INDUCTION OF CEMENTOBLASTS ON THE CERAMIC ARTIFICIAL ROOT SURFACE BY MEANS OF HYDRODYNAMIC BIOMECHANICAL STIMULI

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ABSTRACT

The author has successfully developed a bioceramic artificial root of the gompholic type around which the cementoblast, periodontal ligament and alveolar bone proper can be induced in vivo, for the first time in the world. To disclose causal factors of induction of the peri-root supporting system, synthetic research on bioceramic surfaces behavior in vivo was done from the trilateral view point, i.e., the material, shape, and functional effects of the artificial root upon the surrounding cells.
Animal experiments as well as biomechanical researches, i.e., finite element analysis (FEA) and flow dynamic experiment of a devised model of the masticatory artificial root system with oil pressure were carried out. From the experiments, surface behavior of the ceramics are disclosed to be material effect of chemical elements concomitant with the energy effect, which can induce the gene expression of mesenchymal cells of periostium to differentiate bone morphogenetic protein (BMP), just as a catalyst in a chemical reaction. In conclusion, a peri-root supporting system just like gompholic tooth was developed on the surface of corrugated bioceramic artificial root by hydrodynamic stimuli, i.e., energy, which were fluctuating wave movement evoked by masticatory function.

INTRODUCTION

What are the difference between the tooth system of common reptiles and that of mammals? The former have the ankylotic and latter have the gompholic system. Ankylosis has no root supporting system but osseous adhesion. Gomphosis has major three tooth supporting structures, i.e., cementoblasts, periodontal ligaments and the alveolar bone proper. In conventional research on dental implant, no one has been successful in inducing these three peri-root structures.
From the evolutionary studies on vertebrates, the author has disclosed the gomphosis of the mammalian tooth system had evolved by biomechanical loading of mastication, which is characteristic function of the viscerocranium of the mammals, and which is converted into hydrodynamic flow, i.e., energy. To study causal factors inducing peri-root tissues, i.e., cementoblasts, periodontal ligaments, and the alveolar bone proper from mesenchymal cells, the author observed surface behavior unifying histological studies, observation of SEM and microanalyzer, obtained results of FEA, and model experiment to survey pressure of amplitude vibrations which are converted by masticatory stresses, with oil pressure.
To observe material effect of HA, ZrO_2 and Ti, artificial roots with corrugated forms were developed and they were implanted into adult dogs and adult Japanese monkeys. To observe functional effect, namely energy action, animals were separated into two groups, i.e., with and without mastication function fed by hard and soft diets respectively.

A certain period after operation, implanted artificial root with jawbones were recovered and specimens for light microscopy, SEM, and microanalyzer were prepared.
As a result, only in the functional group with corrugated form, induction of the peri-root supporting apparatus could be observed.
As a biomechanical study, finite element analyses (FEA) of the jawbone specimens in which artificial roots were implanted were carried out. Obtained results were compared with aspects of observed specimens, to disclose what is surface behavior i.e., surface reaction of ceramics.

New method for development of a gompholic artificial root

It is well known that in fractured injuries by inadequate fixation, fracture sites become often pseudarthrosis. The articular system can be easily induced by biomechanical stimuli of movement.
The ankylotic tooth system is known in some reptiles. However even in reptiles, crocodiles have the gompholic tooth system. Even in the ankylotic tooth system, if an erupting tooth suffer long term loading of mastication, the tooth can not adhere with jawbone to develop ankylosis. By these biomechanical stimuli, I conceived of developing the gompholic system.
To develop the gompholic artificial tooth system, the easiest method is to develop an artificial root, on which cementoblasts, periodontal ligaments, and the alveolar bone proper are induced in vivo by recipient mesenchymal cells which can be easily fabricated by technicians.
From clinical study of the masticatory system of the tooth and jawbone, it is known that biomechanical stimuli of fluctuation loading of 0.1 mm to 0.2 mm amplitude vibration are generated by mastication and occlusal movement. This can be attained by precise occlusal condition without contact between the upper and lower teeth and by feeding of soft and hard diets. By these procedures, a gomphlic as well as an ankylotic artificial root can be easily obtained if an artificial root with ideal shape as well as ideal material can be developed.
However, it is very difficult to disclose mechanisms to induce cementoblasts at the both surfaces of root and the alveolar bone proper, and to explain why thin bone of the alveolar bone proper can be induced by these biomechanical stimuli. Causal factors to induce the peri-root system, i.e., cementoblast, periodontal fibrous tissue with capillaries, and the alveolar bone proper can be investigated by analyzing the material, shape, and functional effects of the artificial root.

MATERIAL AND METHODS

Morphological researches by animal experiments

To disclose surface behaviors of ceramics upon the surrounding cells, morphological research by means of animal experiments was carried out. Twelve adult male shepherd dogs and six male adult Japanese monkeys were used in this synthetic experiments developing gompholic artificial roots. Artificial roots of 5 mm diameter with corrugated form of hydroxyapatite were implanted into the premolar site of maxilla and mandibles of them. Artificial roots with jawbones were recovered and specimens for light microscopy, SEM, and microanalyzer were prepared and they were observed. The following is, morphological research observing with light microscope, SEM, and microanalyzer.

1) Light microscopic observation of jawbone regeneration and peri-root supporting structures around gompholic artificial roots and jawbone absorption around ankylotic artificial roots were carried out.
2) Analyses by microanalyzer Kevex 8000 to observe cementoblasts (Fibrous osseous cells) and osteoblasts formation of gompholic artificial roots were carried out.
3) To observe material effect on morphology of mesenchymal cells SEM observation of gompholic artificial root surface of HA, ZrO_2, and Ti were carried out.

Biomechanical studies on shape effect of Artificial root upon the surrounding tissues.

1) FEAs to analyze the shape effect of roots of the jawbone specimens in which artificial roots had been implanted were carried out.
2) Model experiments with the oil pressure system to observe the conversion system of masticatory movement to amplitude vibration were carried out. Based on specimens of artificial roots implanted in jawbone, biomechanical model experiments were carried out.

RESULTS

Morphological researches

1) Satisfactory epithelial and submucosal fibrous tissue attachments resembling those of natural teeth were observed in the undecalcified sections of the artificial roots (AR) of hydroxyapatite (HA), zirconium oxide (ZrO_2), and titanium (Ti) regardless of the different materials at the periodontal region. The modality of epithelial attachment was almost the same in all materials. A layer of connective tissue rich in blood vessels was present in constant width around the artificial roots of hydroxyapatite, zirconium oxide, and titanium, and bone tissue similar to the alveolar bone proper was formed around this layer. At the concave site of the artificial root, the root surface was fixed to the alveolar bone by means of fibrous connective tissues that ran at right or acute angles. In most cases, the bone was indirectly fixed with angled fibrous tissue to the artificial root surface. The layer of fibrous tissue was attached to the root surface with parallel orientation. At the convex site, fibrous connective tissues ran parallel with the artificial root surface. Observation by light microscope and SEM with Kevex 8000 of the mirror-polished surfaces of the dog specimens revealed that in all artificial roots of the three different materials, alveolar bone proper (lamina dura) with trabeculae was observed, which was assumed to coincide with the principal stress trajectories as seen in specimens. However, calcified material similar to cementum or bone attached to the hydroxyapatite artificial root surface exclusively. This finding is substance-specific for hydroxyapatite.
The surface of the sintered hydroxyapatite appeared porous and seemed to remineralize. By SEM, calcified substance was observed attaching to the hydroxyapatite artificial root surface. Calcium and phosphate contents similar to cementum or bone were detected by the Kevex 8000 microanalyzer system. The surfaces of the titanium, and zirconium oxide artificial roots showed no change and lacked calcium substance when studied by Kevex 8000.
At the attached gingival region of hydroxyapatite artificial roots (HA AR), no inflammation was found both in the undecalcified specimen and in the decalcified specimen, and many cells resembling epithelium, were observed by SEM.

At the surface nearer the apex, various attaching structures made of microplatelets were observed. At the surface of the hydroxyapatite artificial root, a laminated layer resembling calcified materials 20 μm in thickness was observed in a 11-month-postop specimen of a dog by SEM. Quite a few cells resembling cementoblasts were observed at the concave surface of clinically-applied hydroxyapatite roots 11 months postop. In some areas of a clinically used 24-month-postop artificial roots, these cells appeared to have been converted to calcified substance. Occasionally, cementoblasts were observed to have been calcified with fibrous tissue. Calcified cementoblasts observed by SEM at the concave surface 24 months postop, were corresponding to the numerous cells seen at the surface of the lamellae with light microscopic specimen of a dog 6 months postop.

2) As a result, the attached substance proved to be a layer of cementum.

Microscopic findings also showed a calcified layer attached to the root surface which was shown to be porous cementum with numerous fibers remineralized, at the surface of the sintered hydroxyapatite by mesenchymal cells. Cementum deposition was substance-specific to sintered hydroxyapatite. At the surface of zirconium oxide or titanium artificial roots, plain lamellae resembling the surface of these roots were observed. By light microscopy and microanalyzer, there were no calcified substances observed at the surface of the zirconium oxide and titanium artificial roots.

Compact lamellae similar to cartilage were observed attached to the zirconium root surface. Although without cement-like substance, the artificial roots of zirconium oxide and titanium were observed as very stable in the mandible and maxilla with fibrous tissue attachment and alveolar bone proper.

3) To observe material effect of HA, ZrO_2, and Ti, shape of attaching cells were compared by SEM findings of the extracted artificial root. On the HA root surface morphologically quite resembling cementoblasts with fibers were observed, while on ZrO_2 and on Ti, flat osteoblasts were observed. Unifying the morphological research it was verified that only on the HA root surface cementoblasts with fibrous ligament could be induced.

Biomechanical studies

1) Biomechanical stresses on the artificial root loaded by occusal movement are converted according to the shape effect and material factors into the principal stress trajectories, which are coinciding with hydrodynamic flow channel in the skeletal system. Parallel stress trajectories at the surface of the artificial root and at that of the alveolar bone proper are converted into hydrodynamic channel concurrent with streaming potential.

The pattern of generating streaming potential can be thought to coincide with stress pattern working at the both surface of the artificial root and alveolar bone proper observed by stress measuring-model experiments using oil pressure.

Immediately after implantation in animal experiments there was no alveolar bone proper around the apex of the artificial root, while new bone formation, osteogenesis concurrent with hematopoiesis could be observed even in jawbone by hydrodynamic energy.

2) From FEA and model experiments using oil pressure, it is assumed that at the both sides of periodontal fibrous tissue space differentiate fibrous osteoblasts, namely cementoblasts from mesenchymal cells by streaming potential. The width of periodontal fibrous tissue with amplitude vibration of 0.1 mm to 0.2 mm was acquired by occlusal movement and also coincides with the total diameter of numerous capillaries being in periodontal fibrous articulation.

Unifying the results of experiments above-mentioned, the results of FEA, model experiments, and observation of specimen by microanalyzer, mechanisms to induce the peri-root system from undifferentiated mesenchymal cells have been disclosed by the author.

DISCUSSION

What is bioceramic surfaces behavior in vivo?
Why can artificial root with functional loading of fluctuating wave motions evoked by mastication induce the peri-root supporting system? And why can not the Wolff's Law of functional adaptation form of the skeletal system be solved by biomechanical studies? The surfaces behaviors of bioceramics in vivo are divided into material, shape, and functional effects and the latter two are biomechanical effects. Because the organisms are constructed by closed water soluble colloid with blood circulation, therefore around the ceramics with peculiar shape, in the surrounding tissue is generated hydrodynamics, just like functional effect of ceramics, i.e., loading-applied to the ceramics. Therefore, shape and functional effect can be understood as biomechanical, i.e., energic reactions, which are evoked by hydrodynamic stimuli.
Biomechanical loading which are controlling Wolff's Law of functional adaptation are also converted in skeletal organs into hydrodynamics. Material effect and hydrodynamics of the skeletal osseous or cartilage system as well as ceramic artificial organ upon the surrounding mesenchymal tissues are the most important for remodeling of cells to change tissues. To investigate the material and hydrodynamic effects of artificial organs, I developed corrugated artificial roots of the same shape of ZrO_2, Titanium (Ti), and hydroxyapatite. They were implanted into the jawbones of the animals. After that I recovered and observed by SEM. On root of ZrO_2 and Ti after 6 months postop, there could be observed no cementum but thick fibrous tissue attached.
On hydroxy-apatite root after 6months postop, there could be observed a maked amount of cementum with fibrous tissue. Ca-ions and phosphates of hydroxyapatite are essential to induce the cementum in early stage.
I analyzed the shape and functional effect concomitant with hydrodynamics with FEM. The jawbone specimens of gompholic as well as ankylotic system in which artificial roots were implanted, showed that new bone formation occurred according to the moderate stress distribution patterns of the Mise's equivalent. Marked destructive remodeling without bacterial infection is developing at continuous part of the principal stress trajectories between artificial root and the alveolar bone.
Considering difference of the function of the artificial root between gomphosis and ankylosis, it is obvious that the former can function as the stress breaking system loading with hydrodynamic wave movement of repeating mastication movement and the latter without the stress breaking system. Therefore destructive remodeling according to the mastication loading takes place in ankylosis. I have disclosed not only material effect as component reaction but also shape and functional effect as hydrodynamic reaction against mesenchymal cells around the ceramic artificial organ.
What reaction can be considered to occur in the mesenchymal cells? It is known that streaming potential can develop by hydrodynamic stream of flowing liquid around the ceramics. Therefore hydrodynamic energies of functional as well as shape effect are converted into streaming potentials.
With elemental reaction of hydroxyapatite component concomitant with steaming potential, it is obvious that the gene expressions of mesenchymal cells are triggered.

As a result, bone morphogenetic proteins (BMP) are induced to differentiate osteoblast concomitant with hemopoietic cells. In some specimens a part of newly generating alveolar bone proper with hemopoietic nest that was observed in bone marrow of the mandible.
Vectors of biomechanical momentum in the osseous skeletal system, which are yielded by functional movement of the skeleton, are necessarily converted into hydrodynamic flows of which orientation can be seen as the principal stress trajectories by FEA.
Therefore, functional movement of the artificial root in the jawbone induce hydrodynamic flow concomitant with streaming potentials around the artificial root, and the potentials trigger the gene expression of mesenchymal cells to generate BMP in the jawbone. After that, the cementoblasts on the artificial root surface with functionally oriented fibrous tissue and thin osseous tissue of the alveolar bone proper are developing.
Thus, the mechanisms of the remodeling system by biomechanical repeated stimuli can be solved for the first time in the world.
In conclusion, a peri-root supporting system just like the gompholic tooth was developed on the surface of ceramic artificial root by using hydrodynamic stimuli, i.e., energy, which were fluctuating wave movement evoked by masticatory function.

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