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Immature porcine knee cartilage lesions show good healing with or without autologous chondrocyte transplantation

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Summary

Objective: The purpose of this study was to find out how deep chondral lesions heal in growing animals spontaneously and after autologous chondrocyte transplantation.

Methods: A 6 mm deep chondral lesion was created in the knee joints of 57 immature pigs and repaired with autologous chondrocyte transplantation covered with periosteum or muscle fascia, with periosteum only, or left untreated. After 3 and 12 months, the repair tissue was evaluated with International Cartilage Repair Society (ICRS) macroscopic grading, modified O'Driscoll histological scoring, and staining for collagen type II and hyaluronan, and with toluidine blue and safranin-O staining for glycosaminoglycans. The repair tissue structure was also examined with quantitative polarized light microscopy and indentation analysis of the cartilage stiffness.

Results: The ICRS grading indicated nearly normal repair tissue in 65% (10/17) after the autologous chondrocyte transplantation and 86% (7/8) after no repair at 3 months. At 1 year, the repair tissue was nearly normal in all cases in the spontaneous repair group and in 38% (3/8) in the chondrocyte transplantation group. In most cases, the cartilage repair tissue stained intensely for glycosaminoglycans and collagen type II indicating repair tissue with true constituents of articular cartilage. There was a statistical difference in the total histological scores at 3 months ($P = 0.028$) with the best repair in the spontaneous repair group. A marked subchondral bone reaction, staining with toluidine blue and collagen type II, was seen in 65% of all animals.

Conclusions: The spontaneous repair ability of full thickness cartilage defects of immature pigs is significant and periosteum or autologous chondrocytes do not bring any additional benefits to the repair.

Key words: Cartilage repair, Autologous chondrocyte transplantation, Periosteum, Animal experiment, Pig, Spontaneous repair, Subchondral bone.

Introduction

It is well known that mature cartilage has poor capacity to heal[1]. Osteochondral lesions can show some repair with fibrocartilage that has durability and biomechanical properties inferior to native articular cartilage[2,3]. Normally, the repair capacity decreases with age. It is possible that immature cartilage has greater intrinsic capacity to heal but the results have been contradictory. Osteochondral lesions of immature rabbits heal well and maintain the repair tissue to maturity[4–6]. Also the superficial cartilage wounds in fetal lambs have been shown to regenerate without scarring[7]. A previous study showed that chondral lesions do not heal in immature or mature dogs, but osteochondral lesions heal similarly in both age groups[8]. Especially deep chondral defects heal poorly in mature rabbits[9–11], goats[12], and dogs[13–17].

Autologous chondrocyte transplantation is a surgical method for cartilage repair[18]. The results have been good in adults and adolescents and the method is gaining wider acceptance[19,20]. The animal experiments of autologous chondrocyte transplantation have been performed with either mature animals[10,12,14,21] or with immature[22] animals but without a control group of spontaneous healing. The aim of the present study was to find out how deep chondral lesions heal in growing animals spontaneously and after

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autologous chondrocyte transplantation. We also wanted to investigate the significance of the periosteum to the repair and compare it to fascia as cover material.

Materials and methods

In this study, 57 skeletally immature pigs (90–100 kg) aged 8–9 months were used. The animals were divided into six groups (Table I); (1) repair with autologous chondrocyte transplantation with periosteum (ACT-P-3) (because of the large individual variation in this group, we performed the same procedure for an additional group of eight pigs to exclude any technical reasons for the variability; this is why the number of animals in this group is larger than in the other groups); (2) repair with periosteum alone (Periosteum-3); (3) repair with autologous chondrocyte transplantation with muscle fascia (ACT-F-3); (4) spontaneous repair (Empty-3); (5) repair with autologous chondrocyte transplantation with periosteum (ACT-P-12); (6) spontaneous repair (Empty-12). The abbreviations show the 3 and 12 months follow-up times used. The pigs were housed at the National Laboratory Animal Center, Kuopio, Finland, in individual pens with floor area of 2×2 m. The experimental design was approved by the local Animal Care and Use Committee. The pigs in the ACT groups were operated twice. First one knee was operated for the cartilage biopsy and 4 weeks later the other knee for the cartilage repair procedure. There were three animals with both operations done on the same knee, however, the results from these animals appeared not to differ from the others. These animals did not have reactive synovitis sometimes seen after recurrent operations in the same joint. For the groups with no chondrocyte transplantation, only one operation was performed.

ANESTHESIA

Before operation, 0.05 mg/kg atropine (Atropin 1 mg/ml, Leiras Oy, Finland) and 8 mg/kg azaperone were given intramuscularly (i.m.). For induction of anesthesia propofol 3 mg/kg (Rapinovet 10 mg/ml, Schering–Plough A/S, Denmark) was used intravenously (i.v.) and continued with 15 mg/kg/h infusion for the duration of the operation. N_2O and oxygen were used for inhalation anesthesia. Cefuroxime (Kefurion, Orion Pharma, Finland) was given preoperatively (3000 mg i.v) for infection prophylaxis and continued for 4 days with a daily dose of 4500 mg.

OPERATIVE PROCEDURES

First operation for groups ACT-P-3, ACT-F-3, and ACT-P-12 was a cartilage biopsy performed through a lateral arthrotomy. An approximately 300 mg cartilage biopsy was taken from the margin of the lateral facet of the patellar groove of the femur to sterile 0.9% NaCl solution. The wound was then closed in layers. The cartilage sample was sent for chondrocyte culture to the Sahlgrenska University Hospital, Gothenburg, Sweden.

In all groups, a 6 mm diameter cartilage lesion was created on the upper part of the lateral facet of the patellar groove of the femur [Fig. 1(A)] with a dermal biopsy punch (Stiefel Laboratories Ltd, Sligo, Ireland) without penetration of the subchondral bone. This was done through a lateral arthrotomy on an unoperated knee. The cartilage was carefully excised down to the cartilage–bone interface with a knife and a curette while avoiding damage and bleeding of the subchondral bone. Then, in groups ACT-P-3, Periosteum-3, and ACT-P-12 an 8 mm (diameter) circular periosteal flap was taken with a biopsy punch from the anteromedial surface of the tibia. The flap was then sutured over the lesion with the cambium layer facing the subchondral bone using 6-0 resorbable suture material (Vicryl, Ethicon Inc.) and fibrin glue (Tisseel Duo Quick, Immuno AG, Vienna, Austria) to seal the seams [Fig. 1(B)]. Through an opening in the seam the precultured chondrocytes (3×10^6 cells in 100 μl of implantation medium) were injected underneath the periosteal flap. The opening was closed with sutures and sealed with fibrin glue. In group ACT-F-3, the 8 mm fascia flap was taken from the quadriceps muscle fascia through the arthrotomy wound and fixed as described. In groups Empty-3 and Empty-12 the lesions were left untreated. The wound was closed in layers. No immobilization was used postoperatively. Flunixin meglumin 2.2 mg/kg i.m. (Finadyne 50 mg/ml,

Table I
The procedures and follow-up times in different groups

Group	Number of samples in histological analysis	Transplanted chondrocytes	Cover	Follow-up time
ACT-P-3	14	Yes	Periosteum	3 months
ACT-F-3	6	Yes	Muscle fascia	3 months
Periosteum-3	7	No	Periosteum	3 months
Empty-3	7	No	No	3 months
ACT-P-12	6	Yes	Periosteum	12 months
Empty-12	6	No	No	12 months

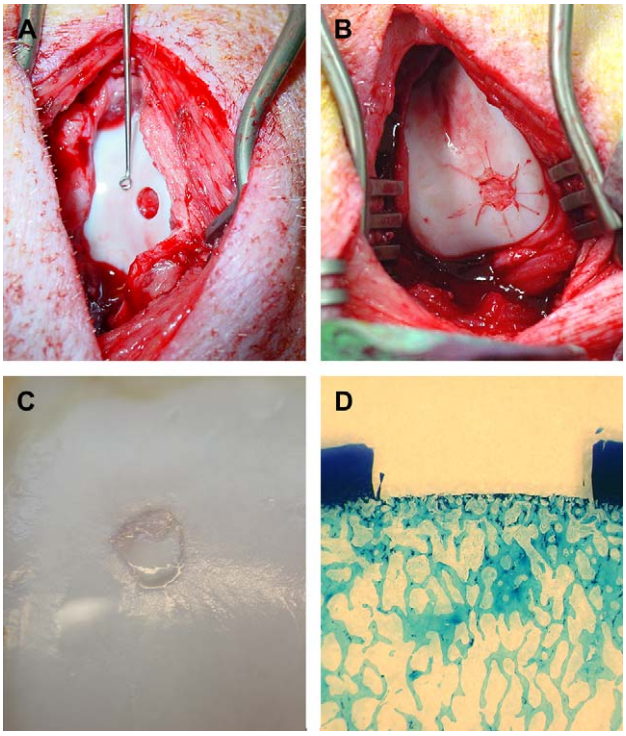

Fig. 1. A 6 mm (diameter) full thickness chondral lesion was made with a biopsy punch and then carefully curetted down to subchondral bone (A), a periosteum patch sutured over the lesion (B), and the same lesion 3 months after autologous chondrocyte transplantation (C). A microscopic image of a fresh chondral lesion showing microscopic penetrations through the subchondral bone plate (D).