DISCLOSURE OF MECHANISMS OF EVOLUTION BY MEANS OF A HYBRID-TYPE TISSUE ENGINEERING SYSTEM

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The definition of the vertebrates is "chordates having bony vertebrae exhibiting various degrees of ossification." During evolution, the skeletal joint system changes with the ossification of skeletal cartilage, including vertebrae.
Therefore, if an artificial skeletal substance can be synthesized, the mechanisms of evolution can be disclosed by means of inducing skeletal joint systems characteristic of mammals. Articulation with leukocyte-hemopoiesis, i.e., the joint system of skeletons, is one of the most important characteristics of mammals. The other key characteristics are the respiratory diaphragm, the lymphovessel system, the homoiothermic system, and the genitourinary system. The circulatory system based on hemopoiesis intermediates between the external respiration system and the internal respiration of mitochondria.
The author has developed a bioceramic artificial bone marrow chamber as well as bioceramic artificial gompholic dental roots by means of a hybrid method by which, in vivo, highly differentiated hematopoietic cells conjugated with osteoblast and cementoblast are induced by means of the gene expression of recipient mesenchymal cells around ceramics via hydrodynamic energy.
The author discovered that the conditions of gene expression was restricted to harmony of shape, material and functional effects of the artificial organ in vivo. The author also disclosed that shape and functional effects are equivalent to biomechanical stimuli: since organisms exhibit inner pressure, hydrodynamic energy acts on the bioceramic organ by means of biomechanical movements of the skeletal structure. The author has verified that hydrodynamic energy is converted into streaming potential and this potential triggers the gene expression of mesenchymal cells around ceramics. This means that a major cause of metamorphoses in evolution is the result of biomechanical stimuli, in a broad sense, because the hemopoietic system in skeletal articulation and the gompholic system are quite restricted in mammals, i.e., the highest evolved stage of the vertebrates.
Following this discovery, the author proposed the Gravity Evolutionary theory whereby the mechanisms of morphological as well as functional evolution can be disclosed as a phenomenon of the metaplasia of organ cells according to Lamark's Use and Disuse theory.

INTRODUCTION

Bones define the vertebrates, and articulations with hemopoiesis, i.e., synovial as well as vertebral joints composed of cartilage and osseous tissue with sufficient blood supply, are one of the most important characteristics of the mammals. [1, 2] Therefore, if artificial bone marrow with hemopoiesis could be developed, the causal factor of hemopoiesis would be disclosed to be the cause of evolution. The definitive organs of the vertebrates are the gut respiration system, i.e., gills and lungs, and the external respiration system.

Cellular respiration, i.e., the internal respiration system, is carried out by mitochondria, which were parasitic prokaryote inside eukaryote resembling aerobic bacteria about 1.8 billion years ago. The cardio-vascular system combines external and internal respiration by the medium of blood and lymph fluid. The bone and external respiratory organs are the definitive structure and system of the vertebrates and they are combined by the articulation system with hemopoiesis, i.e., the generator of blood cells. These systems function for mitochondrial metabolism. After porous sintered hydroxyapatite (HA) granules were developed, the author implanted porous hydroxyapatite chambers in the subcutaneous tissue of mammals. No change in porous hydroxyapatite could be observed. However, the author has been able to develop in vivo artificial bone marrow chambers (ABMC) which were implanted in the muscle of not only mammals, including dogs and Japanese monkeys, but also in archetypical vertebrates as well, i.e., sharks and cyclostomata, which have no hard bones with hemopoiesis. Through these experiments, the cause of morphological evolution was disclosed to be biomechanical stimuli. The author, therefore, proposed the Gravity Evolutionary theory, which is behind Lamarck's Use and Disuse theory: [3, 6]

1. What are vertebrate evolutionary phenomena from the viewpoint of material composition, morphology, function, molecular genetics, and biomechanics?

Here we examine the evolution of vertebrates from the viewpoint of material composition, organs, molecular biology, and physical and chemical factors. In the second revolution of vertebrates, i.e., terrestrialization in the Devonian period, two dramatic changes occurred in the change from water to air as a habitat with a concomitant change in gravitational force (from 1/6G, due to buoyancy, to 1G), as well as in the change from branchial to pulmonary respiration [3, 4]. In addition, the cartilaginous endoskeleton ossified. Salamanders and lungfish are relics of this evolutionary stage. During terrestrialation, drastic changes of biomechanic stimuli occurred in a broad sense. These include energy without mass, e.g., force of gravity as well as substance with mass, e.g., oxygen. They are known as the following five physicochemical stimuli: 1) gravity of 1/6G in water (baoyancy) to 1G (land) or 6 times stronger energy, 2) 0.7% oxygen content in sea water to 21% in air, or 30 times greater, 3) water as a life-medium containing minerals becomes 1/800 lighter as air without minerals, 4) complete "wetness" in water to complete "dryness" in air, and 5) highly viscous water versus extremely low viscosity of air. As the result of gravitational action, dog sharks could adapt by increasing blood pressure as they struggled to try to escape suffocation by returning to water. Consequently, endoskeleton cartilage developed into osseous tissue with bone marrow hemopoiesis. A thirty-fold increase in oxygen caused the sixth gill epithelial membranes to move into the pericardiac sack to develop air sacks (of the lungs during) respiration during the struggle to escape suffocation. Dryness as well as the lack of minerals in the air influenced the placoids (dermal teeth) to develop fur, which are in fact placoids made of collagen without minerals. The high viscosity of water brought about the streamlined shapes of organisms in water. The extremely low viscosity of air brought about common shapes without streamlining after landing. This is epitomized by the changes in morphology and functions in the organs of archetype vertebrates after landing as seen by respiratory gills, the dermal region, and inner skeletons. Metamorphosis is disclosed to be a phenomenon of metaplasia. In pathological terminology, this means a change of cells from one type into another with the same genetic code by biomechanical stimuli, i.e., via physicochemical changes, including energy.

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The constituents of active living organisms, cells, need time in order to remodel themselves. This remodeling of cells over time is termed "cellular regeneration," and the remodeling of the organisms over generations is termed "inheritance." When cells are remodeling, it is essential that genes are duplicated, and this duplication takes a certain amount of time.

While cells are being remodeled by physical and chemical stimuli, including energy acting on the organism, the effects on the genes of the regenerating cells reflect the effect of these stimuli, and subsequently the function or form of the cell will change in proportion to the strength and duration of the stimuli. From this, it can be seen that the morphology and the function of organs in vertebrates can change, as well as the form of the animal itself, even with the same genetic characteristics. In pathology, these phenomena are called metaplasia at the cellular level. This mechanism of change in the skeletal organs as a result of biomechanical stimuli within one generation is known as Wolff's law of functional adaptation. [7]

The mechanism of change in the morphology and function, e.g., development of bone marrow hemopoiesis as well as development of the spleen in vertebrates over a number of generations (phylogeny) is termed evolution. [8]

In mammalian evolution there are three major remarkable changes:

1) Immigration of hemopoietic sites from the gut into bone marrow articulation;

2) Metamorphoses of the gill respiration system into the lungs and various glands; and

3) The establishment of the suckling and masticatory system.

Therefore evolution (metamorphosis) can occur as the result of physical and chemical stimuli that act on the remodeling of vertebrate cells with the same genetic characteristics. Mutation in a "genital cell" may occur only once in several million times during the process of duplication. Therefore, the genetic codes of these genital cells change slowly as far as molecular evolution is concerned, after metamorphoses takes place as a result of stimuli acting on vertebrates.

This is an evolutionary law of vertebrates corresponding to biomechanics. To disclose the mechanisms of evolution, synthetic research methods, i.e., Lamarck's law from the stand point of biomechanics, Haeckel's Biogenetic law from genetics, and experimental evolutionary studies are carried out.

1) Research on Lamarck's Use and Disuse theory concerning evolutionary biomechanics

The biomechanical interpretation of the Use and Disuse theory is that animals in the course of development can change the shape or function of their organs and structures (e.g., skeletons) as the result of repeated biomechanical stimuli which influence these organs and structures.

The theory presented here can therefore be interpreted as an evolutionary theory which takes responses to biomechanical (gravitational) stimuli into account with regard to the use and disuse of organs and structures. These responses are strictly defined as the results of the effects of biomechanical energy that trigger the genetic expression regarding the function of the cells in the organ. From a biomechanical standpoint, the major evolutionary changes during the second vertebrate revolution in evolution (terrestrialization) are bone marrow hemopoiesis which emigrated from the spleen, transformation of gills into lungs, thymus, endocrine and lymphadenoid tissue, and carapas with placoids into dermis with hairs.

These changes can be interpreted as cellular transformation called metaplasia as the result of the response to stimuli provoking use and disuse: the increase of gravity on dry land, and the move to a dry environment from a wet one. As an experiment we can apply these stimuli to neoteny-type Mexican salamanders, which live in water, i.e., adult salamanders having a larval form, which were influenced by these stimuli during the second vertebrate revolution (terrestrialization). If the water surrounding the salamander slowly dries up over a period of some three months, the larval-form imago of the Mexican salamander can be changed from an amphibian to a morphology resembling that of a reptile. Here we see the oxygen-carrying medium change from water to air, and the gravitational force increase to 1G. By the application of these two stimuli, gravity and dryness, cellular transformation can be achieved having the same genetic characteristics as metaplasia.

2) Research on Haeckel's Biogenetic law concerning genetics

"Ontogeny recapitulates (repeats) phylogeny" represents Haeckel's Biogenic law. [9] This means that fertilized ovum develop from protozoa-type mono-cellular organisms via egg-division into four embryonic stages - morula, gastrula, neurula, and branchirula stages. Following this embryonic stage the fetus develops. What does this metamorphosis mean from the standpoint of genetics? Genetically, fertilized ovum and all embryonic stages are always same. However, gene duplications take place. Following that, their morphologies become quite different. Metamorphoses as well as functional changes in ontogeny means that even though they have the same genetic characteristics,[9] metamorphoses and functional changes can occur by means of gene duplications, which is called heterochrony. [10] Therefore, in phylogeny, not only organ but cellular metamorphoses can occur in the same genetic code. This phenomena has been called metaplasia in pathology.

3) Development of the trilateral research method and experimental evolutionary study

Neither Wolff's law, nor the Use and Disuse theory can be verified using conventional research methods, as morphological changes which accompany functional changes take place only as a result of the remodeling of mesenchymal cells. This remodeling takes place as a result of cellular genetic expression triggered by biomechanical stimuli. In order to verify Wolff's law and the Use and Disuse theory in molecular genetics, the author has developed a trilateral research method which integrates morphology, physiology (i.e. metabolism), and molecular biology [11] (including molecular genetics) using biomechanics. The author has also developed research methods for evolution, i.e., experimental evolutionary studies, using this trilateral research on vertebrates at every phylogenetic stage of evolution. From these innovative experiments, it is now possible to establish a solution to the problem of bone marrow hemopoiesis in evolution. In addition, a new concept regarding the immune system, and a new evolutionary theory which depends on response to biomechanical stimuli, are proposed.

2. Verification of the evolutionary system by means of synthetic research methods

(1) In vivo inductions of heterotopic hemopoiesis conjugated with ossification in mammalian muscle were successfully carried out by the author using an artificial bone marrow chamber composed of sintered hydroxyapatite.

Artificial bone marrow chambers made of sintered porous hydroxyapatite (HA) were implanted into the dorsal muscle, the subcutaneous tissue (subcutis) and subperiostium of a rib in several dogs. In both types of tissue, the blood flow from the heart was the same.

However, the lymphoid fluid flow was quite different when the animal was moving. In muscles, hemopoiesis in conjunction with osteogenesis was observed in the chambers, but this was not observable in the subcutis.
Porous HA chambers with bone morphogenetic protein (BMP) were implanted into the subcutis and subperiostium. Subsequent to this, hemopoiesis in conjunction with osteogenesis was observed, even in the subcutis. The difference between hemopoiesis in the muscle and that in the subcutis may lie in streaming potential which is converted from lymphatic fluid flow during the movement of the animal. To determine the streaming potential of sintered HA, the potential was measured with a physiological saline solution having the same pressure as blood in animals. 100 μV was obtained as the value of the streaming potential of HA under 100 cm water pressure. Instead of BMP, a potential of 100 μV was applied to HA chambers which were implanted into the subcutis of dogs. Hemopoiesis in conjunction with osteogenesis occurred in the subcutis, resembling the case of an HA chamber with BMP. Ti chambers with 50-100 μV current were developed and inserted into the subcutis and spleens of dogs. After 4 months the spleens with chambers and the chambers in the subcutis, together with surrounding tissue, were recovered and the specimens were observed. A significant development of leukocytes and lymphocytes was observed in the regions surrounding the Ti chambers implanted into the subcutis. In addition, a definite transformation into collagen-bundle tissue resembling tendon was observed in specimens of the spleen with a Ti electric chamber.
From clinical experiments in dynamic cardiomyoplasty, muscle development from striated muscles to the heart muscle is known to be controlled in proportion to the electricity used.
From these experiments it may be verified that skeletal organs and structures, i.e., tendon, muscle, osseous tissue, bone marrow hemopoiesis, and hematopoietic organs, are induced by electricity, triggering genetic expression from mesenchymal cells as metaplasia.

(2) Developing gompholic artificial dental root by means of biomechanical stimuli

Vertebrate evolution of morphology and function occurs by biomechanical stimuli with the same genetic characteristics. Therefore, artificial roots of mammalian gomphosis or reptilian ankylosis can be easily developed with or without biomechanical stimuli. Development of both mammalian gompholic roots with cementum and ankylotic reptilian roots with sintered compact hydroxyapatite in mammalian jawbones were carried out by means of an experimental evolutionary study method using biomechanical stimuli.
Artificial roots of a corrugated cone type, made from sintered compact hydroxyapatite were developed and implanted into the jawbones of adult dogs and Japanese monkeys. Artificial roots in gompholic and ankylotic conditions were easily obtained from biomechanical stimuli, i.e., with or without applying masticatory loading after the operation.
To investigate the histological differences between gompholic and ankylotic teeth, animal experiments with functional and nonfunctional groups were carried out. To investigate biomechanical differences, stress analyses with conditions modeled to approximate those in the animal experiments were also carried out. Following these, the results were compared. Fibrous tissue and the cementum around the roots with alveolar bone proper were observed in specimens of the functional group. Ankylosis of the artificial roots to the surrounding bone was observed in specimens without occlusal function.

Severe bone destruction was observed in the cortex region around the ankylotic artificial root, on which the masticatory loading was applied for two years after a post-operative lapse of one year.
The pattern of bone destruction in experimental subjects with the usual occlusal function and the finite element analysis (FEA) pattern showed a close correlation. The fibrous junction system around the bioceramic implants plays the most important role, by which the stress is mitigated and dispersed, and the principal stress trajectories are converted into three components: parallel, circulatory, and orthogonal vectors around artificial roots.
It was proved that the effective conversion of the principal stress trajectories depends upon an undulating morphology of the bioceramic artificial root.
These trajectories are equivalent to hydrodynamic waves, which are concomitant with streaming potential.
(3) As a result of the above mentioned synthetic research, the characteristics of mammals are disclosed as follows:

1) Metamorphosis; development of the branchial to pulmonary system with diaphragm;
2) The articulation system with bone marrow hemopoiesis;
3) Complicated interactions between various organs;
4) The suckling and masticatory system; and
5) Development of the sympathetic auto nervous system.

Each item is explained in detail.

1) Metamorphoses of the oral and branchial organs.
① This system regulates basic organs of Selye's stress theory, namely the pituitary-adrenocortical gland system. Animal-characteristic movements, and the alimentary, and genital system, derived from the oral (Ratcke's) and branchial system (into aorta) membrane.
② The branchial gut system, i.e., respiratory membrane and visceral muscles separate into the pulmonary system and the heart muscle, masticatory muscle, tongue, swallowing muscle, ossicle, vocal, and facial expression muscles.
③ In mammals, the lungs develop into a pericardial sac. Therefore, only mammals have a diaphragm.
④ A part of the branchial glands develop into the thymus, parathyroid gland, and tonsillar (the lympho-adenoid system)

2) Bone marrow hemopoiesis in the articulation is complete in mammals. Characteristic bone marrow with marked hemopoiesis in adults are in the dermal bones of the skull, mandible and maxilla with gomphosis articular joints, the vertebrae, iliac bone, and ribs and breast bone (sternum).

3) Organ correlations
Organ correlations first depend on the origin of the derm (endo-, meso-, or exo-), second upon the central nervous system, and third upon the sympathetic nervous system.

4) The suckling and masticatory system, a defining characteristic of mammals comes from these systems. They are supported definitively by visceral respiratory muscles which had been changed into striated muscles controlled by the pyramidal tract.

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