INTRODUCTION:

The repair of an osteochondral defect is progressing with tissue engineering techniques. Interconnected porous hydroxyapatite ceramics (IP-CHA) is a useful scaffold and its composite with growth factors and cultured cells has been studied for the osteochondral repair in animal model. However, its method is complicated and not easy for the clinical treatment. Articular cartilage repair with periosteum has been well studied and performed clinically for more than a decade. The periosteum contains pluripotential mesenchymal stromal cells, bioactive growth factors, and a scaffold onto which other cells and growth factors can be adhered. The osteochondral repair with the IP-CHA and periosteum coverage composite is a simple procedure, however its histological examination has not been reported. The purpose of this study was to examine the effects of the periosteum coverage on the osteochondral repair with interconnected porous hydroxyapatite ceramics (IP-CHA).

MATERIALS AND METHODS:

IP-CHA: The IP-CHA used in this study is commercially manufactured (Toshiba Ceramics, Tokyo) and approved for clinical use as a bone substitute in Japan. It is made from a slurry of hydroxyapatite \( \text{Ca}_10(\text{PO}_4)_6(\text{OH})_2 \) by the “foam-gel” technique. We analyzed its porous structure and reported in a previous study. Scanning electron microscope images (JSM-6330F) revealed regularly aligned pores, thin walls, and interconnecting holes (Fig. 1a). The pore diameter was 100-250\( \mu \)m. Mercury porosimetry (Autopore 9420) demonstrated that the total porosity was 63.6\%, and most of the interporous connections ranged from 10 to 100\( \mu \)m, with a maximum peak at 40\( \mu \)m (Fig. 1b). The shape of the IP-CHA used in this study was a columnar shape (diameter: 4mm, height: 4mm) (Fig. 2a).

Surgical procedure: Forty-eight Japanese white rabbits (average weight, 2.5kg) were used in this study. For anesthesia, 1 mL of pentobarbital (50mg/mL) was administered intravenously, and 5 mL of ketamine (50mg/mL) was administered intramuscularly. Under sterilized condition, a straight 4-cm longitudinal incision was made over the left knee and the joint surface was exposed. An osteochondral defect, 4.1 mm in diameter and 6 mm in depth, were created with a hand-drill in the patellar groove of the distal femur. Rabbit knees were divided into four groups: group I (n=12 knees) received IP-CHA alone; group P (n=12 knees) received IP-CHA with the coverage of periostium; group F (n=12 knees) received IP-CHA with the coverage of the deep-frozen periostium; and group D (n=12 knees) underwent a sham operation with no implantation. In group I, P, and F, all IP-CHA were placed at the subchondral bone level, at 2 mm beneath the adjacent cartilage surface. In group P and F, the 6 mm square periostium was harvested from the medial aspect of the proximal tibia. In group F, the periostium was frozen in liquid nitrogen (-196°C) for 10 minutes and thawed in saline solution to kill cells. In group P and F, the periostium was fixed on IP-CHA, with the cambium layer facing toward the articular surface, by the 4-0 nylon suture running through the 4 bone tunnels Each of the 24 rabbits were sacrificed 4 and 8 weeks after the operation by an intravenous injection of 5 mL of pentobarbital. This animal study was performed in accordance with the “Principles of laboratory animal care” (NIH publication No. 85-23, revised 1985), and was approved by the rules and regulations of the Research Facilities for Laboratory Animal Science, Hiroshima University.

Macroscopic observation: When the animal was sacrificed, the surface of the defect was inspected for color, hardness, continuity to adjacent articular surface, and smoothness. Histological examination: The harvested distal part of the femur was fixed in 10% neutral-buffered formalin. The excised bone was decalcified in formic acid and embedded in paraffin. Sections, 5 \( \mu \)m thick, perpendicular to the joint surface and to the long axis of the femur were made. Sections were stained with hematoxylin and eosin or toluidine blue. The section at the center of the defect was used for the analysis.

RESULTS:

Macroscopic observation: In group P, 4 weeks model, the defect was repaired with a white and hard tissue, showing a smooth surface and good continuity to adjacent articular surface. In group P, 8 weeks model, the color of repaired tissue became close to the adjacent cartilage. In group P, 8 weeks model, a small defect remained on the surface (Fig. 3).

Histological findings: In group P, F, and I, abundant bone tissue was conducted into the pores of IP-CHA. The amount of occupied area by conducted bone in IP-CHA was not significantly different among the 3 groups at 4 and 8 weeks after the operation. The cartilage repair scores of the 4 weeks group were13.2±1.2 in group P, 11.2±1.2 in group F, 8.1±1.3 in group I, 5.4±1.7 in group D, and in the 8 weeks group were 14.1±2.2 in group P, 11.9±2.3 in group F, 9.1±1.7 in group I, 8.8±2.4 in group D. The score in group P was significantly larger than that in group I, both 4 and 8 weeks after the operation. In group P, 8 weeks model, the thick cartilage remained and the subchondral bone formation occurred in the adjacent to the host bone (Fig. 4).

DISCUSSION:

In groups P, F, and I, the abundant bone tissue was conducted into the pores of implanted IP-CHA, and the amount of bone area in IP-CHA was not different among these three groups. This suggested that even in a joint cavity, where the IP-CHA was exposed to the synovial fluid, IP-CHA had good osteoconductivity. For the cartilage repair, group P had the best histological repair among the 4 groups. In group P, 8 weeks model, the thick cartilage remained and the subchondral bone formation occurred adjacent to the host bone, which showed the periostium coverage enhanced the cartilage repair on IP-CHA. The osteochondral repair with the composite of periostium and IP-CHA is a simple technique. The result of this study, showing the good osteochondral repair, indicated that the composite of periostium and IP-CHA is useful for an osteochondral repair.