Adult Stem Cells for Cartilage Tissue Engineering and Regeneration

Faye H. Chen and Rocky S. Tuan*

Cartilage Biology and Orthopaedics Branch, National Institute of Arthritis, and Musculoskeletal and Skin Diseases, National Institutes of Health, Department of Health and Human Services, Bethesda, MD 20892, USA

Abstract: Osteoarthritis (OA) is the most common joint disease and the leading cause of disability in the developed countries. Its clinical manifestations include pain and impairment to movement, and often affect surrounding tissues with symptoms of local inflammation. It is a progressively debilitating disease that is often associated with injury and aging. However, current pharmacological and surgical treatment modalities ultimately fail to stall the progression of OA. Viable treatment options are in need, and current effort of cartilage tissue engineering and regeneration, especially using chondroprogenitor cells, such as adult mesenchymal stem cells (MSCs), has offered hope of eventual success. First, ex vivo MSC cartilage tissue engineering can potentially produce effective replacement constructs for focal cartilage defects to prevent the progression to OA. This paper will review the factors important for cartilage tissue engineering, including cells, scaffold, and environment, as well as current problems and areas that await more research. Secondly, MSCs possess the capacity to function as a systematic regulator, to influence the local environment, *via* direct or indirect interactions, including soluble factors. Through these functions, MSCs can enhance local progenitor cell mediated regeneration, confer immunomodulation and anti-inflammatory effects, which can prove to be critically important in the setting of cell therapy for OA, a degenerative disease with associated local inflammation. Taken together, MSCs, used either as a structural substitute in a tissue engineered construct, or in cell therapy utilizing their modulating functions, or both, present promise in the treatment of OA, although clearly more research is needed to achieve this ultimate goal.

Keywords: Cartilage, tissue engineering, adult stem cell, MSC, regeneration, repair.

INTRODUCTION

OA is the most common type of arthritis. It is estimated that in 2007, 26.9 million Americans aged 25 and older have clinical OA of some joints, with higher percentage of infliction in older population [1]. The total cost of OA is estimated at $28.6 billion dollars per year in the U.S.A. alone [2], with > 200,000 knee replacements performed each year. Since cartilage is an avascular tissue, it has limited intrinsic healing and regenerative capacity. Current pharmacologic treatment has seen limited success, and various surgical procedures, although able to temporarily relieve pain, eventually fail [3]. Given the increasing incidence of OA and increasing life expectancy of the population with higher expectation of better quality of life, there is a growing demand for novel repair strategies. Cartilage tissue engineering seems to offer the best hope in answer to this demand.

Cartilage tissue engineering aims to produce a functional cartilage substitute through combined principles of engineering, biology and medicine. Physiologically, a healthy working articular cartilage serves to withstand and transmit the high stresses with minimal friction in joints during joint motion throughout life. This function depends on the unique mechanical properties of the cartilage tissue, which in turn is endowed by its special extracellular matrix (ECM). For the tissue engineered cartilage to become a successful substitute, it should be able to carry out the same function, which requires that it possesses similar mechanical properties as the native tissue, and presumably similar composition and organization of ECM.

Articular cartilage consists of a solid ECM component as well as a fluid phase of water that can take up to 85% of the total tissue weight [4]. The major collagen, collagen type II, and the aggregating proteoglycan molecules, as well as other smaller non-collagenous proteins, make up the ECM of cartilage. The collagen content and its dense fiber network bestow the tissue a tensile modulus ranging from 1 to 30 MPa. The high density of proteoglycan aggregates (primarily aggrecan) and the high fixed negative charges of their sulfated glycosaminoglycan (GAG) side chains draw counter ions and water into the matrix, resulting in high osmotic pressure, which is restrained by the collage network under pressure. The high content of water thus trapped in the tissue is essential for the function of cartilage: interstitial fluid support can account for more than 90% of the load on the join thus shielding the ECM from damage. Frequently, decreased water content is a telltale sign of weakened cartilage function, and can be used as a marker, along with others, for joint destruction. Due to the critical role of collagen and aggrecan and its GAG side chains to cartilage function, GAG and collagen contents, as well as the expression level of these proteins, have been used as measurements for the successful outcome of the tissue engineered cartilage [4].

There are various approaches to cartilage tissue engineering and regeneration with varying degree of success. Generally speaking, these are categorized into two broad types: the first is ex vivo tissue engineering, in which the tissue is generated and matured *in vitro* before implantation. The second is *in vivo* tissue engineering and regeneration, where cells and constructs are implanted *in vivo* for eventual maturation

*Address correspondence to this author at the Cartilage Biology and Orthopaedics Branch, National Institute of Arthritis, and Musculoskeletal and Skin Diseases, National Institutes of Health, Building 50, Room 1523, MSC 8022, Bethesda, MD 20892-8022, USA; Tel: 301-451-6854; Fax: 301-435-8017; E-mail: Tuanr@mail.nih.gov

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and tissue repair. This includes using cells directly for cell based cartilage regeneration therapy.

EX VIVO CARTILAGE TISSUE ENGINEERING

For *ex vivo* tissue engineering, three factors must come into play in harmony for successful cartilage tissue engineering: responsive cells, supportive scaffolding matrix, and enabling environment. These factors are discussed below.

Scaffold

Scaffolds serve to provide the form and shape and initial mechanical strength, it can also serve as a vehicle for cell delivery, and with the help of embedded factors, can guide the orderly development and differentiation of the neo-tissue. The ideal scaffold should be biocompatible, bioresorbable, biodegradable, porous, permeable, in addition to chondroinductive and chondroproductive. Materials tested so far for cartilage tissue engineering can be derived from natural materials, or can be synthetic polymers, and include protein based materials of fibrin [5], elastin [6], gelatin [7], collagen types I and II [8], silk [9, 10], hydrogels such as agarose [7, 11], alginate [7], polyethylene glycol with chondroitin sulfate [12], photopolymerizing hydrogels [13], and synthetic materials including polyethylene oxide [13], and Poly-L-lactic acid [14], as well as biodegradable nanofibers [15]. Polymeric nanofibrous scaffolds, with diameters of 300-700 nm, are structurally a biomimetic of the native connective tissue ECM, which in itself is nanofibrous in nature. Results from our group have shown that nanofibers can promote favorable chondrocytic responses, specifically, they can promote proliferation and phenotype maintenance of chondrocytes [16] and chondrogenic differentiation of mesenchymal stem cells [15].

Cells

Both chondrocytes, fibroblasts and stem cells, and genetically modified cells, have been used as candidates for cartilage tissue engineering. Since the ultimate goal of cartilage tissue engineering is to generate replacement cartilage tissue with fully differentiated chondrocytes surrounded by its ECM, articular chondrocytes seem to be the ideal candidate cells for this purpose, and they have been indeed used for studies from the beginning efforts of cartilage tissue engineering. In order to have enough cells for engineering a replacement construct, chondrocytes are generally enzymatically released from cartilage where they take up less than 10% of tissue volume, from a minor weight bearing site, and expanded in monolayer, before being put back into the engineering construct or *in vivo*. Chondrocytes from various anatomical sites, including articular, auricular, costal and nasoseptal, have been studied for this purpose. The *in vitro* culturing leads to the rapid de-differentiation of the chondrocytes, a process that is dependent on patient age and gradually becomes irreversible with prolonged passaging [1, 17-19], with end effect of an engineered construct that is inferior to the native articular cartilage in both tissue composition and mechanical properties. Despite these drawbacks, a United States Food and Drug Administration approved chondrocyte cell based cartilage repair procedure, Carticel™ (Genzyme, Cambridge, MA), also known as autologous cartilage transplantation/implantation (ACT or ACI), has been in clinical use since 1997 in the US and 1987 worldwide. It involves harvesting chondrocyte from a non-weight bearing site for *in vitro* expanding, followed by transplanting the chondrocytes to focal defects beneath a periosteal flap [20]. Manifestation of OA is normally an exclusion criteria for ACI [21]. Early patient satisfaction has been reported with this procedure. Of procedures reported to FDA with adverse events, which occur at a minimal rate of 3.8%, graft failure, delamination, and tissue hypertrophy were reported to be the most common incidences. Almost half of the surgical revision was performed due to graft failure [22]. Matrix associated autologous cartilage implantation (MACI) utilizes, for example, collagen type I/III and hyaluronan based scaffolds [23-26]. Recently, a 2-year clinical result was reported on treatment of posttraumatic and focal OA knee cartilage using polymer-based 3-D autologous chondrocyte graft [27]. Short term results showed safety and effectiveness and favorable clinical scores [27, 28]. However, whether MACI offers improvement over ACI in the long term remains to be determined [28].

Stem Cells for Cartilage Tissue Engineering

Theoretically, any cells with chondrogenic potential can be used for this purpose. Embryonic stem cells (ESCs), pluripotent in their differentiation potential into multiple lineages including chondrogenic pathway, are good candidate cells in principle. However, current state of science has not harnessed the control of ESC differentiation specification, e.g., down the chondrogenesis pathways; in addition ESCs have a tendency to form terotomas. Adult stem cells, on the other hand, do not elicit social issues for their potential clinical usage; furthermore, these cells are easy to isolate, capable of *in vitro* expansion to generate sufficient numbers for tissue engineering purposes, and have the propensity to undergo chondrogenic differentiation under appropriate stimulation.

Adult mesenchymal stem cells, or adult multipotent mesenchymal stromal cells (MSC), which are nonhematopoietic stem cells that can differentiate into various mesenchymal lineages, including chondrocyte, osteoblast, and adipocyte, have been identified from various tissues, where they are postulated to exist to carry out the function of replacing and regenerating local cells that are lost to normal turnover, injury, or aging. Originally isolated from bone marrow [29], MSCs can now be isolated from a large number of adult tissues, including adipose [30], periosteum [31, 32], synovial membrane [33, 34], muscle [35, 36], dermis [35], deciduous teeth [37], pericytes [38], peripheral blood [39], bone marrow [40], trabecular bone [41, 42], infrapatellar fat pad [43], and articular cartilage [44-46] (see [47-49] for review). A study that compared hMSCs derived from bone marrow, periosteum, synovium, skeletal muscle and adipose tissue revealed that synovium-derived hMSCs exhibited the highest capacity for chondrogenesis, followed by bone-marrow and periosteum-derived hMSCs [50]. The potentially less invasive nature of obtaining adipose derived stem cells compared to other sources has generated great enthusiasm for using this cell source, however, adipose tissue derived MSCs (ATSCs) seemed to have an inferior chondrogenic potential compared with bone marrow-derived MSCs (BMSCs) [50-53]. Recent studies suggest that the inferior chondrogenic differentiation under standard condition of ATSC is due to the fact that these cells do not express TGFβ type I receptor,

in addition to a reduced expression of BMP-2, -4, and -6 when compared to BMSC [54]. Supplementation with BMP-6 in culture restored the expression of TGFβ type I receptor, and accordingly, supplementation with BMP6 and TGFβ renders the ATSC to undergo chondrogenic differentiation similar to BMSC [54, 55].

In addition to tissue source, donor age, and disease stage can also directly affect MSC yield, rate of proliferation and differentiation potential. Of particular relevance to OA, age, although debatable, and advanced OA disease stage, adversely affect MSCs derived from the bone marrow of patients, with significantly reduced proliferative capacity and chondrogenic activity compared with those from young healthy donors [56-59]. However, irrespective of age or OA disease etiology, it has been found that sufficient number of MSCs with adequate chondrogenic differentiation potential can be isolated [60-62].

Control of MSC Chondrogenesis for Tissue Engineering

It has been realized that the epigenetic status of cells, the heritable trait that does not involve DNA sequences, is important in determining gene expression. Epigenetic modification include DNA methylation, generally associated with gene silencing, and chromatin modification on core histones including acetylation, phosphorylation, methylation, ubiquitination, as well as dynamic histone subunit variation, small interfering RNA regulation, and chromatin remodeling [63-66]. Stem cells are marked epigenetically with their chromatins in a loose or open configuration with greater potential of multi-lineage differentiation than differentiated cells [67-69]. For example, stem cells therefore appear to express, at low levels, genes that are characteristic of various cellular lineages, including chondrocytes, myoblasts, osteoblasts, and hematopoiesis-supporting stroma [70, 71]. This gene expression profile permits the stem cells to readily differentiate along a specific lineage upon appropriate stimulation.

With stem cells poised to differentiate into various lineages on cue, it is very important that the signals to induce differentiation are fine tuned and specific. Our understanding of MSC chondrogenesis is still incomplete, with most of our knowledge derived from chondrogenesis control during the embryonic limb developmental [72, 73], often not differentiating between the two processes of articular cartilage and growth plate cartilage formation, due to the fact that our understanding of how articular cartilage comes into being is still not complete. The standard experimental model of MSC chondrogenesis involves a three dimensional (3-D) culture of MSCs, either as high density cell pellet or micromass culture or in a 3-D scaffold. MSCs that have undergone chondrogenic differentiation assume a chondrocyte-like phenotype characterized by increases in GAG and collagen deposition and expression of collagen type II, aggrecan, and COMP, as well as other cartilage ECM molecules [74-76].

Growth factors that have regulatory effects on MSCs include members of the transforming growth factor (TGF) super family, the insulin-like growth factors (IGFs), the fibroblast growth factors (FGFs), the platelet-derived growth factor (PDGF) and the Wnts. Among these growth factors, TGF-βs, including TGF-β1, TGF-β2, and TGF-β3, as well as BMPs, are the most potent inducers to promote chondrogenesis of MSCs. For hMSCs, TGF-β2 and TGF-β3 were shown to be more active than TGF-β1 in promoting chondrogenesis [75]. BMP2, BMP4, or BMP6, combined with TGF-β3, induced the chondrogenic phenotype in cultured human BMSC pellets, with BMP2 seemingly the most effective [77]. FGF2-supplemented human MSCs showed longer life span with longer telomere size [78], proliferated more rapidly [79], and exhibited greater chondrogenic potential than untreated controls [61, 80, 81]. Wnt signaling pathway protein polymorphism and altered gene expression have recently been implicated in the progression of rheumatoid arthritis and OA [82-86]. Canonical and non-canonical Wnts have been shown to cross-talk with each other in regulating stem cell proliferation and osteogenic differentiation [87, 88]. Canonical Wnt signaling has been shown to enhance MSC differentiation [89] through mechanisms that involve the coordination with TGF-β and BMP signaling pathways [90-92].

Environmental Control

The success of tissue engineering is dependent on the efficient proliferation and differentiation of the functional cell or tissue type, which is under the control of the local environment, consisting of influences that are biological, e.g., various growth factors, and physical, including mechanical stress and oxygen tension. Various forms of mechanical stimuli, including dynamic deformation loading, intermittent hydrostatic pressure, fluid flow, shear stress, and electrical potential, have been demonstrated to be important for the metabolic activities of chondrocytes, and consequently the maintenance of articular cartilage ECM whose function is to withstand high mechanical stress during joint motion (see [4] for review), and have been successfully used to stimulate cartilage ECM production by chondrocytes [4, 93-95]. Chondrogenesis of MSCs is also enhanced by cyclic deformation and hydrostatic loading [96-100]. Another important factor is oxygen tension. Articular cartilage chondrocytes normally exist in a hypoxic (about 5% oxygen) environment. Interestingly, low oxygen tension in this range *in vitro* promotes cartilage-specific matrix production by chondrocytes, as well as chondrogenic differentiation of adult stem cells [100-103]. Oxygen tension has also been used to regulate the expansion of MSCs in culture; and higher levels of telomerase and stem cell marker expression indicate that they maintain their "stemness" state under low oxygen [104-107]. Telomerase activity generally decreases precipitously with extended *in vitro* culture of MSCs [108, 109]. Interestingly, telomerase activity not only is important for stem cell expansion, it is also related to the multipotency of the cells [110]. Overexpression of telomerase reverse transcriptase extends life span of MSCs but restricts differentiation primarily to osteogenesis [110-113]. The complex interplay of various environmental parameters, as well as soluble factors that are important for MSC proliferation and differentiation can be controlled in a closed system of bioreactors. Various bioreactors have integrated the different aspects of the above parameters and they are reviewed elsewhere [114-116].

PROBLEMS ASSOCIATED WITH *EX VIVO* CARTILAGE TISSUE ENGINEERING USING MSCs

There are still numerous problems to be solved, for example, under standard chondrogenic conditions with TGF-β and dexamethasone, the molecular expression signature of

MSC undergoing chondrogenesis is closer to that of the intervertebral disc than articular cartilage of the joint [117], and the differentiation level is inferior to that of chondrocytes, with less matrix contents and lower mechanical strength [118]. Other potential obstacles exist, including cellular senescence and death, hypertrophy, and graft integration (reviewed in [119]).

Hypertrophy

One of the most challenging problem in using MSC for cartilage tissue engineering has been the terminal differentiation to hypertrophy, characterized by high level expression of collagen type X and alkaline phosphatase activity [75, 120-123].

TGF-βs are also involved in terminal differentiation [124]. TGF-β1 can inhibit chick sternal chondrocyte terminal differentiation [125]. In mouse models, Smad3 deficiency accelerates chondrocyte maturation and leads to OA [126]. Ectopic expression of the negative regulator Smurf2 in chondrocytes and perichondral cells accelerated endochondral ossification by stimulating chondrocyte maturation and osteoblast development involving β–catenin signaling [127]. On the other hand, BMP2 can induce terminal differentiation [128, 129], and in chick sternal chondrocytes, this process can be inhibited by the BMP antagonist chordin [129].

During development, hypertrophic maturation of growth plate chondrocytes is under the regulation of a feed back loop involving India hedgehog (IHH) and parathyroid hormone-related protein (PTHrP) [130]. When human bone marrow MSC from OA patients were cultured in a 3-D polyglycolic acid scaffold and differentiated using TGF-β3, cells underwent hypertrophic differentiation as indicated by upregulated expression of collagen type X [62, 131]. When PTHrP was included in the culture at a dose of 1 or 10 μM, significant suppression of type X collagen mRNA expression and alkaline phosphatase activity was seen, without any loss of the cartilage-specific matrix proteins [62]. Interestingly, in a recent study, chondrocytes from the superficial zone of articular cartilage have been shown to inhibit alkaline phosphatase and mineralization of the deep zone chondrocytes, possibly through PTHrP [132], indicating the potential importance of zonal organization in cartilage tissue engineering.

Integration of Tissue Engineered Cartilage with Native Tissue

Chondrocytes have limited migratory ability to infiltrate into neighboring cartilage [133]. Brief enzymatic digestion before tissue implantation seemed to improve integration and interface adhesion [134, 135]. This suggests that matrix organization and composition is important for tissue integration, and in fact it is found that the nature of the surrounding tissues affects the integration of the cartilage [136]. To bind the replacement tissue to the remaining native cartilage tissue, traditionally, collagen crosslinkers and adhesives have been used. A recent *in vivo* study using rabbit and goat models showed that multi-functionalized chondroitin sulfate can be used to chemically bridge biomaterials and host tissue *via* a twofold covalent link leading to increased mechanical stability of the implant and tissue repair in cartilage defects [137].

MSC CELL THERAPY FOR OA

In addition to the traditional ex vivo cartilage tissue engineering with the engineered cartilage as implant, MSCs can also be applied directly *in vivo*. This area of research is not as advanced as *ex vivo* cartilage tissue engineering. MSCs can engraft in multiple organs, preferentially homing to sites of injury, and undergo site specific differentiation. MSCs not only can differentiate into a specific mesenchymal tissue they can also have significant effects on the local cells *via* direct cell-cell contact, or through soluble factors, including growth factors, cytokines and chemokines, e.g., MSCs can stimulate local endogenous stem cells to carry out regenerative function. In addition, MSCs are hypo-immunogenic and exert immunosuppressive and anti-inflammatory effect [138-142].

MSC Trophic Effects

It has been long known that MSCs can be effective feeder layers for hematopoietic cells in supporting their *ex vivo* survival and growth and multilineage differentiation that is necessary for *in vivo* reconstitution [143-145]. Conditioned medium from the stromal cells also support the growth and differentiation of the primitive hematopoietic cells and their clonogenic capacity, suggesting that the paracrine signaling molecules secreted by the stromal cells are important [146, 147]. MSCs secrete a host of growth factors and cytokines, including G-CSF, SCF, LIF, M-CSF, IL-6, and IL-11 [148-151], that are at least partially responsible for their hematopoeitic supportive function. The cytokine and growth factor expression profiles can change depending on the culture conditions and differentiation status [148, 150, 151].

Recent studies on the use of MSCs for treating neurological injury and myocardial infarction have highlighted the functions carried out by MSC without directly structurally contributing to the regenerated tissue. Specifically, although low MSC engraftment and differentiation are observed, substantial functional improvement is achieved. When human bone marrow MSCs were implanted into the dentate gyrus of the hippocampus of immunodeficient mice, the implanted human MSCs markedly increased the proliferation of endogenous neural stem cells, and enhanced their migration and differentiation into mature neurons and astrocytes [152]. This could be due to the fact that human MSCs express neurotrophins not only in culture but also after implantation into the brains of rats and immunodeficient mice [153].

In has been shown that MSCs can also improve tissue repair in disease models of cardiac infarction through paracrine effect [154, 155]. In a rat myocardial infarction model, intramyocardial injection of bone marrow MSCs overexpressing Akt (Akt-MSCs) inhibits ventricular remodeling and restores cardiac function with very low rate of MSC engraftment, low levels of cellular fusion, and differentiation [155]. Akt-MSC conditioned medium also significantly limits infarct size and improves ventricular function relative to controls. *In vitro*, conditioned medium from hypoxic Akt-MSCs markedly inhibits hypoxia-induced apoptosis and triggers vigorous spontaneous contraction of adult rat cardiomyocytes [156]. Recently, the anti-apoptotic effects of MSCs on cardiomyocytes have been attributed to secreted frizzled related protein 2 (sFRP-2), a modulator of Wnt sig-

naling pathways, indicating the importance of Wnt pathway in heart tissue regeneration [157].

That MSCs can aid in the regeneration of OA cartilage through non-structural mechanisms is shown in a goat study carried out by Frank Barry and colleagues [158]. Autologous MSCs, transduced to express GFP, in hyaluronan solution were injected intraarticularly six weeks after surgery. In the MSC treated joints, there is marked regeneration of the medial meniscus, and the degree of cartilage destruction, osteophyte formation and subchondral bone sclerosis were reduced. However, no MSC engraftment was detected in articular cartilage.

When MSCs were co-cultured with degenerated annulus fibrosus (AF) cells, the co-culture pellets were superior in size to all other single culture pellets, with enhanced proteoglycan production [159], thus indicating an interplay between AF and MSCs. Autologous MSCs have been transplanted into degenerating IVD and have been shown to be effective in decelerating disc degeneration in experimental models [160]. In addition, co-culture of nucleus pulposus (NP) cells and MSCs showed that interaction with MSCs can change the phenotype of the NP cells to enhance their effect on the regeneration process [161, 162].

In addition to the observed effects of MSCs on other differentiated cells, other cells can also affect the differentiation of MSCs. For example, chondrocyte and cartilage tissues have been shown to induce and influence the chondrogenesis of both MSCs and ESCs [163-166].

MSC Immunomodulation Function and Anti-Inflammatory Effect

MSCs express characteristic surface major histocompatibility complex (MHC) molecules that enable them to be hypo-immunogenic to evade the host immune elimination. MSCs express low (fetal) to intermediate (adult) MHC class I molecules, and do not express MHC class II molecules on their cell surface [167, 168], although an intracellular pool of MHC class II can be detected, and their surface expression can be stimulated by interferon-γ (IFN-γ) [168]. However, induced surface expression of MHC class II still does not render the MSCs immunogenic as they do not express any co-stimulatory molecules including B7-1 (CD80), B7-2 (CD86) or CD40, and therefore do not activate alloreative T cells [169]. The expression of MHC class I molecules helps to protect MSCs from deletion by natural killer cells. The lack of surface MHC class II expression gives the MSCs the potential to escape recognition by alloreactive $CD4^+$ T cells. After differentiation into adipose, bone and cartilage, MSCs continue to express MHC class I but no class II molecules on their cell surface even under stimulation, and continue to be non-immunogenic [167, 168]. In addition, cytotoxic lymphocytes and natural killer cells do not lyse MSCs [170]. These properties suggest that *in vivo* MSC cell therapy and tissue engineered cartilage construct using MSC in hypoimmunogenic biomaterial scaffolds should not elicit host immune response when transplanted *in vivo*.

MSCs not only evade detection and elimination by the immune system, they can further modulate and suppress alloreativity. *In vitro*, MSCs inhibit T-cell proliferation and activation [169, 171, 172]. Numerous studies have shown that MSCs, their differentiated progenies of adipocytes, osteoblasts, or chondrocytes, do not induce proliferation of allogeneic lymphocytes, even in the face of exogenous co-stimulation, and do not elicit IFN-γ production by human peripheral blood mononuclear cells as a measure of activation [168, 169, 171-174]. Both naïve and memory T cell response in mixed lymphocyte cultures under mitogen stimulation were suppressed [168-173, 175-177]. For example, MSCs have been shown to suppress $CD4^+$ and $CD8^+$ T cells in mixed lymphocyte cultures [169, 171]. MSCs can also induce apoptosis of activated T cells but not resting T cells [178]. Furthermore MSC suppress $CD8^+$ T cell mediated lysis [170, 179]. In addition to the effect of MSCs on T-cells, MSCs can affect dendritic cell differentiation and maturation and interfere with their function [174, 180-183]. MSCs can switch the cytokine secretion profile of dendritic cells to decrease their secretion of pro-inflammatory of IFN-γ, IL-12 and TNF-α, and increase production of IL-10 which is suppressive [174, 177, 181, 182]. MSCs can also alter the phenotype of natural killer cells, and can suppress the proliferation, cytokine secretion and cytotoxicity of these cells against MHC class I targets [182, 184]. MSCs can mediate the aforementioned immunosuppressive functions through either cell-cell interaction mediated inhibition, or through soluble factors that create a local immunosuppressive environment. These factors have been shown to include hepatocyte growth factor, TGF-β1, IL-10, IL-6, prostaglandin E2, and possibly indoleamine 2,3-dioxygnease, although the precise mechanism remains a topic of debate (for review, see [185, 186]). Despite the discrepancies on the mechanism of action, the above studies suggest that MSCs can be transplanted between MHC incompatible individuals.

The *in vitro* studies showing that MSCs possess immunomodulatory and immunosuppressive activities suggest that MSCs can be potentially used *in vivo* for enhancing the engraftment of other tissues (e.g. hematopoietic stem cells), or for the prophylactic prevention and even possibly as a treatment of graft-versus-host-disease (GVHD). In one report, MSCs were used to treat severe steroid-refractory GVHD [187]. In another study, the expected anti-inflammatory effect of MSCs was used to treat therapy-induced tissue toxicity following allogeneic hematopoietic stem cell transplantation with satisfactory results in the majority of the patients [188]. However, in a mouse model, MSCs have been reported to fail to prevent GVHD [189]. Furthermore, using a murine melanoma tumor model, it has been shown that co-transplantation of an MSC cell line (C3H10T1/2) favor tumor growth of subcutaneously injected B16 melanoma cells [190]. However this tumor promoting effect was not observed in another study [191]. The effect of MSCs on tumor growth requires further investigation to rule out the potential side effect of therapeutic use of MSCs.

OA is associated with progressive and often severe inflammation. For tissue engineering or cell therapy to be successful, measures must be taken to control such an inflammatory environment. MSCs can switch the cytokine secretion profile of dendritic cells to decrease secretion of pro-inflammatory IFN-γ, IL-12, and TNF-α, and increase production of IL-10 which is suppressive [174, 177, 181, 182]. In animal models, MSC implantations improve outcomes of renal, lung and cardiac injury, partially by shifting the micro environment at the injury sites from pro-inflammatory to anti-inflammatory [192-196]. MSCs also secrete IL-1 recep-

tor antagonist. In a murine pulmonary fibrosis model, MSC administration was more effective than recombinant IL-1 receptor antagonist delivered *via* either adenoviral infection or osmotic pumps in inhibiting bleomycin-induced increases in TNF-α, IL-1α, and trafficking of lymphocytes and neutrophils into the lung [193]. In a rat model of acute renal failure, intracarotid administration of MSC resulted in significantly improved renal function. Little engraftment or differentiation of the GFP labeled cells was observed. However there seems to be a switch from pro-inflammatory response to anti-inflammatory environment after MSC administration. Expression of the pro-inflammatory cytokines TNF-α, IL-1β, IFN-γ, and inducible nitric oxide synthase was significantly reduced, while expression of the anti-inflammatory IL-10, bFGF, TGF-α, and Bcl-2 was highly upregulated in MSC treated kidneys [192].

SUMMARY AND FUTURE PERSPECTIVES

OA is the most prevalent among degenerative joint diseases and the leading cause of disability, with huge burden to the society; yet so far there exists no cure. MSCs, owing to their multitude properties and characteristics and functions that are yet to be fully discovered and realized, have the potential to be used one day in cell based therapy to prevent cartilage injury progression and relieve patients of their disability and pain. First, due to their expansion and chondrogenesis capacity, MSCs are desirable as the cell source for *ex vivo* cartilage tissue engineering to produce a functional replacement tissue for damaged cartilage, whose successful outcome depends on the optimal interplay between cells with their suitable scaffold in an appropriate environment. Existing challenges including terminal chondrocyte differentiation and functional integration with the host tissue, among others, need to be overcome before the tissue engineered cartilage construct can be used on patients. Aside from their intrinsic differentiation potential, MSCs possess the potential, based on studies from mostly non-cartilage systems, through yet to be clarified mechanisms, to enable the endogenous progenitor cells to carry out the regenerative function, to elicit immunosuppressive effects, and to change their surrounding microenvironment from pro-inflammatory to anti-inflammatory. The combined effects of these functions should be beneficial to the promotion of tissue regeneration under a local inflammatory environment, such as that found in the OA joint. These properties make MSCs a good candidate cell type for cell based therapy of OA. However, research in this field has just started, and much more effort is needed to focused on MSCs on cartilage or the musculoskeletal system before MSCs can be used in clinical cell based therapeutic applications.

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