Chapter 3

Result

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Clinical evaluation:

All animals well tolerated with the surgical procedure and the anesthesia. The recovered rapidly after surgery and there was no evidence of wound infection or wound dehiscence. Ther was no the accidental death of rabbit throughout of this study period. After full recovery, they were able to eat the pellet food and drink water ad libitum.

Gross morphological evaluation:

The macroscopically showed the graft appeared well incorporated in experimental and control site in all specimens. But in group 3 (6 weeks) were better than group 2 (4 weeks) and group 1 (2 weeks), respectively. In the group 1, the graft had been showed the same incoporated in experimental and control site, but less than group 2. The consolidation of Bio-Oss® particle was showed as same as between the experimental and control site in each specimen, but in group 3 the Bio-Oss® particle was showed more consolidation than group 2 and the latter also more consolidation than group 1. The Bio-Oss® particles were still observed in both site of bone defect in all specimens and showed well incoporated with the host bone. The gutta-percha marker around bone defect at both site were observed in all specimens. The guta-percha marker was showed a biocompatible material because their was no sign of infection at the marker. These marker were helpful for located the bone defect margin, although, the bone defect margin of both site could be observed in all specimens. However, the bone defect margin of the specimens in group 1 were obvious to detect than group 2 and group 3, respectively.

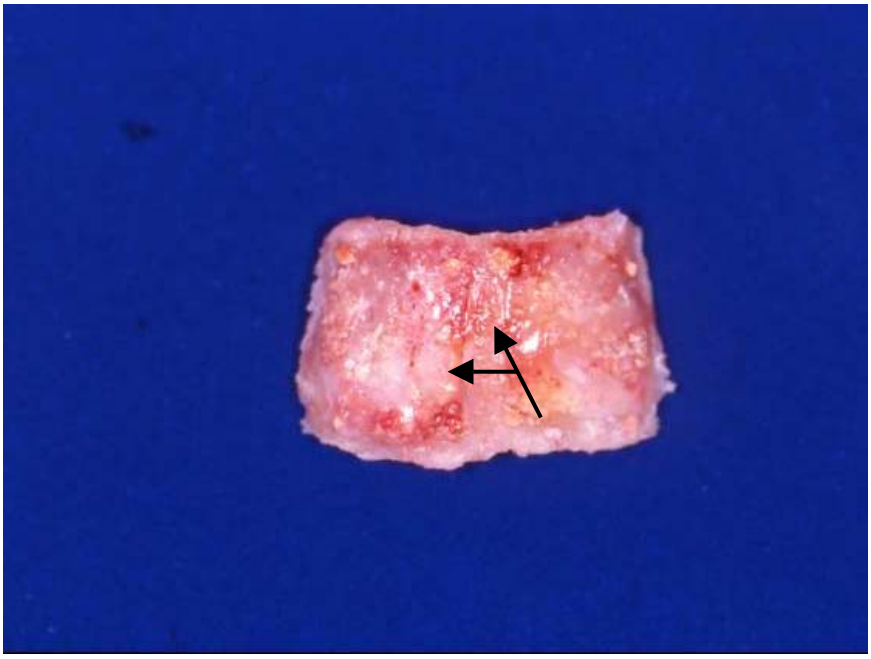


Figure 25. Specimen of rabbit calvarial 2 weeks specimens; arrow indicated the graft-skull margin. The specimen was showed that graft material appeared well incorporated in the experimental and the control site.

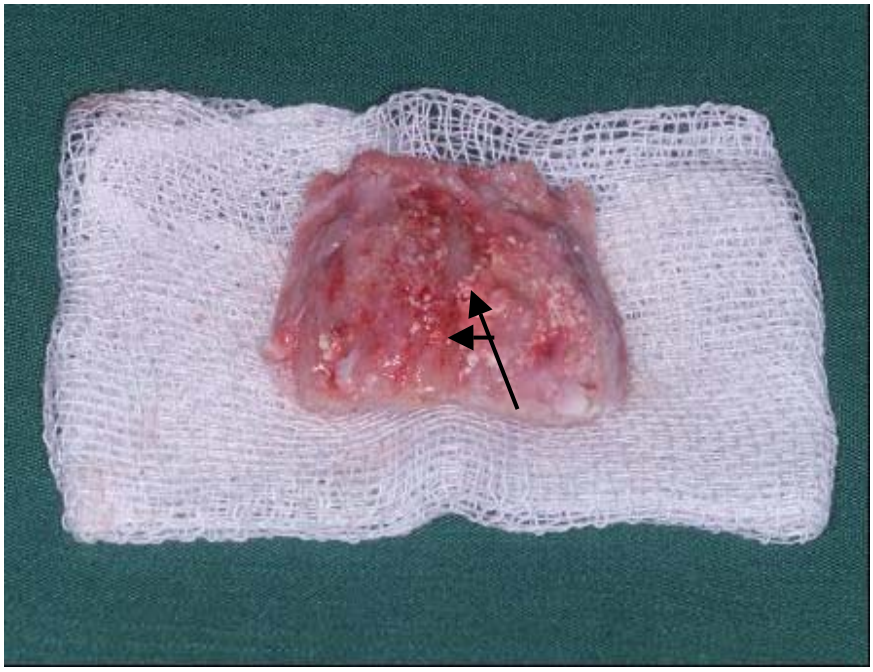


Figure 26. Specimen of rabbit calvarial 4 weeks specimens; arrow indicated the graft-skull margin.

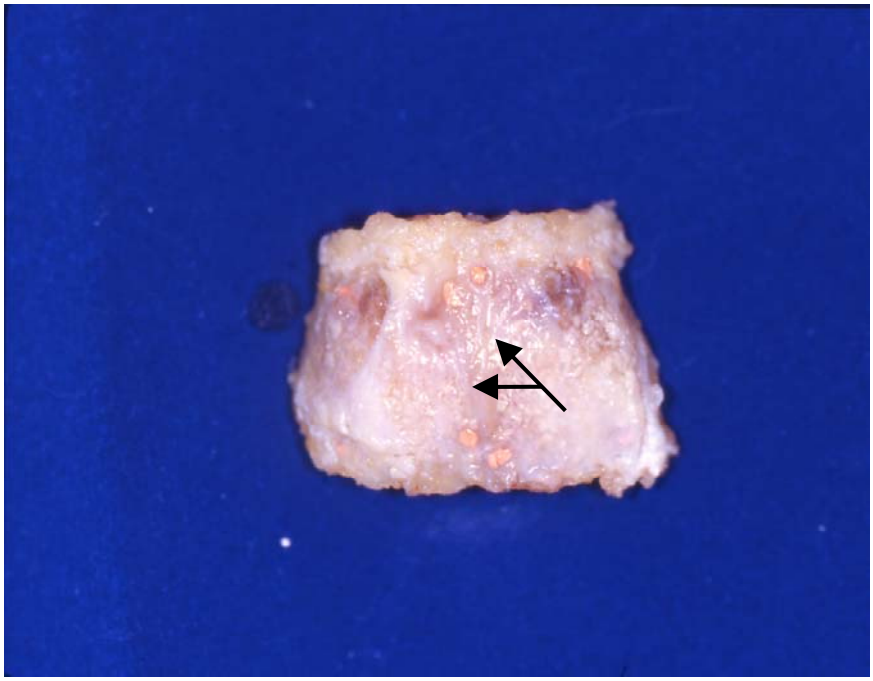


Figure 27. Specimen of rabbit calvarial 6 weeks specimens; arrow indicated the graft-skull margin was still observed.

Radiographic evaluation:

The radiographic results were analyzed as showed in the diagram of the rabbit skull.

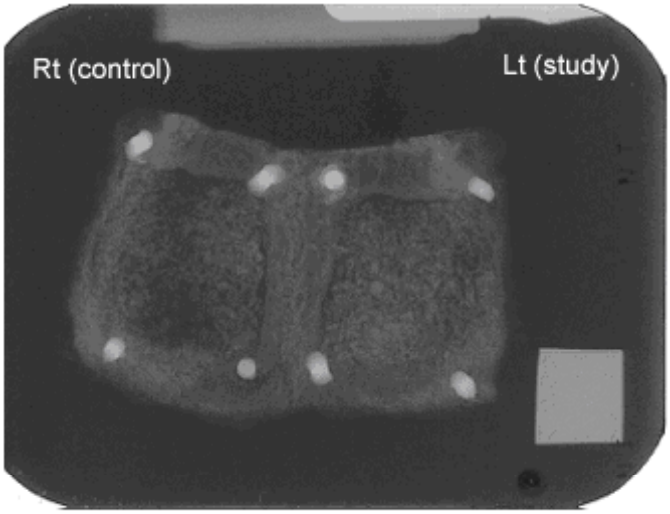


Figure 28. Radiograph taken at 2 weeks. The defect margin and radiopaque area of bone graft materials were observed. The radiograph in the

experimental site was showed a radiopacity as well as the control site.

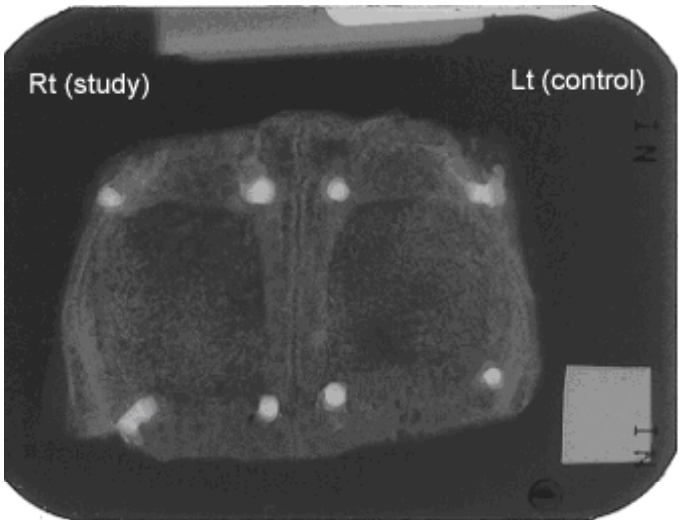


Figure 29. Radiograph taken at 4 weeks. The defect margin and radiopaque area of bone graft materials were observed. The radiograph in the experimental site was showed a radiopacity as well as the control site, but the radiopacity in 4 weeks were slightly more than 2 weeks.

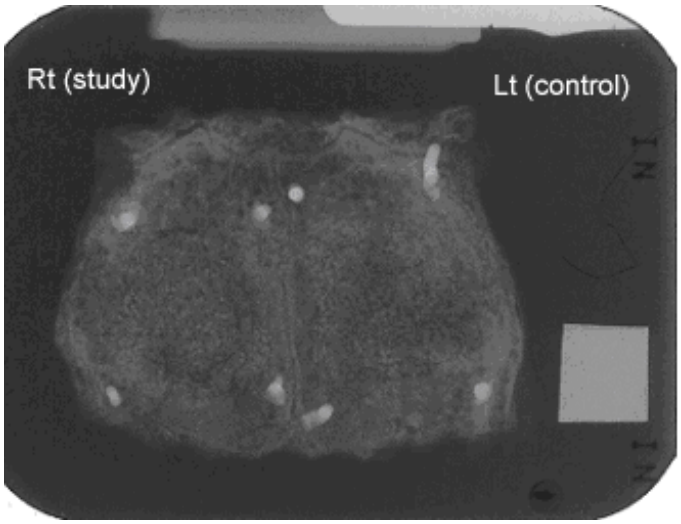


Figure 30. Radiograph taken at 6 weeks. The defect margin was little difficult to detect. The radiopaque area of bone graft materials were observed. The radiograph in the experimental site was showed a radiopacity as well as the control site, but the in 6 weeks were showed greater radiopacity than 4 and 2 weeks, respectively.

During the observation period (2, 4 and 6 weeks), a well-delineated and radiopaque area of bone graft materials were suggestive of a bone defect was observed. The gutta-percha marker could be observed in all specimens.The radiograph in the experimental site was showed a radiopacity as well as in the control site in each specimen. At **4 weeks**, the radiograph was showed a homogenous radiopaque area of the bone defect with showed more radiopacity than 2 weeks. At **6 weeks**, the radiograph was showed a greater homogenous radiopaque area around the bone defect with a density similar to the normal bone tissue. The radiograph in 6 weeks observation period showed greater radiopacity than 4 weeks and the latter showed greater radiopacity than 2 weeks. The area around the bone defect showed visually greater density than the cental portion of the defect in each specimen. The central portion of the defect in 6 weeks showed less radiolucence than 4 and 2 weeks, respectively.

Radiomorphometry analysis:

The results of the radiographic analysis of group1, group2 and group3 are shown in Table 1. **At 2 weeks** postsurgery, mean radiodensity (± SD) for Bio-Oss® with PRP (experimental) and Bio-Oss® alone (control) were 73.6 ± 6.57, 71.87 ± 4.83, respectively. **At 4 weeks** postsurgery, mean radiodensity (± SD) for Bio-Oss® with PRP and Bio-Oss® alone were 75.5 ± 3.27, 74.09 ± 6.68, respectively. In the **6 weeks** postsurgery, mean radiodensity (± SD) for Bio-Oss® with PRP and Bio-Oss® alone were 79.79 ± 14.35, 84.75 ± 11.64, respectively. There was no statistic significant difference ($P>0.05$) between the experimental and control site in each group. The data was showed that the radiodensity in the experimental site was slightly better than the control site in 2 and 4 weeks, but in 6 weeks the radiodensity in the control site was better than the experimental site.

Group	Week	Bone density (Mean ± SD) Bio-Oss®/PRP	Bone density (Mean ± SD) Bio-Oss® alone
1	2	73.60 ± 6.57	71.87 ± 4.83
2	4	75.5 ± 3.27	74.09 ± 6.68
3	6	79.79 ± 14.35	84.75 ± 11.64

Table 1. The data of radiomorphomeric (Bone density) in group 1, group 2, group 3, respectively.

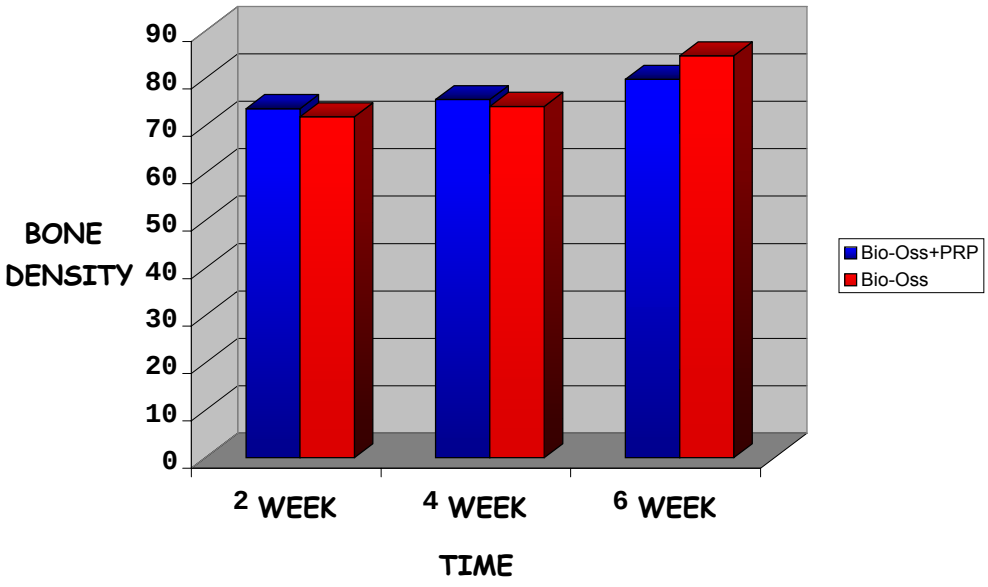


Figure 31. Histogram of the amount of radiopaque new bone with in the defect area at 2, 4 and 6 weeks after surgery.

Histologic evaluation:

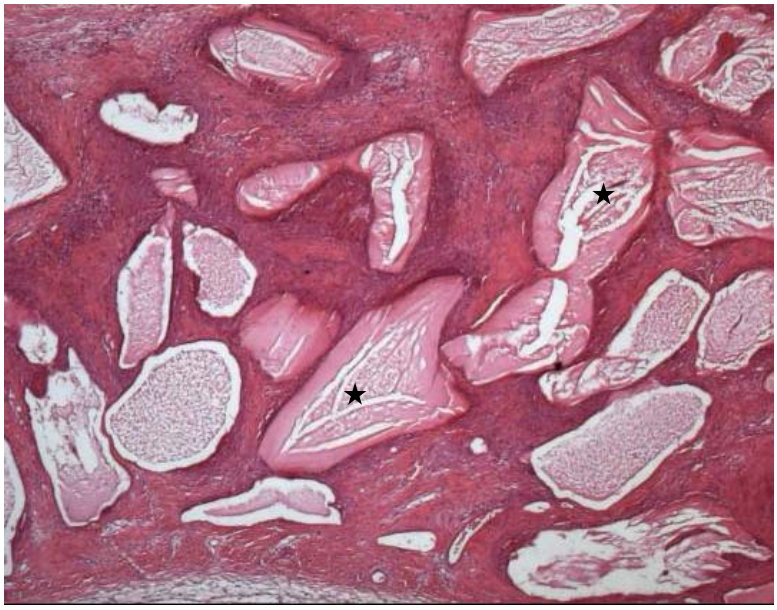
Experimental group:

At **2 weeks** postsurgery, a large number of residual Bio-Oss® particles were observed within fibrous connective tissue in the bone defect. There was slightly inflammatory cell infiltrated in bone defect. At **4 weeks**, the histological observations were similar to the 2 weeks observations, but there showed slightly new bone formation. The histologic revealed that the intimate contact of the osteoblastic cells and the direct apposition of new bone formation around the surface of Bio-Oss® particle. The newly formed bone tissue showed the characterizing centripetal bone growth. The new bone extended from the defect margin and grown into the central portion of the bone defect. There was no inflammatory cell infiltrated in bone defect. At **6 weeks**, a large number of residual Bio-Oss® particles were still observed within bone defect. The histologic showed an increase in bone formation over time, because of the new bone formation that found in 6 weeks was greater than in 4 and 2 weeks, respectively. The newly forming bone or woven bone was in the direct apposition to the Bio-Oss® particles and there was no fibrous capsule layer detectable between them. The newly formed bone tissue showed the centripetal of bone in growth. Therefore, all defects were dominated by woven bone extending from the edges of the defects, while connective tissue occupied the central part of defect. The defect edges could be observed in all specimen in 2, 4 and 6 weeks. The bone tissue was less mature in the central portion and more mature at the periphery of the bone defect. Multiplenucleated giant cell was not found at the border of Bio-Oss® particle. There was not found the resorption of the Bio-Oss® particle in the specimen.

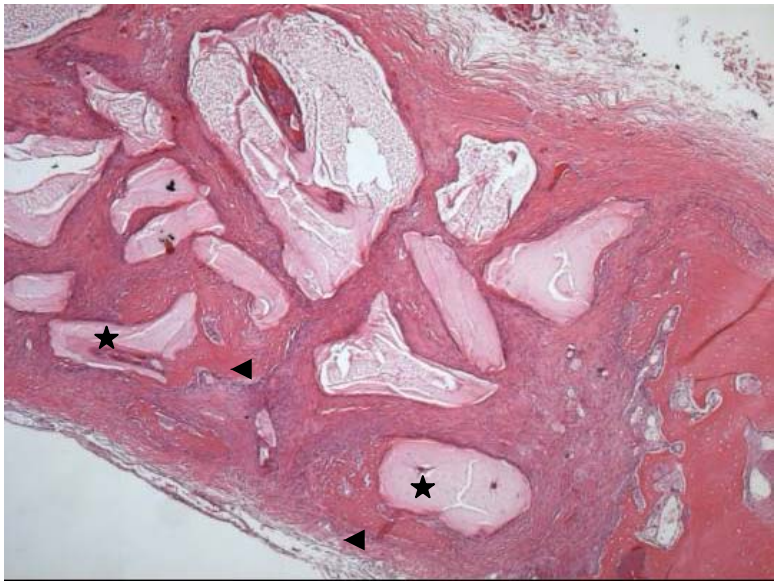
Control group:

At **2 weeks** postsurgery, a large number of residual Bio-Oss® particles were observed within fibrous connective tissue in the bone defect. In addition, the slightly of new bone formation around the surface of Bio-Oss® particle that found in the adjacent area to the defect margins. There was no inflammatory cell infiltrated in the bone defect. At **4 weeks**, the histological observations were similar to the 2 weeks observations, but

the new bone formation was slightly greater than the 2 weeks. The histologic revealed that the intimate contact of the osteoblastic cells and the direct apposition of new bone formation around the surface of Bio-Oss® particle. The newly formed bone tissue showed the characterizing centripetal bone growth. At **6 weeks**, a large number of residual Bio-Oss® particles were still observed within bone defect. The histologic showed an increase in bone formation over time, because of the new bone formation in 6 weeks was greater than in 4 and 2 weeks, respectively. The newly forming bone or woven bone was in the direct apposition to the Bio-Oss® particles. The newly formed bone tissue showed the centripetal of bone in growth. The new bone extended from the defect margin and grown into the central portion of the bone defect. Therefore, all defects were dominated by woven bone extending from the edges of the defects, while connective tissue occupied the central part of defect. The defect edges, also could be observed in all specimen in 2, 4 and 6 weeks. The bone tissue was less mature in the central portion and more mature at the periphery of the bone defect. The newly formed bone was interconnecting the Bio-Oss® particles. Moreover, the histologic examination revealed the amount of new bone ingrowth in the control site was greater than the experimental site. Multiplenucleated giant cell or osteoclast like cell was not found at the border of Bio-Oss® particle. There was not found the resorption of Bio-Oss® particle in the specimen.



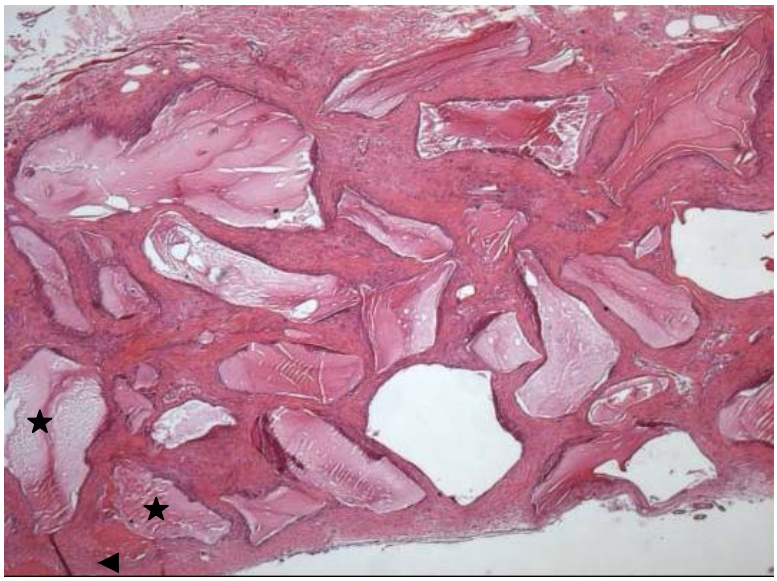
A.



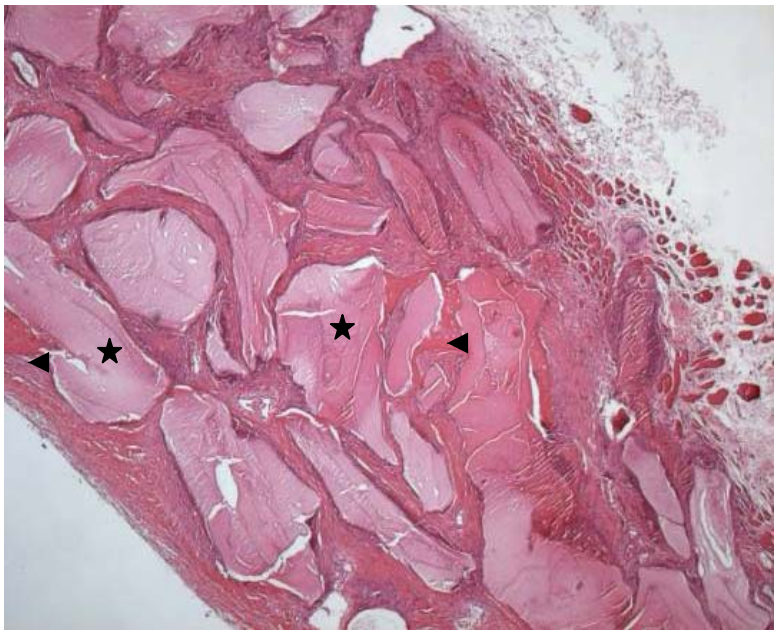
B.

Figure 32. A. Specimen from group1 at 2 weeks (experimental side), demonstrating dense fibrous tissue (arrowheads). The Bio-Oss® particles were seen in the defect (star), (H&E stain, X 50).

B. Specimen from group1 at 2 weeks (control side), demonstrating new bone apposed to Bio-Oss® particles (arrowheads). The Bio-Oss® particles were seen in the defect (star), (H&E stain, X 50).



A.

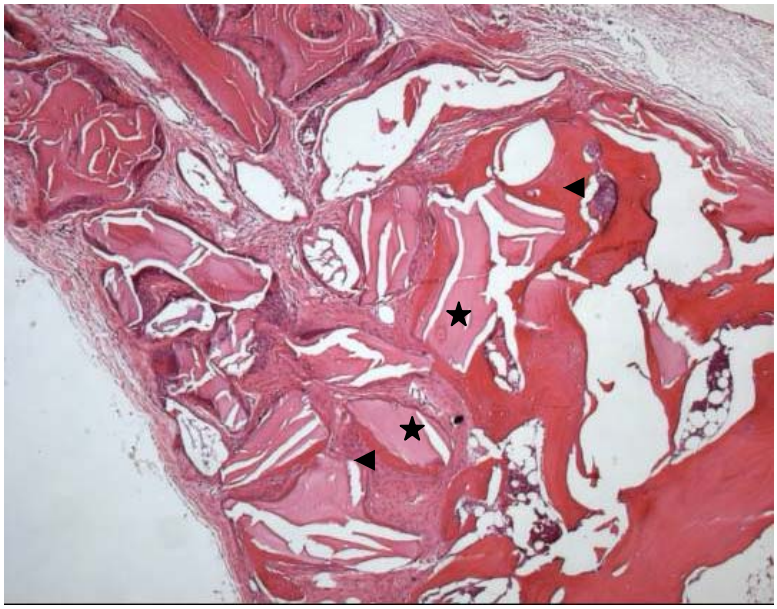


B.

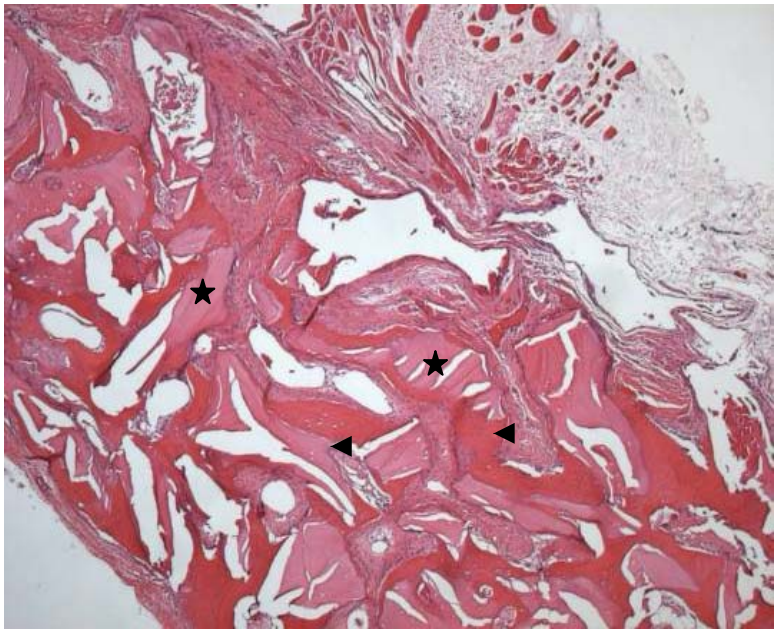
Figure 33. A. Specimen from group2 at 4 weeks (experimental side), demonstrating slightly of new bone formation around the Bio-Oss® particles (arrowheads). The Bio-Oss® particles were seen in the defect (star), (H&E stain, X 50).

B. Specimen from group2 at 4 weeks (control side), demonstrating new bone apposed to Bio-Oss® particles (arrowheads). The Bio-Oss®

particles were seen in the defect(star), (H&E stain, X 50).



A.



B.

Figure 34. A. Specimen from group3 at 6 weeks (experimental side), demonstrating the increasing of new bone formation around the Bio-Oss® particles (arrowheads). The Bio-Oss® particles were seen in the defect (star), (H&E stain, X 50).

B. Specimen from group3 at 6 weeks (control side), demonstrating more newly formed bone apposed to the Bio-Oss® particles (arrowheads). The new bone formation in control side was more than experimental side. The Bio-Oss® particles were seen in the defect (star), (H&E stain, X50).

Histomorphometric analysis:

The results of the histomorphometry analysis are shown in Table 2 . At **2 weeks** postsurgery, mean new bone formation that revealed in % bone area (± SD) for Bio-Oss® with PRP (experimental) and Bio-Oss® alone (control) were 18.67 ± 3.17, 21.76 ± 3.49, respectively, with not statistic significant difference (P>0.05). At **4 weeks** postsurgery, mean % bone area (± SD) for Bio-Oss® with PRP and Bio-Oss® alone were 21.67 ± 3.96, 23.83 ± 10.36, respectively, with no statistic significant difference (P>0.05). In the **6 weeks** postsurgery, mean % bone area (± SD) for Bio-Oss® with PRP and Bio-Oss® alone were 24.36 ± 7.15, 28.70 ± 9.48, respectively, also with no statistic significant difference (P>0.05). The data was showed that the % bone area in the control site was better than the experimental site in 2, 4 and 6 weeks. However, there was no statistic significant difference between the experimental and control site at 2, 4 and 6 weeks.

Group	Week	% Bone area (Mean ± SD) Bio-Oss®/PRP	% Bone area (Mean ± SD) Bio-Oss® alone
1	2	18.67± 3.17	21.76 ± 3.49
2	4	21.67 ± 3.96	23.83 ± 12.36
3	6	24.36 ± 7.15	28.70 ± 9.48

Table 2. Show the data of histomorphomeric (% Bone area) in group 1, group 2, group 3, respectively.

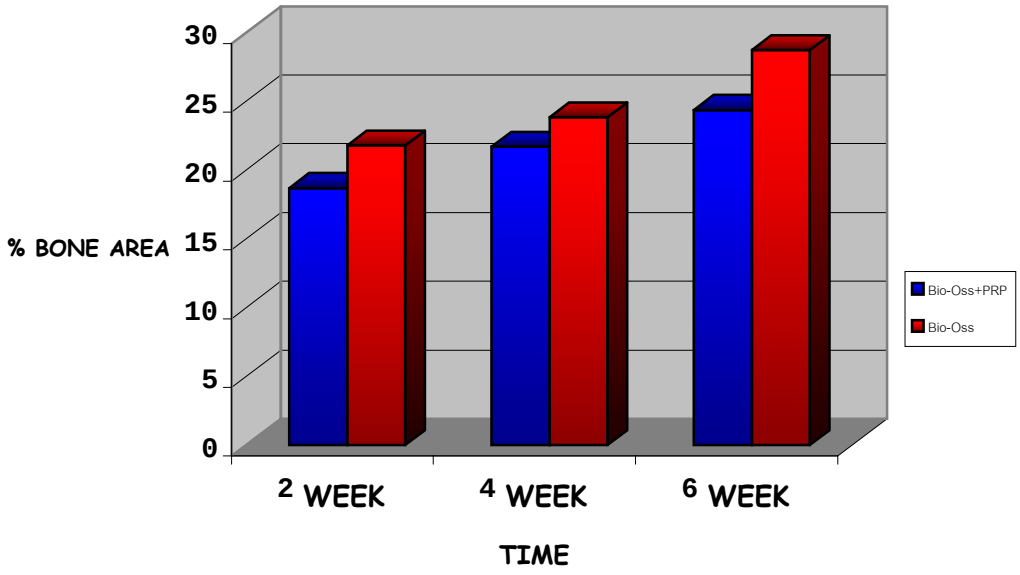


Figure 35. The histomorphometric showed an increase in % bone area over time. The % bone area in the control site was better than the experimental site in 2, 4 and 6 weeks.

Platelet measurement:

Platelet counts confirmed the PRP preparation technique that used in this study produced a source of highly concentration of platelets. The average whole blood platelet count was 227,000 / μl with a range from 154,000 / μl to 274,000 / μl. The average PRP platelet count was 2,935,000 / μl with a range from 1,590,000 / μl to 4,020,000 / μl. Thus, the percentage increase was calculated as 1,297.44 ± 280.37%, with a range from 665.27% to 1,682%. The platelet count of PRP showed a statistically significant increase the quantity of platelet than whole blood ($P<0.05$). The data of whole blood and PRP platelet count are showed in Table 3.

Rabbit No.	Whole blood platelet count (X 10^3)	PRP platelet count	Concentration (%)
1.	274	2850	1040.15
2.	239	1590	665.27
3.	239	4020	1682.01
4.	189	2050	1084.67
5.	180	2510	1394.44
6.	154	2330	1512.99
7.	245	3580	1461.22
8.	241	3030	1257.26
9.	254	3840	1511.81
10.	194	2270	1170.10
11.	246	3780	1536.59
12	269	3370	1252.79
		Mean	1297.44
		SD	280.37

Table 3. Show the data of whole blood and PRP platelet counts of the rabbits.