18 Cartilage Tissue Engineering: Role of Mesenchymal Stem Cells, Growth Factors, and Scaffolds

Mudasir Bashir Gugjoo, Hari Prasad Aithal, Prakash Kinjavdekar, and Amarpal

18.1 Introduction

The articular cartilage, a connective tissue with characteristic structural, biochemical, and metabolic features, furnishes an exceptional resiliency and almost frictionless movement to the diarthrodial joints [1]. The average articular cartilage thickness is at the most a few millimeters with knee thickness being 0.3 mm in rabbits, 0.4–0.5 mm in sheep, 0.6–1.3 mm in dog, 0.7–1.5 mm in goats, and 1.5–2.0 mm and 2.2–2.5 mm in humans. Among the commonly used animals for preclinical studies, horse knee cartilage thickness has closest approximation to human knee cartilage followed by goats [2]. Its composition as well as thickness even vary from joint to joint and with age among species [3]. In general, articular cartilage constitutes three layers/zones with the deep zone separated from subchondral bone by a wavy calcified zone known as tidemark (Fig. 18.1). The three zones bear unique arrangement of matrix and cells. In the superficial zone, the cells are flattened disc-like, while in deeper zones the cells appear more rounded. Collagen arrangement appears parallel to the surface in superficial zone, while it becomes random in middle zone and perpendicular in deep zone. The main proteoglycan, aggrecan, content in superficial zone is limited, while in deeper zone it constitutes a major portion. The tissue ingredients in decreasing order of their concentration include water (approximately 75%), collagen especially type II (15%), proteoglycans (10%), and chondrocytes (<2%) [4]. The collagen provides the tissue strength while the proteoglycans provide functional resistance against compression [5]. The resident cells, chondrocytes that reside in lacunae singly or in groups (cell nests), occupy

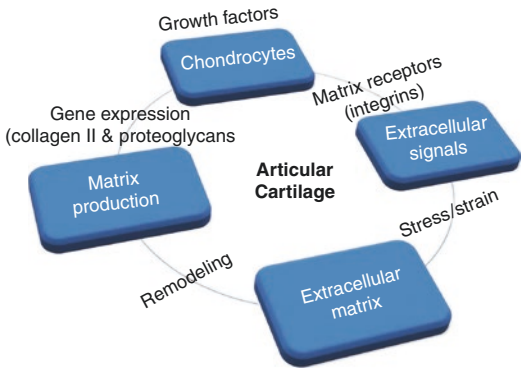

Fig. 18.1 Articular cartilage constitutes three layers/zones with the deep zone separated from subchondral bone by a wavy calcified zone known as tidemark

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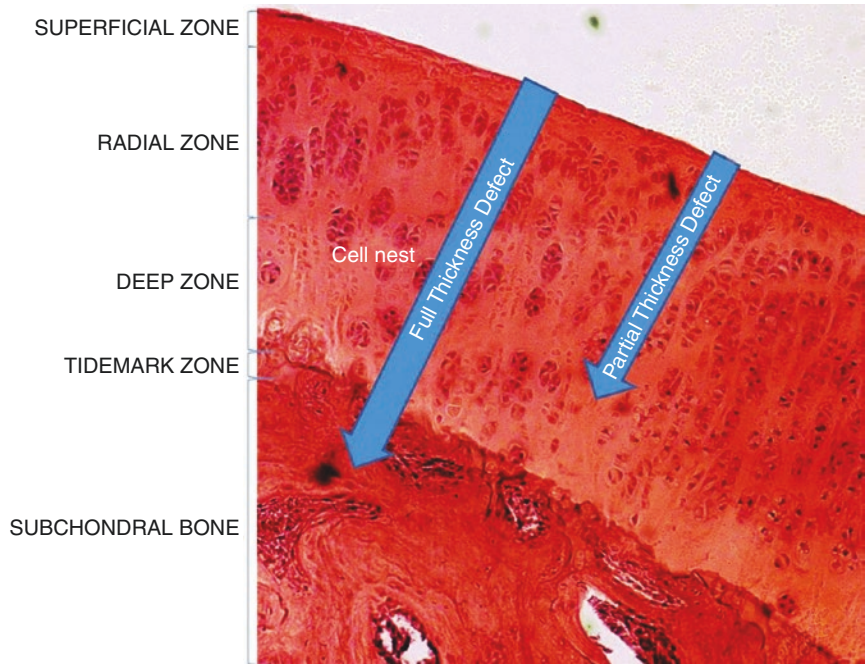

Fig. 18.2 Articular cartilage growth factors. The cells maintain tissue homeostasis through mechanical links generated from extracellular matrix (ECM) via cell surface receptors known as integrins

less than 10% of the tissue. The cells maintain tissue homeostasis through mechanical links generated from extracellular matrix (ECM) via cell surface receptors known as integrins [6]. In addition, the growth factors/cytokines act upon chondrocytes and/synovial cells to secrete proteinases such as aspartic/cysteine/serine and metalloproteinases for tissue homeostasis (Fig. 18.2). Among various proteinases, currently, matrix metalloproteinases that degrade all elements of ECM are considered to carry arthritic degeneration potential [7].

Cartilage is a highly differentiated tissue devoid of any direct blood, lymph, or nerve supply and with a scarce number of less proliferative chondrocytes [8, 9]. Articular cartilage upon damage carries limited regeneration potential. The injury in the form of defects is generally divided into partial- and full-thickness defects with the former confined to the tissue itself and the latter penetrating subchondral bone [10]. Partial-thickness defects do not heal spontaneously as the lesion remains devoid of fibrin clot and thus reparative stem cells. The defects are analogous to fissures or clefts seen in early stages of osteoarthritis [11]. Full-thickness defects though heal spontaneously but with a fibrous tissue that is weaker in structural and mechanical competence [11–15]. Osteoarthritis is a progressive erosion of articular cartilage with about 21.4% of the humans [16] and 20% of dogs [17] affected. The exact pathophysiological basis of osteoarthritis is still disputed but the cardinal signs include inflammation and pain, and the pathognomonic radiological features include articular cartilage thinning characterized by decreased joint space, sclerosis, and osteophyte formation [18, 19]. The pain and subsequent loss of functional activity that arise from an insult to the cartilage and its advancement into osteoarthritis demand advanced techniques for better cartilage rehabilitation [11, 12, 14, 20, 21].

To date no repair procedure has been able to heal the cartilage defects to a satisfactory level. Immediately post-injury the local death of cells hampers matrix production that may integrate with the native tissue. The main aim remains to repair the defects by true hyaline cartilage that has seamless local integration. Numerous invasive procedures such as microfracture [22], subchon-

dral bone drilling [23], lavage, debridement and perichondral arthroplasty [24], periosteal arthroplasty [25], autologous osteochondral transplantation [26], autologous chondrocyte implantation [12, 27, 28], and application of autogenic cancellous bone graft [29, 30] have been attempted for cartilage rehabilitation. The techniques, however, lack true hyaline cartilage repair potential besides being limited to small/medium focal sized osteochondral defects [31]. Autologous chondrocyte implantation (ACI), currently better among the lot, has drawbacks in the form of limited chondrocyte source availability, proneness of the cells to dedifferentiate to fibroblasts, and degeneration in pre-damaged cartilage [32, 33]. In addition, the ageing chondrocytes show declining mitotic and synthetic activity, and synthesize smaller and less uniform aggrecan molecules bearing less functional link proteins [34].

Currently, tissue engineering is being employed to achieve better cartilage rehabilitation. For successful cartilage tissue engineering, various components are required such as cells, growth factors, and three-dimensional matrices. Appropriate cells like autologous chondrocytes or autologous or allogenic stem cells may be implanted. Most of the cell-based therapies currently utilize chondrocytes (approx. 80%), while stem cells constitute only 15% [35]. The limitations associated with ACI mentioned above demand other cell types like stem cells, which are considered to be immunosuppressive. Growth factors incorporated by either viral/nonviral vectors, nucleofection, or direct delivery may regulate directed differentiation. However, the growth factors such as bone morphogenetic proteins (BMPs) direct both bone and cartilage formation and thus need to be regulated at particular step towards chondrogenic lineage [36]. The cells should be implanted on three-dimensional matrices that support the growth and prevent hazardous effect of local environment [10]. Scaffolds, either natural or synthetic, however, bear limitations like early degradation, lack of sufficient porosity, and non-supportive cell growth, and thus the scaffolds that mimic the desired properties of both and exclude the limitations are in demand.

18.2 Mesenchymal Stem Cells

Stem cells (SCs), characterized by the properties of self-renewal, multiplication, immunomodulation, and multi-lineage differentiation potential, are present in almost all the adult tissues of an individual to maintain normal cells, and thus tissue matrix turnover [10, 37]. The stem cells are of various types such as pluripotent (embryonic SCs, and induced pluripotent SCs) or multipotent (mesenchymal stem cells) based upon their potential to differentiate (Fig. 18.3). Pluripotent stem cells carry extended potential to act multipurpose research and clinical tools to understand and model diseases, develop and screen candidate drugs, and deliver cell replacement in regenerative medicine including cartilage [38]. However, limitations in the form of uncontrolled forced expression (iPSCs), and teratogenic effects and ethical issues (iPSCS/ESCs), have restricted their clinical applications [39, 40]. Currently, mesenchymal stem cells (MSCs) carry maximum share among all stem cells both in preclinical and clinical settings in human and veterinary medicine. The cells are easily available, are capable to differentiate, and secrete certain factors that modulate inflammation and promote healing, and in comparison to pluripotent stem cells they have minimal teratogenic and ethical issues associated [39, 41]. The cells are differentiated as per the available local niche/microenvironment and thus contribute to tissue repair or regeneration. Mesenchymal stem cells implanted into osteochondral defects differentiate into chondrocytes [42–44], while MSC-derived cartilage pellets if implanted subcutaneously either disappear [45] or calcify upon vascular invasion [32]. This indicates the role of microenvironment plausibly through cell-surface receptor stimulation by growth factors, extracellular matrix, or direct interaction with surface receptors of other resident cells (chondrocytes) [46–48]. Currently, MSCs are believed to largely act therapeutically by releasing a diverse array of cytokines, growth factors, chemokines, and immunomodulatory proteins, though they may also achieve terminal differentiation [49]. Despite the studies that show immunomodulatory potential of MSCs, two

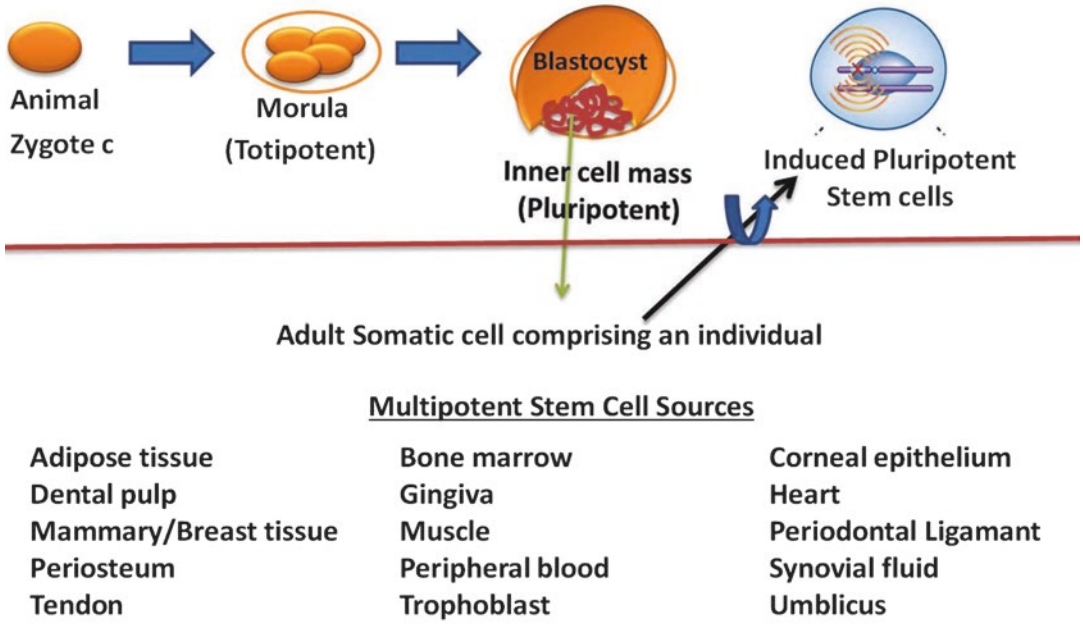

Fig. 18.3 Stem cell sources in animals and humans

recent studies in equines demonstrated development of allo-MSC antibody [50, 51]. One of the studies even showed that the MHC-mismatched MSCs underwent targeted death due to the activation of complement-dependent cytotoxicity. Thus, cautioning about some potential adverse effects that may ensue in addition to the reduced therapeutic efficacy on application of allogenic MSCs [50]. Lack of in-depth understanding in the area demands further steps that need to be deliberated to understand the mechanism(s) behind such differentiation and thereby controlled cell applications.

MSCs that carry maximum share in therapeutics may be derived from almost all the adult tissues (Fig. 18.3) including bone marrow, adipose tissue, embryonic tissue, synovial fluid and membrane, umbilical and peripheral blood, umbilical cord vein, Wharton's jelly, periosteum, muscle, heart, dental pulp, gingiva, periodontal ligament, and mammary tissue [52], each of which carries the potential to differentiate into chondrogenic lineage [36]. Among all the above mentioned sources the most commonly utilized stem cell sources for therapeutics so far have been bone marrow and adipose tissue [53].

Chondrogenic potential of MSCs was first evaluated under in vitro conditions in 1998 employing transforming growth factor-β (TGF-β) and dexamethasone [54]. Further investigations employing various other growth factors such as bone morphogenetic proteins (BMPs), insulin-like growth factor-1 (IGF-1), and parathyroid hormone-related peptide (pTHRP) showed enhanced MSC chondrogenesis [54–57]. However, the in vitro micromass culture method used in such studies may not produce tissue comparable to the native one as the process does not mimic the developmental sequences that actually occur during fetal development. A thorough understanding of embryonic development of the concerned tissue and biological features of the implanted cells is a must-learn criterion for successful cartilage tissue engineering [10]. Recently, under in vitro conditions cartilage tissue was generated approaching hyaline cartilage in physiologic stratification and biomechanical features. This could only be done after recapitulating various developmental processes of mesenchymal condensation via TGF-β1 [58, 59]. The various processes involved include MSC condensation into cellular bodies and condensed

mesenchymal cell bodies (CMBs) followed by chondrogenic differentiation that leads to cartilaginous tissue formation. The CMBs under in vitro conditions have been able to generate tissue comparable to native cartilage on osseous tissue surface and also developed mechanically strong cartilage-to-cartilage tissue interface with complete integration [60].

Variations in MSCs' chondrogenic potential have been observed with respect to their source, culture periods, and age of the donors [53]. Among MSCs from various sources, synovial derived MSCs had better chondrogenic potential and led to formation of a large and heavy cartilage pellet compared to BM-MSCs, AD-MSC, Periosteal-MSC and M-MSCs [61]. In another study that compared BM-MSCs and AD-MSCs, the frequency of colony-forming units reportedly had been three times in the latter compared to the former [62]. In elderly patients, the differentiation potential and proliferation capacity of MSCs are reduced and may affect the healing outcome. The immunomodulation property of MSCs may allow allogenic cells to be used [63, 64]. MSCs are able to maintain their differentiation potential for limited periods with long ex vivo-cultured MSCs manifesting reduced chondrogenic matrix formation, undesired mineralization, and rapid cell death after implantation [32, 65]. The reduced cell population may be compensated by implantation of higher cell density for better cartilage healing as reported in some studies [66, 67]. But it may be noted that higher cell density has chances of more cell apoptosis and thus more inflammation at the site.

18.3 Growth Factors

In healthy cartilage environment various growth factors work either individually or in combination to complement each other for maintenance of cartilage homeostasis [68]. The main roles played by the growth factors are to promote MSC differentiation towards chondrogenic lineage, stimulate chondrocytic matrix synthesis, and decrease catabolic effect of MMPs and cytokines such as interleukin-1 [10, 69–71]. The factors act either at earliest phases to promote chondrocyte proliferation and differentiation like TGF-β [72] or at later stages to promote chondrocyte differentiation rather than initiation of maturation like BMP-2, BMP-4, BMP-6, and TGFβ-3 [73, 74]. To promote MSC differentiation towards chondrogenic lineage, BMP-2 appears superior but has the tendency to promote differentiation towards hypertrophy and osteogenesis characterized by type X collagen and Runx2 expression [72]. Similarly, high intraarticular doses of TGF-β1 have been reported to induce chemotaxis and activation of inflammatory cells tending towards fibrosis and osteophyte formation [72]. To address this issue, combinations of the growth factors have been used either to reduce the activity of each other at certain stage or to complement each other's physiological function. One of the proposals is to co-treat cells with BMP-2 and TGF-β as the latter may potentially prevent differentiation of MSCs into osteogenic lineage [75]. BMP-7 has been reported to inhibit MSC proliferation but does allow proliferation in the presence of TGF-β [76, 77]. Further, growth factors may complement each other and work in synergism. BMP-7 and IGF-1 lead to an enhanced cartilage matrix synthesis [78]. Similarly, IGF-1, IGF-2, and TGF-β regulate each other's gene expression and thus protein production [79]. Further, combination of IGF-1 and TGF- β has better healing potential compared to individual effect as the former is involved in protection of synovium and reduces the synovial thickening depicting lack of chronic inflammation [80]. Limitations in the form of osteogenic synthesis [72], synovial thickening [81, 82], and osteophyte formation [71, 83] as mentioned above may be managed by using growth factors in right combinations and dosages [72, 80, 84].

18.4 Scaffolds

Another criterion for successful cartilage tissue engineering is availability of three-dimensional matrices, as evidences have shown that two-dimensional culture system hardly supports MSCs' chondrogenic differentiation. The micromass culture system as mentioned earlier has failed to recapitulate the cartilage developmental

stages, besides express hypertrophic marker, collagen type X [85]. For cartilage rehabilitation most of the investigators prefer MSC application along with scaffold. This allows cellular growth and prevents them against deleterious effects of local environment. In addition, the cells are retained in situ at the desired locations avoiding the common problem of cell leakage [10]. Selected scaffold is supposed to bear features of biocompatibility, support cellular growth and expansion, and facilitate diffusion and movement, yet maintain adequate mechanical strength and properties till tissue is regenerated and integrated [10, 86–88]. In osteochondral lesions, survival time of scaffold is critical as the neocartilage that replaces it should have preformed subchondral bone to survive in addition to its integration with surrounding native cartilage [89]. Usually the cartilage islands that form during healing fail to survive unless not integrated with the adjacent native cartilage [11].

The scaffold design in cartilage tissue engineering is aimed at maintaining the physical (scaffold architecture, mechanical function, and degradation) and biochemical (relevant to cellular behavior and activity) properties [89]. The matrices evaluated include natural fibrin [43, 90–93], agarose and alginate [86], collagen [94–97], hyaluronan [47, 98–100] as well as synthetic polylactic acid [101–103], polyglycolic acid [104], and polylactic and polyglycolic acid [105, 106]. Natural scaffolds that bear desired biocompatibility, better cell attachment, and differentiation have limitations in the form of availability, ease of fabrication, mesh properties, and controllable biodegradability, in addition to immunological reactions and disease transmission [10]. Synthetic scaffolds in comparison though are modified chemically for desired fabrication, and have better versatility, suitable mesh properties, and controllable degradability, but again fall short with respect to cyto-compatibility and may elicit host response upon release of toxic by-products [86, 87]. To overcome such impediments, hybrid scaffolds have been developed incorporating solid polymer scaffold and hydrogel [10]. The former provides mechanical strength and the latter supports cell delivery resembling the biphasic (solid and liquid phases) nature of cartilage. The cells in hydrogel are maintained in three-dimensional stages and are homogenously distributed in solid polymer scaffold pores [107].

In order to utilize such scaffolds in clinical practice, both in vitro and in vivo studies need to be conducted especially in relation to their biocompatibility and mechanical strength. Apart from the above mentioned scaffold designs, two other types including biomimetic zonal and nonfibrous/nanoporous scaffolds have been developed based on the concept to provide microenvironment comparable to that of native cartilage for the cells [10]. Biomimetic zonal scaffold comprises different zones like that of cartilage in order to mimic the physical properties. The implanted cells thus secrete matrix based on the available environment [108]. Nonfibrous/nanoporous scaffolds constitute nano-size matrix that mimics physicochemical and biological properties of cartilage matrix, and thus tends to develop relevant signals for cellular differentiation (MSCs) and matrix synthesis (from MSCs and chondrocytes) [109]. For creating such scaffolds, numerous fabrication techniques (electro-spinning, chemical etching, particulate clumping, 3D printing, and phase separation) may be employed [10]. Preclinical studies that encapsulated cells in nanofibrous scaffolds by electro-spinning have failed to maintain cell homogeneity and have resulted in cell clumping [110]. 3D printing is currently seen to carry the potential to replicate the cartilage structure. The cells are delivered in a suspension or with a gel as an ink in layer-by-layer process creating an appropriate pericellular environment for the cells located in each cartilage zone [111, 112]. One of the impediments in utilizing the technology in tissue engineering is the need to integrate vascular network for proper nutrient and gas supply. Cartilage, however, being devoid of direct blood, lymph, and nerve supply may act as a good candidate for 3D bioprinting [113]. Direct bioprinting into an ex vivo cartilage defect has resulted in some level of integration into native cartilage and mechanical competence [114]. This demands a detailed analysis of the

fabrication process and its evaluation in preclinical as well as clinical studies.

18.5 Clinical Trials

The successful outcome in clinical settings is the ultimate aim of cartilage tissue engineering. So far the aim is unmet both in veterinary practice and in human medicine though the reports appear promising. The application in animals may provide the basis for human stem cell therapy. In veterinary practice, canines and equines comprise majority of the clinical application studies.

Stem cell therapy in canines has been instituted both in preclinical [115–117] and in clinical settings [118–123]. A single-time, local implantation of the cells in all the studies has been made barring a single study wherein cells were implanted at acupoints [122]. The cells were either applied directly without employing the vehicle [122, 123] or implanted with platelet-rich plasma [120] or hyaluronic acid [124]. All these studies have reported improved healing (pain, visual analog scale, and range of motion) on MSC application with follow-up varying from 1 month [122, 124] and 6 months [120, 121] to 5 years [119–122, 124]. Two comparative studies were conducted involving AD-MSCs versus platelet-rich growth factors (PRGF) [121] and AD-MSC versus stromal vascular fraction (SVF) [122]. In both the studies improved results have been reported with MSCs; however, in the former study MSCs showed better results at 6 months compared to PRGF, while in the latter SVF had better results than MSCs. In another comparative study, vascular endothelial growth factor transgenic BM-MSCs were shown to improve early healing in comparison to simple MSCs [117].

In equines, most of the studies so far have been unable to fetch positive results for better cartilage repair in osteoarthritis patients [71, 125, 126]. Some of the studies, clinical as well as experimental, though have shown beneficial effects in cartilage repair but are mainly on the basis of reduction in pain perception [127–129]. In a clinical study of 40 horses having joint affections treated with BM-MSCs, 77% of the patients returned to work; among them 38% were able to work to the previous condition or exceeded [125]. Currently, the stem cell being implanted is at 2×10^7 concentration in hyaluronan scaffold (22 mg of Hyvisc) (hyaluronate sodium, 3×10^6 Da, Anika Therapeutics, Woburn, MA) [130], prior to which NSAIDs were recommended to reduce joint flare [131].

In human medicine numerous cartilage-related clinical trials implanting stem cells have been registered at http://www.clinicaltrial.gov/. Among them some are completed, while some are in progress. The cells have been injected either locally (intra-articularly) or implanted surgically. All the registered studies located were uncontrolled. The stem cell reported studies are either case series [66, 132–137], case reports [138–144], or comparative [66, 141–148] type. The cell types employed in such studies have been AD-MSCs, bone marrow concentrate, and BM-MSCs with or without the scaffolds. The patient number in case series studies ranged from 4 to 48. The follow-up period of at least 3 months and a maximum of 5 years has been made. An overall improvement in the clinical parameters (Visual Analog Score, Improved Knee and Osteoarthritis Outcome Score, and International Knee Documentation Score), MRI, and histological score in the patients has been reported with no major adverse effect observed on cell application. With respect to the formation of the healing tissue, the variability in outcome was reported. Some of the patients had hyaline-like tissue [135, 140, 145, 149], while others had combination of the hyaline/fibrocartilage [135] or mainly fibrocartilaginous tissue [141]. In a study that compared MSCs versus ACI with equal patient number of 36 in each group, the clinical results were comparable except for improvement in physical functioning of patients in BM-MSC groups [146]. In a study that evaluated dose-dependent healing potential of MSCs, the group of patients that received higher dose (1.0×10^8) had better clinical scores and reduced pain compared to those patients that received lower dose of AD-MSCs (1.0×10^7 and 5.0×10^7) [67].

In clinical settings, variability in lesion type, site, duration of existence, age of the patient, cell

culture techniques, and cell application methods and their number, besides addition of growth factors and scaffolds, have bearing on the outcome, and thus demand controlled studies [10].

18.6 Conclusions and Future Perspectives

Articular cartilage upon damage carries limited regeneration potential. Currently, tissue engineering, employing cells, growth factors, and scaffolds are considered to have the potential to support regeneration and integration of neocartilage with the surrounding native tissue. MSCs especially BM-MSCs and AD-MSCs carry maximum share among all stem cells in cartilage tissue engineering. There is a need to investigate cell source to find out whether only autogenous cells or both autogenic and allogenic/xenogenic cells can be utilized. The cell survival posttransplantation and integration of regenerated tissue matrix with the host native tissue remain the major causes of concern. One of the promising technologies to develop mechanically strong cartilage-to-cartilage interface includes the mesenchymal condensation into cellular bodies under the influence of growth factors. However, more research especially under in vivo conditions is desired in the area to evaluate its actual clinical application. Growth factors form an indispensable part of the tissue engineering and demand further evaluation on the basis of their individual properties as well as combinations including dosages. Scaffold that affects the desired chondrogenesis remains to be elucidated. Newer fabrication technologies that appear promising need to be evaluated and compared against the conventional technologies especially in relation to the maintenance of scaffold mechanical and biological properties. Tissue engineering that appears promising needs to be evaluated with respect to the cell sources; culture methods; concentration; implantation methods; growth factors, their combinations, doses, and frequency; and scaffolds, their sources, design, and type, before it becomes a clinical reality.

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