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An Experimental Evolutionary Study Using Sintered Hydroxyapatite

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Abstract

Hydroxyapatite is characteristic skeletal substance in vertebrates. Evolution is based on morphological changes in the hydroxyapatite-collagen skeleton (Halstead). Roux founded Biomechanics on the correlation between morphogenesis and mechanical factors. The authors propose a trilateral research program in which Haeckel's Biogenetic Law, Roux's biomechanics and Delbrück's molecular genetics are integrated. Using this trilateral approach it is possible to conduct experimental evolutionary research. This experimental procedure is here introduced through the vertebrate-characteristic hydroxyapatite skeleton using biomechanics, which played a crucial role in evolutionary change.

By precise study of the evolution of the hemopoietic organs, gravity, following the landing of vertebrates is known to be a causal factor in bone marrow hemopoiesis [1]. In the same way, careful study of the evolution of the gomphotic tooth shows micromovement of the tooth to be an essential causal factor for gomphosis [2]. Fossil reptiles with the ankylotic tooth used not a masticatory but a prehensile system. During the acquisition of mastication, erupting teeth suffered physiological occlusal stress through the masticatory function. As a result of long-term masticatory function the tooth root could not attain ankylosis, and periroot supportive tissues were induced during eruption of the tooth. These phenomena can be explained by heterochrony, which means a time-phase delay in expressing the gene of somatic cells. Taking the beforementioned viewpoint, the authors carry out experimental evolutionary research, applying biomechanical stimulation to artificial dental roots and artificial bone marrow chambers made of hydroxyapatite.

Key words: biomechanics, evolution, hydroxyapatite, hemopoiesis, cementoblast

Introduction

Haeckel proposed his Biogenetic Law in 1866, which was morphologically established through precise observation and comparison of the ontogeny and phylogeny of vertebrates. Roux, Haeckel's successor, established Biomechanics. He was convinced that embryogenic development as observed in ontogeny could be understood as the genetic expression of phylogeny and that the cause of

evolution was biomechanical stimulation just as in Lamarck's Use and Disuse Theory. Following Roux's concept of Biomechanism for Causal Embryology, the authors developed a program of experimental evolutionary study.

By applying biomechanical stimuli to sintered hydroxyapatite artificial organs, highly differentiated cells with several functions can be induced from undifferentiated mesenchymal cells. The cementum was categorized as fibrous bone by Weidenreich. Therefore, cementoblasts can be easily induced by biomechanics just as osteoblasts can. Also hemopoietic cells can be easily induced in conjugated cell differentiation with osteogenesis. Artificial skeletal chambers made of sintered porous hydroxyapatite chambers are implanted into shark and cyclostomata muscles. Around them hemopoietic nests can be observed.

Materials and Methods

1) Artificial dental roots made of sintered hydroxyapatite (Asahi optical Co. Ltd.) with a corrugated configuration are implanted in dogs and Japanese monkeys. A stress shielding appliance for mastication is set for ankylotic artificial roots. Gomphotic roots are developed by solid diet feeding immediately postop in dogs. In monkeys, free standing artificial roots become ankylotic 3 years postop, even with solid diet feeding. Specimens are observed optically and by scanning election microscope.
2) Artificial bone marrow chambers are fabricated by Asahi Optical Co. Ltd. They are implanted in the dorsal and femoral muscles of dogs and monkeys. They are recovered 1,2,3, and 12 months postop. Histopathological examinations are carried out.
3) Artificial bone marrow chambers fabricated in the National Institute for Research in Inorganic Materials are implanted into the muscles of sharks and cyclostomata, which have no osseous inner skeleton.

Results

1) Not only cementoblasts but also the alveolar bone proper with functionally oriented fibrous tissue can be observed in specimens recovered from dogs fed a solid diet. Ankylotic artificial root with cortical bones, which have very thin osseous tissue (ca. 10μm in thickness) around the apex of the root can be observed in specimens recovered from monkeys with the stress shielding appliance.
2) Marked bone marrow hemopoietic nests with osteogenesis can be observed in artificial bone marrow chambers implanted into the muscles of dogs and monkeys.
3) Hemopoietic nest can also be observed in artificial bone marrow chambers implanted into the muscles of sharks and cyclostomata.

Discussion

Morphological changes in the evolution of vertebrates involves not only the embryological developmental stage but cytological morphogenesis. Namely, development in embryogenesis is evoked through cytological differentiation, which we believe to be induced by biomechanics in a broad sense, i.e., physico-chemical stimuli. Inducement of highly differentiated cells is based upon

gene expression of undifferentiated mesenchyma by combined biomechanical stimuli and the material effect of hydroxyapatite. Through the experiments here reported, highly differentiated cells with specialized functions such as osteoblasts, cementoblasts, chondroblasts, fastia, periosteum, fibroblasts, and hemopoietic cells as well as adipocites can be induced *in vivo* artificially by biomechanical stimuli in conjunction with the material effect of hydroxyapatite [2,3,4]. These biomechanical stimuli applied in experiments can be regarded as equivalent to mechanical as well as material factors in evolutionary changes.

The experiments shows that evolutionary causality can be understood as biomechanical stimuli in a broad sense including gravity, temperature, pressure, light, scent, oxygen, and bicarbonate as well as a variety of nutrients including hydroxyapatite. Therefore, we can easily develop hybrid-type artificial organs such as artificial bone marrow chambers, chambers for tissue culture of liver, pancreas, and immune organ tissue, using newly sintered hydroxyapatite fabricated by a low temperature (40°C) high pressure technique. Bone marrow hemopoiesis took place in evolution as a result of the terrestrialization (landing) of the vertebrates (S. Miki)[1]. Therefore, migration of hemopoietic nests from the visceral-gut-organ (spleen) to the somato-skeletal bone marrow cavity occurs by the mechanical influences on vertebrates of the gravity of I G, which is cancelled in water by buoyancy [1]. This evolutionary change caused by biomechanical responses to gravity in animals is certainly inherited in all vertebrates. Therefore, evolutionary causality is essentially biomechanics just as in Lamarck's Use and Disuse Theory, not natural selection after mutation as Neo-Darwinists claim.

The genetic expression of mesenchymal cells is dependent upon biomechanics. Therefore, biomechanical stimuli can trigger off gene expression in organisms. Heterochrony can be understood as the delay or disuse of gene expression by biomechanical stimuli. Cartilagenous fishes like the shark are archetype pre-vertebrates. Thus the shark has no osseous inner skeleton, and the spleen and liver still constitute the hemopoietic organ. As they have no bone marrow in their system, their mesenchymal cells have no possibility of naturally inducing hemopoietic cells in bone marrow cavities. However, inducement of hemopoietic nests occurs in artificial bone marrow chambers of sintered hydroxyapatite. This phenomenon can be explained as the gene expression of shark mesenchymal cells by the material effect of hydroxyapatite concomitant with streaming potential [5]. Virchow said over a hundred years ago that Dawin's Theory of Evolution could never be proved because it had happened in the archeozoic era. However, evolutionary phenomena can be researched using experimental procedures as Haeckel and Roux both thought. As evolution results from physico-chemical stimuli, evolutionary processes can be studied by means of trilateral experimental procedures.

Conclusion

Experimental evolutionary research using hydroxyapatite was carried out from a trilateral viewpoint of combined procedures. The results show that physico-chemical stimuli, i.e., biomechanics in a broad sense have a causal influence in evolutionary processes.

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