View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Tokushima University Institutional Repository

A subcutaneous tissue reaction in the early stage to a synthetic auditory ossicle (Bioceram®) in rats

Qing Ye*, Katsuichiro Ohsaki†, Kunio Ii*, Dong-jun Li†, Hiroshi Matsuoka†, Satoru Tenshin‡, and Teruko Yamamoto‡

*First Department of Pathology, †Division of Clinical Otology, University Hospital, The University of Tokushima School of Medicine, Tokushima, Japan ; ‡Department of Orthodontics, The University of Tokushima School of Dentistry, Tokushima, Japan ; and ‡Department of Orthodontics, Okayama University Dental School Okayama, Japan

Abstract : The discs of synthetic auditory ossicle (Bioceram®), which are composed of aluminium oxide (Al_2O_3), were implanted subcutaneously in the interscapular region of 16 rats. The implanted specimens were removed at 1, 3, 7 and 14 days after implantation. The decalcified 6 μm thick sections were stained with H.E. and cell types around the implants were counted microscopically. We found that an acute inflammatory reaction occurred at one day, in which macrophages and neutrophiles predominated, and almost disappeared at about 7 days after implantation. Fibrosis began to be observed at 3 days. During this early stage, foreign body giant cells were found in only one specimen at 3 days. These findings, in comparison with those in the controls, showed that the chemical irritation of Bioceram® to the subcutaneous tissue is slight, although the physical and/or chemical irritation of Bioceram® lasts continuously and induces fibrosis around the bioimplant. The results so far suggest that Bioceram® seems to be a satisfactorily biocompatible material, at least within the extent of 2 weeks. J. Med. Invest. 44 : 173-177, 1998

Key Words *: aluminum oxide ceramics ; histology ; cell count ; biocompatible materials ; rats*

INTRODUCTION

Biomaterials are the building blocks of implant technology. Biomaterials used in otolaryngological surgery can be grouped into four categories, on the basis of their material science definition : metals, ceramics, polymers, and composites (1). There had been little study about the histopathological changes in the biomaterial-tissue interface during the early stage of implantation, we therefore decided to investigate the early response of soft tissue to implanted biomaterial. In this study, we chose Bioceram® which contains aluminum oxide ceramics, one of ceramics among those four categories mentioned above, as a experimental bioimplant. However if histologic evaluations of the soft tissue response to biomaterials were merely based on qualitative diagnosis or semiquantitative grading, there would be an inherent weakness due to the subjectivity of the observers. To overcome this drawback, a quantitative approach is preferable (2), so we implanted small, thin discs of Bioceram® subcutaneously in rats and observed the resulting histological reaction quantitatively by light microscopy from 24 hours to 2 weeks after implantation.

Received for publication December 2, 1997 ; accepted January 5, 1998.

[1] Address correspondence and reprint requests to Katsuichiro Ohsaki, M.D., Ph.D., Division of Clinical Otology, University Hospital, The University of Tokushima School of Medicine, Kuramoto-cho, Tokushima 770-8503, Japan and Fax : +81-886-33-7208.

MATERIALS AND METHODS

Implant material characteristics : Dense discs (diameter, 4 mm ; thickness, 1 mm) of Bioceram® (Al_2O_3) were prepared from commercially available synthetic auditory ossicle (Kyocera Co. Ltd., Kyoto). Before implantation, the discs were sterilized in an autoclave at 121℃ for 30 minutes.

Animals and operations : Twenty-four eight-week-old female SPF Wistar rats were divided into two groups. Bioceram® disc were implanted subcutaneously into the interscapular region of 16 rats as a test group. Eight rats underwent the same operation but did not received any implants as a control group. Four rats from the test group and two from the control group were sacrificed at 1, 3, 7 or 14 days after implantation. All surgical procedures were carried out in sterile conditions under general anaesthesia using diethyl ether.

Section preparation and staining : The Bioceram® disc and its surrounding tissue were removed as a single mass and immersed immediately in 10 percent phosphate buffered formalin, then dehydrated in an ethanol series. Seeing that Bioceram® can not be decalcified, and in order to get thin and fine sections, we took Bioceram® discs out of soft tissue mass very carefully without any damage to tissues under microscope before the paraffin embedding procedure (Figure 1). After being embedded in paraffin, the tissue mass was cut into sections 6 μm thick. The

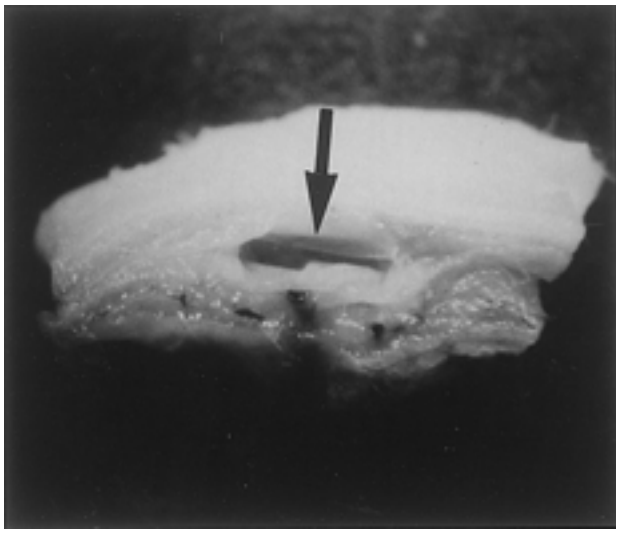

Fig.1. Sample mass of seven days after implantation, showing the disc site (arrow) sheathed with encapsulation of connective tissue (x7.5).

sections were stained with haematoxylin and eosin.

Observation methods : The types and distribution of cells around the implants were photographed (colour slides) under a light microscope. The pictures were enlarged with a slide projector to identify and the different cells were counted. Over 200 cells were counted for each specimen and the percentages of the various component cells were calculated for each group.

RESULTS

The tissue around the implants had two main structures, a layer of acute inflammatory cells, such as neutrophils, macrophages and lymphocytes, and a surrounding layer of fibrous tissue composed of fibroblasts and fibrocytes, and in some cases capillaries. The average percentages of the component cells are shown in Table 1.

Sections from specimens which were removed one day after implantation showed an acute inflammatory response around the implants (Figure 2 a, b). Cell counts revealed a predominance of macrophages (69.7%), followed by neutrophils (19.6%) and lymphocytes (8.7%). Neither

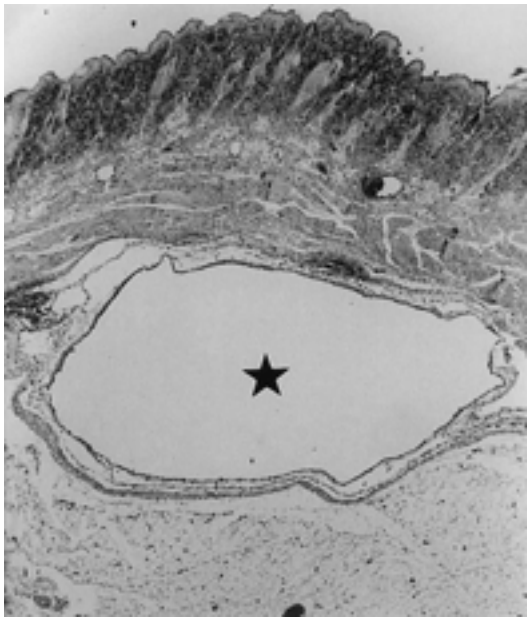

Fig. 2-a. Low power photomicrograph one day after implantation showing a thin layer of inflammatory cells around the Bioceram® disc (★), appearing like a cyst (H.E. x15).

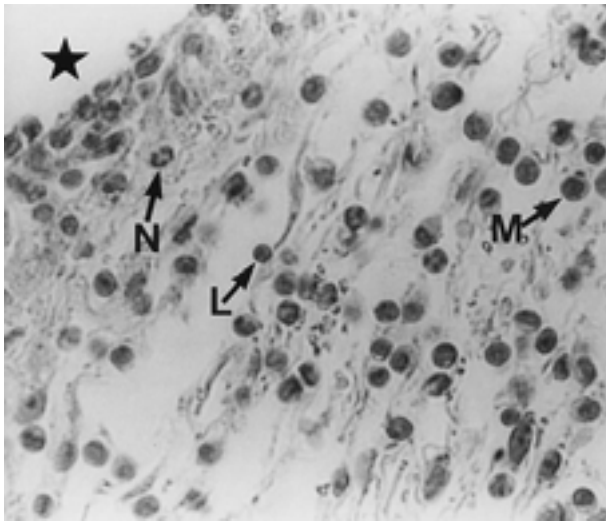

Fig.2-b. High power photomicrograph of the thin layer shown in Fig.2-a. Neutrophils and macrophages are predominant. N : neutrophil, M : macrophage, L : lymphocyte, (★) : Bioceram disc (H.E. x600).

Table 1. Average percentages of component cells in the tissue surrounding implanted Bioceram® discs.

	1 day		3 days		7 days		14 days	
	T	C	T	C	T	C	T	C
Neutrophils	19.6	9.2	0.4	0	0	0	0	0
Macrophages	69.7	85.3	53.3	30.3	19.4	0	3.0	0
Lymphocytes	8.7	3.1	4.4	4.0	2.0	0	1.2	0
Eosinophils	0	0	0	0	0.6	0	0	0
FBGCs	0	0	0.2	0	0	0	0	0
Fibroblasts	0	0	33.1	52.1	52.2	63.3	44.7	58.4
Fibrocytes	0	0	5.5	10.4	23.6	32.8	48.2	39.9
Unidentified	2.0	2.4	3.1	3.2	2.2	3.9	2.9	1.7

FBGCs : foreign body giant cells T : test group (n=4) C : control group (n=2)

fibroblast nor fibrocyte proliferation was observed in any of the specimens.

Specimens taken three days after implantation (Figure 3) showed a sharp decrease in neutrophils to almost zero (0.4%), the components of macrophages and lymphocytes also decreased to 53.3% and 4.4% respectively. In this group, both fibroblasts and fibrocytes began to be observed (33.1% and 5.5%). 0.2% foreign body giant cells (FBGCs) were noticed in one specimen in this group (Figure 4).

Sections from specimens which were removed seven days after implantation (Figure 5) showed a decrease of macrophages to 19.4%. We could not find any neutrophils. The implants were surrounded by a layer of fibroblasts (52.2%) and fibrocytes (23.6%).

Specimens obtained 14 days after implantation (Figure 6) showed that macrophages still remained at a low level (3.0%). The implants were surrounded by a thin layer of fibroblasts (44.7%) and fibrocytes (48.2%), and only a few lymphocytes were still evident (1.2%).

Compared to the test groups, the controls showed the presence of macrophages (85.3%) one day after surgery, and these had completely disappeared by the seventh day after surgery. Neutrophils (9.2%) were evident only one day after surgery. Fibroblasts and fibrocytes increased gradually after three days. The microscopic changes evident in the controls were typical of those normally resulting from surgical intervention.

DISCUSSION

After implantation of a biomaterial, the responses that occur at the interface of the implant and in the surrounding environment are important in determining its biocompatibility. In our study, it revealved that there were two main structures, one was acute inflammatory cells, another one was fibrous tissue and in some cases capillaries, the results we obtained are the same as the finding which other authors described using other sorts of biomaterials such as hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] and bioceramics

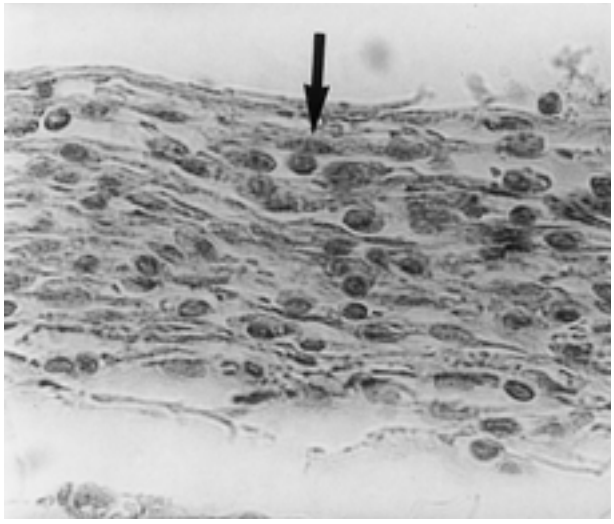

Fig.3. Three days after implantation, showing a decrease in acute inflammatory cells and appearance of fibroblasts (arrow). (H.E. x600).

Fig.5. Seven days after implantation, showing fibroblasts (arrow) (H.E. x600).

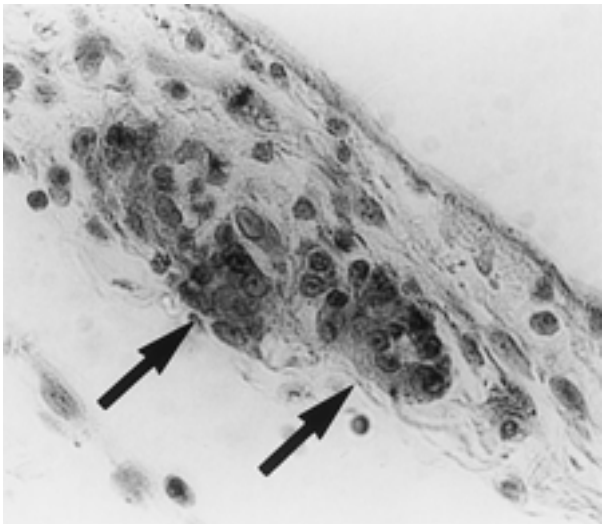

Fig.4. Three days after implantation, showing foreign body giant cells (arrow). (H.E. x600).

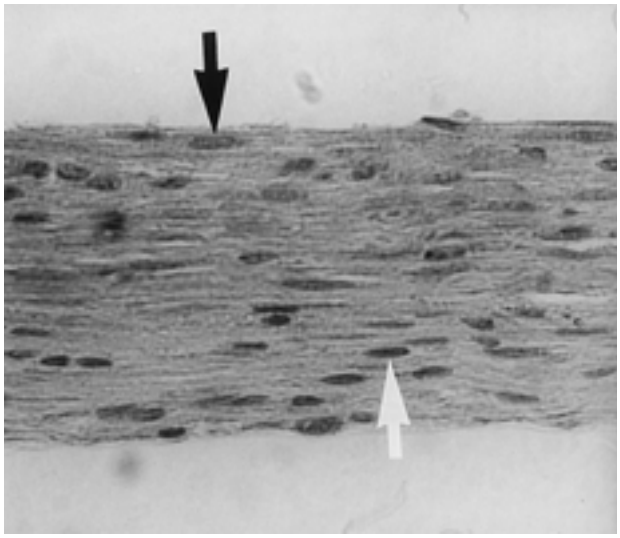

Fig.6. Fourteen days after implantation, showing a layer of fibroblasts (black arrow) and fibrocytes (white arrow). (H.E. x600).

(3, 4, 5). As a kind of synthetic material, Bioceram® is a ceramics, which is made of aluminium oxide (Al_2O_3) (6). It is reported that Bioceram® has merits such as good stability (bioinert), affinity, anti-corrodibility (6). The biological response to an implant can be attributed to acute and chronic inflammatory changes, following the formation of a fibrous capsule, which occurs over a period of time (7). In the present study, the results acquired in the control group within two weeks were compatible with the normal healing of skin wounds. In the test group, the percentages of neutrophils and lymphocytes were approximately two and three-fold greater respectively than those in the control groups. The neutrophils had sharply decreased at three days after implantation, whereas the lymphocytes persisted at a lower level until day 14 after implantation. The peak of macrophages in both groups appeared at the same first day, and these cells had almost disappeared at two weeks after implantation, which was nearly one week later than in the controls. This must have been due to the presence of the implant, and indicated an attempt by the macrophages to induce healing around the bio-material like organ repair following injury (8, 9). There is no doubt that macrophages play a central role in wound repair and tissue reorganization (10). They have been shown to have the ability to control fibroblast activity, and thus indirectly the formation of collagen (8, 9, 2).

The stability of an implant depends largely on the behavior of cells at the material-tissue interface. Since macrophages are cells which are particularly relevant to material compatibility (10), their changes and activities around an implant in the period after implantation may reflect the biocompatibility material (2). Macrophage counts at two weeks after implantation were only three percent. Microscopic observation of the specimens after implantation also confirmed that the Bioceram® discs were encapsulated by a thin layer of fibrous tissue. With respect to the capsule composed of fibroblsts and fibrocytes, fibroblasts began to appear on the third day, increased to a maximum on the seventh day, and decreased at fourteen days. In contrast, fibrocytes continued to increase from three days to fourteen days. The encapsulation of implant material with connective tissue may be considered to be the ideal tissue-implant relation, or may be a good criterion for biocompatibility (2, 11, 12), that is, the results obtained so far suggest that Bioceram® seems to be a satisfactorily biocompatible material.

It is thought that FBGCs are formed through fusion of activated macrophages (13, 14). Macrophages usually appear with inflammatory reaction, but not all the macrophages participate in FBGCs formation. It is generally understood that FBGCs are formed in case of strong foreign body properties of the bioimplant. Result that only few FBGCs (0.2%) were observed in all the specimens (Table 1) after implantation might show that foreign body properties of Bioceram is low.

Some investigators have reported that bioceramic materials such as hydroxyapatite did not induce bone fomation when implanted in soft tissue (3, 15, 16). In our study of tissue reaction to a bioimplant in the early stage, we also did not observe any osteogenesis. As for there will be osteogenesis or not in the long term implantation of Bioceram® in the subcutaneous tissues, it is currently under observation.

REFERENCES

1. DeMane CQ : The development of implants and implantable materials. In : Goldenberg RA, ed. Implants in Otolaryngology. Otolaryngol Clin North Am 28 : 225-234, 1995
2. 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
3. Cui PC, 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
4. Nelson SR, Wolford LM, Lagow RJ, Capano PJ, Davis WL : Evaluation of new high-performance calcium polyphosphate bioceramics as bone graft materials. J Oral Maxillofac Surg 51 : 1363-1371, 1993
5. Di SM, Guizzardi S, Bettini N, Gargiulo G, Savini R : Powdered alumina implants in the experimental animal : a histological study conducted in the rat. Chir Organi Mov 76 : 167-172, 1991
6. Yamamoto E, Iwanaga M : Soft tissue reaction to ceramic ossicular replacement prosthesis. J Laryngol Otol 101 : 897-904, 1987
7. Anderson JM, Miller KM : Biomaterial biocompatibility and the macrophage. Biomaterials 5 : 5-10, 1984
8. Leibovich SJ, Ross R : A macrophage-dependent factor that stimulates the proliferation of fibroblasts in vitro. Am J Pathol 84 : 501-514, 1976
9. Henke C, William M, Jessurun J, Fox J, Harms D, Peterson M, Chiang L, Doran P : Macrophage production of basic fibroblast growth factor in the fibroproliferative disorder of alveolar fibrosis after lung injury. Am J Pathol 143 : 1189-1199, 1993
10. Thomson LA, Law FC, Rushton N : Biocompatibility of diamond-like carbon coating. Biomaterials 12 : 37-40, 1991
11. 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
12. Guy SC, McQuade MJ, Scheidt MJ, McPherson Ⅲ JC, Rossmann JA, Van Dyke TE : In vitro attachment of human gingival fibroblasts to endosseous implant materials. J Periodontol 64 : 542-546, 1993
13. Hassan NF, Kamani N, Meszaros MM, Douglas SD : Induction of multinucleated giant cell formation from human blood-derived monocytes by phorbol myristate acetate in in vitro culture. J Immunol 143 : 2179-2184, 1989
14. Kao WJ, Zhao QH, Hiltner A, Anderaon JM : Theoretical analysis of in vivo macrophage adhesion and foreign body giant cell formation on polydimethylsiloxane, low density polyethylene, and polyetherurethanes. J

Biomed Mater Res 28 : 73-79, 1994

15. El Deeb M, Roszkowski M, El Hakim I : Tissue response to hydroxylapatite in induced diabetic and nondiabetic rats : histologic evaluation. J Oral Maxillofac Surg.48 : 476-481, 1990

16. Nolan P, Templeton P, Mollan RAB, Wilson DJ: Osteoinductive potential of human demineralised bone and a bioceramic on the abdominal musculature of the rat. J Anat 174 : 97-102, 1991