Native joint-resident mesenchymal stem cells for cartilage repair in osteoarthritis

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Abstract | The role of native (not culture-expanded) joint-resident mesenchymal stem cells (MSCs) in the repair of joint damage in osteoarthritis (OA) is poorly understood. MSCs differ from bone marrow-residing haematopoietic stem cells in that they are present in multiple niches in the joint, including subchondral bone, cartilage, synovial fluid, synovium and adipose tissue. Research in experimental models suggests that the migration of MSCs adjacent to the joint cavity is crucial for chonodrogenesis during embryogenesis, and also shows that synovium-derived MSCs might be the primary drivers of cartilage repair in adulthood. In this Review, the available data is synthesized to produce a proposed model in which joint-resident MSCs with access to superficial cartilage are key cells in adult cartilage repair and represent important targets for manipulation in 'chondrogenic' OA, especially in the context of biomechanical correction of joints in early disease. Growing evidence links the expression of CD271, a nerve growth factor (NGF) receptor by native bone marrow-resident MSCs to a wider role for neurotrophins in OA pathobiology, the implications of which require exploration since anti-NGF therapy might worsen OA. Recognizing that joint-resident MSCs are comparatively abundant *in vivo* and occupy multiple niches will enable the optimization of single-stage therapeutic interventions for OA.

'Chondrogenic' OA
A type of osteoarthritis (OA) in which early lesions form in the articular cartilage; distinct from OA that starts in other structures, such as OA that begins following meniscus or bone injury.

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doi:10.1038/nrrheum.2017.182
Published online 9 Nov 2017

The pathogenesis of osteoarthritis (OA) is complex and heterogeneous, with both disease initiation and progression being dependent on multiple joint structures, including cartilage, bone, ligaments, meniscus and synovium[1,2]. Many research articles and reviews have emphasized the role of culture-expanded cellular therapies, scaffolds and drugs in the development of therapies for OA, especially for 'chondrogenic' OA, but there is a paucity of data on the use of native (not culture-expanded) joint-resident stem cells in joint-repair strategies. This Review will focus on 'chondrogenic' OA, in which disease initiation and progression seem to be critically dependent on the articular cartilage. The role of subchondral bone, including the osteochondral junction, is also important in the pathogenesis of OA and has been discussed extensively elsewhere[3]; therefore, our comments on this subject will largely focus to the role of native bone marrow-resident stem cells, especially at sites of cartilage denudation in advanced OA, where such topographically localized cells can directly access the joint cavity.

The pivotal role of articular cartilage loss in OA[4,5] and the recognition that cartilage can be restored, albeit with relatively poor-quality repair tissue, following microfracture techniques in patients with isolated cartilage lesions or following autologous chondrocyte implantation for the treatment of full-thickness lesions[6,7], pointed to the potential importance of cartilage in the development of therapies for OA. These early studies[6,7] suggested that cartilage repair could occur via the actions of highly proliferative cells in close proximity to the cartilage, and were a key impetus for the subsequent culture expansion cellular protocols (first popularised in the 1990s[6]) and for the subsequent joint-repair strategies that used combinations of culture-expanded cells and adjuncts, including scaffolds and pharmaceutical agents[8]. Although it might not be possible to extrapolate the potential benefits of cellular therapy from results in isolated cartilage defects in young individuals to defects in patients with advanced OA, there is evidence that isolated cartilage lesions in skeletally mature individuals increase in severity over time[9–11], suggesting that advances in the treatment of early lesions could help to prevent OA in later life.

Previously, despite spontaneous articular cartilage regeneration being considered unlikely, seemingly misguided reparative responses (in the form of chondro-osteophyte formation) were recognized to occur. In the past few years, the spontaneous repair of full-thickness cartilage defects was noted in humans following joint

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Key Points

- Although historically considered to be very rare cells, native mesenchymal stem cells (MSCs) are actually relatively abundant *in vivo*
- Joint-resident MSCs occupy several bone and joint cavity niches including synovium, adipose tissue and synovial fluid
- Advanced osteoarthritis (OA) is associated with a numerical increase, but functional decline, in MSCs in regions of MRI-determined bone oedema, suggesting direct involvement of MSCs in OA pathology *in vivo*
- The expression of CD271 (also known as low-affinity nerve growth factor receptor) on native bone marrow-resident MSCs might be important in pathological bone changes following anti-nerve growth factor therapy
- In experimental models, there is strong evidence for the involvement of synovium-derived MSCs in cartilage repair following joint injury
- Emerging features of joint-resident MSCs suggests the potential for their use in the development of single-stage therapy to treat large cartilage defects in patients with OA

offloading, either by re-alignment osteotomy[12] or by total joint distraction techniques[13,14], with neither procedure directly breaching the joint cavity. These reparative events did not depend on cell expansion protocols but instead harnessed native joint-resident or periarticular cells in a manner reminiscent of early microfracture methodologies, which also harnessed endogenous reparative capabilities[7]. Importantly, these procedures highlighted the fact that the addition of scaffolds or growth factors was not essential for endogenous repair in chondrogenic OA[15]. As such, in this Review we largely confine our comments to the emerging evidence for a cellular basis for regenerative mechanisms in OA, and focus on cartilage repair.

At the cellular level, spontaneous cartilage regeneration suggests potentially overlapping roles for stem cells from different niches and also for mature chondrocytes (FIG. 1). In this Review, we focus on a subgroup of adult stromal cells that are highly proliferative, clonogenic and capable of multi-lineage differentiation into mesenchymal tissues including bone, cartilage and adipose tissue. As such, these cells are referred to as mesenchymal stem cells (MSCs), alternatively known as mesenchymal stromal cells or marrow stromal cells (when originating from trabecular bone), all of which bear the MSC acronym.

Mesenchymal stem cells

The high proliferative capacity of cultured MSCs and their chondrogenic capabilities have catapulted them to the forefront of cellular therapy development for OA. A large body of literature has accrued on culture-expanded MSCs, which are being trialled as a therapy for OA[16,17], but the combination of expense and limited long-term efficacy still presents a major hurdle to the adoption of this therapy. To make such procedures single-stage, there is interest in using 'off the shelf' allogeneic MSCs. Although allogeneic MSCs might have immunomodulatory effects, they are also associated with potential problems, including loss of functionality following *in vitro* expansion and culture-induced senescence[18]. The culturing of manipulated cells will not be discussed further in this Review as artificially aged *in vitro* cellular therapies might not function efficiently in the hostile environment of the osteoarthritic joint[19].

Understanding of the role of MSCs in OA has been influenced by historical misconceptions about MSCs, which originated from our knowledge of haematology. In the haematopoietic stem cell (HSC) model, a single HSC can repopulate the entire haematopoietic system[20]. Like the HSC, the MSC was also viewed as a rare, highly proliferative, clonogenic, multipotent cell that could circulate systemically to reach remote sites[21]. In hindsight, the shared origin of HSCs and bone marrow-resident MSCs might have resulted in the idea that stem cell progeny can leave the marrow cavity to home to distant sites. In reality, however, apart from being co-housed in the skeleton, both systems are radically different; for example, HSCs are rare, quiescent progenitors that reside in a specific niche, whereas cells with MSC-like characteristics can be readily derived *in vitro* from abundant mature stromal cells, including chondrocytes[22] and adipocytes[23] (FIG. 1). This evidence supports the idea that fully differentiated somatic cells such as chondrocytes might contribute to tissue repair without the need for differentiation from MSCs or some intermediate cell, and challenges traditional stem cell concepts. Moreover, joint-resident cells with a fibroblastic morphology and features of MSCs can occupy multiple tissue niches (FIG. 1). Unlike the HSC model, it is difficult to comprehend how a single MSC could recapitulate the entire skeletal system, and the derivation of an animal model along the same lines as the HSC model is highly improbable.

Bone marrow-resident MSCs. Compared with extraosseous MSCs, our understanding of the biology of bone marrow-resident MSCs is more advanced in terms of phenotype, topography, function and potential therapeutic applications. The bone marrow compartment has an important role in advanced OA and MRI-determined bone marrow oedema is prognostically relevant[5]. Moreover, the theory behind the 'original' stem cell therapy (using microfracture to treat isolated cartilage defects that are thought to be associated with the development of OA[10,11]) was predicated on the idea that bone marrow-resident MSCs percolate through to the cartilage from the bone marrow and act as the cellular building blocks for tissue repair[24]. At birth, the articular cartilage might be indistinguishable from the epiphyseal growth plate in both humans and mice as a result of the secondary epiphyseal cartilage ossification centres having not yet formed[25]. However, since the focus of this Review is on MSCs in adult joint repair, the intricate links between articular cartilage and the adjacent cartilage of the epiphyseal plate destined to become subchondral bone will only be briefly touched upon with regards to the mechanism of cartilage growth during development and during the neonatal period.

Bone marrow-resident MSCs coexist with HSCs, with both cell types able to exert homeostatic control over each other's functions[26]. This unique microenvironment seems to have a profound effect on the physiological demands on MSCs: not only do bone marrow-resident MSCs control host tissue remodelling, homeostasis of adipose tissue in bone and bone repair following fracture, but they also support HSC function, maturation and circulatory egress[27].

Osteotomy
A technique whereby bone is surgically realigned to change the joint alignment and load distribution.

Total joint distraction
A surgical technique in which external fixator devices are placed across the joint to restore the joint space; associated with cartilage repair.

Epiphyseal cartilage ossification centres
Areas of the cartilagenous growth plate at the metaphyseal ends of long bones in which bone formation follows the primary ossification seen in the diaphysis of long bones.

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In other words, bone marrow-resident MSCs can be considered more 'multi-functional' than MSCs in tissues in which HSC support is not a physiological requirement, such as other tissues of the joint. Moreover, native bone marrow-resident MSCs are part of the adventitial reticular compartment (also known as the stromal marrow supportive cellular compartment), which is a functionally mature and abundant cell population[28–30].

The original isolation and characterization of bone marrow-resident MSCs was based on the ability of rare bone marrow-derived cells to adhere to plastic and proliferate *in vitro* to form fibroblastic colonies[31]. In humans, native bone marrow-resident MSCs are characterized by being negative for the expression of haematopoietic cell and endothelial cell markers (for example, CD45 and CD31) and being positive for several other markers such as CD90 and CD73. The most commonly used maker for native bone marrow-resident MSCs is CD271 (also known as TNF receptor superfamily member 16, low-affinity nerve growth factor receptor (LNGFR) or p75 receptor)[32]. Data from the past few years suggest that bone marrow-resident MSCs have diverse embryonic origins (being both neural crest-derived and mesoderm-derived[33]) and that the relative proportions of cells from each origin might depend on several factors, including bone type and stage of development (for example, whether the bone is from a neonate, a child or an adult)[34,35]. In mouse models, Gremlin 1$^+$ progenitor cells, dubbed osteochondroreticular cells, participate in bone repair[35]. In our opinion, the osteochondroreticular cell population represents a more primitive population of MSCs than those carried into the limb bud during the development of the bone marrow niche, with the latter type of MSCs imbued with both tissue regenerative and haematopoietic support capabilities[36]. Although osteochondroreticular cells contribute to fracture repair in murine models, the role of this population in cartilage repair in OA remains conjectural[35].

Bone marrow-resident MSCs are the only type of MSC for which a capacity for self-renewal, in the context of relevant host tissue regeneration, has been demonstrated *in vivo* at the single-cell level[37,38]. A single culture-expanded bone marrow-derived MSC can regenerate a whole ectopic bone organ (termed the bone ossicle), containing not only newly formed bone, but also haematopoiesis-supporting stroma that can later be repopulated by host HSCs[37]. Even though this bone ossicle assay has limitations (such as an inability to recapitulate native mechanical demands on the formed bone or to be used for testing

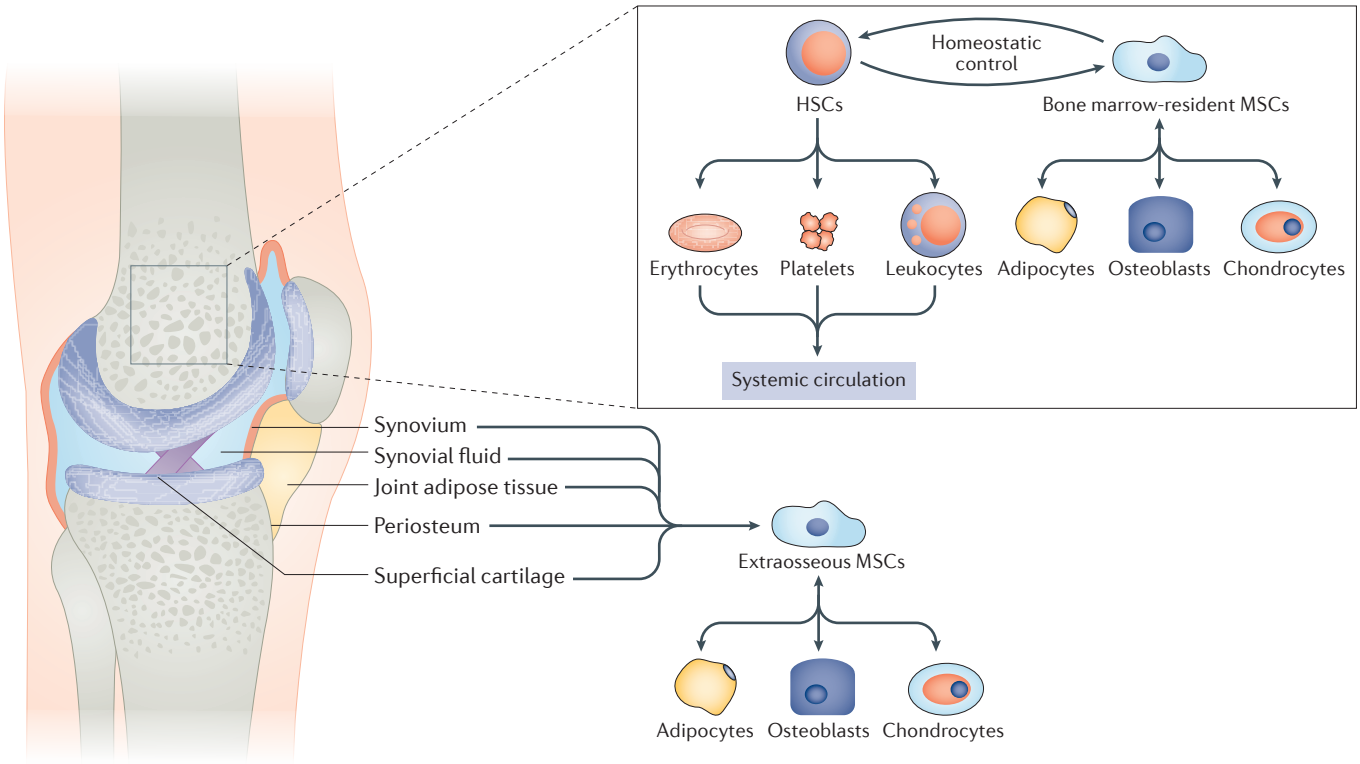

Figure 1 | **Stem cells in the joint.** The hypothesis that mesenchymal stem cells (MSCs) need to access the circulatory system to reach their destination was adapted from the haematopoetic stem cell (HSC) model. Both MSCs and HSCs are found in the bone marrow, but MSCs have also been described in multiple other niches within the joint, including the periosteum, synovium, adipose tissue (for example, the infrapatellar fat pad) and synovial fluid, as well as other periarticular tissues. Given the avascular nature and considerable thickness of some types of cartilage, a model in which multiple local populations of extraosseous MSCs exist with ready access to superficial zone cartilage is superior to the HSC model for the direct repair of cartilage lesions (without the need for systemic circulation or long-range migration of MSCs from the bone marrow). For MSCs, there is ample evidence that mature mesenchymal lineage cells such as adipocytes and chondrocytes can 'dedifferentiate' (indicated by arrows from daughter cell to MSC) into MSCs and exhibit high proliferative capacity and multipotentiality. The recognition that fully differentiated stromal cells can readily adopt MSC-like characteristics following *in vitro* adhesion to plastic argues against the feasibility of discovering specific MSC markers in synovium, cartilage or other joint tissues.

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single, purified, non-culture expanded MSCs), it is the gold standard assay for showing the 'stem cell nature' of bone marrow-derived MSCs. However, the results of such elaborate assays are difficult to translate into the site-specific need for chondrocytes, osteoblasts and osteocytes in OA-related cartilage repair, especially in the context of joint-resident MSCs in early OA, which do not need to provide a supportive role to HSCs.

As previously mentioned, bone marrow-resident MSCs occupy the perivascular niche, as well as the stromal reticular niche[29], where they are known as adventitial reticular cells[39] and form a cellular net, laying down extracellular matrix to support and anchor other cells of the bone marrow. Bone marrow-resident MSCs are thought to be found at the inner surfaces of the bone[40], enabling them to participate directly in bone remodelling processes without the need to migrate to perivascular or reticular locations. Importantly, adult bone marrow-resident MSCs do not follow a chondrogenic differentiation programme unless bone fracture occurs, triggering the endochondral ossification pathway, whereby a provisional cartilaginous tissue (soft callus) is initially formed[41]. In addition, as previously mentioned, bone marrow-resident MSCs are capable of supporting homeostatic, as well as on-demand 'emergency' haematopoiesis[42], the latter property being unique to bone marrow-resident MSCs and not physiologically relevant for joint-resident MSCs required for joint repair strategies.

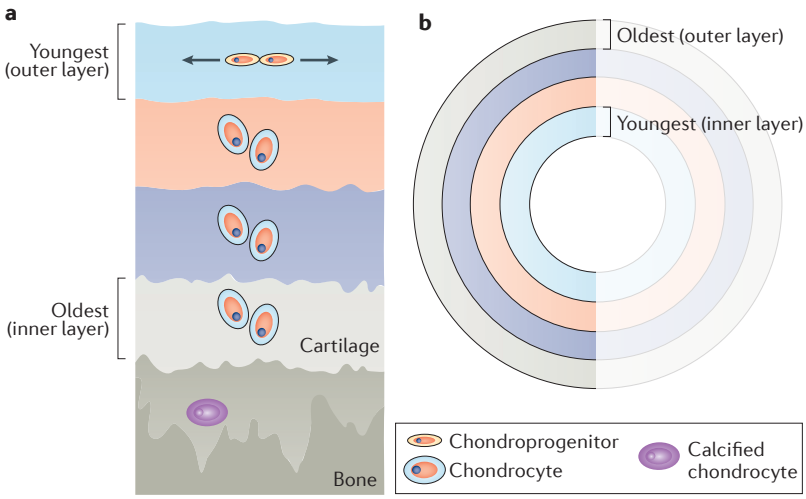

Figure 2 | **Appositional growth versus interstitial growth. a** | For appositional growth, resident chondroprogenitor cells within superficial zone cartilage divide and generate the underlying layers and cartilage matrix. During development, the cells in the superficial layer 'stack up' and the endochondral bone formation that occurs during epiphyseal plate growth results in cells that originated in the superficial zone eventually appearing in the bone matrix. The chondroprogenitor cells are responsible for maintaining the superficial zone progenitor population and might exhibit lateral migration; daughter cells reside in the older, innermost zones found deep within the cartilage, with the outermost zone being the youngest. Mesenchymal stem cells originating from the joint cavity, including the synovial fluid and synovium, can access superficial zone cartilage and complement the actions of these resident chondroprogenitor cells. **b** | In full-thickness cartilage defects that penetrate the underlying trabecular bone, bone marrow-resident mesenchymal stem cells can become exposed. In this setting, interstitial growth or hyperplasia of bone marrow-resident MSC-derived provisional tissue occurs.

Native bone marrow-resident MSCs in OA. The results of bone marrow aspirations showed that native bone marrow-resident MSCs were extremely rare in elderly individuals compared with young individuals, and that these age-related changes mirrored the distribution of age-related diseases including OA and osteoporosis[43]. Knowledge of the *in vivo* phenotype of bone marrow-resident MSCs and the recognition that bone marrow-resident MSCs colocalize with adventitial reticular cells led to the realization that such MSCs might not necessarily be released from the marrow during aspiration[29,44]. More suitable bone marrow digestion protocols were subsequently developed, the results of which indicated that the frequency of $CD271^+$ bone marrow-resident MSCs is in the order of ~1%[30]. This result challenged the concept that bone marrow-resident MSCs are so rare that expansion *in vitro* is necessary before they can be used therapeutically. Indeed, it is now possible to procure good manufacturing practice (GMP)-quality uncultured bone marrow-derived MSCs for orthopaedic applications without resorting to *in vitro* manipulation to bolster MSC numbers, although this technique has yet to be full exploited in humans[45].

Notably, CD271, the most robust surface molecule used for the isolation of bone marrow-resident MSCs, is a nerve growth factor (NGF) receptor[32,46]. The link between angiogenesis and pain in OA is well-established[47], but what is less well-appreciated is the fact that CD271 is typically expressed on bone marrow-resident MSCs in the perivascular niche[40]. Thus, MSCs might not only provide vascular support, but merit consideration as cells that could influence the association of perivascular neuronal ingrowths with osteoarthritic tissue[48,49]. In fact, data from a 2017 study has now shown how mechanical loading can induce the expression of NGF in osteoblasts, resulting in the activation of high-affinity NGF receptor-positive sensory neurons that provide osteogenic cues and facilitate increases in bone mass[50]. These data suggests a clear link between mechanical load, pain pathways and bone formation, which could be a factor in subchondral sclerosis in patients with OA.

Cartilage-resident MSC-like progenitor cells. Our understanding of the biology of cartilage-resident stem cells in health mostly comes from data from animal models, so great care is needed in extrapolating the relevance of this knowledge to patients with OA. The idea that articular cartilage is merely a remnant of epiphyseal cartilage that resisted the advancing front of endochondral ossification and contains residual stem cells is now obsolete, and the associated idea that cartilage regeneration or turnover starts in the deep zone of cartilage seems disadvantageous, as damage typically manifests in the superficial zone in early chondrogenic OA[51]. Studies by the Archer group into the morphology of the mammalian joint during neonatal and post-partum development in which chondrocytes were labelled with intra-articular bromodeoxyuridine indicated that chondrocytes were likely to be replenished from the superficial zone (termed appositional growth), rather than from the deep zone (interstitial growth)[52] (FIG. 2). Subsequent *in vitro* studies from the same group

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confirmed the presence of an MSC-like resident population in the superficial zone[53]. Independent studies demonstrated that during growth, the deep regions of cartilage that are present at the end of the bones at birth are replaced by bone concurrently with the neoformation of articular cartilage adjacent to the joint cavity[54]. Indeed, only the superficial zone cartilage was left unaffected by the remodelling process: following remodelling, the superficial zone contained a cell population that exhibited bidirectional mitotic activity (either horizontal or vertically) and that replenished the pool of cartilage cells by lateral and vertical expansion of the tissue[54].

Elegant cell-fate mapping studies in mice that exploited the fact that embryonic joint interzone cells and superficial chondrocytes both express *Prg4* (encoding lubricin) have confirmed the importance of superficial zone cartilage in tissue homeostasis[55]. Superficial zone cells in young mice served as progenitors for both superficial zone and deep zone chondrocytes in older mice, and not only did the expansion of such cells fill deep zone cartilage with cells, but daughter cells were also found in the underlying subchondral bone of mature animals[55]. Although epiphyseal growth plate chondrocytes and articular chondrocytes arise from distinct progenitor populations, the discovery of daughter cells from superficial zone cartilage in bone validates previous observations of deep zone chondrocytes being able to form bone[54]. In 2017, two subpopulations of cartilage-resident MSC-like progenitor cells were identified in cartilage from patients with OA, one of which exhibited an early senescent phenotype, which possibly reflects a replicative exhaustion following repeated but failed attempts at cartilage repair[56]. The *in vivo* phenotype of these cells has not been defined, but the findings are reminiscent of the loss of proliferative capacity in $CD271^+$ MSCs found in the bone of patients with OA[49]. Together, these studies[49,56] indicate that MSC senescence and an associated loss of potency could be an important facet of OA pathophysiology. The possibility of migrating interstitial cells contributing to chondrocyte clustering has also been noted[57] and tallies well with the aforementioned study[54] that shows how horizontal and vertical cell migration ultimately originates from the superficial zone, providing a mechanistic connection between the superficial zone and repair in deep cartilage regions[55].

Superficial zone chondrocytes express α-smooth muscle actin at greater levels than deep zone chondrocytes, which supports the idea of enhanced migratory activity at sites of fissuring and fibrillation[58]. Notably, bone marrow-resident MSCs also express α-smooth muscle actin *in vivo*[59], but the absence of a robust marker for MSCs in cartilage has hampered a better understanding of the role of putative cartilage-resident MSCs in cartilage repair. In addition, culture-expanded cells derived from the cartilage of patients with OA (of which the precise topographic origin is unknown) are capable of undergoing long-range migration of >1 mm *in vitro*[60]. In the following sections, we consider the evidence that the superficial zone cartilage-resident cells are derived in turn from MSCs that originate in the joint cavity.

Joint-resident MSCs

Spontaneous chondrogenesis (chondromatosis) in the synovium is a well-recognized phenomenon in humans. In an adaptation to high local levels of tissue compression within the joints, articular fibrocartilage lines the surface of bones in elaborate structures (termed synovio-entheseal complexes) at sites where ligaments or tendons compress the adjacent bone[61], and in animal models the implantation of cartilage mitogens in or adjacent to the synovium triggers local synovial chondrogenesis[62]. Collectively, these observations indicate that the joint environment is poised to support chondrogenesis in locations beyond the classically defined opposing articular cartilage surfaces. The native cells responsible for this remarkable synovial chondrogenesis in humans have not yet been identified, so in the following section we discuss potential candidate cells.

Unlike the bone marrow microenvironment (in which all MSCs express CD271[63]), whether all joint-resident (including synovial fluid-resident) MSCs express CD271 is less clear. A unique set of markers that can select the entire highly proliferative multipotent stromal fraction isolated from the synovium and joint adipose tissue has not been universally agreed on; however, some studies have reported markers that can be used to select a distinct MSC subset with high chondro-osteogenic potency from culture-expanded, but not from freshly isolated, synovial cell populations[64]. At present, we would summarize that the literature shows the presence of a phenotypically heterogeneous stromal fraction in joint tissues that exhibit MSC-like activity, and it is therefore rather difficult to make definitive statements about the specific phenotypes of joint-resident MSC populations and their contributions to cartilage repair.

Synovial-resident and joint adipose tissue-resident MSCs. In mice, cells expressing growth/differentiation factor 5 (GDF5) give rise to articular cartilage, ligaments and the inner synovial lining (FIG. 3a), but hardly any contribution has been attributed to these cells in the formation of adjacent long-bone cartilaginous shafts or growth plates, indicating a very close embryologic link between the cartilage and the synovium[55,65]. Joint cavity-related MSCs were first reported in the synovium[66], but it is still unclear whether these cells originate from the superficial synovial lining or are of subsynovial origin, or both. Indeed, the synovium is a potent and rich source of chondrogenic MSCs, which are found at a frequency of ~1%[67,68], a similar frequency to that estimated following bone marrow digestion protocols[30,44] and a far greater frequency than that found in bone marrow aspirates[54].

In rabbit models, the synovium covers the superficial cartilage, and this synovial membrane contributes to cartilage repair[69]. Given the large size of human knee joints, it is unlikely that superficial synovium-resident MSC-like chondroprogenitor cells are able to migrate over the long distances required to reach the site of action. In one mouse model of cartilage injury, spontaneous cartilage repair did not occur *in vivo* but chondrogenic activity was evident at the joint margin[70]. The same study indicated that putative *in vivo* $CD271^+CD44^+$ MSCs

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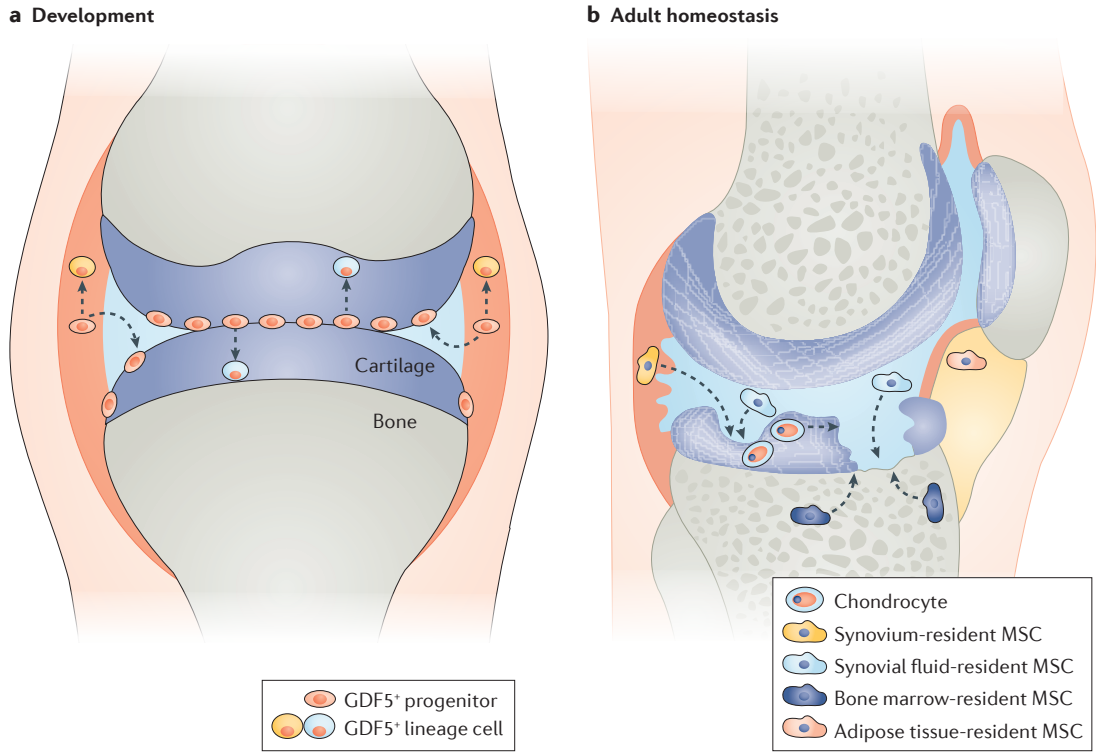

Figure 3 | **Progenitor cells in joint development and cartilage repair. a** | During development, growth/differentiation factor 5 (GDF5)-expressing progenitor cells are responsible for the initial joint cavitation. This population of cells can continue to reach the cartilage surface via periarticular tissues such as the synovium. In early development, appositional growth of cartilage is driven by superficial zone GDF5$^+$ cells. **b** | In adults, it is unclear how bone-marrow resident mesenchymal stem cells (MSCs) could contribute to the repair of superficial cartilage injuries. However, maintenance and repair of superficial (as opposed to full-thickness) injuries could involve resident cartilage progenitor cells or the migration of synovial fluid-resident and synovium-resident MSCs[83]. Indeed, direct migration of MSCs from the synovium and synovial fluid to sites of superficial cartilage injury has been shown experimentally, as has the contribution of synovial fluid-resident MSCs to ligament and meniscus repair[81–83]. Mature chondrocytes from within the cartilage are also capable of proliferation and migration. Lateral migration of adjacent cartilage-resident cells, including chondroprogenitors and chondrocytes, might also have a role in such settings, together with the migration of MSCs from the synovial fluid. Direct migration of native MSCs from the periarticular margins is also possible. For full-thickness cartilage loss, especially with breach of the subchondral plate, bone marrow-derived MSCs might contribute to the repair process from the osseous side. Hence, evidence exists for the migration of chondroprogenitor cells from the joint cavity to the superficial cartilage, both during development and during joint repair in early osteoarthritis.

were found in a location juxtaposed to the joint cavity, whereas a second putative CD271$^+$CD73$^+$ population of MSCs resided in the subsynovium[70]. Furthermore, CD271$^+$ pericytes did not seem to represent a pool of MSCs *in vivo*[70], thus contradicting the hypothesis that all MSCs could be pericytes[71]. Interestingly, human synovial fluid-resident MSCs, which are found topographically near to the synovial lining, also have a CD271$^+$CD44$^+$ phenotype[72]. Joint adipose tissues, including subsynovial fat and the infrapatellar fat pad, are also sources of MSCs[73], as are other joint structures, including the ligaments. A 2016 study provided proof-of-concept evidence that fibrous synovium-derived MSCs can be spontaneously released and might more readily access cartilage than MSCs from subsynovial adipose tissue, because more of the former cell type were released from the synovium in a novel *in vitro* assay[74].

Synovial fluid-resident MSCs. Synovial fluid-resident MSCs were found to exist at a frequency of ~40 cells per million mononuclear cells in synovial fluid from patients with OA, compared with ~1–2 cells per million in synovial fluid from patients with rheumatoid arthritis[63]. Subsequently, the frequency of synovial fluid-resident MSCs was found to be increased in patients with early knee OA with concomitant cartilage defects compared with individuals with knee pain and an absence of such lesions[75]. Another study then directly linked the number of synovial fluid-resident MSCs to the degree of chondropathy (as determined by arthroscopy) and radiographic damage[76]. MSCs are also present in gelatinous Heberden nodes (a very early lesion in hand OA) in radiographically normal joints[77], and in the synovial fluid of healthy individuals[78]. Numbers of synovial fluid-resident MSCs increase in response to

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ligament and meniscal injury[76,79,80] and increased numbers of MSCs have also been reported in superficial zone cartilage[56] and subchondral bone[49] from patients with OA. Collectively, these results suggest a natural, albeit ineffective, potential for repair. Independent studies involving the intra-articular administration of culture-expanded MSCs into synovial fluid have so far shown the successful engraftment of these cells into injured ligaments[79], damaged meniscus[81] and cartilage defects[82,83], providing proof-of-principle that joint-resident MSCs might contribute to the repair of accessible joint structures. Gene profiling of cultured synovial fluid-derived MSCs and comparisons with synovium-resident and bone marrow-resident MSCs suggest that synovial fluid-resident MSCs probably originate in the synovium[76,77]. Given the size of human synovial joints, the relocation of synovium-resident MSCs to the fluid compartment provides a mechanism for 'long-range' movement to access injured cartilage and other tissues.

The identification of MSC populations in multiple tissues within the joint, including the synovium, joint adipose tissue, synovial fluid and superficial zone cartilage, which either occupy the cartilage or are in close proximity to it, challenges the idea that bone marrow-resident MSCs are absolutely necessary for cartilage repair, especially for early lesions. Numerous studies have failed to observe circulatory MSCs in health[84] or in trauma[85], and only limited engraftment of bone marrow-derived MSCs has been observed in joint surface injuries in mouse models[86], providing little support for the idea of a biological role for bone marrow-resident MSCs (mediated by systemic circulation) in cartilage repair (FIG. 3b). The existence of multiple juxta-cartilage sources of MSCs offers a different paradigm to the classic multipotent bone marrow-resident MSC model, which could be further exploited for therapy development.

An MSC model for OA cartilage repair

In contrast to the bone marrow-resident MSC model, supportive roles in osteogenesis or HSC function would not be a requirement for superficial zone MSC-mediated cartilage repair. Indeed, cultured synovial-derived MSCs have consistently good *in vitro* chondrogenic capacity compared with bone marrow-derived MSCs[67]. Moreover, cultured joint cavity-derived MSCs have superior chondrogenetic properties compared with MSCs derived from cultured subcutaneous fat[68]. In 2016, native MSCs obtained from the stromal vascular fraction of subcutaneous fat were used to treat cartilage defects as part of a microfracture procedure in patients with OA, with encouraging short-term results[87]. Whether native MSCs from a non-joint environment will be comparable to native joint-cavity derived MSCs in a therapeutic setting is an open question that needs further investigation.

These collective observations about appositional cartilage growth and the close links between cartilage and synovium raise the question of whether MSCs originating from synovium, joint adipose tissue or synovial fluid could contribute to cartilage repair in humans (FIG. 3b). Studies in a canine model of chondrogenic OA showed that MSCs injected into the synovial fluid are capable of adhering to injured cartilage[82]. Previous studies in goats also indicated that MSCs injected into the synovial cavity following meniscus excision contributed to neo-meniscus formation and integrated into adjacent synovium[81]. Culture-expanded synovium-derived MSCs can also contribute to the repair of full-thickness cartilage defects, although this observation occurred following surgical implantation of MSCs, rather than by a spontaneous repair process[88].

Although many stem cell niches, such as the skin and the gut, have respective epithelial progenitor cells located deep within the tissue, the same does not hold true for cartilage (FIG. 4). Given that cartilage damage can begin superficially, MSCs in the joint cavity are well placed to participate in early repair mechanisms. This model is reminiscent of tooth biology, in which crystals secreted into the mouth cavity from the salivary glands repair the tooth from the outside[89] (FIG. 4).

Three different strands of evidence from animal models strongly support the pre-eminence of synovium-derived MSCs in cartilage repair. First, an 'influx model' was proposed that might be important to joint development, whereby waves of migratory GDF5$^+$ cells replenish developing cartilage, rather than there just being a single layer of such cells in the early interzone[90]; however, the precise periarticular origin of such cells was not defined. Second, a 2017 study showed that *Gdf5* lineage cells in adult mammalian synovium had MSC-like proliferative features *in vitro* and contained chondroprogenitor cells that participated in post-injury cartilage repair *in vivo*[91]. In this model, cartilage repair still took place after the function of cells expressing *Gdf5* was knocked down by the conditional repression of the transcriptional regulator Yap, which might reflect involvement of stem cells from other niches, including bone marrow, owing to the thin nature of murine cartilage, although this possibility was not addressed in this model[91]. Third, another 2017 study in mice showed that the filling of cartilage defects following injury was most notably caused by synovial *Prg4*$^+$ cells, with the authors describing such cells as pioneers for cartilage repair[92].

Ageing and inflammation in MSCs

Analagous to adult stem cells, bone marrow-resident MSCs decline functionally with advancing age[43]. Likewise, several studies have shown that aged human chondrocytes are incapable of generating highly proliferative chondrogenic cells or MSCs *in vitro*, in contrast to the adjacent fat pad, which did contain functional MSCs[93]. The results of other studies suggest that only cells derived from tissues with intrinsic chondrogenic capabilities can make cartilage *in vitro*[94], an idea that cautions against the concept that cells from external sources will be capable of generating robust cartilage *in vivo*. Nevertheless, stromal cells obtained from abdominal lipoaspirates might be capable of contributing to bone repair tissue in humans[95], raising the possibility that non-cartilaginous stromal cells could differentiate along a chondrogenic lineage *in vivo* under the correct environmental cues. It remains to be seen whether stromal

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cells that are not native to the joint could ultimately find a role in one-stage cartilage repair procedures, especially given the abundance of, and underappreciated importance of, joint-resident MSCs.

The potentially negative effects of joint inflammation in OA could detrimentally hinder repair and have been reviewed elsewhere[96]. The evidence that chronic synovial inflammation is ultimately detrimental to joint homeostasis is fairly compelling; however, for tissue injuries elsewhere (including bone fractures), an initial inflammatory reaction is a key part of the repair process. Evidence exists that cultured MSCs derived from an inflammatory joint environment have reduced chondrogenetic potential *in vitro*[97] and an enhanced

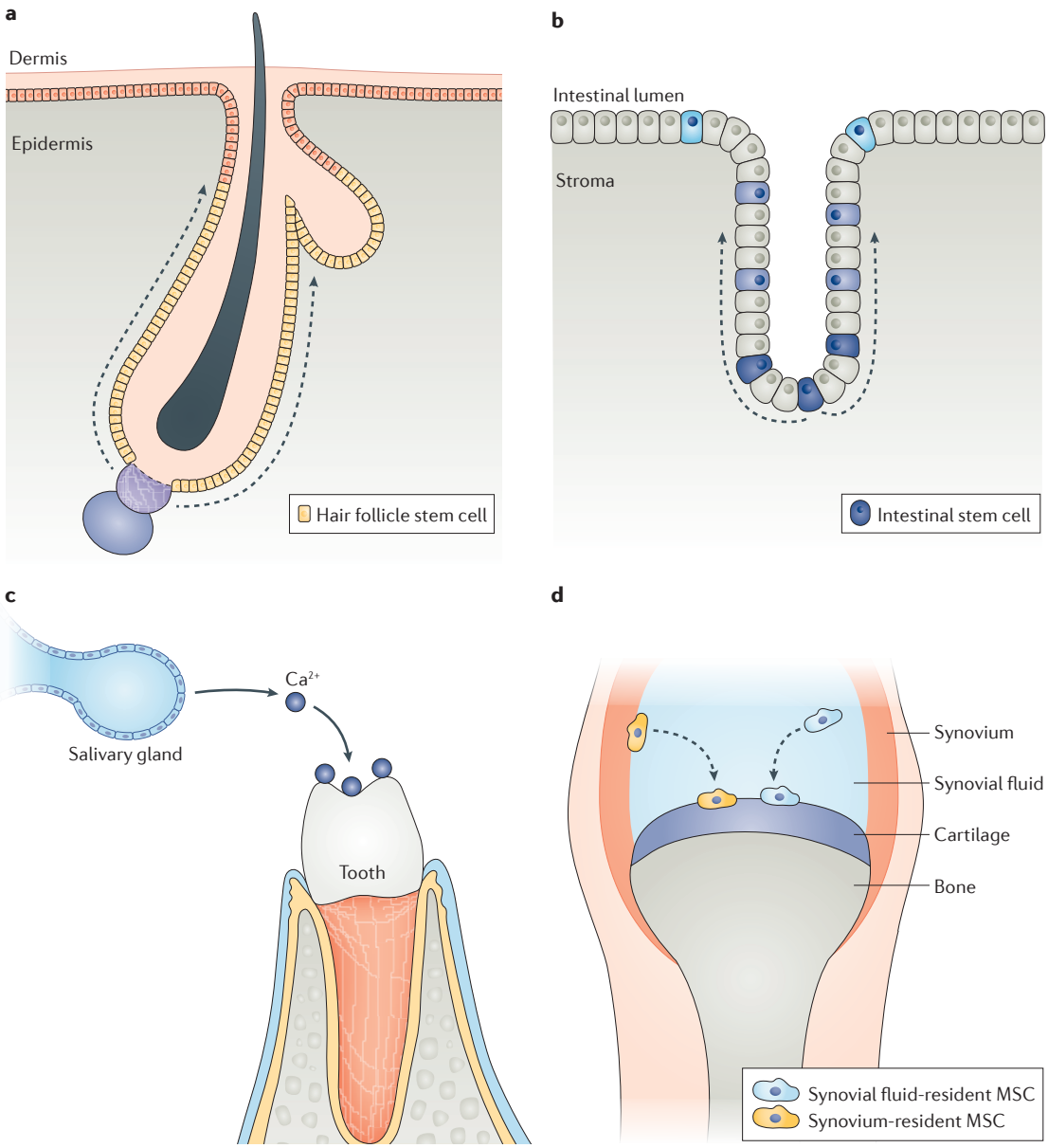

Figure 4 | **Tissue repair mechanisms differ depending on location and tissue microenvironment.** For the skin (part **a**) and gut (part **b**), which are exposed to hostile environments, there is a well-established paradigm for tissue to be repaired by basally located stem cells in the hair follicle and in the intestinal crypts, respectively. These stem cells are protected from the hostile environment. For the repair of (relatively) acellular musculoskeletal tissues, there is a completely different concept. For example, the secreted contents of saliva are involved in superficial tooth repair[89] (part **c**). The discovery that the joint cavity has several mesenchymal stem cell (MSC) niches, including the synovium and synovial fluid, and the demonstration that synovial fluid-resident MSCs can adhere to injured cartilage suggest that a model similar to that of superficial tooth repair could potentially be pivotal in cartilage repair (part **d**). In the case of cartilage repair, matrix-depositing MSCs first adhere to the most superficial tissue, which has the greatest propensity for injury. Osteotomies and joint distraction procedures enable such superficial cell-related repair mechanisms to manifest once abnormal joint mechanics have been corrected.

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pro-inflammatory phenotype[98], although it is unclear whether MSC functionality reverts to 'normal' following the resolution of inflammation. With respect to synovial fluid-resident MSCs, transcriptional dysregulation that correlated with levels of monocyte chemotactic protein 1 resulted in blockade of *in vitro* chondrogenesis[78]. In murine models, inflammation is associated with an increased number of MSCs in adjacent joint fat pads[99], which is consistent with the observation in humans that the proliferation of mesenchymal lineage cells is not suppressed by inflammation[97]. In humans, synovial inflammation might be associated with the degradation of high-molecular-weight hyaluronan, which removes the anti-adhesive coating from synovial fluid-resident MSCs, thereby enabling them to adhere to cartilage[83]. These emerging insights support the idea that a degree of 'controlled inflammation' in the osteoarthritic joint microenvironment might not be detrimental to the repair process, and that the process of inflammation might provide a window of opportunity for initial MSC interactions with injured tissue (FIG. 5a).

Implications for therapy

For bone repair strategies in the clinic, there is a strong interest in the use of native, unmanipulated bone marrow-derived MSCs for one-stage fracture repair procedures, rather than using *ex vivo* culture-expansion protocols[45]. Indeed, one-stage joint repair procedures

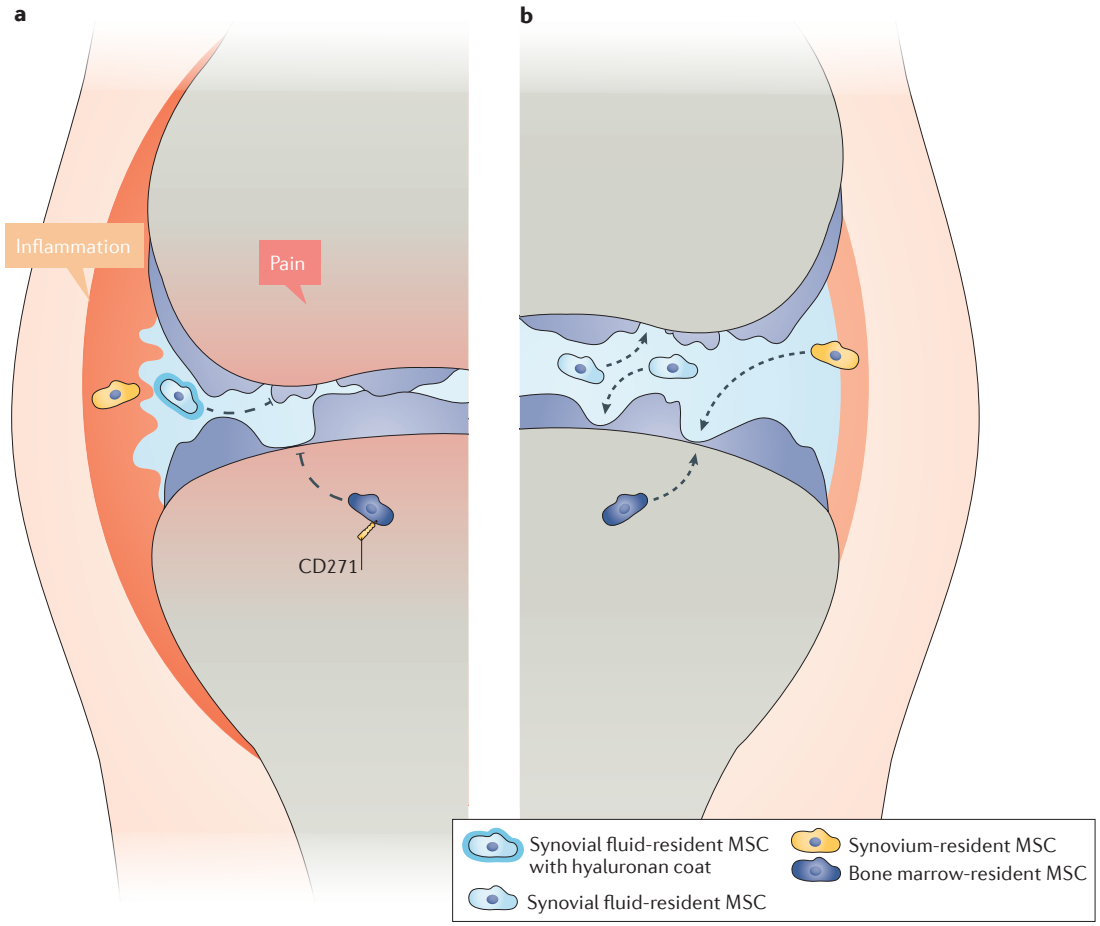

Figure 5 | **Endogenous factors influencing mesenchymal stem cells in adult cartilage repair.** Given the abundance of native or endogenous non-culture expanded mesenchymal stem cells (MSCs) that reside in the joint milieu, it will be important to elucidate the factors that govern MSC function *in vivo*. **a** | Joint inflammation, which is almost always viewed as detrimental in osteoarthritis, might be important for regulating the anti-adhesive hyaluronin coating seen on synovial fluid-resident MSCs. Limited evidence supports the idea that mitogens and growth factors introduced into the joint cavity can augment repair, but the mechanisms involved are poorly understood in humans *in vivo*. Native bone marrow-resident MSCs express CD271 (also known as low-affinity nerve growth factor receptor; LNGFR) and are especially numerous at sites of bone marrow oedema that are associated with a poor prognosis. Whether MSC-related dysfunction and neurotrophin pathways contributes to osteoarthritis awaits further studies. **b** | Joint realignment with the correction of abnormal biomechanical loading is important in cartilage repair, given that realignment osteotomies and joint distraction techniques can lead to spontaneous joint repair. Restoration of the biomechanical forces involved to a 'normal' level (for example, by realignment osteotomy) and alterations in the biochemical environment of the joint, including the loss of the hyaluranon coating from synovial fluid-resident MSCs, might therefore provide a window of opportunity in which joint-resident MSCs can repair tissues.

One-stage fracture repair
A single orthopaedic intervention to correct mechanical instability that optimizes strategies for rapid repair to prevent the need for further interventions.

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that utilize knowledge of the joint microenvironment in conjunction with various factors, including joint mechanics, growth factors, biomaterials, proteases and a knowledge of how to integrate cells with adjacent cartilage and subchondral bone, might be a tenable solution for joint regeneration[100] (FIG. 5b). One-stage techniques aimed at cartilage regeneration have so far relied on bone marrow aspiration and scaffolds[101], rather than on a knowledge of *in vivo* joint-resident MSCs. In a rabbit model, the size of the holes drilled into the marrow had a large effect on the number of fibroblast colony-forming units that appeared in the microfracture-related clot[102]. Indeed, the average number of MSCs released for a 4 mm osteochondral junction breach in this model was ~300 cells[102]. Extrapolating from such animal models, we would surmise that for full-thickness lesions in human OA, bone marrow-resident MSCs might have a substantial role given that numbers of MSCs are increased in the adjacent bone[49].

The highly proliferative nature of MSCs and their chondrogenic differentiation capabilities would seem advantageous for cartilage repair compared with the limited proliferative and differentiation potential of mature chondrocytes, especially in the ageing skeleton. To repair micro-defects in superficial zone cartilage, the use of unipotent chondrocytes or joint cavity-resident MSCs would seem to be perfectly satisfactory. This idea represents a completely different paradigm for joint-repair models, which had been extrapolated from the HSC and bone marrow-resident MSC models, as only limited, unipotent chondrogenic differentiation is required for cartilage repair. The lack of specific MSC markers might attest to the fact that cartilage repair mechanisms can involve many types of cells from diverse locations in and around the joint, as set out in FIG. 3b. Another obvious implication of the multiple niches of MSCs in the joint cavity is that synovial-resident MSCs, rather than bone marrow-resident MSCs, could be used in cartilage repair, an idea that has already been tested in humans in experiments using culture-expanded synovium-derived MSCs[88].

Native CD271⁺ MSCs in OA. In patients with advanced hip OA, native CD271⁺ MSCs are fivefold more abundant in regions of MRI-determined bone marrow oedema compared with adjacent non-oedematous trabecular bone[49]. However, upon expansion in culture, MSCs derived from such oedematous regions had a reduced ability to proliferate and diminished osteogenic capacity. A 2017 microarray study comparing cells derived from bone marrow oedema and non-oedematous lesions from patients with knee OA showed that the former were associated with upregulation of several neuronal growth-related transcripts, the most highly upregulated gene being *STMN2*, which encodes a phosphoprotein that regulates microtubule function and responsiveness to NGF[103]. It remains to be determined whether this finding specifically relates to the MSC populations, which were previously shown to be more abundant in MRI-determined bone marrow oedema lesions[49]. Some studies have also suggested that native bone marrow-resident MSCs express neuronal cell adhesion molecule[104], which was originally used as a marker to define cells of neuronal lineage. Collectively, these findings are noteworthy since NGF blockade has been linked to the development of rapidly progressive OA, a fact that led to a temporary hold being put on all clinical trials of anti-NGF therapy[105–107]. Although rapidly progressive OA has been attributed to a loss of protective pain reflexes and overuse of the joints, the abundance of native bone marrow-resident MSCs in sites of bone marrow oedema and the expression of proteins originally defined in neurogenesis[103] raises the possibility of an elaborate interconnection between pain and tissue regenerative processes. Although not yet established for native bone marrow-resident MSCs, there is comparatively old literature showing how other cells derived from CD271⁺ progenitor cells, such as Schwann cells, contribute directly to tissue repair in an NGF–CD271-dependent fashion[108]. Furthermore, combined use of NSAIDs with anti-NGF therapy seems to increase the risk of rapidly progressive OA[107]. NSAIDs exert an inhibitory effect on MSC differentiation[109], providing support for the notion of an interconnection between anabolic prostacyclins and the NGF pathway in native bone marrow-resident MSC function in OA. Given the abundance of MSCs in hip OA lesions[49] and the known function of NGF in inducing the migration of CD271⁺ cells[110,111], the possibility that pain and tissue regenerative processes converge on native bone marrow-resident MSCs and on the neurotrophin pathways could be considered a hitherto unappreciated mechanism contributing to the role of anti-NGF therapy in the induction of rapidly progressive OA (FIG. 5a).

Conclusions

The perceived challenges to repairing cartilage and adjacent bone, including cell sources, types of scaffolds, lateral integration and bone anchorage[112], are potentially rendered obsolete in many scenarios by the realization that spontaneous MSC-mediated repair can happen *in vivo*, and that native MSCs are relatively abundant in the joint cavity. Additionally, scaffold technologies could be augmented by harnessing knowledge of these abundant sources of native MSCs. The remarkable structural repair demonstrated by total joint distraction procedures and osteotomies, as well as the topographic positioning of MSCs at sites of injury, highlight how intrinsic joint repair might be harnessed. Removing the mechanical load and the destructive forces acting on the damaged cartilage could provide a window of opportunity for joint-resident stem cells to re-establish joint homeostasis. The emergent understanding of native MSCs in the osteoarthritic joint microenvironment and of the ways to coax them to sites of injury (by biophysical or pharmaceutical strategies) has the potential to radically improve cartilage repair strategies. However, abnormal joint biomechanical stress is likely to make the joint environment hostile, so careful consideration of the biomechanics, especially early in the disease course, will be vital to enable native MSC repair strategies to function optimally.

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Acknowledgements
The work of the authors is supported by the National Institute for Health Research (NIHR)–Leeds Musculoskeletal and Biomedical Research Centre.

Author contributions
All authors researched the data for the article, provided substantial contributions to discussions of its content, wrote the article and reviewed and/or edited the manuscript before submission.

Competing interests statement
The authors declare no competing interests.

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