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Original

The Usefulness of Mandibular and Maxillary Bone Derived from Neural Crest as Bone Graft Substitutes

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Abstract: Autografts, which are commonly used for alveolar bone regeneration, often utilize the ilium and jaw bones as alternative bone graft materials. Maxillary and mandibular bones are developmentally derived from neural crest-derived cells (NCDCs), while the majority of trunk and limb bones are derived from mesoblast cells. Consequently, the host bone graft material might differ in developmental origin from the recipient bone. With such a potential mismatch in practical terms, it is unclear whether genuine jaw bone can be regenerated. We hypothesized that bones derived from NCDCs and mesoblast cells show different capacities for *in vivo* bone healing. To investigate this proposal, we undertook bone graft experiments using a murine model. We first perforated a 2-mm diameter area in both the frontal and parietal bones, which are derived from NCDCs and mesoblast cells, respectively; then we grafted various source materials into each bone defect. Mice were euthanized at 2 weeks after grafting, and histological analyses and immunohistochemistry were performed to evaluate differences in bone healing based on the various combinations of graft and recipient bones. The frontal bone was found to heal faster than the parietal bone. Parietal bone defects transplanted with maxillary and mandibular bone grafts exhibited closure, whereas iliac and femoral bone grafts did not result in full healing. Immunostaining for osteopontin also demonstrated good bone regeneration in the parietal bone defects using maxillary and mandibular bone graft materials. These results suggest that maxilla and mandible bones exhibit NCDC properties with an enhanced healing potential. We conclude that maxillary and mandibular bones are effective bone graft and graft bed materials.

Key words: osteopontin, bone graft (s), bone regeneration

Introduction

Bone grafting is usually required for dental implantation and for the treatment of periodontal disease and cleft palate. Bone graft materials include autografts, allografts, xenografts, and

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alloplasts ; however, xenograft and alloplast materials are not of human origin. For example, beta-tricalcium phosphate (β-TCP) alloplasts have been successfully grafted to the floor of the maxillary sinus, wherein the regenerated bone comprised 32% new bone at 1 year after grafting, with a residue of 25% beta-tricalcium phosphate particles[1]. Another example of a commonly used bone graft material is hydroxyapatite (HA). After grafting HA to maxillary bone, the regenerated bone comprised approximately 20% new bone at 9 years after grafting, while the HA residue was approximately 30%[2]. In a xenograft study, bovine bone mineral was grafted to the maxillary sinus, wherein the regenerated bone comprised 27.55% new bone at 1 year after grafting with a bovine bone mineral residue of 27.01%[3]. After grafting to the maxillary bone, the composition of the regenerated bone consisted of 46% new bone at 9 years after grafting with a bovine bone mineral residue of 16%[4]. Under these circumstances, it is questionable whether real jaw bone tissue was reconstructed because the alloplasts and xenografts were not completely replaced with new bone and remained, to some degree, in the jaw bone after reconstruction.

Developmentally all organs consist of endoblast, mesoblast, and ectoderm layers. Most craniofacial bones, including the maxilla and mandible, are derived from ectodermal neural crest-derived cells (NCDCs), except for the ethmoid, temporal, occipital, and sphenoid bones, while most trunk and limb bones, including the ilium, are derived from mesoblasts[5–8]. Regarding the ossification pattern, maxillary and mandibular bones show intermembranous ossification, except for the mandibular cartilage, while most trunk and limb bones show endochondral ossification[9–11]. Consequently, bone grafts with a different developmental origin and ossification pattern from maxillary and mandibular bones are generally used for alveolar ridge augmentation.

A previous study has indicated that development of the craniofacial skeleton is different from that of other bones at the molecular biological level[12]. Indeed, bone marrow cells from the mandible exhibit higher osteogenic potential than those from the long bones[13]. Another study compared *in vivo* transplantation of bone marrow cells obtained from the maxillary, mandibular, and iliac bones, with respect to cellular proliferation, lifespan, growth factor, and protein expression and histology. Bone marrow cells obtained from each source were found to have different properties[14]. Furthermore, cells obtained from different bone sites display site-specific insulin-like growth factor production[15], and we previously reported different gene expression profiles between mandibular and tibial growth cartilage[16]. It has also been suggested that the specificity of each bone site might affect bone regeneration post-grafting[17–19].

The bone matrix formed by post-migratory, craniocerebral NCDCs is histologically different from that formed by bone marrow-derived mesenchymal stem cells (BMSCs). In turn, NCDSs show higher proliferative, osteogenic, and multi-lineage potentials than BMSCs. Such findings suggest that NCDCs are preferable as cell sources for tissue engineering in the craniofacial area[20]. Furthermore, NCDCs have high self-renewal ability in adult tissues[21, 22].

Bone graft materials for repairing maxillary and mandibular bone defects are chosen based on the bone graft material requirements and invasion level, rather than the purpose of bone augmentation. Residual bone graft materials are observed following xenografts and alloplasts,

because the non-replaced materials remain in the bone tissue. Furthermore, allografts and iliac bone have different developmental origins and ossification patterns. Therefore, whether these bone graft materials should be used for jaw bone reconstruction remains controversial.

The aforementioned studies suggest that individual bones have different properties based on their origin, and elicit different healing patterns when bone substitutes are applied. Thus, it is important to choose bone graft materials based on their clinical specificities. Despite this, few studies have evaluated the osteogenic capabilities of different bone graft materials, with a focus instead on differences in developmental origins. In the present study, we compared the osteogenic potential of bone graft materials from different sites.

Materials and methods

Animals

Thirty-five 5-week-old male C57BL / 6J mice were used in this study. Twenty-five mice were randomly divided into five groups ($n = 5$ per group) based on the bone graft material used, as follows : Group 1, ilium group ; Group 2, femur group ; Group 3, maxilla group ; Group 4, mandible group ; Group 5, negative control group with no implanted material. All mice were sacrificed 2 weeks after bone grafting. Bones for grafting were extracted from 10 mice. All animal procedures were approved by The Animal Research Committee of Showa University, Tokyo, Japan (Approval number of animal experiment plan : 12046).

Graft material procedure

All surgical procedures were performed under general anesthesia in sterile conditions. After inhalation of ethyl ether, general anesthesia was achieved with an intraperitoneal injection of pentobarbital sodium. Bones (ilium, femur, maxilla, and mandible) were removed from bones around the teeth, excluding tooth and joint parts. Bones were ground using a mortar.

Surgical procedure

Following anesthesia, an incision was made at the midline of the mouse scalp, from the frontal to the occipital region, and a full-thickness flap was created exposing the calvarial bone. A diamond burr (φ 2.0 mm) was used to perforate an area 2 mm in diameter on the frontal and parietal bones, avoiding perforation of the dura mater. Subsequently, one type of the four materials was grafted into each frontal and parietal bone defect (allograft). The amount of graft material transplanted in all four groups was approximately 0.1 g. The negative control group did not receive implantation of any graft material. All graft materials were transplanted into the defects within 30 min after extirpation. The flap was repositioned and sutured tightly with non-absorbable sutures, covering the bone defect.

Histological procedures

Animals were euthanized 2 weeks after surgery. For all groups, defects were dissected together with the surrounding soft and hard tissues. Section blocks were fixed with 4%

paraformaldehyde, decalcified with Kalkitox (Wako, Osaka, Japan) for 2 days, neutralized with 5% anhydrous sodium sulfate, and then embedded in paraffin. Sections were cut (7 μm), deparaffinized, and stained with hematoxylin and eosin (HE).

Immunohistochemical procedures

Immunostaining with anti-osteopontin was performed 2 weeks after grafting. Sections were cut (7 μm), mounted on slides, deparaffinized, treated to quench endogenous peroxidase, blocked with Protein Block Serum-Free Ready to use (Dako Japan, Tokyo, Japan), incubated with an anti-mouse MAP osteopontin antibody (Cosmo Bio, Tokyo, Japan) at an appropriate dilution, allowed to react with Envision + Kits (Dako Japan) to visualize the DAB staining reaction, and then counterstained with methyl green solution. Sections stained with DAB + SUBSTRATE BUFFER (Dako Japan) appeared as dark brown.

Results

Graft materials from maxillary and mandibular bones heal bone defects faster than those from iliac and femoral bones

Hematoxylin and eosin staining revealed advanced self-healing in the frontal bone control mice (Fig. 1A). In contrast, self-healing was late in the parietal bone control mice, with extensive bone defects confirmed 2 weeks after grafting (Fig. 1B). Interestingly, bone defects in the frontal bone healed regardless of the type of bone graft. The newly formed bones showed abundant bone marrow and an osteoid-like structure. In contrast, bone defects in the parietal bone only healed when jaw bone was used as the graft material, and not when iliac and femoral bones were used. Bone nodules were only observed in the parietal bone defects of the ilium and femur groups. A more positive bone marrow-like structure was observed in the regenerated bone after maxillary bone grafting compared with mandible bone grafting in parietal bone defects.

Immunostaining with anti-osteopontin antibody in the frontal bone defects revealed positive staining in the regenerated bones for all groups (Fig. 2A). In the parietal bone defects, osteopontin staining was observed only in the bone-nodule surface of the ilium and femur groups, with diffused staining in the maxilla and mandible groups (Fig. 2B).

Discussion

We compared the ability of different bone graft materials to heal bone defects by performing transplant experiments, based on the hypothesis that healing would be more successful when the graft material and graft bed had the same developmental origin. Several reports have observed the healing process in rats and mice after applying bone graft materials to bone defects formed in the parietal bones (mesoblast origin), but not the frontal bones (neural crest origin). Furthermore, previous studies have not evaluated differences in developmental origin between frontal and parietal bones when using the cranial bone as a graft bed. In this study, we used the maxilla and mandible (neural crest origin) and ilium and femur (mesoblast origin) as bone

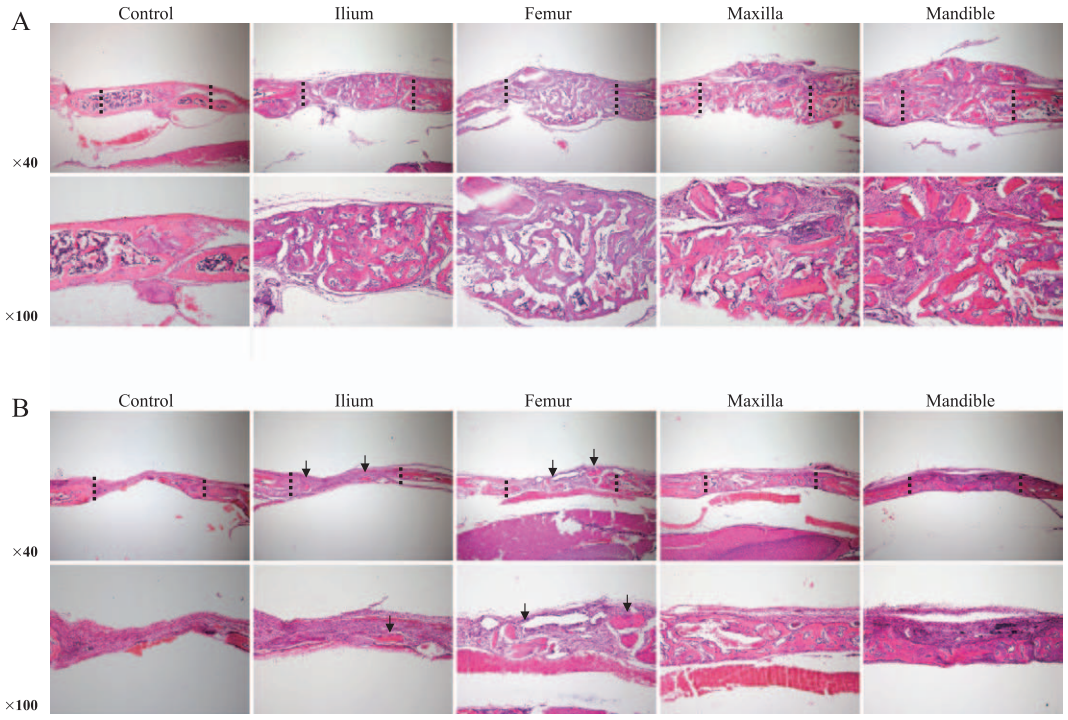

Fig. 1.

A: Histology of bone healing 2 weeks after grafting in frontal bone defects. H&E staining is shown (original magnifications ×40 and ×100). Black dashed line = edge of the bone defect.

B: Histology of bone healing 2 weeks after grafting in parietal bone defects. H&E staining is shown (original magnifications ×40 and ×100). Self-healing of the control bone defect was faster in the frontal bone than in the parietal bone. Bone defects in the parietal bone healed when maxillary and mandibular bones were used as graft materials, while bone defects in the ilium and femur groups remained present. Similarly, bone defects in the parietal bone only healed when jaw bones were used as the graft material, and not when iliac and femoral bones were used. Black dashed line = edge of the bone defect; black arrow = bone nodule.

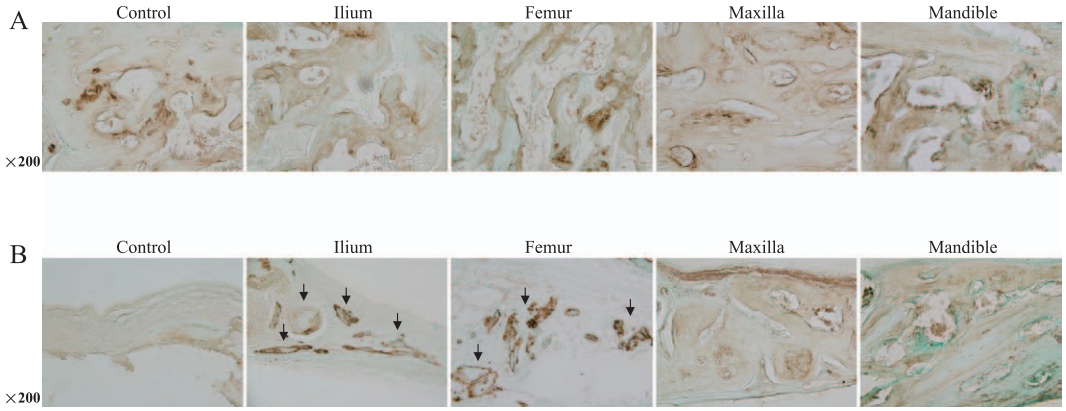

Fig. 2.

A: Immunohistochemical observation of bone graft areas. Sections of frontal bone were stained with anti-osteopontin antibody and counterstained with methyl green 2 weeks after grafting (original magnification ×200). Osteopontin staining was diffuse in the regenerated bones of all frontal bone defect groups.

B: Immunohistochemical observation of bone graft areas. Sections of parietal bone were stained with anti-osteopontin antibody and counterstained with methyl green 2 weeks after grafting (original magnification ×200). Osteopontin staining was diffuse in the regenerated bones of maxilla and mandible groups. Black arrow = bone nodule.

graft materials, and compared their healing ability when the graft bed had the same or different developmental origin.

HE staining analysis revealed that although bone defects in the frontal bone of control mice were not completely closed at 2 weeks, progression of self-healing was observed. Such healing was slower in the parietal bone compared to the frontal bone, with extensive bone defects observed, suggesting that healing ability was greater in the frontal bone. These results are consistent with a previous report by Quarto *et al*[23] who demonstrated increased healing of neural crest-derived frontal bone defects compared with parietal bone defects in mice. In the present study, there was more new bone regeneration in the maxilla and mandible groups than in the ilium and femur groups, suggesting that some factor contained in maxilla and mandible stimulate new bone regeneration. Li *et al*[24] reported that osteoblasts from mouse neural crest-derived frontal bone display a greater proliferative and osteogenic potential, and enhanced activation FGF signaling, compared to osteoblasts from mesoderm-derived parietal bone. In addition, Donovan *et al*[25] showed a two-fold higher level of bone resorption in the ilium than in the calvarial bone 2 months after bone grafting using mesoblast-derived iliac and cranial bones as bone substitutes, while a comparison of bone resorption after 5 months revealed that bone resorption was faster in ischial compared with calvarial bone grafts[26]. These observations together suggest that the residue in the parietal bone defect area in this study was due to resorption of the iliac and femoral bone grafts over time.

We also observed a more defined bone marrow-like structure in the newly formed bone after maxillary bone grafting compared with mandible bone grafting in the parietal bone defects. To this end, Ichikawa *et al*[27] previously reported abundant bone marrow-like tissue in the regenerated tissue of animals receiving transplanted maxillary bone and periosteum.

Osteopontin promotes early differentiation of osteoblasts, their adhesion to bone, and subsequent bone formation. Furthermore, it enhances bone resorption by promoting the adhesion of osteoclasts to the bone surface[28, 29]. To elucidate which bone graft materials might promote osteoblastic differentiation in the present experiments, we used immunohistochemistry for analysis of the distribution of osteopontin in mouse calvariae. In the parietal bone defects, osteopontin localized diffusely in the regenerated bone of the maxilla and mandible groups, with localization observed only on the surface of the small number of regenerated bone nodules in the ilium and femur groups. This finding suggested that maxillary and mandibular bones have high osteogenic potential as bone graft materials.

In conclusion, the present study suggests that the maxilla and mandible exhibit NCDC-like properties with a marked healing ability. The data also suggest that the maxilla and mandible are effective as both bone graft materials and graft beds. In clinical orthodontics, we consider that it is very useful to graft neural crest-derived bones into cleft palates and bone defects for bone augmentation.

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Conflict of interest disclosure

The authors report no conflicts of interest related to this study.

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