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Stem cell and growth factor-based regenerative therapies for reduced osteoarthritis pathogenesis

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Abstract

Regenerative medicine offers the exciting potential of developing alternatives to total joint replacement for treating osteoarthritis (OA). In this article, we highlight recent work that addresses the key challenges of stem cell-based therapies for OA and provide examples of innovative ways in which stem cells can aid in the treatment of OA. Significant progress has been made in understanding the challenges to successful stem cell therapy, such as the effects of age or disease on stem cell properties, altered stem cell function due to an inflammatory joint environment, and phenotypic instability in vivo. Novel scaffold designs have been shown to enhance the mechanical properties of tissue-engineered cartilage and have also improved the integration of newly formed tissue within the joint. Emerging strategies such as injecting stem cells directly into the joint, manipulating endogenous stem cells to enhance regenerative capacity, and utilizing stem cells for drug discovery have expanded the potential uses of stem cells in treating OA.

Keywords: Osteoarthritis (OA), Stem cell, Regenerative medicine

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Introduction

Osteoarthritis (OA) is a prevalent debilitating joint disorder characterized by erosion of articular cartilage, excessive stiffness pain, and crepitus. According to the United Nations estimates, by 2050, 130 million people will be affected by OA throughout the world, out of which 40 million will develop severe OA [1]. As a consequence, a huge economic pressure will be imposed in the treatment and management of OA leading to stress and decreased quality of life. OA is classified as primary and secondary OA; primary OA is associated with

aging, whereas secondary OA is pertinent to disease or other factors [2]. Further, the degradation of a network of collagen and proteoglycan in OA cartilage leads to a loss in tensile strength and shear properties of cartilage [6]. Interestingly, though OA manifests as loss of the articular cartilage, it also includes all tissues of the joint, particularly the subchondral bone. Besides aging, the increase in the level of accumulation of advanced glycation end products (AGEs), oxidative stress, and senescence-related secretory phenotypes are a few reported factors associated with the pathogenesis of OA. The elevated senescent phenotypes in OA reduce the healing properties of cartilage in an aging individual, which might be attributed to oxidative damage and telomere shortening [10]. Aging also severely affects extracellular matrix (ECM) and proteoglycans synthesizing capacity of chondrocytes in OA leading to thinning of the cartilage and decreased water content. Synthesis of irregular and small aggrecans disrupts the structural integrity of aging cartilage and reduces the chondrocytes' response to cytokines [3].

Composition and structure of articular cartilage

Articular cartilage consists of a composite organic matrix that is saturated with water. The water phase of cartilage constitutes 65–85% of the total tissue weight and is important in controlling many physical properties. The dominant structural components of the solid matrix are the collagen molecules (275% by dry tissue weight) and the negatively charged proteoglycans (220–25% by dry tissue weight) [4]. Collagen molecules, principally type II, assemble to form small fibrils and larger fibers with an orientation and dimension that vary throughout the depth of the cartilage layer. The proteoglycans of articular cartilage are large polymers consisting of many aggregating macromolecules known as aggrecan. A single aggrecan molecule consists of a protein core and numerous glycosaminoglycan side chains. Most aggrecan molecules are further bound to a single long chain of hyaluronan to form large proteoglycan aggregates of 50–100#106 MW. The large size and complex structure of the proteoglycan aggregate function to immobilize and restrain it within the collagen network, thus forming the solid matrix of articular cartilage [5]. The proteoglycans are negatively charged due to the presence of carboxyl and sulfate groups on the glycosaminoglycans, and so confer a net negative charge on the cartilage extracellular matrix. As a result, cartilage is highly hydrophilic, with a tendency to imbibe fluid or swell, to maintain mechano–chemical equilibrium. This property significantly contributes to the mechanical function of articular cartilage by generating a large swelling pressure which facilitates load support and tissue recovery from deformations.

There are numerous other molecular species (e.g., types IV and IX collagen, biglycan, decorin) that contribute to the specialized function of articular cartilage The solid matrix of articular cartilage has a highly specific ultrastructure which may be divided into successive

'zones' from the articular surface to the subchondral bone [5]. Collagen fibers in the superficial-most zone of cartilage are densely packed and oriented parallel to the articular surface. This surface zone is also characterized by a relatively low proteoglycan content and a low permeability to fluid flow. In the middle or transitional zone, the collagen fibers are reported to be either random [6] or radially oriented [9] and the proteoglycan content is at a maximum value for the tissue. In the deep zone, adjacent to the zone of calcified cartilage and subchondral bone, the collagen fibers are larger and form bundles that are oriented perpendicular to the bone, and the proteoglycan content is again low [7].

Pathogenesis of OA

At the joint level, many knee structures offer a certain degree of mechanical and functional support to maintain a healthy joint. The subchondral bone (mainly composed of mineralized type I collagen) assists the articular cartilage (mainly constituted of type II collagen and proteoglycans) in providing a surface for joint movement. The menisci provide a significant role in attenuating mechanical forces due to their structure of water, proteoglycans, and collagen. Finally, the synovial fluid to lubricate the articular space is produced by the synovial membrane: it is generally composed of hyaluronic acid and lubricin (also known as proteoglycan 4, PRG4) [8]. Of note, the superficial cartilage layer plays a vital role in constricting water content within the cartilage, acting as a regulator of water content: injuries of early damage to the superficial layer change the water content and, therefore, diminish the load-bearing properties of cartilage.

In the early stages of knee OA, alterations in the structure of collagen and proteoglycans are observed, together with degenerative changes in the meniscal structure. Both of these conditions lead to overcoming the compensatory mechanisms that limit the articular cartilage damage, ultimately, causing meniscal damage and articular cartilage erosions [9]. Of note, the pro-inflammatory role of cytokines has been confirmed as an essential mechanism of articular damage during the early stages of OA. In response to cartilage erosions, chondrocytes first go through a phase of hypertrophic activity to increase the matrix synthesis, producing inflammatory mediators that propagate cartilage degradation [10]. The ultimate stage of cartilage destruction is chondrocyte apoptosis, leading to an imbalance in the synthesis and catabolism of collagen and proteoglycans in favor of catabolism. Inflammatory mediators spread to other joint structures, causing changes in the synovial tissue and subchondral bone causing bone sclerosis and increasing the thickness of the synovial membrane and capsular structures [11].

Ultimately, gaps are produced in the cartilage surface, with free cartilage fragments propagating the synovial inflammatory condition, a condition that further decreases the synthesis of synovial molecules (lubricin and hyaluronic acid). The expression of type II

collagen (a prominent component of cartilage) decreases during chondrocyte growth; therefore, mature chondrocytes are incapable of producing type II collagen de novo [12].

Of note, although the changes in the subchondral bone have been traditionally implicated in the OA pathogenesis at a later stage, recent studies identified this joint structure as one of the first actors in the pathological process [13]. Indeed, recent evidence suggests the existence of specific crosstalk between subchondral bone and articular cartilage. For example, contributing to OA progression has been attributed to altered venous outflow circulation in the subchondral bone, causing physicochemical changes that stimulate osteoblasts to express bone remodeling and cartilage-damaging cytokines [14].

A recent study investigated the relationship between OA subchondral bone-derived exosomes (membrane-derived vesicles implicated in intercellular communication) and chondrocytes. The study showed that in coculture studies, OA sclerotic subchondral bone osteoblast-derived exosomes were internalized by chondrocytes, causing the expression of catabolic genes, as observed in OA cartilage [15]. Therefore, it seems that such exosomes are crucial in OA progression, representing a possible target for OA treatments [16]. As osteoarthrosis is a degenerative disease, the concept of cellular senescence has also been suggested to understand its pathogenesis. Cellular senescence of chondrocytes has been associated with the progressive reduction in cell cycle activity until it eventually stops, apoptosis resistance, and the progressive production of senescence-associated secretory phenotype (SASP) [17]. Different types of molecules can represent SASPs, yet—in the setting of OA—SASPs are all those inflammatory factors previously described (such as cytokines and chemokines). SASPs can be produced not only by chondrocytes, but also by other cells within the OA joint, such as osteoblasts, synovial fibroblasts, and macrophages. For example, TGF-β and IL-6 are both SASP factors that can contribute to chondrocyte aging by activating p15, p21, and p27, therefore, promoting its senescence through the SMAD complex or STAT3 pathway.

Additionally, the release of SASPs by senescent chondrocytes can produce a chemotactic effect on surrounding immune cells; thus, establishing an inflammatory environment further stimulates cartilage degradation [13].

The role of stress and inflammatory factors in biomechanical responses in the osteoarthritis joint

Chondrocytes can respond to direct biomechanical perturbation by upregulating synthetic activity or by increasing the production of inflammatory cytokines, which are also produced by other joint tissues [18]. As mechanisms controlling relationships between joint injuries and biological events that lead to progressive joint degeneration cannot be evaluated over time in patients, both in-vitro and in-vivo models have been used to test hypotheses [19].

The consensus based on in-vitro mechanical loading experiments is that injurious static compression stimulates the depletion of proteoglycans and damage to the collagen network and decreases the synthesis of cartilage matrix proteins, whereas dynamic cyclic compression increases matrix synthetic activity.

In response to traumatic injury, global gene expression is activated, resulting in increased expression of inflammatory mediators, cartilage-degrading proteinases, and stress-induced intracellular signals. Impact injury stimulates the release of reactive oxygen species (ROS) that induce chondrocyte death and activation of stress-induced kinases that upregulate MMP-13, ADAMTS-5, and TNF-α [20]. On the contrary, noninjurious cyclical loading of sufficient magnitude can inhibit IL-1-induced cartilage matrix degradation [21]. Thus, even in the absence of overt inflammation, chondrocytes may respond to mechanical stress by stimulating the expression and/or activities of inflammatory mediators or by inducing inhibitors that serve as feedback modulators [22].

Endogenous stem cells to aid in cartilage repair

An intriguing approach that is gaining traction in the field is to use acellular implants that can manipulate endogenous stem cells to provide regenerative treatments for OA [23]. Building on their previous work showing regeneration of the articular surface by providing a scaffold to guide the homing and differentiation of endogenous stem cells, Recently researcher performed in vitro work to determine which of the candidate stem cell types are most chemotactic, and whether incorporating additional chemotactic factors would enhance cell infiltration into the scaffold [26]. Studies on a cartilage defect model showed that a bilayered instructive scaffold incorporating TGF-β1 in the top layer and BMP-4 in the bottom layer induced appropriate chondrogenic and osteogenic differentiation of endogenous stem cells in a spatially controlled manner.

In an alternative approach to enhance the regenerative environment in an osteochondral defect, modified hydrogels that bind hyaluronic acid were delivered to the site of injury [24]. These hydrogels were able to retain newly synthesized matrix as well as guide the differentiation of stem cells from blood or marrow present in the defect site. The same group also showed that nanofiber scaffolds modified to present chondroitin sulfate preferentially encouraged the synthesis of type II collagen by native cells that infiltrate the scaffold during defect repair. As these approaches are further developed, it will be essential to develop a better understanding of how the properties of endogenous stem cells are altered in response to different manifestations of OA. For example, OA may allow stem cells from the bone marrow to migrate into cartilage due to a compromised tidemark and the overall number and proportion of MSCs in the joint space may be affected by joint injury and OA progression [25].

Revitalization of Cartilage by BMSCs

MSCs derived from bone marrow (BMSCs) are capable enough to differentiate into tissues such as bone and cartilage and mobilize at an injured cartilage site in knee joints thereby assisting in cartilage regeneration in OA. In a study, the intra-particularly transplanted BMSC successfully regenerated injured cartilage in a rabbit model of OA and also improved osteoarthritic symptoms in humans without any major side effects even in the long term. This study demonstrated the possibility of intra-articular injection of MSCs for the treatment of injured articular tissue including anterior cruciate ligament, meniscus, or cartilage [26]. Therefore, if this treatment option is well-established, it may be a minimally invasive procedure compared to conventional surgeries. In a very interesting study, out of the alginate, fibrin-alginate (FA), agarose hydrogel 3D culture, and cell pellet systems, the FA hydrogels and cell pellet promoted chondrogenic differentiation of equine BMSCs, whereas no effect was found in agarose group [84].

However, FA seems a better option than a pellet culture system, as the pellets require a large amount of chondrocytes [27]. Another study established an agarose hydrogel-based model for cartilage regeneration from human BMSCs in the presence of TGF-β3, where the level of chondrogenesis in agarose gel was dependent on the initial density of cells. Furthermore, a scaffold-free human BMSCs-derived cartilage-like sheet matrix has also been developed in the presence of FGF-2 and its efficacy was assessed by transplanting it into an OA rat model. This approach though improved OA condition, the cellular density decreased significantly within 12 months [28]. Further, the limited proliferation ability of the primary BMSCs was overcome by immortalizing them by using human papillomavirus-(HPV-) 16 E6/E7 genes, which showed enhanced chondrogenic potential and long-term survival both in in vitro and in vivo OA mice model. A recent study identified both the promoting as well as the inhibitory role of miR-29b factor in BMSC-based regulation of collagen expression and cartilage regeneration in the OA model. Further, the chondrogenically primed BMSCs have also been demonstrated to promote cartilage regeneration under hypoxia in a sheep model of OA.

However, the effect of oxygen tension was not consistent during ex vivo cartilage regeneration. On the other hand, BMSC also showed enhanced chondrogenesis when seeded on chondrogenic fibrin/hyaluronic hydrogel with improved mechanical strength by adding methacrylic anhydride [29]. Hence, it can also be considered a promising delivery method for cartilage regeneration in OA therapy. Besides, the intra-articular injection of MSCs may also be applied via microfracture through the cartilage and subchondral bone [30]. In a clinical trial (phase I/II), the intra-particularly injected BMSCs among OA patients showed a significant improvement; however, to assess all the clinical parameters, a clinical

phase III study was required. In another clinical study, human BMSCs demonstrated that the optimum level of cell dose (25 million) improved the OA without any major adverse effects. However, at higher doses, knee pain and swelling were observed as adverse effects, which suggested that more clinical studies are required to establish the therapeutic role of human BMSCs in OA treatment [31].

Intra-articular stem cell injection to modify the progression of OA

Thus far this review has focused on stem-cell-based cartilage tissue engineering strategies for end-stage OA, but emerging evidence indicates that the direct injection of stem cells into the joint can boost the normally limited repair and limit destructive processes. A limited clinical trial using ASCs for knee OA showed encouraging early results about improved functionality, but further work is needed to confirm a specific effect of the stem cells [31]. MSC injection to equine joints was effective when delivered during early chemical-induced OA, but the cartilage loss at later stages was too drastic for significant repair even with MSC therapy. Future work will need to continue to improve the specific targeting and retention of MSCs at the cartilage surface to maximize the potential effect [32].

One motivation for direct stem cell injection is that the anti-inflammatory function of stem cells may be effective at preventing or delaying OA if delivered at early stages in the disease process. This is consistent with the role of MSCs in altering the balance of inflammation and regeneration in numerous other injury models. In a recent study, they observed that a single intra-articular injection of purified MSCs could prevent the degenerative changes caused by an articular fracture of the tibial plateau in mice. Similarly, injection of hMSCs into rat joints after meniscectomy prevented the development of subsequent OA at least in part by enhancing meniscal repair by rat cells. Other work also supports the concept that stem cells protect joints from OA by acting on numerous joint tissues, as conditioned medium from stimulated MSCs reduced the gene expression of inflammatory mediators in both cartilage and synovium explants [33].

Conclusion

Stem cell and growth factor injection to achieve a significant therapeutic effect for osteoarthritis (OA) is still debated. For autologous MSCs originally from bone marrow is not established that higher dose intra-articular injections could necessarily result in better therapeutic effects. On the contrary, this leads to conflicting results on which amount is more effective.

Abbreviations

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