

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

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Abstract: To evaluate biocompatibility to tissue in long-term implantation, Bioceram[®] discs made of aluminum oxide (Al₂O₃) were implanted subcutaneously within the interscapular region of 64 rats for six to 20 months. Histological sections stained with haematoxylin and eosin (H&E) and the surface of the implant material were observed using light microscopy. Different cell types and the thickness of fibrous capsules surrounding the implants were examined quantitatively by light microscopy. Small numbers of macrophages (2.8 ± 0.7%) and lymphocytes (2.7 ± 0.9%) were observed at six months after implantation, gradually decreasing to zero at 16, 18 and 20 months. Neither neutrophils nor foreign body giant cells were seen in any specimens. The thickness of fibrous capsules surrounding the implants was closely related to the shape of the implant, but there was no significant change between six and 20 months after implantation. No change in Bioceram[®] surfaces were observed under stereoscopic microscopy from six to 20 months after implantation. The study results indicate that Bioceram[®] is a satisfactory biocompatible material for reconstructive surgery from the viewpoint of long-term tissue response. Present results of experiments with Bioceram[®] are also compared to previous results with Apaceram[®] and different tissue responses of the two materials are discussed. *J. Med. Invest.* 46 : 97-103, 1999

Key words : aluminum oxide, long-term implantation, subcutaneous tissue reaction, histology, rats

INTRODUCTION

The synthetic auditory ossicle (Bioceram[®]) composed of the bio-inert ceramic material aluminum oxide (Al₂O₃) is currently used widely in reconstructive middle ear surgery. Bioceram[®]'s high biocompatibility has been reported (1, 2) and our previous study showed a relative low inflammatory cell response to Bioceram[®] in the early stages after implantation (3). However, histological changes at the Bioceram[®]-tissue interface and surfaces change of implants during long-term implantation have received limited attention. In the present study, small and

thin Bioceram[®] discs were implanted into the subcutaneous tissue of rats with the aim of investigating histological reactions between six and 20 months after implantation. The thickness of the fibrous capsules surrounding Bioceram[®] discs was examined quantitatively by light microscopy; surface changes in Bioceram[®] discs were examined by stereoscopic microscopy. The experimental results on Bioceram[®] were compared with the results of our previous study (4) using bioactive synthetic auditory ossicle (Apaceram[®]) made of hydroxyapatite [Ca₁₀(PO₄)₆(OH)₂].

MATERIAL AND METHODS

Implant material

Dense discs (diameter, 4 mm; thickness, 1 mm) of Bioceram[®] were prepared from commercially

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available synthetic auditory ossicle (Kyocera Co. Ltd, Kyoto, Japan). Before implantation, the discs were sterilized in an autoclave at 121 °C for 30 minutes.

Animals and implantation

Bioceram[®] discs were implanted subcutaneously within the interscapular region of 64 eight-week-old female SPF Wistar rats under general anesthesia (diethyl ether) in a sterile environment. Wounds were closed by suturing.

Histological procedures and observation

Rats were sacrificed quickly in groups of 8 at 6, 8, 10, 12, 14, 16, 18 and 20 months after implantation by general anaesthesia using diethyl ether. The Bioceram[®] discs and surrounding tissue were removed as a single mass and immediately immersed in 10 per cent phosphate-buffered formalin for three days. The Bioceram[®] discs were carefully removed from the tissue mass under Stereoscopic microscopy to minimize damage to the tissue surrounding discs.

Tissue surrounding the discs was dehydrated in an ethanol series. After being embedded in paraffin, 10 to 15 sections (6 µm thick) from each specimen were stained with haematoxylin and eosin (H&E). Five randomly chosen sections per specimen were observed and photographed under light microscopy. Photographic slides were projected and cells were identified and counted. Each specimen had a total of between 202 and 607 cells, and percentages of various cellular components were calculated.

One randomly chosen section per specimen was observed under light microscopy and the thickness of the fibrous capsule surrounding the Bioceram[®] disc was measured. Figure 1 a shows a Bioceram[®] disc divided into : 1) flat portions (upper and lower portions), 2) lateral portions, and 3) ring portions (upper ring-shaped and lower ring-shaped portions). Figure 1 b shows the fibrous capsule attached to the disc surface. Photographic slides of the fibrous capsules were projected on a screen and the thickness of the capsules was measured using an objective micrometer at the same magnification as samples. Average thickness values for each group of 8 rats were calculated at flat portions, lateral portions and ring portions.

Untreated Bioceram[®] disc and Bioceram[®] discs obtained from specimens were observed under stereoscopic microscopy. Statistical analyses were performed on a Macintosh Performa 588 computer with the Excel 5.0 statistical program. Difference

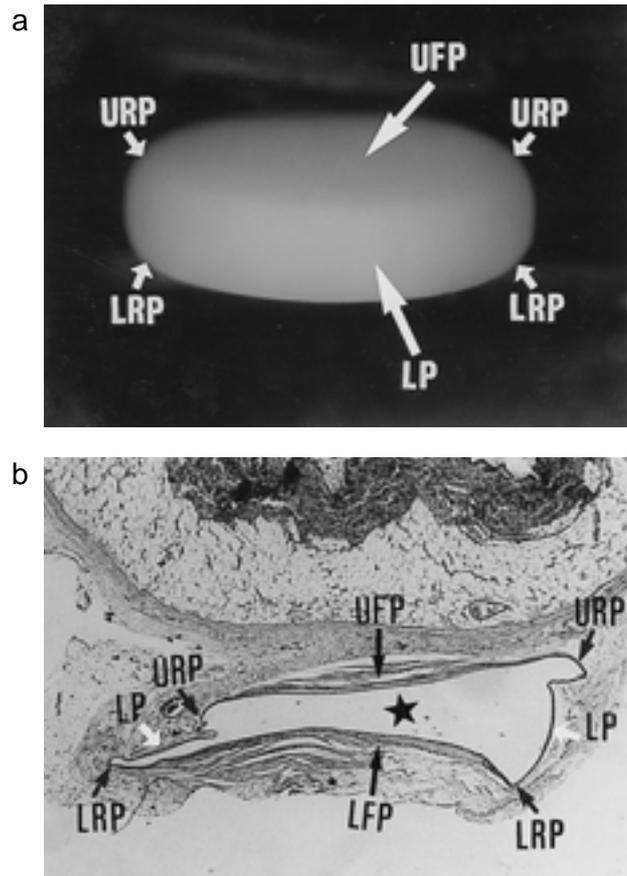


Fig.1. a) Bioceram[®] disc under stereoscopic microscopy. Disc may be divided into three portions : flat portions, lateral portion and ring portions. Arrow UFP identifies upper flat portion ; Arrow LP identifies lateral portion ; Arrows URP identify upper ring-shaped portions ; Arrows LRP identify lower ring-shaped portions (objective lens, x 1.5).

b) Low-power photomicrograph 20 months after implantation showing the fibrous capsule surrounding a Bioceram[®] disc. (H&E ; x 15). Arrow UFP identifies upper flat portion ; Arrow LFP identifies lower flat portion ; Arrow LP identifies lateral portions ; Arrows URP identify upper ring-shaped portions ; Arrows LRP identify lower ring-shaped portions. Star identifies Bioceram[®] disc.

was calculated using Student's *t*-test (two-tailed) with a level of $p < 0.05$ being accepted as significant.

RESULTS

Surface observation of Bioceram[®] discs

Stereoscopic microscopy examination showed no surface changes in the Bioceram[®] discs from six to 20 months after implantation in comparison with untreated Bioceram[®] surfaces

Histological observation

General observation and cell distribution

Fibrous capsules surrounding implant discs were seen in all sections between six and 20 months after

implantation (Fig. 1 b). Capsules were composed of macrophages, lymphocytes, fibroblasts and fibrocytes, as well as collagen, and in some cases, capillaries. Macrophages, lymphocytes and fibroblasts were located close to the Bioceram[®] disc-tissue interfaces. Fibrocytes were located in the outer layers of the fibrous capsules.

Cell population

Table 1 shows the cellular components surrounding Bioceram[®] discs. The proportion of macrophages was $2.8 \pm 0.7\%$ and the proportion of lymphocytes was $2.7 \pm 0.9\%$ at six months after implantation (Fig. 2), gradually decreasing to $0.4 \pm 0.6\%$ for macrophages and $0.1 \pm 0.2\%$ for lymphocytes at 14 months. At 16 months, macrophages and lymphocytes completely disappeared (Fig.3). Percentage of fibroblasts was $25.4 \pm 3.2\%$ at six months, gradually decreasing to $1.8 \pm 1.7\%$ at 20 months. In contrast, fibrocytes increased from $66.7 \pm 2.4\%$ at 6 months to $97.3 \pm 2.2\%$ at 20 months.

The average \pm SD of absolute number of infiltrated cells in Apaceram[®] and Bioceram[®] is shown in Table 2.

Thickness of fibrous capsules surrounding Bioceram[®] discs

Table 3 shows the average thickness of fibrous capsules surrounding Bioceram[®] discs at 1) flat portions, 2) lateral portions, and 3) ring portions. In general, fibrous capsules were thickest at the flat portions and thinnest at the ring portions in every test period. Thickness of fibrous capsules at the flat portions tended to increase from 6 months ($95.1 \pm 16.5 \mu\text{m}$, n=16) to 20 months ($118.2 \pm 48.0 \mu\text{m}$, n=16) after implantation, but the difference

was not significant. Thickness of fibrous capsules changed slightly at the lateral portions from $28.5 \pm 5.0 \mu\text{m}$ to $39.2 \pm 11.1 \mu\text{m}$ and at the ring portions

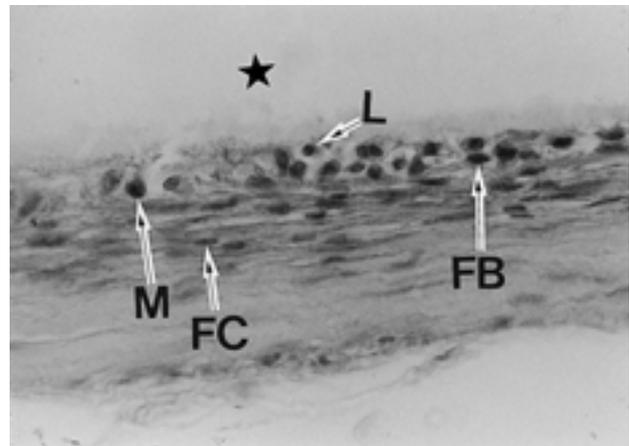


Fig. 2. Six months after implantation, showing macrophages (arrow M), lymphocytes (arrow L), fibroblasts (arrow FB) and fibrocytes (arrow FC). Star identifies Bioceram[®] disc. (H&E x 600)

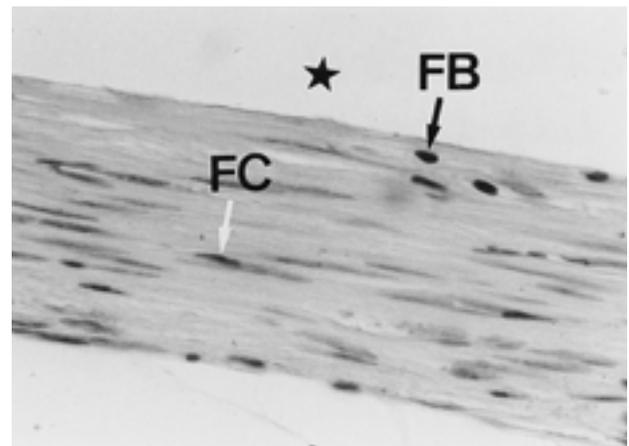


Fig. 3. 16 months after implantation, only fibroblasts (arrow FB) and fibrocytes (arrow FC) remain ; no macrophages nor lymphocytes. Star identifies Bioceram[®] disc. (H&E x 600)

Table 1 . Average percentages of component cells in tissue surrounding Bioceram[®] discs (Mean \pm SD)

	6 Months	8 Months	10 Months	12 Months	14 Months	16 Months	18 Months	20 Months
N	0 \pm 0							
M	2.8 \pm 0.7	2.3 \pm 0.5	1.7 \pm 0.2	1.2 \pm 0.2	0.4 \pm 0.6	0 \pm 0	0 \pm 0	0 \pm 0
L	2.7 \pm 0.9	2.3 \pm 0.7	1.8 \pm 0.5	1.2 \pm 0.2	0.1 \pm 0.2	0 \pm 0	0 \pm 0	0 \pm 0
FG	0 \pm 0							
FB	25.4 \pm 3.2	24.9 \pm 2.3	18.3 \pm 2.9	17.1 \pm 2.0	14.3 \pm 2.4	8.6 \pm 2.3	2.0 \pm 1.2	1.8 \pm 1.7
FC	66.7 \pm 2.4	68.2 \pm 1.5	76.7 \pm 3.3	79.1 \pm 1.6	81.9 \pm 2.4	89.6 \pm 2.5	97.2 \pm 2.0	97.3 \pm 2.2
U	2.4 \pm 1.1	2.3 \pm 0.8	1.5 \pm 0.9	1.4 \pm 0.6	3.3 \pm 1.8	1.8 \pm 1.2	0.8 \pm 0.5	0.9 \pm 0.7

N : neutrophils ; M : macrophages ; L : lymphocytes ; FG : foreign body giant cells ; FB : fibroblasts ; FC : fibrocytes ; U : unidentified cells. 8 rats were sacrificed at each point in time.

Table 2. Average absolute number of infiltrated cells in tissue surrounding Bioceram[®] and Apaceram[®] (Mean \pm SD)

		6 Months	8 Months	10 Months	12 Months	14 Months	16 Months	18 Months	20 Months
M	Ap	36.8 \pm 10.6	44.0 \pm 20.8	27.6 \pm 10.2	28.0 \pm 8.2	32.2 \pm 12.6	24.0 \pm 8.4	15.7 \pm 6.4	13.0 \pm 6.8
	Bio	8.0 \pm 2.4	8.0 \pm 1.9	8.3 \pm 1.9	4.4 \pm 1.7	1.3 \pm 2.1	0 \pm 0	0 \pm 0	0 \pm 0
L	Ap	8.0 \pm 3.8	6.2 \pm 4.2	4.8 \pm 3.5	6.0 \pm 2.6	6.9 \pm 3.7	8.2 \pm 4.3	5.8 \pm 2.8	6.0 \pm 2.2
	Bio	8.0 \pm 2.5	8.1 \pm 2.5	8.0 \pm 2.0	3.6 \pm 1.1	0.3 \pm 0.5	0 \pm 0	0 \pm 0	0 \pm 0
FG	Ap	1.4 \pm 3.2	2.4 \pm 4.3	2.2 \pm 1.6	2.3 \pm 1.4	2.6 \pm 1.8	3.0 \pm 1.4	2.9 \pm 1.2	2.0 \pm 0.8
	Bio	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0

M : macrophages ; L : lymphocytes ; FG : foreign body giant cells ; Ap : Apaceram[®] ; Bio : Bioceram[®]. For Apaceram[®] group, 10 rats were sacrificed at each point in time, except for 20-months (4 rats). For Bioceram[®] group, 8 rats were sacrificed at each point in time.

Table 3. Average thickness of fibrous capsules surrounding Bioceram[®] discs (Mean \pm SD, unit : μ m)

		6 Months	8 Months	10 Months	12 Months	14 Months	16 Months	18 Months	20 Months
F		95.1 \pm 16.5	94.3 \pm 18.2	106.4 \pm 46.5	112.3 \pm 40.0	114.8 \pm 58.7	112.8 \pm 36.2	116.0 \pm 42.3	118.2 \pm 48.0
L		28.5 \pm 5.0	39.0 \pm 6.8	30.6 \pm 5.1	36.1 \pm 10.7	39.2 \pm 11.1	32.4 \pm 9.5	31.8 \pm 8.5	37.3 \pm 10.3
R		8.3 \pm 1.4	10.7 \pm 1.6	9.3 \pm 2.8	9.1 \pm 2.1	9.2 \pm 2.0	8.4 \pm 1.6	8.2 \pm 1.3	9.3 \pm 2.1

F=16 flat portions, 2 portions from each disc (1 upper portion and 1 lower portion) ; L=16 lateral portions, 2 portions from each disc (1 left portion and 1 right portion) ; R=32 ring portions, 4 from each disc (2 upper ring-shaped portions and 2 lower ring-shaped portions). 8 rats were sacrificed at each point in time.

from 8.2 \pm 1.3 μ m to 10.7 \pm 1.6 μ m in the experimental period.

DISCUSSION

Accurate assessment of implant materials requires long-term investigation of the tissue reaction surrounding the implant material and analysis of the physico-chemical changes in the implanted material.

Macrophage reaction to Bioceram[®] at six months and longer after implantation

Clearly, the macrophage is the dominant cell type at the implant surface, playing a major role in cellular response and tissue reaction to the implant (5). Implant stability depends largely on the dynamic behavior of macrophages (6). Macrophages accumulating at the implant-tissue interface can produce various secretions including : 1) chemotactic agents for other cells, 2) growth factors which stimulate production of collagen by fibroblasts, and 3) neutral proteases which may affect the implant surface. Therefore, population and activities of macrophages at the implant-tissue interface may reflect the biocompatibility of a biomaterial (7, 8). In the present

study, macrophages accounted for 2.8 \pm 0.7% at six months after implantation, gradually decreasing to 0.4 \pm 0.6% at 14 months and completely disappearing at 16 months. So, from the viewpoint of cellular response, Bioceram[®] is probably a satisfactory biocompatible material for long-term implantation.

Fibrous capsules surrounding Bioceram[®]

The thickness of fibrous capsules surrounding an implant is also an important indicator in evaluating the biocompatibility of artificial material (2, 8). In the present study on Bioceram[®], the thickness of fibrous capsules from different regions of the same sample differed substantially. Fibrous capsules from flat portions of discs were much thicker than from other portions, and the ring portions were thinnest. These data agree with another reports (9), indicating that capsule formation is closely related to implant shape. Contact of disc surface with tissue is influenced physically and/or chemically by both area and shape (10). Different physical and/or chemical stimulation at the flat portions, lateral portions, and ring portions may cause varying thicknesses of fibrous capsules. Future experiments must investigate precisely how the different portions of a synthetic prosthesis disc affect implant biocompat-

ibility.

The thickness value in the flat portions increased by 24.3% from six to 20 months, but there was no statistically significant difference. The thickness of fibrous capsules surrounding Bioceram[®] discs was stable at least between six and 20 months after implantation, and this is consistent with the report by Boutin et al. (11)

Comparison of Bioceram[®] and Apaceram[®]

Both Bioceram[®] and Apaceram[®] are popular materials in middle ear reconstructive surgery. Tissue response to Apaceram[®] in long-term implantation was investigated in our previous study (4). The results of the present study on Bioceram[®] were compared with the previous results on Apaceram[®], because while Apaceram[®] is a bioactive material, Bioceram[®] is regarded as a bioinert material.

Appearance of macrophages and foreign body giant cells

The number of macrophages surrounding Apaceram[®] was remarkably higher than the number of macrophages surrounding Bioceram[®] (Fig. 4a). A small number of foreign body giant cells (0.5-0.8%) was found at Apaceram[®]-tissue interfaces between six to 20 months after implantation (4). However, no foreign body giant cells were seen in any sections removed from specimens of Bioceram[®] between six and 20 months over the same period of time after implantation. Presumably, tissue reaction to Bioceram[®] from six to 20 months after implantation is milder than tissue reaction to Apaceram[®].

Differences in macrophage and foreign body giant cell responses to Bioceram[®] and Apaceram[®] may be explained by the different physico-chemical properties, biomechanical compatibility, surface texture, and solubility of the two materials to tissue.

Aluminum oxide, a material in the highest state of oxidation, is thermodynamically stable with an ionic structure that creates a hydrophilic surface with high wettability, possibly resulting in low tissue reaction (12). *In vivo* and *in vitro* experimental studies show that aluminum oxide releases few ions into surrounding areas (13). In addition, low stimulus to macrophages is reported (14, 15).

Hydroxyapatite is a calcium phosphate bio-ceramic, the major inorganic component of bone. Hydroxyapatite shows significant ion release *in vitro* and *in vivo* (16-18). Biodegradation (16), demineralization and remineralization of hydroxyapatite have been reported after implantation (17, 18). In

the process of these changes, some particles and ions permeate surrounding tissue and stimulate inflammatory cells. Activated macrophages aggressively fuse to form foreign body giant cells with a few particles in the cytoplasm of macrophages and foreign body giant cells (4, 19). However, aluminum oxide does not create such responses in the living body. Cellular response to aluminum oxide completely disappeared during the later stages of the present experiment. However, a small number of macrophages, lymphocytes and foreign body giant cells were continuously present at Apaceram[®]-tissue interfaces, even up to 20 months after implantation (4).

Another possible explanation for the differences in macrophages and foreign body giant cell responses in Bioceram[®] and Apaceram[®] is that the roughness of implant surfaces is associated with the appearance of macrophages and foreign body giant cells (2). The mechanism is unclear, but rough implant surfaces have resulted in significant increases

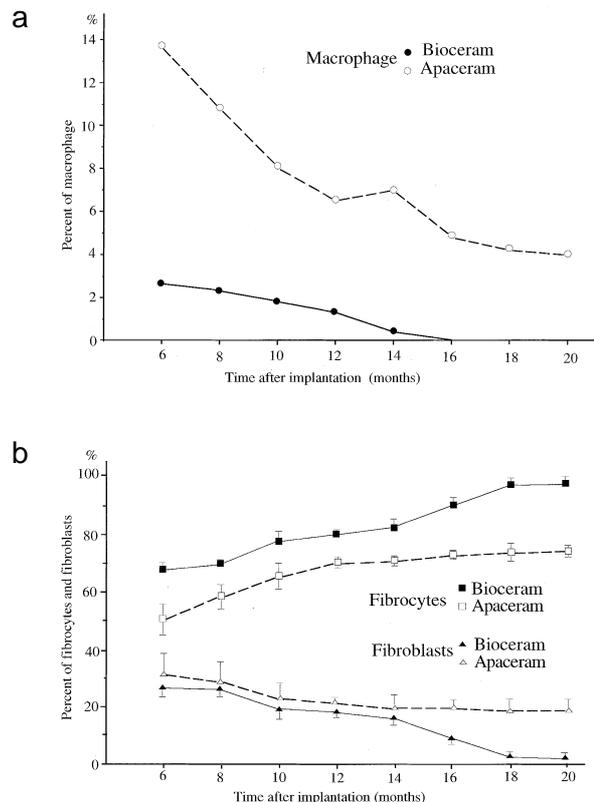


Fig. 4. Comparison in terms of macrophages, fibroblasts and fibrocytes in Bioceram[®] and Apaceram[®] from six to 20 months after implantation. a) Macrophages in Bioceram[®] and Apaceram[®]. b) Fibroblasts and fibrocytes in Bioceram[®] and Apaceram[®]. (Note : mean ± SD in fibrocytes was always p<0.01. At 6, 8 and 10 months after implantation, t-test shows NS for fibroblasts, p<0.05 at 14 months and p<0.01 at 12, 16, 18 and 20 months).

in the proportion of surface covered by macrophages and foreign body giant cells (20-22). In our studies, both the Bioceram[®] and Apaceram[®] discs had a smooth surface before implantation. However, surfaces of Apaceram[®] discs became rough after implantation because of the above-mentioned physico-chemical changes (4, 17). In contrast, examination by stereoscopic microscopy showed no change to the surface of Bioceram[®] discs. The rough surface of Apaceram[®] discs possibly caused macrophages and foreign body giant cells to be continuously present at the implant-tissue interfaces during long-term implantation.

Fibroblasts and fibrocytes

Fig. 4 b shows that the fibroblast level surrounding Apaceram[®] is higher than the fibroblast level surrounding Bioceram[®]. Statistical analyses showed no significant differences at 6, 8 and 10 months, but there were significant differences from 12 to 20 months after implantation ($p < 0.05$). The population of fibrocytes surrounding Bioceram[®] was significantly higher than that surrounding Apaceram[®] at all experimental periods ($p < 0.01$).

Fibrocytes usually demonstrate mature fibrous connective tissue. Thus, Bioceram[®] seems to exhibit a satisfactory tissue-implant relation. Fibroblast ability is mainly controlled by macrophages in wound healing and cellular responses to long-term implants (6, 8). Therefore, different macrophage levels surrounding Bioceram[®] and Apaceram[®] may result in different fibroblastic and fibrocytic reactions.

CONCLUSION

The present study showed eventual disappearance of inflammatory cell response to Bioceram[®] and well-matured connective fibrous capsules surrounding Bioceram[®] discs from six to 20 months after implantation. These results suggest that, from the viewpoint of long-term tissue response, Bioceram[®] has satisfactory biocompatibility for use as an implant material for reconstructive surgery.

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REFERENCES

1. Yamamoto E, Iwanaga M : Soft tissue reaction to ceramic ossicular replacement prosthesis. *J Laryngol Otol* 101 : 897-904, 1987
2. Takeshita F, Morimoto K, Suetsugu T : Tissue reaction to alumina implants inserted into the tibiae of rats. *J Biomed Mater Res* 27 : 421-428, 1993
3. Ye Q, Ohsaki K, Li K, Li DJ, Matsuoka H, Tenshin S, Yamamoto T : A subcutaneous tissue reaction in the early stage to a synthetic auditory ossicle (Bioceram[®]) in rats. *J Med Invest* 44 : 173-177, 1998
4. Li DJ, Ohsaki K, Li K, Ye Q, Nobuto Y, Tenshin S, Takano-Yamamoto T : Long-term observation of subcutaneous tissue reaction to synthetic auditory ossicle (Apaceram[®]) in rats. *J Laryngol Otol* 109 : 702-706, 1997
5. Kao WJ, Zhao QH, Hiltner A, Anderson JM : Theoretical analysis of in vivo macrophages adhesion and foreign body giant cell formation on polydimethylsiloxane, low density polyethylene, and polyetherurethanes. *J Biomed Mater Res* 28 : 73-79, 1994
6. Jacob-LaBarre JT, Assouline M, Byrt T, McDonald M : Synthetic scleral reinforcement materials : I. Development and in vivo tissue biocompatibility response. *J Biomed Mater Res* 28 : 699-712, 1994
7. Anderson JM, Miller KM : Biomaterial biocompatibility and the macrophages. *Biomaterials* 5 : 5-10, 1984
8. 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
9. Matlaga BF, Yasenchak LP, Salthouse TN : Tissue response to implanted polymers : The significance of sample shape. *J Biomed Mater Res* 10 : 391-397, 1976
10. Li DJ, Ohsaki K, Li K, Cui PC, Ye Q, Baba K, Wang QC, Satoru T, Tenshin S, Takano-Yamamoto T : Thickness of fibrous capsule after implantation of hydroxyapatite in subcutaneous tissue in rats. *J Biomed Mater Res*, 1999 (in press).
11. Boutin P, Christel P, Dorlot JM, Meunier A, de Roquancourt A, Blanquaert D, Herman S, Sedel L, Witvoet J : The use of dense alumina-alumina ceramic combination in total hip replacement. *J Biomed Mater Res* 22 : 1203-1232, 1988

12. Christel PS, Biocompatibility of surgical-grade dense polycrystalline alumina. *Clin Orthop* 282 : 10-18, 1992
13. Arvidson K, Fartash B, Mober L-E, Grafström R, Ericsson I : In vitro and vivo experimental studies on single crystal sapphire dental implants. *Clin Oral Impl Res* 2 : 47-55, 1991
14. Harms J, Mäusle E : Tissue reaction to ceramic implant material. *J Biomed Mater Res* 13 : 67-87, 1979
15. Labat B, Chamson A, Frey J : Effects of γ -alumina and hydroxyapatite coatings on the growth and metabolism of human osteoblasts. *J Biomed Mater Res* 29 : 1397-1401, 1995
16. van der Meulen J, Koerten HK : Inflammatory response and degradation of three types of calcium phosphate ceramic in a non-osseous environment. *J Biomed Mater Res* 28 : 1455-1463, 1994
17. Ohsaki K, Shibata A, Yamashita S, Oe M, Wang KQ, Cui PC, Ye Q : Demonstrations of de-and remineralization mechanism as revealed in synthetic auditory ossicle (Apaceram[®]) of rats by laser-Raman spectrometry. *Cell Mol Biol* 41 : 1155-1167, 1995
18. Ohsaki K, Shibata A, Wang KQ, Ohe M, Goto S, Kimura N, Yamamoto A, Yamashita S : Processes of de-and remineralization in vivo and in vitro studied using a synthetic ossicular chain. In : Nakano Y, ed. *Cholesteatoma and Mastoid Surgery*. Kugler Publ, Amsterdam, 1993, pp. 615-621
19. Cui PC, Ohsaki K, li K, Tenshin S, Kawata T : Subcutaneous tissue reaction to synthetic auditory ossicle (Apaceram[®]) in rats. *J Laryngol Otol* 109 : 14-18, 1995
20. Murch AR, Grounds MD, Marshall CA, Papadimitriou JM : Direct evidence that inflammatory multinucleate giant cells form by fusion. *J Pathol* 137 : 177-180, 1982
21. Maxian SH, Zawadsky JP, Dunn MG : Mechanical and histological evaluation of amorphous calcium phosphate and poorly crystallized hydroxyapatite coatings on titanium implants. *J Biomed Mater Res* 2 : 717-728, 1993
22. Salthouse TN : Some aspects of macrophages behavior at the implant interface. *J Biomed Mater Res* 18 : 395-401, 1984