การประเมิน Healing processes ของ Bone Tablet graft (Freeze Dried Bone Graft) ในหนู

An Assessment of The Healing Processes of Bone Tablet (Freeze Dried Bone Graft), Study in Rats

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ABSTRACT

Background : Freeze dried bone graft provide mechanical support and osteoconduction. With the development of preparation of freeze dried bone graft (Bone tablet) for clinical use concerns have been raised as to the osteoconductive properties of such preparations. Several recent studies investigating the histologic appearance of freeze dried bone grafts have questioned their healing processes.

Objective : to assess the healing processes of bone tablet graft in rat femurs.

Materials and Methods : 10 Sprague-dawley rats were performed osteotomy at Rt. femurs and left the defect heal spontaneously. Lt femurs were performed osteotomy and bone graft implantation. The rats were sacrificed at 5 weeks and 10 weeks postoperatively period. Both thighs and fractures included bone grafts were sent for histological examination.

Result : At 5 weeks Rt. femurs (control side) showed periosteal reaction and area of bone healing. Lt. femurs showed fibroblast and multinucleated giant cell around bone graft. No area of bone graft incorporated with host bone was demonstrated. Normal healing of thigh muscle was demonstrated. At 10 weeks. Rt. femurs (Control side) showed new bone formation. Lt. femurs showed new bone formation and bone graft were encapsulated by dense connective tissue. Freeze dried bone graft did not incorporate with host bone.

Conclusions Bone tablet freeze dried bone graft showed foreign body reaction and were not incorporated with host bone. Minimal tissue reaction can be demonstrated. This study can not show complete healing processes of bone graft due to short postoperative period. The healing processes of this bone graft need more studies and more times for conclusions and clinical advantages.

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บทคัดย่อ

บทนำ : Freeze dride bone graft เป็น bone ที่ช่วยพยุงและซ่อมแซมช่องโหว่หรือรอยแตกหักของกระดูก Bone graft ชนิดนี้จะมี Healing process แบบ Osteoconduction สำหรับ Bone tablet greft เป็น freeze dried bone graft ชนิดที่ผลิตโดยธนาคารเนื้อเยื่อแห่งประเทศไทย

วัตถุประสงค์ : เพื่อศึกษา Healing process ของ bone graft ในกระดูก Femur ของหนู

วิธีการศึกษา : ทำการผ่าตัด Osteotomy (one cortex, defect 6 mm.) ในกระดูก Femur ของหนู Spragve-dawley 10 ตัว โดยตำแหน่ง Osteotomy ของกระดูก Femurข้างขวาปล่อยให้มีการหายของกระดูกโดยธรรมชาติ และตำแหน่ง Osteotomy ของกระดูก Femurข้างซ้ายใส่ bone graft แล้วเย็บเนื้อเยื่อหุ้มกระดูกและกล้ามเนื้อคลุมตำแหน่งที่ทำการผ่าตัดเอาไว้ ที่สัปดาห์ที่ 5 และสัปดาห์ที่ 10หลังการผ่าตัดชิ้นเนื้อขาหลังของหนูรวมทั้งกระดูก จะถูกนำมาทำการตรวจชิ้นเนื้อทางพยาธิวิทยา

ผลการศึกษา : 5 สัปดาห์ หลังการผ่าตัด กระดูก femur ข้างขวา แสดงให้เห็นการหนาตัวของเนื้อเยื่อหุ้มกระดูก และมีการสร้างกระดูกขึ้นมาใหม่ กระดูก femur ข้างซ้าย แสดงให้เห็น fibroblast cellและ multinucleated giant cell เข้ามาทำปฏิกริยากับ bone graft ไม่เห็นลักษณะการเชื่อมต่อ (incoporation) ของ bone graft กับ host bone ส่วนกล้ามเนื้อของหนูมีการหายที่เป็นปกติ ที่ 10 สัปดาห์ หลังการผ่าตัดกระดูก femur ข้างขวามีการสร้างกระดูกทดแทนโดยสมบูรณ์ กระดูก femurข้างซ้าย bone graft ส่วนใหญ่ถูกห่อหุ้มด้วยพังผืด (fibrous tissue) และ bone graft บางส่วนถูกห่อหุ้มโดยกระดูกที่สร้างขึ้นมาใหม่ แต่ไม่สามารถบอกได้ว่ามีการเชื่อมต่อกันระหว่าง bone graftโดยสมบูรณ์

สรุป : Bone tablet freeze dried bone graft แสดงให้เห็นถึงปฏิกิริยาที่ก่อให้เกิดพังผืดเป็นส่วนใหญ่ และยังไม่เห็นการเชื่อมต่อของ bone graft กับ host bone ได้ ใน Healing process เบื้องต้นของ bone graft ชนิดนี้ กระบวนการหายโดยสมบูรณ์ของ bone graft ชนิดนี้ต้องการการศึกษาที่นานและมากกว่านี้

FREEZE-DRIED bone is obtained from cortical or cancellous bone or a combination of the two. The bone may be prepared as chips, strips, a block, or as a massive bone graft before freeze-drying. The material may be procured aseptically after the death of the donor or during a surgical operation. It is usual to reconstitute such "aseptic grafts" in an antibiotic solution before use. Alternatively, the graft may be procured about using an aseptic technique and sterilization aimed for by the use of chemicals such as merthiolate solution, ethylene dioxide, or by the use of physical means such as heat or high-energy irradiation.

Experience indicates that the processs of creeping substitution largely accounts for the incorporation of bone autografts and frozen or freeze-dried segmental allografts, but the biomechanical properties of these materials are compromised by resorption as they remodel.[1] The differences in the intensity of resorption and completeness of incoporation that have been recorded in the clinic and laboratory indicate that autograft are superior to allografts, but that freeze-drying greatly improves the allografts performance.[2,3,4]

Bone tablet is one form of freeze-dried bone graft from tissue banking of Siriraj hospital.No studies have characterized the healing processes of this bone graft. Our objectives were to: (1) establish a model of diaphyseal repair such that a large defect would result, (2) induce osseous repair in the defect by implantation of freeze-dried bone graft, and (3) measure the healing processes of induced bone, relating them to the histological characteristics of the repair process.

Materials and Methods

This freeze-dried bone graft was prepared from human frozen bones at $4^{O}C$ such as head of femur, iliac crest, femoral condyes etc.are trimmed and cut into different sizes and shapes. The bone chips are repeatedly washed 3 times with double bio-filtered water using an ultrasonic washing machine to remove bone marrow and fat. The bone chips are submerged in hydrogen peroxide to clean the bone and remove red blood cells. Bonegrafts are submerged for 2 hours in 0.5% sodium hypochlorite solution to in activate HIV and HBV. The bone grafts are then placed in drying chamber of freeze-drying machine,which is pre-cooled to $-50^{O}C$, a vacuum of 10-2 mbar is appllied. It takes about 72 hrs for complete freeze drying, by which time moisture of the bone is not more than 5%.The size of bone graft is 1.2x0.7x0.4 cm. Weight is 450 mg.(Fig1)

A group of 10 (250-350 gm) male Sprague-Dawley rats were followed for five weeks and ten weeks.(Fig2) 10 Sprague-Dawley rats were performed osteotomy at Rt. femur and left the defect heal spontaneously. Lt. femur was

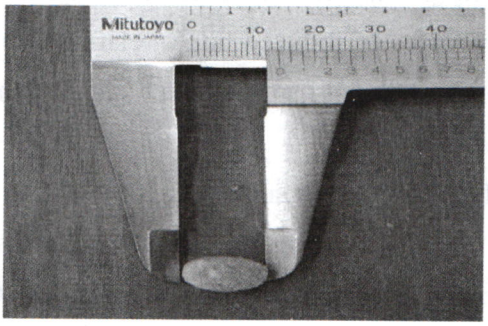
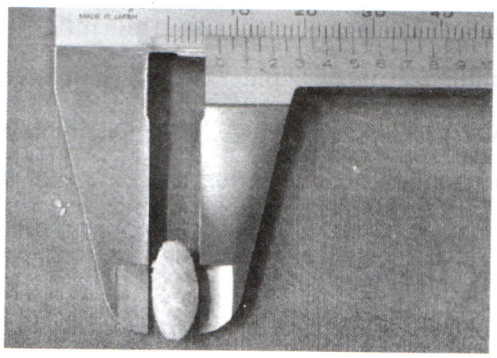


Fig.1.2 bone graft

performed osteotomy and bone graft implantation. The size of defect is 0.6 cm. The rats were sacrificed at 5 weeks and 10 weeks postoperatively period. Both thighs and fractures included bone grafts were sent for histological examination. All of the animals were obtained from one source at one time and were maintained in identical environments. Histological criterions are creeping substitution, osteoinduction (cartilage rests within their vascular channel), only fibrocartilage and fibrous band.



Result

At five weeks Rt.femurs (control side) showed periosteal reaction and area of bone healing(osteocyte in loose matrix).

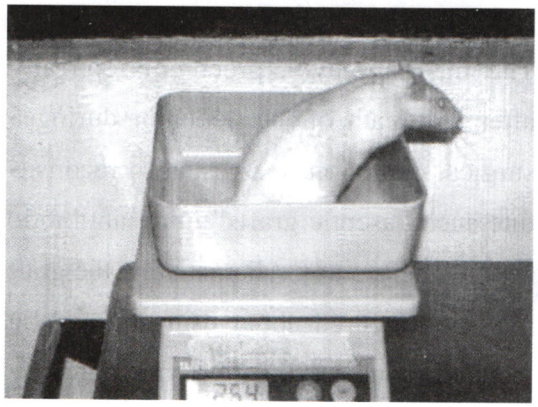


Fig.2 Sprague-Dawley rats

Lt.femur showed fibroblast and multinucleated giant cell around bone graft. No area of peripheral revascularization was demonstrated. Bone graft did not incorporate with host bone(Fig.3,4,5) At ten weeks Rt.femur (control side) showed new bone formation,lamellated bone. Lt. femurs showed new bone formation and bone graft were encapsulated by dense connective tissue. Peripheral revascularization and bone graft incoporation can not be demonstrated at ten weeks period. And minimal tissue reaction from host response can be demonstrated(Fig.6,7,8)

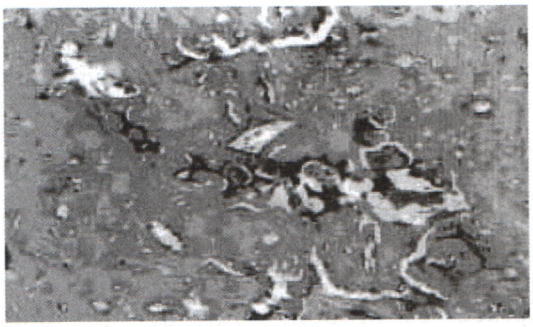


Fig.3 At 5 weeks. Rt. feur showed healing process by immature bone(osteocyte in loose matrix)

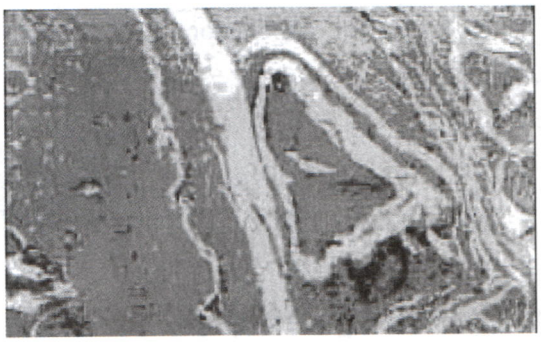


Fig.4 bone graft was encapsulated by fibrous Tissue

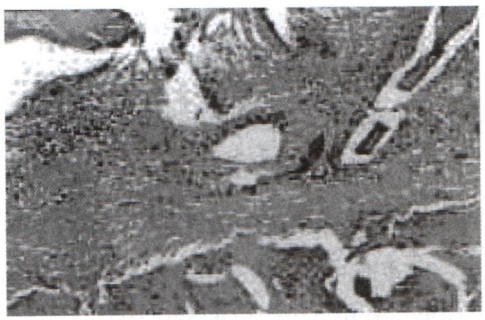


Fig.5 Bone graft in fibrous tissue and host bone

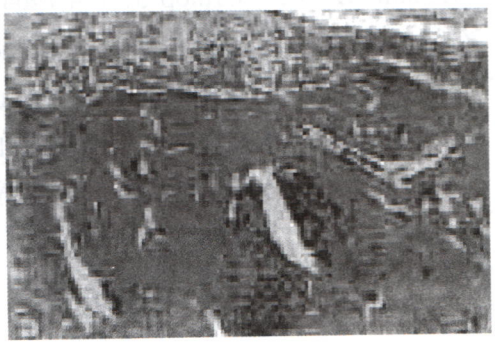


Fig.6 At 10 weeks, Rt. femur showed mature bone at osteotomy site.

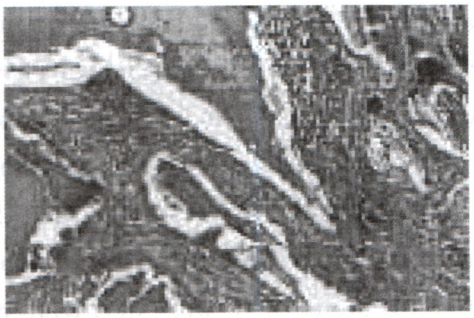


Fig.7 Fibrous tissue encapsulated bone graft[G] and multinucleated gint cell[C] was demonstrated near bone

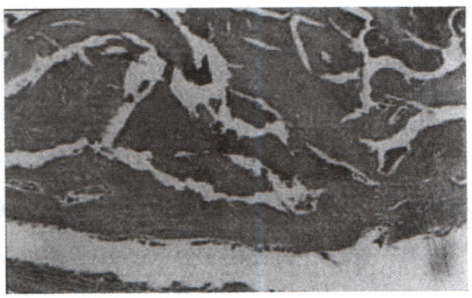


Fig.8 At 10 weeks bone graft was encapsulated by host bone, but no area of incoporation can be demonstated.

Discussion

This experimental model designed for histological evaluation of bone graft healing processes and this model can not study biomechanical strength of bone. Histological fate of freeze-dried bone graft is, for obvious reasons, rarely possible to determine in patients. Again Gresham [5], on the basis of a small number of biopsies, recognized peripheral revascularization, resorption, and replacement of the graft by immature bone and later by osteons. The initial activity over 2-4 months was continued at a much slower rate for many years.[6] And the

rate of freeze-dried bone grafts incoporation were 20-40%.6 The principal problems have been described and include (1) immunogenicity, (2) vascular and cellular invasion, (3) osteogenesis, (4) remodeling,and (5) sterilization. This bone graft have very low immunogenicity and was sterilized by Gamma ray 20 Kgy, so these factor may not limit the healing processes of bone graft. As the physical state of the graft (as particle), the site of implantation can not fixed well. We sutured the periosteum and thigh muscles to cover bone grafts. None of rats died and no infection occured. At the end of study, Rt.femur showed normal bone healing from immature bone (woven bone) to mature bone (lamellated) bone. Lt.femur showed fibroblast and connective tissue encapsulated bone graft. No area of peripheral revascularization and well incoporation was seen. Some area showed host bone encapsulated bone graft (not incoporation). And minimal inflammatory cells can be seen around bone graft.

The question remains why the bone tablet freeze-dried xenograft placed at the host-graft junctions did not discernably benefit bone graft incoporation. It is probable that because these elements were encapsulated early by dense connective tissue, they were effectively sealed off from the immediate graft environment and moved beyond the diffusion.

Conclusion

Bone tablet freeze-dried bone graft showed foreign body reaction and were not incoporated with host bone. Minimal tissue reaction can be demonstrated. This study can not show complete healing healing processes of bone graft due to short postoperative period. The healing processes of this bone graft need more studies and more times for conclusions and clinical advantages.

References

1. Simmons,D.J.:Fracture healing perspectives.Clin.Orthop.200:100,1986.
2. Bonfiglio, M., and Jeter, W.S.: Immunologic response to bone. Clin.Orthop.87:19,1972.
3. Burchardt,H., Jones, H., Glowczewskie, C.,Rudner, C., and Enneking, W.F.:Freeze-dried allogeneic segmental cortical-bone grafts in dogs. J. Bone Joint Surg. 60A:1081,1978.
4. Carr, C.R., and Hyatt, G.W.:Clinical evaluation of freeze-dried bone graft. J. Bone Joint Surg.37A:549,1955.
5. Resham RB: Clin Orthop 37:194,1964
6. Chyun-yu Yang, David J. Simmons, and Renaud Lozano.: The healing of grafts combining freeze-dried and demineralized allogeneic bone in rabbits. Clin Orthop