Mesenchymal stem cells in joint disease and repair

Frank Barry and Mary Murphy

Abstract | Osteoarthritis (OA), a prevalent chronic condition with a striking impact on quality of life, represents an enormous societal burden that increases greatly as populations age. Yet no approved pharmacological intervention, biologic therapy or procedure prevents the progressive destruction of the OA joint. Mesenchymal stem cells (MSCs)—multipotent precursors of connective tissue cells that can be isolated from many adult tissues, including those of the diarthrodial joint—have emerged as a potential therapy. Endogenous MSCs contribute to maintenance of healthy tissues by acting as reservoirs of repair cells or as immunomodulatory sentinels to reduce inflammation. The onset of degenerative changes in the joint is associated with aberrant activity or depletion of these cell reservoirs, leading to loss of chondrogenic potential and preponderance of a fibrogenic phenotype. Local delivery of *ex vivo* cultures of MSCs has produced promising outcomes in preclinical models of joint disease. Mechanistically, paracrine signalling by MSCs might be more important than differentiation in stimulating repair responses; thus, paracrine factors must be assessed as measures of MSC therapeutic potency, to replace traditional assays based on cell-surface markers and differentiation. Several early-stage clinical trials, initiated or underway in 2013, are testing the delivery of MSCs as an intra-articular injection into the knee, but optimal dose and vehicle are yet to be established.

Barry, F. & Murphy, M. *Nat. Rev. Rheumatol*. advance online publication 23 July 2013; doi:10.1038/nrrheum.2013.109

Introduction

Chronic disability in people over 50 years of age is strongly associated with disorders of the musculoskeletal system. Of these conditions, osteoarthritis (OA) of the spine and diarthrodial joints is by far the most common. All joints can be affected in OA, with hand, knee and hip being the major sites.[1] The disease has a striking impact on quality of life and represents an enormous societal and economic cost,[2] a burden that will increase greatly as populations age.[3] OA is not just associated with disability; it has clear links to other conditions, such as neuropathic pain, depression and sleep disorders.[4] Some assessments of disease burden suggest that OA is an important cause of premature death.[2,5]

OA is a complex condition with broad pathology, and is often characterized as a biomechanical disease associated with abnormal joint loading resulting from obesity, joint instability or trauma.[6] Damage to the articular cartilage is a consistent feature, accompanied by changes to the subchondral bone and synovium.[7–9] Progression of the disease involves further degeneration of the articular cartilage, damage to the underlying bone and morphological changes that include subchondral bone thickening, development of cysts, osteophytes and inflammation of the synovium. Enhanced production of proinflammatory cytokines and matrix metalloproteinases accelerates degradation of the articular cartilage.

The synovium seems to play a crucial role in the development of OA of the knee. Synovial inflammation occurs in the majority of patients and is a predictive factor in disease progression.[10] The activity and phenotype of cell populations resident within the synovium affect the maintenance of healthy joints and might also be associated with degenerative changes in OA. For example, infiltration of $CD4^+$ T cells and $CD68^+$ macrophages is substantially increased in the synovium in early compared with late-stage OA,[11] indicating that synovial inflammation is a feature in early disease and might be the initiator of degenerative cascades that lead to tissue destruction. The synovium, however, seems to have two faces to its role in OA, as it might also be the focus of effective repair responses involving endogenous populations of progenitor cells. As discussed in the 'Insights from other cell-based therapies' section of this manuscript, these endogenous synovial MSCs seem to become activated in response to MSC transplantation in the knee.

It is a striking fact that no approved pharmacological intervention, biological therapy or procedure prevents the progressive destruction of the OA joint. All current treatments, without exception, produce symptomatic rather than regenerative results and include pain control with steroidal and non-steroidal anti-inflammatory drugs, viscosupplementation with injections of sodium hyaluronan and a variety of nutraceuticals including chondroitin sulphate, glucosamine, omega-3 fatty acids

Competing interests
F. Barry declares associations with the following companies: Osiris Therapeutics and Orbsen Therapeutics. M. Murphy declares an association with the following company: Osiris Therapeutics. See the article online for details of the relationships.

Regenerative Medicine Institute, National Centre for Biomedical Engineering Science, National University of Ireland Galway, University Road, Galway, Ireland (**F. Barry, M. Murphy**).

Correspondence to: F. Barry frank.barry@nuigalway.ie

Key points

- Osteoarthritis (OA) is associated with progressive and irreversible destruction of joint tissues with no defined aetiology
- All joint tissues contain resident populations of mesenchymal stem cells (MSCs) capable of differentiating into cartilage, bone and other tissues
- OA seems to be associated with changes in the quantity, phenotype, and differentiation potential of resident MSCs
- Transplantation of *ex vivo* preparations of MSCs to the OA joint can evoke a therapeutically useful repair response in animal models of the disease
- The repair effect mediated by delivered MSCs seems to arise as a result of paracrine responses
- Early-stage clinical trials, initiated or underway in 2013, are testing intra-articular injection of MSCs, mostly without scaffold in the knee, but the optimal dose and vehicle have not been established

and other products. None of these compounds has a clinically useful impact on the progressive loss of joint tissues that leads, ultimately, to total joint replacement (TJR).[12] Although TJR is generally successful, resulting in enhanced mobility and reduction of pain, it is nonetheless a major surgical procedure with substantial risk of thrombosis and infection, not to mention the cost in terms of hospital care, physiotherapy and rehabilitation.[13] Thus, TJR usually becomes an option only after structural failure of the joint and after many years of degenerative arthritis.

Speculation about the lack of progress in the development of treatments for OA might encompass factors such as low levels of research funding or lack of public perception about the impact of the disease. Clearly, however, poor understanding of the disease mechanisms, its complex pathology, the lack of biomarkers of early disease and its slow progression all contribute to the absence of therapeutic targets. Signalling pathways, biochemical events and cellular functions that might be involved remain obscure. These factors force us to consider new elements in the biology of the diarthrodial joint that might be important in the progression of OA. There are many reasons to think of OA as a mesenchymal disease, that is, a condition in which the activity, phenotype or mobilization of MSC populations is altered, leading to an absence of repair and increased degenerative changes. This idea is based on the hypothesis that all of the tissues that comprise the healthy joint depend for correct development and homeostasis on the availability and activity of MSCs.

In this Review, we provide an overview of the characterization and phenotypic properties of MSCs, the role of MSCs and MSC-like populations in joint tissues and their potential contribution to joint function. We also discuss the concept that degenerative changes seen in arthritic disease are associated with depletion of MSC reservoirs or alterations in their activity. Finally, we provide a comprehensive review of preclinical data indicating the potential for MSC therapy in the treatment of chronic degenerative joint disease, and outline the approaches being tested in clinical trials.

Mesenchymal stem cells

MSCs are precursors of connective tissue cells and can be isolated from many adult organs. The founder of the field of MSC biology was Alexander Friedenstein, who was the first to isolate fibroblastic cells with the capacity to differentiate into osteocytes from the stromal compartment of bone marrow.[14–16] These plastic-adherent cells were capable of establishing colonies from a single cell, often referred to as colony-forming units fibroblastic (CFU-F). Furthermore, they were able to generate multiple skeletal tissues *in vivo*.[17,18] MSCs have since been isolated and characterized from many other human sources, including adipose tissue,[19–21] skeletal muscle,[22] umbilical cord blood and Wharton's jelly.[23–26] All share the capacity to differentiate into cells of connective tissue lineages *in vitro*, most notably bone, fat, cartilage and muscle. Bone-marrow-derived MSCs are additionally able to provide the stromal support system for haematopoietic stem cells.[27–30]

The wide tissue distribution of MSCs led to the suggestion that the cells are derived from a perivascular niche.[30–32] In support of this idea, the use of prospective isolation techniques identified clonal progenitor cells derived from blood vessels in various human tissues; these cells exhibit multipotentiality and test positive for standard markers of MSCs. Thus, perivascular cells were proposed in 2008 to be the precursors for MSCs,[22] with Caplan writing in an accompanying commentary that all MSCs might be pericytes.[33] In a paper published in 2012,[34] a short-lived, unipotential, profibrotic myofibroblast population derived from a distinct subset of perivascular, proinflammatory stromal cells expressing platelet-derived growth factor receptor α and identified by transient expression of ADAM12 (a disintegrin and metalloprotease 12), was suggested to be involved in the early stages of wound healing in skin and muscle in mice. However, as healing progressed, these unipotential cells were gradually replaced by interstitial mesenchymal cells that were not derived from the ADAM12-positive population.[34] Whether these repopulating stem cells represent a distinct mesenchymal progenitor in the perivascular niche or are derived from an alternative tissue-specific niche remains to be determined. In another mouse study, two nucleoside analogue labels were used to identify synovial cells that were initially slow-cycling but that proliferated after injury to articular cartilage (characteristic stem cell behaviour). Positive for MSC markers and negative for those of haematopoietic and endothelial cells, these stromal cells were distinct from pericytes. Interestingly, co-staining revealed expression of chondrocyte-lineage markers in areas of secondary cartilage metaplasia within the synovium that occurred in some instances as a complication of surgery.[35] This study is discussed further in the 'MSCs in the healthy joint' section of this manuscript.

MSCs in joint tissues

MSCs can be detected in most tissues of diarthrodial joints (Figure 1, Table 1). Joint-resident MSCs in humans were first described in adult human synovial membrane in 2001, by De Bari *et al.*[36] In common with bone-marrow-derived MSCs, these synovial cells have a capacity for self-renewal and the potential to differentiate along the chondrogenic, osteogenic and adipogenic pathways as well as exhibiting apparent sporadic myogenesis

in vitro. They also demonstrate clonal heterogeneity, with individual clonal populations having variable proliferative activity and differentiation potential.[37] When transplanted into T-cell-deficient mice, MSCs derived from human synovium were reported to stimulate repair of the injured tibialis anterior muscle, where the engrafted human cells apparently contributed to the development of myofibres and functional satellite cells.[38] However, subsequent evaluation of the myogenic propensity of synovially derived MSCs, both *in vitro* and *in vivo,* has found scant evidence of this function.[39]

MSCs with a phenotype resembling that of bone marrow MSCs have also been detected in the synovial fluid compartment.[40–42] The number of recoverable MSCs is much greater in synovial fluid samples from patients with rheumatoid arthritis or OA,[41] as well as following ligament injury,[42] than in samples from healthy joints.[43] The yield of cells increases with severity of disease and one possibility is that they originate in the degrading synovium, although this hypothesis has not been verified.[44] Synovial fluid MSCs do seem to have greater clonogenicity and chondrogenic differentiation capacity than those isolated from matched bone marrow.[40] Similarly, synovium-derived MSCs seem to have a more active chondrogenic phenotype than those obtained from bone marrow or the intrapatellar fat pad;[45,46] MSCs from the synovial fat pad maintain typical surface markers and proliferation to high passages.[47]

MSC-like progenitor cells have been reported in the surface zone of adult human articular cartilage.[48] These cells differ from bone-marrow-derived MSCs in that they are selectively isolated by fibronectin binding and have a different chondrogenic propensity (reduced alkaline phosphatase activity and reduced expression of type X collagen). These cells have greater growth potential and higher telomerase activity than dedifferentiated chondrocytes isolated from the same tissue.[49] They may well represent the cartilage reservoir of chondrogenic precursors responsible for maintenance of that tissue.[50–53]

MSCs also evidently reside within the anterior cruciate ligament, migrating out of the tissue when samples are cultured following rupture.[54–57] After detailed study of their characteristics, Cheng *et al.*[58] and Steinert *et al.*[55] found these cells to be almost identical to bone marrow MSCs, although they have profound ligamentogenic potential *in vitro* in addition to the trilineage potential shared by most MSC populations. Meniscus-resident MSCs have also been found; although less extensively characterized than those from other tissues, these cells are efficient colony formers, possess strong chondrogenic activity, and share the same set of typical cell-surface markers as bone-marrow-derived MSCs.[59]

Minor phenotypic differences between joint-resident MSCs might reflect their specific tissue of origin, but current evidence cannot entirely exclude influence from laboratory protocols and culture conditions. Emerging data nonetheless seem to suggest tissue specificity of reparative cell populations; thus, response to joint injury might entail mobilization of local MSC or MSC-like progenitor cell populations with lineage-restricted responses

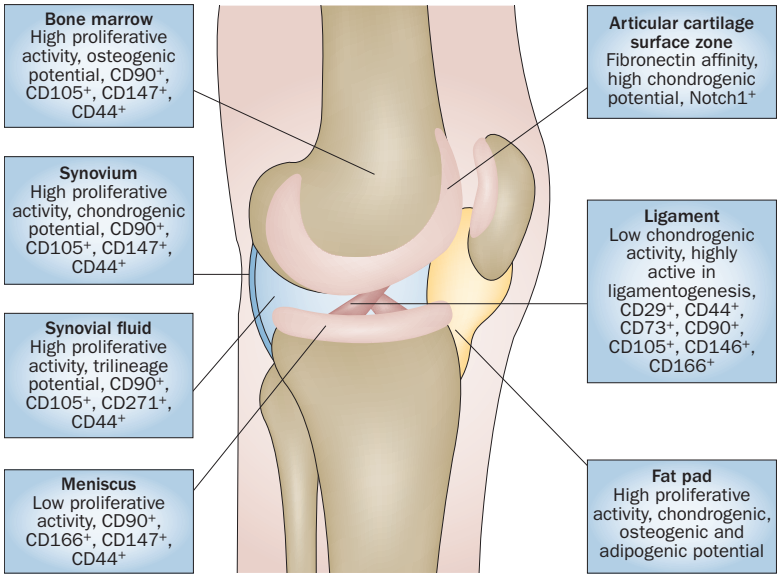

Figure 1 | Characteristics, culture phenotypes and cell-surface markers* of MSCs isolated from tissues within the knee joint. MSCs (or MSC-like progenitor cells) can be isolated from all compartments of the knee joint, and might act as a reservoir of replacement cells to contribute to the maintenance of healthy tissue and/or the response to injury. MSCs might also act as immunomodulatory sentinels to reduce inflammation. Degenerative changes in OA can be partly attributed to the aberrant or defective activity of these local MSC populations. Minor tissue-specific differences in characteristics such as cell-surface markers, proliferative capacity and lineage potential exist between MSCs isolated from different tissues within the knee joint. *The CD molecules named in the figure have full and/or alternative names as follows: CD29, integrin β1; CD44, CD44 antigen; CD73, 5'-nucleotidase; CD90, Thy-1 membrane glycoprotein; CD97, CD97 antigen; CD105, endoglin; CD146, cell surface glycoprotein MUC18; CD166, CD166 antigen; CD271, TNF superfamily member 16. Abbreviations: MSC, mesenchymal stem cell; OA, osteoarthritis.

to injury. This specificity is the case in fracture repair in mice, where Mx1-expressing cells are associated with an osteoblast–osteoprogenitor-restricted fate.[60] In addition, mouse MSC or MSC-like progenitor cells contributing to regeneration of the distal digit (that is, to bone and tendon) were also shown to be lineage restricted, and to reside in local tissue, rather than in the circulation.[61]

MSCs in the healthy joint

The fact that MSCs, or cells with properties very similar to MSCs, can be isolated from every tissue within the diarthrodial joint requires some discussion. A reasonable assumption is that their widespread distribution is associated with key functional characteristics that contribute to the maintenance of healthy tissues or to the response to injury. Scant mechanistic insight from experimental characterization of joint-resident MSCs exists, however, largely because these cells were, until recently, only retrospectively analysed (that is, their properties were revealed after isolation from the tissue). Currently, prospective isolation or *in situ* analysis remains impossible because no cell-specific biomarkers are available. Nevertheless, the nucleoside analogue cell-labelling strategy used by De Bari *et al.*[35] to characterize synovial stem cells and their progeny after joint surface injury has resulted in some progress in mechanistic understanding. Subsets

Table 1 | Characterization and phenotypic properties of MSCs in the diarthrodial joint

Tissue	Characteristics of resident MSCs	MSC markers*	References
Synovial membrane	Stable, proliferative population with high chondrogenic propensity	Positive: CD90, CD105, CD147, CD44 Negative: CD34, CD45, CD117, CD31	De Bari *et al*. (2001)[36]; Sakaguchi *et al*. (2005)[45]; Fan *et al*. (2009)[46]
Meniscus	Slightly lower proliferative activity compared to synovium or bone marrow MSCs	Positive: CD90, CD105, CD166, CD44 Negative: CD34, CD45	Segawa *et al*. (2009)[59]
Ligament (anterior cruciate)	Outgrowth cells from collagenase digests of ACL Less active in chondrogenesis, osteogenesis and adipogenesis compared with bone marrow MSCs Highly active in ligamentogenesis	Positive: CD29, CD44, CD49c, CD73, CD90, CD97, CD105, CD146, CD166 Weakly positive: CD106, CD14 Negative: CD11c, CD31, CD34, CD40, CD45, CD53, CD74, CD133, CD144, CD163	Steinert *et al*. (2011)[55]
Fat pad	Highly proliferative, strong chondrogenic, osteogenic and adipogenic activity	Positive: CD13, CD29, CD44, CD90, CD105 Negative: CD34, CD56, CD271, STRO1	Khan *et al*. (2012)[47]
Cartilage	Isolated from surface zone of articular cartilage, high affinity for fibronectin, strong colony-forming efficiency Active chondrogenic potential with capacity for adipogenic and osteogenic differentiation	Positive: CD49e, Notch1, CD90, STRO-1 antigen‡	Williams *et al*. (2010)[50] Alsalameh *et al*. (2004)[51]
Bone marrow	Highly proliferative, strong chondrogenic, osteogenic and adipogenic activity	Positive: CD13, CD29, CD44, CD90, CD105 Negative: CD34, CD45	Barry & Murphy (2004)[27]

*The CD molecules listed in this table have full and/or alternative names as follows: CD11c, integrin αX; CD13, aminopeptidase N; CD14, monocyte differentiation antigen CD14; CD29, integrin β1; CD31, platelet endothelial cell adhesion molecule; CD34, hematopoietic progenitor cell antigen CD34; CD40, TNF superfamily member 5; CD43, leukosialin; CD44, CD44 antigen; CD45, receptor-type tyrosine-protein phosphatase C; CD49c, integrin α1; CD49e, integrin α5; CD53, leukocyte surface antigen CD53; CD56, neural cell adhesion molecule 1; CD73, 5′-nucleotidase; CD74, HLA class II histocompatibility antigen γ chain; CD90, Thy-1 membrane glycoprotein; CD97, CD97 antigen; CD105, endoglin; CD106, vascular cell adhesion protein 1; CD133, prominin-1; CD144, cadherin-5; CD146, cell surface glycoprotein MUC18; CD147, basigin; CD163, scavenger receptor cysteine-rich type 1 protein M130; CD166, CD166 antigen; CD177, mast/stem cell growth factor receptor Kit; CD271, TNF superfamily member 16. ‡STRO-1 antigen is the as-yet uncharacterized target of monoclonal antibody STRO-1. Abbreviations: ACL, anterior cruciate ligament; MSC, mesenchymal stem cell.

of iododeoxyuridine label-retaining (IdU^+) cells prior to injury displayed an MSC surface phenotype with some interesting differences between cells localized to the synovial lining and the subsynovial tissue niche. In particular, cells positive for both IdU and CD44 were detected in the lining layer, whereas cells positive for IdU and CD73 were found in subsynovial tissue. However, these cells were not labelled with CD146, suggesting that they are phenotypically and functionally distinct from pericytes. Therefore, the suggestion that all MSCs are perivascular in origin does not seem to apply to synovial populations.

Detailed and insightful studies by Mendez-Ferrer *et al.*[62] have shown that MSCs in the bone marrow express the intermediate filament protein nestin, that they exist in close physical association with haematopoietic stem cells (HSCs) and that MSCs and HSCs together form a unique cellular niche which supports the regulation and homing of HSCs. The use of nestin as an *in vivo* marker of MSCs will in the future afford opportunities for evaluation of MSC-associated cellular niches in other tissues.

Given the existence of MSC populations within all joint tissues, it is not difficult to consider how they might contribute to the maintenance of healthy tissues. Two mechanisms seem likely. Firstly, they might provide a reservoir of repair cells that are activated in response to growth, remodelling or repair; secondly, they might act as immunomodulatory sentinels to reduce inflammation or limit the activation of T cells. Both functions are likely to be important.

Whereas MSCs (specifically, a subset of the heterogeneous bone marrow MSC population that expresses interferon-induced GTP-binding protein Mx1) are clearly capable of mobilizing in response to the stress or injury of bone fracture,[60] MSCs or MSC-like progenitor cells in cartilage seemingly lack the capacity for functional repair, given the well-characterized failure of that tissue to regenerate following injury. Potentially, MSC-like cells might reside in cartilage in order to replenish the surface zone proteoglycan lubricin, which is crucial for reducing friction.[63] Indeed, bone marrow MSCs in culture rapidly and dramatically upregulate expression of the lubricin gene upon induction of chondrogenesis (F. Barry and M. Murphy, unpublished observations).

Some further insight into the roles of MSCs in synovial joints has been obtained in studies of joint development in mice. A population of *Gdf5*-expressing MSCs that contributes to articular cartilage and synovial lining formation during development in mice, with little or no contribution to growth plate cartilage or bone, has been described.[64] Whether these cells are related to the MSC-like progenitor cells found in adult human articular cartilage,[48,51] which we discussed in the previous section of this manuscript, remains to be seen. Their potential as an exogenous source of cells for joint surface repair, or to be endogenously mobilized, is also unknown. However, Lee *et al.*[65] demonstrated formation of a surface resembling hyaline cartilage on rabbit humeral heads from which the articular cartilage was excised and replaced with a scaffold infused with transforming growth factor (TGF) β3. The results of this study suggested that cartilage repair involving the mobilization of endogenous populations is possible. Overall, the conclusions that emerge from the

studies described in this section are that MSCs reside within all tissues of the diarthrodial joint, in phenotypically distinct populations, and their function is to contribute to tissue repair and homeostasis.

MSCs in OA

Functional deficiencies of bone-derived MSCs

Various findings evoke possible mechanisms whereby the aberrant or defective activity of MSCs might contribute to the development of OA. For example, Murphy *et al.*[66] demonstrated in 2002 that MSCs isolated from patients with end-stage OA are functionally deficient in terms of their *in vitro* proliferation and differentiation. Obtained from bone marrow during joint replacement surgery and compared with cells from healthy, age-matched controls with no evidence of OA, the OA MSCs were substantially reduced in yield and proliferative activity. Furthermore, their differentiation profile was considerably altered, with reduced chondrogenic and adipogenic activity and increased capacity for osteogenesis. Equivalent loss of function was seen in MSCs isolated from the site of joint replacement surgery (the proximal or distal femur or the proximal tibia) and from a remote site (the iliac crest of the pelvis), indicating the systemic nature of these changes.

These functional deficiencies in OA MSCs can be reversed by supplementation of the culture medium with growth factors such as epidermal growth factor.[67] The inclusion of fibroblast growth factor 2 (FGF2) in growth medium is also beneficial in this context,[68] and Coutu *et al.*[69] showed that MSCs isolated from various tissues on the basis of their *in vivo* expression of FGF receptors 1 and 2 rapidly reached senescence when cultured without FGF2. Conversely, inclusion of FGF2 in the culture medium promoted proliferation and inhibited senescence, via the phosphatidylinositol 3-kinase–AKT and E3 ubiquitin-protein ligase Mdm2 pathways, respectively.[69]

Results from other studies have pointed to both age and disease as factors that influence the phenotype of MSCs. For example, De Bari *et al.*[70] showed that human periosteal MSCs from donors aged <30 years exhibit spontaneous chondrogenic activity in culture, and that this activity is absent in cells from older donors and in cells from young donors that had been extensively subcultured. Jones *et al.*[71] found that MSCs (isolated on the basis of CD271 expression) from trabecular bone samples from healthy donors and patients with OA had equivalent CFU-F capacity, but that the OA MSCs showed an *in vitro* ageing-related loss of proliferation. Together, these observations suggest that MSCs are depleted in the marrow of patients with advanced OA and that their growth factor receptor profile is altered, with higher concentrations of growth factors required to support their proliferation and differentiation. The data also suggest that patient-derived cells might have become senescent and that proliferative potency could be related to the *in vivo* age of native MSCs.[71]

MSC-like cells and progenitors in cartilage

As described above, MSC-like cells can be found in normal and OA human articular cartilage.[48–53] The presence of Notch-1 expression has been associated with these progenitor populations in normal cartilage[48] and in early-passage MSCs;[72] Notch-1 positive cells are found in greater numbers in articular cartilage from patients with OA than from controls, and are primarily located in proliferating clusters of cells.[72] Chondrocyte clusters, a hallmark feature of OA articular cartilage, are thought to result from dedifferentiation and subsequent proliferation of resident chondrocytes,[52,73] although migration of progenitor cells cannot be ruled out as their origin. Indeed, Koelling *et al.*[74] described a migratory multipotent clonal cell population in fibrocartilaginous repair tissue that seemed to have originated from blood vessels that occupy breaks in the tidemark of vascularized cartilaginous tissue from patients with late-stage OA. Expression of the cartilage hypertrophic marker type X collagen, which is upregulated in the middle and deep zones of cartilage from patients with severe OA, can coincide with cluster formation.[75–77] However, pericellular staining for collagens associated with fibrocartilage (types I and III) as well as for type II and type VI collagen is also increased in assays of OA-like chondrocyte clusters in samples from patients with Kashin–Beck disease, with pronounced expression of type I collagen at the surface zone.[76]

In summary, protein expression patterns in OA cartilage cell clusters, as reviewed elsewhere by Lotz *et al.* in 2010,[73] indicate a progenitor cell phenotype and a pattern of abnormal hypertrophic differentiation. Whether these progenitor cell clusters represent an early step in the development of cartilage pathology in OA that is followed by inappropriate terminal differentiation of cells within one cluster, and/or whether distinct cluster types are associated with location or disease stage, is not known. In assessing the role of MSCs or MSC-like progenitor cells in early OA it is interesting that pleiotrophin, which is primarily expressed during development, was detected in clusters in the superficial zone, but not in deeper layers, in the same histological sections of cartilage from patients with OA.[78]

MSCs, TGF-β signalling and cartilage repair

Increased understanding of the interplay between TGF-β and a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5) in modulating the repair response in post-injury OA might help to explain the roles of cartilage-resident or bone-resident MSCs in OA, and why they are unable to achieve an appropriate cartilage repair response.[79] Extensive investigation into protease-mediated destruction of aggrecan—the major proteoglycan component of cartilage—since the 'aggrecanase' cleavage site was discovered in the early 1990s[80] led to the identification in 2005 of ADAMTS5 as the major aggrecanase involved in degradation of mouse cartilage.[81,82] Subsequent studies on the mechanism of decreased cartilage degradation in *Adamts5*$^{-/-}$ mice with experimental OA suggested that lack of this metalloproteinase activity resulted in decreased joint fibrosis and cartilage erosion.[83] In elucidating the role of ADAMTS5 in the degradation of articular cartilage it

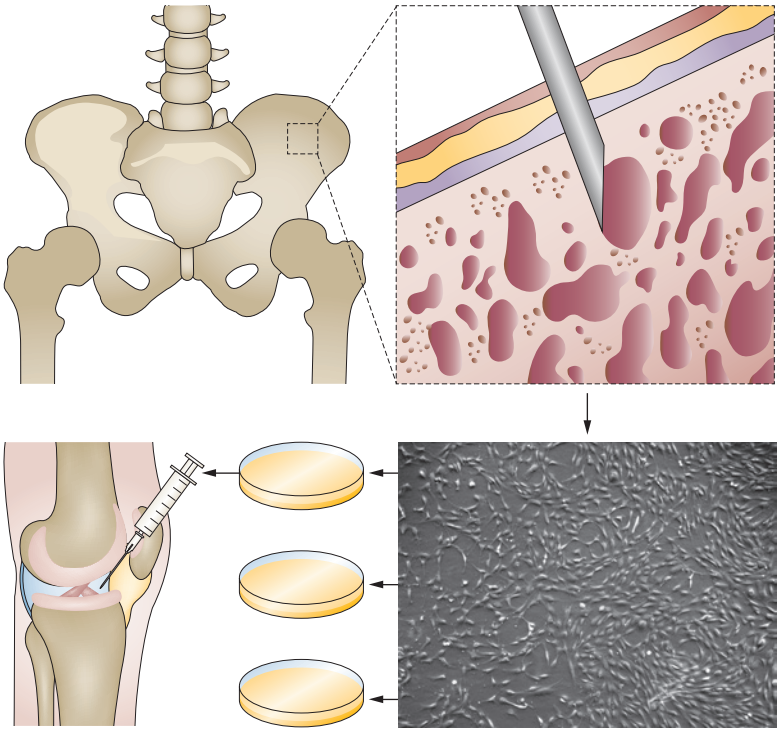

Figure 2 | Cell therapy for the treatment of osteoarthritis using bone marrow-derived MSCs. A bone marrow aspirate of 10–20 ml, usually taken from the iliac crest of the pelvis, is plated onto tissue culture dishes and maintained in a nutrient medium supplemented with growth factors or fetal calf serum. MSCs that adhere to the tissue culture plastic form colonies and undergo many cell divisions. These fibroblastic, adherent cells are further expanded in culture until a large population (10^9–10^{10} cells) is obtained. The cells are detached from the flask, suspended in a vehicle (typically sodium hyaluronan solution) and prepared for injection into the joint space. A typical single dose may consist of $5–50 \times 10^6$ cells in 5 ml of vehicle. Abbreviation: MSC, mesenchymal stem cell.

became apparent that elimination of ADAMTS5 activity mediated a transition from TGF-β1-stimulated fibrosis to chondrogenesis.[84] This finding suggested a binary role for TGF-β associated with either the presence or absence of ADAMTS5. In the presence of ADAMTS5, TGF-β is an inducer of fibrosis, an activity mediated via TGF-β receptor type 1 (also known as Alk5) with phosphorylation of Smad2 and Smad3. In the absence of ADAMTS5, TGF-β is an inducer of chondrogenesis, mediated via serine/threonine-protein kinase receptor R3 (also known as Alk1) with phosphorylation of Smad1, Smad5 and Smad9.[84] All of these effects may involve MSCs, thus suggesting again the role of these cells in the maintenance of healthy joints and in the onset of disease.

MSCs, TGF-β signalling and bone pathology

Proliferation of mesenchymal progenitor cells has been associated with osteophyte formation in mice.[85] Furthermore, MSC-like cells in the periosteum have been shown to respond to TGF-β by phosphorylating Smad2 and Smad3 and promoting endochondral ossification via formation of a cartilage cell intermediate.[86,87] Osteophyte formation might, therefore, represent another consequence of inappropriate recruitment and activation of MSCs in response to the OA milieu.[87,88]

MSC therapy in joint repair

A great deal of attention has been focused on the idea that local delivery of *ex vivo* culture-expanded preparations of MSCs will enhance joint repair, reduce the degenerative changes associated with OA and lead to a successful clinical outcome (Figure 2). This interest was initially provoked by the multipotent nature of the cells and their ability to form cartilage and bone. Furthermore, the evidence we have discussed implicating MSC defects in the OA disease process suggests that replacing defective populations might be of therapeutic value. Favourable results in preclinical models, as discussed in this section, have fuelled efforts in this regard, with MSC-based approaches now at the stage of clinical investigation.

Insights from preclinical models

Much of the early experimental investigation into the therapeutic potential of MSCs was in the treatment of surgically created chondral or osteochondral defects in small animal models.[89–91] Such tissue engineering approaches frequently involved the use of scaffolds of different types, and results were often variable and unimpressive. A more direct, and ultimately more successful, approach was first described by Murphy *et al.*[92] for the treatment of post-traumatic OA in goats. In these studies, resection of the anterior cruciate ligament combined with complete medial meniscectomy in the stifle joint resulted in substantial joint degeneration, with cartilage fibrillation, osteophyte formation and subchondral sclerosis typical of advanced OA. Direct intra-articular delivery of a suspension of goat MSCs then elicited a meniscal repair response resulting in clinical improvement in cell-treated joints compared with controls, with evidence of cartilage protection (Figure 3). Implanted MSCs were detected primarily at the surface of the regenerated meniscus and at other synovial surfaces within the joint, but not in articular cartilage.[92]

The effectiveness of intra-articular delivery of MSCs in the knee has now been tested in a variety of preclinical disease models (Table 2), in organisms including mice,[93] rabbits,[94] rats,[95] Guinea pigs,[96] sheep,[97] dogs,[98] and horses.[99] In these models of surgically induced OA[93–97,99] or clinical lameness,[98] MSC therapy inhibited OA progression. However, results in the horse were restricted to a reduction in prostaglandin E_2 levels in synovial fluid, rather than a clinically significant improvement.[99] As well as surgically induced OA, collagenase-induced OA in the mouse was also modulated by intra-articular injection of adipose-derived MSCs, with considerable cartilage protection and reduced synovial thickening accompanied by an anti-inflammatory response (Figure 3).[100]

For cell-based therapies to be accepted by practitioners and regulators, data must extend beyond proof of concept towards a full understanding of the mechanism of action. Despite the challenges presented by such a complex medicinal product, some insightful steps in elucidating the mechanisms have already been taken. In the goat study by Murphy *et al.*,[92] cell engraftment to articular cartilage could not be detected, and the newly regenerated meniscal tissue consisted almost entirely of

host cells with small numbers of transplanted cells. These observations provided the first evidence to suggest that transplanted MSCs not only act as building blocks for the formation of repair tissue, but also exert effects by different mechanisms, influencing host-cell behaviour via paracrine effects. The implied cascade of secreted, MSC-derived signals that stimulate a repair response in the host is now being gradually unravelled. In one example published in 2012, Horie *et al.*[95] found that human MSCs injected into the injured knee in rats were activated to express a series of genes including Indian hedgehog, parathyroid hormone-like hormone and bone morphogenetic protein 2, resulting in upregulated expression of type II collagen—a repair response—in the host.

Insights from other cell-based therapies

Other approaches to cell-mediated articular repair have emerged, some of which focus on the recruitment of endogenous populations of cells rather than delivery of *ex vivo* preparations. Analysis of such approaches is contributing to our understanding of the effects of therapeutic MSCs. For example, a bioscaffold for surgical replacement of the synovial joint in rabbits, designed by Lee *et al.*[65] and coated with TGF-β3, led to the formation of a useful and structurally sound articular cartilage layer with restoration of function. In comparison with untreated controls, matrix accumulation and production of type II collagen were greater, and cellularity of the TGF-β3-treated articular layer was increased almost threefold. Recruitment of endogenous mesenchymal cells to the repairing articular layer, acting to replenish the depleted chondroprogenitor layers in the tissue, was proposed as the mechanism.[65]

A fascinating approach to the activation of endogenous cells for cartilage repair was reported in 2012.[101] Image-based high-throughput screening enabled the discovery of kartogenin, a novel compound that stimulates chondrogenic differentiation and promotes cartilage repair in collagenase-induced and surgery-induced models of OA in mice. Delivery of kartogenin resulted in an increase in cartilage thickness, improved matrix structure and improved weight-bearing ability, seemingly via increased chondrogenic activation of resident progenitor cells in the cartilage. Kartogenin was shown to bind the FC-1 fragment of filamin-A, disrupting its association with core-binding factor β subunit (CBFβ), part of a heterodimeric transcription factor complex. Treatment of human MSCs with kartogenin caused nuclear localization of CBFβ and the other subunits of this complex, products of *RUNX* genes. These proteins have distinct and crucial roles in joint development, with runt-related transcription factor 1, encoded by *RUNX1*, being important in chondrogenesis.[102,103] Increased nuclear availability of CBFβ led to activation of runt-related transcription factor 1 and its associated network of genes.[101] Thus, a second pathway that activates chondrogenic differentiation of MSCs emerges.

Potentially, the synovium might be the primary responder tissue in joint repair following MSC transplantation,[92,100] with contact between MSCs and synovial cells stimulating the latter to begin a process of regeneration, recapitulating, to some extent, a developmental process in the adult joint. Given that MSCs are the orchestrators of joint development in the embryo, it might be that the key regenerative mechanism of transplanted MSCs resides precisely in their ability to restore a developmental

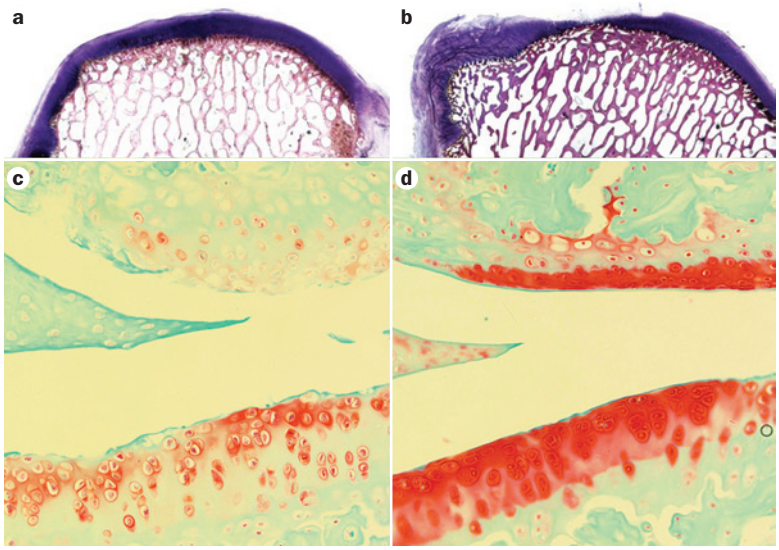

Figure 3 | Examples of MSC therapy used for the treatment of joint lesions or preclinical models of OA. **a** | In surgically-induced unilateral OA in goats, involving complete medial meniscectomy and transection of the anterior cruciate ligament, untreated joints showed considerable loss of articular cartilage, osteophyte formation and subchondral sclerosis in the medial femoral condyle at 6 weeks. **b** | 12 weeks after intra-articular delivery of a single dose of 5×10^6 bone-marrow-derived MSCs, protection of cartilage was evident, with reduced osteophyte formation and subchondral bone change. **c** | In collagenase-induced OA in mice, untreated knee joints showed loss of articular cartilage, changes in chondrocyte cellularity and matrix staining 6 weeks after the induction of degradation. **d** | By contrast, cartilage structure was considerably improved in joints treated with 20,000 bone-marrow-derived MSCs 1 week after induction of OA. Panels a and b reproduced with permission from John Wiley & Sons © Murphy, J. M. *et al. Arthritis Rheum.* **48**, 3464–3474 (2003).[92] Abbreviations: MSC, mesenchymal stem cell; OA, osteoarthritis.

Table 2 | MSC*-induced repair has been shown in various animal models of OA

Disease model	Organism	Outcome	Study
Traumatic OA (knee fracture)	Mouse	Prevention of OA	Diekman *et al.* (2012)[93]
Hemi-meniscectomy	Rat	Meniscal repair	Horie *et al.* (2012)[95]
Unilateral ACL transection	Rabbit	Improved cartilage repair	Toghraie *et al.* (2012)[94]
Spontaneous OA	Hartley strain Guinea pig	Partial cartilage repair	Sato *et al.* (2012)[96]
Collagenase-induced OA	Mouse	Cartilage protection	ter Huurne *et al.* (2012)[100]
ACL transection and medical meniscectomy	Sheep	Reduced OA and meniscal regeneration	Al Faqeh *et al.* (2012)[97]
Microfractured chondral defects	Horse	Enhanced cartilage quality	Frisbie *et al.* (2009)[99]

*MSCs were delivered intra-articularly. Abbreviations: ACL, anterior cruciate ligament; MSC, mesenchymal stem cell; OA, osteoarthritis.

Table 3 | Current clinical trials* of MSCs for the treatment of OA and related joint defects

Trial	Sponsor	Phase; current stage*	Indication	Intervention	Comparator
Treatment of Knee Osteoarthritis With Allogenic Mesenchymal Stem Cells (MSV_allo); NCT01586312[104]	Red de Terapia Celular	Phase I/II; recruiting	Knee OA	Intra-articular injection of 40×10^6 allogeneic MSCs	Intra-articular injection of 60 mg hyaluronan
Treatment of Knee Osteoarthritis With Autologous Mesenchymal Stem Cells (KDD&MSV); NCT01183728[105]	Red de Terapia Celular	Phase I/II; active, not recruiting	Knee OA, Kellgren and Lawrence grade II–IV	Intra-articular injection of 40×10^6 autologous MSCs	None (open-label, single-group safety study)
Intra-Articular Autologous Bone Marrow Mesenchymal Stem Cells Transplantation to Treat Mild to Moderate Osteoarthritis; NCT01459640[106]	National University of Malaysia	Phase II; recruiting	Knee OA, mild to moderate	Single intra-articular implantation of autologous bone marrow-derived MSCs in hyaluronan	None (open-label, single-group safety study)
The Effects of Intra-articular Injection of Mesenchymal Stem Cells in Knee Joint Osteoarthritis; NCT01504464[107]	Royan Institute	Phase II; completed, no results posted	Knee OA	Intra-articular injection of MSCs	Placebo injection
Mesenchymal Stem Cell Transplantation in Osteoarthritis of Hip Joint; NCT01499056[108]	Royan Institute	Phase I; completed, no results posted	Hip OA	MSC injection	None (open-label, single-group safety study)
Allogeneic Mesenchymal Stem Cells in Osteoarthritis; NCT01453738[109]	Stempeutics Research Pvt Ltd	Phase II; active, not recruiting	Knee OA	Intra-articular dose of allogeneic MSCs in 2–4 ml Plasmalyte-A‡ followed by 2 ml hyaluronan	Single intra-articular dose of 2 ml Plasmalyte-A‡
Side Effects of Autologous Mesenchymal Stem Cell Transplantation in Ankle Joint Osteoartritis; NCT01436058[110]	Royan Institute	Phase I; completed, no results posted	Ankle joint OA	Intra-articular injection of MSCs	None (open-label, single-group safety study)
Adult Stem Cell Therapy for Repairing Articular Cartilage in Gonarthrosis; NCT01227694[111]	Banc de Sang i Teixits	Phase I/II; active, not recruiting	Knee OA	Intra-articular injection of 40×10^6 autologous MSCs	None (open-label, single-group safety study)
Autologous Adipose Tissue Derived Mesenchymal Stem Cells Transplantation in Patients With Degenerative Arthritis; NCT01300598[112]	RNL Bio Company Ltd	Phase I/II; completed, no results posted	Knee OA	Intra-articular injection of autologous adipose tissue-derived MSCs. Doses (in 3 ml) listed as: 1×10^7 cells, 5×10^7 cells, 1×10^8 cells	None (open-label, single-group safety study)
Study to Compare the Efficacy and Safety of Cartistem® and Microfracture in Patients With Knee Articular Cartilage Injury or Defect; NCT01041001[113]	Medipost Co Ltd	Phase III; completed, no results posted (Follow-up study, NCT01626677,[114] now recruiting)	Knee cartilage defect or injury	Intra-articular injection of allogeneic umbilical cord blood-derived MSCs	Microfracture treatment
ADIPOA—Clinical Study; NCT01585857[115]	University Hospital, Montpellier	Phase I; recruiting	Knee OA, moderate or severe	Intra-articular injection of autologous adipose-tissue-derived MSCs. Doses (in 5 ml of human albumin): 2×10^6, 10×10^6, 50×10^6 cells	None (open-label, dose-escalating safety study)
Safety and Efficacy Study of MSB-CAR001 in Subjects 6 Weeks Post an Anterior Cruciate Ligament Reconstruction; NCT01088191[116]	Mesoblast, Ltd	Phase I/II; recruiting	Anterior cruciate ligament injury	Single intra-articular injection (into the knee) of MSB-CAR001§ (2 different doses) combined with hyaluronan	Intra-articular injection of hyaluronan
Transplantation of Bone Marrow Stem Cells Stimulated by Proteins Scaffold to Heal Defects Articular Cartilage of the Knee; NCT01159899[117]	University of Marseille	Phase 0; recruiting	Knee cartilage defects	Fresh non-culture-expanded autologous bone marrow-derived MSCs, mixed and activated with protein scaffold	None (open-label, single-group pilot study)

*As of April 2013. ‡Plasmalyte-A is a sterile isotonic buffered salt solution. §MSB-CAR001 is a preparation of MSCs. Abbreviations: MSC, mesenchymal stem cell; OA, osteoarthritis.

milieu in the host joint, providing a complex array of signals that promote growth, cytoprotection, migration, immunomodulation and differentiation.

Approaches in patients with OA

Progress in preclinical studies has led to the initiation of a number of clinical trials (Table 3), several of which are underway during 2013. In 2012, of 13 trials listed in the National Library of Medicine ClinicalTrials.gov website, 11 address treatment of knee OA, 1 is in patients with hip OA and 1 is for ankle-joint OA. The majority of these studies involve the use of autologous, culture-expanded MSCs from bone marrow or adipose tissue. In a few cases, allogeneic cells derived from bone marrow or cord

blood are used. Interestingly, the majority of technical approaches involve intra-articular injection to deliver the cells directly to the synovial fluid compartment using a scaffold-free method. In most instances the vehicle used is hyaluronan, primarily on the basis that hyaluronan is a major component of synovial fluid, and because intra-articular injections of hyaluronan are commonly used in the clinical treatment of OA of the knee. Few other vehicles have been tested, however, either in preclinical or clinical studies, and it is yet to be determined which vehicle(s) may be optimal. In addition, as in every other cell therapy in development, there is uncertainty surrounding the cell dose. The trials listed in Table 3 tested cell injections in doses of $1–4 \times 10^7$ cells in a single injection. Which cell dose will lead to the best outcome cannot be determined until a series of dose-finding studies is carried out. Clearly, the majority of efforts in clinical testing now adopt a scaffold-free approach, indicating that investigative MSC therapy for joint repair has moved away from early principles of tissue engineering that involved cells, scaffolds and growth factors. This streamlined approach is a sensible strategy and is simpler in terms of technical delivery and regulatory approval than multi-component interventions.

Conclusions

OA is associated with progressive and irreversible destruction of joint tissues, and although factors including trauma, obesity and inflammation contribute to its onset, a clear mechanistic origin of the disease remains elusive. All joint tissues contain resident populations of mesenchymal progenitor cells that are capable of differentiating into cartilage, bone and other tissues, which might provide repair cells that help to maintain healthy joints. OA seems to be associated with changes in the quantity, phenotype, and differentiation potential of resident mesenchymal cells. Transplantation of *ex vivo* preparations of MSCs to the the joints of animals with OA seems to evoke a therapeutically useful repair response, apparently as a result of paracrine responses from host cells including progenitor populations residing within the synovium.

The idea that paracrine activities of MSCs might be central to their therapeutic mechanism raises important questions regarding 'potency' measures, such as those proposed in 2006 by Dominici *et al.*[118] These assays rely exclusively on the expression of selected cell-surface markers and on the capacity of the cells to undergo trilineage differentiation. As it becomes increasingly apparent that results of these tests bear no relation to any proposed therapeutic mechanism of action of transplanted MSCs, a new series of tests, most likely related to the profile of secreted factors of transplanted MSCs, will be necessary.

As with all forms of cellular therapy that are under evaluation in 2013, clinical translation has been slow. An emerging database from phase I and II trials will shed further light on the therapeutic utility of intra-articular delivery of MSCs. It might be that these approaches, involving either autologous or allogeneic cells, will provide the long-sought-after disease-modifying therapy for the treatment of OA.

Review criteria

The articles on which this Review is based were selected after a literature survey of the incidence and impact of osteoarthritis, the origins and growth of mesenchymal stem cell therapy, and specific applications of mesenchymal stem cells in joint disease. Articles on the isolation of mesenchymal stem cells from joint tissues were exhaustively searched using a range of terms, as were papers on preclinical studies; the list was updated to November 2012 and includes only full-text papers. Clinical trial information was sourced from www.ClinicalTrials.gov in 2012 and updated to April 2013.

1. Buckwalter, J. A. & Martin, J. A. Osteoarthritis. *Adv. Drug Deliv. Rev.* **58**, 150–167 (2006).
2. Michaud, C. M. *et al.* The burden of disease and injury in the United States 1996. *Popul. Health Metr.* **4**, 11 (2006).
3. Lawrence, R. C. *et al.* Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II. *Arthritis Rheum.* **58**, 26–35 (2008).
4. Gore, M., Tai, K. S., Sadosky, A., Leslie, D. & Stacey, B. R. Clinical comorbidities, treatment patterns, and direct medical costs of patients with osteoarthritis in usual care: a retrospective claims database analysis. *J. Med. Econ.* **14**, 497–507 (2011).
5. McKenna, M. T., Michaud, C. M., Murray, C. J. & Marks, J. S. Assessing the burden of disease in the United States using disability-adjusted life years. *Am. J. Prev. Med.* **28**, 415–423 (2005).
6. Guilak, F. Biomechanical factors in osteoarthritis. *Best Pract. Res. Clin. Rheumatol.* **25**, 815–823 (2011).
7. Findlay, D. M. If good things come from above, do bad things come from below? *Arthritis Res. Ther.* **12**, 119 (2010).
8. Goldring, M. B. & Goldring, S. R. Articular cartilage and subchondral bone in the pathogenesis of osteoarthritis. *Ann. NY Acad. Sci.* **1192**, 230–237 (2010).
9. de Lange-Brokaar, B. J. *et al.* Synovial inflammation, immune cells and their cytokines in osteoarthritis: a review. *Osteoarthritis Cartilage* **20**, 1484–1499 (2012).
10. Ayral, X., Pickering, E. H., Woodworth, T. G., Mackillop, N. & Dougados, M. Synovitis: a potential predictive factor of structural progression of medial tibiofemoral knee osteoarthritis—results of a 1 year longitudinal arthroscopic study in 422 patients. *Osteoarthritis Cartilage* **13**, 361–367 (2005).
11. Benito, M. J., Veale, D. J., FitzGerald, O., van den Berg, W. B. & Bresnihan, B. Synovial tissue inflammation in early and late osteoarthritis. *Ann. Rheum. Dis.* **64**, 1263–1267 (2005).
12. Buckwalter, J. A., Saltzman, C. & Brown, T. The impact of osteoarthritis: implications for research. *Clin. Orthop. Relat. Res.* **427** (Suppl.), S6–S15 (2004).
13. Kurtz, S., Ong, K., Lau, E., Mowat, F. & Halpern, M. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. *J. Bone Joint Surg. Am.* **89**, 780–785 (2007).
14. Friedenstein, A. J., Piatetzky, S. & Petrakova, K. V. Osteogenesis in transplants of bone marrow cells. *J. Embryol. Exp. Morphol.* **16**, 381–390 (1966).
15. Friedenstein, A. J., Chailakhjan, R. K. & Lalykina, K. S. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. *Cell Tissue Kinet.* **3**, 393–403 (1970).
16. Friedenstein, A. J., Chailakhyan, R. K., Latsinik, N. V., Panasyuk, A. F. & Keiliss-Borok, I. V. Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues. Cloning *in vitro* and retransplantation *in vivo*. *Transplantation* **17**, 331–340 (1974).
17. Friedenstein, A. J. Marrow stromal fibroblasts. *Calcif. Tissue Int.* **56** (Suppl. 1), S17 (1995).
18. Owen, M. & Friedenstein, A. J. Stromal stem cells: marrow-derived osteogenic precursors. *Ciba Found. Symp.* **136**, 42–60 (1988).
19. Bunnell, B. A., Estes, B. T., Guilak, F. & Gimble, J. M. Differentiation of adipose stem cells. *Methods Mol. Biol.* **456**, 155–171 (2008).
20. Gimble, J. M., Katz, A. J. & Bunnell, B. A. Adipose-derived stem cells for regenerative medicine. *Circ. Res.* **100**, 1249–1260 (2007).

21. Meliga, E., Strem, B. M., Duckers, H. J. & Serruys, P. W. Adipose-derived cells. *Cell Transplant.* **16**, 963–970 (2007).
22. Crisan, M. *et al.* A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell* **3**, 301–313 (2008).
23. Troyer, D. L. & Weiss, M. L. Wharton's jelly-derived cells are a primitive stromal cell population. *Stem Cells* **26**, 591–599 (2008).
24. Weiss, M. L. *et al.* Human umbilical cord matrix stem cells: preliminary characterization and effect of transplantation in a rodent model of Parkinson's disease. *Stem Cells* **24**, 781–792 (2006).
25. Weiss, M. L. & Troyer, D. L. Stem cells in the umbilical cord. *Stem Cell Rev.* **2**, 155–162 (2006).
26. Flynn, A., Barry, F. & O'Brien, T. UC blood-derived mesenchymal stromal cells: an overview. *Cytotherapy* **9**, 717–726 (2007).
27. Barry, F. P. & Murphy, J. M. Mesenchymal stem cells: clinical applications and biological characterization. *Int. J. Biochem. Cell Biol.* **36**, 568–584 (2004).
28. Delorme, B. & Charbord, P. Culture and characterization of human bone marrow mesenchymal stem cells. *Methods Mol. Med.* **140**, 67–81 (2007).
29. Bianco, P., Robey, P. G. & Simmons, P. J. Mesenchymal stem cells: revisiting history, concepts, and assays. *Cell Stem Cell* **2**, 313–319 (2008).
30. Sacchetti, B. *et al.* Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. *Cell* **131**, 324–336 (2007).
31. Tavian, M. *et al.* The vascular wall as a source of stem cells. *Ann. NY Acad. Sci.* **1044**, 41–50 (2005).
32. Zannettino, A. C. *et al.* Multipotential human adipose-derived stromal stem cells exhibit a perivascular phenotype *in vitro* and *in vivo*. *J. Cell. Physiol.* **214**, 413–421 (2008).
33. Caplan, A. I. All MSCs are pericytes? *Cell Stem Cell* **3**, 229–230 (2008).
34. Dulauroy, S., Di Carlo, S. E., Langa, F., Eberl, G. & Peduto, L. Lineage tracing and genetic ablation of ADAM12$^+$ perivascular cells identify a major source of profibrotic cells during acute tissue injury. *Nat. Med.* **18**, 1262–1270 (2012).
35. Kurth, T. B. *et al.* Functional mesenchymal stem cell niches in adult mouse knee joint synovium *in vivo*. *Arthritis Rheum.* **63**, 1289–1300 (2011).
36. De Bari, C., Dell'Accio, F., Tylzanowski, P. & Luyten, F. P. Multipotent mesenchymal stem cells from adult human synovial membrane. *Arthritis Rheum.* **44**, 1928–1942 (2001).
37. Karystinou, A. *et al.* Distinct mesenchymal progenitor cell subsets in the adult human synovium. *Rheumatology (Oxford)* **48**, 1057–1064 (2009).
38. De Bari, C. *et al.* Skeletal muscle repair by adult human mesenchymal stem cells from synovial membrane. *J. Cell Biol.* **160**, 909–918 (2003).
39. Meng, J. *et al.* The contribution of human synovial stem cells to skeletal muscle regeneration. *Neuromuscul. Disord.* **20**, 6–15 (2010).
40. Jones, E. A. *et al.* Synovial fluid mesenchymal stem cells in health and early osteoarthritis: detection and functional evaluation at the single-cell level. *Arthritis Rheum.* **58**, 1731–1740 (2008).
41. Jones, E. A. *et al.* Enumeration and phenotypic characterization of synovial fluid multipotential mesenchymal progenitor cells in inflammatory and degenerative arthritis. *Arthritis Rheum.* **50**, 817–827 (2004).
42. Sekiya, I. *et al.* Human mesenchymal stem cells in synovial fluid increase in the knee with degenerated cartilage and osteoarthritis. *J. Orthop. Res.* **30**, 943–949 (2012).
43. Morito, T. *et al.* Synovial fluid-derived mesenchymal stem cells increase after intra-articular ligament injury in humans. *Rheumatology (Oxford)* **47**, 1137–1143 (2008).
44. Lee, D. H. *et al.* Synovial fluid CD34$^-$ CD44$^+$ CD90$^+$ mesenchymal stem cell levels are associated with the severity of primary knee osteoarthritis. *Osteoarthritis Cartilage* **20**, 106–109 (2012).
45. Sakaguchi, Y., Sekiya, I., Yagishita, K. & Muneta, T. Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. *Arthritis Rheum.* **52**, 2521–2529 (2005).
46. Fan, J., Varshney, R. R., Ren, L., Cai, D. & Wang, D. A. Synovium-derived mesenchymal stem cells: a new cell source for musculoskeletal regeneration. *Tissue Eng. Part B Rev.* **15**, 75–86 (2009).
47. Khan, W. S., Adesida, A. B., Tew, S. R., Longo, U. G. & Hardingham, T. E. Fat pad-derived mesenchymal stem cells as a potential source for cell-based adipose tissue repair strategies. *Cell Prolif.* **45**, 111–120 (2012).
48. Dowthwaite, G. P. *et al.* The surface of articular cartilage contains a progenitor cell population. *J. Cell Sci.* **117**, 889–897 (2004).
49. Khan, I. M., Bishop, J. C., Gilbert, S. & Archer, C. W. Clonal chondroprogenitors maintain telomerase activity and Sox9 expression during extended monolayer culture and retain chondrogenic potential. *Osteoarthritis Cartilage* **17**, 518–528 (2009).
50. Williams, R. *et al.* Identification and clonal characterisation of a progenitor cell sub-population in normal human articular cartilage. *PLoS ONE* **5**, e13246 (2010).
51. Alsalameh, S., Amin, R., Gemba, T. & Lotz, M. Identification of mesenchymal progenitor cells in normal and osteoarthritic human articular cartilage. *Arthritis Rheum.* **50**, 1522–1532 (2004).
52. Grogan, S. P., Miyaki, S., Asahara, H., D'Lima, D. D. & Lotz, M. K. Mesenchymal progenitor cell markers in human articular cartilage: normal distribution and changes in osteoarthritis. *Arthritis Res. Ther.* **11**, R85 (2009).
53. Fickert, S., Fiedler, J. & Brenner, R. E. Identification of subpopulations with characteristics of mesenchymal progenitor cells from human osteoarthritic cartilage using triple staining for cell surface markers. *Arthritis Res. Ther.* **6**, R422–R432 (2004).
54. Huang, T. F. *et al.* Isolation and characterization of mesenchymal stromal cells from human anterior cruciate ligament. *Cytotherapy* **10**, 806–814 (2008).
55. Steinert, A. F. *et al.* Mesenchymal stem cell characteristics of human anterior cruciate ligament outgrowth cells. *Tissue Eng. Part A* **17**, 1375–1388 (2011).
56. Murray, M. M., Bennett, R., Zhang, X. & Spector, M. Cell outgrowth from the human ACL *in vitro*: regional variation and response to TGF-β1. *J. Orthop. Res.* **20**, 875–880 (2002).
57. Murray, M. M. & Spector, M. The migration of cells from the ruptured human anterior cruciate ligament into collagen-glycosaminoglycan regeneration templates *in vitro*. *Biomaterials* **22**, 2393–2402 (2001).
58. Cheng, M. T., Yang, H. W., Chen, T. H. & Lee, O. K. Isolation and characterization of multipotent stem cells from human cruciate ligaments. *Cell Prolif.* **42**, 448–460 (2009).
59. Segawa, Y. *et al.* Mesenchymal stem cells derived from synovium, meniscus, anterior cruciate ligament, and articular chondrocytes share similar gene expression profiles. *J. Orthop. Res.* **27**, 435–441 (2009).
60. Park, D. *et al.* Endogenous bone marrow MSCs are dynamic, fate-restricted participants in bone maintenance and regeneration. *Cell Stem Cell* **10**, 259–272 (2012).
61. Rinkevich, Y., Lindau, P., Ueno, H., Longaker, M. T. & Weissman, I. L. Germ-layer and lineage-restricted stem/progenitors regenerate the mouse digit tip. *Nature* **476**, 409–413 (2011).
62. Mendez-Ferrer, S. *et al.* Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. *Nature* **466**, 829–834 (2010).
63. Flannery, C. R. *et al.* Articular cartilage superficial zone protein (SZP) is homologous to megakaryocyte stimulating factor precursor and Is a multifunctional proteoglycan with potential growth-promoting, cytoprotective, and lubricating properties in cartilage metabolism. *Biochem. Biophys. Res. Commun.* **254**, 535–541 (1999).
64. Koyama, E. *et al.* A distinct cohort of progenitor cells participates in synovial joint and articular cartilage formation during mouse limb skeletogenesis. *Dev. Biol.* **316**, 62–73 (2008).
65. Lee, C. H. *et al.* Regeneration of the articular surface of the rabbit synovial joint by cell homing: a proof of concept study. *Lancet* **376**, 440–448 (2010).
66. Murphy, J. M. *et al.* Reduced chondrogenic and adipogenic activity of mesenchymal stem cells from patients with advanced osteoarthritis. *Arthritis Rheum.* **46**, 704–713 (2002).
67. Scharstuhl, A. *et al.* Chondrogenic potential of human adult mesenchymal stem cells is independent of age or osteoarthritis etiology. *Stem Cells* **25**, 3244–3251 (2007).
68. Im, G. I., Jung, N. H. & Tae, S. K. Chondrogenic differentiation of mesenchymal stem cells isolated from patients in late adulthood: the optimal conditions of growth factors. *Tissue Eng.* **12**, 527–536 (2006).
69. Coutu, D. L., Francois, M. & Galipeau, J. Inhibition of cellular senescence by developmentally regulated FGF receptors in mesenchymal stem cells. *Blood* **117**, 6801–6812 (2011).
70. De Bari, C., Dell'Accio, F. & Luyten, F. P. Human periosteum-derived cells maintain phenotypic stability and chondrogenic potential throughout expansion regardless of donor age. *Arthritis Rheum.* **44**, 85–95 (2001).
71. Jones, E. *et al.* Large-scale extraction and characterization of CD271$^+$ multipotential stromal cells from trabecular bone in health and osteoarthritis: implications for bone regeneration strategies based on uncultured or minimally cultured multipotential stromal cells. *Arthritis Rheum.* **62**, 1944–1954 (2010).
72. Hiraoka, K., Grogan, S., Olee, T. & Lotz, M. Mesenchymal progenitor cells in adult human articular cartilage. *Biorheology* **43**, 447–454 (2006).
73. Lotz, M. K. *et al.* Cartilage cell clusters. *Arthritis Rheum.* **62**, 2206–2218 (2010).
74. Koelling, S. *et al.* Migratory chondrogenic progenitor cells from repair tissue during the later stages of human osteoarthritis. *Cell Stem Cell* **4**, 324–335 (2009).
75. Blaney Davidson, E. N. *et al.* Elevated extracellular matrix production and degradation upon bone morphogenetic protein-2 (BMP-2) stimulation point toward a role for BMP-2 in

cartilage repair and remodeling. *Arthritis Res. Ther.* **9**, R102 (2007).
76. Guo, X., Thomas, A. & Pirkko, L. A study on abnormal chondrocyte differentiation and abnormal expression of collagen types in articular cartilage from patients with Kaschin-Beck disease [Chinese]. *Zhonghua Bing Li Xue Za Zhi* **27**, 19–22 (1998).
77. Guo, X. *et al.* Abnormal expression of Col X, PTHrP, TGF-β, bFGF, and VEGF in cartilage with Kashin-Beck disease. *J. Bone Miner. Metab.* **24**, 319–328 (2006).
78. Pufe, T., Bartscher, M., Petersen, W., Tillmann, B. & Mentlein, R. Pleiotrophin, an embryonic differentiation and growth factor, is expressed in osteoarthritis. *Osteoarthritis Cartilage* **11**, 260–264 (2003).
79. Plaas, A. *et al.* The relationship between fibrogenic TGFβ1 signaling in the joint and cartilage degradation in post-injury osteoarthritis. *Osteoarthritis Cartilage* **19**, 1081–1090 (2011).
80. Sandy, J. D., Neame, P. J., Boynton, R. E. & Flannery, C. R. Catabolism of aggrecan in cartilage explants. Identification of a major cleavage site within the interglobular domain. *J. Biol. Chem.* **266**, 8683–8685 (1991).
81. Glasson, S. S. *et al.* Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. *Nature* **434**, 644–648 (2005).
82. Stanton, H. *et al.* ADAMTS5 is the major aggrecanase in mouse cartilage *in vivo* and *in vitro*. *Nature* **434**, 648–652 (2005).
83. Li, J. *et al.* Knockout of ADAMTS5 does not eliminate cartilage aggrecanase activity but abrogates joint fibrosis and promotes cartilage aggrecan deposition in murine osteoarthritis models. *J. Orthop. Res.* **29**, 516–522 (2011).
84. Velasco, J. *et al.* Adamts5 deletion blocks murine dermal repair through CD44-mediated aggrecan accumulation and modulation of transforming growth factor β 1 (TGF β 1) signaling. *J. Biol. Chem.* **286**, 26016–26027 (2011).
85. Zhang, Y. W. *et al.* Targeted disruption of Mig-6 in the mouse genome leads to early onset degenerative joint disease. *Proc. Natl Acad. Sci. USA* **102**, 11740–11745 (2005).
86. Blaney Davidson, E. N. *et al.* Resemblance of osteophytes in experimental osteoarthritis to transforming growth factor beta-induced osteophytes: limited role of bone morphogenetic protein in early osteoarthritic osteophyte formation. *Arthritis Rheum.* **56**, 4065–4073 (2007).
87. van der Kraan, P. M., Goumans, M. J., Blaney Davidson, E. & ten Dijke, P. Age-dependent alteration of TGF- β signalling in osteoarthritis. *Cell Tissue Res.* **347**, 257–265 (2012).
88. Anitua, E. *et al.* Relationship between investigative biomarkers and radiographic grading in patients with knee osteoarthritis. *Int. J. Rheumatol.* 747432 (2009).
89. Matsumoto, T. *et al.* Articular cartilage repair with autologous bone marrow mesenchymal cells. *J. Cell. Physiol.* **225**, 291–295 (2010).
90. Wakitani, S. *et al.* Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. *J. Bone Joint Surg. Am.* **76**, 579–592 (1994).
91. Wakitani, S. *et al.* Repair of large full-thickness articular cartilage defects with allograft articular chondrocytes embedded in a collagen gel. *Tissue Eng.* **4**, 429–444 (1998).
92. Murphy, J. M., Fink, D. J., Hunziker, E. B. & Barry, F. P. Stem cell therapy in a caprine model of osteoarthritis. *Arthritis Rheum.* **48**, 3464–3474 (2003).
93. Diekman, B. O. *et al.* Intra-articular delivery of purified mesenchymal stem cells from C57BL/6 or MRL/MpJ superhealer mice prevents post-traumatic arthritis. *Cell Transplant.* http://dx.doi.org/10.3727/096368912X653264.
94. Toghraie, F. *et al.* Scaffold-free adipose-derived stem cells (ASCs) improve experimentally induced osteoarthritis in rabbits. *Arch. Iran. Med.* **15**, 495–499 (2012).
95. Horie, M. *et al.* Intra-articular injection of human mesenchymal stem cells (MSCs) promote rat meniscal regeneration by being activated to express Indian hedgehog that enhances expression of type II collagen. *Osteoarthritis Cartilage* **20**, 1197–1207 (2012).
96. Sato, M. *et al.* Direct transplantation of mesenchymal stem cells into the knee joints of Hartley strain guinea pigs with spontaneous osteoarthritis. *Arthritis Res. Ther.* **14**, R31 (2012).
97. Al Faqeh, H., Nor Hamdan, B. M., Chen, H. C., Aminuddin, B. S. & Ruszymah, B. H. The potential of intra-articular injection of chondrogenic-induced bone marrow stem cells to retard the progression of osteoarthritis in a sheep model. *Exp. Gerontol.* **47**, 458–464 (2012).
98. Guercio, A. *et al.* Production of canine mesenchymal stem cells from adipose tissue and their application in dogs with chronic osteoarthritis of the humeroradial joints. *Cell Biol. Int.* **36**, 189–194 (2012).
99. Frisbie, D. D., Kisiday, J. D., Kawcak, C. E., Werpy, N. M. & McIlwraith, C. W. Evaluation of adipose-derived stromal vascular fraction or bone marrow-derived mesenchymal stem cells for treatment of osteoarthritis. *J. Orthop. Res.* **27**, 1675–1680 (2009).
100. ter Huurne, M. *et al.* Antiinflammatory and chondroprotective effects of intraarticular injection of adipose-derived stem cells in experimental osteoarthritis. *Arthritis Rheum.* **64**, 3604–3613 (2012).
101. Johnson, K. *et al.* A stem cell-based approach to cartilage repair. *Science* **336**, 717–721 (2012).
102. Wang, Y. *et al. Runx1/AML1/Cbfa2* mediates onset of mesenchymal cell differentiation toward chondrogenesis. *J. Bone Miner. Res.* **20**, 1624–1636 (2005).
103. Wotton, S. *et al.* Gene array analysis reveals a common *Runx* transcriptional programme controlling cell adhesion and survival. *Oncogene* **27**, 5856–5866 (2008).
104. US National Library of Medicine. ClinicalTrials.gov [online], http://www.clinicaltrials.gov/ct2/show/NCT01586312?term=NCT01586312&rank=1 (2012).
105. US National Library of Medicine. ClinicalTrials.gov [online], http://www.clinicaltrials.gov/ct2/show/NCT01183728?term=NCT01183728&rank=1 (2012).
106. US National Library of Medicine. ClinicalTrials.gov [online], http://www.clinicaltrials.gov/ct2/show/NCT01459640?term=NCT01459640&rank=1 (2011).
107. US National Library of Medicine. ClinicalTrials.gov [online], http://www.clinicaltrials.gov/ct2/show/NCT01504464?term=NCT01504464&rank=1 (2012).
108. US National Library of Medicine. ClinicalTrials.gov [online], http://www.clinicaltrials.gov/ct2/show/NCT01499056?term=NCT01499056&rank=1 (2011).
109. US National Library of Medicine. ClinicalTrials.gov [online], http://www.clinicaltrials.gov/ct2/show/NCT01453738?term=NCT01453738&rank=1 (2012).
110. US National Library of Medicine. ClinicalTrials.gov [online], http://www.clinicaltrials.gov/ct2/show/NCT01436058?term=NCT01436058&rank=1 (2011).
111. US National Library of Medicine. ClinicalTrials.gov [online], http://www.clinicaltrials.gov/ct2/show/NCT01227694?term=NCT01227694&rank=1 (2011).
112. US National Library of Medicine. ClinicalTrials.gov [online], http://www.clinicaltrials.gov/ct2/show/NCT01300598?term=NCT01300598&rank=1 (2012).
113. US National Library of Medicine. ClinicalTrials.gov [online], http://www.clinicaltrials.gov/ct2/show/NCT01041001?term=NCT01041001&rank=1 (2012).
114. US National Library of Medicine. ClinicalTrials.gov [online], http://www.clinicaltrials.gov/ct2/show/NCT01626677?term=NCT01041001&rank=2 (2012).
115. US National Library of Medicine. ClinicalTrials.gov [online], http://www.clinicaltrials.gov/ct2/show/NCT01585857?term=NCT01585857&rank=1 (2012).
116. US National Library of Medicine. ClinicalTrials.gov [online], http://www.clinicaltrials.gov/ct2/show/NCT01088191?term=NCT01088191&rank=1 (2012).
117. US National Library of Medicine. ClinicalTrials.gov [online], http://www.clinicaltrials.gov/ct2/show/NCT01159899?term=NCT01159899&rank=1 (2013).
118. Dominici M. *et al.* Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* **8**, 315–317 (2006).

Acknowledgements
The authors acknowledge funding support from Science Foundation Ireland (grant number 09/SRC/B1794), the European Union's 7th Framework Programme (grant numbers HEALTH-2007-B-223298 [PurStem], HEALTH-2009-1.4-3-241719 [ADIPOA] and NMP3-SL-2010-245993 [GAMBA]), and the Health Research Board of Ireland.

Author contributions
Both authors made substantial contributions to researching data for the article, discussions of content, writing the article, and review and/or editing of the manuscript before submission.