Chapter 5
Nanomaterials Applications in Cartilage Tissue Engineering

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Abstract Articular cartilage is the smooth layer of soft tissue that covers our bones and allows for the painless movement of our joints. Because of joint pathologies such as arthritis, cartilage can degrade over time in some individuals, causing them to live with considerable pain and reduced mobility. The high prevalence of arthritis and the absence of a cure for osteoarthritis, its most common form, have fueled sustained efforts to develop tissue engineering and regenerative medicine strategies aimed at regenerating cartilage. Despite a number of clinical advances that elicit cartilage repair, true regeneration remains elusive. Recent years have seen an increased use of nanoscale materials in the development of therapies for joint pathologies. Nanomaterials are comparable in scale to the principal building blocks of cartilage extracellular matrix, namely collagen and proteoglycan aggregates. Similarly, nanoparticles are sufficiently small to allow diffusion through the pores of the dense cartilage extracellular matrix and cell targeting. In this chapter, the organization of cartilage's main building blocks will be reviewed from the nano- to macroscale, and sub-micron particles that participate in cell-cell communication will be highlighted. Efforts to design scaffolds incorporating cell-instructive nanoscale features and to tailor the mechanical properties, or even engineer spatial organization, in scaffolds for cartilage repair using nanomaterials will also be discussed. Finally, key design criteria in nanoparticle synthesis to enable targeted therapeutic delivery will be examined.

5.1 Introduction

Articular cartilage is a load-bearing connective tissue that covers the articulating surfaces of bones in synovial joints. It is characterized by a dense and highly hydrated extracellular matrix (ECM) mainly comprising a collagen type II fibrillar network

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interspersed with proteoglycans, as well as other collagens and non-collagenous proteins [1]. The interplay between factors such as composition, orientation and cross-linking of these biomolecules dictates the hydration of the tissue and its mechanical properties [2]. This complex organization allows the tissue to resist shear and tensile forces applied to the joint during articulation while facilitating the absorption and distribution of compression forces transmitted through the joint [3]. Chondrocytes—the resident cells in articular cartilage—are sparsely distributed throughout the tissue, where they remodel the ECM in response to the changing biomechanical environment and help maintain tissue integrity [4].

Under physiological conditions, articular cartilage homeostasis can be maintained throughout life despite age-related changes and the high cyclic loading the tissue sustains. Nevertheless, a large number of factors can cause joint diseases and associated progressive articular cartilage degeneration. These include abnormal loading of a joint due to altered biomechanics (e.g., due to trauma or obesity), as well as degeneration due to genetic, environmental and dietetic factors, among other causes, which may also arise under normal loading conditions [5, 6]. The most common form of joint disease is osteoarthritis (OA), which is estimated to affect approximately 15% of the Canadian population 18 years of age or older [7]. While OA is often characterized in terms of its degenerative effects on articular cartilage, its pathophysiology also involves the other tissues of the affected joint, including the subchondral bone and the synovium [8]. The interplay between these tissues is important in the development and progression of the disease, such that an effective treatment strategy would need to consider and target changes in the joint as a system rather than articular cartilage alone [9, 10]. For example, inflammation of the synovial membrane has been linked with the release of cytokines, such as interleukin 1β (IL-1β) and tumor necrosis factor α, which contribute to the loss of balance between the expression of catabolic enzymes and anabolism in chondrocytes and consequent tissue loss [11].

William Hunter observed more than 250 years ago “when destroyed, [cartilage] is never recovered” [12]. Despite substantial efforts to overcome the limited ability of this tissue to regenerate itself, this early observation holds true today, as there is still no cure available in clinics to interrupt or reverse the progression of OA. As such, treatment modalities aim to either manage the symptoms of the disease or to repair or replace the damaged tissue. Tissue repair is distinct from regeneration: regeneration leads to neo-tissue with similar composition and organization to the native articular cartilage, whereas repair creates tissue with different, typically inferior, properties. Management of symptoms that include swelling, stiffness of the joints and pain is achieved via lifestyle changes through physical exercise and weight loss programs, often combined with the administration of acetaminophen and non-steroidal anti-inflammatory drugs (NSAIDs) [13]. Another non-surgical intervention used to manage the symptoms of cartilage damage is viscosupplementation, the injection of a hyaluronic acid solution into the joint capsule to ease pain and facilitate movement [14]. While disease-modifying OA drugs (DMOAD) remain an unmet need in clinical settings, a number of pharmacological agents targeting OA progression have undergone phase II/III clinical trials in recent years. These include the inducible nitric oxide synthase inhibitor Cindunistat, the IL-1β inhibitor

diacerein, oral salmon calcitonin, and strontium ranelate [15–18]. The clinical data from viscosupplementation intra-articular treatments using hyaluronan is also being analyzed to demonstrate chondroprotective effects [19].

A range of surgical procedures is also available to repair cartilage defects when non-invasive approaches are no longer effective for managing symptoms. The gold standard treatment for patients with advanced OA remains the partial or total replacement of the diseased joint with prosthesis. Total joint arthroplasty is typically very successful for reducing pain and improving quality of life [20]. Nevertheless, failure of orthopaedic implants due to infection, fatigue failure or implant loosening caused by wear debris-induced osteolysis requires technically challenging revision surgeries, rendering this approach less suitable for the treatment of younger patients [20, 21]. These limitations of orthopaedic implants have fueled the development of tissue and cell-based interventions. Mosaicplasty, a surgical procedure that consists of harvesting small cylindrical osteochondral samples from low weight-bearing locations of the joint (autologous) or a deceased donor (allogenic) and transplanting these into the defect has shown encouraging clinical results; however, issues related to donor site morbidity, poor integration of the grafts to surrounding tissues, disease transmission and limited treatable defect size have restrained its use [22–24]. The most common surgical approaches are marrow stimulation techniques (e.g., drilling, abrasion and microfracture) [25]. These comprise methodologies to access the bone marrow by breaching the integrity of the subchondral bone and form a fibrin clot within a debrided cartilage defect. Blood-borne progenitor cells can subsequently infiltrate this fibrin "scaffold" and deposit repair tissue. While these involve a single simple intervention, the resulting fibrocartilage repair tissue is typically mechanically inferior to native articular cartilage and is prone to deterioration within a few years [26]. Another approach is autologous chondrocyte implantation (ACI), which involves the excision of autologous cartilage from non-loading areas of the affected joint in a first surgical intervention, followed by the enzymatic release of chondrocytes from their ECM and their amplification in vitro [27]. The chondrocytes are then implanted back into the debrided cartilage defect under a periosteal graft in a second surgery. The long-term clinical outcome for ACI has been positive [28, 29]. A variety of 3D scaffolds and hydrogels have since been developed as cell delivery constructs and/or templates that instruct tissue formation to improve on the results of microfracture and ACI procedures, some of which have been translated into clinical use [30]. The field of cartilage tissue engineering is rapidly evolving and the technologies being developed are increasingly representative of the complexity of the native tissue they aim to regenerate.

Efforts to identify DMOADs and to develop improved scaffolds for cartilage tissue engineering have paralleled increased incorporation of nanomaterials into cartilage repair strategies. According to the definition provided by the ISO/TS 80004-1:2015, nanomaterials are characterized as having at least one of their external dimensions ranging between 1 and 100 nm, or having internal or surface structures in the same range; however, the term is often applied more loosely to sub-micron scale materials in the literature of many fields including tissue engineering (see Chap. 1). At nanoscale (1–100 nm), materials exhibit unique size-dependent properties that arise

due to a high surface area-to-volume ratio, resulting in a greater relative contribution from surface molecules compared to those in the bulk. These properties provide interesting opportunities to devise novel strategies for biomedical applications, ranging from biomarker detection and in situ imaging to therapeutic delivery and tissue regeneration. Our understanding of the importance of the nanoscale organization level in tissues also offers inspiration for the development of bioinspired nanomaterials that could deliver improved therapeutic efficacy.

In this chapter, we will briefly review the organization of articular cartilage and its main building blocks from the macroscale down to the nanoscale and discuss submicron particles that participate in cell-cell communication. Efforts to design scaffolds incorporating cell-instructive nanoscale features and to exploit unique nanoparticle properties to design scaffolds and hydrogels with unique properties for cartilage tissue engineering applications will be discussed at some length, e.g, approaches to tailor mechanical properties and to engineer spatial organization in biomaterials with nanomaterials. Finally, key findings for the design of nanoparticles to enable targeted therapeutic delivery will be examined.

5.2 Articular Cartilage from Macro- to Nanoscale

Despite having been portrayed as a rather simple tissue from the point of view of its organization due to the absence of vasculature, lymphatic vessels, and nerves, articular cartilage has multiscale structural complexity. Macroscopically, it appears as a smooth, whitish layer of tissue on the articulating surfaces of long bones, where it reaches thicknesses ranging from less than 1–6 mm depending on anatomical position, age, and exercise level [31, 32].

Articular cartilage exhibits depth-dependent anisotropy as pertains to ECM composition, biomacromolecule organization and cross-linking, as well as resulting mechanical properties [33]. This anisotropy is often discussed in the literature as a "zonal" organization, whereby articular cartilage is divided into four zones; from superior to inferior of the joint surface: the superficial (or tangential) zone, the middle (or transitional) zone, the deep (or radial) zone and the zone of calcified cartilage that interfaces with the subchondral bone [3]. Of note, the collagen fibers are aligned parallel to the tissue surface in the superficial zone, transition to a more random orientation in the middle zone, and are perpendicular to the tissue surface in the deep zone [34] (Fig. 5.1a). A number of studies have proposed the existence of additional distinct structural regions in the most superficial zone of articular cartilage, suggesting a higher level of organizational complexity than is generally appreciated [35, 36]. Owing to the avascular nature of articular cartilage, chondrocytes are supplied with nutrients and signaling molecules by diffusion from the synovial fluid, establishing biomolecular gradients that may contribute to generating and maintaining this anisotropic organization [37, 38]. Zonal differences in the phenotypic specification of chondrocytes also exist, with superficial, middle and deep zone chondrocytes having been characterized [39]. These phenotypic differences result in distinct expression

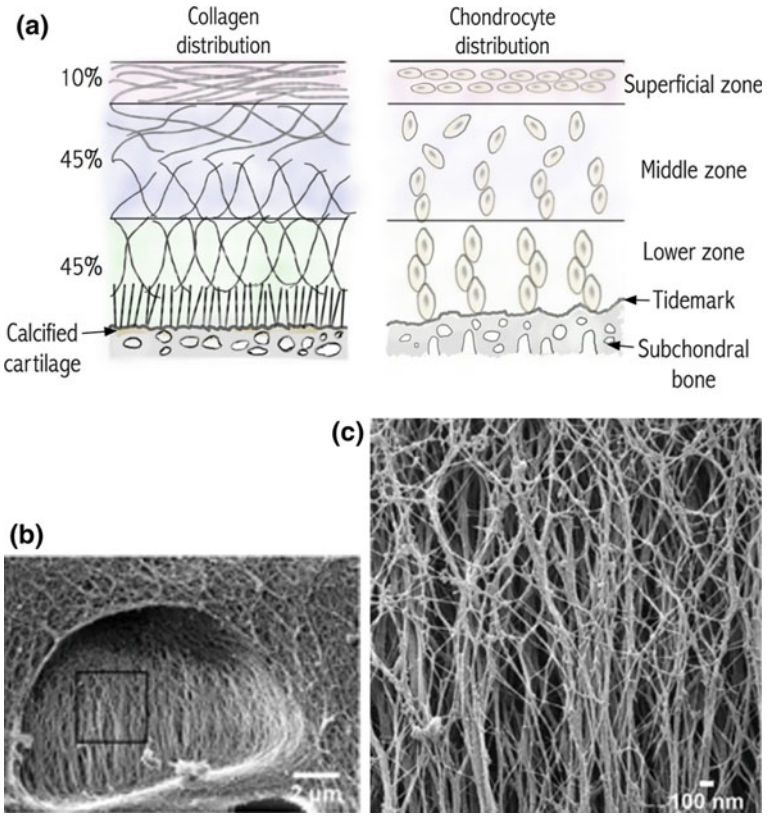

Fig. 5.1 **a** Schematic representation of the zonal organization in articular cartilage. **b**, **c** Helium ion microscope images of the fibrillar extracellular matrix in the pericellular matrix directly interfaced with chondrocytes. Adapted from Vander Berg-Foels et al. [40] Copyright (2012), with permission from John Wiley and Sons

profiles that dictate the compositional and organizational differences between zones and contribute to the mechanical and tribological functions of articular cartilage.

The articular cartilage ECM is also organized differentially with respect to its distance from chondrocytes. The pericellular matrix (PCM) is the thin layer of ECM that directly interfaces each chondrocyte and provides a niche microenvironment for the cells [41]. It has a distinct composition from the remaining cartilage ECM that comprises high proteoglycan content (e.g., perlecan, aggrecan, hyaluronan, and biglycan) and a network of type VI collagen fibrils, a protein that is concentrated in the PCM in cartilage [42]. The chondron, which consists of the chondrocyte and its PCM, has been recognized as the primary biomechanical unit of articular cartilage [43]. Studies suggest that it plays a role in protecting the cell against mechanical loading, as well as in matrix turnover and homeostasis. Important changes have been observed in the mechanical properties and composition of the PCM during OA

pathogenesis [44]. Distal from chondrocytes compared to the PCM, the territorial matrix is composed mainly of thin type II collagen fibrils also arranged around chondrocytes and interspersed with proteoglycan to resist loading and deformation, whereas the interterritorial matrix constitutes the bulk of the tissue with larger type II collagen fibrils oriented with respect to the joint surface rather than around individual cells and interspersed with proteoglycan [41].

These ECM components are self-assembled into nanoscale structures. For example, the collagen fibrils exhibit a broad distribution of diameters, with fibrils as small as 10 and upward of 200 nm having been reported [40, 45]. Fibril dimensions are highly dependent on anatomical site, age, depth from the surface, and disease state. For example, their diameter tends to increase with age but can be reduced in early OA, and large fibrils (upward of 450 nm in diameter) have also been observed in later stages of OA [46]. Multiple groups have also reported a trend towards increased collagen fibril diameter from the superficial zone to the deep zone of the tissue [1, 40, 47]. The interfibrillar spaces in cartilage ECM contain proteoglycan aggregates with hydrodynamic radii ranging from 1000 to 1600 nm [48]. A closer look at cellular interactions with the surrounding PCM highlights the fact that these occur at the nanoscale. Indeed, the PCM collagen network in direct contact with chondrocytes is composed of a majority of fibrils with diameters below 100 nm (Fig. 5.1b, c) [40]. This dense arrangement of ECM components represents a considerable resistance to the diffusion of macromolecules within articular cartilage.

The nature of the cartilage ECM combined with its avascular nature and the fact that chondrocytes are relatively isolated and sparse within the tissue, representing only approximately 2% of tissue volume in adults, have implications for cell-to-cell communication mechanisms. Any signaling molecules carrier passing through the ECM must be of nanometer or at most sub-micron size, as the spacing between collagen fibrils in articular cartilage has been reported to range from 60 to 200 nm [45], while the packing of polyanionic glycosaminoglycan subunits in proteoglycan aggregates is even denser with only a few nanometers between branches [48]. The presence of cellular projections connecting chondrocytes within the ECM has been reported, suggesting a potential pathway for direct cell-to-cell communication [49]. An additional proposed means of communication identified in cartilage is the release of extracellular vesicles (EVs) to shuttle cargos of signaling molecules between cells within a tissue and from one tissue to another. EVs consist of three classes of cell-derived particles: exosomes produced by multivesicular endosomes and ranging in size between 30 and 150 nm, microvesicles formed by cell membrane budding with typically larger sizes ranging between 50 and 1000 nm [50] and apoptotic bodies that have been associated with OA [51]. EVs found in cartilage have been studied extensively in the context of ECM calcification [52]. They have also been associated with inter-tissue signaling within the joint [53]. Given the ability of these nanoparticles to diffuse through the dense ECM of articular cartilage and act as delivery vehicles for bioactive molecules to chondrocytes, continued efforts to understand the mechanisms by which EVs are transported through cartilage, including the importance of size and surface properties, may offer a roadmap for achieving delivery and retention of therapeutic cargo within the tissue.

5.3 Nanomaterials in Cartilage Tissue Engineering Scaffolds

One of the fundamental tenets of the tissue engineering approach, when it was first proposed in the 1980s, was the idea that a micro- or macro-porous biocompatible and resorbable material could be used as a scaffold to guide tissue regeneration [54]. A growing appreciation of the importance of carefully tailoring the microenvironment in a scaffold (or template material [55]) to appropriately modulate the phenotype and fate decisions of cells has driven continued innovation in scaffold/template fabrication. Nanotechnology has taken a position at the leading edge of these efforts to design biomimetic and modulatory biomaterials. Nanomaterial fabrication techniques have been exploited to generate structures analogous to ECM at the nanoscale, a critical dimension in cellular sensing. Nanomaterials have enabled tailoring of the mechanical environment via reinforcement, while high conductivity nanoparticles have been used to facilitate the electrical stimulation of cells. These nanoparticles have also been exploited for controlled delivery of biomolecular signals. Advances in each of these categories for applications in cartilage tissue engineering will be discussed in this section.

5.3.1 Nanoscale Structures in Scaffolds

5.3.1.1 Electrospun Scaffolds

Textile fabrication techniques have been explored extensively for the production of fiber-based scaffolds for tissue engineering, affording the opportunity to mimic some aspects of the fibrous nature of the ECM in tissues. One such technique, termed electrospinning, relies on the generation of an electric field between a polymer solution (typically delivered through a needle) and a collector to draw the solution into a fiber. This drawn solution solidifies on its way to the collector as the solvent evaporates to form a membrane of nonwoven material [56]. Through the careful optimization of process parameters that include polymer concentration, solvent selection, polymer solution flow rate, humidity, voltage differential, needle dimensions, as well as the distance between the needle and the collector, one can produce membranes with average fiber diameters ranging from a few micrometers down to the low nanometer range and fairly narrow distributions. Studies have demonstrated the benefits of these materials for cartilage tissue engineering. For example, chondrocytes seeded onto nanofibrous poly(lactic-co-glycolic acid) (PLGA) membranes with an average fiber diameter of 550 nm exhibited increased proliferation and ECM accumulation compared to those cultured onto flat membranes of the same material [57]. Others showed that mesenchymal stem cells (MSC) seeded onto nanofibrous materials made of poly(caprolactone) (PCL) with an average fiber diameter of 700 nm exhibited enhanced chondrogenesis compared to the cell pellet culture model [58].

A number of groups have exploited the control afforded by electrospinning over fibrous material structures to study the effect of fiber diameter on chondrogenic cell responses, with mixed results. Li et al., reported on the interaction of passaged bovine chondrocytes with electrospun poly(L-lactic acid) (PLLA) scaffolds made of nanoscale (500–900 nm) and microscale (15–20 μm) fibers [59]. They showed increased proliferation and sulfated glycosaminoglycan (sGAG) accumulation, as well as decreased dedifferentiation, on the material made of nanofibers, concomitant with more spherical cell morphology. Others who compared PCL membranes with aligned fibers characterized by average dimensions of 500 and 3000 nm also demonstrated a benefit of sub-micron fibers to the gene expression profile of differentiating MSC [60]; however, the opposite trend was also reported in another study that used nonwoven materials made of 440 and 4300 nm fibers [61]. A detailed study comparing a range of different fiber diameters from 300 nm to 9 μm reported that the larger fiber diameter materials elicited increased chondrogenic differentiation in MSC [62]. The authors suggested that this effect might have to do with the increased pore dimensions in scaffolds characterized by micrometer-scale fibers rather than with the smaller fibers. In support of the suggestion that pore size may play an important role in the cellular responses observed between nanofibrous and microfibrous scaffolds, another group found that the generation of multiphasic scaffolds composed of both nanoscale and microscale fibers held benefits (as measured by increased ECM accumulation) compared with scaffolds that only incorporate microfibers [63]. Taken together, these studies highlight the need for additional work to clarify the effects of electrospun fiber dimensions over the full range of sub-micrometer fibers that can be fabricated and the contribution of pore size on the modulation of cell phenotype. Of particular interest is the study of fiber dimensions comparable to those of the fibrous network in articular cartilage. Controlling the diameter of fibers in electrospun scaffolds has also been shown to provide the opportunity to tune mechanical properties of single fibers [64]. This study, based on atomic force microscopy measurements, highlights the fact that the careful selection of the material-dimension combination is critical for presenting cells with a microenvironment that incorporates native biomechanical cues.

The effect of fiber orientation on the phenotype of chondrogenic cells has also been investigated. Aligned electrospun fibers are typically obtained by using a rotating cylindrical mandrel as a collector and adjusting its rotational speed, whereby a faster rotation will result in increased fiber alignment [65]. One study investigated the effect of aligned or randomly oriented sub-micron fibers on the chondrogenic differentiation of human nasal septum-derived progenitor cells cultured on PLLA/PCL blend electrospun membranes [66]. The authors observed that chondrogenic differentiation was enhanced on aligned fibrous materials, whereas cells on randomly oriented membranes showed increased proliferation. Others have explored the use of aligned nanofibers as an approach to specifically engineer the superficial zone of articular cartilage, demonstrating that aligned fibrous structures drive specification into distinct cellular phenotypes compared to other scaffold structures [60, 67, 68]. Other electrospinning techniques have also been developed to achieve nanofiber

alignment. For example, a collector consisting of two conducting supports separated by a gap was shown to result in fibers bridging the gap in a highly aligned manner [69]. Others have modified the instrumental setup to stabilize the polymer solution fiber emerging from the Taylor cone at the tip of the dispenser [70]. This setup, combined with a digitally controlled moving collector, allowed the production of spatially-defined electrospun mats exhibiting fiber alignment. Another group has proposed a modified rotating collector presenting a circular surface, which was used to generate electrospun materials with circumferential fiber orientation [71]. The resulting material structure mimicked aspects of the meniscus cartilaginous tissue organization and encouraged alignment of MSC along the changing orientation of the nanofibers.

One important setback in the development of electrospun scaffolds for cartilage tissue engineering applications is the fact that the structures generated are essentially organized on a 2D plane, while the fibrous components of articular cartilage ECM exhibit a complex 3D organization with depth-dependent anisotropy. It should also be emphasized that the relatively small pore size characterizing these scaffolds impedes cell migration through the 3D structure, impacting their potential for applications in the repair/regeneration of full-thickness articular cartilage. This is a particularly important problem for materials made with nanofibers with proportionally smaller pores. This situation has led to a number of innovations in the fabrication procedure and cell seeding protocols. As an example, a modified electrospinning setup was used to deposit nanofibers onto the surface of a microfiber, which was subsequently pressed into the desired scaffold shape and density using a piston [72]. Others have developed an approach that involves co-electrospinning the nanofibrous material and sacrificial fibers that can be dissolved to open up the porous structure [73]. Yet another strategy employed the electrospinning apparatus under conditions that enabled "direct writing" to produce a scaffold consisting of struts oriented in such a way as to recreate the general depth-dependent collagen fiber directionality of native articular cartilage tissue, and subsequently electrospinning a fibrous network onto this open scaffold structure [74]. This approach produces full depth anisotropic articular cartilage scaffolds; however, these represent relatively thin (~200 μm) slices of tissue. Stacking and bonding of multiple slices allowed the generation of large constructs. While micrometer fibers were produced in this study, the approach would be amenable to the application of nanofibers onto the scaffold produced by direct writing.

5.3.1.2 Scaffolds Produced by Phase Separation

Other scaffold fabrication techniques have also been used to produce nanofibrous scaffolds for cartilage tissue engineering, albeit not as extensively as electrospinning. Phase separation is one such fabrication technique that exploits the fact that a homogenous polymer solution will separate into polymer-rich and polymer-poor

phases in thermodynamically unstable conditions, including during temperature-induced solidification. Once the solution is frozen, the resulting material can be sublimed to remove the solvent and reveal a porous structure in place of the polymer-poor regions, whereby polymer-rich regions form the scaffold walls. This methodology has been modified to include a gelation step prior to freezing, which results in nanofibrous structures with fiber diameters ranging from 50 to 500 nm [75]. Furthermore, this method offers the opportunity to produce fiber networks in 3D, in contrast with the planar arrangement of electrospun membranes. This fabrication technique has been applied to produce scaffolds for cartilage tissue engineering. For example, Ma and colleagues combined thermally-induced phase separation (TIPS) with solvent casting porogen leaching to generate scaffolds with controllable micron-scale pores and nanofibrous walls [76, 77] (Fig. 5.2). In these studies, sacrificial spherical sugar particles were sintered prior to solvent casting, gelation and freeze-drying to form interconnected pores. A similar approach was used to compare the effect of nanofibrous versus dense scaffold walls on chondrocytes, demonstrating more rounded cell morphology, improved chondrogenic phenotype and increased ECM accumulation on the nanofibrous scaffolds [78]. Another study combined TIPS with 3D printing to yield scaffolds with both macroscopic architecture and nanoscale features [79]. This multiscale scaffold resulted in substantially increased cell adhesion and accumulation of key ECM components (sGAG and collagen). Another group proposed a different strategy to generate nanofibrous scaffolds, employing TIPS with mixture of PLLA and camphene that forms an interpenetrating network [80]. Because of its physical properties, camphene can be removed during the sublimation step to reveal the nanofibrous structure. In this study, chondrocytic phenotype was reduced for

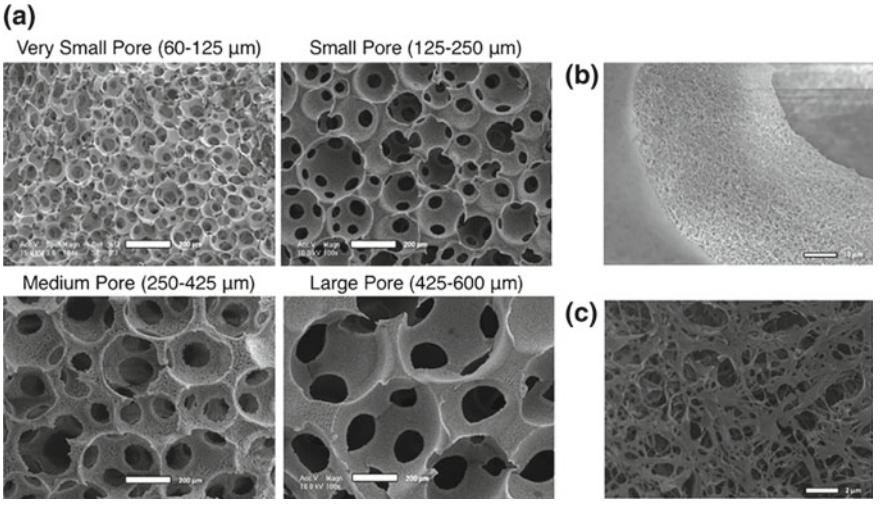

Fig. 5.2 Scanning electron micrographs of scaffolds fabricated by thermally-induced phase separation combined with solvent casting porogen leaching to generate nanofibrous scaffolds walls. Reprinted from Gupte et al. [77] Copyright (2018), with permission from Elsevier

cells in the nanofibrous scaffold compared to cells cultured in scaffolds produced in absence of camphene and characterized by dense, non-fibrous walls. The authors proposed that these results were due to increased cell-material interaction in the nanofibrous scaffold.

5.3.1.3 Self-assembled Supramolecular Structures

The spontaneous self-assembly of molecular building blocks into nanoscale structures, including nanofibers, has also been exploited as a promising strategy for biomaterials fabrication [81]. Through the careful design of these molecular building blocks, often inspired by self-assembly processes occurring in nature, control over intermolecular interactions can be achieved such that supramolecular architectures can be generated. A range of biomolecules that includes peptides, DNA and lipids has been self-assembled and stabilized through non-covalent forces including hydrogen bonds, ionic interactions, and Van der Waals forces. Typically, the fibers produced by self-assembly have diameters of 10 nm or less, representing the lower end of those found in articular cartilage ECM [81, 82]. This also represents a length scale that is more difficult to achieve with electrospinning and phase separation.

The concept of self-assembly has found applications in the design of novel scaffold and hydrogel structures for cartilage tissue engineering applications. Stupp and colleagues have produced self-assembled nanofiber gels from tailored peptide amphiphiles functionalized with transforming growth factor β-1 (TGFβ-1) binding peptide [83]. This gel allowed for a slower release of the growth factor than was observed with non-functionalized materials. The constructs were tested in full-thickness chondral defects treated with microfracture in rabbits to encourage bone marrow stromal cells into the defect. The authors observed that the functionalized gels enhanced articular cartilage regeneration compared to control groups that received an injection of TGFβ-1 or a non-functionalized gel loaded with the growth factor. Others have combined decellularized cartilage matrices (DCM) with nanofibrous gels of self-assembled peptide. Improved cartilage regeneration was demonstrated following implantation of the combined gel/DCM scaffold in rabbit full-thickness defects treated with microfracture compared to microfracture alone, as well as when compared with the microfracture plus the decellularized cartilage matrix scaffold absent the self-assembled nanofibrous gel [84]. Other building blocks have also been used to create constructs incorporating self-assembly. For example, self-assembled DNA-based rosette nanotubes functionalized with the integrin-binding peptide motif RGDSK have been used to produce nanofibrous scaffolds. These scaffolds were shown to support the chondrogenic differentiation of human MSC [85]. These studies highlight the potential of self-assembled nanofibrous scaffolds and hydrogels for cartilage tissue engineering. These highly tunable materials offer the opportunity to tailor the complexity of the microenvironments presented to resident cells and further instruct their responses.

5.3.1.4 Scaffold Surface Nanoroughness

Recognizing the importance of biomimetic surface roughness for controlling cell responses, many groups have proposed surface modification treatments to incorporate topographical features on biomaterials. These techniques have been explored in greater detail for metallic orthopedic implants interfaced with bone; nevertheless, a few studies have investigated the effects of nanoroughness on chondrocyte response. For example, Webster and colleagues used a short NaOH immersion treatment to modify the surface roughness of PLGA scaffolds produced by porogen leaching [86]. The resulting surface topography led to increased chondrocyte attachment, growth and ECM accumulation compared to untreated surfaces. The same group also developed a method to generate nanoroughness on the surface of polyurethane and PCL films by casting the polymer solution onto plasma modified titanium [87]. This surface modification also led to increased chondrocyte attachment and intracellular collagen content. Both of these surface modification protocols caused concurrent production of micro- and nanoscale surface modifications; the extent to which nanoroughness contributed to the observed effects, therefore, remains unclear. In a more fundamental study on the topic, nanotopographical features (nano-pillar, nano-hole, and nano-groove arrays) were produced on PCL surfaces by thermal nanoimprinting [88]. The effects of these surfaces on MSC response were investigated against non-modified surfaces. Nano-pillar and nano-hole arrays exhibited decreased cell proliferation and increased chondrogenic differentiation compared to cells cultured on control surfaces, while nano-grooves led to cell elongation and encouraged phenotypic changes reminiscent of superficial zone chondrocytes. These studies highlight the need for additional work in this area, in order to assess in greater detail how nanoscale topography and roughness can be incorporated with cartilage repair scaffolds to direct cellular responses, as well as responses to microscale versus nanoscale surface modifications.

5.4 Nanoparticle Composites to Tailor Mechanical Microenvironment

5.4.1 Tuning Mechanical Properties

As described previously, articular cartilage is a load-bearing tissue that fulfills primarily biomechanical functions in the body. As such, it displays relatively high mechanical properties compared to other soft tissues and behaves as a viscoelastic material with creep and stress relaxation responses [89]. Efforts to design scaffolds that mimic key aspects of native tissue's mechanical properties have typically involved tradeoffs with scaffold porosity. In the same way, biocompatible hydrogel systems often exhibit mechanical properties that are orders of magnitude lower than those of the native tissue. To address this limitation, nanoparticles have been added to scaffolds

and hydrogels to generate mechanically reinforced composites. A number of different nanoparticles have been used for this purpose in cartilage tissue engineering thus far, including carbon nanotubes [90], Laponite clay particles [91], poly(styrene-acrylic acid) core-shell particles [92], and cellulose nanocrystals [93], to name a few. The reinforcement effect of nanoparticles has been associated with their ability to interact with polymer molecules and form additional cross-links within the resulting structure. An increased surface area leads to greater interaction with the surrounding hydrogel, and nanoscale particles offer the advantage that they exhibit a substantially increased surface area to weight ratio compared to larger particles [94].

5.4.2 Enabling Mechanical Stimulation

The average person takes approximately 2 million steps per year; that is to say that the joints in our leg each typically undergo 1 million loading cycles annually [95]. The importance of mechanical loading in articular cartilage remodeling is well established. Indeed, vigorous physical activity in healthy individuals has been associated with increased cartilage volume and a decreased risk of developing cartilage defects [96]. Biomechanical factors including obesity and injuries leading to joint instabilities, on the other hand, have been associated with increased risks of cartilage pathologies [97]. In vitro studies on chondrocyte and cartilage response to mechanical loading have revealed ranges of stimulation parameters that result in increased tissue formation, while deviation from appropriate loading frequency, strain rate, and amplitude, as well as the loading history have been associated with increased catabolic responses [98]. Substantial efforts in the field have therefore focused on the development of bioreactors to facilitate the application of biologically relevant biomechanical stimulation regimens to induce increased tissue formation in engineered constructs.

Magnetic nanoparticles have been used to stimulate constructs mechanically. These can be incorporated into cells or materials and exposed to a magnetic field to induce strain. For example, magnetic nanoparticles synthesized by *Magnetospirillum sp.* AMB-1 can be efficiently endocytosed by MSC. Exposing the treated to MSC to magnetic fields leads to the application of forces to the cells [99]. Here, the authors showed significantly increased ECM (sGAG and collagen) accumulation and chondrogenic gene expression, in cell pellets subjected to short term physical stimulation (1 h per day for 5 consecutive days) compared to controls at 3 weeks post-stimulation. Although the nanoparticles were not found to be cytotoxic at concentrations below 30 μg/ml, three times above the levels required to achieve cellular magnetization, the long-term safety and clearance of these nanoparticles remains to be clarified. Other groups have incorporated magnetic nanoparticles into hydrogel materials [100, 101]. Ethier and colleagues produced trilayered hydrogels with each zone characterized by a specific agarose concentration and nanoparticle loading [101]. With this approach, the authors were able to produce differential strains in each zone of the construct, mimicking the anisotropic response of native articular cartilage

to mechanical loading. In an interesting study, magnetic nanoparticles were functionalized with an antibody against Frizzled, a receptor for the Wnt signaling pathway, which is of importance in chondrogenesis [102]. These functionalized nanoparticles were incubated with human MSC and shown to bind the Frizzled receptors on their surface. An oscillating magnetic bioreactor was then used to mechanically stimulate the receptor and activate the Wnt pathway. Such an approach could prove powerful for cartilage tissue engineering applications, whereby specific mechanosensitive signaling pathways may be activated without relying on chemicals or drugs. A similar approach had previously been used to activate the potassium channel TREK-1 on the surface of human MSC both in vitro and in vivo, resulting in the upregulation of genes associated with both osteogenesis and chondrogenesis, as well as increased synthesis of ECM components [103]. It should also be mentioned that the application of magnetic fields to chondrocyte cultures, even in the absence of magnetic nanoparticles, can cause cellular responses such as increased proliferation and increased sGAG accumulation [104, 105].

Park and colleagues have proposed an alternative application of magnetic nanoparticles for cartilage tissue engineering [106]. In this study, the authors produced porous microbead-shaped PLGA scaffolds. They used water-in-oil-in-water emulsion templating to achieve microbead structures containing gelatin particles and subsequently leached the gelatin to achieve porosity (Fig. 5.3). The surfaces of the resulting microscaffolds were further functionalized with Fe_3O_4 magnetic nanoparticles, enabling their actuation and deployment to a site of injury under the influence of a magnetic field. These scaffolds were shown to support attachment and chondrogenic differentiation of MSC. Such an approach has the potential for minimally-invasive surgical treatment of joint ailments.

5.4.3 Enabling Electrical Stimulation

Chondrocytes are not considered excitable cells in the same way that neurons and myocytes are; however, these cells are particular in that they exist in a higher osmolarity microenvironment than many other cell types [107]. Furthermore, increasing evidence points to the importance of calcium, sodium, and potassium signaling in chondrocyte and cartilage homeostasis through a complex channelome [108]. Given the importance of charged metal cations in chondrocyte signaling and the presence of voltage-gated ion channels on chondrocyte membrane, electrical stimulation has been investigated extensively for the treatment of cartilage ailments [109]. The responsiveness of chondrocytes to electrical signals was exploited by Webster and colleagues, who demonstrated that loading of conductive carbon nanotubes into polyurethane and subsequent electrical stimulation through the polymer enhanced both chondrocyte adhesion and proliferation compared to neat (unloaded) polymer [110]. The authors further demonstrated that the effect was not only caused by the electrical stimulation and was also due in part to the increased surface nanoroughness resulting from the incorporation of the nanoparticles within the polymer sheets.

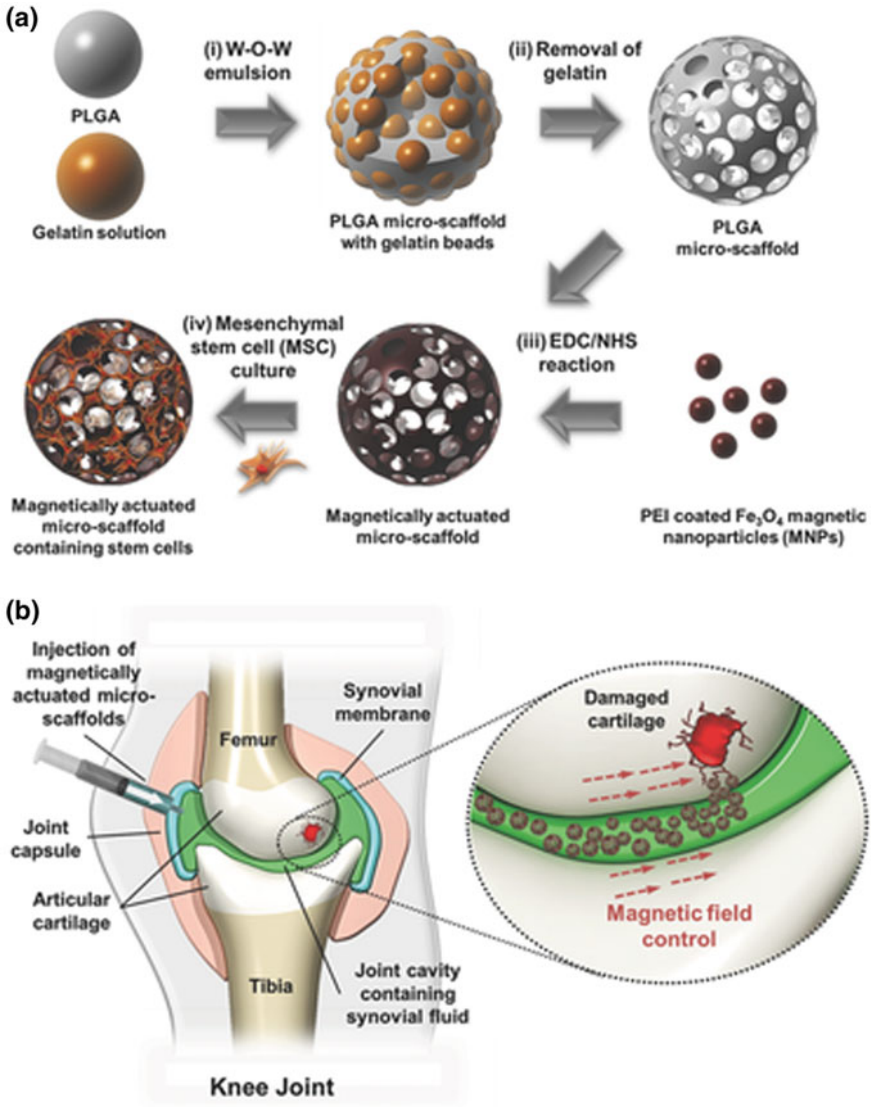

Fig. 5.3 Schematic diagram of a micro-scaffold fabrication process that incorporates magnetic nanoparticles to enable actuation and deployment into cartilage defects. Reprinted from Go et al. [106] Copyright (2017), with permission from John Wiley and Sons

5.4.4 Tailoring the Chemical Microenvironment

While tailoring the physical properties of cellular microenvironments represents an important design consideration for the formation of engineered cartilage, the spatiotemporal availability of biochemical signals has also proven to be equally important in stimulating tissue synthesis and organization. Nanomaterials have been instrumental in achieving increased control over the presentation of biomolecular signals, such as growth factors and small therapeutic molecules, to cells with chondrogenic potential within scaffolds and hydrogels. Park and colleagues took advantage of the specific affinity of heparin for growth factors to develop nanoparticles that deliver TGF-β3 within fibrin hydrogels seeded with MSC [111]. This construct led to significant improvements in chondrogenesis compared to controls, as well as when compared with hydrogels incorporating nanoparticles or the growth factor alone. Other groups have developed systems comprising multiple nanoparticles with distinct growth factor release profiles to integrate a temporal dimension to the release of a suite of growth factors. Nanoparticles have also served to deliver growth factor-rich platelet lysate [112], plasmid DNA-encoding chondrogenic growth factor [113] and bioactive ions [114]. These strategies are typically tailored to achieve sustained delivery of important factors in chondrogenesis and cartilage tissue formation. Furthermore, the uniform distribution of nanoparticles within scaffolds and hydrogels allows to overcome biomacromolecule diffusion limitations within 3D engineered tissues. Diffusion limitations are a major problem in tissue engineering when soluble factors are administered via the culture media, as these limitations can lead to tissue deposition inhomogeneity and significantly altered cellular phenotypes [115, 116]. Nanoparticles have also been used to present ECM signals to resident cells within engineered constructs. This is illustrated by Gibson et al, who produced decellularized ECM nanoparticles originating from a number of tissues, including cartilage. They then introduced the ECM nanoparticles into PCL electrospun scaffolds and investigated the effects on osteogenesis of human adipose-derived stem cells [117].

Biomolecular gradients are important signaling mechanisms that modulate a broad range of cellular responses from proliferation and migration to differentiation. These gradients play crucial roles in development, maintenance and repair of tissues and organs, while also being implicated in many pathological processes. As was previously discussed, biomolecular gradients are hypothesized to play important signaling roles in articular cartilage, owing to fact that nutrients and signaling molecules gain access to the tissue primarily via its superficial aspect (i.e, its surface; see Fig. 5.1a). Generating biologically relevant biomolecular gradients within scaffolds and hydrogels to direct anisotropic tissue organization represents a long-standing challenge in tissue engineering. This is an area where nanoparticles have had an important impact. For example, hydroxyapatite nanoparticles stimulate the osteogenic differentiation of MSC [118]. A number of groups have exploited this effect to generate MSC-containing scaffolds and hydrogels with spatially constrained nanoscale hydroxyapatite particles, and thus drive osteogenesis locally, while encouraging chondrogenesis in areas devoid of nanoparticles [119, 120]. In this way, biphasic constructs

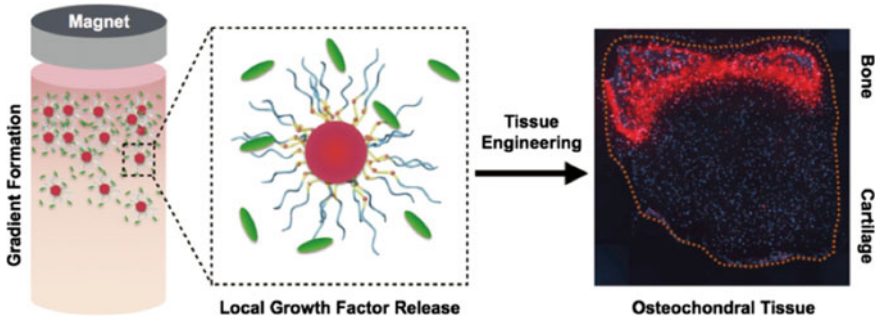

Fig. 5.4 Schematic diagram of procedure to generate biochemical gradients across the depth of hydrogels with heparin-functionalized, growth factor-loaded superparamagnetic nanoparticles. Reprinted from Li et al. [122] Copyright (2018), according to a Creative Commons Attribution License (CC BY)

containing both cartilage and bone, reminiscent of the osteochondral organization present in joints, can be achieved. Radhakrishnan et al. recently proposed a biphasic construct generated by spatially localizing hydroxyapatite and chondroitin sulfate nanoparticles within an alginate poly(vinyl alcohol) hydrogel. The zone loaded with hydroxyapatite nanoparticles generated subchondral bone tissue, while the chondroitin sulfate particles induced cartilage) tissue formation, leading to production of an integrated osteochondral construct [121]. Stevens and colleagues proposed various approaches to generate biomolecular gradients that can be exploited to produce osteochondral hydrogel constructs. In a first study, superparamagnetic nanoparticles were surface-functionalized with heparin, which acted as a reservoir for bone morphogenetic protein 2 [122]. These loaded nanoparticles were incorporated with hydrogel precursors, and the resulting solution was subjected to a magnetic field during the hydrogel cross-linking step. The process generated biomolecular gradients within the hydrogel (Fig. 5.4). In a second study, buoyancy was used to drive the formation of gradients with different types of nanoparticles in a range of hydrogel base materials [123].

5.5 Nanoparticles for Drug Delivery

Many patients with cartilage and joint ailments exhibit advanced signs of articular cartilage degeneration, or substantial injuries to their articular surface that are deemed to be at high risk of degeneration. The recommended course of action is in these cases is typically a surgical intervention. Other patients present early signs of degeneration for which a more conservative approach is favored. For these patients, a number of non-pharmaceutical and pharmaceutical options are available; however, as detailed previously, these therapeutics are aimed at managing symptoms and DMOAD are still an unmet need. Issues with the bioavailability of drugs within the joint space

are increasingly recognized as an important factor in explaining the absence of safe and efficacious DMOAD despite intense efforts in the field. Because of the avascular nature of articular cartilage, the target tissue for many DMOAD candidates, local administration of drugs has been favoured over systemic delivery strategies. Indeed, intra-articular injection provides the opportunity to bypass barriers to drug transport across vascular walls, as well as the ECM of the synovial membrane, and into the synovial fluid. As such, intra-articular injections have been associated with increased local bioavailability for a given administered drug dosage, reduced systemic exposure and thus decreased off-target effects. However, these require administration by practitioners, making this drug delivery strategy costlier and logistically more complex than self-administration strategies, especially for chronic conditions such as arthritis, which require sustained treatment over a period of years to decades. Furthermore, synovial fluid turnover is rapid and injected molecules are typically removed from the intra-articular space via lymphatic drainage in a manner of hours, such that maintaining drug levels within their therapeutic window in the joint is often impractical in clinical settings. Efforts have consequently centered on the development of strategies to increase the retention time of therapeutics within the synovial capsule following intra-articular injection, notably with injectable hydrogels, microcarriers, and nanoparticles. Nanoparticles offer a unique opportunity for drug delivery in the joint as demonstrated by Hubbell and colleagues, who proposed using the articular cartilage matrix as a reservoir for therapeutic molecules and developed nanoparticles that were small enough to penetrate the small pores of articular cartilage and accumulate in its ECM, as well as intracellularly [124]. The authors functionalized the nanoparticles with a short type II collagen-binding peptide that had been identified via phage display to achieve prolonged retention in the cartilage. Since this early effort, a broad range of nanocarriers have been proposed, including cationic and polyelectrolyte nanoparticles, which have exploited the polyanionic nature of the proteoglycan compartments of cartilage to achieve important penetration depths [125].

5.6 Concluding Remarks

Nanomaterials exhibit a host of unique properties due to the increased relative contribution of surface molecules in relation to those composing the bulk material. Furthermore, there is an increased appreciation of the importance of tissue organization at the nanometer scale for cell and tissue functions. These factors have found many applications in tissue engineering and efforts to repair or regenerate articular cartilage are no exception. However, the incorporation of nanomaterials for the regeneration of articular cartilage remains an emerging strategy. A number of techniques that have been thoroughly investigated in other tissue systems have yet to be explored in-depth for articular cartilage. Some of these areas have been highlighted in this chapter. As this field continues to mature, nanomaterial cartilage tissue engineering will undoubtedly help deliver a range of therapeutic solutions to address

joint ailments for a broad spectrum of patients and conditions, from improved early interventions to slow the progress of the disease to the development of implantable materials to resurface damaged and diseased joints.

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References

1. Buckwalter JA, Mankin HJ. Articular cartilage: tissue design and chondrocyte-matrix interactions. Instr Course Lect. 1998;47:477–86.
2. Sophia Fox AJ, Bedi A, Rodeo SA. The basic science of articular cartilage: structure, composition, and function. Sports Health. 2009;1(6):461–8.
3. Poole AR, Kojima T, Yasuda T, Mwale F, Kobayashi M, Laverty S. Composition and structure of articular cartilage: a template for tissue repair. Clin Orthop Relat Res. 2001;(391 Suppl):S26–33.
4. Akkiraju H, Nohe A. Role of chondrocytes in cartilage formation, progression of osteoarthritis and cartilage regeneration. J Dev Biol. 2015;3(4):177–92.
5. Goldring MB, Goldring SR. Osteoarthritis. J Cell Physiol. 2007;213(3):626–34.
6. Lorenz H, Richter W. Osteoarthritis: cellular and molecular changes in degenerating cartilage. Prog Histochem Cytochem. 2006;40(3):135–63.
7. Plotnikoff R, Karunamuni N, Lytvyak E, Penfold C, Schopflocher D, Imayama I, Johnson ST, Raine K. Osteoarthritis prevalence and modifiable factors: a population study. BMC Public Health. 2015;15:1195.
8. Brandt KD, Radin EL, Dieppe PA, van de Putte L. Yet more evidence that osteoarthritis is not a cartilage disease. Ann Rheum Dis. 2006;65(10):1261–4.
9. Scanzello CR, Goldring SR. The role of synovitis in osteoarthritis pathogenesis. Bone. 2012;51(2):249–57.
10. Sharma AR, Jagga S, Lee SS, Nam JS. Interplay between cartilage and subchondral bone contributing to pathogenesis of osteoarthritis. Int J Mol Sci. 2013;14(10):19805–30.
11. Fernandes JC, Martel-Pelletier J, Pelletier JP. The role of cytokines in osteoarthritis pathophysiology. Biorheology. 2002;39(1–2):237–46.
12. Hunter W. Of the structure and diseases of articu-lating cartilages. Philos Trans. 1743;42(470):514–21.
13. Clouet J, Vinatier C, Merceron C, Pot-vaucel M, Maugars Y, Weiss P, Grimandi G, Guicheux J. From osteoarthritis treatments to future regenerative therapies for cartilage. Drug Discov Today. 2009;14(19–20):913–25.
14. Wang CT, Lin J, Chang CJ, Lin YT, Hou SM. Therapeutic effects of hyaluronic acid on osteoarthritis of the knee. A meta-analysis of randomized controlled trials. J Bone Joint Surg Am. 2004;86-A(3):538–45.
15. Hellio le Graverand MP, Clemmer RS, Redifer P, Brunell RM, Hayes CW, Brandt KD, Abramson SB, Manning PT, Miller CG, Vignon E. A 2-year randomised, double-blind, placebo-controlled, multicentre study of oral selective iNOS inhibitor, cindunistat (SD-6010), in patients with symptomatic osteoarthritis of the knee. Ann Rheum Dis. 2013;72(2):187–95.
16. Karsdal MA, Byrjalsen I, Alexandersen P, Bihlet A, Andersen JR, Riis BJ, Bay-Jensen AC, Christiansen C, investigators CC. Treatment of symptomatic knee osteoarthritis with oral salmon calcitonin: results from two phase 3 trials. Osteoarthr Cartilage. 2015;23(4):532–43.

17. Pavelka K, Bruyere O, Cooper C, Kanis JA, Leeb BF, Maheu E, Martel-Pelletier J, Monfort J, Pelletier JP, Rizzoli R and others. Diacerein: benefits, risks and place in the management of osteoarthritis. An opinion-based report from the ESCEO. Drugs Aging. 2016;33(2):75–85.
18. Reginster JY, Badurski J, Bellamy N, Bensen W, Chapurlat R, Chevalier X, Christiansen C, Genant H, Navarro F, Nasonov E and others. Efficacy and safety of strontium ranelate in the treatment of knee osteoarthritis: results of a double-blind, randomised placebo-controlled trial. Ann Rheum Dis. 2013;72(2):179–86.
19. Yu SP, Hunter DJ. Intra-articular therapies for osteoarthritis. Expert Opin Pharmacother. 2016;17(15):2057–71.
20. Bauer TW, Schils J. The pathology of total joint arthroplasty. I. Mechanisms of implant fixation. Skeletal Radiol. 1999;28(8):423–32.
21. Bauer TW, Schils J. The pathology of total joint arthroplasty. II. Mechanisms of implant failure. Skeletal Radiol. 1999;28(9):483–97.
22. Hangody L, Kish G, Karpati Z, Szerb I, Udvarhelyi I. Arthroscopic autogenous osteochondral mosaicplasty for the treatment of femoral condylar articular defects. A preliminary report. Knee Surg Sports Traumatol Arthrosc. 1997;5(4):262–7.
23. Szerb I, Hangody L, Duska Z, Kaposi NP. Mosaicplasty: long-term follow-up. Bull Hosp Jt Dis. 2005;63(1–2):54–62.
24. Bartha L, Vajda A, Duska Z, Rahmeh H, Hangody L. Autologous osteochondral mosaicplasty grafting. J Orthop Sports Phys Ther. 2006;36(10):739–50.
25. Steadman JR, Rodkey WG, Rodrigo JJ. Microfracture: surgical technique and rehabilitation to treat chondral defects. Clin Orthop Relat Res. 2001;(391 Suppl):S362–9.
26. Shapiro F, Koide S, Glimcher MJ. Cell origin and differentiation in the repair of full-thickness defects of articular cartilage. J Bone Joint Surg Am. 1993;75(4):532–53.
27. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med. 1994;331(14):889–95.
28. Brittberg M, Peterson L, Sjogren-Jansson E, Tallheden T, Lindahl A. Articular cartilage engineering with autologous chondrocyte transplantation. A review of recent developments. J Bone Joint Surg Am. 2003;85-A(Suppl 3):109–15.
29. Peterson L, Vasiliadis HS, Brittberg M, Lindahl A. Autologous chondrocyte implantation: a long-term follow-up. Am J Sports Med. 2010;38(6):1117–24.
30. Vinatier C, Guicheux J. Cartilage tissue engineering: from biomaterials and stem cells to osteoarthritis treatments. Ann Phys Rehabil Med. 2016;59(3):139–44.
31. Cohen ZA, McCarthy DM, Kwak SD, Legrand P, Fogarasi F, Ciaccio EJ, Ateshian GA. Knee cartilage topography, thickness, and contact areas from MRI: in-vitro calibration and in-vivo measurements. Osteoarthr Cartilage. 1999;7(1):95–109.
32. Pollock J, O'Toole RV, Nowicki SD, Eglseder WA. Articular cartilage thickness at the distal radius: a cadaveric study. J Hand Surg Am. 2013;38(8):1477–81. Discussion 1482-3.
33. Bergholt MS, St-Pierre JP, Offeddu GS, Parmar PA, Albro MB, Puetzer JL, Oyen ML, Stevens MM. Raman spectroscopy reveals new insights into the zonal organization of native and tissue-engineered articular cartilage. ACS Cent Sci. 2016;2(12):885–95.
34. Jeffery AK, Blunn GW, Archer CW, Bentley G. Three-dimensional collagen architecture in bovine articular cartilage. J Bone Joint Surg Br. 1991;73(5):795–801.
35. Fujioka R, Aoyama T, Takakuwa T. The layered structure of the articular surface. Osteoarthr Cartilage. 2013;21(8):1092–8.
36. MacConaill MA. The movements of bones and joints; fundamental principles with particular reference to rotation movement. J Bone Joint Surg Br. 1948;30B(2):322–6.
37. Spitters TW, Leijten JC, Deus FD, Costa IB, van Apeldoorn AA, van Blitterswijk CA, Karperien M. A dual flow bioreactor with controlled mechanical stimulation for cartilage tissue engineering. Tissue Eng Part C Methods. 2013;19(10):774–83.
38. Zhou S, Cui Z, Urban JP. Factors influencing the oxygen concentration gradient from the synovial surface of articular cartilage to the cartilage-bone interface: a modeling study. Arthritis Rheum. 2004;50(12):3915–24.

39. Coates EE, Fisher JP. Phenotypic variations in chondrocyte subpopulations and their response to in vitro culture and external stimuli. Ann Biomed Eng. 2010;38(11):3371–88.
40. Vanden Berg-Foels WS, Scipioni L, Huynh C, Wen X. Helium ion microscopy for high-resolution visualization of the articular cartilage collagen network. J Microsc. 2012;246(2):168–76.
41. Buckwalter JA, Mankin HJ, Grodzinsky AJ. Articular cartilage and osteoarthritis. Instr Course Lect. 2005;54:465–80.
42. Wilusz RE, Sanchez-Adams J, Guilak F. The structure and function of the pericellular matrix of articular cartilage. Matrix Biol. 2014;39:25–32.
43. Benninghoff A. Form und Bau der Gelenkknorpel in ihren Beziehungen zur Funktion. Der Aufbau des Gelenkknorpels in seinen Beziehungen zur Funktion. Zeitschriftfar Zellforschung und mikroskopische Anatomie. 1925;2(5):783–862.
44. Guilak F, Nims RJ, Dicks A, Wu CL, Meulenbelt I. Osteoarthritis as a disease of the cartilage pericellular matrix. Matrix Biol. 2018;71–72:40–50.
45. Hall BK, Newman S. Cartilage: molecular aspects. Boca Raton, FL: CRC Press; 1991. p. 268.
46. Weiss C. Ultrastructural characteristics of osteoarthritis. Fed Proc. 1973;32(4):1459–66.
47. Poole AR, Pidoux I, Reiner A, Rosenberg L. An immunoelectron microscope study of the organization of proteoglycan monomer, link protein, and collagen in the matrix of articular cartilage. J Cell Biol. 1982;93(3):921–37.
48. Comper WD, Williams RP. Hydrodynamics of concentrated proteoglycan solutions. J Biol Chem. 1987;262(28):13464–71.
49. Mayan MD, Gago-Fuentes R, Carpintero-Fernandez P, Fernandez-Puente P, Filgueira-Fernandez P, Goyanes N, Valiunas V, Brink PR, Goldberg GS, Blanco FJ. Articular chondrocyte network mediated by gap junctions: role in metabolic cartilage homeostasis. Ann Rheum Dis. 2015;74(1):275–84.
50. Tkach M, Thery C. Communication by extracellular vesicles: where we are and where we need to go. Cell. 2016;164(6):1226–32.
51. Hashimoto S, Ochs RL, Komiya S, Lotz M. Linkage of chondrocyte apoptosis and cartilage degradation in human osteoarthritis. Arthritis Rheum. 1998;41(9):1632–8.
52. Golub EE. Role of matrix vesicles in biomineralization. Biochim Biophys Acta. 2009;1790(12):1592–8.
53. Miyaki S, Lotz MK. Extracellular vesicles in cartilage homeostasis and osteoarthritis. Curr Opin Rheumatol. 2018;30(1):129–35.
54. Langer R, Vacanti JP. Tissue engineering. Science. 1993;260(5110):920–6.
55. Williams DF. The biomaterials conundrum in tissue engineering. Tissue Eng Part A. 2014;20(7–8):1129–31.
56. Braghirolli DI, Steffens D, Pranke P. Electrospinning for regenerative medicine: a review of the main topics. Drug Discov Today. 2014;19(6):743–53.
57. Shin HJ, Lee CH, Cho IH, Kim YJ, Lee YJ, Kim IA, Park KD, Yui N, Shin JW. Electrospun PLGA nanofiber scaffolds for articular cartilage reconstruction: mechanical stability, degradation and cellular responses under mechanical stimulation in vitro. J Biomater Sci Polym Ed. 2006;17(1–2):103–19.
58. Li WJ, Tuli R, Okafor C, Derfoul A, Danielson KG, Hall DJ, Tuan RS. A three-dimensional nanofibrous scaffold for cartilage tissue engineering using human mesenchymal stem cells. Biomaterials. 2005;26(6):599–609.
59. Li WJ, Jiang YJ, Tuan RS. Chondrocyte phenotype in engineered fibrous matrix is regulated by fiber size. Tissue Eng. 2006;12(7):1775–85.
60. Wise JK, Yarin AL, Megaridis CM, Cho M. Chondrogenic differentiation of human mesenchymal stem cells on oriented nanofibrous scaffolds: engineering the superficial zone of articular cartilage. Tissue Eng Part A. 2009;15(4):913–21.
61. Bean AC, Tuan RS. Fiber diameter and seeding density influence chondrogenic differentiation of mesenchymal stem cells seeded on electrospun poly(epsilon-caprolactone) scaffolds. Biomed Mater. 2015;10(1):015018.

62. Shanmugasundaram S, Chaudhry H, Arinzeh TL. Microscale versus nanoscale scaffold architecture for mesenchymal stem cell chondrogenesis. Tissue Eng Part A. 2011;17(5–6):831–40.
63. Levorson EJ, Raman Sreerekha P, Chennazhi KP, Kasper FK, Nair SV, Mikos AG. Fabrication and characterization of multiscale electrospun scaffolds for cartilage regeneration. Biomed Mater. 2013;8(1):014103.
64. Jankovic B, Pelipenko J, Skarabot M, Musevic I, Kristl J. The design trend in tissue-engineering scaffolds based on nanomechanical properties of individual electrospun nanofibers. Int J Pharm. 2013;455(1–2):338–47.
65. Li WJ, Mauck RL, Cooper JA, Yuan X, Tuan RS. Engineering controllable anisotropy in electrospun biodegradable nanofibrous scaffolds for musculoskeletal tissue engineering. J Biomech. 2007;40(8):1686–93.
66. Shafiee A, Seyedjafari E, Sadat Taherzadeh E, Dinarvand P, Soleimani M, Ai J. Enhanced chondrogenesis of human nasal septum derived progenitors on nanofibrous scaffolds. Mater Sci Eng C Mater Biol Appl. 2014;40:445–54.
67. Owida HA, Yang R, Cen L, Kuiper NJ, Yang Y. Induction of zonal-specific cellular morphology and matrix synthesis for biomimetic cartilage regeneration using hybrid scaffolds. J R Soc Interface. 2018;15(143).
68. Schneider T, Kohl B, Sauter T, Kratz K, Lendlein A, Ertel W, Schulze-Tanzil G. Influence of fiber orientation in electrospun polymer scaffolds on viability, adhesion and differentiation of articular chondrocytes. Clin Hemorheol Microcirc. 2012;52(2–4):325–36.
69. Li D, Wang YL, Xia YN. Electrospinning of polymeric and ceramic nanofibers as uniaxially aligned arrays. Nano Lett. 2003;3(8):1167–71.
70. Kim GH. Electrospun PCL nanofibers with anisotropic mechanical properties as a biomedical scaffold. Biomed Mater. 2008;3(2).
71. Fisher MB, Henning EA, Soegaard N, Esterhai JL, Mauck RL. Organized nanofibrous scaffolds that mimic the macroscopic and microscopic architecture of the knee meniscus. Acta Biomater. 2013;9(1):4496–504.
72. Thorvaldsson A, Stenhamre H, Gatenholm P, Walkenstrom P. Electrospinning of highly porous scaffolds for cartilage regeneration. Biomacromol. 2008;9(3):1044–9.
73. Baker BM, Shah RP, Silverstein AM, Esterhai JL, Burdick JA, Mauck RL. Sacrificial nanofibrous composites provide instruction without impediment and enable functional tissue formation. Proc Natl Acad Sci USA. 2012;109(35):14176–81.
74. Chen HL, Malheiro ADFB, van Blitterswijk C, Mota C, Wieringa PA, Moroni L. Direct writing electrospinning of scaffolds with multidimensional fiber architecture for hierarchical tissue engineering. ACS Appl Mater Inter. 2017;9(44):38187–200.
75. Ma PX, Zhang R. Synthetic nano-scale fibrous extracellular matrix. J Biomed Mater Res. 1999;46(1):60–72.
76. Hu J, Feng K, Liu X, Ma PX. Chondrogenic and osteogenic differentiations of human bone marrow-derived mesenchymal stem cells on a nanofibrous scaffold with designed pore network. Biomaterials. 2009;30(28):5061–7.
77. Gupte MJ, Swanson WB, Hu J, Jin X, Ma H, Zhang Z, Liu Z, Feng K, Feng G, Xiao G and others. Pore size directs bone marrow stromal cell fate and tissue regeneration in nanofibrous macroporous scaffolds by mediating vascularization. Acta Biomater. 2018;82:1–11.
78. He L, Liu B, Xipeng G, Xie G, Liao S, Quan D, Cai D, Lu J, Ramakrishna S. Microstructure and properties of nano-fibrous PCL-b-PLLA scaffolds for cartilage tissue engineering. Eur Cell Mater. 2009;18:63–74.
79. Prasopthum A, Shakesheff KM, Yang J. Direct three-dimensional printing of polymeric scaffolds with nanofibrous topography. Biofabrication. 2018;10(2):025002.
80. Mahapatra C, Kim JJ, Lee JH, Jin GZ, Knowles JC, Kim HW. Differential chondro- and osteo-stimulation in three-dimensional porous scaffolds with different topological surfaces provides a design strategy for biphasic osteochondral engineering. J Tissue Eng. 2019;10:2041731419826433.
81. Zhang S. Fabrication of novel biomaterials through molecular self-assembly. Nat Biotechnol. 2003;21(10):1171–8.

82. Lim EH, Sardinha JP, Myers S. Nanotechnology biomimetic cartilage regenerative scaffolds. Arch Plast Surg. 2014;41(3):231–40.
83. Shah RN, Shah NA, Del Rosario Lim MM, Hsieh C, Nuber G, Stupp SI. Supramolecular design of self-assembling nanofibers for cartilage regeneration. Proc Natl Acad Sci USA. 2010;107(8):3293–8.
84. Sun X, Yin H, Wang Y, Lu J, Shen X, Lu C, Tang H, Meng H, Yang S, Yu W and others. In situ articular cartilage regeneration through endogenous reparative cell homing using a functional bone marrow-specific scaffolding system. ACS Appl Mater Inter. 2018;10(45):38715–28.
85. Childs A, Hemraz UD, Castro NJ, Fenniri H, Zhang LG. Novel biologically-inspired rosette nanotube PLLA scaffolds for improving human mesenchymal stem cell chondrogenic differentiation. Biomed Mater. 2013;8(6):065003.
86. Park GE, Pattison MA, Park K, Webster TJ. Accelerated chondrocyte functions on NaOH-treated PLGA scaffolds. Biomaterials. 2005;26(16):3075–82.
87. Balasundaram G, Storey DM, Webster TJ. Novel nano-rough polymers for cartilage tissue engineering. Int J Nanomed. 2014;9:1845–53.
88. Wu YN, Law JB, He AY, Low HY, Hui JH, Lim CT, Yang Z, Lee EH. Substrate topography determines the fate of chondrogenesis from human mesenchymal stem cells resulting in specific cartilage phenotype formation. Nanomedicine. 2014;10(7):1507–16.
89. Cohen NP, Foster RJ, Mow VC. Composition and dynamics of articular cartilage: structure, function, and maintaining healthy state. J Orthop Sports Phys Ther. 1998;28(4):203–15.
90. Chahine NO, Collette NM, Thomas CB, Genetos DC, Loots GG. Nanocomposite scaffold for chondrocyte growth and cartilage tissue engineering: effects of carbon nanotube surface functionalization. Tissue Eng Part A. 2014;20(17–18):2305–15.
91. Boyer C, Figueiredo L, Pace R, Lesoeur J, Rouillon T, Visage CL, Tassin JF, Weiss P, Guicheux J, Rethore G. Laponite nanoparticle-associated silated hydroxypropylmethyl cellulose as an injectable reinforced interpenetrating network hydrogel for cartilage tissue engineering. Acta Biomater. 2018;65:112–22.
92. Chen J, An R, Han L, Wang X, Zhang Y, Shi L, Ran R. Tough hydrophobic association hydrogels with self-healing and reforming capabilities achieved by polymeric core-shell nanoparticles. Mater Sci Eng C Mater Biol Appl. 2019;99:460–7.
93. Naseri N, Deepa B, Mathew AP, Oksman K, Girandon L. Nanocellulose-based Interpenetrating Polymer Network (IPN) hydrogels for cartilage applications. Biomacromol. 2016;17(11):3714–23.
94. Wang C, Shen H, Tian Y, Xie Y, Li A, Ji L, Niu Z, Wu D, Qiu D. Bioactive nanoparticle-gelatin composite scaffold with mechanical performance comparable to cancellous bones. ACS Appl Mater Inter. 2014;6(15):13061–8.
95. Bellucci G, Seedhom BB. Mechanical behaviour of articular cartilage under tensile cyclic load. Rheumatology (Oxford). 2001;40(12):1337–45.
96. Racunica TL, Teichtahl AJ, Wang Y, Wluka AE, English DR, Giles GG, O'Sullivan R, Cicuttini FM. Effect of physical activity on articular knee joint structures in community-based adults. Arthritis Rheum. 2007;57(7):1261–8.
97. Andriacchi TP, Favre J, Erhart-Hledik JC, Chu CR. A systems view of risk factors for knee osteoarthritis reveals insights into the pathogenesis of the disease. Ann Biomed Eng. 2015;43(2):376–87.
98. Sanchez-Adams J, Leddy HA, McNulty AL, O'Conor CJ, Guilak F. The mechanobiology of articular cartilage: bearing the burden of osteoarthritis. Curr Rheumatol Rep. 2014;16(10):451.
99. Son B, Kim HD, Kim M, Kim JA, Lee J, Shin H, Hwang NS, Park TH. Physical stimuli-induced chondrogenic differentiation of mesenchymal stem cells using magnetic nanoparticles. Adv Healthc Mater. 2015;4(9):1339–47.
100. Zhang N, Lock J, Sallee A, Liu H. Magnetic nanocomposite hydrogel for potential cartilage tissue engineering: synthesis, characterization, and cytocompatibility with bone marrow derived mesenchymal stem cells. ACS Appl Mater Inter. 2015;7(37):20987–98.
101. Brady MA, Talvard L, Vella A, Ethier CR. Bio-inspired design of a magnetically active trilayered scaffold for cartilage tissue engineering. J Tissue Eng Regen Med. 2017;11(4):1298–302.

102. Rotherham M, El Haj AJ. Remote activation of the Wnt/beta-catenin signalling pathway using functionalised magnetic particles. PLoS ONE. 2015;10(3):e0121761.
103. Kanczler JM, Sura HS, Magnay J, Green D, Oreffo RO, Dobson JP, El Haj AJ. Controlled differentiation of human bone marrow stromal cells using magnetic nanoparticle technology. Tissue Eng Part A. 2010;16(10):3241–50.
104. Stolfa S, Skorvanek M, Stolfa P, Rosocha J, Vasko G, Sabo J. Effects of static magnetic field and pulsed electromagnetic field on viability of human chondrocytes in vitro. Physiol Res. 2007;56(Suppl 1):S45–9.
105. Amin HD, Brady MA, St-Pierre JP, Stevens MM, Overby DR, Ethier CR. Stimulation of chondrogenic differentiation of adult human bone marrow-derived stromal cells by a moderate-strength static magnetic field. Tissue Eng Part A. 2014;20(11–12):1612–20.
106. Go G, Han J, Zhen J, Zheng S, Yoo A, Jeon MJ, Park JO, Park S. A magnetically actuated microscaffold containing mesenchymal stem cells for articular cartilage repair. Adv Healthc Mater. 2017;6(13).
107. Urban JP, Hall AC, Gehl KA. Regulation of matrix synthesis rates by the ionic and osmotic environment of articular chondrocytes. J Cell Physiol. 1993;154(2):262–70.
108. Mobasheri A, Matta C, Uzieliene I, Budd E, Martin-Vasallo P, Bernotiene E. The chondrocyte channelome: a narrative review. Joint Bone Spine. 2019;86(1):29–35.
109. Xu J, Wang W, Clark CC, Brighton CT. Signal transduction in electrically stimulated articular chondrocytes involves translocation of extracellular calcium through voltage-gated channels. Osteoarthr Cartilage. 2009;17(3):397–405.
110. Khang D, Lu J, Yao C, Haberstroh KM, Webster TJ. The role of nanometer and sub-micron surface features on vascular and bone cell adhesion on titanium. Biomaterials. 2008;29(8):970–83.
111. Park JS, Yang HN, Woo DG, Chung HM, Park KH. In vitro and in vivo chondrogenesis of rabbit bone marrow-derived stromal cells in fibrin matrix mixed with growth factor loaded in nanoparticles. Tissue Eng Part A. 2009;15(8):2163–75.
112. Santo VE, Popa EG, Mano JF, Gomes ME, Reis RL. Natural assembly of platelet lysate-loaded nanocarriers into enriched 3D hydrogels for cartilage regeneration. Acta Biomater. 2015;19:56–65.
113. Lu H, Lv L, Dai Y, Wu G, Zhao H, Zhang F. Porous chitosan scaffolds with embedded hyaluronic acid/chitosan/plasmid-DNA nanoparticles encoding TGF-beta1 induce DNA controlled release, transfected chondrocytes, and promoted cell proliferation. PLoS ONE. 2013;8(7):e69950.
114. Mirza EH, Pan-Pan C, Wan Ibrahim WM, Djordjevic I, Pingguan-Murphy B. Chondroprotective effect of zinc oxide nanoparticles in conjunction with hypoxia on bovine cartilage-matrix synthesis. J Biomed Mater Res A. 2015;103(11):3554–63.
115. Albro MB, Nims RJ, Durney KM, Cigan AD, Shim JJ, Vunjak-Novakovic G, Hung CT, Ateshian GA. Heterogeneous engineered cartilage growth results from gradients of media-supplemented active TGF-beta and is ameliorated by the alternative supplementation of latent TGF-beta. Biomaterials. 2016;77:173–85.
116. Albro MB, Bergholt MS, St-Pierre JP, Vinals Guitart A, Zlotnick HM, Evita EG, Stevens MM. Raman spectroscopic imaging for quantification of depth-dependent and local heterogeneities in native and engineered cartilage. NPJ Regen Med. 2018;3:3.
117. Gibson M, Beachley V, Coburn J, Bandinelli PA, Mao HQ, Elisseeff J. Tissue extracellular matrix nanoparticle presentation in electrospun nanofibers. Biomed Res Int. 2014;2014:469120.
118. Li Y, Jiang T, Zheng L, Zhao J. Osteogenic differentiation of mesenchymal stem cells (MSCs) induced by three calcium phosphate ceramic (CaP) powders: a comparative study. Mater Sci Eng C Mater Biol Appl. 2017;80:296–300.
119. Kon E, Delcogliano M, Filardo G, Pressato D, Busacca M, Grigolo B, Desando G, Marcacci M. A novel nano-composite multi-layered biomaterial for treatment of osteochondral lesions: technique note and an early stability pilot clinical trial. Injury. 2010;41(7):693–701.

120. Amadori S, Torricelli P, Panzavolta S, Parrilli A, Fini M, Bigi A. Multi-layered scaffolds for osteochondral tissue engineering: in vitro response of co-cultured human mesenchymal stem cells. Macromol Biosci. 2015;15(11):1535–45.
121. Radhakrishnan J, Manigandan A, Chinnaswamy P, Subramanian A, Sethuraman S. Gradient nano-engineered in situ forming composite hydrogel for osteochondral regeneration. Biomaterials. 2018;162:82–98.
122. Li C, Armstrong JP, Pence IJ, Kit-Anan W, Puetzer JL, Correia Carreira S, Moore AC, Stevens MM. Glycosylated superparamagnetic nanoparticle gradients for osteochondral tissue engineering. Biomaterials. 2018;176:24–33.
123. Li C, Ouyang L, Pence IJ, Moore AC, Lin Y, Winter CW, Armstrong JPK, Stevens MM. Buoyancy-driven gradients for biomaterial fabrication and tissue engineering. Adv Mater. 2019:e1900291.
124. Rothenfluh DA, Bermudez H, O'Neil CP, Hubbell JA. Biofunctional polymer nanoparticles for intra-articular targeting and retention in cartilage. Nat Mater. 2008;7(3):248–54.
125. Bajpayee AG, Grodzinsky AJ. Cartilage-targeting drug delivery: can electrostatic interactions help? Nat Rev Rheumatol. 2017;13(3):183–93.