**Review Article**

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# Cartilage tissue engineering: Role of mesenchymal stem cells along with growth factors & scaffolds

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**Articular cartilage injury poses a major challenge for both the patient and orthopaedician. Articular cartilage defects once formed do not regenerate spontaneously, rather replaced by fibrocartilage which is weaker in mechanical competence than the normal hyaline cartilage. Mesenchymal stem cells (MSCs) along with different growth factors and scaffolds are currently incorporated in tissue engineering to overcome the deficiencies associated with currently available surgical methods and to facilitate cartilage healing. MSCs, being readily available with a potential to differentiate into chondrocytes which are enhanced by the application of different growth factors, are considered for effective repair of articular cartilage after injury. However, therapeutic application of MSCs and growth factors for cartilage repair remains in its infancy, with no comparative clinical study to that of the other surgical techniques. The present review covers the role of MSCs, growth factors and scaffolds for the repair of articular cartilage injury.**

**Key words** Cartilage injury - growth factors - mesenchymal stem cells - scaffolds

## Introduction

The articular cartilage is a unique connective tissue that furnishes the diarthrodial joints with an exceptional resiliency and almost frictionless movement owing to its distinctive structural, biochemical and metabolic characteristics[1]. Cartilage is a highly differentiated tissue with no direct blood, lymph or nerve supply and a scarce number of less proliferative chondrocytes and has limited regeneration potential[2-4]. Articular cartilage is made up of various unique layers, each with unique properties allowing it to be a suitable cushion for weight dispersement[5]. The tissue comprises approximately 75 per cent water, 15 per cent type II collagen, 10 per cent proteoglycans and <2 per cent chondrocytes[6]. Proteoglycans provide resistance against the compression, while tensile strength comes from collagen fibers[7]. Chondrocytes that reside in the lacunae interact with extracellular matrix (ECM) by means of cell surface receptors called integrins. These receptors act as mechanical links between the cells and ECMs and aid in cell homoeostasis (Fig. 1). Many cytokines and growth factors that may be present in diarthrodial joints stimulate chondrocytes and synovial cells to synthesize proteinases such as aspartic, cysteine,

serine and metalloproteinases. The proteinases are normally involved in maintaining the homoeostasis; however, sometimes, these may lead to pathological destruction of articular cartilage involving multiple pathways. Matrix metalloproteinases (MMPs) that can degrade all elements of ECM, have been regarded to be involved in arthritic degeneration of the joint[8].

Articular cartilage defects are generally classified into partial or full thickness, with former confined to the cartilage tissue itself and the latter penetrating the subchondral bone (Fig. 2). In partial thickness defects, the site of lesion remains devoid of fibrin clot and thus of the reparative cells from bone marrow. These lesions do not heal spontaneously and appear similar even after several months and are analogous to the clefts or fissures seen in the early stages of the osteoarthritis (OA)[9]. Full thickness defects, an access to a limited number of reparative cells from bone marrow, result in the formation of fibrocartilage which is weaker in structure and mechanical competence[9-11]. Pain and consequent loss of function resulting from the articular cartilage insult emphasize the need for the development of advanced techniques for improved management of cartilage injury[5,11,12].

Many cartilage repair methods have been developed so far, however, without a satisfactory long-term solution. The main problem that arises is the formation of biomechanically weaker regeneration tissue that lacks integration with the native osteochondral tissue. At the site of injury, death of zone of cells hampers the production of matrix that may integrate laterally with the native cartilage tissue. Surgical techniques such as microfracture[13], subchondral bone drilling[14], lavage and debridement and perichondral arthroplasty[15], periosteal arthroplasty[11,16], autologous osteochondral transplantation[17], autologous chondrocyte implantation (ACI)[12,18-20] and autogenetic cancellous bone grafts[21,22] have been attempted to form a new chondral surface. However, these techniques are limited to a small focal or medium-sized osteochondral defect and lack the potential to regenerate true hyaline cartilage[23]. ACI though has shown some good results, but due to the limited availability of chondrocytes, their proneness to dedifferentiate into fibroblasts and degeneration in pre-damaged cartilage, has limited its usefulness[24,25].

Cartilage rehabilitation should be aimed at elimination of pain and prevention of onset of OA[15,17,26], which can be achieved through the formation of actual hyaline cartilage. Currently, tissue engineering is being considered for better cartilage rehabilitation. For successful tissue engineering, three main components that are required include scaffold, cells and growth factors or cytokines. A scaffold provides a three-dimensional (3D) structure into which cells can grow making them less prone to deleterious environment. Growth factors or cytokines stimulate the cellular pathways for the proper functioning of cells. The present review discusses the possible roles of mesenchymal stem cells (MSCs), growth factors and scaffolds in the process of articular cartilage repair.

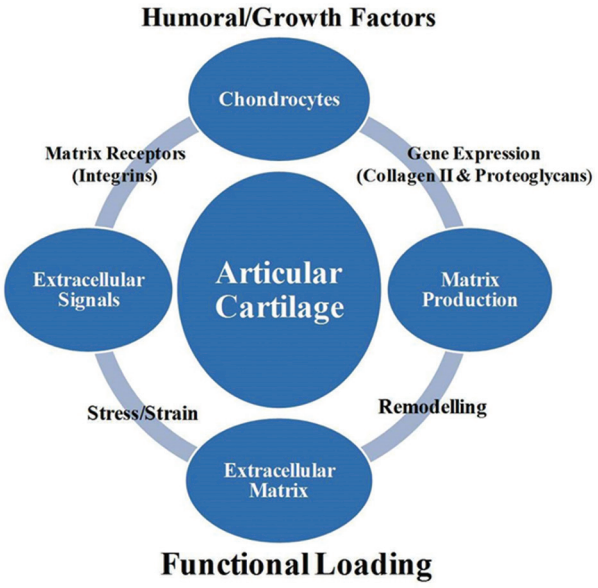


**Fig. 1.** Mechanical signals and humoral factors interaction with chondrocytes for the maintenance of homoeostasis.

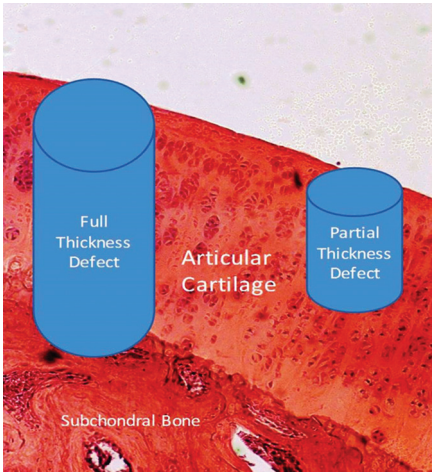


**Fig. 2.** Articular cartilage defects: full thickness penetrating the subchondral bone and partial thickness within the cartilage tissue.

**Mesenchymal stem cells (MSCs)**

Existence of MSCs was first established by Friedenstein *et al*[27], who demonstrated that certain cells present in the bone marrow can differentiate into the bone and cartilage. Later on, several other workers confirmed the finding and reported that MSCs isolated from the bone marrow have potential to proliferate extensively, to self-renew and to differentiate into cells of several lineages including chondrocytes[28-33]. To harvest their potential in cellular therapy, certain criteria were put forth by the International Society for Cell Therapy to confirm the cells as MSCs. The cells that are plastic adherent and express CD105, CD73, CD29 and CD90 surface molecules, but lack the expression of CD45, CD34, CD14 or CD11b, CD79a or CD19 and human leukocyte antigen-antigen D related (HLA-DR) surface molecules and can differentiate towards osteogenic, chondrogenic and adipogenic lineages, are regarded as MSCs[34]. Since MSCs have multiple sources, possess extensive proliferation potential and can differentiate into multiple lineages, these are currently perceived as attractive cell source for experimental and clinical studies in the area of regenerative medicine including cartilage repair[29,35,36].

Johnstone *et al*[28] first evaluated MSCs for chondrogenesis under *in vitro* conditions in 1998 using a specific medium. It was later found that addition of growth factors such as transforming growth factor-beta (TGF-β), bone morphogenetic proteins (BMPs), insulin-like growth factor (IGF) and parathyroid hormone-related peptide (PTHrP) may enhance chondrogenic potential of MSCs under *in vitro* conditions[28,37-40]. Currently, *in vitro* micromass culture method is in vogue to evaluate chondrogenic potential of MSCs. However, it may not produce the cartilage tissue comparable to the native one as the process does not mimic the sequences of cartilage formation that normally occur during foetal development. However, under *in vivo* conditions, MSCs do have a potential to differentiate into chondrocytes, stimulated by the signals arising from the microenvironment of the cartilage[41-44]. MSCs when implanted into osteochondral defects differentiate into chondrocytes[41,45]; however, when cartilage pellets differentiated from MSCs *in vitro* are transplanted subcutaneously, these either disappear[46] or are calcified with vascular invasion[24]. *In vivo* MSC differentiation can be affected by the existing microenvironment, plausibly by the molecular signals generated by other resident cells of the tissue[44,47]. The induction can thus occur by cell surface receptor stimulation, through growth factors, ECM or the direct interaction with the surface proteins of other resident cells (chondrocytes)[48,49]. With the progression in understanding of embryonic development and biological features of stem cells, the tissue engineering approaches also improved. The repaired cartilage tissue approaching to native articular cartilage both in physiologic stratification and biomechanical features has been developed from stem cells under *in vitro* conditions. This has been possible after recapitulating different developmental processes of mesenchymal condensation induced by the growth factors, especially TGF-β[50,51]. During the condensation process, MSCs condense into cellular bodies [condensed mesenchymal cell bodies (CMBs)], undergo chondrogenic differentiation and ultimately form cartilaginous tissue. CMBs after loading onto the osseous tissue were found to generate cartilage on the superficial surface that interfaced with the underlying bone in *in vitro* studies. CMBs also develop mechanically strong cartilage to cartilage interface, leading to the production of seamless interface and thus complete integration[52].

MSCs are generally considered to have a limited potential to undergo chondrogenesis both *in vivo*[43] and *in vitro*[53,54] conditions. This may be due to their limited potential to divide or decrease in number upon apoptosis[54]. This necessitates the implantation of higher cell density for the effective healing of the cartilage. One report has shown better healing upon transplantation of higher cell density compared to lower cell density[43]. Tiwary *et al*[55] implanted $2.96 \pm 0.18 \times 10^6$ mononuclear cells ($2.96 \pm 0.18 \times 10^3$ to $10^4$ MSCs) in the cartilage defect of knee joint and reported better healing compared to control group owing to the presence of the humoral factors.

To treat cartilage defects using MSC transplantation, a vehicle is required to hold them, allow their growth and make them less prone to deleterious environmental effects. Generally, the common problem that arises while transplanting cells into the cartilage defects is their leakage. These cells do not stick at the site of defect, and thus, a scaffold is required for their *in situ* transplantation. Scaffold selection is made on the basis its biocompatibility, ability to be retained at the implantation site and to integrate with the adjacent tissue, sufficient porosity to allow ingrowth of host tissue yet maintain adequate mechanical strength and properties to deliver cells without any toxic effect upon them[56-58]. In osteochondral defects, scaffolds that are

being replaced by neocartilage should survive until two types of tissues, bone and cartilage, are formed[58]. Growth of the superficially placed cartilage depends on the availability of subchondral bone, and if latter is not formed within a requisite period, cartilage regeneration at the superficial surface may be hampered[59]. During healing of articular cartilage, integration of regenerated tissue with that of the adjacent native tissue is another problem. Cartilage islands formed after regeneration fail to survive unless not integrated with the surrounding normal cartilage[9]. Thus, the scaffolds that encourage the growth and survival of implanted cells and also promote the colonization of native cells should be transplanted[60]. Scaffold design for cartilage repair should be aimed at normalizing the biochemical (affecting cellular behaviour and activity) and physical (scaffold architecture, mechanical function and degradability) properties[61]. A number of materials including both natural (fibrin[62-65], agarose and alginate[66], collagen[67-70], hyaluronan[71-73]), or synthetic scaffolds (polylactic acid[74-76], polyglycolic acid[77] and polylactic and polyglycolic acid[78,79]) have been used as scaffolds for cartilage regeneration. Natural scaffolds though bear good biocompatibility (leading to better cell attachment and thus differentiation) but lack in ease of fabrication, suitable mesh properties and controllable biodegradability. Natural scaffolds are also associated with the risk of immunological reactions, disease transmission and are limited in availability. The synthetic scaffolds, chemically modified for desired fabrication, better versatility, suitable mesh properties and controllable biodegradability, lack optimal cyto-compatibility and may also elicit host response upon release of toxic by-products[56,57]. To overcome such limitations, it was desirable to design composite scaffolds that could combine the respective properties of both synthetic as well as natural scaffolds. This has led to the development of the hybrid scaffolds that utilizes the solid polymer backbone (providing mechanical strength) and hydrogel (supporting the cell delivery) resembling the biphasic nature of cartilage, namely, solid and water phases[80]. Hydrogel was found to retain cells in the three dimensional stage in a friendly environment along with their homogenous distribution in the solid polymer scaffold pores[81]. However, such designs demand further *in vitro* as well as *in vivo* investigations, especially with respect to the mechanical strength and biocompatibility, to employ clinically.

Other types of tissue engineered scaffolds, namely, biomimetic zonal scaffold and nanofibrous/nanoporous scaffold, have also been developed to overcome the drawbacks associated with conventional scaffolds, namely, compatibility and functional properties. Zonal scaffolds involve different distinct zones/layers with or without the cells resembling the natural cartilage. This zonal system mimics the physical properties of the native articular cartilage and the cells, if implanted, secrete ECM resembling different layers of cartilage[82]. The biomimetic zonal scaffold technology, although a promising one, is still in its infancy and further investigations are required in its design and fabrication technology. The non-fibrous/nanoporous scaffolds due to their nanosize mimic the biological as well as physico-chemical properties of the native nanosize ECM and thus, play a key role in stem cell and/or chondrocyte growth as well as tissue regeneration[83]. In pre-clinical phase, the cells are encapsulated in nanofibrous scaffolds fabricated by electrospinning. However, the main problem that arises is of cellular homogeneity as the cells get clumped on such fabrication[84]. To avoid cell clumping, other fabrication techniques (particulate leaching, chemical etching, 3D printing and phase separation) warrant investigation.

There are numerous intrinsic or extrinsic growth factors that work independently or complement each other for the maintenance of cartilage homoeostasis[85]. Studies conducted on different growth factors, namely, TGF-β, IGF, BMP, fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF), have shown promising results in cartilage repair[55,86-102]. All these growth factors stimulate chondrocytic matrix synthesis and decrease catabolic effect of MMPs and cytokines such as interleukin-1, except FGF-2 that antagonizes the proteoglycan synthesis and upregulates MMPs[86,88,99]. The growth factors also stimulate MSC proliferation, increase their matrix production and downregulate their collagen type I gene expression. BMP-7, however, has alone been reported to inhibit MSC proliferation but does allow proliferation in the presence of TGF-β[91,93]. Growth factors when used in combinations work synergistically such as BMP-7 and IGF-1 lead to enhanced cartilage matrix synthesis[103]. IGF-1, FGF-2 and TGF-β under *in vitro* conditions regulate their own and each other's gene expression and protein production[104]. It was also demonstrated that the combination of IGF-1 and TGF-β has better healing potential compared to individual effect with IGF-1 involved in protection of synovium, showing reduced thickening depicting lack of chronic inflammation[105].

All these observations suggest that the growth factors have an essential role to play in cartilage tissue engineering. Some drawbacks such as osteophyte formation[89,96] and synovial thickening[87,106] have been reported upon such transplantation which can be managed by standardizing their dosages[107] and using them in right combinations[105].

**Clinical studies on cartilage tissue injury**

Use of MSCs, with or without scaffolds and growth factors, has been reported increasingly for the treatment of cartilage defects. In a case report on a single patient, MSCs were reported to form a hyaline type cartilage tissue with improved arthroscopic score[108]. In another case report, Improved Knee and Osteoarthritis Outcome Score and International Knee Documentation Committee Score were recorded on transplantation of autologous MSCs[109]. In an institutional study, implantation of MSCs along with mononuclear cells and platelets resulted in better visual analogue score (VAS) and increased meniscal and femoral cartilage volume on magnetic resonance imaging (MRI) than the control[110]. MSCs were found as effective as ACI in the management of cartilage defects. A cohort study, comparing MSCs and ACI in 72 patients with almost similar symptoms[111], showed no difference between the groups in terms of clinical outcome, except the physical function that improved over time in MSCs group[111]. Bone marrow-MSCs suspended in a collagen type I gel and transplanted in knee of the osteoarthritic patients showed better arthroscopic and histological scores compared to the control group[70]. The same authors reported improvement in clinical symptoms in three patients[112,113]. Another study wherein five patients with full thickness cartilage (3-12 $cm^2$) were treated with MSCs laden on platelet-rich fibrin glue showed better clinical, arthroscopic and MRI results as compared to the control patients[114]. The efficacy of infrapatellar fat pad-derived MSCs in the treatment of human OA has also been proved in two studies. Intra-articular injection of MSC was found to be safe and provided assistance in reducing pain and improving function in patients with knee OA[115,116].

Among animals, equines and dogs are more prone to the articular cartilage injuries. Autologous fat-derived MSCs clinically evaluated for the treatment of chronic OA in dogs showed improved scores of lameness, pain and range of motions[117]. A multicentre clinical trial was conducted on 39 horses using intra-articular injection of autologous MSCs to treat OA (74% cases of stifle joint), with a follow up of 21 months. Seventy seven per cent were found to resume some work, 38 per cent returning or exceeding the level observed before OA and 38 per cent requiring additional medicinal treatment[118]. Sato *et al*[119] studied the outcome of intra-articular transplantation of MSCs suspended in hyaluronic acid in spontaneous arthritis of Hartley strain guinea pigs. Partial cartilage repair was noted at five weeks post-operation with higher type II collagen and low levels of MMP-13. Migration, differentiation and proliferation of MSCs in the hyaluronic acid in treated animals were also observed. However, there are reports that do not suggest positive outcome following MSC application in human patients. A study on four elderly OA patients (55-65 yr) treated with MSCs therapy did not show any significant knee outcome score, except that the patients could climb number of stairs and had improved VAS[120]. In clinical settings, the differences in the extent of articular lesion, the duration of the lesion, age of the patient, the methods of application, the number of cells used, concurrent use of growth factors and scaffolds, *etc*., may have bearing on the outcome of the treatment. It may, therefore, be imperative to consider all the above-mentioned factors while selecting a patient for MSC therapy and interpreting the results of such therapy.

**Conclusion and future perspective**

MSCs from different sources have shown potential to repair cartilage defects by differentiation into chondrocytes and synthesis of cartilage matrix. Inclusion of suitable growth factors and scaffold may support the regeneration and integration of neocartilage with the surrounding native tissue. The combined and precise use of MSCs, growth factors and scaffolds may offer new modalities that can overcome the limitations associated with currently available surgical techniques. The survival on transplantation and integration of cells with the host tissue remain the major causes of concern. Processes of mesenchymal condensation into cellular bodies under the influence of growth factors may be a promising technology to develop mechanically strong cartilage to cartilage interface leading to the production of seamless interface and complete integration. Further research is needed to investigate the technology under *in vivo* trials for its actual potential of cartilage repair. Moreover, CMBs should be assessed for their phenotypic identity as the cells may lose their identity under *in vitro* conditions. Suitable cell source should also be investigated to find out whether only autogenous cells or both autogenic and allogenic/xenogenic cells

can be utilized for CMBs production. Although scaffold properties clearly affect the chondrogenesis, the exact mechanisms that facilitate such cartilage formation remain to be elucidated. Besides, comparisons between conventional and non-conventional scaffold technology need to be drawn to check the functional benefits of the later. For better comparison of scaffold design, standard mechanical and biological tests should be developed.

***Conflicts of Interest*:** None

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