

EVALUATION OF THE REPAIR OF FULL-THICKNESS ARTICULAR CARTILAGE DEFECTS FILLED WITH AUTOLOGOUS EXOGENOUS FIBRIN CLOT: AN EXPERIMENTAL STUDY IN THE SHOULDER JOINT OF DOGS

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To determine whether the optimizing effect of an exogenous fibrin clot in the repair of full-thickness articular cartilage defects is valid when joint motions are restricted, standard osteochondral defects were constituted in the articular surface of the humeral head in 16 adult dogs. The defects in 8 dogs were packed with fibrin clots that had been prepared exogenously from each animal and the defects of the other animals were left empty. The operated limbs were inactivated for 2 weeks postoperatively and the healing response was then examined using routine histology at 2, 4, 8 and 12-week intervals. Although the clot-filled and control (empty) defects initially healed through proliferation of fibrous connective tissue; the clot-filled defects finally modulated into fibrocartilage with completed subchondral bone formation. The clot-filled defects demonstrated a more advanced reparative tissue which was congruent with the intact articular surface from 4 weeks after the intervention.

Key words: articular cartilage, dog, exogenous fibrin, healing

INTRODUCTION

Clinical and experimental observations have shown that, while hyaline articular cartilage is incapable of producing new tissue to heal partial-thickness (superficial) lesions, full-thickness cartilage defects that perforate into the vascular subchondral bone (osteochondral lesions) are able to heal with fibrous tissue. The fibrous tissue matures with time and eventually undergoes metaplasia to fibrocartilaginous tissue or hyaline-like cartilage (Cheung *et al.* 1980, Desjardins and Hurtig 1990, Furukawa *et al.* 1980, Mankin 1962, Mitchell and Shepard 1976, Richardson and Clark 1990, Thompson 1975). The origin of the healing response in osteochondral lesions is the blood from the subchondral bone that fills the defect with a hematoma and, in turn, forms a clot which acts as a scaffold for the mesenchymal reparative response (Campbell 1969, Mankin 1982). The initial character of the clot varies in response to some intraarticular factors and this may affect the final quality and extent of the reparative tissue. The fibrinolytic enzymes,

produced by synovial membrane cells after a traumatic condition or naturally present in synovial fluid at low concentrations, may dissolve the clot, which is the network of reparative tissue (Kummer *et al.* 1992, Mankin 1974a-b, Roćec *et al.* 2002). The spontaneously forming clot may be diluted by the synovial fluid or dislodged by mechanical forces generated during joint movements (Paletta *et al.* 1992).

Experimental studies have demonstrated that commercial or autologous preparations of fibrin clots could provide sufficient support for a reparative response in avascular tissues such as menisci and articular cartilage (Arnoczky *et al.* 1988, Farkas *et al.* 1989, Grande and Pitman 1988, Henning *et al.* 1990). An experimental study has examined the use of an exogenous fibrin clot in the healing response of osteochondral defects in femoral trochlea of dogs without any restriction of limb movements postoperatively and demonstrated that the healing response in clot-filled defects was more organized than the control defects allowed to heal spontaneously (Paletta *et al.* 1992).

The present study was undertaken to determine whether filling an osteochondral defect with an exogenous fibrin clot could encourage the same superior repair in an other articular surface which has little possibility of dislodgement of the naturally forming clot due to the need for a motionless postoperative period.

MATERIALS AND METHODS

Animals and experimental design: This experimental protocol was approved by the Animal Use Committee and all the animals were treated in accordance with national and local animal welfare legislation based on the European Council Directive. Sixteen adult mongrel dogs with no history of locomotor or joint diseases and weighing 18 to 32 kg were included in the study. Skeletal maturity was insured by radiographic evidence of epiphyseal closure about the elbow. A defect, 4 mm in diameter and 4 mm in depth, was made in the articular surface of the humeral head in the left leg of the dogs. In 8 dogs, the defect was filled with an autologous fibrin clot that had been prepared from the blood of the animal intraoperatively (experimental group). The defects of the other 8 dogs were left empty and served as the control group. The defects were evaluated at 2, 4, 8 and 12-week intervals postoperatively by routine histology.

Preparation of the fibrin clot: At operation, 20 ml of whole blood was obtained from each dog in the experimental group. The blood was placed in a sterile 50 ml glass flask that contained approximately 230 sterile glass beads, 4 mm in diameter, and was gently agitated for approximately 5 minutes and the contents were poured on to a sterile gauze sponge. Under aseptic conditions, the glass beads were removed from the fibrin clot and the clot was rinsed in sterile saline solution to remove the excess of red blood cells. The clot was then gently blotted between sterile gauze sponges to remove excess fluid, and immediately packed in the osteochondral defect.

Surgical technique: After the dogs were anesthetized and shaved of hair in the region of the left shoulder, the articular surface of the humeral head was exposed at the shoulder joint by the craniolateral approach technique as previously

described (Probst and Johnston, 1993). An osteochondral defect, 4 mm in diameter and 4 mm in depth, was drilled in the articular surface of the humeral head in each animal under sterile saline pulverization with a dental drill attached to a dental motor (Figure 1a). The diameter of the spherical tip of the drill was 4 mm and the shape of the tip was used as a guide for standardization of all defects by not allowing drilling deeper than the tip. The joints were lavaged copiously with sterile saline to remove the particles produced during drilling. The defects in the experimental animals were then packed with an exogenous fibrin clot. This was accomplished by placing portions of the clot into the defect with fine toothed forceps. Each portion was then packed down using a blunt dental probe until the defect was filled flush to the surface of the adjacent cartilage with the tightly packed clot (Figure 1b). The defects in the control animals were left empty. The joint capsule and the other tissues were closed as described previously (Probst and Johnston 1993). Postoperatively, the left limbs in both experimental and control animals were inactivated with a Velpau sling for 2 weeks. For reducing pain, all the animals received dipyrone (7 mg/kg) intramuscularly every 6 hours for 3 days.

Figure 1. (a) The procedure of drilling the exposed humeral head with the guidance of the spherical tip () of the dental drill bit. (b) The intraoperative appearance of an experimental defect filled with exogenous fibrin clot () up to the intact articular surface.

Laboratory techniques: At the end of each experimental period, 2 animals from each group were sacrificed with an overdose of barbiturate. The shoulder joint was opened and the proximal portion of the humerus was removed by gigli wire. The defect area was then separated by cutting with a band saw in the longitudinal and transversal planes. The samples were fixed in 10 % buffered formaldehyde, decalcified in 0.1 M buffered EDTA solution (pH 7.0), dehydrated in ethanol and embedded in paraffin. Serial transverse sections of 6 μ m thickness were obtained and stained with toluidine blue (pH 4.5), alcian blue/aldehyde fuchsin (pH

2.5), Mallory's phosphotungstic acid hematoxylin (PTAH) or Masson's trichrome. The sections were then examined under a photomicroscope (BH-2 Olympus).

RESULTS

Two weeks: On gross examination, clot-filled and empty defects were filled with a reddish tissue. Histologically, the experimental defects were consistently smoother and more congruent with the intact cartilage surface than the control defects (Figure 2). The tissues in both experimental and control defects were fibrovascular with mononuclear cells and fibroblasts as the predominant cell types. The experimental sites showed a dense fibrous matrix and were more vascular (Figure 2a). In some sections of the experimental defects a remnant of the exogenous fibrin was still visible at the surface (Figure 2a). The reparative response in control sites was less cellular, with the predominant cell types being red blood cells, especially clustered near to surface of the defect (Figure 2b).

Figure 2. Photomicrographs of the clot-filled (**a**) and control (**b**) defects at 2 weeks (Masson's trichrome X 50). Note that the surface of the defect in (**a**) is more congruent with the adjacent cartilage than in (**b**) and the tissue in (**a**) was more vascular. The remnant of the exogenous fibrin clot (between the two close arrows) was visible in (**a**). The red blood cells (open arrows) clustered near to the surface of the defect in (**b**).

Four weeks: Four weeks postoperatively, all lesions remained filled with reddish tissue. Histological examination of the experimental defects showed fibrous tissue filling the defect near to the intact cartilage surface and the predominant cell type was fibroblasts. There was evidence of new bone formation in the depths of the experimental defects (Figure 3). The control sites were filled with a loosely organized fibrous stroma and the extent of the healing response was more depressed.

Figure 3. Photomicrograph of an experimental defect at 4 weeks (Masson's trichrome X 50). Note the new bone formation in the depths of the defect indicated with closed arrows.

Eight weeks: All experimental defects were filled with a white opaque tissue up to the intact cartilage surface which resembled the surrounding articular cartilage (Figure 4a). Histologically, the predominant cell type in the stroma was chondroid in appearance and the extracellular matrix of this reparative tissue was meta-

Figure 4. Macroscopic (*) and microscopic view of the clot-filled (**a**) and control (**b**) defects at 8 weeks (Toluidine blue X 50). Note the reparative tissue in (**a**) which was metachromatic (mc) and resembling fibrocartilage both microscopically and macroscopically (open arrow). Subchondral bone remodeling (br) was nearly completed in (**a**). The tissue in the control defect was pinkish and being depressed macroscopically (open arrow). New bone formation at lateral edges of the defect in (**b**) is indicated with closed arrows.

chromatic resembling fibrocartilage (Figure 4a). Marked new bone formation in the depths of the defect was observed (Figure 4a). The tissue filling the control defects was pinkish and still depressed, macroscopically (Figure 4b). Histologically this tissue was fibrous and there was evidence of new bone formation at the lateral edges of the defects (Figure 4b).

Twelve weeks: Gross examination revealed that the experimental defects were smooth and congruent with the intact articular cartilage. The tissue filling the defects closely resembled the surrounding articular cartilage in color. Histologically, the experimental defects were completely filled with a metachromatic fibrocartilaginous tissue as at 8 weeks. Moreover, all sections in the experimental group revealed completed subchondral bone formation. Both the gross and histological appearance of the control defects at 12 weeks was similar to that at 8 weeks. The subchondral bone remodeling was still evident except in a few sections (Figure 5).

Figure 5. Photomicrograph of a control defect at 12 weeks (Toluidine blue X 50). Note that the amount of fibrous tissue (ft) in the defect was under the level of the intact cartilage surface (double arrow) and subchondral bone formation was still going on in the depths of the defect (bf).

DISCUSSION

The formation of a hematoma is one of the essential steps of articular cartilage healing after an injury that penetrates into the vascular subchondral bone (Campbell 1969). The hematoma rapidly becomes organized into an enriched fibrin clot. The clot in the lesion has a critical mission that it acts as a scaffold for migration of blood and marrow elements which will modulate into fibroblasts (Mankin 1982). One of the logical theories regarding the influence of a naturally forming clot is the possibility of initial dislodgement due to mechanical forces generated by normal weightbearing and joint motions (Paletta *et al.* 1992). In an experimental study, Paletta *et al.* (1992) demonstrated that filling an osteochondral defect

with an exogenous fibrin clot may promote a more organized and rapid repair, although they did not restrict the joint motions postoperatively.

In the present study, the experimental model of Paletta *et al.* (1992) was generally applied except for the articular surface which the osteochondral defect was created on. Although the experimental studies that focused on the repair of articular cartilage with commercial or autologous preparations of fibrin clots (Farkas *et al.* 1989, Grande and Pitman 1988, Henning *et al.* 1990, Paletta *et al.* 1992) had commonly employed the articular surface of the femoral trochlea, we preferred the articular surface of the humeral head. If the optimizing effect of an exogenous fibrin clot is valuable for osteochondral lesion healing, it might also be considered for other articular surfaces like the humeral head, which has a negligible threat of dislodgement of a naturally occurring clot due to the definite need of a motionless postoperative period.

Our macroscopic and microscopic results demonstrated that the amount of reparative tissue in the spontaneously healed defects was less than in the clot-filled sites at each time interval. Although the quantity of the reparative response was fibrovascular both in clot-filled and empty defects up to 4 weeks, the tissue in clot-filled sites was obviously well vascularised and more cellular. This may partly be due to the mitogenic and chemotactic factors such as fibronectin, thrombin, factor XIII and fibroblast growth factor, which are present in a fibrin clot, as the stimulatory effects of these factors on wound repair are well known (Adolphe *et al.* 1984, Knighton *et al.* 1982, Schlag *et al.* 1989, Weiss and Reddi 1981).

In this study the subchondral bone beneath the clot-filled defects showed relatively more rapid remodeling histologically when compared to the control sites. It is possible that the high content of factor XIII in clot-filled defects resulted in rapid bone remodeling. The finding of Claes *et al.* (1985) that factor XIII produced accelerated healing of fractures in sheep with a high hydroxyapatite concentration and tensile strength, supports this possibility. Moreover, the stimulative effect of fibrin clots on new bone formation has also been mentioned by Paletta *et al.* (1992).

It is of interest that while the time of the first appearance of fibrocartilage formation in clot-filled defects was very similar to the observations of Paletta *et al.* (1992), the quantity of the tissue in the control sites in our study was discordant with their results. Although Paletta *et al.* (1992) observed small islands of fibrocartilage metaplasia in the defects healing spontaneously at 8 weeks and homogeneous fibrocartilage at 12 weeks; in the present study the reparative response in control sites was still fibrous at the end of 12 weeks. The possible cause of these differences in the defects healing spontaneously may be the length of the postoperative period that the joint movements were restricted (15 days in our study). Salter *et al.* (1980) demonstrated that continuous passive motion and intermittent active motion could accelerate fibrocartilage metaplasia during healing of osteochondral defects in rabbits. Although joint motions continuing after an osteochondral injury could be an obvious threat for the initial clot, it seems to be beneficial later. While it is possible that this condition is disadvantageous for articular cartilage lesions of joints that need a certain motionless period, the results of our study

suggest that exogenous fibrin clots may optimize the healing response of osteochondral defects even when joint motions are restricted.

In conclusion, it appears that an autologous exogenous fibrin clot and its associated factors may also hasten the repair of full-thickness articular cartilage lesions in the humeral head. While the superior repair supported by the exogenous fibrin clot is more significant in the shoulder joint than in the knee, further biochemical and biomechanical evaluation of reparative tissue is needed before it is applied generally to assist the healing of full-thickness articular cartilage defects.

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**PROCENA REPARACIJE POTPUNOG DEFEKTA HRKAVICE POSLE PRIMENE
AUTOLOGNOG FIBRINSKOG UGRUŠKA: EKSPERIMENTALNA STUDIJA NA
RAMENOM ZGLOBU PASA**

AVKI S i IZCI C

SADRŽAJ

Cilj ovih eksperimenata je bio da se utvrdi efekat egzogenog fibrinskog ugruška na procese reparacije potpunih defekata hrskavice ramenog zgloba. U grupi od 8 pasa, defekti hrskavice ramenog zgloba su popunjavani autoložnim preparatima ugruška dok je druga grupa od 8 pasa služila kao kontrola. Nakon

operativnog zahvata i mirovanja od dve nedelje vršeno je žrtvovanje po dve životinje 2., 4., 8. i 12. nedelje i izrađeni su odgovarajući histološki preparati. Njihovom analizom je utvrđeno da su u oglednoj grupi pasa defekti hrskavice brže popunjavani novom hrskavicom sa subhondralnim formiranjem novog koštanog tkiva.