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A novel arthroscopic technique for intraoperative mobilisation of synovial mesenchymal stem cells

Running Title: A technique for increasing joint repair potential

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ABSTRACT

Background: Mesenchymal Stem Cells (MSCs) have emerged as a promising candidate for tissue regeneration and restoration of intraarticular structures such as cartilage, ligaments and menisci. However, their routine use is limited in part by their low numbers and the need for methods and procedures outside of the joint or surgical field.

Purpose: To demonstrate feasibility of a technique in which minimally manipulated synovial MSC can be mobilised during knee arthroscopy. Thereby showing proof of concept for the future clinical use of native joint resident MSCs in single-stage joint repair strategies.

Study Design: Descriptive laboratory study

Methods: Patients (n=15) undergoing knee arthroscopy that were free from synovitis or active inflammation were selected. Three samples of irrigation fluid were collected from each patient at inception of the procedure, after an initial inspection of the joint and after agitation of the synovium. MSC numbers were evaluated by colony forming unit-fibroblastic assay. Synovial fluid resident and synovial-mobilised MSCs phenotype was determined by flow cytometry and their functionality by trilineage differentiation. Adhesion of culture expanded mobilised MSCs to fibrin scaffolds was also evaluated to ascertain whether mobilised MSCs might concentrate at site of bleeding.

Results: Normal irrigation during arthroscopy depleted resident synovial fluid MSCs (4-fold decrease, n=15). MSC numbers mobilised using a purpose made device were significantly higher (105-fold) then those mobilised using a cytology brush (median of 5,763 and 54 colonies respectively, p=0.001, n=15). The mobilised cellular fraction contained viable MSCs with proliferative potential and trilineage differentiation capacity for bone, cartilage and fat lineages and cultured daughter cells exhibited the standard MSC phenotype. Following
culture, mobilised synovial MSCs also adhered to various fibrin scaffolds in vitro. The technique was simple and convenient to use and not associated with any complications.

**Conclusions:** Numbers of functional MSCs can be greatly increased during arthroscopy using this technique to mobilise cells from the synovium.

**Clinical Relevance:** This study highlights a novel single-stage technique to increase joint-specific synovial derived MSCs and thereby increase the repair potential of the joint. This technique can be undertaken during many arthroscopic procedures and supports the principle of mobilised MSC integration into microfracture sites and sites of bleeding or targeted repair using fibrin based and other scaffolds.

**Key Terms:** Mesenchymal stem cells, synovium, synovium-derived stem cell, minimally manipulated, single-stage procedure.

**What is known about the subject:** Mesenchymal stem cells have intrinsic regenerative potential. Synovial stem cells are embryological linked to all joint structures and are known as the ‘joint specific’ stem cell. The synovium is the most potent source of chondrogenic mesenchymal stem cells.

**What this study adds to existing knowledge:** Here we demonstrate a simple and reliable technique to increase the numbers of joint specific stem cells using a single-stage procedure. This technique could be used in future arthroscopies to supplement repair or to target mobilised MSCs in combination with scaffolds.
INTRODUCTION

Articular cartilage is essential for synovial joint function, providing a low friction surface to allow joint movement. Cartilage repair, however, is limited due to its avascular, aneural and alymphatic nature. Consequently, management of isolated articular cartilage defects poses numerous challenges and if left untreated are risk factors for developing osteoarthritis (OA) in later life. For more advanced stages of OA, management of the disease comes at a high global and socioeconomic burden secondary to the effects of disability, comorbid disease and expense of treatment. As such there is a need for more effective, earlier treatments.

Advanced treatment of cartilage defects in the form of autologous chondrocyte implantation (ACI) are available. However, ACI is a two-stage procedure, requiring harvest of healthy tissue, tissue processing and cellular expansion outside of the surgical field before re-implantation into the defect site as a second stage surgical procedure. Unfortunately, long-term follow-up results are no better than more conservative microfracture, a much cheaper single-stage procedure. Mesenchymal stem cells (MSCs) have been proposed as an alternative source of reparative cell owing to their ease of harvest, proliferative and differentiation capacity. MSCs are multipotent and internationally recognised by their characteristic adherence to plastic and colony forming capacity, expression of cell surface markers (CD73, CD90 and CD105), lack of expression of CD14, CD34, CD45 and HLA-DR, and their ability to differentiate in vitro into osteoblasts, chondrocytes and adipocytes. Several clinical studies utilising MSCs for treating OA have shown positive results however, similar to ACI, many methodologies still incorporate tissue/cell extraction (either from bone marrow or adipose tissue) prior to culture expansion and re-implantation. Intraarticular injection of culture expanded MSCs has also been hampered by both costs and loss of potency. Other
strategies being explored include the combination of various techniques including scaffolds and fibrin glue for implantation and retention at sites of injury, which looked promising at second look arthroscopy. A major drawback with the aforementioned strategies involving cellular therapy is the need for more than one operative procedure. Recent single-stages strategies towards joint repair includes the use of adipose tissue stromal vascular fraction, which contains MSCs. However, for single-stage cartilage repair and OA, joint cavity MSCs including synovium and synovial fluid are of particular interest as these contain populations of MSCs with superior chondrogenic capacity and may be more readily and conveniently manipulated since these are already in the surgical field. It is now recognised that there are comparatively abundant reservoirs of MSCs in the knee joint cavity and such MSCs might play a role in joint homeostasis and repair. These MSCs are naturally shed from the synovium, are elevated with joint injury and early OA but their repair capacity may be limited due to interactions with synovial fluid hyaluronan which limit their adhesion to cartilage. Current arthroscopic procedures may however inadvertently remove these cells (via saline irrigation) and procedures such as microfracture to encourage MSCs to percolate from the bone marrow may be inefficient as a result of low and variable numbers of MSCs in bone marrow.

In this study, we hypothesised that arthroscopic procedures likely wash away synovial fluid resident MSCs imbued with reparative potential (Figure 1). Our principle aim was to report the feasibility of a technique whereby MSCs are dislodged (mobilised) from the synovium. Thereby increasing numbers of joint cavity MSCs, with access to cartilage, using a simple single-stage procedure, potentially increasing the reparative potential of the joint during a
range of arthroscopic procedures. We sought to determine whether these synovial mobilised MSC (Sm-MSCs) preserved their in vitro MSC phenotype and function. Finally, we asked if Sm-MSCs would rapidly adhere to blood clots and fibrin scaffolds (in vitro) as a surrogate for what could happen in vivo following microfracture or use of a fibrin glue.

**METHODS**

**Patient recruitment**

Patients included in the study were treated arthroscopically for a spectrum knee conditions (Table 1) including ligament, meniscal and cartilage injury, reflecting the varied nature of current practice and procedures which may warrant stem cell treatment. The average age of recruited patients was 32 years, ranging between 18 and 53 (n=15). At the time of arthroscopy, patients were free from joint effusion, synovitis or active inflammation as determined during arthroscopy. Ethical approval for this study was granted by the UK Research Ethics Council and all study participants were recruited following informed written consent.

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* Denotes participants having MSC mobilisation using the STEM Device.

**Retrieval of MSCs from irrigation fluid**

Normal saline was used to inflate the knee joint to adequately visualise all joint compartments. To achieve this, gravity fed saline was irrigated through the capsule to maintain a sufficient intra-articular pressure to obtain arthroscopic views. Irrigation fluid was collected from each patient at three stages during knee arthroscopy. The first sample contained those resident synovial fluid MSCs (SF-MSCs) and was collected by aspirating the initial saline used to irrigate the joint cavity (5-50 ml collected, mean 38 ml). A second sample of fluid was then collected after inspection of the knee compartments to evaluate to what extent native synovial fluid MSCs were being “washed away” during surgery (25-50 ml collected, mean 39 ml). This sample was collected prior to any procedure which breached the
subchondral bone, in settings where this was undertaken. Each sample was placed into a sterile container before transport to the laboratory.

Mobilisation of synovial MSCs

The feasibility of mobilising MSCs from the synovium during arthroscopy, was investigated by collecting a third sample of irrigation fluid after agitation of the synovium to mobilise MSCs. To do this we implemented the use of a cytology brush routinely used for diagnostic purposes to sample cells from mucosal surfaces through natural orifices. Prior to the induction of the cytology brush, saline irrigation was stopped leaving sufficient saline to distend the joint cavity. The cytology brush (Figure 2C, length: 180mm; nylon bristled brush head diameter: 7 mm, Medical Wires and Equipment Ltd, Wilshire, UK) was then introduced into the joint through the medial portal, under arthroscopic guidance the synovial surface of the medial gutter was agitated using the bristled portion for one minute. Mobilised cells were collected by opening the outflow valve of the arthroscope. Samples (n=7, 50-70 ml, mean 53 ml) were collected in a sterile container before transportation to the laboratory.

The cytology brush was difficult to introduce into the knee portal due to its inherent flexibility and was limited in the range of synovium which could be agitated. To further improve upon the capability to mobilise synovial MSCs we used a purpose made device fabricated using medical grade acetal co-polymer to further mobilise MSCs from the synovium, the Stem Cell Mobilising Device or STEM Device (Figure 2D and E). Similar to the cytology brush, this optimised device was sterile, for single-use, and was also introduced through the medial knee portal. The design of the STEM device was modified and tailored for arthroscopic use with a stiffer arm and blunted end to aid insertion and limit potential inadvertent damage to other
joint structures. The design also allowed the STEM device to be guided into the suprapatellar pouch, where a larger area of fibrous synovium could be agitated to maximise MSCs mobilisation. As described above, cells were mobilised by agitating the bristled portion on the synovial surface for one minute. Cells were retrieved by irrigation and aspiration of saline from the joint (n= 8, 50-70 ml, mean 53 ml).

**Retrospective clinical follow-up**

Clinical data for all patients receiving synovial agitation using the STEM device were evaluated retrospectively by a senior soft tissue knee fellow. At the time, this fellow had completed training and had passed the FRCS examination. The surgeon gave his opinion on the likely impact that mobilizing MSCs would have on the expected progression of each participant for their indicated procedure (Table 1). Clinical follow up duration ranged from 6 weeks to a year.

**In vitro growth and quantification of MSC colony number**

Colony forming unit-fibroblastic (CFU-F) assay were used to determine the number of viable MSCs in each sample. Briefly, samples of joint irrigate were centrifuged to pellet cells before resuspending in 10 mL StemMACS expansion media (Miltenyi Biotec, UK). Duplicate 100 mm diameter dishes (containing 2 mL of the cell suspension) for each sample were cultured for 14 days in StemMACS expansion medium with twice weekly media changes, before both dishes were fixed in 3.7% formalin and stained with 1% methylene blue (Sigma-Aldrich, UK). All colonies containing over 50 cells (verified under a microscope) were counted. The remaining sample was either expanded for further assays or frozen. For expansion, cells were cultured in StemMACS expansion medium with twice weekly media changes until cells
reached approximately 90% confluency before being passaged. MSCs were expanded for 3-4 passages, all cells were incubated at 37°C and 5% CO₂.

**Immunophenotype of MSCs**

Flow cytometry was performed on matched culture expanded SF-MSCs and Sm-MSCs (n=3 donors) using standard panel of markers to define cultured MSCs using an LSRII four laser flow cytometer (BD Biosciences, UK). The following antibodies and appropriate isotype controls were used: anti-CD34-allophycocyanin-cyanine (APC), anti-CD19-phycoerythrin (PE), anti-CD45- phycoerythrin-cyanine (PE-Cy7), anti-CD14-fluorescein isothiocyanate (FITC), anti-CD73-PE, anti-CD90-PECy7, and anti-CD105-PE (all from BD Biosciences). Dead cells were discriminated using 4’,6- diamidino-2-phenylindole (DAPI, Sigma-Aldrich, UK). At least 10,000 live cell events were collected for each antibody combination.

**Trilineage differentiation of MSCs**

Trilineage differentiation of donor matched culture expanded SF-MSCs and Sm-MSCs (n=5 donors) was performed using standard protocols. Osteogenic differentiation (medium containing DMEM and 100 mg/ml penicillin/streptomycin from Gibco, UK, 10% FCS (Biosera UK), 10% horse serum (Stem Cell Technologies, UK) 100 nM dexamethasone, 10 mM β-glycerolphosphate and 50 μM ascorbic acid, all from Sigma-Aldrich, UK) was assessed using alkaline phosphatase and Alizarin red staining on days 14 and 21 of culture, respectively. Quantitative analysis of calcium deposition was performed on day 21 according to the manufacturer’s instructions (Calcium assay, Senitial Diagnostics, Italy). Adipogenic differentiation (medium containing DMEM and 100 mg/ml penicillin/streptomycin from Gibco, 10% FCS (Biosera), 10% horse serum (Stem Cell Technologies, UK), 500 μM
hydrocortisone, 500 μM isobutylmethylxanthine and 60 μM indomethacin all from Sigma-Aldrich) was assessed using Oil Red O staining and quantified using Nile red fluorescence on day 21 of culture. Pellet cultures were used to evaluate chondrogenesis (medium containing high glucose DMEM supplemented with 100 U/ml penicillin/streptomycin (both from Gibco), 100 μg/mL sodium pyruvate, 40 μg/mL L-proline, 50 μg/mL L-ascorbic acid-2-phosphate, 1.25 mg/ml BSA, 1× mixture of recombinant human insulin, human transferrin and sodium selenite (ITS+), 100 nM dexamethasone and 10 ng/ml recombinant human transforming growth factor-β3 all from Sigma-Aldrich) on day 21 of culture. For quantitative analysis, glycosaminoglycan (GAG) production was measured (Blyscan assay, Biocolor, UK). Toluidine blue staining was used for qualitative analysis of frozen sections.

**MSC adhesion to biological scaffolds**

Culture expanded Sm-MSCs were used to assess their ability to adhere to various biological scaffolds. Scaffolds were formed from whole blood (WB), platelet rich plasma (PRP) or fibrin glue (FG). Blood from healthy volunteers was collected in sodium citrate tubes. PRP was prepared following centrifugation at 250 x gravity (g) for 10 minutes. Platelets were further concentrated from the supernatant by centrifugation at 1500 x g for 10 minutes, the pelleted platelets were resuspended in 1/5th their original volume using serum. FG scaffolds were formed using bovine purified fibrinogen (23.7 mg/ml) resuspended in StemMACS expansion media. All scaffolds including WB were formed following coagulation by the addition of 100 mM calcium chloride (CaCl₂) and 50U/ml thrombin (Sigma-Aldrich, UK). To each scaffold and each time point (in triplicate), 10,000 Sm-MSCs were added and allowed to adhere for 10, 30 or 60 minutes before the supernatant was removed and any non-adherent cells transferred to tissue culture well. These cells were allowed to attach to the culture well before fixing,
staining with 1% methylene blue and counting. A standard curve using a known number of cells was used to interpolate counted cells as a percentage of initial cell number.

Statistical analysis
Due to the limited number of samples, all data was assumed to be non-parametric. As such paired samples were analysed using Wilcoxon single-rank and non-paired samples using Mann-Whitney U test. The confidence level for each was set at 95%. All statistical analysis was performed using SPSS version 21 (IBM).

RESULTS

Retrieval and enumeration of MSCs from irrigation fluid
CFU-F numbers (a measure of viable MSCs) varied between donors (Figure 2A) with MSC colony number significantly greater (p=0.01, n=15) in the initial irrigate (median of 360, range 1-1,675) compared to the second sample (median of 68, range 4-885). Samples of subsequent irrigation fluid indicate MSC numbers on average fall 4-fold over the course of surgery, suggesting that standard orthopaedic practice depletes the joint of stem cells.

Intraoperative mobilisation of MSCs from the synovium
Resident SF-MSCs were collected as above, and these served as a baseline to assess the ability of the cytology brush to mobilise MSCs from the synovium. The number of resident SF-MSCs in these donors (n= 7) ranged from 3 to 1,235 (Figure 2B). Following synovial agitation using the cytology brush, in comparison to MSC numbers obtained prior to synovial mobilisation, the cytology brush increased MSC colony approximately 5-fold. However, MSC numbers declined relative to the baseline resident synovial fluid population from the initial irrigate,
ranging from 8 to 775. The median number of colonies formed, fell from 256 in the resident population to 54 following synovial agitation, representing a 3.7-fold decrease (not significant).

Intraoperative use of the cytology brush mobilised low numbers of synovium lining MSCs. This could be due to the limited area of synovium which the cytology brush could access, and because this type of device is intended to trap and remove cells from the body. In order to overcome this and to further increase MSC number during surgery, we used a purpose made device (the STEM device, Figure 2C and D) to ascertain if this pool of regenerative cells located in the synovial lining could be further exploited. In each case (n=8), the use of the STEM device reliably and significantly increases the number of MSCs mobilised intraoperatively over those resident in the SF (Figure 1E). Following synovial agitation using the STEM device, the median number of colonies formed were 5,763 (range 225 to 46,500), representing a 10-fold increase over the matched SF resident population (p=0.007, median 531, range 1 to 1,675). In comparison to the cytology brush, the STEM Device mobilised 105-fold greater viable MSCs from the synovial lining during arthroscopy (Figure 2F, p=0.001). Finally, we took a retrospective look at follow-up clinical data for those patients who received MSC mobilisation using the STEM device. From this data, no patient appeared to fare worse than would be expected for their primary surgery had they not undergone MSC mobilisation using this device.

Mobilised MSCs are phenotypically and functionally comparable to SF-MSCs

To determine whether Sm-MSCs were comparable to their resident SF counterparts and withstood the biophysical manipulation, we used standard techniques on culture expanded
donor matched cells to perform immunophenotyping and multi-lineage differentiation. Both SF-MSC and Sm-MSCs were positive for standard MSCs surface markers CD73, CD90 and CD105 and negative for common haematopoietic lineage marker CD14, CD19, CD45 and CD34 (Figure 3A), confirming the feasibility of this procedure to mobilise stem cells during the surgical procedure.

Differentiation of Sm-MSCs towards chondrogenic (Figure 3B), osteogenic (Figure 3C) and adipogenic (Figure 3D) lineages was also compared to donor matched SF-MSCs. In each case, qualitative and quantitative assay demonstrated the differentiation capacity of the mobilised MSCs were comparable to their matched SF-MSCs. These data show Sm-MSCs are comparable to SF-MSCs and consistent with previous literature describing synovium as a source of intra-articular MSCs.

Mobilised MSCs rapidly adhere to biological scaffolds

Finally, having shown how MSC numbers can be reliably increased intraoperatively from the joint synovium, and that these fulfil the requirement of an MSC, we sought to determine if these cells could be targeted to sites of interest via adhesion to relevant biological scaffolds. In these experiments, the ability of mobilised MSCs to adhere to fibrin glue (FG), platelet rich plasma (PRP) or whole blood (WB) clots was evaluated in vitro. Sm-MSCs from a single donor began to adhere to the surface of all three scaffolds within 5 minutes (Figure 4A). By 60 minutes, 89.7% (±6.5%), 88.3% (±3.5%) and 84.0% (±8.3%) of Sm-MSCs were adhered to the FG, PRP and WB clots respectively. To test donor variability, Sm-MSCs from three donors were allowed to adhere to FG scaffolds alone (Figure 4B). Sm-MSCs adhered rapidly with little
variations observed between donors. After 60 minutes 83.5% (±2.6%), 96.7% (±1.0%) and 86.1% (±3.1%) of Sm-MSCs from each donor were adhered to FG scaffold.

DISCUSSION

There is an increasing need for better cartilage and joint repair strategies and MSCs show great promise. To fully exploit these cells, new strategies and methods which limit operative time, cost and tissue manipulation are required. Emerging data from several randomised control trials indicate MSC therapy show promise at least in the short term\textsuperscript{31,48}. However, the majority of trials so far have used culture expanded MSCs from bone marrow or adipose tissue; or other ex vivo manipulation strategies. Here we provide proof of concept, that minimally manipulated MSCs within the synovium can be easily mobilised and their numbers greatly augmented in a simple single-stage procedure as part of knee arthroscopy. Furthermore, since these MSCs are derived from the synovium which is the most potent source of chondrogenic progenitors there is promising potential for utilising this technique to increase the reparative capacity of the knee\textsuperscript{39}.

The discovery of joint resident MSCs highlights potentially new avenues to explore in the treatment of knee intra-articular pathologies\textsuperscript{30}. Studies have shown how numbers of MSC in SF increase in patients with OA and cartilage injury\textsuperscript{19,20,41}, and following ligament and meniscal damage\textsuperscript{28,33}. However, joint irrigation during arthroscopy which serves to aid visualisation of the joint and remove debris, at the same time removes synovial fluid with potentially undesirable effects of reducing SF-MSC numbers\textsuperscript{13}. SF-MSCs are thought to derive from the synovium owing to their superior clonogenic and chondrogenic potential, and shared gene expression profiles\textsuperscript{3,39,41}. The synovium itself holds great potential in providing an abundant
endogenous supply of joint specific MSCs with synovial MSCs are found at a frequency of ~1%, 500 times more numerous than in bone marrow\textsuperscript{11,14,18,39,43}. Additionally, the synovium, cartilage and other joint structures all share a common progenitor which is distinct from that of bone tissue\textsuperscript{26,27}, suggesting the synovium harbours a joint tissue specific stem cell\textsuperscript{16,30,38}. The remarkable cartilage repair seen in OA patients treated using knee joint distraction is testament to the intrinsic regenerative potential of these joint resident cells\textsuperscript{42}. In these patients, significant areas of denuded bone are replaced by intrinsic cartilage repair activity giving functional and clinical benefits\textsuperscript{15,42,47}. It is thought the temporarily altered biochemical and biomechanical environment created by joint distraction favours endogenous joint resident MSC activity and this is in part responsible for this intrinsic repair where no extrinsic growth factors, scaffolds or MSCs have been introduced\textsuperscript{4}.

Synovial MSCs have already been used clinically with promising results, however these studies also required a two-stage procedure and in vitro culture expansion\textsuperscript{1,40}. The purpose of this study was therefore to demonstrate proof of concept for a new single-stage procedure to increase stem cell numbers in the joint thereby avoiding ex vivo manipulation of tissue and cells. Furthermore, our data show that the Sm-MSCs rapidly adhere to biological scaffolds such as FG, PRP and WB clots thus demonstrating relevance in the context of existing procedures such as microfracture (Figure 1), or future practical applications with fibrin base scaffolds.

Characterisation of Sm-MSCs showed consistent phenotype and trilineage differentiation with their donor matched SF counterpart, effectively demonstrating successful mobilisation of joint resident MSCs. In the context of arthroscopic surgery where saline inflation and
irrigation of the joint results in the loss of the SF-MSCs, this technique (when performed with
the purpose made STEM device) not only increases the number and availability of stem cells
(compared to those resident before and during arthroscopy), but (if left in the joint) these
cells then occupy a more suitable biochemical environment being free from high molecular
weight hyaluronan which can limit their interaction with cartilage⁴. Depending on the
arthroscopic procedure used, Sm-MSCs could be aspirated from the joint for loading onto a
scaffold or entrapped in the joint as a final stage procedure.

One of the concerns of stem cell-based cartilage repair is cartilage hypertrophy, which leads
to uneven articular surface. Synovium derived MSCs exhibit superior chondrogenic capacity
with limited potential to hypertrophy compared to MSCs derived from adipose and bone
marrow¹⁶. Data presented here, after use of the STEM device is encouraging, supporting the
potential of this technique and the synovium as a robust and convenient source of MSCs.
Although colony numbers expressed here are low in comparison to the number of
chondrocytes or MSCs currently used in the clinical setting, these Sm-MSC have lost none of
their proliferative or differentiation capacity as a result of culture expansion⁷,¹⁷. Direct
comparison between these colony numbers and those obtained from fresh bone marrow
aspirate are difficult owing to variations in aspiration technique. However, using an optimised
aspiration procedure, where small draw volumes are taken from multiple sites, this would be
equivalent of up to 80 ml of bone marrow⁸,¹⁴. For non-optimised bone marrow aspirates
where larger volumes (~60 ml) are drawn before being concentrated¹² we can equate the
number of colonies mobilised with the STEM device as being equivalent of up to 380 ml. More
research and clinical studies are required to determine whether these Sm-MSCs will lead to
clinically significant results. However, the idea is to achieve cartilage repair with a single
procedure and thus avoid the need for two-stage harvest and cell expansion. This will in turn reduce the burden and risks to the patient, and the cost to the healthcare provider.

LIMITATIONS

We recognise that synovial MSC mobilisation was performed on a limited number of patients with a range of pathologies. These pathologies and the time between injury and surgery may affect MSC numbers and the ability to mobilise them into the joint cavity. The primary purpose of the study was to determine to what extent synovial MSCs could be mobilised during a range of routine arthroscopies. In this study these stem cells were removed from the joint for laboratory analysis and so there was no expected benefit to the patient.

CONCLUSION

Here we show proof of concept that a potent source of joint specific mesenchymal stem cell can be accessed and mobilised during routine arthroscopy. This technique can reliably and rapidly repopulate the knee with synovial MSCs which are viable, with trilineage potential as a single-stage procedure. Further studies are needed to ascertain whether this leads to better joint repair across the spectrum of arthroscopic procedures for damaged cartilage and other joint structures.
Figure 1: A proposed use of this technique to mobilise synovial MSCs and supplement cartilage healing. A-B) Joint irrigation replaces SF leading to a loss of resident SF-MSCs while inflating the knee. C-D) The STEM device is introduced into the joint cavity and maneuvered into the suprapatellar pouch where it physically dislodges cells, including MSCs from the synovial lining. E) These MSCs are released into the cavity where they may supplement those from the bone marrow and participate in repair at sites such as cartilage defects via adhesion to the clot after microfracture.

Figure 2: MSC numbers can be increased during arthroscopy by agitation of the synovium. A) MSC colony number declines during arthroscopy due to loss of resident cells following saline irrigation (n=15, horizontal bars represent medians). B) MSC colony numbers can be recovered following mobilisation from the synovium using a standard cytology brush.
Comparison of the head design and abrasive surfaces of the cytology brush and the purpose made STEM Device. The rigidity of the STEM device, the bullet shaped nose and placement of the projections enable easier insertion into the knee through the soft tissues. D) Comparison of the length and overall design between the cytology brush and the STEM Device (C and D to scale). The increased length and angled head allowed for the STEM device to be maneuvered into the suprapatellar pouch. E) MSC colony number is greatly increased following use of the STEM Device during arthroscopy (horizontal bars represent medians, n=8, below: example of representative CFU-F dishes). F) Colony numbers are significantly increased (over 100-fold) using the STEM Device compared to the cytology brush (n=15, below: example of representative CFU-F dishes).
A

Positive Population (%)

CD73    CD90    CD105    CD34    CD14    CD19    CD45

- Resident MSCs
- Mobilised MSCs

B

Chondrogenesis

Total GAG (μg/pellet)

Toluidine Blue

5 mm

C

Osteogenesis

Total Calcium (μg/mL)

Alizarin Red

Alk. Phos.

9 mm

D

Adipogenesis

Nile:DAPI Ratio

Resident MSCs    Mobilised MSCs

Resident MSCs    Mobilised MSCs

Oil Red O

200 μm
Figure 3: Mobilised MSCs are indistinguishable from resident SF-MSCs. A) Resident and mobilised MSCs have a comparable immunophenotype which is consistent with both synovial fluid and synovium derived MSCs (n=3 matched donors). B-D) Resident and mobilised MSCs have comparable trilineage differentiation capacity as demonstrated by quantitative assays (left, n=5 matched donors) illustrating their chondrogenic, osteogenic and adipogenic potential respectively and qualitative assays (right, representative matched donors).
Figure 4: Mobilised MSCs rapidly adhere to a range of biological scaffolds. A) Mobilised MSCs from a single donor show a rapid ability to adhere to a range of relevant biological scaffolds such as platelet rich plasma (PRP) and whole blood (WB) clots as well as fibrin glue (FG). B) Mobilised MSCs from multiple donors all exhibit the same rapid adhesion to a fibrin glue scaffold (n=3).


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