Journal of Orthopaedic Surgery 2004;12(2):184–190

Musculoskeletal tissue banking in Singapore: 15 years of experience (1988–2003)

A Nather
National University Hospital Tissue Bank, Department of Orthopaedic Surgery, National University of Singapore, Singapore

ABSTRACT

Purpose. To report 15 years' experience of musculoskeletal tissue banking by the National University Hospital Tissue Bank.
Methods. This study describes the development of Singapore's national bone bank since its establishment in 1988. The bone bank's protocol follows guidelines recommended by the American Association of Tissue Banks and the European Association of Tissue Banks using strict donor selection criteria. Informed consent is obtained from all potential donors for tissue procurement and laboratory tests. Detailed medical history, thorough clinical examination, and chart review is performed for consenting donors. Suitable donors are subjected to tests for hepatitis B, hepatitis C, syphilis, and culture/sensitivity test of tissue for aerobic and anaerobic organisms. For living donors, repeat testing for AIDS and hepatitis C is performed at least 180 days after procurement. Tissue procurement is performed under sterile conditions. Small tissues are procured using the 'sterile double jar technique' and long bones using the 'sterile triple wrap technique', both developed by the author. Deep-frozen bones are gamma irradiated at 25 kilograys. Morsellised bones are lyophilised and gamma irradiated. Meticulous preparation for grafts is performed during transplantation. Antibiotic prophylaxis is used for 2 weeks.
Results. The bank maintains a good quality control. In January 2003, it was accredited ISO 9001 status. Up to June 2003, it has procured 440 bones from 440 living donors and 1055 allografts from 63 deceased donors. 854 musculoskeletal transplantations have been performed using tissues processed by the bank. Complication rate encountered was only 2.2%.
Conclusion. The tissue bank provides high-quality allografts for safe tissue transplantations.

Key words: *living donors; muscle, skeletal; tissue banks; tissue donors; transplantation, homologous*

Address correspondence and reprint requests to: Prof Aziz Nather, Director, National University Hospital Tissue Bank, Department of Orthopaedic Surgery, National University of Singapore, 5 Lower Kent Ridge Road, Singapore 119074. E-mail: dosnathe@nus.edu.sg

INTRODUCTION

During the last decade, musculoskeletal tissue transplantation has become well established internationally,[1–3] and tissue banking has gained increasing importance in the Asia Pacific region.[4,5] The awareness of tissue banking and the demand for bone and soft-tissue allografts in the region have increased significantly. This had led to the development of new tissue banks in Malaysia, Indonesia, and most recently, India and South Korea.

Development of the National University Hospital Tissue Bank

The National University Hospital (NUH) Tissue Bank was established in October 1988 as a research bone bank then called the National University of Singapore (NUS) Bone Bank. This was funded by an NUS research grant, RP 880334 'Use of Allografts for Bridging Large Bone Defects', with the author as the principal investigator. The bank was initially equipped with 2 electrical freezers for storage of tissues at -80°C.

There has been tremendous growth in the clinical function of the bank as the national demand for bone and soft-tissue allografts has increased rapidly. In January 1994, the bank received an additional grant of S$239 965 from the Totalisator Board to upgrade itself as a national bone bank to supply bone and soft-tissue allografts to all hospitals in Singapore.[5] It then acquired 4 electrical freezers (-80°C) and 2 lyophiliser units (each with a band saw, shaker bath, lyophiliser, laminar flow cabinet, and vacuum sealer). It started processing lyophilised, gamma-irradiated bone grafts in 1994. Gamma irradiation was first introduced into Singapore in September 1992.[5] The deep-frozen long bones were packed in dry ice (-20°C) in polylite containers and flown to the Malaysian Institute of Nuclear Technology in Bangi, Selangor. There, the bones were subject to gamma irradiation at a dosage of about 25 kilograys, then returned to the bank within 12 hours for re-storage in the freezer at -80°C. For smaller deep-frozen bones procured from living donors, gamma irradiation was performed in a gamma chamber at the Department of Nuclear Medicine in Singapore General Hospital.[5]

In September 1995, the bank was inaugurated as a hospital bone bank and re-named the NUH Tissue Bank.[5] The author became its director under the NUH Administration Board. In 1998, the bank started functioning as a cost centre of the hospital, which was non-profitable, self-sustained and operated independently. The bank continued its focus on maintaining good standards and quality control, together with the whole hospital, it was accredited ISO9001: 2002, ISO14001, and OHSAS18001 in January 2003.

METHODS

Legal status

The development of tissue banking in Singapore started from the Medical (Therapy, Education and Research) Act of 1972,[4,6] in which it stipulates "any person of sound mind and 18 years of age or above may give all or any part of his body for education... transplantation.... The gift takes effect upon death." This 'opting-in' law requires informed consent from the donor, his/her relative, or a next-of-kin. The tissue bank is prohibited from selling tissues procured and processed; however, it is allowed to charge processing costs i.e. costs of procurement, processing, and distribution of tissues.

NUH Tissue Bank Protocol

The NUH Tissue Bank Protocol follows similar guidelines recommended by the American Association of Tissue Banks and the European Association of Tissue Banks.[4,6] These guidelines include strict donor-selection criteria using a detailed medical history and meticulous clinical examination. A chart review is also conducted to exclude any fever. Suitable donors are then subject to laboratory tests for HIV (Anti-HIV_1, Anti-HIV_2), hepatitis B (HbsAg), hepatitis C (Anti-HCV), syphilis (rapid plasma reagin test, if positive, then *Treponema pallidum* haemagglutination assay), and culture/sensitivity test of tissue specimen for aerobic and anaerobic organisms. For living donors, repeat tests are performed for HIV (Anti-HIV_1, Anti-HIV_2) and hepatitis C (Anti-HCV) at least 180 days after procurement. If any of these tests are positive, the specimen is discarded.

Detailed documentation is adopted using Living Donor Form, Consent Form for Living Donor, Deceased Donor Form, Consent Form for Deceased Donor, Wet Processing Form, Dry Processing Form, Radiation Sterilisation Form, Final Inspection Form, and Recipient Form. A meticulous system of documentation is adopted such as individual patient medical record folders maintained by the bank. These are colour coded—red for living donors, blue for deceased donors, and green for recipients. All forms pertaining to each living or deceased donor —Living Donor Form, Consent Form, Laboratory Test Results,

Processing Forms (if any)—are kept in the individual medical record folder for that donor. In addition, log-books are also kept such as Living Donor Log Book, Deceased Donor Log Book, and Recipient Log Book.

Femoral head bone banking

For the first 3 years, the bank's procurements were limited to living donors only.[4,6] The majority of these procurements were femoral heads from elderly patients undergoing hemiarthroplasty for displaced, fractured neck of the femur. Bone slices from patients undergoing total knee arthroplasty for osteoarthritis of the knee were also collected.

Deceased donor procurement

In 1992, the Singapore Ministry of Health developed a Multi-organ and Tissue Procurement System, linking bone procurement with kidney and liver transplantation programmes as well as corneal and skin transplantation programmes.[5] Within this system, national transplant coordinators (kidney transplant coordinators) coordinate the various transplant teams for procurement. These coordinators alert the NUH Tissue Bank when consent for procurement of musculoskeletal tissues from deceased donors has been obtained. With public education programmes running together with the kidney and liver transplantation programmes, the first deceased donor procurement was performed on 15 November 1991 and resulted in the donor donating both kidneys.

Processing of deep-frozen bones and soft tissues

All bones procured from living donors are under sterile conditions using the 'sterile double jar technique' developed by Nather.[4,6] The procured femoral heads or total knee replacement slices are first washed with normal saline in the operating room. A piece of bone is sent for culture and sensitivity tests for aerobic and anaerobic organisms. The procured bone is then put into an inner sterile glass bottle which is then closed with a screw-on cap. The inner bottle is then put into a slightly larger sterile outer glass bottle which has a rubber bung and an air seal lock. The closed, sterile double jar is then passed to the circulating nurse for labelling.

All tissues procured from deceased donors are also under sterile conditions while the donors are still anaesthetised for procurement of kidneys under the Multi-organ and Tissue Donation System in the operating theatres of hospitals.[5] No tissues are procured from the mortuary. Small bones and soft tissues are collected using the 'sterile double jar technique'. Long bones are procured using the 'sterile triple wrap technique' also developed by the author. In this technique, the inner layer is a sterile polyethylene bag (sterilised by gamma irradiation) and the middle layer consists of thick green linen (sterilised by autoclaving). After wrapping with linen, 2 sterile cotton tapes are used for tying the wrap at each end of the bone. The third and outer layer is again a sterile polyethylene bag. The triple wrapped specimen is then passed to the circulating nurse for labelling. Before being wrapped, a small piece of bone from each bone procured is sent for culture and sensitivity tests for aerobic and anaerobic organisms. As an additional safeguard to ensure complete sterility of the grafts, the triple wrapped long bones are gamma irradiated at a dose of 25 kilograys.[5] Soft tissues such as patellar tendon, calcaneum tendon, tibialis anterior tendon, tibialis posterior tendon and fascia lata are collected in sterile double jars and are not subject to gamma irradiation. The sizes of all long bones procured are measured using radiographs (anteroposterior and lateral views) with a graduated ruler. The radiographs facilitate matching between the recipient bone size needed (as seen on similar radiographs traced and faxed to the bank) and the available donor bones.

Processing of lyophilised bone allografts

Our protocol for lyophilisation of bones includes jet lavage of bone with water, dissection of soft tissues from the bone, cutting the bone into smaller pieces of various sizes and shapes with a band saw, chemical processing with 70% ethanol for 3 hours to kill HIV virus, pasteurisation in a shaker bath at 60°C for 3 hours to inactivate HIV virus, and final dissection of soft tissues from the bone pieces in our wet-processing laboratory. The bone pieces are then lyophilised until the residual water content is between 5% and 8%, then packed and labelled under laminar flow conditions in the laminar flow cabinet using vacuum packing in 2 layers of polyethylene bags. A radiation indicator is used in the labelling. Upon receiving radiation, the yellow ferric-ferrous indicator turns into red. These steps are performed in our dry-processing laboratory. The gamma irradiation is then performed at a dosage of 25 kilograys to sterilise the lyophilised tissue grafts. Lyophilised, gamma-irradiated bone allografts are then stored at room temperature.

Preparation of deep-frozen allografts during surgery for transplantation

Surgeons performing the transplantations must have experience in the use of such allografts. Surgeons using them for the first time are assisted by the author to ensure that allografts are prepared properly in order to reduce the complication rate.

For massive bone reconstructions, 2 surgical teams are needed, a recipient team performing the transplantation and reconstruction, and a donor team for meticulous preparation of the donor allograft. A separate trolley with separate instrumentation is necessary for such allograft preparation. The allograft to be used must first be thawed for at least one hour before the start of surgery. It is then soaked for about half an hour in a basin containing one litre of normal saline, 500 mg of ampicillin, and 500 mg of cloxacillin. The donor surgeon then strips completely all periosteum and soft tissues from the cortical surface of the graft. The exact length of the graft needed is measured, marked, and cut using an oscillating saw. Its medullary canal is then reamed using a manual intramedullary nail reamer to remove all the marrow contents. The meticulously cleaned bone is then jet flushed with one to 2 litres of normal saline to remove all blood, debris, and soft tissues until all returning fluid is clear. Finally, the bone is soaked in another litre of normal saline containing 500 mg of ampicillin and 500 mg of cloxacillin for about 30 minutes before it is transplanted.

The same procedure is used for preparing deep-frozen femoral heads. The sterile double jar is allowed to thaw for at least one hour before starting the operation. The femoral head is then soaked in about 200 ml of saline in a kidney dish containing 250 mg of ampicillin and 250 mg of cloxacillin for about 30 minutes on the donor trolley. In addition to removing periosteum and soft tissues from the bone, all articular cartilage must be meticulously removed from the femoral head using a bone nibbler. The bone is cut into small pieces using an oscillating saw and again jet flushed with normal saline. Finally, these small pieces are then soaked in 200 ml of normal saline containing antibiotics for another 30 minutes before being used. If smaller pieces are needed, the bone is shredded using a sterile bone mill.

Postoperative regime

Prophylactic antibiotics are recommended in all cases of musculoskeletal allograft transplantation. Intravenous cefazolin 1 g 6 hourly for 48 hours followed by oral ceporex 500 mg 6 hourly for 2 weeks is given until the wound has healed. A redivac drain is inserted before closing the wound.

Table 1
Living donor procurement

Type of bone procured	No. of living donors
Femoral head	297
Total knee replacement bone slices	138
Humeral head	3
Radial head	1
Rib	1
Total	**440**

Table 2
Deceased donor procurement*

Type of bone/soft tissue procured	Left	Right	Total
Whole femur	28	31	**59**
Proximal femur	31	30	**61**
Distal femur	31	30	**61**
Femoral head	34	34	**68**
Whole tibia	30	31	**61**
Proximal tibia	28	31	**59**
Distal tibia	28	31	**59**
Whole fibula	18	20	**38**
Proximal fibula	7	6	**13**
Distal fibula	7	6	**13**
Iliac crest	35	33	**68**
Patella	8	9	**17**
Whole patella ligament complex	15	14	**29**
Half patella ligament complex	87	89	**176**
Whole calcaneum tendo-achilles	12	15	**27**
Half calcaneum tendo-achilles	21	22	**43**
Tibialis anterior tendon	27	30	**57**
Tibialis posterior tendon	19	20	**39**
Fascia lata	17	16	**33**
Quadriceps tendon	2	2	**4**
Humerus	15	16	**31**
Radius	9	11	**20**
Ulna	9	10	**19**
Total			**1055**

* From a total of 63 deceased donors

RESULTS

Musculoskeletal procurement

Up to 30 June 2003, 440 bones, mainly femoral heads (297), were procured from 440 living donors (Table 1), whereas 1055 bone and soft-tissue specimens were procured from 63 deceased donors (Table 2). Tissues from the lower limbs were procured from all deceased

Table 3
Musculoskeletal transplantations performed

Indication	No. of cases
Spine	
Posterior spinal fusion	132
Anterior spinal fusion	52
Hip	
Revision total hip replacement	113
Primary total hip replacement	3
Avascular necrosis	7
Knee	
Revision total knee replacement	10
Arthrodesis knee	6
Malignant bone lesions	
Intercalary reconstruction	37
Osteoarticular reconstruction	7
Resection arthrodesis	13
Curettage and bone grafting	18
Benign bone lesions	
Curettage and bone grafting	28
Trauma	
Fracture calcaneum	20
Fracture tibial condyle	18
Peri-prosthetic fractures	25
Other fractures	25
Other bone lesions including maxillofacial lesions	102
Soft-tissue reconstruction	
Anterior cruciate ligament, knee	183
Posterior cruciate ligament, knee	32
Lateral collateral ligament, ankle	10
Acromioclavicular joint, shoulder	10
Tendo-achilles reconstruction	1
Ptosis reconstruction, eye	2
Total	**854**

donors. However, bones from upper limbs were procured from only 16 deceased donors. After retrieval of bones and soft tissues, lower limbs are reconstructed with a plastic femur and tibia arthrodesed together at the knee using one roll of a 6-inch plaster of Paris and the soft tissues closed in 2 layers over this plastic bone reconstruction. For the upper extremities, after procurement of bones, reconstruction is performed using plastic humerus, radius, and ulna bones.

Musculoskeletal transplantation

Up to 30 June 2003, 854 bone and soft-tissue transplantations have been performed using soft-tissue allografts procured and processed by NUH Tissue Bank (Table 3). Of the 854, 184 were performed for spinal fusions, 123 for hip surgery, 75 for malignant bone lesions and 88 for bone trauma. 238 patients underwent soft-tissue reconstructions, the majority of which (183) were arthroscopic anterior cruciate ligament reconstruction using the patella-ligamentum patella-tibial tuberosity complex. Of the 854 musculoskeletal transplantations, 450 were performed in NUH, 127 in Tan Tock Seng Hospital, 101 in Singapore General Hospital, 13 in Alexandra Hospital, 9 in Kandang Kerbau Women's and Children's Hospital, 8 in Changi General Hospital, 2 in the National Cancer Centre, 2 in the National Eye Centre and 142 in private hospitals. Over the past 4 years, the number of transplantations performed annually in Singapore has increased to about 100 to 120 transplantations per year.

Complications

Complications were encountered in 19 (2.2%) of the 854 musculoskeletal transplantations performed. These included 9 (6.8%) of the 132 cases undergoing posterolateral spinal fusion, of which 2 had deep infections, 2 had superficial infections, and 5 had pseudoarthroses with implant failure; all these 9 complications were treated successfully, of which the 2 deep infections were salvaged using radical debridement, removal of allografts, and aggressive antibiotic therapy. Other complications included one case that have persistent serous discharge from the thoracic wound of an anterior spinal reconstruction using a lyophilised femoral ring allograft for a burst fracture of the first lumbar vertebra; the sterile discharge settled after 2 weeks. Six (10.5%) complications were encountered in 57 cases undergoing massive bone reconstruction for limb-salvage surgery for malignancy, of which 2 had non-salvageable deep infections and underwent above-knee amputation and 4 had superficial infections and were successfully treated. Other complications included one case of deep infection of a fibula allograft used to augment a short traumatic below-knee stump which could not be saved and resulted in above-knee amputation, one case of implant failure in an elderly woman with delayed union of the humerus treated with plating and onlay fibula allograft, and one case of methicillin-resistant *Staphylococcus aureus* superficial infection in a patient with delayed union of the femur treated with autografts supplemented by onlay femoral cortical allograft.

DISCUSSION

The outcome of allograft transplantation depends on several factors such as quality control of tissue grafts processed by the tissue bank, the surgeon's understanding of the biological and biomechanical

behaviour of allografts, proper selection of indications for the use of allografts, the choice of the most appropriate type of allograft for each indication, and the use of antibiotic prophylaxis to prevent infection.

The NUH Tissue Bank has good quality control and was accredited the ISO 9001 certification. It provides high-quality tissue allografts by adopting a protocol of strict donor selection criteria and meticulous documentation. Indeed, it is well recognised that good documentation is the key to quality control.[7,8]

To ensure good quality control, tissues procured from both living and deceased donors are under sterile conditions in the operating room.[9–11] The femoral heads from living donors are stored using the 'sterile double glass jar technique'.[4,6] This technique saves storage space in the freezers and is easy to handle compared to techniques that involve an inner jar and then wrapping with 3 sterile hand towels and a sterile plastic bag.[12] The 'sterile triple wrap technique' for storage of long bones procured from deceased donors uses an inner sterile polyethylene layer because polyethylene does not stick to bone. If linen is used as the inner layer, it will stick to the bone after freezing for some time and will be difficult to remove. Furthermore, polyethylene is used as the outer layer because it is waterproof and can protect the middle linen layer from getting wet.

During deceased donor procurement, it is extremely important to perform proper reconstruction of the extremities in order to give the utmost respect to the deceased and to allow for open casket funerals. The bank uses plastic bones for reconstruction of extremities.

The NUH Tissue Bank Protocol involves using gamma irradiation at a dose of 25 kilograys for sterilisation of both deep-frozen and lyophilised bone allografts and for inactivation of the HCV virus.[13]

The NUH Tissue Bank aims to reduce the complication rate by restricting the transplantation of musculoskeletal allografts to surgeons who are experienced with the use of such allografts. Care is taken to ensure that surgeons transplanting these grafts are familiar with the meticulous preparation techniques required and employ prophylactic antibiotics to reduce the infection rate. The overall complication rate in our series is small—2.2 % (19 of 854 recipients). Infection is the most common complication encountered—11 out of 19 cases. It is also the most common complication reported by several other authors including Lord et al.[14] (11.7%), Mankin et al.[15] (13.2%), and Tomford et al.[16] (5%). The actual complication rate varies depending on the type of transplantation performed. Although our complication rate of 6.8% for posterior spinal fusions is relatively low, that for massive bone reconstruction in limb-salvage surgery for bone malignancy is as high as 10.5%. Ortiz-Cruz et al.[17] reported a much higher complication rate of 24% in 104 cases, whereas Fox et al.[18] reported a complication rate of 39% in 137 cases for similar limb-salvage surgery using massive bone allograft reconstruction.

CONCLUSION

The NUH Tissue Bank is a high-quality tissue bank accredited with ISO 9001 certification. It is the national bone bank and provides high-quality musculoskeletal tissue allografts to all hospitals in Singapore. Up to June 2003, 854 bone and soft-tissue transplantations have been performed using tissue grafts procured and processed by the bank. The complication rate encountered in this series is small.

ACKNOWLEDGEMENTS

The author thanks the National University of Singapore for providing the research grant, RP 880334 'Use of allograft for bridging large bone defects', and the Totaliser Board for granting S$239 965 for the project 'Bone and ligament allograft transplantation in Orthopaedic Surgery', as well as Ms Jamaliah Baharim, Mr Chris Lee Choon Wei and Dr Wang Lihui for rendering secretarial assistance and Mr Tan Boon Kiat for providing the photographs.

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