Original Article

Histological assessment of porous custom-made hydroxyapatite implants 6 months and 2.5 years after cranioplasty

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Abstract

Background: In cranial reconstruction, the features of artificial bone differ. Custom-made porous hydroxyapatite (HAp) implants for cranioplasty have been used all over the world because of their good cosmetic, biocompatibility, and osteoconductive properties. Surgical techniques were analyzed, and histological assessment of new bone formation in the hydroxyapatite was performed.

Methods: Over a 6-year time period, 41 patients underwent cranioplasty using a custom-made three-dimensional hybrid pore structured hydroxyapatite (3DHPoHAp) implant. The surgical techniques and histological evaluations of 3DHPoHAp in 2 cases, removed 6 months and 2.5 years after cranioplasty, are described.

Results: Using 3DHPoHAp, cranioplasty was successfully performed for all patients. The implant fit the bone defect exactly, and surgical manoeuvres were simple and easy. All implants were firmly fixed using a titanium plate, and postoperative infection occurred in 1 patient (2.4%). New bone formation was seen in 2 cases 6 months and 2.5 years after cranioplasty. Osteoblasts were progressing to the stoma at various depths, and bone tissue had ripened. Furthermore, lamellar structure was observed in the case at 2.5 years.

Conclusions: In this study, there was a low infection rate, and new bone formation was seen *in vivo* after cranioplasty. This study also demonstrated that the 3DHPoHAp implant is a good candidate for cranial bone implants because its good osteoconductivity and biocompatibility.

Key Words: Cranioplasty, hydroxyapatite, osteoconductivity, osteointegration, traceability

INTRODUCTION

Selection of artificial bone, including autologous bone, and reconstruction of cranial bone for cranioplasty have been entrusted to each medical institution.[20] Because of the different features of each artificial bone, each medical institution has decided to select artificial bone based on various factors including cost. Two cases in which histological evaluation of bone formation was

performed after cranioplasty using three-dimensional hybrid pore structured hydroxyapatite (3DHPoHAp) implants, that were manufactured in Japan, are presented here. Moreover, this is the first clinical report of the histological evaluation of new bone formation 6 months after cranioplasty using Hap implants.

MATERIALS AND METHODS

From June 2009 to December 2014, 41 cranioplasty procedures were performed at our institution using 3DHPoHAp. The bone defects were divided into three categories by size, namely, small-sized category, including defects <150 cm^2 (2 cases); medium-sized category, including defects 150–350 cm^2 (18 cases); and large-sized category, including defects >350 cm^2 (21 cases). This study was approved by the local ethics committee (approval no. 3368); patient consent was not required because this was a retrospective study.

Patients' ages ranged from 31 to 85 years (mean, 65.3 years) with no sex predominance. The average period until cranioplasty was 34.8 days. There were no spontaneous fractures, rejections, or reabsorption. The features of the cases are summarized in Table 1.

Design of artificial bone and the surgical technique in our institute

In the present study, the design of the artificial bone and the surgical techniques were as follows: Confirmation and antibiotic selection for systemic infection; setting the size and shape of the artificial bone using 3DCT; sufficient exposure of the autologous bone edge; cleaning the operative field and foreign body removal, including artificial dura mater; and fixation using a titanium plate and screw. Finally, in principle, antibiotics for postoperative infection control were not used except during surgery.

Manufacturing process of 3DHPoHAp

An aqueous solution of phosphoric acid was poured into a suspension of purified calcium hydroxide, which resulted in HAp slurry. The slurry was sprayed and dried using the spray-dry method to produce a fine spheroid powder. HAp powder was homogenized in water, and a water soluble polymer was added to the slurry as a binder. The slurry was gel-formed and dried. The green ceramic was shaped into a specific shape by a milling machine and then sintered at 1200°C in air. The water soluble polymer included in the green ceramic was removed by the sintering process, and pores were formed. Porosity, pore structure, and interconnecting pore size were controlled by the synthetic conditions, grain size, sintering temperature, and mixed polymer. It has been reported that APACERAM® (HOYA Technosurgical Corporation, Tokyo, Japan) has a suitable pore structure for bone regeneration and mechanical strength when these parameters are controlled.[16,23] A porous, custom-made HAp cranial plate was designed with data obtained from 3DCT of the whole skull including the bone defect by mirroring images of the contralateral normal skull. The plate was machined using a computer-aided design/computer-aided manufacturing (CAD/CAM) system, and it was then sintered at 1200°C in air.[14,15] This product has pores with diameters of 100–500 μm connected in a 3D matrix by small pores called "interconnecting pores" with diameters of 0.5–2.0 μm.[9]

The 3DHPoHAp used in this study was an artificially synthesized ceramic with 40% porosity, which is currently the most commonly used cranial plate reconstruction material in Japan. This type of custom-made HAp was created by HOYA Technosurgical Corporation in 2004.

Access and cost of 3DHPoHAp

The use of custom-made HAp in Japan is not special, with each institution deciding its use. Information on 3DCT and the opinion of the neurosurgeon are important for artificial bone preparation, and in particular, the shape, curvature, and the fixed part of the artificial bone are determined. The 3DHPoHAp setting period up to cranioplasty depends on the size, however, it can be created in approximately 14 days from order. Finally, the 3DHPoHAp is sterilized packed with high-pressure steam sterilization method according to the domestic regulations in Japan. Then, it is delivered to the medical institution on the day before the surgery.

Regarding the cost of artificial bone in Japan, all artificial bones including custom-made HAp are set at a fixed price as medical equipment. The cost for artificial bone is determined by the total amount paid based on the size of the artificial bone, the patient's private income, medical cost, etc., as determined by the insurance plan. The cost of 3DHPoHAp paid by the patient is approximately $180 to $800.

Table 1: Summary of patients treated with 3DHPoHA for cranioplasty

No. of Patients	41
Age, years (mean/range)	65.3/31-85
Men: Women	21:20
Initial diagnosis (no. of cases)	
Subarachnoid hemorrhage	12
Intracerebral hemorrhage	12
Cerebral infarction	11
Acute subdural hematoma	5
Brain contusion	1
Duration from decompression to cranioplasty, days, (mean/range)	34.8/17-58
Complications: wound infection (case 2)	1

RESULTS

Illustrative cases

Case 1

A 39-year-old man suffered a massive ventricular hemorrhage with immediate coma. The day after admission, he was diagnosed with Moya Moya disease by brain angiography. Although he underwent ventricular drainage of the intraventricular haemorrhage, 1 week after admission, brain herniation developed due to cerebral infarction. He then underwent immediate decompressive craniectomy using artificial dura, and intracranial pressure was controlled. After decompressive surgery, his consciousness recovered gradually. He then underwent cranioplasty using a 3DHPoHAp implant. After cranioplasty, he significantly improved by rehabilitation treatment after hospital discharge.

Six months after prosthesis implantation, left encephalo-myo-synangiosis was performed to improve cerebral blood flow to the Moya Moya disease. It was found that the 3DHPoHAP plate had fused tightly to the cranium and could be excised in parts using a drill and luer [Figure 1]. The hydroxyapatite plate had adhered to the dura mater without an artificial dura. Finally, the plate was replaced.

Histological findings of Case 1

The cranial implant was fixed in 10% neutral formalin and dehydrated in alcohol. Sections approximately 400-μm thick were cut using a crystal cutter (Marutoh Co., Tokyo, Japan), ground to a thin section of approximately 50 μm, and stained with hematoxylin-eosin stain and toluidine blue. Light microscopy showed that the new bone was formed not only in the margin of the implant in direct contact with the skull but also in non-contact areas such as the implant vault or inside the pores. New bone was formed mainly parallel to the implant on the surface and in the perpendicular direction along the pores. Overall, the formation was three-dimensional. Bone formation on the dural side of the hydroxyapatite plate was more extensive than on the skin side [Figure 2]. A specimen

for scanning electron microscopic analysis was prepared from the embedded implant. On examination, osteons were formed in the pores of the implant on the dural side. One pore contained one osteon. Haversian canals were found in the centres of the osteons. A new vessel was connecting pores between large pores [Figure 3].

Case 2

A 49-year-old man was transferred to our hospital after emergency surgical treatment with decompression for putaminal haemorrhage in a foreign medical institution. On admission to our hospital, he had meningitis due to postoperative complications, along with hydrocephalus due to the infection. After treatment of the meningitis and hydrocephalus was performed in our hospital, he eventually improved and was able to uneventfully undergo cranioplasty by 3DHPoHAp. However, postoperatively, 3DHPoHAp was removed from the surgical wound defect because of his own scratching 2.5 years later. Therefore, because the artificial bone was exposed, it was removed to avoid infection. The hydroxyapatite plate had fused tightly to the cranium without artificial dura and could be excised using a drill and luer during the procedure. It was excised in pieces in the same manner as in Case 1 [Figure 4].

Histological findings of Case 2

Histological sections were processed in the same manner as in Case 1. New bone formation was more mature than that seen after 6 months in the pores of the plate between the cranium and implant. Light microscopy showed that new bone was formed mainly parallel to the implant on the surface and in the perpendicular direction along the pores. The artificial bone tissue layer plate structure was observed. Scalloping that was absorbed into mature bone with a lamellar structure on the surface of the new bone was recognized as osteoid, suggesting thriving bone formation. Artificial bone and bone tissue boundaries in
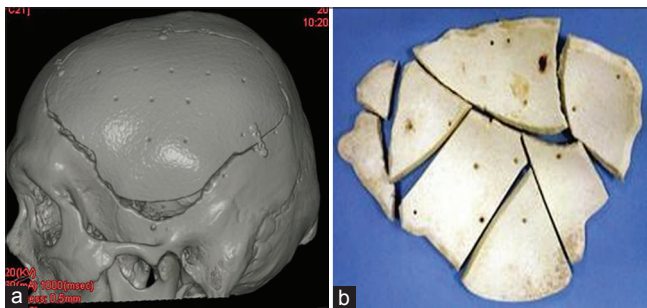

Figure 1: 3DCT (a) of 3DHPoHA implant after decompression and photograph (b) of removal surgery. EMS (encephalo-myo-synangiosis) surgery after massive infarction due to Moya Moya disease (Case 1; implantation period: 6 months)
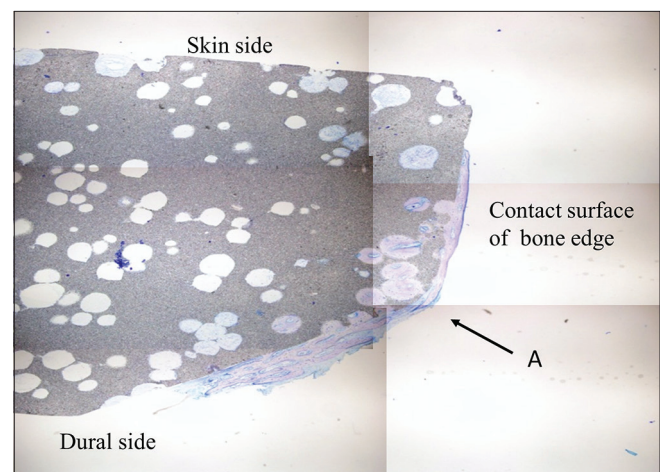

Figure 2: Bone tissue attached to the artificial bone. (black arrow, point A) Also near the surface of the artificial bone, bone tissue is present in the pores

the vicinity of the absorption fossa and osteoclast-like tissue were observed [Figures 5 and 6].

DISCUSSION

The purpose of this study was to histologically evaluate the new bone formation of HAp implants. It has previously been shown that a prosthesis made of a HAp implant induces new bone formation in experimental animal models. According to previous reports of histological investigations, HAp implants demonstrated osteointegration in animal model studies.[3,10,11,24,25] Clinically, a HAp implant is a commonly used material for cranioplasty worldwide. The reason for this is its good osteoconductivity. Osteoinduction is the process by which osteogenesis is induced. It is a phenomenon regularly seen in any type of bone healing process. Osteoinduction implies the recruitment of immature cells and the stimulation of these cells to develop into preosteoblasts.[1,20]

In a bone healing situation, such as a fracture, the majority of bone healing is dependent on osteoinduction.[2] Osteointegration is the stable anchorage of an implant achieved by direct bone-to-implant contact[16,21] and bone formation by the dura.[6,12]

Also related to intensity, it is important to have a biocompatible material with suitable porosity in a HAp implant. Then, an appropriate spatial distribution of suitable holes with the correct size and shape to allow migration of osteoblasts from the living bone to occur results in osteointegration.

Therefore, these properties of implants will lead to the biocompatibility of the artificial bone. HAp implants have generally been noted to have many advantages in some past reports.[8,10,19,22,27]

However, in comparison with other artificial bone, there is not much evidence for osteointegration of HAp implants for humans *in vivo*.[4,19] One of the reasons for this is that there are few reports of new bone formation. New bone formation *in vivo* using HAp implants was observed in only 4 cases.[4,5,12] Information regarding the implant was confirmed in some of the reports,[4,5,11,13,15,20,21] however, there were only limited descriptions of properties such as porosity and thickness of the artificial implant.

Figure 4: 3DCT (a) of 3DHPoHA implant after decompression and photograph (b) of removal surgery. Epidural abscess after cranioplasty following decompressive surgery for intracranial haemorrhage. (Case 2; implantation period: 2.5 years)
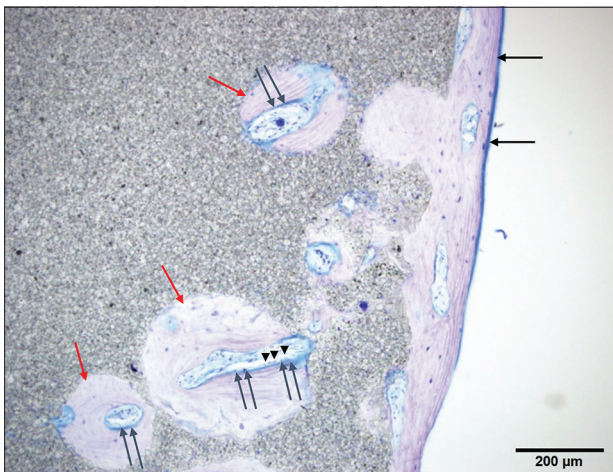

Figure 3: Histological view of point A in Figure 2 (Enlarged view). Well-developed osteoid is observed on the surface (black arrow). The artificial bone and scaffolding show active bone formation. Bone tissue is formed in the pores near the surface (red arrow). Osteoid (double blue arrow) and osteoblasts (arrowhead) are also observed. Bone formation is in progress
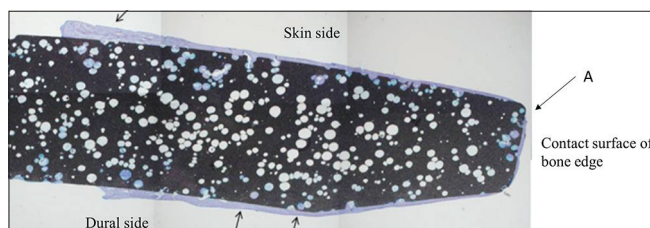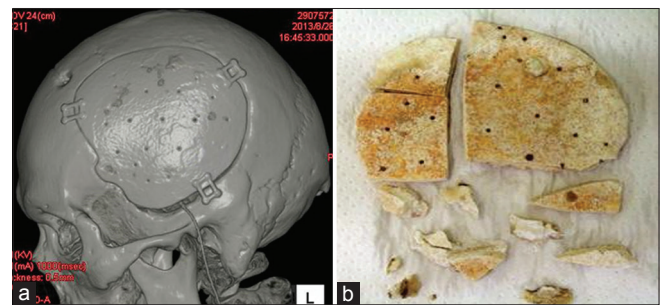

Figure 5: Bone tissue attached to the artificial bone (black arrow, point A). Formation of new bone over the surrounding artificial bone is observed (small arrow)
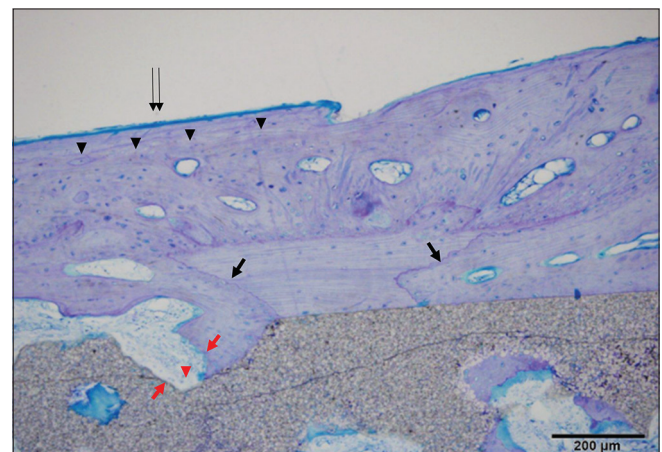

Figure 6: Histological view of point A in Figure 5 (enlarged view). The artificial bone tissue layer plate structure is observed. Osteoid is observed on the bone surface (black double arrows). Scalloping that has been absorbed into mature bone with a lamellar structure observed (black arrow) on the surface of the new bone is recognized as osteoid (black arrowheads), suggesting thriving bone formation. Artificial bone and bone tissue boundaries in the vicinity of the absorption fossa (red arrow) and osteoclast-like tissue are observed (red arrowhead)

Including the porosity of the implant, Marco *et al.* demonstrated new bone formation with a HAp implant after cranioplasty. They histologically evaluated three different porosities of the implants used in two cases. The porosities of the three different implants were 48.8%, 42.9%, and 55.8%. With the implant with a porosity of 42.9%, there was no new bone formation 6 months after cranioplasty. As one of the reasons for this, they noted shortened period of cranioplasty and explantation.[5]

In the present cases, new bone formation was observed at 6 months and 2.5 years after cranioplasty using 3DHPoHAP implants because the implant had porous characteristics and there was binding of the artificial bone and the marginal cranium edge. The implant used was formed of a 3D porous body with various pore sizes, taking into account new bone formation. Therefore, the porosity of the implants was 40%, with 5-mm thickness to provide sufficient strength.[14,23] The reason for this is the stable production of porosity and thickness of HAp implants, which is important for the retention of new bone formation.

In the perioperative period, the implant was designed by 3DCT, and the boundary between the artificial bone and the marginal cranium edge was as small as possible, which significantly increases the contact surface of both. As for the operative technique, the remaining connective tissue on the marginal cranium edge and the epidural membrane were removed and washed sufficiently. The small titanium plates were fixed by screws.

Regarding the timing of cranioplasty after decompression, there are some opinions that 2–6 months to cranioplasty are required to avoid postoperative infectious complications;[17,18,26] most cases in the present study were performed within less than 2 months. However, among the postoperative complications in the present study, there were no skin infections, other than the one case due to scratching by the patient himself (Case 2 in the present series).

When comparing the present approach to the use of autologous bone, the problems with the autologous bone use are the complicated preservation method and deformation, and it is clear that there is a particular risk of infection, as well as with artificial bone. Of course, autogenous bone is ideal for cranioplasty. However, because there are not a few infections of autologous bone,[7,19] it is necessary to consider and analyze a large amount of data following cranioplasty, including that with artificial bone.

Furthermore, due to the vulnerability of HAp implants, it has been reported that they cannot be used for large bone defects. However, there is no problem for a fixed defect, providing brain protection for activities of daily living of the patients in the present study. Therefore, the strength of Hap implants for large defects needs to be examined in the future.

This research has some limitations. Because only Hap implants were used in cranioplasty, comparisons with other artificial bones, including other porous hand-made Hap implants, were not possible. When using HAp implants, a long period of observation is needed, and there is a need to consider several issues in the future. With respect to HAp implants, there is no correct answer for selecting artificial bone. However, for artificial bone, which is created in the same manner, to be used, a system of product traceability is needed.

Finally, the main issue is that HAp implants are manufactured in different ways in each country. Therefore, constant evaluation of postoperative outcomes is difficult. Thus, information about artificial bone will become very important in the future. Neurosurgeons must understand the product to demonstrate its effectiveness, and they must cooperate in sharing information to improve management of patients treated with artificial bone implants.[27]

CONCLUSIONS

The present histological study of 2 3DHPoHAp plates removed after use for 6 months and 2.5 years, respectively, showed fusion of the 3DHPoHAp to the cranium and new bone formation, mainly on the dural side and in a 3D matrix within pores. 3DHPoHAp showed osteointegration and lamellar organization in the two cases presented. The 3DHPoHAp implant appears to be an excellent candidate as a cranial bone substitute due to its osteoconductivity and biocompatibility.

Conflicts of interest
There are no conflicts of interest.

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