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Adipose derived mesenchymal stem cell therapy in the treatment of isolated knee chondral lesions: design of a randomised controlled pilot study comparing arthroscopic microfracture versus arthroscopic microfracture combined with postoperative mesenchymal stem cell injections

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ABSTRACT

Introduction: The management of intra-articular chondral defects in the knee remains a challenge. Inadequate healing in areas of weight bearing leads to impairment in load transmission and these defects predispose to later development of osteoarthritis. Surgical management of full thickness chondral defects include arthroscopic microfracture and when appropriate autologous chondrocyte implantation. This latter method however is technically challenging, and may not offer significant improvement over microfracture. Preclinical and limited clinical trials have indicated the capacity of mesenchymal stem cells to influence chondral repair. The aim of this paper is to describe the methodology of a pilot randomised controlled trial comparing arthroscopic microfracture alone for isolated knee chondral defects versus arthroscopic microfracture combined with postoperative autologous adipose derived mesenchymal stem cell injections.

Methods and analysis: A pilot single-centre randomised controlled trial is proposed. 40 participants aged 18–50 years, with isolated femoral condyle chondral defects and awaiting planned arthroscopic microfracture will be randomly allocated to a control group (receiving no additional treatment) or treatment group (receiving postoperative adipose derived mesenchymal stem cell treatment). Primary outcome measures will include MRI assessment of cartilage volume and defects and the Knee Injury and Osteoarthritis Outcome Score. Secondary outcomes will include further MRI assessment of bone marrow lesions, bone area and T2 cartilage mapping, a 0–10 Numerical Pain Rating Scale, a Global Impression of Change score and a treatment satisfaction scale. Adverse events and cointerventions will be recorded. Initial outcome follow-up for publication of results will be at 12 months. Further annual follow-up to assess long-term differences between the two group will occur.

Ethics and dissemination: This trial has received prospective ethics approval through the Latrobe University Human Research Ethics Committee. Dissemination of outcome data is planned through both national and international conferences and formal publication in a peer-reviewed journal.

Trial registration number: Australia and New Zealand Clinical Trials Register (ANZCTR Trial ID: ACTRN12614000812695).

BACKGROUND

The management of intra-articular chondral defects presents a challenge to clinicians. The capacity of articular cartilage to repair, particularly after skeletal maturity, is limited.[1 2] Incomplete healing in areas of weight bearing leads to impairment in load transmission and several studies have indicated a predisposition to later development of degenerative osteoarthritis.[3 4]

Cartilage regeneration has an inherently low healing potential due to the avascular nature of cartilage and hence lack of systemic regulation.[1] In the absence of bleeding, no

fibrin clot or network is developed to act as a scaffold for tissue repair and the release of inflammatory mediators and other cytokines involved in the stimulation of cellular migration and proliferation is limited. This leaves the existing latent chondrocytes to facilitate the healing mechanism without external stimulus.[1]

Treatment options for chondral defects range from conservative to surgical interventions, with the choice of treatment dependent on the stage of the lesion (partial vs full thickness), site of the lesion and also the patient's clinical presentation. Surgical management of traumatic and/or degenerative chondral defects includes arthroscopic debridement, microfracture/osteoplasty and when appropriate autologous chondrocyte implantation (ACI) or matrix-induced autologous chondrocyte implantation (MACI). These latter methods are technically difficult and can be associated with a high failure rate.[5 6] Procedures intending to 'unload' the affected area of the knee, such as realignment osteotomy, can be used in combination with the above.

Microfracture has become a commonly practised surgical technique to assist in stimulating a healing response. This technique involves making multiple holes (microfractures) into the subchondral plate at the site of a full thickness chondral defect. This exposes bone marrow derived pluripotent cells to the articular surface and creates an environment amenable to healing.[7] Multiple studies have successfully shown a cartilaginous response at the sites of microfracture, yet histology has confirmed that this tissue is fibrocartilage rather than the hyaline cartilage typical of normal articular surfaces.[8 9] While evidence suggests effective short-term functional improvement of knee function following microfracture, long-term results are inconclusive. Inadequate defect filling and poor load bearing quality of fibrocartilage have been postulated as reasons for poor long-term outcome.[10 11]

A growing understanding of the pathology of chondral defects and their inherent inability to heal has seen increased focus on the area of regenerative medicine. Mesenchymal stem cells (MSCs) have an intrinsic role in tissue repair and regeneration and display plasticity and multipotency; being able to differentiate towards osteoblasts, chondrocytes and adipocytes.[12] These cells are present in bone marrow, peripheral blood, skeletal muscle, heart muscle and adipose tissue.[13]

Recent work has demonstrated that autologous MSCs can differentiate into cartilage and bone supporting their potential in the treatment in degenerative chondral lesions and osteoarthritis.[14 15] The capacity of MSCs to influence the disease process and healing mechanism may be achieved however through an immunomodulatory and paracrine mechanism rather than their differentiation capability and pluripotentional nature.[16]

MSCs are observed to suppress inflammatory T-cell proliferation, and inhibit maturation of monocytes and myeloid dendritic cells resulting in an immunomodulatory and anti-inflammatory effect.[16] They also produce essential cytokines such as transforming growth factor β, vascular endothelial growth factor and epidermal growth factor and secrete an array of bioactive molecules that stimulate local tissue repair.[12 17 18]

Further research highlighting the proinflammatory cytokines involved in the destruction of hyaline cartilage and development of degenerative osteoarthritis has identified the potential of MSCs as a disease modifying agent due to their immunomodulatory/anti-inflammatory properties.[19]

Cartilage regrowth has been shown in animal models with chondral defects after treatment with MSCs.[20 21] Significant improvement in lameness and range of motion in dogs following intra-articular stromal cellular injections compared to control has been observed.[22] Further, histological analysis has indicated better hyaline cartilage repair in goat chondral defects treated with microfracture/subchondral drilling and postoperative bone marrow aspirate injections than when treated with microfracture alone.[23] Enhanced tissue repair with histological confirmation of improved proteoglycan content has also been shown after the use of intra-articular MSC injections following microfractured chondral defects in horses.[24]

In the human literature, there is growing evidence supporting the efficacy of MSC therapy for chondral defects. A randomised control trial has assessed the efficacy of intra-articular injections of peripheral blood MSCs following arthroscopic subchondral drilling (a variant of microfracture) of knee chondral defects.[25] Histological and MRI analysis at 18 months showed a statistically significant improvement in cartilage quality when compared to subchondral drilling without MSCs. As observed by the authors, limited follow-up (18–24 months) and confounding variables of multiple active treatments (ie, HA and MSCs) means that further research is required. It is also not known whether the use of alternative sources of MSCs (ie, adipose derived) would lead to similar outcomes.

Preliminary research on MSCs was carried out using bone marrow derived cells and there is increasing evidence of the use and possible benefits of bone marrow concentrates in the treatment of degenerative knee conditions.[26] However, the multicellular mononuclear make up of these preparations and combined use of biological carrier mediums such as platelet-rich plasma means that it is difficult to determine which component may have caused any observed effect. Further, bone marrow harvest procedures are uncomfortable and can yield inconsistent numbers of MSCs.[27]

The investigation of MSCs in the treatment of various conditions including cartilage lesions continues to grow. The National Institutes of Health lists 367 current trials in the area of MSCs.[28] Importantly, based on current clinical trial outcomes, MSC therapy appears safe. A recent systematic review and meta-analysis of trials involving a total of 1012 participants receiving intravascular MSC therapy for various clinical conditions including

ischaemic stroke, Crohn's disease, cardiomyopathy, ischaemic heart disease and graft versus host disease, did not identify any significant adverse events other than transient fever.[29] Patients were followed up in some studies for over 90 months. This meta-analysis included both autologous and allogeneic MSCs and also expanded/cultured cells.

MSCs due to their multipotential and ability to differentiate along a chondrogenic lineage, coupled with their immunomodulatory properties show increasing promise in offering a disease modifying treatment and may have significant impact on disease progression.

We hypothesise that the use of autologous adipose derived MSCs injected following arthroscopic knee microfracture will improve the quality of repair with hyaline cartilage like repair tissue, more complete filling of the articular defect and superior integration of the repair into surrounding cartilage when compared to microfracture alone. We hypothesise that pain and activity capability should parallel the hyaline cartilage changes.

Isolated autologous adipose derived MSCs have been chosen as they are immunocompatible, lack the ethical concerns associated with embryonic stem cells, have been shown to be safe and are relatively easy to harvest.[29–34] Adipose derived MSCs have been shown to be up to 500 times more prevalent that bone marrow mesenchymal stem cells when comparing an equivalent volume of tissue (lipoaspirate vs bone marrow aspirate).[34]

While past publications have indicated superior chondroprogenitor potential of bone marrow derived MSCs, this has been an inconsistent finding with further research indicating comparative chondrogenic ability of MSCs from either bone marrow or adipose tissue.[32–34] As the role of MSCs in healing and regeneration may be more through paracrine and immune-modulatory pathways, chondrogenic differentiation may be of less importance than first anticipated.[16]

The proposed phase II pilot study will evaluate the effectiveness and safety of autologous adipose derived MSCs following microfracture in knee joint chondral defects. Results will assist in determining the importance and relevance of performing a larger phase III trial. As mesenchymal stem cells derived from different locations may have different properties this project will also determine whether adipose derived MSCs have similar effect to that of peripheral blood MSCs observed in previous publications.[35]

This study is a preliminary step in the process of obtaining evidence on which practitioners and their patients with knee chondral defects can make important treatment selection decisions. Should the results of this trial be promising, future research, building on the foundation of this current project, may have the potential to significantly reduce the economic burden of knee joint chondral defects and later early onset osteoarthritis on healthcare systems around the world.

METHODS

Study design

This will be a single centre pilot randomised controlled trial. An overview of the process of the trial is presented in figure 1.

Setting

Assessment for inclusion into the study will be conducted at the Melbourne Stem Cell Centre, Melbourne, Australia. Participants randomised to the treatment group will undergo all treatment at this location. The treatment will include a liposuction stem cell harvest and postarthroscopy stem cell injections.

Arthroscopy and microfracture will be performed within the hospitals where the referring orthopaedic surgeon has formal accreditation. The arthroscopy forms part of the accepted standard treatment of the participant and is external to the study. The arthroscopy will be performed by the referring surgeon and will not be limited to a single surgeon.

Eligibility and screening

Participants currently planning arthroscopic microfracture will be sought via advertising to orthopaedic specialists. Potential participants will then undergo preliminary screening by a study doctor via telephone according to the eligibility criteria in box 1. Those found to be potentially eligible will be invited to attend a baseline assessment prior to the planned arthroscopy to confirm eligibility and gain informed consent to participate.

Baseline assessment

Baseline assessment will involve a subjective and physical examination including: history, observation (posture/gait), active movement testing, palpation and passive tests for structural integrity of the knee ligaments and menisci. The preoperative diagnostic MRI which confirmed an isolated cartilage defect will be viewed to assess relevant eligibility criteria as indicated above.

Participants will receive formal written information about the requirements of the study and invited to provide informed and written consent. Participants will then complete baseline online questionnaires (see section Outcome Measures) using the software program Clinical Intelligence and undergo a further preoperative baseline MRI. The Orebro Musculoskeletal Pain Questionnaire will also be completed. This questionnaire has shown to be reliable and valid for detecting individuals at risk of developing persistent pain.[37] It asks questions relating to a variety of known risk factors for the development of persistant pain. This questionnaire will be used in the current study to assess the potential impact of psychosocial factors on participants' outcome. It will not be used to exclude patients from the trial.

Randomisation and allocation

Eligible participants who provide written consent to participate will be randomised into one of two treatment

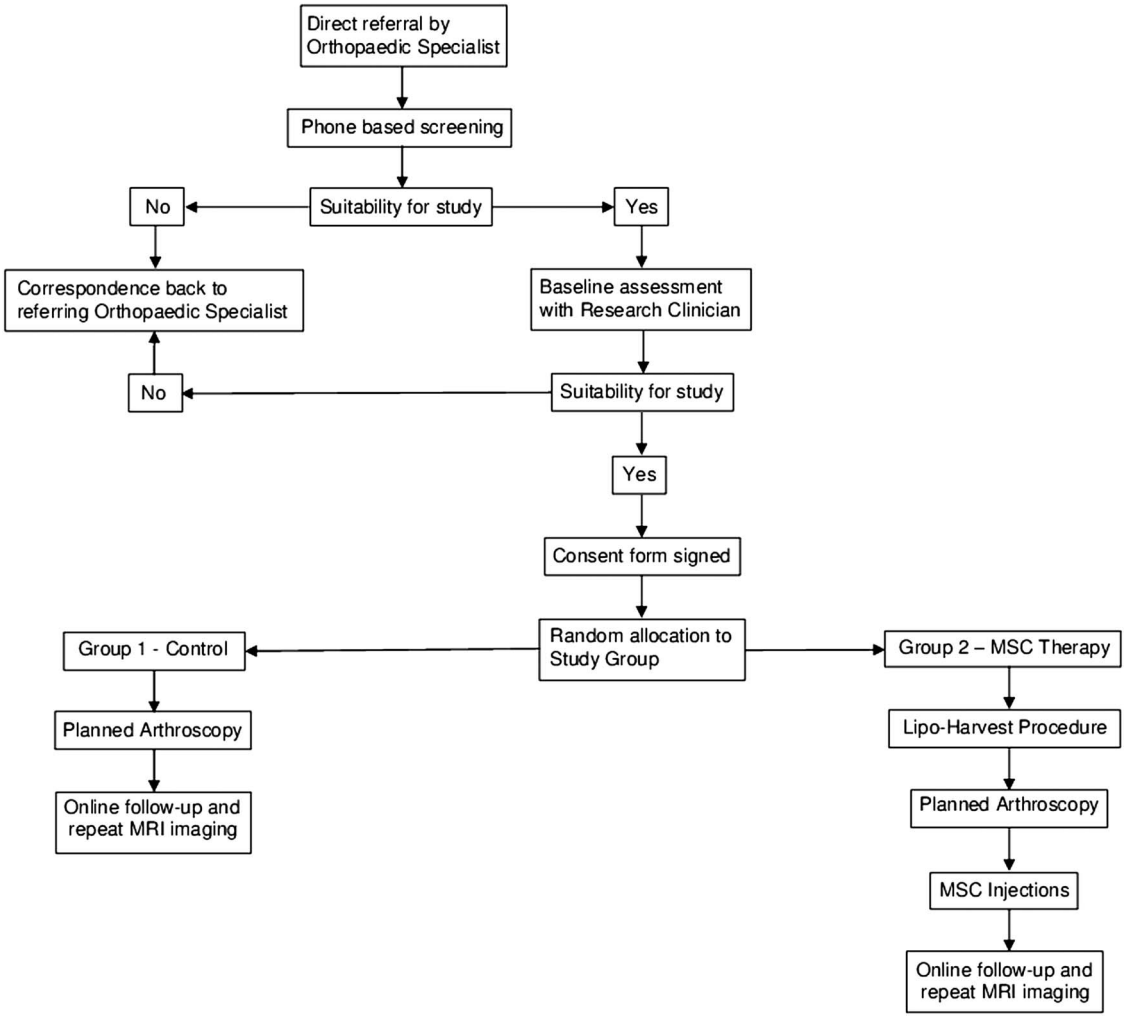

Figure 1 Overview of study.

groups. The first will receive no additional postoperative treatment. The second will receive postoperative intra-articular MSC injections. Using a web-based automated random number generator (http://www.randomizer.org) a treatment schedule will be prepared in advance by a designated researcher. This researcher will have no contact with any participants throughout the trial and will not be involved in the recruitment, screening, assessment, enrolment or treatment process.

Allocation of participants in accordance with the randomisation schedule will be undertaken by a study administrative assistant. During the trial, the administrative assistant will be the only person with access to the allocation spreadsheet. To enrol a participant, the study doctor will email/fax the consenting participant's name and date of birth to the administrative assistant. These details will be entered into the allocation spreadsheet and the study doctor notified of the treatment allocation.

Treatment protocols

Arthroscopic microfracture

All participants will undergo a planned arthroscopic microfracture for an isolated chondral lesion. The technique used for microfracture will be dependent on the surgeon performing the procedure. While this may potentially affect outcome and is an accepted limitation it has been purposefully chosen to allow recruitment of participants from across the orthopaedic community.

Control group

Those participants randomly allocated to the control group will undergo their arthroscopic procedure and microfracture alone of the isolated cartilage lesion as planned by their referring orthopaedic specialist. As part of the trial there will be no additional postoperative treatment apart from routine review according to the requirements of the referring surgeon.

Treatment group—adipose derived mesenchymal stem cell injections

Participants randomly allocated to the treatment group will undergo an abdominal liposuction harvest prior to their scheduled arthroscopy.

A course of antibiotics will start the day prior and for 4 days postliposuction. While the relative risk of infection

Box 1 Eligibility criteria

Inclusion criteria

1. Aged 18–50 (inclusive). As isolated full thickness chondral defects are often traumatic in nature it is likely that this age restriction represents the majority of patients who undergo microfracture.
2. Preoperative MRI diagnosis of an isolated full thickness knee cartilage defect of the medial or lateral femoral condyle
3. Planned arthroscopic microfracture
4. Sufficient English skills to complete the questionnaires required for the study, as well as to understand the instructions given by the study doctors.
5. No plans at the time of enrolment to undergo additional non-orthopaedic surgery in the following 3 months. This criterion is aimed at avoiding cointerventions that may confound the results of the study. While involvement in the project will not strictly prevent participants from undertaking such interventions if required, we will exclude volunteers who already have such procedures scheduled

Exclusion criteria

1. Pregnancy (accepted contraindication as no safety data on this population).
2. Have other causes of their knee symptoms suspected to be due to serious pathology such as tumour or referral from the hip or lumbar spine. These conditions are not under investigation within the current project.
3. Bleeding disorder—that is, haemophilia (accepted contraindication as no safety data on this population).
4. MRI confirmed displaced meniscal tear.
5. MRI confirmed cruciate ligament deficiency.
6. Radiological confirmed grade III-IV degenerative osteoarthritis—Kellgren Lawrence Classification.[36]
7. History of cancer.
8. History of atypical chronic pain syndrome—that is, chronic regional pain
9. Current medications include anticoagulation therapy that cannot be ceased prior to liposuction
10. Immunodeficiency
11. History of systemic illness or significant organ impairment/failure (ie, renal failure).
12. History of allergy to any substances used within the treatments

is low, the use of antibiotic prophylaxis is regarded as routine clinical practice.[38] The lateral abdominal region will initially be anaesthetised using tumescent fluid comprising of 30–40mL of 2% lignocaine with 1 mL of 1:1000 adrenaline, buffered using 8.4% bicarbonate (1 mL), and suspended in a saline solution (total 1000 mL). Using a 4 mm lipoaspiration canula, up to 60 mL of adipose tissue and tumescent fluid will be aspirated. The contents of these aspirations will be collected in a sterile medical grade single use filter. The study doctor performing the lipoharvest will be appropriately certified to perform liposuction.

All patients will be reviewed 1 week after the cell harvest procedure by the study doctor. Participants will also be provided with the study doctor's contact details to discuss any concerns following the procedure.

MSCs will be derived from the lipoharvest and isolated and expanded as detailed below. Participants will then receive autologous MSC injections at 4 weekly intervals following the microfracture (1, 5, 9 and 13 weeks) for the initial four injections, followed by a 6-month injection.

At each visit, the knee will be prepared using standard sterile procedures. The subcutaneous area of the injection site will be anaesthetised using 2 mL of 1% lignocaine and then approximately 40 million adipose derived MSCs will be injected into the knee joint using a lateral patella-femoral approach. Ultrasound guidance will be used to confirm intra-articular placement of the needle.

Isolation and expansion of autologous adipose derived mesenchymal stem cells

Isolation and expansion of autologous adipose derived MSCs will be undertaken using previously published techniques.[39]

The lipoaspirate, in a sterile single use filter, will be processed in a sterile environment in a Biological Safety Cabinet (BSC) Class II and using strict aseptic techniques. All the equipment used is qualified and validated for aseptic use in cell culture and all reagents and buffer used will be sterile, qualified and validated for cell culture use.

Cells will be characterised by flow cytometry (FACS) using four surface markers for MSCs as indicated by the International Society for Cellular Therapy: CD 90, CD44, CD 73 and CD105 as positive markers and CD 34 and CD45 as negative surface markers for MSC.[40]

Dosages containing approximately 40 million autologous MSCs each will be frozen individually in sterile cryovials in approved cell safe cryoprotectant media by a validated control rate freezing technique and stored in liquid nitrogen until required.[41 42]

On start of treatment, a single dose of cells will be thawed at 37°C in a sterile water bath and centrifuged to remove cryoprotectant media. The pelleted cells will then be mixed with 3 mL of sterile clinical grade injectable normal saline and injected into the knee of the patient as outlined above.

Outcome measures

Primary and secondary outcome measures will be obtained through use of MRI and questionnaires (see table 1).

Following arthroscopy and microfracture, all participants will complete online follow-up questionnaires at 1, 3, 6 and 12 months postarthroscopy. A follow-up MRI will be performed at 12 months to determine change in cartilage volume and quality of cartilage formation in the area of microfracture.

Ongoing annual follow-up beyond 12 months will be pursued to indicate long outcome differences between the two groups.

While it is accepted that histological analysis would provide best evidence of quality of tissue regeneration, repeat non-therapeutic arthroscopy for the purpose of performing a biopsy was felt to be both an obstacle to ethics approval and also participant recruitment and hence not included in the study design.

Magnetic resonance imaging

MRI will be assessed by a trained investigator who will be blinded to treatment allocation. Independent random cross checks will be performed by a second trained investigator. Both investigators will be blinded to the participants' treatment allocation. 3 T MRI with volumetric three-dimensional (3D) gradient and fast spin echo sequences will be performed.

Knee cartilage volume will be determined using a previously validated method.[43] Image processing will be performed using the Osiris software (Digital Imaging Unit, University Hospital of Geneva, Switzerland). Volume of the individual cartilage plates (medial and lateral femoral condyles, medial and lateral tibial plateaus and patellar) are manually isolated from the total volume by drawing disarticulation contours around the cartilage boundaries on each section. The data is re-sampled by bilinear and cubic interpolation for the final 3D rendering. The volume of the particular cartilage plate is determined by summation of the pertinent voxels within the resultant binary volume. Coefficient of variation for cartilage volume measures has been shown to be 2.1% for medial tibia and 2.2% for lateral tibia.[43]

Semiquantitative measures of cartilage defects will be obtained using a modified International Cartilage Repair Society (ICRS) score:

- Grade 0: normal cartilage;
- Grade 1: focal blistering and intracartilaginous 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.

A cartilage defect must be present in at least two consecutive slices for it to be measured. Significant cartilage defects will be defined as a cartilage defect score of ≥2 at any site within that compartment. Previous research has confirmed both intraobserver and interobserver reliability (expressed as intraclass correlation coefficient, ICC) using this technique.[44]

Bone marrow lesions (BMLs) will be measured. BMLs are defined as areas of increased signal intensity within the subchondral bone region in either the distal femur or the proximal tibia.[45] A lesion is identified if it appears on two or more adjacent slices. Lesions will be classified as 'small' (grade 1) if they encompass less than one-quarter of the width of medial or lateral compartment, or 'large' (grade 2) if they encompass at least one-quarter of the width of the medial or lateral compartment and appear on three or more slices.[45] This method of analysis has been validated in previous publications that have documented significant intraobserver reliability.[45]

Previous research has confirmed that tibial plateau bone area correlates with progression of osteoarthritis.[46] The cross-sectional areas of medial and lateral tibial plateaus will be directly measured from axial images transformed from sagittal images using OsiriX software. Osteophytes, if present, will not be included in the area of interest. Coefficient of variation for the medial and lateral tibial plateau bone areas are 2.3% and 2.4%, respectively.[46]

In addition to assessing morphological change, the study will use the novel method of MRI T2-relaxation time cartilage mapping to assess cartilage quality. T2 relaxation time gives a quantifiable value to the ability of free water protons to move and to exchange energy within a cartilage matrix.[47] Increased water content as a result of cartilage pathology may increase T2 relaxation time.

Questionnaires

Both groups will complete follow-up questionnaires at 1, 3, 6 and 12 months and will be recorded on software program Clinical Intelligence. Data will be de-identified.

Table 1 Outcome measures

Outcome measure	Measurement point (months)
Primary outcome measure	
1. MRI analysis ▶ Cartilage volume ▶ Cartilage defect grade (grade 0–4)	0, 12
2. Knee Injury and Osteoarthritis Outcome Score	0, 1, 3, 6, 12
Secondary outcome measure	
1. MRI analysis ▶ Bone marrow lesion (grade 1–2) ▶ Bone area ▶ T2 cartilage mapping	0, 12
2. Numerical Pain Rating scale (0–10)	0, 1, 3, 6, 12
3. Patient global impression of change(7-point Likert scale)	1, 3, 6, 12
4. Treatment Satisfaction scale (5-point Likert scale)	1, 3, 6, 12
5. Pain medication in previous week (yes/no, type and dose)	1, 3, 6, 12

1. The Knee Injury and Osteoarthritis Outcome Score (KOOS) consists of five subscales being pain, other symptoms, function in daily living, function in sport and recreation and knee-related quality of life. Standardised answers to questions are given (5-point Likert scale) which each question assigned a score of 0–4. A normalised score (100 indicates no symptoms and 0 indicates maximum symptoms) is calculated for each subscale. It is a reliable and valid scoring system is intended to be used for assessing knee injury that may result in post-traumatic knee osteoarthritis.[48]
2. A 0–10 Numerical Pain Rating Scale (NPRS), which asks participants to rate their knee pain intensity over the previous week. The NPRS has been validated for use in people with knee osteoarthritis.[48]
3. Patients Global Impression of Change (PGIC) Scale where participants are asked to indicate any overall change in their condition since the start of the trial. Measures of global effect are a recommended outcome measure for clinical trials.[49]
4. Treatment Satisfaction Scale. Participants' satisfaction with their treatment, will be assessed using a 5-point Likert scale.
5. Pain medication taken in the previous week will be recorded at follow-up intervals 1, 3, 6 and 12 months. Measuring medication intake of participants is considered a useful outcome measure and it also allows medication intake to be evaluated as a potential confounding factor or cointervention.[50]

Adverse events

Participants will be asked to identify any adverse, harmful or unpleasant events attributable to the treatment they received in the trial. Further, the study doctor will record in their standardised clinical notes any observed adverse events during the course of treatment and follow-up. Serious adverse events which require protocol modification will be formally communicated to the Human Research Ethics Committee, investigators, trial participants, trial registries and other relevant parties.

Participants will be provided with ancillary and post-trial care for any injury sustained as a result of their involvement in the trial.

Participant compliance and cointerventions

The number of treatment sessions attended by each participant, injections received and the number of missed or cancelled appointments, will be recorded by the study doctor. Information regarding the nature and degree of cointerventions will be obtained from participants on each follow-up outcome questionnaire. Further, any cointerventions at time of arthroscopy (ie, partial meniscectomy) will also be recorded.

Data integrity

Data entered by the participant using the online software program Clinical Intelligence is automatically checked for omissions and outliers to identify potential data entry errors. If such errors are identified the study administrator will be prompted by the program to clarify these areas with the participant. All questionnaire data will be exported into a computer spreadsheet by a study administrator.

Data and Safety Management Committee

An independent group of experts/peers will form a Data and Safety Management Committee (DSMC). The members of the DSMC will serve in an individual capacity and provide their expertise and recommendations. The primary responsibilities of the DSMC will be to:

1. Periodically review and evaluate the accumulated study data for participant safety, study conduct and progress, and, when appropriate, efficacy.
2. Make recommendations to HREC concerning the continuation, modification or termination of the trial.

Blinding

Participants will not be blinded during the course of this trial and control subjects will not receive a placebo injection. This has been decided as one of the primary outcome in MRI findings and will not be subject to placebo effect. Importantly the independent investigators assessing the MRI results will be blinded to the participants' treatment allocation. Placebo injections were also felt to be an unnecessary intervention as they would not be risk-free.

Data analysis

The researcher performing the task of data analysis will be blinded to the group allocation. A primary focus of the data analysis will be to determine any intergroup treatment effect difference (with 95% CIs) at each of the follow-up points (1, 3, 6 and 12 months postarthroscopy). SPSS V.21, will be used to conduct analysis using a two-tailed hypothesis with α set at 0.05.

Continuous data sets will be analysed using linear mixed models (with the group×time interaction estimating the treatment effect) as they are robust in longitudinal data analysis and are able to account for correlations associated with repeated measurements.[51–53] As recommended by the revised CONSORT statement, the mixed models will adjust for the baseline score of the outcome of interest.[54] Original data will be analysed using the Mann-Whitney U test.

Participants in each group will be dichotomised according to whether they achieved the minimum clinically important difference of the outcome or not at each point of follow-up. From this the risk ratio, risk difference and number needed to treat will be calculated together with 95% CIs.[54 55] Statistical significance will be determined by χ^2 analysis. For these purposes, the minimum clinically important difference will be defined as 10/100 for the KOOS,[56] 2/10 for the NRS pain scales,[56] at least 'much improved' on the global rating of

change scale[57 58] and 'very satisfied' on the treatment satisfaction scales.[58]

Where possible, missing data will be managed using a restricted maximum likelihood estimation within the linear mixed models.[59] To determine whether the results would differ if missing data were replaced using the last observation we will undertake a secondary sensitivity analysis using a last observation carried forward method.

Sample size

There is insufficient data published to date on the effects of autologous MSC on knee cartilage defects. As such sample size calculations are not possible. The study will aim to recruit 40 participants with 20 patients allocated to each group. Published guidelines recommend that pilot studies be undertaken to allow trial protocols to be tested under study conditions prior to evaluation in a full randomised controlled trial.[60]

The results of this limited phase II trial will also assist in determining the relevance and possible benefits of performing a significantly larger phase III randomised controlled trial requiring far greater financial assistance/funding.

Dissemination of outcome results

Clinical Investigators will review participants at completion of follow-up and discuss and explain outcome results. The results will be submitted for publication in a peer-reviewed journal and presented to both the medical and scientific community at suitable international scientific conferences.

ETHICS AND REGISTRATION

The trial has been prospectively registered with the Australian New Zealand Clinical Trials Registry (Trial ID: ACTRN12614000812695).

DISCUSSION

In this randomised pilot study we aim to compare the results of arthroscopic microfracture for isolated chondral defects (accepted surgical practice) versus microfracture with postoperative adipose derived MSC therapy. We hypothesise that postoperative intra-articular injections of adipose derived MSCs will result in better (hyaline-like) cartilage formation at the site of microfracture and therefore have greater efficacy in prevention of secondary osteoarthritis.

Primary outcome measures will be by both quantitative MRI analysis and the KOOS questionnaire. Secondary outcome measurements will include various validated pain and function questionnaires and further MRI analysis including novel cartilage quality assessment using T2 cartilage mapping techniques.

Patients will not be blinded. Both groups undergo routine microfracture treatment. The control group do not receive placebo injections postarthroscopic microfracture. While this may be perceived as a limitation and may effect some outcome measures, this will not affect primary outcome MRI assessment. Further, it was felt that intra-articular postoperative placebo injections presented an unacceptable risk.

Current research indicates that the use of peripheral blood mesenchymal stem cell therapy postarthroscopic microfracture may result in increased type II collagen deposition and development of hyaline-like cartilage. We aim to confirm this observation using adipose derived MSCs and further explore the appropriate protocol for postoperative MSC administration.

We hope to complete enrolment for the trial by mid-2016 with all 12-month follow-up data expected by mid-2017.

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Contributors JFr, JFo, RB, DB and LH were involved in conception of the study. JFr, JFo, RB, AR, DB, AH, LW, FC, LH, CN and KS designed the study protocol. JFr, JFo, DB, AH and LW were involved in the literature search. AH designed the statistical analysis strategy.

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Competing interests JFr, DB, LH and RB are partners within Melbourne Stem Cell Centre at which the trial will take place. JFr, DB, RB and LH are associated with Magellan Stem Cells and are part of its Medical Advisory Committee.

Ethics approval La Trobe University Human Research Ethics Committee.

Provenance and peer review Not commissioned; externally peer reviewed.

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