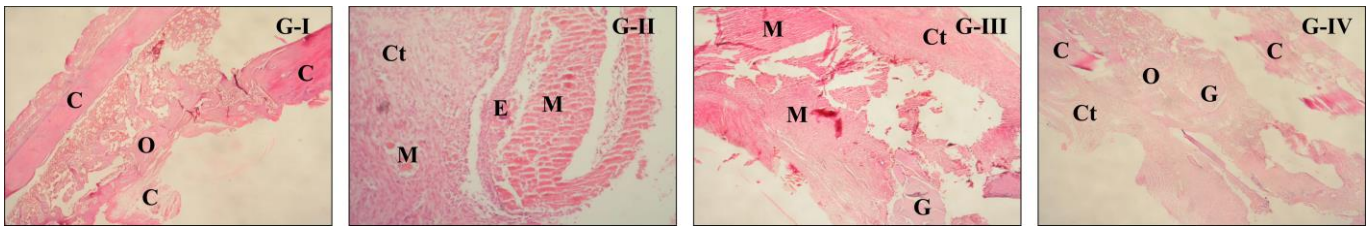

Figure 3. 21st-day histologic sections (HEX40) (C:cortex, O:osteogenesis, Ct:connective tissue, M:membrane, E:enflamation, G:graft)

Day 45

Active bone formation was observed in the control group. New bone formation had progressed further in Group II, Group III, and Group IV. Vascular structures and connective tissue were observed in all groups, with no foreign body reaction, and it was determined that membrane resorption had been initiated. In Group IV, the newly formed bone was associated with porous structures, and the osteogenesis was graded moderate to good (Fig. 4).

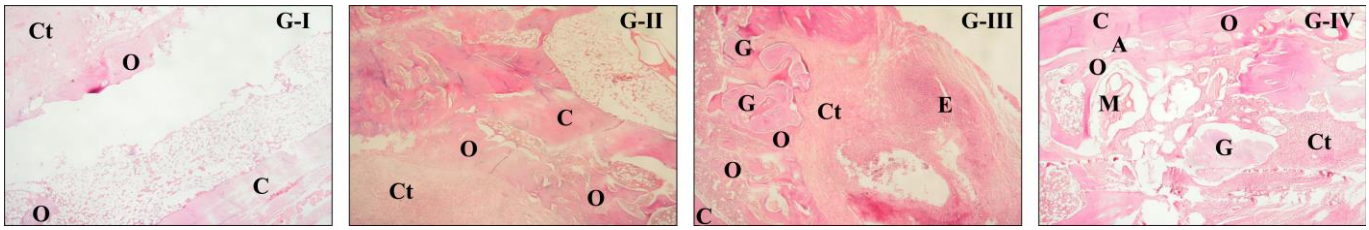

Figure 4. 45th-day histologic sections (HEX40) (C:cortex, O:osteogenesis, Ct:connective tissue, M:membrane, E:enflamation, G:graft, A:attachment)

Day 90

Osteoblastic activity progressed further in the control group, and the new bone formation was graded as moderate. In Group II and Group III, osteoblastic activity and new bone formation were in progress, with physical attachment of graft and bone cortex observed. Collagen membrane resorption was in progress, connective tissue was diminishing, and no foreign body reaction was present. In Group IV, osteogenesis was almost complete, and new bone formation was more advanced than in Group III. Furthermore, osteogenesis in Group III had progressed further than in Group I and Group II (Fig. 5).

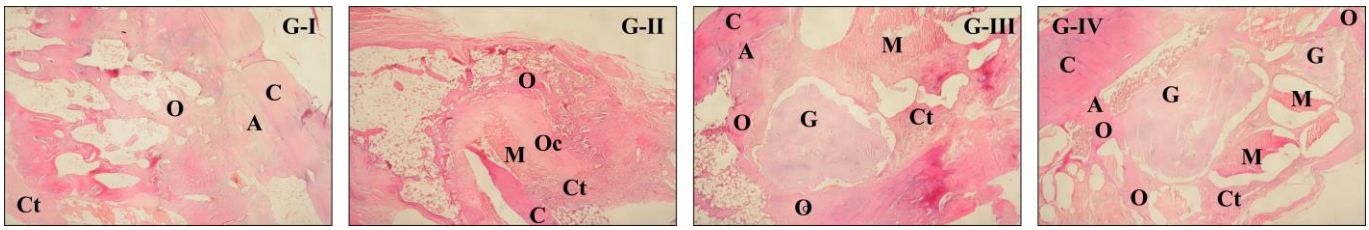

Figure 5. 90th-day histologic sections (HEX40) (C:cortex, O:osteogenesis, Oc: osteochondral ossification, Ct:connective tissue, M:membrane, E:enflamation, G:graft, A:attachment)

Discussion

When the studies are examined on guided bone regeneration, some controversies are encountered. While studies are defending the positive effect of this technique, some studies suggest that it does not benefit osteogenesis (8, 9, 25). In the early stages of our study, the groups with collagen membrane (II, III, and IV) had significantly better osteogenesis than the control group. This result supports the idea that the membrane creates a barrier, inhibiting fibrotic healing and causing an increase in osteogenesis.

The most important factor preventing the success of bone healing is the migration of the connective tissue to the defect area. Soft tissue invasion hinders osteogenesis. The biocompatible and occlusive physical barriers prevent the ingrowth of fibroblasts and generate a compartment that enables the osteogenic and angiogenic cells originated from the medullar spaces to restore those defects with new bone tissues (25). In our study, new bone formation in the membrane-applied groups was statistically more significant than the control group.

When the samples were examined, biomaterials did not cause any inflammation, foreign tissue reaction. It was observed that the collagen membrane + HA application gave better results compared to the control group. However, group III results were not significantly different from group II except for the 10th-day results. The 21st day shows that there was no statistically significant difference between any groups regarding osteogenesis.

The appearance of new bone formation around the graft material on the 45th day shows that HA helps osteogenesis with its osteoconductive properties, as proven previously (26). And general evaluation of bone healing, group III showed significance compared to group I. While there was no difference between groups II and III ($p > 0.05$), results were close to group IV. Similar results have been obtained from previous studies. (13, 27, 28)

In the late phases (90th days), groups II, III, and IV had significantly better osteogenesis. The new emerging bone tissue was also better in quality and quantity than the control group. Moreover, in histological evaluations, any of the hydroxyapatite, type-I collagen membrane, and PRP did not cause any inflammation, foreign body reactions, fibrous encapsulation, and forming a biocompatible physical attachment with the bone tissue. PRP supplemented group scores were significantly better than the other groups. This result was attributed to the presence of a growth factor in the PRP and the resulting osteoblastic increase in the area of the defect.

According to the study results, in-group and intergroup comparisons, the group in which PRP was used gave statistically significant results compared to the other groups.; however, the healing of bone defects was almost excellent except for the control group. Besides, none of the biomaterials caused any inflammation, foreign body reactions, or fibrous encapsulation.

It has been reported in the literature that the use of PRP has positive effects as well as no clinically significant results. Marx et al. reported that PRP secretes PDGF, VRGF, TGF-1, and TGF-β isomers. Using growth factors rapidly reproduces the small number of stem cells, which can transform into different cells and ensure active wound healing. After all, many studies have been conducted and published on PRP in oral and maxillofacial surgeries (16, 29). Simman *et al*. evaluated the repair of fractures in rats and reported that PRP enhances the healing both histologically and radiographically (24). Guzel et al. reported that PRP has promoted fracture healing and accelerate the histological union (30). Penteado et al, Messora et al, and Fennis et al. reported that PRP positively affects osteogenesis (31–33). Similar results with the literature were obtained in this study. In clinical studies, Taschieri et al. reported that PRP gave better results on the 7th day but in the long-term period, soft tissue and wound healing in implant applications after extraction was the same as the

control group (34). Del Fabro et al. intraosseous periodontal defect treatment (35), Kassolis et al. sinus lifting surgeries (36) can be given as an example of PRP's positive effects.

Vaishnavi et al. evaluated 20 subjects with HA, PRP, HA+ PRP, and control. They argued that the PRP+ HA group has better results in bone regeneration (37). Marcacci et al. concluded that PRP with ß-TCP was not indicated and gave better results with autogenous bone and HA grafts in their systematic review (38). On the other hand, Pocaterra et al. and Lemos et al. concluded that PRP administration did not contribute to implant survival in their meta-analysis (20, 39)

Conclusions

In conclusion, within the limitations of this study, we propose that collagen membrane, HA, and PRP can be used safely and effectively to facilitate bone regeneration when needed. We believe that PRP plays a crucial role in regenerative treatment due to its growth factor, osteoinductive potential, low cost, and ease of application. Although PRP applications are currently in use, there are still some controversial reports on their effectiveness. At this point, we propose that protocols for the standardization of PRP and its clinical use should be developed. To this end, long-term randomized clinical studies should be conducted.

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Ethical Approval: Ethics committee approval was received for this study from Dicle University, Faculty of Dentistry in accordance the World Medical Association Declaration of Helsinki, with the approval number: 2006/43.

Peer-review: Externally peer-reviewed.

Author Contributions: Conception – Ç.Ç.G.; Design –B.G.; Supervision – Ç.Ç.G.; Materials – Ç.Ç.G.; Data Collection and/or Processing – Ç.Ç.G.; Analysis and/or Interpretation – B.G.; Literature Review – Ç.Ç.G; Writer – B.G.; Critical Review – B.G.

Conflict of Interest: No conflict of interest was declared by the authors.

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