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Diversity of multinucleated giant cells by microstructures of hydroxyapatite and plasma components in extraskeletal implantation model

Kota Morishita [a,b], Eri Tatsukawa [b], Yasuaki Shibata [b,1], Fumio Suehiro [c], Masanobu Kamitakahara [d], Taishi Yokoi [d,2], Koji Ioku [e], Masahiro Umeda [a], Masahiro Nishimura [c], Tohru Ikeda [b,*]

[a] Department of Clinical Oncology, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8588, Japan
[b] Department of Oral Pathology and Bone Metabolism, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8588, Japan
[c] Department of Prosthodontics, Kagoshima University Graduate School, 8-35-1 Sakuragaoka, Kagoshima 890-8544, Japan
[d] Graduate School of Environmental Studies, Tohoku University, 6-6-20 Aramaki, Aoba-ku, Sendai, Miyagi 980-8579, Japan
[e] Department of Chemistry, Faculty of Economics, Keio University, 4-4-1 Yokohama, Kanagawa 223-8521, Japan

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ABSTRACT

Foreign body giant cells (FBGCs) and osteoclasts are multinucleated giant cells (MNGCs), both of which are formed by the fusion of macrophage-derived mononuclear cells. Osteoclasts are distinct from FBGCs due to their bone resorption ability; however, not only morphological, but also functional similarities may exist between these cells. The characterization and diversity of FBGCs that appear in an *in vivo* foreign body reaction currently remain incomplete. In the present study, we investigated an *in vivo* foreign body reaction using an extraskeletal implantation model of hydroxyapatite (HA) with different microstructures. The implantation of HA granules in rat subcutaneous tissue induced a foreign body reaction that was accompanied by various MNGCs. HA granules composed of rod-shaped particles predominantly induced cathepsin K (CTSK)-positive FBGCs, whereas HA granules composed of globular-shaped particles predominantly induced CTSK-negative FBGCs. Plasma, which was used as the binder of ceramic granules, stimulated the induction of CTSK-positive FBGCs more strongly than purified fibrin. Furthermore, the implantation of HA composed of rod-shaped particles with plasma induced tartrate-resistant acid phosphatase (TRAP)-positive MNGCs in contrast to HA composed of globular-shaped particles with purified fibrin, which predominantly induced CTSK-negative and TRAP-negative typical FBGCs. These results suggest that CTSK-positive, TRAP-positive, and CTSK- and TRAP-negative MNGCs are induced in this subcutaneous implantation model in a manner that is dependent on the microstructure of HA and presence or absence of plasma.

Statement of Significance

We attempted to elucidate the mechanisms responsible for the foreign body reaction induced by the implantation of hydroxyapatite granules with different microstructures in rat subcutaneous tissue with or without plasma components as the binder of ceramic granules. By analyzing the expression of two reliable osteoclast markers, we detected tartrate-resistant acid phosphatase-positive multinucleated giant cells, cathepsin K-positive multinucleated giant cells, and tartrate-resistant acid phosphatase- and cathepsin K-negative multinucleated giant cells. The induction of tartrate-resistant acid phosphatase-positive multinucleated giant cells was plasma component-dependent while the induction of cathepsin K-positive multinucleated giant cells was influenced by the microstructure of hydroxyapatite. This is the first study to show the conditions dividing the three kinds of multinucleated giant cells in the foreign body reaction.

Abbreviations: FBGC, foreign body giant cell; MNGC, multinucleated giant cell; HA, hydroxyapatite; CTSK, cathepsin K; TRAP, tartrate-resistant acid phosphatase; MMP, matrix metalloproteinase; RANKL, receptor activator of nuclear factor kappa-B ligand; M-CSF, macrophage colony-stimulating factor; DC-STAMP, dendritic cell-specific transmembrane protein; IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor.

* Corresponding author.
E-mail address: tohrupth@nagasaki-u.ac.jp (T. Ikeda).
[1] Present address: Department of Histology and Cell Biology, Nagasaki University Graduate School of Biomedical Sciences, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan.
[2] Present address: Materials Research and Development Laboratory, Japan Fine Ceramics Center, 2-4-1 Mutsuno, Atsuta-ku, Nagoya 456-8587, Japan.

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1. Introduction

Foreign body giant cells (FBGCs) and osteoclasts are both multinucleated giant cells (MNGCs) generated by the fusion of mononuclear progenitor cells derived from a monocyte-macrophage lineage. Osteoclasts are easily distinguished from FBGCs due to their bone resorption ability [1]. Osteoclasts are one of the most important regulators of bone metabolism and are essential for maintaining the homeostasis of bone volume and calcium concentrations in serum. Osteoclasts dissolve bone mineral, chiefly composed of hydroxyapatite (HA) with acid synthesized by their acidification enzymes, such as carbonic anhydrase and proton ATPase [2]. Bone matrix proteins are chiefly digested by matrix metalloproteinase-9 (MMP-9) and cathepsin K (CTSK), both of which degrade collagen, the major component of the bone matrix. Hence, these enzymes are important markers for identifying osteoclasts [3–5]. Tartrate-resistant acid phosphatase (TRAP) and calcitonin receptors have also been identified as important markers of osteoclasts [6]. In the presence of macrophage colony-stimulating factor (M-CSF), osteoclasts are induced by the stimulation of receptor activator of nuclear factor kappa-B ligand (RANKL) through its receptor, RANK, which is expressed in mononuclear macrophages [7–9]. RANK binds with RANKL to stimulate the transcriptional factor NFATc1, which leads to the induction of multinucleated osteoclasts [10,11]. Therefore, RANK and NFATc1 are also important markers of osteoclasts [9]. Multinucleation is considered essential for bone resorption; however, the phenotype of mice deficient in the dendritic cell-specific transmembrane protein (DC-STAMP) or osteoclast stimulatory transmembrane protein (OC-STAMP), both of which are key molecules in cell fusion for the formation of MNGCs, revealed bones with bone marrow cavities in spite of almost no multinucleated osteoclasts existing [12,13]. These findings indicate that mononuclear preosteoclasts have the ability to resorb bone similar to multinucleated osteoclasts.

DC-STAMP- and OC-STAMP-deficient mice have a disorder for multinucleation of not only osteoclasts, but also FBGCs [12,13]. FBGCs have been classified into several groups based on their *in vivo* locations and morphological characteristics; however, the functional diversity of FBGC remains largely unclear. One of the reasons for the difficulties associated with analyzing FBGCs is that there are very limited numbers of useful specific markers to identify these cells [14–16]. FBGCs were previously shown to be induced *in vitro* by the stimulation of interleukin (IL)-4 and/or IL-13 in the presence of granulocyte-macrophage colony stimulating factor (GM-CSF) or M-CSF [17,18]. The biological significance of FBGCs and osteoclasts differs and previous studies also suggested that the biogenesis of FBGCs and osteoclasts are mutually exclusive [19–23]; however, part of the biological mechanism to form MNGCs is similar between these cell types [15].

We have been investigating the repair of bone defects implanted with ceramics using animal models. In the course of our research, HA composed of rod-shaped particles has been synthesized using an applied hydrothermal method [24,25]. HA synthesized with a hydrothermal process (HHA) and HA synthesized by conventional sintering (SHA) have been implanted into bone defects and the resulting biological responses were compared. Our findings showed that the number of osteoclasts was significantly larger in the region implanted with HHA than in that implanted with SHA. Furthermore, a significantly larger amount of bone was induced in the region implanted with HHA than in that implanted with SHA [26,27]. These findings suggest that HHA exhibits more potent osteoclast-homing activity than SHA, which results in the formation of a larger amount of bone in the implanted region. When implanted into bone defects, the calcium phosphate ceramics have been shown to be attached with osteoclasts and involved in the bone generated by osteoblasts [26–28]. This means that calcium phosphate ceramics tend to be recognized as bone equivalent materials rather than foreign bodies if they are surrounded by a large amount of bone. In the extraskeletal tissue, calcium phosphate ceramics are usually recognized by FBGCs as foreign bodies. When calcium phosphate ceramics are implanted into extraskeletal soft tissue, a foreign body reaction may also be influenced by their microstructures, and their microstructures may control certain phenotypes of FBGCs. However, information regarding this issue is still limited [29,30], and the implantation of calcium phosphate ceramics with different microstructures into extraskeletal soft tissue may contribute to examinations on the diversity of FBGCs. In addition, it was suggested that mononuclear osteoclast precursor cells circulate in the blood stream and settle on the surface of bone tissue [31], and HHA and/or SHA may act as scaffolds of osteoclast precursor cells and osteoclast-like MNGCs may be detected on the surface of these implants. In the present study, we implanted HHA and SHA granules into rat subcutaneous tissue and compared foreign body reactions in order to evaluate whether the microstructure of HA influences the phenotype of FBGCs around these implants.

2. Materials and methods

2.1. Preparation of ceramic granules

A total of 13.5 g of α-TCP powder (Taihei Chemical Ind. Co., Ltd., Osaka, Japan) was mixed and kneaded with 67.5 g of 10% gelatin solution, and dropped into a stirred oil bath heated to 80 °C. The bath was then chilled on iced water and spherical α-TCP/gelatin granules were formed. The granules were separated from the oil,

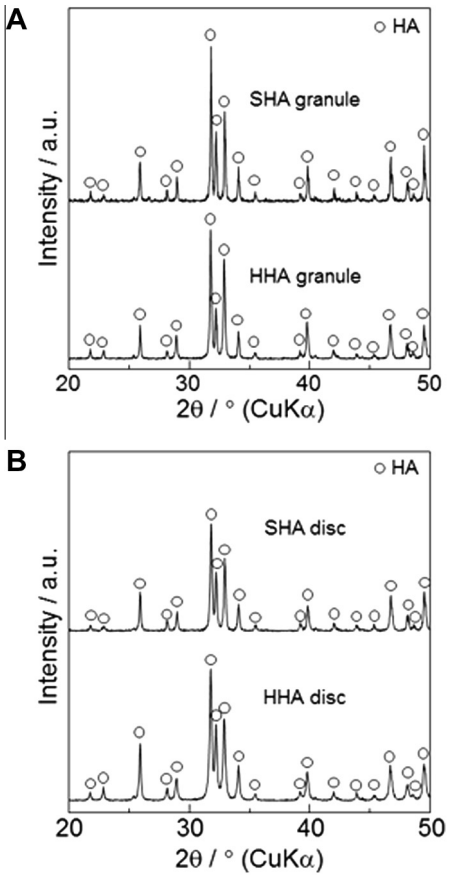

Fig. 1. X-ray diffractometry of HHA and SHA granules and discs used in this study.