DIFFERENTIATION INTO HEMOPOIETIC CELLS FROM MESENCHYMAL CELLS IN POROUS CERAMIC BONE MARROW CHAMBER SURFACE IN VIVO BY MEANS OF HYDRODYNAMICS

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ABSTRACT

Using sintered porous hydroxyapatite, artificial bone marrow chambers were developed by the author, in which mesenchymal cells were differentiated into hemopoietic cells in muscle. For this work, the author received an award from the 32^{nd} Congress of the Japanese Association of Artificial Organs. Using adult shepherd dogs (35kg), two groups of experiments were carried out; 1, Artificial bone marrow chambers were implanted into subcutaneous tissue in two dogs; 2, artificial bone marrow chambers were implanted into dorsal muscles of the two dogs. After 6 months, the artificial bone marrow chambers were recovered under general anesthesia. These specimens were observed with light microscopy. In all chambers implanted into subcutaneous tissue, no hemopoietic cells were observed. In all chambers implanted into dorsal muscles extirpated, marked hemopoietic nests were observed in porous sites of sintered hydroxyapatite. Several experiments were carried out to find out the phenomena of highly differentiated functional cells by bioceramic skeletons. The author hypothesizes that hydrodynamics are converted to streaming potential at the surface of the ceramics.
In conclusion, the author has developed in vivo artificial bone marrow chambers by means of surface reaction of ceramics using hydrodynamics.

INTRODUCTION

Phylogenically it is well known that in evolutionary process heamopoietic bone marrow had developed during the second revolution of the vertebrates, i.e., landing.
During landing inner skeleton of cartilage had changed into bone with haemopoietic marrow. Therefore causal factor of development of bone marrow hemopoiesis can be found by experimental evolutionary studies, which the author has developed by means of biomechanical stimuli.
To disclose inducement of highly differentiated functional cells at the surface of bioceramics, it is necessary to know surface behavior of mesenchymal cells against ceramic artificial bone. Therefore, it is necessary to analyze trilateral effects, i.e., material, shape and functional effect of the ceramic artificial organ upon surrounding mesenchymal cells. As material for artificial bone marrow i.e. material effect, phosphate and mineral ions are essential for surface behavior of mesenchymal cells as components to induce hemopoiesis.
Certain shapes including surface condition of porous sintered ceramics of artificial skeletal organ influence upon mesenchymal cells as hydrodynamic stimuli in moving muscles. In the subcutaneous tissue no strong movement of fluid as in muscle is maintained except in capillary circulation. Therefore in subcutaneous tissue no marked shape or functional effect of ceramic organ was observed as a surface reaction of mesenchymal cells, except material effect.

Functional effect of an artificial organ implanted into muscles means the muscle movements, which influence the surface of ceramic chambers by hydrodynamics of blood and lymph.
After that the author carried out several experiments to find out the phenomena of highly differentiated functional cells by ceramic skeletons.
From experiments, the author analyzed and determined the causal factors for developing bone marrow homopoiesis in vivo in muscles.

EXPERIMENTS

1) Experimental evolutionary study using chondrichthyes
To find out physical change in landing inducing bone marrow hemopoiesis, artificial landing an hour a day for 10 days of two Triakis (shark) and two Heterodontus japonicus (dog shark) were carried out. After that they were compared with control shark in physical and morphological changes.

2) Animal experiment to analyze trilateral effect
To observe material and functional effect of artificial bone marrow chambers (ABMC) made of sintered porous hydroxyapatite(HA) and porous tricalciums phosphate (TCP) were implanted into subcutaneous tissue and dorsal muscles of dogs. For shape effect sintered porous HA and compact HA were implanted into dorsal muscles of dogs.

3) Measured of streaming potential of HA
It is well known that bone is generated by streaming potential and bone can be generated by 10mV potential in orthopaedic treatment.
Therefore streaming potential of HA with $NaHPO_4$ (0.005F) + NaH_2PO_4 (0.005F) solution with 0 to 180 cm. water pressure was measured.

4) Artificial bone marrow systems made of titanium electrode with 10 mV were developed and implanted into subcutaneous regions muscle, and spleen of dogs. They were implanted into dorsal muscle of sharks, which had no bone marrow hemopoiesis.

5) Artificial bone marrow chamber of HA and TCP with BMP (bone morphogenetic protein) were implanted into subcutaneous regions of dogs.

RESULTS

1) It was disclosed that elevated blood pressure in shark 15cm Hg in water up to 30cmHg in land was major factor to induce bone marrow hemopoiesis.

2) As material effect of HA and TCP upon mesenchymal cells implanted in subcutaneous region, no reaction but macrophages around both materials was observed. As functional effect of HA and TCP implanted in muscles marked hemopoietic cells as well as osteogenesis could be observed (Fig. 1). As shape effect no marked hemopoiesis and osteogenesis could observed around compactly sintered HA and TCP but marked hemopoiesis concomitant with osteogenesis could be observed in pore site of porously HA sintered as well as TCP.

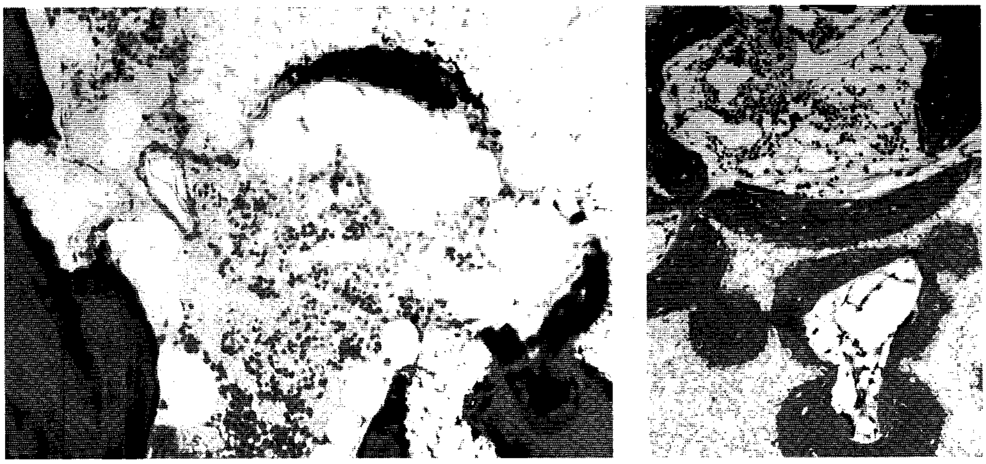

Fig.1 Hemopoiesis in artificial bone marrow chamber (A.B.M.C.)
Left: TCP Right: HA

3) Streaming potential of HA as well as TCP were measured as shown in Table 1. Around titanium electrode implanted into subcutaneous as well as is muscle, marked leukocytes hemopoiesis without ossification could be seen. Around the electrode chamber implanted into the spleen of dogs marked degeneration into fibrous skeletal tissue from hemopoietic spleen cells were observed. (Fig. 2)

Table 1

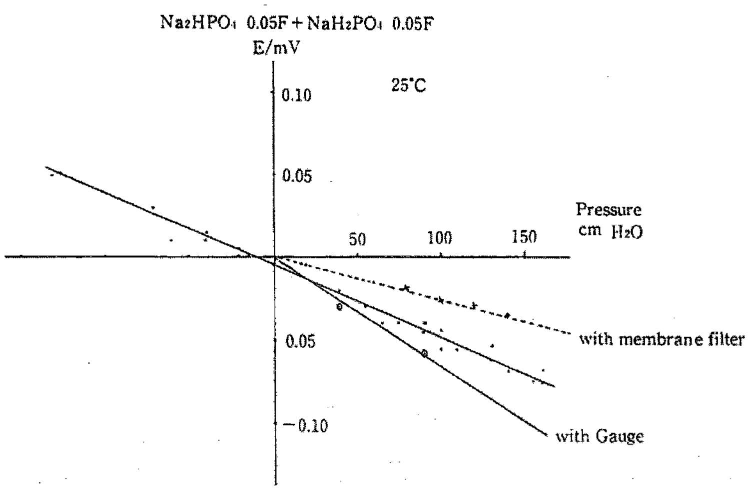

Table1 Sfreaming potential of HA generated by hydrodynamics with $NaHPO_4$ (0.005F) + NaH_2PO_4 (0.005F) solution

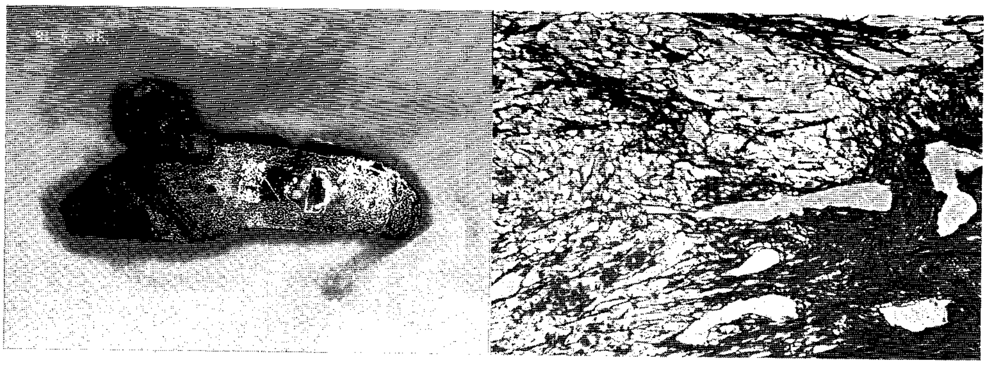

Fig.2 Ti electrode A.B.M.C. implanted in spleen(L) Tissue of the spleen around it (R)

4) The upper sites of spine in sharks dorsal muscle marked hemopoietic nest with osseous tissue could be observed, in which titanium electrodes were implanted.
5) In artificial chamber of HP and TCP with BMP marked hemopoiesis concomitant with bone formation in subcutaneous region could be seen. (Fig. 3)

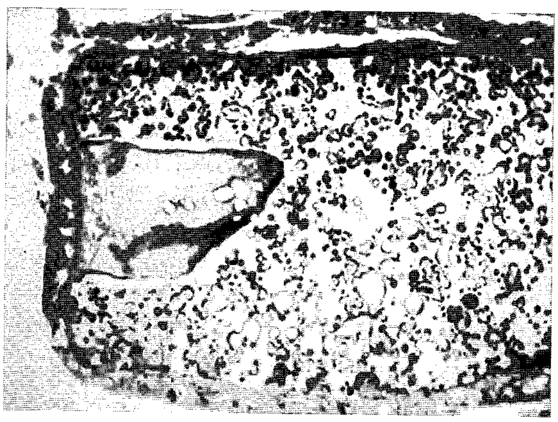

Fig. 3 A.B.M.C. implanted into subcutaneous tissue with BMP 2months after implantation

DISCUSSION AND CONCLUSION

From the series experiments followings are disclosed; all biomechanical stimuli influenced upon the skeletal system of animals are converted into hydrodynamics concomitant with streaming potentials in organisms. Because organisms are constructed by water-soluble colloid, therefore biomechanical movement of animals essentially generate streaming potential in organisms.
The streaming potentials trigger gene expression of the mesenchymal cells to induce BMP and by the cytokine differentiate hemopoietic cells and osteoblasts from undifferentiated mesenchymal cells. By 10 mV current even in shark muscles

hemopoietic nest with osteoblasts can be induced by metaplasia, which have no hard bone in inner skeleton but placoids (dermal teeth). In spleen 10mV current induce fibrous skeletal tissue from hemopoietic cells. In subcutaneous region 10 mV current can induce only lenkocytes but not osteoblast. Electrode with HA in subcutaneous tissue can induce hemopoietic nest concomitant with osseous tissue. Therefore, calcium, phosphate and streaming potential are essential to induce BMP. Even in jawbone, around artificial root streaming potential induces cementoblasts (osteoblast with fibrous tissue) and the alveolar bone proper concomitant with hemopoietic nests. (Fig. 4) Therefore, the mechanisms in induction of hemopoietic nest with osseous tissue by artificial bone marrow chamber in muscles and the mechanisms in induction of lamellated ossification with hemopoiesis of the peri-root supportive system around artificial root in jawbone are quite the same. (Fig. 5)

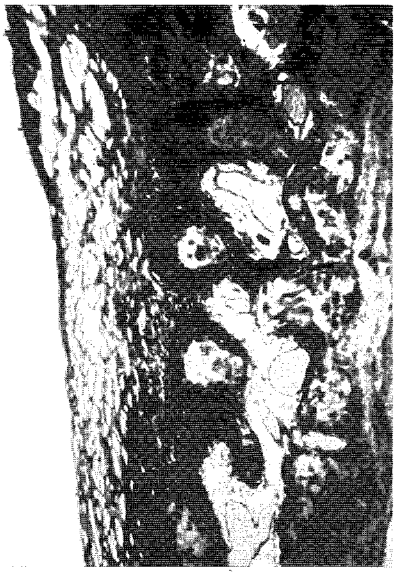

Fig. 4 Peri-root tissue formation around artificial root:
Cementogenesis and lamellar bone formation are observed concomitant with hemopoiesis.

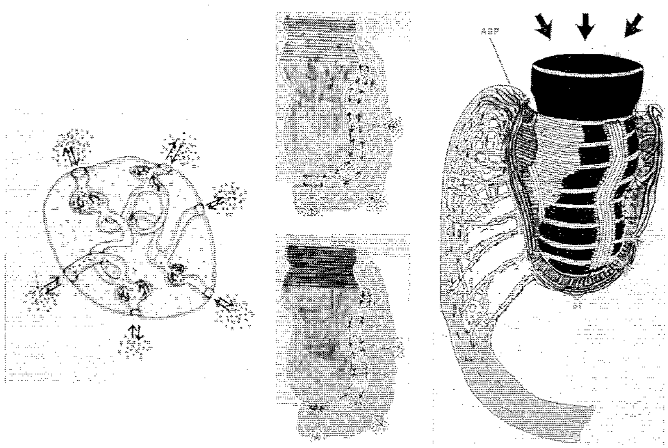

Fig. 5
Hemopoiesis as well as pri-root tissue formation are carried out by same mechanism concurrent with hemopoiesis.

Biomechanical stresses (stimuli) applied to ceramic artificial organ implanted in vivo are converted in organisms into hydrodynamics, which are synthesized not only biomechanical fluid dynamics brought about shape effect of ceramic organ and hydrodynamics generated by heart pump. The synthesized hydrodynamic flows are concurrent with streaming potential and they approximate to vector of the principal stress trajectories of the biomechanical stresses (stimuli). According to the vector osteoblasts concurrent with hemopoiesis as well as osteoblasts develop.
All water-soluble colloids are electrified, therefore, hydrodynamic flows of blood essentially are concomitant with streaming potential. Current is the electron-transferring system, therefore any kind of current in vivo can induce hemopoiesis concurrent with ossification in sufficient existence of calcium ions and phosphate.
Under the ankylosis system of artificial root and bone no hydrodynamic flow develop around the root in jawbone during occlusal function. The principal stress trajectories run continuously though the joint border of the implant and bone which have different material constants. Implant failure or bone destruction occurs by different material constants under repeating loading.
The functional biomechanical stresses applied to the skeletal system of animals in repetition of stresses by hydrodynamics as well as streaming potentials. This is the realities of the Wolff's Law of functional metamorphosis of the bone.
As conclusion, in organisms biomechanical stimuli are converted to hydrodynamic energy concomitant with streaming potential, which can induce BMP to differentiate bone marrow hemopoietic cells and osteoblasts as metaplasia in skeletal mesenchymal tissue.

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