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STRATEGIES FOR IMPROVED TARGETING OF THERAPEUTIC CELLS: IMPLICATIONS FOR TISSUE REPAIR

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Abstract

Multipotent mesenchymal stem cells (MSCs) have been suggested as a suitable cell source for cell-based treatments for diseases such as osteoarthritis due to their ability to differentiate towards chondrogenic and osteogenic lineages. MSCs can be obtained from a variety of tissue sources, are scalable for mass-production and immuno-privileged enabling their use for allogeneic cell therapy. However, recent pre-clinical studies and clinical trials point to the necessity of increasing engraftment and efficacy of MSCs. This review explores how cell surface modification of the cells can improve homing of MSCs and summarises the use of nanoparticles to enable gene delivery by stem cells as well as facilitate *in vivo* imaging. The use of advanced biomaterials and how they can be applied to reduce the overall dose of MSCs during therapeutic interventions while achieving optimal targeting efficiency of cells to the diseased sites are addressed. Particular attention is paid to methods that improve engraftment of MSCs to cartilage and research describing combinatorial approaches of particle-based cell therapies for improved regeneration of this tissue is reviewed. The use of such approaches will add to the array of potential regenerative therapeutics for treatment of osteoarthritis.

Keywords: Mesenchymal stem cells; chondrocytes; nanoparticles; targeting approaches; cell surface markers; cartilage repair; osteoarthritis; regenerative medicine.

Introduction

Mesenchymal stem cells

Mesenchymal stem or stromal cells (MSCs), originally identified in bone marrow, are multipotent adult stem cells capable of self-renewal and differentiation (Pittenger *et al.*, 1999). Although originally thought to be restricted to bone marrow, MSCs have been isolated from trabecular bone (Nöth *et al.*, 2002), adipose tissue (Yoshimura *et al.*, 2006), synovium (De Bari *et al.*, 2001), skeletal muscle and cord blood (Wagner *et al.*, 2005). They have the capacity to differentiate into cells of connective tissue lineages including bone, fat and cartilage (Pittenger *et al.*, 1999). Whether MSCs differentiate in the context of tissue repair or exert their therapeutic effects via trophic or paracrine factors at the site of the damaged tissue (Caplan and Correa, 2011), successful engraftment and retention of the cells is prerequisite. The potential of MSCs to regenerate damaged tissue has also been attributed to the presence of chemokine receptors on the surface of MSCs enabling these cells to migrate towards gradients of growth factors secreted by damaged tissues (Mirosou *et al.*, 2011) or tumours (Song and Li, 2011). This migration has recently been exploited to mark breast cancer for radionuclide treatment using sodium iodide symporter transduced MSCs as the targeting vehicle for tissue destruction (Dwyer *et al.*, 2011). Despite the various mechanisms enabling MSC homing, the majority of implanted cells are trapped in draining organs (liver, lung and spleen) and only a very small fraction migrate to sites of tissue damage, with rates of engraftment depending on the method of administration and disease models (Freyman *et al.*, 2006; Wilson *et al.*, 2010; Curley *et al.*, 2011). Indeed, the inherent variability in bio-distribution of MSCs post implantation has been described as a potential impeding factor in translation of MSC-based therapies to clinical practice (Web ref. 1). Thus, several cell targeting efforts have been examined, as illustrated in Fig. 1, to improve the delivery and retention of MSCs at the desired tissue/site of injury post injection which will be reviewed here with a focus on novel strategies that include engineered cell homing mechanisms and incorporation of nanomaterials.

Improving MSC homing/targeting with cell surface modifications

Improving the targeting and engraftment of MSCs is of the utmost importance to their potential use as a cellular therapy and for the progression of MSC-based therapies to the clinic. An extensively investigated approach is modification of the cell surface by expressing receptors

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appropriate to the desired site of injury. This targeting system is primarily based on many of the previously published immunotargeting studies and more recently in cancer therapeutics (Table 1). Enhancing the tumour targeting abilities of MSCs to achieve site-specific retention has been the focus of several recent studies. Komarova *et al.* (2010) examined whether over-expression of a tumour specific artificial receptor to erbB2 on transplanted MSCs could increase their homing in an ovarian tumour xenograft model and a transient transgenic mouse model that expressed erbB2 in the lungs. Increased targeting affinity of MSCs to erbB2 expressing cells was shown in both *in vivo* models presenting a strategy that could be applicable to target MSCs to other tissue types (Komarova *et al.*, 2010). In the same way, Balyasnikova *et al.* (2010), investigated the use of modified MSCs for specific cell targeting to human xenograft malignant gliomas. These authors focussed on over expression of a mutated version of the epidermal growth factor receptor (EGFRvIII), a distinctive hallmark of primary malignant glioblastomas. MSCs were genetically modified to express EGFRvIII specific single chain antibodies on the cell surface to enhance their targeting capabilities to EGFRvIII expressing tumours. This strategy proved successful for cell specific targeting both *in vitro* and *in vivo* and prolonged retention in gliomas (Balyasnikova *et al.*, 2010). Enhanced accumulation of MSCs was also described in a model of pancreatic carcinoma where tumour stroma-induced CCL5 overexpression by the MSCs was utilised to visualise enhanced reporter gene activity under the control of a CCL5 promoter and transgenic herpes simplex virus thymidine kinase expression reduced the growth of the tumour (Zischek *et al.*, 2009). Similarly, a very interesting paper demonstrated genetic modification of MSCs resulting in overexpression of the chemokine receptor CXCR4 on their surface. These authors showed enhanced homing of these cells to ischemic myocardium following systemic administration but more significantly improved post-myocardial infarction recovery of left ventricular demonstrating clinical efficacy in a rat model (Cheng *et al.*, 2008).

Integrins are essential for tumour metastasis and angiogenesis (Ruegg and Alghisi, 2010) and the manipulation of integrin receptors and their effects on cell migration, invasion and homing has also been explored. In particular, integrin $\alpha 4$ has been shown to play an important role in homing of haematopoietic stem cells (HSC) to bone since transplanted HSC failed to engraft when bone marrow was incubated with anti-integrin $\alpha 4$ antibody (Zanjani *et al.*, 1999). Based on this, a study by Kumar and Ponnazhagan hypothesised that enrichment to the progenitor phenotype and bone homing signal on *ex vivo* cultured MSCs could enhance reconstitution in bone after systemic transplantation. Transient, ectopic expression of integrin $\alpha 4$ on MSCs increased their homing to bone, but even more importantly, decreased the number of cells that engrafted in lung (Kumar and Ponnazhagan, 2007). This is noteworthy as aggregation of MSCs in the lung is an unremitting unease associated with intravenously administered MSCs. A seminal study, recently published by Guan *et al.* (2012) exploited the expression of integrins

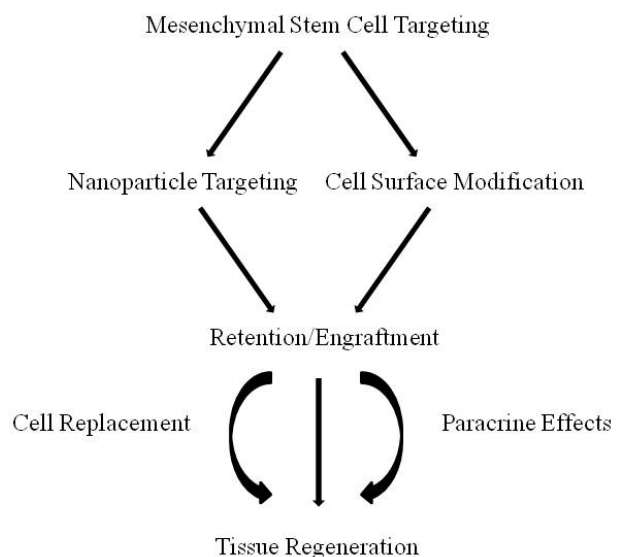


Fig. 1. Schematic depicting the steps required for MSC targeting. Cell surface modifications and incorporation of nanomaterials are two common methods used to enhance targeting of MSCs. It is proposed that once MSCs are retained at the desired site of action they can aid in the process of tissue repair either by replacing damaged cells/tissue or exerting their therapeutic effects via paracrine/trophic mediators.

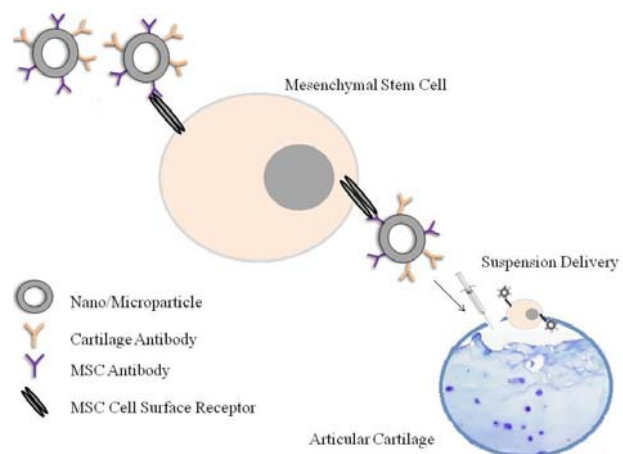


Fig. 2. Nano/microparticle mediated MSC targeting to articular cartilage. To enhance targeting to articular cartilage, nanoparticles can be bi-functionalised with antibodies to therapeutic cells such as MSCs and degraded cartilage on the premise that co-localisation of cells at the site of damage will enhance tissue repair.

on MSCs to direct these cells to the bone surface by using a unique dual targeting molecule, LLP2A-ALE. They developed a synthetic peptidomimetic ligand (LLP2A) against integrin $\alpha 4\beta 1$, thereby binding MSCs and to this conjugated a bisphosphonate (alendronate, Ale) with a high affinity for bone. Using several pre-clinical models including a xenotransplantation model they demonstrated efficient homing and retention of MSCs to bone. Furthermore, following injection of MSCs they found improved bone formation rates at both the endocortical and trabecular surfaces whilst bone formation rates at

Table 1. MSC cell targeting in immunotherapy

Cell Source	Vector/Plasmid	Tumor type	Targeting outcome	Reference
mMSC	(Ad-RGDFKN)	C26 (H-2d) - murine colon adenocarcinoma cell line, LLC (H-2b) Lewis lung carcinoma cell line	MSC/RGDFKN efficiently targeted tumoral tissue and suppressed the number of lung metastases	(Xin <i>et al.</i> , 2009)
mMSC	CCL5-TK	Orthotopic Pancreatic Carcinoma Model	Enhanced targeting of MSCs to tumor environment using the CCL5 promoter with selective expression of the therapeutic gene	(Zischek <i>et al.</i> , 2009)
hMSC	AdRGDpK7. C6.5luc	SKOV3ip1 (human ovarian carcinoma cell line) and Ovarian xenograft mode	Enhanced MSC-AR binding <i>in vitro</i>	(Komarova <i>et al.</i> , 2010b)
hMSC	scFvEGFRvIII	U87 glioma cells (expressing EGFRvIII)	Prolonged retention in EGFRvIII expressing glioma compared to control groups	(Balyasnikova <i>et al.</i> , 2010)

the periosteal surface was also improved in the group of animals that received LLP2A-ALE. This study elegantly demonstrated efficient MSC targeting to bone and could potentially transform MSC treatment for bone degenerative diseases (Guan *et al.*, 2012).

Other mechanisms whereby HSC or haematopoietic progenitor cells (HSPCs) home to bone include the expression of E-selectin ligands (Frenette *et al.*, 1998; Katayama *et al.*, 2003) and CXCR4 (Sharma *et al.*, 2011) the receptor for CXCL12. Sackstein and co-workers modified the surface of MSCs by enzymatically converting the native CD44 glycoform, endogenously expressed on MSCs, to confer potent E-selectin/ L-selectin binding affinity (Sackstein *et al.*, 2008). This innovative approach to mimic HSPC function on the MSC surface resulted in infiltration of the marrow within hours post-infusion. These findings establish that the HCELL glycoform of CD44 confers tropism to bone and reveals a readily translatable approach for programming cellular trafficking by chemical engineering of glycans (Sackstein *et al.*, 2008; Sackstein, 2012). In another study, MSCs were mixed with biotinylated lipid vesicles and fusion of the lipids with the MSC resulted in efficient biotinylation of the MSC surface. After the addition of streptavidin and biotinylated sialyl Lewis X (SLeX) carbohydrate motifs to the modified MSC surfaces, improved rolling interactions compared to unmodified MSCs were observed on P-selectin surfaces, one of the initiating steps of firm adhesion and of cell homing. Thus, modification of the cell surface with lipid vesicles can be used to efficiently immobilise adhesion ligands and potentially target systemically administered cells to sites of inflammation (Sarkar *et al.*, 2010).

Surface functionalisation by conjugating antibodies is also an attractive approach to promote cell targeting and innovative work by Lee and co-workers described an interesting and more importantly a clinically effective approach. A bispecific antibody (BiAb) combination was constructed and examined for its ability to increase the targeting of human CD34⁺/CD45⁺ HSCs to infarcted rat myocardium. In this approach, an anti-human common

leukocyte antigen (CD45) antibody was chemically linked to anti-rat myosin light chain (MLC) antibody. The cells were armed with the bispecific antibody, injected intravenously 2 days after transient ligation of the left anterior descending artery and were found to specifically localise in the infarcted region of the rat heart. These cells engrafted and persisted with some undergoing transdifferentiation to adopt a myocyte-like morphology while others localised to vascular structures. Most importantly, improvements in cardiac function were also observed in the animals that received the BiAb functionalised CD34⁺/CD45⁺ haematopoietic cells *versus* animals that received unarmed CD34⁺ cells (Lee *et al.*, 2007).

Addressin, also known as mucosal vascular addressin cell adhesion molecule 1, is an extracellular protein of the endothelium of venules. The protein encoded by this gene is an endothelial cell adhesion molecule that interacts preferentially with the alpha 4 beta 7 integrin or L-selectin on myeloid cells to direct leukocytes into mucosal and inflamed tissues (Berg *et al.*, 1993; Berlin *et al.*, 1993; Tanida *et al.*, 2011). Ko and colleagues investigated the premise that coating MSCs with anti-addressin antibodies could achieve efficient delivery of MSCs to sites of inflammation. Using an *in vivo* model of inflammatory bowel disease, they successfully showed that cell coating with the specific antibody showed increased efficacy of treatment and increased delivery of stem cells to inflamed organs (Ko *et al.*, 2010). This group have also looked at improving MSC targeting to endothelial cells using a similar strategy (Ko *et al.*, 2009).

Kean *et al.* (2012) more recently reported on a novel single step peptide based targeting strategy for directing MSCs to ischemic myocardium. They assessed the cell homing ability of MSCs modified with a variety of targeting peptide ligands in a mouse myocardial infarction reperfusion model. Histological analysis of the ischemic heart tissue demonstrated enhanced localisation of the peptide-targeted MSCs to sites of damaged tissue compared to unmodified MSCs (Kean *et al.*, 2012).

Table 2. Examples of particles and their applications in biomedical research and therapy.

Type of particle	Characteristics of particle	Applications	Reference
Lipid- based particles	Consist of aqueous compartment surrounded by lipid bilayer membrane; particles have adjustable sizes, surface chemistry and target specificity	Gene delivery (Lipoplexes) for the genetic modification of cells in biomedical research Antibody-targeted lipidic nanotherapeutics	(Resina <i>et al.</i> , 2009, Kirpotin <i>et al.</i> , 2012)
Dendrimer & Polymers	Dendrimers: Macromolecules with highly-branched three-dimensional structure composed of a core, branches and terminal end groups e.g. Polymer Poly(propylene sulphide) (PPS) with a size of 31-38 nm; able to penetrate the extracellular matrix	Especially Polyamidoamine (PAMAM) and Poly(propyleneimine (PPI) dendrimers have been widely used in gene delivery Biofunctional polymer nanoparticles for intra-articular targeting and retention in cartilage	(Xu <i>et al.</i> , 2010, Rothenfluh <i>et al.</i> , 2008)
Viral vectors	Several types of viruses can be distinguished from each other based on their biology, size and specificity to hosts (e.g. adenoviruses, adeno- associated viruses, lentiviruses and hybrids thereof)	Cancer therapy and gene delivery	(Gomez-Manzano <i>et al.</i> , 2012)
Antibody- or peptide conjugated particles	Peptides or antibodies or fragments thereof can be obtained from phage display or hybridoma technology	e.g. Nanobodies as therapeutics and imaging tools	(Vaneycken <i>et al.</i> , 2011)

Modification of the cell surface of cells has been shown to be feasible as a strategy to increase targeting efficiencies and shows great promise for the development of treatment options for a wide variety of diseases. However, there are several limitations associated with cell surface modifications to enhance homing and retention of MSCs. Firstly, genetic modification of the cells, especially in the case of viral vectors, raises potential safety and regulatory concerns and gives rise to questions as to the applicability in translational approaches. Furthermore, the addition of surface modifications by covalent conjugation, while useful, has potential to impair functions of membrane proteins or even trigger signalling events when the surfaces are densely modified thereby potentially altering receptor binding efficiency. Consequently, alternative methods for improved and safer MSC targeting are required.

MSCs and nanotechnology

Nanoparticles are currently used for a variety of purposes and have emerged as valuable tools not only in diagnostic imaging, drug delivery therapy and for non-viral gene therapy (Arias, 2011; Herranz *et al.*, 2011; Parveen *et al.*, 2012) but also for cell targeting. One of the main advantages of nanoparticles is the ability to modify their physical, chemical, and biologic properties. This flexibility results in enormous functionality where the particles can be designed for a variety of different systems depending on the application. Table 2 lists some nanoparticles types and their potential use in biomedical research or therapy.

Current uses of nanoparticles in gene delivery and imaging of MSCs

Nanoparticles have a number of attributes that make them good candidates for specific gene delivery. Of these, the ease at which DNA can be conjugated onto the particle surface, which can then be easily introduced into the cell by endocytosis, is critical. Several recent studies have utilised nanoparticles to deliver genes of interest to MSCs. Yang and co-workers developed non-viral biodegradable polymeric-DNA nanoparticles for delivery of hVEGF gene to hMSCs to promote angiogenesis *in vivo* (Yang *et al.*, 2010). Nanoparticles have also been investigated as a delivery method to induce differentiation of hMSCs. Co-incubation of MSCs with biodegradable PLGA particles containing differentiation factors allowed for sustained intracellular and extracellular delivery of dexamethasone. Controlled release properties of these particles had the capacity to promote differentiation of particle-carrying cells, as well as neighbouring and distant cells not containing any particles (Sarkar *et al.*, 2011). Introduction of a combination of SOX5, 6, and 9 genes complexed with PEI-modified PLGA nanoparticles (Park *et al.*, 2010; Park *et al.*, 2011) and biodegradable PLGA nanoparticles to mediate SOX9 gene delivery to human mesenchymal stem cells (hMSCs) was used to induce chondrogenesis (Kim *et al.*, 2011). Recent developments in nanotechnology have also benefited *in vivo* tracking and labelling of MSCs. Examples of nanoparticles used in these studies include magnetic, iron-oxide and superparamagnetic iron oxide nanoparticles (Huang *et al.*, 2005; Jing *et al.*, 2008; Ai *et al.*, 2009; Loebinger *et al.*, 2009).

Nanoparticles and targeting

As previously mentioned, the failure of injected MSCs to engraft to targeted tissues in therapeutically effective numbers is a major barrier with current stem cell therapies. While nanoparticles are generally targeted to receptors on the surfaces of cells and taken up via several pathways (Kumari and Yadav, 2011), the ability to alter the size and surface functionalities create possibilities for other uses such as targeting studies. For successful application in cell targeting, nanoparticles need to be carefully designed to correctly express an optimal number of ligands on their surfaces for interaction with receptors. Cell specific interactions of nanoparticles can be achieved by attaching relevant ligands on their surfaces. Although, few studies have been performed on the targeting ability of nanoparticles and MSCs, several reports on targeting other cells sources have been reviewed extensively, especially in the areas of cancer therapy (Egusquiaguirre *et al.*, 2012) or in cardiovascular applications (Chorny *et al.*, 2011).

A novel method of magnetic targeting was investigated by Cheng and co-workers. Cardiosphere-derived cells were labelled with superparamagnetic microspheres, injected intramyocardially and demonstrated increased engraftment and functional benefit following administration to an ischemic region of the heart. Interestingly, magnetic targeting not only resulted in accumulation in the heart, but there was less migration of the cells to other organs, achieving more localised delivery (Cheng *et al.*, 2010). Magnetic labelling of synovium-derived cells and their targeted accumulation in the patellar groove has also been shown in a rat osteochondral defect model (Hori *et al.*, 2011). This novel method has potential to improve MSC cell therapy, increasing cell retention at the desired site; moreover, it offers the potential for rapid translation into clinical applications. In a similar study, PEG-PLGA based nanoparticles were utilised to investigate whether they could achieve brain-specific drug delivery after modification with a 12 amino acid peptide motif (Pep TGN) isolated from a phage display library. Enhanced brain accumulation efficiency together with lower accumulation in liver and spleen following intravenous injection was observed with Pep TGN conjugated nanoparticles in comparison to control PEG-PLGA nanoparticles without a targeting peptide. This methodology resulted in significant brain selectivity and thus potential for targeted drug delivery across the blood brain barrier (Li *et al.*, 2011).

Targeting methods to improve engraftment of MSCs to cartilage

A more recent approach is to facilitate homing of cells to tissues that are not accessible via the vasculature, such as cartilage as illustrated in Fig. 2. Hyaline articular cartilage is a remarkably durable tissue, however, once damaged it has limited capacity for self-repair due to its avascular nature with the ultimate outcome being the development of osteoarthritis (OA). Tissue engineering has emerged as a very attractive approach to cartilage repair utilising natural (collagen, hyaluronan, silk protein and chitosan) and synthetic biomaterial scaffolds such as poly(L-lactic acid), as well as allogeneic and autologous sources of cells and chondroinductive growth factors. Although MSCs

have been described as an ideal cell candidate source for cartilage regeneration (Coleman *et al.*, 2010), it is true that chondrocyte transplantation has become a clinical reality whereas MSC therapy has not. Autologous chondrocyte implantation (ACI) with a requirement for two invasive procedures generally addresses focal cartilage lesions, although two recent reports offer some promise for its use in treatment of complex larger lesions (>10 cm²) or for patients with early OA where treatment of an average lesion area of ~10 cm² size delayed the need for joint replacement in 92 % of the study cohort (Minas *et al.*, 2010; Ossendorf *et al.*, 2011). Intra-articular injection of targeted therapeutic cells whether chondrocytes, MSCs or other progenitors for cartilage repair represents a less invasive strategy to open knee surgery and could feasibly address larger cartilage lesions in the early OA joint. MSCs delivered to the joint after induction of OA in a caprine model engrafted at the synovial capsule and periosteum and not to fibrillated articular cartilage limiting their therapeutic potential (Murphy *et al.*, 2003). Specific targeting of potentially therapeutic cells such as MSCs to the fibrillated articular cartilage associated with OA may improve outcomes of this debilitating disease and Table 3 summarises relevant literature for cell or nanoparticle therapies in cartilage repair.

There are many biomarkers (Kraus *et al.*, 2011) including several possible ligands for OA cartilage targeting, such as extracellular matrix components and degraded collagen neoepitopes, to promote engraftment of cells to damaged cartilage. As previously discussed, genetic modification of cell surfaces is not the most favourable approach; hence, alternative options have been investigated. Dennis *et al.* (2004) modified the surface of pre-chondrocytes with antibodies to keratan sulphate, chondroitin-4-sulphate and collagen type II in an attempt to promote the adherence of progenitors to cartilage defects. This was one of the first studies to demonstrate the feasibility of using a "painting" technique to coat cells with antibodies or peptides that would promote the binding to cartilage extracellular matrix (Dennis *et al.*, 2004).

A key biological obstacle to hyaline cartilage formation using therapeutic cells is loss of transplanted cells from the desired site. The use of nanoparticles to improve targeting of cell-based therapeutics is a very appealing approach as ligands or homing devices that specifically bind to the surface receptors at the target site can be linked to the carrier particles to enhance their specificity. Furthermore, nanoparticles could provide a three-dimensional environment for transplanted cells and enable maintenance or differentiation to the desired chondrocytic phenotype by incorporation of differentiation factors (Bouffi *et al.*, 2010; Liu *et al.*, 2011). It is evident that nanoparticles have potential use as drug delivery vehicles as shown by Rothenfluh *et al.* (2008). This group developed an efficient targeting system using polymeric nanoparticles composed of poly(propylene sulphide) immobilised with a novel ligand specific for articular cartilage (Rothenfluh *et al.*, 2008). They demonstrated that nanoparticles with a diameter of 31-38 nm could enter the articular cartilage extracellular matrix, while larger particles of 96 nm size could not. This study also highlighted

Table 3. Overview of leading approaches of particle/cell based therapies in cartilage regeneration.

Title of Paper	Author	Cells used	Particles used
Stem cell therapy in a caprine model of osteoarthritis	(Murphy <i>et al.</i> , 2003)	Mesenchymal stem cells	-
Targeted delivery of progenitor cells for cartilage repair	(Dennis <i>et al.</i> , 2004)	Chondrocytes	-
Hyaluronate-covered nanoparticles for the therapeutic targeting of cartilage	(Laroui <i>et al.</i> , 2007)	Chondrocytes	Hyaluronan particles
Biofunctional polymer nanoparticles for intra-articular targeting and retention in cartilage	(Rothenfluh <i>et al.</i> , 2008)	-	Poly(propylene sulphide) (PPS)
Cellular and extracellular programming of cell fate through engineered intracrine-, paracrine-, and endocrine-like mechanisms	(Sarkar <i>et al.</i> , 2011)	Mesenchymal stem cells	Poly lactide-co-glycolic acid (PLGA) particles
Nanofibrous hollow microspheres self-assembled from star-shaped polymers as injectable cell carriers for knee repair	(Liu <i>et al.</i> , 2011)	Chondrocytes	Star-shaped poly(L-lactic acid) (SS-PLLA)

the issue of nanoparticle size and potential therapeutic application. 31-38 nm nanoparticles were internalised by chondrocytes and may be useful for gene or drug delivery applications. In the context of using such nanoparticles for therapeutic cell targeting in OA, larger nanoparticles would be more suitable in localising cells to the cartilage surface. Ideally, targeting nanoparticles would need to infiltrate the fibrillated cartilage and thus be retained at the site of injury whilst avoiding internalisation by their cell load.

Although cartilage possesses cells that are capable of both mitotic division and chondrogenic differentiation, failure of spontaneous repair is common in adult animals and humans. Therefore, targeted delivery of progenitor cells may enhance engraftment and repair of the degraded cartilage surface. Homing of endogenous cells to the surface of articular cartilage has been demonstrated previously and presents an opportunity for enhancement using nanoparticles. Hunziker and Rosenberg (1996) showed that digestion of proteoglycans at the surface of articular cartilage using chondroitinase ABC, followed by insertion of a fibrin clot containing transforming growth factor β 1, increased the migration of cells to the defect (Hunziker and Rosenberg, 1996). Similarly, regeneration of the articular surface of a synovial joint was demonstrated by homing of endogenous cells (Lee *et al.*, 2010). Animals that received TGF β 3-infused bioscaffolds regained weight bearing ability and locomotion 3-4 weeks after surgery compared to control bioscaffolds. Furthermore, TGF β 3-infused bioscaffolds were fully covered with hyaline cartilage in the articular surface with uniformly distributed chondrocytes in a matrix with collagen type II and aggrecan and significantly greater thickness. These findings suggest that the articular surface of the synovial joint has potential to regenerate without cell transplantation possibly through the recruitment of endogenous cells and modulation by appropriate factors such as TGF β 3.

Similarly, nanofibrous hollow microspheres were self-assembled from star-shaped polymers as injectable cell carriers for knee repair (Liu *et al.*, 2011). This group hypothesised that these nanofibrous hollow spheres

would mimic the structural features of collagen fibres, a major component of the extracellular matrix surrounding cartilage, and provide an appropriate scaffolding material to promote cell migration, proliferation and ultimately facilitate in tissue regeneration and integration with the host. It was demonstrated that these nanofibrous hollow microspheres could help regulate cellular function and tissue regeneration by mimicking the nanoscale architecture of the extracellular matrix; the microspheres were functional as a micro-carrier for chondrocytes to facilitate high quality hyaline cartilage regeneration in a mouse implantation model and in a critical-size rabbit osteochondral defect-repair model (Liu *et al.*, 2011).

Laroui *et al.* (2007) developed hyaluronate-covered nanoparticles for the therapeutic targeting of cartilage based on the affinity of hyaluronic acid (HA) to bind the CD44 receptor expressed on chondrocytes (Laroui *et al.*, 2007). These HA particles have the potential to actively target chondrocytes and potentially improve lubrication as well as joint regeneration. Since hyaluronan has previously been shown to contribute to wound healing (Murashita *et al.*, 1996) and the migration of mesenchymal and epithelial cells (Chen and Abatangelo, 1999), the presence of such particles in MSC targeting could potentially increase the natural repair process by recruiting these cells to the site of tissue repair or regeneration.

Thus, the use of delivery vehicles such as nanoparticles in cell therapy for cartilage regeneration is advantageous because they contribute to the penetration and localisation of the cells in the region of implantation while providing beneficial scaffolding to the cells, which aids in the regeneration of the damaged tissue. Moreover, these nanoparticles can contain or be tethered to peptides, growth factors, anti-inflammatory cytokines or even small molecule drugs, a process sometimes referred to as 'functionalisation' to provide micro-environmental cues augmenting the recruitment of endogenous progenitor or stem cells in addition to promoting the survival of the cells *in vivo* with the ultimate goal to enable transplanted cells to remain viable and functional in their new environment.

The development of biocompatible nanoparticles would represent an off the shelf product that could ultimately be combined with the desired therapeutic cell for cartilage repair. However, translation of targeting methods for tissue repair will critically depend on the availability of cells and biomaterials (industrial scalability), the capacity to avoid immunological side effects and comply with current international regulatory standards along with cost efficiency. It remains to be seen which of the targeting approaches described herein will ultimately become the method of choice in clinical approaches for tissue repair.

Conclusions and Future Perspectives

In the past 10 years tissue engineering and MSC therapy have become the focus of many research efforts in the treatment of cartilage defects. However, significant limitations have hindered the progress of these therapeutic strategies. In particular, the engraftment and retention of MSCs remains an issue to be resolved. Development of tools for enhancing MSC retention within the joint and specifically targeting articular cartilage is required if this cellular therapy is to be of benefit. The characteristics of nanoparticles including their small size, high surface-to-volume ratio and high surface functionality makes them ideal for use in MSC targeting to ensure adequate delivery of pertinent cells to the requisite tissues. However, a better understanding of the stem cell niche within the joint is needed to achieve efficient MSC targeting to cartilage. Essentially, once the tissue microenvironments are identified that provide favourable sites for MSC engraftment and we understand the signals that retain MSCs within their niche, progress can be made in manipulating biomaterials/nanoparticles to ensure site specific MSC targeting.

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Discussion with Reviewers

Reviewer II: Has there been much investigation into how these cell surface modifications affect cell function?

Authors: Whilst most studies discussed in this review investigated the effects of cell surface modifications on cell viability, very few examined potential changes in downstream cell signalling or indeed changes in gene expression profiles. Sackstein *et al.* (2008, text reference) did examine the impact of SLeX modification on MSC proliferation, adhesion and differentiation with no change in cell phenotype observed. With regards to whether or not the addition of biomaterials to cells alter cellular function: Stephan *et al.* demonstrated that coupling approximately 150 nanoparticles, each with a diameter of 200 nm to the surface of a T cell with a typical diameter of 7 µm did not compromise their proliferative response following co-culture with dendritic cells, nor were they toxic to the cells (Stephan *et al.*, 2010, additional reference). Similarly, Swiston *et al.* investigated the effects of polymer patches on T cell viability and their ability to migrate and did not observe any adverse effects (Swiston *et al.*, 2008, additional reference). However, in response to your question, we do think that studies using microarrays, chemokine arrays and other bioinformatic approaches should be added as more vigorous analyses to ensure that these modifications have no long term effects on cell function.

Reviewer II: Is anyone using these surface coating technologies to impact a cellular response other than just making the cells more adherent?

Authors: Cell surface coating/engineering technologies are also widely used to help mask cells from immune responses and safeguard against immune rejection following cell transplantation. A term coined “Protein Painting” employs the use of glycosylinositol phospholipid (GPI) moieties to anchor proteins to the outer membrane of a cell. Notohamiprodjo *et al.* used such an approach by genetically rendering the T cell activator RANTES immune-inhibitory by introducing a GPI-anchored version of the construct into the external membrane of endothelial cells to protect the vasculature from acute immune rejection (Notohamiprodjo *et al.*, 2006, additional reference). Additionally, the covalent attachment of mPEG onto lymphocytes effectively blocked MHC class II-mediated, allospecific T-cell activation. Significant attenuation of antigen-specific and memory cell-dependent activation and proliferation was observed which was most likely due to altered cell-cell interaction via reduced recognition and binding (Murad *et al.*, 1999, additional reference). Alternatively, biocompatible nanoparticles or related biomaterials have been modified with peptide ligands to enhance the differentiation potential of stem/ progenitor cells. Re'em *et al.* found increased chondrogenesis of hMSCs on alginate scaffolds immobilised with RGD. This resulted in stronger activation of Smad-dependent (SMAD2) and Smad-independent (ERK1/2) signalling pathways *vs.* un-modified controls suggesting that

inductive cues essential for stem cell differentiation can be efficiently provided from biomaterial platforms (Re'em *et al.*, 2010, additional reference).

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