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Stem cell-based therapeutic strategies for cartilage defects and osteoarthritis

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The gold standard cell therapy for repair of articular cartilage defects is autologous chondrocyte implantation, with good outcomes long-term. Mesenchymal stromal/stem cells (MSCs) from bone marrow or connective tissues such as fat are being pursued as alternatives for cartilage repair, and are trialled via intra-articular administration in patients with knee osteoarthritis. Early-phase clinical studies concur on safety and provide some promising insight into efficacy, but the mechanism of action remains unclear. Recent studies implicate extracellular vesicles as important mediators of MSC action, offering exciting therapeutic prospects. Our increasing understanding of the mechanisms underlying intrinsic articular cartilage maintenance and repair fosters hope that novel/repurposed therapeutics could elicit repair through activation of endogenous stem/progenitor cells to maintain healthy joints and prevent osteoarthritis.

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Introduction

Osteoarthritis (OA) is a common degenerative joint disease, characterised by progressive cartilage breakdown, subchondral bone sclerosis and aberrant bone outgrowths (osteophytes). Traumatic joint lesions increase the risk of OA. Advances in the regenerative treatment of early cartilage lesions could help to prevent OA. This review discusses cell-based approaches for the repair of cartilage lesions and the treatment of OA (Figure 1).

Cartilage repair

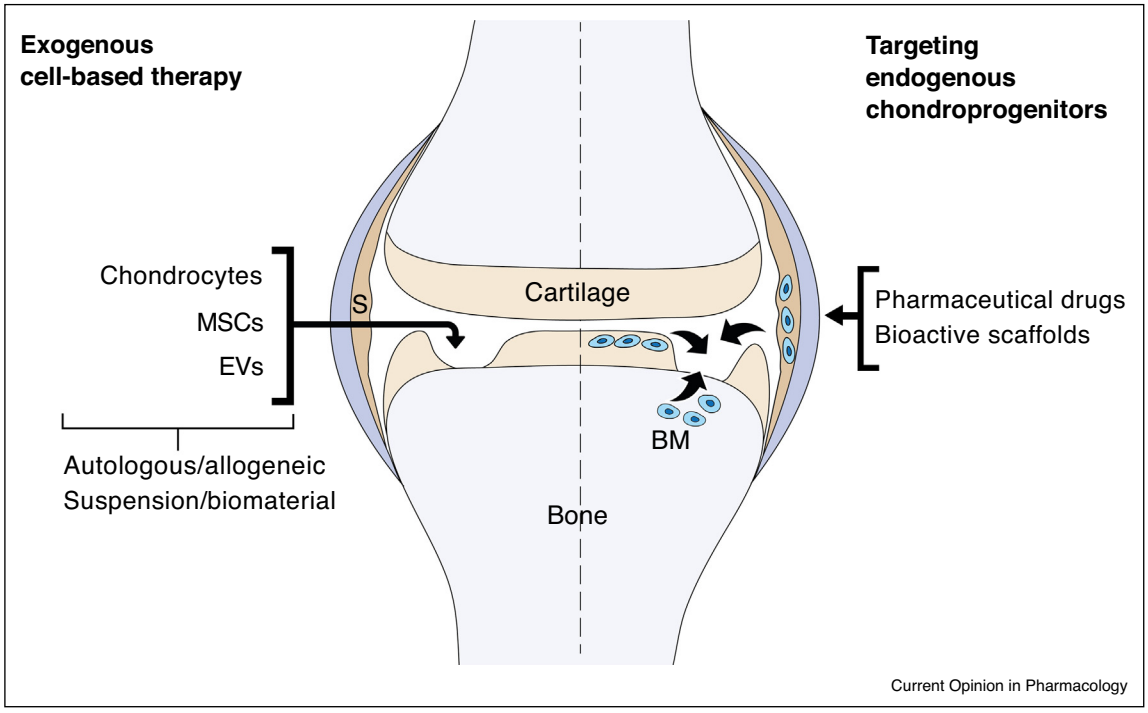
A commonly performed surgical procedure is microfracture, a marrow stimulation technique that allows communication between the joint space and subchondral bone marrow to release mesenchymal stromal/stem cells (MSCs) from the marrow that form a repair tissue. However, particularly in defects larger than 2 cm^2, the repair tissue with microfracture frequently undergoes degeneration over time with the formation of scar-like fibrous tissue or even replacement with bone [1].

Autologous chondrocyte implantation (ACI) was first described in 1994 by Brittberg and colleagues, who reported symptomatic relief in 14 out of 16 patients with lesions of the femoral condyle at 2 years follow-up [2]. A cartilage biopsy is obtained from a healthy area of the patient's articular cartilage, chondrocytes are isolated and expanded in culture, and are implanted in the cartilage defect either in suspension under a periosteal flap or synthetic membranes, or in three-dimensional matrices [3]. Clinical trials have confirmed the good clinical outcome of ACI. In 118 patients at 12 and 18 months following either ACI or microfracture, clinical outcome was similar in both groups but ACI was associated with increased structural repair [4]. At 5 years, clinical outcomes were again comparable [5]. However, ACI was more effective in a subgroup of patients who had undergone the procedures close to presentation of symptoms [6]. Results from up to 20 years follow-up have demonstrated that ACI is an effective and durable solution for the treatment of large joint surface lesions of the knee [7,8•].

However, variability in structural outcome after ACI has been reported, with some patients showing repair tissue consisting of disorganised fibrocartilage [9]. Chondrocytes dedifferentiate during culture-expansion to a fibroblast-like phenotype and lose their capacity to form stable hyaline cartilage *in vivo* [10], which may underpin the variability in structural outcome. The use of chondrocytes expanded under conditions that preserve their cartilage-forming potency may enhance joint surface regeneration with the formation of hyaline-like cartilage repair tissue [11]. Of interest, chondrocytes derived from the nasal septum, with known capacity to generate hyaline-like cartilage, have been successfully used for knee cartilage defect repair in ten patients [12••], but large controlled trials are warranted to assess efficacy.

MSCs are easy to grow in culture and have chondrogenic ability, and are therefore considered an alternative cell source for cartilage repair. MSCs have been isolated from bone marrow [13,14], and most connective tissues including periosteum [15,16], synovium [17,18], and adipose tissue [19]. Numerous preclinical studies have supported

Figure 1



Regenerative therapeutic strategies for cartilage defects and osteoarthritis. Exogenous cell-based therapy entails delivery of autologous or allogeneic cells such as chondrocytes or mesenchymal stromal/stem cells (MSCs), or extracellular vesicles (EVs), either in suspension or seeded in a biomaterial. Alternatively, endogenous chondroprogenitors, which reside in synovium (S), bone marrow (BM) and cartilage itself, could be targeted with pharmaceutical drugs or bioactive scaffolds to trigger or enhance intrinsic repair.

the use of MSCs in joint resurfacing [20], and studies in humans revealed a variable structural outcome, ranging from hyaline-like cartilage to fibrous tissue [21]. Notably, autologous bone marrow MSCs were shown to be non-inferior to chondrocytes in clinical outcomes at 24 months in an ACI-like procedure [22], although longer-term follow-up and more robust assessment of structural outcome are needed to draw definitive conclusions.

An important consideration is the tissue source of MSCs for cartilage repair, and whether MSCs from non-joint environments such as the stromal vascular fraction of visceral fat would be comparable to MSCs derived from joint tissues. MSCs from bone marrow appear to have high propensity to undergo chondrocyte hypertrophy and bone formation [23,24] and thus may not be ideal for the repair of articular cartilage. Superiority of MSCs from synovium for cartilage formation *in vitro* when compared with MSCs from other tissues including bone marrow and periosteum has been reported [25]. The differences in potency could be related to MSC ontogeny and distinctive molecular programmes of embryonic formation of the native tissues from which the MSCs are obtained. Lineage tracing studies in mice have demonstrated that articular cartilage and synovium have a common developmental origin from the Gdf5-expressing cells of the embryonic joint interzone. Gdf5-traced MSCs resident in the adult knee were found to display joint progenitor activity and ability to repair articular cartilage [26••,27••]. Promising data using synovium-derived MSCs have been reported in preclinical and clinical studies [28•,29,30].

Notably, chondroprogenitors derived from the surface zone of articular cartilage using differential adhesion to fibronectin, showed ability to maintain chondrogenic potency upon extensive expansion [31], and formed a cartilage-like repair tissue in a chondral defect in a goat model [32]. Human studies are awaited.

Interventions consisting of implantation of stem/progenitor cells seeded in smart biomaterials, with the addition of cartilage-promoting growth factors, are also being pursued to support adequate repair [33], but such combinations render the path to clinical application complex.

Osteoarthritis

Intra-articular injection of bone marrow MSCs in goats, in which the medial meniscus was excised and the anterior cruciate ligament was resected, resulted in regeneration of the medial meniscus and decreased development of secondary OA compared with untreated animals [34]. This and many other subsequent preclinical studies

[20] have constituted the foundation for cultured MSCs being trialled as therapy in patients with OA, mostly in the knee. Recent systematic reviews of phase I/II clinical trials (not always controlled or blind) concluded that MSCs, obtained most commonly from bone marrow or adipose tissue and injected intra-articularly into the knee, are overall safe and well tolerated. Furthermore, MSCs can improve pain and function of the knee joint, with scattered histological data suggesting formation of hyaline-like cartilage repair tissue [35,36]. A meta-analysis of 11 small trials of MSC therapy for knee OA, including a total of 582 knee OA patients, reported improvements across a range of clinical outcome measures [37]. However, the efficacy of these therapies cannot be determined until large randomised controlled trials are carried out. While most studies have used autologous cells, allogeneic MSCs appear to have an acceptable safety profile [38•,39••]. Their use would be advantageous over autologous procedures by allowing manufacturing of large batches of off-the-shelf MSC products, which would enhance consistency and decrease the costs of cell therapy.

To circumvent the hurdles of culture expansion such as costs and variability, fresh bone marrow aspirate concentrate would provide a readily available autologous cellular product for intra-articular delivery, and has been shown to be safe in 25 patients with knee OA [40]. Protocols for digestion of fresh bone marrow aspirate have been developed for enrichment of clinical grade $CD271^+$ MSCs for orthopaedic applications [41], but potency of the cellular product and clinical feasibility need to be determined.

In summary, cell therapy has proven safe but large controlled studies are needed to confirm whether cell therapy can improve pain and induce structural benefit in OA. Standardisation of cell product manufacturing (including potency assessment), frequency and method of delivery, and definition of target patient populations through stratification will be necessary to determine efficacy and allow meaningful comparisons of clinical study outcomes.

MSC-derived extracellular vesicles

The mechanism of action of MSC therapy remains unclear. Evidence that injected MSCs contribute directly to joint tissue repair is limited. MSCs could modulate the joint environment and mediate intrinsic tissue repair via paracrine signals. Recent studies have reported that MSC-derived extracellular vesicles (EVs) can promote cartilage repair and protect against OA-induced cartilage degeneration [42,43••,44,45••]. EVs are small, membrane-enclosed particles that are released from cells either through budding from the cell membrane (microvesicles) or through fusion of endosomal multivesicular bodies with the plasma membrane (exosomes). They contain biologically active signalling molecules and can bind to target cells via ligand-receptor interactions. They can activate intracellular signalling from the plasma membrane or can be internalised resulting in intracellular delivery of their cargo.

Single intra-articular injections of mouse bone marrow MSC-derived exosomes or microvesicles were both similarly effective in protecting against the development of collagenase-induced OA in mice compared to injection of whole MSCs [42], suggesting that EVs may be major paracrine factors mediating the protective effects of MSCs. Weekly intra-articular injections of exosomes from MSCs generated from human embryonic stem cells resulted in improved repair of critical-sized osteochondral defects in the femoral groove in immunocompetent rats [43••,44]. Rat chondrocytes *in vitro* internalised the exosomes and increased their proliferation and migration rate which was at least in part mediated via rapid activation of AKT and ERK through CD73-mediated adenosine signalling [44].

Other recent studies showed that intra-articular administration of exosomes derived from cultured rat synovial MSCs partly prevented cartilage damage in a trauma-induced rat OA model, and this OA protective effect was enhanced with exosomes from miR-140-5p overexpressing synovial MSCs [45••]. *In vitro* experiments showed synovial MSC-derived exosomes to contain high levels of Wnt5a/b, which upregulated Yap in exosome-treated chondrocytes to promote proliferation and migration but downregulated synthesis of cartilage matrix proteins [45••]. The latter was overcome by loading exosomes with miR-140-5p [45••], a pro-chondrogenic miRNA important for maintaining cartilage homeostasis [46,47], showing amenability of the EV cargo to be modified for optimal targeted delivery to enhance efficacy.

Biodistribution of EVs following intra-articular delivery is not known. Although EVs can attach to and penetrate the cartilage, at least in an inflammatory arthritis model [48••], EVs may be subject to rapid clearance from the joint space. Encapsulating EVs in a suitable biomaterial to prevent rapid clearance and achieve sustained effects could facilitate clinical translation, and is being explored for cartilage repair [49•]. Similar to cell therapies, it will be crucial for therapeutic use of EVs to ensure high-quality, standardised EV production and purification methods compatible with clinical application.

Targeting joint-resident MSCs

Since MSC populations can be derived from multiple tissues in the joint, including synovium [18], infrapatellar fat pad [50], synovial fluid [51,52] and the cartilage itself [31], why joint-resident MSCs would fail to repair damaged cartilage remains unclear. Their repair capacity may be progressively impaired in ageing, and a reduction in MSC populations and their proliferative and

differentiation capacity has been postulated in OA [53]. An increased number of MSCs in the synovial fluid [54] and bone marrow lesions of OA patients [55•] suggests that an MSC-mediated attempt to repair would occur albeit ineffectively, which may be due to a hostile environment or impaired MSC regenerative function or senescence [55•,56•]. An in-depth understanding of the role of MSCs in joint homeostasis, remodelling, repair and OA pathogenesis is therefore needed.

Particularly at early disease stages, the prospect to support MSCs in their function to maintain joints as healthy as possible while preventing progression of damage is exciting. The use of bioactive scaffolds or therapeutics that elicit an effective tissue repair response through activation and mobilisation of endogenous stem/progenitor cells, without the need to administer exogenous cells, would be easier to implement clinically and likely to encounter fewer regulatory hurdles. A study showing that intra-articular injection of kartogenin protected against OA development in mice, possibly via modulating endogenous stem cells to promote repair [57], provided insights regarding novel cell-free regenerative therapy for OA.

The lack of an exclusive marker for MSCs in joint tissues has hampered studies of joint-resident MSCs [58]. Recently, genetic tools allowing lineage tracing in mice have elucidated the ontogeny of MSCs in adult tissues while providing means to locate and monitor these cells in health and disease. It has thus become clear that MSCs in bone marrow in adult life primarily support and regulate haematopoiesis through interactions with haematopoietic stem cells [59] and replenish osteoblasts [60]. In parallel, stem/progenitor cell kinetics in joint development, homeostasis and repair are being unravelled, providing previously unappreciated clues for cartilage regeneration/repair. Three independent studies have demonstrated that Prg4-traced cells in the joint have properties of progenitor cells during postnatal life [27••,61•,62], but their natural healing potential remains uncertain. Two independent studies have shown that the Prg4-lineage, and the Gdf5-lineage deriving from the embryonic joint interzone, which are at least partly overlapping in the adult joint, contribute chondroprogenitors to cartilage repair in mice [26••,27••]. Such lineage cells are present in both the synovium and the superficial zone of cartilage, and the question as to where the progenitors are located remains to be addressed. Contribution from the superficial zone cannot be excluded, since superficial zone cells are able to migrate to the site of injury in cartilage explants [63]. However, the absence of detectable proliferation of those lineage cells in cartilage and, instead, their considerable expansion to underpin the synovial lining hyperplasia following injury [26••,27••], have seeded the idea that the chondroprogenitors that contribute to cartilage repair would originate from the synovium. Of interest, inactivation of the transcriptional co-factor Yap in the Gdf5 lineage abrogated the synovial lining hyperplasia and reduced the contribution of Gdf5-traced cells to cartilage repair, but cartilage repair could still take place [26••], suggesting a simultaneous and/or compensatory recruitment of chondroprogenitors from other lineages, which may include the Leptin receptor lineage in bone marrow [64].

MSCs have also been found in the synovial fluid, and comparative gene profiling showed the MSCs from synovial fluid to be closer to MSCs from synovium than to MSCs from bone marrow, suggesting that they might originate in the synovium [54]. Whether MSCs percolate in the fluid to become available at the site of injury is yet to be proven.

Conclusions

Cell-based therapies are increasingly becoming available for the repair of joint surface lesions and OA. A one-fits-all solution is unlikely and instead the type of regenerative intervention will depend on targeting the right patient through personalised medicine and OA stratification. While progress in our understanding of mechanism of action has been made and several clinical trials are ongoing, the journey towards routine regenerative medicine applications for OA patients in clinic remains complex. Together with the trial-and-error clinical approaches, evidence-based refinements will be implemented as guided by our increasing knowledge of the regenerative biology of the synovial joints.

Conflict of interest statement

Nothing declared.

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