CHAPTER 4

RESULTS

4.1 Preparation of PHA membrane

The mcl-PHA was extracted from the bacteria cells and cast into a film. Twelve uniformly-sized pieces were cut from a large piece of the PHA cast. The PHA was transparent and elastomeric (Figure 4.1).

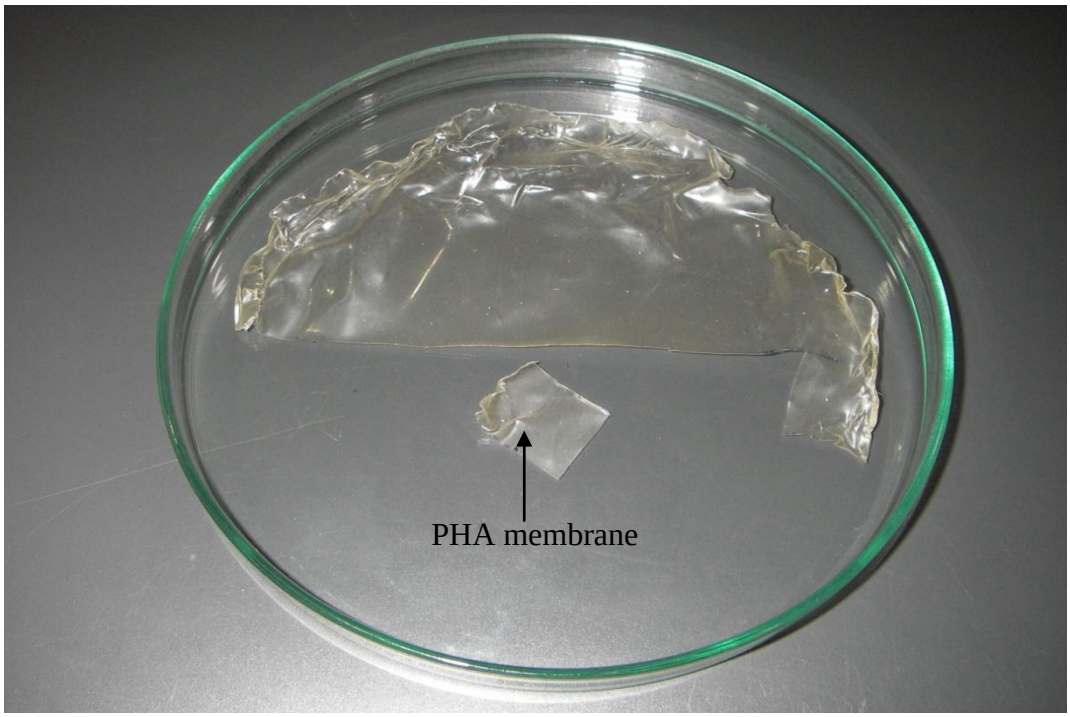

Figure 4.1 PHA film in a Petri dish – final product used for insertion.

4.2 Clinical and physical examination

All animals remained in fairly good health throughout the course of the experiment.

4.3 Critical size defect

All the defects were created with the size mentioned earlier (15 mm × 10 mm) except for Rabbit 3 and Rabbit 9. The defects in these two rabbits were created at approximately 10 mm × 10 mm because of their relatively small jaw bone structure.

4.4 Observations of the test animals and implanted PHA

During three weeks of observation (after the insertion of PHA), Rabbit 2 was found to have bitten its left leg. The abnormal behaviour of this rabbit was probably caused by the tingling effect of ketamine. However, the rest of the rabbits did not exhibit any abnormal behaviour.

The appearance of the skin overlying the mandibular defect could be observed in most of the animals (Figure 4.2).

Gross examination of the harvested mandibles (Figure 4.3) did not show evidence of swellings or infection in all the animals except Rabbit 1, which had a swelling adjacent to the defect on the right side of the mandible.

It was noted that the mandibular incisors in some of the rabbits were lengthened and deformed. The left incisor in Rabbit 5 was found broken while the

right incisor was still intact. The incisors on the harvested defect of Rabbit 7 (Figure 4.4) were mobile and discoloured. It could also be observed that the left incisor of Rabbit 8 has an apical fracture despite having both incisors remaining firm.

It was found at 12 weeks that, PHA films which were inserted in Rabbit 1, Rabbit 2 and Rabbit 3 were clinically not visible; while the PHA films inserted in the rest of the rabbits remained intact, when examined between 3 to 9 weeks, (Figure 4.5 – 4.8). However, the physical appearance of the films in the rabbits had changed from a transparent yellowish film to a yellowish opaque film. Besides that, the films observed from 3 to 9 weeks were brittle compared to the films before insertion which was elastomeric.

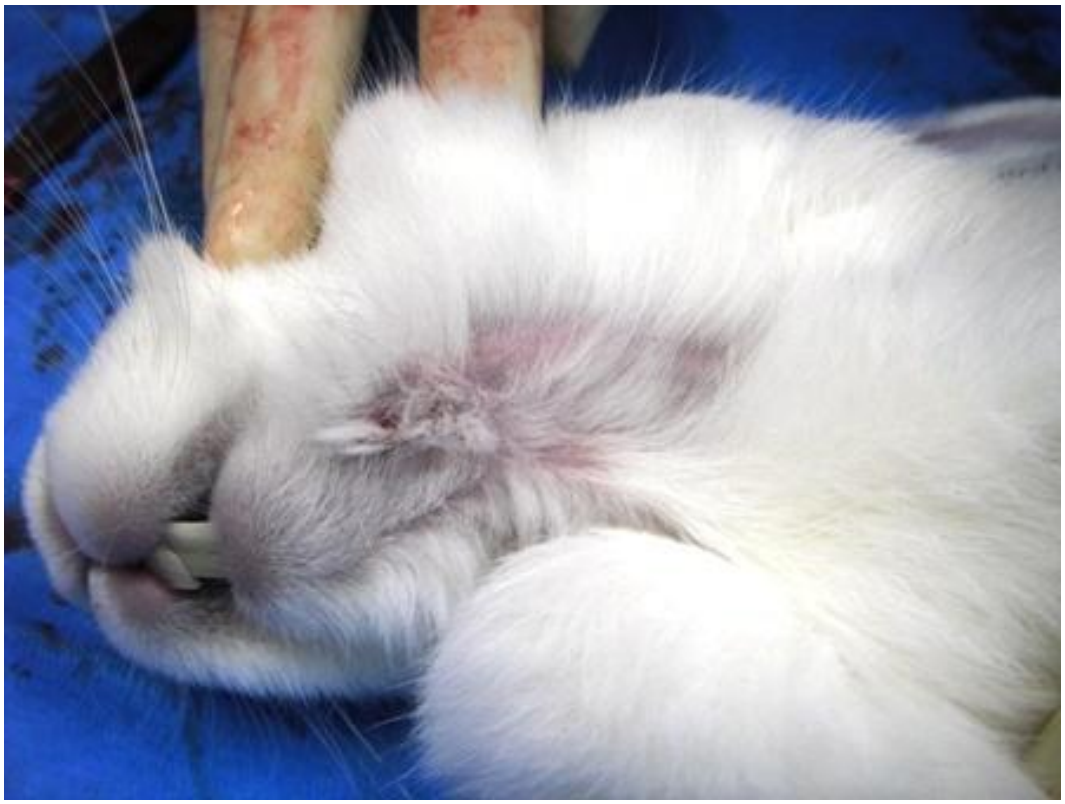

Figure 4.2 Rabbit 9 at 6 weeks. Physical appearance of the skin overlying the mandibular defect observed in most of the animals.

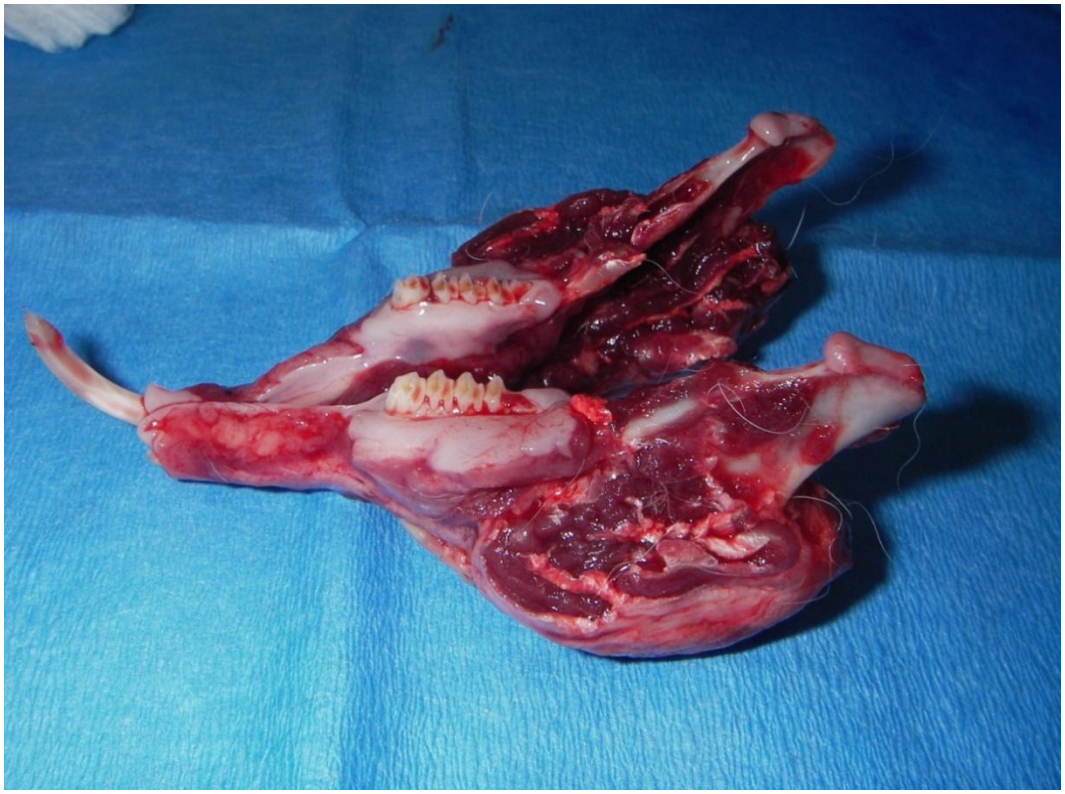

Figure 4.3 The harvested specimen of Rabbit 5 showing the defect and its surrounding tissues.

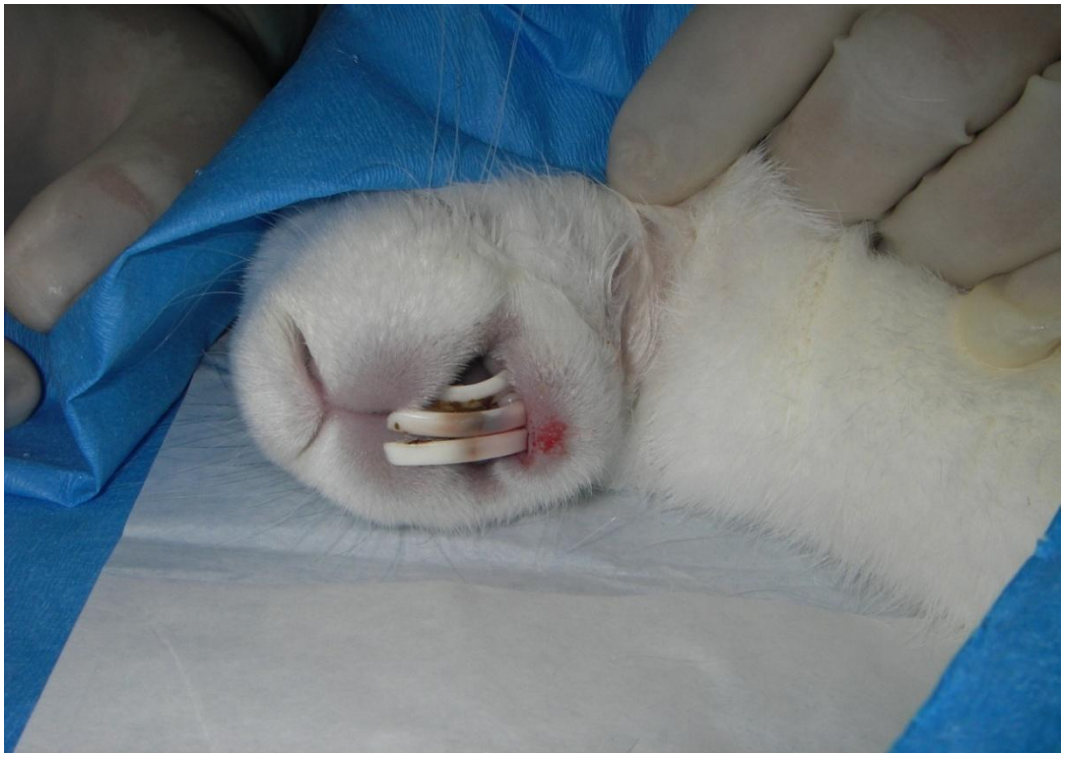

Figure 4.4 Rabbit 7 at 6 weeks. The incisors were mobile and discoloured.

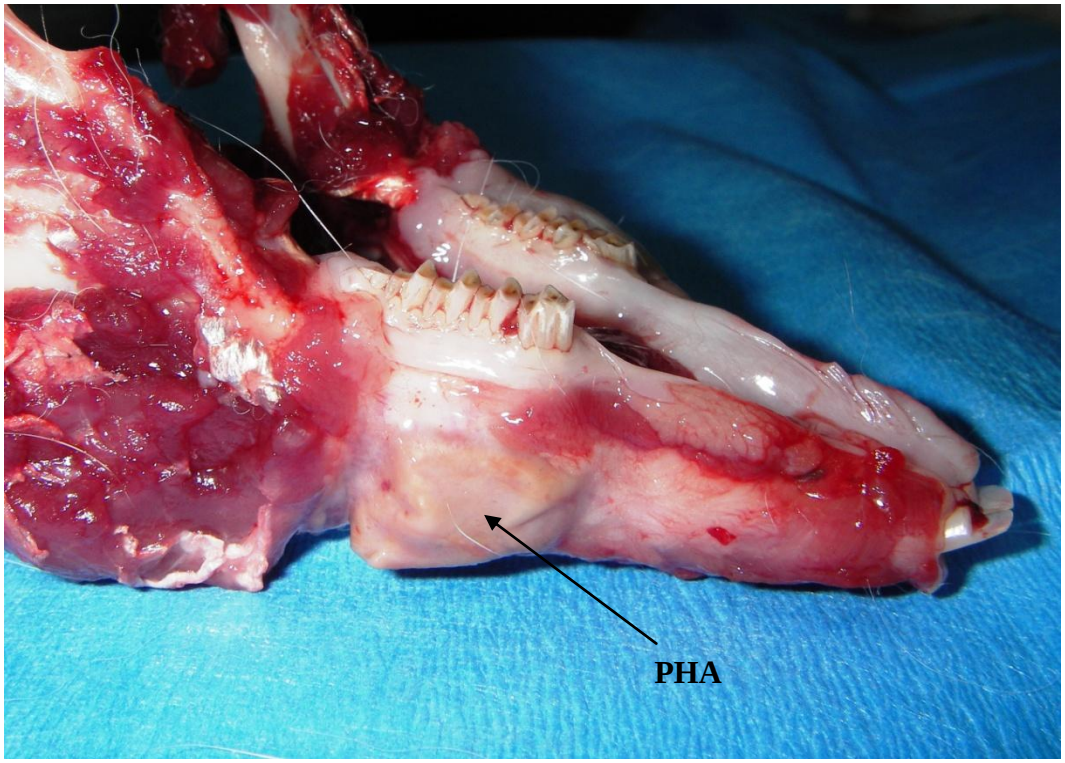

Figure 4.5 The mandible of Rabbit 4, harvested at week 9. The location of PHA was indicated.

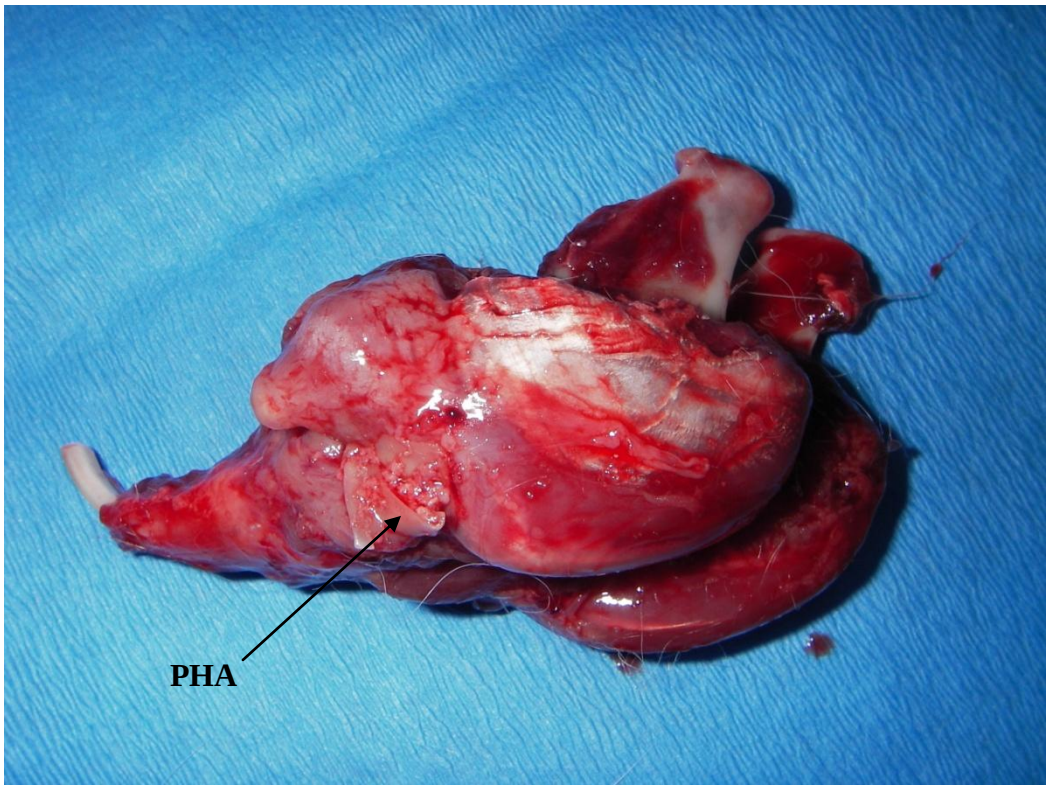

Figure 4.6 PHA remained mostly intact in this specimen of Rabbit 11 harvested at 3 weeks.

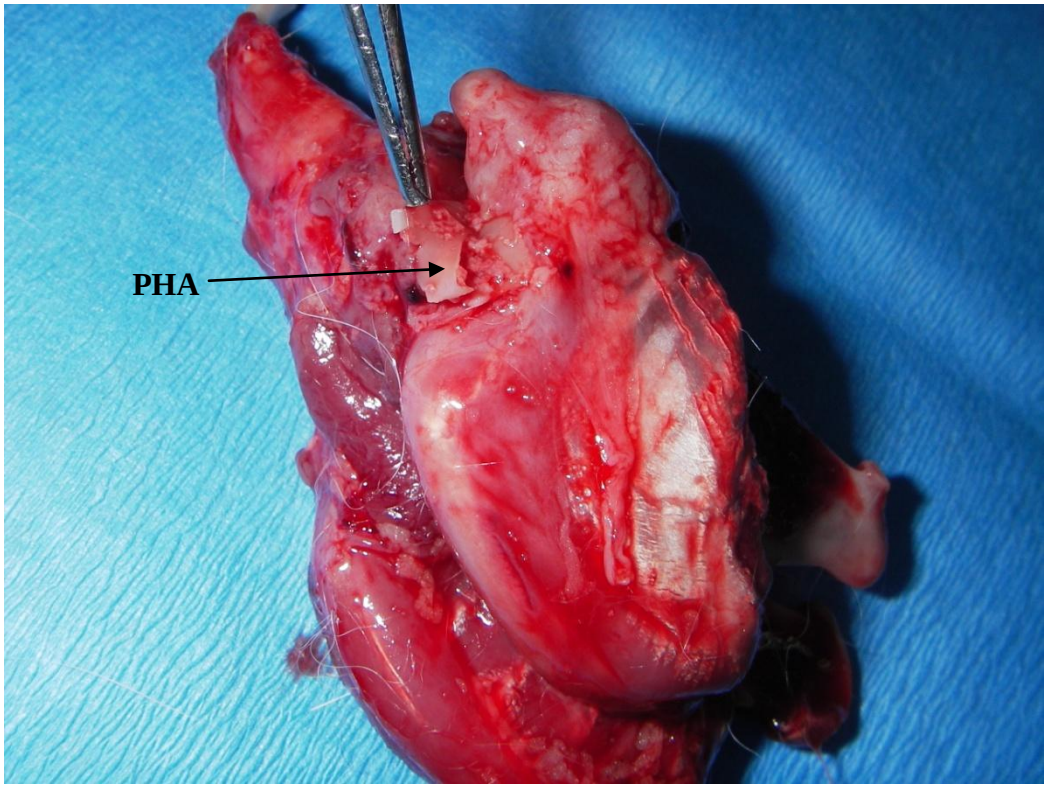

Figure 4.7 Vertical view of the mandible of Rabbit 11 harvested at 3 weeks. The appearance of PHA is indicated.

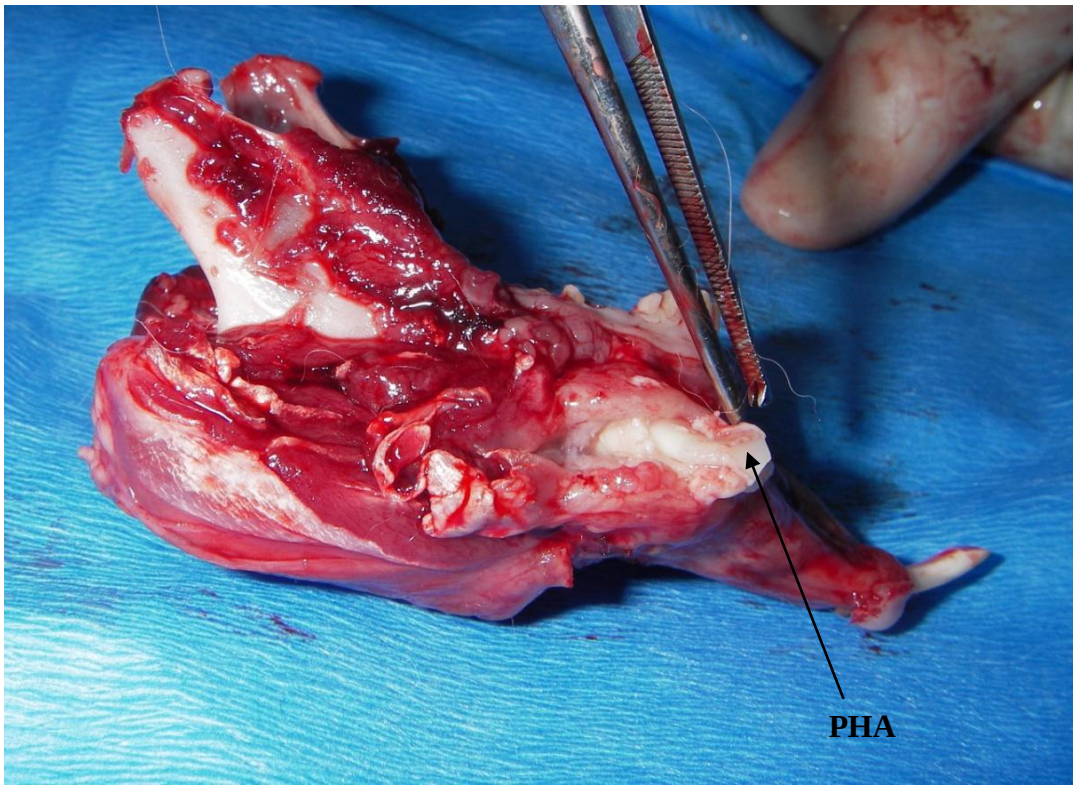

Figure 4.8 Appearance of defect in Rabbit 12 harvested at 3 weeks.

4.5 Histological findings

Histological examination of all sections from the PHA grafted defects and controls did not show any adverse host tissue response. There was no evidence of chronic inflammation, foreign body giant cell reaction nor osteoclastic activity. Instead, in the PHA grafted sites there was a variable amount of new trabecular bone ingrowth into the bone defects harvested at different time intervals. However residual PHA material was still observed within these defects even at 12 weeks post-implantation.

In the control sites, the defects were largely empty. Incipient new bone was sometimes encountered at the host bone interface. Scant fibrous connective tissues were also observed.

4.6 Histomorphometric findings

Mean scores on new bone volume percentage relative to host bone, residual PHA grafted material; connective tissues at specific time intervals for each animal are detailed in Table 4.1 and Table 4.2.

Table 4.1 Average mean new bone volume scores for PHA and control groups at specific time intervals

Rabbit	Time interval (weeks)	PHA group		Control	
		Side of defect	Mean new bone volume	Side of defect	Mean new bone volume
1	12	Right	3.04	Left	2.44
2		Left	3.81	Right	1.40
3		Right	2.67	Left	1.25
Average mean			**3.17**		**1.70**
S.D.			**0.58**		**0.65**
p-value			**0.04** < 0.05		
4	9	Right	1.74	Left	0.67
5		Left	1.50	Right	0.91
6		Right	1.00	Left	0.44
Average mean			**1.41**		**0.67**
S.D.			**0.38**		**0.24**
p-value			**0.04** < 0.05		
7	6	Left	2.00	Right	0.00
8		Left	0.60	Right	0.00
9		Left	1.60	Right	0.31
Average mean			**1.40**		**0.10**
S.D.			**0.72**		**0.18**
p-value			**0.04** < 0.05		
10	3	Left	0.89	Right	0.14
11		Right	1.00	Left	0.32
12		Right	0.80	Left	0.44
Average mean			**0.90**		**0.30**
S.D.			**0.10**		**0.15**
p-value			**0.00** < 0.05		

Table 4.2 Comparison of average mean new bone volume between PHA groups and negative controls according to the time intervals respectively

Time interval	Average mean new bone volume ± Standard Deviation	
	PHA group	Negative control
12 weeks	3.17 ± 0.58	1.70 ± 0.65
9 weeks	1.41 ± 0.38	0.67 ± 0.24
6 weeks	1.40 ± 0.72	0.10 ± 0.18
3 weeks	0.90 ± 0.10	0.30 ± 0.15

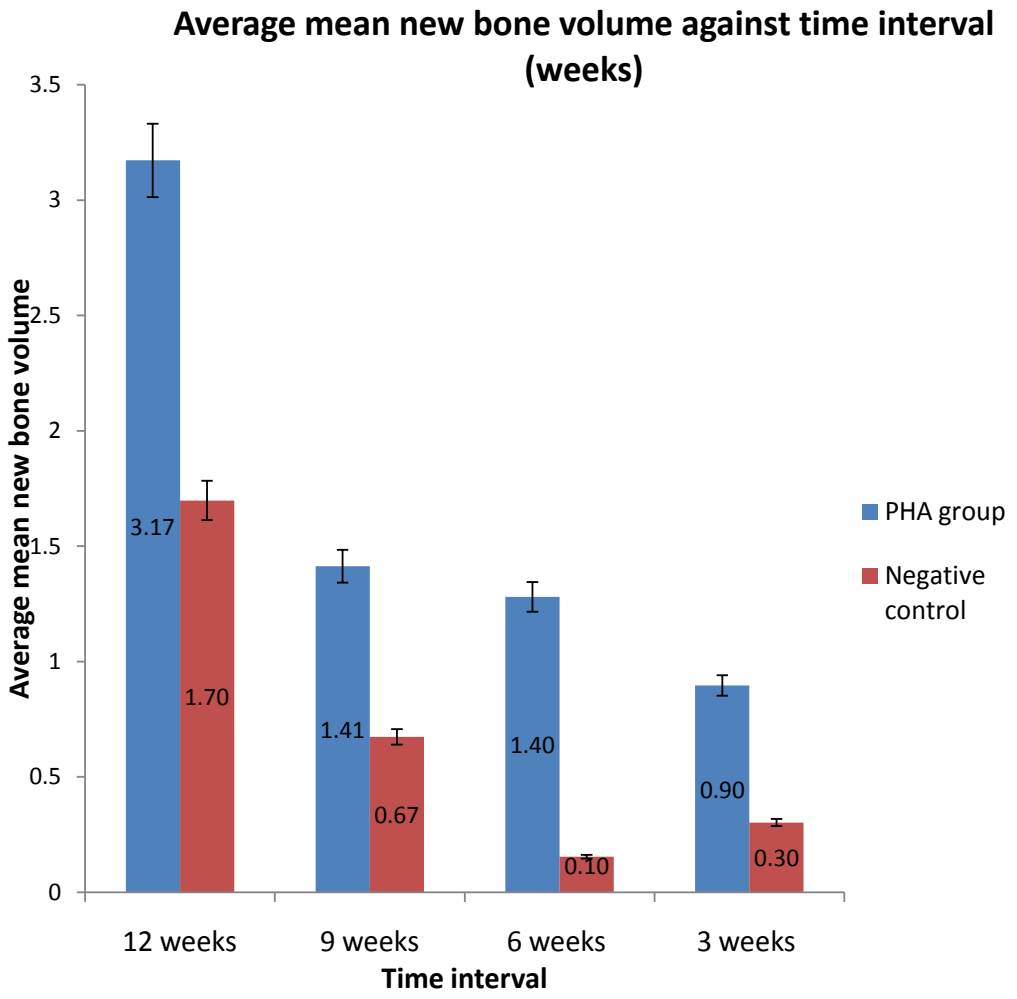

Figure 4.9 Comparison of mean new bone volume between PHA and control groups at 3- , 6-, 9- and 12-week healing intervals.

Figure 4.9 shows the comparison between the average mean new bone volume for PHA group and control against time intervals of 3- , 6- , 9- and 12 weeks. The average mean new bone volume for PHA group gradually increased with increasing time interval.

4.7 Photomicrographs of PHA groups and controls

As mentioned in Chapter 3, the thin sections were viewed and photomicrographs were captured with a digital camera attached to the microscopic system. Some of the photomicrographs of PHA groups followed by control groups are shown below.

4.7.1 PHA groups

Figure 4.10 showed that new bone were formed in the presence of PHA on the periphery of the defect at 12-weeks healing interval for Rabbit 2. In figure 4.11, histological new bone can be seen along the host bone of the defect grafted with PHA at 9-weeks healing interval for Rabbit 4. More new bone is present horizontally from the host bone. The photomicrograph of a defect implanted with PHA for Rabbit 7 at 6-weeks healing interval showed that a channel of a new bone is localized on the periphery of the host bone (Figure 4.12). Besides that, histological new bone trabeculae could be seen along the periphery of the host bone for Rabbit 10 which was grafted with PHA at 3-weeks healing interval. There were traces of PHA which could be viewed on the defect across the newly

formed bone (Figure 4.13). Figure 4.14 showed the presence of new bone formed along the periphery of the host bone for Rabbit 11 which was grafted with PHA at 3-weeks healing interval.

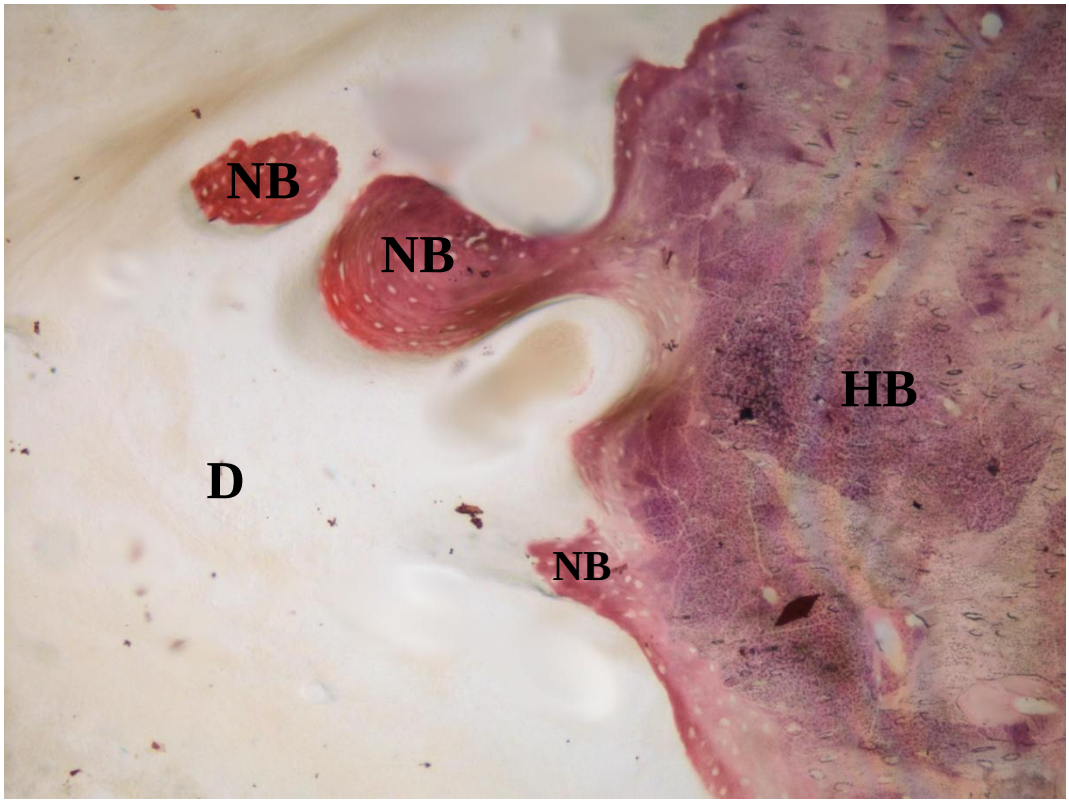

Figure 4.10 Photomicrograph of the defect grafted with PHA at 12 weeks for Rabbit 2. (NB = new bone, HB = host bone, D = Defect). Haematoxylin and Eosin stain, original magnification: × 200.

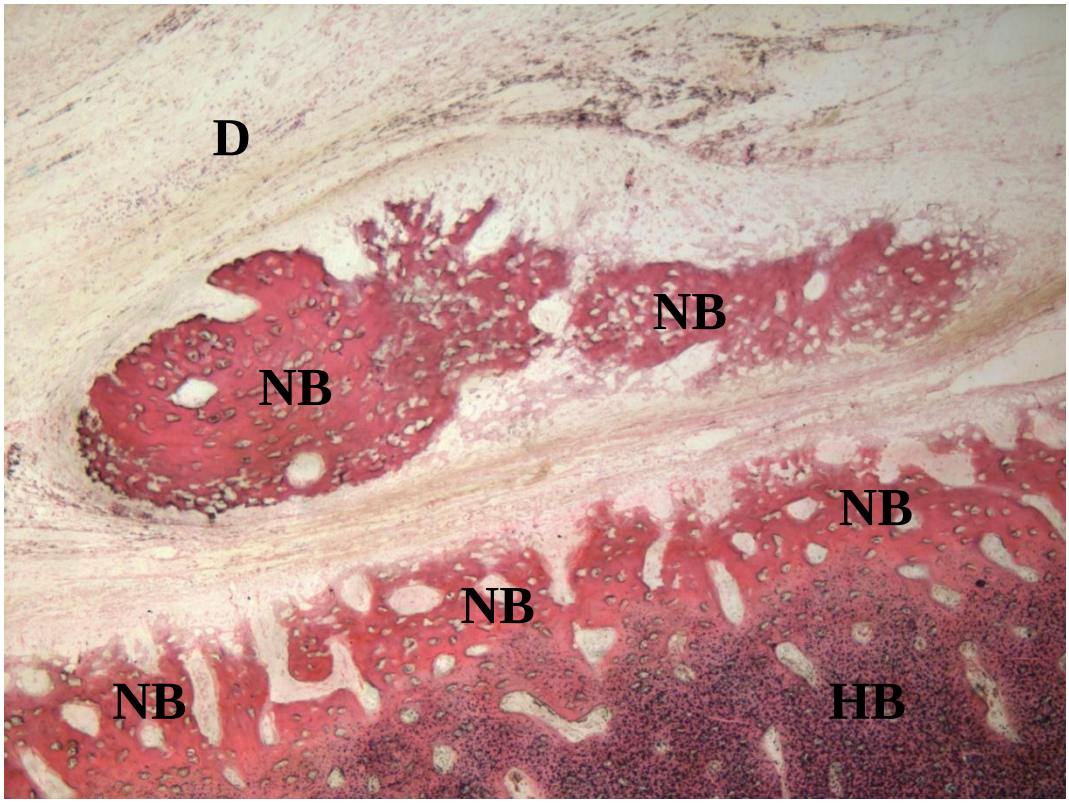

Figure 4.11 Photomicrograph of the defect grafted with PHA at 9 weeks for Rabbit 4 (NB = new bone, HB = host bone, D = defect). Haematoxylin and Eosin stain, original magnification: × 200.

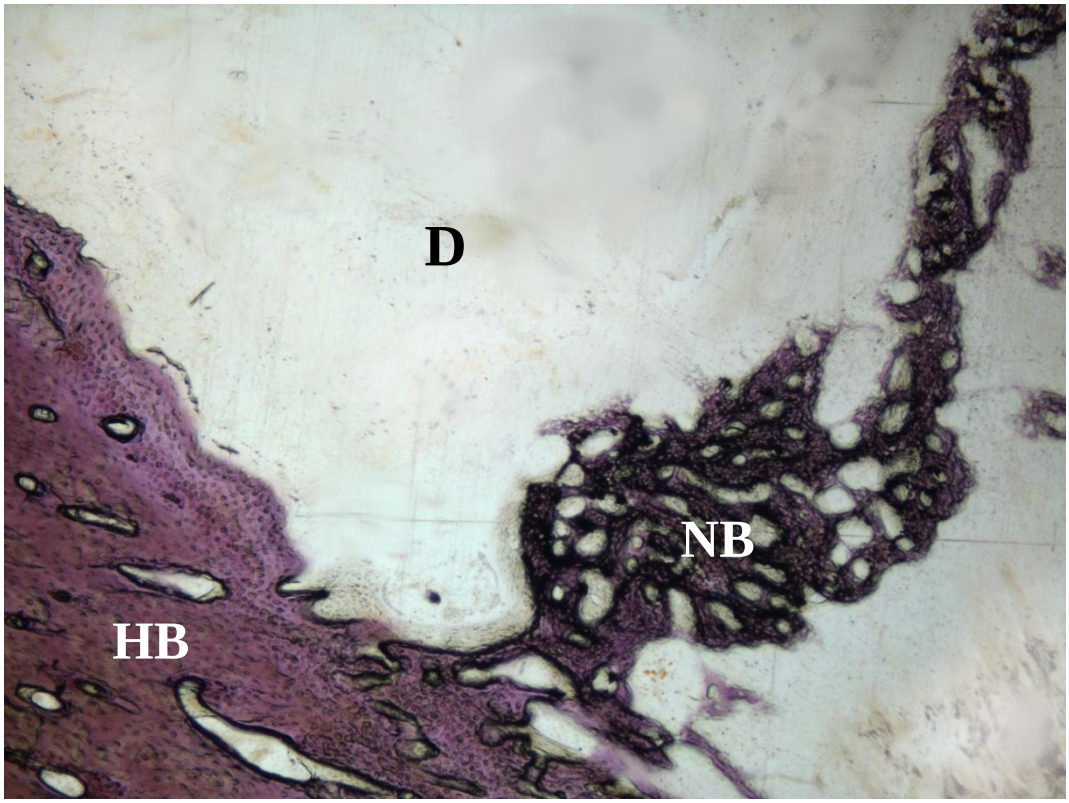

Figure 4.12 Photomicrograph of the defect grafted with PHA at 6 weeks for Rabbit 7 (NB = new bone, HB = host bone, D = defect). PAS stain, original magnification: × 200.

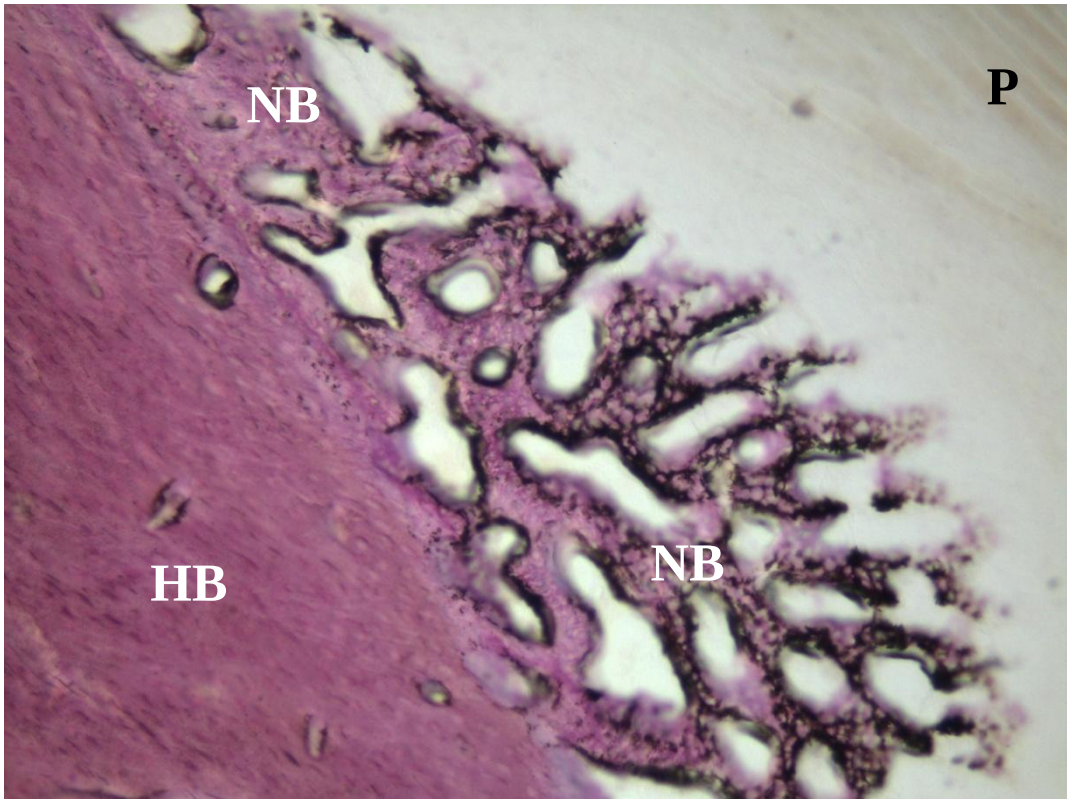

Figure 4.13 Photomicrograph of the defect grafted with PHA at 3 weeks for Rabbit 10 (NB = new bone, HB = host bone, P = PHA). PAS stain, original magnification: × 200.

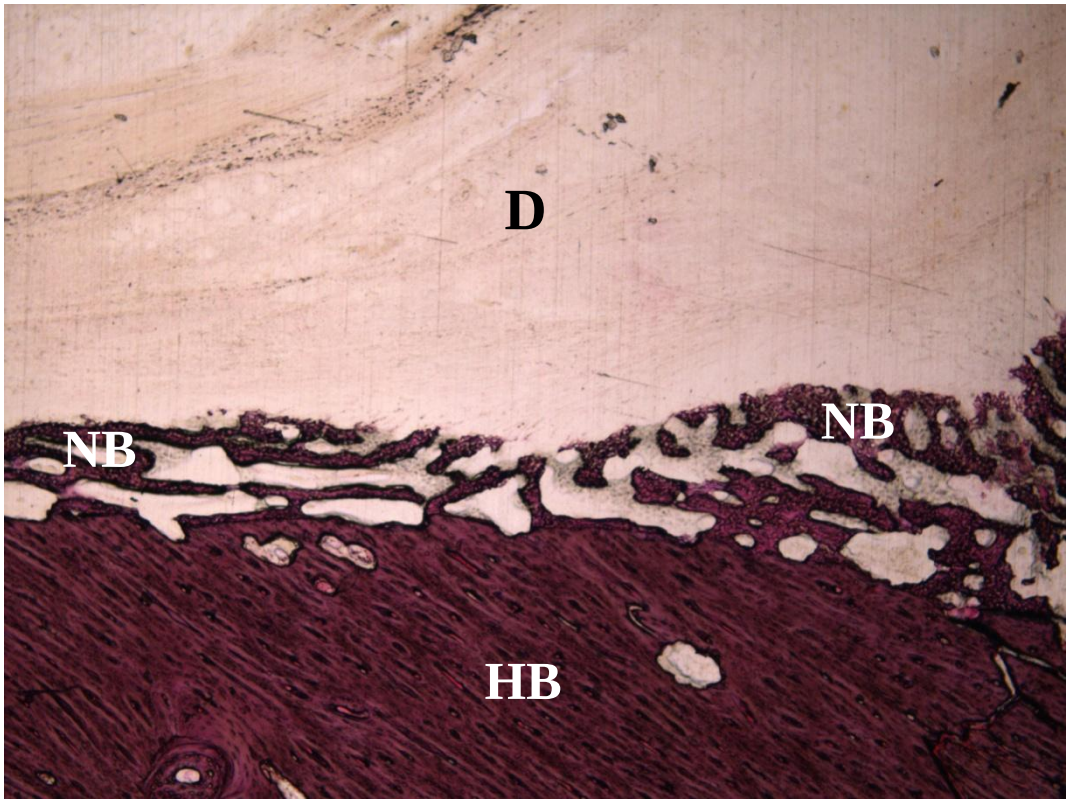

Figure 4.14 Photomicrograph of the defect grafted with PHA at 3 weeks for Rabbit 11 (N = new bone, H = host bone). PAS stain, original magnification: × 100.

4.7.2 Controls

Presence of minimal new bone which is stained in light purple comparatively were formed on the periphery of the host bone of a negative control defect for Rabbit 1 at 12-weeks healing interval (Figure 4.15). Presence of matured bone without any traces of newly formed bone could be viewed in the photomicrograph of a negative control defect for Rabbit 2 at 12-weeks healing interval (Figure 4.16). Figure 4.17 showed the presence of histological matured bones and no new bone were formed for the negative control defect at 3 weeks for Rabbit 10. Similarly, another site of negative control defect for Rabbit 10 at 3

weeks showed the presence of matured bone only (Figure 4.18). Besides that, Figure 4.19 showed the photomicrograph of a negative control defect at 3-weeks healing interval for Rabbit 11. A residual host bone was found among the fibrous tissues. However, there were no traces of new bone formed.

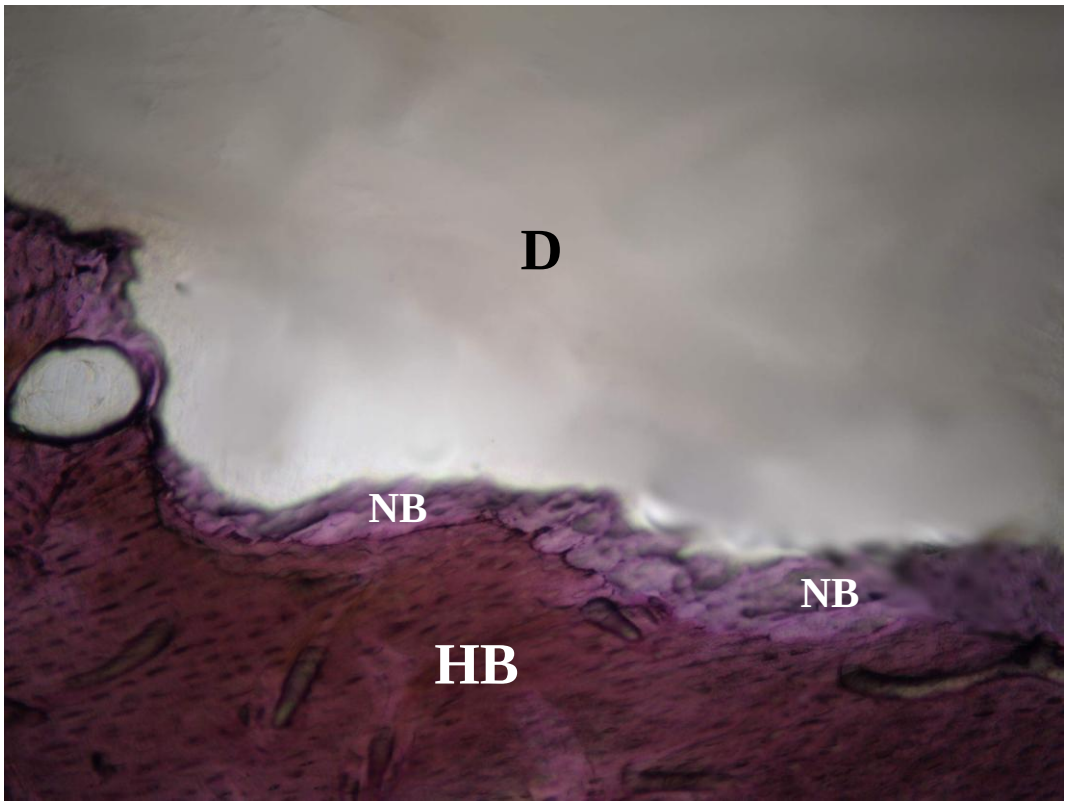

Figure 4.15 Photomicrograph of a negative control defect at 12 weeks for Rabbit 1 (NB = new bone, HB = host bone, D = defect). PAS stain, original magnification: × 100.

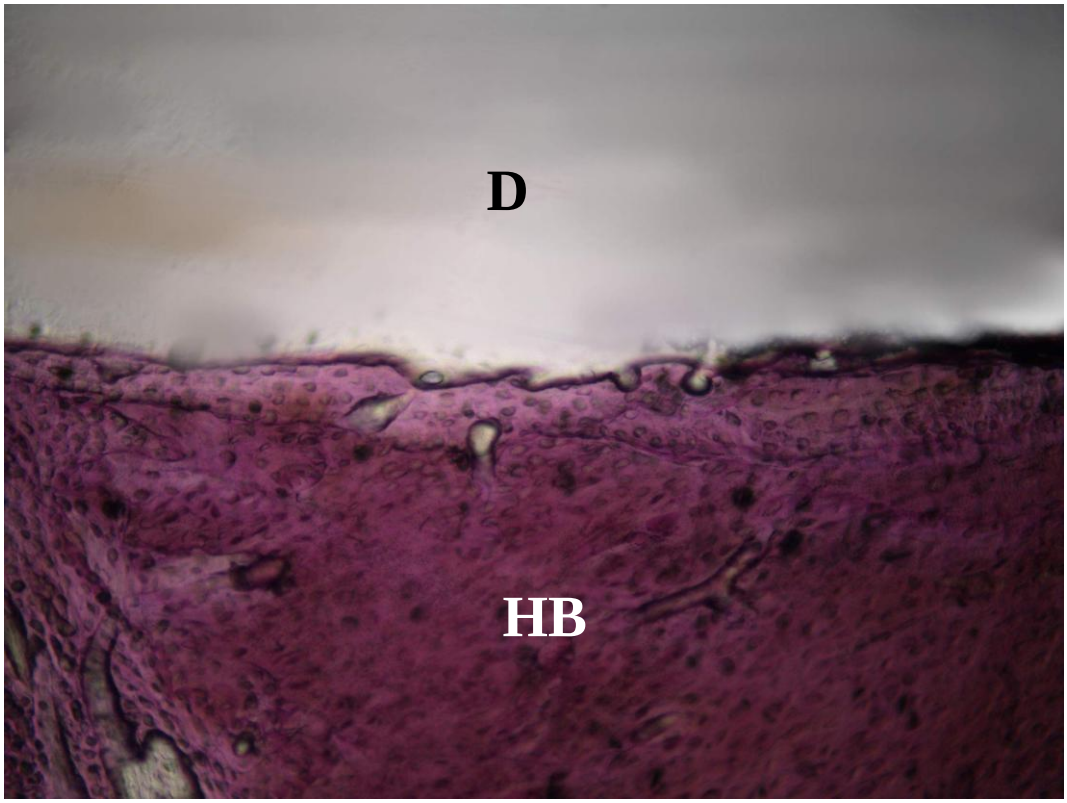

Figure 4.16 Photomicrograph of a negative control defect at 12 weeks for Rabbit 2 (HB = host bone, D = defect). PAS stain, original magnification: × 100.

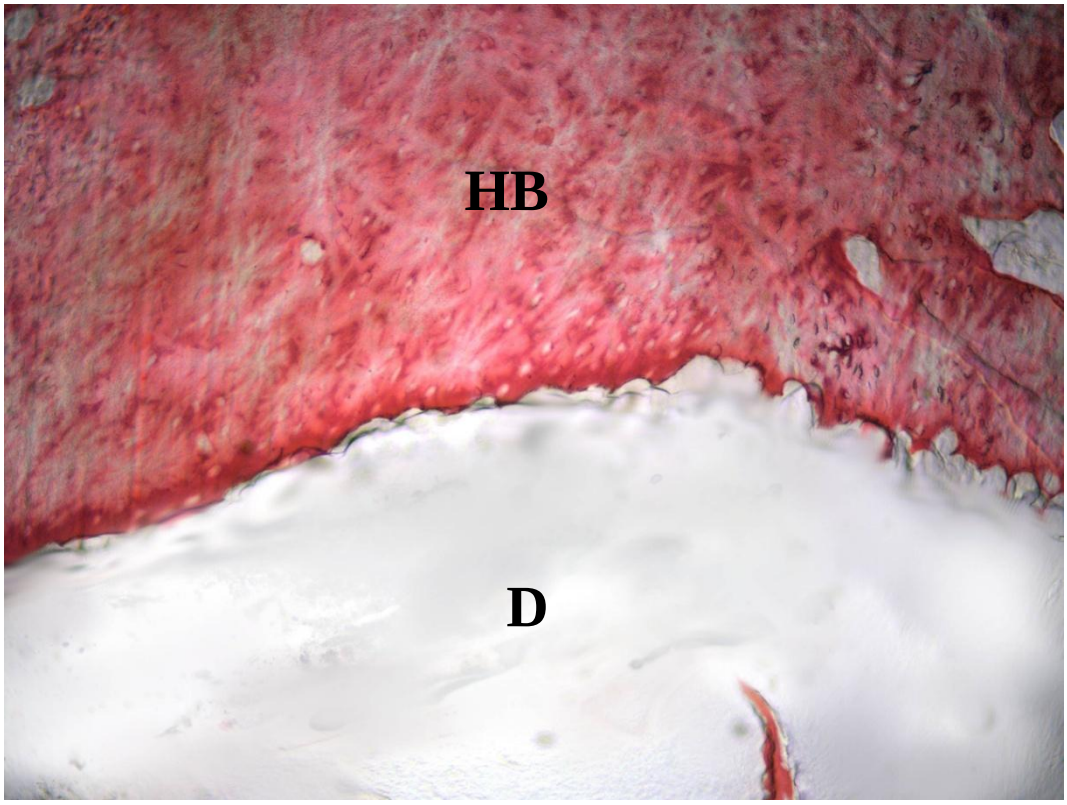

Figure 4.17 Photomicrograph of a negative control defect at 3 weeks for Rabbit 10 (HB = host bone, D = defect). Haematoxylin and Eosin stain, original magnification: × 100.

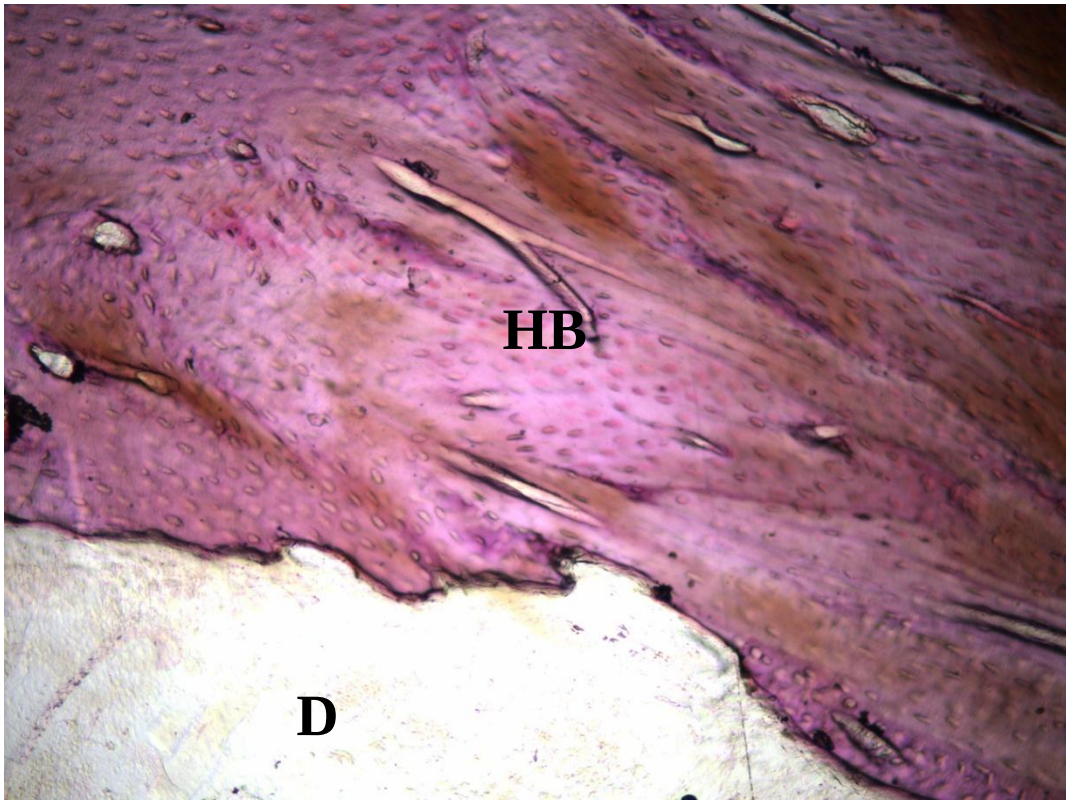

Figure 4.18 Photomicrograph of a negative control defect at 3 weeks for Rabbit 10 (HB = host bone, D = defect). PAS stain, original magnification: × 100.

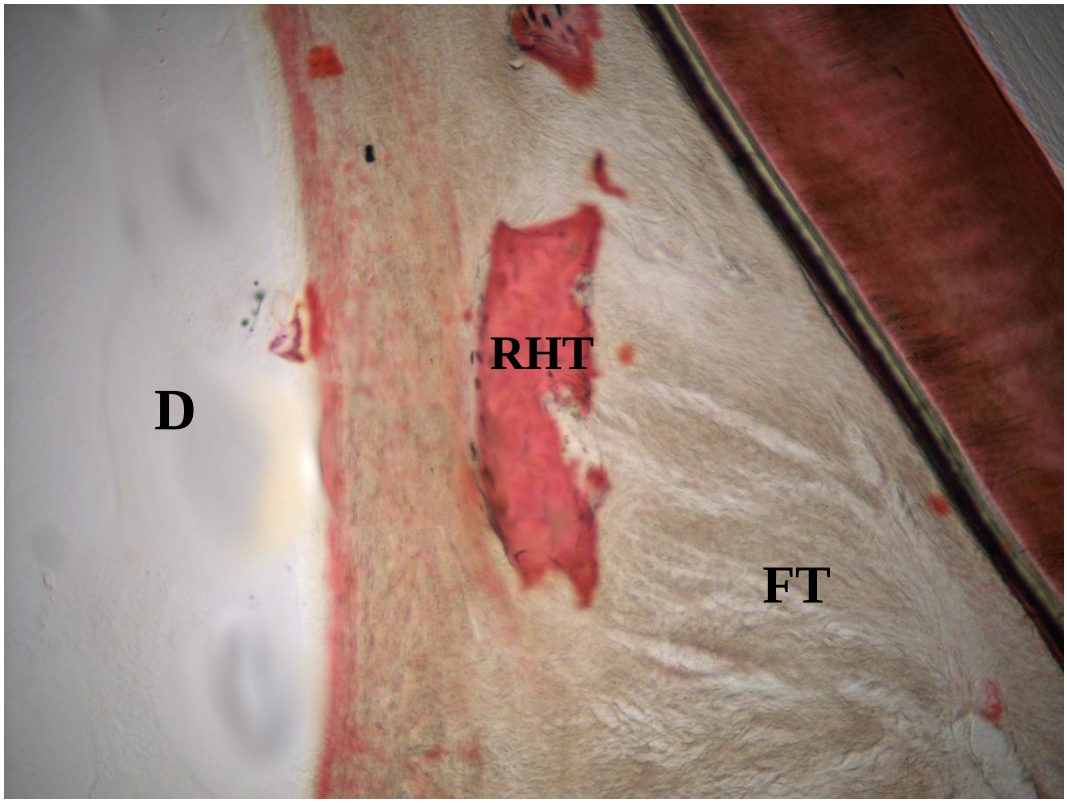

Figure 4.19 Photomicrograph of a negative control defect at 3 weeks for Rabbit 11 (FT = fibrous tissues, RHT = residual host bone, D = defect). Haematoxylin and Eosin stain, original magnification: × 100.