DEVELOPMENT OF REVOLUTIONIZING METHOD FOR CREATING HYBRID-TYPE ARTIFICIAL ORGANS USING CERAMICS BY MEANS OF ELECTRIC ENERGY

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SUMMERY

Revolutionizing method for creating hybrid-type ceramic artificial organs was developed by means of biomechanical energy, for the first time in the world which was converted into streaming potential, which induced BMP(bone morphogenetic protein) gene expression of recipient cells in vivo. Using sintered hydroxyapatite, I developed in vivo artificial bone marrow chambers, as well as artificial roots, on which hemopoietic cells concomitant with osteoblasts (cementoblasts) were induced in the muscle and jawbone from undifferentiated mesenchymal cells as metaplasia by means of hydrodynamic energy. In the osseous system, it is known that in the Haversian system, streaming potential is generated by the hydrodynamics of the blood stream not only during cardiac circulation and muscle movement but also during repeating movement of the osseous system. I consider that hydrodynamics converted to streaming potential at the surface of ceramics just as in the osseous Haversian system. To verify this, the streaming potential of sintered hydroxyapatite was measured with physiological saline solution. Subsequently the author hypothesizes that with a streaming potential of 5~10mV, the gene expression of the mesenchymal cells is triggered to induce BMP(bone morphogenetic protein) by which mesenchyme can differentiate into osteoblasts concomitant with hemopoieteic cells. This is metaplasia induced by streaming potential, which triggers the gene like a catalyst in a chemical reaction. After that, I developed an artificial bone marrow chamber using titanium electrode with a 5~10mV current. They were implanted into the subcutaneous, muscle tissues and spleen of dogs, as well as into the muscles of Triakis (shark) which has no bone marrow hemopoietic nests. In all cases, hemopoietic nests were induced around the electrode chambers except the spleen. In conclusion, in vivo induction of osteoblasts and cementoblasts on the ceramic surface concomitant with hematopoietic tissues from mesenchymal cells can be verified to be metaplasia by means of surface reaction of ceramics, i.e., surface behavior induced by the ceramic components concomitant with streaming potential generated by hydrodynamic energy.

1. INTRODUCTION

The definitive substance of the vertebrates is the osseous tissue, therefore if synthetic ceramic osseous tissue, i.e., sintered hydroxyapatite (HA) can be developed, the riddle of evolution of the vertebrates can be read. From the precise research on phylogeny, I comprehended immigration of the hemopoietic system from the gut to the bone marrow developed during the second revolution of the vertebrates, i.e., the landing and the gompholic tooth system during the third revolution, i.e., the birth of the mammals. To verify the former I developed experimental evolutionary research meth-

ods using Mexican Salamanders (Axolotls), thus I evidenced that in axolotls development of osseous tissue with bone marrow hemopoiesis from cartilage occurred during landing by elevated blood pressure. In recent research, several papers concerning molecular mechanisms of electrically stimulated NGF (nerve growth factor) expression were reported. After that I hypothesized the causal factor developing the both skeletal systems of the hemopoiesis and gomphosis is biomechanical hydrodynamic energy, which is converted into the streaming potential, and the potential concomitant with material effect of HA triggered the gene expression of mesenchymal cells to generate bone morphogenetic protein (BMP). To verify the causal factor of induction of highly differentiated skeletal cells from mesenchyme, surface behavior of two kinds of ceramic organ, i.e., bone marrow and root in the muscle and bone were investigated respectively. As a result, metaplasia of mesenchymal muscle cells or periostium cells occurred by BMP developing bone marrow hemopoiesis as well as the gompholic system. Thus, molecular mechanisms of evolution can be explained as the metaplasia of cells. To evidence this hypothesis, I conceived revolutionizing method to develop hybrid-type ceramic artificial organs, which were implanted into the large muscle and the jawbones, to both of which the repeating biomechanical loads were applied. In organisms, the energy of biomechanical loading of skeletal system is converted into hydrodynamic flow concomitant with streaming potential. I considered that the mechanisms to develop hybrid-type artificial organs by biomechanical stimuli were dependent upon the surface reaction, i.e., the surface behavior of the material and energy effect, i.e., streaming potential, which can induce highly differentiated osseous as well as hemopoietic cells as metaplasia from the recipient own stem cells.

2. New methods to develop hybrid-type artificial organs *in vivo* by means of biomechanical energy.

From the viewpoint of vertebral evolution, by observing results of experimental evolutionary studies and surveying from the stand points of biomechanics, the highly differentiated skeletal system, i.e., hemopoietic bone marrow as well as the gompholic articulation system can be understood to have been evoked by means of biomechanical movement of structures in organism according to the Use and Disuse Theory. This biomechanical movement of skeletal organs is convèrted into hydrodynamic energy concomitant with streaming potential. Therefore, causal factors to develop hybrid-type artificial ceramic organs can be understood as surface reactions, i.e., surface behavior of mesenchymal cells, which can be analyzed as trilateral aspects of material (components), shape, and functional effect. Material effect of ceramic surface means elemental or chemical effect of ceramic components upon differentiation of mesenchymal cells. Functional effect means energy effect of various kinds of biomechanics loading on ceramic organs upon the surrounding mesenchymal cells. Shape effect depends upon function of ceramic organ, because organisms are constructed of the closed system of hydrodynamic fluid medium. Therefore, around the ceramic organ with peculiar shapes implanted into organisms, there take place definite hydrodynamic conditions, which are generated by functional loading on ceramic organ. The loading stresses are dispersed by the shape of the ceramic organ and scattered stresses distribute around the ceramic organ in tissue. These distribution patterns of the principal stress trajectories as well as the Mise's equivalent mean shape effect. The principal stress trajectories coincide with vectors of hydrodynamics concomitant with streaming potential. As well, the Mise's equivalent pattern means dis-

tribution patterns of strength of stresses. At the end of trilateral chain reaction of ceramic surface upon mesenchymal cells, there takes place electrically stimulated BMP gene expression of mesenchymal cells. As a result mesenchymal cells forming cartilage, muscle, tendon, and periosteum change into osteoblasts as well as cementoblasts concurring with hematopoietic cells. These phenomena are called metaplasia in pathology and these metaplasias are the molecular mechanisms of evolution.

3. Development of the artificial bone marrow chambers

3-1. Artificial bone marrow chamber of hydroxyapatite and Ti electrode

I developed several types of artificial bone marrow chambers made of sintered compact and porous HA, which were implanted into the dorsal muscle of dogs. @These artificial organs implanted into the subcutaneous tissue, induce neither hemopoietic cells nor osteoblasts, but macrophages. On porous sintered HA implanted into the deep dorsal muscle, marked osteogenesis concomitant with hematopoietic cells could be observed according to channel of pore in sintered HA. Hetero-topical osteognesis, i.e., in the muscle without osseous tissue started from 3 months after implantation. Hematopoietic cells were observed by electro microscopy. Porous HA chambers with BMP extracted from cattle-bone were implanted into the subcutaneous tissue of dogs and their specimens recovered, and observed. Quite similar patterns of osteogenesis with hematopoiesis, compared with HA chambers which were implanted in muscle without BMP, could be seen. Based on this hemopoiesis concurring with osteogenesis in subcutan with BMP, I concluded that hemopoiesis in ceramic chamber implanted in the muscle was caused by BMP gene expression of mesenchymal cells induced by streaming potentials. After study of streaming potential by Pollack, I measured streaming potential of sintered HA during flow of physiological saline solution with 80-120 cm water pressure. The potential was detected 5~100mV for generating hematopoiesis concurring osteogenesis in vivo.
After that titanium chamber of electrode with 10mV developed and implanted into the subcutaneous tissue of dogs. One month after implantation, a marked quantity of white blood corpuscles, i.e., leukocyte-hemopoiesis could be observed. Leukocyte is considered to have been brought about by material effect of titanium.

3-2. Experimental Evolutionary Study using titanium electrode

Ti electrode chambers were implanted into the dorsal muscle of Triakis (shark). 3 months after operation, chambers with the surrounding muscle were recovered to make preparations for light microscopic observation. Marked hemopoietic nest formation with ossification could be observed above the vertebrae. As the shark has no osseous tissues of inner skeletons, therefore, this successful inducement of bone marrow hemopoiesis means the induction of inner bone in hetero-species which have no osseous and hemopoietic system in bone marrow. Through this experiment the causal factor of evolution of the vertebrates can be verified as biomechanical hydrodynamic stimuli, which are converted into streaming potential, by which gene expression of mesenchymal cells are triggered. This phenomenon is called metaplasia. Thus, not only in mammals but in chondrichthyes hemopoiesis can be induced by 10mV current. From these researches, I concluded that BMP was induced by streaming potential in muscle from undifferentiated mesenchymal cells, and these cells differentiated into osteoblasts concurring with hematopoiesis.

4. Development of gompholic artificial

Researches on formation of the peri-root structures, i.e., the alveolar bone proper, cementum, and periodontal ligament by biomechanical hydrodynamics were carried out. The author invented animal experiments to disclose the mechanisms differentiating peri-root structures by biomechanical hydrodynamics applying masticatory movement with fluctuation of 0.1~0.2 mm width. Using same shaped AR, the ankylotic system was developed by complete rest of roots with soft diets. Biomechanical experiments were also carried out to verify conversion mechanism of hydrodynamic energy into electric current. Details are described in research on the artificial root in this symposium. By these synthetic researches it was evidenced that on and around the artificial root surface with fluctuating movement of 0.1~0.2 mm width, parallel and orthogonal flows were evoked, which were equivalent to the principal stress trajectories. After that on both sides of the surface, i.e., the root and the alveolar bone proper cementoblasts, namely osteoblasts with fibers developed in concurrence with hemopoiesis. Thus fibrous ligament-articulation, i.e., gomphosis also developed two kinds of osseous structures, i.e., the cementum as well as the alveolar bone proper, by means of hydrodynamics around the ceramic root with smooth surface.

5. Discussion and conclusion

The later 20century, it was said that to induce osteoblasts concomitant with hemopoietic nests as well as cementoblasts with hemopoiesis from mesenchymal muscle tissue are impossible. Conventionally the bone marrow hemopoietic system has been used to analyze what cytokine is necessary to differentiate hemopoiesis from stem cells, after that, in vitro these cytokines has been added into tissue culture of stem cells. Although even numerous cytokines have been found for hemopoiesis, in vitro development of bone marrow hematopoietic chamber has not been successful. Even in vivo, in the muscle none have been successful to develop the hemopoietic system heterotopically namely, out of the bone tissue. Conventional researches to develop the gompholic systems are tried to culture the articulation system in vitro vigorously and after that the systems are tried to implant in animals. These are divided into two ways. The first is to develop total tooth from embryonic tooth germ, and the second is to develop an artificial dental root with having cementoblasts with periodontal ligament. In the former method, it takes much time, and to control shape and size are quite difficult. As well, the latter, to develop in vitro cementoblasts, i.e., osteoblasts with fibrous ligaments is absolutely difficult.

Conventionally, life science has been studied from the stand point of "the law of constancy of mass". From the view point of "the law of energy conservation", I researched the evolutional process of the vertebrates, especially that of second revolution of the terrestrialization, i.e., landing, and discovered the major causal factor of the evolutional changes during landing to be biomechanical reaction of organisms against increased gravity action, i.e., energy. In this stage we have to realize what the energy is. In 20 century, the law of energy conservation was discovered, and the mass-energy conversion has been carried out as atomic energy. Therefore, it is obvious that the energy is substance without mass. Ignoring the energy as the causal factor of the evolution, trilateral riddles of the vertebrates, i.e., the evolutionary cause, the immune system, and the development of bone marrow hemopoiesis are never read. I have read the trilateral riddles by developing HA artificial bone marrow chambers as well

as HA gompholic artificial root by biomechanical stimuli. Researching the evolution of the vertebrates from the view point of "the Law of energy conservation", especially the second revolution of the landing and the third revolution, i.e., the birth of the mammals, the author hypothesized that the bone marrow hemopoietic and gompholic system, which were the characteristic newly differentiated system in each evolutionary stages respectively, had evolved by biomechanical loading, to which organisms reacted to survive. To verify the hypothesis, the author developed the artificial ceramic hemopoietic chamber and artificial ceramic root and carried out biomechanical studies with experiments.

In conclusion, the mechanisms of in vivo induction of osteoblasts and cementoblasts on ceramic surface concomitant with hematopoietic tissues from mesenchymal cells has proved to be same as metaplasia by means of investigating surface reaction of ceramics, namely surface behavior induced by electric current of streaming potential generated from hydrodynamic energy.

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