Mesenchymal stem cell therapy combined with arthroscopic abrasion arthroplasty regenerates cartilage in patients with severe knee osteoarthritis: a case series

Julien Freitag^{*,‡,1,2,3} , James Wickham¹ , Kiran Shah^{2,4} , Douglas Li^{2,5}, Cameron Norsworthy⁶ & Abi Tenen^{2,3,7}

¹School of Biomedical Sciences, Charles Sturt University, Orange, NSW, Australia

²Magellan Stem Cells, Box Hill, Victoria, Australia

³Melbourne Stem Cell Centre, Box Hill, Victoria, Australia

⁴Department of Chemistry and Biotechnology, Swinburne University of Technology, Melbourne, Victoria, Australia

⁵Orthopaedics Sports Arthroplasty, Melbourne, Victoria, Australia

⁶Orthosport Victoria, Melbourne, Victoria, Australia

⁷School of Primary Healthcare, Faculty of Medicine, Monash University, Monash, Victoria, Australia

*Author for correspondence: julien.freitag@mscc.com.au

‡The corresponding author is the guarantor of the submission

Aim: To evaluate the safety and efficacy of adipose-derived mesenchymal stem cell (ADMSC) therapy in combination with arthroscopic abrasion arthroplasty (AAA) in advanced knee osteoarthritis (OA). **Materials & methods:** 27 patients with Grade IV OA of the knee underwent AAA and ADMSC therapy (50 × 10⁶ ADMSCs at baseline and 6 months). Clinical outcome was assessed over 36 months. Structural change was determined using MRI. **Results:** Treatment was well tolerated with no serious adverse events. Clinically significant improvements in pain and function were observed. Reproducible hyaline-like cartilage regeneration was seen in all participants. **Conclusion:** ADMSC therapy combined with AAA in Grade IV OA results in reproducible pain, functional and structural improvements. This represents a joint preservation technique for patients with advanced OA of the knee.

Trial registration number: ACTRN12617000638336

Lay abstract: **Aim:** In this study, we planned to test the safety and benefit of stem cell therapy in patients with advanced knee osteoarthritis (OA) when combined with keyhole knee surgery. **Materials & methods:** 27 patients with advanced knee OA received keyhole knee surgery and stem cell therapy. We measured the clinical effects of this treatment for 36 months and assessed any changes to the structure of the knee joint during that time using MRI scanning. **Results:** Patients tolerated the treatment well and there were no serious complications caused by it. Patients also experienced significant reduction in knee pain and improvement in knee function. Cartilage regeneration was also seen in all patients. **Conclusion:** Stem cell therapy combined with keyhole knee surgery in advanced OA leads to reduced pain, increased knee function and regrowth of cartilage. These results suggest this technique could in the future be used in patients with advanced knee OA to prevent the need for knee replacement surgery.

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Osteoarthritis (OA) is a chronic and progressive condition, which can result in significant pain and loss of function. Symptomatic OA, while often regarded as an age-related condition, is not only a disease of the elderly as it has a prevalence rate of 10% of males and 18% of females over the age of 45 years [1–4]. Alarming, there has been an observed increase in the number of patients undergoing total joint replacement under the age of 65 [5].

Conservative pharmaceutical management of knee OA is aimed at symptomatic control and does not halt progressive joint degeneration. Furthermore, these therapies demonstrate often only modest benefits and may have unwanted side effects [6–9]. Surgical total knee replacement (TKR), while the accepted treatment option for symptomatic knee OA uncontrolled by conservative therapies, has an unpredictable outcome with persistent pain or loss of function in up to 20% of patients at 12 months and is associated with a significant complication rate of 2% [10–12].

Structurally OA is recognized as a loss of load-bearing cartilage and may include osteophyte formation, subchondral sclerosis and cyst formation and periarticular synovitis [13]. While initially considered a ‘wear and tear’ causality, we now understand that OA is the result of an imbalance between anabolic/reparative and catabolic/proinflammatory pathways with the instigation of this imbalance associated commonly with abnormal mechanical forces operating at the joint [14–16]. Chondrocyte cell senescence as a result of mechanical or biological stressors has been shown to be accompanied by an observed increase in a cascade of inflammatory cytokine expression leading to a procatabolic state with resultant matrix degradation and development of OA [17].

The ability of mesenchymal stem cells (MSCs) to differentiate along a mesodermal lineage including into bone and cartilage has seen considerable research interest in their potential to assist in musculoskeletal repair and regeneration [18–20]. It is, however, their observed anti-inflammatory role and expression of anabolic trophic cytokines through both paracrine and cell-to-cell interaction that are now considered to be their mechanism of action and that are responsible for their observed potential therapeutic role in the management of OA [21–25].

Both preclinical and early clinical trials have indicated benefits of MSC therapy in OA. Early Phase I and II trials using expanded and isolated MSC therapies have shown both pain and functional improvements and evidence of disease modification with radiological confirmed OA stabilization, improvement in cartilage quality and cartilage regrowth [26,27]. In addition to this, we have recently published a randomized controlled trial on the use of autologous adipose-derived MSC (ADMSC) therapy in moderate knee OA with evidence of clinically significant pain relief and effective structural stabilization of articular cartilage [28].

Surgical techniques used to promote structural regeneration at the site of Grade IV (full-thickness) chondropathologies with resultant pain and functional improvements include arthroscopic microfracture and arthroscopic abrasion arthroplasty (AAA). Many clinicians have questioned the ongoing validity of operative techniques such as microfracture arguing that it destroys the gross structure of the subchondral plate, may promote the formation of subchondral cysts, and leads to only fibrocartilage regrowth with an observed decrease in clinical outcome within 2 years [29–32]. In contrast, Sansone *et al.* documented successful long-term results of AAA with an observed positive outcome of 67.9% of patients at 20-year follow-up [33]. Such long-term outcome has not always been observed with other studies indicating less reproducible benefit from AAA with progression of disease within 3 years of follow-up and conversion to a TKR in a significant number of patients [34].

Past research has shown histopathology of fibrocartilage formation after bone marrow stimulation techniques including AAA or microfracture [35–37]. As fibrocartilage lacks the load-bearing properties of hyaline cartilage, it is postulated that it may fail to significantly alleviate pain and also fail to delay progressive degeneration which would explain the inconsistent long-term outcomes observed with these techniques. Some authors have suggested that the application of cellular therapies including MSCs may improve the surgical outcome of microfracture or AAA by assisting in the conversion of regenerative fibrocartilage to mature hyaline-like cartilage [38]. In support of this, we have recently reported the successful outcome of AAA in combination with ADMSC therapy in the treatment of isolated full-thickness chondral defects of the knee with observed hyaline-like cartilage regeneration using noninvasive MRI T2-mapping techniques [39].

Despite advances in both medical and surgical techniques, the treatment of broader grade IV knee OA remains a TKR. Unfortunately, while cartilage regeneration techniques hold promise, several studies have highlighted that regenerative surgical methods used in focal cartilage defects have failed to have reproducible benefit in broader more advanced OA particularly where corresponding load-bearing surfaces have full-thickness cartilage loss [40,41]. Importantly, and in contrast to these previous trials, we have previously published a promising case report whereby AAA in combination with high tibial osteotomy and ADMSC therapy in the treatment of advanced knee OA successfully resulted in long-term pain and functional improvement and sustained structural hyaline-like cartilage regeneration [42].

Therefore, the primary aim of this case series is to assess both the tolerability of ADMSC therapy in conjunction with AAA in the treatment of diffuse x-ray confirmed severe Grade IV OA of the knee and to confirm efficacy

Figure 1. Flow chart of participant enrollment and progress through the trial.
 ADMSC: Adipose-derived mesenchymal stem cell; MOCART: MRI Observation of Cartilage Repair Tissue.

through validated pain and functional outcome measures. A secondary objective is to assess any observed cartilage regeneration and the quality of that cartilage regeneration.

Materials & methods

Trial design

This prospective case series study represents a subgroup of participants within a broader case series on ADMSC therapy in the management of arthritis and has been approved by the Human Research Ethics Committee of Charles Sturt University (Australian New Zealand Clinic Trials Registry – ACTRN12617000638336). All participants underwent a formal eligibility screening process and completed written informed consent.

Participants underwent AAA performed by an experienced orthopedic knee specialist. Intra-articular injections of ADMSCs were performed within a private practice setting within 1 week of knee arthroscopy, with an additional planned second injection at 6 months. Participants were recruited over a 2.5-year time period with 32 participants formally enrolled. Two participants were lost to follow-up. An additional two participants were excluded due to additional treatment (other than ADMSC therapy) to the knee being undertaken within the follow-up period that may have influenced/improved outcome, and a further participant was excluded due to unrelated knee surgery during the time course of follow-up. A final sample size of 27 participants was formally followed-up during the period of the trial (Figure 1).

Table 1. Eligibility criteria.

Inclusion criteria
<ol style="list-style-type: none"> 1. Diagnosis of OA/chondropathology 2. Radiological grading of Grade IV OA of the knee using the Kellgren and Lawrence system[†] 3. Age <65 years[†] 4. 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 5. Sufficient English skills to complete the questionnaires required for the study
Exclusion criteria
<ol style="list-style-type: none"> 1. Age <18 years 2. Pregnancy 3. Breast feeding 4. Have any other causes of their symptoms suspected to be due to serious pathology such as tumor or referral from the spine 5. History of inflammatory arthropathy[†] 6. Current cancer 7. History of significant organ impairment/failure (i.e., renal failure) 8. History of allergy to any substances used within ADMSC preparation and treatment
[†] Specific criteria for this subgroup case series within the broader case series. ADMSC: Adipose-derived mesenchymal stem cell; OA: Osteoarthritis.

Participant selection

Participants met the inclusion criteria for a broader encompassing Human Research Ethics Committee and registered case series assessing ADMSC therapy in the treatment of arthritis. In addition to the inclusion criteria for this broader registered case series, all participants had radiologically confirmed advanced Grade IV knee OA and were under the age of 65 (Table 1). All participants were assessed as suitable for TKR, based on pain and functional limitation and severity of OA.

Baseline eligibility assessment included a musculoskeletal examination and diagnostic imaging (i.e., x-ray and MRI). Prior to recruitment, participants were given additional written information regarding the trial and completed a formal written informed consent form if they wished to proceed.

Autologous ADMSC preparation

Harvest procedure

While adult MSCs may be sourced from various tissues, adipose tissue was chosen due to the ease of harvest and the relative abundance of MSCs (up to 10% of total stromal vascular fractions of the digested adipose tissue) [43]. In addition to this, ADMSCs have been observed to exhibit reproducible chondrogenic differentiation potential [44].

The harvesting of adipose tissue via a minimally invasive liposuction (lipoharvest) has been previously described in past publications [28,45,46]. In brief, the procedure was performed under local anesthetic control with the lipoaspirate collected using either a manual syringe suction technique or via mechanical suction into a sterile single-use Shippert Tissu-Trans Collection Filter container (Shippert Medical, CO, USA). Immediately after collection, the lipoaspirate was transferred to a clean room laboratory facility on site (Magellan Stem Cells, Victoria, Australia) for further processing.

Isolation & expansion of MSCs

The laboratory protocol for the isolation and expansion of ADMSCs has been previously published [28]. Processing of ADMSCs was performed within a clean room facility with ISO5 air quality or greater with laboratory staff performing all manual tasks within Class II Biological Safety Cabinets. Culture-expanded ADMSCs were stored within cryovials under cryopreservation conditions using a validated control rate-freezing method. Cryovials were stored in liquid nitrogen until required [47,48].

Isolated cell populations were characterized using flow cytometry with Fluorescence-Activated Cell Sorting in accordance with standards established by the International Society of Cellular Therapy [49]. Samples from each participant 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.

Additional quality control testing for microbial growth/contamination by an independent pathology provider was performed on all isolated culture-expanded ADMSC participant samples.

Carrier media

At time of injection, MSCs were suspended in an autologous biological carrier media. The autologous biological carrier media was either autologous conditioned serum (ACS) or autologous platelet lysate (APL). Decision of which carrier media to use was based upon available resources at the time of injection.

ACS was prepared by the initial withdrawal of a total of 27 ml of whole blood via venepuncture – collected within 3 × 9 ml sterile S-Monovette® clotting activator tubes (Starstedt, Numbrecht, Germany). The S-Monovette tubes were incubated at 38°C for a period of 24 h after which they underwent centrifugation at 1000 rpm for 5 min. After successful separation of the whole blood into serum and cellular layers, the serum layer was removed and filtered through a 0.2-micron syringe filter (PALL, NY, USA), resulting in a final acellular sample of ACS.

APL preparation was achieved with the initial withdrawal of 25.5 ml of whole blood via venepuncture – 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). Using a bench-top centrifuge, the blood underwent an initial soft spin at 1000 rpm for 10 min. The separated plasma layer was collected within a sterile tube and underwent a second hard spin at 3500 rpm for a total of 5 min, resulting in the formation of a platelet concentrate plug and Platelet Poor Plasma (PPP). Excess PPP was removed and discarded leaving only 6 ml of PPP and the platelet plug. Using gentle manual agitation, the platelet plug was reconstituted within the reduced PPP volume creating a platelet-rich plasma (PRP) preparation. Calcium chloride (0.2 ml at 10%) was added to the PRP and incubated at 37°C for 10 min. This resulted in the formation of a fibrin clot that was allowed to retract and resorb over time. The final preparation was passed through a 0.2-micron syringe filter (PALL), producing an acellular and sterile APL.

Autologous carrier media preparations were stored at -20°C for later use at the time of ADMSC therapy.

Intervention

Arthroscopic abrasion arthroplasty

Arthroscopic knee surgery was performed by an experienced orthopedic knee specialist. Surgery was conducted under general anesthetic and lower limb vascular tourniquet control. An initial arthroscopic examination was performed with assessment of chondral surfaces, meniscus and any related intra-articular pathology.

Areas of unstable cartilage underwent chondroplasty using an arthroscopic shaver. Limited resection/debridement of displaced or complex meniscal tears were performed where suitable. AAA was undertaken following methods previously described by Johnson [35]. Using a 4–4.5 mm burr, arthroscopic abrasion was performed to areas of full-thickness cartilage loss with exposed bone debrided down to the subchondral plate until adequate capillary bleeding was observed (Figure 2).

Abrasion was performed to the margins of native cartilage and was performed on all surfaces accessible and with full-thickness chondral loss. Where full-thickness loss involved corresponding femoro-tibial or patello-femoral surfaces (often described as ‘kissing’ lesions), both surfaces underwent abrasion.

Postoperatively, participants were placed in a continuous passive motion device. The continuous passive motion was set at 0°–90° and participants were allowed to increase the passive range of motion based on symptoms. Participants were discharged home on the following day.

Injection method

Participants underwent an intra-articular injection of 50×10^6 ADMSCs within 1 week of their arthroscopic surgery and again at a planned 6 month time point (average time point of 2nd injection: 6 months + 4 weeks). On the day of injection, cryopreserved cells were thawed in a prewarmed sterile water bath. All cryoprotectant were removed through a process of repeat centrifugation and washing in chilled sterile phosphate-buffered saline. The resultant cell pellet was resuspended in 3 ml of carrier media. Prior to injection, cell number and viability were confirmed using a Muse Cell Analyzer (Merck, Millipore, MA, USA) (Table 2).

All intra-articular injections were performed under ultrasound guidance with the area of injection prepared using standard sterility protocols. Prior to injection of ADMSCs, 2 ml of 1% lidocaine was infiltrated superficially to the joint capsule. At the time of baseline injection up to 25 ml of hemarthrosis, a result of the AAA was aspirated and discarded. Under direct ultrasound visualization and using a superolateral approach, the resuspended autologous ADMSCs in 3 ml of carrier media were injected intra-articularly into the knee joint cavity.

Figure 2. Arthroscopic abrasion arthroplasty. Intraoperative arthroscopic pictures showing (A) Broad Grade IV osteoarthritis with full-thickness chondral loss involving both the medial femoral condyle and medial tibial plateau, (B) arthroscopic chondroplasty of unstable margins, (C) arthroscopic abrasion arthroplasty of the femoral condyle with exposure of bleeding subchondral bone and (D) arthroscopic abrasion arthroplasty of the tibial plateau with exposure of bleeding subchondral bone.

Table 2. Average cell count and viability confirmed using a Muse Cell Analyzer.			
Baseline ADMSC injection		6-month ADMSC injection	
Cell count (million)	Cell viability (%)	Cell count (million)	Cell viability (%)
52.22 (6.02)	94.39 (3.8)	52.07 (5.84)	94.63 (3.9)
All values in this table are mean (standard deviation). Mean data (n = 27 patients) are presented with standard deviations in brackets.			
ADMSC: Adipose-derived mesenchymal stem cell.			

Postoperative rehabilitation
Medial or lateral unicompartmental OA

In the period following AAA participants were advised to strictly use crutches with touch weight-bearing only through the operative side for 4 weeks. After 4 weeks, participants were allowed to transition to partial weight bearing and then to weight bearing as tolerated. Participants were fitted with a unicompartmental unloading knee brace (Ossur Unloader One, Ossur, Reykjavik, Iceland) and instructed to wear the brace for all partial to full-weight-bearing activities for the following 3 months.

Patello-femoral OA

In the immediate postoperative period, participants were instructed on the use of crutches for symptomatic relief. Participants were able to transition to full-weight bearing as tolerated. Weight-bearing flexion was increased as

Table 3. Outcome measures.

Outcome measure	Measurement point (months)
Primary outcome measure	
1. Adverse events	0, 6, 12, 24, 36
2. Numeric Pain Rating Scale	0, 6, 12, 24, 36
3. Knee Injury and Osteoarthritis Outcome Score	0, 6, 12, 24, 36
4. Western Ontario and McMaster Universities Osteoarthritis Index	0, 6, 12, 24, 36
5. Patient Global Impression of Change	36
Secondary outcome measure	
1. MRI analysis	
– MOCART score	12, 36
– T2 cartilage mapping	12, 36
MOCART: MRI Observation of Cartilage Repair Tissue.	

tolerated. This was consistent with previously published postoperative rehabilitation by Saw *et al.*, where they performed arthroscopic microdrilling and intra-articular peripheral blood progenitor cell therapy [50].

Combined medial & lateral compartment OA

In the period following AAA, participants were advised to strictly use crutches with touch weight-bearing only through the operative side for 6 weeks. At 6 weeks, participants were allowed to transition to partial weight-bearing and finally to full-weight bearing as pain allowed. Participants in this group had an extended period of touch weight-bearing as they were unable to be fitted with a unicompartmental unloading knee brace due to abrasion being performed on both the medial and lateral compartments.

During the period of reduced load, all participants were instructed on a lower limb muscular activation and passive range of motion program with active range of motion achieved 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. Beyond the period of reduced load bearing, participants were advised on a progressive lower limb-strengthening program where physiotherapy was encouraged. Early postoperative active range of motion exercises with progression to partial load bearing were advised due to previously documented benefits associated with cartilage health and maturation [51–53].

Outcome measures

The primary end points of this trial were to assess both the safety/tolerability of ADMSCs over a 36-month period in combination with AAA, and also to determine the long-term pain and functional changes as measured by the Numeric Pain Rating Scale (NPRS), the Knee Injury and Osteoarthritis Outcome Score (KOOS), the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and the Patient Global Impression of Change (PGIC) (Table 3).

In addition to this, a secondary end point was to determine the potential structural benefit and hence joint preservation result. Structural change was assessed using MRI with recording of the MRI Observation of Cartilage Repair Tissue (MOCART) score and assessment of cartilage quality through the use of the validated noninvasive method of T2 relaxation time mapping.

Questionnaires

The software program Clinical Intelligence (Melbourne, Australia) was used to record outcome questionnaire results. Questionnaire-based outcome measures were recorded at baseline, 6, 12, 24 and 36 months and included:

1. NPRS – participants were asked to rate their average pain intensity over the past week on an 11-point scale of 0–10 [54];
2. KOOS – consisting of five 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 [55];

3. WOMAC – a validated quality of life score in patients with symptomatic OA [56]. The Global WOMAC score was presented as an inverse percentage so to be comparable with the KOOS subscales;
4. PGIC – a self-reported measure that reflects the participant's belief about the efficacy of treatment [57]. PGIC is a 7-point scale with participants rating change as 'very much improved/completely recovered', 'much improved', 'minimally improved', 'no change', 'minimally worse', 'much worse' or 'very much worse'.

Magnetic resonance imaging

The area of baseline-diagnosed Grade IV OA was visually assessed using MRI at 12 months and again after 36 months (average 38 months). MRI was performed on a 1.5T or greater MRI using a dedicated knee coil placed centrally over the knee with the patient positioned centrally within the magnet bore. Images were taken in coronal, sagittal and axial planes using proton density and proton density fat-saturated sequences. For participants who had additional T2 mapping, MRI was performed on Phillips Ingenia, and Achieva 3.0T systems (software release 5.3 or above) with the image protocol consisting of a 2D fast spin-echo T2-mapping sequence using the following acquisition parameters: repetition time/echo time, 2000 ms/11, 22, 33, 44, 55 and 66 ms; bandwidth, 241.1 Hz/pixel; number of excitation, 6; field of view, 160 mm; slice thickness, 2.5 mm; acquisition time, 6 min and 50 s.

Quantitative assessment of structural cartilage regeneration was performed using MOCART and T2-mapping techniques:

1. 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 [58];
2. T2 mapping – MRI T2 relaxation time cartilage mapping is a validated noninvasive method used to assess the quality of cartilage and has been used in previous studies to comment on the structural outcome of cartilage regeneration techniques [39,58–62]. For unicompartmental tibio-femoral OA, T2 relaxation time values were compared against an area of native cartilage within the contralateral compartment of the joint. T2 values greater than 100 ms were excluded from analysis as they are understood to represent tissue other than cartilage (i.e. synovial fluid) or result from chemical shift artefact [63,64].

Sample size

No sample size calculation was performed.

Statistical analysis

Statistical analysis was performed using SigmaPlot 10 graphical and statistical software (Systat Software, Inc., CA, USA). Quantitative outcome measures of NPRS, WOMAC and KOOS were assessed by One-Way Repeated Measures ANOVA to determine mean differences in time (baseline vs 6, 12, 24 and 36 months) for each outcome measure. The objectives were to determine if mean differences occurred when comparing 6, 12, 24 and 36 months data points to baseline measurements.

When statistically significant ($p < 0.05$) interactions between the different time points were apparent an All Pairwise Multiple Comparison Procedure (the Holm–Sidak method) was employed to determine where the specific mean differences for time were apparent with the overall significance level set at 0.05.

A simple linear regression analysis was also performed between baseline NPRS score and NPRS pain relief at 3 years.

Adverse events

Adverse events (AEs) were defined as an undesirable clinical occurrence in a participant which were not present prior to treatment or which increased in severity after treatment and were recorded during the follow-up period. AEs were additionally defined as related or unrelated to the treatment and also expected or unexpected. AEs were graded in regard to severity:

Mild – a transient symptom or clinical sign resolved with use of simple interventions including simple analgesia and which did not interfere with the subject's usual activity.

Moderate – a symptom which may require treatment including regular analgesia (i.e., opiate analgesia) and which limits the subject's usual daily activity.

Table 4. Demographic characteristics.	
Demographic characteristics	Data
Total number (n)	27
Gender (male/female)	18/9
Age (years; mean $\pm$ SD)	53.6 $\pm$ 6.7
Height (m; mean $\pm$ SD)	1.78 $\pm$ 0.1
Weight (kg; mean $\pm$ SD)	87.4 $\pm$ 16.8
BMI (kg/m ² ; mean $\pm$ SD)	27.7 $\pm$ 5.3
Past knee surgery	20
Past meniscus resection	18
ACL deficiency	3
Site of Grade IV OA:	
– Medial compartment	17
– Lateral compartment	2
– Patello-femoral compartment	6
– Multicompartment	2
Hypertension	3
Hypercholesterolemia	7
Diabetes	Nil
Smoker	Nil
ACL: Anterior cruciate ligament; OA: Osteoarthritis; SD: Standard deviation.	

Severe – a symptom which may require use of regular analgesia and significantly impacts on the subjects' usual activity and extends for greater than 2 weeks.

Serious – defined as an untoward medical occurrence resulting in a life-threatening condition, or requiring hospitalization and/or results in chronic and significant disability or results in death.

Results

Demographic characteristics

In this case series, 32 participants were formally enrolled. Two participants were excluded from analysis due to additional interventions that may have improved/impacted outcome and a further participant was excluded due to unrelated knee surgery during the time course of follow-up. Two participants were lost to follow-up. The final sample size of the case series was 27 participants and consisted of nine females and 18 males aged between 32 and 63 years of age (mean: 53.6 years) (see Table 4). Twenty of the 27 participants had a past history of knee arthroscopy with five participants having a history of anterior cruciate ligament (ACL) rupture and later ACL reconstruction. While three of the 27 knees had image-confirmed ACL deficiency, no participant had a history of mechanical instability. The site of OA was primarily the medial compartment (17 participants) with six participant knees having patello-femoral OA, two participants with lateral compartment OA and two participants having multicompartmental grade IV OA (involving the medial and lateral compartments $\pm$ patello-femoral compartment).

Participants had a mean BMI of 27.7 with 7 of the 27 participants categorized as obese. Three participants had a history of hypertension and seven participants had a history of hypercholesterolemia. All participants were nonsmokers. All participants used simple analgesia and/or oral nonsteroidal anti-inflammatories as required.

Pain & function

Consistent and progressive improvement in pain and function was seen across the period of follow-up. A single participant failed treatment and underwent TKR after 12 months of follow-up. Data beyond 12 months represents that of the remaining 26 knees.

Pain, as measured by NPRS, showed clinical and statistically significant improvement against baseline at all time points of follow-up, improving from a mean (SD) score of 5.6 (2.7) at baseline to 1.5 (1.4) at 36 months (Figure 3). This equated to a mean reduction in pain levels of 73.2%. Improvement in NPRS expressed against baseline pretreatment scores showed a clinically significant and robust positive correlation within the case series (r

Figure 3. Numeric pain rating scale. Mean data from 27 patients with error bars representing 95% confidence intervals.
*Indicates a significant difference between that time point and baseline. As a single participant failed treatment and underwent total knee replacement after 12 months of follow-up data beyond 12 months represents that of the remaining 26 participants.
AAA: Arthroscopic abrasion arthroplasty; MSC: Mesenchymal stem cell.

Table 5. Patient global impression of change at 36 months (n = 26).	
PGIC at 36 months	Percentage of participants
Very much improved	23.3%
Much improved	69.2%
Slightly improved	3.8%
No change	0%
Slightly worse	3.8%
Much worse	0%
Very much worse	0%

PGIC: Patient global impression of change.

– 0.82; $p < 0.001$) with the value for the linear regression line slope being 0.66 (a slope of 1 indicating complete resolution of pain) (Figure 4).

Improvement seen in NPRS was reflected in the KOOS subscale analyses with clinical and statistically significant improvement in all subscales. Importantly, values continued to improve until final follow-up at 36 months (Figure 5). The Quality of Life subscale improved by an average of 119.1% with even larger improvements seen in the sport and recreation subscale of 161%. Global WOMAC score expressed as an inverse percentage correlated with the improvements seen in the KOOS subscales with an improvement of 55.2% (Figure 6).

Of the 26 participants who completed 36 months of follow-up, satisfaction with treatment, as represented by PGIC, indicated overall satisfaction with 92.5% of participants indicating that they were ‘very much improved’ or ‘much improved’ at completion of follow-up at 36 months. A single participant indicated that they had progressed and were ‘slightly worse’ at final follow-up (Table 5).

Figure 4. Simple linear regression of baseline numeric pain rating scale pain score versus numeric pain rating scale pain relief at 3 years. The pain relief was calculated by taking away the 3-year NPRS pain score from the baseline NPRS pain score. Values for the linear regression line slope (0.66) and linear regression coefficient (r) are depicted. Group mean data are depicted. Significance of the associated variables is ($p < 0.001$) with the power of the performed test with alpha 0.05 = 1. A 'perfect' treatment with slope of 1.00 is shown for comparison. NPRS: Numeric pain rating scale.

Figure 5. Knee injury and osteoarthritis outcome scores for the five knee injury and osteoarthritis outcome subscales for patients who received an arthroscopic abrasion arthroplasty + mesenchymal stem cells at baseline. *Indicates significant differences ($p < 0.05$) at 6, 12, 24 and 36 months for all KOOS subscales when compared with their baseline KOOS score. Mean data from 27 participants with error bars representing 95% confidence intervals. As a single participant failed treatment and underwent total knee replacement after 12 months of follow-up data beyond 12 months represents that of the remaining 26 participants. ADL: Function in daily living; KOOS: Knee Injury and Osteoarthritis Outcome score.

Figure 6. Global Western Ontario and McMaster Universities Osteoarthritis Index score. Mean data from 27 patients with error bars representing 95% confidence intervals.
*Indicates significant differences ($p < 0.05$) at 6, 12, 24 and 36 months when compared with baseline score. Note that the WOMAC score is represented as an inverse score to allow direct comparison to the Knee Injury and Osteoarthritis Outcome score analysis. As a single participant failed treatment and underwent total knee replacement after 12 months of follow-up data beyond 12 months represents that of the remaining 26 participants.
AAA: Arthroscopic abrasion arthroplasty; MSC: Mesenchymal stem cell; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index.

Table 6. Minimal clinically important difference at follow-up time points in comparison to baseline data.				
Outcome measures	6 months	12 months	24 months	36 months
NPRS	75	89.3	92.6	92.6
WOMAC	74.1	82.1	96.3	92.6
KOOS (pain improvement)	76.9	85.2	96.2	92.3
KOOS (symptom improvement)	66.7	75	88.9	81.5
KOOS (ADL)	70.4	78.6	96.3	92.6
KOOS (Sport & Rec)	75	82.1	96.3	92.6
KOOS (QoL)	67.9	82.1	100	82.1
Averages	72.3%	82.1%	95.2%	89.5%

All data are in percentages and expressed as the percentage of subjects who registered a minimal clinically important difference when compared with their baseline value. Note that an minimal clinically important difference equates to a decrease of 1 point or more on an 11-point numeric pain rating scale and an increase of 8 points or more on a 100-point KOOS or WOMAC score.
ADL: Function in daily living; KOOS: Knee Injury and Osteoarthritis Outcome score; QoL: Quality of life; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index.

Importantly, improvements observed in pain and function were both statistically significant but also clinically significant with 89.5% of participants achieving a minimal clinically important difference (MCID) at 36 months when averaging all outcome measures (Table 6).

Structural outcome

MRI was performed at 12 and 36+ months and showed successful and sustained cartilage regeneration (Figure 7). A single participant was unable to have MRI due to a spinal implant. Of the 26 participants who had MRI at

Figure 7. Sequential coronal and sagittal MRI proton density images of a participant's knee at baseline, 12 and 36+ months.

Table 7. MRI observation of cartilage repair tissue score. Average MRI observation of cartilage repair tissue score per compartment.		
Compartment	12 months (mean $\pm$ SD), n = 26	36+ months (mean $\pm$ SD), n = 21
Medial compartment	73.8 $\pm$ 9.6	75 $\pm$ 13.4
Lateral compartment	80.0 $\pm$ 0.0	80.0 $\pm$ 7.1
Patello-Femoral compartment	71.0 $\pm$ 6.5	76.0 $\pm$ 6.5
Multicompartmental	72.5 $\pm$ 3.5	70.0 $\pm$ 0.0
Overall average MOCART score	73.7 $\pm$ 8.6	75.5 $\pm$ 11.1
Bold is used to highlight the overall score. MOCART: MRI observation of cartilage repair tissue; SD: Standard deviation.		

12 months, a total of 21 of these participants had a further MRI at 36+ months of follow-up. Assessment of cartilage repair using the MOCART score indicated considerable cartilage repair at 12 months with a mean MOCART score of 73.7 out of a maximal score of 100. Improvement was maintained at 36+ months with a mean MOCART score of 75.5. Results were comparable for areas of Grade IV OA that had undergone AAA and ADMSC therapy in the medial, lateral and/or patella-femoral compartments when averaged individually (Table 7). See [Supplementary Table 1](#) for additional MOCART analysis.

Of the 26 participants, 18 had MRI T2-weighted imaging performed at 12 months with 14 having additional T2-weighted imaging at completion of follow-up at 36+ months. In those participants with unicompartmental medial or lateral compartment OA, average T2 scores in the deep, intermediate and superficial zones compared favorably with a chosen sample area of native cartilage of the contralateral compartment taken at 12 months (see Table 8 & Figure 8). Comparison of overall average 12 and 36+ month values indicated progressive maturation of regenerative cartilage (average 41.7 and 40.0 ms comparatively).

Two of the participants had marked genu-varum on initial clinical examination with degree of varus angulation measured by long-leg mechanical axis x-ray performed at baseline and again at 12 months. Radiological assessment confirmed a correction of 3.0° and 3.1° after 12 months, respectively (Figure 9).

Complications & AEs

Participants experienced expected discomfort and limitation in daily activities due to the postoperative period of touch weight-bearing and use of crutches following AAA. No unexpected or serious AEs occurred as a result of the surgical intervention of AAA. A reproducible self-limiting flare of discomfort and swelling was observed following ADMSC injections. This responded to conservative management including ice and oral analgesics including oral nonsteroidal anti-inflammatories. No serious or unexpected AEs relating to ADMSC therapy were observed. See [Supplementary Tables 2–4](#) for a complete list of graded AEs.

Figure 8. Example of sequential MRI T2 mapping of the area of cartilage regeneration at 12 and 36+ months. Formal T2 mapping was done over a number of slices to include the entire area of regeneration. This analysis indicated improved maturation over time with values comparable to T2 values in areas of native cartilage.

Figure 9. Long leg mechanical axis x-ray of a participant at baseline and 12 months.

Table 8. MRI T2 relaxation time values within compartments having undergone arthroscopic abrasion arthroplasty in combination with adipose-derived mesenchymal stem cell therapy.

Compartment	MRI T2 relaxation time values		
	12 months	Native cartilage at 12 months	36+ months
Medial compartment:			
– Deep zone	38.4 (9.4)	33.0 (9.0)	38.9 (12.0)
– Intermediate zone	40.7 (8.8)	39.3 (9.8)	35.7 (9.2)
– Superficial zone	49.4 (9.1)	47.4 (10.25)	44.6 (6.8)
– Average	42.8 (10.1)	40.0 (11.3)	39.7 (10.1)
Lateral compartment:			
– Deep zone	39.1 (9.3)	29.4 (3.0)	37.2 (7.9)
– Intermediate zone	38.3 (5.2)	36.8 (7.1)	37.4 (5.5)
– Superficial zone	47.3 (2.8)	47.3 (8.9)	45.5 (6.9)
– Average	41.5 (7.4)	37.8.6 (9.8)	40.0 (7.3)
Patello-femoral compartment:			
– Deep zone	40.0 (3.0)		45.0 (6.7)
– Intermediate zone	37.2 (2.4)		37.5 (2.9)
– Superficial zone	38.9 (2.73)		38.7 (4.3)
– Average	38.7 (3.1)		40.4 (6.2)
All compartments:			
– Deep zone	38.8 (8.4)		40.7 (10.4)
– Intermediate zone	39.5 (7.3)		36.5 (7.1)
– Superficial zone	46.9 (8.2)		42.7 (6.6)
– Average	41.7 (8.7)		40.0 (8.5)

Native cartilage values were recorded over the central weight-bearing region of the contralateral femoral condyle that did not undergo surgical intervention. Average values are in milliseconds with standard deviations in brackets.
ADMSC: Adipose-derived mesenchymal stem cell.

Discussion

The increasing prevalence of symptomatic OA is of considerable concern with the rate of TKR expected to grow by 600% between the years 2005 and 2030 [65]. Current conservative measures are aimed at symptomatic control rather than disease modification with total joint arthroplasty accepted as the treatment of choice for those patients who fail conservative interventions. Alarming, an increasing number of total joint replacements are being performed on patients under the age of 65 [5]. This is additionally concerning when recognizing that the risk of requiring revision TKR is 2.5-times higher when primary TKR is performed under the age of 65 [66].

In this case series study, 27 participants under the age of 65, with severe Grade IV OA and otherwise suitable for consideration of TKR, underwent AAA in combination with ADMSC therapy. Surprisingly despite sequential recruitment, there were far greater male participants with a male to female ratio of 2:1. Treatment was well tolerated with no related serious AEs associated with either AAA or ADMSC therapy (both ADMSC treatment and the lipoharvest procedure). Following AAA participants experienced a period of discomfort and reduced mobility due to the requirement of touch weight-bearing with use of crutches. No unexpected AEs were documented as a result of surgery. ADMSC therapy was associated with an observed self-limiting period of swelling and/or pain that responded to oral analgesics and/or application of ice. As these AEs have been documented in past research, they were recorded as expected AEs [25–27,39].

Improvement was observed in pain and functional assessments consistently throughout the time course of follow-up. Pain, as assessed by NPRS, improved by 65.2% at 12 months and continued to improve until final follow-up at 36 months with final mean improvement of 73.2%. Improvement in NPRS, KOOS and WOMAC scores were reflected both statistically ($p < 0.05$) and clinically with MCID values. Importantly, Function in Daily Living and Quality of Life KOOS subscales improved by 51.7 and 119%, respectively, when comparing baseline values to the final 36 month time point. Pain improvements, compared with baseline, were both statistically significant ($p < 0.05$) at all time points and remained clinically significant at 36 months (MCID). It is important to recognize that

a single patient of the enrolled 27 participant case series failed AAA and ADMSC therapy and underwent TKR after 12 months. Results after 12 months therefore reflect analyses of the remaining 26 participants.

Improvements observed in pain and function were mirrored by consistent and stable chondral regeneration at 12 and 36+ month follow-up time points as assessed by MRI analysis. MOCART assessment indicated sustained improvement until final follow-up. Limited subchondral cyst formation was observed in some participants in zones beneath the area of chondral regeneration or at the interface of regenerative and native cartilage. These cysts were stable between 12 and 36+ months and have been observed in other cartilage graft techniques including Matrix-induced Autologous Chondrocyte Implantation and microfracture [67].

In addition to structural improvements, quality of cartilage regeneration as measured by quantitative T2-weighted imaging was indicative of hyaline-like cartilage morphology. Participants with unicompartmental Grade IV OA (medial or lateral compartments) showed regenerative cartilage with T2-mapping values comparable to an area of native cartilage within the contralateral compartment. Importantly, of the 18 participants who had T2 mapping performed at baseline, 14 participants had additional T2 mapping performed at 36+ months with additional improvement in T2 values suggestive of progressive cartilage maturation. This observation is similar to that observed in our past research assessing the same technique for focal full-thickness chondral pathology whereby improved T2-mapping values over time are suggestive of reduced water content and improved collagen anisotropy consistent with progressive cartilage maturation [39].

In this study, areas of Grade IV chondral loss that had not undergone AAA failed to show chondral regeneration. This suggests that abrasion, with disruption of the arthritic sclerotic subchondral bone until capillary bleeding is observed, and the resultant migration and proliferation of bone marrow-derived MSCs, is an important requirement in achieving chondral regeneration. In addition, it is understood that the release of chemotactic cytokines at the site of abrasion arthroplasty – including TGF and CXCL chemokines – assist in the migration of intra-articular injected MSCs to the site of abrasion [68,69]. In effect, the observed chondral regeneration has benefited from both the combined reparative effect of local endogenous bone-marrow-derived MSCs and the intra-articular injection of ADMSCs; a double-dosing of MSCs. The relative paucity of MSCs within bone marrow aspirate potentially explains the marked improvement in repair tissue quality with hyaline-like cartilage repair observed in this trial with introduced intra-articular MSCs. This is in direct comparison to the fibrocartilaginous repair tissue seen when bone marrow stimulation techniques are used in isolation [35–37].

It is unclear whether in this trial the ADMSCs directly integrated into the repair site and differentiated along a chondrocyte lineage as documented in preclinical trials assessing bone marrow stimulation techniques or assisted regeneration through a paracrine mechanism with release of reparative cytokines [70,71]. While some preclinical trials have shown successful engraftment of MSCs at the site of chondral repair, other trials have failed to show this and/or have indicated reduction in MSC number over time [72,73]. Further research with use of cell-labeling techniques including magnetic tagging would allow for additional assessment of cell migration, integration and retention [74,75].

In this case series study, an autologous biological carrier media of either ACS or APL was used in which to suspend MSCs at the time of injection. Previous research has indicated improved expression of anti-inflammatory cytokines including IL-1ra and anabolic growth factors such as TGF- β by MSCs in the presence of a blood-derived carrier media [76,77]. In addition, TGF- β has been shown to upregulate expression of collagen type II, reduce expression of type I collagen and also assist with migration of stromal cells to the site of injury [68]. However, as AAA results in a postsurgical hemarthrosis, it is debatable whether the use of a biological carrier media offers additional benefit in regard to MSC action.

It is recognized that the use of an autologous blood-derived biological carrier media may have had additional benefit with past studies showing reproducible pain and functional improvements with the use of ACS or PRP in the treatment of knee OA [68,78]. Importantly, the use of ACS or PRP in symptomatic OA involves multiple weekly to monthly intra-articular injections, whereas the treatment protocol used in this trial involved only an injection at baseline and again at 6 months. In addition, Bastos *et al.* have previously assessed the benefit of MSC therapy in combination with PRP versus MSC therapy in isolation and indicated no observable additional improvement [79]. This suggests that the use of a biological carrier media, particularly with the aforementioned post-surgical hemarthrosis, is unlikely to offer additional benefit to MSC therapy alone.

In regard to limitations, this trial is understandably limited by its nature as a case series study with its lack of a suitable control group undergoing AAA without additional ADMSC therapy. Prior research assessing AAA in isolation has, however, shown inconsistent results with poor cartilage regeneration and early conversion to TKR

within 2–5 years [34]. Alternatively, in this trial, while a single participant underwent TKR after 12 months, 92.5% of the remaining 26 participants indicated either a very much improved, or much improved, global impression of their knee at 36 months as expressed in their PGIC, thus inferring the additional benefit gained from combining AAA with ADMSCs. MRI assessment showed retained cartilage regeneration with progressive improvement in cartilage maturation at 36+ months of follow-up.

Importantly, it is notable that 18 of the participants had a history of meniscal resection that is a clear risk factor for secondary OA and hence it is accepted that further long-term follow-up is required to show sustained and stable structural benefit. In addition to this, while radiological follow-up indicated partial correction of femorotibial mechanical axis (Figure 9) persistent varus or valgus malalignment is understood to result in increased medial or lateral compartment loading and risk of progression in OA. Further research is required to determine the reproducible corrective ability of AAA combined with MSC therapy and whether those with significant baseline malalignment may benefit from additional realignment surgery including distal femoral or high tibial osteotomy [42].

An additional limitation of the trial is the lack of histopathology assessment of regenerative cartilage. Repeat arthroscopy and chondral biopsy could not be clinically justified and unfortunately chondral samples were not taken of the participant who underwent TKR after 12 months. Despite this limitation, it is argued that T2-weighted imaging as used in this trial is a validated and noninvasive quantitative assessment of regenerative cartilage with the comparable values to native cartilage indicating similar cartilage anisotropy and hyaline-like properties.

Our previous published trials assessing the use of ADMSC therapy for moderate knee OA, although providing significant pain and functional improvement, did not document the same robust cartilage regeneration or outcome measure results as seen in this study [27,39]. Despite the lack of a control group, the pain, functional and structural results seen in this case series against the previously well established and published limitations of bone marrow stimulation techniques alone represent convincing ‘real-world evidence’ of efficacy.

Conclusion

This case series study illustrates the novel and successful treatment of advanced Grade IV OA of the knee with AAA in combination with ADMSCs. No serious AEs were observed and both AAA and ADMSCs were well tolerated by all participants. Both statistical and clinically significant improvement in pain and function was consistently observed throughout the follow-up period. Structural analysis confirmed stable hyaline-like cartilage regeneration and was comparable to results observed using the same technique for focal full-thickness cartilage lesions. This case series indicates that AAA in combination with ADMSC is an effective joint preservation technique in patients with advanced knee OA who are unsuitable for TKR due to age or coexisting co-morbidities.

Translational perspective

Research in the area of regenerative medicine continues to grow with the use of MSC-based therapies in the management of musculoskeletal conditions including OA showing particular promise with documented safety and efficacy in early trials. This case series study is the first to successfully show the use of ADMSCs in conjunction with a current surgical technique, AAA, in the treatment of advanced knee OA with resultant joint preservation and prevention of need for TKR. Given the increasing impact of OA with observed significant debility and economic impact, MSC therapy, whether used in isolation or in combination with surgical management techniques represents an exciting therapeutic advancement.

Author contributions

All the authors were involved in conception of the study and designed the study protocol. J Wickham was involved in developing the statistical analysis strategy.

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Financial & competing interests disclosure

J Freitag and A Tenen are clinic partners within Melbourne Stem Cell Centre. J Freitag, K Shah, D Li and A Tenen are affiliated with Magellan Stem Cells and are part of its Clinical and Scientific Advisory Committee. J Freitag is the chief clinical officer of Magellan Stem Cells. K Shah is the chief scientist 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 coauthors 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.

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 Clinic Trial Registry (Trial ID: ACTRN12617000638336). In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Data sharing statement

Individual deidentified participant data, which underlie results reported in this article, will be available upon publication. The study protocol will also be available. 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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Summary points

- Osteoarthritis (OA) is a leading cause of disability with a prevalence rate of 10% of males and 18% of females over the age of 45 years.
- Alarming, there has been an observed increase in the number of patients undergoing total knee replacement under the age of 65.
- Surgical total joint replacement remains the accepted treatment for knee OA uncontrolled by conservative therapies and is not without complication with up to 20% of patients experiencing persistent pain and loss of function at 12 months.
- Previous clinical research has supported the use of mesenchymal stem cell therapy in conjunction with arthroscopic abrasion arthroplasty in the treatment of focal cartilage lesions.
- This is the first case series to assess the use of adipose-derived mesenchymal stem cell (ADMSC) therapy in conjunction with arthroscopic abrasion arthroplasty in the treatment of broad advanced OA of the knee.
- Clinically, significant pain and functional improvement was observed over a 36-month follow-up period.
- Quantitative MRI analysis using validated T2-weighted imaging showed hyaline-like regenerative cartilage with values approaching that of native cartilage.
- No related serious adverse events were observed and ADMSC therapy was well tolerated.
- Autologous ADMSC therapy represents an exciting joint preservation therapy with the delay or prevention of need for later total knee replacement.

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