

Ultrasonography and Radiography Evaluation of the Cartilage Graft in Repair of Experimentally Induced Radial Bone Defect in Rabbit

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Abstract: We would like to thank to the Faculty of Specialized Veterinary Sciences research council. Science and Research Branch of Islamic Azad University, Punak Tehran for approval and financial support to finish this project. **Problems statement:** The purpose of this research was to determine the biological effect of cartilage graft as a bone defect filler and osteogenetic stimulation to speed up bone healing too. **Approach:** Sixteen adult male New Zealand white rabbits having body weight ranged from 3.0-3.5 Kg. Under general anesthesia, a segmental full thickness bone defect of 10 mm in length was created in the middle of the right radial shaft in all rabbits. They were divided into two groups of 6 rabbits each. Group I was considered as control and the fractured site was fixed using finger bone plate with 4 screws, whereas the ear cartilage of 1×1 cm graft was used to fill the gap after fracture fixation in Group II. Rabbits in two groups were subdivided into 2 subgroups of 1 and 2 months duration with 4 rabbits in each. Radiography and two dimensional and color Doppler sonography were done before and after creating defects and on 15, 30 and 60 days to evaluate local reaction as far as new blood vessels network and callus formation are concerned. **Results:** On the radiographs during the whole process, bone repair in Group I was not as perfect as those in Group II samples and trace of internal callus filled the gap incompletely in 60 days in Group I, whereas in Group II internal callus almost was formed on 30 days and in addition intercortical callus was seen supporting to cover and filled the gap completely in this group. Sonographic findings confirmed the protrusion of newly formed blood vascular network in 30 days in Group I and from 15 days in Group II and remarkably increased till end of observation period. **Conclusion:** Cartilage graft is suitable alternative bone filler and radiography and sonography are reliable techniques to trace local reaction at proper time.

Key words: Cartilage, sonography, radial bone, radiography, vascular network

INTRODUCTION

Although the skeleton is strong enough but occasional fractures has been reported many times. The healing processes of the fractures are main clinical problem in human and animals (Barden and Brinker, 1976; De Lutio di *et al.*, 1993; Dickens *et al.*, 1994; Fery-Lemonier *et al.*, 1993; Anast *et al.*, 1958; Friedrich and Volkenstein, 1991). Considering the non-healing hypothesis and long term healings many investigations have been performed about the case in recent decades. The main attempts of these investigations were precipitation in healings, osteopathy and battening capability. In the last two decades osteografts and replacements in healing spongy bones and compact bones in osteopathy has prevail (Morgan and Leighton, 1997). Clinical consequences of grafts

depends on many factors, such as grafts type, osteopexy and fracture position. All kinds of osteografts assimilate in body and assimilation processes in spongy bones are faster than compact bones. The most prevalent grafts are autografts, allograft, xenografts and synthetic substances. In spite of widespread osteobanks around the world there are still many questions about allograft immunology and repatterings to be answered. Many synthetics have developed in osteopathy and replacements. They had been used as implements in osteoectomy that investigated experimentally on animals (Toal, 1994). They enforce ossification, three dimensioned vascular genesis, capillary genesis and host intramembranous ossification in grafts. Considering restriction in replacements, it seems that using biological substances such as host cartilage tissue, fresh or reserved in limb banks should be examined in

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bones impairments, because of its osteogenesis capability and tissue interchanges. This investigation evaluates the cartilage grafts using sonographic and radiographic techniques.

MATERIALS AND METHODS

This study was performed in accordance with the Islamic Azad University Law on animal experimentation. Rabbits were treated accordingly to animal welfare legal regulations.

Sixteen adult male New Zealand rabbits having body weight from ranged from 3.5-3.0 Kg were given general anesthesia using combinations of acepromazine 1 mg kgbw⁻¹ intramuscularly as premedication (Karon Co. Iran) and anesthesia was induced intramuscularly using ketamine 35 mg Kg⁻¹ (Alfasan Woerden-Holland) with combination of xylazine 5 mg Kg⁻¹ (Afasan Woerden-Holland). Under restrict aseptic condition, skin incision was given on the latero medial surface of right radial bone and after separation of connective tissues and fascia a segmental full thickness bone defect of 10 mm in length was created in the middle of the right radial shaft in all rabbits (Fig. 1). They were divided into two groups of 8 rabbits each (Fig. 2). Group (I) was considered as control and the fractured site was fixed using finger bone plate with 4 screws, whereas the ear cartilage of 1×1 cm graft was used to fill the gap after fracture fixation in Group (II) (Fig. 2). Rabbits in two groups were subdivided into 2 subgroups of 1 month and 2 months duration with 4 rabbits in each. Radiography and two dimensional and color Doppler sonography were done before experiment, after fixation and on 15, 30 and 60 days and interpretation were done by considering dislocation of graft, new-bone formation and new blood vascular network formation.

Post-operative care: Antibiotics, penicillin G procaine 40000 IU kg⁻¹, i.m. bid dexamethasone 0.6 mg Kg⁻¹, Vitamin B Complex 0.2 mg Kg⁻¹ and analgesic agent such as Tramadol hydrochloride 5 mg Kg⁻¹, i.m. bid were administered for 3 post-operative days. The lateral and cranio-caudal radiographs were taken before experiment, after fixation and on 15, 30 and 60 days:

- Radiographic evaluation was done using the healing indices such as:
 - The probable inchoate internal callus
 - The probable inchoate external callus
 - The probable inchoate intercortical callus

- Osteo inductions in adjacent bones
- The amount of cartilage opacity in defective bone and its role in osteoconductivity during healings process
- The amount of healing callus in defective bones
- Sonographic evaluations were performed by Sonosite Titan Equipment. Linear probe in 10 Megahertz frequency and imbuing topical gel used in sonography

RESULTS

No operative or postoperative complications were encountered. All of the rabbits tolerated operation well and survived until the final study period. No wound opening or infections were observed. Radiographically keeping in view the parameters were used to evaluate the major changes revealed no sign of infection on the edges of each defect and both groups permitted stabilization of the defected bones and maintenance of correct position. The new bone formation and local reaction in form of biodegradation of cartilage in the bone defects in Group II could be clearly reflected by the gradually replacing with new bone invasion. On the radiographs during the whole process, bone repair in Group I (Fig. 3) was not as perfect as those in Group II samples (Fig. 4, Table 1) and trace of internal callus filled the gap incompletely in 60 days in Group I, whereas in Group II internal callus almost was formed on 30 days and in addition intercortical callus was seen supporting to cover and filled the gap completely in this group (Fig. 5, Table 2). Sonographic findings confirmed the protrusion of newly formed blood vascular network in 30 days in Group I (Fig. 5, Table 3) and from 15 days in Group II and remarkably increased till end of observation period (Fig. 6, Table 4).

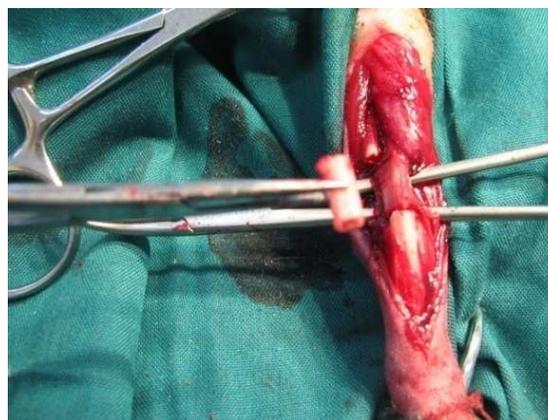


Fig. 1: Removal of cut piece of bone

Table 1: Radiographic findings in Group I at different intervals

Day	0	15	30	60
Soft tissue	Swelling soft tissue	Decrease of swelling soft tissue	No swelling soft tissue	No swelling soft tissue
Osseous	No internal callus	No internal callus	No internal callus	No internal callus
Tissue	No external callus	No external callus	No external callus	No external callus
	No intercortical callus	No intercortical callus	No intercortical callus	No intercortical callus
	To empty defect from callus	To empty defect from callus	To empty defect from callus	Relative to fill defect from callus
	No osteo induction	No osteo induction	No osteo induction	No osteo induction

Table 2: Radiographic findings in Group II at different intervals

Day	0	15	30	60
Soft tissue	Swelling soft tissue	Decrease of swelling soft tissue	No swelling soft tissue	No swelling soft tissue
Osseous	No internal callus	No internal callus	Internal callus	Internal callus
tissue	No external callus	No external callus	No external callus	No external callus
	No intercortical callus	No intercortical callus	No intercortical callus	Intercortical callus
	To empty defect from callus	To empty defect from callus	To empty defect from callus	Completely to fill defect from callus
	No osteo induction	No osteo induction	No osteo induction	No osteo induction

Table 3: Findings of two dimensional sonography and color Doppler in Group 1

Day	0	5	30	60
Ultrasonography	Defect: Hypo echogenicity	Defect: Hypo echogenicity	Defect: Increase echogenicity	Defect: Increase echogenicity
B-mode	Defect: No vascular blood	Defect: No vascular blood	Defect: Very small vascular blood	Defect: Increase vascular blood
Ultrasonography color doppler				

Table 4: Findings of two dimensional sonography and color Doppler in Group 2

Day	0	15	30	60
Ultrasonography	Defect: Hypo echogenicity	Defect: Increase echogenicity	Defect: Increase echogenicity	Defect: Hyper echogenicity
B-mode	Defect: No vascular blood	Defect: Very small vascular blood	Defect: Increase vascular blood	Defect: Many vascular blood
Ultrasonography color doppler				



Fig. 2: Fixation of radial bone and packing of defect using ear cartilage



Fig. 4: Radiograph of group II at 60 days

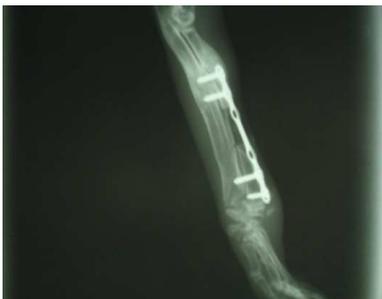


Fig. 3: Radiograph of control group at 60 days

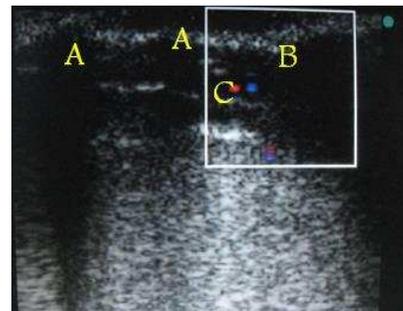


Fig. 5: Sonography of control showing (A) screw; (B) defect

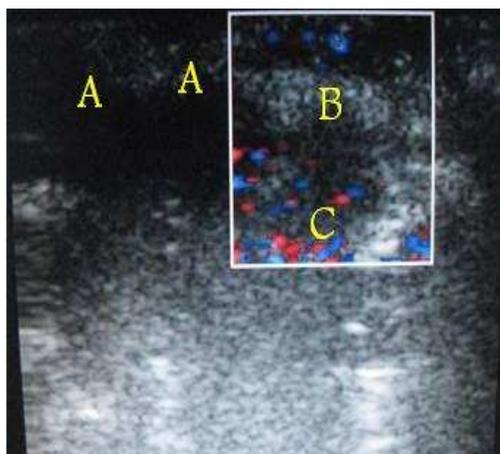


Fig. 6: Sonography of group II showing (A) screw; (B) defect

DISCUSSION

Radiology is a most important diagnostic test in the investigation of skeletal disease because good radiographic contrast is naturally provided between the bone and surrounding soft tissues permitted detection of rather small but clinically important changes (Barden and Brinker, 1976). Bone is a living system with number of functions and skeleton develops first as a cartilage model which is then replaced with bone by process of endochondral ossification (Mafulli *et al.*, 1992; Sjostrom, 1998; Toal, 1994). As to evaluate the viability of biologically active freshly harvested ear cartilage as a graft to have dual role beside filling bone defect to stimulate and speed up healing capacity of fractured sites. In this study using freshly obtained cartilage tissue from the base of ear showed to have enough tough texture to be rolled and fill the gap without having any tendency to slip out. As per expected no local reaction was seen during first two weeks in both groups but sonographically sprouting small blood vessels network in Group II at end of two weeks in Group II with radiographic findings of intercortical callus formation within 30 days were good indication for local reaction of cartilage graft to act as stimulator to provide suitable bed for blood vessels penetration as compared to Group I (Graf *et al.*, 1988; Hamanishi *et al.*, 1994; Mende *et al.*, 1986; Moss and Mowatt, 1983; Williamson *et al.*, 1991) The ossification radiographically and pathologically being reported (Haktanir *et al.*, 2005) in diced cartilage grafts used in cranial defects in rabbits. Findings of this study confirmed biological activity of cartilage despite of

having self-blood vessels limitation. Using cartilage due to autogenicity, ease of shaping absence of post operative displacement and complication, inherent viability and ossification properties but slow in resorption rates will be a suitable biological graft for most of the lost fragments in skeletal system (Abiri *et al.*, 1989; Eyres *et al.*, 1993; Hamanishi *et al.*, 1994). Despite of recommendation made for different synthetics materials to be used as a source of osteogenesis but the results of this study radiographically and sonographically confirmed the positive local effects of ear cartilage as a bone filler and local stimulator to increase blood vessels and inner and intercortical callus formation. In conclusion. Investigators suggest the use of ear cartilage for local bone construction in which bone deformities may cause aesthetic and functional disabilities (Haktanir *et al.*, 2005).

CONCLUSION

Cartilage graft is suitable alternative bone filler and radiography and sonography are reliable techniques to trace local reaction at proper time.

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