Experimental Studies on Reimplantation of Autogenous Bone Decalcified by Combined HCl and Ultrasonic Exposure

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Hironaka R, Inokuchi T and Ninomiya H. Experimental studies on reimplantation of autogenous bone decalcified by combined HCl and ultrasonic exposure. *Oral Med Pathol* 2004; 9:7-11, ISSN 1342-0984

Decalcified bone implants induce new bone formation. In this experiment, the autogenous bone implant of canine mandible was treated by HCl under ultrasonic exposure for rapid decalcification. The aim of this study was to investigate the osteoinduction property of this rapidly decalcified bone reimplanted to the original site. The decalcified bone implant exhibited a similar degree of new formation to that of the fresh bone graft which was not subjected to decalcification. The results demonstrate that the combinational use of HCl and ultrasonic exposure for rapid decalcification treatment preserves the regenerative capacity of bone to be implanted.

Key words: ultrasonic decalcification, immediate reimplantaion of bone, osteoinductive factors

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Introduction

In surgical treatment of cancerous lesions of the mandible, loss of mandibular continuity due to segmental resection of the tumorous mandibular bone leads to severe post-operative impairment, both morphologically and functionally. Recent years have seen immediate mandibular reconstruction conducted through fresh bone autograft of the iliac bone, rib, or fibula. Advantages of this technique include 1) fewer operations and a shorter duration of treatment in comparison with secondary reconstruction, 2) reduction of the physical, mental, and economic burdens on the patient, and 3) avoidance of scar contracture at the surgical site, resulting in earlier post-operative functional and aesthetic success. Various problems can also be cited, however, such as 1) difficulty in establishing the three-dimensional morphology of the mandible, 2) the need for surgical intervention of the bone graft donor site, with resultant possibility of deformation and functional impairment, and 3) limitations on the amount of bone that can be obtained (1-3).

There are also reconstruction methods involving transplants of bone other than by autograft, namely allograft and xenograft. The merits of transplants using allogenic banked bone are that 1) sufficient quantities of

bone can be obtained, and 2) bone can be selected so as correspond with transplant size and shape requirements. Disadvantages, however, are 1) slow replacement with new growth in comparison with autograft, due to the remaining presence of immunogenicity, and 2) the danger of viral infection such as HIV or hepatitis (4).

Meanwhile, experimentation has been conducted for some time involving techniques in which bone that has been invaded by cancer cells is resected, the cancer cells eliminated, and the resected bone subsequently reimplanted. The use of resected autogenic bone is a superior method for mandibular reconstruction in light of the following factors: 1) anatomically optimal morphology and function can be recovered, 2) there is no need for surgical intervention at other regions of the body, 3) transplant immunity is not an issue, and 4) there is no danger of viral infection. Obviously, however, complete devitalization of the cancer cells in the resected bone is essential in such cases (3, 5-10).

Decalcification treatment of the bone to be implanted facilitates the exposure of osteoinductive factors involved in the bone, thereby facilitating osteoinduction. However, decalcification normally takes considerable time, and is difficult to accomplish during the period avail-

able for surgery. Actual clinical application of this technique therefore requires a secondary operation.

Although cortical bone transplants are marked by the maintenance of sufficient bone strength, new bone formation is extremely gradual. Even in the case of fresh autogenic bone implantation, remodeling of the implanted cortical bone does not proceed for a long period afterwards (1). Furthermore, the regenerative capacity of bone declines with aging (11).

In contrast, the use of bone that has been subjected to surface decalcification allows the undecalcified interior portion of the bone to maintain its mechanical strength, while the decalcified surface is observed to promote osteoinduction (12-14). Nevertheless, application of decalcification treatment in the immediate reimplantation of resected autogenic bone requires a more rapid decalcification technique.

Attention has been attracted by the decalcification-promoting effect in hard tissue obtained through the combined use of ultrasound as applied in the preparation of histopathological specimens (15-17). Our previous study indicated that only the surface of bone was decalcified by 0.6N HCl for one hour in combination with ultrasound exposure (18).

The aim of this study was to evaluate healing of autogeneous bone implant decalcified by HCl under ultrasonic exposure.

Materials and Methods

Animals

Four adult mongrel dogs were used, each weighing approximately 15kg. The dogs were young to middle age, as determined by the condition of their dentition, physical status and coat of hair.

Preparation of autogenous bone implant

An extraoral incision was made to the position corresponding to the mandibular first molar, and two segments of 1 ¥ 2cm block bone were taken from both sides of the inferior border of the mandible under intravenous general anesthesia with pentobarbital sodium (25mg/kg). The inferior neurovascular bundle was removed from the harvested bone segment (Fig. 1). For the bone segment from the one side, dehydration was performed by means of ascending ethanol (70%, 80%, 90%, and 100%). After defatting with diethyl ether, decalcification was conducted using 200ml of 0.6N HCl / 1g bone for 1h at 18-22°C under exposure of ultrasonic generator (Bransonic 221 ultrasonic washer, 47,000Hz, Branson, Japan) (18). The bone segment from the opposite side was kept in the saline solution as the untreated control bone.

Reimplantation of bone implant

The decalcified bone and the untreated control bone segments were reimplanted in the original sites, and fixed

with 0.4mm stainless steel wire.

The dogs were euthanized by overdose of pentobarbital sodium at 2, 4, 6, and 8 weeks post-operatively.

Tissue preparation

After soft X-ray imaging, the mandibles were processed for preparation of paraffin sections. Histopathological examination was conducted by means of hematoxylin-eosin staining, as well as PAM staining (19) for observing polysaccharide deposition in new bone formation.

Results

Soft X-ray observation

At 2 weeks post-operatively, radiopacity resembling fine fibers was observed spreading in the direction of the reimplanted bone, extending from the recipient bone marrow site in the control. Radiopacity was seen at the interface between the recipient bone and the decalcified implant as well, although a somewhat lesser extent (Fig. 2).

At 4 weeks, the control and decalcified implant similarly showed X-ray opacity on the outer side of the recipient bone. Fibrous structures of new bone were noted at the interface between the recipient and reimplanted bone in both cases, and this was more substantial than that observed at the 2 week point (Fig. 3).

At 6 weeks, the outer side of the recipient bone in both cases was characterized by the fibrous structures of new bone, and the increased opacity at the interface was evident.

At 8 weeks, new bone formation was observed to extend from the interface to the medullary space of reimplanted bone in both the control and ultrasonically decalcified implants (Fig. 4).

Histological findings of the reimplanted bone

At 2 weeks post-operatively, the decalcified implant

Fig. 1: Surgical sites.

The two autogenous osseous implants, 2¥1 cm in size, were taken from the right and left inferior border of the mandible. For the bone segment from the one side, decalcification was conducted by HCl under ultrasonic exposure. The bone segment from the opposite side was untreated. Then both segments were reimplanted in the original sites, right and left, respectively.

Fig. 2: Soft X-ray 2 weeks after reimplantation of canine mandible.

A; Untreated control implant
B; Decalcified implant

Radiopacity resembling fine fibers is seen, extending from the recipient bone marrow site to the implant in the control. Radiopacity in the interface is noted in the decalcified implant as well.

was incorporated to the recipient bone resulting from new bone formation on the inner side of the interface. New trabecular growth was seen in the medullary space of the reimplanted bone. Inflammatory cell migration was noted in the adjacent tissues, including fibrous cells, fibroblasts, histiocytes, and foreign body giant cells (Fig. 5).

At 4 weeks, the control showed new bone formation on the outer side of recipient bone. New trabecular growth was more active in the control case in the medullary space than in the decalcified reimplant. The decalcified reimplant showed thin bone formation on the outer side of recipient bone. Resorption of the decalcified portion appeared. Inflammatory cell migration disappeared, and osteoclasts and osteoblasts were seen.

At 6 weeks, new bone formation in the interface with both the decalcified and control implant presented a fine bone matrix from the outer side and bridging accompanied by stroma from the inner side, indicating

Fig. 3: Soft X-ray 4 weeks after reimplantation of canine mandible

A; Untreated control implant
B; Decalcified implant

New bone formation on the periosteal surface of the host bone is exhibited. Bridging between the host bone and the implant is apparent.

Fig. 5: 2 weeks after reimplantation of canine mandible. Decalcified bone (PAM stain. Original magnification, $\times 4$) Incorporation is found between the recipient bone and the reimplanted bone due to new bone formation on the inner side of the interface. New trabecular formation is seen in the medullary space of the reimplanted bone. (R: reimplanted bone, NB: new bone)

Fig. 4: Soft X-ray 8 weeks after reimplantation of canine mandible

A; Untreated control implant
B; Decalcified implant

The decalcified implant is observed to be similar in appearance to the untreated control, and fine structure of radiopacity indicating new bone formation is seen within the medullary space of the implant.

Fig. 6: 6 weeks after reimplantation of canine mandible. Decalcified bone (PAM stain. Original magnification, $\times 8$) New bone formation in the interface presents a fine bone matrix from the outer side. A bridge-like appearance is accompanied by stroma from the inner side. (R: reimplanted bone)

Fig. 7: 6 weeks after reimplantation of canine mandible. Decalcified bone (PAM stain. Original magnification, $\times 20$) Resorption of the decalcified portion and immature new bone formation is noted on the inner side of the implant. (R: reimplanted bone, NB: new bone $\blacktriangle$: immature new bone)

Fig. 8: 8 weeks after reimplantation of canine mandible. Decalcified bone (PAM stain. Original magnification, $\times 16$) New bone growth is seen in the interface. A low level of cell composition is presented in the newly formed bone matrix. (R: reimplanted bone, NB: new bone)

Fig. 9: 8 weeks after reimplantation of canine mandible. Decalcified bone (HE stain. Original magnification, $\times 20$) Newly formed bone from the recipient site is joining with the cortical region on the inner side of the reimplanted bone. (R: reimplanted bone, NB: new bone)

maturity and cortification of the new bone (Fig. 6). In the medullary space of this bone implant, new trabecular formation was seen from the recipient bone in the direction of the mandibular lower margin. The periphery of the new bone was characterized by palisaded arrangements of osteoblasts, and proliferative extension of the new vasculature was noted in the interstitial tissues. Advanced resorption of the decalcified portion and immature new bone formation were noted on the inner side of the reimplanted bone (Fig. 7).

At 8 weeks, fusion of the implant to the host bone at the osteotomy site, with a low level of cell composition in the interstitial tissues, and with a fine bony structure resembling the cortical bone at the recipient site, was shown in both the reimplantation cases (Fig. 8). Furthermore, new bone formation proceeded from the recipient bone marrow in the direction of the medullary space of the reimplanted bone, and new bone incorporated with the implant was observed (Fig. 9). The medullary space was occupied by new bone for both implants.

Discussion

Bone deposition, which appeared to have resulted from periosteal reaction, was observed on the recipient site for both implants. At the interface, bridge formation which resulted from new bone growth in both the untreated and decalcified bone implant was present.

New bone formation was seen within the reimplanted bone in both cases of implants. Periosteal bone deposition on the outer side of the implant was also seen in both cases.

In the implantation of a fresh autograft, such as the untreated bone in the present study, new bone formation is carried out by osteogenetic cells that survive the initial implant period, with subsequent induction of osteogenetic cells from the mesenchymal cells in the recipient bed, and further creation of new bone, which results in the most rapid and complete bony healing (1). As for the devitalized bone implant, because osteogenetic factors are closed up inside the bone, osteoinduction of these factors would not be expected (1).

While decalcification treatment devitalizes osteogenetic cells of the bone, it serves to expose osteoinductive factors on the bone surface, and these factors promote osteoinduction.

The rapidity of healing is an issue of major clinical significance in the context of bone implantation. Tsuruki *et al.* (22, 23) have carried out comparison of histological changes between fresh and freeze-thaw treated autografts in canine mandibles, and have reported the same process and rapidity of healing for both of these instances.

The present study indicates that the healing capacity of decalcified bone implant is comparable with the fresh bone graft.

The advantages of surface decalcified bone (12-14)

are mainly that 1) mechanical strength is preserved by keeping the interior of the bone intact, and 2) surface decalcification enhances osteoinductive capacity. That is, the morphology and function of the mandible can be preserved, while healing through osteoinduction can be anticipated. Recent years have seen consideration of reconstruction using banked bone. Osteoinductive capacity of bone implant could be heightened and new bone formation improved by means of surface decalcification (13, 14, 24, 25).

In summary, the combinational use of HCl and ultrasonic exposure for rapid decalcification treatment preserves the regenerative capacity of bone to be implanted.

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(Accepted for publication September 12, 2003)