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Long-term observation of subcutaneous tissue reaction to synthetic auditory ossicle (Apaceram®) in rats

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Abstract

The present study evaluates histological characteristics of the soft tissue response to long-term implantation of Apaceram® discs composed of dense hydroxyapatite in rats. Discs were implanted into the subcutaneous tissue of 76 rats for six to 20 months. Decalcified histological sections stained with haematoxylin and eosin (H & E) and Mallory's azan were examined. Different cell types surrounding implants were counted. The greatest proportion of macrophages was found at six months (13.5 per cent). This proportion gradually decreased to four per cent at 20 months. Small numbers of lymphocytes and foreign body giant cells were observed in every group, but neither neutrophils nor osteogenesis were observed in any specimens. Results of the present study and previous related studies indicate that despite reappearance of a small number of macrophages six months after implantation, Apaceram® is useful for reconstructive surgery.

Key words: Hydroxyapatites; Rats

Introduction

Synthetic auditory ossicle (Apaceram®) composed of hydroxyapatite (HA) is currently used widely in reconstructive middle-ear surgery. HA is a material of bioactive and biodegradable ceramics (Daculsi, 1988), consisting largely of inorganic constituents of mature bone (Williams *et al.*, 1989) and is one of the most biocompatible materials available today (Grote, 1990; Goldenberg, 1992). Our previous study examined soft tissue response to thin Apaceram® discs implanted in rats for up to 10 months (Cui *et al.*, 1995). In that study, however, macrophages in the proportion of 18.9 per cent reappeared at six months after implantation and a few foreign body giant cells were observed at the Apaceram®-tissue interface at 10 months after implantation. To clarify this reappearance of a slight macrophage reaction at six months, we investigated long-term tissue reaction to Apaceram® from six months to 20 months after implantation. Thin Apaceram® discs were implanted subcutaneously in rats to study the histological reaction quantitatively by light microscopy.

Materials and methods

Implant material characteristics

Dense Apaceram® discs (diameter, 4 mm; thickness, 1 mm) were prepared from commercially available synthetic ossicle (Asahi Optical Co. Ltd., Tokyo), composed of HA (Ca_{10} $(PO_4)_6$ $(OH)_2$). Before implantation, discs were sterilized in an autoclave at 121 °C for 30 minutes.

Animals and surgical procedure

Apaceram® discs were implanted subcutaneously into the interscapular region of 76 eight-week-old female SPF Wistar rats. The rats were sacrificed at six, eight, 10, 12, 14, 16, 18 or 20 months after implantation. All surgical procedures were carried out under general anaesthesia (diethyl ether) in a sterile environment.

Section preparation and staining

The Apaceram® disc and surrounding tissue was removed as a single mass, and immediately immersed in 10 per cent phosphate-buffered formalin for three days. The mass was then decalcified for at least seven days using five per cent formic acid. Implants were dehydrated in an ethanol series, embedded in paraffin, and cut into 6-μm-thick sections. Sections were stained with haematoxylin and eosin (H & E stain) and Mallory's azan.

Observation methods

Slides of the cells surrounding the implants were photographed under light microscopy to determine the types and distribution of cells. The slides were

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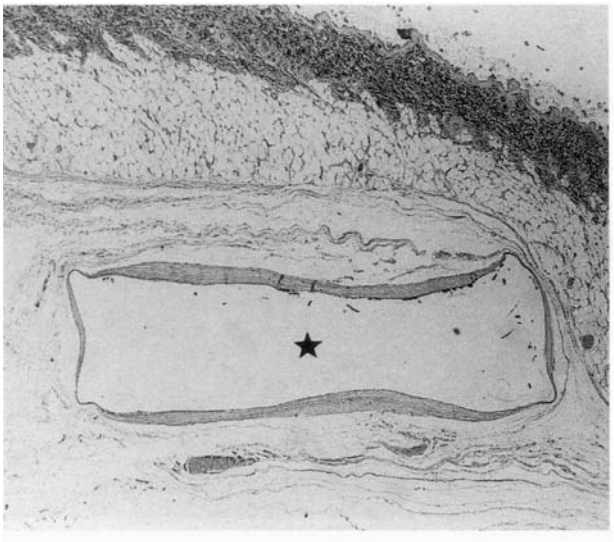

FIG. 1a

Low-power photomicrograph 6 months after implantation, showing fibrous capsules formed around Apaceram® disc (★). (H & E; × 15)

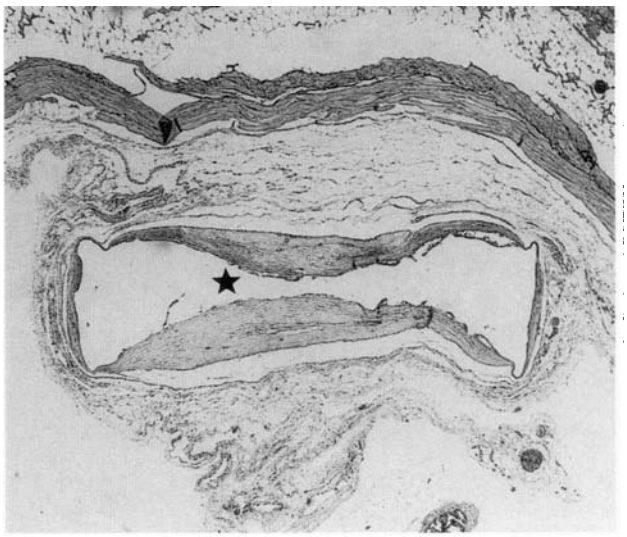

FIG. 1b

Low-power photomicrograph 20 months after implantation, showing fibrous capsules formed around Apaceram® disc (★). (H & E; × 15)

projected and the cells were counted and identified. The content of various component cells was calculated at the most representative regions of each section.

Results

Cell population surrounding Apaceram®-tissue interface

Sections from specimens removed between six and 20 months after implantation showed fibrous capsules surrounding the Apaceram® disc (Figure 1a, b). Foreign body giant cells (FBGCs), macrophages, lymphocytes and fibroblasts were observed at the interface between Apaceram® discs and tissue as well as in the surrounding area. Fibrocytes were observed in the outer layer of the fibrous capsule. Neutrophils were not seen, nor osteogenesis observed in any specimens. Table I lists the component cell proportions of sections.

The proportion of macrophages was highest (13.5 per cent) six months after implantation (Figure 2), decreasing to 10.8 per cent at eight months and four per cent at 20 months (Figure 3). The macrophage proportion decreased sharply from 13.5 per cent at six months to 6.4 per cent at 12 months after implantation, and then gradually stabilized (Figure 4a).

The percentage of FBGCs fluctuated little, on average 0.6 per cent. Lymphocytes comprised approximately 1.7 per cent (1.2 to 2.9 per cent) in all groups, regardless of elapsed time (Figure 4a). A small number of rather flat FBGCs were also observed at the Apaceram®-tissue interface of each group (Figure 5a, b).

Fibroblasts accounted for 30.4 per cent of the cell population at six months, decreasing to 18.1 per cent at 20 months. In contrast, the fibrocyte population increased from 51.4 per cent at six months to 73.8 per cent at 20 months (Figure 4b).

Fibrous capsule of Apaceram® disc

Figure 1 shows the fibrous capsules that surrounded Apaceram® discs after implantation. Such fibrous capsules were composed of macrophages, lymphocytes, FBGCs, fibroblasts, fibrocytes, and capillaries. The thickness of the fibrous capsules (FCT) tended to increase with time. The average FCT was calculated at the thickest part of both the upper and lower surfaces of the Apaceram® discs. After implantation, the FCT increased rapidly from six months (117.8 μm, n = 8) to 10 months (177.1 μm, n = 7) and then more slowly after 12 months, to a maximum (213.1 μm, n = 4) at 20 months. Mallory's azan staining revealed that the inner thin layer of the fibrous capsule was composed of macrophages, FBGCs and fibroblasts. A thick

TABLE I

CELL POPULATION BETWEEN 6 AND 20 MONTHS AFTER IMPLANTATION (UNIT: %)

	6 Months	8 Months	10 Months	12 Months	14 Months	16 Months	18 Months	20 Months
Neutrophils	0	0	0	0	0	0	0	0
Macrophages	13.5	10.8	8.1	6.4	7.0	4.7	4.3	4.0
Lymphocytes	2.9	1.5	1.2	1.4	1.5	1.6	1.6	1.9
FBGCs	0.5	0.6	0.8	0.5	0.7	0.6	0.8	0.6
Fibroblasts	30.4	27.4	22.5	20.1	18.9	18.3	18.4	18.1
Fibrocytes	51.4	57.6	65.1	69.9	70.3	72.6	73.3	73.8
Unidentified	1.3	2.1	2.3	1.7	1.6	2.2	1.6	1.5

FBGCs: foreign body giant cells. Sample size for each group is n = 10; except 20-month group which was n = 4.

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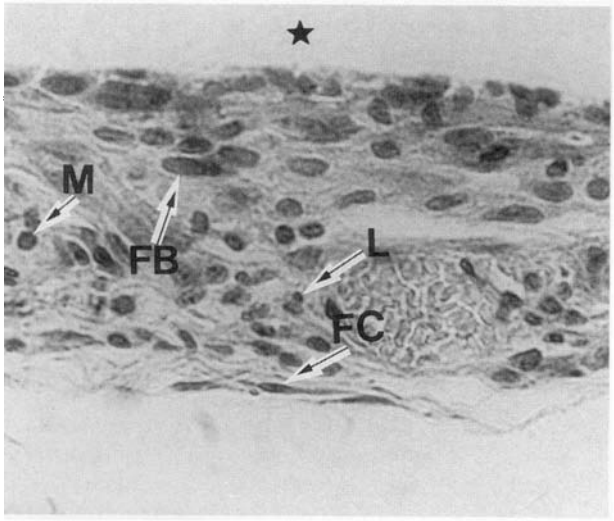

FIG. 2
Six months after implantation. Macrophages (M), lymphocytes (L), fibroblasts (FB) and fibrocytes (FC). ★: Apaceram® disc. (H & E; × 600)

layer of fibrocytes was also found in all specimens (Figure 6).

Discussion

Macrophage reaction six months and longer after implantation

The present study investigated chronic tissue reaction to a synthetic auditory ossicle (Apaceram®) with respect to the biocompatibility of dense HA as a middle ear implant in rats. We previously examined rats for tissue reaction to Apaceram® discs from one day to 10 months after implantation. We found that macrophages disappeared one month after implantation and reappeared (18.9 per cent, n = 4) at six months after implantation. Macrophage levels decreased to 8.5 per cent (n = 5) at 10 months after implantation (Cui *et al.*, 1995). In the present study, macrophages (13.5 per cent, n = 10) were

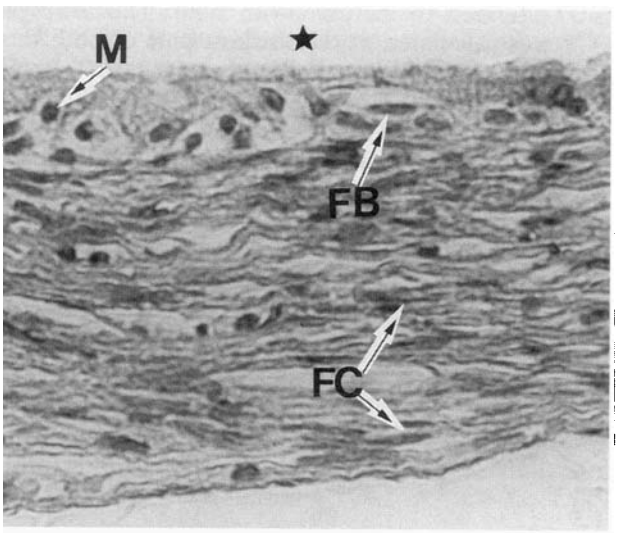

FIG. 3
Twenty months after implantation. Decrease in macrophage and fibroblast populations, and increase in fibrocyte population. ★: Apaceram® disc. (H & E; × 600)

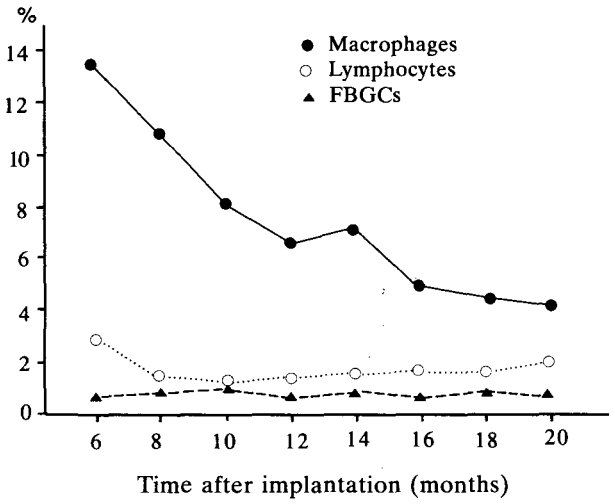

FIG. 4a
Changes in population of inflammatory cells after implantation.

present at six months and longer after implantation. But the macrophage level gradually decreased to 10.8 per cent at eight months and to four per cent at 20 months after implantation (see Table I).

One possible explanation for an increased proportion of macrophages six months after implantation may be deposition of amorphous material adjacent to the interface of the capsule. This deposition may indicate biodegradation (Cui *et al.*, 1995), as many implant-derived particles in the cytoplasm of macrophages were observed by transmission electron microscopy between one week and one year after implantation (Grote *et al.*, 1986). Daculsi (1988) suggested that biodegradation is characterized by changes in the physiochemical properties of material after implantation. Biodegradation or demineralization of implanted HA has been reported (den Hollander *et al.*, 1991; Ohsaki *et al.*, 1993; Buskinsky *et al.*, 1994).

Another explanation for increase in numbers of macrophages at six months is demineralization and remineralization on the surface of Apaceram®. In

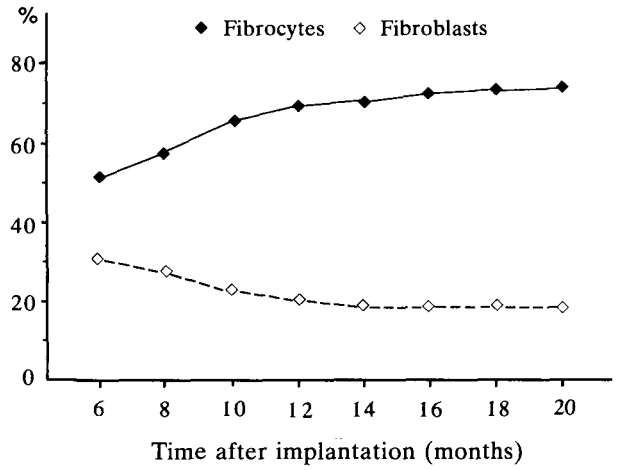

FIG. 4b
Changes in population of fibroblasts and fibrocytes after implantation.

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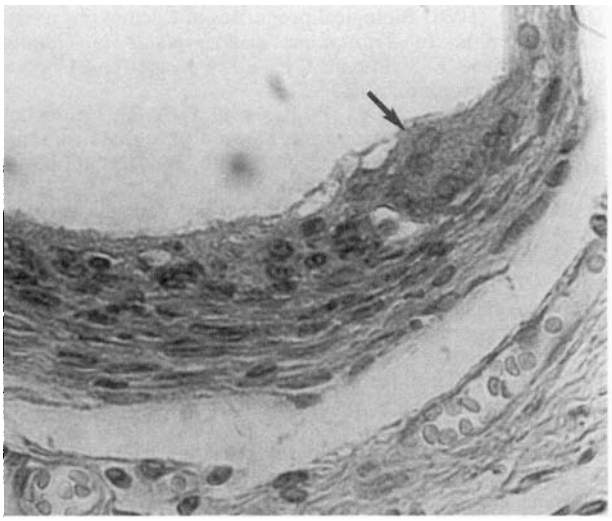

FIG. 5a
Six months, foreign body giant cells (arrow) at Apaceram® disc-tissue interface. (H & E; × 600)

the *in vitro* experiment the process of demineralization of HA powder was measured by an ion-meter (Thomann *et al.*, 1990). Conductivity measurement shows that the rate of dissolution of Apaceram® in saline solution is approximately 700 times faster than the dissolution of Apaceram® in distilled water (Ohsaki *et al.*, 1995).

Laser-Raman spectrometry study of the *in vivo* experiment shows ions moved to subcutaneous tissue and then, as a local part became saturated with these ions, remineralization was improved by deposition of phosphate ions (Ohsaki *et al.*, 1995). In a previous study (Cui *et al.*, 1995) and the present study, at six, eight, 10, and 14 months after implantation the particles were observed in the cytoplasm of macrophages at the Apaceram®-tissue interface in cases of incomplete decalcification. These particles of redeposition on the disc surface were the initial species for the development of crystal (in the process of remineralization) and were very small (Moore,

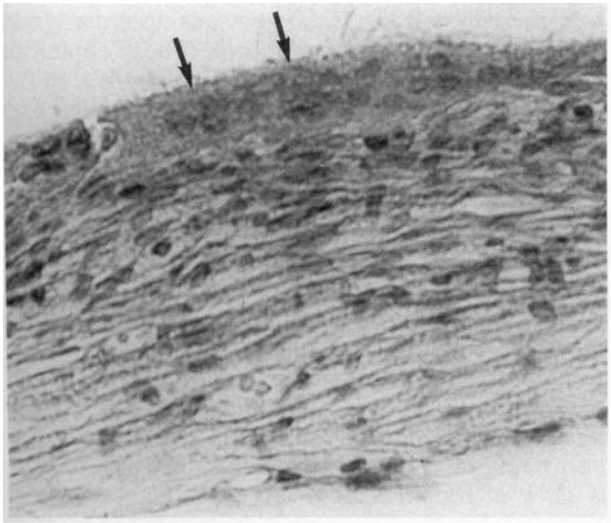

FIG. 5b
Twenty months after implantation, foreign body giant cells (arrow) at Apaceram® disc-tissue interface. (H & E; × 600)

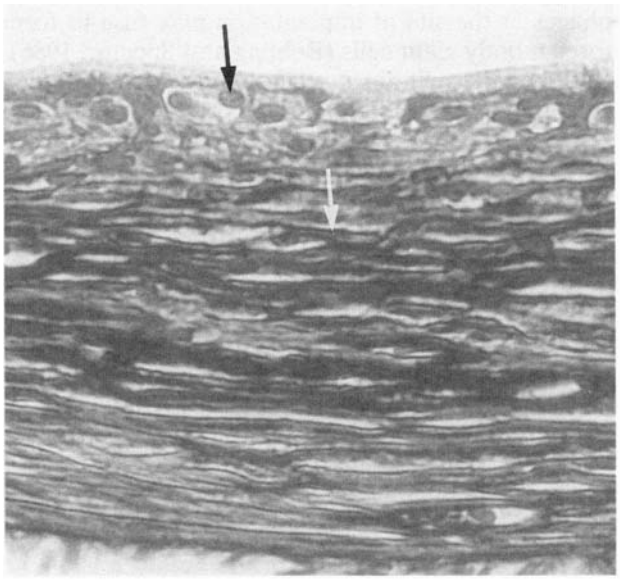

FIG. 6
Fibrous capsule at 12 months after implantation. A thin layer of macrophages and fibroblasts (black arrow), and a thick layer of fibrocytes (collagen tissue) stained blue (white arrow). (Mallory's azan; × 600)

1962). These species were structurally unstable. Therefore, macrophages could ingest these particles easier than particles which originated from dense discs unaffected by subcutaneous tissue reaction. Demineralization and remineralization caused physical-chemical changes of the implanted disc surface, resulting in macrophage reaction. But with increase of time after implantation, FCT increased and fibrous capsules surrounding the discs became more dense. So, increase of FCT and density of collagen could restrict diffusion of ions through dense fibrous capsules contacting with the interface regions between Apaceram® and the capsule. Also, the balance between demineralization and remineralization would be maintained under a restricted environment of ion diffusion. As shown in Table I, the number of macrophages decreased and were almost constant after 16 months.

Roles of macrophage and FBGC

Long-term biological response clearly indicates that the macrophage is the dominant cell type at the implant surface and that stability of an implant depends largely on the dynamic behaviour of macrophages (Jacob-LaBarre *et al.*, 1994). Thus, biocompatibility of the material may be reflected in what occurs in the area surrounding the implant following implantation. Macrophages have a number of functions in wound healing and cellular responses to long-term implants, including phagocytic activity and the ability to control fibroblast activity. Therefore, macrophages may indirectly influence the formation of collagen (Anderson and Miller, 1984; Salthouse, 1984). Macrophages secrete a variety of products including: (1) chemotactic agents for other cells, (2) growth factors that stimulate production of collagen by fibroblasts, and (3) neutral proteases that may affect an implant surface. Persistent macro-

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phages at the site of implantation may fuse to form foreign body giant cells (Behling and Spector, 1986). Based on these findings, macrophages appear to play a central role in the tissue reaction to implants.

FBGCs were observed at the interface of Apaceram®-tissue in all specimens. Table I and Figure 3 show that the percentage of FBGCs remained essentially constant from six to 20 months after implantation (0.6 per cent average). FBGC formation represents a common phenomenon that occurs in reaction to foreign bodies. However, the mechanism involved in FBGC formation remains unclear. FBGCs may form through the fusion of activated macrophages (Smetana, 1987; Abe *et al.*, 1987). Furthermore, macrophage adhesion, FBGC formation, and other stages of inflammatory response might be modulated by chemical composition and physical properties of implanted materials (Kao *et al.*, 1994).

Conclusion

Inflammatory cell reaction surrounding Apaceram® discs is relatively slight and formation of fibrous capsules occurs about six to 20 months after implantation. Tissue reaction appears to reach a steady state 16 months after implantation. The present study suggests that Apaceram® has satisfactory biocompatibility for use as an implant material for reconstructive surgery.

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