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Review

Mesenchymal stem cell - based tissue engineering strategies for repair of articular cartilage

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Summary. Restoration of articular cartilage function and structure following pathological or traumatic damage is still considered a challenging problem in the orthopaedic field. Currently, tissue engineering-based reconstruction of articular cartilage is a feasible and continuously developing strategy to restore structure and function. Successful articular cartilage tissue engineering strategy relies largely on several essential components including cellular component, supporting 3D carrier scaffolding matrix, bioactive agents, proper physical stimulants, and safe gene delivery. Designing the right formulations from these components remain the main concern of the orthopaedic community. Utilization of mesenchymal stem cells (MSCs) for articular cartilage tissue engineering is continuously increasing compared to use of chondrocytes. Various sources of MSCs have been investigated including adipose tissue, amniotic fluid, blood, bone marrow, dermis, embryonic stem cells, infrapatellar fat pad, muscle, periosteum, placenta, synovium, trabecular bone, and umbilical cord. MSCs derived from bone marrow and umbilical cord are currently in different phases of clinical trials. A wide range of matrices have been investigated to develop tissue engineering - based strategies including carbohydrate-based scaffolds (agarose, alginate, chitosan/chitin, and hyaluronate), protein-based scaffolds (collagen, fibrin, and gelatin), and artificial polymers (polyglycolic acid, polylactic acid, poly(lactic-co-glycolic acid), polyethylene glycol, and polycaprolactone). Collagen - based scaffolds and photopolymerizable PEG - based scaffolds are currently in different phases of clinical trials. TGF-$ß_1$, TGF-$ß_3$, BMP-2, and hypoxic environment are the recommended bioactive agents to induce optimum chondrogenesis of MSCs, while TGF-$ß_1$, TGF-$ß_3$, SOX-9, BMP-2, and BMP-7 genes are the best candidate for gene delivery to MSCs. Electromagnetic field and the combination of shear forces/dynamic compression are the best maturation-promoting physical stimulants.

Key words: Tissue Engineering, MSCs, Articular Cartilage

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Introduction

In a typical diarthrodial joints, articular cartilage is a highly resilient connective tissue that covers the end of long bones to provide a lubricating surface for frictionless movement (McCormick et al., 2008; Ahmed and Hincke, 2010). This hyaline-type cartilage composed mainly of chondrocytes surrounded by extracellular matrix (ECM) (Aigner and Stove, 2003). ECM is synthesized and secreted by the chondrocytes and is composed mainly of type II collagen, aggrecan, chondroitin sulfate and other glycosaminoglycans, which are responsible for articular cartilage-specific biomechanical properties (Ahmed and Hincke, 2010; Ahmed and Hincke, 2013). The loss of cartilaginous tissues due to pathological conditions such as rheumatoid arthritis (RA) and osteoarthritis (OA) or

traumatic conditions such as intra-articular fracture and cartilage damage following ligament injury is a major challenge to orthopaedic surgeons (Aigner and Stove, 2003; Beris et al., 2005). Because of its avascular nature, articular cartilage has very low innate capability for self-repair and regeneration. Consequently, injury to cartilage usually heals through scar tissue formation composed mainly of fibrocartilage (Ahmed and Hincke, 2010). Fibrocartilage has substandard biomechanical properties compared to hyaline cartilage and progressively degenerates with time, resulting in permanent loss of structure and function leading to severe pain. These factors in concert lead to disability (Aigner and Stove, 2003; Ahmed and Hincke, 2010).

The number of RA and OA patients is continuously increasing worldwide as the elderly population grows. Articular cartilage damage due to RA and OA is the major cause of disability in both the developed and developing world (Richardson et al., 2010). It has been shown that arthritis affected approximately 70 million U.S. adults in 2005. It is sobering that in 2000, the estimated economic burden of arthritis in the United States was $60 billion and it is expected to increase to $100 billion by 2020 (Ahmed and Hincke, 2010).

Articular cartilage defects are classified as either partial or full-thickness defects (Beris et al., 2005). Partial - thickness defects are confined to the cartilage; therefore, they have no access to bone marrow-derived stem cells and subsequently lack the ability to heal spontaneously (McCormick et al., 2008). Usually, this type of defect progress to degeneration of the articular surface and can lead to a variety of problems such as swelling and mechanical symptoms (Detterline et al., 2005). In contrast, full - thickness cartilage defects penetrate the subchondral bone which allow recruitment of bone marrow-derived stem cells leading to spontaneous healing through the formation of fibrocartilaginous tissue (Beris et al., 2005; McCormick et al., 2008).

A wide variety of strategies have been widely developed to restore the structure and functions of injured cartilage. These strategies range from reducing the pain and swelling, to repair of cartilage through the formation of fibrocartilage (i.e., reparative procedures), and to a variety of restorative procedures (Ahmed and Hincke, 2010). Although all these strategies promote pain relief and enhanced joint function, they fail to restore the native structure and biomechanical properties of articular cartilage (Kessler et al., 2008). In addition, some of the currently used strategies are associated with technical difficulties and adverse results (Ahmed and Hincke, 2010); therefore, researchers have focused on reconstructing cartilage *in vitro* using tissue engineering strategies.

Tissue engineering is a promising strategy for restoration of cartilaginous tissue after injury, and has the potential to improve the quality of life of millions of patients and delay future medical costs related to joint arthroplasty and associated procedures (Ahmed and Hincke, 2013). Various tissue engineering strategies depend largely on four essential components: functionally active cell source, a supporting three - dimensional (3D) carrier/scaffolding matrix, proper bioactive factors, and a maturation promoting mechanical environment (Kessler and Grande, 2008; Ahmed and Hincke, 2013). A tissue -engineered cartilage substitute should have similar biphasic properties as native cartilage (i.e., fluid phase [80% water and <1% electrolytes] and solid phase [10-20% collagen II and 5-10% proteoglycans]) after remodeling (Temenoff and Mikos, 2000). In addition, the regenerated tissue should occupy entirely the defect site and integrate optimally with the surrounding native cartilage to withstand *in vivo* mechanical forces (Peretti et al., 2006). In the following sections we will summarize the most widely used cell sources, scaffolding materials, bioactive molecules, and maturation environment that are used either experimentally or clinically to develop tissue engineering - based cartilage repair strategies.

MSCs versus chondrocytes as a cell source

Finding the best cell source is an absolute requirement for a successful tissue engineering application (Ahmed and Hincke, 2013). The cellular component should be healthy, viable, easily accessible, manipulable, nonimmunogenic, and nontumorigenic (Song et al., 2004). The cells should also have stable phenotype and should respond appropriately to the bioactive factors with subsequent production of the typical chondrocytic markers (Kerker et al., 2008). The most widely used cell sources for cartilaginous tissue engineering are chondrocytes and mesenchymal stem/stromal cells (MSCs) (Ahmed and Hincke, 2013). Chondrocytes are the first relevant and most widely used cell source; however, the long culturing period required before implantation leads to formation of cartilaginous repair tissue that is of fibrous nature (Temenoff and Mikos, 2000). In addition, it has been shown that chondrocytes implanted into experimental osteochondral lesions filled the defect with new cartilage ECM, but failed to integrate with the subchondral plate because the chondrocytes did not mature to become hypertrophic chondrocytes (precursors of subchondral bone) (Ahmed et al., 2011). Alternatively, MSCs have emerged as an important tool in the field of regenerative medicine since 1970s due to their easy-accessibility and abilities to self-renew and to differentiate into several tissue lineages including cartilage, bone, and adipose tissues (Arita et al., 2011). Since, obtaining sufficient number of cells is problematic in cartilage tissue engineering (Peretti et al., 2006), the cellular component should be easily expandable to provide a sufficient quantity of tissue for the regeneration process (Song et al., 2004). MSCs have been harvested in large quantities from a wide range of tissues in the human body (Arita et al., 2011). In addition, they can be expanded many times without

affecting the nature of the tissue formed after implantation into chondral defects (Ahmed et al., 2011). Furthermore, MSCs implanted into experimental osteochondral lesions showed proper integration with the subchondral plate (Yan and Yu, 2007). Since full - thickness cartilage defects involve both cartilage and subchondral bone, an ideal cell source for tissue engineering - based repair strategies should have the capability to generate these two very distinct skeletal tissues. MSCs-based approaches would be appropriate for developing constructs at the cartilage - bone interface and to develop ligament and meniscus substitutes (Ahmed et al., 2011). Finally, MSCs can be obtained in autologous form in a minimally invasive procedure and comply with the key ethical issues as compared to embryonic stem cells; therefore, they have been widely used in different clinical trials (Ahmed and Hincke, 2013).

Different sources of MSCs

Mesenchymal stem cells for cartilage tissue engineering have been derived from many sources including bone marrow (Kisiday et al., 2008; Erickson et al., 2009; Duval et al., 2012; Re'em et al., 2012; Gadjanski et al., 2013; Randau et al., 2013), adipose tissue (Kessler and Grande, 2008; Estes et al., 2010; Jakobsen et al., 2010), synovium (Park et al., 2005; Pei et al., 2009; Fan et al., 2010), blood, periosteum (Tuan et al., 2003; Ahmed and Hincke, 2010), amniotic fluid (Park et al., 2011a; Ahmed and Hincke, 2013), trabecular bone (Tuan et al., 2003; Giannoni et al., 2012), infrapatellar fat pad (Buckley et al., 2010), placenta (Li et al., 2012), muscle (Tuan et al., 2003; Kessler and Grande, 2008; Andrades et al., 2012), umbilical cord (Wharton's jelly) (Esposito et al., 2013), dermis (Tuan et al., 2003; Ahmed and Hincke, 2010), and embryonic stem cells (Zhang et al., 2013).

Although scientists have focused their research on utilizing adult mesenchymal stem cells derived from the bone marrow (BM-MSCs) for chondrogenesis, isolation of MSCs from the bone marrow is accompanied by pain at the donor site and other possible various complications such as inflammation and bleeding. In addition, the number of cells that can be obtained from the bone marrow is limited. However, adipose tissue-derived MSCs (AD-hMSCs) from adults pose no ethical problems and potentially could provide large number of cells (Jung et al., 2010). In this context, AD-MSCs have been differentiated into chondrogenic MSCs, and then combined with fibrin glue (FG) for subcutaneous injection into nude mice to explore the feasibility of whether cartilage can be generated *in vivo*. These MSCs were found to proliferate and form new cartilage suggesting that formation of cartilaginous tissue from such a cell source *in vivo* can be achieved (Jung et al., 2010). In a related study, MSCs derived from bone marrow, adipose tissue, or amniotic fluid, were encapsulated in fibrin hydrogel and then evaluated for their capacity for differentiation *in vitro* and *in vivo*. The three different types of hMSCs encapsulated in fibrin hydrogels produced chondrocytes as indicated by high expression of cartilage specific genes and proteins (Park et al., 2011a). In contrast, in a third study, AD-MSCs were compared to BM-hMSCs for their cartilage forming potential. Both cell types were encapsulated in a commercial fibrin hydrogel. AD-MSCs showed much weaker potential for chondrogenesis compared to BM-MSCs; these results weaken the value of adipose tissue as a source of MSCs (Im et al., 2005). Alternatively, MSCs extracted from synovial fat pad may have better chondrogenic potential than BM-MSCs. Compared to bone marrow, synovial fat pad is reported to give a higher yield of adherent colony forming cells. Obtaining a large number of cells at harvest has the potential advantage of reducing costly and time-consuming tissue culture expansion that risks cell contamination. There is also less pain and morbidity associated with the harvest of synovial fat pad cells compared with bone marrow cells (Khan et al., 2010).

Carrier / scaffolding matrix

Tissue engineering - based strategies for repair of articular cartilage require an artificial ECM (matrix) in which the cells can propagate and differentiate with subsequent new tissue generation. Typically, scaffolding matrix for articular cartilage tissue engineering should mimic the effect of native cartilage ECM on cell proliferation, cell-to-cell interaction, and differentiation (Kerker et al., 2008; Ahmed and Hincke, 2010). In addition, the matrix must be biodegradable and promote superior cell adhesion (Ahmed et al., 2008). Furthermore, the scaffold should be biocompatible and stable for an adequate length of time until being replaced gradually by cartilage - like ECM secreted from the cells (i.e. remodeling). Moreover, they must facilitate uniform cell distribution, retain the cells at the lesion site, and promote optimum integration with the surrounding native cartilage. Finally, the scaffold should have sufficient mechanical properties to withstand *in vivo* forces (Kerker et al., 2008; Ahmed and Hincke, 2010). A wide range of scaffolding matrices have been evaluated for tissue engineering - mediated cartilage repair including protein based (collagen, fibrin, and gelatin), carbohydrate based (agarose, alginate, chitosan, and hyaluronan) (Kessler and Grande, 2008), and synthetic polymers such as polyglycolic acids (PGA), polylactic acid (PLA), copolymers of glycolic and lactic acids (PLGA) (Ahmed and Hincke, 2013), poly(ethylene glycol) (PEG) (Ahmed and Hincke, 2010), and poly (ε-carpolactone) (PCL) (Abrahamsson et al., 2010).

Carbohydrate-based scaffolds

Because of the stimulatory effect of glycosaminoglycan (GAG), a long unbranched carbohydrate, on chondrogenesis, the utilization of

carbohydrate-based scaffolds is a relevant strategy to induce chondrogenesis (Iwasaki et al., 2004).

Agarose

Agarose is a typical naturally-occurring polysaccharide that has been frequently utilized as a delivery vehicle for drugs and living cells. In addition, it is utilized as a scaffolding matrix for different tissue engineering applications including progenitor cell chondrogenesis (Sakai et al., 2007). It has been shown that BM-MSCs - seeded agarose hydrogels culture promoted ECM synthesis and accumulation (Kisiday et al., 2008). In addition, functional chondrogenesis as indicated by increased mechanical properties was observed in BM-MSCs - seeded agarose hydrogels (Erickson et al., 2009). In general, chondrogenic cells require an appropriate biomechanical stimulation to produce functional cartilage. However, applying direct mechanical stimulation is not always a suitable strategy to achieve this goal. It has been reported that transient adenosine 5'-triphosphate (ATP) treatment of cartilaginous constructs made of BM-MSCs encapsulated in agarose hydrogels in the fourth week of cultivation can improve functional mechanical properties in terms of equilibrium and dynamic compressive moduli (Gadjanski et al., 2013). Alternatively, in an *in vitro* experiment carried out in a sliding contact bioreactor to recapitulate the mechanical stimuli arising from physiological joint loading, short-term sliding contact of BM - MSCs - seeded agarose promoted enhanced chondrogenic gene expression in a manner dependent on both the axial strain applied and transforming growth factor-ß supplementation. Long-term sliding contact significantly improved the tensile properties of the constructs and elicited alterations in type II collagen and proteoglycan accumulation (Huang et al., 2012). Finally, infrapatellar fat pad (IFP)-derived MSCs encapsulated into agarose hydrogel and cultured under low oxygen tension (5%) in the presence of TGF-$ß_3$ showed enhanced chondrogenesis leading to superior mechanical functionality compared to chondrocytes-seeded agarose constructs cultured under identical conditions (Buckley et al., 2010).

Alginate

Alginate is a brown algae - derived material that has been used widely in a variety of applications including cell encapsulation, drug delivery and macromolecule immobilization (Ghahramanpoor et al., 2011; Randau et al., 2013). It has been demonstrated that MSC - seeded alginate constructs cultured and maintained with TGF-$ß_3$ promoted improved accumulation of collagen II and GAGs (Xu et al., 2008). Similar results were observed when synovium - derived MSCs (SD-MSCs) or adipose - derived MSCs (AD-MSCs) - seeded alginate constructs were cultured in the presence of BMP-2 (Park et al., 2005) or TGF-$ß_3$/BMP-2 combination (Estes et al., 2010), respectively. Alternatively, a modified form of alginate known as affinity binding alginate-sulfate scaffold was utilized for the presentation and sustained release of TGF-$ß_1$ to the seeded BM-MSCs. Such presentation of TGF-$ß_1$ led to MSCs chondrogenic differentiation as indicated by deposition of collagen type II. These chondrogenically differentiated MSCs with type II collagen and aggrecan typical of the articular cartilage were observed when these TGF-$ß_1$ affinity-bound constructs were subcutaneously implanted in nude mice (Re'em et al., 2012). In another study, chondrogenially differentiated BM - MSCs (TGF-$ß_1$- mediated induction for 3 weeks) that were encapsulated into alginate beads and subjected to dexamethasone treatment for 5 weeks have been shown to terminally differentiate and subsequently can be used to generate a model of endochondral ossification (Randau et al., 2013). Alginate is characterized by a non toxic nature, easy solution to gel transition procedure and low cost; however, the inferior biomechanical properties limit its utilization in various tissue engineering applications. To overcome this drawback, esterification of the alginate polysaccharide backbone by octadecyl chains has been demonstrated to enhance gel strength that is accompanied by a more stable structure in physiological solution. This hydrophobically - modified alginate gel supported MSCs chondrogenic differentiation (Ghahramanpoor et al., 2011). In general, the induction of the chondrocyte phenotype requires serum or growth factors such as TGF-$ß_s$ or BMP. However, the use of these bioactive agents raises important ethical and regulation problems and may also enhance osteoblastic differentiation. When BM-MSCs were encapsulated into alginate beads and cultured under the effect of hypoxic environment (5% oxygen tension) or HIF-1α (ectopically expressed in MSCs) in the absence of any growth factor, chondrocytic phenotype was induced *in vitro* and *in vivo* as indicated by type II collagen and aggrecan expression (Duval et al., 2012). A common practice in tissue engineering is to combine two or more biomaterials to incorporate advantages of the different materials (Ahmed et al., 2008). BM-MSCs encapsulated into alginate that was blended with fibrin at various blend ratios were evaluated for physical properties including tensile and dimensional stability and biological properties including cell proliferation and accumulation of chondrocytic markers. The fibrin component offered gel extensibility and promoted cell proliferation, while alginate offered gel biostability and supported GAGs and collagen II production and chondrogenic gene expression. In this study, BM-MSC differentiation has been shown to vary between fibrin and alginate regions of blended scaffolds. In addition, it provides insight into the development of heterogeneous engineered tissues by control of the cell-scaffold interactions through manipulating the scaffold composition (Ma et al., 2012). As indicated above, BM-MSCs have been shown to produce superior expression of chondrocytic markers *in vitro*. However, the

utilization of these cells *in vivo* has not been fully explored. It has been shown that, application of MSCs encapsulated into alginate for the treatment of full-thickness cartilage defects created in a rabbit model led to superior tissue regeneration when compared to cartilage defects that were untreated as indicated by histological and immunohistochemical analysis (Dashtdar et al., 2011; Tay et al., 2012). Likewise, implantation of BM-MSCs seeded onto alginate/(poly(lactic-co-glycolic) acid (PLGA) constructs into rabbit full-thickness articular cartilage defects resulted in creation of cartilage-like tissue, which resembled the adjacent normal cartilage as indicated by the accumulation of aggrecan (Reyes et al., 2013).

Chitosan and chitin

Chitosan is a natural polymeric biomaterial derived from the shells of crustaceans, such as shrimp and crabs, and has been used as a clotting agent for treatment of hemorrhage (Cascio and Sharma, 2008). Chitosan is the deacetylated derivative of chitin. Chitosan alone or in combination with a wide variety of scaffolds has been used extensively in the tissue engineering of articular cartilage (Di Martino et al., 2005). When encapsulated into a copolymer of water-soluble chitosan and thermosensitive poly(N-isopropylacrylamide), BM - MSCs have been shown to differentiate into chondrocytes *in vitro*; and this construct can be injected *in vivo* below the lower critical solution temperature, which then can gelate and demonstrates expression of chondrocytic markers (i.e., GAGs and collagen II), particularly collagen II (Cho et al., 2004). Native articular cartilage is subjected to synovial fluid flow during normal joint function. The effect of shear stress, caused by perfusion of the medium (fluid flow) by a flow-perfusion bioreactor through a construct of BM-MSCs seeded onto chitosan/poly(butylene terephthalate adipate) fibre mesh, on the differentiation process, was investigated. Chondrogenic differentiation, as indicated by improved ECM deposition and collagen type II production, was observed in the bioreactor samples when compared to the static controls (Alves da Silva et al., 2011). It has been shown that chitosan modification of the porous elastromeric poly L-lactide-co-ε-caprolactone (PLCL) scaffold improved the cell compatibility of the PLCL scaffold as indicated by even distribution of the seeded MSCs and improved attachment without significant alteration of the physical elastomeric properties of PLCL along with formation of cartilage tissue of better quality (Yang et al., 2012a). As tissue engineering-mediated repair of the osteochondral defect involves simultaneous regeneration of bone and cartilage, MSCs seeded in a bi-layered gene-activated osteochondral scaffold consisting of plasmid TGF-$ß_1$-activated chitosan-gelatin scaffold for chondrogenic layer and plasmid BMP-2-activated hydroxyapatite/chitosan-gelatin scaffold for osteogenic layer was evaluated as a strategy for regeneration of complex tissues. This bi-layered integrated scaffold induces MSCs in the different layers to differentiate into chondrocytes and osteoblasts *in vitro*, and simultaneously supports articular cartilage and subchondral bone regeneration in the rabbit knee osteochondral defect model (Chen et al., 2011a). A scaffold composed of chitosan and ß-glycerophosphate known as BST-CarGel is used as adjunct to microfracture to stabilize the blood clot and retain MSCs in the cartilage lesion. BST-CarGel is liquid at room temperature and solidifies at human body temperature. It was introduced for clinical application by Biosyntech (Quebec, Canada) (Cascio and Sharma, 2008). The technique involves mixing fresh autologous blood with BST-CarGel in the operation room, which then delivered to the holes created by microfracture and the surrounding prepared defect (Kerker et al., 2008).

Hyaluronate and Hyaff®11

Because of its multiple functions in regulating and stabilizing the internal environment of cartilage, hyaluronate (hyaluronic acid [HA]) is a promising scaffold to promote cartilage repair (Ahmed and Hincke, 2010). The chondrogenic potential of MSCs derived from bone marrow (BM) and adipose tissue (AT) in combination with hyaluronate under the effect of TGF-$ß_1$ has been evaluated in an *in vitro* study. HA - seeded BM-MSCs showed improved expression of the chondrocytic markers collagen IIα_1 and aggrecan. In addition, chondrogenesis in HA scaffolds was more efficient using BM-MSCs than AD-MSCs (Jakobsen et al., 2010). It has been hypothesized that increasing the MSCs seeding density in a novel photocrosslinkable HA would promote functional maturation. BM-MSCs encapsulated into this HA form under the effect of dynamic culture conditions resulted in improved functional properties, as indicated by the increased compression modulus (Erickson et al., 2012). In a related study, BM-MSCs were encapsulated into the same photocrosslinkable HA and transiently exposed to high dose of TGF-$ß_3$. Acute exposure to this high dose of TGF induces functional and long-term differentiation of stem cell populations (Kim et al., 2012). It has been shown that transplantation of BM - MSC - seeded hyaluronate sponge into rabbit osteochondral defects resulted in considerable regeneration of cartilage, which was very similar in nature to the surrounding cartilage, especially when HA hydrogel was loaded with fibroblast growth factor-2 (FGF-2) (Kayakabe et al., 2006). Alternatively, when MSCs suspended in chemically modified hyaluronan and gelatin (Thiolated forms of both) and injected into an osteochondral defect created in rabbit knee, they cross-linked *in situ* to form a hydrogel which then completely filled the defect with elastic, firm, translucent cartilage and showed superior integration of the repair tissue with the normal cartilage (Liu et al., 2006). Similarly, SD - MSCs encapsulated into injectable type I collagen/hyaluronic acid/fibrinogen

(COL/HA/FG) composite gel was investigated *in vivo* for the repair of damaged rabbit articular cartilage. This COL/HA/FG construct with encapsulated cells produced a hyaline-like cartilage regeneration tissue, as indicated by dense Safranin-O staining and positive type II collagen immunostaining (Lee et al., 2012). In contrast, combining BM with fibronectin-coated hyaluronan-based sponge (ACP™, Fidia Advanced Biopolymers Sre, Abano Terme, Italy) does not have a remarkable effect on the medium and long-term outcomes of the regeneration process when compared to bone-marrow free ACP. However, the outcomes of osteochondral defects filled with ACP hydrogel were superior to that of nontreated defects (Solchaga et al., 2002).

HYAFF®11 is the benzylic ester of hyaluronan (Radice et al., 2000), and has been shown to provide a good environment to support the chondrogenic differentiation of MSCs (Facchini et al., 2006). Rabbit MSCs seeded onto HYAFF®11 showed good adhesion and proliferative properties along with elaboration of the chondrocytic marker collagen II *in vitro* (Ahmed and Hincke, 2010). In addition, implantation of cell-seeded or unseeded HYAFF®11 scaffolds into osteochondral lesions did not elicit any immune reaction and promoted the healing process compared to nontreated lesions (Radice et al., 2000). In a similar study, tissue that regenerated after implantation of the MSCs - seeded HYAFF®11 sponge showed better cellular density and better integration with the surrounding cartilage than the cell-free sponge (Gao et al., 2002).

Protein - based scaffolds

Collagens

Collagen-based hydrogels have been extensively used in tissue engineering applications because of their easy manipulability and low immunogenicity (Galois et al., 2004; Stark et al., 2006). In addition, collagen can achieve high seeding efficiency and good cell adhesion due to its hydrophilicity and the presence of bioactive domains in its structure (Chen et al., 2003, 2004). Further, as a component of the ECM, collagen has been reported to regulate the chondrocytic phenotype and chondrogenesis both *in vitro* and *in vivo* (Galois et al., 2004; Stark et al., 2006). However, collagen hydrogels cannot withstand *in vivo* forces and fails to maintain the required shape *in vitro* due to its poor mechanical properties (Chen et al., 2003, 2004). In addition, its rapid degradation has been documented as a drawback both *in vivo* and *in vitro* (Meinel et al., 2004).

However, it has been demonstrated that *in vitro* seeding of MSCs into a collagen I scaffold preserves cell viability and morphology along with elaboration of the chondrocytic markers (Schulz et al., 2008). In addition, MSCs exhibited condensation and contraction necessary for cartilage histogenesis with enhanced glycosaminoglycan and collagen type II accumulation (Ng et al., 2011). Further, it has been shown that MSCs differentiated into chondrocytes *in vitro* when seeded onto a stimulatory collagenous biomaterial consisting of multilayered collagen I/II/III matrix (Geistlich, Wolhusen, Switzerland) (Vavken et al., 2010). Collagen I has being combined with other materials to incorporate the advantages of both (Ahmed et al., 2008). Collagen type I modification of a poly(lactic-co-glycolic acid) (PLGA) scaffold resulted in increased histological biocompatibility (Mouthuy et al., 2013). In addition, MSCs encapsulated within the matrix of HA -collagen II fibrils have a higher proliferation rate than cells grown within the unmodified fibrils (Chen et al., 2009). In an *in vitro* study, a multiphasic composite scaffold with an upper collagen I fibre layer seeded with MSCs for articular cartilage repair and a lower polylactic acid (PLA) part for bone repair in the presence of TGF-β_1 was investigated. Homogeneous cell distribution and chondrogenic differentiation as indicated by chondrocyte-like appearance of cells along with accumulation of proteoglycan and collagen type II-rich ECM was observed in the upper collagen layer (Heymer et al., 2009). Further, MSCs suspended in collagen I gel which was then seeded onto poly(ε-caprolactone) (PCL) scaffolds resulted in chondrogenesis of the cells along with regional mineralization at the interface between soft, newly formed engineered cartilage, and stiffer underlying PCL scaffold (Abrahamsson et al., 2010).

In a rabbit knee full-thickness cartilage lesion, transplantation of MSCs - loaded collagen I scaffold showed the most hyaline cartilage, highest histological scores and superior compressive modulus. Moreover, it showed a good integration with the subchondral bone and adjacent cartilage (Qi et al., 2012). Similarly, MSCs seeded into genipin cross-linked type II collagen scaffold was evaluated in a rabbit full-thickness cartilage defects and resulted in chondrocyte-like cells, with lacuna structure and corresponding ECM found in the repaired sites without apparent inflammation (Chen et al., 2011b). Alternatively, transplantation of *in vitro* predifferentiated MSCs embedded into collagen I hydrogel (Arthro Kinetics, Esslingen, Germany) into osteochondral lesions (i.e. matrix - associated autologous chondrocyte transplantation with predifferentiated MSCs) resulted in significantly better histological scores with morphological characteristics of hyaline cartilage such as columnarization and presence of collagen type II (Zscharnack et al., 2010). In a related study, implantation of MSCs in combination with bi-layered collagen I/II/III into ovine chondral defects promoted better repair and formation of hyaline-like tissues (Dorotka et al., 2005). Chondro-Gide by Geistlich (Wolhusen, Switzerland) is a bi-layered porcine collagen type I/III membrane that has been utilized clinically in a single-stage procedure after microfracture and this is known as autologous-matrix-induced chondrogenesis (AMIC). Chondro-Gide membrane in this case provides the scaffold for growth and multiplication of stem cells released after microfracture procedure (Cascio and Sharma, 2008). Satisfactory outcomes have been reported after 2 years

of follow-up during the first clinical assessment of 32 patients treated with AMIC in combination with microfracture, in terms of defect filling, functional improvement, pain reduction, and patient satisfaction (Steinwachs et al., 2008). Alternatively, VeriCart (Histogenics, Waltham, MA) has been introduced to the market as an adjuvant to microfracture. VeriCart relies upon the same principle as BST-CarGel; however, a double-structured collagen scaffold is utilized in VeriCart instead of the chitosan and ß-glycerophosphate scaffold utilized in BST-CarGel (Ahmed and Hincke, 2010). Finally, a concentration-gradient (CG) collagen type I transplanted into a full-thickness cartilage defects created in rabbit knee model has been shown to promote migration of MSCs to the centre of the lesion site as indicated by histological grading score and increased number of proliferating cells (Mimura et al., 2008).

Fibrin

Fibrin is a biopolymer of the monomer fibrinogen. Fibrin and fibrinogen have critical roles in blood clotting, fibrinolysis, cellular and matrix interactions, the inflammatory response, wound healing, and neoplasia. The fibrinogen/fibrin system has been used extensively in a wide range of clinical settings (Ahmed and Hincke, 2013). Fibrin can be isolated autologously from patients and fabricated into a hydrogel scaffold (Ahmed et al., 2008). Other features of fibrin scaffolds include biodegradability, biocompatibility, high seeding efficiency, and uniform cell distribution (Ahmed et al., 2007). Because of the good manipulability of fibrinogen and its role in the natural healing process, it is a promising choice as both scaffolding and delivery agent for cells with regenerative capacity (Ahmed and Hincke, 2013).

Encapsulation of MSCs into fibrin glue (FG) promoted the chondrogenic process as indicated by enhanced accumulation of aggrecan and collagen II along with chondrocyte-like morphology of the differentiated cells (Ahmed et al., 2011). In the same study, encapsulation of MSCs in platelet-rich fibrin glue (PR-FG) led to initially increased expression of collagen II; however, no difference was observed between FG and PR-FG after long term *in vitro* culture. In a related study, the encapsulation of BM-MSCs into commercially available fibrin glue in the presence of TGF-$ß_1$ has been shown to influence mesenchymal condensation which preceded chondrogenic differentiation. These fibrin encapsulated cells differentiated into chondrocytes that secreted aggrecan and collagen II. This study was completed by testing fibrin construct in the cartilage phase of the biphasic osteochondral constructs. Fibrin supported superior cartilage growth with higher cellularity, total glycosaminoglycan (GAG) and collagen II levels (Ho et al., 2010). Constructs of BM-hMSCs encapsulated into fibrin/polyurethane scaffold were evaluated as an optimized environment to promote chondrogenesis of MSCs *in vitro*. This fibrin/polyurethane scaffolds promoted chondrogenesis of MSCs comparable to that of MSCs pellets, and chondrogenesis was dependent on the initial seeding density (Li et al., 2009a).

Recently, BM-MSCs encapsulated into a commercially available FG were transplanted into a rabbit osteochondral defect model resulting in defect filling by a repair tissue that has similar biomechanical properties and durability to the surrounding cartilage (Berninger et al., 2013). Alternatively, cartilage fragments isolated from osteoarthritic knee were evaluated as a factor to promote chondrogenic differentiation of MSCs. In this study, intact non-injured parts of cartilage tissues obtained during total knee arthroplasty were mixed with immortalized BM-hMSCS and encapsulated into a fibrin gel, followed by subcutaneous implantation into nude mice. Histological analysis showed formation of neocartilage-like structures in the cartilage fragment - fibrin - MSC constructs, with elevated type II collagen gene expression (Chen et al., 2012). In an extensive study, BM-MSCs encapsulated into a fibrin hydrogel containing heparinized nanoparticles (NPS) loaded with TGF-$ß_3$ have been evaluated *in vitro*, nude mouse, and in a rabbit defect model for chondrogenesis and cartilage repair. The *in vitro* and *in vivo* results indicated that transplanted MSCs together with TGF-$ß_3$ may constitute a clinically efficient method for the regeneration of hyaline articular cartilage (Park et al., 2011b). Alternatively, SD-MSCs in combination with fibrin have been seeded into non-woven polyglycolic acid (PGA) mesh and incubated for a month in a rotating bioreactor system with growth factor cocktails before evaluation for cartilage repair *in vivo*. After six months of implantation into a rabbit knee osteochondral defect, this construct promoted defect filling with smooth hyaline-like cartilage that was integrated with the surrounding native cartilage. High expression of collagen II and GAGs along with the absence of collagen I and inflammatory signs were observed (Pei et al., 2009). In an *in vivo* study, PR-FG prepared by the cell saver centrifuge system, in combination with autologous BM-MSCs, was evaluated clinically in human subjects. Autologous BM-MSCs were culture expanded, placed on PR-FG intraoperatively, and then transplanted into full-thickness cartilage defects of femoral condyles under an autologous periosteal flap. All patients exhibited significant improvement by objective criteria over the follow-up period. At this point, MRI examination revealed complete defect fill and complete surface congruity with native cartilage in more than half of the patients (Haleem et al., 2010). Finally, photopolymerizable poly(ethylene glycol) (PEG)-modified fibrinogen, known as PEGylated fibrinogen or Gelrin C (Regentis Biomaterials, Haifa, Israel), that crosslinks *in situ* after exposure to UV light is now in the stage of clinical trails. Gelrin C is also considered as an adjunct to microfracture or osteochondral defect filler, and its rate of degradation depends on the degree of

PEGylation (McNickle et al., 2008).

Gelatin

Gelatin is produced commercially through boiling and hydrolysis of collagen-containing raw material such as bones and skins, mainly of cows and pigs (Ahmed and Hincke, 2010). In comparison to collagen, gelatin has lower immunogenicity and cost. However, it maintains some of the positive collagen properties such as adhesion, differentiation, and proliferation capabilities (Lien et al., 2009). Gelatin has been widely used for cartilage tissue engineering, especially in combination with other scaffolding materials to combine the advantages of both (Ahmed and Hincke, 2010).

A PLGA-gelatin/chondroitin/hyaluronate (PLGA-GCH) hybrid scaffold with TGF-$ß_1$-impregnated microspheres (PLGA-GCH/MS-TGF), in combination with MSCs, has been evaluated *in vitro* and *in vivo*. PLGA-GCH/MS-TGF significantly augmented proliferation of MSCs and GAG accumulation. When implanted and differentiated into a rabbit knee chondral defect, MSCs seeded on PLGA-GCH/MS-TGF showed better chondrocyte morphology, integration, and subchondral bone formation as indicated by histology grading score (Fan et al., 2007). Similarly, the effect of hybrid microspheres (MS) composed of gelatin TGF-$ß_1$-loaded MS and chitosan MS on the AD-MSCs differentiation into chondrocytes was evaluated by *in vivo* pellet culture and in a rabbit full-thickness cartilage defect. The hybrid microspheres showed superior mechanical properties and promoted improved collagen II expression in the cell pellet culture. In addition, the pellet of AD-MSCs/hybrid MS promoted cartilage regeneration in the cartilage defect, as indicated by histological analysis (Han et al., 2010).

Artificial biodegradable polymers

Synthetic biodegradable polymers combine some important characteristics such as controllable degradation rate, high reproducibility, high mechanical strength, and easy manipulation into specific shapes (Chen et al., 2003; Ahmed and Hincke, 2010). However, the cell recognition signals are missing in such scaffolds. In addition, they posses relatively hydrophobic surfaces that are not ideal for efficient cell seeding (Chen et al., 2003).

Polyglycolic acid (PGA)

Polyglycolic acid (PGA) is a highly crystalline, hydrophobic linear polyester (Zwingmann et al., 2007). When MSCs were seeded onto PGA scaffolds and cultured under the effect of LE135 (a low molecular weight synthetic inhibitor of the retinoic acid receptor), there was a dose-dependent formation of cartilage, demonstrated both histologically and by biochemical analysis of the collagen component of the ECM. It has been demonstrated that inhibition of retinoic acid receptor ß promotes chondrogenic differentiation of MSCs independent of the SOX-9 pathway and in the absence of serum and growth factors (Kafienah et al., 2007a). A composite scaffold composed of polyglycolic acid-hydroxyapatite (PGA-HA) and autologous mesenchymal stem cells (MSCs) was investigated *in vivo* to promote repair of osteochondral defects in a rabbit model. The implantation of a MSC-PGA-HA composite demonstrated hyaline cartilage and a complete subchondral bone formation. In addition, significant integration of the newly formed tissue with surrounding normal cartilage and subchondral bone was observed (Zhou et al., 2008).

Polylactic acid (PLA)

PLA is a linear polyester that is less crystalline but more hydrophobic than PGA due to the presence of an extra methyl group (Zwingmann et al., 2007). It has been shown that PLA fibrous scaffold maintained a robust scaffold structure upon incubation in a physiological solution, based on macroscopic and SEM observations. In addition, PLA scaffolds supported the highest rate of proliferation of seeded MSCs (Li et al., 2006). Cartilage constructs fabricated by press-coating PLA blocks onto high-density cell pellets of MSCs have been evaluated *in vitro*. Histochemical analysis showed that the press-coated pellets formed cell layers composed of morphologically distinct, chondrocyte-like cells, surrounded by a sulfated proteoglycan-rich ECM. In addition, cartilage-specific marker genes and proteins are detected in such constructs (Noth et al., 2002).

Construct of MSCs seeded into PLA have been investigated for the repair of full-thickness cartilage defects created in a rabbit model. Defects repaired by these constructs showed hyaline-like cartilage tissue formation. In addition, cartilage defects treated with these MSCs constructs appeared to have better cell arrangement, subchondral bone remodeling, and integration with surrounding cartilage (Yan and Yu, 2007). Alternatively, the capacity of a triple composite consisting of an interconnected porous hydroxyapatite (IP-CHA), recombinant human bone morphogenetic protein-2 (rhBMP-2), and a synthetic biodegradable polymer PLA-PEG as a carrier for rhBMP-2, to induce the regeneration of articular cartilage, has been evaluated in a rabbit full-thickness cartilage defects. After implantation of this triple composite, the defects were completely repaired via migration of MSCs from the surrounding bone marrow and formation of hyaline-like tissue with mature matrix and columnar organization of chondrocytes (Tamai et al., 2005).

Poly(lactic-co-glycolic acid) (PLGA)

Combining lactic acid with glycolic acid to form PLGA has been shown to prolong the degradation time of the hybrid, probably because glycolic acid is more

hydrophilic than lactic acid (Ahmed and Hincke, 2010). It has been shown that modification of natural nanofibrous articular cartilage ECM (ACECM) with PLGA enhanced the mechanical strength of ACECM. This composite scaffold promoted improved cell proliferation and viability *in vitro* after seeding of MSCs (Zheng et al., 2011). Transplantation of MSCs-seeded PLGA into rabbit osteochondral defects showed significant cartilage regeneration as indicated by gross morphology, mechanical properties, histological examination, and the accumulation of chondrocytic markers (Han et al., 2008). Bilayered porous scaffolds of PLGA with different pore sizes have been fabricated and combined with MSCs to determine the optimal pore size to facilitate the repair of osteochondral defects in a rabbit model. The different PLGA porous scaffolds in combination with MSCs all supported regeneration of articular cartilage, but the best results observed in cell-seeded PLGA scaffold with 100-200 μm pores (Duan et al., 2013). The potential of 3D PLGA/nano-hydroxyapatite (PLGA/NHA) scaffold in combination with MSCs for cartilage regeneration was investigated *in vitro* and *in vivo*. Improved viability, proliferation, attachment, and distribution of MSCs in PLGA/NHA scaffolds were observed *in vitro*. The application of this construct to rabbit osteochondral defects promoted the formation of smooth hyaline-like cartilage with abundant GAGs and collagen type II deposition along with improved defect filling (Xue et al., 2010). There is a major problem in using PLGA as a scaffolding material: its degradation products (e.g., glycolic acid and lactic acid) are observed to cause an intense inflammatory reaction after *in vivo* implantation (Ahmed and Hincke, 2010).

Poly(ethylene glycol) (PEG)

PEG is a synthetic polymer that has wide biotechnological applications. It is a highly biocompatible material and used widely for many medicinal purposes. The neutral and noninteractive nature of PEG facilitates ECM secretion from cells after their encapsulation (Ahmed and Hincke, 2010). Another advantage of the PEG-based scaffold is that it can be laminated by adding a second layer before complete crosslinking of the first layer, which creates zonal patterns resembling the organization of articular cartilage (Temenoff et al., 2002). It has been shown that the mechanical properties of PEG-based scaffolds are dependent on the mesh size. Different mesh sizes can be achieved by changing the PEG molecular weight, concentration, or the crosslinking density (Temenoff et al., 2002; Ahmed and Hincke, 2010). PEG dimethacrylate and PEG diacrylate are the most widely used PEG derivatives as they can crosslink via photoinitiator with UV light (i.e., photopolymerizable PEG). One advantage of the photocrosslinkabe derivatives is that they can be used for *in situ* application in a minimally invasive procedure such as liquid injection (Nguyen and West, 2002; Ahmed and Hincke, 2010). It has been demonstrated that encapsulation of MSCs into photopolymerizable PEG resulted in uniform distribution of cells and enhanced accumulation of chondrocytic markers (Buxton et al., 2007). Likewise, encapsulation of SD-MSCs into the same photopolymerizable PEG promoted high cell viability and chondrogenesis *in vitro* (Fan et al., 2010). The capacity of BM-MSCs encapsulated into this hydrogel to form cartilaginous tissue was explored in an immunodeficient mice model. After implantation, this construct promoted the formation of cartilaginous tissue that stained intensively to safranin O and expressed immunolocalized collagen II (Alhadlaq and Mao, 2005). Chondrogenic differentiation of MSCs encapsulated into poly(ethylene glycol) hydrogel having noncovalent HA-binding capabilities (i.e. HA- interactive scaffolds) was evaluated for cartilage formation *in vitro* and in a rat articular defect model. This HA-binding hydrogel produced the best cartilage *in vitro* as indicated by biochemical, histological, and gene expression analyses. The *in vivo* study demonstrated improved cartilage tissue production in defects treated with the HA-interactive hydrogel as indicated by histological analysis (Unterman et al., 2012).

The incorporation of negatively charged molecules such as chondroitin sulfate into PEG results in better mechanical properties, as gauged by compressive modulus results (Stuart and Lubowitz, 2006). The photoreactive form of PEG in combination with chondroitin sulfate (bioadhesive) is now utilized in the clinical setting (ChonDux, introduced to the market by Cartilix [Foster City, CA]). The product is applied in conjunction with microfracture to enhance the cartilage repair process through promotion of chondrogenic differentiation and cartilage tissue formation by BM-MSCs. The product is applied in a liquid form that solidifies upon exposure to UVA light (Cascio and Sharma, 2008). Recently, a pilot clinical study was initiated in 18 patients with focal cartilage defects on the medial femoral condyle to evaluate human safety and clinical feasibility of the poly(ethylene glycol) diacrylate (PEGDA) hydrogel system in combination with the standard microfracture surgery. For translation to the joint environment, a chondroitin sulfate adhesive was applied to covalently bond and adhere the hydrogel to cartilage and bone tissue in articular defects. Treated patients achieved significantly higher levels of tissue fill, increased tissue organization, less pain, and increased knee function scores compared to controls (Sharma et al., 2013).

Polycaprolactone (PCL)

PCL is a semicrystalline, hydrophobic, biodegradable polyester polymer characterized by an extended resorption time that makes it a promising scaffold for cartilage tissue engineering (Li et al., 2006; Shafiee et al., 2011). The PCL - based fibrous scaffold

exhibited a robust structural integrity upon incubation in a physiological solution, based on macroscopic and SEM observations. In addition, it supported desirable cell responses of the seeded MSCs (Li et al., 2006). Chondrogenic differentiation of MSCs on oriented nanofibrous PCL scaffolds was explored in an *in vitro* study. Electrospun and oriented PCL scaffolds (500 or 3000 nm fiber diameter) were created, and MSCs were cultured onto these scaffolds. Based on assessment by the chondrogenic markers, use of the nanofibrous PCL scaffold (500 nm) appears to enhance the chondrogenic differentiation of MSCs (Wise et al., 2009).

Since, mechanical load and the inflammatory environment constitute the major problems during treatment of large cartilage defects by implantation strategy, a 3D woven PCL scaffold seeded with MSCs was developed to promote chondrogenesis and to have satisfactory mechanical properties in the presence of the pro-inflammatory cytokine interleukin-1 (IL-1). MSC-seeded PCL scaffolds cultured in chondrogenic conditions synthesized a functional ECM rich in collagen and proteoglycan content, along with having sufficient mechanical properties. However, the presence of IL-1 limited matrix accumulation and inhibited any increase in mechanical properties (Ousema et al., 2012). Alternatively, 3D loosely or tightly woven PCL were combined with MSC in static dish or oscillating bioreactor to engineer mechanically functional cartilage constructs *in vitro*. Chondrogenesis of constructs were verified histologically by rounded cells within a hyaline-like matrix that immunostained for collagen type II. The mechanical properties of the construct approached normal articular cartilage for tightly woven PCL cultured in bioreactors. In addition, bioreactor culture yielded constructs with improved collagen content and homogenous matrix (Valonen et al., 2010).

A biodegradable PCL nanofibrous scaffold seeded with MSCs was evaluated for the repair of swine model full-thickness cartilage defects. The MSCs-seeded constructs showed enhanced mechanical properties and complete repair via formation of hyaline cartilage-like tissue, along with restoration of smooth cartilage surface (Li et al., 2009b). Alternatively, the potential of poly(vinyl alcohol)/polycaprolactone (PVA/PCL) nanofiber scaffolds seeded with BM-MSC for cartilage tissue engineering was investigated *in vitro* and *in vivo*. The PVA/PCL scaffolds supported proliferation and chondrogenic differentiation of MSCs *in vitro*. Moreover, rabbit full-thickness cartilage defects treated with cell-seeded PVA/PCL scaffolds showed improved healing (Shafiee et al., 2011). Finally, a mixture of MSCs and fibrin gels was seeded onto elastic mechano-active poly(lactide-co-caprolactone) (PLCL) and subjected to continuous compressive deformation in a chondrogenic medium was evaluated for cartilage repair *in vivo*. After subcutaneous implantation into nude mice, the application of dynamic compression and the 3D environment of the hybrid scaffolds induced MSCs to differentiate into chondrocytes with phenotype stability and enhanced GAGs production, subsequently improving the quality of cartilaginous tissue formed *in vitro* and *in vivo* (Jung et al., 2009).

Clinically investigated matrices and cell sources (Table 1)

A wide variety of strategies have been widely developed to restore the structure and functions of injured cartilage. These strategies range from reducing the pain and swelling, to repair of cartilage through the formation of fibrocartilage (i.e., reparative procedures), and to a variety of restorative procedures, including tissue-engineering-based strategies (Detterline et al., 2005; Ahmed and Hincke, 2010). Reparative strategies aim at initiating bleeding from the subchondral bone, which permits the migration of bone marrow (BM) stem cells to the site of injury along with blood clot formation, leading to repair tissue formation composed mainly of fibrocartilage (Ahmed and Hincke, 2010). This is why reparative procedures are commonly referred to as marrow stimulation techniques. Fibrocartilage has inferior mechanical properties to that of hyaline cartilage. However, it covers the exposed underlying bone, which subsequently reduces pain and

Table 1. Mesenchymal stem cells and the associated scaffolding materials investigated clinically during tissue engineering of articular cartilage.

Study no.	MSCs Source	MSCs type	Associated Matrix	Commercial Name	Clinical Phase	Status
1	Bone Marrow	Autologous	Chitosan/ß-Glycerophosphate	BST-CarGel	III	Completed
2	Bone Marrow	Autologous	Collagen I/III	Chondro-Gide	II/III	Recruiting
3	Bone Marrow	Autologous	Collagen	VeriCart	I/II	Ongoing
4	Bone Marrow	Autologous	PEGylated Fibrinogen	Gelrin C	I/II	Recruiting
5	Bone Marrow	Autologous	PEG/ Chondroitin Sulfate	ChonDux	II/III	Ongoing
6	Bone Marrow	Autologous	Collagen I	-	I	Completed
7	Bone Marrow	Autologous	Commercial Scaffold	-	I	Ongoing
8	Bone Marrow	Autologous	Proper Scaffold	-	II/III	Recruiting
9	Umbilical Cord	Allogeneic	Viscous Polymer	CartiStem®	III	Completed
10	Umbilical Cord	Allogeneic	Viscous Polymer	CartiStem®	III Follow-up	Recruiting
11	Bone Marrow	Autologous	-	-	I/II	Ongoing
12	Bone Marrow	Allogeneic	-	-	I/II	Recruiting

swelling (Detterline et al., 2005). Reparative procedures include arthroscopic abrasion arthroplasty, microfracture, subchondral drilling, and spongialization (Ahmed and Hincke, 2010). A wide range of commercially available products have been developed and used as an adjunct to the microfracture reparative strategy including BST-CarGel, Chondro-Gide for AMIC, VeriCart, Gelrin C, and ChonDux. These products are in different phases of clinical trials in Europe and the United States. BST-CarGel has completed phase III clinical trials (Stanish et al., 2013), while chondro-Gide (Steinwachs et al., 2008; Anders et al., 2013) and Chondux (Spiller et al., 2011) are still in phase II/III clinical trials. Gelrin C and VeriCart are currently in phase I/II clinical trials (Ahmed and Hincke, 2010).

MSCs from different sources in combination with various supporting matrix are currently in different phases of clinical trials. Autologous transplantation of BM-MSCs mixed with collagen I scaffold for patients with knee cartilage defects and osteoarthritis has completed phase I clinical trials (Safety and efficacy) at the Royan Institute, Tehran University of Medical Sciences, Iran (Miremadi et al., 2013). Alternatively, comparing the treatment efficacy of autologous MSCs versus chondrocytes following implantation in combination with a commercially available scaffold for full-thickness cartilage defect is currently under clinical trial phase I investigation at Ullevaal University Hospital, Oslo Norway (Shenaq et al., 2010). A third study, currently in phase II/III clinical trial at Cairo University, Egypt, aims at investigating whether implanting autologous BM-MSCs in combination with suitable scaffold in patients suffering from early osteoarthritis or cartilage defects is an effective treatment strategy (Shenaq et al., 2010; Gupta et al., 2012). WJ-MSCs in combination with a viscous polymer that is commercialized under the name CartiStem® have been investigated clinically in two studies (Shenaq et al., 2010; Barry and Murphy, 2013). The first study aimed at comparing the efficacy and safety of Cartistem® versus microfracture in patients with knee articular cartilage injury or defect, and has completed phase III clinical trial at Korea University, Seoul, South Korea (Shenaq et al., 2010; Barry and Murphy, 2013). The second study, currently in phase III clinical trials, is a complementary follow-up study for patients involved in the first study, in order to investigate the long term safety and efficacy of Cartistem® versus microfracture until 5 years post-treatment timepoint (Barry and Murphy, 2013). Finally, in two related studies, either allogeneic (MSV_allo) or autologous BM-MSCs have been evaluated for the treatment of osteoarthritis at University of Valladolid, Spain. These two studies are currently in phase I/II clinical trials (Gupta et al., 2012; Barry and Murphy, 2013).

Table 2. Different bioactive agents evaluated experimentally during tissue engineering of articular cartilage.

Bioactive agent	Concentration range	Commonly used concentration
ATP	60 μM	60 μM
BMP-2	10 - 500 ng/mL	10 ng/mL
BMP-4	10 - 500 ng/mL	10,500 ng/mL
BMP-6	10 - 500 ng/mL	500 ng/mL
BMP-7	10 - 200 ng/mL	10,100,200 ng/mL
Dexamethasone	10^{-7}M	10^{-7}M
FGF-2	0.5 -10 ng/mL	10 ng/mL
FGF-18	10^{-9} M	10^{-9} M
GDF5	100 ng/mL	100 ng/mL
Hypoxic environment	5% O_2	5% O_2
IGF-1	10-100 ng/mL	100 ng/mL
LE135	1 μM	1 μM
PTHrP	1 nM -10 μM	1 nM
TD-198946	10^{-7} M	10^{-7} M
TGF-ß$_1$	10-50 ng/mL	10 ng/mL
TGF-ß$_2$	0.5-10 ng/mL	5,10 ng/mL
TGF-ß$_3$	5-10 ng/mL	10 ng/mL
LiCl (ß-Catenin activator)	5 mM	5 mM

Bioactive agents (Table 2)

Bioactive agents should have the capability to induce tissue regeneration. Ideal bioactive factors should promote proliferation, differentiation, and maturation of the cellular component. In addition, they should have chemotactic activity and should maintain sufficient ECM production by the cells (Leo and Grande, 2006; Ahmed et al., 2008). A wide range of bioactive agents have been investigated during the development of tissue-engineered cartilage substitutes and shown to regulate chondrogenesis in MSCs, including TGF-ß$_1$, TGF-ß$_2$, TGF-ß$_3$, BMP-2, BMP-4, BMP-6, BMP-7, FGF-2, IGF-1 and parathyroid hormone-related protein (PTHrP) (Ahmed and Hincke, 2010). Other bioactive factors that have been shown to be critical for MSCs - dependent cartilage regeneration include dexamethasone (Randau et al., 2013), adenosine 5'-Triphosphate (ATP) (Gadjanski et al., 2013), thienoindazole derivative (TD-198946) (Yano et al., 2013), stromal derived factor-1ß (SDF-1ß) (Mendelson et al., 2011), growth and differentiation factor-5 (GDF5) (Feng et al., 2008), retinoic acid receptor (RAR) inhibitor (LE135) (Kafienah et al., 2007a), FGF-18 (Davidson et al., 2005), hypoxic environment (5% O_2) (Buckley et al., 2010; Duval et al., 2012), and TGF/ß-Catenin activation (Yang et al., 2012b).

TGF-ß$_1$ and TGF-ß$_3$ are the most widely applied bioactive molecules during the development of cartilaginous substitutes and have been shown to promote chondrogenesis (Ahmed and Hincke, 2010), especially in 3D cultures where chondrogenesis is facilitated (Im et al., 2006). BMP-2 (Park et al., 2005; Tamai et al., 2005; Reyes et al., 2013) and low oxygen tension (Buckley et al., 2010; Duval et al., 2012) (hypoxic environment; 5% O_2) have been also used widely for induction of chondrogenesis in MSCs. BMP-2 can induce chondrogenic differentiation of adult stem

cells, comparable to the TGF-ß family members, in both monolayer and 3D cultures (Park et al., 2005). In a related study in alginate matrix, BMP-2 and BMP-7 induce chondrogenic differentiation of MSCs better than TGF-$ß_1$ (Kurth et al., 2007). In addition, a combination of TGF-$ß_2$ and BMP-7 promotes chondrogenesis of MSCs in pellet culture better than a combinations of TGF-$ß_2$ and BMP-2, TGF-$ß_2$ and BMP-6, or each growth factor separately (Kim and Im, 2009). Alternatively, it has been shown that a combination of TGF-$ß_3$ and BMP-2 induces chondrogenesis in hMSCs pellet culture better than TGF-$ß_3$ and BMP-4, TGF-$ß_3$ and BMP-6, or TGF-$ß_3$ alone (Sekiya et al., 2005). However, a combination of TGF-$ß_3$ and BMP-6 (Indrawattana et al., 2004; Hennig et al., 2007) or TGF-$ß_3$ and IGF-1 (Indrawattana et al., 2004) are effective inducers of chondrogenesis in MSC pellet, as well as monolayer culture (Indrawattana et al., 2004; Takagi et al., 2007). FGF-2 increases, while TGF-$ß_2$ decreases, MSCs proliferation in monolayer culture (Im et al., 2006). Alternatively, It has been shown that combining IGF-1 with TGF-$ß_1$ induced chondrogenic differentiation of MSCs into chondrocyte-like cells and promoted expression of chondrocytic markers in monolayer culture (Xiang et al., 2007). Similarly, combining IGF-1 with TGF-$ß_2$ resulted in increased production of proteoglycans in MSC pellet culture compared to TGF-$ß_2$ alone (Im et al., 2006). It has also been revealed that combining TGF-$ß_3$ with PTHrP promoted chondrogenesis of MSCs and resulted in decreased expression of collagen X (hypertrophic marker), collagen I (fibrocartilage marker), and alkaline phosphatase (bone marker) without affecting the other hyaline cartilage markers (i.e., collagen II and aggrecan) (Kafienah et al., 2007b).

The hypoxic environment induces a chondrocyte phenotype in MSCs in the absence of exogenous growth factors (Duval et al., 2012). In addition, low oxygen tension enhanced chondrogenesis of MSCs in the presence of TGF-$ß_3$, leading to superior mechanical functionality (Buckley et al., 2010). Alternatively, transient ATP treatment has been demonstrated to improve functional mechanical properties of cartilaginous constructs based on chondrogenic cells (Gadjanski et al., 2013). Dexamethasone increases GAGs accumulation and induces terminal differentiation of the chondrogenically differentiated MSCs (Randau et al., 2013). In contrast, the thienoindazole derivative TD-198946 induces chondrogenic differentiation of MSCs without promoting endochondral ossification (Yano et al., 2013). TGF-$ß_3$ and/or SDF-1ß have been demonstrated to recruit MSCs and to induce chondrocytic markers expression including aggrecan and collagen II (Mendelson et al., 2011). Similarly, GDF5 induces chondrogenesis of MSCs as indicated by improved accumulation of aggrecan and collagen II (Feng et al., 2008). Likewise, the low molecular weight synthetic inhibitor of the RAR alpha and RAR beta receptors (LE135) can induce chondrogenic differentiation of MSCs in a comparable way to TGF-$ß_3$ in monolayer, and when seeded onto 3D PGA scaffold (Kafienah et al., 2007a). Alternatively, FGF-18 as a selective ligand for FGF receptor 3 (FGFR3) promotes chondrogenic differentiation and cartilage matrix production in mesenchymal cells isolated from embryonic mice limb buds (Davidson et al., 2005). Finally, temporal co-activation of the TGFß signaling pathway with ß-catenin resulted in the enhancement of chondrogenic differentiation of MSCs (Yang et al., 2012b).

Physical stimulants

An appropriate mechanical environment plays a crucial role during the normal development and homeostasis of cartilage, while excessive physical forces can lead to cartilage damage. Articular cartilage is subjected to a combination of compressive, tensile and shear stresses; consequently, it is likely that compression forces are not sufficient as a mechanical signal to generate a cartilage-like tissue *in vitro* (Ahmed and Hincke, 2013). Cartilage is normally subjected to physical pressures ranging between 1 and 20 MPa at a frequency of 0-1 Hz (Ahmed and Hincke, 2010). A wide range of physical stimulants have been evaluated during the development of MSCs-based articular cartilage tissue engineering strategies, including electromagnetic fields (EMFs) (Ongaro et al., 2012; Esposito et al., 2013), dynamic compression (Jung et al., 2009), combination of shear and dynamic compression (Pelaez et al., 2009; Li et al., 2010a,b; Schatti et al., 2011), sliding contact (Huang et al., 2012), fluid flow (Alves da Silva et al., 2011), hydrostatic pressure (Miyanishi et al., 2006; McMahon et al., 2008) and low intensity ultrasound (LIUS) (Ebisawa et al., 2004; Ongaro et al., 2012).

Treatment of WJ-MSCs with pulsed electromagnetic field (PMEF) resulted in increased cell division and cell density along with a reduction in the time required for differentiation and ECM deposition (Esposito et al., 2013). In addition, treatment of SD-MSCs pellets with EMFs in the presence of TGF-$ß_3$ counteracts the IL-1ß-induced inhibition of chondrogenesis, as indicated by enhanced expression of the chondrocytic markers aggrecan and collagen II (Ongaro et al., 2012). Alternatively, TGF-$ß_3$ - induced chondrogenesis of MSCs pellet, and subsequent expression of chondrocytic markers, were both improved with LIUS (Ebisawa et al., 2004; Lee et al., 2007). Further, LIUS has been shown to inhibit apoptosis that was observed when MSCs were seeded into alginate scaffold with TGF-$ß_1$ (Lee et al., 2007).

Application of dynamic compression to BM-MSCs seeded onto hybrid scaffolds of fibrin and PLCL promoted the chondrogenic differentiation of MSCs, maintained their phenotypes, and enhanced GAGs accumulation (Jung et al., 2009). The effect of mechanical load on the differentiation of BM - MSCs (suspended into fibrin and seeded into biodegradable

polyurethane scaffolds) under the effect of exogenous TGF-ß$_1$ has been investigated (Li et al., 2010a). Mechanical load (surface motion superimposed on cyclic compression) stimulated chondrogenesis of hMSCs compared to the unloaded scaffolds, with a much stronger effect on gene expression at lower TGF-ß$_1$ concentrations. In the absence of TGF-ß$_1$, mechanical load stimulated gene expression and protein synthesis of TGF-ß$_1$ and TGF-ß$_3$. Thus, mechanical load promotes chondrogenesis of hMSCs through the TGF-ß$_1$ pathway by upregulating TGF-ß gene expression and protein synthesis (Li et al., 2010a). In a related study, a combination of shear and dynamic compression was applied to constructs made of fibrin/polyurethane composites in which human MSCs were encapsulated without exogenous growth factor. The application of shear superimposed upon dynamic compression led to significant increases in chondrogenic gene expression. In addition, sulfated glycosaminoglycan and collagen II were only detected when compression forces were applied in combination with shear forces (Schatti et al., 2011). In a similar study, BM-hMSCs in combination with fibrin/polyurethane composites were subjected to various mechanical loads to determine the effect of compression, surface rotation frequency and axial compression magnitude on the induction of cartilage-specific gene expression and protein synthesis in the presence of TGF-ß$_1$. Application of dynamic compression and surface shear (1 h/day for 1 week) led to enhanced chondrogenesis of hMSCs compared to no load controls. The load frequency and compression amplitude were positively correlated with the development of chondrogenic characteristics (Li et al., 2010b). In a fourth study, the capability of fibrin hydrogels to support chondrogenesis of BM-hMSCs under cyclic compression was evaluated. Different fibrin concentrations and stimulus frequencies were assessed for impact on viability, proliferation and chondrogenic differentiation of hMSCs, demonstrating a threshold in these parameters for maintaining cellular viability within scaffolds. This study confirmed the suitability of fibrin hydrogels for supporting cyclic compression-induced chondrogenesis of mesenchymal stem cells (Pelaez et al., 2009). Finally, sliding contact was applied to a MSCs-seeded agarose construct to recapitulate the mechanical stimuli associated with physiological joint loading. Sliding contact has been shown to improve chondrogenic gene expression in the presence of TGF-ß$_3$. In addition, it improved tensile properties along with enhanced collagen II and proteoglycan accumulation (Huang et al., 2012).

MSCs in cell pellets subjected to intermittent hydrostatic pressure in the presence of TGF-ß$_3$ showed enhanced production of cartilage-associated ECM molecules (Miyanishi et al., 2006). In addition, application of continuous cyclic tensile loading to MSC-seeded collagen I-GAG scaffold led to increased synthesis of GAGs, while application of uniaxial static mechanical constraint resulted in reduced production of GAGs (McMahon et al., 2008). Alternatively, the effect of fluid flow on the chondrogenic differentiation of BM-MSCs seeded onto CPBTA hybrid scaffold in the presence of TGF-ß$_3$ was evaluated *in vitro*. Shear stress caused by this fluid flow was able to promote the differentiation process, as indicated by enhanced ECM deposition and type II collagen accumulation, along with reduction in the time required for differentiation (Alves da Silva et al., 2011).

Gene delivery (Table 3)

To further improve the quality of the regenerated cartilage tissue, new genetic information can be delivered to the cellular component, which subsequently

Table 3. Different transgenes and the delivery techniques employed during development of MSCs - based tissue engineered articular cartilage substitutes.

Transgene	MSCs Source	Delivery Technique	Study Type	Resultant Effects
Bcl-xL	Bone Marrow	Liposome	*In vitro & In vivo*	Improved cell viability and cartilage defect healing.
BMP-2	Bone Marrow	Adenovirus	*In vitro*	Superior production of cartilage-specific markers including aggrecan and SOX-9.
BMP-7	Bone Marrow	Liposome	*In vitro*	Improved production of cartilage-specific markers.
BMP-7	Bone Marrow	Retrovirus	*In vivo*	Satisfactory production of GAGs and the formation of hyaline-like regeneration tissue.
CTGF	Bone Marrow	Adenovirus	*In vitro & In vivo*	Promotion of chondrogenic differentiation and hyaline - like cartilage regeneration.
Endostatin	Bone Marrow	Liposome	*In vitro*	Improved production of cartilage-specific markers.
IGF-1	Bone Marrow	Liposome	*In vitro*	Enhanced production of cartilage-specific markers.
SOX - 5, 6, 9	Bone Marrow & Adipose Tissue	PEI/PLGA NP	*In vitro & In vivo*	Differentiation into mature chondrocytes and hyaline - like cartilage regeneration.
SOX-9	Bone Marrow	Adenovirus	*In vitro & In vivo*	Promotion of chondrogenic differentiation and superior tissue repair.
SOX-9	Bone Marrow	Electroporation	*In vitro & In vivo*	Promotion of chondrogenic differentiation.
TGF-ß$_1$	Bone Marrow	Liposome	*In vivo*	Hyaline-like cartilage formation along with adequate defect filling and integration.
TGF-ß$_1$	Bone Marrow	Adenovirus	*In vitro*	Upregulation of cartilage-specific genes and improved chondrogenesis.
TGF-ß$_1$/IGF-1/ BMP-2	Bone Marrow	Adenovirus	*In vitro*	Superior expression of cartilage specific markers (GAGs and Collagen II).
TGF-ß$_3$	Bone Marrow	Adenovirus	*In vitro*	Enhanced production of cartilage-specific markers.
TGF-ß$_3$/ shRNA	Synovium	Lentivirus - Adenovirus	*In vitro*	Improved chondrogenesis (Collagen II, aggrecan, and COMP) & depletion of collagen I expression.

regulates the regeneration process at the cellular and molecular levels. This can be achieved through either viral or nonviral vectors (Kim et al., 2006). The most widely used viral vectors include adenovirus, adeno-associated virus, foamyvirus, herpes simplex virus, lentivirus, and Moloney murine leukemia virus (Kim et al., 2006; Steinert et al., 2008). The nonviral vectors include plasmid DNA in liposomes, naked DNA, and DNA delivered via Ca/P precipitation, electroporation, gene gun, or injection (Kim et al., 2006; Steinert et al., 2008). Viral vectors have higher transfection efficiency than the nonviral vectors. In addition, the delivered gene is expressed longer in the viral versus nonviral vectors (Kim et al., 2006; Saraf and Mikos, 2006). However, the use of such gene transfer strategies has major safety concerns because viral vectors may acquire replication competence, as well as the risk of insertional mutagenesis (retroviral vectors) and the development of undesirable host immune responses (adenoviral vectors) (Elsler et al., 2012). In addition, nonviral vectors are easier to synthesize and have lower immunogenicity than the viral vectors (Kim et al., 2006; Saraf and Mikos, 2006).

Gene delivery through viral vectors

Over-expression of TGF-ß$_3$ (Hao et al., 2008) and BMP-2 (Palmer et al., 2005) in BM-MSCs via adenoviral vectors resulted in enhanced production of cartilage-specific markers (Ahmed and Hincke, 2010). In a related study, the co-delivery of TGF-ß$_3$ gene (to promote chondrogenesis) and small hairpin RNA (shRNA) gene (to silence collagen type I gene expression) to SD-MSCs via lentiviral and adenoviral vectors, respectively, resulted in improved chondrogenesis as indicated by expression of collagen II, aggrecan, and cartilage oligomeric matrix protein (COMP) along with reduced collagen I expression (Zhang et al., 2012). Alternatively, the effect of adenoviral-mediated overexpression of BMP-2 on chondrogenesis of BM-MSCs encapsulated into fibrin/polyurethane scaffold was evaluated *in vitro*. It has been shown that transduction with BMP-2 gene resulted in profound upregulation of aggrecan and SOX-9 genes. However, to improve collagen II expression, mechanical stimulation should be combined with BMP-2 gene transfer (Neumann et al., 2013). In another study, bioactivity of adeno-associated virus (AAV) - cytomegalovirus promoter (CMV) - TGF-ß$_1$ (AAV-CMV-TGF-ß$_1$) released from diluted (50%) and undiluted (100%) FG (Tisseel/Tissucol) was assessed by measuring induction of cartilage-specific gene expression in BM-MSCs. AAV-TGF-ß$_1$ released from diluted FG transduced hMSCs efficiently and subsequently higher concentrations of bioactive TGF-ß$_1$ and greater upregulation of cartilage-specific gene expression were observed compared with MSCs from undiluted FG. This study demonstrated that diluted FG promoted enhanced release of bioactive AAV-TGF-ß$_1$, efficient transduction, and improved chondrogenesis of BM-MSCs (Lee et al., 2011). The effect of adenovirus-mediated co-delivery of the three transgenes (IGF-1, TGF-ß$_1$, and BMP-2) on the chondrogenesis of BM-MSCs in pellet culture was investigated in an *in vitro* study. Co-expression of IGF-1 and TGF-ß$_1$, BMP-2, or both at low doses, resulted in larger pellet size, higher GAGs accumulation, stronger proteoglycans and collagen II staining, and greater expression of cartilage-specific markers genes, than with either transgene alone (Steinert et al., 2009). However, in a related study overexpression of IGF-1 in MSCs did not result in significant improvement in expression of cartilage-specific markers (Palmer et al., 2005).

The effect of adenoviral vector - mediated transduction of BM-MSCs with SOX-9 gene on chondrogenesis was evaluated *in vitro* and *in vivo*. SOX-9 promoted chondrogenic differentiation of BM-MSCs in monolayer and PGA scaffold effectively. In addition, the implantation of BM-MSCs transduced with SOX-9 gene, in combination with PGA scaffold into rabbit full-thickness cartilage defects, resulted in superior tissue repair and accumulation of cartilage specific ECM along with expression of many chondrogenesis marker genes (Cao et al., 2011). However, in a related *in vitro* study, transduction of BM-MSCs with SOX-9 failed to improve GAGs synthesis, revealing that mechanical load must necessarily be combined with SOX-9 over-expression in order to promote cartilage ECM accumulation (Kupcsik et al., 2010). Alternatively, implantation of BMP-7 over-expressing BM-MSCs (retroviral-mediated), in combination with a 3D supporting PGA matrix, into a rabbit chondral defect, led to satisfactory production of GAGs and the formation of hyaline-like regeneration tissue (Grande et al., 2003). Finally, constructs consisting of BM-MSCs transfected (adenoviral-mediated) with connective tissue growth factor (CTGF), that were seeded on NaOH treated PLGA, have been evaluated *in vitro* and *in vivo*. The CTGF-modified BM-MSCs/NaOH-treated PLGA construct promoted chondrogenic differentiation *in vitro* and showed superior hyaline-like cartilage regeneration, similar to normal cartilage when implanted in rabbit full-thickness cartilage defects (Zhu et al., 2013).

Gene delivery through non-viral vectors

It has been shown that overexpression of IGF-1 (Elsler et al., 2012), BMP-7 (Bai et al., 2011), and endostatin (Sun et al., 2009) (DNA plasmid in liposome) in BM-MSCs resulted in enhanced production of cartilage-specific markers. BM-MSCs transfected with plasmid DNA encoding TGF-ß$_1$ and seeded onto fibrin/PLGA sponge (Wang et al., 2010), poly-L-lysine coated PLA (Guo et al., 2006), or chitosan (Guo et al., 2007) were evaluated for the repair of full-thickness cartilage defects created in the rabbit model. The implantation of these constructs into cartilage defects promoted the formation of hyaline-like cartilage that

filled the defect site and integrated well with the surrounding cartilage and subchondral bone (Guo et al., 2006, 2007; Wang et al., 2010). Alternatively, BM-MSCs have been transduced by plasmid DNA encoding the anti-apoptotic protein Bcl-xL to prevent cell death and improve implantation efficiency in a rabbit model. Bcl-xL - engineered MSCs, in combination with collagen scaffold, resulted in improved cartilage healing both morphologically and histologically (Hu et al., 2010). It has been shown that SOX-9 transfected BM-MSCs (electroporation-mediated), encapsulated within alginate/chitosan polysaccharide capsules, promoted the chondrogenic process *in vitro* and *in vivo* as indicated by positive expression of SOX-9 and collagen type II, along with accumulation of cartilage-specific matrix (Babister et al., 2008). Likewise, BM-MSCs and AD-MSCs transfected with SOX5, 6, and 9 genes (SOX Trio) and encapsulated in a fibrin hydrogel exhibited similar chondrogenic-specific gene expression and protein synthesis. Chondrogenic genes and proteins were more highly expressed in SOX Trio expressing cells than in untransfected cells. Both *in vitro* and *in vivo* analyses revealed that fibrin hydrogel-encapsulated, both cultured or transplanted cells transfected with the SOX Trio, successfully differentiated into mature chondrocytes and could be used for the reconstruction of hyaline articular cartilage (Yang et al., 2011). It was concluded that TGF-$ß_1$, SOX-9, BMP-7, BMP-2, and TGF-$ß_3$ are the best candidates and the most widely used transgenes during the development of tissue-engineered cartilage substitute.

Conclusions (Fig. 1)

Ideally, successful cartilaginous constructs should achieve a number of goals. First, synthesis of the cartilage-specific ECM components that will assemble to provide the same architecture as native cartilage ECM is required. Secondly, the construct should integrate completely with the surrounding native tissue. Thirdly, the mechanical properties of the construct must be comparable to those of native cartilage in order to withstand *in vivo* forces. Finally, introduction / implantation in a minimally invasive fashion is desirable. MSCs - based cartilage tissue engineering are the most promising strategies to fulfill these goals. MSCs derived from bone marrow, synovium, and umbilical cord blood are the most promising cell source. BM-MSCs can be obtained autologously to eliminate the need for primary surgery to harvest chondrocytes. In addition, MSCs can be obtained from a young healthy donor and used allogeneically to avoid propagation of a disease state that might exist in patient cells. Further, MSCs can be genetically manipulated to express a wide variety of growth factors and ECM molecules. TGF-$ß_1$, TGF-$ß_3$, SOX-9, BMP-2, and BMP-7 are the best candidate transgenes for genetic modification of MSCs. Of all tested materials, photocrosslinkable matrices consisting of both natural and synthetic materials along with collagenous materials are the most promising scaffolding material, since they can be combined with MSCs and gelate *in situ* under UVA light in a minimally invasive procedure. At this time, photopolymerizable

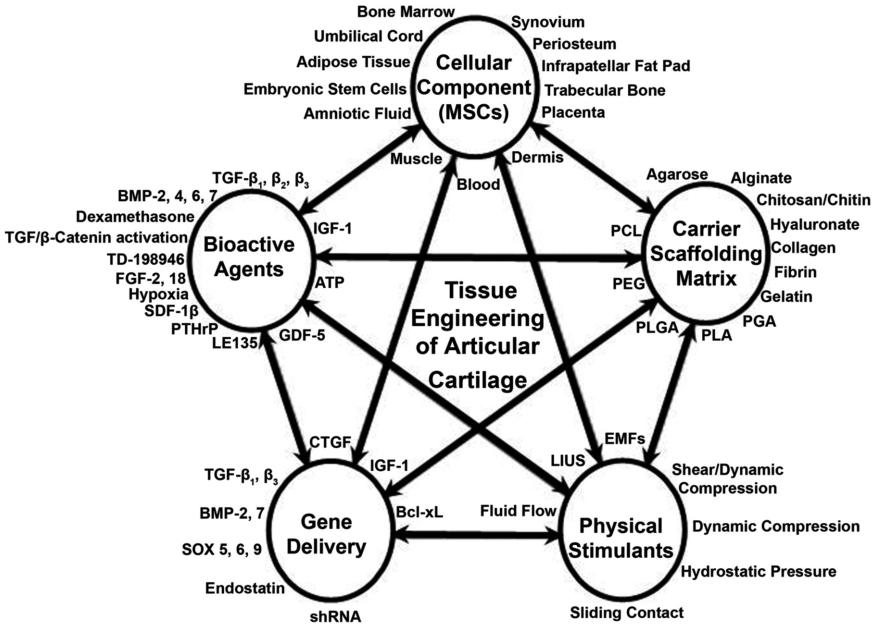

Fig. 1. Different components of MSCs - based tissue engineering of articular cartilage.

PEG - based scaffolds, including Chondux and Gelrin C, and collagen - based scaffolds, including Chondro-Gide and VeriCart, are good candidates. Amongst growth factors, TGF-$ß_1$, TGF-$ß_3$, BMP-2, and hypoxic environment seem to be the best bioactive factors for articular cartilage tissue engineering. However, choosing the best combination of these bioactive factors requires a better understanding of the cell signaling involved in chondrogenesis, in order to optimize proliferation and maturation of the cellular component, maximize the secretion of proper ECM component, and induce secretion of enzymes required to remodel the supporting matrix. The best maturation-promoting physical stimulants include EMF and shear forces/dynamic compression combination.

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