The Usage of Tissue Allograft in Thailand

YOUNGYUDH VAJARADUL, M.D., CHAROEN CHOTIGAVANICH, M.D., PRAMOTE DUMAVIBHAT, M.D., DHAIVADEE DULAYAJINDA, M.D., CHOOKIAT ASAVANICH, M.D., SOMMART KEOROCHANA, M.D., PRAKIT THIENBOON, M.D., CHOOPONGSE SENCHANTICHAI, M.D.

Bangkok Biomaterial Center, Pan-Asiatic Tissue Bank Organization.

Faculty of Medicine Siriraj Hospital, Bangkok, Thailand.

ABSTRACT: This is an initial report of tissue allograft transplantation from Bangkok Biomaterial Center. During December 1984 to May 1987, the tissue allograft transplantation was instituted in 191 cases, (102 male and 89 female). There were bone allograft, dura mater, fascia lata, amniotic membrane and cornea. The methods of procurement of the tissue allograft are lyophilization, deep freezing and fresh preservation. For lyophilization is to wash and rinse by biofiltered water and irradiation with 2.5 M rad after 72 hours lyophilization. The result yielded satisfactorily with 2.6 per cent complication occurred only from bone allograft transplantation.

The usage of tissue allografts in surgery now adays, is very well documented with excellent result.[1-14] The follow up of numerous patients who underwent tissue allografts transplantation were satisfactory. There is no convincing evidence to show contraindication for the implimentation of the tissue allografts. The cost for running tissue bank is not high and finally the cost of those tissues will be minimal, comparing with that of the commercially available or the artificially made implant substituting the allografts.

The Department of Orthopaedic Surgery, Faculty of Medicine, Siriraj Hospital, Mahidol University started the project of processing the lyophilized bone allograft at the beginning of 1979 and in June of that year the first operation using human lyophilized bone allograft was performed. In 1980 the research grant from Mahidol University was given to the investigators and finally, there were 70 patients having preserved lyophilized bone, fascia lata transplantation with 7.15 per cent failure rate reported in the 8th WPOA meeting in Bangkok on 29th November 1985.[14] Prior to that report this project received award from the National Research Council of Thailand. The Faculty of Medicine Siriraj Hospital established the tissue bank on 6th December 1984, which is named "The Bangkok Biomaterial Center", under the Royal Patronage of Her Royal Highness Princess Galyanivadhana. There are excutive, steering and condultative committees from various departments and various hospitals and institutes. At the very beginning the sterilization of the tissue allograft was carried out by ethylene oxide gas. After the establishment of Bangkok Biomaterial Center, and up to the present time sterilization technique was changed, utilizing 2.5 M rads irradiation of Gammatron Company.

In Thailand there are 4 methods to procure bone allografts; 1. deep freezing and sterile preserved, 2. lyophilization or freezed dried method, 3. wet preservation of bone (e.g. Cloward operation), 4. chemical dried preserving method, the so called A.A.A. bones. The common soft tissue allografts, which has been using in Thailand are lyophilized fascia lata and dura mater. Both tissues are widely used in ophthalmology and general surgery for retinal detachment and hernia repair.

Amniotic membrane is the new tissue allograft that comes into tissue banking project for the need of biological dressing in burn patient. At the beginning, fresh aminiotic membrane was used but the problems of fresh preservation for sterility and amniotic tissue became autolysis. The freezed dried amniotic membrane is therefore more convenient in the utilization and storage. The recent activity on fresh cornea graft is to preserve its liability, for keratoplasty.

To procure the freezed dired tissue allograft, the tissue, including bone, fascia, tendon and dura mater, will be obtained from newly dead bodies (within 24 hours) who had no previous history of communicable disease, malignancy, blood diseases, infection or having received any radioactive substances and the age should not be more than 50 years. (Table 1)

MATERIALS AND METHODS

One hundred and ninety one patients including 102 male and 89 female, age ranged 1-86

TABLE 1
Tissue Allegraft Procured in Thailand.

1. Bone
   - deep freezed – sterile preserved
   - freezed dried — gas sterilization / irradiation
   - wet preserved:– skull from craniotomy for cloward operation
   - – chemical dried preserved (A.A.A.)
2. Soft Tissues
   - fascia lata — fresh preserved / freezed dried
   - dura
     - freezed dried
   - amniotic membrane
     - fresh preserved
     - freezed dried
   - cornea graft (fresh preserved)

TABLE 2
Age and Sex of Allograft Patients. (Dec. 16, 1986 – May 18, 1987).

Age (year)	Male	Female	Total
0 – 10	11	10	21
11 – 20	17	17	34
21 – 30	18	12	30
31 – 40	18	10	28
41 – 50	16	14	30
51 – 60	13	14	27
61 – 70	7	9	16
71 – 80	2	2	4
81 – 90	–	1	1
Total	102	89	191
Average age (x)	34.07	36.12	35.03
Standard deviation (SD)	18.51	20.68	19.56

The t-test shows no significant different between male and female average age ($p > .05$)

years, with average age of 35 years, received various kinds of graft. (Table 2)

The follow up time was 1-21 months. The segmental freezed dried bone allograft were used in 129 patients, (65 male and 64 female). In 2 male patients both segmental and chip bone allograft were used. The application of soft tissue allografts were freezed dried dura mater, freezed dried fascia lata, freezed dried amniotic membrane and fresh preserved cornea. There were 64 patients 39 male and 25 female, received those soft tissue allograft. (Table 3)

TABLE 3
Type of Tissue Allograft.

Type of allograft	Male	Female	Total
Bone			
– freeze dried chip allograft	47	55	102
– freeze dried Cloward allograft	14	5	19
– segmental allograft			
– deep freezed	2	2	4
– freezed dried	–	1	1
– chemical dried preserve (A.A.A.)	2	1	3
Freeze dried dura mater	7	3	10
Freeze dried fascia lata	9	14	23
Freeze dried amniotic membrane	21	8	29
Cornea	2	–	2
Total	104*	89	193*

*2 male patients being used both segmental and chip bone allograft

Evaluation of the post operative patients consisted of both clinical and laboratory assessment. The laboratory evaluations were of two blood routine examinations, namely CBC and ESR, and x-ray examination. CBC and ESR would be achieved preoperatively and post-operatively, every week for 4 consecutive weeks and there after the CBC and ESR study would be carried out monthly.

There were 21 patients with the diagnosis of cervical spondylosis who underwent anterior spinal fusion, 19 cases had CLOWARD operation and 2 cases had ROBINSON operation. The freezed dried bone chip allograft were mostly used in posterior spinal fusion, for fractures, spinal stenosis with instability, ankylosing spondylitis, scoliosis, old unstable fracture spine, kyphosis, and spondylolisthesis. The highest benefit from using freezed dried bone allograft is in the scoliosis patient undergoing posterior spinal fusion who requires considerable amount of bone graft and needs no additional incision to obtain the bone. (Table 4)

The other common application of freezed dried bone allograft was to fill the gap due to bone loss in upper extremity fractures. There were 6

TABLE 4
Operative Patient Using Freezed Dired Bone Allograft.

No.	Diagnosis	Operation	Male	Female	Total
	Spine				
1.	Cervical spine	Robinson anterior spinal fusion	1	1	2
	(C-spondylosis)	Cloward operation	14	5	19
2.	Fracture of body T_{12} with complete cord injury	Total laminectomy T_{12} and posterior spinal fusion	2	1	3
3.	Old fracture lumbar spine	Posterior spinal fusion	3	2	5
4.	Ankylosing spondylitis	Total laminectomy & posterior spinal fusion	1	–	1
5.	Spinal stenosis (L-S)	Total laminectomy and posterior spinal fusion (freeze dried bone allo-graft + autograft)	4	4	8
6.	Idiopathic scloiosis	Posterior spinal fusion			
		Luque instrumentation	1	9	10
		Harrington rod – instrumentation	1	3	4
		Zilke instrumentation	–	1	1
7.	Kyphosis $T_3 - T_5$	Posterior spinal fusion	1	–	1
8.	L-spondylosis	Posterior spinal fusion	–	2	2
9.	Spondylolisthesis	Total laminectomy and posterior spinal fusion	1	10	11
	Total		29	38	67

TABLE 5
Operative Patient Using Freezed Dried Bone Allograft.

No.	Diagnosis	Operation	Male	Female	Total
	Upper extremity				
1.	Non-union fracture humerus	ORIF + freeze dried bone allograft	1	1	2
2.	Mal-union Colles' fracture	Corrective osteotomy + freeze dried allo-graft	1	5	6
3.	Impacted fracture neck radius	Disimpaction + freeze dried bone allograft	–	1	1
4.	Osteolytic lesion humerus	Curettage & freeze dried bone allograft	1	–	1
5.	Delayed fracture left forearm bones	ORIF + freeze dried bone allograft	1	–	1
6.	Crush injury left hand	ORIF + freeze dried bone allograft	1	–	1
	Total		5	7	12

ORIF = Open reduction internal fixation

impacted fractures of the lower end of radius (Colles' fracture) underwent surgical correction and needed allograft to pack into the gap in order to gain radial length. (Table 5)

There were 5 cases of loosening of total endoprosthesis (MUELLER type) required revision and supplemented with bone graft. Segmental freezed dried allograft was implanted in one instance, the other 4 patients had been placed with chip bone allografts following the insertion of new prosthesis. (Table 6)

There were 32 cases of lower extremity affection underwent various kinds of surgical intervention requiring implementation of bone grafting

TABLE 6
Operative Patient Using Freeze Dried Bone Allograft.

No.	Diagnosis	Operation	Male	Female	Total
	Hip				
1.	Total hip prosthesis loosening	Revision + freeze dried bone allo-graft (segmental bone allograft)	3	2	5
		Arthrodesis	1	–	1
	Total		4	2	6
	Lower extremity				
2.	Fracture femur with bone loss				
	– supracondylar fracture	ORIF + freeze dried bone allograft	3	1	4
	– trochanteric fracture	ORIF + freeze dried bone allograft	2	1	3
	– shaft fracture	OIRF + freeze dried bone allograft	1	–	1
3.	Shortening of femoral shaft due to post chronic osteomyelitis	Bone lengthening and freeze dried bone allograft	1	1	2
		External fixator and freeze dried bone allograft	1	–	1
4.	Non-union lower end femur	Replating and bone grafting	–	1*	1
5.	T.B. knee joint	Arthrodesis + freeze dried bone allograft	–	1	1
6.	Old fracture patella and fracture condyle of femur	ORIF of Patellar fascial arthroplasty (freeze dried dura mater allograft)	1	–	1
7.	Traumatic arthritis (LT) knee	Arthrodesis + freeze dried bone allograft	–	1	1
8.	Genu varum knee	Corrective osteotomy	1	–	1
9.	Chronic osteomyelitis tibia with non-union	Curettage filling bone defect with freeze dried bone allograft	1	–	1
10.	Old fracture tibia				
	– with non-union	– ORIF + freeze dried bone allograft	7	1	8
	– with bone loss	– ORIF + freeze dried bone allograft	4	3	7
	Total		22	10	32

ORIF = Open reduction internal fixation.
*Dead within 24 hours postoperatively

procedures. Freeze dried bone allografts were used in conjunction with various types of surgical management of bone tumors. (Table 7) There were 11 cases of bone tumors and tumor like lesion namely giant cell tumor 5 cases, osteosarcoma 3 cases, and fibrous dysplasia 3 cases. Chip bone graft was used to pack the cavity and segmental graft was utilized to bridge the defect following the resection of the tumor. Freezed dried dura mater allograft was used as an interposing material for patellofemoral arthroplasty following the reduction of patellar fracture. (Table 8)

Dura mater was used to repair abdominal hernia in 8 cases. One child underwent arthroplasty of the hip joint for old dislocation, in which dura mater was also utilized as an interposing material. Freeze dried fascia lata allograft were used in retinal detachment and congenital ptosis in 23 cases, 9 male and 14 female. (Table 8) For biological dressing, the freezed dried amniotic membrane were used in 22 burn patients. The same material was also utilized in various patients sufferin from numbers of several problems, namely skin defect due to compound fracture, after resection of giant all tumor, contracture of little finger who required Z plasty for which the graft was used to replace. Two cases of old tuberculosis of the hip joint, in whom arthroplasty was performed by using 50 layers of freezed dried amniotic membrane. (Table 9)

Fresh preserved cornea was used in 2 instances for keratoplasty; one patient developed

TABLE 7
Operative Patient Using Freeze Dried Bone Allograft.

No.	Diagnosis	Operation	Male	Female	Total
	Bone tumor & tumor like lesion				
1.	Giant cell tumor				
	– middle finger	Resection + freeze dried bone allograft	–	1	1
	– femur	ORIF + freeze dried bone allograft	–	1	1
	– tibia	ORIF + freeze dried bone allograft	1	2	3
2.	Osteosarcoma femur	ORIF + segmental freeze dried bone allograft + autograft	–	1	1
	Osteosarcoma tibia	Resection arthrodesis of knee (femoral turn down) + freeze dried bone allograft	1	1	2
3.	Fibrous dysplasia				
	– humerus	Curettage + freeze dried bone allograft	1	–	1
	– hip	Curettage + freeze dried bone allograft	–	1	1
	– tibia	Curettage + freeze dried bone allograft	1	–	1
	Total		4	7	11

ORIF = Open reduction internal fixation.

TABLE 8
Operative Patient Using Freeze Dried Dura Mater, and Fascia Lata Allograft.

No.	Diagnosis	Operation	Male	Female	Total
1.	Dura				
	– hernia (indirect H., incisional H., umbilical H., gastrochisis)	Repair with freeze dried dura mater allograft	5	3	8
	– dislocation hip	Open reduction with Chiari osteotomy and 1 K.W. fixation + freeze dried dura mater allograft arthroplasty in acetabulum	1	–	1
	Total		6	3	9
2.	Fascia				
	– retinal detachment	Buckling operation with freeze dried fascia lata allograft	4	1	5
	– congenital ptosis	Sling operation with freeze dried fascia lata allograft	5	13	18
	Total		9	14	23

H = hernia
K.W = Kirschner wire

homograft reaction post therapeutic keratoplasty for Moorevis ulcer, and perforation hypopyon corneal ulcer in another case. (Table 10)

RESULT AND DISCUSSION

The result of using tissue allograft was satisfactory. Only 2 cases were complicated with infection, 2 cases with bone graft breakage. One patient died postoperatively due to unrelated condition. The patient died within 24 hours postoperatively. The diagnosis of this patient was non-union of lower end of femur. The operation was replating and the application bone graft. (Table 11)

The advantage of using tissue allograft is to reduce morbidity of the patient,[6] especially in children and in old age individual whose autograft are not available or due to poor quality of bone stock.

TABLE 9
Operative Patient Using Aminotic Membrane.

No.	Diagnosis	Operation	Male	Female	Total
1.	Amnion				
	Burn				
	– flame	Dressing with freeze dried amniotic membrane	8	3	11
	– scald	Dressing with freeze dried amniotic membrane	6	3	9
	– electric	Dressing with freeze dried amniotic membrane	1	–	1
	– chemical	Dressing with freeze dried amniotic membrane	–	1	1
	Total		15	7	22
2.	Skin defect				
	– chronic skin loss compound fracture	Dressing at donor site (partial thickness skin graft) with freeze dried amniotic membrane	3	–	3
	– Giant cell tumor of leg with chronic skin defect	Resection of tumor mass + dressing with freeze dried amniotic membrane	1	–	1
	– flexion contracture 5th finger with chronic skin defect	Dressing with freeze dried amniotic membrane	1	–	1
3.	Joint interposing				
	– Old T.B. hip join	Arthroplasty with freeze dried amniotic membrane (50 layers)	1	1	2
	Total		6	1	7

TABLE 10
Operative Patient Using Preserved Cornea Graft.

No.	Diagnosis	Operation	Male	Female	Total
	Cornea (fresh preserve)				
	– homograft reaction post therapeutic keratoplasty-Moorevis ulcer	Penetrating keratoplasty with cornea allograft	1	–	1
	– perforation hypopyon corneal ulcer	Penetrating keratoplasty with cornea allograft	1	–	1
	Total		2	–	2

TABLE 11
Complications.

	Cases
1. Infection (posterior spinal fusion in spondylolisthesis)	2
2. Bone graft breakage	2
3. Dead	1
Total	5

Freezed dried tissue allograft contained a fair amount of moisture. In bone allograft the moisture value was approximately 5 per cent w/w.[14]

The procedure of tissue allograft preparation is economical as no expensive solvents are used to remove the fat and blood cells from the bone. They are simply washed out with biofiltered water and distalled water at the last rinsing. Freezed drying in lyophilizer is accomplished for 72 hours followed by packaging and 2.5 M. rad irradiation. Our results are satisfactory. There are only 5 complicated bone allograft transplanted cases out of

191 cases (2.6%).

This method of the procurement of tissue allograft may be appropriate technique in Thailand.

To use the lyophilized bone allograft, the recommended method is not to reconstitute or rehydrate because the authors believe that the reconstitution of bone graft will be accomplhished themselves by the recepient body fluid. However, some studies suggested to mix the bone allografts with bone marrow substance.[3] The best quality bone graft is cortico-cancellous type in a massive one because it can bear weight better than pure concellus bone graft. The result of using cortico-cancellous chip bone allograft is satisfactory and having low rate of complication. The method of procurement of the tissue allograft by washing and rinsing with biofiltered water without using any solvents of chemical substance yielded satisfactory result as in our 70 cases reported in the 8th WPOA congress in 1985.[14]

We started using fresh deep freezing bone allograft since 10th May 1987. Most of the reported cases utilizing with this massive fresh deep freezed bone allograft had shown good result. There were 8 cases that needed segmental bone allografts, all gave satisfactory results. There was one case of 19 year old male patient with osteosarcoma at the left proximal part of tibia. The operation was enbloc resection of upper end of tibia, and replaced by intercalary massive bone allograft with the length of 13.5 cm., fixed by plating. Further follow up is needed for the final out come. Another massive lyophilized bone allografts were used to feel the gap for segmental bone loss. There were 2 cases in this study. One case was a 16 years old patient with chronic osteomyelitis of the femur after compound fracture. The other patient underwent reconstitution of femoral neck in the revision of total hip prosthesis, and required a segmental bone graft to replace it.

The trend of utilizing segmental deep freezing boe allograft is now increasing to save the limb, and we are in the position of providing the graft upon the request.

Chip bone graft was used in several purposes with a high successful rate. They were used to fill the defect in non-union for osteogenesis, to obliterate the bony cavity following curettage in tumor or tumor like lesion of the bone. Chip bone graft exhibited satisfactory result in this initial study. The healing time was rapid. New bone formation incorporated with the graft was evident roentgenographically as early as 4 weeks, which occurred in the majority of children.

The first soft tissue allograft transplantation in Bangkok Biomaterial Center was achieved on December 6, 1984. At the beginning, dura mater and fascia lata were procured and preserved by mean of lyophilization and ethylene oxide gas sterilization. There after the sterilization method was changed to irradiation. The result of soft tissue allograft transplantation was also quite satisfactory, there was no serious complication in our early work. It is quite evident that cryobiological technique plays a role in biocompatibility. The soft tissue allograft itself, however could be completely absorbed at certain period of time after transplantation. In the contrary it is hard to find complete bone resorption in our cases, since the bone allograft is too hard to be demineralized and absorbed. Furthermore the cortical bone allograft transplantation will provide stability in conjunction with internalfixation in certain instances which had entertained the successful result. The lyophilized amniotic membrane is now proved from the result in this study that it could substitute the fresh prepared amniotic membrane. It is of convenience and sterility for usage. The fresh cornea preservation and skin culture are now in the process of development for future clinical application.

CONCLUSION

The clinical result of using tissue allograft in 191 patients with the average age of 35 years old, during December 1984 to May 1987, 1-21 months of follow up period, were presented from the Bangkok Biomaterial Center. The results were satisfactory. There was only 2.6 per cent of complications which were all of bone allograft transplantation. There was none from soft tissue allograft. The method of tissue allograft procurement and sterilization could be an appropriate technology for Thailand and probably could be utilized to other neighbour countries due to its simplicity, economical, and good result.

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