

CHAPTER FOUR

RESULTS

Radiography. Retained deciduous teeth at the experimental sites were not present in the 10 dogs. The two artificial defects (1st and 3rd holes) are PRP and control groups. The rest (2nd and 4th holes) of the artificial defects are expanded polytetrafluoroethylene (ePTFE) membrane and PRP-ePTFE membrane groups which are not included in this thesis. Radiographic images of mandibular bones obtained at different periods are shown in figure 8-12. Bone density at surgical sites is gradually increasing when longer sacrificed period animals were evaluated.

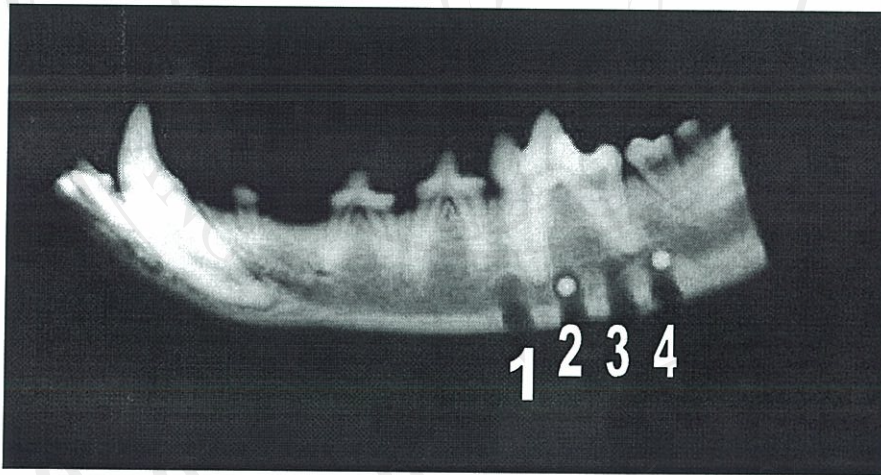


Figure 8 Radiographic finding of mandibular bone in dog sacrificed at 2 weeks period.

(1 = PRP , 3 = control)

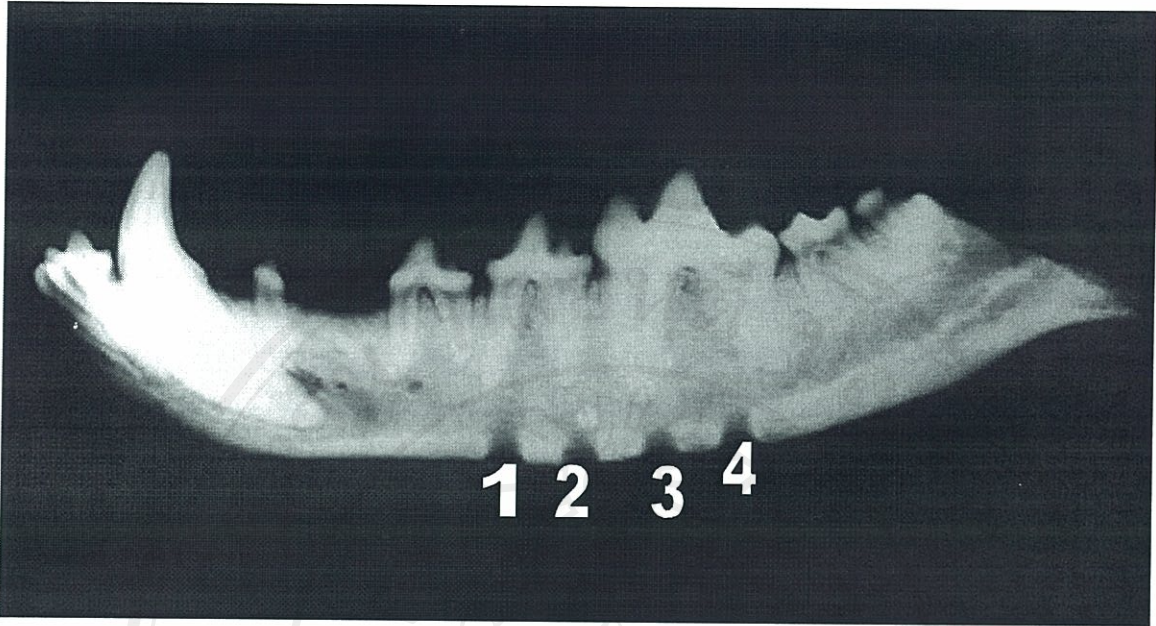


Figure 9 Radiographic finding of mandibular bone in dog sacrificed at 4 weeks period.
(1 = PRP , 3 = control)

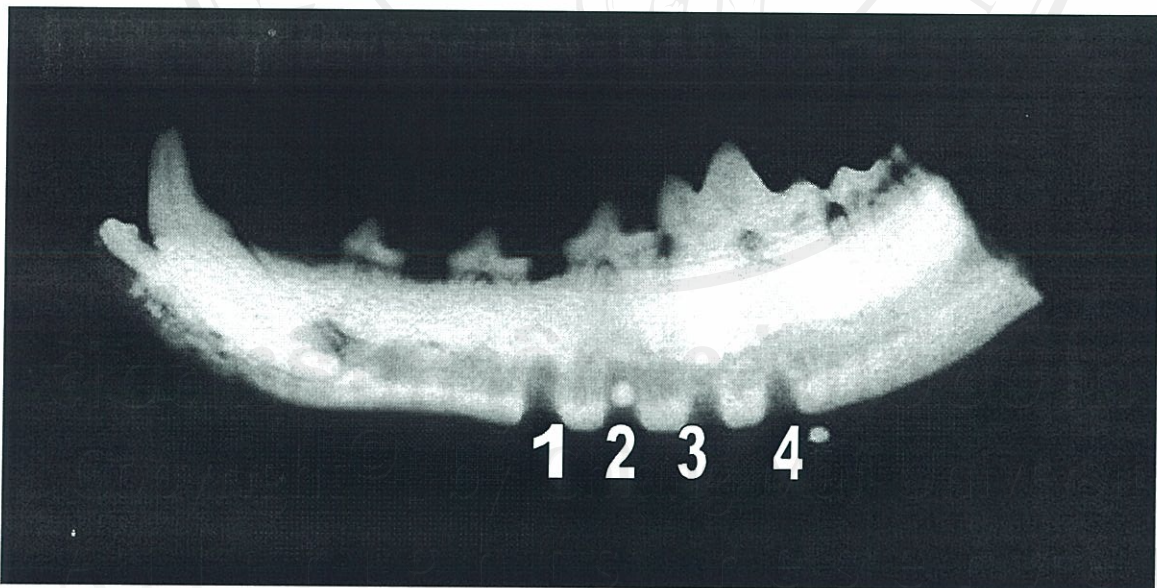


Figure 10 Radiographic finding of mandibular bone in dog sacrificed at 6 weeks period.
(1 = PRP , 3 = control)

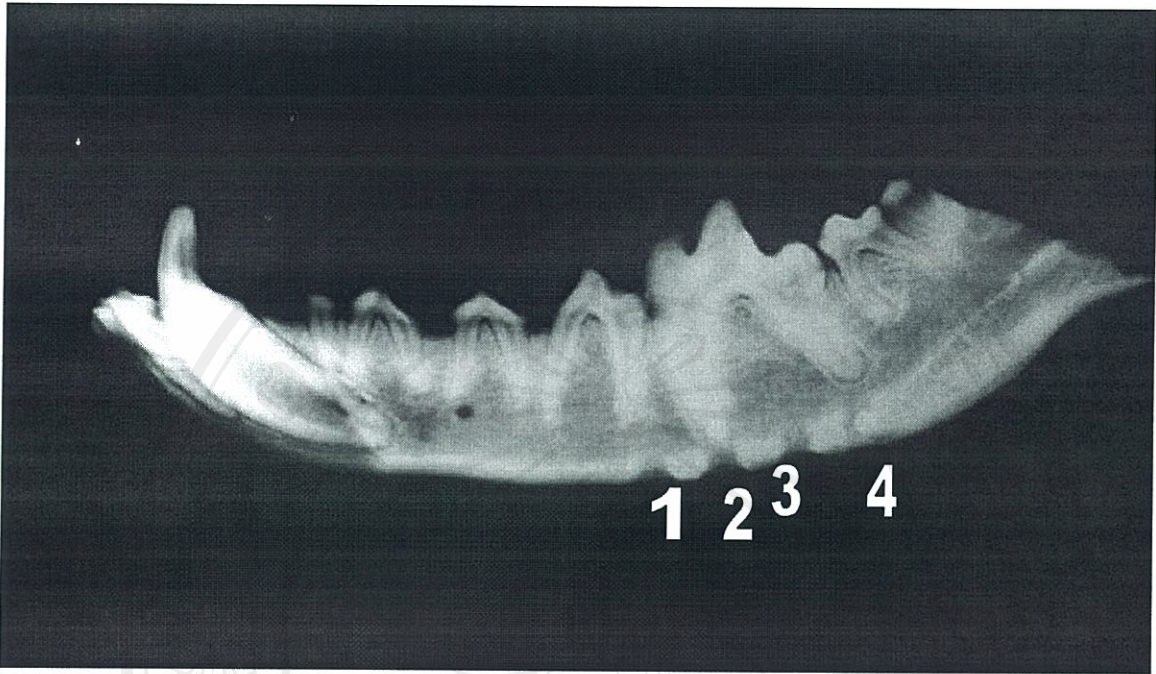


Figure 11 Radiographic finding of mandibular bone in dog sacrificed at 8 weeks period.
(1 = PRP , 3 = control)

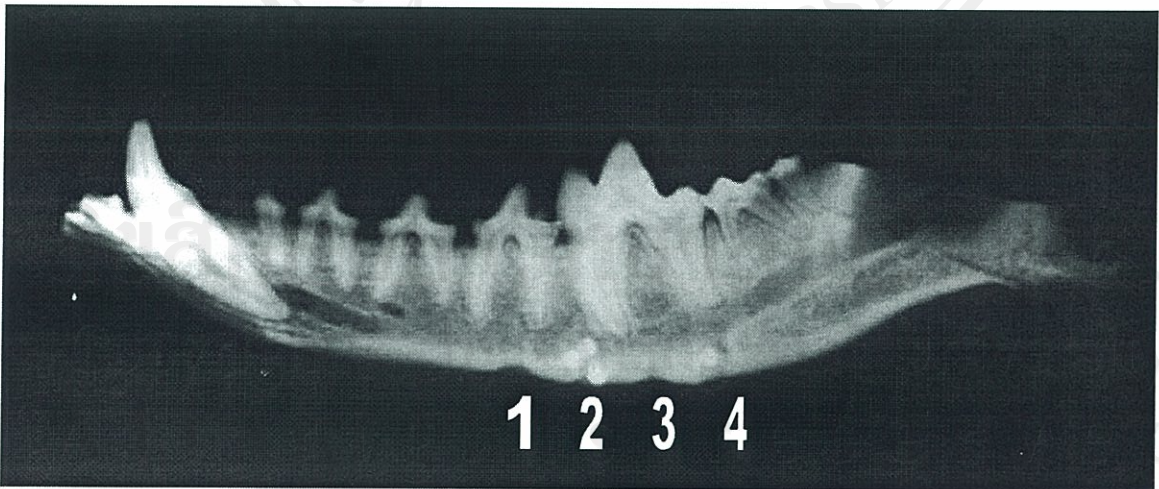


Figure 12 Radiographic finding of mandibular bone in dog sacrificed at 12 weeks period.
(1 = PRP , 3 = control)

Platelet Count Study. Platelet count performed on each experimental dog yielded a mean platelet count value of 146595, with a range of 102000 to 195750. The average PRP platelet count was 518900, with a range of 201000 to 711100. These values confirmed the platelet sequestration ability of the process and quantified the average concentration as 356.16 % of baseline platelet counts (Table 2).

Table 2: The platelet count of fresh blood and platelet-rich plasma

Experimental dogs	Baseline platelet count	PRP platelet count	Percent of increased platelet
1	102000	201000	197.05 %
2	183000	598000	326.77 %
3	159900	630500	394.03 %
4	153400	520000	338.98%
5	195750	598000	305.49 %
6	153400	545400	355.54%
7	139100	520000	373.83 %
8	114400	711100	621.59 %
9	140000	513000	366.42 %
10	125000	352000	281.60 %
	$\bar{x}_1=146595$	$\bar{x}_2=518900$	$\bar{x}_3=356.16$

Histological Evaluation. This study presents the healing process and bone regeneration of both control and PRP groups at different periods. In all animals the healing process of both extraoral soft tissue and bone defect progressed uneventfully. None of the animals showed changes in behavior nor signs of pain as well as for general infections. Neither wound infections nor fistulas were presented. Structures of histological sections including nerve, artery, vein, entrance of artificial defect, periosteum, trabecular bone, local compact bone, and woven bone or new bone formation are shown in figure 13.

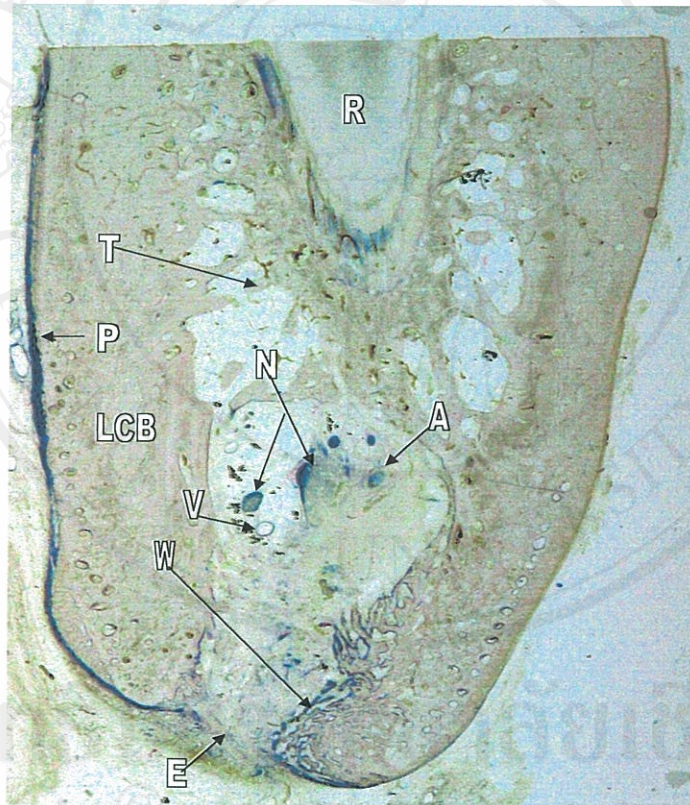


Figure 13 Structure of mandibular bone.

(R) Root, (T) trabecular bone, (P) Periosteum, (LCB) Local compact bone, (N) nerve, (A) Artery, (V) Vein, (W) Woven bone, and (E) Entrance to defect

Specimens at 2 weeks

PRP group. All defects revealed new bone formation slightly starting from the internal walls of the defect.(figure 14) Neither inflammatory cells nor bacteria were found around the artificial defects. Collagen was still present in the artificial defects. (figure 15) Overlying soft tissue tends to cover the entrance of the defect.

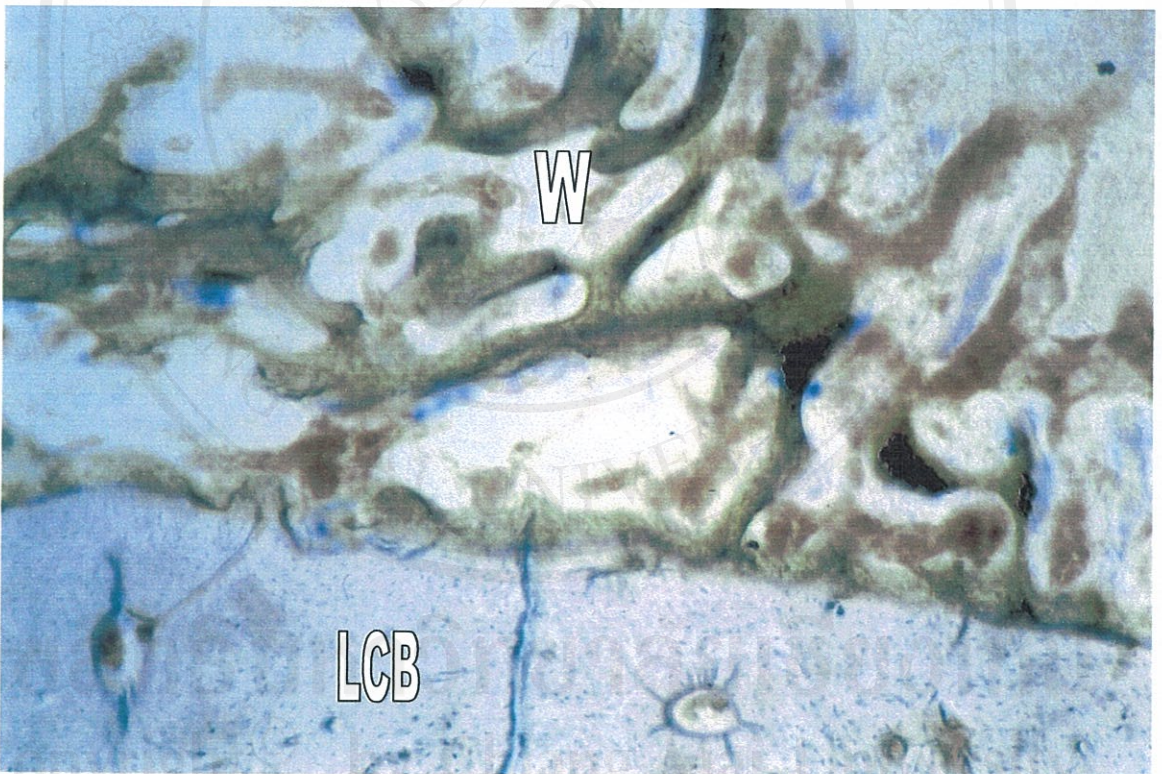


Figure 14 New bone formation or woven bone (W) and local compact bone (LCB)

of specimen in PRP group at 2 weeks period.

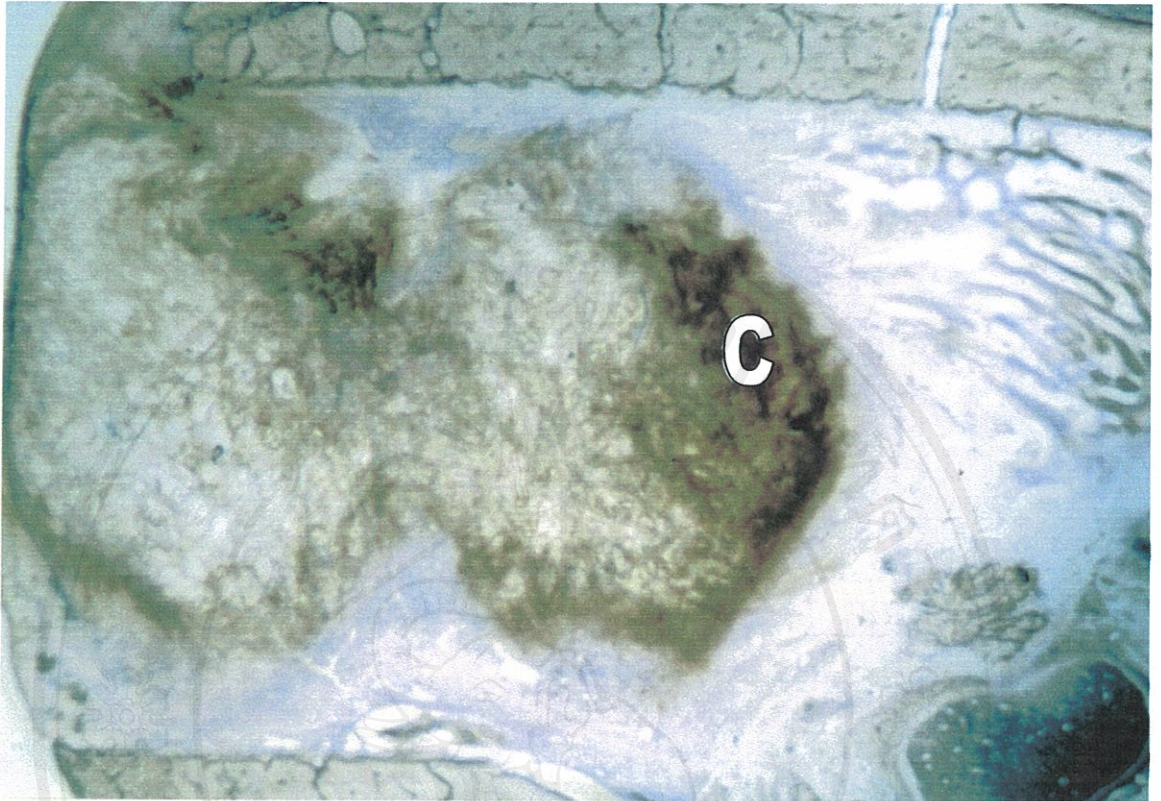


Figure 15 Collagen in artificial defect of specimens in PRP group at 2 weeks period.

Control Group. New bone formation of control defects at 2 weeks was not obvious. Inflammatory cells and bacteria were not found around the artificial defects. Collagen still presented in the artificial defects as in PRP defects at the same period. Angiogenesis was slightly present in the defect. Overlying soft tissue tends to cover the entrance of the defect.

Specimens at 4 weeks

PRP group. All defects revealed more condensed new bone formation compared to the specimens obtained after 2 weeks. Woven bone of PRP group at 4 weeks was obviously seen in artificial defects. The new bone formation was found not only in the

internal walls of the defect but also under the periosteum. Neither inflammatory cells nor bacteria were found around the artificial defects.

Control group. The budding of new capillaries into the defect(angiogenesis), a primary part of all wound healing, was still present in control group at 4 weeks (figure 16) All defects revealed new bone formation slightly starting from the internal walls of the defect. Woven bone of control group at 4 weeks was obviously less than in artificial defect of PRP group. New woven bone formation began at the walls of the bone defects. Inflammatory cells and bacteria were not found around the artificial defects.

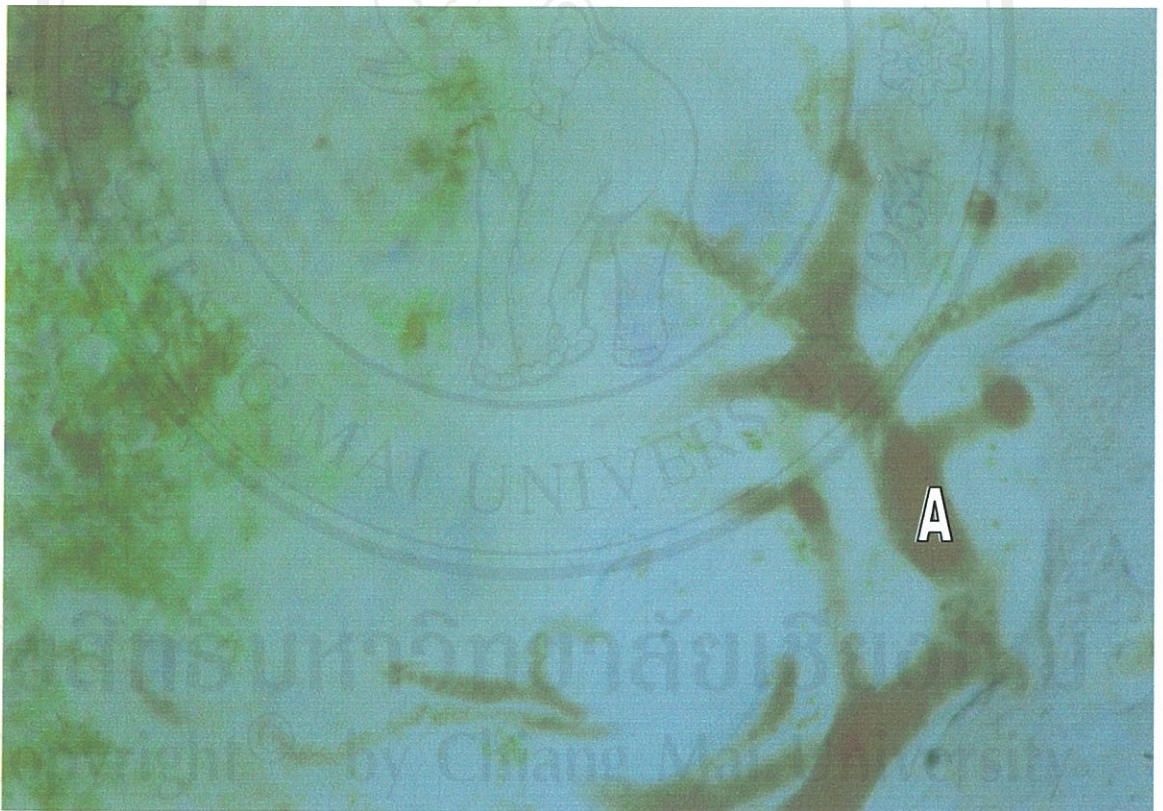


Figure 16 Angiogenesis (A) of specimens in control group at 4 weeks period.

Specimens at 6 weeks

PRP group. New bone formation in the defect of PRP group at 6 weeks seemed to be not different when compared to the control defect at the same period. The woven bone was found not only in the internal walls of the defect but also under the periosteum. Neither inflammatory cells nor bacteria were found around the artificial defects. The entrance of the defect was covered by the extraoral soft tissue.

Control group. The new bone formation was found not only in the internal walls of the defect but also under the periosteum. Connective tissue was still present in the control defect but was not found in the PRP defect. Control defect revealed a layer of fibrous tissue and woven bone formation similar to PRP defect at the same period. Overlying soft tissue was present around the entrance of the defect (figure 17).

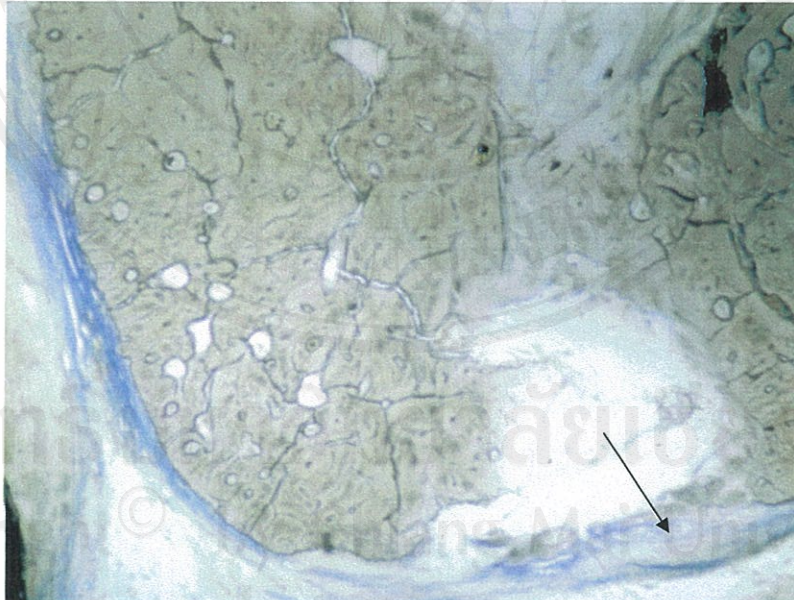


Figure 17 Soft tissue (arrow) of specimen in control group at 6 weeks period.

Specimens at 8 weeks

PRP group and control group. Neither inflammatory cells nor bacteria were found around the artificial defects. Descriptively, the new bone formation of PRP and control groups seemed to be not different. All defects revealed more condensed bone compared to the specimens obtained at the former periods (Figure 18). Newly formed bone beneath as well as below both the periosteum and the entrance of the defect revealed a vascularized and more condensed bone. The entrance of the defect was covered by the extraoral soft tissue and periosteum.

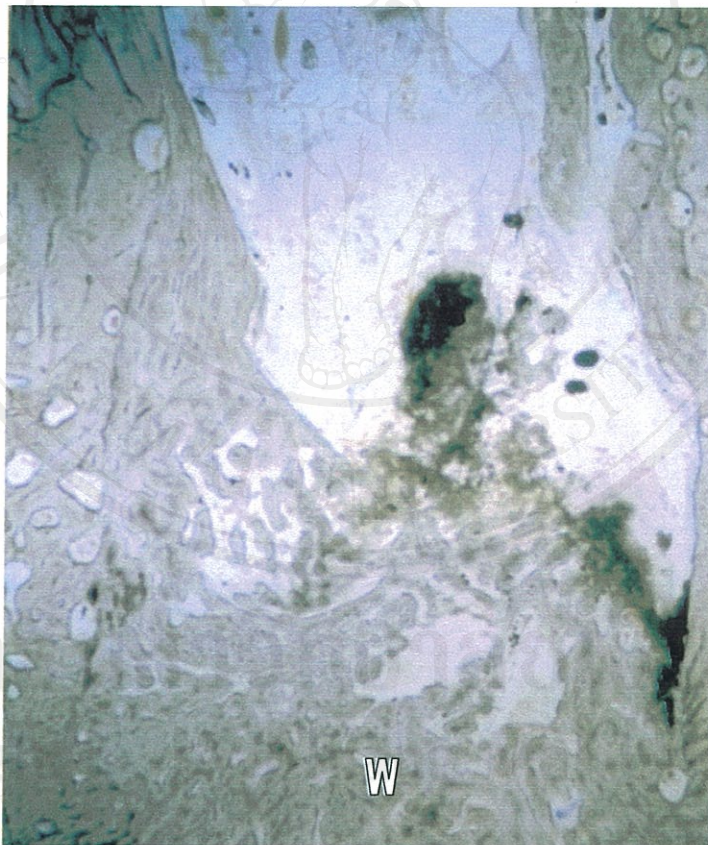


Figure 18 New bone formation or woven bone (W) of specimen at 8 weeks period.

Specimens at 12weeks

PRP group and control group. The healing pattern of PRP and control groups revealed similar finding. Descriptively, the new bone formation of these two groups seemed not to be different. The maturation of the new bone was seen in both experimental defects and control defects. The entrance to the defect of both PRP and control group was covered by not only the overlying soft tissue but also by newly formed bone. The artificial defects were completely filled by new bone formation (figure 19). Inflammatory cells and bacteria were not found around the artificial defects.

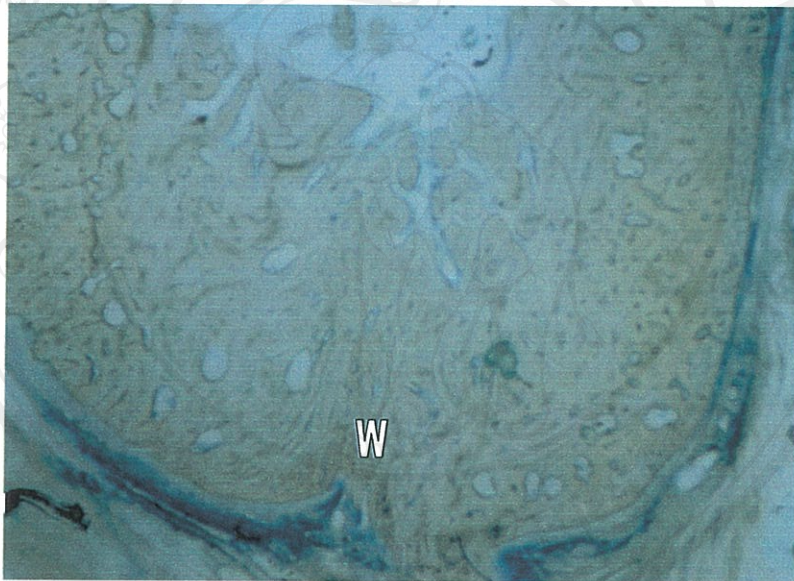


Figure 19 New bone formation or woven bone (W) of specimen at 12 weeks period.

The healing process and new bone formation at 2 weeks, 4 weeks, 6 weeks, 8 weeks and 12 weeks of both PRP and control groups are compared histologically in figure 20.

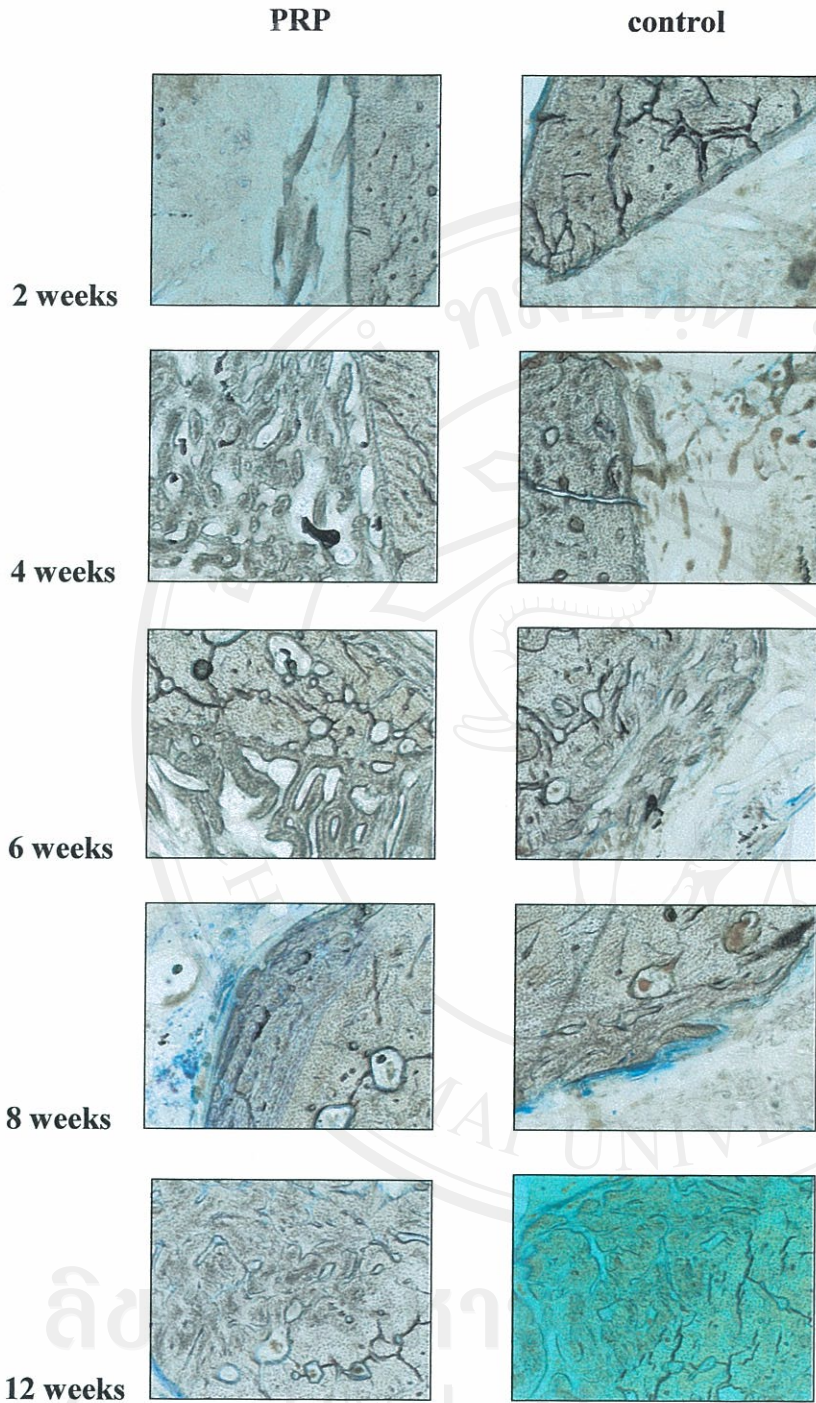


Figure 20: Histological findings of the bone healing at different periods in PRP and control groups.

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Histomorphometrical Results. Percent of bone regeneration (woven bone) measured from all slides are presented in table 3. The results of the histomorphometric study, which are illustrated in table 4 indicate that bone regeneration between PRP and control group at different periods show no statistical significant differences. The number of slides of PRP and control group at different period were also presented in table 4.

Table 3: Percent of woven bone measured by Image J program (version 1.24)

Slide number	Prp at 2 wks	Control at 2 wks	Prp at 4 wks	Control at 4 wks	Prp at 6 wks	Control at 6 wks	Prp at 8 wks	Control at 8 ks	Prp at 12 wks	Control at 12 wks
1	4.22	6.9	13.06	24.39	1.14	0.85	15.98	11.41	15.52	7.96
2	8.04	7.16	7.54	15.67	4.46	15.14	15.21	10.92	13.89	7.77
3	10.69	2.97	7.06	33.26	17.74	4	13.21	10.46	8.37	10.36
4	8.15	1.9	29.46	24.85	12.38	0.2	10.77	4.12	19.93	16.17
5	11.19	4.7	14.95	14.3	2.31	7.32	4.08	0.85	12.31	18.87
6	2.45	7.12	13.32	4.93	18.09	19.22	4.66	2.73	14.64	28.1
7	3.11	6.92	23.76	6.3	14.53	14.3	7.6	13.55	12.18	16.54
8	4.74	8.68	18.68	7.19	19.27	15.18	18.39	6.17	11.82	22.74
9	10.75	6.25	18.87	8.84	17.54	13.9	10.24	2.86	15.56	-
10	6.05	4.7	21.21	5.59	7.13	16.58	10.66	3.18	13.59	-
11	7.14	3.57	10.89	12.84	4.26	9.77	10.38	4.1	14.08	-
12	1.95	6.42	8.89	19.76	15.01	8.45	-	10.19	19.03	-
13	3.94	7.97	3.71	10.1	15.62	5.28	-	15.1	18.76	-
14	3.16	3.74	4.08	24.95	9.47	1.28	-	21.09	-	-
15	4.17	6.94	6.64	13.82	13.17	4.13	-	11.52	-	-
16	1.33	9.36	-	13.51	15.28	20.87	-	7.77	-	-
17	-	3.89	-	15.66	16.35	2.97	-	15.85	-	-

Table 3: Percent of woven bone measured by Image J program (version 1.24) (cont.)

Slide number	Prp at 2 wks	Control at 2 wks	Prp at 4 wks	Control at 4 wks	Prp at 6 wks	Control at 6 wks	Prp at 8 wks	Control at 8 wks	Prp at 12 wks	Control at 12 wks
18	-	4.45	-	11.47	17.21	4.61	-	19.39	-	-
19	-	4.46	-	10.83	-	29.05	-	12.55	-	-
20	-	2.43	-	8.49	-	21.73	-	-	-	-
21	-	-	-	13.7	-	12.06	-	-	-	-
22	-	-	-	9.72	-	17.26	-	-	-	-
23	-	-	-	8.33	-	8.73	-	-	-	-
24	-	-	-	-	-	11.32	-	-	-	-
25	-	-	-	-	-	8.75	-	-	-	-
26	-	-	-	-	-	10.49	-	-	-	-

Table 4: Mean and standard deviation of woven bone in PRP and control group at different periods

period	PRP group	Control group	p Value
2 weeks	5.69 +/- 3.26 (16)	5.53 +/- 2.12 (20)	0.861
4 weeks	13.48 +/- 7.63 (15)	13.85 +/- 7.24 (23)	0.880
6 weeks	12.28 +/- 5.93 (18)	10.90 +/- 7.24 (26)	0.510
8 weeks	11.02 +/- 4.50 (11)	9.67 +/- 5.85 (19)	0.518
12 weeks	14.59 +/- 3.25 (13)	16.06 +/- 7.22 (8)	0.599

NS = non significance (p<0.05)