A New Recommendation for Handling and Care of Human Allogenic Bone Graft (at Siriraj Hospital) to Reduce Post-Operative Complication

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Background: *Bangkok Biomaterial Center is the only allogenic bone bank in Thailand, supplying the bones to all medical centers. From a report, a certain number of post-operative complications were found.*

Objective: *To compare pre-protocol revision and post-protocol revision success rates and complications associated with the use of allogenic bone grafts relative to perioperative handling and care of allogenic bone.*

Material and Method: *This retrospective comparative study was conducted at the Bangkok Biomaterial Center, Faculty of Medicine Siriraj Hospital, Mahidol University. All registration forms and surgical follow-up reports relating to the use allogenic bones procured from our bone bank between 2005 and 2015 were reviewed. New recommendations for the use of our allogenic bones were established in 2009. Results and complications after allogenic bone transplantation between 2005 and 2008 were compared with results and complications after transplantation to a new protocol between 2009 and 2015. Descriptive analysis and analysis of variance were used to evaluate the data.*

Results: *Data of 825 patients who underwent deep frozen allogenic bone transplantation and 1,541 patients who underwent freeze-dried allogenic bone transplantation were retrospectively reviewed. Overall, the complication rate was reduced from 14.83% in the pre-protocol revision period to 5.15% in the period after the new recommendations for perioperative graft handling were established and implemented.*

Conclusion: *New recommendations for the handling and care of allogenic bone during the perioperative period significantly reduced post-operative complications in patients who received transplantation with deep frozen allogenic bone. The infection rate in patients who received allogenic bone graft was very low.*

Keywords: *Bone, Bone banks, Allogenic bone, Bone transplantation*

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Bangkok Biomaterial Center, under the patronage of Her Royal Highness Princess Galyani Vadhana Krom Luang Naradhiwas Rajanagarindra, Department of Orthopaedic Surgery, Faculty of Medicine Siriraj Hospital, Mahidol University, was established in 1984[1]. Bangkok Biomaterial Center is Thailand's only source for allogenic bone. Between 1984 and 2015, there were 526 deceased donors (436 males and 90 females, with an age range of 10 to 80 years) and 8,556 live donors (1,505 males and 7,051 females, with an age range of 11 to 88 years). One thousand six hundred thirty four pieces of deep frozen allogenic bone and 39,106 pieces of freeze-dried allogenic bone were recovered and banked. Allogenic bone grafts from these stored specimens were used in 6,302 patients (3,119 males and 3,183 females, with an age range of 5 to 86 years). We reported on use and success rates relative to allogenic bone grafts procured from the Bangkok Biomaterial Center in the year 2000[1]. The success rate in that report was 85% with complications found in 15% of transplant patients. Infection rate was 5.1% in patients who received deep frozen allogenic bone grafting, which was comparable to results reported in other studies[2-4]. Ongoing review of procedures and performance is necessary to ensure patient safety and improved outcomes. Accordingly, the objective of this study was to compare pre-protocol revision (prior to 2009) and post-protocol revision (2009 and later) success rates and complications associated with the use of allogenic bone grafts relative to perioperative handling and care of allogenic bone.

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Material and Method

Registration forms and reports describing postoperative results of allogenic bone transplantation and complications between 2005 and 2015 were retrospectively reviewed. Patient follow-up period ranged from three months to 5.5 years. The results of bone union, surgery-related complications, and feedback reports from surgeons were studied. Subgroup analysis was performed, comparing between the deep frozen allogenic bone group and the freeze-dried allogenic bone group. Descriptive analysis and analysis of variance (ANOVA) were used to evaluate the data.

There were 852 patients (415 males and 437 females, with an age range of 4 to 81 years) who underwent transplantation with deep frozen allogenic bone, and 1,541 patients (760 males and 781 females, with an age range of 6 to 84 years) who underwent freeze-dried allogenic bone transplantation in this study. The process for allogenic bone graft recovery and post-operative recommendations given to users by the Bangkok Biomaterial Center were revised in 2008 and implemented in 2009. For purposes of evaluating the effect of these changes, postoperative data collected from 2005 to 2008 were compared with postoperative data collected from 2009 to 2015.

There are two different types of allogenic bone available at our bone bank, 1) deep frozen allogenic bone (Fig. 1), and 2) freeze-dried allogenic bone (Fig. 2). The steps of bone recovery that we used between 2005 and 2015 are presented in Table 1 and 2[5]. During step 2 of allogenic bone recovery, if blood tests show positive for blood transmitted diseases, all collected bones from that donor are rejected. Rejected allogenic bones and soft tissue undergo cremation according to the religious traditions of the donor. During the bone storage process, a certain number of bones from each donor are sampled for microbiological study at the third and sixth month after recovery and all cultures must be negative. If a culture is positive, all bones from that donor are rejected and cremated. Between 2005 and 2015, there were 64 deceased donors and 5,999 live donors who satisfied the donor criteria and their recovered bones were banked for allogenic bone transplantation.

When a surgeon requires allogenic bone from our center for reconstructive surgery, the request is made by telephone, e-mail, or letter. The request is then reviewed and allogenic bone selection is performed according to size, side, and amount of bone required. The allogenic bone specimen that most closely matches the requested characteristics is then delivered or picked up by the division, department, or faculty of the surgeon who requested the bone. Allogenic bones must be used as soon as possible on the day of delivery or no later than the day after delivery. The new recommendations for perioperative graft handling were implemented in 2009 and they are described in Table 3. The revised recommendations are sent directly to the surgeon via e-mail and/or a hard copy is enclosed with the allogenic bone delivery. In some conditions or settings, photographs of the selected allogenic bones are also sent to the requesting surgeon via e-mail. Both deep frozen and freeze-dried allogenic bone grafting were followed up similarly.

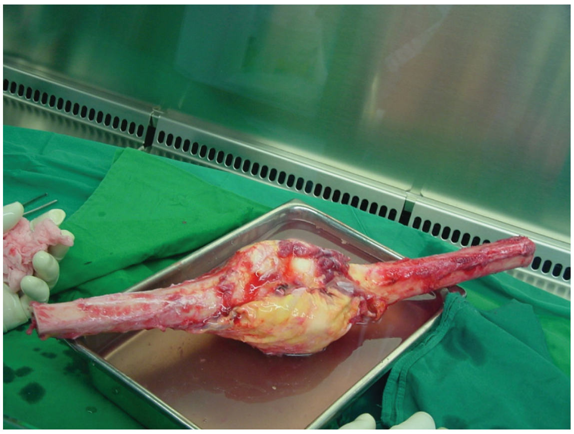

Fig. 1 Deep frozen allogenic bone.

Fig. 2 Freeze-dry allogenic bone in the gamma sterilized package.

Table 1. Steps of deep frozen allogenic bone recovery (2005 to 2015)

1.	Collecting bone allograft and venous blood for possible blood transmitted diseases from the deceased donors under sterile techniques
2.	Temporary preservation of the grafts in the quarantine chambers at -70 degrees Celsius
3.	If the blood tests revealed negative results the bone allograft recovery was continued
4.	Cleansing out the bone marrow and unnecessary soft tissues
5.	Rapid deep frozen at -80 degrees Celsius
6.	Radiation sterilization at 25 KGy
7.	X-ray, tagging and preservationthe recovered grafts at -80 degrees Celsius

Table 2. Steps of freeze-dry allogenic bone recover (2005 to 2015)

1.	Collecting the chip allogenic bone grafts under sterile technique
2.	Venous blood was also collected from the donors for possible blood transmitted diseases
3.	Temporary preservation of the grafts in the quarantine chambers at -70 degrees Celsius
4.	If the blood tests revealed negative results the bone allograft recovery was continued under sterile techniques
5.	Mechanical cleansing and ultrasonic cleansing in a sterile chamber
6.	Cutting the graft in to about 0.5x1.0x2.0 cm pieces
7.	Freeze-drying under sterile techniquesfor3 days
8.	Vacuum packaging
9.	Radiation sterilization at 25 KGy
10.	Tagging and preserving at room temperature

Table 3. Recommendation for clinical use (2009 to 2015)

1.	Slow thawing to room temperature before the surgery
2.	No direct contact between bone forceps and the deep frozen allogenic bone graft during the surgery, some sterile cushion must be used to protect the grafts
3.	Intramedullary fixation should be the first choice of osteosynthesis
4.	When plate and screws are used for osteosynthesis, minimal number of screws for fixation must be use and lock plate and screws are preferred
5.	Autogenic bone grafting must be added at the junction between host bone and allogenic bone allograft at the first surgery or mixed with freeze-dry allogenic bone graft to increase the volume of bone replacement
6.	For freeze-dry allogenic bone graft manual packing was recommended and hammering on freeze-dry allogenic bone graft should be avoided
7.	Prolong use of antibiotics for at least 4 weeks after the surgery should be carried out in large deep frozen allogenic bone grafting
8.	External immobilization should be carried out until radiographic bone union was observed

After transplantation of the bone graft, the surgeon must fill out and submit the allogenic bone graft registration forms[6]. Staff from our center maintained close telephone and e-mail contact with the surgeon to monitor the results of bone incorporation and complications, and to elicit feedback that is used for ongoing process improvement. Normally scheduled center-to-surgeon contact is every three months during the first year and every six months until the graft incorporation was found, which ranged between two and five years in the deep frozen allogenic bone group and six to twelve months in the freeze-dried allogenic bone group.

Results

Data relative to allogenic bone use and characteristics, transplant recipient characteristics, and post-transplant complications are shown in Table 4 and 5. Deep frozen allogenic bones were used in 852 patients during the study period after the new recommendations were delivered in 2009. Of those, 410 patients (48%) had adult reconstructive surgery, 290 patients (34%) underwent bone tumor surgery, and the remaining 152 patients (18%) had other orthopedic conditions. Complications were found in 81 patients or 9.51% (Table 4). Freeze-dried allogenic bones were used in 1,541 patients, as follows, spinal surgery (840 patients, 54%), bone tumor surgery (324 patients, 21%), pediatric orthopedic surgery (180 patients, 12%), and other orthopedic operations (197 patients, 13%). Complications of the use of freeze-dried allogenic bone were found in 30 patients or 1.96% (Table 5).

Complications were significantly higher before the new recommendations were implemented in 2009 than in the period after implementation (14.83% down to 5.15%, respectively) (Table 4). Allogenic bone fractures and implant failures were clearly reduced, which might reflect better allogenic bone handling and osteosynthetic techniques (Table 6). Infection rate in the deep frozen allogenic bone group decreased from 0.7 to 0.4%, but the difference did not achieve statistical significance. There was no significant reduction in the complication rate in the freeze-dried allogenic bone group after the new recommendations were implemented (Table 5). No infections were reported in the freeze-dried allogenic bone group after the implementation of the new recommendations.

Discussion

The demand for and use of allogenic bone in clinical settings is increasing. New surgical concepts and instrumentation used in the surgical reconstruction of the musculoskeletal system are the main factors influencing this increasing demand for allogenic bone[7]. Paradoxically, donations of allogenic bone are decreasing due to lowered awareness and increasingly complicated and prohibitive laws and regulations. In an effort to reverse this trend, our center has worked

Table 4. Results of the use of deep frozen allogenic bone grafts

Year	Deep frozen bone		Numbers of patients with main complications	Percentage of patients with main complications	*p*-value
	Numbers of the grafts	Numbers of the patients			
2005	111	98	11	13.88	<0.05
2006	106	101	24		
2007	129	122	11		
2008	122	104	13		
Sub total	468	425	59		
2009	76	69	9	5.15	
2010	76	67	4		
2011	42	40	1		
2012	60	55	3		
2013	52	52	1		
2014	80	74	2		
2015	73	70	2		
Sub total	459	427	22		
Total	927	852	81	9.51	

Table 5. Results of the use of freeze-dry allogenic bone grafts

Year	Freeze-dried bone		Numbers of patients with main complications	Percentage of patients with main complications	*p*-value
	Numbers of the grafts	Numbers of the patients			
2005	1,651	145	3	2.12	>0.05
2006	1,519	139	4		
2007	1,041	106	1		
2008	1,085	129	3		
Sub total	5,296	519	11		
2009	1,277	139	1	1.85	
2010	1,194	116	1		
2011	912	129	6		
2012	1,238	137	4		
2013	790	116	3		
2014	1,295	171	2		
2015	1,235	214	2		
Sub total	7,941	1,022	19		
Total	13,237	1,541	30	1.96	

Table 6. Types of complication after using deep frozen and freeze-dry bone allograft

Types of complication	Deep frozen bone (n = 81/852)		Freeze-dry bone (n = 30/1,541)	
	2005 to 2008 (n = 59)	2009 to 2015 (n = 22)	2005 to 2008 (n = 11)	2009 to 2015 (n = 19)
Graft fracture	11	3	-	-
Infection	3	2	2	-
Delayed union	20	9	7	19
Nonunion	8	5	2	-
Implant failure	17	3	-	-

Some patients experience more than one complication

to increase the number of live and deceased donors by increasing community education, expanding hospital affiliations, and collaborating with Buddhist monks and temples. Live donor bone donations from hip fractures and arthroplasty have been the major source of freeze-dried allogenic bone chips. The increasing rate of many serious blood transmitted diseases has reduced the supply of available allogenic bones. This limited supply of screened and disease-free allogenic bone increases the need for and importance of proper bone recovery, handling, and care during the perioperative and postoperative periods.

The handling of allogenic bone during the perioperative period is a sensitive and determining factor. From our previous study, deep frozen bones have about 30% less strength than fresh bones and freeze-dried bones have about 90% less strength than fresh bones(8). Gamma sterilization used during the last step of graft recovery can weaken both organic and inorganic graft structures(9). For these reasons, recovered allogenic bones must be handled with care to reduce the chance of allogenic bone fracture. Deep frozen allogenic bone cannot heal by itself. Creeping substitution of allogenic bone from recipient bed to the allogenic bone fracture site must occur in order for allogenic bone to heal. At the end of that process, the allogenic bone fracture is replaced by new bone. The healing process in allogenic bone fractures is lengthy. Fractured deep frozen allogenic bone usually results in collapse and resorption of the allogenic bone, delayed union, nonunion, and/or implant failure. Screw fixation with a minimal number of screws is recommended in allogenic bone transplantation to lessen the chance of longitudinal graft fracture(10). External immobilization and delayed weight bearing also help to lessen the rate of implant failure. By proactively educating the surgeons who use our allogenic bones, we were able to reduce the failure rate of deep frozen allogenic bone grafts (Table 1 and 3). Education to the surgeons who use our allogenic bone graft should be continued and the recommendations of the use of the allogenic graft should be concerned with minimizing complications.

As compared to infection rates reported from other studies, the 0.04 to 0.07% rates found in our study were rather low(2-4). There are some factors that may have influenced this outcome. First, in our recommendations, we strongly advise surgeons not to use deep frozen allogenic bone grafts in cases with infection or previous infection. Second, if deep frozen bone samples reveal positive bacterial culture, all bones from that donor are immediately excluded and destroyed. Third, we use gamma sterilization at the final step of allogenic bone recovery. Lastly, to minimize post-operative infection, prolonged use of antibiotics for at least four weeks after large deep frozen allogenic bone transplantation is recommended in every patient even though the patients had no infection before the operation(11).

A significant number of patients in both allogenic bone type groups had delayed union or nonunion. Gamma sterilization might destroy some bone morphologic proteins and other cytokines, both of which play a major role in allogenic bone incorporation(12,13). Research is focused on the biological improvement of the allogenic bones provided by our center is ongoing.

The failure rate of freeze-dried allogenic bone grafts was not significantly reduced between periods in this study, even though our recommendations were revised and the surgical technique was improved. To improve the biological quality of our freeze-dried allogenic bone graft specimens and decrease graft failure rate, investigations involving the pre-coating of bones with materials like fibroin of Thai silk worm are currently ongoing(14).

This study has some mentionable limitations, including 1) the retrospective nature of the study,

2) incomplete follow-up after 5.5 years after the operation in most of the patients, 3) limited number of available post-operative radiographs, 4) inadequate details relating to surgical management of complications after allogenic bone transplantation, and 5) feedback from only a small percentage of allogenic bone transplant surgeons.

Conclusion

After the implementation of the new recommendations for perioperative handling and care of allogenic bone, the failure rate of transplanted deep frozen allogenic bone was significantly reduced. Additional studies should be conducted in freeze-dried allogenic bone biology in order to reduce complications and improve clinical outcomes.

What is already known this topic?

Allogenic bone transplantation is a common surgical technique for reconstructive surgery in the musculoskeletal system with acceptable results. However, graft fractures, delayed bone union and implant failure were still reported at a certain rate.

What this study adds?

Better allogenic bone handling and proper fixation by the use of our new recommendation could lessen complications in the use of deep frozen allogenic bone grafting.

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Potential conflicts of interest

None.

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ผลการปรับเปลี่ยนข้อแนะนำการใช้กราฟกระดูกจากมนุษย์เพื่อป้องกันภาวะแทรกซ้อน

สารเนตร์ ไวคกุล, ธัญวรรณ อาศนสุวรรณ์, สหชาติ มาฆะดิลก, จิราภา พลายเล็ก, รัตน์หทัย เมืองธรรม, ยงยุทธ วัชรดุลย์

ภูมิหลัง: *ศูนย์เนื้อเยื่อชีวภาพกรุงเทพ ในพระอุปถัมภ์ กรมหลวงนราธิวาสฯ เป็นสถานที่เดียวที่ให้บริการกราฟกระดูกจากมนุษย์ในการผ่าตัดทางคลินิก พบว่ามีรายงานภาวะแทรกซ้อนจากการใช้กราฟกระดูกนี้ซึ่งควรได้รับการพัฒนา*

วัตถุประสงค์: *เปรียบเทียบการเกิดภาวะแทรกซ้อนโดยรวมจากการใช้กราฟกระดูกจากมนุษย์ระหว่างก่อนและหลังการใช้ข้อแนะนำใหม่ที่พัฒนาขึ้นในการดูแลและจับต้องกราฟกระดูกจากมนุษย์ระหว่างผ่าตัด*

วัสดุและวิธีการ: *เป็นการศึกษาย้อนหลังในรายงานผลการการติดตามการใช้กราฟกระดูกจากมนุษย์ที่ผลิตโดยศูนย์เนื้อเยื่อชีวภาพกรุงเทพฯ ในพระอุปถัมภ์สมเด็จพระเจ้าพี่นางเธอเจ้าฟ้ากัลยาณิวัฒนา กรมหลวงนราธิวาสราชนครินทร์ ภาควิชาศัลยศาสตร์ออร์โธปิดิกส์ คณะแพทยศาสตร์ศิริราชพยาบาล ด้วยข้อแนะนำการดูแลและการใช้กราฟกระดูกจากมนุษย์ได้ปรับปรุงใหม่ใน พ.ศ. 2552 จึงได้นำข้อมูลผลและภาวะแทรกซ้อนจากการใช้กราฟกระดูกก่อนการใช้ข้อแนะนำใหม่ระหว่าง พ.ศ. 2548 และ 2551 เปรียบเทียบกับผลและภาวะแทรกซ้อนจากการผ่าตัดลักษณะเดียวกันหลังการใช้ข้อแนะนำใหม่ระหว่าง พ.ศ. 2552 และ 2558 การวิเคราะห์ผลเปรียบเทียบใช้สถิติชนิดบรรยาย และ analysis of variance*

ผลการศึกษา: *ศึกษารายงานจากผลการผ่าตัดผู้ป่วยโดยการใช้กราฟกระดูกชนิดแช่แข็ง 825 ราย และผลการผ่าตัดโดยใช้กราฟกระดูกชนิดทำให้เย็นจัดและแห้ง 1,541 ราย ภาวะแทรกซ้อนโดยรวมลดลงจากก่อนการใช้ข้อแนะนำใหม่ร้อยละ 14.85 ลงมาเป็นร้อยละ 5.15 หลังการใช้ข้อแนะนำใหม่ เมื่อแยกการวิเคราะห์พบว่าภาวะแทรกซ้อนจากการใช้กราฟกระดูกชนิดทำให้เย็นจัดและแห้งลดลงบ้างแต่ไม่มีความสำคัญทางสถิติ*

สรุป: *การใช้ข้อแนะนำใหม่ที่เน้นการระมัดระวังการดูแลและการใช้กราฟกระดูกจากมนุษย์ระหว่างผ่าตัดมีผลให้ภาวะแทรกซ้อนจากการใช้กราฟกระดูกจากมนุษย์ชนิดแช่แข็งลดลง และพบอัตราการติดเชื้อจากการผ่าตัดต่ำ*