International Orthopaedics (SICOT) (1996) 20: 147–152

International Orthopaedics

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Preparation of bank bone using defatting, freeze-drying and sterilisation with ethylene oxide gas

Part 2. Clinical evaluation of its efficacy and safety

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Accepted: 8 September 1995

Summary. *We used bone allograft treated by defatting in chloroform and methanol, freeze-drying and sterilisation with ethylene oxide gas in operations on 396 patients. The purpose of defatting and freeze-drying is to facilitate subsequent sterilisation by eliminating a barrier to the diffusion of ethylene oxide gas into bone, to lower the residual levels of the ethylene oxide and its toxic by-products after sterilisation, to eliminate alloantigens and to make storage at room temperature possible. Postoperative infections confirmed by a positive bacterial culture occurred in 2 of the 396 patients receiving the allograft, which was prepared under clean, but not sterile, conditions, one of which was thought to be due to dehiscence of the wound, rather than to the allograft. There were also 3 probable infections. Histological sections of the area around the interface of the allograft and its bony bed showed: (1) osteoblasts lining the surface of the dead cortical bone of the graft with appositional new bone; (2) ingrowth of new bone into the Haversian canals, and (3) little infiltration of inflammatory small round cells. These findings indicated the ability of the bone to support new bone formation and to eliminate antigens. The low incidence of infection confirmed the efficacy of this method of sterilisation.*

Résumé. *Nous avons établi une méthode pratique et efficace pour la stérilisation d'allogreffes osseuses. Cette méthode se compose de: 1) dégraissage dans du chloroforme et du méthanol, 2) lyophilisation et, 3) stérilisation avec gaz d'oxyde d'éthylène. Le but du dégraissage et de la lyophilisation est de faciliter une stérilisation ultérieure en éliminant la barrière à la diffusion du gaz d'oxyde d'éthylène dans les os, d'abaisser les niveaux résiduels de l'oxyde d'éthylène et de ses sous-produits toxiques après la stérilisation, d'éliminer les iso-antigènes et de rendre possible un stockage à la température de la pièce. L'efficacité et la sécurité de cette méthode ont été évaluées par: 1) la capacité de stérilisation d'os infectés prélevés à partir du foyer d'une ostétomyélite chronique active chez six patients, 2) la pénétration du gaz d'oxyde d'éthylène dans des têtes fémorales humaines traitées par cette méthode ou par d'autres, telle que la lyophilisation ou la congélation-décongélation, et 3) l'élimination de l'oxyde d'éthylène et de ses sous-produits toxiques, chlorhydrine d'éthylène et éthylène glycol, provenant de l'os traité par cette méthode ou d'autres, telle que la lyophilisation ou la congélation-décongélation. Tous les échantillons d'os provenant d'une ostéomyélite chronique sont restés bactériologiquement stériles après traitement par cette méthode. Lorsque des têtes fémorales traitées avec cette méthode ont été exposées à l'oxyde d'éthylène, le gaz a suffisamment pénétré dans la zone centrale en quelques heures seulement. Les niveaux résiduels de l'oxyde d'éthylène et de ses sous-produits toxiques dans les os traités par cette méthode ont été bien plus faibles que ceux se trouvant dans les os lyophilisés ou congelés-décongelés, et ont rapidement diminués dans l'air ambiant. Ces résultats indiquent qu'un dégraissage antérieur et une lyophilisation ont permis de faciliter la pénétration du gaz d'oxyde d'éthylène dans l'os durant la stérilisation et*

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l'élimination de l'oxyde d'éthylène et de ses sous-produits toxiques après stérilisation. La préparation dans des conditions propres, mais non stériles, et un stockage à la température de la pièce permettent de rendre le système de banque des os plus pratique et efficace.

Introduction

We devised the method of preparing and sterilising bone, which is described in the preceding paper, and have used it since 1981. The defatting, freeze-drying and subsequent exposure to ethylene oxide gas does not require large equipment. Our preliminary study showed the possibility of achieving good clinical results [7].

The purpose of the present study was to evaluate clinically the efficacy and safety of these defatted, gas-sterilised bone allografts.

Materials and methods

The bone was obtained and treated sequentially under clean, but not sterile, conditions as in the preceding paper. The types of graft prepared were: (1) thick cortical bone from the diaphysis of the femur or tibia; (2) thin cortical bone from the metaphysis of the same bones; (3) chips of cancellous bone in 1 cm cubes, and (4) cylindrical-shaped diaphysis of the fibula. Massive whole bone was not used in this series.

Defatted, gas-sterilised bone allograft was prepared as bank bone at the Osaka University Medical School. The study comprised 396 patients who underwent operations performed by one of the two authors between November 1981 and December 1991. All were carried out in a standard operating room. The allografts were not placed in infected, or previously infected, sites. Prophylactic antibiotics were given routinely. A blood test was done at one day, one week and one month after operation. At least once a month during the first 6 months, the patients were assessed for the signs of infection such as pain, erythema, swelling or discharge, and radiographs inspected for evidence of bone resorption around the allograft. When such signs were present, an additional blood test and bacterial culture of any drainage or aspirate was carried out.

A definite infection was present when there were physical or radiological signs and a positive culture. A probable infection was diagnosed when there were signs of infection, persistent leucocytosis and raised C-reactive protein, but negative cultures.

Histological sections of the area around the interface of the allograft and its bony bed specimens were examined in 7 patients (Table 2).

Follow up was for an average of 5 years, 2 months (range from 9 years, 4 months to one year, 5 months).

Results

Thick cortical bone allografts were used in 215 patients; 2 (0.9%) had a definite infection and 3 (1.4%) probable infection. Thin cortical strips or bone chips were used in 181 patients and there were no cases of infection. Table 1 classifies the procedures, with details of the allografts used, in relation to the incidence of definite and probable infections.

Table 1. Clinical incidence of postoperative infection; patients classified by surgical procedures and sizes and types of allograft

Procedure	Patients
Cylindrical thick cortical bone taken from diaphysis of the tibia or femur	
10 cm long or more	
Proximal tibial or distal femoral replacement combined with endoprosthesis for osteosarcoma or recurred giant cell tumor	7 (DI = 1[a])
Less than 10 cm long	
Revision of total hip or knee arthroplasty	11
Subtotal resection of lumbar vertebral body and anterior interbody fusion	5
Thick cortical bone plates taken from diaphysis of the tibia or femur and/or cylindrical fibula	
10 cm long or more	
Repair of defect made by resection and curettage of giant cell tumor	15 (PI = 1)
Postero-lateral lumbar fusion	5 (DI = 1)
3 to 10 cm long	
Postero-lateral lumbar fusion	7
Posterior lumbar fusion with internal fixation	14
Repair of bone defect in fracture of limbs	16 (PI = 1)
Repair of donor site for iliac autograft	4
Less than 3 cm	
Posterior lumbar interbody fusion	31 (PI = 1)
Posterior lumbar interbody fusion with internal fixation	67
Anterior lumbar interbody fusion	7
Anterior cervical interbody fusion	5
Correction osteotomy in limbs	12
Repair of donor site for iliac autograft	9
Subtotal	215 (DI = 2, PI = 3)
Thin cortical bone plates taken from metaphysis of the tibia or femur	
Revision of total hip or knee arthroplasty	18
Repair of bone defect in fracture of limbs	12
Chips of cancellous bone	
Revision of total hip or knee arthroplasty	27
Shelf arthroplasty of hip	12
Repair of donor site for tibial autograft	21
Repair of defect made by curettage of benign bone tumors	91
Subtotal	181 (DI = 0, PI = 0)
Total	396 (DI = 2, PI = 3)

The values given are the number of patients, with the number of those with postoperative infection in parentheses (DI: definite infection, PI: probabe infection). When two or more pieces of bone were used in a patient, the size and thickness of bone indicated above are for the largest and thickest

[a] The infection in this patient is considered to be closely related to wound necrosis and dehiscence of incision, and not associated with surgical procedures or allograft

Table 2. A list of patients who underwent biopsy of the allograft

Case No.	(Age (years)	Sex	Diagnosis at the time of grafting	Surgical procedure using allograft	Type of allograft used	Period between grafting and biopsy	Event giving an opportunity for biopsy	Sequence after grafting	Histological findings of biopsy specimens
1	39	man	Giant cell tumour of distal femur	Repair of defect made by resection and curettage	Struts of cortical bone	5 months	Arthroscopy for knee pain	normal	See Fig. 1
2	39	man	Non-union of femoral fracture	Repair of donor site in iliac crest	Cylindrical fibula	7 months	Removal of plate and screws	normal	See Fig. 2
3	38	woman	Eosinophilic granuloma of proximal femur	Filling defect made by curettage	Chips of cancellous bone	8 months	Curettage of tumour of ilium on the same side	normal	Ingrowth of new bone and partial resorption of dead bone
4	25	man	Lumbar disc herniation	Discectomy and posterior fusion	Hemi-cylindrical fibula	1 year & 4 months	Removal of wire	failed, non-union	No ingrowth of new bone and little resorption of dead bone
5	15	man	Osteosarcoma of proximal tibia	Endoprosthetic replacement combined with allograft	Cylindrical tibia	2 years & 3 months	Resection of glioblastoma of the brain	normal	Scanty dead bone remnant within living new bone
6	12	woman	Fibrous dysplasia of proximal femur	Repair of defect made by curettage	Struts of cortical bone	5 years & 2 months	Removal of wire	normal	Scanty dead bone remnant within living new bone
7	42	man	Recurrent giant cell tumour of distal femur	Repair of defect made by resection and curettage	Struts of cortical bone	5 years & 8 months	Correction osteotomy for valgus deformity of knee	normal	Living new bone surrounding dead bone remnant

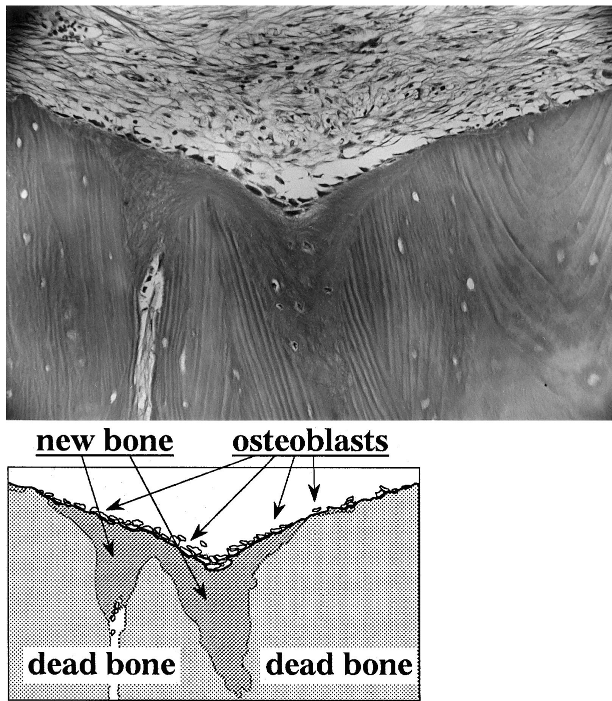

Fig. 1. Histological sections of the defatted, gas-sterilized cortical bone allograft obtained from a thirty-nine-year-old man (Case 1, Table 1) five months after grafting. Osteoblasts with appositional deposits of new bone are noted. No infiltration of inflammatory small round cells is observed (hematoxylin and eosin, ×123)

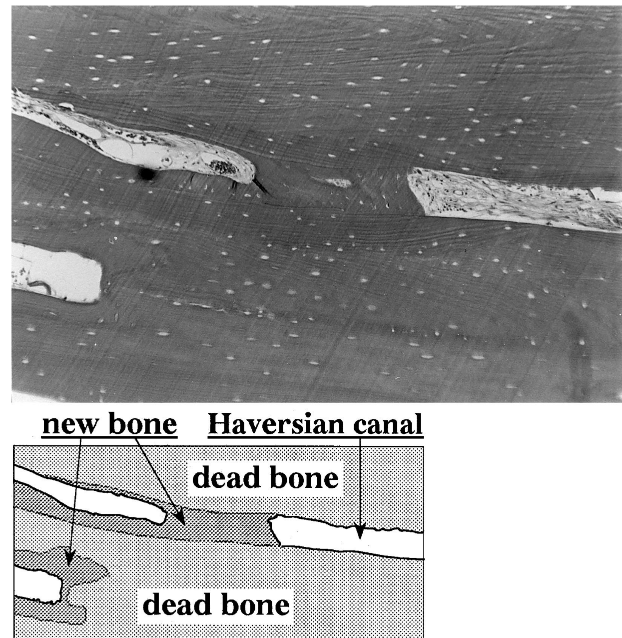

Fig. 2. Histological sections of area around the interface of the recipient bone bed and the defatted, gas-sterilized fibular bone allograft obtained from a thirty-nine-year-old man (Case 2, Table 1) seven months after grafting. Ingrowth of new bone into Haversian vascular channels is noted (hematoxylin and eosin, ×25)

Definite infections

One of the 2 patients with definite infections developed drainage from the wound with intermittent fever 3 days after posterolateral lumbar fusion using long thick pieces of cortical bone. Staphylococcus aureus was cultured. The allograft was removed and the infection cleared.

The other patient had a proximal tibial endoprosthetic replacement for osteosarcoma and long cylindrical cortical bone had been added. Wound necrosis developed with dehiscence 3 days after operation and staphylococcus epidermis was grown. The complication was considered to be due to the dehiscence of the wound and not the surgical procedure or the allograft. The infection cleared after removal of the allograft and endoprosthesis.

Probable infections

One of the 3 patients with probable infections had a posterior lumbar interbody fusion with thick cortical bone. Low back pain developed 18 months after operation and radiographs showed resorption of bone around the graft. Bacteria were not grown from a biopsy specimen. Intravenous antibiotics were given and the signs of infection disappeared. The resorbed bone repaired spontaneously and there was no recurrence 5 years later.

The second patient had resection and curettage of a giant cell tumour of the proximal tibia and long thick cortical bone was used. Erythema, swelling of the wound and leucocytosis developed after the operation. Bacteria were not cultured in fluid aspirated from the wound and the knee. The local signs cleared after 5 weeks.

The third patient had undergone open reduction of a tibial plateau fracture using thick cortical bone. There was intermittent fever with persistent swelling and local heat, but no discharge. Bacteria were not cultured in aspirate from the knee and the wound.

The results indicate that of the total of 396 bone allografts infection occurred at most in 4 (1.0%) patients (the patient whose infection was related to wound dehiscence is excluded). Of the 215 thick cortical bone grafts, 4 (1.9%) developed infection. Thin cortical strips or bone chips were used in 181 patients with no cases of infection, definite or probable.

Biopsy specimens

The histological findings in the area around the interface between allograft and recipient bone are given in Table 2. Those obtained after 6 months showed osteoblasts lining the surface of dead cortical bone with deposits of appositional new bone (Fig. 1), and ingrowth into Haversian vascular channels (Fig. 2). Those obtained a few years after grafting had living new bone surrounding the dead bone, as in normal bone graft incorporation. Little or no round cell infiltration was seen in either case.

Discussion

Limbs amputated for trauma or ischaemic necrosis have a greater risk of transmitting bacteria to recipients than when strictly screened cadaveric bone is used, and bacterial contamination may not always be detected by the culture of swabs. If bone from such limbs is used for grafting, efficient sterilisation is essential as in the method we have described. In spite of the use of amputated limbs and preparation under unsterile conditions, we found evidence of infection in only 1.0% of patients at most, and only with thick cortical allografts. This may be explained by the relatively greater invasiveness of the operation compared to when thin cortical bone or cancellous chips are used.

Defatting in organic solvents, such as chloroform and methanol, reduces the antigenicity of the allograft [9, 19], and our biopsy specimens showed little infiltration with inflammatory round cells.

Another concern is whether demineralisation of the allograft favourably affects bone repair in humans. In lower mammals, such as rodents, demineralisation with hydrochloric acid enhances osteoinduction in allografts and new bone is induced even in non-ossifying tissues [17, 18, 19] as a result of the bone morphogenetic properties of the matrix. Experiments in higher mammals have however shown that demineralised bone has failed to induce new bone formation in muscles [2], and does not enhance it in orthotopic sites [14]. Our clinical data showed that allografts, which are completely demineralised and exposed to ethylene oxide gas, were completely resorbed without new bone formation when they were placed in paraskeletal sites in humans [6]. These data provide sufficient reason for excluding demineralisation from the preparation of allografts used in patients.

Sterilisation with ethylene oxide at 30 °C for 4 h does not affect the results of repair of skull defects replaced with canine skull autograft [12], but at 37 °C for 4 to 6 h, which completely eliminated bacillus subtilis spores, demineralised bone is incapable of bone induction in rats [1, 11]. These results are not contradictory if it is assumed that ethylene oxide sterilisation has an adverse effect on osteoinduction of the bone matrix, but no effect on osteoconduction, and that the osteoinductive properties of the matrix are already much less effective in higher mammals, such as dogs, monkeys and humans, than in lower mammals, such as rodents. There is no evidence that demineralised bone induces sufficient new bone formation in nonskeletal tissues of humans, although semi-purified morphogenetic bone protein induces new bone in muscles of monkeys [10]. In squirrel monkeys, allogeneic or autogenic demineralised bone matrix does not induce extraskeletal bone formation [2]. Our biopsy showed that the defatted gas-sterilised bone, whose ability to induce new bone in nonskeletal tissues may have been lost, supported new bone formation on its surface. This positive result in humans must be due to properties other than osteoinduction, and osteoinductive properties may contribute little to new bone formation in allografts used clinically. Therefore, ethylene oxide sterilisation, though capable of reducing osteoinduction, does not necessarily have harmful effects on bone formation in clinical allografts. The role of osteoinduction in humans will be elucidated when pure bone morphogenetic protein on appropriate carriers, with higher inductive properties than those of bone matrix, becomes available.

Donor screening is considered effective in preventing human immunodeficiency virus (HIV) transmission, but a risk remains. Organs, or fresh-frozen bone, taken from a thoroughly screened antibody negative HIV infected donor have consistently transmitted HIV to recipients. The donations were most probably made during the period between infection and antibody seroconversion [15] which ranges from 4 weeks to 6 months in most cases [5]. Sterilisation further reduces the risk of transmission. Storage in a dried state at room temperature for many days, or treatment with alcohol for some minutes, inactivates HIV [13]. Drying, or treatment with solvents such as alcohol, ether or chloroform, usually inactivates the enveloped virus. Each procedure of defatting, freeze-drying and exposure to ethylene oxide gas, as used in our method, may be capable of inactivating HIV.

There have been a few cases reported of transmission of Creutzfeldt-Jacob disease (CJD) through dura mater allografts [8, 16], but none

through bone allografts. The CJD virus has usually been found in neural tissues, and only infrequently in non-neural tissues in humans [3], so the risk of transmission by bone is small. The CJD virus is resistant to most disinfectants, including ethylene oxide [4] and autoclaving is not always effective. Since there is no laboratory screening test, donors with neurological illness or dementia (the primary symptoms of CJD) should be excluded. The disease usually occurs in older people [16], so donors should be under 50 years of age.

Our method is safe and biologically acceptable. The bone can be procured in unsterile conditions and stored and transported at room temperature, making the procedure easy and cheap.

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