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Rabbit articular cartilage defects treated by allogenic chondrocyte transplantation

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Abstract Articular cartilage defects have a poor capacity for repair. Most of the current treatment options result in the formation of fibro-cartilage, which is functionally inferior to normal hyaline articular cartilage. We studied the effectiveness of allogenic chondrocyte transplantation for focal articular cartilage defects in rabbits. Chondrocytes were cultured in vitro from cartilage harvested from the knee joints of a New Zealand White rabbit. A 3 mm defect was created in the articular cartilage of both knees in other rabbits. The cultured allogenic chondrocytes were transplanted into the defect in the right knees and closed with a periosteal flap, while the defects in the left knees served as controls and were closed with a periosteal flap alone, without chondrocytes. Healing of the defects was assessed at 12 weeks by histological studies. Allogenic chondrocyte transplantation significantly increased the amount of newly formed repair tissue ($P=0.04$) compared with that found in the control knees. The histological quality score of the repair tissue was significantly better ($P=0.05$), with more hyaline characteristics in the knees treated with allogenic chondrocytes than in the control knees. Articular cartilage defects treated with allogenic chondrocyte transplantation result in better repair tissue formation with hyaline characteristics than those in control knees.

Résumé Nous avons étudié l'efficacité de la transplantation de chondrocytes allogènes pour des pertes de substances localisées chez le lapin. Une perte de substance de 3 mm a été créée au niveau des 2 genoux. Des chondrocytes ont été cultivés in vitro à partir de

prélèvements fait sur les genoux de lapin blanc de Nouvelle Zélande. Les chondrocytes cultivés ont été transplantés dans le defect du genou droit et enfermés sous un lambeau périosté tandis que le genou gauche servait de contrôle avec un lambeau périosté sans chondrocyte. La cicatrisation cartilagineuse a été étudié histologiquement à 12 semaines. La transplantation de chondrocytes allogènes augmente significativement la quantité de tissu de réparation en comparant aux genoux de contrôle ($p=0.04$). Le score histologique est meilleur que dans le groupe de contrôle.

Introduction

Articular cartilage injuries have a very limited potential to heal, because they are avascular [2], and, thus, over a period of time may lead to secondary arthritis [4]. Articular cartilage defects are associated with pain and joint dysfunction [14].

Various treatment options have been tried for articular cartilage defects. Articular resurfacing techniques that have been used to help repair cartilage include subchondral drilling, abrasion, and the procedure termed “spongialisation” (excision of diseased cartilage and subchondral bone, leaving well-vascularised cancellous bone exposed) [9, 13]. The tissue that results from these reparative techniques is disorganised fibrocartilaginous tissue with type I collagen fibres [10, 18] which is unable to restore the biomechanical properties of normal articular cartilage [6].

Arthroscopic debridement and lavage may provide symptomatic relief for a limited time [12], although a controlled trial of debridement in patients with osteoarthritis of the knee showed the outcomes to be no better than those of treatment with a placebo [19]. Periosteal or perichondrial grafts have been used for treatment of localised cartilage defects in animals. However, the results of transplantation with both periosteum and perichondrium are inconsistent [8, 23, 24]. Thus, a variety of strategies has been employed for managing articular cartilage defects of the knee. These treatments are not always effective, and, when they are, the benefits may only be transitory [25].

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Unsuccessfully treated cartilage damage may progress to degenerative disease states and result in the need for a total knee replacement [1, 25].

In recent years the surgical implantation of chondrocytes [autologous chondrocyte transplantation (ACT)] into damaged areas has been seen as an alternative option and is currently under investigation as a potential improvement over the current strategies for the management and treatment of articular cartilage defects [25]. In this study we evaluated the transplantation of in vitro expanded allogenic chondrocytes for treatment of articular cartilage defects.

Materials and methods

Chondrocyte isolation and culture

We harvested cartilage from the knee joints of an adult New Zealand White rabbit under sterile conditions by taking small slices of cartilage with a surgical knife. The cartilage was washed three times with Dulbecco's modified Eagle's medium (DMEM, Hi Media) and further sliced into small pieces. The pieces were digested overnight at 37°C in a 5% carbon dioxide (CO₂) incubator with 0.25% collagenase (CLS-II, Worthington) in DMEM. Chondrocytes were

Table 1 Modified histological grading scale for repair of articular cartilage defects

Category score	Points
1. Filling of defect relative to surface of normal adjacent cartilage	
111–125%	1
91–110%	0
76–90%	1
51–75%	2
26–50%	3
<25%	4
2. Integration of repair tissue with surrounding articular cartilage	
Normal continuity and integration	0
Decreased cellularity	1
Gap or lack of continuity on one side	2
Gap or lack of continuity on two sides	3
3. Matrix staining with safranin O-fast green	
Normal	0
Slightly reduced	1
Moderately reduced	2
Substantially reduced	3
None	4
4. Cellular morphology of cartilage above original tidemark	
Normal	0
Mostly round cells with the morphology of chondrocytes	
>75% of tissue with columns in radial zone	0
25–75% of tissue with columns in radial zone	1
<25% of tissue with columns in radial zone (disorganised)	2
50% round cells with the morphology of chondrocytes	
>75% of tissue with columns in radial zone	2
25–75% of tissue with columns in radial zone	3
<25% of tissue with columns in radial zone (disorganised)	4
Mostly spindle-shaped (fibroblast-like) cells	5
5. Architecture within entire defect (not including margins)	
Normal	0
1–3 small voids	1
1–3 large voids	2
>3 large voids	3
Clefts or fibrillations	4
6. Architecture of surface	
Normal	0
Slight fibrillation or irregularity	1
Moderate fibrillation or irregularity	2
Severe fibrillation or disruption	3

isolated by centrifugation at 1,800 rpm for 3 min and washed twice with culture medium containing DMEM, 10% foetal bovine serum (FBS), 50 µg/ml ascorbic acid, 200 µg/ml streptomycin, 200 units/ml penicillin and 0.8 µg/ml amphotericin. The chondrocytes were counted in a haemocytometer and then resuspended in 12 ml of culture medium in a 75 cm² culture flask (Medox) and kept in a 5% CO₂ incubator. The culture medium was changed every two days. The chondrocytes formed a cell sheet when they became confluent and were isolated by trypsin treatment (0.25% trypsin EDTA, Sigma). The cell sheet was washed twice with DMEM.

Creation of articular cartilage defects and transplantation of chondrocytes

Seven adult New Zealand White rabbits (average weight 2 kg) were used. They were given food and tap water ad libitum. All animals were kept in separate cages and allowed to move freely. Surgery on the rabbits was performed under general anaesthesia using thiopental sodium (40 mg/kg) administered intra-peritoneally. In each rabbit both knees were shaved and disinfected with 70% alcohol. The right knee joint was exposed, the patella was dislocated laterally, and a defect 3 mm in diameter was created in the centre of the trochlear groove to subchondral bone with a circular stainless steel punch. Care was taken not enter the subchondral bone and cause bleeding. A periosteal flap with a diameter of 4–5 mm was harvested from the lateral side of the lateral femoral condyle. The cultured allogenic chondrocytes were transferred into the defect and closed with the periosteal flap (using octyl-cyanoacrylate), with the cambium layer facing the defect. The same procedure was repeated on the left side without chondrocyte transplantation. Thus the defect in the right knee of each rabbit was transplanted with chondrocytes and covered with a periosteal flap, while the defect in the left knee of each rabbit served as control and was covered with periosteal flap without chondrocytes. The knee joints were closed in layers and the skin was closed with absorbable suture material. The rabbits were followed up for a period of 12 weeks.

Histological evaluation

At the end of 12 weeks the rabbits were killed with thiopental sodium (70 mg/kg). The lower end of the femur was excised and fixed in 10% buffered formalin for three days. Each specimen was decalcified and embedded in paraffin. Sections 4 µm thick were prepared and stained with safranin O-fast green and haematoxylin and eosin.

The quality of the repair tissue in the articular defect that was treated with chondrocytes and covered with a periosteal flap was compared with that of the defect that was covered with a periosteal flap without chondrocytes by the modified histological grading scale described by Pineda et al. [22] (Table 1). The sections were graded according to:

(1) filling of defect relative to surface of normal adjacent cartilage; (2) Integration of repair tissue with surrounding articular cartilage; (3) matrix staining with safranin O-fast green; (4) cellular morphology; (5) architecture within the entire defect (not including margins); (6) architecture of surface.

Statistical analysis

We used the Mann–Whitney U test. Statistical significance was considered when the *P* value was ≤0.05.

Results

One rabbit died during anaesthesia and another died postoperatively. The right knee of one rabbit was excluded, since there were signs of septic arthritis.

Histological findings

There was significantly better filling of the defect relative to the surface of normal adjacent cartilage in the knees that had chondrocyte transplantation than in those with periosteal covering alone (*P*=0.043). The knees containing the transplants showed more round cells with the morphology of chondrocytes (Fig. 1). There was better integration of repair tissue with surrounding articular cartilage on the transplanted side (Fig. 2). Matrix staining with safranin O-fast green was also better on the transplanted side, being normal or slightly reduced (*P*=0.054, Fig. 3). The architecture within the defect, in terms of number of voids, and also the architecture of the surface of the defect, in terms of irregularities, was not significantly different between the two knees. The overall histological score of the repair tissue was significantly better for the transplant-containing knees than for the knees with periosteal covering alone (*P*=0.05, Table 2).

Fig. 1 Articular cartilage defect area treated with allogenic chondrocyte transplantation at 12 weeks, showing hyaline characteristics. Haematoxylin and eosin, ×200

Fig. 2 Histological evaluation at 12 weeks after allogenic chondrocyte transplantation. There was better integration of repair tissue with surrounding articular cartilage. $\times 100$

Fig. 3 Articular cartilage defect area treated with allogenic chondrocyte transplantation at 12 weeks, showing hyaline characteristics. Safranin O-fast green, $\times 200$

Discussion

Until recently, efforts to induce cartilage healing and regeneration have been directed toward enhancing the natural healing potential of cartilage. In recent years much research has been done on the use of cultured chondrocytes in treating articular cartilage defects. Autologous chondro-

cyte transplantation has shown varying results in various clinical trials [3, 11, 16, 21]. Since current evidence is subject to the inherent weaknesses of case series or reports, a Cochrane review says that autologous chondrocyte transplantation must currently be considered as a technology under investigation whose effectiveness is yet to be determined in well designed and conducted clinical trials [25]. The results of ongoing randomised clinical trials will help improve this situation [25].

In our study, articular cartilage defects treated with allogenic chondrocytes resulted in significantly better repair tissue formation than those with periosteal covering alone. The repair tissue formed after allogenic chondrocyte transplantation had a significantly better histological score, more hyaline characteristics and better integration with surrounding articular cartilage than that with periosteal covering alone.

Allogenic transplantation of chondrocytes has been used with some success in experiments involving rabbits. Kawabe and Yoshinao [15] identified an immune rejection response in rabbits, which was associated with premature degeneration of the newly formed healing tissue. Noguchi et al. [20] found no difference in the healing of osteochondral defects in rats that had been treated with isogenic chondrocytes compared with those that had been treated with allogenic chondrocytes, both of which were carried in collagen gels.

Despite the strong expression of histocompatibility antigens on chondrocytes, cartilaginous tissues seem to be protected from immunological attacks because the chondrocytes are surrounded by matrix, which has little antigenicity and is avascular [5, 7, 17].

Use of allogenic, cultured, cartilage cells can be advantageous over autologous methods, if the success rates of both the procedures are equivalent. An allogenic approach would require only a single surgical procedure, as compared to autologous methods, which requires two procedures: one to obtain tissue from which to isolate the cells to make the implant, and one to implant the autograft. The morbidity of two operations and anaesthetics can be avoided. The costs involved for operations and autologous cultures can be avoided in allogenic transplantation, especially in developing countries. Allogenic cartilage

Table 2 Histological score of the repair tissue

Question	Left knees (control) Median score (minimum, maximum)	Right knees (study) Median score (minimum, maximum)	<i>P</i>
Filling of defect relative to surface of normal adjacent cartilage	4.0 (1.0, 4.0)	0.5 (0.0, 3.0)	0.043
Integration of repair tissue with surrounding articular cartilage	3.0 (2.0, 3.0)	2.0 (0.0, 3.0)	0.10
Matrix staining with safranin O-fast green	2.0 (0.0, 4.0)	0.0 (0.0, 1.0)	0.054
Cellular morphology	5.0 (1.0, 5.0)	1.0 (0.0, 5.0)	0.1
Architecture within entire defect (not including margins)	1.0 (0.0, 4.0)	0.0 (0.0, 3.0)	0.418
Architecture of surface	1.0 (0.0, 1.0)	1.0 (0.0, 1.0)	0.655
Total score	16.0 (5.0, 19.0)	5.0 (2.0, 13.0)	0.050

cells would be available as needed and would satisfy the large clinical demand.

Though allogenic chondrocyte transplantation in rabbits has shown reasonable success, further studies on immune responses are necessary before being translated to humans. We have shown that allogeneically cultured cartilage cells in vitro lead to repair of chondral defects in rabbits. There was no evidence of mononuclear cell infiltrates suggesting host-versus-graft reaction. Hence, allogenic chondrocyte culture and transplantation is a viable option that needs further evaluation in humans.

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