Joint distraction and movement for repair of articular cartilage in a rabbit model with subsequent weight-bearing

T. Nishino, F. Chang, T. Ishii, T. Yanai, H. Mishima, N. Ochiai

From University of Tsukuba, Ibaraki, Japan

We have previously shown that joint distraction and movement with a hinged external fixation device for 12 weeks was useful for repairing a large articular cartilage defect in a rabbit model. We have now investigated the results after six months and one year. The device was applied to 16 rabbits who underwent resection of the articular cartilage and subchondral bone from the entire tibial plateau. In group A (nine rabbits) the device was applied for six months. In group B (seven rabbits) it was in place for six months, after which it was removed and the animals were allowed to move freely for an additional six months. The cartilage remained sound in all rabbits. The areas of type II collagen-positive staining and repaired soft tissue were larger in group B than in group A.

These findings provide evidence of long-term persistence of repaired cartilage with this technique and that weight-bearing has a positive effect on the quality of the cartilage.

Osteoarthritis (OA) is associated with loss of movement at a joint owing to cartilage degeneration. Joint distraction, using a bridging external fixation device (EFD) across the joint, offers the potential for repair.\(^1,2\) It has been shown that mechanical stimulation through joint distraction and/or joint movement promotes the repair of articular cartilage.\(^3,5\) In 2005 we described a model of a large defect of the articular cartilage in rabbits fitted with a hinged EFD.\(^6\) In that experiment, a full-thickness defect in the cartilage of the tibial plateau and subchondral bone was created by resection using an oscillating saw, exposing trabecular bone. We proposed that the space created by distraction would provide a suitable environment for mesenchymal cells and proliferation which, with movement of the joint, would establish a physiological setting where mechanical and hypoxaemic stress, plus nutrient diffusion, would promote cell growth and differentiation into articular cartilage. Using this model we evaluated the short-term effects on cartilage repair for up to 12 weeks after the operation, during which the EFD maintained the joint distraction and movement. The advantages of this procedure are that exogenous factors, carriers and transplanted cells are not needed. However, the long-term results are important if it is to be considered for clinical use.

We now present more recent investigations on the longer term results at six months and one year after the operation. We anticipate that this procedure could be successfully applied in the treatment of OA.

Materials and Methods

A total of 16 Japanese white male rabbits (Tokyo Experimental Animals, Tokyo, Japan) which were 16 weeks old, with a mean weight of 3.1 kg (2.96 to 3.28) were studied. Following a two-week period of acclimatisation, the rabbits were anaesthetised with intravenous pentobarbital (50 mg/kg body-weight), and an originally constructed Ilizarov-type half-ring EFD with a hinge was fixed to the left knee of each rabbit using fine wires (Fig. 1). The EFD was adapted to the size and anatomy of the rabbits, as in previous studies.\(^6,7\) The patella was everted once the EFD was loosened, the cruciate and collateral ligaments were divided and the menisci resected. A 3 mm deep, full-thickness osteochondral defect was then made by excising the entire surface of the tibial plateau using an oscillating saw. The space formed at the femorotibial joint by the resected osteochondral defect was maintained by the EFD. After the operation, movement was unrestricted and the rabbits were allowed free access to food and water. They were divided into two groups. In group A \(n = 9\), the distraction and movement with the EFD were maintained for six months, and the animals were then killed. In group B \(n = 7\) the distraction and movement were maintained for six...
months after which the devices were removed under sedation with an intramuscular injection of medetomidine hydrochloride (67 μg/kg body-weight) and midazolam (0.67 mg/kg body-weight). These rabbits were then allowed to move without restriction for a further six months, and killed one year after surgery. All rabbits were killed with a lethal injection of pentobarbital sodium. The study was approved by the University Committee for Animal Experimentation.

**Computed tomography.** This was used for morphological observations of the proximal tibia using a LaTheta LCT-100 scanner (Aloka, Tokyo, Japan). Immediately after the rabbit was killed, the distal femur and proximal tibia were explored and a segment of bone was taken 20 mm from the centre of the knee joint. The excised knee joints were positioned in almost complete extension and secured in a 45 mm imaging tube. Then imaging was performed to obtain 40 sagittal slices, approximately 0.5 mm thick from each knee joint. The excised joints were then immersed in 10% buffered formalin. From the 40 images, the middle slice of each medial and lateral tibial plateau was selected, having been determined by the shape and size of the adjacent femoral condyle. The surface characteristics of the proximal tibia were defined as regular or irregular, where regular surfaces had continuity over the whole plateau, whereas irregular surfaces had gaps or defects in any part of the surface.

**Histological evaluation.** At necropsy, the appearances were examined macroscopically and photographed. The proximal tibia was resected and fixed in 10% neutral buffered formalin for at least one week and decalcified with 0.5 M of ethylenediaminetetraacetic acid solution and mounted in paraffin. Then, 5 μm thick sagittal sections were taken at the mid-portion of the medial and lateral tibial plateau for histological analysis. The specimens were stained with haematoxylin and eosin, and safranin-O/fast green. For immunohistological staining, sections were deparaffinised with xylene and rehydrated with decreasing concentrations of ethanol solutions. They were incubated with 3% H2O2 for ten minutes, and proteinase K (Dako, Glostrup, Denmark) for ten minutes to block the activity of endogenous peroxidase. Further incubation was then undertaken with monoclonal mouse antibodies for type I and type II collagens (type I: Sigma, St. Louis, Missouri; type II: Oncogene, Darmstadt, Germany) at the optimal dilution in 0.05 M tris hydroxymethyl aminomethane (tris) buffered saline for one hour. The sections were rinsed with 0.05 M tris buffered saline three times for five minutes each, and then incubated with a hypersensitive chemical detector Envision and System labelled polymer HRP (Dako) for 30 minutes. They were rinsed three times with tris buffered saline and then incubated with diaminobenzide (DAB) solution (Dako) for a few minutes. The central one-third of the regenerated

![Figure 1a](image1a.png)  ![Figure 1b](image1b.png)  ![Figure 1c](image1c.png)

Figure 1a – Anterior view of a left knee secured with the external fixation device; Figure 1b – flexion and Figure 1c – extension of the knee.
tissue was assessed and scored blindly using a grading scale modified from the International Cartilage Repair Society (ICRS) visual histological assessment scale.6 The original ICRS scale comprised six criteria8 to which we added additional points, namely staining of type I and type II collagens (Table I).

<table>
<thead>
<tr>
<th>Feature</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Surface</td>
<td>Smooth/continuous: 3, Discontinuous/irregularities: 0</td>
</tr>
<tr>
<td>II Matrix</td>
<td>Hyaline: 3, Mixture: hyaline/fibrocartilage: 2, Fibrocartilage: 1, Fibrous tissue: 0</td>
</tr>
<tr>
<td>III Cell distribution</td>
<td>Columnar: 3, Mixed/columnar-clusters: 2, Clusters: 1, Individual cells/disorganised: 0</td>
</tr>
<tr>
<td>IV Cell population viability</td>
<td>Predominantly viable: 3, Partially viable: 1, &lt; 10% viable: 0</td>
</tr>
<tr>
<td>V Subchondral bone</td>
<td>Normal: 3, Increased remodelling: 2, Bone necrosis/granulation tissue: 1, Detached/fRACTure/cALLus at base: 0</td>
</tr>
<tr>
<td>VI Cartilage mineralisation (calcified cartilage)</td>
<td>Normal: 3, Abnormal/inappropriate location: 0</td>
</tr>
<tr>
<td>VII Type I collagen staining of the matrix</td>
<td>Normal or nearly normal: 3, Slight: 2, Moderate: 1, Abundant: 0</td>
</tr>
<tr>
<td>VII Type II collagen staining of the matrix</td>
<td>Normal or nearly normal: 3, Moderate: 2, Slight: 1, None: 0</td>
</tr>
</tbody>
</table>

Histomorphometric study. Digital images of the sections were obtained, and the repaired articular surface on the reformed tibial plateau was defined. The area of repaired soft tissue, stained by safranin-O followed by type II collagen antibody, was measured using the public domain analysis software available from the National Institute of Health website,9 according to the method described previously.6 In the repaired soft tissue, the areas of fibrous tissue, synovial tissue and meniscus-like tissue attached to the articular capsule were excluded from the measurement area. The sections were examined and scored blindly by a researcher (TY) familiar with the histological evaluation of repaired cartilage. In order to clarify the relationship between the CT scan and histological evaluation, the results from both were compared statistically. Statistical analysis. Statistical significance was determined using the Mann-Whitney U test for differences in the surface continuity of the proximal tibia on CT, and the modified ICRS visual histological assessment scale for each criterion. For comparisons of the areas between the two groups of the repaired soft tissue, the safranin-O-positive area and the type II collagen-positive area were compared using Welch’s t-test. The ICRS visual histological assessment scale was compared using the Mann-Whitney U test, and regenerated soft tissue, safranin-O-positive area, and type II collagen-positive area were compared to the CT findings using Welch’s t-test. For comparison of previously published modified ICRS scores with those of the current study, the Kruskal-Wallis test was used. Statistical analyses were performed using the software package Statcel (OMS, Saitama, Japan), and a p-value < 0.05 was considered significant.

Results
Visual inspection. The animals had almost normal movement both during EFD fixation and after its removal. In most rabbits the synovial tissue and fluid were macroscopically normal when the joints were exposed, although two joints out of seven in group B had bony ledges shaped at the anterior and posterior edges of the joint of each femoral condyle. In all joints, regenerated white soft tissue was observed on the surface of the tibial plateau that had been resected during the operation. The central zones on the medial and lateral plateau facing the femoral condyles were concave. Exposed subchondral bone was observed macroscopically in only one joint out of seven in group B. There was evidence of meniscus-like tissue peripherally, and cruciate-like tissue in the intercondylar notch. Ulceration of cartilage was sometimes observed on the articular surface of the femoral condyle. Ectopic bone formation was not observed in any specimen. No other differences were apparent on visual inspection.

CT imaging analysis. CT images showed 11 regular and seven irregular surfaces in group A and 11 regular and three irregular surfaces in group B (Fig. 2). There was no significant difference (Mann-Whitney U test, p = 0.403) in the rate of irregular surface between the two groups. There was only one joint in the whole series that appeared irregular on the medial and lateral halves of the plateau, occurring in an animal in group A. On all other occasions where irregularity was found it had occurred on either the medial or the lateral side in isolation.

Histological findings. In group A (Figs 3 and Fig. 4a to f) there was a small amount of fibrous tissue and repaired soft
tissue. In the regenerated soft tissue, the cartilage matrix stained weakly with safranin-O just above newly formed subchondral bone. Most of the tissue stained with safranin-O was also stained with type II collagen antibody and poorly stained with type I collagen antibody. In a few sections fibrous tissue was abundant, without staining for either antibody. In contrast, in group B (Figs 3 and 4 g to l) most of the surface was covered with repaired tissue on safranin-O staining. In contrast to group A, safranin-O staining was more extensive and thicker, and some cells had

CT images showing sagittal morphological findings in the knee joint. The surface continuity of the proximal tibia is regular in (a) and (c), and irregular in (b) and (d).

Photomicrographs showing histological findings stained with safranin-O in all specimens (magnification × 12.5; 18 photomicrographs for group A and 14 for group B).
Photomicrographs showing histological findings from group A (a to f) and group B (g to l). Safranin-O staining (a, g magnification × 12.5; b, h magnification × 40; c, i, ix magnification × 400) and immunohistological staining of type I (d, j magnification × 100) and type II (e, k magnification × 40; f, l magnification × 400) collagen. The left side indicates anterior in all photomicrographs.

<table>
<thead>
<tr>
<th>Table II. Mean and SD of the scores of groups A and B according to the modified International Cartilage Repair Society scale</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>Group A (SD)</td>
</tr>
<tr>
<td>Group B (SD)</td>
</tr>
<tr>
<td>p-value*</td>
</tr>
</tbody>
</table>

*The difference was not statistically significant in each criterion (Mann-Whitney U test)

a columnar arrangement. The surface showed extensive positive staining for type II collagen antibody; there was almost no staining for type I collagen antibody.

In the modified ICRS visual histological assessment scale, the mean scores, except for criterion IV, of group B, were greater than those of group A, but there were no significant differences between the two groups (Table II).

**Histomorphometric.** The areas of repaired soft tissue and type II collagen-positive staining were significantly larger in group B than in group A (Welch’s test, p = 0.034 and 0.042). The area of safranin-O positive staining was larger in group B than in group A, although the difference was not statistically significant (p = 0.07) (Fig. 5).

**Comparison between CT and histological findings.** Table III shows the relationship between surface continuity on CT and the modified ICRS visual histological assessment scale. All criteria scored significantly higher in the regular group than in the irregular group, except for criterion IV.
There was no difference in the area of repaired soft tissue between the regular and the irregular groups, although safranin-O-positive and type II collagen-positive areas were significantly larger in the regular group than in the irregular group (Welch’s t-test) for repaired soft tissue and type II collagen-positive staining (Fig. 6).

**Discussion**
This study demonstrated that the extensive cartilaginous repair that developed following joint distraction and movement which was present at six months in rabbits showed superior histomorphometric findings after an additional six months. There were significant differences (Welch’s t-test) for safranin-O-positive staining and type II collagen-positive staining.

---

**Table III.** Mean and SD of the scores according to the modified International Cartilage Repair Society scale between the two groups classified by CT imaging

<table>
<thead>
<tr>
<th></th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular (n = 22) (SD)</td>
<td>1.09 (1.48)</td>
<td>1.50 (0.60)</td>
<td>1.41 (0.85)</td>
<td>2.91 (0.43)</td>
<td>2.09 (0.43)</td>
<td>2.45 (1.18)</td>
<td>1.86 (0.64)</td>
<td>2.05 (0.49)</td>
</tr>
<tr>
<td>Irregular (n = 10) (SD)</td>
<td>0.00 (0.00)</td>
<td>0.60 (0.52)</td>
<td>0.30 (0.48)</td>
<td>2.80 (0.63)</td>
<td>1.20 (0.42)</td>
<td>0.30 (0.95)</td>
<td>0.90 (0.74)</td>
<td>1.10 (0.57)</td>
</tr>
<tr>
<td>p-value*</td>
<td>0.03</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
<td>0.81</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

* Mann-Whitney U test
months of weight-bearing. We believe that changes in the mechanical environment with physiological loading after removal of the EFD promoted differentiation from repaired soft tissue to cartilaginous tissue.\textsuperscript{10,11}

There have been only a few reports on the long-term results of cartilage regeneration through differentiation of mesenchymal cells. Wakitani et al\textsuperscript{12} observed that regenerated cartilage derived from bone marrow mesenchymal cells at 24 weeks after autologous transplantation was slightly thinner than at 12 weeks in a full-thickness defect in the rabbit. With the joint distraction and movement procedure, the repaired cartilage remained for six months and did not show loss of thickness due to wear after an additional six months, despite free weight-bearing.

CT was used for morphological observation of the bone. A regular bony surface on CT showed superior histological and histomorphometric findings to an irregular surface. The regularity of subchondral bone, which is critical to cartilage repair,\textsuperscript{13} results in a stable nutritional supply to articular cartilage and articular congruity. However, loosening and bending of the wires of the EFD would have generated the irregular surface of the proximal tibia and inhibited cartilage repair.

Yanai et al\textsuperscript{6} studied joint distraction and movement for 12 weeks using the same apparatus and in the same manner (n = 6) as in the present study. Comparing their published mean scores (SD) at 12 weeks after the EFD fixation of I, 1.25 (1.54); II, 1.25 (0.75); III, 1.25 (0.97); IV, 2.83 (0.58); V, 1.42 (0.51); VI, 0.50 (1.17); VII, 1.08 (0.79), and VIII, 1.08 (0.10) for each criterion of the modified ICRS visual histological assessment scale to those of the present study, showed no significant difference among the three groups after 12 weeks of EFD, after six months (group A), and after 12 months (group B) (I p = 0.669, II p = 0.082, III p = 0.267, IV p = 0.948, V p = 0.101, VI p = 0.130, VII p = 0.181, VIII p = 0.141). In contrast, for the measured areas of the repaired soft tissue, safranin-O-positive staining and type II collagen-positive staining were significantly smaller at six months than at 12 weeks (Fig. 7), indicating that, 12 weeks of fixation is more suitable than six months for cartilage repair in this model.

These results show that control of the mechanical environment is also crucial to cartilage repair. The absence of mechanical stress during joint distraction is one of the important factors affecting the actual repair of cartilage.\textsuperscript{14} However, prolonged absence of mechanical stress weakens the cellular biochemistry and matrix synthesis, erodes the collagen network of cartilage and newly differentiated cartilage derived from bone marrow mesenchymal cells degenerates in this situation. According to Hung et al,\textsuperscript{15} nine weeks’ continuous distraction in the knee joint of a rabbit using an EFD caused morphological changes in the chondrocytes prior to degeneration of the cartilage. Changing the mechanical environment by removing the EFD improves the situation. The comparison between the 12-week and the six-month groups clearly shows that if the distraction is too lengthy it is detrimental to the quality of the cartilage.

The results of this study indicate that this situation can be reversed if the mechanical environment is improved. This observation is supported by in vitro models in which changes in mechanical stress occur at the cellular level.\textsuperscript{16} Cellular perception of mechanical stress within cartilaginous tissues is an important modulator of chondrocyte function. This is particularly the case in articular cartilage, where the sensing of mechanical forces by chondrocytes leads to profound changes in the health and function of the joint.\textsuperscript{17,18} Mechanical stresses sensed by chondrocytes are often referred to as mechano-electrophysiological events,\textsuperscript{19} and these co-ordinate with other environmental, hormonal and genetic factors to regulate the metabolic activity of chondrocytes and their contribution to the maintenance of the extracellular matrix.\textsuperscript{20}

We initially wanted to use a control group with the EFD applied for one year. However, in some preliminary experiments the pins became loose or broke, and the experiments failed when the EFD was applied for more than six months. We applied full weight-bearing quickly in group B, but in actual clinical rehabilitation programmes patients are allowed a gradual return to weight-bearing after operations for cartilage repair. There is little evidence to guide us on the appropriate manner in which loading should be restored to repaired cartilage.

Joint distraction and movement for six months made the repaired cartilage atrophic compared to our previous results up to 12 weeks, although subsequent weight-bearing after six months of joint distraction and movement, improved cartilage repair. In order to develop the technique for clinical use, further research will be required to clarify the timing of EFD removal and how to stage the re-introduction of weight-bearing.

We thank Dr A. Watanabe and Dr T. Ogawa (Department of Orthopaedic Surgery, Graduate School of Comprehensive Human Sciences, University of Tsuchiya) for their technical assistance.

No benefits in any form have been received or will be received from any commercial party related directly or indirectly to the subject of this article.

References


