A comparative assessment of cartilage and joint fat pad as a potential source of cells for autologous therapy development in knee osteoarthritis

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Objectives. The utility of autologous chondrocytes for cartilage repair strategies in older subjects with osteoarthritis (OA) may be limited by both age-related and disease-associated decline in chondrogenesis. The aim of this work was to assess OA Hoffa’s fat pad as an alternative source of autologous chondroprogenitor cells and to compare it with OA chondrocytes derived from different areas of cartilage.

Methods. Cartilage and fat pad tissue digests were obtained from 26 subjects with knee OA and compared with normal bone marrow (BM) mesenchymal stem cells (MSCs) with respect to their in vitro colony-forming potential, growth kinetics, multipotentiality and clonogenicity. Flow cytometry was used to investigate their MSC marker phenotype.

Results. Expanded cultures derived from eroded areas of cartilage were slightly more chondrogenic than those derived from macroscopically normal cartilage or chondro-osteophytes; however, all cartilage-derived cultures failed to maintain their chondrogenic potency following extended expansion. In contrast, OA fat pads contained highly clonogenic and multipotential cells with stable chondrogenic potency in vitro, even after 16 population doublings. Standard colony-forming assays failed to reflect the observed functional differences between the studied tissues whereas flow cytometry revealed higher levels of a putative MSC marker low-affinity growth factor receptor (LNGFR) on culture expanded fat-pad derived, but not cartilage-derived, MSCs.

Conclusions. In contrast to OA cartilage from three different sites, OA Hoffa’s fat pad contains clonogenic cells that meet the criteria for MSCs and produce multipotential cultures that maintain their chondrogenesis long term. These findings have broad implications for future strategies aimed at cartilage repair in OA.

Key words: Mesenchymal Stem Cells, Osteoarthritis, Cartilage.

Osteoarthritis (OA) is the most common degenerative arthritic condition and represents a disease where the prospect of stem cell therapy offers considerable hope [1–3]. Thus far, culture-expanded autologous chondrocytes (also termed articular cartilage-derived dedifferentiated chondrocytes, ADDCs) have shown promise but their use is largely limited to young patients with isolated chondral lesions [4]. Notably, normal ADDCs exhibit many features of mesenchymal stem cells (MSCs), including high rates of proliferation, clonogenicity, multipotentiality and MSC marker phenotype [5–7].

Although the immediate goals of cellular therapies in OA are the treatment of solitary chondral defects in younger subjects that may otherwise have normal joints, this cohort represents only a small fraction of the global knee OA load. By far the greatest burden of disease is in older subjects where a prominent feature of the disease includes significant denudation of articular cartilage over the femoral condyles and where knee replacement surgery represents the only available treatment option. It is envisaged that the introduction of stem cells, either by intra-articular injection or embedded in scaffolds, with or without growth factors, could lead to future cartilage repair strategies in OA. However, before this becomes a possibility, there are many hurdles to overcome and chief amongst these is the best stem cell source and whether it is autologous or allogeneic [3, 8–12].

Whilst considerable MSC activity has been shown in articular cartilage from normal subjects younger than 40 yrs old [7, 13], good preservation in chondrogenesis may be lacking in older subjects with OA since an age-related decline in chondrogenesis is a feature of normal ADDCs [13]. It has also been suggested that most of the stem cell activity in normal cartilage is confined to the superficial layer [14], which is destroyed in OA [15]. Furthermore, a reduction in the MSC activity, and particularly in the chondrogenic capacity, was found even from remote locations (in the bone marrow) in advanced OA [16], although this view has recently been challenged [17]. Therefore, a suitable cell source for autologous cells for OA therapy development in older subjects with OA has not yet been well defined.

Despite the apparent reduction in cartilage MSC activity, increased joint remodelling with the formation of chondro-osteophytes and loose bodies are common in OA, which suggests good potential for the preservation of repair capacity. One explanation for this could be that other tissues sources of MSCs are present within the joint. Indeed, our previous work has documented an increased number of MSCs in OA synovial fluid (SF) compared with rheumatoid arthritis (RA) [18]; whether this reflects their relative increase in OA compared with normal homoeostasis is yet to be established. Nevertheless these findings raise the possibility that some other tissue within the joint cavity may harbour a population of MSCs, even in advanced OA.

The aim of this study was to compare the cartilaginous joint structures involved in knee OA to Hoffa’s fat pad-derived MSCs for their proliferation and multipotentiality, and particularly for their relative capacity to produce cartilage. Our findings showed that chondrogenic cultures that fulfilled the criteria for MSCs, including good chondrogenesis, could be generated from the fat pad, but not from OA cartilage. Moreover, polyclonal fat pad-derived cultures retained superior preservation of chondrogenic capacity on extended passaging. These data suggest that the fat pad could be a useful autologous source for cellular therapy development in OA.

Materials and methods

Patients and normal controls

Clinical material was obtained from 26 patients with knee OA (median age 75 yrs). The diagnosis of OA was confirmed based
on clinical and radiographic features pre-operatively and on the intra-operative findings where denudation of articular cartilage was universally noted. Ethical approval for the study was obtained from the Calderdale and Huddersfield NHS Trust and United Leeds Teaching Hospitals. Informed patient consent was obtained for all samples used in this study. None of the patients had a history of inflammatory arthritis or crystal-induced arthritis. Samples collected at joint arthroplasty were transported to the laboratory in normal saline solution. Normal bone marrow (BM) was used as a ‘gold-standard’ control for the MSC activity. BM aspirates were obtained from the posterior iliac crest on 33 donors (median age 19 yrs). Following separation with Lymphoprep (Nycomed, Oslo, Norway) BM mononuclear cells (MNCs) were collected and cryo-preserved in liquid nitrogen prior to culture. Negative control skin fibroblasts were obtained from the American Type Culture Collection (ATCC) (Tedddington, UK).

**Tissue processing, digestion and primary culture**

Cartilage was harvested from femoral condyles (the anterior, posterior and lateral areas), both from regions that were macroscopically normal (MN) and immediately adjacent to erosion (E) (Fig. 1A and B, top panels). The articular cartilage in the MN area had a smooth shiny surface whereas E areas showed clear macroscopic evidence of erosion. In sampling cartilage, care was taken to avoid contamination with synovium or areas of early osteophyte formation. For necartilage (N), osteophytes and loose bodies cartilagous surfaces only were processed, which was achieved by removing the outer layers of tissue to avoid disruption of the bony structures. From the Hoffa’s fat pad, tissue was harvested from the interior areas of the capsule excluding vascular areas and synovial regions.

Dissected tissue was cut into small (~0.5 mm³) pieces and digested overnight at 37°C using 0.25% collagenase (StemCell Technologies, Canada). After digestion, collagenase was removed by a single wash in sterile phosphate-buffered saline (PBS), followed by two further washes in Dulbecco-modified Eagle medium (DMEM) supplemented with 2% fetal calf serum (FCS) (all from Invitrogen, Paisley, UK). For primary culture, 5 × 10⁶ trypan blue-negative cells were plated in 25 cm² flasks (SLS, Nottingham, UK) in DMEM/10% FCS, supplemented with standard antibiotic mixture (100 units/ml penicillin and 100 μg/ml streptomycin) (both from Invitrogen). Next day media were changed to standard MSC expansion media, which contained DMEM, antibiotics and 10% FCS, previously selected for its support of MSC expansion (Mesencult, Stem Cell Technologies).

BM MNCs were seeded at 5 × 10⁶/25 cm² flask and grown in a similar manner. Growing cultures were fed twice weekly with 1/2 media changes. Initial confluent cultures were designated ‘passage 0’ (p0). Flow cytometry and differentiation assays were performed on minimally expanded (p1, ~11 population doublings, PDs) cultures. For the assessment of culture growth kinetics equal numbers of passages to ~16 PDs was performed. Changes in tripotential differentiation capacity were determined in cartilage- and fat pad-derived cultures at 11 and 16 PDs.

**Colony-forming unit-fibroblast (CFU-F) assay**

For the CFU-F assay, 0.5 × 10⁶ trypan blue negative collagenase-released cartilage and fat pad-derived cells were seeded in triplicate wells of a 6-well cluster plate in standard MSC expansion medium and fed twice weekly. On day 14, colonies were fixed in 1% paraformaldehyde (BDH), stained with 1% crystal violet (Sigma) and scored. BM MNCs were plated at the same seeding density and scored on day 14.

**Quantitative in vitro assays of MSC tri-potentiality**

Osteogenic differentiation was induced by placing cells in standard osteoinductive conditions as previously described [19]. The extent of osteogenesis was measured as the amount of calcium produced at the end of the culture (day 21), using a commercial calcium kit (DLC, Charlottetown, Canada). Adipogenic differentiation was induced as previously described [19]. For quantitative analysis of adipogenesis, triplicate cultures of 5 × 10⁴ cells were seeded onto 13 mm diameter sterile coverslips in 24-well cluster plates. At the end of 3-week culture accumulation of lipid vacuoles was visualized with 0.5% Oil Red (Sigma). Coverslips were counterstained with Harris’s Haematoxylin (Surgipath, Peterborough, UK), mounted on glass slides using Aquamount (VWR Lutterworth, UK) and the extent of adipogenesis in individual cells was ranked, based on the percentage of cytoplasm filled with fat globules (0–24, 25–50, 51–75, 76–100%, for grades 1, 2, 3 and 4, respectively). A grade 1 staining was observed in negative control skin fibroblasts and was considered to be of a background level. The data were presented as a percentage of fat-laden cells (with grades 2–4) in relation to all nucleated cells present on a coverslip. Chondrogenic assay was performed as previously described, with 2.5 × 10⁶ cells used to initiate each pellet [19, 20]. The amount of sulphated glycosaminoglycans (sGAG) (in microgram/pellet) produced after 3-week culture was measured in Alcian blue binding assay (IDS, Boldon, UK) [18].

**Cell cloning**

Cell cloning was performed by limiting dilution of freshly isolated cells from the three different cartilage sources and fat pad, seeded at 10, 5, 1 and 0.5 cells per well (in a 96-well plate). Microscopy was performed to ensure that clones were only taken from wells where a single cell had attached and proliferated. Clones were passaged through to a 25 cm² flask (equivalent to 22 PDs) and their tripotency was then tested, as described above.

**Flow cytometry for the assessment of standard MSC markers**

MSC marker phenotyping was performed on p1/11 PDs cultures as previously described [19]. CD133-FITC and CD105-PE were from Serotec (Kidlington, UK), CD45-FITC and CD34-PE were from DAKO (Ely, UK), CD106-PE, CD73-PE, CD146-PE, CD166-PE, CD44-PE, CD151-PE and low-affinity growth factor receptor (LNGFR)-PE were from BD Bioscience (Oxford, UK). CD133-PE was from Miltenyi Biotec (Bisley, UK), TGF-β RI-PE
was from R&D Systems (Abingdon, UK) and all isotype-specific negative controls were from Serotec. D7-FIB-PE was labelled in-house from purified D7-FIB (Serotec). Three-colour flow cytometry was performed on a BD FACScan. Dead cells were gated out based on propidium iodide exclusion (Sigma). All flow cytometry data were analysed with CellQuest or WinMDI v2.8 (Scripps Research Institute, La Jolla, CA, USA).

**Immunohistochemistry and cytochemistry of cartilage**

To investigate the quality of cartilage tissue in MN and E areas, whole depth sections were embedded in OCT (VWR) and snap frozen in liquid nitrogen. Cryostat sections (3 µ- and 4 µ thick) were mounted on superfrost slides (Surgipath) and dried overnight at 37 °C. To visualize cartilage proteoglycans, sections were fixed in methanol and stained with Toluidine blue (Sigma) as previously described [19]. To examine the retention of collagen II in the different areas of cartilage, immunohistochemical staining was performed as previously described [19]. All slides were examined using a LEICA DMLB [Leica Microsystems (UK) Ltd], images were captured with an Insight 4.2 camera and analysed using Spot software (Optivision, Ossett, UK).

**Statistical analysis**

Degrees of osteo-, chondro- and adipogenesis, CFU-F frequencies and proportions of marker-positive cells were first compared using Kruskal–Wallis test to look for overall significance. Mann–Whitney U test was then applied to assess the pair-wise significant differences. P-values were adjusted for significance according to the number of multiple comparisons used. Since this was a pilot study, descriptive statistics were employed to describe trends that failed to reach statistical significance. Matched analysis of chondro- and adipogenesis of OA cartilage- and fat pad-derived cultures at 11 PDs vs 16 PDs was performed using non-parametric Wilcoxon test. The SPSS v12 was used to analyse data.

**Results**

**Histological appearance of macroscopically normal and eroded cartilage**

MN cartilage from OA subjects lacked the superficial layer associated with healthy cartilage from young individuals [21] and showed evidence of fibrillation and superficial proteoglycan depletion (Fig. 1A, bottom panel). The cartilage obtained from sites immediately adjacent to erosions (E) showed a greater degree of proteoglycan depletion, fibrillation and chondrocyte loss (Fig. 1B, bottom panel).

**Growth characteristics of cultures derived from different sites of OA cartilage and from Hoffa's fat pad**

We initially determined the growth characteristics of cells derived from MN, E and N cartilage. No significant differences were noted in the number of colony-forming cells in freshly digested cartilage and fat pad using the standard CFU-F assay (Fig. 2A). Colony-forming capacity of OA cartilage in all three sites, and in the fat pad was comparable to that of normal BM donors; however, condyle cartilage (both MN and E) demonstrated a considerably higher degree of donor-to-donor variability (Fig. 2A).

Consistent with the CFU-F data, no significant differences were found in growth kinetics of polyclonal cultures derived from primary digests of MN, E or N cartilage (Fig. 2B), at least during the first 5 weeks of culture (up to p6, equivalent to ~16 cumulative PDs). Average population doubling time after reaching confluence at ~9–11 PDs remained the same for all three types of cartilage studied (one division/4 days). The Hoffa’s fat pad-derived cultures showed similar initial growth kinetics as did the control BM MSCs (Fig. 2B).

**MSC tri-potentiality of early-passage cultures from different sites of OA cartilage and from Hoffa’s fat pad**

The failure of intrinsic cartilage repair in the areas of erosion could be due to a defect in chondrogenic potential of remaining resident cells. Conversely, neocartilage from loose bodies and osteophytes could retain a potent chondrogenic capacity. To investigate this, we compared tri-potentiality of early passage (pl, corresponding to ~11 PDs) cultures derived from digested MN, E and N cartilage and Hoffa’s fat pad using quantitative in vitro differentiation assays and used a group of normal BM MSCs as a positive control of differentiation (Fig. 3).

First, the median chondrogenic capacity of cultures derived from all cartilaginous tissues was 2–5-fold above normal BM MSCs, however, these differences failed to reach statistical significance after correcting for multiple analysis (Fig. 3A). In agreement with published data, these data confirmed that E cartilage was not deficient in its chondrogenic capacity [22]. In fact, its chondrogenic drive was 2–3-fold higher than MN and N cartilage (Fig. 3A). Cultures derived from Hoffa’s fat pad had a median level of chondrogenesis above that of MN, N cartilage.
and BM MSC controls (1.6-, 1.7- and 3.6-fold, respectively), however, due to large donor-to-donor variability in all OA samples these differences also failed to reach statistical significance. Importantly, 80% of fat pad-derived cultures were more chondrogenic than control BM MSCs (Fig. 3A).

Second, the osteogenic capacity of different types of OA cartilage was compared, and no significant differences were found either between the three different areas of cartilage and fat pad or between OA cartilage, fat pad and normal BM MSCs (Fig. 3B). Finally, when the adipogenic capacity of all cultures was investigated, all cartilaginous cultures demonstrated significantly lower levels of adipogenesis compared with normal BM MSCs (P < 0.001, 0.001 and 0.002 for MN, E and N cartilage, respectively) (Fig. 3C). Amongst OA cartilaginous cultures, neo-cartilage was the least adipogenic (6- and 5-fold, compared with MN and E cartilage, respectively). Fat pad-derived cultures showed significantly higher levels of adipogenesis compared with all three areas of cartilage and BM MSC controls (P < 0.0001, 0.004, 0.001 and 0.001 for MN, E and N cartilage and BM MSCs, respectively). Cultured skin fibroblasts used as a negative control of differentiation did not display tri-potentiality, as shown previously [19, 23].

These data, therefore, showed some MSC activity in all areas of OA cartilage and fat pad. Areas of cartilage erosion were more likely to display higher levels of chondrogenesis (measured by higher sGAG content) compared with MN and N cartilage, possibly reflecting some local attempt at cartilage repair. As expected, fat pad-derived cells were the most adipogenic, however, very high levels of chondrogenesis (above control BM MSCs) were observed in 80% of cultures tested.

**Lack of multipotentiality at the single cell level in OA cartilage and superiority of Hoffa’s fat pad**

In the next set of experiments we compared chondrogenic capacities of cartilage- and fat pad-derived cells grown for ~11 PDs (equivalent to cultivation for less than 3 weeks) and 16 PDs (equivalent of cultivation for >1 month). As described in the previous section, the chondrogenic capacity of OA fat pad-derived cultures at 11 PDs was similar, and often superior, to OA cartilage (Fig. 4A). Microphotographs of representative pellets illustrate one such case where the fat pad-derived sample (Fig. 4B, right panel) was more chondrogenic than a matched cartilage-derived pellet (Fig. 4B, left panel). When matched cartilage and fat pad-derived cultures were compared at 11 PDs, the fat pad-derived cells were more chondrogenic in two out of three patients (data not shown).

Whereas OA cartilage completely lost its chondrogenic re-differentiation capacity at 16 PDs (Fig. 4A, grey bars), fat pad-derived cultures demonstrated less prominent decline in their chondrogenic capacity at 16 PDs (Fig. 4A, empty bars). Although some 2-fold loss in chondrogenesis was found in fat pad-derived cultures, the actual level of chondrogenesis at 16 PDs remained within the normal BM range (sGAG production between 1 and 5 µg/pellet). Similar trends were found for osteogenesis (data not shown). Fat pad-derived cells were more adipogenic than cartilage at both 11 PDs and 16 PDs and no significant differences in adipogenesis were found between 11 PDs and 16 PDs. However, in chondrogenic differentiation conditions no aberrant adipogenesis in fat pad-derived pellets was found (data not shown).

Clonogenic cells identified in the CFU-F assay are thought to contain a mixed population of highly proliferative MSCs and less proliferative transit-amplifying progenitors [14]. True MSCs are normally identified at the single-cell-level using limiting dilution techniques and mesenchymal lineage differentiation assays on clones expanded to a minimum of 10 PDs [5, 7, 24]. Single-cell-derived clonogenic cultures could not be generated from OA cartilage digests but matched OA Hoffa’s fat pad digests and yielded several bi- and tri-potential clones (Fig. 4C). This suggested that although OA cartilage retained good proliferative capacity, it was likely driven by transit-amplifying progenitors and not by true highly proliferative MSCs.

**Surface marker analysis of early passage cultures from OA cartilage and fat pad**

The expression of certain surface receptors, particularly CD105, was previously suggested to be indicative of the cells’ responsiveness to chondrogenic induction [25]. Co-expression of CD105 and CD166 was also used to identify MSCs in normal and OA articular cartilage [26, 27]. When we compared the expression of CD105 and CD166 on early passage (11 PDs) cultures derived from MN, E or N OA cartilage or from OA fat pads, no significant differences were found (Fig. 5, Table 1). CD105 is an accessory receptor for the TGF-β receptor complex interacting with the signalling receptors TGF-βRI and TGF-βRII [28]. No TGF-βRII expression was found in any of the samples tested, however, this may be due to the known fact that its expression is highly dependent on cell growth conditions [29]. Similarly, no differences were observed for...
CD73, a well-recognized MSC marker [30], which was uniformly expressed in all the cultures, whereas CD34, CD133 and CD45, were uniformly negative. More recently, the expression of CD44 and CD151 was proposed to be characteristic for human chondrocytes with enhanced chondrogenic potential [31], but tested on our set of samples, no differences in their expression were found (Table 1). Moreover, in OA cartilage-derived cultures the levels of CD44 and CD151 expression did not diminish following extended passaging to ~16 PDs even though their chondrogenic capacity plummeted. Specifically, CD44 remained present on 100% of cells, and cells expressing CD151 increased slightly from 96 ± 2% at 11 PDs to 98 ± 1.5% at 16 PDs (n = 4). As documented previously, the number of cells expressing CD146 were higher in BM MSCs compared with both cartilage and fat pad-derived cultures [32, 33]. Interestingly, fat pad-derived cultures expressed higher levels of LNGFR, the in vivo marker of MSCs in human BM and synovial fluid (Fig. 5, Table 1). We had previously demonstrated that during culture expansion of BM MSCs LNGFR expression is lost [17]; remarkably, however, it remained on a subpopulation of fat pad-derived cultures.

Discussion

Many hurdles have to be overcome with respect to therapy development for chondral defects in advanced OA. This work explored one of these issues, namely optimal sources for autologous cells for cartilage repair. The existing evidence suggests an age-related loss of chondrogenic capacity of ADDCs, the currently preferred cells for cartilage repair [13, 22]. The data presented in this study demonstrate functional and immunophenotypic evidence for an MSC population in OA joint Hoffa’s fat pad, but not in OA cartilage. Specifically, we found considerable MSC activity at the single-cell level from fat pad-derived cells but none of the cartilaginous structures. Therefore, Hoffa’s fat pad could represent a useful source of regenerative cells for cartilage repair in OA. As there is evidence for some spontaneous joint repair in OA, we initially evaluated three distinct knee articular cartilage sites including chondro-osteophytes for MSC activity. When we compared sites of complete cartilage erosion to macroscopically normal cartilage, we found comparable proliferation, however, a trend was found for an increased chondrogenic potential in the eroded areas, suggesting that such sites may indeed activate some ‘compensatory mechanisms’ aimed to restore chondrogenesis and replenish depleted proteoglycans. In this regard, our findings support the concept that such sites of cartilage injury may serve as ‘signalling centres’, augmenting local chondrogenesis and, perhaps, also influencing more distant remodelling processes [34]. Our data are also in agreement with previously published reports demonstrating no reduction in proliferation and proteoglycan production in affected areas of OA cartilage compared with ‘unaffected’ cartilage [22].

A novel aspect of this work resides in the fact that sites of neochondrogenesis (N) in vivo did not have a higher chondrogenic capacity compared with MN cartilage.

In addition to performing clonogenic assays for resident MSCs, we deliberately assessed early passage polyclonal OA cartilage- and Hoffa’s fat pad-derived cultures. From the perspective of OA therapy development in older subjects shorter cultivation would be desirable for a number of reasons, including minimizing the risks of genetic instability. Additionally, it would reduce the time from cartilage biopsy to cell implantation, and also lessen the recognized loss of potency following expansion and implantation [35]. In these experiments, the differences in chondrogenesis between the four different OA tissues (fat pad and three cartilage areas) failed to reach statistical significance due to very large donor-to-donor variability in all the tissues studied, but overall, the fat pad-derived cultures had comparable (and in many cases superior) chondrogenesis to the cartilage-derived cultures.

We were unable to generate single MSC-derived highly proliferative cultures from OA cartilage in this study, indicating an inability to extract ‘true’ MSCs. The presence of MSCs in OA cartilage remains controversial. It has been previously shown possible to generate single-cell-derived multipotential clones from normal young individuals in some studies [5-7] but not in the others [35], suggesting limited expandability of cartilage-derived MSCs even in health. In OA cartilage, the presence of cells with putative MSC phenotype was previously documented [26, 27, 36] and their frequency was suggested to be increased compared with normal cartilage, however, this remains to be proven using clonal cultures derived from single sorted cells. The data presented in this work are in agreement with previous studies that showed some preservation of chondrogenesis in low-passage polyclonal ADDCs [22, 35]. A potential concern of the clinical use of OA cartilage-derived cultures, however, is the lack of true clonogenic MSCs in such grafts, which could be associated with poor long-term graft survival following in vivo implantation.

![Fig. 4. Functional superiority of Hoffa's fat pad at polyclonal and single-cell level.](https://academic.oup.com/rheumatology/article-abstract/46/11/1676/1785713/data/file)
Fig. 5. Cell surface phenotype of minimally expanded (grown for 11 PDs) cultures derived from OA cartilage and from OA Hoffa’s fat pad (FP) compared with control BM MSCs. Histograms for a representative sample are shown. Markers—grey-shaded histograms, isotype control antibodies—empty histograms. No surface marker was found sufficiently differentially expressed to be selected as a potential indicator of observed functional differences between all the cultures studied.
FGF receptor 3, BMP2 and Col2A1 were proposed in the past as flow cytometry-based assays are reproducible, automated and study [31]. Cell surface markers represent the easiest solution, ADDCs is well-recognized and we confirmed this in the present study [31]. We observed enrichment for clonogenic and chondrogenic cellular fractions in vivo MSCs, even after culture expansion for LNGFR expression on a high proportion of fat pad-derived MSCs, even after culture expansion for LNGFR expression on a high proportion of fat pad-derived MSCs [18, 19]. In the present study, we have observed that synovial fluid MSCs express LNGFR for BM MSCs as well as polyclonal cultures from fat pad with good molecular markers predictive of its chondrogenicity in vivo and in vitro and to prospectively purify the fat pad MSC population. Further studies are also needed to determine whether the observed lack of MSC activity in articular cartilage may be a contributory factor to disease progression in OA.

In contrast to cartilage, the ability to generate clonogenic MSCs as well as polyclonal cultures from fat pad with good chondrogenesis, even at high PDs, suggests that the fat pad has resident MSCs even in advanced OA. As shown previously, even in OA, Hoffa’s fat pad contains true highly proliferative and multipotient MSCs [37]. This is distinct from MSCs from other sites in OA where chondrogenesis may be defective [16]. We have also demonstrated that extended cultivation of Hoffa’s fat pad-derived cells leads to a slower loss in their chondrogenesis compared with OA cartilage. Although there are other potential sources of chondrogenic progenitors including bone marrow MSCs [17], the Hoffa’s fat pad offers a number of practical advantages, including familiarity and accessibility for the orthopaedic surgeon. In addition, the potential yield of cells is greater compared with BM by virtue of the fat pad size. There is considerable interest in synovium as another source of MSCs for OA cellular therapy development [11], but it is a very heterogeneous in OA. It may vary in thickness, show fibrotic or haemorrhagic changes or inflammation, the latter of which in itself may be detrimental to stem cell function [38]. In comparison to knee joint synovitis, which is common and variable in extent in OA, the recognition of inflammatory changes in Hoffa’s fat pad is less frequent [38]. This factor, the consistent location, size and accessibility of the fat pad could make it a good source for cell therapy development in OA.

The present study also provides some intriguing immunophenotypic data as to the identification of the fat pad MSC in vivo. We and others have demonstrated that LNGFR is highly specific for BM MSCs in vivo [19, 23, 39]. Additionally, we have shown that synovial fluid MSCs express LNGFR in vivo [18]. Moreover, we documented that BM MSCs lose LNGFR following proliferation in culture and that it is not re-acquired by MSCs during expansion [18, 19]. In the present study, we have observed LNGFR expression on a high proportion of fat pad-derived MSCs, even after culture expansion for ~11 PDs. Based on these data, it is probable that fat pad MSCs may also express LNGFR in vivo. Studies are ongoing to prospectively isolate LNGFR positive cells in the fat pad and to determine whether they are enriched for clonogenic and chondrogenic cellular fractions in numbers sufficient to be used directly as cell therapy without prior culture expansion.

Besides the optimal source of repair cells, the other important issue remains an ability to predict a chondrogenic outcome from a given cell population. Large donor-to-donor variation in OA ADDCs is well-recognized and we confirmed this in the present study [31]. Cell surface markers represent the easiest solution, as flow cytometry-based assays are reproducible, automated and highly objective. In relation to chondrogenesis-related markers, FGF receptor 3, BMP2 and Col2A1 were proposed in the past [40], but no well-characterized commercial antibodies are yet available to monitor these markers at the protein level. Another marker of chondrogenicity of ADDCs, CD105, was recently proposed, based on its ability to facilitate TGF-β-transduced signals [25]. However, most researchers consider it as a common MSC marker, and this was confirmed in our previous work [18, 33]. Similarly, the expression of other putative chondro-predictive markers, namely CD44 and CD151 [31], did not differ in the tissues tested in this study, nor did it reflect the loss of chondrogenic capacity following extended passageing of chondrocytes. Although it may be possible to select potential surface markers predictive of chondrogenesis using global genomics approaches, it is likely that molecules involved in distinct signalling pathways such as TGF-β/BMP or Wnt, may have a higher predictive value [34].

In conclusion, this study showed that cells that meet the full criteria for MSCs, namely clonogenicity and multipotentiality were absent in OA cartilage, irrespective of the source, but could be readily isolated from Hoffa’s fat pads. The Hoffa’s fat pads may therefore represent a good source for autologous MSCs and our findings may facilitate the exploration of stem cell therapy in older subjects with OA. Further work is needed to establish robust molecular markers predictive of its chondrogenicity in vivo and in vitro and to prospectively purify the fat pad MSC population. Further studies are also needed to determine whether the observed lack of MSC activity in articular cartilage may be a contributory factor to disease progression in OA.

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