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THERAPEUTIC NANOMATERIALS FOR CARTILAGE REGENERATION

Elif Arslan, Seher Ustun Yaylaci, Mustafa O. Guler, and Ayse B. Tekinay

Institute of Materials Science and Nanotechnology, National Nanotechnology Research Center (UNAM), Bilkent University, Ankara, Turkey

4.1 INTRODUCTION

Cartilage tissue is present throughout the human body and is mainly responsible for load bearing, lubrication, and articulation (Fox et al., 2009; Han et al., 2011). It is categorized under three types: hyaline cartilage, elastic cartilage, and fibrocartilage. The tissue is avascular, aneural, and alymphatic, which causes its low capacity for self-repair following damage (Mahmoudifar and Doran, 2012). Cartilage is secreted by cartilage-specific cells, called chondrocytes, that comprise 3–5% of adult cartilage (Taipaleenmaki, 2010). During embryonic development, mesenchymal stem cells (MSCs) surround the cartilage in the deep region of the perichondrium and subsequently differentiate into chondrocytes (Slomianka, 2009). Besides its cellular components, cartilage also has an extracellular matrix (ECM) that is largely responsible for the load-bearing function of the tissue (Taipaleenmaki, 2010). The primary extracellular constituents of cartilage are the fibrillar collagen network and proteoglycans (Han et al., 2011). Specifically, type II collagen is the primary collagen fibril found in the cartilaginous ECM. Other

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collagens found in cartilage are the types III, VI, IX, X, XI, XII, and XIV, all of which contribute to the mature extracellular microenvironment of the tissue (Eyre, 2002). Proteoglycans are also a major element of the cartilage ECM, with aggrecan, the glycosaminoglycans (GAGs) chondroitin sulfate and keratan sulfate, hyaluronan, and the glycoprotein lubricin (PRG4) occurring commonly in the tissue matrix (Lin et al., 2005). The load-bearing, articulative, and lubricant properties of cartilage also depend heavily on the water content of the tissue. Water is highly abundant in cartilage and comprises 80% of its total weight. Significant amounts of water are integrated within the collagen matrix in interfibrillar spaces (Lu et al., 2006). The interfibrillar water forms a gelatinous structure and assists in spreading the pressure gradient across the tissue, thus relieving the solid matrix from excessive mechanical stress (Mow et al., 1980, 1992). The ability of healthy cartilage tissue to endure significant weight loads is attributed to two basic mechanisms of water: the frictional resistance of water flow and the pressurization of water within the matrix (Fox et al., 2009).

The joints of the human body are composed of articular cartilage (AC), synovial membranes, ligaments, subchondral bone, and menisci (Clouet et al., 2009). AC is the main focus of this chapter and indeed most cartilage regeneration studies, since it is commonly affected by diseases and traumatic injuries that necessitate its therapeutic regeneration. AC is a heterogeneous type of cartilage and contains different types of material organizations and matrix components across its structure. It is composed of four tissue layers that are horizontally stacked across the tissue surface and can be categorized with respect to their ECM composition and cellular morphology (Stroebel, 2007). The superficial zone forms 10–20% of AC tissue by volume, consists of flattened chondrocytes and tightly packed collagens (mainly types II and IX), and is predominantly responsible for the protection of the underlying layers from shear stresses, as well as providing tensile strength to the tissue (Fox et al., 2009). The middle (transitional) zone forms 40–60% of cartilage tissue; is composed of round chondrocytes, thicker collagen fibrils, and proteoglycans; and assists in resisting compressive forces (Fox et al., 2009). The deep zone forms 30% of the tissue and contains the thickest collagen fibers observed in cartilage. It is rich in proteoglycans and largely responsible for creating the resistance of cartilage against compressive forces (Fox et al., 2009). The last layer, called the tidemark, is located between the deep zone and the calcified part of cartilage; it displays a high proteoglycan content that also allows it to resist compression. In addition to the layers of cartilage proper, there is an additional calcified layer between the bone and cartilage. This intermediary layer integrates the two tissue types and contains hypertrophic chondrocytes.

While structural differences can be used to classify cartilage into four strata, compositional, collagen-based, and positional differences also exist within the cells and matrix elements of a given cartilage layer. Three types of cartilage ECM are recognized: pericellular, territorial, and interterritorial (Fox et al., 2009). The pericellular matrix is a thin layer that is mostly composed of proteoglycans, noncollagenous proteins, and glycoproteins and is responsible for supporting chondrocytes. It is also suggested that this layer may play a role in establishing the signal transduction networks required for the proper functioning of cartilage tissue (Eggli et al., 1985). The pericellular matrix is encompassed by the territorial matrix, which is composed mainly of a network of thin collagen fibrils that spread throughout the cartilage tissue. It has been suggested that this matrix is responsible for the resistance of cartilage against mechanical stresses and that it bears the majority of the mechanical load on the tissue (Engel et al., 1969). The interterritorial region is the largest among the three regions of the cartilage ECM and contributes the most to the biochemical composition and function of the AC. This matrix has abundant proteoglycan content in the form of collagen bundles, which are arranged in parallel in the superficial zone, perpendicular on the surface of the deep zone, and in intermediate angles for middle zone collagens (Mow and Guo, 2002).

The physiological metabolism of cartilage tissue is as crucial as its structural and biocompositional features, and its investigation is vital for the determination of processes underlying the diseases, defects, and repair mechanisms of cartilage. Cartilage metabolism is mainly anaerobic, since it is an avascular and alymphatic tissue. Nutrition is provided by diffusion from the synovial fluid, as the cartilage matrix contains pores of approximately 6 nm in diameter and is therefore limited in its capacity for material exchange (Maroudas, 1968; Mow et al., 1992). Chondrocytes are the main effectors of the metabolic activities of the tissue, despite their scarcity (~3–5% of cartilage tissue). They are also responsible for the synthesis, maintenance, and repair of the ECM (Buckwalter and Mankin, 1997). Several factors and molecules surround the chondrocytes and affect their ECM synthesis and degradation. For example, proinflammatory cytokines play a role in regulating the catabolic and anabolic metabolisms of chondrocytes and affect their capacity to synthesize and degrade ECM molecules (Buckwalter and Mankin, 1997). Growth factors, on the other hand, are involved in the regulation of proteoglycan synthesis: Although there is a dearth of information about the mechanisms and cellular pathways behind their effects, several growth factors are known to be important for cartilage proteoglycan synthesis. These include transforming growth factor beta-1 (TGF-β1), insulin-like growth factor, interleukin-1, and tumor necrosis factor-α (Fox et al., 2009).

The homeostasis of cartilage tissue contributes to its structural stability and depends on the healthy turnover of its ECM components; thus, deficiencies in turnover mechanisms commonly lead to disease. Enzymes responsible for the degradation of ECM molecules include metalloproteinases and cathepsins (Dejica et al., 2008; Woessner, 1991). The physiological metabolism of cartilage is also modified according to its stage of growth. Chondrocytes rapidly synthesize ECM components and expand the new tissue during embryonic development, which necessitates high rates of activity. However, the chondrocytes of adult cartilage tissues are at a steady state and function only to replace degraded or damaged matrix materials (Buckwalter, 1995).

There is a constant cross-communication between chondrocytes and their ECM, through which various functions of the tissue are performed. Chondrocytes are responsible for the turnover of the ECM, while the ECM facilitates nutrient exchange and determines which molecules can pass through the matrix to reach the chondrocytes. This function of the ECM depends on its composition and organization, especially on its proteoglycan components (Buckwalter and Mankin, 1997). The ECM itself also imparts signals in a more direct manner by transferring mechanical signals (e.g., joint loading) to the chondrocytes (Buckwalter, 1995).

Overall, a thorough characterization of the biomechanical function of cartilage is necessary to understand the nature of the tissue and the mechanisms behind its degeneration and regeneration. As such, it is unsurprising that cartilage tissue regeneration is one of the most comprehensively investigated topics in tissue engineering, especially in light of the fact that osteochondral defects and joint diseases are severely debilitating disorders with no known cures. Osteoarthritis (OA) in particular is the most common joint disease in the world and primarily affects the population aged over 60 (Arden and Nevitt, 2006; Clouet et al., 2009). OA has a detrimental effect on several regions of cartilage tissue, including AC, the synovium, and subchondral bone. It often manifests as a decrease in the thickness of AC, the growth of bone on the joint margins, and the alteration of the molecular components of the synovial fluid (Clouet et al., 2009). Although many treatment methods have been developed for OA, the complete amelioration of its effects has so far been outside the reach of modern medicine. In clinics, regular therapies for OA include weight reduction, physical therapy, drug treatments (e.g., pain relievers), and the administration of chondroprotective molecules; more severe conditions may necessitate surgical interventions such as arthroplasty and osteotomy (Clouet et al., 2009). These treatments are only able to alleviate the symptoms of OA and cannot reverse the condition itself; as such, significant advances in regenerative medicine

and tissue engineering are necessary for the development of prospective clinical treatments and therapeutics for osteochondral diseases.

4.2 CURRENT TREATMENT METHODS FOR CARTILAGE INJURIES

Defects in AC can result from traumatic injury or pathological degeneration. The capacity of cartilage to repair injury is limited, and when not treated, such defects can progress to degenerative arthritis. Various repair strategies are currently used in the treatment of cartilage defects. Arthroscopic techniques, such as lavages and debridements, aim to clean out the defect site and alleviate pain associated with the inflammation of the cartilage tissue (Hunziker, 2002). These procedures are considered to be palliative, as they only provide short-term relief by removing unstable cartilage flaps and preventing inflammation; they do not restore the function of the damaged site (Shannon et al., 2001). An arthroscopic lavage involves the washing of the joint space for the removal of blood or loose debris, while debridement is the removal of unstable cartilage flaps. The main targets of these procedures are patients with very small cartilage defects (Lewis et al., 2006).

Other arthroscopic techniques aim to utilize intrinsic repair capacity of cartilage and include drilling and microfractures. Both of these techniques consist of the penetration and subsequent bleeding of subchondral bone to induce fibrocartilage formation. They rely on the formation of a blood clot at the defect site by stimulating the bone marrow, resulting in the deposition of a mechanically weak fibrocartilage tissue at the defect site. The microfracture technique is used for defects smaller than 2.5 cm^2 and involves the removal of the unstable cartilage for the creation of a well-defined defect environment that is surrounded by healthy cartilage (Bhosale and Richardson, 2008). In this procedure, a microscopic awl is used to penetrate subchondral bone without damaging its integrity. The defect is then filled with clot material from the subchondral bone, which contains MSCs that will oversee the subsequent repair of cartilage tissue. The outcome of the treatment depends mainly on how well the patient can avoid overexerting the cartilage tissue with excessive weight loads. Clinical data suggest that 75% of patients display short-term clinical improvements; however, functional deterioration of the defect site begins within 24 months in 48–80% of cases (Gobbi et al., 2014). Low numbers of MSCs, or their dilution by synovial fluid, can result in a low regeneration capacity and early deterioration following the procedure. Adequate regeneration therefore

depends on the optimization of the number of cells extracted from the subchondral bone, which is performed by controlling the depth and numbers of the holes produced on the cartilage surface.

Grafting is the preferred way of treating larger cartilage defects and consists of the removal of healthy cartilage tissue from nonweight-bearing areas of the knee, followed by the transplantation of the removed tissue into the defect site (Görtz and Bugbee, 2007). Multiple plugs may be required depending on the depth and the extent of the defect site. Grafts are self-secured to the defect site without using any adhesives; their success depends heavily on whether draft plugs from nonload-bearing regions are able to withstand the stresses applied to the weight-bearing parts of the tissue. Another concern about the treatment of cartilage injuries through osteochondral grafting is the negative effect the procedure has on the viability of cells at the margins of the grafts. Cells in these regions are more prone to be affected by stress, and the resulting cell death may lead to the degeneration of the tissue over time (Boscainos et al., 2008). In addition, the dead space between the graft and the native tissue affects the integrity of repaired cartilage.

A new generation of tissue repair strategies involving the external administration of autologous or donor cells into the defect site has been developed to replace damaged cartilage with biomechanically stronger and functional hyaline cartilage. Autologous chondrocyte implantation (ACI) is one such strategy and utilizes the patient's own chondrocytes. It is conducted in three stages: Chondrocytes are first arthroscopically extracted from nonload-bearing parts of cartilage, subsequently expanded in an *in vitro* environment until sufficient numbers of cells are obtained, and finally reimplanted back into the defect site and secured with a periosteal graft taken from the tibia (Brittberg et al., 1994). The use of the patient's own chondrocytes prevents immune responses, and the isolation of chondrocytes can be performed through a minor biopsy operation, which reduces potential complications at the donor site. However, the operations involved are complex, and the procedure necessitates a longer recovery time to allow for the maturation of new cartilage tissue (Dehne et al., 2009). Graft hypertrophy is one of the most pronounced postsurgery complications of this technique.

Second-generation ACI use restorable collagen matrices instead of periosteal flaps. A combination matrix of collagens I and III has been shown to reduce graft hypertrophy and the number and depth of incisions necessary for the operation (Tuan, 2007). However, the use of artificial scaffolds increases the risk of immune reaction. Technical constraints during the culture and transplantation of cells, such as nonhomogeneous

distribution of chondrocytes, leakage of the transplanted cell suspension due to gravity, and the risk of dedifferentiation of chondrocytes, limit the usefulness of this technique for the production of functional hyaline cartilage and create large variations in the success of the operation (Vavken and Samartzis, 2010).

These difficulties have led to the development of a third generation of ACI, composed of biomechanically stable and functional tissue matrices that integrate better into host tissues (Bartlett, 2005). Use of scaffold-based techniques has some major advantages over scaffold-free techniques: Scaffolds can better assume the three-dimensional (3D) structure of the defect, provide a suitable substrate for cellular adhesion, ensure the homogeneous distribution of cells, and prevent leakage during transplantation. Matrix-induced autologous chondrocyte implantation (MACI) is an example of scaffold-based techniques in clinical use (Kon et al., 2011, 2013). Unlike second-generation ACI methods, cells are seeded onto 3D porcine-derived type I/III collagen matrices and cultured for an additional 3 days prior to implantation. These matrices are designed in a way that one side is porous and allows the infiltration of MSCs, while the other side exhibits high mechanical strength and has a low-friction surface that allows easier integration into the chondral cavity. The membrane patch is secured by a fibrin glue during implantation, eliminating the need for tedious watertight sutures. Materials used in MACI include collagen type I/III, hyaluronan (Hyalograft®-C, HYAFF®11; Fidia Advanced Biopolymers, Abano Terme, Italy) and fibrin (Tissucol, Baxter, Austria) scaffolds (Iwasa et al., 2008).

In vitro studies have demonstrated that the use of bilayer collagen membranes presents an adequate environment for cell attachment and chondrogenic differentiation (Gigante et al., 2007). However, there are no clinical findings that clearly prove the superiority of MACI over existing techniques (Memon and Quinlan, 2012): Indeed, no differences were found in the arthroscopic appearances, histological observations, and rates of tissue hypertrophy between collagen I/III-based ACI-C and bilayer collagen-based MACI grafts used in the treatment of symptomatic cartilage defect cases (Bartlett et al., 2005). MACI is nonetheless an attractive treatment method, as it provides a means of arthroscopic implantation that reduces invasiveness and operation times. Nonetheless, chondrocytes tend to lose their differentiated states during clonal expansion, which adversely affects their ability to synthesize functional cartilage ECM and results in large variances in the outcomes of ACI and MACI treatments. Three days of culture in collagen matrices may result in the formation of only an immature tissue environment, leading to the redifferentiation of cells and a corresponding

decrease in their matrix synthesis capability (Schulze-Tanzil, 2009). Longer culture periods may permit chondrocytes to synthesize their own matrix, resulting in more mature and stable tissue replacements.

4.3 TISSUE ENGINEERING EFFORTS

Existing treatment techniques are insufficient for the long-term repair of cartilage and need to be complemented with engineered systems containing cartilage-mimetic material architectures, inductive signals, or stem cells to produce reproducible, safe, and functional tissue constructs. There are four main approaches for cartilage regeneration: cultured cell implantation, engineered tissue construct implantation, scaffoldless tissue regeneration, and guided tissue regeneration. Cartilage growth, development, and repair are dependent on both biomechanical and biological signals. *In vitro* culture environments that attempt to induce cartilage repair or recapitulate the structural and biochemical elements of cartilage environment can therefore increase the success of implantation. Scaffold materials can directly alter the sensory landscape of the cells they contain, in addition to providing mechanical cues necessary for chondrogenic differentiation. As such, a great variety of factors should be considered in tandem for the design of optimal cartilage scaffolds. In a general sense, the material should allow the transport of nutrients and waste products, facilitate the migration and development of cells, possess an intrinsic biocompatibility, and provide the biochemical signals necessary for the survival and maintenance of chondrocytes.

Scaffold surface characteristics have a direct role on controlling the adhesion, migration, and differentiation of cells (Ma et al., 2005). Material properties such as scaffold morphology, hydrophilicity, and surface charge should therefore be considered for the development of functional scaffolds that can interact with the surrounding environment. An additional factor in the design of cartilage scaffolds is the time of implantation, that is, whether the construct is set to be implanted immediately or following an *in vitro* culture period. Constructs implanted immediately should be stable in the physical environment of cartilage and protect the seeded cells until neotissue formation is completed. However, this kind of structural integrity and durability is not particularly necessary for cell-laden constructs matured in *in vitro* environments, since newly formed tissue itself may be able to withstand the native, high-shear cartilage environment. The material should also be able to fill the defect site and integrate with the surrounding native tissue for the implantation to succeed. In addition, degradation rate

of scaffolds in native tissue should be in concert with the formation rate of neotissue, as faster degradation results in shape retention issues, while slow degradation intervenes with the ingrowth of neotissue.

Material source (natural or synthetic), physical properties, biofunctionality, and tissue integration capability can affect the choice and design parameters of a scaffold. A wide range of natural and synthetic polymers have been investigated in cartilage tissue engineering efforts (Chung and Burdick, 2008; Kim et al., 2012; Makris et al., 2014). Natural materials are preferred due to their low costs and similarity to the cartilage ECM, which allows their natural degradation, facilitates their interaction with cells through functional groups, and ensures that the scaffold will be biocompatible. Agarose, alginate, hyaluronic acid, fibrin glue, chitosan, type I and II collagen, and reconstituted tissue matrices are among the natural materials used in cartilage tissue engineering (Zhao et al., 2013). However, natural polymers lack the mechanical properties necessary to withstand the high-stress environment of cartilage and tend to undergo rapid degradation following implantation. Synthetic polymers, in contrast, provide tailorable physical and chemical properties through different synthesis methods and can be processed in different sizes and shapes. In addition, they present low immunogenic responses and toxicity and tend to have lower batch-to-batch variances compared to natural materials.

4.3.1 Natural Polymers

Agarose and alginate are both derived from algae and provide a biocompatible 3D environment that has been shown to preserve the rounded morphology of chondrocytes. Alginate and agarose are continuous hydrogel-based materials that can transmit applied forces to encapsulated chondrocytes and allow the uniform distribution of seeded cells; this property has led to their use in studies investigating effects of dynamic loading on cell behavior (Chahine et al., 2009; Grogan et al., 2012; Henrionnet et al., 2012; Mauck et al., 2015). However, alginate is not degraded rapidly in the body and interferes with growth of neotissue. Covalent crosslinking of alginate can be performed to tune the mechanical properties, degradation kinetics, and swelling ratio of alginate (Kuo and Ma, 2001). Photocrosslinkable alginate systems allow noninvasive implantation of scaffolds that can fill defects of any size and geometry. In a recent study, alginate was modified with 2-aminoethyl methacrylate (AEMA) and crosslinked under 365 nm UV light with the help of a photoinitiator, creating hydrogels with adjustable degradation kinetics based on the rate of methacrylation (Jeon et al., 2009).

Collagen types I and II are the most abundant proteins in the native cartilage ECM and have been used in the development of bioactive scaffolds. Collagen-derived materials bear inherent biological cues that facilitate cell attachment and can be remodeled by cells during their development. Like other natural materials, collagen has to be processed to decrease its antigenicity before use. This process removes immunogenic components within collagen and crosslinks the remaining atellopeptides by aldehyde or carbodiimide chemistry. It can easily be processed into various physical configurations, such as tubes, sheets, fleeces, and sponges; however, gels and fibers are the forms most commonly studied in cartilage tissue engineering (Parenteau-Bareil et al., 2010). The predominant collagen of cartilage is type II collagen, and chondrocytes seeded in type II collagen scaffolds were previously demonstrated to maintain their phenotype. However poor availability and undesirable mechanical properties of type II collagen limit its use in tissue engineering studies.

Hyaluronan is a major GAG component of cartilage and synovial fluid and helps in the lubrication of joints. It is a nonsulfated GAG and is involved in cell differentiation, ECM organization, and cell motility (Parenteau-Bareil et al., 2010). Hyaluronan can be derived from various animal tissues or produced by microbial fermentation. Hyaluronan can be injected to fill defects in any shape and is therefore suited for less invasive applications. However, hyaluronan is quickly degraded *in vivo* by cell-secreted hyaluronidases. As with agarose and alginate, hyaluronan and hyaluronic acid can be crosslinked to produce porous solid platforms with higher resistances to degradation (Jin et al., 2010). Aqueous solutions of hyaluronic acid can be crosslinked through covalent crosslinking or photocrosslinking. HYAFF®'7 and HYAFF®11 are the ethyl and benzyl esters of hyaluronan, respectively, and remain intact in the body for around 2 months prior to their degradation by the hydrolysis of their ester bonds (Grigolo et al., 2002).

Fibrin is a protein involved in the blood clotting process and used as an adhesive in surgery. It is composed of fibrinogen monomers. The fibrinogen molecule has two sets of three polypeptide chains and is solidified through the binding of thrombin. The stability and mechanical integrity of the resulting fibrin gel are dependent on pH and the concentrations of fibrinogen and calcium ions. Fibrin gels can be derived from the patient's own blood and subsequently utilized as autologous scaffold. Because of its complete biodegradability and injectable forms, it is popular in various tissue engineering applications. However, it needs to be blended with different materials to enhance its mechanical properties.

Chitosan is the deacetylated form of chitin, one of the most abundant polysaccharides in nature. This polysaccharide has an intrinsic antibacterial

ability, triggers mild immunological reactions, and has a structure similar to GAG molecules. Its highly cationic nature and resemblance to GAGs allow it to bind growth factors and adhesion proteins. Chitosan is degraded by deacetylation, and the host tissue progressively metabolizes the sugar units detached from its structure. The degradation rate of chitosan scaffolds can be modified during processing by altering the number of acetyl units. Similar to fibrin, it can be injected into target tissues due to its unique temperature-dependent gelation property: It is liquid at room temperature and gel at physiological temperature. Generally, chitosan is combined with other materials, such as poly(lactic-*co*-glycolic acid) (PLGA) and hyaluronan, to improve chondrocyte attachment, proliferation, and matrix synthesis (Jayakumar et al., 2010).

4.3.2 Synthetic Polymers

Synthetic polymers show predictable chemical and physical properties and can be customized easily to meet specific requirements, including degradation rates, mechanical properties, and biological activities. In addition, synthetic polymers are generally cheaper than natural polymers, can be produced in large quantities, and have longer shelf lives (Gunatillake and Adhikari, 2003; Kim and Mooney, 1998).

Short-chain saturated aliphatic polyesters, including poly(glycolic acid) (PGA), poly(lactic acid) (PLA), and PLGA copolymers, are commonly used for scaffold materials because of their biodegradability and the US Food and Drug Administration (FDA) approval for their clinical use. Their degradation products are monomeric glycolic acids or lactic acids and can be resorbed *in vivo*. However, these products alter the local pH, resulting in insufficient tissue ingrowth and triggering inflammatory responses. Total degradation takes place within 24 months and accompanies the loss of mechanical durability. These polymers can be produced in different shapes and forms, but for cartilage tissue engineering purposes, they are generally utilized as nonwoven meshes or feltlike forms (Gunatillake and Adhikari, 2003).

PGA is a hydrophilic polymer and enables the attachment of chondrocytes onto its surface. However, its acidic degradation products adversely affect cell proliferation, and polymers implanted into the defect site undergo rapid degradation, losing 50% of their mass within 2 weeks (Middleton and Tipton, 2000). For these reasons, applications of PGA are limited. PLA is similar to PGA, although its extra methyl group renders it more hydrophobic. PLA also degrades slower than PGA, allowing the deposition of a replacement ECM in the defect site before the total loss of

its mechanical integrity. PLGA is a copolymer composed of PGA and PLA monomers. The overall material properties are dependent on the ratio of each polymer (Maher et al., 2011).

PCL is another polymer approved by the US FDA. It has a longer degradation time compared to PGA/PLA/PLGA; however, it also has poor wettability characteristics and cellular interaction potential (Middleton and Tipton, 2000).

4.3.3 Composite Materials

Single-phase homogeneous scaffolds cannot fully replicate the structure and function of cartilage or withstand the mechanical stresses at the defect site until the formation of new tissue. The disadvantages associated with natural and synthetic materials can be overcome by combining two or more polymers into a single material. The use of multiple polymers can improve the interaction of the scaffold material with native tissue and therefore enhance cartilage regeneration. Inert polymers in particular are coated or blended with biofunctional natural polymers in order to enhance cellular attachment, proliferation, and matrix synthesis. For example, it has been reported that filling the empty fraction of PLGA with chondrocytes in fibrin glue creates a material that exhibits homogeneous cell distribution and better infiltration capacity during neotissue formation (Ameer et al., 2002). In another study, the immobilization of hyaluronic acid on PLGA scaffolds was shown to enhance chondrocyte attachment and differentiation and also to prevent the dedifferentiation of chondrocytes (Yoo et al., 2005). The bioactivity of scaffolds can be improved by the incorporation of bioactive molecules, including growth factors, adhesion proteins, and short peptide sequences into their structure. The most important growth factors used in cartilage regeneration and tissue engineering approaches are the transforming growth factor β (TGF-β) family members, particularly TGF-β1 and TGF-β3. Various *in vitro* and *in vivo* studies proved that the use of TGF-β assists in the maintenance of the chondrocyte phenotype and stimulates the synthesis of a collagen II-rich ECM (Blunk and Sieminski, 2002; van der Kraan et al., 1992). Other growth factors used for the enhancement of cartilage regeneration are insulin-like growth factor I (IGF-I), basic fibroblast growth factor (bFGF) (FGF-2), bone morphogenetic proteins (BMPs), and hedgehog (hh) and wingless (Wnt) proteins.

In situ delivery of growth factors involves the covalent or noncovalent binding of the factors to the scaffold. Noncovalent binding is achieved by the physical entrapment of the growth factor within the scaffolding material. In one study, bFGF was immobilized onto the surface of PLGA after carbon

dioxide plasma treatment, which provided the formation of ionic binding sites for positively charged FGF and greatly enhanced its binding to the polymer matrix (Shen et al., 2008). Covalent binding allows the prolonged release, slow degradation, and cellular internalization of the growth factor. Functional groups that are incorporated to polymers by physical/chemical binding or copolymerization can be used for the conjugation of growth factors onto the scaffold material (Cabanas-dan et al., 2014; Lee et al., 2011).

Many research efforts focus on replicating tissue function in terms of both the structural architecture and mechanical and biological functions. Self-assembling peptides are used extensively to provide short amino acid-based biological signals to cells. Unlike long chains, the use of short chains does not sterically hinder active domains and increases the stability of the structure (Hartgerink et al., 2001). In one study, researchers designed peptide amphiphile (PA) molecules that display a TGF-β-binding epitope to sequester endogenously released TGF-β. When implanted with MSCs, TGF-β-binding PA significantly enhanced the recovery of microfracture-treated cartilage defects without the addition of exogenous growth factors (Shah et al., 2010). As an alternative approach, the incorporation of cell-interacting sequences to the scaffold material is also used to elicit specific cellular responses. Methacrylated hyaluronic acid hydrogels have been functionalized with N-cadherin-mimetic peptides to induce chondrogenic differentiation of MSCs, and results showed that the conjugation of N-cadherin-mimetic peptides promoted chondrogenesis and cartilage-specific ECM production of MSCs (Bian et al., 2013).

4.3.4 Physical Stimuli

Conventional cell culture methods lack the biochemical and structural characteristics of the ECM. As such, it is hard to obtain highly organized, layered, and differentiated constructs. Nutrient diffusion and waste transport issues, especially the accumulation of anabolic products, result in fluctuations in the metabolic state of the medium and affect the homogeneity of scaffolds in static cultures (Dunkelman et al., 1995). However, mechanical stimulation can be used during the conditioning of cell-seeded constructs in *ex vivo* environments to improve the mechanical and functional properties of resulting constructs (Vunjak-Novakovic et al., 1999). Cartilage is a tissue that responds to mechanical stimuli, and its maintenance and development are dependent on strong mechanical forces. It has been shown that ECM synthesized by chondrocytes under dynamic conditions display better compositions and zonal organization (Vunjak-Novakovic et al., 1999). Bioreactors have been developed to apply mechanical loading regimens and

create reproducible and homogeneous tissue constructs. Functional tissue engineering efforts have consequently focused on the modulation of dynamic compression, shear stress, and hydrostatic pressure through bioreactors, with the aim of producing cartilage-mimetic constructs under a well-defined set of mechanical conditions (Mabvuure et al., 2012). Compressive loading is the main mechanical stimulus in the native cartilage environment, especially in articulating joints. In joints, the surface of the cartilage on one side physically compresses the opposing cartilage in a continuous manner. The most commonly used bioreactors are compression bioreactors, which generally use platens to contact the construct surface. Dynamic compression, in which the loading is cyclical, has better outcomes than static compression in terms of ECM synthesis and chondrocyte proliferation rate. The combination of dynamic loading and growth factor application on agarose constructs has demonstrated that the two stimuli have a synergistic effect on the ECM synthesis (Mauck et al., 2003).

The other type of loading exerted on chondrocytes in their native environment is hydrostatic pressure. As mentioned previously, cartilage is a highly hydrated tissue, and mature cartilage is comprised primarily of water (70–80% by weight). During joint function, stress imparted on cartilage is distributed on cartilage surface homogeneously by water entrapped in the tissue; this effect is enhanced by the small effective pore size of cartilage tissue. As stress continues to be applied on the joint surface, water is expelled from the tissue and synovial fluid transmits the mechanical stress to water, thus decreasing friction and dissipating energy. During its normal function, cartilage is exposed to 3–10 MPa of stress at a frequency of around 1 Hz. Tissue engineering efforts using 0.1 and 15 MPa and 0.05 and 1 Hz pressures and frequencies have produced positive results for chondrocyte phenotype maintenance (Athanasiou et al., 2013). However, the application of constant hydrostatic pressure over long periods of time results in low cellular viability and matrix synthesis (Elder and Athanasiou, 2009). For that reason, in addition to pressure and frequency, the duration of hydrostatic pressure needs to be optimized for each construct or explant system.

4.4 CLINICAL THERAPEUTICS FOR CARTILAGE REGENERATION

Age-related problems spearhead the list of cartilage-related joint disorders. Although more common in the elderly, joint problems can also be seen in younger individuals due to traumatic accidents or obesity (Bosnakovski

et al., 2006; Clouet et al., 2009). Cartilage injuries are hard to treat due to the low self-regeneration capacity of the tissue and the tendency of such injuries to deteriorate over time. Current treatments against OA incorporate both pharmacological and alternative treatments. Although severe cases cannot be treated with alternative methods alone, nonpharmacological approaches such as weight reduction, patient education and self-management, referral to a physical therapist, aerobics, muscle strengthening, walking aids, thermal modalities, transcutaneous electrical nerve stimulation, and acupuncture may assist in the recuperation process of arthritic patients (Hochberg et al., 2012). Pharmacological therapies, however, are common and indeed necessary for more advanced forms of OA. Two types of drugs—slow acting and fast acting—are prescribed for OA treatment (Table 4.1). In addition, several drug candidates are still under development. Novel analgesic and anti-inflammatory drugs, for example, aim to efficiently reduce joint pain while eliminating the adverse effects of currently prescribed drugs. Moreover, a class of molecules called disease-modifying osteoarthritis drugs (DMOADs) are currently under investigation for their ability to reduce tissue deformation. Finally, chondroprotective drugs such as growth factors and hormones are also under development for potential use in OA therapy (Clouet et al., 2009) (Table 4.2).

4.5 CONCLUSIONS AND FUTURE PERSPECTIVES

The unique structure and indispensable function of cartilage tissue render it difficult to develop new therapeutic approaches for osteochondral defects and diseases. Even though several clinical strategies are currently used in the treatment of such injuries, no method is so far capable of facilitating the long-term repair of cartilage tissue. At the cellular level, however, the application of stem cells onto defect sites appears to be promising, as MSCs have a high differentiation capacity and can secrete cartilage-specific ECM materials under the right conditions. However, difficulties in cell isolation, stem cell heterogeneity, and potential hypertrophy of the defect site and limited reproducibility in academic studies limit the use of stem cells in clinical applications. Tissue engineering strategies also hold great promise for the development of novel therapeutics and treatments, although few have made it into clinical use. Recent efforts have focused on improving current scaffold designs through the use of multipurpose materials that incorporate physical and biochemical cues to improve cell adhesion, survival, and maintenance. In addition, mechanical properties are as important as biochemical cues for the development of functional scaffolds

TABLE 4.1 Current Pharmacological Drugs Used in Clinics

	Drug	Function	Side Effects	References
Fast-Acting Drugs	Acetaminophen (paracetamol)	Analgesic	High doses of acetaminophen (up to 4 g/day) can trigger adverse hepatic events in patients with hepatic insufficiency	Hochberg et al. (2012)
	Nonsteroidal anti-inflammatory drugs (NSAIDs) Cyclooxygenase-2 (Cox-2) inhibitors	Analgesic	Gastrointestinal, renal, and cardiovascular toxicity	Ayral (2001), Gerwin et al. (2006), Glass (2006), Michael et al. (2010)
	Glucocorticoids (intra-articular injection)	Analgesic	Adverse metabolic effects	Michael et al. (2010)
	Opioids	Analgesic	Wide range of adverse effects, such as gastrointestinal problems (nausea, vomiting, and constipation), alteration in cognitive function, dependence, and respiratory depression	Glass (2006)

	Drug	Origin	Function	References
Slow-Acting Drugs	Glucosamine	Natural precursor of GAGs; stimulates GAG and collagen synthesis by chondrocytes	Reduces the rate of joint space narrowing	Derfoul et al. (2007), Pavelká et al. (2002)
	Chondroitin sulfate	Major component of cartilaginous ECM	Decreases the activity of catabolic enzymes in osteoarthritic cartilage and stimulates the synthesis of GAGs and collagens	McAlindon et al. (2000)
	S-Adenosyl methionine (SAM)	Small nonprotein metabolite—a coenzyme involved in methyl group transfers between enzymes	Increases the synthesis of GAGs in articular chondrocytes, suggesting that it may be able to aid in the repair of damaged cartilage through this mechanism. Oral administration of SAM induces a significant decrease in pain and improvements in joint function, comparable to that of NSAID	Najm et al. (2004)
	Hyaluronic acid (HA)	Polysaccharide ubiquitously found in ECMs	Used as viscosupplementation through intra-articular injections, it restores the viscoelastic and tribologic properties of the synovial fluid. Also shown to be chondroprotective, as it is able to stimulate the production of TIMPs in chondrocytes	Brockmeier and Shaffer (2006), Simon and Jackson (2006), Wang et al. (2004)

TABLE 4.2 Potential Future Therapeutics in Preclinical and Clinical Trials

	Drug Type	Function	References
Analgesic and Anti-Inflammatory Drugs	COX/LOX inhibitor	Anti-inflammatory activity with no detrimental effects on blood pressure and good gastrointestinal tolerability and safety	Clouet et al. (2009), Qvist et al. (2008), Steinmeyer and Konttinen (2006)
	Cyclooxygenase-inhibiting nitric oxide donators (CINODs)		
	NSAIDs		
	Transient receptor potential vanilloid subfamily 1 receptor agonist (TRPV1)		
	Serotonin–norepinephrine reuptake inhibitor		
	Bradykinin B2 receptor antagonist		
Disease-Modifying Osteoarthritis Drugs (DMOADs)	Matrix metalloproteinases (MMPs)	Slowing of OA-associated cartilage degeneration by targeting catabolic enzymes or cytokine-activated signaling cascades	Clouet et al. (2009), Pelletier and Martel-Pelletier (2007), Qvist et al. (2008)
	ADAMTS-5		
	Cathepsin K		
	IL-1-converting enzyme (ICE)		Chevalier et al. (2005)
	p38 MAP kinase	Synthetic inhibitors of various signaling pathways implicated in the physiopathology of OA, such as MAP kinase	Pelletier and Martel-Pelletier (2007), Qvist et al. (2008)
	NF-κB		
	Osteoprotegerin	Bone resorption	Tat et al. (2009)

Chondroprotective Drugs	TGF-β FGF (e.g., FGF-18) BMPs (BMP-2 and BMP-7)	Growth factors involved in cartilage repair	Blaney Davidson et al. (2007), Moore et al. (2005), Hayashi et al. (2008)
Novel Scaffolds	Collagen I/II-based hydrogels Polymer-based hydrogels Peptide-based nanofiber hydrogels	Biodegradable three-dimensional matrices incorporating both biochemical and physical clues. Clinical use limited but still present, including cases such as • Bone marrow MSCs incorporated in a collagen gel transplantation in an athlete with a grade IV cartilage defect • A poly(ethylene glycol) diacrylate (PEGDA) hydrogel used in a pilot clinical study alongside microfracture surgery in focal defects of 15 patients	Kuroda et al. (2007) Johnstone et al. (2013), Nguyen et al. (2011), Shah et al. (2010)

capable of facilitating the regeneration of cartilage, and nanomaterials can be designed to provide the physical cues necessary for the maintenance of chondrocytes. Native ECM-like and fiber-reinforced hydrogels are good material candidates for scaffold-incorporated approaches.

New design strategies also concentrate on the anatomical and structural complexity of cartilage tissue for advanced treatments. The heterogeneity of cartilage defects and diseases is another obstacle that is faced in the treatment of these injuries, and novel tissue engineering approaches can be optimized depending on whether the defect in question is osteochondral or solely chondral, leading to the creation of therapeutic platforms that are specific to a given type and severity of cartilage injury (potential future therapeutics in preclinical and clinical trials is summarized in Table 4.2). Overall, new strategies in the field of cartilage regeneration focus on the unique biochemical and physical properties of native cartilage to design novel tissue constructs that are decorated with cartilage-specific signals and display suitable anatomical geometries and mechanical properties for the treatment of large tissue defects.

REFERENCES

Ameer, G.A., Mahmood, T.A., and Langer, R. (2002). A biodegradable composite scaffold for cell transplantation. J. Orthop. Res. *20*, 16–19.

Arden, N. and Nevitt, M.C. (2006). Osteoarthritis: epidemiology. Best Pract. Res. Clin. Rheumatol. *20*, 3–25.

Athanasiou, K.A., Darling, E.M., Hu, J.C., DuRaine, G.D., and Reddi, A.H. (2013). Articular Cartilage (CRC Press, Boca Raton, FL).

Ayral, X. (2001). Injections in the treatment of osteoarthritis. Best Pract. Res. Clin. Rheumatol. *15*, 609–626.

Bartlett, W., Skinner, J.A, Gooding, C.R., Carrington, R.W.J., Flanagan, A.M., Briggs, T.W.R., and Bentley, G. (2005). Autologous chondrocyte implantation versus matrix-induced autologous chondrocyte implantation for osteochondral defects of the knee: a prospective, randomised study. J. Bone Joint Surg. Br. *87*, 640–645.

Bhosale, A.M. and Richardson, J.B. (2008). Articular cartilage: structure, injuries and review of management. Br. Med. Bull. *87*, 77–95.

Bian, L., Guvendiren, M., Mauck, R.L., and Burdick, J.A. (2013). Hydrogels that mimic developmentally relevant matrix and N-cadherin interactions enhance MSC chondrogenesis. Proc. Natl. Acad. Sci. U.S.A. *110*, 10117–10122.

Blaney Davidson, E., Van der Kraan, P., and van Den Berg, W. (2007). TGF-β and osteoarthritis. Osteoarthr. Cartil. *15*, 597–604.

Blunk, T. and Sieminski, A. (2002). Differential effects of growth factors on tissue-engineered cartilage. Tissue Eng.. *8*, 73–84.

Boscainos, P.J., Gross, A.E., and Kellett, C.F. (2008). Articular defects of the knee. 4, 1–13.

Bosnakovski, D., Mizuno, M., Kim, G., Takagi, S., Okumura, M., and Fujinaga, T. (2006). Chondrogenic differentiation of bovine bone marrow mesenchymal stem cells (MSCs) in different hydrogels: influence of collagen type II extracellular matrix on MSC chondrogenesis. Biotechnol. Bioeng. *93*, 1152–1163.

Brittberg, M., Lindahl, A., Nilsson, A., Ohlsson, C., Isaksson, O., and Peterson, L. (1994). Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N. Engl. J. Med. *331*, 889–895.

Brockmeier, S.F. and Shaffer, B.S. (2006). Viscosupplementation therapy for osteoarthritis. Sports Med. Arthrosc. Rev. *14*, 155–162.

Buckwalter, J.A. (1995). Activity vs. rest in the treatment of bone, soft tissue and joint injuries. Iowa Orthop. J. *15*, 29–42.

Buckwalter, J.A. and Mankin, H. (1997). Articular cartilage: degeneration and osteoarthritis, repair, regeneration, and transplantation. Instr. Course Lect. *47*, 487–504.

Cabanas-dan, J., Huskens, J., and Jonkheijm, P. (2014). Chemical strategies for the presentation and delivery of growth factors. J. Mater. Chem. B *2*, 2381–2394.

Chahine, N.O., Albro, M.B., Lima, E.G., Wei, V.I., Dubois, C.R., Hung, C.T., and Ateshian, G.A. (2009). Effect of dynamic loading on the transport of solutes into agarose hydrogels. Biophys. J. *97*, 968–975.

Chevalier, X., Giraudeau, B., Conrozier, T., Marliere, J., Kiefer, P., and Goupille, P. (2005). Safety study of intraarticular injection of interleukin 1 receptor antagonist in patients with painful knee osteoarthritis: a multicenter study. J. Rheumatol. *32*, 1317–1323.

Chung, C. and Burdick, J.A. (2008). Engineering cartilage tissue. Adv. Drug Deliv. Rev. *60*, 243–262.

Clouet, J., Vinatier, C., Merceron, C., Pot-vaucel, M., Maugars, Y., Weiss, P., Grimandi, G.L., and Guicheux, J.R.M. (2009). From osteoarthritis treatments to future regenerative therapies for cartilage. Drug Discov. Today *14*, 913–925.

Dehne, T., Karlsson, C., Ringe, J., Sittinger, M., and Lindahl, A. (2009). Chondrogenic differentiation potential of osteoarthritic chondrocytes and their possible use in matrix-associated autologous chondrocyte transplantation. Arthritis Res. Ther. *11*, R133.

Dejica, V.M., Mort, J.S., Laverty, S., Percival, M.D., Antoniou, J., Zukor, D.J., and Poole, A.R. (2008). Cleavage of type II collagen by cathepsin K in human osteoarthritic cartilage. Am. J. Pathol. *173*, 161–169.

Derfoul, A., Miyoshi, A.D., Freeman, D.E., and Tuan, R.S. (2007). Glucosamine promotes chondrogenic phenotype in both chondrocytes and mesenchymal

stem cells and inhibits MMP-13 expression and matrix degradation. Osteoarthr. Cartil. *15*, 646–655.

Dunkelman, N.S., Zimber, M.P., Lebaron, R.G., Pavelec, R., Kwan, M., and Purchio, A.F. (1995). Cartilage production by rabbit articular chondrocytes on polyglycolic acid scaffolds in a closed bioreactor system. Biotechnol. Bioeng. *46*, 299–305.

Eggli, P.S., Herrmann, W., Hunziker, E.B., and Schenk, R.K. (1985). Matrix compartments in the growth plate of the proximal tibia of rats. Anat. Rec. *211*, 246–257.

Elder, B.D. and Athanasiou, K.A. (2009). Hydrostatic pressure in articular cartilage tissue engineering: from chondrocytes to tissue regeneration. Tissue Eng. Part B Rev. *15*, 43–53.

Engel, A., Larsson, T., and Skandiakoncernen, (1969). Aging of connective and skeletal tissue. Symposium October 1–3, 1968 (Stockholm, Nordiska bokhandelns).

Eyre, D. (2002). Collagen of articular cartilage. Arthritis Res. *4*, 30–35.

Fox, A.J.S., Bedi, A., and Rodeo, S.A. (2009). The basic science of articular cartilage: structure, composition, and function. Sports Health *1*, 461–468.

Gerwin, N., Hops, C., and Lucke, A. (2006). Intraarticular drug delivery in osteoarthritis. Adv. Drug Deliv. Rev. *58*, 226–242.

Gigante, A., Bevilacqua, C., Ricevuto, A., Mattioli-Belmonte, M., and Greco, F. (2007). Membrane-seeded autologous chondrocytes: cell viability and characterization at surgery. Knee Surg. Sports Traumatol. Arthrosc. *15*, 88–92.

Glass, G.G. (2006). Osteoarthritis. Dis. Mon. *52*, 343–362.

Gobbi, A., Karnatzikos, G., and Kumar, A. (2014). Long-term results after microfracture treatment for full-thickness knee chondral lesions in athletes. Knee Surg. Sports Traumatol. Arthrosc. *22*, 1986–1996.

Görtz, S. and Bugbee, W.D. (2007). Allografts in articular cartilage repair. Instr. Course Lect. *56*, 469–480.

Grigolo, B., Lisignoli, G., Piacentini, A., Fiorini, M., Gobbi, P., Mazzotti, G., Duca, M., Pavesio, A., and Facchini, A. (2002). Evidence for redifferentiation of human chondrocytes grown on a hyaluronan-based biomaterial (HYAFF s 11): molecular, immunohistochemical and ultrastructural analysis. Biomaterials *23*, 1187–1195.

Grogan, S.P., Ph, D., Sovani, S., Pauli, C., Chen, J., Hartmann, A., Colwell, C.W., Lotz, M.K., and Lima, D.D.D. (2012). Effects of perfusion and dynamic loading on human neocartilage formation in alginate hydrogels. Tissue Eng. Part A *18*, 1784–1792.

Gunatillake, P.A. and Adhikari, R. (2003). Biodegradable synthetic polymers for tissue engineering. Eur. Cell. Mater. *5*, 1–16.

Han, L., Grodzinsky, A., and Ortiz, C. (2011). Nanomechanics of the cartilage extracellular matrix. Annu. Rev. Mater. Res. *41*, 133–168.

Hartgerink, J.D., Beniash, E., and Stupp, S.I. (2001). Self-assembly and mineralization of peptide-amphiphile nanofibers. Science *294*, 1684–1688.

Hayashi, M., Muneta, T., Ju, Y.-J., Mochizuki, T., and Sekiya, I. (2008). Weekly intra-articular injections of bone morphogenetic protein-7 inhibits osteoarthritis progression. Arthritis Res. Ther. *10*, R118.

Henrionnet, C., Wang, Y., Roeder, E., Gambier, N., Galois, L., Mainard, D., Bensoussan, D., Gillet, P., and Pinzano, A. (2012). Effect of dynamic loading on MSCs chondrogenic differentiation in 3-D alginate culture. Biomed. Mater. Eng. *22*, 209–218.

Hochberg, M. C., Altman, R. D., April, K. T., Benkhalti, M., Guyatt, G., McGowan, J., and Tugwell, P. (2012). American College of Rheumatology 2012 recommendations for the use of nonpharmacologic and pharmacologic therapies in osteoarthritis of the hand, hip, and knee. Arthritis Care Res. *64*, 465–474.

Hunziker, E.B. (2002). Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects. Osteoarthr. Cartil. *10*, 432–463.

Iwasa, J., Engebretsen, L., Shima, Y., and Ochi, M. (2008). Clinical application of scaffolds for cartilage tissue engineering. Knee Surg. Sports Traumatol. Arthrosc. *17*, 561–577.

Jayakumar, R., Prabaharan, M., Nair, S.V., and Tamura, H. (2010). Novel chitin and chitosan nanofibers in biomedical applications. Biotechnol. Adv. *28*, 142–150.

Jeon, O., Bouhadir, K.H., Mansour, J.M., and Alsberg, E. (2009). Photocrosslinked alginate hydrogels with tunable biodegradation rates and mechanical properties. Biomaterials *30*, 2724–2734.

Jin, R., Moreira Teixeira, L.S., Dijkstra, P.J., van Blitterswijk, C.A., Karperien, M., and Feijen, J. (2010). Enzymatically-crosslinked injectable hydrogels based on biomimetic dextran-hyaluronic acid conjugates for cartilage tissue engineering. Biomaterials *31*, 3103–3113.

Johnstone, B., Alini, M., Cucchiarini, M., Dodge, G.R., Eglin, D., Guilak, F., Madry, H., Mata, A., Mauck, R.L., and Semino, C.E. (2013). Tissue engineering for articular cartilage repair—the state of the art. Eur. Cell. Mater. *25*, e67.

Kim, B.-S. and Mooney, D.J. (1998). Development of biocompatible synthetic extracellular matrices for tissue engineering. Trends Biotechnol. *16*, 224–230.

Kim, S.H., Park, D.Y., and Min, B.-H. (2012). A new era of cartilage repair using cell therapy and tissue engineering: turning current clinical limitations into new ideas. Tissue Eng. Regen. Med. *9*, 240–248.

Kon, E., Filardo, G., Condello, V., Collarile, M., Di Martino, A., Zorzi, C., and Marcacci, M. (2011). Second-generation autologous chondrocyte implantation: results in patients older than 40 years. Am. J. Sports Med. *39*, 1668–1675.

Kon, E., Filardo, G., Matteo, B., Di Perdisa, F., and Marcacci, M. (2013). Matrix assisted autologous chondrocyte transplantation for cartilage treatment a systematic review. Bone Joint Res. *2*, 18–25.

Kuo, C.K. and Ma, P.X. (2001). Ionically crosslinked alginate hydrogels as scaffolds for tissue engineering: part 1. Structure, gelation rate and mechanical properties. Biomaterials *22*, 511–521.

Kuroda, R., Ishida, K., Matsumoto, T., Akisue, T., Fujioka, H., Mizuno, K., Ohgushi, H., Wakitani, S., and Kurosaka, M. (2007). Treatment of a full-thickness articular cartilage defect in the femoral condyle of an athlete with autologous bone-marrow stromal cells. Osteoarthritis Cartilage *15*, 226–231.

Lee, K., Silva, E.A., and Mooney, D.J. (2011). Growth factor delivery-based tissue engineering: general approaches and a review of recent developments. J. R. Soc. Interface *8*, 153–170.

Lewis, P.B., McCarty, L.P., Kang, R.W., and Cole, B.J. (2006). Basic science and treatment options for articular cartilage injuries. J. Orthop. Sports Phys. Ther. *36*, 717–727.

Lin, Y., Luo, E., Chen, X., Liu, L., Qiao, J., Yan, Z., Li, Z., Tang, W., Zheng, X., and Tian, W. (2005). Molecular and cellular characterization during chondrogenic differentiation of adipose tissue-derived stromal cells in vitro and cartilage formation in vivo. J. Cell. Mol. Med. *9*, 929–939.

Lu, Y., Parker, K.H., and Wang, W. (2006). Effects of osmotic pressure in the extracellular matrix on tissue deformation. Philos. Trans. R. Soc., A *364*, 1407–1422.

Ma, Z., Ph, D., Kotaki, M., and Inai, R. (2005). Potential of nanofiber matrix as tissue-engineering scaffolds. Tissue Eng. *11*, 101–109.

Mabvuure, N., Hindocha, S., and Khan, W.S. (2012). The role of bioreactors in cartilage tissue engineering. Curr. Stem Cell Res. Ther. *7*, 287–292.

Maher, S.A., Ph, D., and Lowman, A.M. (2011). Hydrogels for the repair of articular cartilage defects. Tissue Eng. Part B Rev. *17*, 281–299.

Mahmoudifar, N. and Doran, P.M. (2012). Chondrogenesis and cartilage tissue engineering: the longer road to technology development. Trends Biotechnol. *30*, 166–176.

Makris, E.A., Gomoll, A.H., Malizos, K.N., Hu, J.C., and Athanasiou, K.A. (2015). Repair and tissue engineering techniques for articular cartilage. Nat Rev Rheumatol *11*, 21–34.

Maroudas, A. (1968). Physicochemical properties of cartilage in the light of ion exchange theory. Biophys. J. *8*, 575–595.

Mauck, R.L., Nicoll, S.B., Seyhan, S.L., Ateshian, G.A., and Hung, C.T. (2003). Synergistic action of growth factors and dynamic loading for articular cartilage tissue engineering. Tissue Eng. *9*, 597–611.

Mauck, R.L., Soltz, M.A., Wang, C.C.B., Wong, D.D., Chao, P.G., and Ateshian, G.A. (2015). Functional tissue engineering of articular cartilage through dynamic loading of chondrocyte-seeded agarose gels. J. Biomech. Eng. *122*(3), 252–260.

McAlindon, T.E., LaValley, M.P., Gulin, J.P., and Felson, D.T. (2000). Glucosamine and chondroitin for treatment of osteoarthritis: a systematic quality assessment and meta-analysis. JAMA *283*, 1469–1475.

Memon, A.R. and Quinlan, J.F. (2012). Surgical treatment of articular cartilage defects in the knee: are we winning? Adv. Orthop. *2012*, 528423.

Michael, J.W.-P., Schlüter-Brust, K.U., and Eysel, P. (2010). The epidemiology, etiology, diagnosis, and treatment of osteoarthritis of the knee. Dtsch. Arztebl. Int. *107*, 152.

Middleton, J.C. and Tipton, A.J. (2000). Synthetic biodegradable polymers as orthopedic devices. Biomaterials *21*, 2335–2346.

Moore, E., Bendele, A., Thompson, D., Littau, A., Waggie, K., Reardon, B., and Ellsworth, J. (2005). Fibroblast growth factor-18 stimulates chondrogenesis and cartilage repair in a rat model of injury-induced osteoarthritis. Osteoarthr. Cartil. *13*, 623–631.

Mow, V.C. and Guo, X.E. (2002). Mechano-electrochemical properties of articular cartilage: their inhomogeneities and anisotropies. Annu. Rev. Biomed. Eng. *4*, 175–209.

Mow, V.C., Kuei, S.C., Lai, W.M., and Armstrong, C.G. (1980). Biphasic creep and stress relaxation of articular cartilage in compression: theory and experiments. J. Biomech. Eng. *102*, 73–84.

Mow, V.C., Ratcliffe, A., and Robin Poole, A. (1992). Cartilage and diarthrodial joints as paradigms for hierarchical materials and structures. Biomaterials *13*, 67–97.

Najm, W., Reinsch, S., Hoehler, F., Tobis, J., and Harvey, P. (2004). S-Adenosyl methionine (SAMe) versus celecoxib for the treatment of osteoarthritis symptoms: a double-blind cross-over trial. [ISRCTN36233495]. BMC Musculoskelet. Disord. *5*, 6.

Nguyen, L.H., Kudva, A.K., Guckert, N.L., Linse, K.D., and Roy, K. (2011). Unique biomaterial compositions direct bone marrow stem cells into specific chondrocytic phenotypes corresponding to the various zones of articular cartilage. Biomaterials *32*, 1327–1338.

Parenteau-Bareil, R., Gauvin, R., and Berthod, F. (2010). Collagen-based biomaterials for tissue engineering applications. Materials (Basel). *3*, 1863–1887.

Pavelká, K., Gatterová, J., Olejarová, M., Machacek, S., Giacovelli, G., and Rovati, L.C. (2002). Glucosamine sulfate use and delay of progression of knee osteoarthritis: a 3-year, randomized, placebo-controlled, double-blind study. Arch. Intern. Med. *162*, 2113–2123.

Pelletier, J. and Martel-Pelletier, J. (2007). DMOAD developments: present and future. Bull. Hosp. Jt. Dis. *65*, 242.

Qvist, P., Bay-Jensen, A.-C., Christiansen, C., Dam, E.B., Pastoureau, P., and Karsdal, M.A. (2008). The disease modifying osteoarthritis drug (DMOAD): is it in the horizon? Pharmacol. Res. *58*, 1–7.

Schulze-Tanzil, G. (2009). Activation and dedifferentiation of chondrocytes: implications in cartilage injury and repair. Ann. Anat. *191*, 325–338.

Shah, R.N., Shah, N.A., Lim, M.M.D.R., Hsieh, C., Nuber, G., and Stupp, S.I. (2010). Supramolecular design of self-assembling nanofibers for cartilage regeneration. Proc. Natl. Acad. Sci. U. S. A. *107*, 3293–3298.

Shannon, F., Devitt, A., Poynton, A., Fitzpatrick, P., and Walsh, M. (2001). Short-term benefit of arthroscopic washout in degenerative arthritis of the knee. Int. Orthop. *25*, 242–245.

Shen, H., Hu, X., Bei, J., and Wang, S. (2008). The immobilization of basic fibroblast growth factor on plasma-treated poly(lactide-co-glycolide). Biomaterials *29*, 2388–2399.

Simon, T.M. and Jackson, D.W. (2006). Articular cartilage: injury pathways and treatment options. Sports Med. Arthrosc. Rev. *14*, 146–154.

Slomianka, L. (2009). Blue Histology—Skeletal Tissues—Cartilage. http://www.lab.anhb.uwa.edu.au/mb140/corepages/bone/bone.htm (accessed on October 28, 2015).

Steinmeyer, J.R. and Konttinen, Y.T. (2006). Oral treatment options for degenerative joint disease-presence and future. Adv. Drug Deliv. Rev. *58*, 168–211.

Stroebel, S. (2007). Engineering of cartilage tissue constructs in a 3-dimensional perfusion bioreactor culture system under controlled oxygen tension. In Naturwissenschaftlichen (Basel, Universität Basel), pp. 134.

Taipaleenmaki, H. (2010). Factors regulating chondrogenic differentiation. In Medical Biochemistry and Genetics (Turku, Finland University of Turku).

Tat, S.K., Pelletier, J.-P., Velasco, C.R., Padrines, M., and Martel-Pelletier, J. (2009). New perspective in osteoarthritis: the OPG and RANKL system as a potential therapeutic target? Keio J. Med. *58*, 29–40.

Tuan, R.S. (2007). A second-generation autologous chondrocyte implantation approach to the treatment of focal articular cartilage defects. Arthritis Res. Ther. *9*, 109.

Van der Kraan, P., Vitters, E., and van den Berg, W. (1992). Differential effect of transforming growth factor beta on freshly isolated and cultured articular chondrocytes. J. Rheumatol. *19*, 140–145.

Vavken, P. and Samartzis, D. (2010). Effectiveness of autologous chondrocyte implantation in cartilage repair of the knee: a systematic review of controlled trials. Osteoarthr. Cartil. *18*, 857–863.

Vunjak-Novakovic, G., Martin, I., Obradovic, B., Treppo, S., Grodzinsky, A.J., Langer, R., and Freed, L.E. (1999). Bioreactor cultivation conditions modulate the composition and mechanical properties of tissue-engineered cartilage. J. Orthop. Res. *17*, 130–138.

Wang, C.-T., Lin, J., Chang, C.-J., Lin, Y.-T., and Hou, S.-M. (2004). Therapeutic effects of hyaluronic acid on osteoarthritis of the knee. A meta-analysis of randomized controlled trials. J. Bone Joint Surg. Am. *86*, 538–545.

Woessner, J.F. (1991). Matrix metalloproteinases and their inhibitors in connective tissue remodeling. FASEB J. *5*, 2145–2154.

Yoo, H.S., Lee, E.A., Yoon, J.J., and Park, T.G. (2005). Hyaluronic acid modified biodegradable scaffolds for cartilage tissue engineering. Biomaterials *26*, 1925–1933.

Zhao, W., Jin, X., Cong, Y., Liu, Y., and Fu, J. (2013). Degradable natural polymer hydrogels for articular cartilage tissue engineering. J. Chem. Technol. Biotechnol. *88*, 327–339.