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SUCCESSFUL INDUCEMENT OF HYBRID TYPE ARTIFICIAL BONE MARROW USING BIOCERAMICS IN VARIOUS VERTEBRATES

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ABSTRACT

A trilateral research method integrating morphology (Goethe), molecular biology (Delbrück), and Biomechanics (Roux) was developed using bioceramics. Based on this research method, cell differentiation was induced around bioceramics by biomechanical stimuli. The authors already reported the successful inducement of hemopoietic nests with sintered hydroxyapatite (HA) artificial bone marrow chambers implanted in mammals (dogs and monkeys.) In this paper, through the trilateral research method, artificial inducement of bone marrow hemopoiesis in muscles is reported using dochi-zame (chondrichthyes) and hagfish (cyclostomata), which have no inner skeletons of bone with marrow but HA bone only in placoids, and represent the phylogenical stage of archetype vertebrates. Successful inducement of hematopoietic fields with osteoid tissue in hydroxyapatite chambers was obtained in muscles of shark and cyclostomata. Through this experiment inducement of hemopoiesis with osteoid tissue can be understood by biomechanics conjugated with the material effect of HA.

INTRODUCTION

For an experimental hemopoietic field, muscles were selected, and for bone substitution, sintered porous tubular HA artificial bone was used. The authors tried to induce artificially a hematopoietic field in muscles of dogs and Japanese monkeys using sintered porous tubular hydroxyapatite[1]. As a result, in the pore sites of tubular hydroxyapatite artificial bone implanted in the dorsal muscles, a marked differentiation of osseous tissue with bone marrow cell clusters of the hematopoietic field could be observed[2].

This work was awarded the originality prize of the 32nd Japanese Society of Artificial Organs in October, 1994. To study evolutionary mechanisms of bone marrow hemopoiesis, hematogenesis in artificial bone marrow chamber made of sintered hydroxyapatite (HA) was tried using archetype vertebrate, i.e., chondrichthyes and cyclostomata, which had no inner skeleton of hydroxyapatite but cartilage. Hemopoiesis conjugated with osteoid tissue genesis could be observed around artificial HA skeleton implanted into muscles of archetype vertebrates.

MATERIALS AND METHODS

Six adults dochi-zame (chondrichthyes) and three adult hagfish (cyclostomata) were used for experiments. For anesthesia 100ppm para-amino-benzoate was used. In each

dorsal muscle of 6 sharks 3 HA artificial bone marrow chambers (Asahi Optical Co. Led.)and 3 chambers of collagen-HA composite chamber (National Institute for Research in Inorganic Material) were implanted and recovered 2, 3, and 4 months after implantation. In each dorsal muscle of 3 hagfish HA artificial bone marrow chamber (Sangi Co. Ltd.) was implanted and recovered 1 and 2 mouths postop.

RESULTS

In all specimens recovered from six chondrichthyes and 3 cyclostomata metaplasia into hemopoietic nests around HA chambers was observed from muscle fibers. Fig. 1 shows collagen-HA composite chamber implanted into shark dorsal muscle, undecalcified specimen, 2 months postop.

Fig. 2 shows osteoid tissue with hemopoiesis and metaplasia of muscle fibers into hematopoietic cells around sintered HA implanted, 4 months. Fig. 3 shows muscle and subcutaneous tissue under HA placoids without hematopoietic tissue (control). Fig. 4 shows sintered HA chamber (Sangi Co. Ltd.) implanted into dorsal muscle of hagfish, one month postop. No inflammatory reaction can be observed. Fig. 5 shows hemopoiesis in pore sites of sintered porous HA chamber implanted in dorsal muscle of hagfish.

DISCUSSION

In mammals around implanted artificial bone marrow chamber of HA in muscle hemopoiesis with osteogenesis cloud be surely induced, 2 months postop. As the control in tubular HA chamber implanted into subcutis of dogs, neither inducement of hemopoiesis nor osseous tissue occurred. However, in the same chamber with electricity (15μA) implanted in subcutis of dogs, marked hemopoiesis with ossification could be observed[2].

Fig. 1 HA chamber in shark

Fig. 2 Hemopoiesis in osteoid tissue in shark

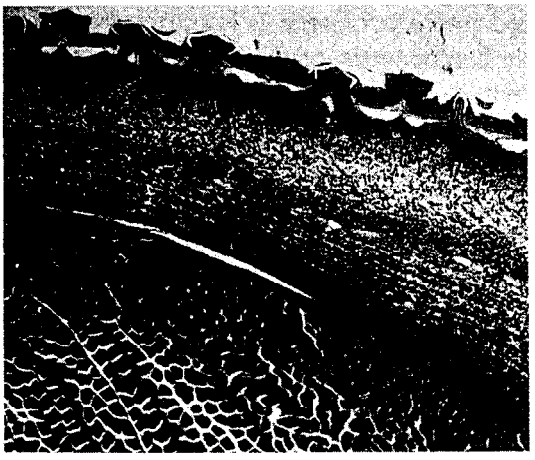

Fig. 3 Normal HA placoids and muscles of shark

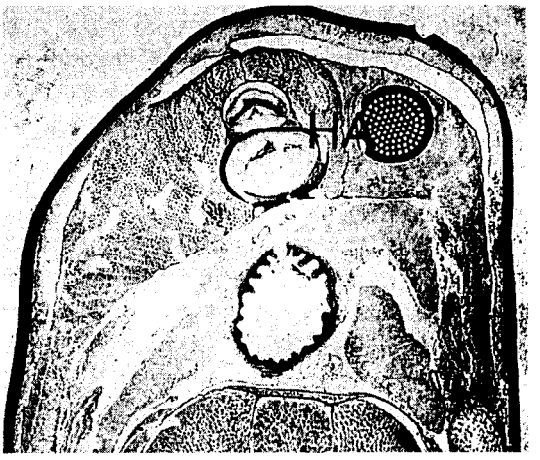

Fig. 4 HA chamber in hagfish muscle

Fig. 5 Hemopoietic nest around HA chamber in hagfish

Also, around the titanium chamber with electricity (15μA) implanted in subcutis of dogs, marked inducement of leukocytes and lymphocytes without ossification could be observed. From results of these series of experiments the following conclusion was obtained: the inducement of hemopoiesis conjugated with ossification occurred through gene expression of mesenchymal cell by HA. The biomechanical stimuli evoked in muscle is converted into streaming potential. Without biomechanical movement in subcutis no hemopoiesis inducement occurs around HA chamber. But with electricity it occurs even in subcutis. Inducement of hemopoiesis conjugated with ossification from undifferentiated mesenchymal cells needs essentially not only calcium and phosphate ions but electricity for gene expression in undifferentiated cells, in which nucleotide metabolism and tissue respiration are active. The cause of immigration of the hemopoiesis from the gut system i.e., the spleen into the bone marrow in higher mammals is thought to be a response to the gravity in terrestrial life[3]. The chondrichthyes evolved into amphibian by terrestrialization, during which chondroskeleton changed into osseous tissue with bone marrow cavity. Hemopoietic nests immigrated from the spleen into the bone marrow cavity. From this immigration process of hemopoiesis evolutionary transformation can be understood as response to changing of biomechanical stimuli of gravity upon organisms. To prove this hypothesis the following experiments were carried out: Artificial bone marrow chambers of conventionally sintered HA chamber (Asahi Optical Co. Ltd.) and

composite of HA and collagen sintered with high-pressure gas technique (National Institute of Inorganic Materials) were implanted into Dochizame (Chondrichthyes) and hagfish (Cyclostomata) osteoid formation with hemopoiesis could be observed. These results show that gene expression of mesenchymal cells were controlled by streaming potential conjugated with material effect of HA.

CONCLUSION

The hemopoiesis inducement in artificial bone marrow chamber was successfully carried out using archetype vertebrates. From the experiments evolution of bone marrow hemopoiesis can be demonstrated to occur as a response to gravity in terrestrialization of archetype vertebrates.

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REFERENCES

1. Nishihara, K., Tange, T., Hirota, K. and Kawase, K., Bio-Medicals and Engineering, Vol.4, No.1, 1994, 61-65.
2. Nishihara, K., Jpn J Artif Organs, Vol. 24, No. 1, 1995, 6-12.
3. Nishihara, K., J Oromax Biomech, Vol.1, No. 1, 1995, 79-87.