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Elastic cartilage grafting in canine radial fracture

Saifzadeh, S.[1*]; Hobbenaghi, R.[2] and Hodi, Sh.[3]

[1]Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Urmia, Urmia, Iran; [2]Department of Pathobiology, Faculty of Veterinary Medicine, University of Urmia, Urmia, Iran; [3]Graduated from Faculty of Veterinary Medicine, University of Urmia, Urmia, Iran

[*]**Correspondence:** S. Saifzadeh, Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Urmia, Urmia, Iran. E-mail: s.saifzadeh@mail.urmia.ac.ir

Summary

Bone has a capability to repair itself when it is fractured. Repair involves the generation of intermediate tissues, such as fibrous connective tissue, cartilage and woven bone, before final bone healing can occur. The process of cartilage-to-bone transition (CBT) is a key for the achievement of rigid bone healing during fracture repair. We tested this potential for elastic cartilage using a long bone defect model in dog. Eight sexually intact female mongrel dogs, 4.57 ± 0.53 years old and weighing 11.48 ± 0.63 kg, were studied. After an ostectomy of the midshaft radius, bone healing was evaluated over an 8-week period in control dogs (n = 4) and dogs in which autologous grafts of auricular cartilage were inserted into the bone defects (n = 4). Quantitative radiographic assessment was conducted every 2 weeks. Eight weeks post-operative, qualitative histopathologic analysis was performed on the operated radii. Furthermore, histological grading was done using the Ulutas *et al*., scoring system. Experiment dogs had more advanced radiographic healing of ostectomy sites. The defects with elastic cartilage implants were bridged completely with new bony spicules originated from the implants. Transformation of elastic cartilage clusters to mesenchymal connective tissue and bony spicules was obvious in the experiment group. Significant differences were observed for cellular morphology [3 ± 0.82 (experiment) vs. 1.75 ± 0.5 (control)] and cartilage integration [2 (experiment) vs. 1 (control)] at ostectomy sites between the studied groups. This study demonstrated that by using the ostectomy gap model, autologous auricular cartilage enhanced the radiographic and histopathologic aspects of bone healing in dogs.

Key words: Elastic cartilage, Auricular, Ostectomy, Bone healing, Dog

Introduction

Bone is a dynamic biological tissue composed of metabolically active cells that are integrated into a rigid framework. The healing potential of bone, whether in a fracture or fusion model, is influenced by a variety of biochemical, biomechanical, cellular, hormonal, and pathological mechanisms (Kalfas, 2001). Bone grafts often are necessary to provide support, fill voids, and enhance biologic repair of skeletal defects. Materials and techniques currently used for bone replacement/repair conform to the current paradigm, relying on bone or bone products to produce bone or induce bone formation (Montufar-Solis *et al*., 2004). Bone harvested from donor sites is the gold standard for this procedure. Although autogenous bone is the standard that all alternatives must meet or exceed, autograft has significant limitations, including donor site morbidity and inadequate amount (Parikh, 2002). Furthermore, the process of harvesting, isolating, and grafting of autologous bone is invasive and time consuming (Scott-Burden *et al*., 2002). Yet, nature forms and heals most of the skeleton by ossification of a cartilaginous model (Montufar-Solis *et al*., 2004). Repair involves the generation of intermediate tissues, such as fibrous connective tissue, cartilage and woven bone, before final bone healing can occur. The intermediate tissues serve to stabilize the mechanical environment and provide a scaffold for differentiation of new tissues (Lacroix and Prendergast, 2002). The process of cartilage-to-bone transition (CBT) is a key for the achievement of rigid bone healing during fracture healing (Nakase *et al*., 1998).

One potential source of chondrocytes is auricular cartilage, which can be harvested by a minimally invasive technique that preserves cell viability, decreases surgical time, and minimizes post-operative complications. Besides, auricular chondrocytes are abundant, readily accessible, and easily and efficiently harvested (Scott-Burden *et al*., 2002). Over the past 20 years, the experimental use of auricular elastic cartilage has expanded considerably. Efforts have been devoted to the auricular elastic cartilage as a potential material for penile reconstruction (Yoo *et al*., 1998), lining the luminal surfaces of cardiovascular prostheses (Scott-Burden *et al.*, 2002), in-utero tracheal augmentation (Fuchs *et al*., 2002), laryngoplasty (Caballero *et al*., 2004), and nucleus polposus repair (Gorensek *et al*., 2004).

To our best knowledge, there are no reports describing autologous elastic cartilage implanted directly in a long bone defect. In this study, we tried to assess an alternative approach in the field of bone repair by investigating the ability of auricular elastic cartilage autograft to support long bone formation.

Materials and Methods

Surgical procedures

Eight sexually intact female mongrel dogs, 4.57 ± 0.53 years old and weighing 11.48 ± 0.63 kg, were studied. Dogs were determined to be healthy on the basis of physical and orthopedic examination findings and normal CBC and serum biochemistry results. Cranio-caudal and medio-lateral radiographs were taken before surgery to exclude abnormal radiographic findings. Dogs were randomly assigned to experiment (n = 4) or control (n = 4) groups. The experimental protocol was approved by the Veterinary Clinical Sciences Committee at Urmia University. Dogs were premedicated with atropine (Darou pakhsh, Tehran, Iran) (0.04 mg/kg, intramuscularly [IM]) and acepromazine (Hoogsrraten, Belgium) (0.1 mg/kg, IM). Anaesthesia was induced with sodium thiopental (Biochemie GmbH, Vienna, Austria) (10 mg/kg, 2.5% intravenously) and maintained with halothane after intubation. Right radius and left pinna were selected for creation of midshaft ostectomy and elastic cartilage harvesting in each dog. The limb and pinna were clipped and prepared for aseptic surgery.

The limb was draped, and an approach to the craniomedial radius was made. A Kelly hemostat was passed between the radius and ulna to protect the ulna from damage during osteotomy. A Gigli wire was used to create an ostectomy, leaving a 2-mm gap between bone ends. The pinna was draped, and a piece of auricular cartilage was trimmed as described previously in canine cosmetic otoplasty technique (Slatter, 2002). The auricular elastic cartilage was removed from the surrounding dermal tissue by delicate dissection. An oval-shaped piece of the cartilage (1 × 1.5 cm^2), according to the dimensions of ostectomy recipient site, was resected and placed directly into the radial bone defect of dogs in experiment group. The area was lavaged. Closure was routine. Immobilization was achieved by application of fiberglass cast from mid-humerus to digits. All dogs received sodium ampicillin (Zakaria Pharmecutical Co., Tabriz, Iran) (25 mg/kg, intravenously, every 6 hrs), and gentamycin sulfate (Darou pakhsh, Tehran, Iran) (5 mg/kg, intravenously, every 24 hrs) for 5 consecutive post-operative days. Diazepam (Chemi Darou, Iran) (0.2 mg/kg, IM) was administered every 6 hrs after surgery for 24 hrs and as needed thereafter to control pain and discomfort.

Radiographic studies

Cranio-caudal and medio-lateral radiographs were taken immediately after surgery and at 2, 4, 6, and 8 weeks post-operatively under sedation with acepromazine (0.1 mg/kg). Periosteal callus size was measured directly on medio-lateral radiographs using a radiographic bone callus index method based on Millis *et al*., (1998). A caliper was used to measure the maximal length and width of bony callus at four points on the medio-lateral radiographs: (1) the cranial and (2) caudal aspects of the proximal radius and (3) the cranial and (4) caudal aspects of the distal radius. The radiographic bone callus index was

determined by summing the eight measurements (Millis *et al*., 1998).

Histopathologic studies

During week 8 of the study, dogs were euthanized by intravenous administration of sodium thiopental solution. Ostectomized radii were harvested, and dissected from surrounding soft tissues, with care taken to preserve the callus around the ostectomy site. The defect areas were then carefully removed en bloc and fixed in 10% neutral buffered formalin for 5 days. Specimens were then decalcified in 5% formic acid solution for 7 days. Decalcified specimens were dehydrated in graded alcohols (80% to 100%) and then embedded in paraffin. Five-micrometer paraffin sections were stained with haematoxylin-eosin. The bone defect and the cartilage implant were evaluated under light microscope. The characteristics of the regenerated tissue and its relation with the surrounding tissues were scored according to Ulutas *et al*., (2005). This new scaling system has two categories:
(1) Cellular morphology: 100% fibrous tissue: 0 point; fibrous tissue + mesenchyme (less): 1 point; fibrous tissue + mesenchyme (more): 2 points; mesenchyme + bone tissue (less): 3 points; mesenchyme + bone tissue (more): 4 points and 100% bone: 5 points.
(2) Integration with the adjacent bone: no integration on the defect edges: 0 point; one edge integrated: 1 point and both edges fused: 2 points.

The points of both groups were calculated using the scale above. Total scores and mean values were calculated for each group.

Statistical analysis

The results of cellular morphology and integration scaling were statistically evaluated using Kruskal-Wallis test. Comparison of bone callus indices between the two groups was made using Student's t-test. The level of statistical significance was set at $p<0.05$.

Results

Radiography

Bony callus developed at the ostectomy sites in the experiment dogs by week 4 and became more pronounced at 6 and 8 weeks. In contrast, control dogs had relatively little bony callus at the 8-week study period. The bone callus index was significantly greater in experiment dogs than in control dogs by week 4. These differences were even more pronounced 6 and 8 weeks after surgery (Table 1).

Table 1: Mean (±SD) bone callus index of experiment and control dogs

Week	Group	No.	Callus index (mm), mean (±SD)	P-value
2	Control	4	11.1 (3.67)	P>0.05
	Experiment	4	14.1 (5.12)	
4	Control	4	34.4 (5.50)	P= 0.000
	Experiment	4	82.5 (6.64)	
6	Control	4	39.3 (6.29)	P= 0.000
	Experiment	4	93 (7.15)	
8	Control	4	44.4 (5.94)	P= 0.000
	Experiment	4	96.3 (6.87)	

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Control group: at week 8, the defects in radii were filled with mesenchymal connective tissue, hyaline cartilage, and newly formed bone. The bony spicules are attaching to the surrounding compact bone through tiny fissures formed at the defect edges. Irrespective of several attachment foci of bony spicules, the areas of incomplete integration are obviously visible at both edges of the defect. The new bone was observed to have reached both edges of the defect incompletely (Fig. 1). Thus, the spontaneous bony repair of control bone defects was limited to the immediate edges of the osseous gap.

Experiment group: for up to 8 weeks in vivo, autologous elastic cartilage implants were well tolerated by the host. There was no inflammatory or foreign body giant cell reaction. Implanted elastic cartilage remained viable and retained a rather normal structure throughout the period of observation. There were prompt proliferation of mesenchymal tissue originated from the elastic cartilage clusters, followed by differentiation of the cells to osteoblasts and the synthesis of new bone. Cellular hyperplasia was seen at the edges of the elastic cartilage clusters, which was differentiated to mesenchymal connective tissue and a large amount of new bony spicules (Fig. 2).

The external callus and repair tissue

volume was not identical in the control and experiment radii. The quantity of callus formed by the implantation of elastic cartilage, at the end of the experimental period, appeared visually to be more than that formed in the control group.

Fig. 1: The histopathologic view of the control group at the 8th week (H&E, ×37). Newly synthesized bone (bony spicules, BS) is observed, indicating differentiation of the hyaline cartilage (HC) and mesenchymal connective tissue (MT). Note to the attachment foci of bony spicules (black thin arrows) through tiny fissures (white thin arrows) formed at the surrounding compact bone (SB) edges, areas of incomplete integration (black thick arrow), and the new bone which is spatially separated from the lower defect edge

Fig. 2: The histopathologic view of the experiment group at the 8th week (H&E, ×45). Differentiation of the implanted elastic cartilage (EC) to mesenchymal connective tissue (MT) and bony spicules (BS) is shown

Cellular morphology and integration scores of the implanted elastic cartilage based on Ulutas *et al*., (2005) and its comparison with the control group were summarized in Table 2. A significant difference was visible regarding the cellular morphology between the studied groups ($P<0.05$). According to that, the experiment group revealed a mean Ulutas score of 3 (±0.82) regarding the cellular morphology in comparison to the control group (Ulutas score of 1.75 ± 0.5), which indicates a transition from mostly mesenchymal to mostly bony area. Moreover, significant difference ($P<0.05$) was seen between the studied groups grading the integration of repair tissue to surrounding bone. The Ulutas integration score showed a marked increase in experiment dogs, meaning that the defect filling had reached the level of the surrounding bone and fused with the both edges of the defect.

Table 2: Results of the quantitative assessment of the regenerated tissue and its relation with the surrounding tissues (scored according to Ulutas *et al.*, 2005)

Dogs	Groups			
	Control		Experiment	
	CM	Int.	CM	Int.
1	2	1	3	2
2	2	1	4	2
3	2	1	3	2
4	1	1	2	2
Mean (±SD)	1.75 (±0.5)	1 (±0.0)	3[a] (±0.82)	2[b] (±0.0)

CM: cellular morphology; Int.: integration; [a] $p = 0.0012$ and [b] $p = 0.0019$

Discussion

Ostectomy of the radius, leaving the ulna intact, is a model that reliably produces nonunion or greatly delayed fracture healing in dogs. Although the ulna acts as a splint and allows weight bearing, excess motion occurs at the ostectomy site, and a nonunion fracture develops (Millis *et al*., 1998). The results obtained in the control group dogs in this study support these findings.

Because many bones form by bone replacement of a cartilage model, i.e. endochondral ossification, cartilage can be used to repair bone (Nguyen *et al*., 1998). As the first step in proving the feasibility of this concept, we harvested, isolated, and inserted canine autologous auricular cartilage in the model of bone defect, and studied its potential adherence to the bone surfaces and participation in bone healing.

Although the radii of dogs receiving elastic cartilage graft did not completely reach bony union during the 8-week study period, there was progressive healing and some areas had bridging callus by the end of the study period, whereas bones of control dogs displayed little healing, indicative of a developing nonunion. The radiographic callus index provided a quantitative measure of bone healing and further substantiated the greater degree of bone healing in the experiment dogs. The amount and progression of callus production suggest that experiment dogs likely would bridge the ostectomy sites in time, although we cannot be certain of this without studies of longer duration.

The results showed tissue integration between autologous elastic cartilage and surrounding cortical bone at the ostectomy site. The difference between integration scores in both groups (Table 2), might explain this finding. Although attachment foci were present only at one defect edge in the control dogs (with mean integration score 1), complete integration of the regenerated tissue to both defect edges was obvious in the experiment dogs (with mean integration score 2). Since elastic cartilage is avascular, the metabolism of its chondrocytes is dependent on the vascular supply of the perichondrium (Michaels, 2002). While the perichondrium of implanted elastic cartilage has been differentiated to mesenchymal tissue in this study, the engraftment and integration of the cartilage was probably due to nourishing of the implant by the surrounding mesenchymal tissue.

Greater cellular morphology score of the regenerated tissue in the experiment dogs (Table 2) suggested more advanced stage of healing in this group in compare to the control. Differentiation of chondrocyte to osteoblast in this study was thought to be due to the osteogenic ability of perichondrium. Interactions between bone and cartilage formation are critical during

growth and fracture healing and may influence the functional integration of osteochondral repair process (Case *et al*., 2003). Case *et al*., (2003) demonstrated that tissue-engineered cartilage constructs, implanted into a well-vascularized bone defect will support direct appositional bone formation and that bone formation is significantly influenced by the viability of chondrocytes within the constructs and the local mechanical environment in vivo.

Histopathologic studies revealed no inflammatory reaction against elastic cartilage transplants. The elastic cartilage implanted in this study had autogenous source and was compatible with the surrounding tissues (Ulutas *et al*., 2005), which may explain this finding.

In view of the fact that the present experiment is the first study on the elastic cartilage potential in promotion of bone healing, our results are not comparable with those of previous works (Montufar-Solis *et al*., 2004). A study with cartilage by Cao *et al*., (1998) investigated an effective approach to the creation of autologous tissue-engineered elastic cartilage in the shape of a human nipple. The feasibility of creating natural penis prostheses of auricular cartilage, useful in children with ambiguous genitalia and patients undergoing penile reconstruction was investigated by Yoo *et al*., (1998). In-utero tracheal augmentation in an ovine model was studied by Fuchs *et al*., (2002) with autologous free grafts of auricular elastic cartilage. Caballero *et al*., (2004) reported successful integration of autologous auricular cartilage in vocal folds of 15 New Zealand White rabbits to achieve medialization of the paralyzed vocal fold. Gorensek *et al*., (2004) described nucleus pulposus repair with cultured autologous elastic cartilage derived chondrocytes in 6 New Zealand White rabbits.

Our study showed that autologous auricular cartilage can be implanted successfully into the long bone defects, resulting in engraftment and function. However, further studies are needed to prove that autologous elastic cartilage would be an appropriate means of providing enhanced structural support after implantation into the long bone defects.

It is also necessary to have study duration long enough to adequately assess the stages of fracture healing. Studies of longer duration are necessary to confirm that complete bony union occurs in the experiment dogs and that controls develop nonunion or have greatly delayed healing.

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