Chapter 6
Nanomaterials for Cartilage Regeneration

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Abstract Repair and regeneration of cartilage tissue has always posed difficulties owing to its avascular, aneural structure and its sparsely distributed cellular arrangement within a dense extracellular matrix. This is why damage to cartilage tissue such as acute trauma, repetitive trauma, inflammatory disease, or wear due to aging eventually results in osteoarthritis. In addition to being a challenging disease for both patients and physicians, osteoarthritis is also a significant public health issue that requires the attention of healthcare planners. In the osteochondral tissue damage, it is important to provide a tissue scaffold and support biosignaling molecules due to its dense extracellular structure as well as cell-based treatments. Therefore, the importance of nanomaterials in tissue regeneration studies is gradually increasing. Nanomaterials are defined as structural elements smaller than 100 nm in at least one dimension, and they offer us the ability to control various properties of materials by assembling them at nanometric proportions. With their excellent biomimetic and physicochemical properties, nanomaterials open up new possibilities and horizons as integration, interaction and signaling in structural and cellular dimensions. This chapter will discuss the opportunities provided by nanomaterials in cartilage regeneration.

6.1 Introduction

In the musculoskeletal system, cartilage is a light blue or white bright tissue that covers the end-bone of all synovial joints. It allows movement of the joints due to its low-friction gliding surface, increased compressive strength, and bio-lubrication. Any damage to the chondral tissue might harm not just the cartilage but also the subchondral osseous structures. Because of this, chondral pathologies may be generally described as "osteochondral injuries." These injuries are commonly encoun-

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tered conditions in orthopedic practice; and they cause pain, swelling, and loss of range-of-motion in the affected joint. Joint instability may also be evident, due to accompanying ligamentous or capsular injuries. Such processes, influenced by numerous other etiological factors, usually lead to osteoarthritis (OA). While OA prevalence increases dramatically with age, it can be encountered in all age groups. It is more prevalent in groups such as soldiers, professional athletes, and females. Current approach to cartilage injury includes conservative and surgical treatment options; mainly utilizing bone marrow stimulation techniques (microfracture), autograft and allograft transplantations (mosaicplasty), transplantations of autologous chondrocytes, and application of bioactive agents (Simon & Jackson, 2018). All of these treatments, however, are still far from achieving the desired curative outcomes, and their usage is subject to significant limitations and obstacles.

The goal of treatment in joint cartilage injury is to ensure the formation of new cartilage tissue that is similar to healthy hyaline cartilage. This requires the restoration of the extracellular matrix, existence of healthy chondrocytes, and presence of appropriate morphogenetic signals. Unfortunately, our treatment options are still very limited in terms of ensuring these factors. Regenerative medicine is a branch of medical science that is still in development, and cartilage regeneration currently receives a great deal of attention from researchers. The main goal of nanomaterial-focused tissue engineering is to discover new treatment options using biocompatible, biodegradable, and bioactive materials in order to restore or regenerate one or more of these three components that ensure cartilage viability. Nanomaterials are versatile components that are able to mimic the surface characteristics of extracellular matrix elements, thereby providing a wide array of possibilities for tissue engineering efforts. The high level of interest in this field of research accurately reflects the immense potential for improvement. This chapter will specifically focus on the role of nanomaterials-based tissue regeneration research in cartilage injury, with a discussion of the possibilities and future goals.

6.1.1 Cartilage Tissue

During the embryonic period, in the 5th week of gestation, some mesenchymal cells form the blastema. These cells begin producing cartilage matrix and then reside within this specialized matrix and are called respectively chondroblasts and chondrocytes. Eventually, the mesenchymal tissue encircling the blastema forms the perichondrial membrane (Bhosale & Richardson, 2008).

Cartilage structures form the temporary skeleton until the onset of ossification, and hypertrophic chondrocytes eventually form the bone tissue. During this process, cartilage also differentiates into various types according to their matrix structures. The four types of cartilage in the human body are elastic, fibro-cartilage, fibro-elastic, and hyaline cartilage. Hyaline cartilage is the type that covers the bone surfaces in synovial joints, connects the ribs and the sternum, and supports the trachea. It is the most abundant type of cartilage in the human body, and we shall call the type that covers the joint surfaces “articular cartilage” (Bhosale & Richardson, 2008; Simon & Jackson, 2018).

6.1.2 Articular Cartilage

Articular Cartilage is an extremely specialized, white-blue colored, smooth, tough, multilayered tissue. It has a water content of over 70% and its main organic components are collagen II and aggrecan. Collagen fibrillary structure provides tensile strength, while proteoglycan aggrecans and hydrophilic glycosaminoglycans allow the water to change compartments when bearing weight, thereby ensuring pressure resilience. The joint surface also has a very low friction coefficient due to biolubricants produced by the cartilage and synovial cells, such as lubricin and hyaluronic acid (S. R. Goldring & Goldring, 2016). Articular cartilage does not have a direct blood, lymphatic, or neural supply; instead, it receives nutrients from surrounding tissues through diffusion. Apart from providing unique biomechanical properties, this complex and multilayered architecture of the articular cartilage also causes great difficulties when it comes to repair and regeneration. There are differences between these layers not only limited to matrix structure but also between their chondrocyte phenotypes and the functional properties of these components.

6.1.2.1 Zonal Structure

Articular cartilage has a highly organized structure composed of four distinct zones, which are identified as the superficial (tangential) zone, middle (transitional) zone, deep (radial) zone, and calcified zone.

The superficial (tangential) zone contains flattened chondrocytes that are elongated and lie parallel to the surface. Also parallel to the surface are the densely deposited type II collagen fibers, and the type I collagen content is minimal. This zone constrains resistance to shear stress and swelling pressures imposed by the negatively charged glycosaminoglycans (GAGs). This allows the cartilage to retain its shape under pressure (Simon & Jackson, 2018). The surface of the superficial zone does not contain any cells and a distinct layer that is several hundred nanometers thick, called "lamina splendens," forms the joint surfaces. While it is currently assumed that this layer provides a low friction surface for the cartilage, its actual role is still poorly understood (Camarero-Espinosa, Rothen-Rutishauser, Foster, & Weder, 2016).

The middle (transitional) zone is where shear forces from the superficial layer transform into compressional forces. Cell density in this region is low and the chondrocytes, which now take a spherical shape, express large amounts of collagen II and proteoglycans. The collagen fibers are arranged in random orientation, and this zone has the richest proteoglycan content (S. R. Goldring & Goldring, 2016; Simon & Jackson, 2018).

In the deep (radial) zone, the chondrocytes begin to assume oval shapes. Collagen fibers are thicker and perpendicular to the subchondral bone, and are distributed to resist load compression. In this zone, the cell density is decreased, while the proteoglycan concentration is increased (Camarero-Espinosa et al., 2016; Simon & Jackson, 2018).

The calcified zone is a thin layer of tissue between the subchondral bone and the cartilage. Some of its chondrocytes are completely encapsulated in calcified lacunae and their metabolic activity is minimal. Type X collagen is also present in the calcified zone. This zone is far more dense and mineralized compared to the adjacent subchondral bone, and it also contains the tidemark that separates the calcified and non-calcified cartilage. The calcified tidemark and subchondral zone cut off the cartilage completely from bone marrow blood supply. During joint movement and loading, the calcified zone transforms shear stress into compressive and tensile stresses (S. R. Goldring & Goldring, 2016).

Underneath the calcified cartilage, there is the subchondral bone that is mostly similar to cortical bone. This zone gives way to cancellous bone that is more porous and metabolically active.

6.1.2.2 Chondrogenesis

Chondrocytes, the main cell type in cartilage, are distributed within a dense extracellular matrix. Near the joint surface, the ratio of chondrocytes to matrix is 1.65%, while in deeper zones this can reach to 2.6% (Hunziker, Quinn, & Häuselmann, 2002). Chondrocytes are located in small spaces called lacunae, similar to osteocytes. However, the specialized cytoplasmic structures seen in osteocytes that allow communication are not present in chondrocytes. Mature chondrocytes lose their migration, proliferation and repair abilities as they settle in their matrix. At the same time, the potential for matrix production is limited by the synthesis and stimulation of certain types of proteoglycans and the growth factor response is reduced (Simon & Jackson, 2018).

Compared to other cell types, chondrocytes reside in a lower oxygen tension environment. Oxygen tension in areas close to the articular surface is around 10%, while near the deep zone it is less than 1%. Intracellular survival factors such as hypoxia-inducible factor 1α (HIF1α) ensure the cell viability of these cells in such hypoxic environments (S. R. Goldring & Goldring, 2016).

Chondrogenesis starts with the production of collagen I, III and V by the mesenchymal cells. Chondroprogenitor cell differentiation, however, requires the expression of the cartilage specific collagens II, IX, and XI. Proliferative chondrocytes express collagen VI and matrilin 1. Matrix restructuring involves the activities of matrix metalloproteinases (MMP) 9, 13, and 14. One of the earliest signals triggering chondrogenesis is the expression of transforming growth factor-β (TGF-β). Also involved in chondrogenesis in complex ways are fibroblast growth factor (FGF), bone morphogenetic protein (BMP) and Wnt signaling pathways that serve to regulate the development of the skeletal system (M. B. Goldring, 2012). For this reason, TGF-β is important for both natural cartilage development and cartilage tissue engineering approaches. TGF-β is produced by chondrocytes as part of a larger molecular complex, and then stored in the extracellular matrix. Afterwards it undergoes an activation process and is released from storage as activated-TGF-β. The active form induces the chondrocytes to produce extracellular matrix components such as type II

collagen and proteoglycans (especially aggrecan) (M. J. Chen et al., 2019). Spagnoli et al. have demonstrated that TGF-β signaling is essential for joint morphogenesis (Spagnoli et al., 2007). Because of its effects upon progenitor cells and its critical importance in joint and growth plate development, TGF-β has the potential to become one of the target components for the treatment of osteoarthritis (T. Li et al., 2012; T. Li, Chubinskaya, et al., 2019; Longobardi et al., 2012).

6.1.3 Articular Cartilage Injury

Degeneration of articular cartilage (arthritis) is a commonly encountered clinical scenario. Its treatment involves difficulties for both patients and physicians, as cartilage has no blood supply and low regeneration potential. Increasing life expectancies, prevalence of senile osteoarthritis, and numerous diseases that affect the joints in every age group lead us to conclude that arthritis is a public health issue. Osteoarthritis affects 10–12% of all humans, and it is the most common musculoskeletal system disease in the world. This ratio increases to 49.7% among people over the age of 65. In the United States, over one million total joint arthroplasties are performed annually, and this number is expected to reach four million by 2030 thanks to an aging population and increased obesity prevalence (Etkin & Springer, 2017; Medvedeva et al., 2018).

Cartilage insufficiency can be described as the deterioration of the balance between destructive forces on the joint and synthesis of the extracellular matrix, in favor of destructive forces (Armiento, Stoddart, Alini, & Eglin, 2018). The exact mechanisms of this decay are still not fully understood.

We can classify cartilage injury into three groups in terms of the depth of the damage: (1) Group, only affecting the superficial or middle zones; (2) Group, injury reaching all the way down to the subchondral bone but not penetrating into the bone marrow; and (3) Group, injuries that have reached the bone marrow (Simon & Jackson, 2018). Each of these categories has different clinical symptoms, findings, treatment options, and treatment response characteristics.

At the present, progressive degeneration of the joint cartilage as a result of trauma and degenerative diseases is most commonly named as “osteoarthritis” (OA). OA can be divided into primary and secondary forms: Primary OA is observed when there is no underlying abnormality, while secondary OA, by definition, involves a primary cause that damages the extracellular matrix of the cartilage. Various risk factors for OA have been described, including trauma, obesity, high-impact physical activity, joint malalignment, age, gender, hypermobility syndromes (Ehlers–Danlos), degenerative joint diseases (Perthes), and metabolic diseases (diabetes mellitus). Until recently, OA was considered to be a disease that was limited to the wear and tear of joint cartilage. However, new molecular pathophysiological knowledge indicates that it is a disease that involves all components of the joint (Loeser, Goldring, Scanzello, & Goldring, 2012). In response to cartilage injury, paracrine and autocrine mechanisms act to disrupt the regular chondrocyte functions.

Catabolic enzymes such as matrix metalloproteinases (MMPs) 1, 3, 13 and a-disintegrin-and-metalloproteinase-with-thrombospondin-motifs (ADAMTS) 4, 5 play a part in furthering the degeneration. While these processes initially serve to activate the chondrocytes to speed up their metabolism, they end up contributing to a catabolic process with decreased proteoglycan and type II collagen synthesis. This, in turn, decreases the capability of cartilage to hold water and lowers its compression resistance, resulting in further cartilage damage (Armiento et al., 2018; Martel-Pelletier et al., 2016).

6.1.4 Treatment Options

The main goal of current medical and surgical therapies in chondral injuries is to relieve the symptoms. Anti-inflammatory analgesic medication and rest comprises the early treatment options, while later options include physical exercise and activity modifications. In the next stage, an attempt to stimulate cartilage regeneration is made through intra-articular injections and arthroscopic surgery.

Microfracture method is the most common of these surgeries. It can be applied in defects where subchondral bone tissue is intact and the surrounded cartilage is healthy. This minimally invasive surgery arthroscopically opens holes in the defect area until marrow material enters the joint (Fig. 6.1). Fibrocartilaginous cartilage tissue is formed after a long period of weight-bearing restriction postoperatively. However the symptoms return quickly due to the biomechanical weakness of fibrocartilage tissue.

Another method is osteochondral autograft or allograft transplantation (mosaicplasty). This method involves the transfer of full-thickness cartilage and subchondral bone tissue to the defect area (Fig. 6.2). Allograft transplantation offers a wider range of possibilities in terms of size and shape since the graft is removed from a cadaver, while immune response and disease transmission are the main drawbacks. On the other hand, autografts have problems such as limited donor tissue and donor site morbidity (hematomas, inflammation, deterioration of articular function).

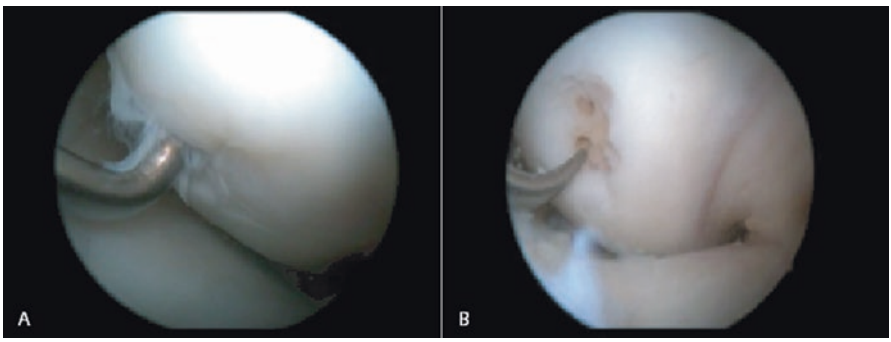

Fig. 6.1 Arthroscopic intra-articular view. (**a**) Chondral injury, (**b**) Microfracture

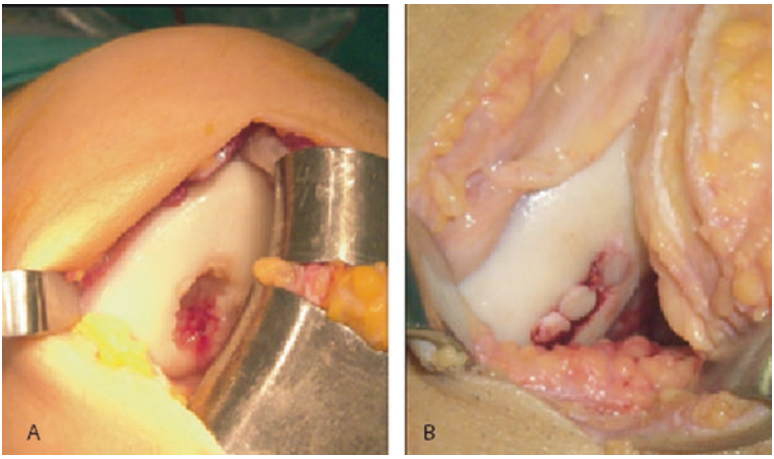

Fig. 6.2 Intraoperative view. (**a**) Osteochondral injury, (**b**) Autograft transplantation (mosaicplasty)

Autologous Chondrocyte Implantation (ACI) and Matrix-induced Autologous Chondrocyte Implantation (MACI) are two-stage surgical procedures that are used for the treatment of OA. First surgery collects healthy chondral tissue and these cells are induced to proliferate in vitro. In ACI, cultured cells are directly applied to the defect area and periosteal or synthetic collagen patches are then used to cover them. In MACI, the cells are cultured on a scaffold and inserted into the defect site with fibrin glue. These methods are partially successful, but the excessive proliferation of cells from the periosteal flap (leading to osseous overgrowth of the defect cavity) and the time-consuming and expensive nature of the two-stage procedure remain the most significant problems associated with these treatment methods (Kwon et al., 2019; Zylińska, Silmanowicz, Sobczyńska-Rak, Jarosz, & Szponder, 2018).

All these regenerative methods require donor material, are invasive, and require long-term postoperative treatment. If these methods fail, total joint arthroplasties are widely used for pain relief and restoration of function. In arthroplasty surgeries, joint surfaces are cut and internal prostheses consisting of metal and polyethylene components are applied to bone surfaces. While complications due to infection and poor surgical technique are seen in the early period, the biggest problem in the late period is the need for revision surgery because of aseptic loosening.

As is evident, none of these treatments can achieve true joint regeneration, and major surgical procedures often pave the way for later invasive interventions. As all these treatments are costly and do not result in full improvement, treatment always continues through various modalities. Regenerative therapies, a new alternative, aim to restructure cartilage with a different approach in order to obtain healthy cartilage tissue. Although there is still much to be done on this subject, research efforts continue to make progress. In the following section, the current state and goals of nanomaterial-based approaches in cartilage regenerative treatment will be discussed.

6.2 Nanomaterials and Cartilage Tissue Engineering

The main methods used in the treatment of damaged cartilage tissue aim to relieve the symptoms by alleviating pain and restoring function, while not focusing on the tissue structures at the core of the problem. Regenerative treatments, however, are focused on the formation of new cartilage tissue that is identical to the original tissue (Armiento et al., 2018). Tissue engineering is a promising approach in the field of regenerative medicine. It requires interdisciplinary work of various scientific disciplines such as engineering, material science, biology, and chemistry. Tissue engineering today is founded upon cells, scaffolds, and signals (the tissue engineering triad) (Zhang, Hu, & Athanasiou, 2012). However, tissues have a complex hierarchical arrangement from the nano level to the micro and macro levels. This affects tissue biology, the transition between layers, and the regulation of tissue interactions. The macro level determines aspects like biocompatibility, biodegradability and mechanical properties. Micro level manages tissue architecture, surface chemistry, surface stiffness, cell migration, nutrient delivery, and vascularization. On the nano scale, the functions of bioactive factors, cell adhesion, mineralization and gene expression are regulated (Santo, Gomes, Mano, & Reis, 2012). Since natural tissues achieve homeostasis through these nano- and micro-level interactions, attaining this complex structure is also crucial for tissue engineering. Therefore, nanomaterials, with their excellent physicochemical structures and biomimetic properties, have attracted great interest in improving cell growth and function and facilitating and directing tissue regeneration (Eslahi, Abdorahim, & Simchi, 2016).

In conventional tissue engineering, cells planted in tissue scaffolds are first statically cultured. They are then transferred to a bioreactor that cultures them under loads similar to the tissue. This immature tissue is expected to undergo remodeling after implantation into the body. The current approach, however, aims to avoid the cell seeding and maturation stages of these methods. By implanting only the scaffold and performing microfractures in order to recruit mesenchymal stem cells from the bone marrow, it is expected that adequate mesenchymal stem cells differentiation can be stimulated (Camarero-Espinosa et al., 2016). Nanomaterials also offer new possibilities for the production, transport, release and timed-activation of bioactive substances. These bring a wealth of approaches for cartilage regeneration, allowing researchers to try new combinations.

6.2.1 Biomaterials

Biomaterials are composed of mainly ceramics, metals, and polymers that can be obtained naturally or synthetically. The biomaterials that are designed to be used for cartilage tissue engineering should be biocompatible, allowing cell retention, allowing the passage of bioactive substances, and able to support cell viability, proliferation and secretory activities (Vinatier & Guicheux, 2016). Since osteochondral

tissues are connective tissues that are regularly subjected to loads, the scaffolds used must be made of materials that can withstand mechanical loading. As a result, rigid polymers have been used for three-dimensional structural support, while hydrogel scaffolds are more suitable as cell carriers. Composite biomaterials are still being developed in order to mimic the multilayered hierarchical architecture of the cartilage tissue, which includes a special osteochondral interface with bone tissue (Manoukian et al., 2018).

Natural polymers used as cartilage scaffolds are physiological and nontoxic materials which usually have bioadhesive surfaces for cells. However, they are mechanically weaker due to their rapid degradation profiles. Some of the natural polymers being studied extensively are: polysaccharide structures (chitosan, hyaluronan, alginate, agarose, chondroitin, methylcellulose); and protein structures (collagen, gelatin, fibrin, silk, keratin). Collagen and hyaluronan adapt and degrade more easily since they are a part of natural cartilage tissue (Eslahi et al., 2016; Vinatier & Guicheux, 2016).

Synthetic polymers offer stronger mechanical structures, ease of processing, and can be sterilized. Unfortunately they are less biologically active, degrade poorly, and might cause a stronger inflammatory response. Some synthetic polymers that have been used as cartilage scaffolds are poly(ethylene glycol) (PEG), polylactide (PLA), poly(L-lactide) (PLLA), poly(lactic-co-glycolic acid) (PLGA), poly(ε-caprolactone) (PCL), poly(vinyl alcohol) (PVA), polyurethanes (PUR), polyglycolide (PGA), and PGA/PLA copolymers (Eslahi et al., 2016; Manoukian et al., 2018).

6.2.2 Nanomaterials and Scaffolds

Nanomaterials are emerging as versatile components that can control conventional tissue engineering approaches structurally, mechanically and chemically at the nano level through the use of nanofabrication technologies. They can exhibit a high level of cellular compatibility and bioactivity by enabling molecular interactions with the cell, while providing unique mechanical, optical, electrical, and magnetic properties to better control cell functions. Nanomaterials can also mimic extracellular matrix surface properties such as energy and topography. All these features have led researchers to study nanomaterials to be used as tissue scaffolds, biomolecular carriers or biosensor nanovehicles (H. Chen et al., 2013).

Cartilage tissue scaffolds can be built as hydrogels, fibrous meshes or foam (sponges) depending on their preparation techniques. Hydrogels are water-swollen polymers or protein structures that are physically, chemically or hybrid cross-linked. While chemically cross-linked hydrogels have covalent bonds, physically cross-linked hydrogels are bound by non-covalent (hydrogen bonding, hydrophobic, and electrostatic) weak molecular interactions. Hydrogels are currently the most popular cartilage scaffolds and are being studied extensively. This is because they are easy to apply and they can be injected or applied through minimally invasive surgery such as arthroscopy. The hydrogels can easily fill lesion areas within the joint

and quickly adapt to the surrounding healthy tissue. They have a high water content allowing the diffusion of nutrients and waste. Cells within these gel structures can become embedded, as opposed to simple adhesion, and they can differentiate in the direction of a chondrogenic phenotype. Commonly used polymers are PEG, agarose, alginate, hyaluronan, collagen, and chitosan, which can provide high biocompatibility and potential biodegradability. However, hydrogels are mechanically weak, especially for the cartilage surfaces of the load-bearing joints; thus, efforts are still being made to increase the mechanical strength of hydrogels (Camarero-Espinosa et al., 2016; Eslahi et al., 2016; J. Li, Chen, et al., 2019).

Reducing the gel to nano scales is one of the key strategies to increase the response of hydrogel dynamics to stimuli. The large surface-to-volume ratios obtained by the addition of nanoparticles to the structure strengthen the mechanical properties as well as improving function (J. Li, Chen, et al., 2019). However, the number of nanostructuring techniques that can be used for this purpose are limited due to the high water content of hydrogels. But self-assembly of hydrophobic segments of polymers in aqueous environments can still take place. Nanoparticle hydrogels (nanogels), nanofiber hydrogels and hybrid hydrogels constitute promising materials that are being researched extensively for this purpose.

Among the techniques that are used for the production of nanofiber hydrogels, molecular self-assembly and electrospinning techniques are tested extensively by the researchers. The extracellular matrix mimicking biomolecular structures of self-assembled supramolecular materials enable them to support cell adhesion, proliferation, and migration through their bioactive interactions. Among self-assembling materials, peptide nanofiber gels are emerging as an alternative treatment for cartilage tissue. Yaylacı et al. have shown that glycosaminoglycan-mimetic peptide nanofibers can support chondrogenic differentiation in mesenchymal stem cells, and hyaluronic acid mimetic self-assembled peptide nanofiber gels may enhance cartilage regeneration (Yaylaci et al., 2016). Likewise, Arslan et al. investigated the therapeutic effects of a hybrid peptide nanofiber-hyaluronic acid membrane they developed in an in vivo rat OA model. They demonstrated that this hybrid nanofiber membrane was more effective than the commercially available gels they compared it to, and that the hybrid peptide nanofiber-hyaluronic acid membrane could be a suitable alternative for the treatment of OA (Arslan et al., 2018).

Scaffolds produced by electrospinning technique are obtained from polymer solutions through the use of high-voltage collectors, thereby forming a non-woven fabric made of fibers. These simple yet robust structures also enable the discovery of new material combinations, since they also allow the co-spinning of multiple synthetics or biological materials. The surfaces of the scaffolds produced through electrospinning can also be tailored to have surface modifications, chemical modifications, and add biologically active materials. Combined materials can be made with growth factors and biological signal proteins. Electrospinning is a carefully studied topic for 3D scaffold production, which is particularly important for joint cartilage (Zhou, Chyu, & Zumwalt, 2018). Mahboudi et al. fabricated a nanofiber-based polyethersulfone scaffold via electrospinning, and they were able to demonstrate osteochondral differentiation of human bone marrow mesenchymal stem cells

on this scaffold (Mahboudi et al., 2018). In another study, Erisken et al. loaded two bioactive substances on a poly(ε-caprolactone) scaffold they produced with the electrospinning technique. Added to the scaffold were insulin (for chondrogenic differentiation) and β-glycerophosphate (for mineralization). They showed that human adipose-derived stromal cells cultured in this scaffold for 8 weeks were heavily directed towards chondrogenic differentiation (Erisken, Kalyon, Wang, Örnek-Ballanco, & Xu, 2011). Yu et al. applied a bioactive resveratrol—PLA—gelatin porous nano-scaffold that they built with the electrospinning technique to a rat cartilage defect. They showed that the scaffold was able to heal the defect and demonstrated how this structure affects the repair through the PI3K/AKT signaling pathway (Yu et al., 2018).

Nano-level controllable composite tissue scaffolds are being studied extensively in cartilage tissue engineering. These composite structures offer new horizons and possibilities not only for cartilage, but also in the formation of a cartilage-bone hierarchical interface. Nanomaterials are also used in tissue engineering to produce and distribute tissue stimulating agents such as growth factors and peptides. Chahine et al. evaluated the biocompatibility of single-wall carbon nanotubes (SWNTs) in articular cartilage tissue engineering in 2D and 3D composite tissue scaffolds. They showed that SWNTs increased GAG content in composite scaffolds, and that it biomechanically increased pressure resistance and tensile modulus. They demonstrated that SWNTs may be able to provide functionalization with bioactive molecules and biomechanical strengthening (Chahine, Collette, Thomas, Genetos, & Loots, 2014). In another study, Ribeiro et al. developed biofunctional hierarchical scaffolds consisted of a horseradish peroxidase (HRP)-cross-linked silk fibroin (SF) cartilage-like layer (HRP-SF layer) fully integrated into a HRP-SF/ZnSr-doped β-tricalcium phosphate (β-TCP) subchondral bone-like layer (HRP-SF/dTCP layer) aimed at regenerating chondral and subchondral tissue. Human osteoblasts (hOBs) and human articular chondrocytes (hACs) planted on this bilayer hierarchical structure demonstrated sufficient integration, proliferation, and appropriate ECM production to the respective interfaces. With this study, they showed that osteochondral-like tissue formation can be promoted with appropriate stimuli in a culture system (Ribeiro et al., 2019). Karami et al. designed a composite scaffold that would firmly adhere to tissues such as cartilage and meniscus. This hydrogel system consisted of poly(ethylene glycol) dimethacrylate, alginate, and nanofibrillated cellulose. They showed that their composition provided a significant increase in cartilage adhesion compared to the existing commercial tissue adhesives (Karami et al., 2018).

6.3 Summary and Future Outlook

Studies on regenerative medicine are advancing with great momentum, challenging our current perceptions of medicine. Our focus is now shifting from alleviation of symptoms to the full regeneration of tissue. We are also witnessing the most promising advances for cartilage, one of the most challenging tissues for regeneration.

The scaffold necessary for the regeneration of cartilage tissue is slowly taking shape; involving new composite structures with bioactive extracellular matrix mimicking materials, and chondrocytes differentiated according to the multilayered architecture required by the natural tissue. As we have seen in the recent studies above, developments in this area have reached an exciting level. However, there are still issues ahead, such as the hierarchical relationship of cartilage tissue with bone and joint fluid, the complex unforeseen interactions that may be observed in vivo, and the unique biomechanical and lubricative properties of the cartilage tissue. These still pose difficulties to be overcome by researchers of this field, and the biomechanical shortcomings of current scaffolds is another problem encountered in clinical practice.

Cartilage tissue is very difficult to reach with medications administered systemically. This is why intra-articular drug administrations are an important subject, and this also poses various hardships. Nanomaterials can also be used to overcome the difficulties in distributing intra-articular medications. Moreover, the ability of nanostructures to overcome extracellular matrix and cell barriers provides great advantages in drug distribution and diffusion (Brown, Kumar, & Sharma, 2019; Wang et al., 2018). Currently, the effective delivery of drugs is limited to indirect pharmacological measures. However, imaging agents added to nanoemulsions will soon be able to monitor the distribution, release, and efficacy of drugs in vivo. This area called *Theranostics* enables delivery, treatment, and imaging with the same molecule using nanomaterials and bioimaging technologies (Herneisey et al., 2016; Patel, Beaino, Anderson, & Janjic, 2015).

All these issues are related and interacting with each other. Future research goals range from regeneration of damaged tissue, to the manufacture of organs and extremities. This subject stands before all humanity as a common goal to be striven towards in order to promote human welfare.

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