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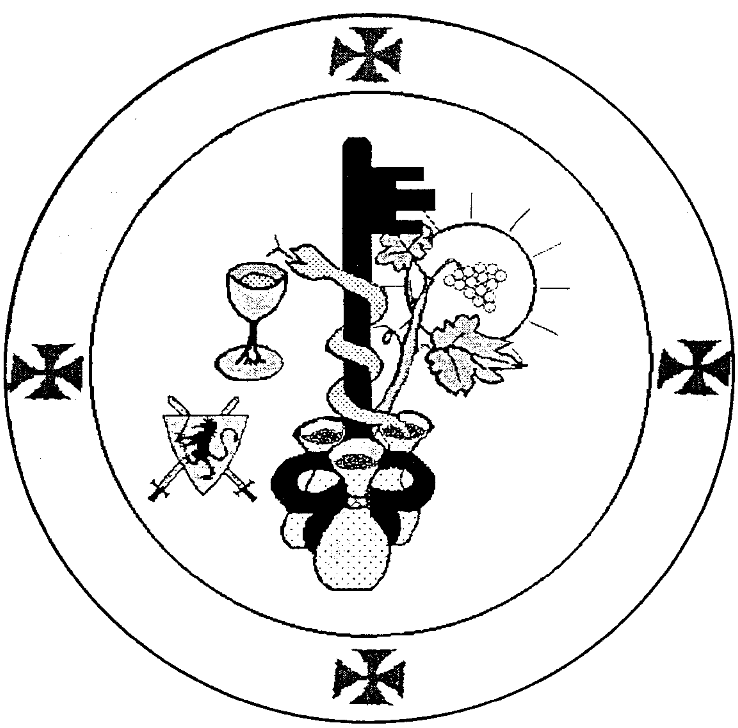

DRUGS DELIVERY SYSTEMS

(materials, know-how, new technologies, principles)

To be held at the Congress Hall of BANCA DI ROMAGNA
Via P. Costa, 3 – Faenza - Italy

March 9-11, 2000

FINAL PROGRAM

Evidence-Based Evolutionary Research and Development of the Practical Phylogenetics: Verification of the Gravity-corresponding Evolutionary Law by Means of Biomaterials

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From studies of developing artificial bone marrow chambers with hematopoiesis the author evidenced that the evolution of hemopoiesis in endoskeletons is dependent upon correspondence of organisms against the gravitational force. After that the author proposes the Gravity-corresponding Evolutionary Law in vertebral phylogeny. Metamorphosis of the endoskeleton is known as functional adaptation named Wolff's Law within a generation which is in accordance with repeated biomechanical stimuli. The Wolffs Law is in accordance with Use and Disuse Theory of Lamarck through phylogenetic generations.

In this paper comparative anatomy concerning skeletons between the amphibian, reptiles, and the mammals are carried out. Skeletal morphology of the vertebrates depends upon not only modality of repeated movements of skeletal organs (inner factors) but biomechanical stimuli influencing outer side upon organisms (outer factors by Lamarck, i.e., environmental factors). Therefore, if metamorphosis can be observed among same animals of the same phylogenic stage, there should be differences of inner or outer factors during evolution between these animals. Differences in the skeletal and muscle system between amphibian as well as reptiles and mammals can be seen as follows: 1) The teeth and jawbone system 2) The branchial arch system 3) The ossiclls of the auditory system 4) The collarbone 5) The diaphragum and the lung system 6) The nostrils and masticatory muscle system

Experimental evolutionary studies are carried out using chondrichthyes Triakis, Heterodontus, and neoteny-type Mexican salamanders through artificial terrestrialization. The formation of the diaphrugm and the lungs correlation with pericardial sack as well as collarbone are investigated and divergence of the mammals and the amphibian, reptiles is evidenced. As conclusion through comparative anatomy it is evidenced that origins of mammals and reptiles are different at the stage of archetype vertebrates before terrestrialization, i.e., chondrichthyes. Not only skeletons in phylogeny but also the evolutionary processes of the lungs as well as the tongue are quite different between the amphibian as well as reptiles and the mammals.

Invited Lecture

Studies on the Evolutionary Processes of the Bone as Natural D.D.S. of Biological Ceramics

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The definition of the vertebrates is a chordate having bony backbone i.e., vertebrae with different degree of ossification. Therefore, skeletal substances of connective tissue composed with the collagen, the cartilage and the bone are definitive substances of the vertebrates. The bone is mineralized fibrous collagenous connective tissues. The bone can be seen as natural biological D.D.S. made of ceramics. Hydroxyapatite is mineral of the bone, of which phosphate offers pyrophosphate for energy metabolism as well as nucleic acid metabolism in genetic substances. The calcium ions of hydroxyapatite acts as essential substance for the entire metabolism in organisms. The osseous tissues are remodeled by the action of the time, biomechanical stimuli and by nutritional condition of the organisms. Calcium ion, phosphate, collagen, and sulfate are delivered from the bone and cartilage during remodeling. The bone is definitive substance of the vertebrate and the tooth system as well as tooth morphology is the criteria of phylogenetic category in animals. What is the origin of the tooth and the bone? "To known the essential function of the organ is to inquire that of origin in phylogenetics." This is the orthodox way to investigate the morphology established by the famous poet and scientist Goethe. The bone derived from specialized tooth or the tooth can be seen as specialized bone. Because, at the initial stage of archetype vertebrate original skeletal substances were exoskeleton placoids which had been unified calcified organ of the bone and tooth in chondrichthyes. Moreover, in stage of prochordata organisms have placoids on the surface of derma, which is detected cartilage with sulfer of chondroitin by microanalyzer. Evolution of exoskeletons, endoskeletons, and splanchro skeletons in phylogenic stage are studied from the initial stage of prochordata up to the mammals.

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Development of Revolutionizing Biomaterials Substituting Various Mammalian Organs by Means of Sintered Bioceramics

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Keywords: Collagen-Hydroxyapatite Composite, Experimental Evolutionary Studies, Genetic Expression, Hemopoiesis, High-Pressure Technique, Ontogeny, Phylogeny, Tissue-Immune System, Xenotransplantation

Abstract

Development of biomaterials substituting various mammalian organs can be carried out by means of experimental evolutionary studies using collagen -hydroxyapatite composite, derived from adult cattle.

The evolution of the tissue-immune system can be studies by compound-ceramics of collagen-hydroxyapatite composite. Collagen-hydroxyapatite composite was sintered by high-pressure technique using collagen extracted from cattle skin, which had antigenicity [1,2]. Artificial bone marrow chambers were fabricated with the sintered collagen-hydroxyapatite composite. Experimental evolutionary studies using mammals (dogs) and chondrichthyes (sharks) were carried out implanting the chambers into their muscles [3,4]. The result showed that around the collagen composed chambers implanted into dorsal muscle of dogs, marked cell differentiation as well as dedifferentiation with atypia could be observed, which resembled a part the digestive tract of intestine histologically. Around the chambers implanted into dorsal muscle of sharks hemopoietic nests could be observed, which were quite similar to those induced by the chambers of conventionally sintered hydroxyapatite. Hemopoiesis and osteoid formation 4 months after surgery were observed around the collagen-apatite chamber implanted in the shark muscle as well as in upper site of vertebral cartilage of the spinal cord. No bone marrow in the cartilaginous tissue in upper site of the spinal cord is evident in control sharks. Xenotransplantation of skin, i.e., skin grafts between sharks of different kinds of species, as well as between sharks and xenopus (amphibian), sharks and mammals (rat) are carried out. All of them are successful and chimera placoids between them are developed. After that, the author successfully carried out xenotransplantation of various organs of chondrichthyes into those of dogs [5-7].

Introduction

The bone is definitive substance of the vertebrates. Therefore, using synthesized artificial osseous biomaterials trilateral riddles of the vertebrates, i.e., the evolutionary system, immune system, and development of bone marrow hemopoiesis can be read. Self and not-self immunology is in vogue in these days. However, this concept is defined only in tissue immunity. Present research aims to prove the development of tissue immunity via genetic expression by the gravity during terrestrialization. In ontogeny, embryo has no tissue immunity, which is called immuno-tolerance. During research on phylogeny concerning bone marrow hemopoiesis the authors disclose that the function of human leukocyte antigen (HLA) is induced through the second evolution of the vertebrates, i.e., in terrestrialization conjugated with emergence of bone marrow hemopoiesis. The authors propose hypothesis in development of tissue immunity by increased gravity of 1G during landing or delivery from 1/6G in sea water or in amniotic fluid through phylogeny as well as ontogeny, to which organisms correspond by increasing blood pressure.

Materials and Methods

(1) Hydroxyapatite(HA) was sintered with collagen by high pressure gas technique at 40℃ with water. Artificial bone marrow chambers made of collagen-HA composite made of high-pressure sintering were fabricated by National Institute for Research in Inorganic Materials; collagen with antigenicity was extracted from cattle skin [1, 2].

(2) These chambers were implanted into mammals (dogs) as well as chondrichthyes (sharks). These were recovered 3 months postoperatively to make specimens. They were observed and these histopathological findings were compared.

(3) The following xenotransplantations between archetype and higher vertebrates are carried out.

A-Shark cartilage and muscle transplantation to two adult dogs (beagles).

B-Intestines of sharks to these of two adult dogs (shepherds).

C-Corneas of sharks to these of dogs

D-5 cases of a part of brains of sharks to these of rats

E-5 cases of a part of spine of cyclostomata to a part of rat femur nerves

Results

Collagen-hydroxyapatite composite was sintered by high-pressure low temperature technique using collagen extracted from cattle skin, which had antigenicity. Artificial bone marrow chambers were fabricated with the sintered collagen-hydroxyapatite composite. Experimental evolutionary studies using mammals (dogs) and chondrichthyes (sharks) were carried out implanting the chambers into their muscles. The artificial bone marrow chambers were implanted in dogs as well as in sharks of dorsal muscle.

The result showed that around the collagen composed chambers implanted into dorsal muscle of dogs, marked tissue differentiation with atypical cells could be observed, which resembled epithelium of digestive tract of the intestine histologically. Around the chambers implanted into dorsal muscle of sharks hemopoietic nests could be observed, which were quite similar to those induced by the chambers of conventionally sintered hydroxyapatite. From the experiments the authors disclosed chondrichthyes have no HLA (human leukocyte antigen) even though they have MHC (major histocompatibility complex). Therefore archetype vertebrates are in immuno-tolerance just like embryo of higher animals. To verify immuno-tolerance of chondrichthyes xenotransplantation of skin (skin graft) of sharks into mammals (rat) are carried out. After that, the author carried out xenotransplantation of corneas and brains of chondrichthyes into those of dogs as well as rats. All xenotransplantation between corneas (Figs 1-5) of sharks and those of dogs as well as xenotransplantation between a part of brains and those of rats (Fig 6) are successfully carried out. Neither rejection nor infection occurred. Histopathological findings show successful xenotransplantation of the intestine between dogs and sharks.

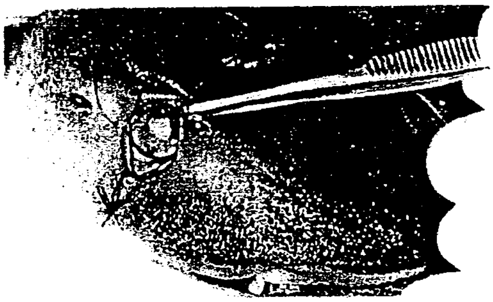

Fig. 1 Shark eye is being extirpated.

Fig. 2 Extirpated shark eye.

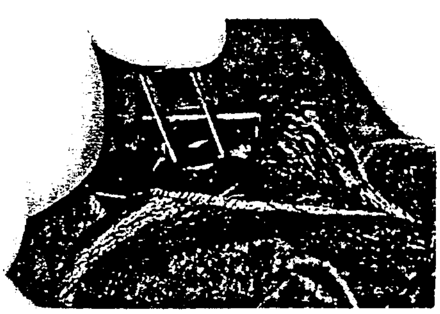

Fig. 3 Cornea of dog is being extirpated by trephine.

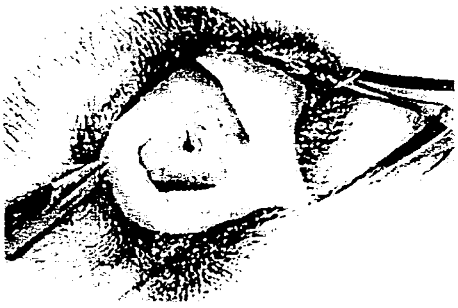

Fig. 4 Transplanted shark cones into dog eye, 2 weeks postop.

Fig. 5 Transplanted cornea, 3 months postop

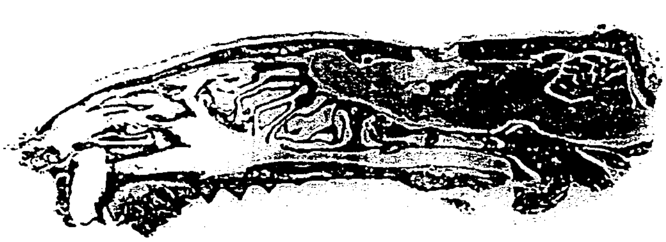

Fig. 6 Successful xenotransplantation of shark brain into that of rat, 6 months postop

Discussion and Conclusion

Haeckel proposed in his biogenetic law that ontogeny recapitulates phylogeny. Mammalians embryo has no tissue immunity, i.e., immuno-tolerance, even embryo has major histocompatibility complex (MHC). According to the biogenetic law archetype vertebrates chondrichthyes naturally has no tissue immunity. In sharks the major histocompatibility complex (MHC) of class I as well as class II are well known to exist. Therefore, in archetype vertebrates MHC is masked in genetic expression just as immuno-tolerance in embryos of higher animals.

As conclusion genetic expression of MHC is triggered by the gravity, which triggers the development of bone marrow hemopoiesis in phylogeny as well as in ontogeny. Without genetic expression of MHC xenotransplantation can be successfully carried out between the archetype vertebrates and mammals. Therefore MHC exists not to distinguish self or not-self in organism but to function for cellular level digestion. From these experiments revolutionizing new biomaterials substituting allograft of various organs are developed for mammals.

Acknowledgments

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Comparative Studies on Tissue Reaction of Newly Sintered and Conventionally Sintered Hydroxyapatite

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Keywords: Bone Guided Regeneration, Hydroxyapatite-Collagen Composite, Lamina Limitans, Nonstoichiometric, Ossiculoplasty, Stoichiometric Formula

Abstract
Comparative Studies on tissue reaction of newly sintered and conventionally sintered hydroxyapatite are carried out. Conventionally, hydroxyapatite sintering has been aimed at stiff, stable apatite due to chemical bonding with osseous tissue. However, direct bonding of sintered hydroxyapatite with bone may easily be destroyed under severe repeated loading just like osseointegration of metal because of differences in elastic moduli from each other. Therefore, development of stiff, stable hydroxyapatite makes no sense just as in the case of bioinert metal for biomechanical substitution. The merit in medical application of synthesized hydroxyapatite is to provide materials easily utilized and remodeled for surrounding living osseous tissue. Collagen and calcified substance are essential for bone remodeling. It is well known that the apatite phase found in living bodies exists as very small crystallites, which are bonded with organic, high-molecular polymers such as collagen. The biological apatite phase also contains bonded water, carbonate ions, and many other inorganic minor components. If it is possible to prepare an implant made of hydroxyapatite-collagen composite similar to biological apatite, the biological effects of the implant may be different from those of conventionally sintered hydroxyapatite [1-5]. Research on tissue reaction of conventionally sintered HA were carried out through co-operation between Research Institute for Ceramics Technology of the Italian National Research (IRTEC-CNR) and Experimental Surgery Division of "Rizzoli" Institutes of Bologna.

Introduction
From standpoint of the bone evolution as biological bioceramics stoichiometric as well as nonstoichiometric hydroxyapatite (HA) were sintered by high-pressure gas technique. After that they were implanted into dorsal muscles of dogs. Comparative studies on tissue reactions between biologically and conventionally sintered ceramics implanted were carried out. The definition of the vertebrates is a chordate having bony backbone i.e., vertebrae with different degree of ossification. Therefore, skeletal substances of connective tissue composed with the collagen, the cartilage and the bone are definitive substances of the vertebrates. The bone is mineralized fibrous collagenous connective tissues. At the initial stage of archetype vertebrates original skeletal substances were exoskeleton placoids which had been unified as calcified organs of the bone and tooth in

chondrichthyes. Moreover, in stage of prochordata organisms have placoids on the surface of derma, which is detected cartilage with sulfer of chondroitin by microanalyzer. The bone is the most important substance of the vertebrates. In evolution the origin of the bone is biological ceramics sintered in low temperature by enzyme with water, which are mineralized in nonstoichiometric condition. Sintering of calcium-deficient hydroxyapatite has already been reported, which bonded water at the calcium-deficient site, and was sintered up to fully dense bodies at 300°C under a pressure of 600 MPa[1]. In the presence of collagen in an aqueous phase, we tried to synthesize the hydroxyapatite by means of reaction between an aqueous solution of phosphoric acid and a calcium-hydroxide suspension. A diluted collagen solution was mixed with an aqueous solution of phosphoric acid and was poured slowly into a calcium-hydroxide suspended aqueous phase. No collagen was found in the mother liquid thus formed. All collagen in the solution was found to be collecting in the precipitate. Five hundred grams of commercially available collagen solution was diluted up to 8 liters and mixed with 0.6 mole of phosphoric acid. The CaO was crushed into fine powder and mixed with water. The Ca (OH) aqueous suspension thus formed was mixed, and the collagen-phosphoric acid mixed solution was added slowly. The precipitate was filtered and partly dried until suitable water content formed. Then, it was mounted in a metal capsule. The capsule was evacuated and sealed by welding. It was thin kept for 8 hrs at 200 MPa at 40°C. The resulting apatite-collagen composite was 1.75g/ml in density, 2 GPa in Young's modulus, and 6.5MPa in compression strength. The specimen could be cut by a knife, and was stable against immersion in water. The physical property of this type of complex may change according to composition and treating conditions. The sample was implanted in dogs and histologically evaluated.

Materials and Methods

From the standpoint of evolution the authors developed compact stoichiometric and nonstoichiometric HA as well as collagen-composed HA of compact type by high pressure sintering technique with water. Instead of the enzyme action of natural bone synthesis high pressure sintering technique with water were applied and sintered in 40-300 degree C. These biological bioceramics were sintered in National Institute for Research in Inorganic Materials and implanted into subcutis and muscles of dogs and monkeys as well as experimental evolutionary researches implanting into dorsal muscle of sharks were carried out in faculty of medicine, University of Tokyo.

(1) Comparative histopathological studies on conventional stoichiometric hydroxyapatite and new type hydroxyapatite sintered by high pressure gas technique

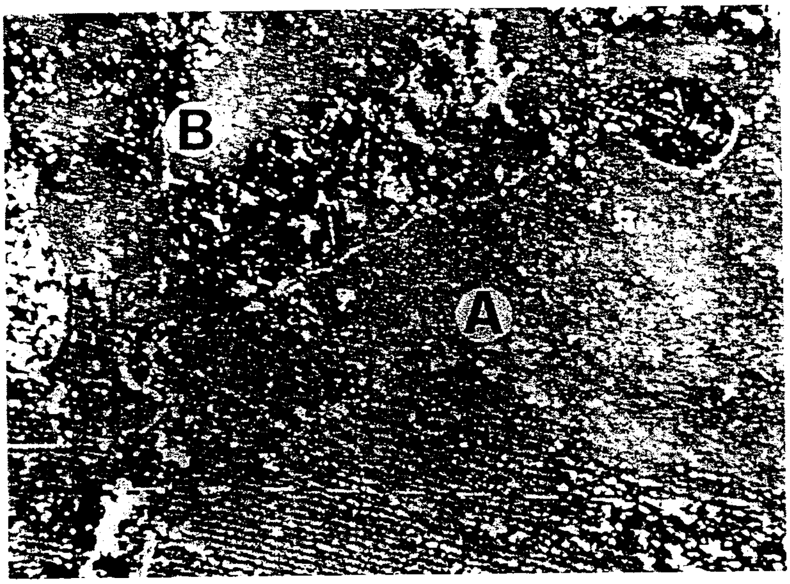
1) For a preliminary experiment, the following studies were carried out. Conventionally sintered stoichiometric porous hydroxyapatite plates and dense new type plates of stoichiometric and nonstoichiometric hydroxyapatite were implanted in the bone and muscle of a dog to compare histological tissue reactions for 8 weeks. For conventional stoichiometric hydroxyapatite, porous hydroxyapatite plate (40% porosity) made by ASAHI Optical Co. Ltd. was used. Dense new type of stoichiometric and nonstoichiometric hydroxyapatite were sintered in the National Institute for Research in Inorganic Materials. Undecalcified polished specimens for SEM and EPMA were made. They were then observed and compared.

2) Research on tissue reaction of conventionally sintered HA were carried out through co-operation between Research Institute for Ceramics Technology of the Italian National Research (IRTEC-CNR) and Experimental Surgery Division of "Rizzoli" Institutes of Bologna.

(2) Experiments on pressure sintering of apatite-collagen composite)

Five hundred grams of commercially available collagen solution (concentration 2 wt%, isoelectric point 9, pH3) were diluted up to 8 liters and mixed with 0.6 mole of phosphoric acid. The $CaCO_3$, 1 mole was kept at 900°C in air for 10 h. The CaO thus formed was crushed in a mortar into fine powder and mixed with 3 liters of water. The Ca(OH)2 aqueous suspension thus formed was mixed vigorously, and collagen-phosphoric acid mixed solution was slowly added at room temperature to the aqueous suspension. In this case, the mixing ratio of collagen to hydroxyapatite was 1 to 10. The precipitate thus formed was filtered and partly freeze-dried until the water content of the precipitate became suitable for sintering. Then, the precipitate was mounted in a metal capsule. The capsule was evacuated and sealed by welding, after which it was kept for 8 h at 200 MPa, 40°C.
The specimen thus formed was examined by both 5 MHz sound velocity measurement and compression strength measurement using INSTRON model 1123. The cross head speed was 0.5mm/min. during the measurement.
Sintered apatite-collagen composites were implanted in dorsal muscles of a dog; 8 weeks after implantation, specimens were recovered, prepared, and observed.

Results
Studies by SEM observation on comparison of conventional hydroxyapatite and new type hydroxyapatite sintered with high-pressure gas technique revealed the following:
Conventionally sintered porous hydroxyapatite was observed as amorphous in the cutting surface. Therefore, the grain could not be detected. Porous hydroxyapatite plate and newly formed bone fused well. However, conventionally sintered apatite plate did not fuse with fibrous tissue. In the interface between the porous apatite plate and fibrous tissue, Ca. 2μm space was observed, wherein some small spots of connection of tissue and apatite could be observed. On the contrary, dense apatite plates sintered with high-pressure gas technique fused with soft tissue firmly. Both new types of apatite (stoichiometric and nonstoichiometric) were observed constructed with ultrasmall grains by SEM. Nonstoichiometric apatite of high pressure gas technique was observed having very weak fusion with soft tissue. Almost all parts of the fusion were disrupted by artifact of specimen treatment.
The results of pressure sintering of the apatite-collagen composite were as follows:
After pressure treatment, the metal capsule made from lead was removed. In every run, the specimen was slightly yellow and solid. When the water content of a specimen during pressure treatment was about 10 wt%, for example, the resultant solid specimen was stable in air, but unstable in water. When the specimen was immersed in liquid water, it broke vigorously into small pieces. When the water content was near 50 vol%, the pressure-treated specimen was stable in liquid water. The solid feature of the specimen was unchanged during a period of one month of immersion in water at room temperature. The results were compared with apatite powder compact treated under the same conditions as those of the apatite-collagen composite, i.e., 200 MPa, 40°C, and 8 h of run duration, and the presence of liquid water. The apparent density of the apatite powder compact thus obtained was 2.1g/ml, and was hard and brittle. In this case, without collagen, the load-displacement curve was almost a straight line until it broke, while in the case of the collagen-apatite composite, the load-displacement line showed small and varying gradients, indicating large deformation of the specimen. These results coincided with the sound velocity measurement, from which 2 GPa of Young' modulus and 6.5 MPa in compression strength were calculated. The collagen-apatite composite could be cut with a razor blade. The average size of the apatite crystal was 10 nm in diameter and 40 nm in length.



The figure shows a slide-sample of an implant of a HA ceramic cylinder (made in a femur of rabbit) extracted after 6 months (after sacrifice of the animal). The implantation was made into mid-diaphiseal defect of the femur of 6 rabbits and the analyses gave substantially the same results. The HA cylinders were fired at 1240°C for 3 hours. The animals were allowed to move freely in its cage immediately after surgery. The implant (together with the surrounding bone and tissue) was removed after 6 months after implantation and fixed in a 10% formalin solution (adjusted to neutral pH by buffer mixture of sodium phosphate salts) and then embedded into methylmetacrylate. The specimen for instrumental examinations were sectioned parallely to the cylinder axis by a diamond circular saw (Leitz) to obtain sections with a thickness of about 50-100 μm. The sections were examined through a scanning electronic microscope (Cambridge). It is also visible the border of about 1-2 μm thick constituted with the *lamina limitans* material.

Discussion

New type hydroxyapatite apatite sintered with high-pressure gas technique proved to have excellent histocompatibility with fibrous and osseous tissue. Fusion of apatite to soft tissue is the most important property of any biomaterials. It was found that the stoichiometric hydroxyapatite became nonstoichiometric *in vivo*. On implantation of hydroxyapatite, it has been shown that the bond formation between the implanted hydroxyapatite and tissue of living bodies needs some induction period, about 4-5 weeks. In hard tissue of living bodies, it has been shown that the hydroxyapatite phase is complex in chemical composition. It is, therefore, doubtful that this induction period is long enough to change the implanted hydroxyapatite. It has been shown that stoichiometric hydroxyapatite is one of the thermodynamically stable phases of the system CaO-P2O5-H2O. But for nonstoichiometric hydroxyapatite, no stable field has been given. This means that the solubility of stoichiometric hydroxyapatite in aqueous solution is lower than other metastable phases such as nonstoichiometric hydroxyapatite. Due to this character, sintered stoichiometric hydroxyapatite can keep its shape long-term in living bodies, being an excellent material for implantation. From this experiment, it was found that stoichiometric hydroxyapatite sintered bodies change their composition to nonstoichiometric in living bodies. It seems important that the shape of implanted sintered bodies not be changed in spite of their compositions being changed. Sintering temperature suitable for hydroxyapatite is known to be 1200_100°C. Under a pressure of 200 MPa, a fully dense sintered specimen can be obtained at 800°C. Presence of water in the system lowers the sintering temperature of hydroxyapatite. In a stream of steam at 300°C and atmospheric pressure, grain growth of hydroxyapatite can be found, and also a powder compact of hydroxyapatite shows small shrinkage in volume due to its sintering.

Previously, we reported that calcium-deficient hydroxyapatite, which has bonded water at the calcium-deficient site, sinters up to full density at 300°C under a pressure of 600 MPa. At that time, 300°C was the lowest limit to obtain a stable sintered specimen. At 200°C or lower, full density could not be obtained and strain remained in the pressed powder by treating it at 600 MPa. It was found that such an obtained specimen, placed in air, gradually broke into small pieces. When specimens with remaining strain were dipped into water at room temperature, they broke vigorously into small pieces within several seconds. The water content in these apatite specimens was determined. It was found that these specimens were intensely dried. The bonded water molecules in the calcium-deficient site had been partly lost. In the present experiment, therefore, wet apatite powder was used for the pressure treatment. The pressure-treated apatite specimen was thus obtained by pressure treatment at room temperature. It seemed obvious that in liquid water, the strain in the apatite phase due to the pressure treatment could be released at room temperature. This phenomenon led us to the further possibility of pressure-sintering of hydroxyapatite mixed with organic compounds at room temperature.

These results also suggest that the higher pressures make it possible to obtain a collagen-apatite composite of higher density, as well as higher strength at room temperature.

Resort to the use of ceramic hydroxyapatite (HA) as biomaterials is justified for application in bone, particularly in the maxillofacial and orthopaedic sectors, and also in dental and otorinolaringoiatric ones. Chemically speaking, the substance with this name corresponds to the stoichiometric formula $Ca_5(PO_4)_3OH$ (according to IUPAC standard rules) [in the following R-HA] taken as reference in biomedical field when speaking of substituted HA. In fact it belongs to the wider family of apatites where every ionic constituent can be totally or partially substituted by others with formation of apatitic solid solutions [in the following SS-HA, more or less crystalline]. Due to their very high insolubility in water (for values of pH $\geq$ 7) and its instability in acid environment, the apatites exist in practice only in the crystalline state. As concerns substitution that can occur in the particular case of SS-HA for biological use, PO_4^{3-} group can be substituted by *e.g.* CO_3^{2-} and HPO_4^{2-}; OH^- one by *e.g.* F^- (which confers higher stability to the crystalline lattice), but even by CO_3^{2-} (which on the contrary weakens it); Ca^{2+} by *e.g.* Na^+, K^+, Sr^{2+}, *etc* [6].

Fibroblasts grow very well on the surface of non-resorbable calcium-phosphates bioceramics, and particularly on SS-HA ones, on which they adhere with flat shape and with the tendency to enter the porosity if present [7,8]. This is particularly important in ossicular implants of HA bioceramic prosthesis (TORP or PORP). The implanted prosthesis tends to become covered with an epithelial tissue which is like to the natural one. Unfortunately, the amount of fibrous tissue formed all around the surface of the ossiculoplasty device is excessive and makes heavy it. This behavior was attributed to the presence of some small amorphous phase [9], however present in a sintered body.

Osteoblasts grow well on the surface of SS-HA bioceramics, however with a kinetic rate of growth slower than that of fibroblasts. Consequently, in some cases (as in the regeneration of the dental alveolar crest) to avoid a mixed growth of the two cellular species together, (apart from the use of specific growth and anti-growth factors) the zone of the soft tissue is separated with proper nanoporous membranes from that of the bone, giving rise to the so called *bone guided regeneration*.

On an implant of HA bioceramic, a deposition of a big amount of mineralised neoformed bone occur particularly in the wide and irregular cavities; many neoformed bone trabeculae adhere directly on the external surface. Only in some zones, a slight (apparently connective) inter-gap layer interposes between the surface of HA bioceramic and neoformed bone. A close contact between

neoformed bone and surface of the ceramic is however produced [10]. Many wide vascular cavities may be observed in the neoformed bone closely near the surface of HA bioceramics. Such cavities decrease their diameter in time until 12 months [11] on the basis of a decreased request of metabolic exchanges (due to lowering of cellular activities). At the end the HA ceramic surface may exhibit many indentations completely filled with new bone depositions (experiences on sheep femurs). Typical well formed osteocitary cavities appear all around HA bioceramics implants and even closely to the ceramic surface [12].
The goal was to accelerate the process of bone formation and to anchor the device to the bone as fastest as possible, also trying to obtain the greatest percentage of direct contact points too.

Conclusion

It is obvious that conventionally sintered HA above 1000 degree C is almost bioinert, so no tissue reaction can be observed in subcutis. As conclusion tissue reaction of biologically sintered HA is excellent to induce leukocytes hemopoiesis.

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