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## The influence of tissue microenvironment on stem cell–based cartilage repair

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### Abstract

Mesenchymal stem/progenitor cells and induced pluripotent stem cells have become viable cell sources for prospective cell-based cartilage engineering and tissue repair. The development and function of stem cells are influenced by the tissue microenvironment. Specifically, the local tissue microenvironment can dictate how stem cells integrate into the existing tissue matrix and how successfully they can restore function to the damaged area in question. This review focuses on the microenvironmental features of articular cartilage and how they influence stem cell–based cartilage tissue repair. Also discussed are current tissue-engineering strategies used in combination with cell-based therapies, all of which are designed to mimic the natural properties of cartilage tissue in order to achieve a better healing response.

### Keywords

cartilage repair; stem cells; progenitor; chondroprogenitor; microenvironment; articular cartilage

### Introduction

Articular cartilage is aneural and lacks vasculature—ideal for minimizing the occurrence of inflammation and preventing the sensation of pain during joint loading. However, these conditions are not conducive to tissue repair because of the lack of a systemic blood supply. As such, articular cartilage heals poorly and there is much focus on finding effective methodologies to restore its structure and function following cartilage injury. Given its complex architecture, articular cartilage is challenging to reengineer. Current cell-based cartilage repair strategies utilize both mature and progenitor/stem cells. For instance, autologous chondrocyte implantation (ACI) implants presumably healthy mature chondrocytes from non-load-bearing regions of a joint into a cartilage defect.<sup>1,2</sup> On the other hand, for smaller cartilage defects, microfracture surgery is performed to drill through the

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### Conflicts of interest

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subchondral bone in order to create a fibrin clot that facilitates the migration of bone marrow–derived mesenchymal stem cells (BM-MSCs) into the cartilage to promote healing.<sup>3</sup> Both methods are conventionally used for cartilage repair but have been reported to result in the formation of fibrous or hypertrophic repair tissues,<sup>4–6</sup> which can deteriorate over time because of poor mechanical properties in comparison to hyaline cartilage. Indeed, while there is certainly room to improve the cell sources currently being considered for cartilage repair strategies, the aforementioned findings strongly suggest that the local tissue microenvironment ultimately plays the most crucial role in regulating the development and function of these cells. The questions arise as to what happens to cells that are implanted into a highly inflammatory or osteogenic microenvironment, and whether they will develop into fibrocartilage or hypertrophic cartilage, respectively. As such, it is important to recognize that regulating the tissue microenvironment may be another point of control that can be used to dictate the cartilage repair response.

## The functional simplicity and biological complexity of articular cartilage

### Development

Articular cartilage is a product of long bone formation, which occurs through the highly regulated process of endochondral ossification (Fig. 1). During early embryonic limb development, cells of the mesenchymal lineage condense to form the limb bud. These mesenchymal chondrocyte precursors will highly express SOX-5/SOX-6/SOX-9 and undergo chondrogenesis-producing types II/IX/XI collagen and aggrecan, all of which are components of the cartilage extracellular matrix (ECM).<sup>7–9</sup> These cells proliferate longitudinally from proximal to distal end, as they are coordinated by Indian hedgehog (IHH) and Wnt signaling to form a cartilage template that will eventually be replaced by bone.<sup>10,11</sup> Articular cartilage represents the remains of the cartilage template from which the fully formed long bone developed.

### Function and organization

The anatomical function of articular cartilage is to bare load and minimize friction during joint movement, which is mostly reflected in its structural heterogeneity, organization, and cellularity. Compared to many other tissues, it has a high water content and consists mainly of an extracellular matrix that is produced by a sparse population of chondrocytes<sup>12</sup>—two features that contribute to the compressive resistance or characteristic “bounce” exhibited by this tissue. Although outwardly appearing as a relatively simple tissue, the architecture of articular cartilage is, in fact, elegantly complex. Articular cartilage of the knee and hip are approximately 1.5–2.55 mm and 1.35–2 mm thick, respectively.<sup>13</sup> Articular cartilage is also compartmentalized into several zones (superficial zone, middle zone, deep zone, and calcified cartilage),<sup>14</sup> and there is a distinct heterogeneity in both ECM and cellular organization between the zones. As such, each can be thought to have a different microenvironment and cellular organization that is best suited for its anatomical function.

For instance, the superficial zone makes up 10–20% of the full articular cartilage thickness in healthy adults and consists of an ECM that is organized in parallel to the articular surface to capitalize on the tensile strength of its network of collagen fibrils.<sup>12,15</sup> This is important

for preventing the tissue from tearing during physical stress. The articular surface has a higher cellularity than the rest of the cartilage and houses cells that produce a lubricating proteoglycan, known as lubricin (PRG4),<sup>16,17</sup> which minimizes surface friction generated by diarthrodial joint movement. In clear contrast to the superficial zone, the middle zone, which makes up 40–60% of the full cartilage thickness, shows a columnar organization of cells and collagen fibrils of larger diameter.<sup>18</sup> While the superficial zone mostly consists of some proteoglycans among collagen types II/IX, the middle zone has a relatively larger proteoglycan content, which helps with water retention. The large area and composition of the middle zone is well adapted to its primary function of providing compressive resistance during joint loading. Furthermore, the deep zone represents approximately 30% of the entire cartilage thickness and has essentially the same function as the middle zone but provides even more compressive resistance, as it has the densest ECM of the aforementioned zones.<sup>19</sup> Deep zone chondrocytes are large, hypertrophic, and sparsely distributed. This zone has the largest collagen fibrils in articular cartilage tissue, organized longitudinally, and completely perpendicular to the articular surface to maximize compressive resistance against mechanical stress. Unlike in the other zones, the deep zone chondrocytes produce type X collagen, which is mostly found in the calcified cartilage below the tidemark. The calcified cartilage is a transitory sector of tissue that separates cartilage from the subchondral bone tissue.

### Local stem/progenitor cells

In the last two decades, important discoveries have been made with respect to mesenchymal progenitor cells that can be isolated from adult mesenchymal tissues. The existence of these progenitor cells was first proposed by Hayes *et al.* on the basis of their observation that cartilage exhibits appositional growth during development.<sup>20</sup> Mesenchymal progenitor cells have been found in many areas of the joint, including cartilage, synovial lining, bone marrow, infrapatellar fat pad, meniscus, and even tendons,<sup>21–29</sup> and it has been proposed that they may be quiescent remnant cells from the developing limb bud mesenchyme.<sup>30</sup> Cartilage-derived mesenchymal progenitors make up a small percentage of all cells in the cartilage tissue<sup>31</sup> and reside side-by-side with the more abundant chondrocytes throughout cartilage; however, they are most abundantly found in the superficial zone, specifically in the articular surface.

Cartilage-derived mesenchymal progenitor cells differ from chondrocytes in many ways. For example, they have been reported to proliferate faster *in vitro* than mature articular chondrocytes and exhibit several common mesenchymal progenitor cell surface markers, including CD49e, CD90, CD105, CD166, and Notch1.<sup>22,23,32,33</sup> Specific subsets of progenitors found on the articular surface are also capable of homing/migrating to areas of tissue that have been impacted or damaged.<sup>34</sup> Most importantly, these cells are multipotent and have the capacity to differentiate in a chondrogenic microenvironment and form cartilage-like tissue.<sup>23</sup> The presence of these cells opens up new possibilities for cartilage tissue repair and, in the process, calls attention to the potential importance of the stem cell niche in the cartilage healing response.

## Changes to stem cell niche

### In injury

Sustained injury as well as chronic diseases, such as osteoarthritis (OA), can result in substantial changes to the architecture of articular cartilage tissue. As expected, these changes can significantly affect the stem cell niche, which is best described as the local tissue microenvironment of resident stem/progenitor cells. For instance, a direct cartilage injury that occurs at the articular surface extending into the neighboring middle zone will not only compromise its primary function of resisting compressive forces, but will also result in an acute inflammatory response that will begin changing the local tissue microenvironment. This inflammatory response to articular cartilage injury primarily involves the production of interleukins, in particular interleukin-1 (IL-1), IL-6, IL-17, and IL-18, as well as tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ).<sup>14,35,36</sup> The production of these cytokines is not exclusive to joint cartilage tissue; indeed, much of it comes from the neighboring joint synovium, which, unlike cartilage, is vascularized and allows circulating immune cells, such as macrophages, to enter the joint and further heighten the immune response. In addition to the acute immune response brought on by cartilage injury, it would also result in the immediate disruption and chronic breakdown of the pericellular matrix, leading to the release of collagen, hyaluronan, aggrecan, and fibronectin cleavage fragments.<sup>14</sup> Such neopeptides have previously been shown to perpetuate cartilage breakdown by promoting the production of collagenases, aggrecanases, reactive oxygen species (i.e., H<sub>2</sub>O<sub>2</sub>, hydroxyl radicals), and nitric oxide (NO).<sup>37,38</sup>

### In osteoarthritis

Many of the same conditions persist in OA cartilage. In addition to the active breakdown of the ECM by the matrix metalloproteinase (MMP) family of collagenases and ADAMTS family of aggrecanases, COX-2 and prostaglandins are actively produced by OA chondrocytes.<sup>36</sup> Additionally, due to chronic inflammation and tissue wear-and-tear, higher grades of OA present with complete erosion of the articular surface. Furthermore, in contrast to a cartilage injury, OA is more defined by its osteogenic features. Chondrocyte hypertrophy,<sup>39</sup> osteophyte formation,<sup>40</sup> and changes to the subchondral bone and bone marrow<sup>41</sup> are hallmarks of OA, many of which influence the immediate tissue microenvironment of OA cartilage. The subchondral bone undergoes striking changes during OA pathogenesis. The bone begins to thicken and bone marrow edema can be clearly observed. The subchondral bone also becomes more permeable, and bone morphogenetic proteins (BMPs) and members of the transforming growth factor  $\beta$  (TGF- $\beta$ ) super family produced by osteoblasts potentially leak into cartilage, favoring terminal differentiation of chondrocytes and osteophyte formation.<sup>42</sup> Overall, such events promote a microenvironment that favors chondrocyte hypertrophy and osteogenesis (Fig. 2). Hypertrophic chondrocytes exhibit elevated RUNX2 expression resulting in the production of type X collagen, which accumulates at, or just above, the tidemark and becomes incorporated into the OA cartilage ECM. Increased integration of type X collagen into the cartilage ECM not only alters the pericellular microenvironment of local cell populations, but also alters the mechanical properties of the tissue itself by facilitating mineralization.<sup>43,44</sup>

During OA, there are also a greater number of mesenchymal progenitors that are present in the articular cartilage.<sup>21,32</sup> It is speculated that this increase in progenitor cell number could be due to the activation and proliferation of once dormant progenitor cells in the tissue as a last-resort attempt to recover from tissue erosion. It has also been speculated that the increasing permeability to the subchondral bone may allow the migration of bone marrow stromal cells to penetrate into the cartilage. In both scenarios, the inflammatory and hypertrophic microenvironment of OA cartilage may decrease the likelihood that these cells will undergo chondrogenesis properly to replenish the damaged/eroded cartilage tissue. In such a case, the increasing presence of progenitor cells, which are entrained by the microenvironment of OA cartilage, may not be sufficient to restore tissue homeostasis.

## Repairing damaged cartilage

### Considerations for stem cell–based cartilage repair

Despite its aforementioned limitations, the use of autologous chondrocytes for articular joint cartilage repair is clinically practiced. However, in patients who have OA or are in the early stages of OA development, the utility of using autologous chondrocytes for cartilage repair is not the best option since these cells may already exhibit pathogenic features that limit their ability to repopulate the defect and produce new and healthy cartilage tissue. In such situations, mesenchymal progenitor cells with chondrogenic potential may represent a desirable alternative cell type for use in autologous cell implantation.

Although chondrogenic progenitor cells are regarded as being biologically primed for chondrogenesis, they are not entirely lineage restricted, and, as such, they exhibit enough plasticity to differentiate along other mesenchymal cell lineages, namely, osteogenic and adipogenic lineages.<sup>23,45</sup> The local tissue stem cell niche is a critical feature that can dictate which path to maturation these cells will ultimately take. Similarly, when seeking to utilize stem cells in cell-based therapy for cartilage repair/regeneration, it is essential to consider the effects of the local tissue microenvironment into which these stem cells are being introduced. From a tissue-engineering perspective, there are two important factors to consider in this regard. First, a viable source of stem/progenitor cells that offer a biological repertoire that complements the desired path of differentiation is required. Second, one must consider how to provide the best stem cell niche for these cells to mature. There is currently a great need to develop regulatory strategies that can be used in conjunction with stem cells to promote the most desirable repair response. Such strategies include anything that will favorably alter the local stem cell niche, including the use of recombinant growth factors, implementation of artificial/biological scaffolds, and even the use of small interfering RNAs (siRNAs) to attenuate further damage.

### Cell sources

**Mesenchymal progenitor cells**—Mesenchymal progenitors are proposed to be a good fit for cartilage repair since they are self-renewing and exhibit high proliferative capability, in comparison to mature articular chondrocytes. Progenitor cell sources under consideration for use in cartilage defect repair include the bone marrow, joint fat pad, periosteum, synovium, and even non-weight-bearing regions of articular cartilage. BM-MSCs are the

most studied source of these cells that are currently clinically used for cartilage repair and have been found to be a good cell source for progenitor cell–based cartilage repair because of their relative abundance in the body and their ability to be easily induced into differentiating down the chondrogenic lineage in culture by simply treating with growth factors, such as TGF- $\beta$ 1. Indeed, there are many comparative studies where BM-MSCs are used as the gold standard to which lesser known progenitor cell sources are held. For example, BM-MSCs have been compared to adipose tissue–derived progenitor cells of the infrapatellar fat pad, and, remarkably, the cells from the infrapatellar fat pad of OA patients retain the same chondrogenic properties as those in nondiseased tissue.<sup>46</sup> Although adipose tissue is relatively expendable in the joint, making it a convenient source of cells for cell–based therapeutic approaches, there is also evidence suggesting that adipose-derived progenitors generally have reduced chondrogenic differentiation potential, relative to MSCs.<sup>47,48</sup>

Another suitable progenitor cell source that has been investigated is the periosteum,<sup>49,50</sup> which, like bone marrow, is considered to be a built-in reservoir of progenitor cells and mediates bone growth and fracture healing. These cells can nevertheless be induced into chondrogenesis under the correct culture condition and used in cartilage-tissue repair strategies.

Interestingly, the joint synovium contains progenitor cells that are reported to have even higher chondrogenic potential than those derived from adipose tissues, periosteum, and bone marrow.<sup>51</sup> It has been previously postulated that the increased chondrogenic potential of synovium-derived progenitors may be attributed to their close proximity to the articular cartilage stem cell niche.<sup>52</sup> This is likely true considering that articular cartilage–derived progenitors exhibit high chondrogenic potential.

A recent study by Jiang *et al.* demonstrated that mature chondrocytes can be induced into a progenitor cell–like state via low-glucose 2D-culture conditions that promote expression of the early MSC marker, CD146.<sup>53</sup> While these cells are similar to MSCs, they exhibited higher chondrogenic potential than MSCs. The authors used these cells to successfully repair the cartilage defects in 15 patients, strongly suggesting that the cartilage-derived progenitor cells from committed mature chondrocytes have future potential use for cartilage regenerative therapies.

OA cartilage was once considered an unlikely source of chondrogenic progenitor cells given the pathological characteristics of this tissue. However, it is now known that OA cartilage consists of a larger-than-normal pool of progenitor cells,<sup>22</sup> some of which are promising potential cell sources for tissue repair. It has been demonstrated that articular cartilage from late-stage OA patients contains a population of chondrogenic progenitors, in which inhibition of hypertrophic transcription factor RUNX2 results in elevated expression of the master chondrogenesis regulator SOX-9.<sup>54</sup>

**Induced pluripotent stem cells**—Induced pluripotent stem cells (iPSCs) have become a potentially viable cell source for cartilage tissue repair. These cells are mature adult cells, typically from the skin, which have been genetically reprogrammed into a pluripotent stem

cell state by transducing them with factors present in embryonic stem cells (ESCs)<sup>55</sup> and can be induced into differentiating along all three germ-line lineages. They can also be modified to become committed to a specific germline.<sup>56</sup> It has been confirmed that human ESCs can be induced into differentiating along the chondrogenic lineage using *in vitro* culture conditions that mimic cartilage microenvironmental conditions and endogenous growth factors.<sup>57,58</sup> However, the remarkable plasticity of iPSCs is simultaneously an advantage and a disadvantage when considering these cells as a viable cell source for tissue repair. Yamashita *et al.* recently demonstrated that suspension culture conditions can be used to generate chondrogenic cells from human iPSCs.<sup>59</sup> While some studies have reported that ESCs express little in the way of off-target cell markers,<sup>57</sup> other studies have reported that the use of iPSCs in cartilage defect repair can result in instances of immature teratoma formation as part of the repair tissue when the transplanted cells are not completely homologous with the genome of the host.<sup>60,61</sup> In this regard, in comparison to mesenchymal progenitors isolated from mature tissue sources, iPSCs must be regulated more tightly to ensure that they differentiate in a desirable fashion.

### Creating the best stem cell niche for cartilage repair

Given the important role of stem cells in maintaining cartilage homeostasis and the great potential of stem cells in cartilage repair, researchers have developed the use of natural extracellular matrix proteins, scaffolding, and combined growth factors for stem cell attachment, growth, and regulation of cell differentiation. In the following sections, we discuss how each of these factors combines with stem cells in improving cartilage regeneration. Table 1 lists these factors and their desirable qualities.

**Extracellular matrices**—Decellularized ECMs (dECMs) are used for cartilage repair because they have little to no cytotoxicity<sup>62</sup> and contain many of the natural structural components that modulate cell attachment, growth, and differentiation.<sup>63</sup> The dECM can be used as a scaffold that closely mimics the natural tissue matrix in which cells can reside and function. The successful use of the dECM for promoting cartilage repair embodies the importance of preserving the local tissue microenvironment for improving cellular function, as demonstrated by a recent study using the dECM in combination with adipose-derived stem cells to achieve a superior cartilage healing response, resulting in the production of repair tissue that closely resembles articular cartilage in molecular and biomechanical properties.<sup>64</sup> Using a canine model, a study by Yang *et al.* also confirmed that decellularized osteochondral scaffolds can successfully induce primary canine BM-MSCs to produce repair tissue with a stiffness (70.77% of normal cartilage) and glycosaminoglycan content (74.95% of normal cartilage) comparable to that of native cartilage.<sup>65</sup>

The idea behind using the dECM in this capacity is to preserve as much as possible the natural architecture of the tissue while “evicting” the resident cells in order to repopulate with cells that are more suitable to facilitate tissue repair. There are mainly two categories of the dECM: tissue-derived and cell-derived matrices. To obtain a dECM that retains its natural structural architecture, native cells are removed by physical and chemical means, such as exposure to freeze/thaw cycles and treatment with Triton X-100.<sup>64</sup> Tissue-derived matrices can be further divided into allografts and xenografts. Allograft dECMs are obtained

from a donor that is the same species as the host, whereas xenograft dECMs are obtained from a donor of a different species. Additionally, Cheng *et al.* have demonstrated that the cartilage tissue matrix can be mechanically homogenized and lyophilized to produce scaffolds that promote chondrogenic differentiation of adipose-derived stem cells.<sup>66</sup> Although the natural tissue architecture is not well preserved when using a lyophilized ECM to build a scaffold, this study makes it clear that the presence of the cartilage ECM alone can be sufficient to induce chondrogenesis.

Alternatively to using whole dECMs, individual ECM proteins can be used to accomplish many of the same functions. The best example of this is the use of collagen as the active component of sponges to promote adhesion and chondrogenesis of cells.<sup>67</sup> Collagen is biocompatible and its degradation products have low cytotoxicity. Type I collagen sponges have been used to deliver growth factors, such as insulin and fibroblast growth factor (FGF), which not only increases proteoglycan content, but also results in a repair response that produces new tissue that more closely resembles hyaline cartilage instead of fibrocartilage.<sup>67</sup> Likewise, the cartilage matrix protein matrilin-3 is shown to stimulate chondrogenesis of progenitor cells,<sup>68,69</sup> suggesting that it too carries potential for future development into a biologically active component of engineered matrices. Matrilin-3 is highly expressed during development by proliferating chondrocytes in the growth plate, and ATDC5 chondroprogenitor cell proliferation and differentiation in the presence of matrilin-3 demonstrate that specific matrix molecules can be used to regulate cell behavior.<sup>69</sup> While it is clear that matrilin-3 is not highly expressed in articular cartilage, there is evidence to suggest that its presence provides a chondrogenic microenvironment for progenitor cells, which promotes their differentiation along the chondrocyte lineage.<sup>68,69</sup> Matrilin-3 also has anti-inflammatory properties that inhibit several downstream targets of the IL-1 $\beta$  pathway;<sup>70</sup> a BMP-2 pathway antagonist has been shown to ultimately repress collagen X expression and chondrocyte hypertrophy.<sup>71</sup> All of these features contribute to future consideration of matrilins in cartilage tissue repair strategies, as they currently hold promise for helping to provide an optimal microenvironment that promotes chondrogenesis, while simultaneously inhibiting chondrocyte hypertrophy and terminal differentiation.

**Synthetic scaffolding**—As discussed above, cartilage is a connective tissue with a dense ECM, low cell density, and no vasculature. The first challenge in cartilage regeneration is creating an environment suitable for stem cell homing and growth. In this manner, cell growth scaffolding is critical. Synthetic scaffolding materials used for cartilage tissue repair and engineering are designed to be low in cytotoxicity and often emulate various features of the natural cartilage microenvironment in order to facilitate a better healing response from chondrocytes and progenitor/stem cells.

One of the most commonly used scaffolding is biodegradable polymers, which have advantages in the precise control of chemical composition, crystallinity, molecular weight, molecular weight distribution, as well as easily fabricated microstructure and macrostructure (including porosity).<sup>72,73</sup> However, since all polymers are covalently linked, biodegradation may be an issue. Some high-molecular weight polymers present a high cytotoxicity.<sup>74</sup> Therefore, polymers with biodegradable linkage or components are the favored scaffolding materials. For instance, polylactide-co-glycolide (PLGA) is a widely used, U.S. Food and



Drug Administration (FDA)-approved polymer for stem cell adhesion and growth in cartilage tissue engineering. PLGA was originally fabricated for biodegradable sutures in surgeries. Since then, its potential in many applications, such as medical devices, drug delivery, and tissue engineering, has attracted much attention. PLGA is the co-polymer of PGA (polyglycolide) and PLA (polylactide).<sup>73</sup> Its ester bonds can degrade via hydrolysis, and, subsequently, the long polymer chains gradually break into small-molecular weight pieces in the physiological environment, which are eventually cleared by circulation (the degradation mechanism is shown in Fig. 3). The hydrolysis of polymers is closely related to the molecular weight and degree of polymerization. Many factors can increase the degradation rate of a polymer, such as high water content, large surface area, small particle size, lower molecular weight, and higher surface area. The regeneration of cartilage is a slow process because of its avascular nature. Thus, the degradation rate of the polymers for cartilage repair should occur at an appropriate rate—neither too fast nor too slow. The optimal rate would complement and mimic as much as possible the natural low rate of turnover that is characteristic of cartilage ECM. Numerous studies have made an effort to control the degradation rate of the polymer scaffolding.<sup>75–77</sup> However, one of the complications associated with many hydrolysis polymers (e.g., PLGA) relates to the acidic degradation byproducts, which can lower the local tissue pH and result in cell and tissue necrosis.<sup>78</sup>

In addition to synthetic polymers, hydrogels extracted from natural materials are very popular scaffolding for cartilage repair. Hydrogels contain polymeric networks of branched molecules and are colloidal gels when water is the dispersion medium. Owing to their high water content, hydrogels present a degree of flexibility that is very similar to natural soft tissues, especially the polysaccharide family hydrogels, such as alginate and agarose, which have a similar chemical formula as glycosaminoglycans (one of the major components of natural cartilage ECM). This close similarity to glycosaminoglycans may account for the success that has been attributed to the use of alginate and agarose in cartilage defect repair applications. In addition, hydrogels exhibit high biocompatibility with cartilage stem cells, low cytotoxicity,<sup>79–81</sup> and do not undergo hydrolysis through ester bonds. Although they usually take longer to degrade than hydrolysis polymers (e.g., PLGA), they are suitable for cartilage, a slow regeneration tissue.<sup>82–84</sup> More importantly, they do not produce acidic byproducts during degradation, which greatly increase the potential for clinical applications. Both synthetic polymers and natural hydrogels can benefit from the recent development of nanotechnology to improve the surface and structure, as well as to lower inflammatory responses.<sup>84</sup> They can also improve select protein adsorption capability, which is important for mediating cell adhesion and function.<sup>85</sup> Furthermore, nanofiber hydrogels can mimic the collagen orientation and alignment of cartilage; the biomimetic architecture can promote chondrogenic differentiation of mesenchymal stem cells, improving cartilage regeneration.<sup>86,87</sup>

Most recently, newer developments in cell scaffolding involve bioinspired materials, which can be derived and engineered from either DNA/ RNA or protein/peptides and usually mimic the chemistry, structure, and assembly of natural biomolecules, presenting excellent biocompatibility and biodegradability. For example, rosette nanotubes are a class of biomimetic self-assembled supramolecular structures, for which the basic building blocks

are derived from guanine and cytosine DNA base pairs with the linkage of a lysine side chain.<sup>88</sup> Their units undergo a hierarchical process to form a six-membered supermacrocycle by the formation of 18 hydrogen bonds under physiological conditions; the rosettes then form a stable stack, with an inner channel that is 11 Å in diameter, on the basis of electrostatic force, base-stacking interactions, and hydrophobic effects. It was reported that such nanotubes have a similar size and morphology to collagen, enhanced protein absorption, and stem cell and chondrocyte functions.<sup>89</sup> For these reasons, rosette nanotubes have future potential for cartilage tissue engineering and repair. Interestingly, the structure of these bioinspired materials contains a hydrophobic core (aromatic rings) and a hydrophilic surface (lysine side chains), allowing their self-assembly to incorporate a variety of hydrophobic drugs into a stable water medium in order to improve cell differentiation and functions.<sup>90,91</sup> Other examples of bioinspired materials are those that are peptide based, including nanostructured biomaterials that are genetically selected and/or design peptides with specific binding to functional solids. They tailor their binding and assembly characteristics, develop bifunctional peptide/protein genetic constructs with both material binding and biological activity, and use these constructs as molecular synthesizers, erectors, and assemblers.<sup>92</sup> For example, a self-assembling peptide RADA16 (Ac-RADARADARADARADA-COHN2) was engineered to form a 3D scaffolding for cell growth.<sup>93</sup> When it was exposed to physiological salt conditions, the peptide formed a hydrogel due to the formation of ionic bonds and a hydrophobic interaction. The authors stated that the nanofiber structure resembles natural collagen, with a fiber diameter of 5–10 nm, and enables cell proliferation more than any other synthetic scaffold, such as PLGA.

In short, scaffolding suitable for cartilage regeneration should be biocompatible and biodegradable and should support stem cell adhesion and growth. Some materials may also promote differentiation, but a well-mediated chondrogenesis process can only be achieved by adding growth factors. One shortcoming of synthetic scaffolding, such as PLGA, is that—unlike natural materials, such as collagens, that are better at mimicking features of the surrounding cartilage matrix, including biodegradability—its breakdown products can be relatively more cytotoxic to cells that they are designed to harbor.

**Growth factors**—Growth factors, another critical component in regenerative medicine, can regulate stem cell differentiation to control which cells they become and how fast they differentiate. Specifically for cartilage regeneration, TGF- $\beta$ , insulin-like growth factor-1 (IGF-1), and BMPs are the most commonly used growth factors.

IGF-1 is a small protein containing only 70 amino acids, but it plays an important role in regulating DNA synthesis in multiple cell types, including chondrocytes. It accounts for most of the chondrocyte stimulation that is induced by serum<sup>94</sup> and has a positive effect on chondrocyte proliferation and proteoglycan and type II collagen synthesis.<sup>95,96</sup>

TGF- $\beta$ , a 25-kDa homodimeric protein, has three homologous isoforms (TGF- $\beta$ 1, TGF- $\beta$ 2, and TGF- $\beta$ 3), for which sequences have been identified in all mammalian species.<sup>97</sup> TGF- $\beta$  plays an important role in cell proliferation and differentiation, including chondrogenesis, bone formation, angiogenesis, neuroprotection, and wound repair.<sup>98–100</sup> Especially for cartilage, TGF- $\beta$  controls the production of extracellular matrices by stimulating the

synthesis of collagens, fibronectin, and proteoglycans,<sup>101,102</sup> exerting positive effects on cartilage regeneration. However, TGF- $\beta$  can also induce undesired side effects, such as inflammatory responses and osteophyte formation in articular cartilage defects, if present in the knee joint for too long.<sup>103</sup>

BMPs, consisting of 30 members, are a subfamily of the TGF super family and play an important role in mediating chondrocyte and osteoblast growth and differentiation. The most widely studied BMPs are BMP-2, BMP-4, and BMP-7, which can promote chondrogenesis and stimulate proteoglycan synthesis.<sup>104</sup> They can also induce chondrogenic differentiation of mesenchymal stem cells.<sup>105</sup> Although BMPs are used for cartilage repair, they have also been found to stimulate osteoblast growth and osteogenic differentiation. In addition, they appear to be less potent than TGF- $\beta$ 1 in promoting proteoglycan synthesis in the joint.<sup>106</sup>

There are two obstacles in using these growth factors in regenerative medicine: (1) the short half-life *in vivo*—for example, TGF- $\beta$  has a 30-min half-life<sup>107</sup> in the body, and IGF-1 in the body has an even shorter half-life (10–12 min);<sup>108</sup> and (2) the high cost in producing and maintaining the full-length recombinant protein. Growth factors, especially TGF- $\beta$  and BMP-2, have a hundred amino acids and complex 3D structures, and are easy to deactivate during shipping and storage. In addition, physicians have to administer an extremely high dose of the growth factors to achieve desired clinical outcomes, making growth factor therapy very expensive. Nowadays, scientists apply different approaches to identify the bioactive area of growth factors and use shorter peptides to achieve similar functions as the full-length growth factors.<sup>109,110</sup> Moreover, many of the short peptides have been modified onto the cell growth scaffolding, greatly enhancing their half-life, delivery, and efficacy.<sup>111–113</sup> The use of growth factors in combination with scaffolds/dECM is an excellent strategy to try to mimic the structural and bioactive conditions of native articular cartilage. Although this strategy is not perfect in that not every element of native healthy cartilage is represented in the resulting synthetic microenvironment, it has helped to enhance the healing response and produce repair tissue that is comparable to hyaline cartilage.

## Conclusions

Understanding the stem cell niche is critical in cell-based tissue repair strategies because it determines the developmental fate of stem cells. As such, recent advances in stem cell-based cartilage repair strategies have come from paying close attention to the role of the tissue microenvironment in the healing process. Biocompatible scaffolding can facilitate stem cell attachment and growth, while growth factors promote chondrogenesis and enhance cellular functions. Further advancement in this field will depend on the ability to find new and innovative approaches to the combined use of stem cells, growth factors, and biomimetic scaffolds in order to emulate the native cartilage architecture, its biological composition, and its mechanical properties.

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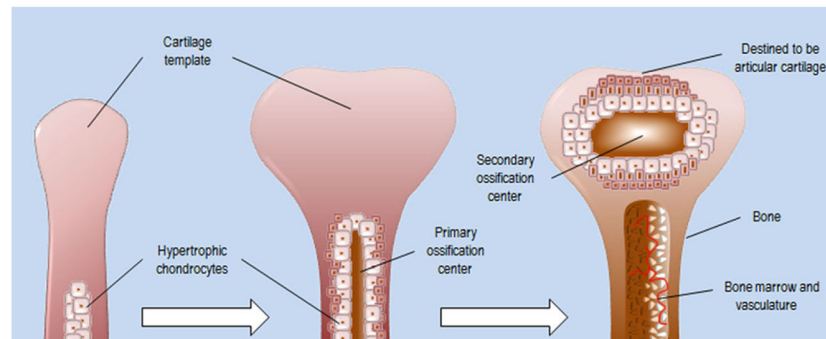
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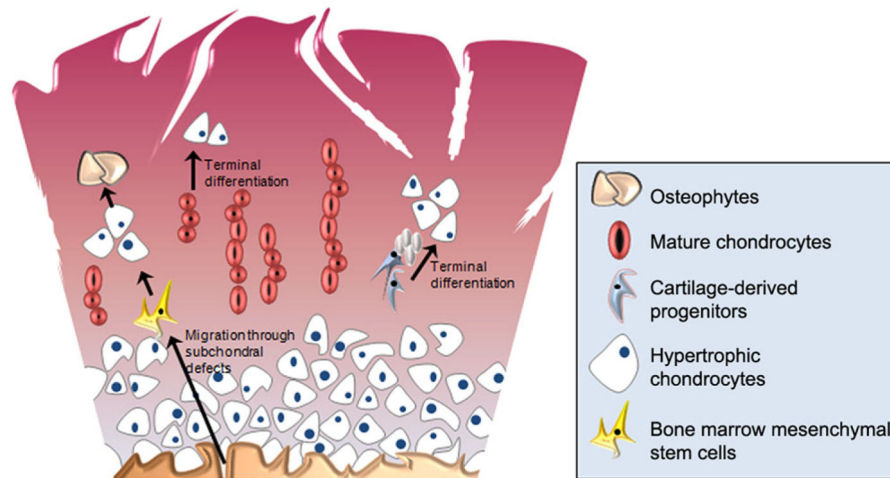
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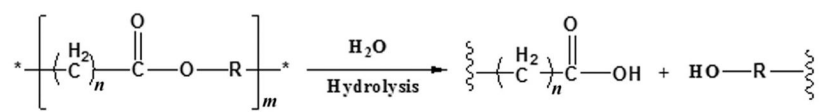


**Figure 1.** Endochondral ossification and articular cartilage. During the process of endochondral ossification, chondrocytes of the cartilage template, from which long bones will form, become hypertrophic and are replaced by osteoblasts. Articular cartilage tissue represents the remnants of the cartilage template from which long bones developed.



**Figure 2.**

Osteoarthritis cartilage microenvironment promotes cellular hypertrophy and terminal differentiation. Cartilage tissue homeostasis is thrown off balance during osteoarthritis, as the combined effects of inflammatory factors and degradative enzymes erode cartilage tissue, resulting in matrix remodeling and changes to the subchondral bone. These events affect the cartilage stem cell niche, potentially altering the differentiation of local and migratory progenitor cell populations and ultimately leading to cellular hypertrophy and/or heterotopic bone formation.



**Figure 3.**  
Mechanisms of the hydrolysis of ester bonds during the degradation of polymers.

**Table 1**

Components with current or prospective use in cartilage tissue repair

<b>Stem cell type</b>	<b>Desirable qualities</b>
Bone marrow mesenchymal stem cells	Relatively abundant
Cartilage-derived mesenchymal progenitors	High colony-forming efficiency
Induced pluripotent stem cells	Most abundant
<b>Extracellular matrices</b>	<b>Desirable qualities</b>
Decellularized extracellular matrix (ECM)	Acts like natural scaffolding
	Promotes cell attachment
	Helps maintain stem cell niche
Individual matrix proteins	Depending on which protein is used, they can promote cell attachment, proliferation, and chondrogenesis
<b>Scaffolds</b>	<b>Desirable qualities</b>
Synthetic biodegradable polymers	Helps maintain tissue infrastructure
	Biodegradable to allow for replacement by natural ECM over time
Hydrogels	Flexible/malleable
	Low cytotoxicity
	High water content, similar to that in natural cartilage tissue
Biomimetic materials	Mimics natural molecules and structures found in cartilage tissue
<b>Growth factors</b>	<b>Desirable qualities</b>
Transforming growth factor $\beta$ 1	Promotes cell proliferation, chondrogenesis, and ECM synthesis
Insulin-like growth factor 1	Promotes cell proliferation and ECM synthesis
Bone morphogenetic factor	Stimulates proteoglycan synthesis