Targeted cell delivery for articular cartilage regeneration and osteoarthritis treatment

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Osteoarthritis (OA) is one of the main causes of pain and disability worldwide. In recent years, numerous efforts have been made in pharmaceutical and surgical therapies for OA management. The therapeutic features of mesenchymal stem cells (MSCs), have led to numerous preclinical and clinical trials that confirmed the efficacy of cell therapy as treatment for OA. Recent works have attempted to customize cell participation in tissue regeneration using site specific targeting approaches. Targeting approaches are based on direct modifications to the surface of MSCs or indirect modifications on an engineered nanomediator. Here, we provide a comprehensive review of the advances in targeted cell delivery and define the priorities for future work in terms of OA and cartilage regeneration.

Introduction

Degenerative joint disease or osteoarthritis (OA), a predominant cause of joint pain worldwide, will be the fourth-leading cause of disability by 2020. OA has a progressive prevalence as most people over the age of 65 exhibit some degree of OA [1]. Self-reported and symptomatic knee OA account for 15.7% of men and 8.8% of women, whereas the prevalence of radiographic OA is reported to be 45.1 and 33.4% for men and women, respectively [2]. Limited therapeutic approaches for OA and inability of current pharmaceutical and operative therapies to successfully prevent OA progression makes it necessary to develop new therapeutic strategies. Cell or stem cell therapy, particularly mesenchymal stem cells (MSCs), is a potent strategy in medicine that can render a biological solution for tissue regeneration and repair. Thus far, numerous studies have used various cells and cellular compartments in clinical and preclinical settings [3]. However, efficient delivery of exogenous cells to the site of injury and the ability to intensify their contribution to the regenerative process remains challenging for cell therapy [4]. Here, we review recent advances in targeted cell therapy approaches for tissue repair, with a particular focus on articular cartilage (AC). This review describes the structure and function of healthy hyaline AC and OA, as well it covers the current strategies that enhance cell contributions to tissue repair. We mainly focus on targeting approaches that include cell-surface modifications and nanoparticle (NP)-mediated tissue targeting with a discussion of their successes, limitations, and hopes for future cell delivery.

Articular cartilage (AC): structure and function

The articulating ends of bone surfaces of all joints are covered by a specialized avascular and aneural type of connective tissue (see Glossary) known as hyaline AC. AC provides a lubricating low-friction surface to avoid wear [5]. AC is typically characterized by low cell density (1–5% of chondrocytes) and an abundant extracellular matrix (ECM). AC is structurally divided into four zones: superficial, transitional, middle, and calcified.

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The main feature of the superficial layer zone is the presence of disc-shaped chondrocytes that produce all of the matrix components. This layer contains abundant type II collagen and a small amount of type I collagen at the very superficial layer, and a low proteoglycan content. This layer contains the lubricin (proteoglycan 4; PRG4) and most of proteins responsible for joint lubrication [5]. The transitional layer is characterized by decreased cellular density, presence of round chondrocytes and increased proteoglycan content [6]. The middle zone contains lower proteoglycan content and less spherical chondrocytes with a columnar arrangement. In the calcified zone, a proteoglycan-free matrix surrounds the small number of rounded hypertrophic chondrocytes. The tidemark is the boundary between the middle and calcified layers (Fig. 1).

The hydrodynamic property of AC arises from type II collagen for tensile strength and proteoglycan (PGs), which provides compressive strength. Various types of collagen fibrils (i.e collagen types II, VI, IX, X, and XI) constitute an extensive network which provide the mechanical integrity of cartilage [7]. The presence of negatively charged PGs (e.g. aggrecan) and glycosaminoglycan (GAG) (e.g. hyaluronic acid (HA), chondroitin sulfate (CS)) within the ECM makes cartilage tissue absorb water, which creates the swelling pressure. Altogether, AC provides decreased friction and a shock-absorber layer allowing for frictionless joint movement.

Osteoarthritis (OA): damaged articular cartilage

OA is a heterogeneous degenerative joint disease that results from disturbances of catabolic, anabolic, and inflammatory pathways. Risk factors of OA are endogenous (genetic background, aging, inflammatory mediators, and obesity) and environmental (mechanical stress) [8]. With aging, a primary risk factor, cartilage extracellular components (proteoglycan and collagen type II) and chondrocytes undergo age-related metabolic changes, which result in an imbalance between matrix synthesis and degradation [9]. Various risk factors may simultaneously change the biomechanical properties of cartilage tissue, resulting in pain and joint dysfunction (and therefore loss of mobility). Given the absence of vasculature, hyaline cartilage regeneration does not usually occur. Lack of progenitor cells migration and/or absence of them within the degenerated cartilage are responsible for the lack of cartilage defects healing. Therefore, providing the cartilage lesions with progenitor cells could accelerate the regenerative process that is fundamental for cell therapy.

Current status of osteoarthritic and damaged articular cartilage treatment: efficiency of cell and stem cell therapy

Current therapeutic approaches for early and moderately early OA include non-pharmacological methods, pharmaceutical treatments, and surgical intervention – all of which fail to repair cartilage lesions [10]. They merely relieve pain for a limited period and lack the capability to prevent disease progression. Over the last two decades, cell or stem cell therapies have emerged as a potent strategy to be used in regenerative medicine and tissue engineering. The first report of cell therapy in the clinical setting was published in 1994 when autologous chondrocyte implantation (ACI) was used for OA treatment with relatively promising results [11]. Moradi *et al.* conducted a 14-year follow-up study to examine the effect of ACI on 23 patients with advanced OA (grade IV). They observed that ACI improved clinical parameters and led to partial restoration of joint function in more than 50% of patients [12]. Similarly, another research group assessed first-generation ACI in

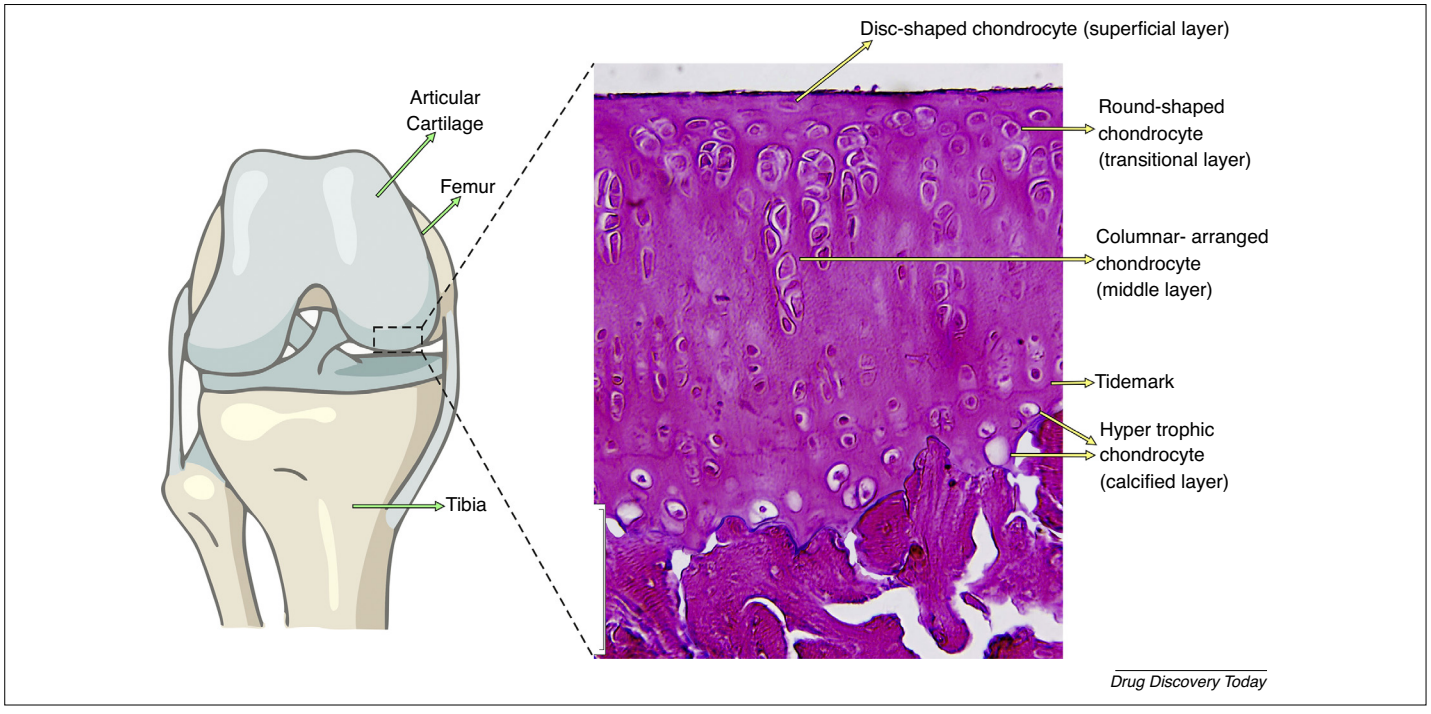

FIGURE 1

The multi-zonal structure of articular cartilage (AC). Hematoxylin and eosin (H&E) staining of histological section taken from adult rat knee joint (femoral condyle joint surface) are shown. Different structures, chondrocyte shape and orientation indicate the four distinct zones in AC. (Scale bar = 100 μm).

23 patients and observed survival rates and clinical improvements during 20 years of follow up [13]. ACI does not elicit the host immune response and minimizes donor-site morbidity compared to autologous osteochondral implantation. However, inability to restore the articular congruity and poor histological score of repaired cartilage was observed in different studies [14,15]. This might allocated to the fact that during *in vitro* culture, chondrocytes undergo dedifferentiation and lose their capability to produce hyaline cartilage and form fibrocartilage with less long-term mechanical resistance, which affects the clinical outcomes of ACI [10].

MSCs, with their high differentiation potential into mesodermal lineages such as chondrocytes, the ability to adjust the immunomodulatory status of the injury via trophic factors and ease of accessibility from a variety of adult tissues, would be the ideal candidates for stem cell therapy in cell-based AC repair approaches [16]. Contributions of MSCs in tissue regeneration are mainly associated with their capacity to secrete trophic factors that mediate apoptosis and cell death, vascularization, endogenous cell recruitment and immune response modulation [3,17]. Many basic and translational studies with MSCs have been conducted *in vitro* and in small (i.e. mouse, rat, and rabbit) and large (i.e. pig, dog, sheep, horse) animal models to develop the concept of cartilage tissue regeneration for clinical applications [18]. The results of these animal studies are translated to clinical practice. Several clinical trials have evaluated therapeutic and regenerative potential of MSCs derived from bone marrow, adipose tissue and synovium [19,20] (Table 1). Centeno *et al*., examined the efficacy of autologous bone marrow MSCs (BM-MSCs) in a patient with degenerative knee cartilage disease. They observed promising clinical and MRI improvements in a short-term follow-up (24 weeks) [21]. Accordingly, MSCs have the capability to decrease pain, improve joint physical function and develop the hyaline like cartilage.

In terms of MSC safety, except some rare mild infection and pulmonary embolism, no malignancy or acute adverse effects was reported following MSCs transplantation [22]. Lalu *et al*. claimed that besides intra-articular MSC therapy, intravascular MSCs administration, has not been associated with severe side effects, except transition fever in some patients [23]. Moreover, the low immunogenicity of MSCs enable its allogeneic sources to be used in stem cell therapy for tissue regeneration. Since the behavior of MSCs is donor-age dependent, allogeneic MSCs are preferable in diseases like OA, which mostly impacts involved aged people (Fig. 1). All in all, MSCs are being considered as a ''safe cell'' so that it is rational to preserve MSC therapy for OA and AC defects.

Cell engraftment in the target tissue profoundly affects the quality of cell therapy. Several animal studies represented the participation of MSCs in regeneration of AC lesions [24], which in turn resulted in improvement of clinical outcome and pain relief. However, there is no evidence for efficient hyaline cartilage restoration. We believe this is a consequence of the inadequate direct contribution of MSCs to cartilage regeneration.

How can mesenchymal stem cell participation be enhanced in tissue repair?

The robustness of MSCs urged scientists to develop novel strategies to enhance its contribution in the regeneration process [25–27] (Fig. 2). Cell density, an appropriate cell source, time and location for cell implantation, genetic modification, pretreatment of MSCs by inflammatory cytokines, and cell implantation along with hydrogels and NPs are some approaches used to enhance the efficiency of cellular therapy. Below, we provide a detailed description of different parameters which affect the efficacy of MSCs therapy and we could improve MSC outcome by controlling these parameters.

Cell density

Cell density is one of the criteria that affects the success rate of cell therapy. An appropriate cell density precludes unwanted complications in cell therapy techniques. For example, high cellular density may cause tumorigenesis or repair failure may occur due to a low number of injected cells. Different cell densities have been used in clinical and preclinical studies. Preclinical studies, regardless of the type of animal model, have used 8.0×10^5 to 8.0×10^7 cell/cm^3 for regeneration of AC defects [27]. Clinical studies have used a range of MSCs from 1 to 2.5×10^6 MSCs/cm^3 [28]. Few clinical studies have discussed the number of delivered cells in systemic or local transplantation for cartilage regeneration. It has been observed *in vitro* that only high densities of MSCs seeded in hyaluronic acid hydrogels could produce functional hyaline cartilage [29]. Approaches that enable site-specific delivery of cells to the damaged tissue enable the use of the lowest possible cell density since they can prevent the off-target attachment of cells.

Time modality

Injection time appears to be of utmost importance for cell therapy efficacy. Factors involved in immunosuppression and the homing capability of MSCs are induced by high concentrations of inflammatory cytokines at the injury site. Interleukin (IL)-1β, tumor necrosis factor (TNF)α, IL-6, IL-15, IL-17, and IL-18 comprise well-known cytokines seen during an inflammatory response [30]. MSCs primed with these proinflammatory cytokines could improve their homing and immunomodulatory functions [31]. Thus, it may be that the administration of MSCs in the inflammatory phase of the disease (inflammatory response phase) could improve cell delivery outcomes.

Mode of cell administration

Generally, there are two main methods for MSC transplantation: systemic (intravenous (IV) or intra-arterial (IA) injection) and local. Cell-mediated treatment options for OA have been limited to local administration. It is a valuable approach for OA as a disease that involves all of the synovial tissues such as cartilage, synovial membrane, and subchondral bone. Given the distinct histological and anatomical characteristics of AC, particularly its avascular nature, systemic injection is not an efficient method and local injection is the more direct way for AO cell therapy. AC is surrounded by a unique milieu — the synovial space. Local injection of MSCs in the synovial space enables the surface of the OA cartilage, which lacks access to stem cell sources, to be exposed to a high number of MSCs. In addition, this fluid space that contains synovial fluid provides for local cell injection without tissue damage at the site of the injection.

TABLE 1

Recent 5-years clinical trials with mesenchymal stem cells (MSCs) for treatment of articular cartilage defects without any targeting approaches.

Stem Cell	Autologous vs. Allogeneic	Number of Cells	Co-treatment	Patient No./ fallow- up (months)	Type of cartilage lesion	Mode of cell delivery	Results	Ref.
BMSCs	Allogeneic	LD: 50×10^6, HD: 150×10^6	HA	55/24	Knee OA	IA injection	Pain improvement and MRI evidence for meniscus regeneration in both doses vs. control	[82]
ADSCs	Autologous	3.7×10^6	–	37/26.5	Knee OA	Arthroscopy direct cell deposition	Improved IKDC, ICRS, KOOS and VAS scores in patients with small lesion size and BMI $\leq$ 27.5 kg/m^2	[83]
ADSCs	Autologous	LD: 10×10^6 MD: 50×10^6 HD: 100×10^6	–	18/6	Knee OA	IA injection	Improved pain, function and WOMAC score in high dose group confirmed by MRI and second look arthroscopy, histological evidence for hyaline like cartilage regeneration in high dose group.	[84]
BMSCs	Allogeneic	40×10^6	–	30/12	Knee OA	IA injection	Significant improvement in algofunctional indices and cartilage quality in MSCs group vs. control.	[85]
BMSCs	Autologous	40×10^6	–	12/24	Knee OA	IA injection	Remarkable improvement in clinical and quantitative MRI outcome during 2 years	[86].
BMSCs	Autologous	N	–	19 MACI 18 BMC/ 36	Knee cartilage patellofe moral chondral defect	Surgical delivery	Both MACI and BMAC revealed significant improvement in MRI and histological scores.	[87]
BMSCs	Autologous	N	MAST HA matrix	40 ACI 40 BMC/48	OLTs	Surgical delivery	ACI and MAST was equally effective for the treatment of OLT. Lower costs and better results for the 1 step procedure in MAST.	[20]
SDSCs	Autologous	47.2×10^6		10/48	Knee chondral defect	Surgical delivery	Significant clinical improvement, positive MRI results, and formation of hyaline like tissue	[88]
ADSCs	Autologous	4.3×10^6	SVF/FG scaffold vs. PRP/SVF	20 SVF-FG 20 SVF-PRP/286	Isolated focal defects in knee OA	Surgical delivery vs. IA injection	Significant improvement was reported in both groups. Better clinical results and IKDC score at 28 months after surgery for MSC s + FG.	[89]
BMSCs	Autologous	5×10^6	–	17/30	Hip, Knee, Ankle OA	IA injection	Improved VAS and WOMAC scores was revealed by MRI.	[90]
BMSCs	Allogeneic	25, 50, 75 and 150×10^6	HA	60/12	Knee OA	IA injection	Improvement of VAS, ICOAP, and WOMAC-OA scores only in 25 million cell dose group.	[19]

TABLE 1 (*Continued*)

Stem Cell	Autologous vs. Allogeneic	Number of Cells	Co-treatment	Patient No./ fallow- up (months)	Type of cartilage lesion	Mode of cell delivery	Results	Ref.
BMSCs	Autologous	8-9 $\times$ 10^6	–	3/60	Knee OA	IA injection	Movement and pain parameters was improved at 6 months. Global assessment were better at 5 years.	[91]
BMSCs	Autologous	N	MAST HA matrix	56/36	OLTs and ankle OA	Surgical delivery	Gradual clinical outcome improved during 36 months, Higher BMI and OA degree had attenuated healing outcome.	[92]
ADSCs	Autologous	N	SVF/FG scaffold	24/279	Isolated focal defects in OA knee	Surgical delivery	Significant improvement in IKDC, clinical and MRI scores/ outcome.	[93]
ADSCs	Autologous	N	MFX/ FG/SVF vs. MFX	40 MFX/ SVF/FG 40 MFX/27.4	Knee chondral defects	Surgical delivery	Better score for KOOS, pain and MRI for SVF compared to control SVF.	[94]
ADSCs	Autologous	LD: 2 $\times$ 10^6 MD: 10 $\times$ 10^6 HD: 50 $\times$ 10^6	–	18/6	OA	Single IA injection	No serious adverse events were reported 6 month post-injection. Four patients experienced transient knee joint pain. Low-dose ASCs significantly improved the pain levels and function.	[95]
ADSCs	Autologous	14 $\times$ 10^6	–	6/12	OA	Single IA injection	Statistically significant improvement in WOMAC and VAS scores.	[96]
BMSCs	Allogeneic	N	10 or 20% autologous chondrons	10/12	OA	Single defect site specific Implantation	Statistically significant improvement in clinical and MRI outcomes at 12 months, hyaline like regenerated cartilage originated only from autologous chondrons.	[97]
BMSCs	Autologous	LD: 10 $\times$ 10^6 HD: 100 $\times$ 10^6	HA	30/48	Knee OA	Single IA injection	Statistically significant improvement in VAS and WOMAC scores.	[98]

Abbreviations: BMSCs, bone-marrow-derived mesenchymal stem cells; LD, low dose; MD, mid dose; HD, high dose; IA, intra-articular injection; BMI, body mass index; HA, hyaluronic acid; *IKDC,* International Knee Documentation Committee; KOOS, Knee Injury and Osteoarthritis Outcome Score; *VAS,* visual analog scale; MACI, matrix-induced autologous chondrocyte implantation; ICOAP, intermittent and constant osteoarthritis pain; BMAC, bone marrow aspirate concentrate; MRI, magnetic resonance imaging; MAST, matrix-associated stem cell transplantation; OLT, osteochondral lesions of the talus; SDSCs, synovium-derived stem cells; ADSCs, adipose-derived stem cells; SVF, stromal vascular fraction; FG, fibrin glue; MFX, microfracture; PRP, platelet-rich plasma; WOMAC, Western Ontario and McMaster Universities Arthritis Index; N, not determined.

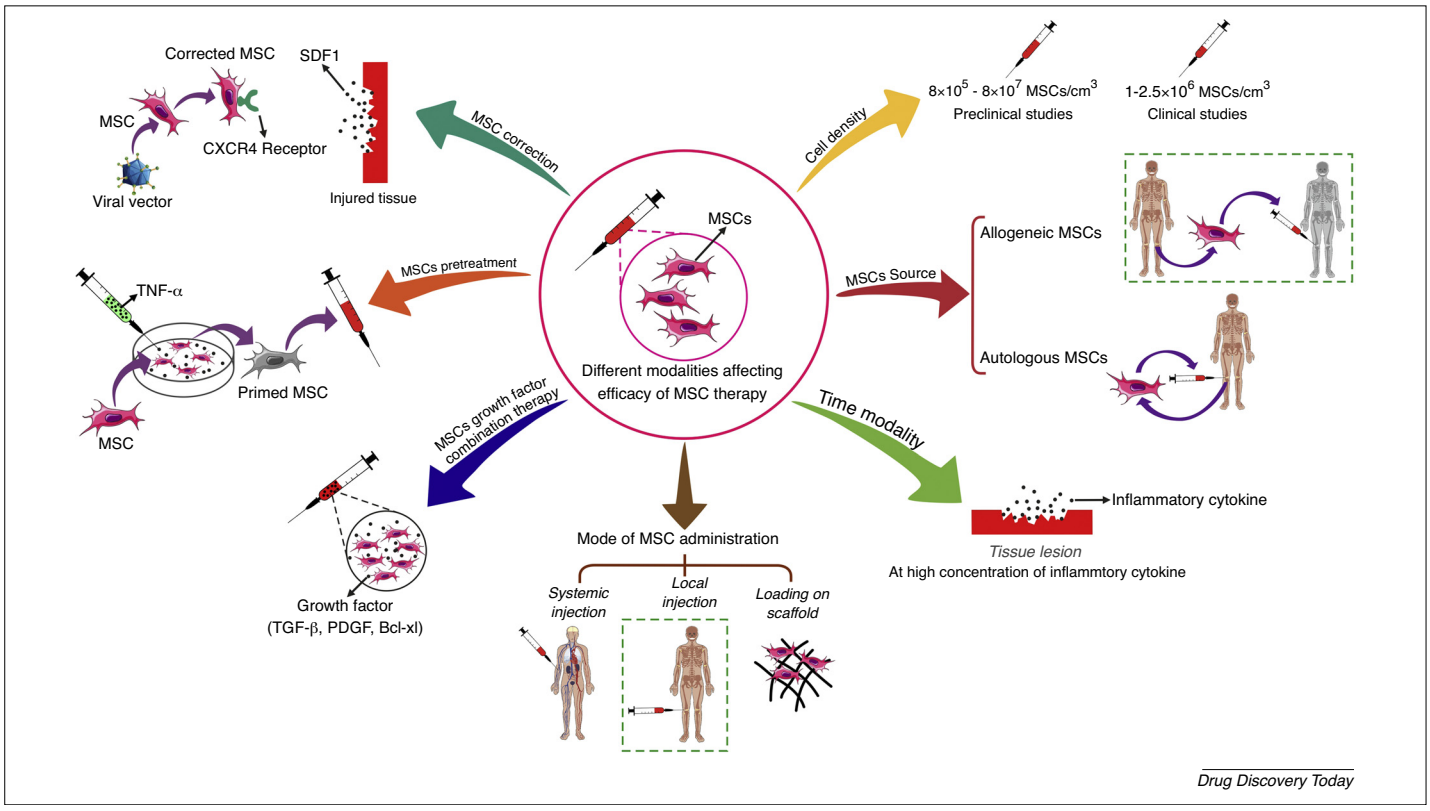

FIGURE 2

Schematic representation of factors that affect the efficacy of MSC therapy: focus on articular cartilage (AC) defects. Dotted squares represent preferable methods for treatment of osteoarthritis.

Genetic modification of mesenchymal stem cells

MSC gene correction in cell therapy approaches has been performed to promote their ability to secrete the desired factors and/or differentiation toward a particular fate such as to improve their chondrogenic efficiency in treatment of defected cartilage [32]. MSCs have been transfected with different genes involved in MSCs homing or migration such as the C–X–C chemokine receptor type 4 (CXCR4) [33,34]. Despite encouraging outcomes of cartilage regeneration in the preclinical setting; safety issues that include decreased MSCs viability; adverse immune response; mutagenesis and activation of oncogenes; preclude the use of genetically modified cells in the clinic.

Gene therapy techniques to perform mesenchymal stem cell correction

Gene correction is usually performed via viral or non-viral techniques. The most frequently used viral vectors for modification of human MSC genes are adenoviral and retroviral vectors. The retroviral vectors have high efficiency for long-term expression of modified genes, although they are integrated into cell DNA and therefore have mutagenesis potential. By contrast, the nonintegrative adenoviruses are much safer, yet with lower transgenic efficiency [35]. Despite the higher efficacy of viral vectors, their clinical application has been hampered by the safety concerns such as host immune response due to presence of viral genetic materials, high infectivity and production cost. In addition to viral based gene correction, genetic modification via non-viral vectors (such as cationic polymer and liposome) or physical methods (such as DNA microinjection) has been successfully performed and demonstrated lower host immune response, toxicity and oncogenicity [36].

In the case of cartilage regeneration, several attempts have been made to transfect MSCs using viral vector for a list of potentially useful cDNAs [e.g. transforming growth factor (TGF)-β, fibroblast growth factor (FGF), insulin-like growth factor (IGF-1), bone morphogenic proteins (BMPs), and epidermal growth factor (EGF)] [37]. Experimental data revealed that these genetically modified MSCs have been able to promote cartilaginous tissue formation after transplantation into AC defect. In a study, Peng et al., co-modified MSCs with IGF-1 and BMP-7, which resulted in increased expression of collagen II and improvement of rabbit knee AC lesion [38]. Recently, Yang et al. have applied a combination of MSC gene modification and tissue engineering to treat a full-thickness damaged joint cartilage in rats. They loaded modified MSCs which were transfected with adenovirus containing C-type natriuretic peptide (CNP) gene, onto chitosan/silk fibroin porous scaffold to repair the AC lesion. The findings revealed promising histological repair for focal AC defect [39].

In recent years, clustered regularly interspersed short palindromic repeat (CRISPR) technology has been developed to achieve gene inactivation, without the possibility of tumourigenicity, that can be delivered as RNA, plasmid, virus and protein. Given the multifactorial pathogenesis of OA, this technique has not been used directly for OA treatment. Indirectly, Yang *et al.* generated a stable chondrocyte lineage in which aggrecan gene was knocked out. They reported that generated cell line was able to stimulate host immune response and attenuated chondrosarcoma development after subcutaneous injection [40].

Mesenchymal stem cell conditioning

The process of MSC differentiation depends on the presence of special chemicals and a range of GFs [41]. MSC–GF interactions occur during the differentiation process or before the start of differentiation in order to form polarized cells that have increased differentiation potential. It has been reported that pretreatment of MSCs with inflammatory cytokines such as TNFα and IL-1β, as (NF)-κB pathway inducers, enhanced the survival rate, increased differentiation and migration capacity, as well as increased engraftment into the target tissue followed by transplantation [42].

Mesenchymal stem cell/growth factor combination therapy

The combination of various GFs with MSCs have been used as an efficient strategy to improve cell therapy efficacy [43]. Various types of factors most often used in the preclinical setting include cartilage-derived morphogenetic protein 1, TGF-β, BMP-2, anti-apoptotic proteins (Bcl-xL), connective tissue growth factor, platelet-derived growth factor (PDGF), IGF-1 and SOX 5, 6, 9 [44,45]. The results from almost all of these studies indicate a promising improvement of cartilage defects; particularly more-effective treatment of large osteochondral lesions. Recently, Nandeesh *et al.*, conducted a study to examine the effect of stem cells with platelet-rich growth factors in clinical setting for cartilage regeneration. They reported improvement of cartilage regeneration including cartilage regrowth and motor function after 2-year follow-up [46].

How does the body control migration of mesenchymal stem cells? A clue for targeted cell delivery

To date, numerous efforts have been made to increase the efficiency of MSC therapy; however, cell engraftment efficacy into the damaged target tissue remains low. Reports have confirmed that the survival rate of MSCs at the damaged site is shorter than the time that MSCs need to contribute to the healing process [47]. In addition to the primary issues with cell therapy, two other challenges affect the outcome of current cell therapies. First, inefficient integration between therapeutic cells and the damaged tissue. Next, lack of a targeted delivery system to transfer cells towards the injured tissue.

Upon tissue injury in human body, MSCs naturally migrate stochastically or mechanically towards injured/inflamed sites, though the underlying mechanisms are unknown. Local vasodilation changes passively regulate MSC migration, whereas mechanical conduction is mediated by soluble chemokines and GF gradients whose related receptors are present on the MSC surface. Among these receptors, the importance of the CXCR4 and its ligand SDF-1 have been highlighted. It has been shown that SDF-1 up-regulates at the injured site and affects the MSC migration in a dose-dependent manner [48].

Regeneration of cartilage tissue through homing of endogenous cells has been reported in defective AC, including in OA [49]. Such reports have suggested that the synovial tissues have the potential for endogenous stem cell recruitment, which results in partial regeneration even in the absence of exogenous cell transplantation. Nevertheless, these mechanisms cannot provide a guarantee for autonomous regeneration. Systemically administered MSCs have been shown to aggregate at the site of damaged tissue [50]. Therefore, modulation of the homing process would increase cell delivery towards the target tissue.

Future trends to address challenges with cell and stem cell therapy of osteoarthritis

Cell-targeting approaches

Despite the mentioned mechanisms (stochastic or mechanistic mechanisms) that contribute to endogenous homing of MSCs, the majority of exogenous cells (>99%) are unable to reach and engraft inside the target [51]. As a result, it is evident that endogenous chemotactic mechanisms alone have inefficient delivery of exogenous administered cells (Fig. 2A). Cell-based therapies should have highly efficient cell delivery approaches for cell migration, adhesion and growth prior to the use of MSCs in the clinical setting [52]. Different strategies employed to enhance the homing of MSCs to desired tissues are described below. Moreover, the opportunity of each strategy for future AC regeneration and OA treatment are discussed.

Cell surface modifications

Surface modifications involve any modification that leads to expression of particular receptors on the surface of MSCs with binding affinity to specific ligands on the target site [53]. In recent years, this approach has been utilized as an immune targeted cancer therapy to increase the tumor-targeting capability of MSCs [54,55].

Antibody-mediated surface modification

Cell surfaces are functionalized through conjugation by bispecific Abs, whereby an Ab against the specific antigen on target tissue conjugates to another Ab that has an affinity to a certain surface marker of the therapeutic cells [56,57]. Lipidated protein G followed by Ab incubation, also known as ''cell painting'', is an alternative method for bispecific Ab. In the lipidation method, either a palmitated-conjugated protein A (PPA) or B (PPB) is attached to the Fc domain of the Ab [58]. In a study by Dennis *et al.*, the cell painting method, as lipidated protein G, was intercalated into the pre-chondrocyte cell membrane and subsequently incubated with Abs to cartilage matrix antigens. They observed that painted cells preferentially bound to the cartilage explants in the full thickness defect rabbit model without any adverse effect on cell viability and chondrogenic potential [59]. Similarly, *in vivo* injection of armed MSCs with Abs against AC matrix antigens, would potentially increase the adhesion of intra-articular-injected MSCs to fibrillated cartilage and prevent the off-target attachment of cells to other articular receiving surfaces (Fig. 2B).

Peptide mediated surface modification

Cell targeting peptides (CTPs) provide the possibility to target the tissue of interest with a high degree of specificity [60]. CTPs have the ability to be easily conjugated to different carriers such as NPs or cells to deliver various therapeutic agents, particularly for cancer therapy. Several research groups have exploited CTPs for treatment and diagnosis of colorectal, pancreatic, lung, gastric, prostate and breast cancers with significant therapeutic outcomes [54,55]. Numerous reports have discussed AC repair using CTPs. Pi *et al.* identified a short peptide sequence (CAP: DWRVIIPPRPSA) with the binding affinity to chondrocytes. They introduced a non-viral vector in which the florescent-labeled CAP was covalently conjugated to polyethylenimine (PEI) and subsequently injected into rabbit knees to target hyaline cartilage. They showed that higher concentrations of fluorescein isothiocyanate (FITC)-labeled CAP compared to scrambled peptide were attached to chondrocytes. In addition, green fluorescent protein (GFP) gene delivery by CAP–PEI indicated significant cartilage targeting transfection [61]. Similarly, Cheung *et al.* used a phage

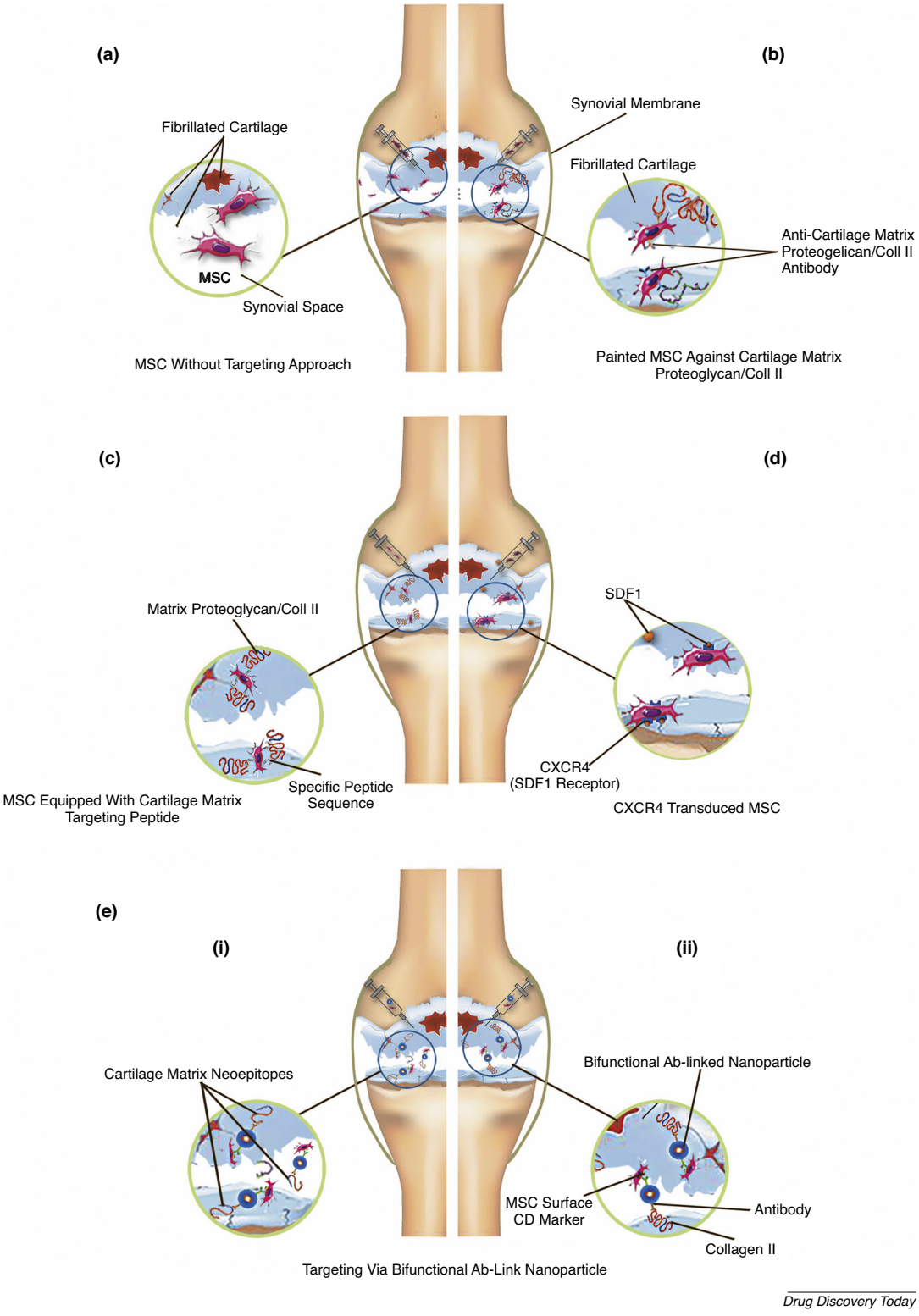

FIGURE 3

Potent approaches for targeted mesenchymal stem cell (MSC) delivery toward fibrillated articular cartilage (AC). (a) MSC therapy without targeted approach. Intra-articular injected MSCs attach to different accepting surfaces in the articular spaces, which include synovial membrane, ligaments, and bone, or are suspended in synovial fluid. Only a small portion of the cells are attached to the injured cartilage. (b) ``Cell painting" technique. MSC surface functionalization through conjugation by bispecific antibodies (Abs). An Ab against the specific targeted site's antigen (anti-cartilage matrix proteoglycan/Col II) is conjugated to another Ab which targets a certain surface marker that belongs to therapeutic cells/MSCs. (c) Peptide mediated surface modification. Cell targeting peptides (CTPs) as high potential targeting ligands can be easily conjugated to different carriers as nanoparticles (NPs) or cells and deliver various therapeutic agents or cells to appropriate targets/receptors. When the fibrillated cartilage is targeted, MSCs can be supplied with cartilage matrix targeting peptides and attach to the cartilage ECM. (d) Genetically mediated surface modification. MSCs are transduced with genetic materials [C–X–C chemokine receptor type 4 (CXCR4); SDF1

display library to identify two 12-amino-acid cartilage-binding peptide sequences (RLDPTSYLRTFW and HDSQLEALIKFM) that specifically bind to both the chondrocyte and the cartilage ECM. They used the culture chondrocyte system instead of immobilization of a specific chondrocyte protein on the solid surface to simulate the chondron (chondrocyte and its pericellular matrix) microenvironment [62]. Their results showed a strong attachment of the peptide to the chondrocyte surface. In 2015, Hu *et al.* developed a small construct to deliver a cathepsin D protease inhibitor, pepstatin A, as a drug for reduction of cathepsin D protease activity and deceleration of cartilage degradation. In this model, the drug carrier, a 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid amide, was conjugated with drug and a peptide sequence against collagen II. Local injection of this complex into the knees of mice showed increased drug retention time of ~20-times longer than scrambled peptide (7 days versus 6–8 h). Consequently, this construct successfully inactivated cathepsin D protease in targeted knee AC [63].

Merits of CTPs include highly specific targeting, ease of synthesis, small size, low molecular weight, and high biocompatibility. There is the possibility to concurrently attach multiple ligands to the cell surface or other carriers. The unique features of CTPs, among other targeting techniques would make them promising for prospective cell delivery in the clinical setting. Specifically for AC, hyaline cartilage ECM and chondrocyte-specific peptides could be integrated to MSCs surface and injected into articular space (Fig. 2C). Although no cytotoxicity has been observed regarding their application as a surface modification tool, further studies are needed to confirm their clinical safety.

Genetically mediated surface modification

Genetically modified cells have the ability to express specific ligands against target tissue, leading to their localization in the desired site [64]. Given the important role of the CXCR4–SDF-1 axis in endogenous homing routes, the efficacy of this strategy has been addressed in several studies. For example, CXCR4-transduced MSCs were used in an infarcted heart [65] and bone loss in an ovariectomized mouse [66], which homed towards the SDF1 gradient. In these studies, MSC trafficking was increased at the injury site, which accelerated tissue regeneration. A literature search has revealed no outstanding attempt regarding surface modification by genetic manipulation for the therapeutic cells that home to either focal or diffuse cartilage defects. The inflammatory status caused by either trauma or during OA development in joints may provide the possibility for genetically modified MSCs to migrate toward proinflammatory cytokines and localize there (Fig. 3d). Nevertheless, there are several issues associated with the cell transfection process required for gene therapy, such as time and financial cost, low efficacy, and oncogenicity.

Selectin-mediated surface modification

Vascular lining endothelial cells naturally express an inducible cell adhesion molecule, P-selectin (CD62P), in response to various pathologic mediators. P-selectin in turn mediates leucocyte rolling and recruitment via attachment to their respective surface ligands [67]. Xia *et al.* were the first to fucosylate the surface of human cord blood cells to increase their engraftment in bone marrow as a consequence of binding to P-selectin and E-selectin [68]. Liao *et al.* created genetically engineered MSCs to express two homing ligands and succeeded in delivery of the anti-inflammatory cytokine IL-10 to inflamed tissue [33]. Selectin-directed cell targeting has not been applied in orthopedic diseases because this homing mechanism is related to the vascular network. Cartilage, an avascular tissue, cannot be targeted by this strategy. Nevertheless, it is proposed that selectin-directed cell targeting could be beneficial once cells are systemically injected for cartilage regeneration, which would increase cell density in the articular space around the fibrillated cartilage.

Targeted cell delivery via incorporation of nanoparticles

There are several drawbacks associated with cell-surface modifications that hamper their translation to the clinic. For example, covalent conjugation of Abs or peptides to the cell membrane can affect the function of membrane proteins and probably alter the signaling patterns, which would result in decreased ligand–receptor binding and possible changes to the cell fate [53]. Therefore, it is essential to develop safe cell targeting approaches.

NPs are widely used in various medical fields, including drug delivery, gene therapy, imaging and targeted drug or cell therapy. NPs exhibit unique size-dependent physicochemical properties. They have the flexibility to withstand various modifications on their surface and acquire distinct biological and physicochemical properties for a variety of applications. Likewise, homing ligands could be attached to the NP surface that specifically bind to respective receptors at the target site. NPs create a 3D microenvironment for exogenous or endogenous cells at the injury site, which enables them to interact efficiently interact with ECM components, adjacent cells, or even GF — all of which are requisite for cell engraftment and differentiation [69,70]. Currently, many commercial NPs are available for use in therapeutics for cancer and tissue regeneration purposes.

NPs for targeted cell delivery must be carefully engineered to correctly express the relevant ligands with optimal density. To date, several studies have been conducted that deliver MSCs using NPs towards targeted tissues such as the heart, spine, eyes and liver [71,72]. Recruitment of iron NP-labeled MSCs that use an external magnetic force has been extensively used by recent studies. Cheng *et al.*, labeled cardio-sphere-derived cells with superparamagnetic microspheres and directly injected them into a rat model of an infarcted myocardium. The results revealed enhanced cell retention time and engraftment into the infarcted zone in the group that used superimposed magnetic force [73].

In terms of target AC, the cell delivery technique should provide more cell numbers engrafted in cartilage metrics, which increase cell participation in tissue regeneration. NPs, particularly magnetic NPs,

receptor] which enable them to localize around the SDF1 density range. (e) MSC delivery by incorporation of bifunctional Ab-linked NPs. (i) Against cartilage matrix neo-epitopes. In this approach, the central nanoparticle core covalently attaches to two types of Abs — one against the surface CD marker of the MSC and the other against cartilage matrix neo-epitopes. However, some of these neo-epitopes are released into the synovial fluid and may cause ectopic binding of the MSC-carrier nanoparticle to floating neo-epitopes. (ii) Against cartilage matrix proteoglycan/collagen II. A second type of Abs which bind to the surface of MSC-carrier NPs can be against cartilage matrix components (proteoglycan or collagen II). These intact ECM proteins are trapped within the cartilage matrix and can accurately direct the MSC-carrier NPs toward the hyaline cartilage tissue.

have been utilized to direct and track MSCs within fibrillated cartilage [74]. Feng *et al.* used superparamagnetic iron oxide particle (SPIO)-labeled MSCs and external magnetic force to deliver MSCs to a centrally created defect in human osteochondral fragments *in vitro*. MRI and histological analysis confirmed higher numbers of cells after the application of a magnetic force at the defect site [75]. In a preclinical study, the same technique was used to conduct the labeled MSCs into a full-thickness cartilage defect created in the center of the patella. The results confirmed the role of exogenous MSCs in cartilage repair as evidenced by improved histological score [76].

Considerations in articular cartilage cell and drug targeting: nanobiotechnology-based treatment options

Different MSC-receptive tissues exist in the osteoarthritic articular space that include the bone, synovial membrane and possibly cartilaginous fragments that float within the synovial fluid. Thus, combined cell therapy with nano targeting approaches could be an appealing strategy to achieve an appropriate outcome. Until now, various nanocarriers such as micelles, liposomes, and NPs have been used for drug delivery purposes for degenerated AC [77,78]. As a novel approach, these nanocarriers as well as nanoghosts (NGs) would be conjugated to Abs, peptides, or other ligands against specific cartilaginous markers to enhance targeting and eventually result in a positive clinical outcome. There are a number of distinct candidate markers, known as neoepitopes, attributed to osteoarthritic cartilage that could be considered for specific targeting of cartilage tissue. Neoepitopes are generated through cartilage degradations by matrix metalloproteinases (MMPs), and include VDIPEN and NITEGE, keratan sulfate proteoglycan epitope, C-propeptide of type II collagen (CPII), aggrecan 846 epitope, collagenase-generated cleavage epitope from type II collagen (CIIC) and crosslinked peptides from the C-telopeptide domain of type II collagen (col II CTX) [79,80]. Most of these neoepitopes are considered blood and synovial fluid markers for biochemical diagnosis of cartilage-degeneration-associated diseases such as OA. These enzyme generated neoepitopes are locally generated at the surface of ulcerous cartilage; thereafter, they release into synovial fluids and then into the bloodstream. The existence of these markers in blood makes them inappropriate for site-specific targeting (Fig. 3e i). Selection of compounds that account for a large percentage of cartilage volume, such as collagen type II that constitutes 10–30% of the wet weight of normal AC or proteoglycans (chondroitin sulfate and keratan sulfate comprise 3–10%) would be a safer and more feasible alternative (Fig. 3eii). More importantly, binding of MSCs to collagen II fibrils would improve the integration between tissue-engineered-to-native cartilage surfaces, which is considered to be one of the major challenges with cell therapy.

Nanocarrier size is another important feature of NPs for AC targeting. Nanocarriers <38 nm in size have the capability to penetrate into the ECM of cartilage or enter into chondrocytes, whereas NPs larger than 96 nm do not [81]. Another promising point is that contrary to the surface-modification-directed targeting, cell delivery via incorporation of NPs also allows endogenous MSCs, alongside exogenous cells, to be trapped and bind to the target site. In other words, NPs conjugated by targeting tools could independently mediate the delivery of endogenous recruited MSCs without any exogenous MSCs administration.

Concluding remarks

In OA, lack of an efficient cell delivery system is one of the major issues for production of normal hyaline AC. It is anticipated that improvements of targeting technology in the context of molecular biology could facilitate OA treatment. Several clinical and experimental studies have demonstrated the potential use of targeted cell therapy for cartilage defects. Nonetheless, enquires that must be addressed include the management of integration of MSCs into fibrillated cartilage. The necessity of increased MSC engraftment to fibrillated cartilage which increases the cell contribution to tissue repair is discussed in new approaches. Additionally, it is important to know which strategy is preferred to move to the clinic setting — direct targeting with MSC surface modification or indirect targeting via incorporation of engineered NPs. Targeting techniques are based on modifications directly on the surface of MSCs or indirectly on an engineered nanomediator, and lead to expression of appropriate superficial ligands against respective specific receptors at the target site. Next, it is necessary to determine which markers of healthy hyaline cartilage would be more reliable to increase the therapeutic targeting efficiency of AC lesions. To have a targeting approach with elevated selectivity, specific markers of the tissue of interest are needed. Targeted therapy strategies usually rely on unique features of specific damaged, but not healthy, tissues. However, most unique markers of degenerated AC (neoepitopes of cartilage degradations) are not reliable to design targeting routes due to their release from cartilage. Therefore, only when cells are to be injected locally in the articular space, which is the main common composition of hyaline cartilage, would type II collagen appear to be the more reliable ligand for direct or indirect binding to therapeutic cells. In addition, binding the cells to cartilage ECM may improve the challenge of cell integration into cartilage remnants. In the future, it would be appealing to develop a targeting system to trap the endogenous cells to accelerate tissue regeneration, even in the absence of exogenous cells.

GLOSSARY

Connective tissue Tissue of mesodermal origin is located between other tissues and consists of three main components: ground substance, fibers, and cells. Its multiple roles include maintenance of the body's structural integrity and protection of organs.
Proteoglycans They are present in connective tissue as a major component of an animal's extracellular matrix. Proteoglycans are formed from core proteins that bind to mucopolysaccharide groups.
Microfracture This surgical technique is considered the gold standard therapy to treat cartilage defects. Microfractures are most often performed for knee joints. This minimally invasive procedure generates tiny fractures in the subchondral bone to access bone marrow cell sources.
Cell therapy Also known as cytotherapy, is the local or systemic injection of a medicinal product that contains cellular material (intact, living cells) into a patient.
Growth factors Extremely heterogeneous proteins that can determine a cell's fate via regulation of various cellular processes of cell survival, proliferation, differentiation, and migration by endocrine, paracrine or autocrine mechanisms.
Vascularization Vasculogenesis or angiogenesis, means to make or become vascular, and develop blood vessels and capillaries in an organ or tissue.
Cell recruitment The activation of additional numbers of responsive cells toward a natural cell population in tissues following increased intensity of a stimulus.
Allogenicity Cells, tissues, or organs should be allogeneic for successful transplantation from a genetically different donor to a recipient of the same species.
Inflammatory cytokines Or proinflammatory cytokines are a type of signalling molecule produced predominantly by immune cells. They are positive mediators of inflammatory reactions.
Gene correction A genetic engineering tool for repair and mutation of DNA in the genome of a living organism to generate a new specific sequence as well as discover new gene functions.
Cell engraftment One of the steps in successful cell/stem cell therapies. Cell engraftment occurs when the transplanted cells/stem cells are accepted by the body and incorporate into the target tissue to generate new differentiated cells, extracellular matrix, and develop regenerated tissue. The transplanted cells/stem cells may be taken from a patient or from a donor.
Functionaliztion The addition of new capabilities or functions to a material by changing its surface chemistry. For example: the addition of a functional group to a material's surface.
Bispecific antibodies Bispecific antibodies or immunoglobulins contain two different antigen-binding sites that simultaneously bind to two different types of antigen.
Fibrillated cartilage A cartilage with initial degenerative morphologic changes caused by osteoarthritis. In the early stages of the disease, the cartilage surface splits into vertical clefts or forms fibrils between groups of cartilage cells.
Nanoparticles Inorganic materials with all dimensions less than 100 nm. Compared to larger particles, nanoparticles have a greater surface area per weight by which they have unique material characteristics as well as practical applications in various areas.

Conflicts of interest

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