

# Role of autologous chondrocyte transplantation in articular cartilage defects: An experimental study

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## ABSTRACT

**Introduction:** Injuries of articular cartilage (AC) have very limited potential to heal, because they are avascular and this may subsequently lead to secondary arthrosis. Autologous cultured chondrocytes transplantation can be used to create hyaline or hyaline-like repair in a cartilage defect area. The purpose of this study was to repair artificially created full-thickness AC defects in 20 rabbit knee joints with autologous cultured chondrocytes.

**Materials and Methods:** An AC defect of 3 mm was created on the lateral condyle of both tibiae. The defect was filled with autologous chondrocytes cultured *in vitro* and fixed with fibrin, at a later stage on the left side. The right knee acted as a control. The rabbits were sacrificed after 3, 6, and 12 weeks of transplantation and the reparative tissues were analyzed macroscopically and histologically.

**Results:** Histological scores of the cultured autologous chondrocyte transplanted knees were significantly better than the control knees at 3, 6, and 12 weeks following the transplantation. Integration of repaired tissue with adjacent cartilage, hyaline characteristics of repaired tissue, maturity of cartilage, and cellularity increases with duration and is significant in chondrocytes-transplanted defects compared to control. The histological scores also become better with increasing duration of followup.

**Conclusion:** Transplantation of autologous chondrocytes cultured *in vitro* and fixed with fibrin is effective in repairing AC defects.

**Key words:** Cartilage, cultured chondrocyte transplantation, articular cartilage defects

## INTRODUCTION

Articular cartilage (AC) being avascular has very limited potential to heal following injuries.<sup>1</sup> Cartilage injuries or damages cannot be left untreated as they, over time, progress to symptomatic joint degeneration.<sup>2</sup> Treatment options range from palliative (debridement) to reparative (marrow stimulation) to restorative (osteochondral mosaicplasty and autologous chondrocyte implantation [ACI]). All of these techniques claim to improve the clinical status compared with the preoperative state.<sup>3</sup> Arthroscopic debridement and lavage may provide symptomatic relief for a limited time, however, the long term results are comparable

to placebo treatment.<sup>4,5</sup> Osteochondral mosaicplasty is limited by the amount of graft that can be harvested.<sup>3</sup> ACI involves the biological replacement of AC, first reported clinically by Brittberg *et al.*<sup>6</sup> which is an acceptable treatment option in appropriately indicated patients with symptomatic chondral defects. Studies regarding the use of autologous cultured chondrocytes in treatment of cartilage defects are limited.<sup>3,5,7-9</sup> Many publications in literature, not favoring the use of ACI when compared to abrasive techniques<sup>7-9</sup> are in stark contrast to the excellent results of ACI seen in *in vitro* studies and *in vivo* animal studies. This is also reflected by the fact that only one commercial ACI methodology has gained approval from the Food and Drug Administration (FDA) in the US to date and the first and only approval (Carticel) being given in 1997. Hence, there is a need to go back to the drawing board to perfect our techniques in use of ACI. In this study, we have evaluated the role of autologous cultured chondrocytes in healing artificially created cartilage defects in rabbit knee. This has been compared to control rabbits in histological terms in which cartilage defects have been created but no intervention has been done.

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Access this article online	
Quick Response Code:	Website: www.ijoonline.com
	DOI: 10.4103/0019-5413.108878

## MATERIALS AND METHODS

### Selection of animals

Twenty adult rabbits of average 2-2.5 kg weight were

chosen. They had free access to standard diet (pellets) and water. None of the rabbits had any deformity or malalignment of the limbs.

The principles of laboratory care, feeding, and sacrifice of animal were followed as defined under Indian Council of Medical Research guidelines on care of experimental animals. The study was approved by the Animal Ethics Committee of our institute.

### **Creation of articular cartilage defects**

Twenty adult rabbits were used. They were given food (pellets) and tap water for nutrition. Surgeries on the rabbits were performed under anesthesia using ketamine and midazolam administered intramuscularly. In each rabbit, both the knees were shaved and disinfected with spirit. The knee joint was exposed, the patella was dislocated laterally, and a defect 3 mm in diameter was created in the center of the lateral tibial condyle up to the subchondral bone with a circular stainless steel biopsy punch [Figures 1 and 2]. Care was taken to take out only the cartilage and not to damage

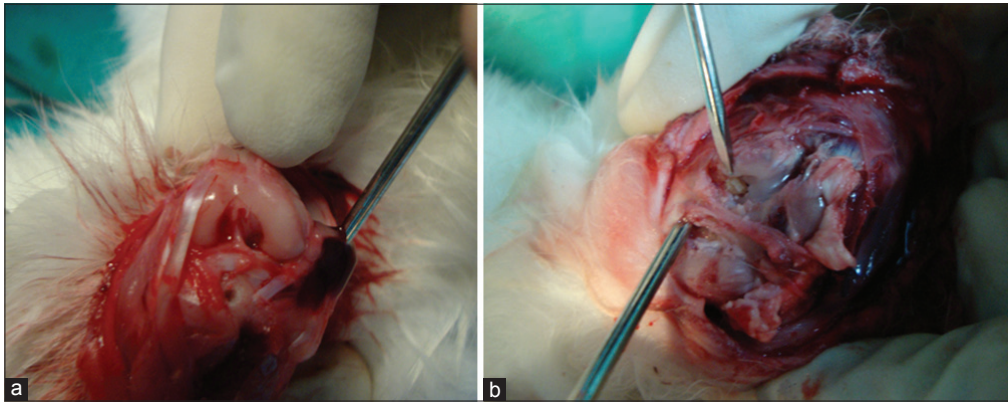
the underlying bone. The wound was closed in layers.

### **Chondrocyte isolation and culture**

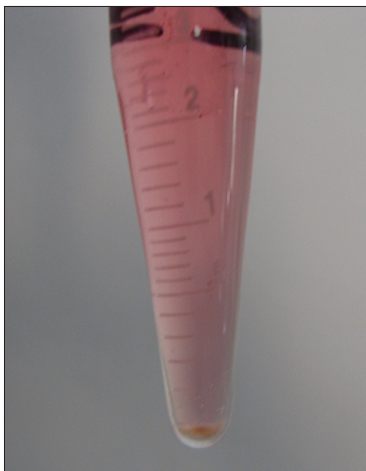
The cartilage was washed three times with Dulbecco's modified Eagle's medium (DMEM) and further sliced into small pieces. The pieces were digested overnight at 37°C in a 5% carbon dioxide (CO<sub>2</sub>) incubator with 0.25% Worthington collagenase type II (CLS-II), in DMEM. Chondrocytes were isolated by centrifugation at 1800 rpm for 3 min and washed twice with culture medium containing DMEM, 10% fetal bovine serum (FBS), 50 µg/ml ascorbic acid, 200 µg/ml streptomycin, 200 units/ml penicillin, and 0.8 µg/ml amphotericin. After centrifugation, supernatants were discarded and pellets were used for transplantation [Figure 2].

### **Transplantation of chondrocytes**

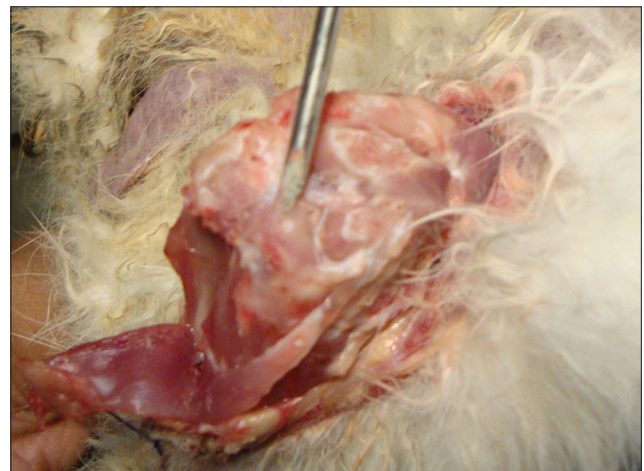
Chondrocytes were transferred into the defect by a second operation [Figure 3] on the left knee only and fixed with fibrin glue. The transplantation was usually performed 7 days following the creation of defect. Each rabbit



**Figure 1:** Peroperative photograph showing (a) Defect created in the lateral condyle of tibia of rabbit knee (b) final gross picture of chondrocyte transplanted knee at 12 weeks posttransplantation



**Figure 2:** Pellets containing *in vitro* cultured chondrocytes after centrifugation



**Figure 3:** Peroperative photograph showing defect being filled with cultured chondrocytes

was operated separately and transplanted with its own chondrocytes expanded in culture. The same procedure was repeated on the right side without chondrocyte transplantation. Thus, the defect in the left knee of each rabbit was transplanted with chondrocytes and fixed with fibrin glue while the defect in the right knee of each rabbit served as control.

### Histological evaluation

At the end of 3, 6, and 12 weeks 6, 7, and 7 rabbits were sacrificed, respectively, with thiopental sodium (70 mg/kg). The upper end of the tibia was excised and fixed in 10% buffered formalin for three days. Each specimen was decalcified and embedded in paraffin. Sections 4- $\mu$ m thick were prepared and stained with hematoxylin and eosin.

The quality of the repair tissue in the articular defect that was treated with chondrocytes and fixed with fibrin glue was compared with that of the defect that was without chondrocytes by the modified histological grading scale described by Pineda *et al.*<sup>10</sup> [Table 1].

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

Category score	Points
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
Integration of repair tissue with surrounding AC	
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
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 (disorganized)	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 (disorganized)	4
Mostly spindle-shaped (fibroblast-like) cells	5
Architecture of surface	
Normal	0
Slight fibrillation or irregularity	1
Moderate fibrillation or irregularity	2
Severe fibrillation or disruption	3

AC = Articular cartilage

Mann Whitney U test was used for statistical comparison of Pineda scores scores. *P* value of < 0.05 was considered significant.

### RESULTS

The amount of repaired tissue and filling of defects was more on the transplanted side. It had better integration of repaired tissue with normal cartilage compared to control. It had more round cells with morphology of chondrocytes (hyaline cartilage) (less score according to Pineda *et al.* scoring) [Figure 4a] while the other side had spindle-shaped cells [Figure 4b] (fibrocartilage) (higher score according to Pineda *et al.* scoring) and few round cells. The maturity of cartilage and hyaline characteristics were better observed after 6 and 12 weeks [Table 2].

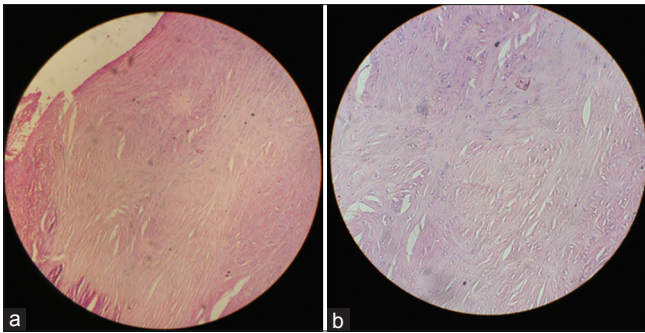
The differences in the scores in all four variables at 3, 6, and 12 weeks were statistically significant (*P* < 0.05).

### DISCUSSION

AC injury is a common disorder of joints which can affect people of all ages, resulting in a spectrum of clinical presentations. Although AC has a poor ability to regenerate itself, there is potential for repair. Until recently, efforts

**Table 2: Comparison of Pineda<sup>10</sup> *et al.* histological grading scores between the autologous chondrocyte transplanted [left] and control knee [right]**

Grading	Mean ( <i>n</i> =6)	
	Left knee (study)	Right knee (control)
After 3 weeks		
Filling of defect relative to surface of normal adjacent cartilage	1.33	3.67
Integration of repair tissue with surrounding articular cartilage	2.00	3.00
Cellular morphology	2.67	4.33
Architecture of surface	2	2.67
Total	8.00	13.67
Mean ( <i>n</i> =7)		
After 6 weeks		
Filling of defect relative to surface of normal adjacent cartilage	1.25	3.00
Integration of repair tissue with surrounding articular cartilage	2.00	2.75
Cellular morphology	2.25	4.50
Architecture of surface	1.00	1.75
Total	6.50	12.00
After 12 weeks		
Filling of defect relative to surface of normal adjacent cartilage	1.00	3.00
Integration of repair tissue with surrounding articular cartilage	1.50	2.50
Cellular morphology	2.00	3.50
Architecture of surface	1.00	2.00
Total	5.50	11.00



**Figure 4:** (a) Histology of chondrocyte transplanted knee at 12 weeks showing abundance of chondrocytes (round cells with central nuclei) (b) histology of control knee at 12 weeks showing more of fibrocytes (spindle shaped cells)

to induce cartilage healing and regeneration have been directed toward enhancing the natural healing potential of cartilage. In recent years, much research has been carried out on the use of cultured chondrocytes in treating AC defects. Autologous chondrocyte Implantation (ACI) has shown varying results in various clinical trials. In our study, AC defects treated with autologous chondrocytes resulted in significantly better repair tissue formation than those without transplantation. The repair tissue formed after ACI had a significantly better histological score [Pineda *et al.*] [Table 2], more hyaline characteristics, and better integration with surrounding normal AC.

Several methods have been used to improve regeneration potential of AC by implanting cell or tissue phenotypes that have chondrogenic potential.<sup>7-9</sup> Most of the recent approaches for cartilage repair of chondral defects may include one or more of the following techniques:<sup>7-9</sup> Providing a matrix, carrier, or support for cells to facilitate or direct synthesis of new cartilage; Providing a new population of cells to express chondrocytic phenotype to synthesize new cartilage – this source may be an autogenous or allogeneic tissue graft, or cells manipulated in culture; and inducing existing chondrocytes to more actively repair the defect through enhanced proliferation, migration, and synthesis, often through application of soluble regulators (cytokines or growth factors).

Though ACI has been used in human subjects since 1994,<sup>6</sup> the results of this procedure in human subjects as reported in the literature, are not very satisfactory. There are two generations of ACI in practice. First-generation techniques inject chondrocytes under a periosteal or fibrin patch. Second- and third-generation techniques include combinations of autologous or allogeneic chondrocytes, minced cartilage, scaffolds or matrices, and growth factors.

Magnussen *et al.*<sup>7</sup> did a systematic review to study whether “advanced” cartilage repair techniques (osteochondral

transplantation or ACI) showed superior results when compared with abrasive techniques for the treatment of isolated AC defects. They found a total of five randomized controlled trials (RCT)<sup>7</sup> and one prospective comparative trial<sup>7</sup> that met their selection criteria and they concluded that no technique had been shown to produce superior clinical results for treatment of AC defects with the available followup. They stated that, “any differences in outcome based on the formation of articular rather than fibrocartilage in the defect may be quite subtle and only reveal themselves after many years of followup.”

In a RCT of 80 patients randomized to either ACI or microfracture of the knee (an arthroscopic marrow stimulation procedure), Knutsen *et al.*<sup>8,9</sup> reported no significant differences in the treatment groups at 2-year followup in macroscopic and histologic findings.<sup>8</sup> The physical component score of the Short Form [SF]-36 was worse in the ACI group, which the authors suggest may be related to the greater surgical involvement. Five-year followup on all 80 patients revealed nine failures (23%) for both groups.<sup>9</sup> There was a trend ( $P = 0.10$ ) for earlier failure in the ACI group (26 vs. 38 months).

The methodology of use of ACI still needs refining in terms of ability to produce hyaline cartilage, filling up large cartilage defect etc., judging by the moderate clinical results of ACI when used in human subjects. *In vitro* research is an important tool for gaining knowledge of the interaction of cells, scaffolds, cytokines, and mechanical stimuli, but mimicking and duplicating the processes which take place during AC development and repair are virtually impossible. Hence, there is still a strong need for animal models in tissue engineering and experimental surgery to evaluate the expected impact of new cell-based strategies on AC repair.<sup>1</sup> This justifies the use of animal studies in perfecting our techniques in use of ACI and promoting our understanding of the behavior of cultured chondrocytes *in vivo*.

Granade *et al.*<sup>11</sup> reported successful repair of full thickness cartilage defects following implantation of cultured autologous chondrocytes in rabbit. Using knee joints of New Zealand white rabbits, a baseline study was carried out to determine the intrinsic capacity of cartilage for healing defects that do not fracture the subchondral plate. The second experiment examined the effect of autologous chondrocytes grown *in vitro* on healing rate of defects. The results were evaluated using both qualitative and quantitative light microscopy. Macroscopic results from grafted specimen displayed a marked decrease in synovitis and other degenerative changes. In defects that had received chondrocytes transplants; a significant amount of cartilage (82%) was reconstituted compared to grafted controls (18%).

In our study, healing of defects was assessed at 3, 6, and 12 weeks by gross and microscopic examination. Amounts of repaired tissues were more in chondral defects treated by chondrocytes compared to controls. Histological scoring was more significant in chondrocytes-transplanted defects at 3, 6, and 12 weeks and also significance increased with duration.

In our study, the defects were filled with chondrocytes and fixed with fibrin. Fibrin is formed from a reaction between fibrinogen and thrombin producing a natural three-dimensional matrix which has favorable biodegradability characteristics producing nontoxic physiological substances. Fibrin was obtained from rabbit's blood, drawn from its ear lobe. After the blood had clotted the plasma was separated from the clot and the clot containing the fibrin was used as a fibrin fixation. Fortier and Nixon *et al.*<sup>12</sup> have successfully used fibrin composites as three dimensional scaffolds to support chondrocytes and mesenchymal stem cells *in vitro* and equine cartilage defects *in vivo*. The same group further showed that chondrocytes in a fibrin composite could be up-regulated by insulin-like growth factor (IGF-1) producing a greater amount of tissue with increased extracellular matrix and collagen protein.

Plonczak *et al.*<sup>13</sup> repaired full-thickness AC defects in rabbit knee joints with autologous chondrocytes cultured *in vitro* and placed into the knee on a polysulfonic membrane. At 8 weeks after the operation, the reparative tissue was analyzed macroscopically and histologically. At 8 weeks after the operation, the surfaces of the reparative tissue were smooth, and the defects were filled with mature hyaline cartilage in five cases. In two cases, the reparative hyaline cartilage was immature and there was worse integration of grafted tissue into the adjacent normal cartilage. In two cases, the surface of the grafted area was irregular, and the reparative tissue was disintegrated. We found round cells with morphology of chondrocytes in transplanted chondral defects, compared to control which had few round cells and majority of spindle cells. Repaired cartilage after 3 weeks was immature and maturity increases after 6 weeks and then after 12 weeks. Integration of repaired cartilage with surrounding cartilage is significant compared to control. This study shows that cartilage healing with autologous cultured chondrocytes progressed faster (12 weeks) when culture medium containing DMEM, FBS, ascorbic acid, streptomycin, penicillin, and amphotericin is used in culture. Fibrin glue fixation to cover the defect had also helped in hasten the process of healing.

Boopalan *et al.*<sup>14</sup> used allogeneic chondrocyte transplantation for focal AC defects in knee joints of rabbits. Healing of the defects was assessed at 12 weeks by histological studies. Allogeneic 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 allogeneic chondrocytes than in the control knees. We have used autologous chondrocytes in our experimental model to avoid immunogenic reaction. Allogeneic chondrocytes might be rejected from the defect due to an immune response. Kawabe and Yoshinao<sup>15</sup> identified an immune rejection response in rabbits, which was associated with premature degeneration of the newly formed healing tissue. Noguchi *et al.*<sup>16</sup> found no difference in the healing of osteochondral defects in rats that had been treated with isogenic chondrocytes compared with those treated with allogeneic, both of which were carried out in collagen gels.

We found filling of defects more after 12 weeks, compared to those after 3 and 6 weeks. It was more significant in chondrocytes-transplanted defect compared to control. The overall histological score also becomes better than control with increasing duration in all rabbits. Breinan *et al.*<sup>17</sup> reported no difference between implants of periosteum with autologous chondrocytes compared with periosteum alone 12-18 months after surgery. However, in a substantial number of animals, unintentional penetration of the subchondral bone had occurred.

We found that the results were promising and excellent in experimental study in rabbit. A trial of chondrocytes implantation can be tried in chondral defects of humans although proliferation rate of human chondrocytes is much slower than that of rabbit chondrocytes. Long term followup of such patients show better results as confirmed by following study of autologous chondrocytes implantation.

Autologous chondrocytes implantation represents a promising treatment modality in the surgical management of AC defects when used judiciously in laboratory setting in rabbits. Short to medium term results reported to date have shown largely positive outcomes, and long term followup is awaited.

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**How to cite this article:** Rastogi A, Srivastava P, Iqbal Z, Kumaraswamy V, Singh RP. Role of autologous chondrocyte transplantation in articular cartilage defects: An experimental study. *Indian J Orthop* 2013;47:129-34.

**Source of Support:** Nil, **Conflict of Interest:** None.

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