ELSEVIER

Bone 32 (2003) 291–296

BONE

www.elsevier.com/locate/bone

Removal of the surface layers of human cortical bone allografts restores in vitro osteoclast function reduced by processing and frozen storage

R. Kluger,[a] W. Bouhon,[b] H. Freudenberger,[b] A. Kröner,[a] A. Engel,[a] and O. Hoffmann[b,*]

[a] *Department of Orthopaedics, SMZO Donauspital, A-1220 Langobardenstrasse 122, Vienna, Austria*
[b] *Department of Pharmacology and Toxicology, University of Vienna, A-1090 Althanstrasse 14, Vienna, Austria*

Received 17 June 2002; revised 29 October 2002; accepted 30 October 2002

Abstract

A major complication of cortical bone grafting is nonunion at the host-graft junction. Many factors are thought to be involved in successful engraftment including the quality of the graft and the host response to it. In particular, the recipient osteoclasts (OCs) play a critical role by resorbing the engrafted bone. Thus, effective engraftment may depend on the inherent biological properties of the bone graft, which subsequently correlates with early and effective OC resorption. Normally, bone grafts are stored and processed by freezing, freeze-drying, irradiation, and lipid extraction. We sought to determine whether processing and storage affected bone quality, as evaluated by OC bone resorption. Cortical bone specimens from six human donors were either fresh, frozen at −75°C, or had undergone combinations of freezing at −75°C, freeze-drying, lipid extraction, irradiation, and treatment with hydrogen peroxide. Bone slices of 0.5-mm thickness taken from the surface, beneath the surface, and at a depth of 7.5 mm were incubated with isolated rabbit OCs and resorption lacunae were measured. We observed highest OC activity with fresh bone followed by frozen, partially processed, and fully processed bone. When allografts were stored at −75°C for 12 months, there was up to a 4.2-fold reduction in OC activity on the surface layer. Additionally, we found reduced OC activity upon the outer surface bone compared to the inner layers. Removal of more than 0.5 mm of frozen and processed bone significantly improved OC activity. These results imply that inner bone layers of stored and processed bone allografts are protected against degradation of bone matrix components, except when frozen for extended periods of time. Taken together, these data suggest that bone allografts should be stored for less than 1 year and require the removal of at least 0.5 mm from their surface prior to transplantation.

Keywords: Bone transplantation; Bone bank; Tissue preservation methods; Bone grafting; Osteoclasts; Bone resorption

Introduction

Mineralized human cortical bone allografts have been used in orthopedic and trauma surgery for more than 50 years. In the majority of cases, massive frozen allografts [1–5], freeze-dried allografts [6–8], and allografts that had undergone a variety of additional preservation and sterilization techniques [9–12] yield good clinical results. However, one major complication of cortical bone grafting is nonunion at the host-graft junction [13]. The causes of nonunion include immunological mismatching of donor and recipient [4,5], lack of stability of the graft to the host bone construct [14], and adjuvant chemotherapy and radiation of the recipient [2,13]. An additional cause for nonunion may be related to reduced allograft quality resulting from processing and storage in bone banks. In support of this notion, there are data demonstrating that immunologically matched fresh allografts incorporate faster than matched processed bone grafts [14,15]. Osteoclasts (OCs) play an essential role in the incorporation of bone grafts by initiating remodeling of the graft by the resorption of bone. Moreover, OCs promote recanalization and cancellization of the graft and in collaboration with osteoblast (OBs) bridge bone and induce partial union [2,7,10]. Several studies have shown that both

* Corresponding author. Department of Pharmacology and Toxicology, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria.
E-mail address: oskar.hoffmann@univie.ac.at (O. Hoffmann).

8756-3282/03/$ – see front matter
doi:10.1016/S8756-3282(02)00980-8

OB and OC activity of the recipient are reduced when tested on processed and stored animal [14–18] or human bone [6,19,20]. Thus, successful engraftment depends upon effective bone resorption by OC, which appears to correlate with the quality of the bone graft.

To determine the effects of preservation and sterilization on mineralized cortical animal bone, OC attachment and resorption has been tested by using the pit assay [21]. Increased OC activity appears to correlate with improved incorporation of fresh compared to devitalized cortical bone in vivo [14,15,22]. However, studies addressing OC function as a means of determining quality of bone are few [15,18,21,22] and have not shown effects tested such as time of storage and storage temperature on human mineralized cortical bone grafts.

The aim of this study was to determine whether processing and storage of bone allografts affects bone quality, as evaluated by in vitro OC bone resorption. Thus, we correlated bone allograft quality with net OC activity/function, which ultimately relates to the ability of OCs to attach and resorb the graft. Rabbit OC activity was evaluated on stored and processed human cortical bone allografts compared to fresh bone. Although this is a xenogenic model, high rates of bone resorption due to immunologic mismatch do not play a crucial role because T lymphocytes are not present in cell and bone preparations. Using this technique, we demonstrate that all forms of processing and storage reduce OC activity. Furthermore, we show that the removal of at least 0.5 mm of the surface of the bone allografts that were frozen and/or processed improves OC function, thus illustrating the need for further preparation of bone allografts prior to transplantation.

Material and methods

Preparation of allografts

All cortical bones were obtained from the bone bank of the SMZ-Ost Danube Hospital under the guidelines of the European Association of Tissue Banking. The thickness of cortical bone in the samples ranged between 8 and 10 mm. Transverse femoral cortical bone rings (length = 25 mm) were cut using a low-speed diamond saw (Isomet; Buehler Ltd, Lake Bluff, IL). Samples were derived from each donor and prepared as follows: Fresh, samples stored at +4°C for up to 24 h; frozen, samples stored at −75°C for 6 weeks; frozen-fully processed, samples stored at −75°C for 6 weeks following processing with lipid extraction, lyophilization, hydrogen peroxide treatment, and gamma irradiation (25 kGy); frozen-partially processed group A, initial freezing at −75°C, followed by lipid extraction, and gamma irradiation (25 kGy), or group B, hydrogen peroxide treatment and lyophilization. Briefly, lipids were extracted by diethylether renewed three times for at least 6 h. The specimens were then rinsed with 70% ethanol for 3 h, 50% ethanol for 3 h, and 30% ethanol for 4 h. Bones were then washed with distilled water for 2 h and kept in a solution of 3% hydrogen peroxide for 2 h. Bones were freeze-dried for five consecutive days. The final residual moisture was less than 5% of final dry weight. Lyophilized specimens were packed under vacuum and argon gas in glass bottles with rubber caps. Bone specimens were sterilized with 25 kGy of cobalt 60 irradiation. To investigate the effect of long-term storage, frozen specimens from a single donor were kept at −75°C for up to 12 months and studied at the time points indicated. Transverse bone slices of 0.5-mm thickness were used in all experiments and separated into the following groups: surface layer, subsurface layer, 0.5 mm below the surface; and inner layer, 7.5 mm below the surface. All reagents used were of analytical grade (Merck, Darmstadt, Germany).

Preparation of OCs

OCs were isolated from the long bones of 5-day-old New Zealand White rabbits by the method of Chambers et al. [23]. Briefly, bones were minced and gently vortex mixed in αMEM (Sigma Chemical Co., Vienna, Austria) containing 1% penicillin/streptomycin (Sigma). The OC-enriched cell fraction was recovered by centrifugation at 400*g* for 5 min at 4°C. Enriched OCs from one rabbit were redispersed in 15 ml of medium before transfer. OC concentration prior to transfer was approximately 1–2% of the enriched cell suspension.

Pit assay

Single-cell suspensions of enriched OCs (600 μl) were transferred onto the bone specimens and incubated for 2 h in αMEM plus 5% fetal bovine serum. Contaminating, nonattaching, and dead cells were then removed by washing with medium. The remaining attached OCs were incubated on bone slices for an additional 48 h. At the end of the culture period, attached OCs were removed by immersing the bone slices in 70% isopropanol (Sigma) and placing them in an ultrasonicator (Transsonic 570; Elma, Singen, Germany). The bone slices were then washed in distilled water, stained with 0.1% toluidine blue, and stored dry until pit area measurement. The number and area of OC resorption lacunae in the air-dried specimens were measured by using a Nikon Optiphot microscope with a 10×/0.30 NA objective under oblique illumination. Six images per bone slice were taken using a Sony video camera (DXC-151AP) and digitized by a frame grabber attached to a Windows 98SE PC system (Fig. 1). Pit areas were traced by using a digitizing tablet and Adobe Photoshop 5.0. The sum of the number of pits and pit areas per image for the six selected images was calculated by using NIH image 1.62 (National Institute of Mental Health, Bethesda, MD) and presented as the percentage of the area of bone resorption of the total image

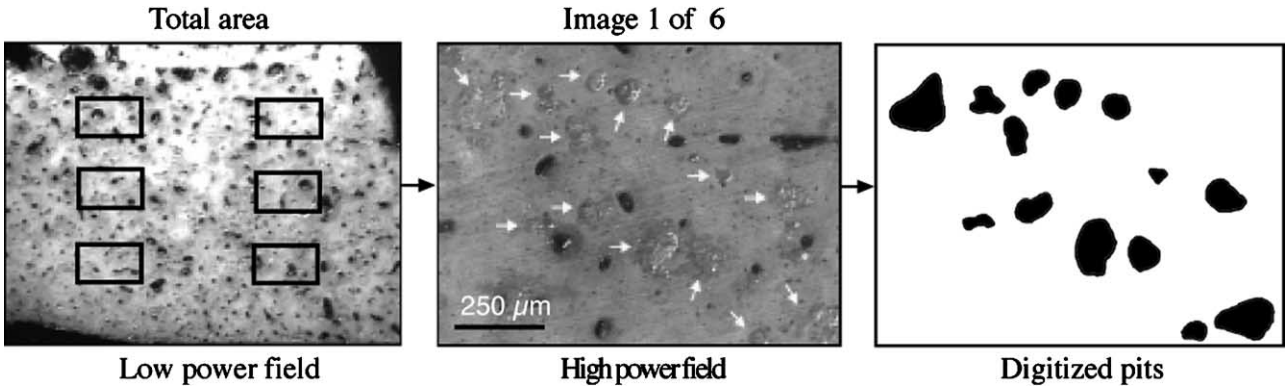

Fig. 1. Quantification of resorption pits on human bone specimens. Human bone slices were inspected under low-power magnification. Six centrally located regions of equal size (1250 × 820 μm) were selected for the quantification of osteoclast bone resorption. Digital images were taken of the selected regions at high magnification (100×) and areas of resorption (pit area) were traced by using Adobe Photoshop. Pit area tracings from all regions were transferred to NIH Image software where pit surface areas from all six regions were individually quantified and calculated as the mean percentage of the total surface area.

area. Pit numbers per bone slice approximately represent the number of OC per bone slices.

Statistical analysis

Data are presented as values of the mean percentage of area of resorbed bone ± SEM. All experiments were repeated at least twice. Statistical analysis was determined by using analysis of variance. Statistical differences between groups were determined by Fisher's LSD test and considered significant at $P < 0.05$.

Results

Fresh allografts maintain bone quality

Since fresh bone grafts are reported to incorporate more efficiently, we tested OC function on fresh allografts stored for less than 24 h at 4°C. Rabbit OCs were incubated for 48 h on fresh bone slices that were prepared from the surface, subsurface, and inner layers. We observed vigorous bone resorption that was maintained at each level of bone evaluated (Fig. 2), suggesting that fresh bone maintains its integrity. These data support the notion that bone quality correlates with OC activity.

Reduced OC activity on frozen versus fresh allografts

To determine whether frozen bone allografts maintain their quality, we directly compared OC resorption on fresh and frozen bone samples. As expected OC activity on frozen bones ($n = 30$) was between 2.5- and 1.4-fold lower than on fresh bones (Fig. 2). All three layers of frozen bone displayed reduced pit area compared to the corresponding layers in fresh bone ($P < 0.01$). The reduction of OC activity in frozen bones was approximately 60% for surface, 53% for subsurface, and 28% for inner layers compared to positive fresh allograft bone slices. As OCs were incubated farther away from the frozen bone surface, we observed increased activity. There was a 96% increase in the resorbed area upon the inner compared to the surface layer demonstrating that OC activity on the inner layers was restored to 72% of fresh bone levels. These data demonstrate that there is reduced bone quality on the surface layers of frozen bone.

Long-term frozen allograft storage reduces OC bone resorption

Approximately one-half of all bone implants are massive allografts that are stored frozen for up to 5 years. To further analyze the effect of frozen storage on bone quality, we tested OC activity on bones stored at −75°C for 6, 9, and 12 months. We found that there was an inverse correlation

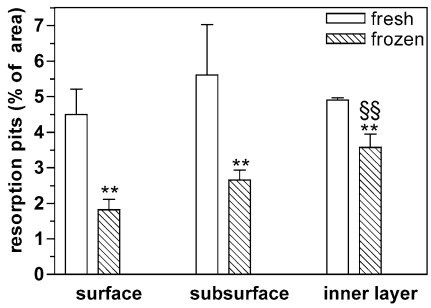

Fig. 2. Osteoclast (OC) activity on fresh and frozen bone allografts. Bone allografts were stored for 24 h at 4°C ($n = 12$) or for 6 weeks at −75°C ($n = 30$) before use. Bone slices from surface, subsurface, and inner layers were incubated with rabbit OCs for 48 h at 37°C. OCs were then washed from the bone and pit area was quantified. These data are presented as the mean percentage of area of resorbed bone ± SEM for two individual experiments. **Significant differences ($P < 0.01$) between fresh and frozen bones for all layers. §§Significant differences ($P < 0.01$) between surface and inner layers of frozen bone.

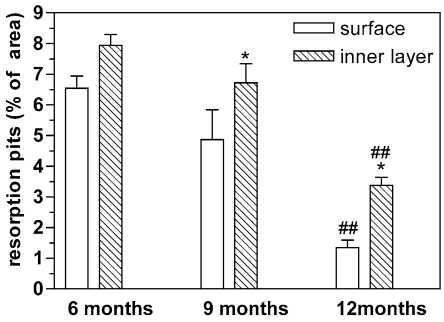

Fig. 3. Effect of long-term storage at −75°C on frozen bone allografts. Bone allografts were stored for 6, 9, and 12 months ($n = 4$) at −75°C before use. Bone slices from the surface and inner layers were incubated with rabbit osteoclasts (OCs) for 48 h at 37°C. OCs were then washed from the bone and pit area was quantified. These data are presented as the mean percentage of area of resorbed bone ± SEM for two individual experiments. *Significant differences ($P < 0.05$) between inner bone layer and surface layer at 9 and 12 months. ##Significant differences ($P < 0.01$) at 6 and 12 months between surface and inner layers.

between OC activity and duration of frozen storage (Fig. 3). The results indicate that there is reduced bone quality in bone stored longer than 9 months. Similar differences were observed for surface compared to inner layers following long-term frozen storage ($P < 0.05$). These results illustrate that long-term frozen storage severely reduces bone quality. Though it is known that the incorporation of long-term stored frozen bone is often successful [2], we speculate that reduced OC activity might decrease the internal repair mechanism at the host-allograft interface.

Allograft processing reduces bone quality

Bone banks process a considerable number of bone allografts because they are thought to maintain bone quality, reduce immunological reactions, and allow for storage at room temperature. We tested the effects of common processing methods used on OC activity. To determine whether full processing affected OC resorption, we incubated OC on bone slices treated by defatting with ether and ethanol, incubation with hydrogen peroxide, freeze-drying followed subsequently by gamma irradiation for preservation, and sterilization. We observed that OC activity on bones that were fully processed was 57% lower compared to stored frozen controls (Fig. 4). Additionally, subsurface layers and inner layers of processed bone revealed 42% and 49% lower OC activity compared to the corresponding frozen bone layers. However, as observed with frozen bone allografts, the inner layers induced improved OC resorption compared to surface layers ($P < 0.05$), demonstrating higher bone quality in the inner layers of bone despite the combination of full processing and frozen storage.

While many bone banks fully process bone specimens, partial processing is also used. To further analyze the effect of processing on bone quality, we tested OC activity on partially processed bone allografts. We compared frozen

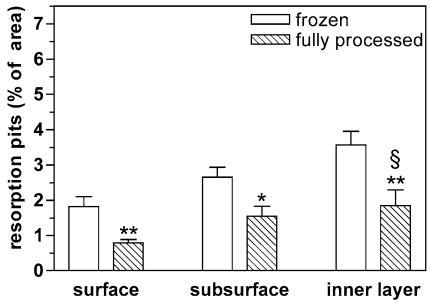

Fig. 4. Effect of full processing on osteoclast (OC) activity on allografts. Bone allografts were stored for 6 weeks at −75°C ($n = 13$) or fully processed by treatment with freezing, ether/ethanol, hydrogen peroxide, lyophilization, and irradiation ($n = 18$) before use. Bone slices from surface, subsurface, and inner layers were incubated with rabbit OCs for 48 h at 37°C. OCs were then washed from the bone and pit area was quantified. These data are presented as the mean percentage of area of resorbed bone ± SEM for two individual experiments. Differences between frozen layers are consistent with Fig. 2. **Significant differences ($P < 0.01$) between frozen surface and fully processed surface. *Significant differences ($P < 0.05$) between frozen subsurface and fully processed surface. **Significant differences ($P < 0.01$) between frozen inner and fully processed inner layers. §Significant differences ($P < 0.05$) between fully processed surface and inner layers.

bone with bone that had been frozen and treated with ether and ethanol, and irradiation (group A), or bone that had been frozen and treated with hydrogen peroxide and lyophilization (group B). Partial processing reduced OC resorption compared to unprocessed frozen bone (Fig. 5). While partial processing in treatment group A revealed a similar pattern of improved OC activity on the inner versus the surface layer, this was not observed for group B. These data demonstrate that partial processing is less damaging to bone than full processing.

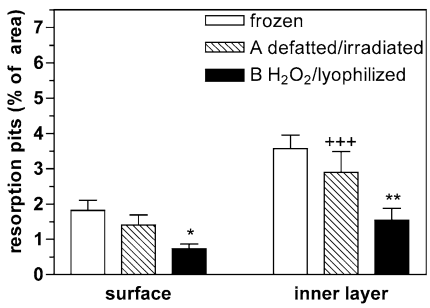

Fig. 5. Effect of bones with partial processing. Bone allografts were stored for 6 weeks at −75°C ($n = 13$) or partially processed in group A by treatment with freezing, ether/ethanol, and irradiation ($n = 18$) and group B by treatment with freezing, hydrogen peroxide, and lyophilization ($n = 18$) before use. Bone slices from surface and inner layers were incubated with rabbit osteoclasts (OCs) for 48 h at 37°C. OCs were then washed from the bone and pit area was quantified. These data are presented as the mean percentage of area of resorbed bone ± SEM for two individual experiments. +++Significant differences ($P < 0.001$) between inner layer and surface layer of partial processing (A); *Significant differences ($P < 0.05$) and **significant differences ($P < 0.01$) between surface and inner T layer of partial processing (B) and frozen bone.

Discussion

Using the in vitro pit assay, we demonstrated that storage and processing of bone allografts reduces OC activity, a finding, which may be an important factor causing host-graft nonunion. We found that fresh bone allografts at all bone layers were associated with the highest OC activity, supporting the notion that OC activity in vitro is correlated with bone quality. Our findings support previous histological data in an allogeneic rat model showing that fresh retrieved matched-bone allografts are incorporated at a higher rate and more completely than frozen devitalized matched bone [14]. Furthermore, rabbit OC resorption on fresh mouse calvarial bone was significantly higher than on bone that had been devitalized by repeated freezing and thawing [22]. In contrast to these results, others have observed that fresh bone is resorbed less well than frozen bone [21]. These experiments, using the pit assay, demonstrated that freeze-thawing seemed to preserve bone matrix and allowed for improved OC attachment and spreading. We suspect that these disparate results are due to two factors. First, fresh bone grafts in the above study were used within 1 h of retrieval, and second, the experiments were done with rabbit rather than human bone [21]. It has been suggested that the mechanism underlying the augmented ability of OCs to initiate resorption on fresh versus frozen bone is associated with the presence of viable osteocytes [15]. However, this has been challenged by the finding that the number of viable osteocytes did not alter the mean volume/area ratio of pits between fresh bone and frozen/thawed devitalized bone [21]. While the mechanism remains controversial, our data illustrate OC activity is higher on fresh compared to frozen bone allografts.

Whereas fresh bone resulted in high OC activity, we predicted that frozen storage conditions decrease bone quality. Optimal duration and temperature for the storage of bone has not been well established despite that frozen, stored human cortical bone is widely used in orthopedic and trauma surgery for massive structural allografts [2,4,5] and strut grafts [1,3]. We observed that bone devitalized by freezing had significantly reduced OC activity compared to fresh bone after 6 weeks of storage at −75°C. However, when we tested bone quality at 9 and 12 months, we found a further reduction in OC activity. In addition, we observed that inner bone layers induced higher OC resorption compared to surface layers, indicating that degradation of matrix components proceeds faster at the surface layers of frozen bone.

The bone bank regulations recommend temporary bone storage of 6 months at −20°C to −40°C and for permanent storage up to 5 years below −40°C [24]. In support of short-term storage, one study showed that OC stimulation in an OB-OC coculture was similar for bones stored for 2 weeks at either −20°C or −80°C [19]. However, when grafts were stored at −20°C there was a marked reduction in bone quality compared to 6 months at −80°C [19]. Taken together, these data imply that frozen bone allografts lose their ability to induce OC resorption when stored for more than 6 months at temperatures above −80°C.

Bone banks routinely use further processing and freezing to devitalize and store bone allografts. Many specimens are frozen, frozen and irradiated [9,10], freeze-dried [6–8], freeze-dried and irradiated [12], or lipid-extracted, freeze-dried, and gas-sterilized [11] and in combinations of these with optional use of hydrogen peroxide. We found that bone treated with freezing and thawing, lipid extraction with ether and ethanol, hydrogen peroxide, consecutive lyophilization, and terminal irradiation resulted in low OC function. These data support previous studies showing that processing procedures result in reduced bone quality, as evaluated by the capacity to absorb work and strength [25] and diminished bone engraftment [6,14–17]. However, we found that while partially processed bone allografts induced higher OC resorption compared to full processing, they were lower than frozen bone. In particular, partially processed bone from group B consisting of freezing, treatment with hydrogen peroxide, and lyophilization impaired OC activity in the two layers of the bone specimen. In contrast, partial processed bone from group A, which consisted of freezing, lipid extraction, and irradiation decreased OC function similar to frozen bone, thus demonstrating that this processing method was less harsh on the bone. Interestingly, bone specimens that had undergone the same treatment combination as group A had no inhibitory activity on OB function [20]. It is tempting to speculate that the underlying mechanism of processing is the oxidation of bone matrix components important for cellular adhesion such as integrins [26], and that damage of these matrix proteins impairs OC attachment and resorption.

While processing and freezing had detrimental effects on the surface of bone allografts, OC activity was higher on the inner layers compared to the outer most surface layers of processed bone and in bone frozen at −75°C. Thus, our data support the clinical practice of suitably tailoring bone allografts at the time of implantation to more closely coapt the host and grafted bone [1,8,12]. Our findings demonstrate that the removal of more than 0.5 mm of bone surface results in higher bone quality, and may correlate with the quantity of OC attracting and stimulating proteins [27] and ultimately will improve engraftment.

In summary, we demonstrated that fresh bone promotes the highest OC function, suggesting that OC activity relates to bone quality. Furthermore, we show that frozen and processed bone loses its integrity on the surface layers but maintains inner layer quality. Moreover, we observed that long-term storage of bone at −75°C further reduces OC activity. These in vitro findings suggest that short-term storage and removal of bone surface prior to implantation will improve allograft success.

Acknowledgments

We thank Barbara Berger and Hannelore Kadlec for technical assistance and Drs. Paula H. Stern and Michelle M. Epstein for critical reading of the manuscript. This work was partly funded by a grant from the Mayor of the City of Vienna.

References

[1] Chandler HP, Tigges RG. The role of allografts in the treatment of periprosthetic femoral fractures. AAOS Instr Course Lect 1998;47: 257–64.
[2] Enneking WF, Campanacci DA. Retrieved human allografts: a clinicopathological study. J Bone Joint Surg Am 2001;83:971–86.
[3] Molinari RW, Bridwell KH, Klepps SJ, Baldus C. Minimum 5-year follow-up of anterior column structural allografts in the thoracic and lumbar spine. Spine 1999;24:967–72.
[4] Muscolo DL, Ayerza MA, Calabrese ME, Redal MA, Santini Araujo E. Human leukocyte antigen matching, radiographic score, and histologic findings in massive frozen bone allografts. Clin Orthop 1996; 326:115–26.
[5] Strong DM, Friedlaender GE, Tomford WW, Springfield DS, Shives TC, Burchardt H, Enneking WF, Mankin HJ. Immunologic responses in human recipients of osseous and osteochondral allografts. Clin Orthop 1996;326:107–14.
[6] Becker W, Urist M, Becker BE, Jackson W, Parry DA, Bartold M, Vincenzzi G, De Georges D, Niederwanger M. Clinical and histologic observations of sites implanted with intraoral autologous bone grafts or allografts. 15 human case reports. J Periodontol 1996;67:1025–33.
[7] Emerson RH Jr, Malinin TI, Cuellar AD, Head WC, Peters PC. Cortical strut allografts in the reconstruction of the femur in revision total hip arthroplasty. A basic science and clinical study. Clin Orthop 1992;285:35–44.
[8] Martin GJ Jr, Haid RW Jr, MacMillan M, Rodts GE Jr, Berkman R. Anterior cervical discectomy with freeze-dried fibula allograft. Overview of 317 cases and literature review. Spine 1999;24:852–9.
[9] Gouin F, Passuti N, Verriele V, Delecrin J, Bainvel JV. Histological features of large bone allografts. J Bone Joint Surg Br 1996;78:38–41.
[10] Hamer AJ, Suvarna SK, Stockley I. Histologic evidence of cortical allograft bone incorporation in revision hip surgery. J Arthroplasty 1997;12:785–9.
[11] Kakiuchi M, Ono K. Defatted, gas-sterilised cortical bone allograft for posterior lumbar interbody vertebral fusion. Int Orthop 1998;22: 69–76.
[12] Zasacki W. The efficacy of application of lyophilized, radiation-sterilized bone graft in orthopedic surgery. Clin Orthop 1991;272: 82–7.
[13] Hornicek FJ, Gebhardt MC, Tomford WW, Sorger JI, Zavatta M, Menzner JP, Mankin HJ. Factors affecting nonunion of the allograft-host junction. Clin Orthop 2001;382:87–98.
[14] Stevenson S, Li XQ, Davy DT, Klein L, Goldberg VM. Critical biological determinants of incorporation of non-vascularized cortical bone grafts. Quantification of a complex process and structure. J Bone Joint Surg Am 1997;79:1–16.
[15] Kamijou T, Nakajima T, Ozawa H. Effects of osteocytes on osteoinduction in the autogenous rib graft in the rat mandible. Bone 1994; 15:629–37.
[16] Aspenberg P, Tagil M, Kristensson C, Lidin S. Bone graft proteins influence osteoconduction. A titanium chamber study in rats. Acta Orthop Scand 1996;67:377–82.
[17] Dziedzic-Goclawska A, Ostrowski K, Stachowicz W, Michalik J, Grzesik W. Effect of radiation sterilization on the osteoinductive properties and the rate of remodeling of bone implants preserved by lyophilization and deep-freezing. Clin Orthop 1991;272:30–7.
[18] Matsuda M, Satoh Y, Ono K. Scanning electron microscopic and light microscopic observations on morphological changes of freeze-dried bone implantation in rats: comparison with fresh autogenous bone transplantation. Histol Histopathol 1992;7:393–403.
[19] Mejdahl S, Hansen CA, Skjodt H, Reimann I. Human bone bank allografts stimulate bone resorption and inhibit proliferation in cultures of human osteoblast-like cells. Acta Orthop Scand 1998;69: 63–8.
[20] Moreau MF, Gallois Y, Basle MF, Chappard D. Gamma irradiation of human bone allografts alters medullary lipids and releases toxic compounds for osteoblast-like cells. Biomaterials 2000;21:369–76.
[21] Kingsmill VJ, Boyde A, Jones SJ. The resorption of vital and devitalized bone in vitro: significance for bone grafts. Calcif Tissue Int 1999;64:252–6.
[22] Shimizu H, Sakamoto M, Sakamoto S. Bone resorption by isolated osteoclasts in living versus devitalized bone: differences in mode and extent and the effects of human recombinant tissue inhibitor of metalloproteinases. J Bone Miner Res 1990;5:411–8.
[23] Chambers TJ, Revell PA, Fuller K, Athanasou NA. Resorption of bone by isolated rabbit osteoclasts. J Cell Sci 1984;66:383–99.
[24] Woll JE, Kasprisin D, editors. Standards for tissue banking. American Association of Tissue Banks; Library of Congress Catalog Card Number 2001;84-7269.
[25] Hamer AJ, Strachan JR, Black MM, Ibbotson CJ, Stockley I, Elson RA. Biochemical properties of cortical allograft bone using a new method of bone strength measurement. A comparison of fresh, fresh-frozen and irradiated bone. J Bone Joint Surg Br 1996;78:363–8.
[26] Raynal C, Delmas PD, Chenu C. Bone sialoprotein stimulates in vitro bone resorption. Endocrinology 1996;137:2347–54.
[27] Kaneko H, Arakawa T, Mano H, Kaneda T, Ogasawara A, Nakagawa M, Toyama Y, Yabe Y, Kumegawa M, Hakeda Y. Direct stimulation of osteoclastic bone resorption by bone morphogenetic protein (BMP)-2 and expression of BMP receptors in mature osteoclasts. Bone 2000;27:479–86.