Evaluation of autologous adipose-derived mesenchymal stem cell therapy in focal chondral defects of the knee: a pilot case series

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Aim: To evaluate the safety, pain, functional and structural improvements after autologous adipose-derived mesenchymal stem cell (ADMSC) therapy in combination with arthroscopic abrasion arthroplasty in focal chondral defects of the knee. Methods: Eight patients with a focal full thickness chondral defect of the knee underwent arthroscopic abrasion arthroplasty followed by postoperative intra-articular injections of autologous ADMSCs (50 x 10⁶ ADMSCs at baseline and 6 months). Clinical outcome was assessed using numeric pain rating scale, Knee Injury and Osteoarthritis Outcome Score and the Western Ontario and McMaster Universities Osteoarthritis Index. Structural outcome was determined by magnetic resonance imaging. Outcome was assessed over 24 months. Results: No serious adverse events occurred. Participants observed clinically significant improvement in pain and function. Magnetic resonance imaging analysis showed cartilage regeneration with T2 mapping values comparable to hyaline cartilage. Conclusion: Arthroscopic abrasion arthroplasty in combination with intra-articular ADMSC therapy results in reproducible pain, functional and structural improvements with regeneration of hyaline-like cartilage.

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Keywords: cartilage • chondral defect • mesenchymal stem cells • osteoarthritis • regeneration

It is recognized that articular cartilage has a limited capacity to heal with several studies indicating that isolated chondral defects predispose patients to later development of early onset and progressive osteoarthritis (OA) [1–4]. The management of focal chondral defects of the knee can be particularly challenging and is influenced by factors including patient age, partial or full thickness chondral loss and the site of the lesion.

Pain and functional limitation as a consequence of a chondral defect and the additional concern regarding later development of early onset OA has meant surgical intervention is often considered. These techniques include, but are not limited to, arthroscopic debridement/chondroplasty, bone marrow stimulating techniques including microfracture/microdrilling and arthroscopic abrasion arthroplasty and graft techniques such as autologous chondrocyte implantation (ACI) or osteochondral autograft transplantation. However, none of these surgical interventions guarantee a long-term sustained improvement in pain, function and structural improvement of the affected joint.

Microfracture was developed during the early 1980s and has become a commonly used surgical technique in the treatment of an isolated chondral defect. The technique involves the drilling or punching of holes through the
subchondral plate and is thought to encourage migration of bone marrow derived pluripotent cells to the articular surface with later cartilage repair. More recently however, some clinicians have questioned the ongoing validity of this operative technique, arguing that it destroys the gross structure of the subchondral plate, may promote subchondral cyst formation, is associated with increased pain in a significant portion of patients and stimulates only fibrocartilage, with observed decrease in clinical outcome within 2 years [5–8].

Arthroscopic abrasion arthroplasty is a modification of the open Magnusson debridement arthroplasty and was pioneered by Lanny Johnson [9]. The use of abrasion arthroplasty for full thickness cartilage lesions has been associated with prolonged functional improvement with Sansone and colleagues noting sustained positive functional outcome at follow-up of 20 years in 89.5% of patients under the age of 50 years and in 55.7% of patients aged 50 or older [10]. Results were significantly better in patients with lesions less than 4 cm². This observation has led some authors to postulate that arthroscopic abrasion is a preferred bone marrow stimulation technique over microfracture [11]. Such outcomes however are not consistent with other articles citing less reproducible outcome with progression of disease within 3 years of follow-up and early conversion to total knee replacement [12].

Past research has shown histopathology of fibrocartilage formation post-microfracture or abrasion arthroplasty at second-look arthroscopy and subsequent biopsy [9,13,14]. As fibrocartilage lacks the load bearing properties of hyaline cartilage it is postulated that it may fail to significantly alleviate pain and delay progressive degeneration. The reduced load bearing properties of fibrocartilage and often observed inadequate defect filling have been postulated as a cause for poor long-term outcome.

Chondrocyte transplantation techniques including ACI and matrix induced ACI (MACI) have shown encouraging results in both preclinical and clinical trials with hyaline-like cartilage formation and observed long-term durability [15–18]. Such interventions however are technically difficult, are additionally limited by observed chondral donor site morbidity and at times show poor integration with surrounding native cartilage, early degeneration and inconsistent functional outcome.

An improved understanding of the pathology of chondral defects and the limitations observed in current surgical management techniques has seen renewed interest in the area of regenerative medicine. The ability of mesenchymal stem cells (MSCs) to differentiate along a mesodermal lineage including into bone and cartilage has led to interest in their potential role in assisting cartilage repair [19–21]. It is however their observed immunomodulatory role and expression of anabolic cytokines through both paracrine and cell-to-cell interaction that is now considered their primary mechanism of action and likely role in tissue repair [22–26].

MSCs may directly modulate the cytokines responsible for progressive cartilage degeneration seen in focal chondral lesions through the suppression of inflammatory T-cell proliferation, inhibition of monocyte maturation and resultant inhibition of the inflammatory cascades involving cytokines including IL-1, TNF-α and matrix metalloproteinases [27]. In addition to inhibition of inflammatory pathways, MSCs secrete several anabolic cytokines including TGF-β, VEGF and EGF, and may lead to local tissue repair through stimulation of trophic pathways [23,24,28].

Clinically, Gobbi and colleagues have shown the successful application of bone marrow derived MSCs within a collagen matrix in the treatment of full-thickness cartilage lesions of the knee [29]. Magnetic resonance imaging (MRI) indicated complete healing in 80% of trial participants with later histological analysis showing type II collagen and hyaline-like cartilage morphology. In addition to this some authors have suggested that the application of cellular therapies including MSCs may assist in the conversion of regenerative fibrocartilage following bone marrow stimulation techniques such as microfracture or abrasion arthroplasty to mature hyaline-like cartilage [30]. Both preclinical and early clinical trials have supported this hypothesis with intra-articular injections of MSCs in conjunction with surgical microfracture/microdrilling showing significant improvement in articular cartilage repair with hyaline-like cartilage regeneration and type II collagen shown on histopathology [31,32].

The aim of this case series is to assess both the safety/tolerability and efficacy of adipose-derived MSCs (ADMSCs) therapy in conjunction with arthroscopic abrasion arthroplasty in the treatment of focal full thickness chondral defects of the knee.

**Materials & methods**

**Trial design**

This prospective case series forms part of a broader case series approved by the Human Research Ethics Committee of Charles Sturt University. The case series is registered on the Australian New Zealand Clinic Trial Registry UK trial number 17041704 Regen. Med. (2020) 15(6) future science group
Table 1. Eligibility criteria.

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
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<tbody>
<tr>
<td>1. Isolated full thickness (Grade IV) chondropathology of the medial or lateral femoral condyle as determined by the modified ICRS score†</td>
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<tr>
<td>2. Primary conservative treatment already undertaken including analgesia/anti-inflammatory medication, an attempted prescribed exercise and weight management program, and biomechanical adjustment including bracing if appropriate as prescribed by a physiotherapist, podiatrist or medical practitioner</td>
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<tr>
<td>3. Sufficient English skills to complete the questionnaires required for the study</td>
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<table>
<thead>
<tr>
<th>Exclusion criteria</th>
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<tbody>
<tr>
<td>1. Age &lt;18 years</td>
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<tr>
<td>2. Pregnancy</td>
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<tr>
<td>3. Breast feeding</td>
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<tr>
<td>4. Have any other causes of their symptoms suspected to be due to serious pathology such as tumor or referral from the spine</td>
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<tr>
<td>5. Current cancer</td>
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<tr>
<td>6. History of significant organ impairment/failure (i.e., renal failure)</td>
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<tr>
<td>7. History of allergy to any substances used within ADMSC preparation and treatment</td>
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</table>

†Specific criteria for this sub-group case series within the broader case series.
ADMSC: Adipose-derived mesenchymal stem cell; ICRS: International Cartilage Repair Society.

(ACTRN12617000638336). All participants underwent an eligibility screening process and completed a formal written informed consent form.

All participants underwent arthroscopic abrasion arthroplasty and intra-articular autologous ADMSC therapy. Intra-articular injections of ADMSCs were performed within a private practice setting and within 1 week of knee arthroscopy and again at 6 months. A total of 8 participants were enrolled. Participants were followed up for a period of 24 months.

Participant selection

As indicated, this prospective case series represents a subgroup of participants in a broader case series assessing the outcome of ADMSC therapy in arthritis/chondropathology. The participants in the case series met the inclusion criteria for the broader encompassing case series but specifically had MRI confirmed areas of focal isolated full thickness chondropathology (rather than diffuse arthritic change) which was assessed as amenable to arthroscopic abrasion arthroplasty (see Table 1). Grading of chondropathology was assessed according to modified International Cartilage Repair Society (ICRS) grading (see Outcome Measures) [33].

Baseline assessment included a musculoskeletal examination and diagnostic imaging (i.e., MRI) to determine eligibility. All participants received written information regarding the trial and completed a formal written informed consent form.

Autologous ADMSC preparation

Harvest procedure

Adipose tissue was chosen as a source of MSCs due to the ease of harvest, the relative abundance of MSCs (up to 10% of total stromal vascular fractions of the digested adipose tissue) and the observed chondrogenic potential of ADMSCs [34,35].

A minimally invasive liposuction (lipoharvest) was performed under local anesthetic tumescence control as previously described in past publications [36–41]. Lipoaspirate was collected using either a manual syringe suction technique or via mechanical suction into a sterile single use container (Shippert Medical, CO, USA). Lipoaspirate was transferred directly after collection to a clean room laboratory facility (Magellan Stem Cells, Victoria Australia) for further processing.

Isolation & expansion of MSCs

ADMSCs were isolated and expanded using previously published protocols [32–37]. All processing was performed within a clean room facility with ISO5 air quality or greater and within Class II Biological Safety Cabinets. ADMSCs were cryopreserved within cryovials using a validated control rate freezing method and stored in liquid nitrogen until required [42,43].

Isolated cell populations were confirmed as ADMSCs in accordance with standards established by the International Society of Cellular Therapy [44]. Using flow cytometry with Fluorescence-Activated Cell Sorting, isolated
cells were assessed for the presence of MSC specific surface markers CD90, CD73 and CD105 and the absence of hematopoietic surface makers CD14, CD19, CD34 and CD45 (Table 2).

In addition to Fluorescence-Activated Cell Sorting analysis, all isolated ADMSC participant samples underwent independent sterility testing for microbial growth/contamination.

**Carrier media**

MSCs were suspended in an autologous biological carrier media which was either autologous conditioned serum (ACS) or platelet-rich concentrate (PRC; Supplementary Table 2). As participant recruitment occurred over considerable time, ACS or PRC was chosen dependent upon availability of resources.

ACS preparation involved the withdrawal of a total of 27 ml of whole blood via venipuncture and collected within 3 × 9 ml sterile S-Monovette® clotting activator tubes (Starstedt, Numbrecht, Germany). The tubes were placed in an incubator at 38°C for 24 h and later centrifuged at 1000 rpm for 5 min with resultant separation of the whole blood and the serum. The serum layer was removed and filtered through a 0.2 micron syringe filter (PALL, NY, USA) producing an acellular sample of ACS.

PRC preparation involved the withdrawal of 25.5 ml of whole blood via venipuncture and collected within 3 × 8.5 ml ACD (trisodium citrate 22.0 g/l, citric acid 8.0 g/l and dextrose 24.5 g/l) BD Vacutainers (BD, NJ, USA). The blood underwent an initial soft spin at 1000 rpm for 10 min using a bench top centrifuge. The separated plasma containing the platelets was then transferred to a sterile tube which subsequently underwent a second hard spin at 3500 rpm for a total of 5 min resulting in formation of a platelet concentrate plug and platelet poor plasma (PPP). Excess PPP was removed and discarded leaving only 6 ml of PPP and platelet concentrate. Using gentle manual agitation the platelet plug was reconstituted within the PPP creating a platelet-rich plasma (PRP) preparation. The PRP was activated using the addition of 0.2 ml of calcium chloride (10%) and incubated at 37°C for 10 min, creating a fibrin clot. This was allowed to retract and resorb over time. The final preparation was passed through a 0.2 micro syringe filter (PALL, NY, USA) producing an acellular and sterile PRC.

Both autologous media preparations were stored at -20°C for later use at time of MSC therapy.

**Intervention**

*Arthroscopic abrasion arthroplasty*

Surgery was performed by an experienced orthopedic knee specialist under general anesthetic and lower limb vascular tourniquet control. An arthroscopic examination was performed with chondroplasty to areas of unstable cartilage and limited resection/debridement of displaced or complex meniscal tears, if present, using a 3 mm arthrosopic shaver. Arthroscopic abrasion arthroplasty was undertaken following methods previously described by Johnson et al. [9]. Abrasion was achieved using a 4–4.5 mm burr with areas of exposed bone eburnated down to the subchondral plate until capillary bleeding was observed (Figure 1).

In the immediate postoperative period, participants were placed in a continuous passive motion device until discharge on the following day. The continuous passive motion was set at 0° to 90° and participants were allowed to increase the passive ROM based on symptoms.

*Injection method*

At the time of injection, the cryopreserved cells were thawed in a prewarmed sterile water bath, with cryoprotectant removed through centrifugation and washing in chilled sterile phosphate buffered saline. The resultant cell pellet was resuspended in 3 ml of autologous carrier media. Cell number and viability was assessed using a Muse Cell Analyzer (Millipore Sigma, MA, USA; Table 3).

Participants underwent an initial intra-articular injection of ADMSCs within 1 week of their arthroscopic surgery and again at 6 months. At the time of injection, the participant’s knee was prepared using standard sterility protocols.
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Figure 1. Arthroscopic abrasion arthroplasty. Intraoperative arthroscopic pictures showing (A) loose chondral body, (B) a full thickness chondral defect – modified International Cartilage Repair Society Grade IV – with unstable chondral border, (C) arthroscopic chondroplasty of unstable margins, (D) final area of abrasion arthroplasty after use of a 4 mm burr with exposure of subchondral bone.

Table 3. Average cell count and viability confirmed using a Muse Cell Analyzer.

<table>
<thead>
<tr>
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<th>Baseline ADMSC injection</th>
<th>6 Month ADMSC Injection</th>
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<tbody>
<tr>
<td>Cell count (million)</td>
<td>Mean (Standard deviation)</td>
<td>Cell count (Million)</td>
</tr>
<tr>
<td>Cell viability (%)</td>
<td>51.5 (2.9)</td>
<td>97.3 (1.2)</td>
</tr>
<tr>
<td>Cell viability (%)</td>
<td>97.3 (1.2)</td>
<td>96.1 (2.5)</td>
</tr>
</tbody>
</table>

See Supplementary Table 3 for individual participant cell count and viability data.

ADMSC: Adipose-derived mesenchymal stem cell.

and 2 ml of 1% lidocaine infiltrated superficially to the joint capsule. Using a superolateral patella approach and under ultrasound guidance the resuspended autologous ADMSCs were injected into the intra-articular knee space. Prior to the first injection up to 15 ml of hemarthrosis effusion, a result of the arthroscopic abrasion arthroplasty, was aspirated and discarded.

Postoperative rehabilitation

Following arthroscopic abrasion arthroplasty the participants were limited to touch weight bearing with the use of crutches for a period of 4 weeks. During this time all participants were advised on a lower limb muscular activation program and commenced active range of motion using a stationary bike at minimal or no resistance. Participants were advised to complete 30–60 min of active range of motion exercises at least twice per day.

After 4 weeks, all participants were allowed to transition to partial weight bearing and then to weight bearing as tolerated. All participants were fitted with a uni-compartmental unloading knee brace (Ossur Unloader One, Ossur, Reykjavik, Iceland) and instructed to wear the brace for all partial to full weight bearing activities during the first 3 months postoperatively.

Outcome measures

Primary outcome aims of this trial were to assess the safety/tolerability of ADMSCs in combination with arthroscopic abrasion arthroplasty and also pain and functional changes as measured by the numeric pain rating scale (NPRS), the Knee Injury and Osteoarthritis Outcome Score (KOOS) and the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). A secondary outcome of structural improvement was assessed using MRI with observation of modified ICRS grading at site of abrasion, MRI Observation of Cartilage Repair Tissue (MOCART) score and use of the validated noninvasive method of T2 relaxation time for cartilage quality (Table 4).

Questionnaires

Outcome questionnaires were recorded using the software program Clinical Intelligence (Clinical Intelligence, Melbourne, Australia) and were recorded at baseline, 1, 6, 12, 18 and 24 months.
Table 4. Outcome measures.

<table>
<thead>
<tr>
<th>Outcome measure</th>
<th>Measurement point (months)</th>
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<tbody>
<tr>
<td><strong>Primary outcome measure</strong></td>
<td></td>
</tr>
<tr>
<td>1. Adverse events</td>
<td>0, 1, 6, 12, 18, 24</td>
</tr>
<tr>
<td>2. Numeric Pain Rating Scale</td>
<td>0, 1, 6, 12, 18, 24</td>
</tr>
<tr>
<td>3. Knee Injury and Osteoarthritis Outcome Score</td>
<td>0, 1, 6, 12, 18, 24</td>
</tr>
<tr>
<td>4. Western Ontario and McMaster Universities Osteoarthritis Index</td>
<td>0, 1, 6, 12, 18, 24</td>
</tr>
<tr>
<td><strong>Secondary outcome measure</strong></td>
<td></td>
</tr>
<tr>
<td>1. MRI analysis</td>
<td>0, 12, 24</td>
</tr>
<tr>
<td>– Modified ICRS grade</td>
<td></td>
</tr>
<tr>
<td>– MOCART score</td>
<td></td>
</tr>
<tr>
<td>– T2 cartilage mapping</td>
<td></td>
</tr>
</tbody>
</table>

ICRS: International Cartilage Repair Society; MOCART: MRI Observation of Cartilage Repair Tissue.

- NPRS – participants were asked to rate their average pain intensity over the past week on an 11 point scale of 0–10 [45].
- KOOS – consisting of 5 subscales – pain, symptoms, function in daily living, function in sport and recreation and knee related quality of life. A score is allocated to all subscales with 100 indicating no symptoms and 0 indicating maximal symptoms [46].
- WOMAC – a validated quality of life score in patients with symptomatic OA [47]. The Global WOMAC score is presented as an inverse percentage so to be comparable to the KOOS subscales.

Magnetic resonance imaging

MRI was performed with a dedicated knee coil on a 1.5 T or greater MRI. Proton density and proton density fat saturated (PDFS) images were taken in coronal, sagittal and axial planes. The area of chondropathology prior to, at 12 months and after 24 months (average 29 months) post-treatment was visually assessed by MRI using the modified ICRS grade and also the validated MOCART score [33,48]. The MOCART score assesses the area of cartilage repair and surrounding tissue over nine categories with a maximal total score of 100 indicating complete hyaline like cartilage repair. The modified ICRS grade was allocated using the below criteria -

- Grade 0: normal cartilage
- Grade 1: focal blistering and intra-cartilaginous low-signal intensity area with an intact surface and bottom
- Grade 2: irregularities on the surface or bottom and loss of thickness of less than 50%
- Grade 3: deep ulceration with loss of thickness of more than 50%
- Grade 4: full thickness cartilage wear with exposure of subchondral bone.

In addition to semi-quantitative assessment, cartilage quality at the site of abrasion was quantitatively assessed using the validated technique of MRI T2 relaxation time cartilage mapping [49,50]. T2 relaxation time values were compared against an area of native cartilage within the contralateral femoral condyle of the joint.

Sample size

This study represents a limited pilot case series to assess the safety/tolerability and efficacy of arthroscopic abrasion arthroplasty in combination with ADMSCs. No sample size calculation was performed.

Adverse events

An adverse event (AE) was defined as an undesirable clinical occurrence in a participant which was not present prior to treatment or which increased in severity after treatment and was observed during the period of follow-up. AEs may be related or unrelated to the treatment and may be expected or unexpected. AEs were assessed in regards to severity:

- Mild: a transient symptom or clinical sign that does not interfere with the subject’s usual activity and is resolved with use of simple interventions including simple analgesia.
- Moderate: a symptom which limits the subject’s usual daily activity and/or requires symptomatic treatment including regular analgesia (i.e., opiate analgesia).
- Severe: a symptom which causes severe discomfort and/or significantly impacts on the subject’s usual activity and extends for ≥2 weeks.
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Results

Demographic characteristics
The case series consisted of four females and four males aged between 23 and 52 years of age (mean 36 years). Three participants had a past history of anterior cruciate ligament (ACL) rupture and had undergone prior ACL reconstruction. All knees were clinically stable on examination. Participants had a mean BMI of 25.24 with no participants categorized as obese (see Supplementary Table 4 for individual participant BMI data). All patients were otherwise well with no history of diabetes or smoking. A single patient had hypertension for which they took an antihypertensive and their blood pressure was well controlled. All participants used simple analgesia and/or oral nonsteroidal anti-inflammatories as required.

Site of the chondral lesion was evenly distributed between the medial compartment and lateral compartment with a single lesion involving the lateral tibial plateau. Average size of the Grade IV chondral lesion was 17.1 mm (length/sagittal plane) × 9.5 mm (width/coronal plane).

Pain & function
Consistent and progressive improvement in pain – as measured by NPRS – was documented over the period of follow-up with a mean (standard deviation) pain score of 5.6 (2.8) improving to 2.3 (2.2) at 24 months equating to a 60% reduction in pain levels (Figure 2). Variability among participants was observed in clinical recovery time following arthroscopic abrasion arthroplasty with 1 participant recording increased pain from baseline during the first 6 months of follow-up. This patient had an additional comorbidity of a past anterior cruciate ligament rupture with subsequent ligament reconstruction and incomplete recovery which may have influenced their expected outcome.

KOOS subscale analysis indicated consistent improvement in all subscales with values continuing to improve until final follow-up at 24 months (Figure 3). Sport and recreational activity subscale improved by an average of 111% with quality of life (QoL) increasing by over 50%.

Global WOMAC score expressed as an inverse percentage reflected the improvements seen in KOOS (Figure 4).
Figure 3. Knee Injury and Osteoarthritis Outcome Score. Unidirectional error bars represent 95% CI. KOOS: Knee Injury and Osteoarthritis Outcome Score; QoL: Quality of life.

Figure 4. Global Western Ontario and McMaster Universities Osteoarthritis Index Score. Unidirectional error bars represent 95% CIs. Western Ontario and McMaster Universities Osteoarthritis Index is represented as an inverse score to allow direct comparison to Knee Injury and Osteoarthritis Outcome Score analysis. WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index.

Structural outcome
MRI assessment at 12 and 24+ months showed successful regeneration of articular cartilage in all 8 participants (Figure 5). Modified ICRS score assessment showed improvement from Grade IV to Grade 0 or 1 in all participants at 12 and 24+ months of follow-up. Where abrasion arthroplasty was performed good integration was observed...
between regenerated cartilage and native cartilage. MOCART score showed considerable cartilage repair at 12 months with a mean score of 79 out of a maximal 100. Additional improvement was observed after 24 months with a mean MOCART score of 89 (see Supplementary Table 5 for itemized MOCART score allocations). Results were comparable in lesions of the medial femoral condyle, lateral femoral condyle and lateral tibial plateau (LTP).

MRI T2-weighted imaging was performed after 24 months. Average T2 scores in the deep, intermediate and superficial zones compared favorably with chosen sample area of native cartilage of the contralateral femoral condyle (see Table 5 & Figure 6). Two participants had T2-weighting imaging also performed at 12 months. Comparison of average 12 month and 24+ month values indicated progressive maturation of regenerative cartilage (average 44.6 and 42.7 ms comparatively).
Table 5. Magnetic resonance imaging T2 relaxation time values. Native cartilage values were recorded over the central weight bearing region of the contralateral femoral condyle. Average values are in milliseconds with standard deviations in brackets.

<table>
<thead>
<tr>
<th>Cartilage zone</th>
<th>MRI T2 relaxation time values 24 months</th>
<th>Native cartilage at 24 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep zone</td>
<td>35.9 (15.9)</td>
<td>33.3 (12.6)</td>
</tr>
<tr>
<td>Intermediate zone</td>
<td>40.3 (9.5)</td>
<td>38.4 (11.2)</td>
</tr>
<tr>
<td>Superficial zone</td>
<td>45.4 (16.9)</td>
<td>47.0 (15.1)</td>
</tr>
<tr>
<td>Overall</td>
<td>40.6 (14.1)</td>
<td>39.6 (13.0)</td>
</tr>
</tbody>
</table>

Figure 6. Example of sequential magnetic resonance imaging T2 mapping of the area of cartilage regeneration at 12 and 24 months. Formal T2 mapping was done over a number of slices to include the entire lesion and area of regeneration. This analysis indicated improved maturation over time with comparable T2 values to native cartilage.

Complications & AEs
Participants experienced expected pain and limitation in daily activities due to the postoperative period of touch weight bearing and use of crutches. A self-limiting flare of pain and swelling following ADMSC injections was observed which responded to conservative management including ice and simple analgesia including oral nonsteroidal anti-inflammatories. One participant noted discomfort and swelling lasting 3 weeks which was recorded as a severe AE. No serious AEs were observed. See Supplementary Table 6 for a complete list of graded AEs.

Discussion
The management of isolated chondral defects remains difficult. Current surgical techniques such as microfracture are associated with fibrocartilage formation and result in inconsistent pain, functional and structural results with anticipated progression to early degenerative OA. Other interventions involving graft techniques such as MACI, while showing promising long-term follow-up, are limited by technical difficulty, donor site morbidity and inconsistent integration with surrounding native cartilage.

In this case series, arthroscopic abrasion arthroplasty in combination with ADMSC therapy was observed to be a safe and well tolerated procedure. While participants experienced a self-limiting period of discomfort and swelling
following ADMSC therapy this was an expected AE and has been documented in past trials [32,37,38,40,41]. No serious AEs were observed.

Participants showed improvement across the course of follow-up as measured by quantitative pain and functional outcome scores. Variability was seen in recovery from arthroscopic abrasion arthroplasty with one participant recording worse pain and functional scores during the first 6 months of follow-up. The observed variability in recovery may relate to the size and position of the focal chondral lesion, additional intervention at arthroscopy (i.e., partial meniscal resection/debridement) and other pre-existing comorbidities (i.e., ACL reconstruction).

Improvement in pain and function was mirrored in structural improvement with chondral regeneration observed at 12 and 24+ months follow-up time points. All patients had improvement of a modified ICRS score from 4 to 1 or 0 at both 12 and after 24+ months. MOCART assessment indicated progressive improvement until final follow up. T2-weighted imaging values performed after 24 months were comparable to values taken in an area of central weight bearing native cartilage of the contralateral femoral condyle. In two patients who had T2 mapping performed at 12 and after 24 months, T2 mapping values indicated progressive cartilage maturation with reduced water content and improved collagen anisotropy. This observation is not dissimilar to ACI techniques whereby long-term follow up has shown greater type II collagen and hyaline-like properties at 24 versus 12 months.

Final T2 mapping values after 24 months of follow-up compared favorably against studies assessing alternative surgical techniques. In a recent randomized controlled trial assessing the role of microfracture versus microfracture in combination with a biological scaffold (BST-CarGel) T2 mapping values at 12 months in both groups showed greater difference to native cartilage values than that observed in this case series [51]. Results in this trial were most comparable to past research involving MACI techniques with T2 mapping values approaching that of native cartilage [52].

In this case series the MSCs were suspended in an autologous biological carrier media of either ACS or PRC. A blood derived biological carrier media was chosen due to previously published studies indicating observed benefits in increased expression by MSCs of anti-inflammatory cytokines including IL-A receptor antagonist (IL-1ra) and anabolic growth factors such as TGF-β [53-55]. TGF-β has been shown to reduce expression of collagen type I, upregulate expression of collagen type II and also assist with migration of stromal cells to site of injury [55]. As arthroscopic abrasion arthroplasty results in a post-operative haemarthrosis – with release of blood derived growth factors including TGFβ at the site of abrasion/bleeding – the use of a biological carrier media may not have been of greater benefit over an isotonic electrolyte solution. It is noted that both ACS and PRC therapy have been documented in past studies to improve pain and function in the treatment of knee OA [55,56]. In this case series participants only received a single injection at baseline and again at 6 months, whilst treatment protocols of ACS and PRC typically involve multiple injections over weeks. It is however accepted that both ACS and PRC may have had additional effect to that of ADMSC therapy in regards to participant outcome and observed pain and functional improvements.

This trial is limited by its nature as a case series. A randomized controlled trial design with comparison of arthroscopic abrasion arthroplasty against arthroscopic abrasion arthroplasty in combination with MSC therapy would be more definitive. In addition, it is accepted that cartilage morphology would be best assessed by histopathology. As histopathology analysis would require repeat arthroscopy and chondral biopsy this could not be clinically justified. Importantly T2-weighted imaging allows a validated noninvasive quantitative assessment of regenerative cartilage against native cartilage with comparable values indicating similar cartilage anisotrophy and hyaline-like properties.

Whether ADMSCs directly integrated into the repair site and/or differentiate along a chondrocyte lineage is debatable. While some preclinical trials have shown successful engraftment of MSCs at the site of chondral repair this has not been consistently observed with some trials indicating reduction in MSC number over time [57,58]. It is proposed that the release of chemotactic cytokines at the site of abrasion arthroplasty – including TGF and CXCL chemokines – may assist in the migration of intra-articular injected MSCs to the site of abrasion with subsequent integration within the repaired tissue together with the secretion of a plethora of growth factors by these cells [55,59]. Use of cell labeling techniques including magnetic tagging would allow for additional assessment of cell migration, integration and retention and would be of benefit in future formal trials [60,61].

Conclusion
This pilot case series illustrates the novel and successful treatment of isolated focal chondral lesions of the knee with arthroscopic abrasion arthroplasty in combination with ADMSCs. Treatments were well tolerated with no serious AEs and participants experienced clinically significant pain, functional and structural improvement. Structural
analysis was comparable to more complicated graft techniques such as MACI with improved regenerative cartilage quality in comparison to other surgical techniques including microfracture.

Translational perspective
The use of MSC therapy in the management of chondropathology and OA is an emerging field in regenerative medicine. The pilot study is the first to show the successful use of ADMSCs in conjunction with a current surgical technique, arthroscopic abrasion arthroplasty, to enhance healing and result in hyaline-like cartilage formation. MSC therapy promises to improve the surgical management of musculoskeletal complaints with improved long-term outcome. The results of this pilot case series highlighting safety and preliminary efficacy mean that additional well-structured research with direct comparison against conventional treatment controls is justified.

Summary points

- The management of isolated focal chondral defects pose a challenge to treating clinicians.
- Isolated focal chondral defects are associated with significant pain and functional limitation and predispose patients to early onset degenerative osteoarthritis.
- Current surgical management options including microfracture and arthroscopic abrasion arthroplasty are limited due to fibrocartilage formation, poor defect filling, early degeneration and at times persistent pain and functional limitation.
- Chondral graft techniques such as autologous chondrocyte implantation can be associated with improved long-term results but are technically difficult, require multiple invasive surgeries and may result in donor site morbidity and poor integration with native cartilage.
- Previous preclinical and early clinical studies have supported the use of mesenchymal stem cell therapy in conjunction with past surgical management techniques including microfracture/microdrilling.
- This is the first case series to assess the use of adipose-derived mesenchymal stem cell (ADMSC) therapy in conjunction with arthroscopic abrasion arthroplasty.
- Clinically significant pain and functional improvement was observed over a 24 months follow-up period.
- Quantitative MRI analysis using validated T2-weighted imaging showed hyaline-like regenerative cartilage with values approaching that of native cartilage.
- No serious adverse events were observed and ADMSC therapy was well tolerated.
- Autologous ADMSC therapy represents an exciting development which may increase the success of existing surgical management of isolated focal chondral defects.

Author contributions
J Freitag, K Shah, J Wickham, D Li, C Norsworthy, A Tenen were involved in conception of the study. J Freitag, K Shah, J Wickham, D Li, C Norsworthy, A Tenen designed the study protocol.

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Financial & competing interests disclosure
J Freitag, A Tenen are clinic partners within Melbourne Stem Cell Centre. J Freitag, D Li, A Tenen are associated with Magellan Stem Cells and are part of its Clinical and Scientific Advisory Committee. K Shah is the Chief Scientific Officer at Magellan Stem Cells. The study was co-sponsored by Melbourne Stem Cell Centre and Magellan Stem Cells. Members of their Clinical and Scientific Advisory Board have been involved in the study conception and design and are listed as co-authors of this paper. Interpretation of results, and subsequent submission and publication decisions have been made independent of the sponsors. Mesenchymal stem cell therapy was performed within a private medical facility and funded by the patients/participants. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. No writing assistance was utilized in the production of this manuscript.
Evaluation of autologous adipose-derived mesenchymal stem cell therapy in focal chondral defects of the knee: a pilot case series

Ethical conduct of research
Ethics approval was given for the study by the Human Research Ethics Committee of Charles Sturt University and registered prospectively on the Australian and New Zealand Clinical Trial Registry (Trial ID: ACTRN12617000638336). In addition, informed consent has been obtained from the participants involved.

Data sharing statement
Individual de-identified participant data which underlie results reported in this article will be available upon publication. The study protocol will also be available upon publication. Material will be accessible to investigators whose proposed use of data has been approved by an independent review committee and for data meta-analysis. Requests are to be directed to the corresponding author of the article.

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