JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 5 (1994) 194–199

Tissue compatibility of polylactic acids in the skeletal site

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This study was undertaken to examine the osseous tissue compatibility of polylactic acid (PLA) with properties of bioabsorbability and plasticity. Two kinds of granular PLA with different molecular weight (PLA-H: 28 000 MW, PLA-L: 10 600 MW) were implanted into rat tibiae and light microscopic sections were prepared at 1, 2, 4, 8, 16, and 24 weeks following surgery. The tissue compatibility of the PLAs was compared with that of hydroxyapatite (HAP) which is a non-absorbable biocompatible material. After implantation, both PLAs were gradually absorbed and replaced by marrow tissue at 24 weeks. PLA-H degraded slower than PLA-L. New bone formation was observed around each PLA by 4 weeks, and a vast amount of bone deposited on PLA-H at 16 weeks. Phagocytic reactions to the PLAs were noted, however, severe inflammation was not seen. HAP was not absorbed and bone surrounded it throughout the experiment. These results indicate good tissue compatibility of PLA as a bioabsorbable material at the skeletal site.

1. Introduction

Polylactic acid (PLA) is a biomaterial of absorbable synthetic polymer type [1]. It easily undergoes hydrolytic de-esterification *in vivo*. The degraded products of lactic acids are incorporated into metabolic pathways and excreted as water and carbon dioxide, thus PLA does not remain in the body as a foreign material. PLA can be formed in various shapes by industrial processings. In addition, various mechanical strengths can be given to PLA according to the molecular weight, component and shape [2].

These properties make PLA an interesting material as a surgical device. At present, it is already in clinical use as a surgical suture. Moreover, PLA is expected to be used as a plate for fracture fixation [3], an artificial tendon [4], a blood vessel [5], and a delivery substance for various drugs [6–8]. With regard to dental applications of PLA, it is thought to be a useful device in fixation of jaw fractures, filling of bone defects, and augmentation of the alveolar ridge. In these usages, PLA will achieve its object in the period required, then it will be absorbed and replaced by the proper tissues, and the second operation to remove the PLA will be eliminated.

When PLA is used for the purposes described above, good tissue compatibility in the skeletal site is required. However, there are few reports on the osseous tissue compatibility of PLA [9, 10]. Most studies have only discussed the soft tissue compatibility, mechanical strength, and absorption rate of PLA as a surgical suture [1, 11, 12].

In this study, in order to investigate the usefulness of PLA as a surgical device in the skeletal site, we examined the osseous tissue compatibility of PLAs with different molecular weight. The tissue reactions of PLAs were compared with those of hydroxyapatite (HAP) which is known to be a non-absorbable implant material with good tissue compatibility in the skeletal site.

2. Materials and methods

2.1. Implant materials

Two kinds of PLA with different molecular weight, and HAP were examined in this study. The abridged name, molecular weight, form, and size of each material are shown in Table I.

TABLE I The implant materials used in this study

Material	Molecular weight	Form	Size of granule (μm)	Abridged name
Polylactic acid	28 000	Granular	100–400	PLA-H
Polylactic acid	10 600	Granular	100–400	PLA-L
Hydroxyapatite	—	Granular	297–500	HAP

2.2. Preparation of the PLAs

The PLAs were prepared by the Center of Biomedical Engineering, Kyoto University [1]. They were obtained by condensation polymerization of lactic acid (90% aqueous solution) under reduced pressure. Resultant polymers were ground and sieved into granular form. The molecular weight was determined by gel permeation chromatography.

2.3. Procedure of implantation and histological evaluation

Thirty-six male Wistar strain rats, 8 weeks old (mean body weight 270 g), were used in this study. They were anesthetized with Nembutal® (Abbot Lab., USA). An incision was made in both hind legs. Covering soft tissues and muscles were displaced, and the anterior surface of the tibia was exposed. A cavity through the

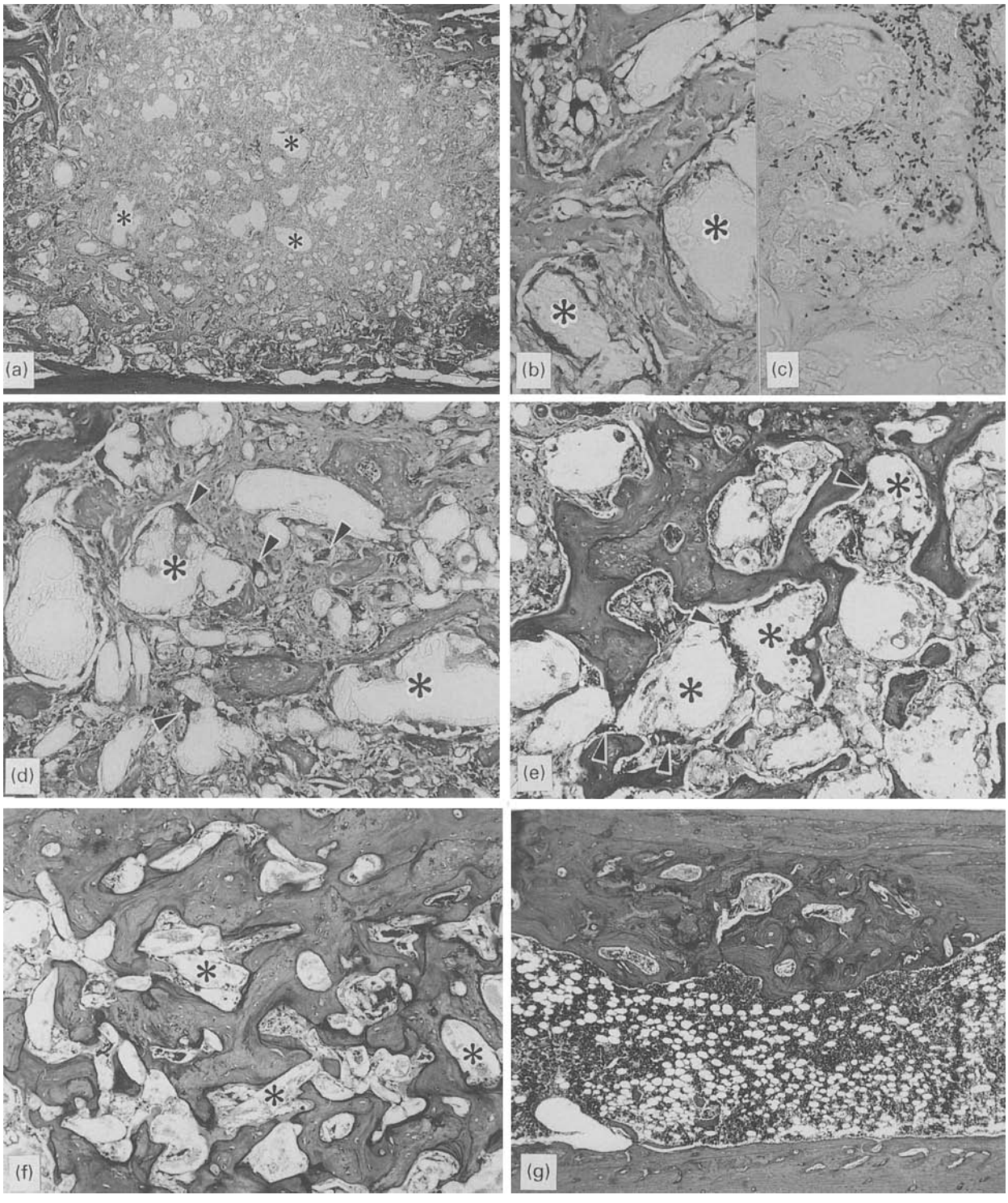

Figure 1 Histological findings of PLA-H implanted into rat tibia. (a) At 1 week, the PLA-H granules (*) are closely packed in the cavity (× 10). (b) In the peripheral portion, granulation tissue with new bone formation proliferates among the granules (*) (× 50). (c) In the centre portion, hemorrhage and fibrinous exudate are observed (× 50). (d) At 2 weeks, formation of immature bone is observed among the PLA-H granules (*). Foreign body giant cells (arrowheads) and macrophages infiltrate (× 25). (e) At 4 weeks, the new bone increases in amount and maturity. Foreign body giant cells (arrowheads) phagocytose the PLA-H granules (*) (× 25). (f) At 16 weeks, the PLA-H granules (*) are surrounded by a mature bone tissue. The amount of PLA-H decreases (× 25). (g) At 24 weeks, the PLA-H granules are completely absorbed and replaced by a bone marrow tissue with a fatty tissue. Corresponding to the remodelling, new bone trabeculae disappear (× 10).

cortex into the marrow space was made with a round bar (2 mm in diameter). After irrigation and hemostasis with saline and 3% H_2O_2, the implant materials were filled tightly into the cavity. The covering soft tissues were replaced and the wound was sutured. At 1, 2, 4, 8, 16, and 24 weeks after the operation, the complete tibiae were removed. There were four samples per time period for each material. These samples were fixed with 10% neutral buffered formalin. After decalcification, they were trimmed and embedded into paraffin, then serial sections of 4.5 μm thickness were prepared. The sections were stained with hematoxylin and eosin for light microscopic observation.

2.4. Measurement of the remaining amount of the materials

To assess the remaining amount of materials, four serial sections from each sample, a total of 16 sections per experimental period, served for histomorphometrical analysis.

The histological sections were viewed with a microscope using a 10X objective lens. The pictures of these sections were taken with a 3.3X photographic lens, and then the images transferred to a microcomputer screen. The areas occupied by the remaining materials were delineated using a personal image analysis system (LA-550®, PIAS, Japan). The mean relative amount of these areas from each period were computed.

3. Results

3.1. Histological findings

3.1.1. Tissue reaction to PLA-H

At 1 week after implantation, the PLA-H granules were closely packed in the cavity prepared in the rat tibia (Fig. 1a). In the peripheral portion of the cavity, granulation tissue with a small amount of new bone formation proliferated among the granules. Slight infiltration of macrophages was also seen in the granulation tissue (Fig. 1b). Proliferation of the granulation tissue did not extend to the centre portion of the cavity and hemorrhage and fibrinous exudate were observed among the PLA granules (Fig. 1c). At 2 weeks, granulation tissue proliferated through the cavity. Immature new bone actively formed in the granulation tissue. Phagocytic reaction of foreign body giant cells and macrophages was observed around the granules (Fig. 1d). At 4 weeks, the new bone increased in amount and

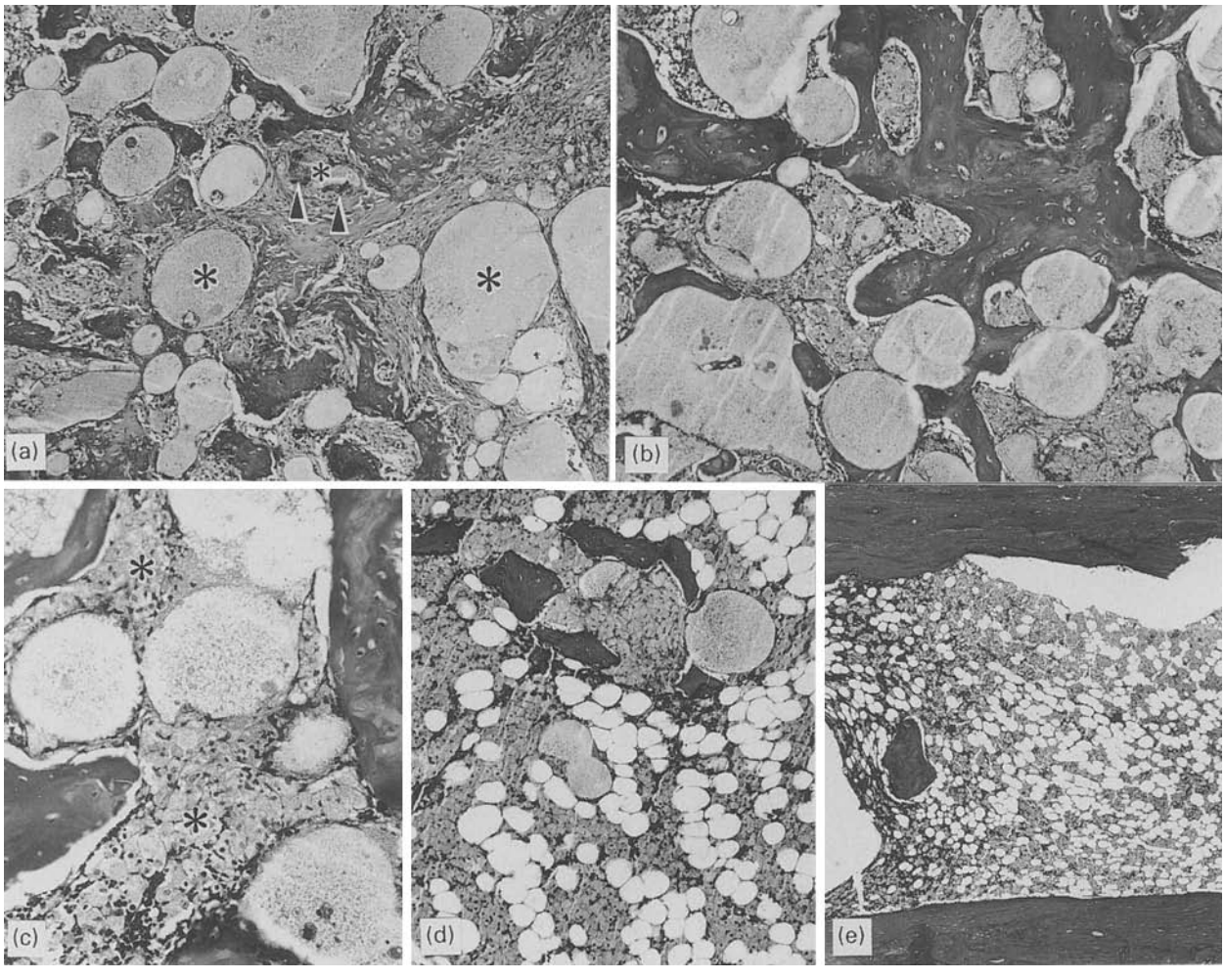

Figure 2 Histological findings of PLA-L implanted into rat tibia. (a) At 2 weeks, formation of immature bone is seen among the PLA-L granules (✳). Foreign body giant cells (arrowheads) phagocytose the PLA-L granules) (× 25). (b) At 4 weeks, the new bone increases in maturity (× 25). (c) Numbers of macrophages (✳) infiltrate among the PLA-L granules (× 50). (d) At 16 weeks, the cavity is occupied by a granulation tissue with diffuse infiltration of macrophages and a fatty tissue. The new bone disappears (× 25). (e) At 24 weeks, the PLA-L granules are completely absorbed and replaced by a bone marrow tissue with fatty tissue (× 10).

maturity. The phagocytic reaction to the PLA was continuously seen. The amount of the granules decreased a little (Fig. 1e). At 16 weeks, most of the PLA-H granules were surrounded by mature bone tissue. Foreign body giant cells were still observed on the granules, but the phagocytic reaction to PLA-H was reduced. The amount of PLA-H decreased apparently, as compared with that at 4 weeks (Fig. 1f). At 24 weeks, the granules were completely absorbed and replaced by bone marrow tissue with fatty tissue. Corresponding to the absorption, new bone trabeculae remodelled and disappeared (Fig. 1g).

3.1.2. Tissue reaction to PLA-L

Early tissue reactions to PLA-L were essentially the same as those to PLA-H. Briefly, granulation tissue with immature bone was observed at 2 weeks (Fig. 2a), and the new bone increased in maturity at 4 weeks. The new bone tissue was equal in amount to that formed around the PLA-H granules (Fig. 2b). However, the phagocytic reaction, especially infiltration of macrophages, was more prominent in PLA-L than in PLA-H (Fig. 2c). From 8 weeks on, absorption of the granules was apparent. At 16 weeks, the granules considerably decreased in amount and the new bone remodelled and disappeared (Fig. 2d). At 24 weeks the PLA-L granules were completely absorbed and replaced by bone marrow tissue with fatty tissue (Fig. 2e).

3.1.3. Tissue reaction to HAP

At 1 week, granulation tissue with slight infiltration of lymphocytes proliferated among the granules through the cavity (Fig. 3a). Formation of small amount of new bone was observed at the periphery of the cavity (Fig. 3b). At 2 weeks, new bone tissue formed actively among the HAP granules. Occasionally foreign body giant cells were seen, but the phagocytic reaction to HAP was less than to either PLA. At 4 weeks, the HAP granules were firmly surrounded by bone tissue. The amount of new bone was more than that with either PLA. A small amount of hematopoietic marrow tissue was seen in the bone. Phagocytic reaction to the HAP granules was not seen (Fig. 3c). Thence, the new

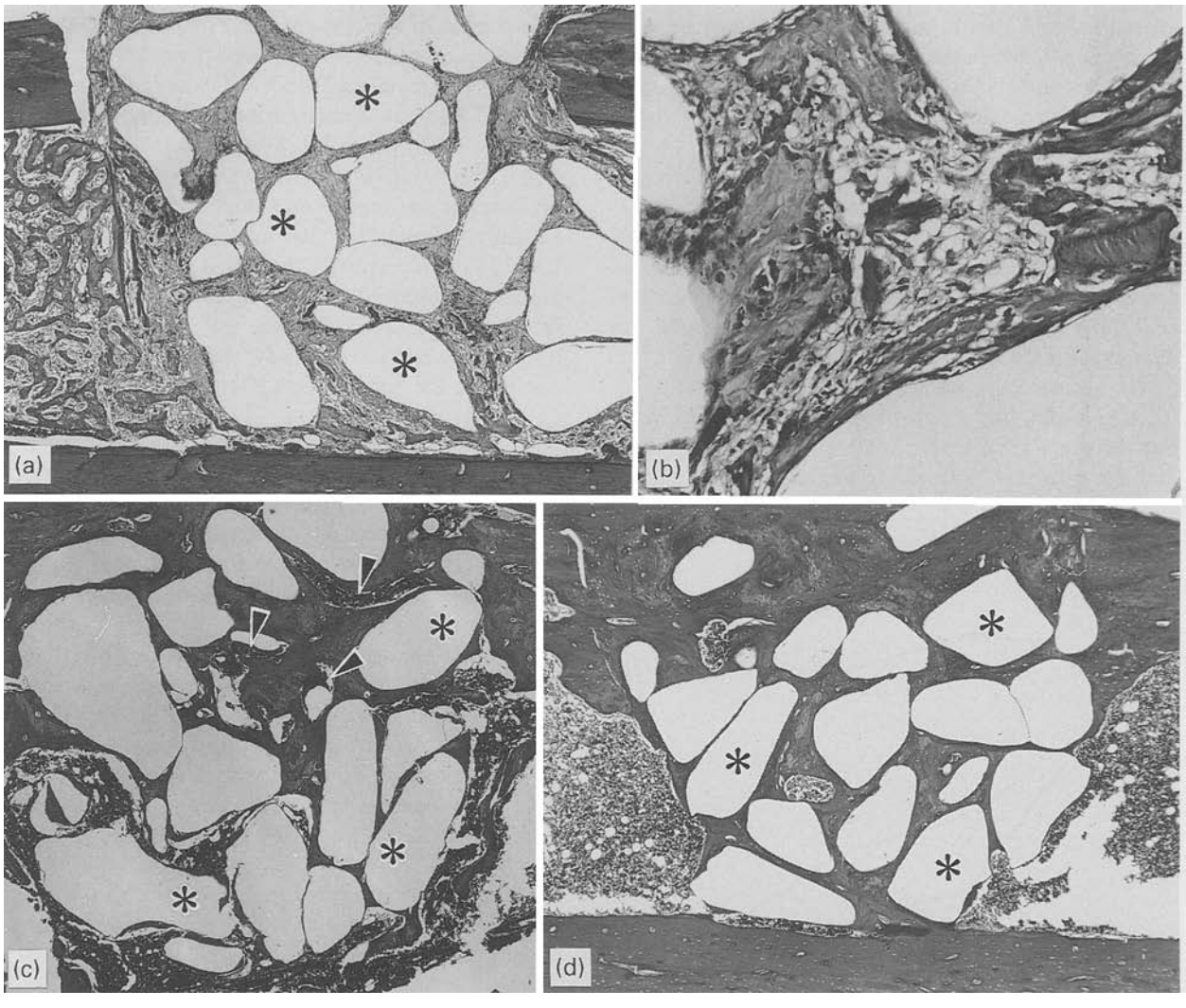

Figure 3 Histological findings of HAP implanted into rat tibia. (a) At 1 week, granulation tissue proliferates among the HAP granules (∗) through the cavity (× 10). (b) Formation of small amount of new bone is observed at the periphery of the cavity (× 25). (c) At 4 weeks, the HAP granules (∗) are firmly surrounded by bone tissue. A small amount of hematopoietic marrow tissue is seen in the bone (arrowheads) (× 10). (d) At 24 weeks, the HAP granules (∗) are surrounded by a mature bone tissue. The HAP granules are not absorbed (× 10).

bone increased in maturity. At 24 weeks, the HAP granules were surrounded by mature bone tissue. The amount of HAP granules did not change throughout the experiment (Fig. 3d).

3.2. Histomorphometrical analysis (Fig. 4)

The remaining amount of both PLAs gradually decreased and PLAs were completely absorbed at 24 weeks. The absorption rate of PLA-L was higher than that of PLA-H. No obvious change of the remaining amount of HAP could be observed throughout the experiment.

4. Discussion

HAP is known as a bioactive material which can bind with bone tissue chemically [13] and has been widely accepted as a useful biomaterial [14]. In this study, the good osseous tissue compatibility of HAP was substantiated as indicated by formation of mature bone tissue surrounding the HAP granules and minimal inflammatory reaction. On the other hand, HAP was not absorbed and still remained at 24 weeks. An ideal biomaterial for use in bone fracture fixation or bone defect filling should be rigid, non-inflammatory, non-allergenic, and should remain until sufficient healing has occurred. When this stage is reached, the material should be removed and replaced by the proper tissues, otherwise it remains as foreign material in the body [15].

In this study, we histologically examined the compatibility of PLA in bone tissue. Formation of immature bone was observed around the PLA granules at 2 weeks. At 4 weeks, the new bone tissue increased in amount and became mature. Especially, in the case of PLA-H, a vast amount of mature lamellated bone was seen around the PLA granules at 16 weeks. The bone tissue occasionally showed direct contact with the PLA granules. Although there was phagocytic reaction to the PLAs, severe infiltration of neutrophiles and lymphocytes was not observed at any experimental period of the present study. Moreover, the PLAs were gradually absorbed and replaced by bone and bone marrow tissue. These findings indicate that PLA is acceptable as a useful bioabsorbable material in the skeletal site.

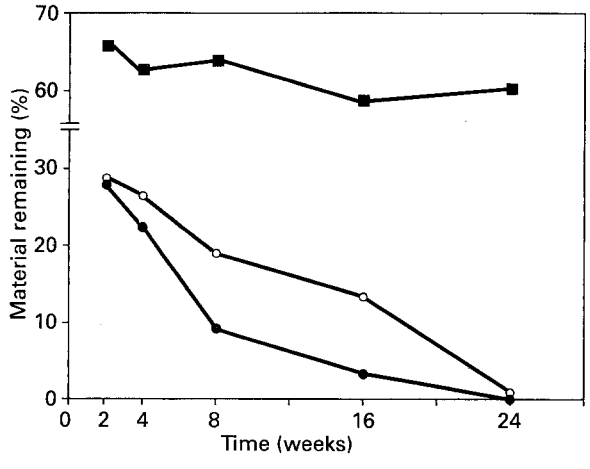

Figure 4 The remaining amount of the materials. The PLAs gradually disappear and are completely absorbed at 24 weeks. PLA-L shows quicker absorption than PLA-H. HAP is not absorbed and remains at 24 weeks (○ PLA-H; ● PLA-L; ■ HAP).

From the comparison of tissue reactions by the two kinds of PLA, it was shown that more severe phagocytic reaction accompanied by diffuse infiltration of macrophages was seen in PLA-L than in PLA-H. This seems to indicate that tissue reactions to the PLAs differ, depending on their molecular weight. PLA-L, a low molecular weight polymer, easily underwent liquefaction by tissue fluid and showed quick degradation after the implantation. Therefore, we speculate that PLA-L caused acute phagocytic reaction with diffuse infiltration of macrophages. On the other hand, because PLA-H maintained crystallization and did not degrade in the early periods, an encapsulation of PLA-H by the newly formed bone was observed in the long term.

From the results of this study, PLA which shows slower degradation seems to have a better tissue compatibility in bone tissue. The degradation rate of PLA is thought to depend on its molecular weight, size of granule, and shape [9]. These factors should be controlled in clinical use if PLA is to be an acceptable biomaterial in the skeletal site.

Studies on the use of PLA as a drug delivery system have been carried out [6–8]. We examined the possibility of PLA as a carrier of bone morphogenetic protein (BMP) which is a bone inducing protein [16]. It is known that BMP needs an appropriate carrier, because the amount of new bone induced by BMP is strongly affected by the substance combined with BMP [17]. We showed that PLA did not inhibit the inductive potential of demineralized bone in which BMP was included. Taking into consideration the results obtained in this study, the use of a BMP/PLA composite for bone defects is a good possibility.

5. Conclusions

Two kinds of PLA with different molecular weight were implanted into the cavity prepared on rat tibiae and the osseous tissue compatibility of PLA was examined histologically. The following observations were made.

1. New bone was formed around the PLAs.
2. There was no severe inflammatory reaction to the PLAs at any time period of the experiment.
3. The PLAs were gradually absorbed and replaced by bone and bone marrow tissue.
4. The inflammatory reactions and the absorption rate were influenced by the molecular weight of PLA.

These observations strongly indicate that PLA is acceptable as a useful bioabsorbable material in the skeletal site.

References

1. S.-H. HYON, K. JAMSHIDI and Y. IKADA, in "Polymer as biomaterials", edited by S. W. Shalaby, A. S. Hoffman, B. D. Ratner and T. A. Horbett (Plenum, New York, 1984) p. 51.

2. R. K. KULKARNI, K. C. PANI, C. NEUMAN and F. LEONARD, *Arch. Surg.* **93** (1966) 839.
3. D. E. CUTRIGHT and E. E. HUNSUCK, *Oral Surg.* **33** (1972) 28.
4. J. ARAGONA, J. R. PARSONS, H. ALEXANDER and A. B. WEISS, *Clin. Orthop.* **160** (1981) 268.
5. L. AUDELL, S. BOWALD, C. BUSCH and I. ERIKSSON, *Acta Chir. Scand.* **146** (1980) 97.
6. Y. IKADA, S.-H. HYON, K. JAMSHIDI, S. HIGASHI, T. YAMAMURO, Y. KATUTANI and T. KITSUGI, *J. Controlled Release* **2** (1985) 179.
7. C. G. PITT, A. R. JEFFCOAT, R. A. ZWEIDINGER and A. SCHINDLER, *J. Biomed. Mater. Res.* **13** (1979) 497.
8. A. D. SCHWOPE, D. L. WISE and J. F. HOWES, *Life Sci.* **17** (1975) 1877.
9. D. E. CUTRIGHT, B. PEREZ, J. D. BEASLEY, W. J. LARSON and W. R. POSEY, *Oral Surg.* **37** (1974) 142.
0. D. E. CUTRIGHT, E. E. HUNSUCK and J. D. BEASLEY, *J. Oral Surgery* **29** (1971) 393.
11. H. DARDIK, I. DARDIK, A. KATZ, R. B. SMITH, B. H. SCHWIBNER and H. LAUFMAN, *Surgery* **68** (1970) 1112.
12. D. E. CUTRIGHT, J. D. BEASLEY and B. PEREZ, *Oral Surg.* **32** (1971) 165.
13. M. JARCHO and C. H. BOLEN, *J. Mater. Sci.* **11** (1976) 2027.
14. P. N. GALGUT, I. M. WAITE, J. D. BROOKSHAW and C. P. KINGSTON, *J. Clin. Periodontol.* **19** (1992) 570.
15. J. M. BRADY, D. E. CUTRIGHT, R. A. MILLER and G. C. BATTISTONE, *J. Biomed. Mater. Res.* **7** (1973) 155.
16. H. SAITOH, T. TAKATA, H. NIKAI, H. SHINTANI, S.-H. HYON and Y. IKADA, *J. Oral Rehabil.* (1993, in press)
17. K. BESSHO, N. TANAKA, J. MATSUMOTO, T. TAGAWA and M. MURATA, *J. Dent. Res.* **70** (1991) 171.

Received 23 June
and accepted 14 September 1993