Pergamon

Bio-Medical Materials and Engineering, Vol. 4, No. 1, pp. 000–000, 1994

0959-2989/94 $6.00 + .00

0959-2989(94)E0027-F

DEVELOPMENT OF HYBRID TYPE ARTIFICIAL BONE MARROW USING SINTERED HYDROXYAPATITE

KATSUNARI NISHIHARA,* TSUYOSHI TANGE,† KAZUSHI HIROTA‡ and KIYOSHI KAWASE§

*Department of Oral Surgery, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan
†Department of Pathology, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan
‡National Institute for Research in Inorganic Materials, 1-1 Namiki, Tsukuba, Ibaragi 305, Japan
§Kawase Veterinary Hospital, 1-64-11 Sakura, Setagaya-ku, Tokyo 156, Japan

Abstract—*In vivo* inducement of hybrid-type artificial bone marrow with hemopoietic inductive microenvironment (HIM) in sintered hydroxyapatite (HA) chamber was carried out. This research is important to disclose the mechanisms of hemopoiesis and is useful for clinical application. In the evolution of vertebrates, cartilage of the inner skeleton changed into bone, having biomechanical properties to form bone marrow cavities. The hemopoietic nests immigrated into the cavities from the spleen. We should be able to induce hemopoietic nests in a hydroxyapatite chamber in place of bone, if we can find optimal structural conditions. Therefore, we tried to artificially induce a hematopoietic field in muscles using sintered porous tubular hydroxyapatite and new type hydroxyapatite plate made by high-pressure gas technique. As a result, not only in the pore sites of tubular hydroxyapatite artificial bone, but at the surface of the new type hydroxyapatite plate implanted in the dorsal muscles, marked differentiation of bone marrow cell clusters of the hematopoietic field could be observed.

Key Words—artificial bone marrow, hemopoietic inductive microenvironment (HIM), hydroxyapatite chamber, high-pressure gas technique, hybrid-type

INTRODUCTION

BONE HAS characteristic architectural structure, including bone cortex, cancellous bone, and bone marrow. In such architecture of a bone marrow cavity, HIM is commonly induced. The bone marrow structure evolved from inner skeletal cartilage after the second evolution of vertebrates (1). After that, the hemopoietic nests immigrated into bone marrow cavities (hydroxyapatite-collagen chamber), from the spleen (collagen chamber) (2). For an experimental hemopoietic field, muscles were selected, and for bone substitution, conventionally sintered porous tubular HA artificial bone and dense HA plates made by a high-pressure gas technique were used from the following viewpoints: (a) Bone marrow with active hemopoietic potency is generally found in skeletal bone in the muscles; (b) bone is considered to be calcified connective tissue with hydroxyapatite; (c) from a phylogenical point of view, the hemopoietic field immigrated from the spleen or liver to a lumen of a skeletal chamber made of HA and collagen, i.e., bone marrow cavity (2).

As an initial experiment, tubular artificial bones made of porous sintered HA were prepared and implanted into the femur of an adult dog. Thereafter, tubular artificial bones of the same type were implanted in dorsal muscles of other dogs. Then, plates of sintered HA made by the newly developed method, i.e., stoichiometric and nonstoichiometric dense HA plates made by high-pressure gas technique, were implanted in the dorsal muscles of dogs. After phlebotomy, these materials were recovered and specimens were prepared and evaluated microscopically.

MATERIALS AND METHODS

Tubular HA artificial bone (stoichiometric, 20% porosity), 14mm in diameter and 25mm in length with 7-mm diameter lumen, was prepared by Asahi Optical Company Ltd (Tokyo). Granules of HA (Apaceram G-M Asahi Optical Co.) were also prepared. Stoichiometric and nonstoichiometric dense HA plates were prepared by high-pressure gas technique in the National Institute for Research in Inorganic Materials. Pressure and temperature ranges used were 600–800 MPa and 300–400°C, respectively (3). The aforementioned materials were implanted in an adult dog (shepherd) of about 30 kg, as follows: (a) The femur was cut off and the lumen was enlarged to fit the sterilized tubular HA artificial bone. This artificial bone was inserted (Fig. 1), and the femur with the implant was fixed with an A-O plate; (b) the sterilized tubular HA artificial bone was filled with HA granules to prevent dead space growing after implantation, then sunk into venous blood of the recipient dog. After that, the artificial bone was implanted into dorsal muscles; (c) stoichiometric and nonstoichiometric, dense, new type HA plates were implanted into dorsal muscles of several dogs. Eleven weeks postop, a 100 ml phlebotomy was carried out to activate the hemopoietic field. One week after phlebotomy, specimens for histopathological examination were prepared and evaluated microscopically.

RESULTS

From these experiments, the following results were obtained: (a) The femur had normal mechanical function with fixation (A-O plate) after tubular artificial bone implantation. No fracture nor infection was observed postoperatively in the femur. Bone marrow cell regeneration was observed in the lumen of the implanted HA tubular artificial bone (Fig. 2); (b) no infection was observed postoperatively in the sites of implants in dorsal muscles. At the surface of the implanted tubular HA artificial bone, marked blood vessel formation was observed. In the lumen of the artificial bone, marked collagenous fibrous tissue with formation of a reticulo-endothelial system resembling tissues was observed. In fibrous tissue occupying the pore sites of the artificial bone, cell differentiation of hemopoietic nests was

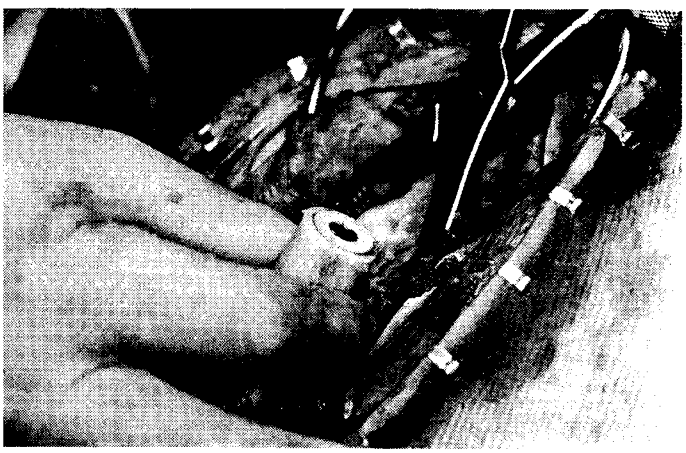

FIG. 1. During the operation, the femur was cut off and the lumen was enlarged, then tubular porous apatite artificial bone was inserted.

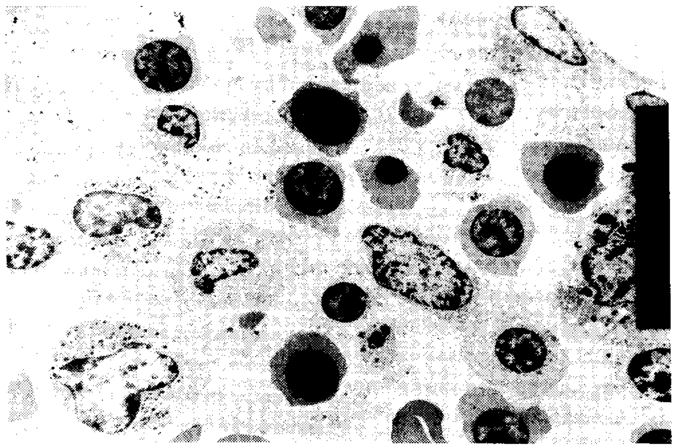

FIG. 2. Electron microscopic observation of regenerated bone marrrow hemopoiesis in tubular HA artificial bone. Erythroblastic islet surrounding central macrophage was observed with granulocytes all around (3 months postop).

clearly observed without any inflammatory cell reaction (Fig. 3); (c) at the outer surface of the collagenous tissue around both the stoichiometric and nonstoichiometric new type HA plates, cell differentiation of the hematopoietic nest was clearly observed with marked blood vessel formation (Figs. 4 and 5). However, no inflammatory cell reaction was observed histopathologically around the HA plates.

DISCUSSION

It has been reported that bone marrrow cells regenerate in grafted decalcified femur in subcutaneous tissue (4). It has also been reported that a composite of apatite ceramics and syngeneic rat bone marrow cells induce intensive bone formation in the porous site of

FIG. 3. At the central part of fibrous tissue occupying the pore site of tubular HA artificial bone, hematopoietic nests regenerated (arrow). HA: Hydroxyapatite.

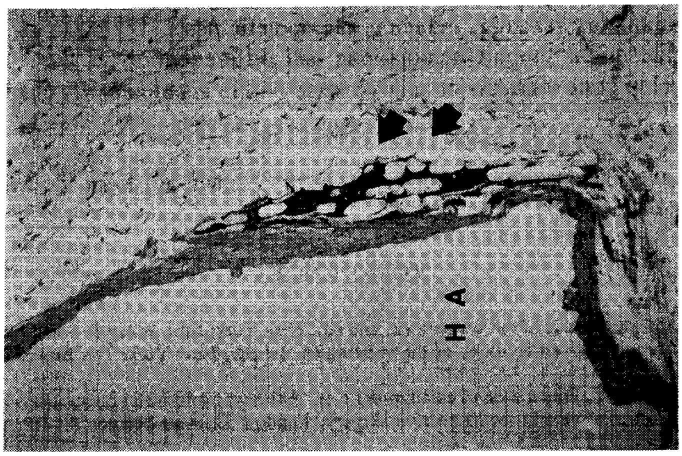

FIG. 4. A hematopoietic nest (arrows) occurred near the outer surface of collagenous tissue covering dense HA plate made by high-pressure gas technique adjacent to adipose tissue. HA: Hydroxyapatite.

ceramics in the subcutaneous tissue of rats (5,6,7). In these studies, without marrow cells, no bone formation in apatite ceramics was observed (6). These experiments aimed at osteogenesis. On the contrary, our interest concerns not osteogenesis but bone marrrow formation because there has been no report so far concerning bone marrrow induction in ceramics chamber *in vivo* leading to the development of artificial bone marrrow of the hybrid type.

From these experiments, it was found that the material properties of sintered synthetic HA were very important for inducement and differentiation of the hemopoietic nest. The difference between conventionally sintered HA and that of the new type is the size of the synthetic HA crystal. The latter is so small that the size of each crystal is almost equal to that of natural bone. In our experiments, it was proved that *in vivo* bone marrrow cells with hemopoiesis could be induced artificially with the use of HA bone implantation in muscle. Moreover,

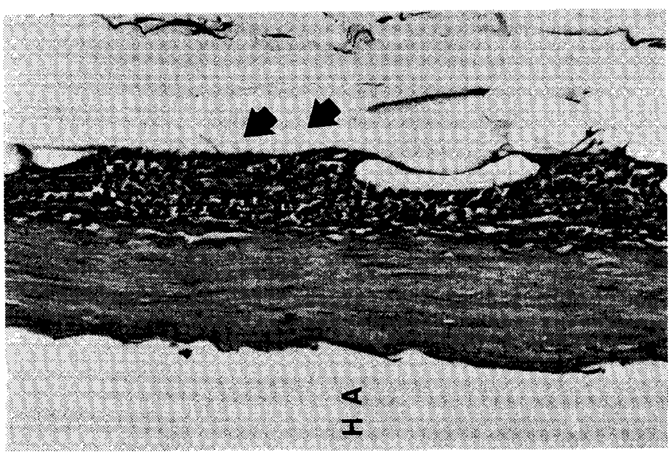

FIG. 5. Hemopoietic nest was observed at outer surface of collagenous tissue around sintered HA plate (arrows). Around hematopoietic nest, no inflammatory cell infiltration was observed. HA: Hydroxyapatite.

sintered HA by high-pressure gas technique similar in crystalline size to natural bone did not cause any inflammatory reaction, inducing hemopoietic nests.

Hydroxyapatite might support hemopoietic cell differentiation *in vivo*. Therefore, it is possible to develop bone marrrow on a large scale within a sintered HA chamber connected to an artery.

Acknowledgement—This work was supported by a Grant-in-aid from the Ministry of Education of Japan (03557107).

REFERENCES

1. Torrey, T. W. Morphogenesis of the vertebrates, 2nd ed., New York: John Wiley & Sons, Inc.; 1967:144–210.
2. Nelsen, O. E. Comparative embryology of the vertebrates. 1st ed., New York: The Blakiston Company, Inc.; 1953:733–736.
3. Hirota, K.; Hasagawa, Y. Preparation of calcium-deficient hydroxyapatite-metal composites. In: Vincenzini, P., ed. Ceramics in substitutive and reconstructive surgery. London: Elsevier Science Publishers BV; 1991:137–145.
4. Mori, M.; Miwa, A.; Mori, S. Regeneration of bone marrow in grafted decalcified femur of C3H mice: The role of bone and bone matrix. Recent Adv. RES Res. 25:36; 1985.
5. Friedenstein, A. J. Precursor cells of mechanocytes. Int. Rev. Cytol. 47:327–355; 1976.
6. Owen, M. Lineage of osteogenic cells and their relationship to the stromal system. In: Peck, W. A., ed. Bone and mineral research 3. London: Elsevier Science Publishers BV; 1985:1–25.
7. Ohgushi, H.; Goldberg, V. M.; Caplan, A. Heterotopic osteogenesis in porous ceramics induced by marrow cells. J. Orthop. Res. 7:568–578; 1989.

(*Received* 15 *February* 1994)