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Histologic Evaluation of Human Alveolar Sockets Treated With an Artificial Bone Substitute Material

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Abstract

This study involved a histologic, enzyme histologic, immunohistologic, and three-dimensional microstructure evaluating the extent of osteogenesis and repair in the human alveolar extraction socket achievable with an artificial bone substitute. After tooth extraction in 7 patients, extraction sockets were filled with Mastergraft (15% hydroxyapatite, 85% β -tricalcium phosphate complex). Radio-micrographs and histologic examinations were performed on samples obtained during dental implant placement procedure. On micro-computed tomography, new bone was observed in all collected samples, and osteogenesis was observed to have taken place around the artificial bone substitute. Histologically, active osteogenesis was found throughout the region observed. Addition of new bone around the Mastergraft was observed, and osteoblast-like cells were present. Cells that had partially invaded the artificial bone included tartrate-resistant acid phosphate-positive and CD34-positive cells. These findings indicate that the Mastergraft artificial bone induced osteogenesis in the jawbone and seemed effective for repairing bone defects.

Keywords

β -Tricalcium phosphate; hydroxyapatite; osteoblast; osteoclast; human alveolar socket repair

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With the progress made in nanotechnology and construct/scaffold engineering in recent years, intraoral deficiencies with hard and soft tissues due to disease or trauma can be reconstructed by auto-grafting or allografting.

The adaptation and use of these grafting procedures have been associated with problems such as surgical invasion at secondary donor sites of tissue harvest and insufficient volume of tissue from donor sites. Interest has focused on the development of artificial materials for tissue augmentation to solve these problems. In the field of dental surgery and implant dentistry, the development of artificial bone has gained attention as an effective means to restore form and function in patients with loss of bone volume and contours due to trauma or disease.

Many histologic and radiographic evaluations of bone tissue formed by artificial bone grafts for defects of jaws have been reported using animal models. Several studies looked at composite grafts with β -tricalcium phosphate (β -TCP) and bone marrow in a rat calvarial defect model. One study used histologic and immunohistologic evaluations to observe the course of proliferation and differentiation of bone marrow cells and osteogenesis in the defect, reporting that angiogenesis plays an important role in osteogenesis.¹ In another study, Kojima et al² induced osteogenesis in the calvaria of rats using a bone regeneration induction method consisting of a combination of bone-filling material atellocollagen and bovine hydroxyapatite (HA) granules and thermoplastic bioabsorbable plates. They reported that osteogenesis was promoted. Fujita et al³ embedded a block of HA and β -TCP subperiosteally in the parietal region of rats and histologically compared β -TCP and HA osteogenesis. The bone formed was membranous bone, and they reported that HA showed higher osteogenesis capability than β -TCP. These reports all showed good bone repair using artificial bone. However, very few reports have histologically examined the course of bone repair around artificial bone in the human jawbone. The details of this process remain unclear.

In this study, bone cores were harvested to evaluate the new bone regenerated by using a HA- β -TCP mixture as a new grafting material to augment tooth sockets in preparation for dental implant placement. We preliminarily examined the osteogenesis marker osteopontin (OPN), osteoclast marker tartrate-resistant acid phosphate (TRAP) and the angiogenesis marker CD34 in cells.

Materials and Methods

Patients

This study included 7 patients in good general health (2 men and 5 women; mean age, 55.9 $\pm$ 18.5 y; range, 35–82 y). All patients required tooth extractions for reasons such as advanced periodontitis or tooth trauma and replacement with dental implants. Informed consent was obtained from all patients for all phases of surgical treatment. Grafts were performed using Mastergraft (Medtronic, Minneapolis, MN), a composite consisting of 15% HA and 85% β -TCP. This material has a porosity rate of 80%, a mean pore size of 500 μ m, and an interconnected diameter of 125 μ m.

This study was approved by the ethics committee of the Research Institute of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences (Okayama, Japan), and the ethics committee of the University of California at Los Angeles.

Artificial Bone Filling Method and Bone Harvesting

All patients underwent tooth extraction under local anesthesia (2% lidocaine + 1:100,000 epinephrine). The sockets of extracted teeth after complete removal of infected granulation tissue were filled with Mastergraft granules so that the sockets were impacted. The sockets were then covered with cellulose sponge (Spongostan; Johnson & Johnson, Birkering, Denmark) and sutured with 4-0 absorbable chromic suture⁴ (Figs. 1A, B).

Hard Tissue Harvesting

When the primary dental implant operation was performed at 4 to 5 months after socket augmentation, cylindrical bone core 7 mm in length was harvested using a 2-mm trephine drill (Figs. 1C, D). As a control, 5 core samples were harvested from the normal alveolar bone site.

Observation of Bone Microstructure on Micro-Computed Tomography

The microtrabecular bone structure of tissue specimens was observed three-dimensionally using a micro-computed tomographic (CT) scanning device (Scan × mate-A080; Comscan Technom, Yokohama, Japan). Imaging conditions were as follows: tube current, 0.1 mA; tube voltage, 60 kV. Three-dimensional images were constructed from two hundred 10-μm slice tomographic images.

Histologic, Enzyme Histologic, and Immunohistochemical Observations

Specimens were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C for 24 hours and then decalcified in 5% ethylenediaminetetraacetate in 0.1 M phosphate buffer (pH 7.4) for 20 days. After dehydration in a graded ethanol series and embedding in paraffin, sections cut to a thickness of 6 μm were mounted on glass slides and rehydrated. Some slides were stained with hematoxylin and eosin for light microscopic observation. The remaining slides were immersed in 5 mM periodic acid for 10 minutes to inhibit endogenous peroxidase before blocking with 10% bovine serum in phosphate-buffered saline (PBS) for 30 minutes at room temperature. Slides for detection of CD34, as a marker of vascular endothelial cell surface antigen, were exposed to anti-CD34 monoclonal antibody (Nichirei Biosciences, Inc, Tokyo, Japan). Slides for OPN, as a marker of bone formation, were exposed to anti-OPN polyclonal antibody (donated by Dr Nakamura, Matsumoto, Japan) and diluted 1:1000 in PBS containing 3% bovine serum albumin at 4°C overnight. After incubation, slides were exposed to Histofine Simple Stain MAX-PO (Multi) (Nichirei Biosciences, Inc) for 60 minutes at room temperature. After washing in PBS, slides were incubated for 5 minutes at room temperature in a solution containing 0.05% 3,3'-diaminobenzidine tetrahydrochloride, 0.01% hydrogen peroxide, and 0.05 M Tris-HCl (pH 7.6) to visualize the immune complexes. Sections were counterstained with hematoxylin. As a positive control, rabbit and mouse IgG preimmune serum were used to confirm immunoreaction. For a negative control, some slides were incubated with 3% bovine serum

albumin in PBS instead of the primary antibody. To detect osteoclastic cells, TRAP staining^{5,6} was performed.

Results

Radiographic Observations Using Micro-CT

In harvested tissue specimens, new bone formation matching the shape of the extraction sockets was observed, including radiopaque structures of artificial bone granules in the regions examined. Mean calcified volume index was $32.4\% \pm 2.98\%$. Control site showed $44.2\% \pm 2.04\%$ calcified volume (Figs. 2A, B).

Histologic Examinations

New bone formation was observed in all tissues examined. Mastergraft granules remained in the extraction sockets, and eosinophilic new bone formation was observed around the Mastergraft granules. Fibrous connective tissue was observed around the new bone (Fig. 3A). Osteocytes were present in the bone tissue around the Mastergraft, and partial new bone formation as a continuation of new bone surrounding the Mastergraft was observed within the Mastergraft in the magnified photo (Fig. 3B). In the control group, few osteoblastic cells were seen around bone (Fig. 3C).

Immunohistologic and enzyme histochemical examinations showed CD34-positive vascular endothelial cells were present. In regions with partial rupture of Mastergraft granules, infiltration of cells from surrounding fibrous connective tissue was observed (Figs. 4A and 5A). Infiltrating cells showed partially both CD34- and TRAP-positive reactivity (Figs. 4B and 5B); no OPN labeling was seen in these cells (Fig. 5C). Osteopontin-positive osteoblasts were not observed around the new bone, but OPN was observed in the regions surrounding new bone (Fig. 5C). No infiltration of inflammatory cells, granulation tissue, or scar tissue was observed in any of the tissues examined. In the control group, there were no CD34-, TRAP-, and CD34-positive cells (data not shown).

Discussion and Conclusions

Bone repair using HA and β -TCP has been reported in several animal studies.^{2,7} At present, these substances are widely applied clinically as artificial bone filler to replace autologous bone in orthopedic and plastic surgery. The Mastergraft used in human jawbones in the current study is an artificial bone composite consisting of 15% HA + 85% β -TCP. β -Tricalcium phosphate shows good bioaffinity and, when grafted in bone defects, is absorbed and substituted in the surrounding bone.⁸ Hydroxyapatite is a material with biomechanical properties similar to natural bone but is not absorbed into the surrounding bone and remains in the filled area.

In a similar research on different materials, Carmagnola et al⁹ used substitute bone of bovine origin to fill sockets after tooth extraction in humans and reported that a very small amount of new bone was found around the substitute bone; however, no invasion of cells into the artificial bone or findings of connected active osteogenesis was reported. However, to the

best of our knowledge, no histologic evaluation of Mastergraft artificial bone in the human alveolar socket has previously been reported.

Among the findings that we obtained in the current study, micro-CT indicated that new bone with low radiopacity matching the extraction sockets was present around the Mastergraft. On micro-CT, the microtrabecular structure of the bone can be measured three-dimensionally. Moy et al¹⁰ analyzed bone using HA only or an HA and chin bone composite in a maxillary sinus augmentation procedure. They reported that the calcified region accounted for 20.3% with HA alone and 44.4% with the HA–chin bone composite. Such data have not been reported for extraction sockets. An environment such as extraction sockets represents a special healing environment and verification of osteogenesis in comparison with maxillary sinus grafting is necessary.

In the current study, bone was harvested 4 to 5 months after socket grafting, and the process of bone healing was observed histologically, immunohistologically, and enzyme histologically. With hematoxylin-eosin staining, new bone formation was clearly observed around the Mastergraft granules in the extraction sockets as a whole under low magnification. Under higher magnification, osteoblast-like cells and bone formed from these cells were observed in the new bone around the Mastergraft granules. Moreover, invasion of cells into the Mastergraft granules was also observed. Because it is well known that TRAP-positive cells showed staining characteristic of osteoclasts, enzyme histologic observations showed TRAP-positive osteoclast-like properties in these invading cells. The TRAP-positive cells showed staining characteristic of osteoclasts. In addition, some cells to invade into Mastergraft granules showed CD34-positive labeling. The level of CD34 expression is said to be reduced as cells mature and differentiate and is known as a marker of vascular endothelial cells.^{11,12}

CD34 shows an inherent immune reaction with vascular endothelial cells. These cells were connected with bone formed around the Mastergraft, and vascular endothelial cells and osteoclasts were observed in the initial stage of osteogenesis. Osteogenesis is presumably achieved by induction of these cells.

Osteopontin is produced by preosteoblasts, osteoblasts, osteocytes, and osteoclasts.^{13–15} This protein plays an important role in inducing differentiation and functional expression of osteogenesis-related cells and shows a high affinity for HA.^{16,17} Osteopontin is thus considered to be closely related to the early process of calcification of calcified tissue^{17,18} and is also a marker of osteogenic cells. Osteopontin-positive cells were not observed in tissue that invades the Mastergraft but were observed in the regions surrounding osteogenesis.

Previously performed histologic observations using a rat calvarial defect model found that osteoclasts induced from grafted bone marrow cells in composite grafts with β -TCP appeared at an early stage after transplantation. These cells promoted proliferation and differentiation of osteoblasts and vascular cells at graft sites and are reportedly closely involved in osteogenesis in β -TCP artificial bone.^{1,19}

In the healing process of human tooth sockets in the current study, osteoclast-like cells and vascular endothelial cells were seen within the Mastergraft granules, suggesting these cells may affect subsequent osteogenesis. Osteoclasts are adsorbed on the artificial bone grafted in the human body at an early stage after grafting, leading to absorption of the artificial bone. By activation, many cytokines that activate osteoblasts are released and are actively involved in osteogenesis. In the process of fracture healing, osteoclasts appear around the damaged bone and undifferentiated mesenchymal cells appear around the fracture-induced osteoblasts and vascular cells.²⁰ Therefore, in Mastergraft grafted in human extraction sockets, osteoclasts that have infiltrated into the Mastergraft and vascular endothelial cells adsorbed on the Mastergraft may be involved in subsequent osteogenesis by osteoblasts.

Osteoconduction is a biologic process that provides grafted sites with passive osteogenesis via incorporation of newly formed blood vessels and osteogenic cells into grafts from the graft matrix, and these findings suggest that Mastergraft has this capability.

Given the small number of subjects in the current study, drawing a definitive conclusion is difficult. However, ongoing research will increase the number of subjects in the future, and additional investigations will be performed to determine the osteogenic activity of Mastergraft. In this study, the course of osteogenesis of the jawbone in human extraction sockets at 4 to 5 months after extraction using Mastergraft was observed histologically. Active osteogenesis together with osteoblasts, osteoclasts, and vascular endothelial cells was observed around the Mastergraft granules. On the basis of these findings, good bone repair is obtainable with Mastergraft in human extraction sockets.

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Figure 1.

Intraoperative photograph. A, Mastergraft granules placed into the extracted socket as a graft material. B, Mastergraft granules were covered with a cellulose sponge. C, Bone core samples were taken from the grafted sites at 4 months after grafting. D, Installed implant into the grafted site at 4 months after grafting.

Figure 2. Micro-CT scan of core samples. A, Core samples from Mastergraft granules. Clear white radiopaque images show Mastergraft granules, and dark radiopaque shows new bone formation. B, Core samples from normal alveolar bone.

Figure 3.

Histologic observation of core samples taken from Mastergraft granules–augmented extraction sites and normal alveolar bone. A, New bone formation is seen around and between Mastergraft granules (MG). B, In regions with partial rupture of Mastergraft granules (MG), infiltration of cells from the surrounding bone (Bone) was observed. C, The normal alveolar bone (Bone) is seen. Bars, 300 μ m (A, C); 50 μ m (B).

Figure 4.

Histologic and immunohistologic observation of core samples taken from Mastergraft granule-augmented extraction sites. A, New bone formation is seen around and between Mastergraft granules (MG). Osteoblasts are located on newly formed bone (Bone) surface (arrowheads). B, CD34-positive reactivity was observed in cells invading Mastergraft granules (MG). Bars, 50 μm.

Figure 5.

Histologic, immunohistologic, and enzyme histologic observations of core samples taken from Mastergraft granule-augmented extraction sites. A, Cells invade the Mastergraft granule (MG) from the surrounding bone (Bone). B, Cells (arrows) into the Mastergraft granule show TRAP-positive labeling. C, Osteopontin reactivity was observed in the regions surrounding new bone (arrowheads). Cells into the Mastergraft granule show no OPN labeling. Bars, 25 μ m.