1）発表論文　A）英 文

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D2-2A Artificial Inducement of Bone Marrow Hemopoiesis by Electric Bio-Chamber of Titanium

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ABSTRACT

Authors disclosed that remodeling of skeletal organ by biomechanical stimuli is controlled by treaming potentials, which is converted from repeating nechanical loads in organisms (1). Therefore, emodeling of osseous tissue conjugated with bone narrow hemopoiesis can be controlled by electric device. 'he authors developed titanium (Ti) electric bio-hamber with 10μA current. This research is based on rilateral research, which is integrated by means of iomechanics, molecular genetics in remodeling, norphology, and metabolism (2,3). Using bio-chamber rith electricity or bioceramics we can disclose the nechanisms of Wolff's law of functional adaptation of one, i.e., the system of remodeling in skeletal organ (4).

NTRODUCTION

According to the mechanism of adaptation sclosed, it can be understood that shape of organism nanges under use and disuse theory. Therefore ansformation of the shape can be transmitted to the ext generation, not only by genetic cord of hard formation of DNA but by the fixed usage of skeleton in ganisms (1). The fixed usage of body is transmitted by lucation, i.e., soft information to the next generation other organisms. Therefore, morphological character n be transmitted even with same genetic cord (hard formation of homeobox), if the soft information are ansmitted to next generations (1). Applying the ilateral research on archetype vertebrates, the thors also developed experimental evolutionary udies (1,4). By implanting electric bio-chamber into arks (chondrichthyes), which have no osseous inner eletons with bone marrow but only cartilage ones, hemopoietic nest resembling bone marrow can be induced in vertebral cartilage. From this fact Neo-Darwinism of evolution can be completely denied. Use and disuse theory of Lamarck is re-established as biomechanics-corresponding evolution (1,4).

Fig.1 Bio-chamber of sintered HAP with a 10μA current for implantation into mammalian subcutis

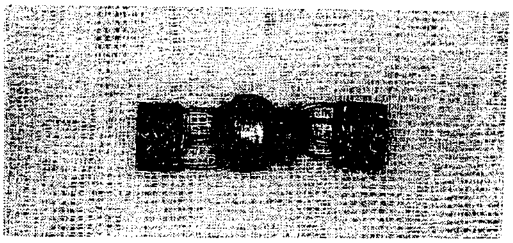

Fig. 2 Bio-chamber of Ti with 10μA current for shark muscle

MATERIALS, METHODS, AND RESULTS

The following experimental evolutionary studies were carried out using artificial skeletal organs.

1) Development of several kind of artificial bio-chambers;

① Artificial bone marrow chambers made of conventionally sintered HAP by Asahi Optical Co. and Sangi Co. for implantation into muscle of animals (5-7)

② Artificial bone marrow chambers made of sintered HAP (Fig. 1) and titanium (Ti) mesh (Fig. 2) with a

10μA current by National Inst. for Research in Inorganic Mat (10) for implantation into subcutis of animals (4).

2) Implantation of the artificial bone marrow chambers of HAP and Ti with or without a 10 μA current into subcutis of dogs.

3) Implantation of HAP and Ti bio-chamber with or without a 10 μA current into muscles of sharks (8).

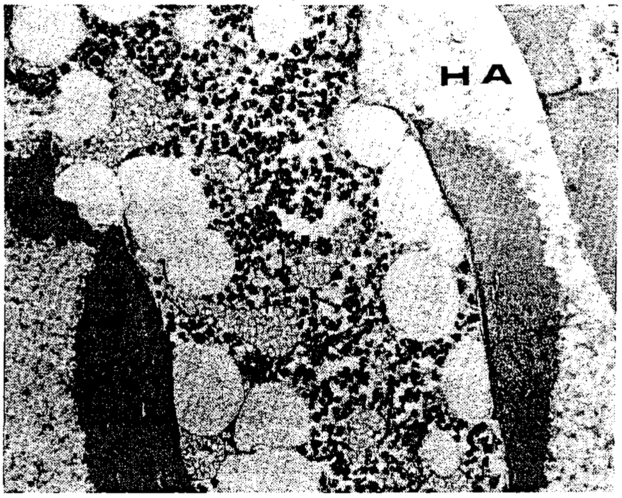

Fig. 3 Hematopoiesis induction in HAP bio-chamber, 12 months after implantation in muscle of Japanese monkey

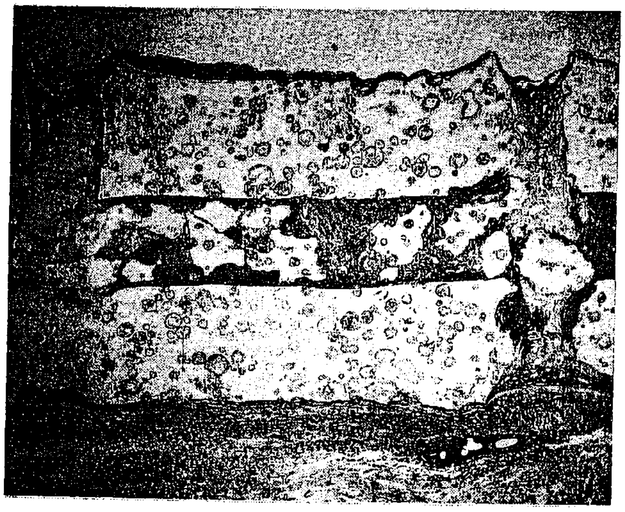

Fig. 4 Hemopoiesis induction in the HAP bio-chamber with 10μA current implanted in subcutis of a dog, 4 months

Artificial induction of hemopoiesis was carried out successfully in each of the newly developed chambers for mammals and chondrichthyes. Marked leukocyte and lymphocyte induction was seen around the Ti bio-chamber with a 10μA current in a dog 4 months after implantation. Leukocyte production induced from undifferentiated mesenchymal cells by the electrical current around the Ti was observed.

Figure 3 shows induction 12 months after surgery of hemopoiesis in conjunction with osteogenesis by the HAP bio-chamber implanted in a Japanese monkey. Osteogenesis as well as hemopoiesis could be observed around the chamber.

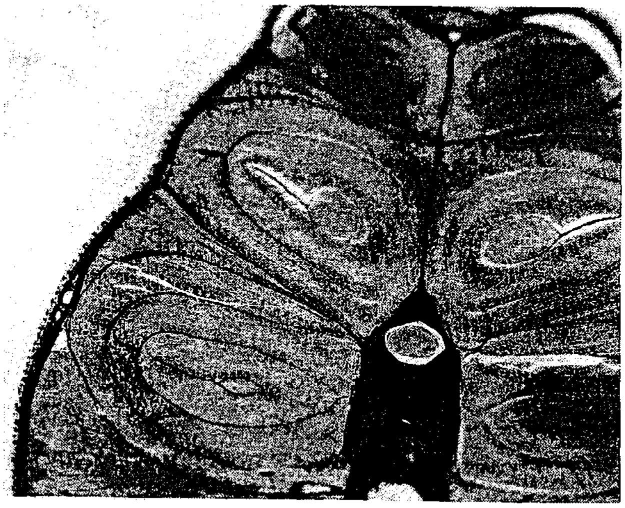

Fig. 5 Cross section of a shark for control

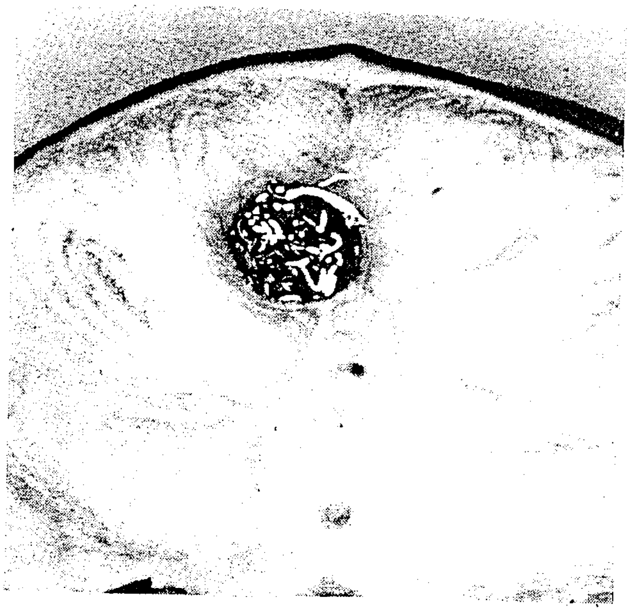

Fig. 6 Cross section of a shark with Ti bio-chamber

Figure 4 shows induction 4 months after surgery f hemopoiesis in conjunction with osteogenesis by the IAP bio-chamber with 10μA current (Fig. 1) implanted ıto subcutis of a dog. Hemopoiesis and osteoid ɔrmation were observed 4 months after surgery around ıe HAP bio-chamber implanted in the shark muscle.

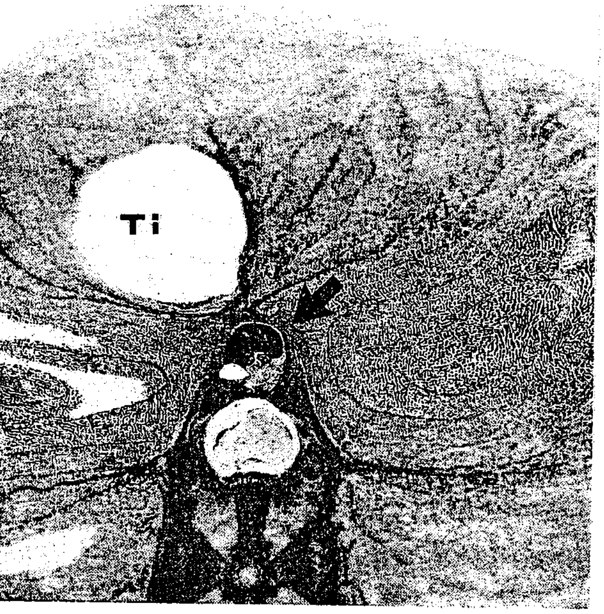

Fig. 7 Cross section of a shark with Ti bio-chamber implanted, 4 months after surgery

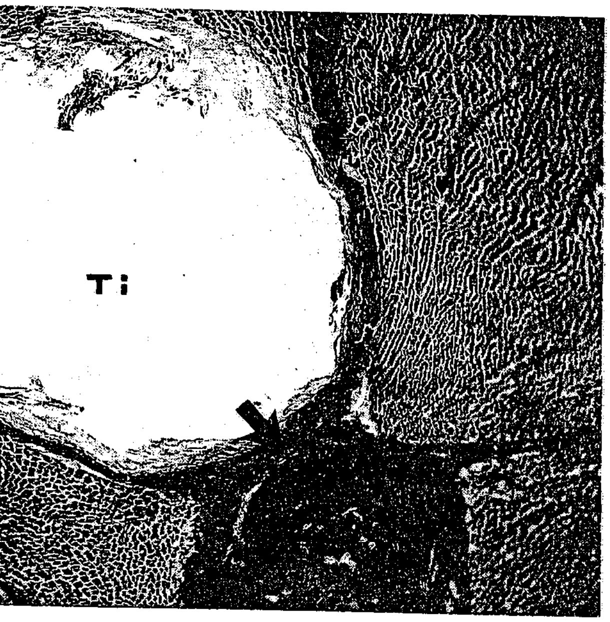

Fig. 8 Hemopoietic marrow induction (arrow), 4 months after surgery

Figure 5 shows a cross section for control of a shark (dochi). No bone marrow in the cartilaginous tissue around the spinal cord (arrow) is evident. Figure 6 shows a cross section of a shark with a Ti bio-chamber with a 10μA current (Ti), 4 months after surgery.

Figure 7 shows the histological findings associated with the dorsal cartilage with hemopoietic marrow induction (arrow) by the adjacent Ti bio-chamber (Ti). Figure 8 shows histological findings of induced hemopoietic marrow in the dorsal cartilage.

DISCUSSION

The authors developed a hybrid-type artificial bone marrow chamber that uses sintered hydroxyapatite (HAP) which was able to induce hemopoietic nest formation in conjunction with osteogenesis heterotopically in mammalian muscle but not subcutaneous tissue (3-10). This process has been believed to proceed in bio-chambers by heterotopical hemopoiesis that is induced by the streaming potential of sintered HAP, which trigger genetic expression of mesenchymal cells to drive cytocaine of the bone morphogenetic protein (BMP). In order to investigate this hypothesis, the following preliminary experiments were carried out:

1) Transplantation of an HAP bio-chamber with BMP into the subcutis (1). 2) Transplantation of an HAP bio-chamber with a 10 μA current. 3) Measuring the streaming potential of sintered HAP using a physiological saline solution (1). 4) Transplantation of a titanium (Ti) mesh with a 10μA current into the subcutis (1).

The preliminary results demonstrated that biomechanical stimuli were converted into streaming of the organic body fluid, ultimately resulting in the induction of a streaming potential (1). Therefore, the author concluded that heterotopical hemopoiesis in the bio-chamber is induced by genetic expression of the mesenchymal cells. Based on this assumption, Ti artificial bio-chambers with a 10μA current were developed.

To further investigate this theory from a biomechanical perspective, a trilateral research method that integrates morphology (Goethe), molecular biology (Delbrück), and physiology (Bernard) was developed. Based on this research method, the mechanisms associated with morphogenesis and function appear to originate from genetic expression, which are driven by biomechanical stimuli such as environmental factors.

An experimental evolutionary study in which the trilateral research method is employed was first proposed by the present authors in 1995 (2,3). In this study, biomechanical stimuli are applied to biomaterials in sintered hydroxyapatite chambers that have been implanted into various animal muscles during different phylogenetic stages. A hybrid-type artificial immune organ that induces hemopoietic bone marrow tissue was developed successfully not only in mammals but also in chondrichthyes (sharks), which are relicts of the archetype that does not possess bone marrow tissue in their internal skeletons (4).

The use and disuse theory proposed by Lamarck has been revived as a result of recent molecular genetic studies. Alberch reported that the recapitulation theory (Haeckel) could be explained by the heterochrony of genetic expression (11). The use and disuse theory can also be explained by heterochrony, because all the functions of muscle cells and osteocytes are controlled through gene expression of mesenchymal and neural cells, which are triggered by physicochemical stimuli (also considered to be biomechanical stimuli) that affect the topical cells of the organism (1). Based on this trilateral research, evolutionary changes in vertebrate morphology can be understood as a series of biomechanical events, as described by Lamarck (1). Thus, evolutionary changes can be induced at the cellular level in the mesenchyma of heterospecies through biomechanical stimuli. This approach is referred to as an experimental evolutionary study (1).

The substance that defines the vertebrate structure is the hydroxyapatite-collagen complex skeleton that forms bone and teeth (2,3). Vertebrates can be further distinguished by the presence of hemopoiesis in the bone marrow cavity of higher reptiles and mammals. This is not seen in lower vertebrates such as chondrichthyes and cyclostomata. Structural differences also allow mammals to be distinguished from lower vertebrates. These consist of a highly evolved viscerocranium with gomphotic teeth and jawbones (2,3). Combining the understanding of the importance of hydroxyapatite in vertebrates with the effects of biomechanical stimuli, an experimental evolutionary research model can be developed at the cellular level (1). Using such a model, a new technique in tissue engineering called the hybrid-type artificial immune organ has been developed (7). If evolution is the product of biomechanical stimuli as proposed by Lamarck, heterospecific and heterotopical evolutionary changes should be possible to induce at the cellular level in the mesenchyma (7). The present paper investigates the correlation between the immunity system and evolutionary changes. Furthermore, this paper presents a new concept of immunology that involves a cytological digestion system (4), a new theory of biomechanics-responsive evolution (1), and a simple theory to explain biological reactions.

The present results suggest that the trigger for hemopoiesis induction in conjunction with osteogenesis is a streaming potential (12-15) which is converted from fluid flow that occurs due to repeated optimal loading. Cellular functions can be categorized as follows. 1) metabolism, 2) skeletal construction, 3) muscle activity, 4) planocytes and phagocytes for cytological respiration and digestion, 5) signal transmission of neural cells, 6) absorption of nutrients and oxygen, 7) excretion of catabolites, 8) support of metabolites for epithelial cells Almost all cells are capable of cell division and remodeling. Osseous cells function as a support against mechanical stress. They form the basic skeleton of the organism in response to biomechanical stimuli. Almost all cellular functions are ultimately carried out through genetic expression of each cell.

Morphology, metabolism, and remodeling are all products of cellular function. These cellular functions are also products of genetic expression of cells. Through trilateral research that integrates morphology, metabolism, and molecular genetics of remodeling with responses to biomechanical stimuli, these three different categories can be studied as the same molecular genetic phenomenon with three different features.

Thus, genetic expression appears to be under the control of the general biomechanical stimuli. The present results demonstrate that the migration of hemopoiesis from the spleen into the bone marrow cavity occurred as a result of gravity that became a factor during the terrestrialization period of evolution. The only difference between archetype vertebrates and higher vertebrates is blood pressure. Mammals have substantially higher blood pressure than do sharks. By loading a mechanical stress of 1G, blood pressure should increase dramatically in sharks that have very low blood pressure in water with approximately 1/6 G.

During terrestrialization, archetype vertebrates (sharks) initially experienced difficulty breathing due to the lack of respiration without water, and writhed in response to the suffocation. As a result, blood pressure increased. During surgery, bleeding was not apparent in most areas of shark muscle. Thus, gravity appears to be the primary influence on the elevation of blood pressure during terrestrialization. This in turn induced a higher streaming potential (12) than that in the water environment. Therefore, hemopoietic nest migration appears to be a direct consequence of the introduction of gravity.

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