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Novel techniques

Stem cell transplantation for the treatment of osteochondral defects of the knee: Operative technique for a single-stage transplantation procedure using bone marrow-derived mesenchymal stem cells

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A B S T R A C T

Background: Autologous chondrocyte implantation (ACI) is a NICE-approved technique to regenerate hyaline cartilage in chondral and osteochondral defects (OCDs). The drawbacks of ACI include that it requires a two-stage approach, involves a lengthy rehabilitation process and is expensive. Bone marrow harvest with mesenchymal stem cell transplantation using a single-stage procedure and an accelerated rehabilitation programme has been developed to overcome this. The aim of this paper is to describe the surgical technique for stem cell transplantation of the knee for OCDs with reference to case examples.
Methods: The surgical technique for stem cell transplantation of the knee for OCDs is described, with reference to three cases. Magnetic resonance imaging was performed at six months postoperatively.
Results: The surgical technique is described in this paper. The three patient cases described all improved clinically with reduced pain and improved function at a minimum of six months follow-up.
Conclusions: Stem cell transplantation has the potential to produce favourable outcomes for patients with osteochondral defects of the knee. This single-stage approach and accelerated rehabilitation is associated with reduced financial costs. A long-term prospective study of this technique is currently underway at our institution and randomised controlled trials are planned to demonstrate the effectiveness over other techniques.

1. Introduction

Osteochondral defects (OCDs) of the knee are a relatively common problem and may be challenging to treat [1]. Articular cartilage is avascular and consequently has limited regenerative potential. The avascularity is speculated to limit progenitor cell infiltration, which is necessary for cartilage regeneration [2,3]. Adequate treatment of OCDs is essential to prevent progressive tissue loss and ultimately degenerative joint disease. Traditional methods of treatment such as microfracture or

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mosaicplasty are generally reserved for smaller defects (i.e. <2 cm^2) and result in fibrocartilage formation, which has inferior biomechanical and biochemical properties to hyaline cartilage and over time undergoes degeneration [2,4,5].

Cell-based therapies have been developed to achieve cartilage regeneration. Autologous chondrocyte implantation (ACI) is a technique that has been shown histologically to result in the formation of hyaline or hyaline-like cartilage [5,6]. Graft survival of greater than 80% at 10 years and improved clinical outcomes compared with mosaicplasty or microfracture have been demonstrated with ACI techniques [7]. A disadvantage of ACI is that it requires a two-stage approach, firstly to harvest the chondrocytes from a non-weight-bearing area of the patient's knee and a second procedure to implant these after a period of culture-expansion in vitro. Additionally, the rehabilitation programme may last for approximately 12 months [8], and the cost of the entire procedure is prohibitively expensive. In contrast, bone marrow aspiration and stem cell transplantation may be performed as a single-stage procedure.

This paper describes the surgical technique for stem cell transplantation of OCDs of the knee and an accelerated rehabilitation protocol used in our institution. The cases of a number of patients treated with this technique, with a minimum of 18 months follow-up, are illustrated to clarify the text.

2. Methods

2.1. Indications

Stem cell transplantation is indicated for OCDs of the knee in symptomatic, motivated patients between 15 and 55 years of age. The knee should be mechanically stable with normal alignment. Where there is malalignment, either tibiofemoral or patellofemoral, this needs to be corrected. Contraindications include inflammatory arthropathy, generalised osteoarthritis (greater than Outerbridge grade 1), smoking and a body mass index greater than 35 [9].

2.2. Surgical technique

The procedure is performed under general anaesthetic, usually with a femoral or adductor canal nerve block. A diagnostic knee arthroscopy may be performed prior to bone marrow harvest to confirm the OCD and to exclude any other intra-articular pathology, but is often not necessary with up-to-date high-resolution cross-sectional imaging. Bone marrow may be harvested from the posterior superior iliac spine with the patient in the lateral decubitus position using a powered bone marrow aspiration system (OnControl® Bone Marrow Biopsy System) or a Jamshidi needle [10]. Alternatively, the bone marrow may be harvested from the anterior superior iliac spine with the patient remaining in a supine position. This negates the need for turning the patient in order to re-prep and drape. The quantity and quality of the harvested stem cells is not affected and the procedure is more streamlined. Sixty millilitres of bone marrow is harvested (Figure 1).

A tourniquet is then inflated and the knee is accessed via a mini-arthrotomy. A sharp scalpel is used to precisely cut the junction between the defective cartilage and the healthy surrounding tissue. This technique is used to avoid any injury to healthy tissue (Figure 2).

The cells are centrifuged in the operating theatre using the Autospin™ bone marrow concentration system (Magellan, Arteriocyte Medical Systems Inc.). Single use, sterile-packed containers are used to ensure sterility. The cycle time is approximately 17 min. A spin speed of approximately 2800 RPM is used to separate 93–97% of the red cells. A speed of 3800 RPM is used to concentrate the buffy coat. The centrifuged cells are then aspirated into a syringe. A collagen scaffold manufactured

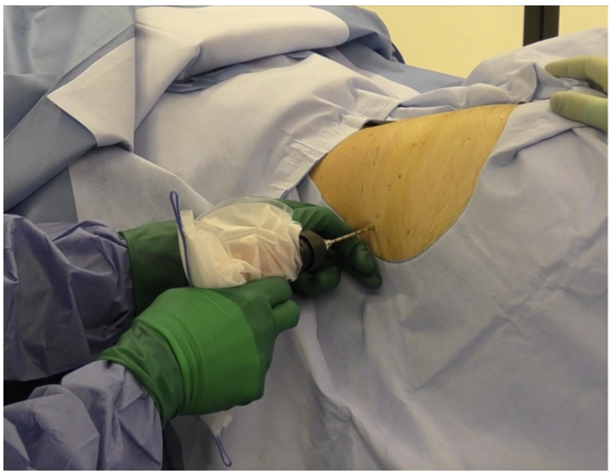

Figure 1. Bone marrow harvest from the posterior superior iliac spine using the OnControl® Bone Marrow Aspiration System.

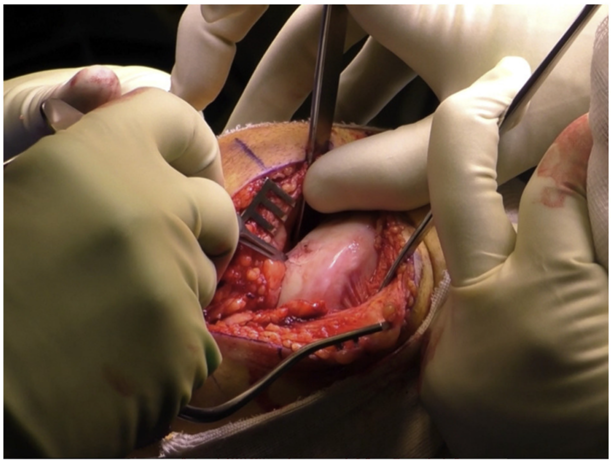

Figure 2. The junction between the defective cartilage and the healthy surrounding tissue is cut with a scalpel.

from type 1 equine collagen (Syngenit™ Biomatrix) is cut to match the size and shape of the chondral defect (Figure 3). This scaffold has a monolayer construction and a multi-dimensional crosslink structure. The scaffold is then soaked with the stem cells for two minutes (Figure 4). Using sterile forceps, the membrane may be turned over to ensure full saturation. A sample of the aspirate is taken to calculate the cell numbers using a Muse (Merck) cell analyser. This provides real-time feedback on mesenchymal stem cell number and viability. The cell count generally varies between approximately 7×10^7 and 4×10^8 depending on the aspiration technique and the number of nucleated cells in the bone marrow aspirate (patient-dependent).

The scaffold soaked with stem cells is implanted into the defect (Figure 5) and secured in place with autologous fibrin (Figure 6). This fibrin 'glue' is derived from a sample of the patient's venous blood taken intraoperatively by the anaesthetist. Thirty millilitres of the patient's blood is placed into three vactubes. Two of the vactubes contain anticoagulant citrate dextrose solution (ADCA), which is used to produce fibrinogen, whilst the third does not and the resultant clot will be stimulated to release thrombin serum. The tubes are centrifuged at a speed of 3500 RPM rating (Regen Lab SA). The processing time is five minutes for tubes containing ADCA (fibrinogen production) and 10 min for the non-ACDA tube (thrombin serum production). The fibrinogen and thrombin serum are aseptically transferred into the sterile field and the thrombin serum is combined with 0.5 mL of calcium gluconate to act as a source of calcium ions. The material transitions from a low viscosity to a dense fibrinogen membrane over a period of several minutes, which is patient-dependent. The process is accelerated when the material comes into contact with the Type 1 collagen membrane and bone marrow aspirate. This 'fibrin glue' contains a variable number of platelet-derived growth factors (TGF-B, PDGF, bFGF, IGF, VEGF, EGF) and creates a niche microenvironment for cartilage regeneration allowing fibroblast proliferation and collagen synthesis, as well as offering some protection against proteolytic degradation.

The knee is cycled between flexion and extension to confirm that the implant is stable. The knee joint and subcutaneous tissues are then closed in a standard manner and an extension brace is applied at the end of the procedure.

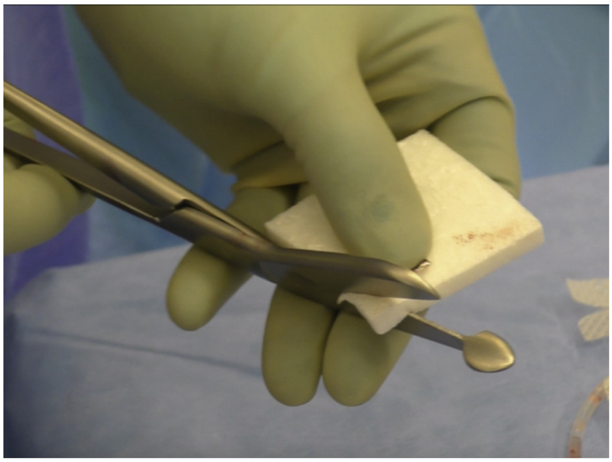

Figure 3. A type 1 collagen scaffold is cut to match the size and shape of the chondral defect.

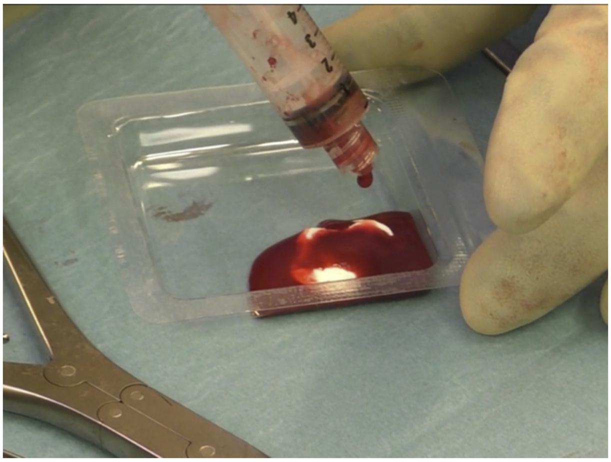

Figure 4. The scaffold is soaked with stem cells.

2.3. Postoperative rehabilitation

The knee is locked in an extension brace for the first week postoperatively. Range of movement exercises then begin after one week. Closed chain active or active-assisted range of movement knee flexion and extension, closed chain quadriceps, stationary quadriceps, co-contraction of the quadriceps and hamstrings and low resistance isometric exercises are com-

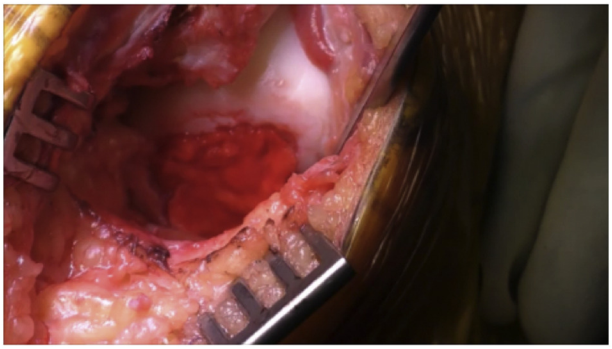

Figure 5. The scaffold soaked with stem cells is implanted into the osteochondral defect.

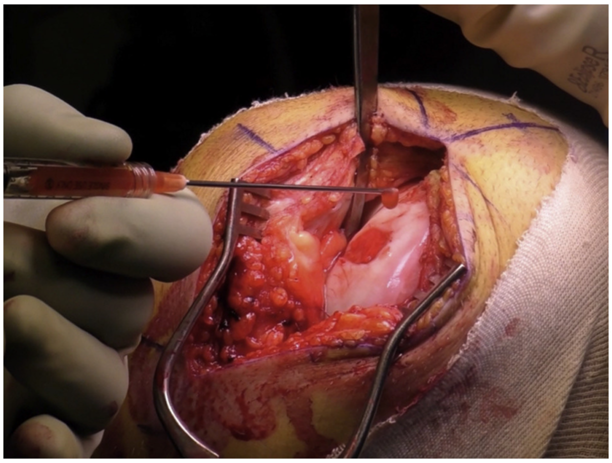

Figure 6. Autologous fibrin glue is used to secure the scaffold in place.

menced. Stationary cycling, with low resistance, may be introduced from week four postoperatively as tolerated. For patellar or trochlear grafts, patients are allowed to bear weight immediately with the knee locked in extension for four weeks. Range of movement exercises are commenced from 0 to 90 degrees of flexion until four weeks postoperatively. From four to six weeks, the brace is unlocked gradually for weight-bearing as control allows, with the aim of not wearing the brace from six weeks postoperatively. For femoral condyle or tibial plateau grafts, patients may bear weight with the protection of crutches for six weeks and progress with range of movement as symptoms allow, aiming for full range of movement at six weeks. Patients can be weaned from the brace at six weeks as control allows. After six weeks, when normal movement has returned, physiotherapy is directed at improving strength and balance/proprioception. This includes the introduction of resistance work with bands or weights. The aim is to return to all activities including full participation in sports by six months postoperatively.

2.4. Outpatient follow-up

Patients are reviewed routinely in the outpatient clinic at approximately six weeks, three months, six months and one year. A magnetic resonance imaging (MRI) scan of the knee is performed at six months to assess the cartilage repair and subchondral bone. If the MRI demonstrates good graft integration then the patient is permitted to resume full activity.

3. Results

A 38-year-old female patient was referred with a five-year history of bilateral knee pain. She had previously undergone arthroscopic chondroplasty of bilateral patellofemoral OCDs, but continued to experience severe pain. MRI revealed chondral defects of the lateral aspect of the trochlear of both knees.

The patient underwent stem cell transplantation of the left knee. The defect measured 2.88 cm^2 after debridement. At six months postoperatively she reported that her symptoms had greatly improved and her symptoms were minimal. An MRI scan showed integration of the graft and she returned to her normal activities (Figure 7). At 19 months postoperatively she remained satisfied with the results and proceeded to undergo right knee stem cell transplantation. She is currently in the early postoperative period following that procedure.

A 24-year-old male graphic designer was referred with a two-year history of left knee pain that started following a knee injury involving a twisting mechanism at work. He underwent open fixation of an OCD of the medial femoral condyle. The OCD subsequently failed to heal. He then underwent an arthroscopy a year later with removal of the fragment and microfracture. On presentation, he was unable to bear weight on his left lower limb due to pain and mobilised with crutches. He was unable to work as some of his work involved heavy lifting.

He underwent stem cell transplantation with bone graft. The lesion measured 3.74 cm^2 after debridement. The defect was deep to the subchondral bone and tricalcium phosphate was used as a bone graft substitute to the base of the defect. At six months postoperatively, he reported a significant improvement in his symptoms, with his knee being pain-free at rest with only a vague discomfort after activity. His MRI scan at six months showed maturation of the hyaline-like cartilage (Figure 8).

Restoring knee stability and alignment has previously been shown to be crucial in cartilage regeneration. A number of patients have had concurrent ligament reconstructions (anterior cruciate ligament, posterior cruciate ligament or medial patellofemoral ligament reconstructions) or osteotomies (high tibial osteotomy, distal femoral osteotomy or tibial tubercle osteotomy).

We believe the ideal patient is young, with good regenerative potential, and has had no previous intervention (Figure 9). A 26-year-old chef was referred with intermittent knee pain and locking of the knee following a skate boarding injury. Plain radiographs revealed a large loose body in the suprapatellar pouch. The chondral defect appeared to have arisen from the lateral femoral condyle. The patient underwent removal of the loose body and stem cell transplantation of a 4.41 cm^2 lateral femoral condyle OCD. At six months follow-up his pain had resolved and he returned to work.

4. Discussion

Bone marrow aspirate and stem cell transplantation is a relatively novel technique, which remains in its infancy. There are clear advantages in regenerating articular cartilage with only one procedure and at a cost saving compared with ACI. There is limited clinical data on outcomes following stem cell transplantation. Haleem et al. reported a technique where autologous bone marrow-derived stem cells were culture-expanded and transplanted in femoral condyle chondral defects [11]. Their technique differed from the technique described in this paper as they harvested only 20 mL of bone marrow from the iliac crest and due to the low stem cell numbers obtained, culture-expansion was required, thus necessitating a two-stage technique [11]. Buda et al. reported a single-stage technique using a hyaluronic acid membrane filled with 2 mL of an autologous bone marrow concentrate [10]. Their case series included patients with either medial or lateral femoral condyle defects, in contrast to ours where condylar and patellofemoral defects are both addressed (with re-alignment if necessary). Their technique also involved harvesting of blood pre-operatively the day before surgery to provide platelet-rich fibrin gel [11], whereas our technique involves fibrin gel being obtained from blood taken from the patient intra-operatively. They also performed the procedure arthroscopically whereas we believe a mini-arthrotomy is necessary to accurately debride the

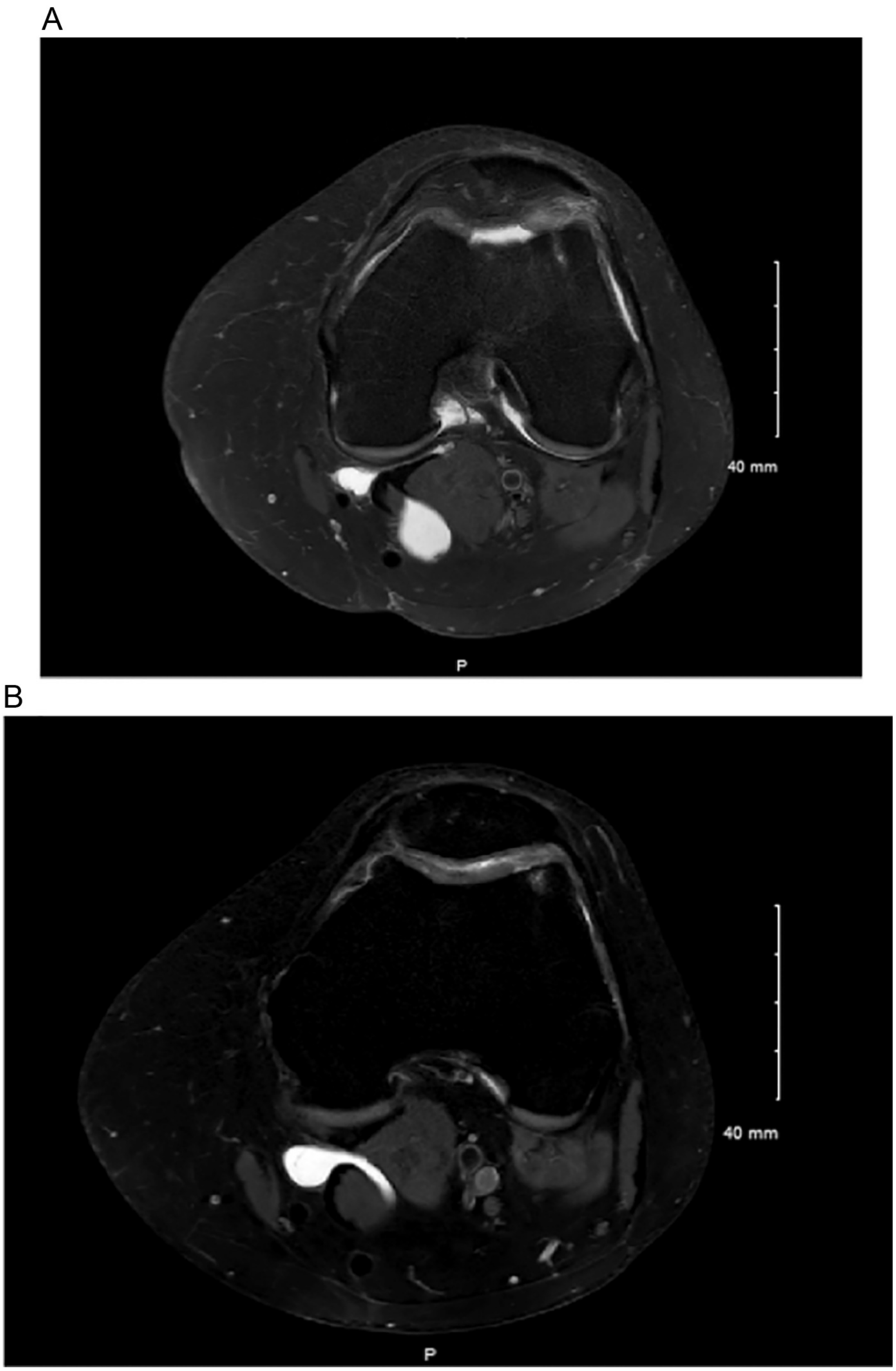

Figure 7. (a). Pre-operative MRI showing a lateral trochlear OCD. (b). Six-month post-operative MRI showing graft integration.

defect and to ensure secure fixation with fibrin glue. It is unclear if this may have accounted for the fact that Buda et al.'s postoperative rehabilitation schedule involved starting full weight-bearing and exercises focusing on recovery of muscular function at 10 weeks postoperatively [11], compared with our accelerated rehabilitation programme with these activities being commenced after the first postoperative week. This is considered important as many of our patients are young active patients and compliance with a more lengthy rehabilitation programme may be variable.

The current National Institute for Health and Care Excellence (NICE) guidance recommends ACI as an option for treating symptomatic chondral defects of the knee only if the patient has not had previous cartilage reparative surgery, based on evi-

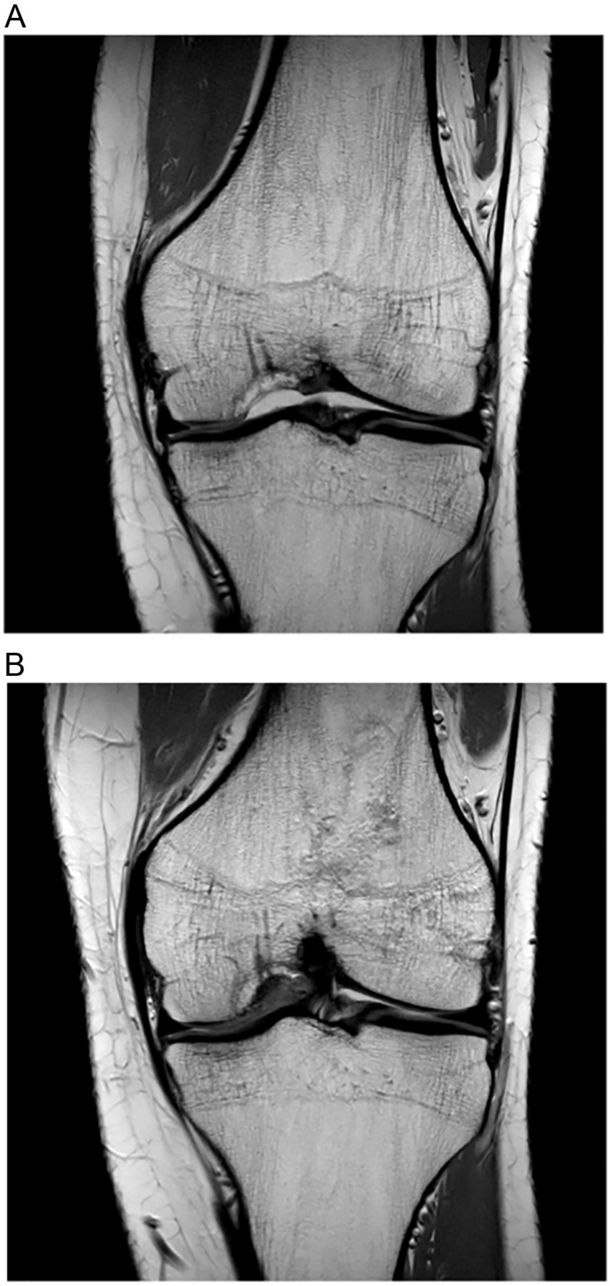

Figure 8. (a). Pre-operative MRI showing a medial femoral condyle OCD. (b). Six-month post-operative MRI showing graft integration.

dence that ACI is likely to be more successful as a first-line surgical treatment [12]. Previous reports or case series of stem cell transplantation of the knee have also focused on using this as a first-line treatment. Therefore, this suggests that the major role of stem cell transplantation would be as a first-line treatment. Previous microfracture has been associated with a three times greater failure rate of ACI [13,14]. Future research stratifying patients according to whether or not they have had previous cartilage regenerative/reparative surgery would be required to determine the efficacy in both groups.

Alternative methods of delivering stem cells to OCDs have been reported. Animal studies and a human clinical case report on the use of intra-articular injection of mesenchymal stem cells to treat OCDs have shown successful cartilage regeneration [15–19]. Intra-articular stem cell injections have been used primarily in patients with more diffuse degenerative changes where a stem cell transplant would not be an option [20–22]. Park et al. reported improved clinical outcomes in one patient using a composite of umbilical cord blood-derived allogeneic mesenchymal stem cells and a hyaluronic acid hydrogel [23]. Umbilical cord blood-derived mesenchymal stem cells were used due to their good expansile capacity, the ease and non-

Figure 9. (a). Pre-operative radiographs showing a lateral femoral condyle OCD and a large loose body in the suprapatellar pouch. (b). Six-month post-operative MRI showing graft integration.

invasive nature of collection as well as their potential immunomodulatory properties. Hyaluronic acid, being a component of normal cartilage, may have an effect on cartilage regeneration but in this study it was primarily used to deliver and hold the stem cells in place. The cause of the clinical benefit experienced in this case is less clear as subchondral drilling was also performed prior to implantation of the stem cells [23].

Alternative sources of stem cells have been proposed including the use of adipose-derived cells [24]. Adipose tissue may be obtained from abdominal liposuction, although this may be associated with donor-site morbidity and would be logistically less straightforward as many orthopaedic surgeons would not perform the harvesting procedure. The infrapatellar fat pad is being investigated as a source of adipose-derived stem cells and would overcome these issues [25,26]. Synovium is another alternative source of stem cells being studied. Synovium-derived stem cells are isolated from the synovial membrane, which is attached at the surface of articular cartilage. As a result, it is hypothesised that they may have the ability to enhance chondrogenic potential [3,27].

Limitations of this paper include the fact that the cell count is variable depending on the aspiration technique and patient factors. Data on the cell counts is being collected in order to have a data set for future use in the correlation to patient outcomes. Information on the optimum mesenchymal stem cell number/mm^2 is currently not available. Furthermore, growth factor components of the 'fibrin glue' are not controlled and may affect the results. Additionally, the patients presented in this paper did not undergo second look arthroscopy to determine the integrity of the grafts or biopsy for histological confirmation of hyaline cartilage formation, which reduces the ability to draw firm conclusions regarding the histological success of the technique. However, there are ethical concerns associated with performing additional investigative surgical procedures in patients who have improved clinically, particularly in the presence of MRI scans showing good graft integration. A longer-term prospective study of this technique including objective clinical outcome scores is currently in progress in our institution and the results will be presented in the near future. Randomised controlled trials comparing this technique to either microfracture, a cell-free scaffold or NICE-approved ACI are planned to determine the efficacy of the procedure.

Author agreement

The authors confirm that this paper has not been published previously and it is not under consideration for publication elsewhere.

Ethics statement

The authors confirm that only non-identifiable patient images have been included in this paper and the relevant consent was obtained prior to taking clinical photographs.

Declaration of competing interest

None.

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