DEVELOPMENT OF SUCCESSFUL XENOTRANSPLANTATION SYSTEM BY MEANS OF COLLAGEN-HYDROXYAPATITE COMPOSITE: BONE IS THE ION-PROTEIN-PHOSPHATE DELIVERY SYSTEM

K. Nishihara and *J. Tanaka

Department of Oral Surgery, Faculty of Medicine, University of Tokyo 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655 and *National Institute for Research in Inorganic Materials, 1-1 Namiki, Tsukuba-city, Ibaragi-prefecture 305-0044, Japan

ABSTRACT

The development of the tissue-immune system can be studies by composite-ceramics, i.e., artificial bone as the protein delivery system. 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 collagenhydroxyapatite 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 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

Artificial bone marrow chambers made of collagen (with antigenicity)-hydroxyapatite (HAP) composite were developed and implanted into mammals as well as chondrichthyes. After that authors disclosed that archetype vertebrates chondrichthyes are in immuno-tolerance. Therefore, xenotransplantation between sharks and mammals can be done. Various organs and tissues of chondrichthyes are successfully transplanted into mammals. From these experiments new biomaterials are developed substituting organs or tissues.

The authors have successfully carried out skin grafts between different kinds of sharks, from Heterodantus japonicus (dog-shark) to Triakis scyllia (dochi shark). Skin grafts can also be carried out successfully from cyclostomata (hagfish) to rats, and the corneas of sharks can be successfully transplanted to the eyes of dogs. In addition, a part of the intestines and brains of sharks can be succcssfully transplanted to these of rats and dogs.

MATERIALS AND METHODS

(1) HAP was sintered with collagen by high pressure gas technique at 40° C with water. Artificial bone marrow chambers made of collagen-HAP 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 for histopathological observation[5-7].
(3) The following xenotransplantation between archetype and higher vertebrates are carried out.
A-Shark cartilage and muscle transplantation to two adult dogs (beagles).
B-Intestines of sharks to that of two adult dogs (shepherds).
C-Cornea of sharks to that of dog.

RESULTS

The collagen-hydroxyapatite (artificial cartilage) artificial bone marrow chambers were implanted in shark muscle (Figure 1). Hemopoiesis and osteoid formation 4 months after surgery were observed around the hydroxyapatite implanted, in the shark muscle as well as in vertebral cartilage. The hemopoietic nests as well as osteoid formation were quite resembled with those of conventionally sintered HAP. On the other hand, in dog muscles around the artificial cartilage implanted marked tissue differentiation with atypical cells that resembled epithelium of the digestive tract could be observed, which were thought to be cytological digestion of the collagen delivered from proteins delivery system of the sintered HAP (Figure 2-A, B). From these results the authors found sharks were in immuno tolerance. All xenotransplantation between organs of sharks and those of dogs are successfully carried out. Neither rejection nor infection occurred. Histopathological findings show successful xenotransplantation bf the intestine between dogs and sharks.

DISCUSSION

Collagen composed HAP is considered some kind of materials-delivery system. So, it can be supposed the even collagen with antigenicity can be composed into HAP without antigenicity or with weak antigenicity. Prior to sinter HA-collagen composite with high pressure gas technique following two working hypothesis are set; 1) Collagen-composed HAP is digested in vivo slowly, therefor, no antigenicity exert in mammals and chondrichthyes. 2) Collagencomposed HAP is digested in vivo slowly, however, weak reaction against low antigenicity exert in mammals and chondrichthyes which are masked by slow materials-delivery system of HAP. After that the artificial bone marrow chambers of collagen-composed HAP were implanted into dorsal muscle of mammals (dogs) and chondrichthyes (sharks). In the dog muscles around the chambers weak reactions against low antigenicity were observed in shark muscles. On the other hand no reaction against antigenicity was observed. From experiments of successful inducement of hemopoiesis with ossification of cartilage by collagen-HAP composite (artificial cartilage) implanted into dorsal muscle of sharks, it is disclosed that archetype vertebrates of chondrichthyes, i.e., sharks have no tissue immunity against cattle collagen with antigenicity. It is known that sharks have MHC. Namely primitive vertebrate chondrichthyes are know to possess genomes of major

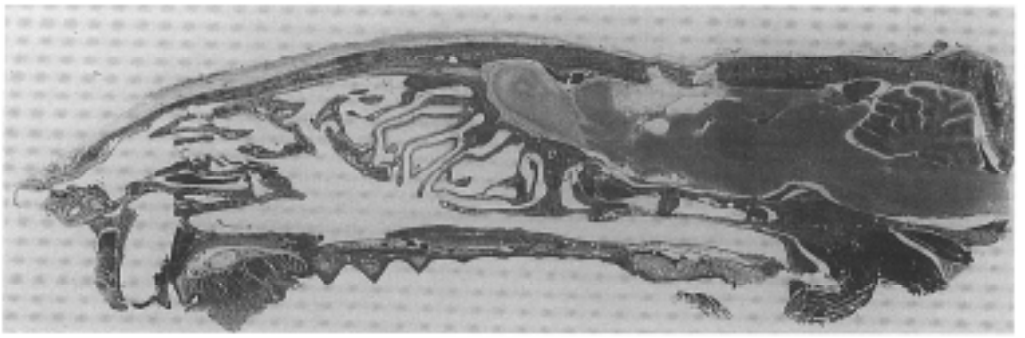

Figure 3 Successful xenotransplatation of brain of shark into that of rat, 3 month post op.

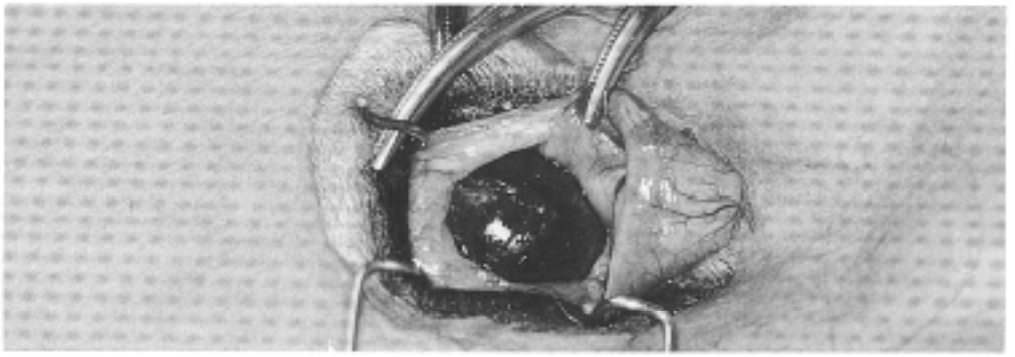

Figure 4 Successful zenotransplantation of shark cornea to that of dog.

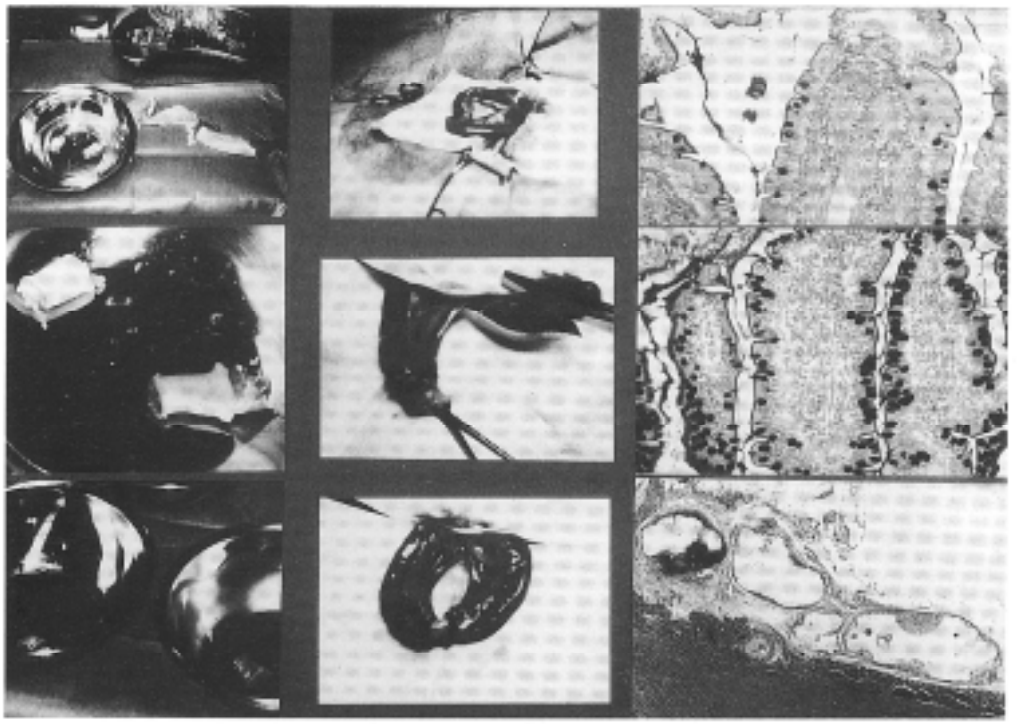

Figure 5 Successful zenotransplantation of shark intestine into that of dog Goblet cells resembling that of shark can be observed.

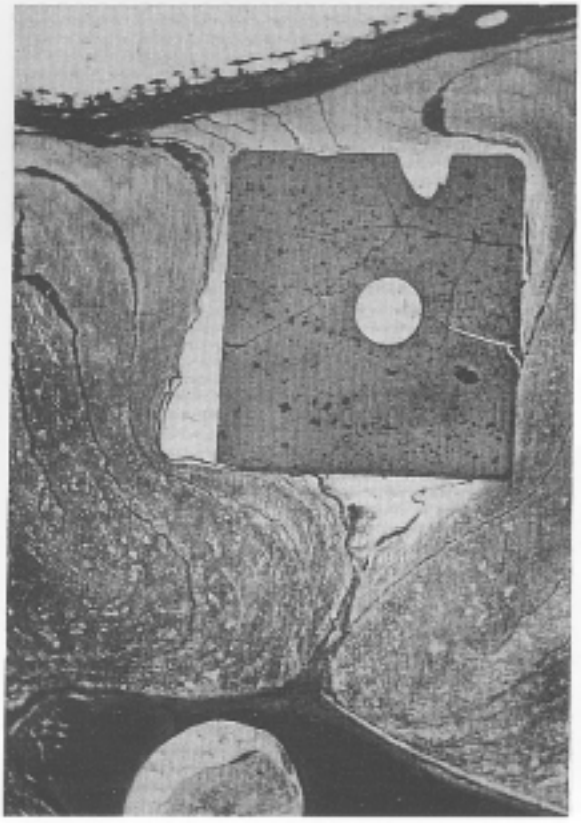

Figure 1
Collagen-composite HAP implanted into dorsal muscles of Triakis, by which hemopoietic nests are induced.

Figure 2
A - Cell differentiation around collagen-composite, implanted into a dog 6 months postop.

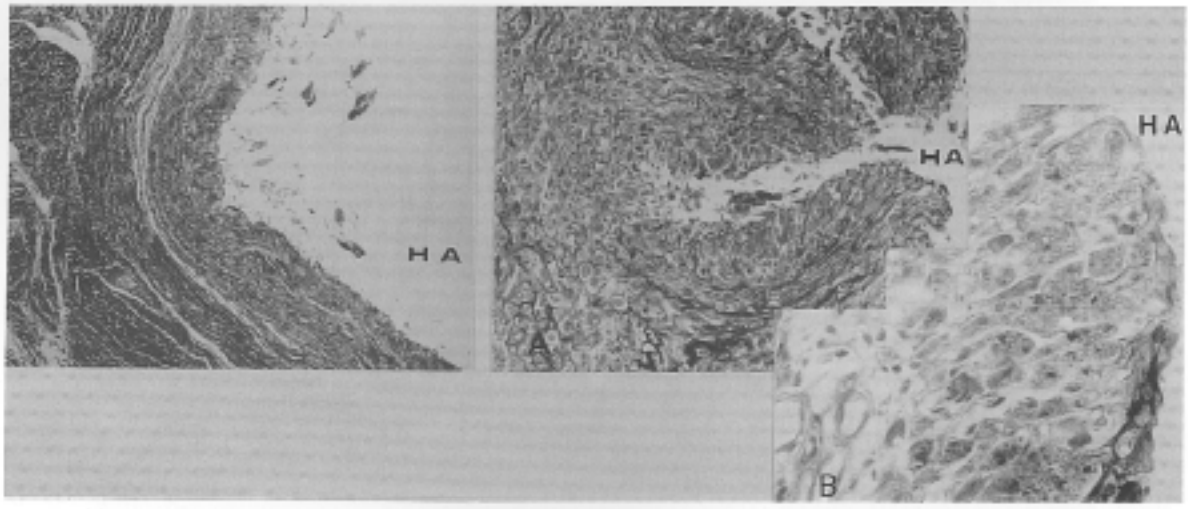

Figure 2
B - Cell differentiation resembling tissue of digestive tract, which are induced y collagen composite HAP (Protein Delivery System)

histocompatibility antigen complex (MHC), but the authors have disclosed that they have no tissue immunity. This means that they are in immuno-tolerance just as the embryo of higher animals such as the reptile, aves, and mammals. From the experiments the authors discovered chondrichthyes have no HLA (human leukocyte antigen) even though they have MHC (major histocompatibility antigen complex). Therefore archetype vertebrates are in immuno-tolerance just like embryo of higher animals. These facts coincide with the Biogenetic Low of Haeckel in tissue immunity. After terrestrialization of chondrichthyes not only hemopoietic nest immigration from the gut system to bone marrow occurred but tissue immunity by MHC was generated through reactions of sharks against increased gravity. To verify immune-tolerance of sharks xenotransplantation of xenopus and cyclostomata derma as well as rat skin to that of sharks are carried out for preliminary experiment. Successful skin graft with chimera placoids formation between heterodontus and xenopus is fulfilled. After that xenotransplantations of shark brains (Figure 3) cornea (Figure 4), muscle and intestine (Figure 5) to those of rats and dogs are successfully performed. From these successful xenotransplantation, the major function of MHC (HLA) is found to be the cytological digestion system mainly functioning for tissue remodeling in the organism's own cells of organs, and partly to digest parasites as well as transplanted imported tissue. From these experiments new biomaterials substituting allograft of various organs are developed.

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