Histological reaction of auditory bulla bone to synthetic auditory ossicle (Apaceram®) in rats

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Abstract : To investigate the biocompatibility of a synthetic auditory ossicle to host bone, small thin Apaceram® disks composed of dense hydroxyapatite were implanted under the periosteum of the left auditory bulla in 32 rats for periods ranging from 1 day to 270 days. A sham operation performed on 10 rats served as a control. Decalcified histological sections stained with hematoxylin and eosin were observed using light microscopy. The experiment showed : 1) a time-dependent mature fibrous connective tissue surrounding the Apaceram® disk, 2) no evidence of inflammatory reaction caused by the implant from 90 days after implantation until the end of the experiment, 3) no evidence of osteolysis by osteoclasts caused by the implant, and 4) direct contact of bone to the implant on the bone-disk interface at 180 and 270 days after implantation. The findings suggest that Apaceram® has a high degree of implant biocompatibility, making it a satisfactory substitute biomaterial for otological reconstructive surgeries. J. Med. Invest. 47 : 56-60, 2000

Key words **:** *hydroxyapatite ; auditory bulla bone ; implant ; biocompatibility ; rats*

INTRODUCTION

In the early 1960s, otologists experimented with inert materials to reconstruct the auditory ossicular chain (1). Various biomaterials are presently used in clinical practice (2, 3), including hydroxyapatite (HA), the main constituent of the mineral matrix of bone. HA is relatively new, but widely applied as a substitute biomaterial because of its biocompatibility and mechanical and chemical properties (2, 4, 5).

New applications of biomaterials require testing to ensure safety and efficacy. Testing needs to be designed for specific implant requirements and varies widely according to implant type (2). Our previous studies showed histological reactions to HA in the subcutaneous tissue and in the mucosa of the middle ear of rats (6, 7). However, histopathological changes on the HA-bone interface have received limited attention. The present study concerned the interaction between the implant material and host bone in the auditory bulla of rats for various periods of implantation between 1 day and 270 days.

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MATERIALS AND METHODS

Implant material

Dense disks (diameter, 3mm ; thickness, 1mm) of Apaceram®, shown by X-ray diffraction analysis to be 99.66% HA [$Ca_{10}(PO_4)_6(OH)_2$] and 0.34% CaO, were prepared from commercially available synthetic auditory ossicle (Asahi Optical, Tokyo). Before implantation, the disks were sterilized in an autoclave at 121℃ for 30 minutes.

Surgical technique

All experimental animal procedures were approved and monitored by the Institute of Animal Care and Use Committee of the University of Tokushima, and performed according to the institutional guidelines on the care and use of laboratory animals.

A total of 42 eight-week-old specific pathogen free female Wistar rats weighing 130-150 grams were used. Thirty-two rats underwent surgical procedures as an implant group and 10 rats underwent sham operations as a control group. Surgery was carried out under general anesthesia using diethyl ether in sterile conditions.

An incision approximately 7 mm long was made 3-4 mm behind the postero-superior left auricle of each rat. The auditory bulla was exposed and its periosteum was separated to expose the bony surface. This bony surface was lightly scratched using a small blunt blade to induce an injury similar to injury in otological reconstructive surgery. Scratching was minimized to avoid fractures of thin bulla bone. The Apaceram® disk was implanted under the periosteum of the auditory bulla, directly contacting the scratched bony surface (Fig. 1). Then, the wound was closed with one or two stitches.

Four rats from the implant group and two rats from the control group were sacrificed at 1, 3, 7, 14 and 30 days after surgery. The remaining animals (all from the implant group) were sacrificed in groups of four at 90, 180 and 270 days after implantation. Rats were quickly sacrificed using diethyl ether under general anesthesia and were then decapitated.

Prepared sections and staining

The heads of the rats were immediately immersed in 10% phosphate-buffered formalin for three days. Then, the ear drum was punctured by a needle ($23G \times 1_{1/4}$) to allow decalcifying solution to irrigate the middle ear and the auditory bulla with the Apaceram® disk and the heads were sufficiently decalcified with Decalcifying Solution A, adjusted by the Plank Rychlo method (Wako Pure Chemical, Osaka). After three days of decalcification, the auditory bulla and surrounding implanted tissue were dissected and dehydrated in an ethanol series, embedded in paraffin and cut into 6 μm thick sections. For each specimen, 10 to 15 sections were made and stained with haematoxylin and eosin (H & E).

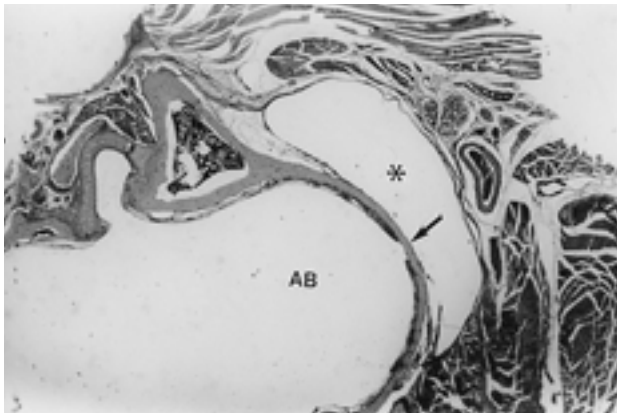

Fig. 1. Specimen from implant group at 24 hours showing : Apaceram® disk space : *, auditory bulla cavity : AB, and auditory bulla bone : arrow (H & E ; original magnification×5).

Observation method

For each specimen, five randomly chosen sections of the auditory bulla and soft tissue surrounding the implants were observed and photographed (color slide) under a light microscope. Color slides (original magnification×200) of cell distribution around implants were enlarged by projecting slides on a screen to identify and count the different cells. Between 180 and 240 cells were counted for each specimen and percentages of the various component cells were calculated.

RESULTS

Table 1 shows the average percentage of component cells in tissue surrounding the implanted Apaceram® disk.

1. Specimens examined within 30 days after surgery

1-1. Implant group

Sections from specimens removed one day after implantation showed an acute inflammatory response around the implants (Fig. 2). Cell counts revealed a predominance of neutrophils (81.2%), followed by macrophages (14.4%) and lymphocytes (3.5%).

At 3 days after implantation, all specimens showed a sharp decrease in neutrophils (15.9%), whereas macrophages had increased significantly (66.2%) and lymphocytes had increased slightly (5.9%). In addition, both fibroblasts and fibrocytes began to appear [fibroblasts (7.9%) ; fibrocytes (2.4%)].

At 7 days after implantation, specimens showed mild inflammatory response around the Apaceram® disks. Between the implant surfaces and soft tissue and between the implant surfaces and the bulla

Table 1. Average percentage of component cells in tissue surrounding implanted Apaceram® disk.

	1 day		3 days		7 days		14 days		30 days		90 days	180 days	270 days
	I	C	I	C	I	C	I	C	I	C	I	I	I
Neutrophils	81.2	80.6	15.9	11.7	0	0	0	0	0	0	0	0	0
Macrophages	14.4	15.0	66.2	58.5	19.0	0	2.7	0	1.6	0	0	0	0
FBGCs	0	0	0.9	1.6	1.6	0	0	0	0	0	0	0	0
Lymphocytes	3.5	3.5	5.9	5.2	2.2	0	1.2	0	1.3	0	0	0	0
Fibroblasts	0	0	7.9	16.7	58.9	68.6	53.5	56.1	39.1	35.7	23.4	20.7	10.3
Fibrocytes	0	0	2.4	4.7	17.0	27.3	41.5	41.9	57.1	62.5	75.0	78.2	88.4
Unidentified cells	0.9	0.9	0.8	1.6	1.3	4.1	1.1	2.0	0.9	1.8	1.6	1.1	1.3

FBGCs : foreign body giant cells
I : implant group (n=4) C : control group (n=2)

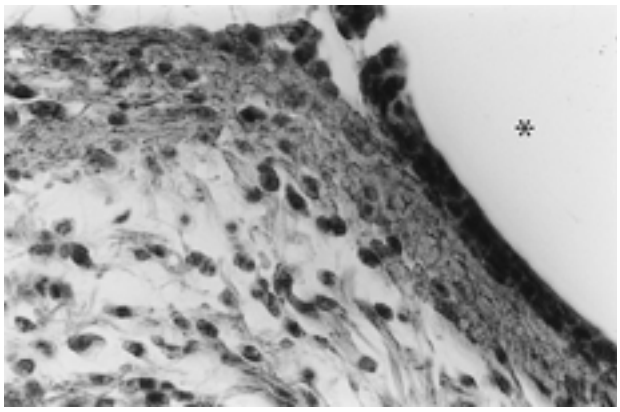

Fig. 2. Specimen from implant group at 24 hours showing an acute inflammatory reaction, evidenced by infiltration of neutrophils and macrophages. * : Apaceram® disk space (H & E ; original magnification×200).

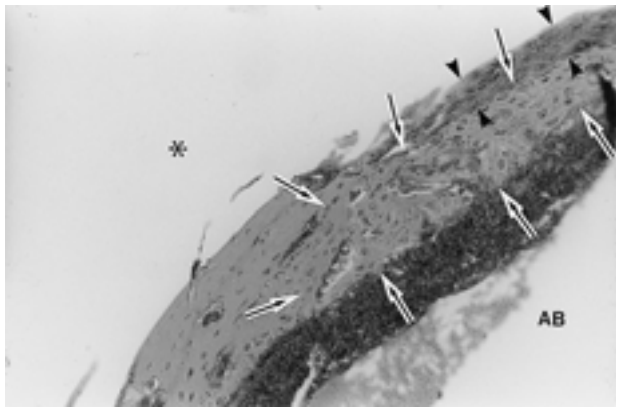

Fig. 3. Specimen from implant group at 14 days showing new bone formation (area outlined by arrows) and a fibrous connective tissue layer (area outlined by arrow heads) at the bone-implant interface. * : Apaceram® disk space. AB : auditory bulla cavity (H & E ; original magnification×50).

bone there was fibrovascular tissue containing fibroblasts (58.9%), fibrocytes (17.0%), macrophages (19.0%) and a few foreign-body giant cells (1.6%). A small amount of new bone formation was observed on the bone surface where the periosteum had been elevated. Osteoclasts were not observed

At 14 days after implantation, implants were surrounded by a thin layer of fibroblasts (53.5%) and fibrocytes (41.5%), while macrophages remained at a low level (2.7%) and there were few lymphocytes (1.2%). On the bone-implant interface new bone formation was observed, evidenced by a number of osteoblasts (Fig. 3). Osteoclasts were not observed.

At 30 days after implantation, specimens showed fibrovascular tissue consisting of collagen fibers with fibroblasts (39.1%) and fibrocytes (57.1%). This fibrovascular tissue was more mature than that found at two weeks. Small numbers of macrophages (1.6%) and lymphocytes (1.3%) surrounded the implants. On the bone-implant interface, newly formed bone was more mature than that found at two weeks (Fig. 4). Osteoclasts were not observed.

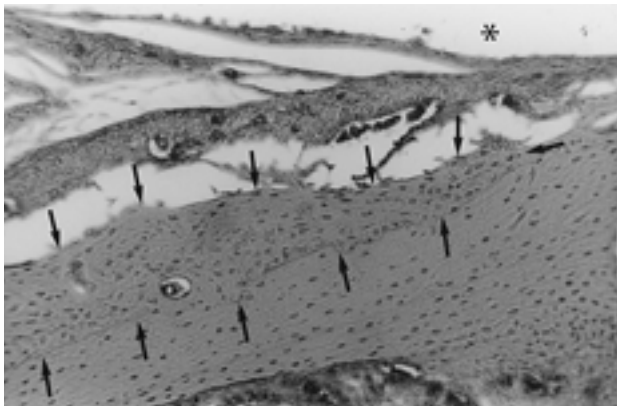

Fig. 4. Specimen from implant group at 30 days showing the maturity of newly formed bone (area outlined by arrows). * : Apaceram® disk space (H & E ; original magnification×50).

1-2. Control group

Changes in inflammatory reaction were generally similar to that in the implant group, but inflammatory cells had completely disappeared at 7 days

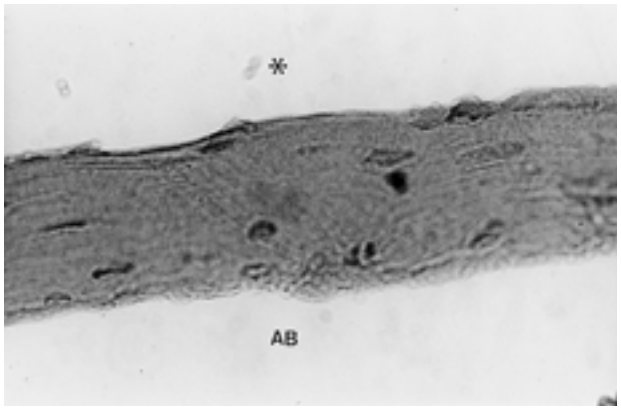

Fig. 5. Specimen from implant group at 270 days showing the direct contact of bone to the implant and the normal bone structure at the bone-implant interface. * : Apaceram® disk space. AB : auditory bulla cavity (H & E ; original magnification×200).

after surgery. Fibroblasts and fibrocytes increased after 3 days. From 14 days to 30 days, fibroblasts decreased, but fibrocytes increased. At 7 days after surgery, a small amount of new bone formation was observed on the bone surface where the periosteum had been elevated. However, at 14 days, there was no evidence of new bone fomation. Osteoclasts were not observed.

2. From 90 days to the end of implantation

From 90 days after implantation, on the soft tissue-implant interface, fibrovascular tissue became dense. Fibroblats decreased gradually. Fibrocytes increased gradually, reaching a maximum at 270 days. Almost all macrophages and lymphocytes disappeared.

From 180 days to the end of implantation, on the bone-implant interface, there was direct contact of bone to implant and the bony structure in this area was similar to the bony structure in other areas of the bulla (Fig. 5).

DISCUSSION

Animal experiments provide valuable information concerning implant biocompatibility (8), which has recently been defined as" the ability of a material to perform with an appropriate host response in a specific application "(9). The present study investigated implant biocompatibility of Apaceram® in the auditory bulla of rats and the host response to Apaceram® was the reaction of bone and soft-collagenous tissue to the implants.

Reaction of soft collagenous tissue surrounding the implant

From 1 to 3 days after surgery, the inflammatory response was slightly higher in the implant group than in the control group. But the number of neutrophils in the implant group did not differ significantly from the number of neutrophils in the control group, suggesting that the effects of implantation were consistent with normal surgical intervention (10).

At 7 days after surgery, changes in inflammatory reaction in the control group indicated normal recovery from tissue injury. The implant group showed similar changes and mild inflammatory reaction suggested that the soft tissue had adapted well to the implant in a short period of time.

From 7 days to 30 days after surgery, the implant group showed a gradual decrease in macrophages, suggesting their important role in the fibroproliferative tissue response to injury (11, 12). Gradual maturity of fibrovascular tissue and a continuous low level of lymphocytes indicated that normal wound healing was mildly disturbed by the implant. The control group showed no evidence of inflammatory reaction from 7 to 30 days after surgery when observation was ended.

Regarding the fibrosis stage (from 30 days after surgery), implant stability depends largely on the reaction at the material-tissue interface, where each component of the fibrovascular proliferation is required for successful wound healing and tissue remodeling (13). Fibrosis is an important criterion in evaluating the biocompatibility of artificial materials (6, 8, 12, 14). The present study indicated the satisfactory implantation of Apaceram® due to the appearance of time-dependent mature fibrous connective tissue and no evidence of inflammatory reaction from 90 days after implantation until the end of the experiment.

Reaction at the bone-implant interface

At 7 days after surgery, in both the implant and control groups, a small amount of new bone formation appeared on the bone surface where the periosteum had been elevated, suggesting normal healing of bone tissue from the scratched lesion.

At 14 days after surgery, the control group showed no evidence of new bone formation. In the implant group, at the bone-implant interface, new bone formation was observed suggesting that Apaceram® caused an increase in the underlying bone tissue. However, from 30 days to the end of the experi-

ment, at the bone-implant interface, there was no evidence of continuous new bone formation and the maturity of newly formed bone found at 2 weeks suggested that Apaceram® only caused a slight increase in the underlying bone tissue during the first 2 weeks of implantation.

During implantation, there was no evidence of osteolysis by osteoclasts caused by the implant confirming the implant biocompatibility. Furthermore, our study indicated the high degree of implant biocompatibility of Apaceram® with host bone because there was direct contact of bone to implant at 180 and 270 days after implantation, and the bony structure in this area was similar to the bony structure in other areas of the bulla.

With respect to histological evaluation of the decalcified sections from the implant-bone interface, the present study suggests that Apaceram® is highly biocompatible with host bone. Combined with findings from previous studies of response to dense hydroxyapatite granules and porous hydroxyapatite in the mucosa of the middle ear of rats (7, 8), there is strong evidence that hydroxyapatite in general, or Apaceram® in particular, is a satisfactory biomaterial for application in reconstructive surgeries in the middle ear.

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REFERENCES

1. Wehrs RE : Hydroxyapatite implants for otologic surgery. Otolaryngol Clin North Am 28 : 273-286, 1995
2. DeMane CQ : The development of implant and implantable materials. Otolaryngol Clin North Am 28 : 225-234, 1995
3. Saitoh A, Tsuda Y, Bhutto IA, Kitaoka T, Amemiya T : Histologic study of living response to artificially synthesized hydroxyapatite implant : 1-year follow-up. Plast Reconstr Surg 98 : 706-710, 1996
4. Friedman CD, Costantino PD, Jones K, Chow LC, Pelzer HJ, Sisson GA : Hydroxyapatite cement, II. Obliteration and reconstruction of the cat frontal sinus. Arch Otolaryngol Head Neck Surg ; 117 : 385-389, 1991
5. Magliulo G, Ronzoni R, Vingolo GM, Cristofari P : Reconstruction of old radical cavities. Am J Otol 13 : 288-291, 1992
6. Cui P-C, Ohsaki K, Ii K, Tenshin S, Kawata T : Subcutaneous tissue reaction to synthetic auditory ossicle (Apaceram®) in rats. J Laryngol Otol 109 : 14-18, 1995
7. Ye Q, Ohsaki K, Ii K, Li D-J, Zhu C-S, Tenshin S, Takano-Yamamoto T : Histological reaction to hydroxyapatite in the middle ear of rats (submitted to Arch Otolaryngol Head Neck Surg [Chicago])
8. Grote JJ, van Bliterswijk CA, Kuijpers W : Hydroxyapatite ceramic as middle ear implant material : Animal experimental result. Ann Otol Rhinol Laryngol 95 (Suppl 123) : 1-5, 1986
9. Christel PS : Biocompatibility of surgical-grade dense polycrystalline alumina. Clin Orthop 282 : 10-18, 1992
10. van der Meulen J, Koerten HK : Inflammantory response and degradation of three types of calcium phosphate ceramic in a non-osseous environment. J Biomed Mater Res 28 : 1455-1463, 1994
11. Leibovich SJ. Ross R : A macrophage-dependent factor that stimulates the proliferation of fibroblast in vitro. Am J Pathol 84 : 501-513, 1976
12. Anderson JM, Miller KM : Biomaterial biocompatibility and the macrophage. Biomaterials 5 : 5-10, 1984
13. Rubin PAD, Nicaeus TE, Warner MA, Remulla HD : Effect of sucralfate and basic fibroblast growth factor on fibrovascular ingrowth into hydroxyapatite and porous polyethylene alloplastic implants using a novel rabbit model. Ophthal Plast Reconstr Surg 13 : 8-17, 1997
14. Therin M, Christel P, Meunier A : Analysis of the general features of the soft tissue response to some metals and ceramics using quantitative histomorphometry. J Biomed Mater Res 28 : 1267-1276, 1994