SHORT COMMUNICATION

Histological and histometric observations of rat jaw bone after implantation of synthetic hydroxyapatite

Nobuyoshi Takeshita, Masahisa Inoue, Tetsundo Nojima,
Osamu Ishida, Masako Yamasaki, Yasumasa Miyake
and Yoshinori Nagai

*Department of Oral Pathology, Okayama University Dental School
2-5-1 Shikata-Cho, Okayama 700, Japan
(Chief: Prof. Noriyuki Nagai)*

[Accepted for publication : January 14, 1991]

Key words : Synthetic hydroxyapatite / dissolution / Biomaterial / rat jaw bone / histometry

Introduction

Synthetic hydroxyapatite (HAP) is known as an excellent biomaterial for bone defects. It is also established that HAP implanted into the osseous tissue shows osteoconductivity which means bone growth-guiding properties, and is completely embedded in the newly formed osseous tissue^{1,2)}. After the synthesis HAP is sintered at temperatures in the range of from 200 to 1300 °C to produce a dense material. Sakae *et al.*³⁾ reported that the crystallinity of HAP was relatively related to the sintering temperature. There are however, few reports about the fate of HAP sintered at several temperatures after the intraosseous implantation.

In order to study the fate of HAP in long-term intraosseous implantation, the present study reports histological and histometric observations of the rat jaw bone after the implantation of HAP sintered at 200, 900 and 1250 °C.

Materials and Methods

HAP used in the present study was synthesized by a wet method, and was dried or

sintered at 200, 900 and 1250 °C (Taihei Kagaku Sangyo Co. Ltd., Osaka). Then, each HAP was pulverized and sieved to a granule size of 0.3-0.5 mm. The series of HAP were designated HAP200, HAP900 and HAP1250.

Forty-eight male Wistar rats (body weight, about 200 g) were anesthetized with the intraperitoneal injection of pentobarbital sodium. After an incision along the lower border of the bilateral mandibles and retracting the overlying skin, masseteric muscle, and periosteum, the outer surface of the jaw bone was exposed. By using a slowly rotating dental round bur mounted in a dental handpiece, bone cavities, 1×2×2 mm were prepared bilaterally in the molar apical region of the mandibles. HAP was implanted densely in to these cavities just after they had been rinsed with physiological saline, and then the skin was closed with nylon sutures. At 4, 6, 8, and 16 weeks after the experiment the animals were anesthetized and killed by an intracardial perfusion with 2 % glutaraldehyde-2 % paraformaldehyde solution in 0.1 M phosphate buffer, pH 7.4. The right and left mandibles were dissected out and were demin-

Fig. 1 Photomicrographs of rat mandibles 16 weeks after implantation of HAP 200 (a), 900 (b), and 1250 (c). H-E, $\times 150$.

neralized with 10 % EDTA, pH 7.4 for 2-3 weeks.

For the light microscope, tissue blocks were dehydrated through a graded series of ethanols and then embedded in paraffin by routine methods. Then, serial sections, $4\ \mu\text{m}$ in thickness were stained with hematoxylin-eosin. For the histometric study, photomicrographs

with a final magnification of $200\times$ were printed in black-white from 50 random fields from each of the observation periods. A 42-point test grid (Weibel *et al.*⁴⁾) was superimposed on the photomicrograph to designate a total unit area of $1.0\ \text{mm}^2$ at $200\times$ magnification. The volume percentage HAP, osseous tissue and other tissue (fibrous tissue, bone marrow) was determined by tallying the point-hits on these components separately for each observation period of HAP200, 900 and 1250. Then, the mean of each volume percentage was registered.

Results and Discussion

With the light microscope, HAP200, 900 and 1250 was already embedded in newly formed mature osseous tissue at observation periods ranging from 4 to 16 weeks. The interface between HAP1250 and newly formed osseous tissue was distinctly observed, but the interface between both HAP200 and HAP 900, and newly formed osseous tissue was not well-defined throughout the observation periods. Moreover, at 16 weeks after the intraosseous implantation HAP200 granules were irregular in size as compared with those at 4 weeks. Granules of both HAP 900 and 1250 showed, however, little change in size 4 to 16 weeks after the intraosseous implantation (Fig. 1 a, b, c).

Histometric results are presented in Table 1. When compared with HAP900 and HAP 1250, per cent value for HAP200 in the unit area markedly decreased at 16 weeks more than that for HAP200 at 4-8 weeks, and also the per cent value for the osseous tissue embedding HAP200 increased. Changes of per cent value for HAP900 in the unit area resembled that for HAP1250: percent value for both HAP900 and 1250 showed a slight decrease from 4 weeks to 16 weeks after the intraosseous implantation.

The present results that HAP200 implanted in the rat jaw bone was partially replaced by the newly formed osseous tissue in a long-term period, but HAP900 and 1250 was not. This could show that the dissolution of HAP 200 had occurred in the long-term intraosseous implantation. The dissolution of some synthetic apatites has also been reported⁵⁻⁷⁾.

Table 1 Histometric differences between rat jaw bones implanted HAP200, 900, and 1250

	HAP200			HAP900			HAP1250		
	HAP	Bone	others	HAP	Bone	others	HAP	Bone	others
4W	57.1	25.4	17.5	55.0	19.5	25.5	52.1	24.5	23.4
6W	55.1	28.5	16.4	52.1	23.8	24.1	50.3	27.7	22.0
8W	52.3	31.6	16.1	47.4	31.2	21.4	49.9	30.6	19.5
16W	21.4	55.6	23.0	41.5	39.9	18.6	44.1	38.1	17.8

(% : unit area = 1.0 mm²)

Sakae *et al.*³⁾ using the same series of HAP as this study reported that although HAP 200 showed a typical peak of hydroxyapatite by X-ray powder diffraction, HAP crystals prepared at low temperature had some structural deficiencies such as lattice defects. Such crystal defects resulting from the sintering processes affect its *in vivo* dissolution of HAP⁷⁾.

This study also suggests that a dissolution of HAP implanted in the osseous tissue is caused by a low degree of HAP crystallinity. The present study did not show, however, how newly formed osseous tissue replaced degraded HAP200. Further studies of this point are necessary before firm conclusions can be drawn.

References

- 1) Nagai, N., Takeshita, N., Shirasuga, H. and Akiba, M.: Histopathological studies of implantation of hydroxyapatite in the rat jaw bone. Jpn J Oral Maxillofac Surg. **31** : 1352-1361, 1985. (in Japanese).
- 2) Jarcho, M.: Biomaterial aspects of calcium phosphates. Properties and applications. Dent Clin North Am. **30** : 25-47, 1986.
- 3) Sakae, T., Davies, J. E., Frank, R. M. and Nagai, N.: Crystallographic properties of a series of synthetic hydroxyapatite. J Nihon Univ Sch Dent. **31** : 458-463, 1989.
- 4) Weibel, E. R., Kistler, G. S. and Scherle, W. F.: Practical stereological methods for morphometric cytology. J Cell Biol. **30** : 23-38, 1966.
- 5) Arends, J. and Jongebloed, W. L.: Dislocations and dissolution in apatites. Theoretical considerations. Caries Res. **11** : 186-189, 1977.
- 6) Jongebloed, W. L., Berg, P. J. and Arends, J.: The dissolution of single crystals of hydroxyapatite in citric and lactic acid. Calcif Tissue Res. **15** : 1-9, 1974.
- 7) Daculsi, G., LeGeros, R. Z. and Mitre, D.: Crystal dissolution of biological and ceramic apatites. Calcif Tissue Int. **45** : 95-103, 1989.