Decreasing Strength of Bone Allograft after Recovery and Preservation

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Bone allograft is a commonly used implant for reconstruction in the orthopedic surgery. The strength of grafts is one of the most important properties.

The study was conducted to find out the effects of the bone allograft recovery and preservation toward the strength of bone in the conditions of 1) being deeply frozen meanwhile having the rapid temperature change, 2) being deeply frozen and having the slow temperature change, 3) being freeze-dried and eventually having the gamma radiation sterilization.

Sixteen fresh similar sized porcine femurs were used as the samples for the strength test. They were divided into 4 groups and each group consisted of four femurs: two right and two left. Group I was the control. The bones in Group II underwent the state of being deeply frozen meanwhile having the rapid temperature change during the preparation. In Group III, the bones underwent the state of being deeply frozen and having the slow temperature change during the preparation. Group IV was the freeze-dried group. Before using the compression load to the subjects, all of them were placed in the moist chamber until their bone temperature remained at room temperature. Then, all the samples were pressed down by the three-point bending, single load named Shimutzu AGB 2000 until the fracture occurred. The compression load was applied to the middle of the bone and to the other two fixed points which were designed at 10 cm away from both sides of the middle point. The load was applied at the rate of 1 mm per second under the ambient temperature of 25 degrees Celsius and 55% humidity. The maximum weights of each group was recorded and compared with the others by using Student-t-test.

The control group was the strongest as its fracture happened at 675.90 $\pm$ 5.11 Kg. The bone strength of the deeply frozen group that had the rapid temperature change was 467.21 $\pm$ 3.02 Kg while the one that had the slow temperature change was 467.30 $\pm$ 2.90 Kg. There was no significant difference in terms of the strength between the bone under the rapid temperature change and the one under the slow temperature change while being prepared. The freeze-dried group yielded the weakest bone strength; the bone was broken at 61.17 $\pm$ 4.21 Kg.

The process of bone graft preparation resulted in weakening the strength of bone for approximately 30% in the deeply frozen condition and approximately 90% in the freeze-dried group. Surgeons should know the changes in the strength of the bone allograft and hand the bone grafts with care. Furthermore, they must select the proper type of bone grafts with proper indication.

Keywords: *Bone banks, Bone transplantation, Transplantation, homologous*

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In the massive allogeneic bone transplantation, the strength of the allograft very helpfully withstands the muscle force, body weight and force of implants. Most processes of the allograft recovery and preservation reduce the graft strength down to a certain level[1-3]. The different steps of graft preparation and preservation result in the different final outcomes of the graft strength. The weakened bone allograft can cause graft fractures after the operation as the bone graft cannot heal by itself. Handling the allograft improperly also produces the graft fractures. Bangkok Biomaterial Center under the patronage of Princess Kalayaniwatana, Department of Orthopedic Surgery, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand has supplied more than 10,000 pieces of bone allograft to many orthopedic centers all over Thailand since 1978. The users reported that 3 to 8% of fractures were found after the bone allograft was used in surgeries. The majority of the fractured grafts occurred in the lower limbs where the grafts were under a certain level of load. The strength of the grafts could be a major factor of bone graft fractures. Thus, this study focused on finding out the changes of the allogeneic massive bone graft strength after the process of graft recovery and preservation.

Material and Method

Sixteen male porcine femurs weighing 100 to 150 Kg were used in the study. They were randomly divided into 4 groups. Four femurs were in each group: two right and two left. Group I, the freshly wet bones, was the control group. The freshly wet bones were under the three-point bending, continuously single load at the displacement rate of 1 mm per second until the fracture occurred by using Shimatzu Universal Testing Machine AGB 2000. The tests were carried out in the same environment at the ambient temperature of 25 degrees Celsius and in the humidity of 55%. The distance between the fixed Point One and Two was 20 cm and the other compression point was in the middle of the distance of the first two fixed points. The amount of weights at the fracture occurrence point of each bone was recorded. The mean and standard deviation were calculated and used as the referent data.

The bones in Group II were cleaned in the normal saline solution and frozen at -70 degrees Celsius for 7 days. Then, the bones were immediately incubated in Jouan Incubator at 56 degrees Celsius in the phosphate buffer solution pH 7.0 after they were removed from the freezer. The incubation took 4 hours to inhibit the possible bacterial and viral contamination in the bones. After the incubation, the bones were repacked under the sterile technique and preserved in dry ice. The bones underwent the radiation sterilization by using the gamma ray at the absorptive dose of 35 KGy meanwhile the bones were cooled down by dry ice. After the sterilization, the bones were preserved at -70 degrees Celsius for 3 months. Then, the bones were removed from the freezer and placed in the moist chamber until the temperature of the bones was equal to room temperature that was 25 degrees Celsius. The bones were tested by the three-point bending, single load like Group I. The amount of weights at the fracture occurrence point of each bone was recorded and the data were also collected and calculated like Group I.

The bones in Group III were cleaned in the normal saline solution and frozen at -70 degrees Celsius for 7 days. Then, the bones were removed from the freezer and left until their temperatures were equal to room temperature. The bones, consequently, were incubated in Jouan Incubator at 56 degrees Celsius in the phosphate buffer solution pH 7.0 for 4 hours like Group II. After the incubation, the bones were repacked under the sterile technique and preserved in dry ice. The bones underwent the radiation sterilization by using the gamma ray at the absorptive dose of 35 KGy meanwhile the bones were cooled down by dry ice. After the sterilization, the bones were preserved at -70 degrees Celsius for 3 months. Then, the bones were tested by the three-point bending, single load at room temperature like Group II. The amount of weights at the fracture occurrence point of each bone was recorded and the data were also collected and calculated like Group I.

The bones in Group IV were cleaned in the normal saline solution and frozen at -70 degrees Celsius for 7 days. Then, the bones were removed from the freezer and left until their temperature were equal to room temperature. The bones, consequently, were incubated in Jouan Incubator at 56 degrees Celsius in the phosphate buffer solution pH 7.0 for 4 hours like Group III. After the incubation, the bones underwent the freeze-dried treatment by using Lyovac GT 2 for 72 hours. After the freeze-dried process, the bones were firstly repacked and sealed under the vacuum condition by Henkovac Vacuum Pack, and then sterilized by the gamma radiation at the absorptive dose of 35 KGy. This sterile technique took 12 hours. After the sterilization, the bones were tested by the three-point bending load at room temperature like Group I. The amount of weights at the fracture

occurrence point of each bone was recorded and the data were also collected and calculated like Group I.

Results

The load at the fracture occurrence point of the bones in Group II, *i.e.* deeply frozen bone in the state of rapidly changing the bone temperature was 467.21 ± 3.02 Kg. In Group III, the fracture load amount of deeply frozen bone under the condition of slowly changing the bone temperature was 467.30 ± 2.90 Kg. The load amount causing the fractures in the bones of Group IV, *i.e.* freeze-dried bone was 61.17 ± 4.21 Kg. The weight of this group was significantly the lowest. It was only 10% of the amount at the fracture occurrence point of the control group, or Group I (fresh bone) that was 675.90 ± 5.11 Kg (Table 1). The average weights at the fracture occurrence points in Group II and III were one-third of Group I. However, there was no significant difference of the weights between Group II and Group III that were 467.21 ± 3.02 Kg and 16 Kg respectively.

Discussion

The aims of studying the bone allograft recovery and preservation were to get rid of possible bacterial and viral contamination and also to minimize antigenicity of the bone grafts. However, most steps of the graft recovery and preservation could weaken the bone grafts. The deeply frozen process produced micro ice crystals that caused micro-cracks in the bones[4]. The repetition of freezing protocol also produced more micro-cracks that could highly weaken the bones[5]. While incubating the samples at 56 degrees Celsius to destroy possible bacterial and viral contamination, the autolysis of all cell types also happened. This autolysis weakened the non-mineral parts of the bone. Finally, the radiation sterilization by gamma ray directly weakened the grafts[6]. Through the process of treating freeze-dried bones, water in the bones was mostly removed. This process significantly decreased the elastic property of the bone that led to low bone strength[7].

The process of graft recovery and preservation: freezing the bones deeply, incubating them at 56 degrees Celsius and providing them the gamma radiation sterilization significantly reduced the bone strength about 30% in Group II and Group III. However, both rapid and slow bone temperature changes with the determined rate between -70 and 56 degrees Celsius revealed that there was no significant effect on the strength of the bones in both Group II and Group III (Table 1). The deeply frozen bones can be suitable implants in the area that needs the graft strength at a certain level such as plate and screws or intra-medullary nail. The findings in this study were different from Moreno and Forriol's report[4]. Both of them found that the ultimate strength of frozen bones was higher than the fresh ones. They froze the bones at -20 degrees Celsius for 60 days whereas this research used the freezing level at -70 degrees Celsius. Furthermore, the frozen bones in their study were not exposed to the radiation sterilization while this study used this technique. The differences in the freezing temperatures and sterilization techniques might be the major factors influencing the final ultimate strength.

The freeze-dried bones lost most of their strength. This result implies that the freeze dried bones can be used as chip grafts to fill the bone defect while the rest of the recipient bones have to provide adequate strength. In some particular conditions, incorporating freeze-dried chip grafts in an implant using the metal brace in order to produce the rigid fixation, enhance the rapid bone healing and protect the newly born bone to have well-shaped bone contour. The successful incorporation between the recipient bone and the graft can be observed by the radiographic study.

Bangkok Biomaterial Center is a main supplier of allogenic bones for the orthopedic use in Thailand. The surgeons using the bone grafts from the Center must realize these data. The preserved bone grafts are

Table 1. The load amount at the fracture occurrence point under the three-point bending, single load until the tested bones broke

Type of bone	Load at fracture occurrence point (Kg)
Control group	675.90 ± 5.11
Deeply frozen bone meanwhile having the rapid change of bone temperature	467.21 ± 3.02
Deeply frozen bone and having the slow change of bone temperature	467.30 ± 2.90
Freeze-dried bone	61.17 ± 4.21

necessarily handled with care. During the operation, using minimal optimum force is suggested when grasping a bone graft with bone holding forceps to avoid the graft fractures. Furthermore, after fixing the bone grafts in the lower extremities, weigh bearing should be avoided until the incorporation of the graft and host is detected by postoperative radiograph. Bone graft fractures can be prevented if these precautions are followed.

Conclusion

The load amount at the fracture occurrence point of deeply frozen bone grafts decreased 30% compared with the weight used in the fresh bone group. The strength of freeze-dried bone grafts was only 10% of the fresh one.

References

1. Hofmann C, Schadel-Hopfner M, Berns T, Sitter H, Gotzen L. Influence of processing and sterilization on the mechanical properties of pins made from bovine cortical bone. Unfallchirurg 2003; 106: 478-82.
2. Pelker RR, Friedlaender GE, Markham TC. Biomechanical properties of bone allografts. Clin Orthop Relat Res 1983; 54-7.
3. 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.
4. Moreno J, Forriol F. Effects of preservation on the mechanical strength and chemical composition of cortical bone: an experimental study in sheep femora. Biomaterials 2002; 23: 2615-9.
5. Conrad EU, Ericksen DP, Tencer AF, Strong DM, Mackenzie AP. The effects of freeze-drying and rehydration on cancellous bone. Clin Orthop Relat Res 1993; 279-84.
6. Anderson MJ, Keyak JH, Skinner HB. Compressive mechanical properties of human cancellous bone after gamma irradiation. J Bone Joint Surg Am 1992; 74: 747-52.
7. Bashaiwoldu AB, Podczeck F, Newton JM. A study on the effect of drying techniques on the mechanical properties of pellets and compacted pellets. Eur J Pharm Sci 2004; 21: 119-29.

ความแข็งแรงของกระดูกในคลังกระดูก

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กราฟกระดูกที่ได้จากมนุษย์ เป็นวัสดุที่มีประโยชน์อย่างมากในการปลูกกระดูกทดแทนส่วนที่มีพยาธิสภาพ ความแข็งแรงของกราฟกระดูกเป็นคุณสมบัติที่สำคัญในการยึดตรึงกราฟให้ติดกับกระดูกที่ปกติได้อย่างมั่นคง การเตรียมกราฟกระดูกมีหลายขั้นตอน ซึ่งทำให้ความแข็งแรงของกราฟลดลง การศึกษานี้มีจุดประสงค์เพื่อให้ทราบว่า การเตรียมและการเก็บรักษากราฟกระดูกที่ใช้อยู่เป็นประจำในศูนย์เนื้อเยื่อชีวภาพกรุงเทพฯในพระอุปถัมภ์ฯ ทำให้ความความแข็งแรงของกราฟลดลงมากน้อยเพียงไหน การศึกษานี้ใช้กระดูกต้นขาหมูสดที่มีน้ำหนักตัวประมาณ 100 ถึง 150 กก. จำนวน 16 ข้าง โดยแบ่งกระดูกต้นขาออกเป็น 4 กลุ่มๆละ 4 ชิ้น เป็นกระดูกต้นขาด้านซ้าย 2 ชิ้น และข้างขวา 2 ชิ้น กลุ่มที่หนึ่งเป็นกลุ่มควบคุม นำกระดูกสดมาทดสอบแรงกดให้งอทางด้านข้างชนิด 3 จุด โดยใช้เครื่องทดสอบคุณสมบัติทางกลศาสตร์ชิมัสซึ เอจีบี 2000 การกดทำที่กึ่งกลางลำกระดูก และจุดตรึงอยู่ห่างออกไปข้างละ 10 ซม. ใช้แรงกดชนิดต่อเนื่องด้วยความเร็ว 1 มม. ต่อวินาที บันทึกข้อมูลแรงกดที่ทำให้กระดูกหักของกระดูกทุกชิ้นแล้วนำมาคำนวณหาค่าเฉลี่ย กลุ่มที่สองเป็นกลุ่มกระดูกที่แช่แข็งที่ -70 องศาเซลเซียส และมีการเปลี่ยนแปลงอุณหภูมิเร็ว กลุ่มที่สามเป็นกลุ่มกระดูกที่แช่แข็งที่ -70 องศาเซลเซียสและมีการเปลี่ยนแปลงอุณหภูมิช้า ส่วนกลุ่มที่สี่เป็นกลุ่มที่ทำให้แห้งภายใต้ความเย็น ผลการทดลองพบว่า กระดูกในกลุ่มที่สอง สาม และสี่ มีความแข็งแรงลดลงกว่ากลุ่มที่หนึ่งอย่างมีนัยสำคัญ แต่ไม่พบความแตกต่างระหว่างกลุ่มที่สองและกลุ่มที่สาม ทั้งสองกลุ่มนี้มีความแข็งแรงลดลงประมาณร้อยละ 30 ส่วนกลุ่มที่สี่ความแข็งแรงลดลงเหลือเพียงร้อยละ 10 ของกระดูกสด การใช้กราฟกระดูกต้องทำผ่าตัดด้วยความนิ่มนวล ควรหลีกเลี่ยงการใช้แรงกดจากคีมจับกระดูกที่มากเกินไปกราฟกระดูกอาจหักได้ กระดูกที่ผ่านการทำให้แห้งมีความแข็งแรงน้อยมากไม่ควรให้กราฟชนิดนี้ต้องรับน้ำหนัก