

## Bone Healing in a Critical-Size Defect Using Platelet-Rich Plasma

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**Purpose:** To evaluate the effect of platelet-rich plasma (PRP) on bone healing in a critical size osteotomized defect of the rabbit mandible.

**Materials and Methods:** Twelve adult female New Zealand White rabbits were randomized to one of two treatment groups: group A had an osteotomy with addition of a bone graft, and group B had an osteotomy without a bone graft. Regardless of treatment group, one side in each rabbit was randomly selected to receive PRP on one side as an internal control. Bilateral 1.0 x 0.5 cm mandibular inferior border osteotomies were performed in each animal, 0.5 cm anterior to the antegonial notch to create critical-size defects. The osteotomy sites were evaluated by histologic and radiographic analyses for bone healing at 1, 2 and 3 months following surgery.

**Results:** A four point ordinal scale was used to compare healing, based upon radiographic density, radiographic height, and histologic height of new bone formation. Group A rabbits showed significantly shorter healing times compared to Group B rabbits. A pair-wise analysis indicated that the addition of PRP did not increase the overall score of any measured parameter, at any time interval ( $p>0.9$ ).

**Conclusions:** In the rabbit osteotomy model, bone grafting (group A) significantly improved healing in comparison to no bone grafting (group B). In critical sized defects of the mandible, an increased radiographic and histologic bone density and height were seen at 1, 2, and 3 month intervals in the bone graft group; however, the addition of PRP did not appear to provide any statistically significant benefit to healing in either group.

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## **Bone Healing in a Critical-Size Defect Using Platelet-Rich Plasma**

It has been established that various growth factors are released by platelets that have been shown to enhance bone healing. The production of platelet-rich plasma (PRP) by gradient centrifugation allows clinicians to produce high concentrations of platelets and these growth factors. Platelet-derived growth factor (PDGF), transforming growth factor- $\beta$  (TGF- $\beta$ ), and insulin-like growth factor -I (IGF-I) are thought to be key factors in proliferation and differentiation of mesenchymal cells into osteocytes. Vascular endothelial growth factor (VEGF) has been shown to be a key signaling factor in angiogenesis. All these growth factors released from the  $\alpha$ -granules of platelets work in concert to promote the regeneration of bone and its supportive vasculature after injury. Clinicians have long searched for an alternative to harvesting of autogenous bone grafts due to the associated morbidity. Additionally, means of augmenting the volume and contour of autologous grafts is often desired. Preparation of PRP has the appeal of being readily available and resulting in relatively low morbidity. However, results from past experimental studies using PRP in animal models have been contradictory. Fennis<sup>6</sup> et al analyzed the radiographic and later histologic changes induced by adjunctive PRP in critical defects of goat mandibles (N=28) repaired with autologous bone grafting. This study demonstrated statistically superior healing in the PRP group at 6 and 12 weeks, but showed no statistical improvement radiographically at 3 weeks. This result is somewhat discouraging as a normal healing without PRP would be expected to occur by six weeks, and therefore healing analyzed as superior at these later times may be inconsequential. Fennis<sup>6</sup> et al subsequently analyzed subjects histologically and found improved bone union and callus formation histologically in the PRP group at 3 weeks.

Aghaloo<sup>2</sup> et al also investigated whether healing was enhanced by PRP in noncritical defects of the rabbit cranium (n=15) with and without bone grafting. The results showed no statistical benefit to the addition of PRP in these subjects. While subtle enhancements in bone healing have been elucidated, further testing is needed to determine if there exists statistically significant benefits from platelet-rich plasma and if its clinical application will result in improved bone healing not only radiographically and histologically, but also in terms of clinical outcomes. The purpose of this study is to determine the effects of platelet-rich plasma (PRP) on bone healing in a critical-size defect of the rabbit mandible.

## **Materials and Methods**

### **SURGICAL PROCEDURE**

The University of Nebraska IACUC reviewed the protocol and approved this animal study. Twelve adult female New Zealand White rabbits were randomized to one of two general treatment groups that had an osteotomy with osteotomy of the mandible performed in a standard fashion by the same surgeons. Group A received an osteotomy with addition of a bone graft (particulated bone from the osteotomy site), and Group B received an osteotomy without bone graft. Regardless of treatment group, one side in each rabbit was randomly selected to receive PRP added to the osteotomy site either with the bone graft in Group A, or alone in Group B (Table 1). After appropriate intramuscular anesthesia with xylazine (5-7 mg/kg) and ketamine (35-45 mg/kg), and following the administration of local anesthesia, the mandible was shaved and prepped

with betadine solution. An extraoral skin incision was made along the inferior border of the mandible bilaterally. Dissection was carried down through the subcutaneous and muscle layers to the periosteum. The periosteum was then incised and reflected to expose the underlying mandible. Using a rotary handpiece, bilateral standardized 1.0 x 0.5 cm mandibular inferior border osteotomies were created in each animal at 0.5 cm anterior to the antegonial notch to create critical defects (Figure 1). In Group A subjects, osteotomized bone was collected, particulated and auto-grafted into the sites bilaterally. Group B subjects received no bone graft in either site. All subjects in both groups received PRP on one side, chosen randomly, as an internal control group. In summary, Group A received bone graft alone on one side (Figure 2) and bone graft with PRP on the other side (Figure 3), while Group B had an empty defect on one side, and an empty defect with PRP alone on the other side (Figure 4). The PRP preparation consisted of cannulation of an ear vein for collection of a 20 cc blood sample that was mixed with 4 mL citrate dextrose (1:5 ratio) to achieve anticoagulation. The mixture was then placed in a Cell Factor Technologies, Inc. centrifuge (Biomet Co. Warsaw, IN) and spun using the manufacturer's instructions to separate the platelet rich plasma (PRP) layer from the platelet-poor plasma (PPP) and red blood cell fractions. A mixture of 10 mL of 10% calcium chloride and 10,000 U of topical thrombin was prepared. After the PPP was removed from the chamber, one milliliter of the CaCl/thrombin mixture was added to the remaining six milliliters of PRP and agitated with air in preparation to add to one side in each treatment group. Lastly, the soft tissues were re-approximated and closed in a layered fashion with interrupted 4-0 resorbable polyglactin sutures and skin staples.

## SAMPLE EVALUATION

The subjects in each group were randomized to 3 time periods for bone healing. Animals were randomly chosen and sacrificed at each time interval: 1, 2 and 3 months. The mandibles were harvested en-bloc and radiographs were taken of each sample at each time period. Specimens were then fixed in 10% buffered formaldehyde and decalcified with formic acid. Following dehydration and paraffin embedding, the specimens were sectioned at 4  $\mu$ m and stained with H&E. The specimens were examined by light microscopy. The osteotomy sites were evaluated by histologic and radiographic analysis for bone healing at each time point.

## DATA EVALUATION SCALES

At each time interval, a four point ordinal scale was used to compare healing based on three criteria: radiographic density, radiographic height, and histologic height.

Radiographic density was rated as follows: 1 = no bone formation in defect, 2 = density is less than adjacent cancellous bone, 3 = density is greater than or equal to adjacent cancellous bone, but less than cortical bone, and 4 = density is greater than or equal to density of adjacent cortical bone. The radiographic height and histologic height were assigned values as follows: 1 = 0-25% fill of height in defect area, 2 = 26-50% fill, 3 = 51-75% fill, 4 = 76-100% fill.

## STATISTICAL ANALYSIS

To evaluate the enhancement in healing with the addition of PRP within each rabbit, comparisons of the median scale measurements of each side were made using non-parametric paired Wilcoxon signed rank test. Between animal comparisons of the median scale measurements were also made using a non-parametric 2-group Wilcoxon rank sum test.

## **Results**

The ordinal scale was used for the evaluation of radiographic height and density, and histologic height (Table 2).

### **RADIOGRAPHIC RESULTS**

Radiographic height and density was rated by one blinded investigator on the four point ordinal scale described above. There were no significant height differences seen between group A subject sites with and without PRP ( $p>0.9$ ) or group B subject sites with and without PRP ( $p>0.9$ ). The scores tended to be higher for group A subjects versus group B subjects ( $p=0.001$ ). This demonstrated the positive effect of the particulate bone grafting (Figures 5 and 6). There were no significant density differences seen between the group A subjects with and without PRP ( $p>0.9$ ) or group B subjects with and without PRP ( $p=0.3$ ). There were, however, marginally higher density scores when comparing group A with PRP subjects to group B subjects with and without PRP ( $p=0.06$ ,  $p=0.09$ ).

These trends were not statistically significant ( $p>0.05$ ), but were not seen when comparing group A subjects without PRP to group B subjects overall ( $p=0.2$ ).

## HISTOLOGIC RESULTS

After sample processing, the height of the bone fill in the defect was analyzed microscopically and rated by one blinded investigator using the previously described scale. There were no significant height differences seen between group A subject sites with or without PRP ( $p>0.9$ ) or group B subject sites with or without PRP ( $p>0.9$ ). As was seen in the radiographic samples, the scores tended to be higher for all group A subjects versus the group B subjects with PRP ( $p=0.007$ ) and without PRP ( $p=0.02$ ) (Figures 7 and 8).

## Discussion

The use of biological signaling molecules and growth factors to provide a more favorable environment for wound repair and bone healing has led to intense interest by many clinicians and researchers. Platelet rich plasma (PRP) has been applied in multiple studies on bone and soft tissue healing. The results of these studies have been mixed and it remains unclear if there is any significant enhancement in healing provided by PRP.

Marx<sup>4,7</sup> evaluated a series of cancellous marrow grafts placed in continuity defects of the human mandible, and found consistently increased bone healing at 2, 4, and 6 month

intervals on panoramic radiography when compared to controls. At least one endosseous implant was ultimately placed in each cancellous graft after maturity, which allowed for a core sample to be taken for histomorphometric analysis. The results of the histomorphometry also showed greater trabecular bone area for grafts treated with PRP than grafts alone. In an animal pilot study, Aghaloo<sup>2</sup> reported on the use of PRP in rabbit cranial bone defects. Treatment groups consisted of bone graft, bone graft with PRP, PRP alone, and no treatment. Defects were evaluated radiographically and histologically at 1, 2 and 4 months post-operatively. Results did not show a significant difference for defects treated with the addition of PRP. As in the present study, defects treated with bone and bone with PRP tended to show significant improved bony healing. On radiographic evaluation, it was noted that defects treated with PRP alone actually tended to show less bone density than no treatment, although this was not statistically significant. The addition of PRP to allograft materials as well as autogenous bone has been reported. In another rabbit cranial defect study, Aghaloo<sup>3</sup> evaluated the effects of PRP autogenous bone, Bio-Oss, freeze-dried bone, and freeze-dried demineralized bone. Autogenous bone was again found to be the most ideal grafting substrate although Bio-Oss with PRP did show increased histomorphometric bone healing compared with Bio-Oss alone. This is an interesting finding since it was previously assumed that PRP exerted its effects through growth factor mediation on living graft material. The question of sufficient platelet concentration has previously been raised as well. An in-vitro study by Choi<sup>5</sup> actually found that high concentrations of PRP could suppress alveolar bone cell viability and proliferation. PRP concentrations of 1% to 5% appeared to enhance bone growth in-vitro, while a PRP concentration of 100% was reported to be toxic to

cultured cells. Although the present study did analyze the platelet concentration, it is believed that the manufacturer's protocol allowed for an adequate density of platelets to be obtained in the PRP. If there is a therapeutic range of PRP concentration, future studies may evaluate not only the platelet count present in the sample but the ideal PRP concentration within the graft.

Certain limitations of this present study should be considered. Regarding the osteotomy design, the size and placement of the osteotomy was limited due to the dimensions of the rabbit mandible itself, and proximity to the tooth-bearing region, however it was fairly predictable that a 1.0 x 0.5 cm defect would fail to heal completely in an adult rabbit in the specified time frame. Statistical limitations may include the lack of adjustments made for multiple within and between-group comparisons, and, as a result, significant and non-significant p-values must be interpreted cautiously. The results of this study showed Group A (with bone graft) subjects had significantly shorter healing times and improved overall healing in comparison to Group B (no bone graft) rabbits when specimens were analyzed post-mortem both radiographically and histologically. These findings support the use of particulate bone grafting in order to promote complete osseous healing in osteotomy/osteotomy sites. Unfortunately, given the tendency for group A subjects to have near complete healing at one month, and the time points chosen at 1, 2, and 3 months, there was little data for interval analysis generated by the study's low sensitivity ordinal scale. Perhaps, some benefits from PRP would have been realized if samples were also analyzed at the 2 week time period, related to early changes as a result of the addition of PRP. Additionally, the four point ordinal scales used in this study would prevent subtle differences from being seen as significant,

if in fact this were the case. Since the false positive error rate was most likely high as a consequence of the small sample size, it was important that a greater difference would be necessary to change the rating between compared subjects. Also, the evaluator would be more likely to reproducibly score the sample with the correct rating. Still, these scales are not validated to ensure a correlation between the rating and the actual height or density measurements, nor were they shown to be reliable such that the analysis repeated by the same investigator or by different researchers would result in the same rating. The experimental variable, the addition of PRP to osteotomy site, was included in one side of each subject for internal control. The split-side design of this study also provides strong evidence of any differences, since each side would be expected to heal equally unless there was a significant variable that enhanced or inhibited the healing process. Although PRP was added to one side in each subject, multiple pair-wise analyses failed to demonstrate any significant difference between the two sides in any of the twelve subjects. It is possible that bone healing in rabbits would be much less dependent on platelet-released growth factors than healing in humans, but the appropriate bone sigma was taken into consideration when the sacrifice times were determined. Despite these issues, there remains the need for further research to demonstrate the effectiveness of PRP in bony and soft tissue healing responses. Based upon the results of this study, it could be concluded that PRP could not be used in place of a particulate bone graft in a critical-sized defect of the rabbit mandible. Furthermore, when PRP is used in conjunction with a bone graft, healing results may only be minimally improved, making the addition of PRP a remaining questionable clinical decision.

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## Figures

1. Inferior border osteotomy of the rabbit mandible (1.0 x 0.5 cm).
2. An example of a Group A animal with the addition of bone graft to the defect.
3. An example of a Group A animal with bone graft and PRP in the defect.
4. An example of a Group B animal with PRP only in the defect.
5. Radiograph of a Group A animal, bone graft and PRP, at 8 weeks, showing complete bone formation (radiographic height and density scores of 4/4).
6. Radiograph of a Group B animal, PRP alone, at 8 weeks, showing incomplete bone formation (radiographic height and density scores of 2/4).
7. Histologic specimen of a Group A animal, bone graft and PRP, at 8 weeks, showing mature bone formation (histologic score of 4/4).
8. Histologic specimen of a Group B animal, PRP alone, at 8 weeks, showing incomplete bone formation (histologic score of 2/4).

## Tables

1. Distribution of animals in groups, based upon whether bone graft or PRP was used in the defect.
2. Mean ratings of specimens for radiographic height, radiographic density, and histologic height using the four-point ordinal rating scale.

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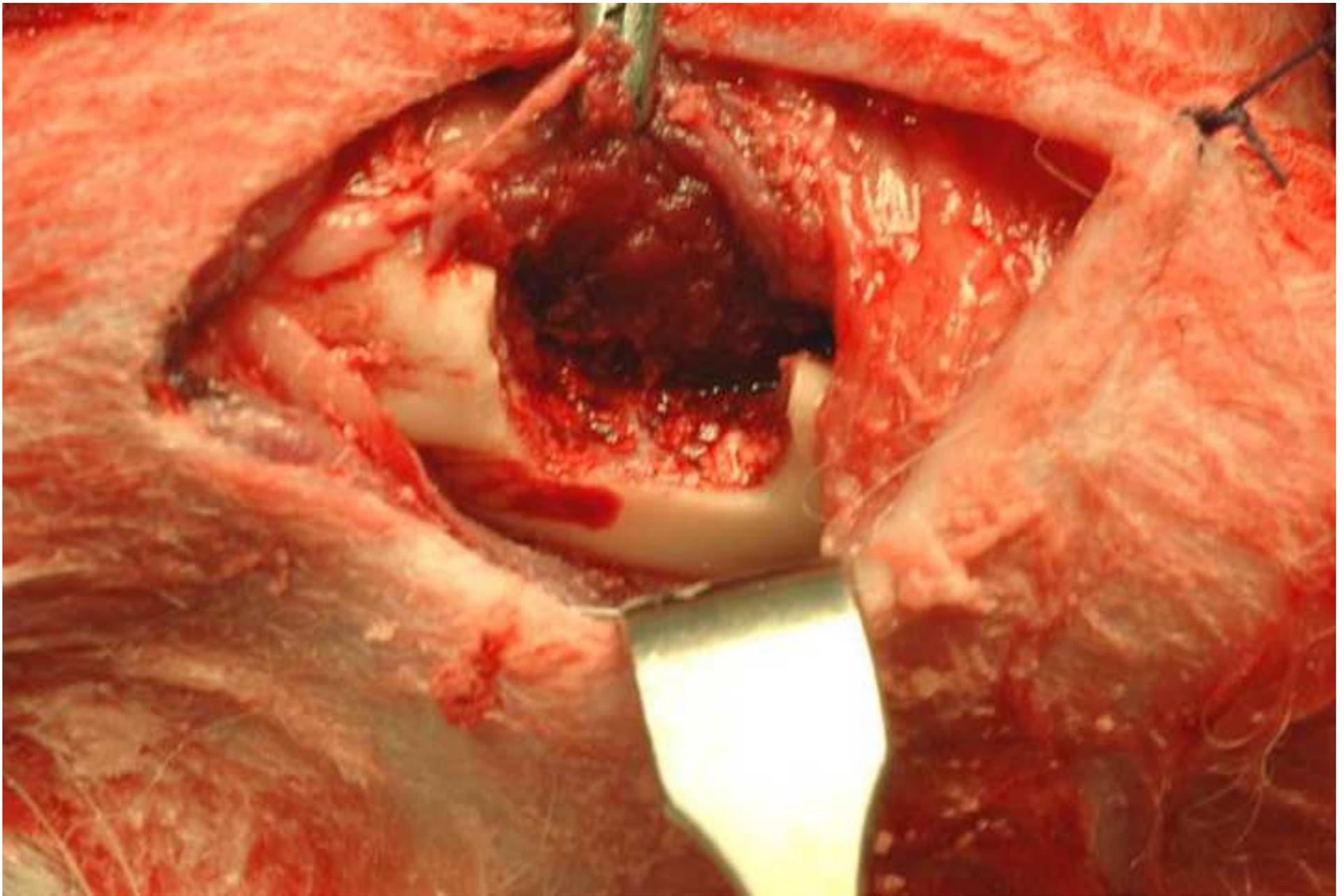
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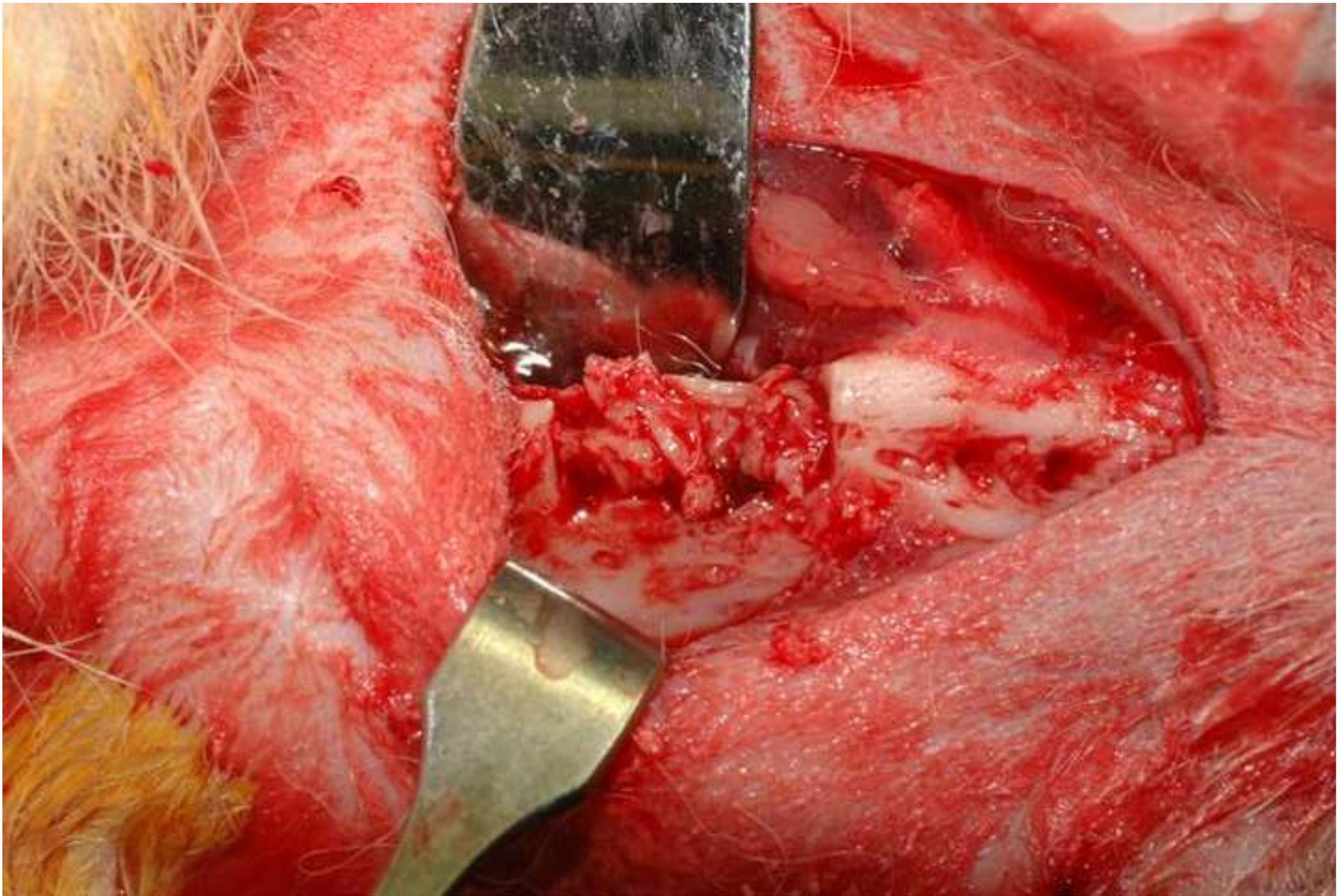
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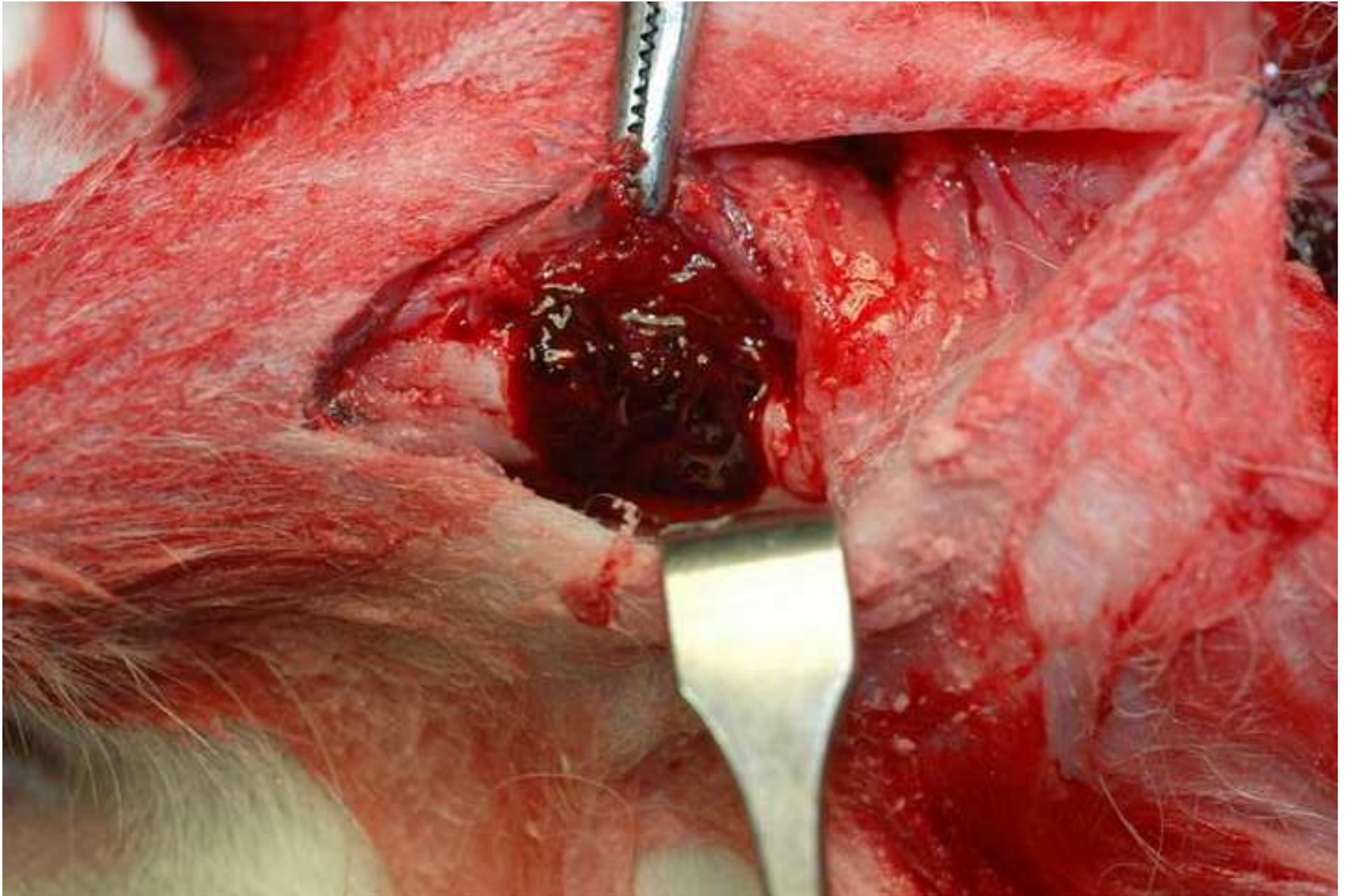


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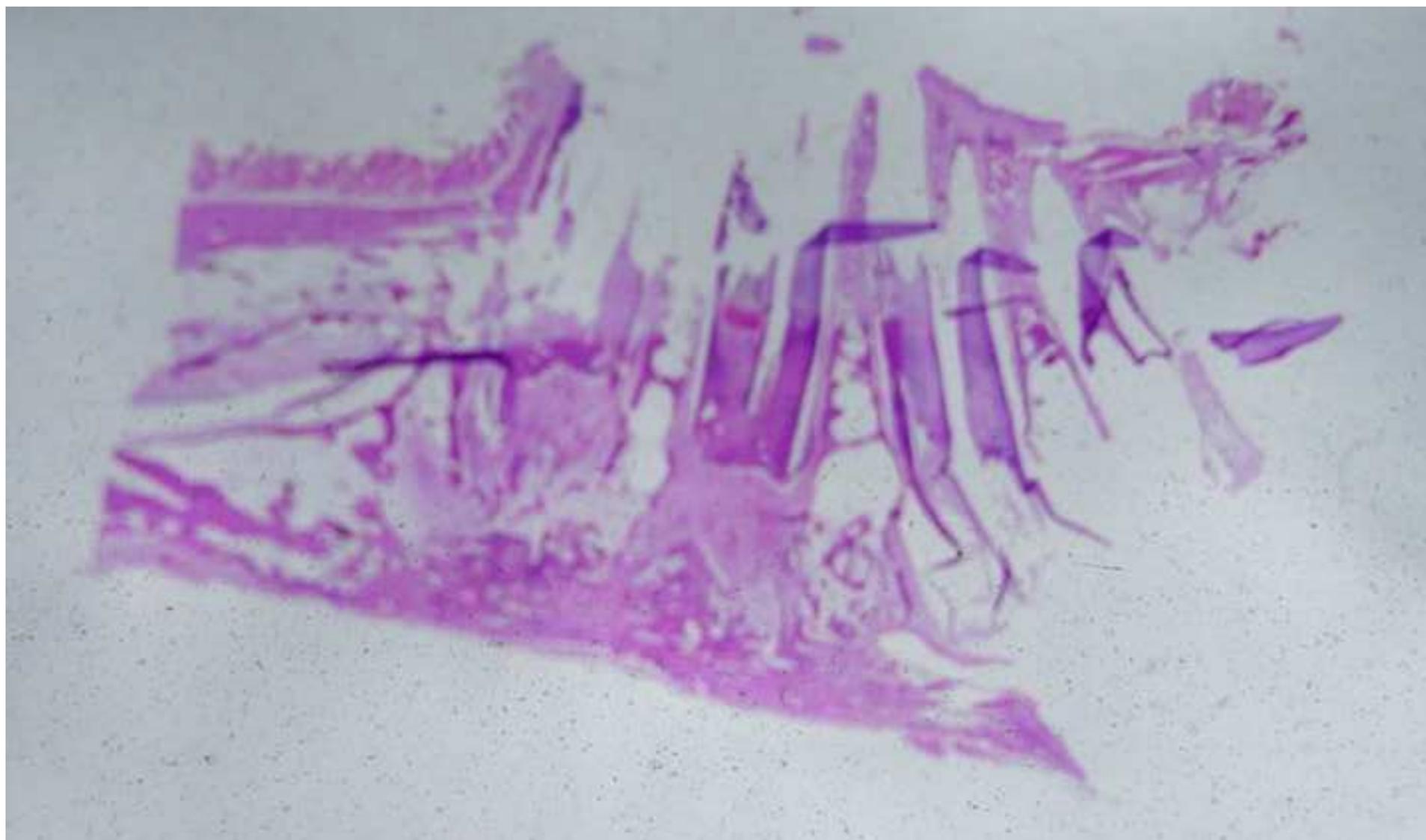


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Figure 7

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**Figure 8**  
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**Table 1**

	Bone graft	PRP
Group A (n=6)	Bilateral	Unilateral
Group B (n=6)	None (empty defect)	Unilateral

Table 1. Distribution of animals in groups, based upon whether bone graft or PRP was used in the defect.

Table 2

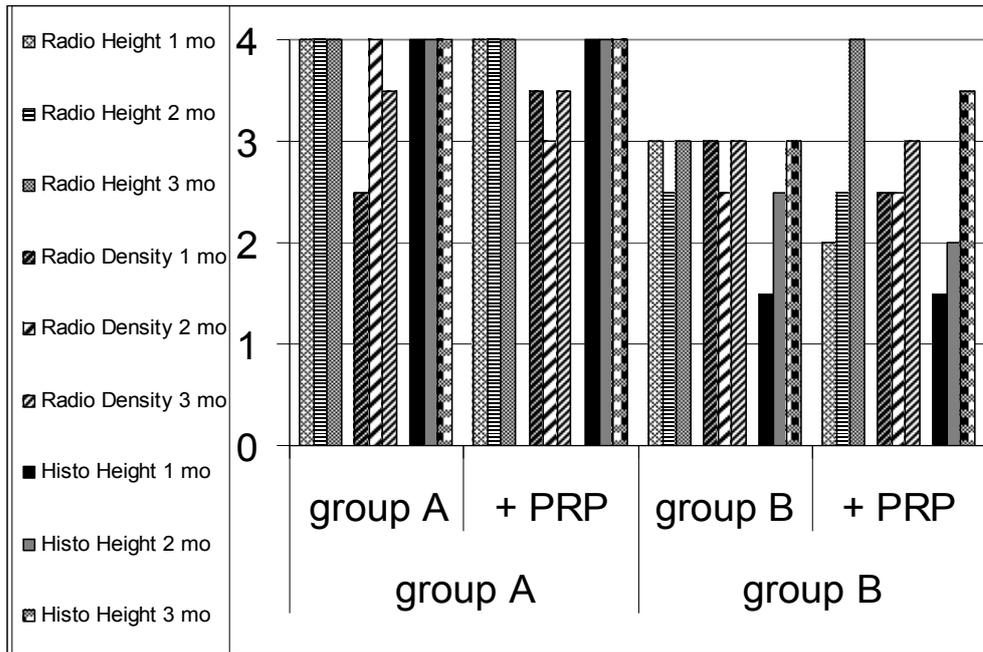


Table 2. Mean ratings of specimens for radiographic height, radiographic density, and histologic height using the four-point ordinal rating scale.