Study on Developing Artificial Bone Marrow Made of Sintered Hydroxyapatite Chamber

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ABSTRACT

Among various possible ways to study hemopoietic inductive microenvironment (HIM), we chose a unique way by developing artificial bone marrow made of a sintered hydroxyapatite chamber. For preliminary study, the following experiments were carried out using dogs: 1) To observe bone marrow reaction to hydroxyapatite (apatite) and zirconium oxide (ZrO_2), artificial roots made of these materials were implanted into the bone marrow; 2) To observe the component effect of sintered apatite upon mesenchymal tissue, apatite granules were applied to a dental pulp chamber clinically; 3) Sintered apatite plates were placed into rib bone marrow; and 4) A section of femur with marrow was removed, after which tubular-type apatite artificial bone was implanted and fixed with an A-O plate. After a fixed period, specimens were made and then histopathologically examined.

INTRODUCTION

From an evolutional standpoint, it is known that HIM had definitely existed in an organ nearest the digestive tract, for example, liver or spleen, in an early stage of phylogenesis. In the second revolution of vertebrates, certain animals abandoned their water environment. Animals had to withstand gravity in activity, and the internal skeleton changed to bone tissue from cartilage.

Bone tissue essentially has a special biomechanical property in that the osteon, the unit of bone tissue, is formed according to repeated principal stress trajectories.

For this property, there gave rise to cavities, i.e., a bone marrow cavity in skeletal bone tissue. In this cavity there occurred a hemopoietic inductive microenvironment(HIM). From this fact of phylogenesis, bone marrow in a chamber made of bioceramics can be artificially induced from mesenchymal tissue, if we can determine the optimal condition of hemopoietic microenvironment in the bone marrow cavity.

The authors carried out experimental studies on developing artificial bone marrow. For preliminary studies, we observed bone marrow reaction to dense sintered apatite and ZrO_2. Then we also observed the component effect of porous sintered apatite upon pulpal mesenchymal tissue of the tooth. A chamber was formed in tooth crown pulp by bur, after which apatite granules were applied(1).
Sintered porous apatite plates were also implanted in rib marrow and tissue reaction was observed. To induce bone marrow tissue in artificial bone, tubular apatite artificial bone was implanted in the femur of a dog. After 2 months, specimens were made and examined microscopically. From these experiments, apatite chambers with active bone marrow structures were to be developed as a model for studying HIM.

METHODS AND MATERIALS

1) Artificial roots made of sintered dense apatite and ZrO_2 were implanted into bone marrow of the mandible of a dog. Artificial roots were implanted deeply enough so that the occlusal surface reached the gingival level. Therefore, no strong occlusal load was applied. After 18 months, decalcified and undecalcified specimens were prepared and observed microscopically and by SEM.
(2) Apatite granules were clinically applied to dental pulp chambers of intact teeth, which were formed by bur. These teeth were to be extracted for orthodontic or pericoronitis treatment. Crowns of the teeth were drilled and coronal pulps were removed by sterilized burs. After that, apatite granules 100μm in diameter were applied to cover the injured pulp surface. Then the granules were covered with apatite cement (Yamahachi Co.). After 3 and 6 months, the teeth were extracted, and tissue formation in the chamber was observed histopathologically.
(3) Apatite porous plates (5mm x 1.8mm with 200μm pores, 20% porosity) were implanted in rib bone marrow just below the rib cartilage. In another case, the same porous apatite plates were implanted outside the rib cartilage, and the former were compared to them. After fixed intervals, (4w-52w) specimens were prepared and microscopically observed.
(4) Tubular apatite artificial bone (20% porosity) was implanted in the femur of a 30kg adult dog. The femur with the implant was fixed with an A-O plate. After 11 weeks postop, a 100ml phlebotomy was carried out. Then one week after phlebotomy, 12 weeks after implantation, decalcified and undecalcified specimens were prepared and evaluated microscopically.

RESULTS

From these experiments, the following results were obtained:

1) Dense apatite was in some part fused directly with bone, but in another part in contact with bone marrow tissue with a fibrous tissue interface (Figure 1). The interfaces between the apatite and calcified tissue resembling bone or cementum appeared porous when observing undecalcified specimens by SEM. The interface between the apatite and fibrous tissue resembling periodontal ligament also appeared porous (Figure 2). At the surface of ZrO_2, cartilage-like tissue was seen outside attaching in some part to the bone marrow structure. In an other part, contacting bone marrow was observed with fibrous tissue interface having normal reticulo-endothelial structure.

2) In the pulp chamber, marked calcified tissue formation rich in blood vessels was observed (Figures 3-5). At the coronal side of the chamber, mesenchymal tissue resembling a reticuloendothelial system (RES) rich in blood vessels was observed in some cases (Figure 4). In the apical side of the pulp, the mesenchymal tissue turned into a collagenous bundle with enlarged blood vessels (Figure 6).

3) In bone marrow of the rib, the sintered apatite plates dissolved and changed drastically in appearance microscopically after 12 months of implantation (Figures 7, 8). Marked cartilage proliferation was observed (Figures 7, 8) compared to the control Figure 9). The apatite was surrounded by calcified tissue. The bone marrow showed no difference from normal rib marrow. Apatite plates in the outer surface of the rib cartilage showed no marked change microscopically 12 months postop (Figure 10).

4) Tubular apatite bone was implanted in femur (Figure 11). Figure 13 shows excised specimens including tubular artificial bone. During fixation, no fracture was observed postoperatively. Figure 12 is a 6-week-postop radiograph. Figure 14 is a radiograph of the specimens 12 weeks postop. Bone marrow formation was observed in the implanted apatite artificial bone (Figure 13).

DISCUSSION

From a preliminary histopathological survey of artificial root implantation, normal bone marrow structures could be built around apatite and ZrO_2 artificial roots surrounded cementum-like calcified or cartilage-like tissue. From these findings, components of apatite and ZrO_2 are assumed to have no influence upon bone marrow structure reconstruction, i. e., no significant foreign-body-capsule formation was observed.

In the pulpal chamber, calcified tissue with vessels resembling the RES regenerated. This regeneration process was assumed to occur as

follows: Immediately after chamber formation with a sterilized bur, no tissue existed there at all. For a while, bleeding occurred. At that time, apatite granules were applied to the chamber to cover the amputated pulp tissue. After that, the apatite granules were covered with apatite cement. In this stage, the chamber was filled with apatite granules with coagulum. The mesenchymal cells adjacent to the coagulum were assumed to differentiate to form granulation tissue with capillaries in the same way as observed in injury healing processes. Later, from mesenchymal cells in granulation tissue, cellular calcified tissue was assumed to be derived in the atmosphere of dental pulp surrounded by dentin. Mesenchymal tissues in pulp near the root apex of the tooth distant from apatite granules were found to change into tissues rich in a collagenous bundle with enlarged blood vessels (Figure 6). Through these healing processes, blood vessels potentially forming an RES were supposed to remain in the calcified tissues. From these experiments, it was ascertained that some tissues could be regenerated around apatite granules in a chamber made of calcified substance.

From these results, we assumed that it was possible to derive bone marrow in tubular apatite artificial bone. In this atmosphere in dental pulp, apatite granules induced calcification from the mesenchymal cells. On the contrary, porous apatite plates in rib bone marrow were observed to dissolve and change in appearance with marked rib cartilage proliferation 12 months postoperatively. The atmosphere in rib bone marrow and dental pulp are assumed to be quite different. However, a bone marrow structure with an RES could be observed around the dissolved apatite.

It is reported that a composite of apatite ceramics and syngeneic rat marrow cells induced intensive bone formation in the porous region of the ceramics in the subcutaneous region of rats, (2,3). However, without marrow cells, no bone formation in apatite ceramics was observed (2). These experiments involved osteogenesis. On the contrary, our interest concerned not osteogenesis but bone marrow formation. Although it has been reported that growth of bone marrow cells on porous ceramics can occur in vitro, there is no report concerning bone marrow induction in ceramics chamber in vivo.

In tubular apatite artificial bone filled with coagulum, bone marrow could be derived with trabeculae. From these findings, it is assumed possible to develop bone marrow in an artificial bone chamber made of apatite which is connected to an artery. We found that the apatite chamber can be utilized for an artificial organ in vivo, in which specifically differentiated organ cells can be cultured.

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

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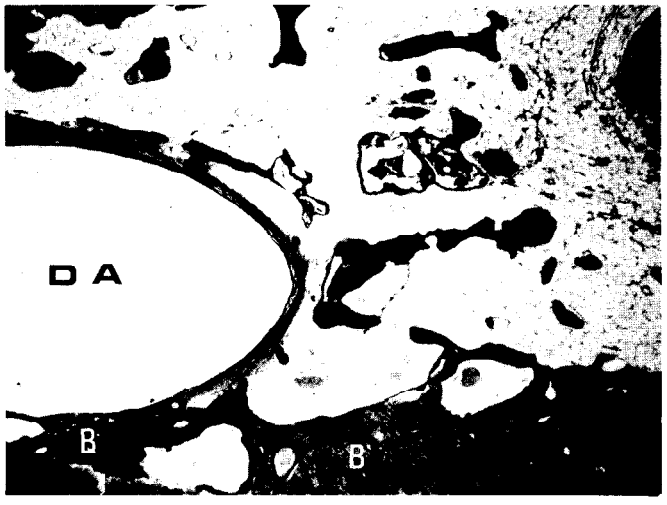

Figure 1. Dense Apatite (DA) in bone marrow. In some parts apatite fused directly with bone (B). In other parts in contact with bone marrow tissue with a fibrous tissue interface.

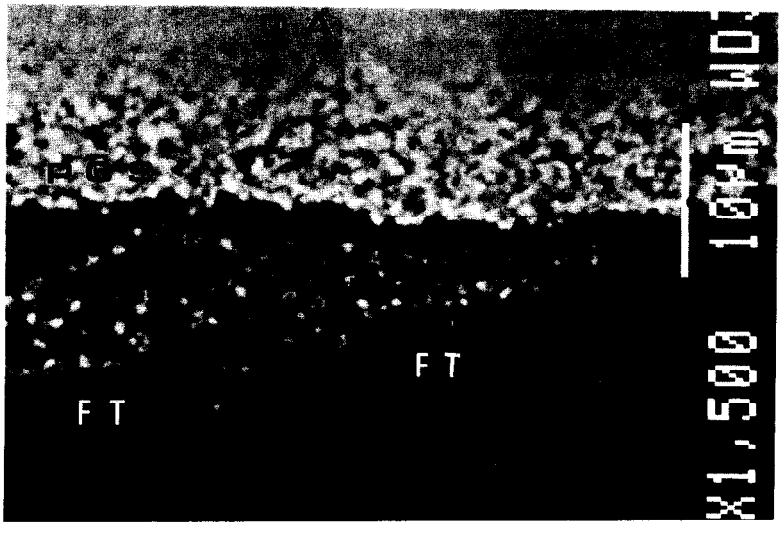

Figure 2. Interface between dense apatite (DA) and fibrous tissue (FT) with porous calcified substance (PCS).

Figure 3. Pulpal chambers containing apatite granules covered with apatite cement (AC), decalcified specimens.

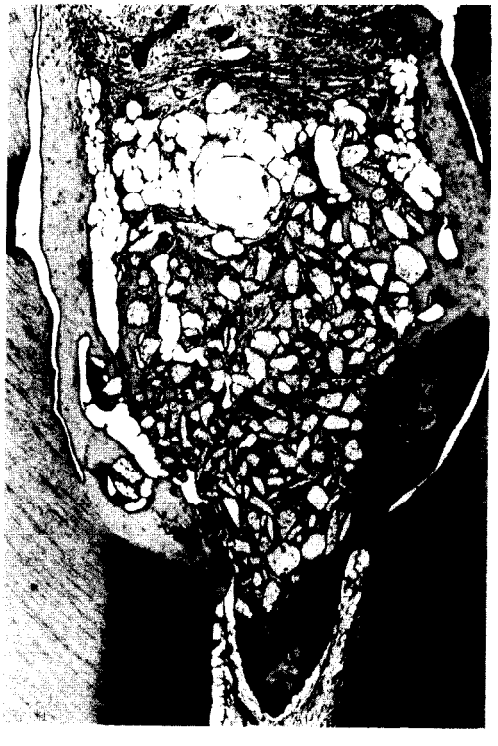

Figure 4. Calcified tissue formation near pulpal tissue in chamber with RES rich in blood vessels at the upper side.

Figure 5. Part of Figure 4. enlarged

Figure 6. Pulpal change in collagen-rich tissue with enlarged blood vessels near apex.

Figure 7. Absorbed apatite plate in rib. Enlarged cartilage with normal bone trabeculae formation inside is observed with normal bone marrow structure 12 months after implantation.

Figure 9. Rib bone marrow with cartilage covering. (control)

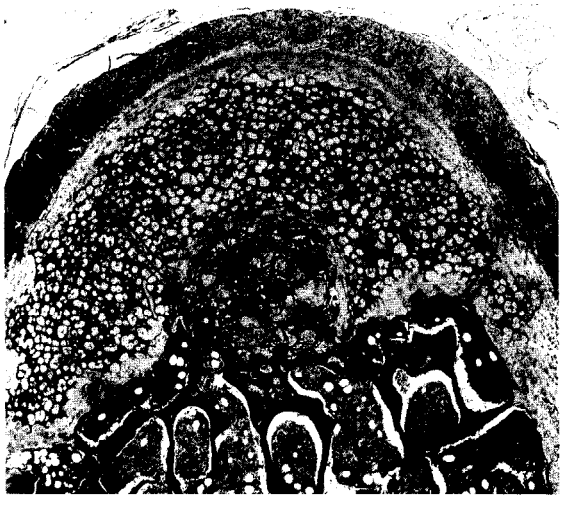

Figure 8. Dissolved and calcified apatite in rib marrow 12 months after implantation.

Figure 10. Apatite implanted into outer surface of rib cartilage 12 months after implantation. (control)

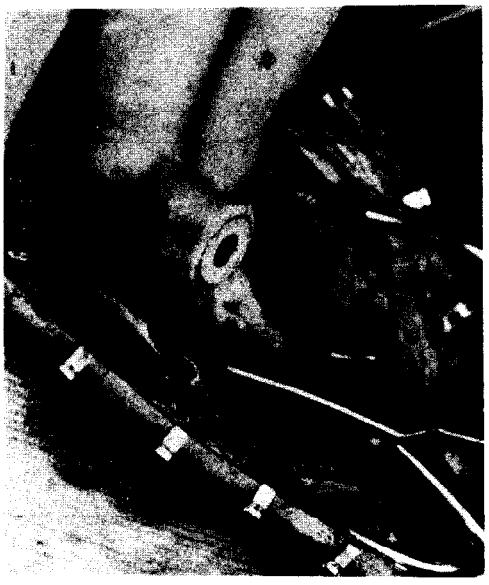

Figure 11. Tubular apatite artificial bone implanted in femur.

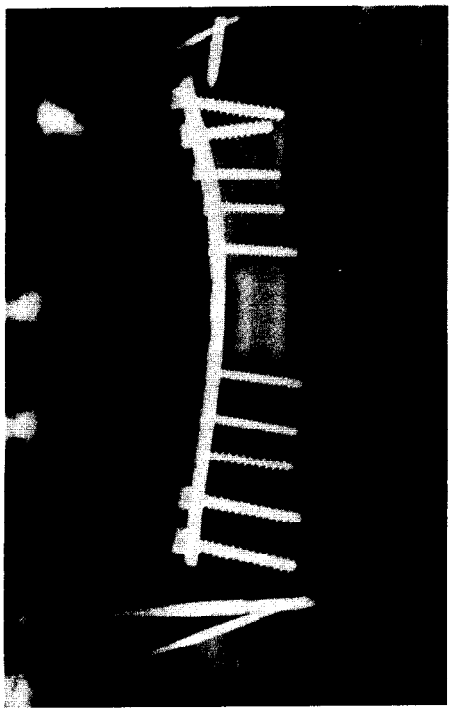

Figure 12. Tubular apatite artificial bone implanted in femur, radiograph, 6 weeks postop.

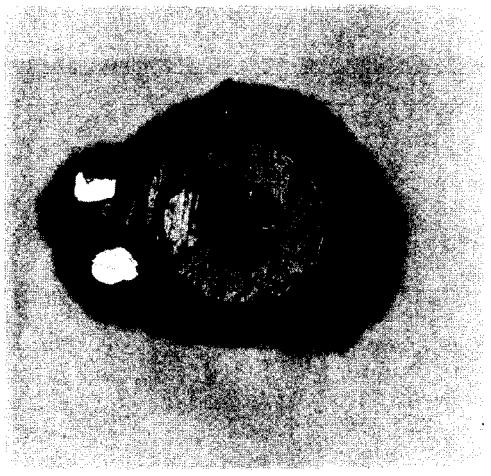

Figure 13. Specimen of implanted tubular apatite artificial bone. Bone marrow formation is observed.

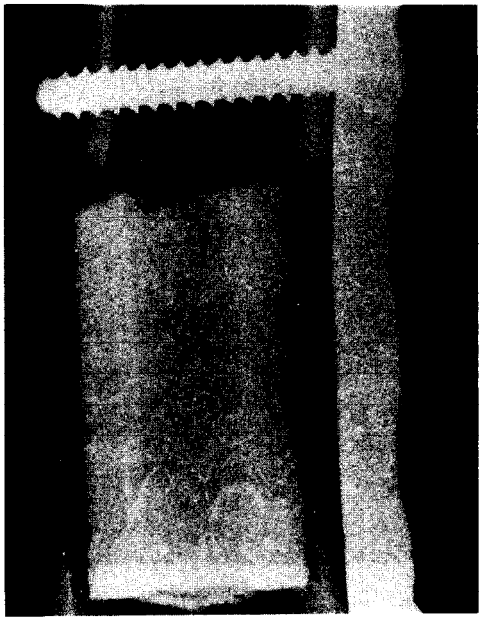

Figure 14. Radiograph of specimen 12 weeks after implantation.