The Effect of Fish Bone Powder and Human Lyophilized Platelet on Bone Healing in Rabbit Model

Abstract

**Background:** Acceleration of bone healing is one of the most challenging issues in orthopedic science. This study aimed to evaluate bone healing process with the application of fish bone powder and human lyophilized platelet (prepared with a novel protocol) in the rabbit animal model.

**Materials and Methods:** This study was carried out on 20 male New Zealand white rabbits (12 month old), divided into four equal groups as control, fish bone powder, lyophilized platelet and a combination of fish bone powder and lyophilized platelet. After exposing the radius, a bone segment (10 mm) was cut from the bone, and the empty space was left empty in the control group but filled with the mentioned biomaterials in other groups. Radiographs of each rabbit were taken on the 14th, 28th, 42nd, and 56th post-operative days to evaluate bone formation, union and remodeling of the bone defect. All animals were euthanized on the 56th post-operative day for histopathological evaluation.

**Results:** Radiological evaluation showed a significant difference between the lyophilized platelet group (P=0.02) and the control (P=0.007) and the fish bone powder (P=0.005) on 56th post-operative day, where the lyophilized platelet group was superior, compared to other groups. Moreover, the histopathological evaluation revealed a significant difference between the control group (P=0.01), the fish bone powder (P=0.03) and lyophilized platelet group (P=0.01), where treatment groups were superior, compared to the control group on 56th post-operative day. Nonetheless, there was no evidence of graft rejection in all groups.

**Conclusion:** According to the results of the study, using lyophilized platelet could accelerate the bone healing process in rabbit and has the potential for use in medicine.

**Keywords:** Orthopedics, Bone healing, Biomaterial, Xenograft, Lyophilized Platelet, Fish Bone Powder

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Introduction

Bone healing is one of the most challenging issues, occupying the human mind for a long time. Injuries such as cervical dislocation, skull fracture, and compound fracture are shown on a papyrus discovered by Edwin Smith in ancient Egypt (1). In addition, wooden and fabric bone scaffolds found inside the mummies discovered from ancient Egyptian tombs showed some of the most basic orthopaedic surgeries (2). Even after thousands of years, scientists are still searching for materials and methods that can accelerate the process of bone healing. While human beings’ welfare has increased due to technological and industrial advancements, these amenities have caused some complications. For instance, complications such as fractures have increased with the development of motor vehicles. Bone fracture is one of the common injuries of the musculoskeletal system, imposing medical costs on insurance companies, the government and even the injured person. On the other hand, they result in physical inability in people and possibly mental disorders in the injured and their families (3, 4). Other disorders of the musculoskeletal system include trauma, tumors, osteitis, delayed union and...
non-union, osteotomies, and joint stability, all of which can cause major bone defects. To solve these problems, scientists have suggested various approaches, including autografts, allografts, xenografts and alternatives to bone graft. The golden standard for a bone graft is using your own bones due to having inherent properties of osteoconductivity, osteogenicity, and osteoinductivity. However, bone extraction can lead to complications in the patient and the removal site. Bone resection sites for autograft include ileum clamp, the upper end of the humerus, and tibia. It is notable that bone resection from the mentioned sites requires additional surgery, which increases the possibility of pain, infection, fracture, blood loss and the limited amount of harvested tissue. In general, allografts are easily accessible and there is no concern regarding the amount of extracted tissue. Nonetheless, most concerns are related to the increased possibility of communicable diseases. Bone graft alternatives include metals (e.g., platinum and titanium) and polymers (e.g., polyethylene and polymethylmethacrylate), which are mostly proposed as a tissue alternative rather than a tissue regeneration agent. On the other hand, xenograft is defined as the transplantation of fluids, cells, tissues or organs from one species to another, such as the transfer of fish skin to horses. Xenografts have been suggested as attractive options for use in orthopedic injuries due to limitations in access and risk of transmission of allograft-related communicable diseases and lack of assurance of integrity and biodegradability of synthetic composites. In general, xenograft transplantations are more convenient options, compared to allografts, due to scarcity and costs. As biological scaffolds, xenografts contain extracellular matrix, as well as laminin, collagen, elastin and fibronectin, showing chemical and mechanical properties similar to human tissue, which can create an alternative to tissue defect by host body through providing a supportive structure. The skeleton of the fish is made of either cartilage or bone. In addition, the bone skeleton of the fish contains type I collagen, non-collagen proteins, and bone growth-inducing factors and can be used as an affordable and available xenograft in fracture healing. Bones in mammals consist of four types of cells: osteoblasts, osteoclasts, osteocytes, and osteoprogenitor cells. One of the functions of the bones is to provide a mechanical structure that protects the organs in the ventricular cavities from injury. In addition, bones play a fundamental role in the generation of blood cells and the metabolism of ions. Calcitonin is involved in helping to regulate levels of calcium and phosphate in the blood, opposing the action of parathyroid hormone. For instance, low calcium levels in the blood stimulate parathyroid hormone secretion, thereby increasing and decreasing the activities of osteoclasts and osteoblasts, respectively, which ultimately lead to bone resorption and normal blood calcium levels. These hormones are also effective in urinary calcium, phosphate and sodium excretion. The water around the fish is an unlimited source of calcium and they can absorb the calcium in the water directly, unlike terrestrial vertebrates. Therefore, if the fish diet contains a limited amount of calcium, they can continue their normal growth without any disturbance to the bone. Teleost fish (e.g., Salmon and Tilapia), are more evolved, compared to Holostei fish, and have a unique bone known as “the acellular bone”. Due to lack of osteocytes in its structure, the acellular bone is used in the bone graft to minimize immune response at the transplantation site in order to avoid transplant rejection. In the past, scientists have considered this type of bone dead from a metabolic perspective. Nonetheless, recent
research indicates the role of this bone in calcium metabolism and is now considered a living tissue\(^{(10)}\). Blood platelets are small, non-nucleated elements with a limited lifespan (10 days), which are formed with the fragmentation of megakaryocytes in the bone marrow. One of the major effects of these platelets is the repair of blood vessels, which has been well studied in patients with hemophilia and even in topical applications on damaged tissues. According to scientists, this restorative effect of platelets on blood vessels is due to granular platelet components rich in healing factors\(^{(17)}\).

To date, more than 300 molecules have been found in the platelet secretory substances. Dense granules often contain small molecules such as serotonin, ADP, and polyphosphates. Alpha granules contain a set of proteins that form the huge part of platelet secretions. These include blood factors (e.g., V, VWF, and factor, fibrinogen), angiogenesis factors (e.g., angiogenin and VEGF), anti-angiogenic factors (e.g., angiotatin and PF4), growth factors (e.g., PDGF, bFGF, and SDF1\(\alpha\)), proteases (e.g., MMP2 and MMP9), and necrotic factors (e.g., TNF\(\beta\) and TNF\(\alpha\))\(^{(18)}\). In addition, mRNAs are responsible for protein construction, which remain in platelets with the origin of megakaryocytic cytoplasm\(^{(19)}\).

Therefore, platelets are the source of biologically active proteins such as growth factors that accelerate injury healing and can be used to accelerate the pace of bone defect healing. Platelets can be found in a variety of platelet products such as platelethphesis, washed platelets, frozen platelets, cold-stored platelets, platelet-derived microparticles (PMP), platelets affected by chemicals, platelet-rich plasma (PRP), and lyophilized platelets\(^{(20)}\). In some studies, researchers were able to increase bone tissue construction by combining PRP as an osteogenic stimulus full of factors such as PDGF, VEGF, TGF-\(\beta\)1, TGF-\(\beta\)2, and IGF, and other materials such as Coral Reefs in the Persian Gulf. Meanwhile, conflicting results have been obtained in some other studies\(^{(5, 21, 22)}\).

Similar to other studies, different animal species can be used to carry out orthopedic studies. Nevertheless, there are inter-species and intra-species differences in terms of the biochemistry, biomechanics and anatomy of normal bones and the process of healing, which does not necessarily reflect the process of bone healing in humans. For instance, sheep have a spongy and dense bone, which undergoes rearrangement at a rate similar to that of human tissue. However, they have plexiform bone tissue (similar to woven bone tissue) and encompass fewer Haversian canals. This difference also exists in bone composition, and this plexiform bone is rarely seen in humans. On the other hand, while dog bone is similar to human bone tissue in terms of texture, structure, and architecture, it is a combination of lamellar and plexiform bones, the rearrangement of which is very diverse and their biomechanical properties are different. Nonetheless, rabbit bone is rapidly rearranged and the Haversian canal in the rabbit is similar to that of humans while mice lack the Haversian canal. The radius and the ulna constitute as the bones of the forearm in rabbits, where the ulna bone acts as a splint when a part of the radius bone is removed and there is no need for internal or external stabilization equipment. On the other hand, rabbits are a good option for orthopaedic studies due to small size, easy manipulation, ability to be kept in a small space, low purchasing costs, and simple maintenance, and feeding. Therefore, we used rabbits in the present study to evaluate the bone healing process\(^{(23, 24)}\). This study aimed to evaluate the effect of fish bone powder and lyophilized platelet separately and a combination of both on bone healing in the rabbit model.

**Method**

The present study was conducted in the surgery and radiology department of School of Veterinary Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran in spring...
and summer of 2019. The research was approved by the ethics committee of the university, and the researchers adhered to all animal rights during the study. In addition, the study was carried out based on the basics of working with animals approved by the research council of the school (code: 170/996).

**Preparation of Lyophilized Platelet**
A fresh bag of platelets was obtained from the Blood Transfusion Organization of Chaharmahal and Bakhtiari Province and transferred to the laboratory for lyophilization at a temperature of 22°C (20). Afterwards, the platelets were poured into falcon tubes and transferred to a freezer-driver device that creates a porosity state in the material in question with its vacuum pump and lowers the temperature to minus 50°C during freezing. Following that, the lyophilized platelet was kept in the freezer until surgery.

**Preparation of Fish Bone Powder**
Eight alive Tilapia fish with a weight of 150±50 gr were received from the Isfahan University of Technology and transferred to the animals’ house in Shahrekord University. The fish were euthanized one week before surgery, and meat and fascia-free spine and rib bones were separated with a sterile method. Ultimately, the samples were washed with 0.9% normal saline, dried at room temperature and powdered in a porcelain mortar. The fish bone powder was kept in a refrigerator until surgery and was sterilized by a sterile nano device.

**Preparation of Animal Species and Storage Conditions**
In this study, we used 20 male New Zealand white rabbits with a weight of 2±0.4 kg and age of 12 months, which were kept in the animal house of Shahrekord University for 15 days to adjust to the new environmental, nutritional, weather and light conditions. Notably, the animals had free access to food and water during this period, and parasite decontamination was carried out by treating the samples with a 0.4 mg/kg dose of ivermectin (Iver 1%) on the first day, which was repeated two weeks later.

**Grouping and Performing Surgery**
The rabbits were randomly divided into four five-sample groups of control, fish bone powder, lyophilized platelet, and a combination of fish bone powder and lyophilized platelet. Light anesthesia was achieved by intramuscular injection of a combination of xylazine and ketamine hydrochloride (2 and 10 mg/kg doses, respectively), followed by the use of an inhaled anesthesia machine containing isoflurane and oxygen to induce surgery anesthesia and continue anesthesia in animals (26). In the next stage, aseptic surgery was performed on the right hand skin, where a five-cm cut was made on the anterior-internal surface of the radius, a 10-ml bone segment was removed, and the bone defect was filled based on the above grouping. After placing the transplantation materials, the muscles and skin were sutured in a single layer with simple suturing pattern and Vicryl suture (number 0-2). Rabbits were injected intraperitoneally with 10% enrofloxacin at a dose of 10 mg/kg daily for three days.

**Evaluation**
Rabbits were evaluated at clinical, radiological and histopathological levels.

**Clinical Evaluation**
Rabbits were examined daily to assess the operation site and pay attention to the status of inflammation, infection, wound healing, bleeding, and weight gain on the target organs.

**Radiological Evaluation**
Radiology graphs of lateral-mid views of the arms of all rabbits were prepared on the 14th, 28th, 42nd and 56th post-operative days. The film distance from the X-ray source was approximately 90 cm and the radiograph settings were adjusted to 44 kV and 4 mAs. Moreover, the modified Lane-Sandhu radiological scoring system was applied to evaluate and rate healings in the radiology graphs (Table 1) (5,10).
**Table 1. Evaluation and grading of healing in radiological graphs using Sandhu and Lane deformed system**

<table>
<thead>
<tr>
<th>Bone formation</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper union</td>
<td>2</td>
</tr>
<tr>
<td>Lower union</td>
<td>2</td>
</tr>
<tr>
<td>Rearrangement</td>
<td>2</td>
</tr>
<tr>
<td>Maximum score</td>
<td>10</td>
</tr>
</tbody>
</table>

**Histopathological Evaluation**

The rabbits were kept for eight weeks and were anesthetized on the 56th day by intermuscular injection of xylazine and ketamine hydrochloride (doses of 5 and 40 mg/kg, respectively). In the end, the animals were euthanized by intrathecal injection of sulfate magnesium. Afterwards, samples were obtained from the transplant site in a way that the upper and lower end of the bone tissue remained intact. The samples were placed in 10% formalin for 10 days to establish tissue fixation. Formalin was then removed from the containers and 10% nitric acid was added for demineralization for one week. After these processes, the samples were washed with water and processed by an autotechnicon device. The processes of clarification, paraffinization, wax infiltration, and molding were carried out in the next stage. Tissue sections were cut to a thickness of five microns using a microtome machine, and the samples were stained by hematoxylin and eosin, followed by attaching the lamell to the slide using ethylene adhesive.

After performing the above steps, the slides were examined by light microscopy. In the next stage, the histopathological evaluation of the slides was carried out using the Emery grading system. This grading system is, as follows: zero points if the defect is empty, one point if the defect is filled only with fibrous connective tissue, two points if the fibrosis tissue is dominant in the combination of fibrosis tissue and fibrocartilage, three points if the fibrocartilage tissue is dominant in the combination of fibrosis tissue and fibrocartilage, four points if there is only the fibrocartilage tissue, five points if the fibrocartilage tissue exist along with the bone tissue and the fibrocartilage tissue is dominant, six scores, if the fibrocartilage tissue is along with bone tissue and the bone tissue, is dominant, and seven scores if the defect has only bone tissue.

**Statistical Analysis**

Data analysis was performed in SPSS version 24 using the nonparametric Kruskal-Wallis test and Mann-Whitney U. In addition, a P-value of less than 0.05 was considered statistically significant.

**Results**

**Clinical Evaluation**

After complete recovery from anesthesia, swelling, laminitis, as well as lack of weight gain and mobility of hand were observed after the surgery. However, these syndromes disappeared seven days after the surgery. It is worth noting that no disease or death was observed during the research.

**Diagnostic Imaging Results**

Evaluation of radiographies on the 14th and 28th days showed no significant difference between the research groups (figures 1 and 2). On the 42nd post-operative day, radiography assessments demonstrated a significant difference between the groups of fish bone power and the combination of lyophilized platelet and fish bone powder (P=0.04), and the groups of lyophilized platelet and the combination of fish bone powder and lyophilized platelet (P=0.008). Nonetheless, poorer results were obtained in the combination group, compared to the groups of fish bone powder and lyophilized platelet. In addition, no significant difference was observed between the control and other groups on the 42nd post-operative day (Figure 3 and Table 2). Compared to other groups, the lyophilized platelet showed a significant superiority on the 56th post-operative day. In addition, no significant difference was observed between the control and other groups on the 42nd post-operative day. In addition, the level of significance between the group of lyophilized platelet and control, fish bone powder and the combination groups was P=0.02, P=0.007, and P=0.005, respectively. On this day, no other significant difference was observed among the research groups (Figure 4 and Table 2). The best evidence
of osteogenesis was observed in the lyophilized platelet group at week eight when the highest possible score (i.e., 10) was obtained in all figures in the mentioned group. The figures show the formation, uniformity, and rearrangement of bone completely (Figure 4-C).

Figure 1. Radiography images 14 days after the surgery. A) Control group, B) Fish bone powder group, C) Lyophilized platelet group, and D) A combination of fish bone powder and lyophilized platelet

Figure 2. Radiograph images on the 28th post-operative day, A) Control group, B) Fish bone powder group, C) Lyophilized platelet group, and D) The combination of fish bone powder and lyophilized platelet group

Figure 3. Radiograph images on the 42nd post-operative day, A) Control group, B) Fish bone powder group, C) Lyophilized platelet group, and D) The combination of fish bone powder and lyophilized platelet group

Figure 4. Radiography images on the 56th post-operative day, A) Control group, B) Fish bone powder group, C) Lyophilized platelet group, and D) The combination of fish bone powder and lyophilized platelet group
The non-parametric Kruskal-Wallis test was conducted and the difference between the groups was significant in different weeks (P<0.05). However, the Mann-Whitney U test was performed when P<0.05.

A significant difference (P=0.04) was observed between the fish bone powder group and the combination of fish bone powder and lyophilized platelet group in the sixth week, where the group of fish bone powder had a higher impact, compared to the combination of fish bone powder and lyophilized platelet group.

A significant difference (P=0.008) was observed between the lyophilized platelet group and the combination of fish bone powder and lyophilized platelet group in the eighth week, where the lyophilized platelet group had a higher impact, compared to the combination of fish bone powder and lyophilized platelet group.

A significant difference (P=0.02) was observed between the control and lyophilized platelet groups in the eighth week, where the lyophilized platelet group had a higher impact, compared to the control group.

A significant difference (P=0.007) was observed between the fish bone powder and lyophilized platelet groups in the eighth week, where the lyophilized platelet group had a higher impact, compared to the fish bone powder group.

A significant difference (P=0.005) was found between the lyophilized platelet group and the combination of fish bone powder and lyophilized platelet group in the eighth week, where the lyophilized platelet group had a higher impact, compared to the combination of fish bone powder and lyophilized platelet group.

**Histopathology Results**

On the 56th post-operative day, the histopathological assessments demonstrated a weaker performance in the control group, compared to other groups (P<0.05). According to the results, a significant difference was observed between the control and fish bone powder groups, the control and lyophilized platelet groups, and the lyophilized platelet and the combination of fish bone powder and lyophilized platelet groups (P=0.01, P=0.03, and P=0.01, respectively). However, the control group had a weaker performance, compared to the other groups, while no significant difference was observed between the other groups. In the control group, the lyophilized platelets prevent bone union (Figure 5-A). In other groups, the combination of fibrocartilage and bone tissues completely filled the defective space (Figure 5-B, C, D, and E). While thick trabecular bone and raw fish bone pieces were found in the bone powder group, there were no traces of bone marrow canal formation. On the other hand, there was evidence of bone marrow canal formation and progression in the combination and lyophilized platelet groups. In the combination group, the raw pieces of fish bone powder prevented the greater progress of bone marrow (figures 5 & 6).

### Table 2. Total radiography scores of bone healing at specified intervals after surgery based on the median (minimum-maximum)

<table>
<thead>
<tr>
<th>Group</th>
<th>Control Fish bone powder</th>
<th>Lyophilized platelet</th>
<th>Combination of fish bone powder and lyophilized platelet</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>4 (6-3)</td>
<td>3 (4-3)</td>
<td>3 (4-1)</td>
<td>0.2</td>
</tr>
<tr>
<td>4</td>
<td>6 (9-3)</td>
<td>6 (7-3)</td>
<td>6 (9-6)</td>
<td>0.2</td>
</tr>
<tr>
<td>6</td>
<td>6 (9-3)</td>
<td>7 (10-6)</td>
<td>9 (10-8)</td>
<td>0.02</td>
</tr>
<tr>
<td>A</td>
<td>6 (10-4)</td>
<td>8 (9-6)</td>
<td>10 (10-10)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

### Table 3. Total histopathological scores of bone healing on the 56th post-operative day based on the median (minimum-maximum)

<table>
<thead>
<tr>
<th>Group</th>
<th>Control Fish bone powder</th>
<th>Lyophilized platelet</th>
<th>Combination of fish bone powder and lyophilized platelet</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>3 (4-2)</td>
<td>5 (6-5)</td>
<td>6 (6-5)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

- The non-parametric Kruskal-Wallis test was performed and the difference between the groups was significant (P<0.05), which led to performing the Mann-Whitney U test.
b- There was a significant difference between the control and fish bone powder groups, the control and lyophilized platelet groups, and the control and the combination groups (P=0.01, P=0.03, and P=0.01, respectively). However, the control group had a weaker performance, compared to the other groups.

Figure 5. Histopathological sections of control and transplantation groups on 56th post-operative day with x10 magnification. A) control group, B) fish bone powder group, C) lyophilized platelet, D and F) group of the combination of fish bone powder and lyophilized platelet. Each of the symbols in the sections above is indicative of a specific tissue: ★ Lyophilized platelet, ➤ bone marrow canal, ➔ fish bone section, ➤ old bone, ➤ new bone.
Figure 6. A histopathological section of the groups of the combination of fish bone powder and lyophilized platelet with different magnifications, stained with haematoxylin and eosin. A) x5 magnification, B) x10 magnification, and CO x40 magnification equal to the chondroplasia region. Each of the symbols in the section above is indicative of a specific tissue:

- magnified area
- old bone
- new bone
- bone marrow canal
- the area experiencing chondroplasia
- chondroblast lacunae

Discussion

In the present study, we used fish bone powder and lyophilized platelet separately and in combination with each other. Our goal was to assess the effect of fish bone powder as a scaffold, lyophilized platelet as a bone stimulus, and the combination of fish bone powder and lyophilized platelet on the rabbit model. In the histopathological sections of fish bone powder group, thick trabecular bone formation was observed while there was no evidence of bone marrow canal formation. In addition, raw fish bone pieces were present in the defective site. In a retrospective study by Mia et al. (2009), the use of head of some fish (e.g., *Anabas testudineus*) along with *Cissus quadrangularis* L. stem and dried fish along with the whole plant of *Gnaphalium luteoalbum* L. was useful in bone fracture healing (28). Ozawa and Suzuki (2002) and Yunoki et al. (2004) attempted to
construct biomedical ceramics using fish bone, which has a major phase of hydroxyapatite (29, 30). In 2013, Oteyaka et al. used the powdered fish heads (species of argyrosomus regius) after the transition to the hydroxyapatite phase for bone graft (31). In 2016, Orian et al. used salmon fish bone and its demineralized bone matrix for healing in rat. According to the results, all members of the fish bone graft group showed a thick layer of woven bone along with the irregular trabecular bone. In addition, blood vessels were well developed and bone marrow was properly formed. Moreover, the fish bone graft group had more osteochondral tissue at the defective tissue site. However, the comparison of biomechanical parameters showed no significant difference. Notably, raw fish bone pieces were present at the graft site (10).

In the present study, we observed fibrocartilage tissue, trabecular bone, cortical bone, and even bone marrow canal progress in the lyophilized platelet group. The complete formation of bone modulus canal was also observed in the radiology images on the 56th day. Nash et al. (1994) were the first researchers who used the platelet-derived growth factors of foreign origin in rabbit tibia. In the mentioned study, radiographic, biomechanical (three-point flexural test), and histopathological assessments showed the superiority of the treatment group with these factors (26). In 1998, Marx et al. demonstrated the presence of at least three growth factors (PDGF, TGF-b1, and TGF-b2) in PRP, which had receptors in the spongy bone marrow cells. These scholars believed that the condensation of released growth factors could be achieved by activating platelets using compounds such as calcium chloride mixed with 10,000 units of bovine thrombin and starting the clotting process. They used autologous platelet-rich plasma to repair autologous bone defects in bone repair for the first time, demonstrating the positive effects of adding PRP on bone amount and quality (32).

Gerard et al. (2006), Meymandi Parizi et al. (2011) and Orian et al. (2011) showed that PRP has excellent osteogenic effects and is capable of repairing critical bone defects owing to its regenerative effects in their studies on dogs and rabbits by adding PRP to insular bone graft, coral reefs in the Persian Gulf, and hydroxyapatite, respectively (5, 22, 32). In 2017, Alidadi et al. used platelet gel and gelatin scaffold, indicating that the independent use of the gel had a higher impact on bone healing (34). In another study by Forum et al. (2002), it was reported that adding PRP to grafts led to no significant increase in the formation of new bones (21). According to the author’s information, this study was conducted globally for the first time in two dimensions: first, an inventive method for releasing healing agents found in platelets, and second, the effects of this platelet product on bone healing.

In hematological studies to obtain platelet-lyophilized platelets, the platelets were washed with 1.8% paraformaldehyde, then frozen in 5% albumin, and finally lyophilized. These processes protect platelets from freezing damage (35). Since we did not use cryopreservatives in contrast to other studies, this superior osteogenesis of lyophilized platelet might be due to the elimination of paraformaldehyde and cytotoxic effects (36). In addition, the platelet would be destructed and biologically active molecules and proteins would be released without the use of this compound. Comparison of radiograph results in the eighth week showed the significant superiority of the lyophilized platelet group, compared to the other groups. However, histopathological results were indicative of the significant preference of the treatment groups, compared to the control group.

Conclusion: in the end, the radiological and histopathological comparison of the research groups in terms of healing process demonstrated the superiority of the lyophilized platelet group, which had an effect on osteogenesis, bone tissue regeneration, and bone medullary canal formation.
Therefore, it is recommended that the lyophilized platelet powder be produced and combined with compounds such as demineralized bone matrix, the biodegradability of which has been confirmed, to evaluate its osteogenesis effects along with another substance that has the property of bone conduction. In addition, it is suggested that fully milled fish bone powder be applied so that the phagocytosis effects of the local immune system on these bone fragments can be overcome, the sections could be analyzed and would not inhibit the formation of the bone marrow canal. The use of the cartilaginous fish powder might have faster and better biodegradation effects, compared to the fish bone skeleton, and be able to move the healing process forward.

**Conflicts of Interest**
None declared.

**References**