Histological evaluation of internally-fixed osteochondral lesions of the knee

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We evaluated the histological changes before and after fixation in ten knees of ten patients with osteochondritis dissecans who had undergone fixation of the unstable lesions. There were seven males and three females with a mean age of 15 years (11 to 22). The procedure was performed either using bio-absorbable pins only or in combination with an autologous osteochondral plug. A needle biopsy was done at the time of fixation and at the time of a second-look arthroscopy at a mean of 7.8 months (6 to 9) after surgery.

The biopsy specimens at the second-look arthroscopy showed significant improvement in the histological grading score compared with the pre-fixation scores (p < 0.01). In the specimens at the second-look arthroscopy, the extracellular matrix was stained more densely than at the time of fixation, especially in the middle to deep layers of the articular cartilage.

Our findings show that articular cartilage regenerates after fixation of an unstable lesion in osteochondritis dissecans.

Fixation of the osteochondral fragment to the osteochondral defect is a method of treatment for osteochondritis dissecans (OCD).1-3 However, the outcome of this procedure remains controversial because the likelihood of healing of the osteochondral fragment largely depends upon its quality.4 It is essential therefore that the histological changes which occur in the osteochondral lesion after fixation are well understood.

Internal fixation has been shown to be an effective method of treatment for unstable and displaced lesions in OCD.5-10 Although this method has been evaluated in terms of the functional and radiological outcome using clinical analysis,5-10 MRI and second-look arthroscopy, to date there have been no reports of the evaluation of the histological findings of the OCD lesion before and after fixation. Our aim therefore was to assess the histological changes before and after internal fixation of the OCD lesion. Our hypothesis was that an unstable osteochondral fragment might show regeneration of cartilage after internal fixation even if it had already deteriorated.

Patients and Methods

We obtained the approval of the Local ethical committee of our university and informed consent from all the patients and their parents.

Between 2003 and 2006, ten consecutive patients (ten knees) with unstable lesions of OCD were treated by open reduction and internal fixation. All the procedures were performed by one of two senior surgeons (MO or NA) involved in the study. There were seven males and three females with a mean age of 15 years (11 to 22). They were followed up for a mean of 23 months (12 to 30). Pre-operatively, all the patients had been restricted in their daily or sporting activities. Seven had experienced locking of the knee and six were unable to run because of pain in the knee. Two patients had limited range of movement and nine did not participate regularly in athletics. The mean duration of symptoms before surgery was 5.2 months (3 to 12). None of the knees had previous surgery. The operative details of the patients are given in Table I. Standing anteroposterior, lateral and skyline radiographs of the knee and CT and MR scans had been obtained pre-operatively. In six knees, bony fragments were noticed in the joint cavity separated from the site of the defect. Six lesions involved the medial femoral condyle, two the lateral femoral condyle and two the patellar groove. In one of the knees with a lesion in the patellar groove, repair of the medial patellofemoral ligament was also performed. In five patients, the distal femoral physe was open at the time of diagnosis. The operative details including the location of the lesion, the stability of the fragment, the number of pins used and associated procedures such as autogenous osteochondral grafting were recorded (Table I).
Free fragments and their original beds were identified in cases of ‘in situ’ detachment. In four cases in which the articular cartilage was partially separated (‘in situ’ detachment) the fragment was hinged open. The bed was then debrided of fibrous tissue or calcified cartilage to expose the intact subchondral bone. If the subchondral bone was remarkably sclerotic, an autogenous osteochondral plug of diameter 4.5 mm or 8.5 mm (Mosaicplasty, Smith and Nephew, Andover, Massachusetts), obtained from the unloaded area of the femoral condyle was inserted through the centre of the lesion with the intention of encouraging the circulation of blood to the subchondral bone. Subsequently, poly-L-lactide (PLLA) pins of diameter 2 mm (Neofix; Gunze, Kyoto, Japan) were inserted around the periphery until a rigid and stable construct was obtained. In six knees with detached lesions, the free fragments were always larger than the original defects and had to be trimmed by approximately 2 mm to 3 mm around the periphery to fit the defect. The fragment was reduced and transfixed temporarily by two Kirschner (K)-wires of 1.0 mm diameter while compressing the fragment manually.

Definitive fixation was achieved by PLLA pins of 2.0 mm diameter which were inserted into pre-drilled channels of 2.0 mm diameter through the fragment in divergent directions. A needle biopsy was performed with a 14.5 gauge biopsy needle at almost the centre of the fragment and a bio-absorbable pin was inserted into the remaining channel. The heads of the pins were placed at 2 mm below the level of the surrounding articular surface to avoid impingement. The K-wires were then removed and bio-absorbable pins inserted into the channels to promote stability of the fragment. The knee was immobilised in an above-knee cast at 30° of flexion for two to three weeks. The patients were encouraged to begin quadriceps strengthening exercises immediately after surgery. After removal of the cast, they then proceeded to passive and active range-of-movement exercises. Partial weight-bearing was allowed at three to four weeks, followed by full weight-bearing at eight weeks. Full participation in sports was restricted for up to six months depending on the clinical and radiological progress.

**Operative techniques.** Standard anterior portals were created and a diagnostic arthroscopy was initially performed. Free fragments and their original beds were identified in detached cases, and the stability of the osteochondral fragments examined in cases of ‘in situ’ detachment. The knee was then exposed through a small parapatellar arthrotomy. In four cases in which the articular cartilage was partially separated (‘in situ’ detachment) the fragment was hinged open. The bed was then debrided of fibrous tissue or calcified cartilage to expose the intact subchondral bone. If the subchondral bone was remarkably sclerotic, an autogenous osteochondral plug of diameter 4.5 mm or 8.5 mm (Mosaicplasty, Smith and Nephew, Andover, Massachusetts), obtained from the unloaded area of the femoral condyle was inserted through the centre of the lesion with the intention of encouraging the circulation of blood to the subchondral bone. Subsequently, poly-L-lactide (PLLA) pins of diameter 2 mm (Neofix; Gunze, Kyoto, Japan) were inserted around the periphery until a rigid and stable construct was obtained. In six knees with detached lesions, the free fragments were always larger than the original defects and had to be trimmed by approximately 2 mm to 3 mm around the periphery to fit the defect. The fragment was reduced and transfixed temporarily by two Kirschner (K)-wires of 1.0 mm diameter while compressing the fragment manually.

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**Follow-up examination.** A second-look arthroscopy was performed to evaluate macroscopic healing at a mean of 7.8 months (6 to 9) after surgery. All the patients agreed to a second-look arthroscopy despite being asymptomatic. A needle biopsy was performed at a site as close as possible to the previous biopsy site and repaired tissues were evaluated histologically and immunohistochemically. At the final follow-up the knees were examined for range of movement, effusion and tenderness. Plain radiographs and MR scans were obtained to assess the union of the fragment. All the patients were asked to express an opinion on pain and function using the Lysholm scoring system.11

**Histological examination.** Each needle biopsy specimen was fixed with 10% buffered formalin for one day. Specimens were then decalcified with 0.25 methylenediaminetetra-acetic acid in phosphate buffered saline (PBS) at a pH of 7.5, dehydrated in graded alcohol, and embedded in paraffin wax. They were cut sagittally into sections 5 μm thick and stained with Safranin O/Fast Green. Histological sections were graded according to the scale described by Mankin et al12 (Table II) by a pathologist (KA) who was not provided with any information on the patients. In this grading system, the articular cartilage is evaluated for structure (0 to 6 points), cells (0 to 3 points), Safranin O staining (0 to 4 points), and tidemark integrity (0 to 1 points) (0 point for normal cartilage) (Table II).

Expression of type-II collagen within biopsy specimens was analysed immunohistochemically. Sections (5 μm) were cut, air-dried, deparaffinised, rehydrated, and incubated for three minutes in PBS. In order to abolish endogenous peroxidase activity, they were incubated with 0.3% enzymatically digested by testicular hyaluronidase type VIII (0.2% in PBS, pH 7.4) for 30 minutes at 37°C. They were then incubated overnight in a 1:100 dilution of type-II...
collagen antibody (Oncogene Research Products, San Diego, California) at 4°C. Immunoreactivity was detected by the serial incubation of sections. The signal was developed as a brown reaction product with the use of peroxidase substrate 3, 3’-diaminobenzidine, and H2O2. The sections were then counterstained with Harris haematoxylin and dehydrated, cleared and mounted.

**Statistical analysis.** This was performed using Statview 5.0 (SAS Institute, Cary, North Carolina). All data were shown as the mean SEM. The histological scores relating to loose bodies and in situ detachment before and after fixation were assessed using analysis of variance (ANOVA) with Bonferroni/Dunn post hoc comparison. A p-value < 0.01 was taken to be statistically significant.

**Results**

**Clinical and radiological evaluation.** The mean size of the defect was 399 mm² (80 to 900). In three knees, the fragment was fixed by the combination of an osteochondral plug (4.5 mm or 8.5 mm in diameter) taken from the ipsilateral femoral trochlear and the use of bio-absorbable PLLA pins (mean 3.3 pins, 1 to 7). In the other seven knees, the fragment was transfixed by PLLA pins alone (mean 4.0 pins, 2 to 7). There was no intraoperative complications related to the insertion of the pins. Two patients (2 knees) were found to have a lateral discoid meniscus and underwent a partial lateral meniscectomy at the same time. Union of the fragment was obtained in all knees, with no pain or locking. Serial radiographs revealed no redisplacement of the fragment. None of the patients treated by bio-absorbable pins had synovitis during the follow-up period. At the final follow-up, all were satisfied and there was no swelling or limitation of movement of the knee. The mean Lysholm score improved from a pre-operative value of 70.5 points (58 to 89) to 98.3 points (90 to 100) at the final follow-up. MR scans obtained at the second-look arthroscopy showed that the image intensity of the fixed fragment was similar to that of the surrounding normal articular cartilage, although the channels of the PLLA pins could be seen (Figs 1 and 2).

**Arthroscopic evaluation.** At the second-look arthroscopy, in all ten knees, although the fragment was stable to probing and had an intact smooth surface, we could distinguish between the original lesion and the normal surrounding tissue or osteochondral plug because the border was slightly concave (Figs 1 and 2). In seven knees, there was complete integration of the border of the osteochondral fragment with the surrounding articular surface. In two knees with an intact smooth surface, the fragment had fibrillation on its surface. In one knee, the surface of the fragment was soft. There were no post-operative complications.

**Histological evaluation.** The loose bodies at the time of fixation showed a tendency towards breakdown of layered structure of normal cartilage. The extracellular matrix was stained weakly by safranin O and immunohistochemical staining showed the presence of type-II collagen. However, as regards the osteochondral lesions in in situ detachment, the extracellular matrix was well stained although there was breakdown of the normal cartilage. At the second-look arthroscopy, the extracellular matrix was stained more densely than at pre-fixation especially in the middle to deep layers. In addition, the layered structure showed improvement compared with that at pre-fixation (Figs 3 and 4).

The mean histological score in the loose-body group was 8.5 (SEM 1.0) before and 1.5 (SEM 0.8) after fixation indicating a statistically significant improvement (p < 0.01). The mean histological score in the in situ detachment group was 4.5 (SEM 0.6) before and 1.0 (SEM 0.8) after fixation which was also statistically significant (p < 0.01). Statistically significant differences were noted between both groups in the histological scores before fixation (p < 0.01), whereas the difference after fixation was not significant (p = 0.44; Fig. 5).

**Discussion**

There are several surgical techniques available for the treatment of unstable OCD lesions in the knee.1-3,13 They include removal of the fragment and curettage of the crater, replacement and stabilisation of the fragment by internal fixation, allograft replacement, osteochondral autograft transplantation and autologous chondrocyte transplantation. The goal of these procedures is to produce a smooth articular surface of hyaline-like cartilage. We believe that if an unstable osteochondral fragment or

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**Table II. The modified histological grading scale of Mankin et al12**

<table>
<thead>
<tr>
<th>Structure grade</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td>Surface irregularities</td>
<td>1</td>
</tr>
<tr>
<td>Pannus and surface irregularities</td>
<td>2</td>
</tr>
<tr>
<td>Clefts to transitional zone</td>
<td>3</td>
</tr>
<tr>
<td>Clefts to radial zone</td>
<td>4</td>
</tr>
<tr>
<td>Clefts to calcified zone</td>
<td>5</td>
</tr>
<tr>
<td>Complete disorganisation</td>
<td>6</td>
</tr>
</tbody>
</table>

| Safranin O staining                   |       |
| Normal                                | 0     |
| Slight reduction                      | 1     |
| Moderate reduction                    | 2     |
| Severe reduction                      | 3     |
| No dye noted                          | 4     |

| Tidemark integrity                   |       |
| Intact                                | 0     |
| Crossed by blood vessels              | 1     |

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loose body is left in the joint with adequate conditions for fixation, internal fixation should be the preferred method of treatment because it preserves the natural contour of the articular surface.

The principles of internal fixation are the achievement of stable fixation, the promotion of a blood supply to the base of the osteochondral fragment and bone grafting at the base to promote healing with the articular cartilage over the

Case 8. A 16-year-old male with osteochondritis dissecans of the medial femoral condyle. a) Sagittal pre-operative MR scan showing a large lesion affecting the medial femoral condyle. b) Pre-fixation arthroscopic photograph showing complete detachment of the osteochondral lesion. c) Photograph of a biopsy specimen taken using a 14.5 gauge biopsy needle. d) Sagittal MR scan seven months after surgery showing consolidation of the fragment. The channel of the poly-L-lactide pin is still visible. e) Second-look arthroscopy seven months after surgery showing a smooth cartilage surface and no detachment.
osteochondral fragment being left as intact as possible. Recently, bio-absorbable pins or screws have been used for fixation of the osteochondral fragment with favourable results in unstable lesions.\(^{1,2,14-16}\) Derwin et al\(^1\) undertook internal fixation of fragments in nine skeletally mature OCD patients using PLLA rods, which resulted in radiologically stable lesions in eight. Din et al\(^9\) described 12 knees with stable osteochondral lesions treated by early fixation using bio-absorbable pins and drilling. Within six months all the lesions had united on MRI and at a mean of 32.4 months all the patients were satisfied with the outcome with no swelling or restriction of movement of the knee.

Most studies on the surgical treatment of OCD have evaluated the findings clinically and radiologically and in most a second-look arthroscopy has been performed. Only a few have examined the histological findings after fixation of the osteochondral fragment. Prokop et al\(^{17}\) studied 36 sheep with osteochondral fractures of the femoral condyle. After fixation using bio-absorbable pins, they were followed up radiologically and histologically for three years. Bone union was achieved in all knees and bio-absorbable pins did not lead to clinically significant inflammatory reactions in the joint. Touten et al\(^{18}\) evaluated 18 rabbit knees histologically and immunohistochemically after internal fixation of loose bodies to the osteochondral defects using bio-absorbable pins. They found that although histologically there was deterioration in isolated osteochondral loose bodies and repaired tissue after fixation to the defect which was related to the duration of isolation of the fragment, some loose bodies showed regeneration of cartilage after fixation. However, to date there have been no reports of the histological findings before and after fixation in patients with OCD.

In our study we demonstrated some regeneration of cartilage after fixation, showing improvement in the layered structure of the cartilage and an abundant
extracellular matrix. To our knowledge, our study is the first demonstration of regenerative change in the articular cartilage layer within an OCD lesion after its fixation which has been supported by histological evidence. The second-look arthroscopy biopsy specimens of the loose bodies and the "in situ" detachment lesions showed an abundant extracellular matrix compared with that at pre-fixation, especially in the middle to deep layers near the subchondral bone. The layered structure of cartilage also improved compared with that at pre-fixation. This suggests the possibility that even a loose body whose cartilage has deteriorated may demonstrate some
regeneration if the fragment is fixed in a stable fashion. Although we have no obvious explanation for these findings, it is possible that stable fixation leads to improvement in the nutrition of the cartilage and the subchondral bone. The deteriorated cartilage within the osteochondral fragments could regenerate through normal biomechanical conditions, including movement of the joint and weight-bearing. Therefore, if loose bodies are identified in the joint, internal fixation should be the preferred method of treatment. By contrast, Milgram\(^4\) reported in a study of 119 patients that degenerative calcification occurred in all osteochondral fractures which had been detached for more than three weeks.

There were several limitations to our study. First, the number of patients was too small to obtain definite conclusions. Although informed consent was obtained from all the patients and their parents, we limited the indications for biopsy according to the existence of loose bodies and the hope of an early return to sport, because the needle biopsy at the second-look arthroscopy is invasive. Secondly, the biopsies before and after internal fixation were not taken from exactly the same site, but from nearby. Therefore, differing biomechanical or biological conditions may have given rise to histological differences. Finally, although we mainly investigated the OCD lesions before and after internal fixation radiologically and histologically, biochemical evaluations were not performed. Additional biomechanical or biological studies, including quantitative analysis are therefore needed.

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

References


