Review

# Cartilage engineering: a crucial combination of cells, biomaterials and biofactors

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**Injuries to articular cartilage are one of the most challenging issues of musculoskeletal medicine due to the poor intrinsic ability of this tissue for repair. The lack of efficient modalities of treatment has prompted research into tissue engineering combining chondrogenic cells, scaffold materials and environmental factors. The aim of this review is to focus on the recent advances made in exploiting the potential of biomaterial-assisted cell therapy for cartilage engineering. We discuss the requirements for identifying additional specific growth factors and evaluating the optimal combination of cells, growth factors and scaffolds that is able to respond to the functional demand placed upon cartilage tissue replacement in clinics. Finally, some of the major obstacles encountered in cartilage engineering are discussed, as well as future trends in clinical applications.**

## Introduction

Articular cartilage is a highly specialized tissue that reduces joint friction at the extremities of long bones. It consists of chondrocytes, some progenitor cells [1] and an extracellular matrix (ECM) that is composed of a network of collagens, in particular type II collagen, which gives the tissue its shape and strength, and proteoglycans, which give resistance to mechanical stress [2]. When damaged, the articular cartilage has a limited capacity for repair due to the absence of vasculature, which would allow progenitor cells from the blood or the bone marrow to enter the tissue. These limitations have prompted researchers and clinicians to develop surgical methods to restore cartilage surfaces. Some good results have been obtained using approaches that rely on mechanical penetration of the sub-chondral bone to stimulate marrow entry inside the lesion, on periosteum and perichondrium grafts or on autologous chondrocyte transplantation [3]. Unfortunately, none of these approaches has provided a complete and reproducible solution to the problem. The latest strategies rely on cell-based therapies, which are currently in the developmental or preclinical stages, and involve biomaterials that have been seeded with chondrocytes or progenitor cells and/or chondrogenic factors.

## Cells sources and environmental factors for cartilage engineering

### *Cell sources*

Among the various cell types that have been contemplated for cartilage tissue engineering, chondrocytes from hyaline cartilage, which constitutes the mature and functional articular cartilage, have been considered the logical cells of choice [4]. Chondrocytes are indeed the cells that are responsible for secretion of the ECM, which is composed of proteoglycans and collagens and which gives the tissue its structure and strength. These cells are mainly isolated from articular cartilage, but chondrocytes recovered from nasal cartilage have also been proposed as a promising cell source [5]. Although chondrocytes have been widely used for cartilage repair, these cells suffer from two major concerns: their instability in monolayer culture and the rareness of the donor tissue.

Recently, multipotent mesenchymal stromal cells or mesenchymal stem cells (MSCs) have been considered as an attractive source of cells for cartilage engineering owing to their ease of availability and their high capacity of *in vitro* expansion. MSCs are mainly isolated from bone marrow or adipose tissue. They are characterized by their capacity to adhere to plastic, their phenotype ($CD73^+$, $CD90^+$, $CD105^+$, $CD14^-$ or $CD11b^-$, $CD19^-$ or $CD79\alpha^-$, $CD45^-$ and $HLA\text{-}DR^-$; with CD34 being expressed solely by adipose-derived MSCs) and their potential to differentiate into adipocytes, chondrocytes and osteoblasts [6]. MSCs also exhibit the potential to differentiate into other cell types [7]. More recently, these cells have also been described as immunoregulatory cells because they were shown to be able to escape immune recognition and to inhibit the host defence mechanisms (for a review, see [8]).

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### Role of the three-dimensional environment and biomaterials

It is well established that cells reside, proliferate and differentiate inside the body within a complex three-dimensional (3D) environment. In articular cartilage, chondrocytes are surrounded by an abundant ECM, which is composed of a highly hydrated complex network of molecules. In contrast, isolated chondrocytes will lose their differentiated phenotype in two-dimensional (2D) culture [9]. The dedifferentiation process is accompanied by a shift towards a fibroblast-like phenotype. which is characterized by an increased expression of type I collagen and the adoption of a spindle shape [10]. However, this process is reversible because dedifferentiated chondrocytes can recover their differentiated phenotype when they are relocated into a 3D environment [11–13]. This observation confirms that the 3D environment is a pivotal factor that has a significant role in supporting or in restoring the chondrocytic phenotype. 3D environments have therefore been used for either culturing of chondrocytes or for promoting the chondrogenic differentiation of MSCs using pellet [14] or micromass [15] culture systems, which help to form aggregates of MSCs. Under these conditions, MSCs are packed or seeded in high cell density to mimic the mesenchymal condensation observed during embryologic chondrogenesis [16] and to promote cell–cell contact [17].

Biomaterials have also been used for both the 3D culture and implantation of chondrogenic cells. These biomaterials serve as scaffolds and can be classified into natural biomaterials, which are further distinguished as protein-based and polysaccharide-based biomaterials, and into synthetic biomaterials. An overview of used biomaterials is provided in Table 1. Among the protein-based biomaterials, membranes formed of type I and III collagens [18] are clinically available for autologous chondrocyte implantation; such membranes include MACI® (Verigen, Leverkusen, Germany), Maix® (Matricel, Hezoenrath, Germany) and Chondro-gide® (Geistlich Biomaterials, Wolhusen, Switzerland). Atelocollagen® (Koken Co. Ltd, Tokyo, Japan) is a gel made of type I collagen from which telopeptides containing antigenic determinants have been removed. This collagen gel enables the 3D culture and *in vivo* implantation of human autologous chondrocytes [19] and of bone marrow MSCs [20]. Of the polysaccharide-based biomaterials, Hyalograft® C, a tissue-engineered graft, consists of autologous chondrocytes that are associated with a hyaluronic-acid-based matrix termed HYAFF-11® (Fidia Advanced Biopolymers, Abano Terme, Italy). This concept has shown a clinical improvement of cartilage function in humans [21]. Among the synthetic biomaterials, Bio-Seed®-C (BioTissue Technologies, Freiburg, Germany) is a porous 3D scaffold made of polyglycolic acid (PGA), polylactic acid (PLA) and polydioxanone that has been seeded with autologous chondrocytes embedded within fibrin gel [22]. Bio-Seed®-C has been reported to induce the formation of hyaline cartilage, which is associated with a significant clinical improvement of joint function. Despite encouraging clinical results, the above-mentioned matrices suffer a major limitation in that that they all require a surgical incision into the joint to be implanted. In this context, the development of injectable biomaterials that are suitable for mini-invasive transplantation of chondrogenic cells remains challenging for researchers.

**Table 1. Main biomaterials used for the 3D culture and transplantation of chondrogenic cells in cartilage tissue engineering**

| Matrices | | Cells | |
|---|---|---|---|
| **Type** | **Material** | **Chondrocytes** | **MSCs** |
| Protein-based | Collagen | [18] | [20] |
| | Fibrin | [81] | [82] |
| Polysaccharide-based | Alginate | [83] | [86] |
| | Chitosan | [84] | N/A |
| | Hyaluronic acid | [21] | [87] |
| | Cellulose | [85] | N/A |
| Synthetic | PLGA (poly[lactic-co-glycolic acid]) | [88] | N/A |
| | PLA (polylactic acid) | N/A | [90] |
| | PEG (polyethylene glycol) | [89] | [91] |

Hydrogels are a new class of biomaterials that could potentially be injected transcutaneously into joints. These biomaterials are composed of a viscous polymer made of synthetic or natural hydrophilic macromolecules, which are able to form a hydrogel after physical, ionic or covalent crosslinking [23]. Hydrogels exhibit a high water content close to that found in cartilage and therefore mimic the 3D environment of cells in cartilage [24]. The chondrogenic differentiation of MSCs has also been demonstrated with most of the above-mentioned scaffolds (see Table 1). However, it is still debatable whether the transplantation of fully differentiated MSC-derived chondrocytes or of pre-commited cells is indeed required for successful cartilage repair. Future advances in the development of 3D scaffolds, such as hydrogels that might be able to support *in vivo* chondrogenesis, could help to address this issue and should be the subject of further research efforts.

### Role of biophysical stimuli

*Oxygen tension.* As mentioned above, articular cartilage is avascular and, consequently, chondrocytes receive oxygen and nutrients via a passive diffusion from the synovial fluid [25]. Articular chondrocytes naturally experience low oxygen tension, with the oxygen concentration in the articular cartilage varying from 1 to 7%. The adaptation of chondrocytes to low oxygen tension is mediated by transcription factors, such as hypoxia inducible factor (HIF) [26]. HIF is a heterodimer that consists of the subunit HIF-1α or -2α and the aryl hydrogen receptor nuclear translocator (ARNT) subunit, also known as HIF-1β. Whereas HIF-1β is stable in normoxic conditions, HIF-1α and -2α are unstable and are rapidly degraded through the ubiquitin proteasome pathway [27]. Under hypoxic conditions, HIF-1α and -2α are stabilized and translocate from the cytoplasm to the nucleus, where they heterodimerize with ARNT to bind to the hypoxic responsive element (HRE), thereby initiating the transcription of hypoxia-specific genes [28]. HIF-1α has been shown to be essential for growth arrest and survival of chondrocytes [29]. Nevertheless, it has been suggested that HIF-2α, but not HIF-1α, is essential for the hypoxic induction of the human articular chondrocyte phenotype [30].

Hypoxia has also been shown to increase the synthesis of ECM proteins in cultured chondrocytes *in vitro* [12]. This

positive effect of reduced oxygen tension is further corroborated by a hypoxia-induced chondrogenic differentiation of MSCs derived from bone marrow or adipose tissue [31,32]. These data suggest that low oxygen tension is a key regulatory factor of proliferation, differentiation and activity of chondrogenic cells. Interestingly, hypoxia has also recently been suggested to inhibit the expression of type X collagen, which is the major marker of chondrocyte hypertrophy, during the chondrogenesis of epiphyseal chondrocytes [33] and of adipose-derived MSCs [34], thereby preventing the potential calcification of engineered cartilage. This result strongly suggests that hypoxia is a useful tool for articular cartilage tissue engineering, where chondrocyte hypertrophy and subsequent bone formation has to be avoided.

*Mechanical stimuli.* Under physiological conditions, articular cartilage is subjected to various mechanical stimuli, such as hydrostatic pressure, as well as compressive and shear straint. The composition and structural organization of the ECM of articular cartilage are responsible for its biomechanical properties. The type II collagen network confers to cartilage its strength against tensile forces, and the highly hydrated proteoglycans provide its compressive resistance [35]. Interestingly, physiological loading is a pivotal factor influencing the chondrogenic differentiation of MSCs during articular cartilage development. In addition, mechanical stimuli on chondrocytes have been reported to be essential for the maintenance of cartilage integrity [36]. Varying loads that have been applied on cartilage tissue engineering constructs resulted in a different structural organization of cartilage ECM proteins, such as collagens and glycosaminoglycans [37]. Given that mechanical stimuli widely influence cartilage formation, they are an important factor to take into account in the development of cartilage engineering products. To address this issue, the above-cited parameters have been integrated into bioreactors, in which specific physicochemical parameters, mechanical stimuli and fluid flow can be controlled and applied to cell-seeded scaffolding biomaterials. The different bioreactor systems used to mechanically stimulate tissue engineering constructs have been reviewed by Schulz [38]. A bioreactor that is able to accommodate the environmental factors mentioned above would be a crucial tool for cartilage engineers and would help to move tissue engineering from the laboratory to the bedside.

## Requirement for growth factors

Apart from an appropriate scaffold, implantation of MSCs will also require the use of growth and differentiation factor(s) that will induce specific differentiation pathways and the maintenance of the chondrocyte phenotype (Figure 1). Several growth and differentiation factors that are involved in regulating cartilage development and homeostasis of mature articular cartilage have been identified (Figure 2). However, long-term successful cartilage repair still requires identification and application of those specific growth factors that are able to induce and maintain the chondrocytic phenotype. In the following sections, we present the current knowledge on the roles of five families of factors that are particularly relevant for cartilage formation and highlight the need for a better understanding of the complex molecular events that are involved in the different pathways.

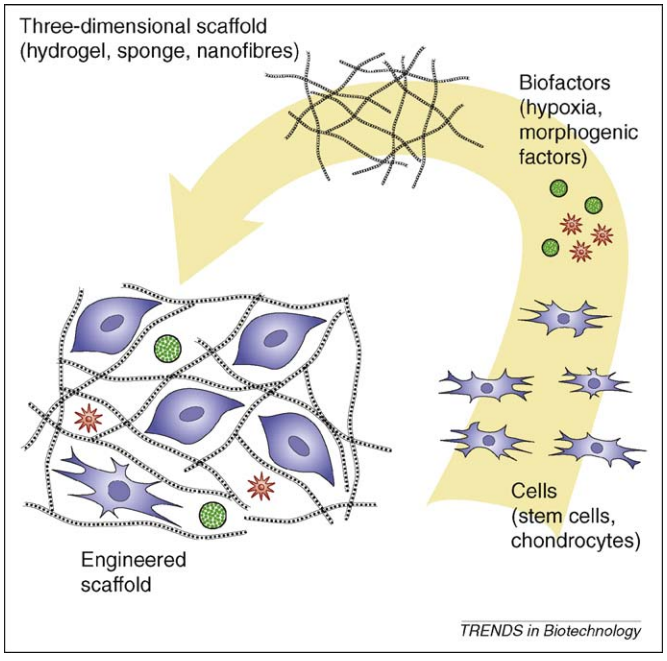


**Figure 1**. Requirement for a combination of cells, biofactors and scaffolds for cartilage formation. Cells, such as chondrocytes or mesenchymal stem cells (MSCs), are expanded *ex vivo* and subsequently mixed with morphogens (growth and differentiation factors in hypoxic environment) on a 3D scaffold to initiate differentiation. The engineered scaffold will lead to cartilage formation after cells have differentiated, either after a period of *ex vivo* culture or after implantation *in vivo*.

### *The transforming growth factor-β superfamily*

The transforming growth factor (TGF)-β family of polypeptides includes TGF-β, bone morphogenetic proteins (BMPs), activins and inhibins. These molecules initiate signalling from the cell surface by interacting with type I and type II receptors, depending on the ligands they bind [39]. Upon ligand binding, the type II receptor activates the type I receptor, which phosphorylates the downstream mediators: Smads 1, 5 and 8 after BMP activation and Smads 2 and 3 after TGF-β- and activin-binding, respectively. The phosphorylated Smads associate with Smad 4 and translocate into the nucleus, where they participate in gene transcription [39].

The TGF-β family includes five members (TGF-β1–5), which are predominantly produced in bone and cartilage. Active TGF-β1, 2 and 3 are generally considered to be potent stimulators of proteoglycans and of type II collagen synthesis in chondrocytes and are able to induce the chondrogenic differentiation of MSCs *in vitro* (for review see [40]). *In vivo*, TGF-β1 can induce the chondral differentiation of MSCs to form ectopic cartilage and was able to repair a full-thickness cartilage defect by improving chondrocyte integration into the endogenous tissue [41]. However, direct injection of TGF-β or of TGF-β-expressing adenoviruses resulted in side effects in the joints, such as osteophyte formation, swelling and synovial hyperplasia [42], suggesting that a tightly coordinated regulation of TGF-β is needed to control chondrogenesis.

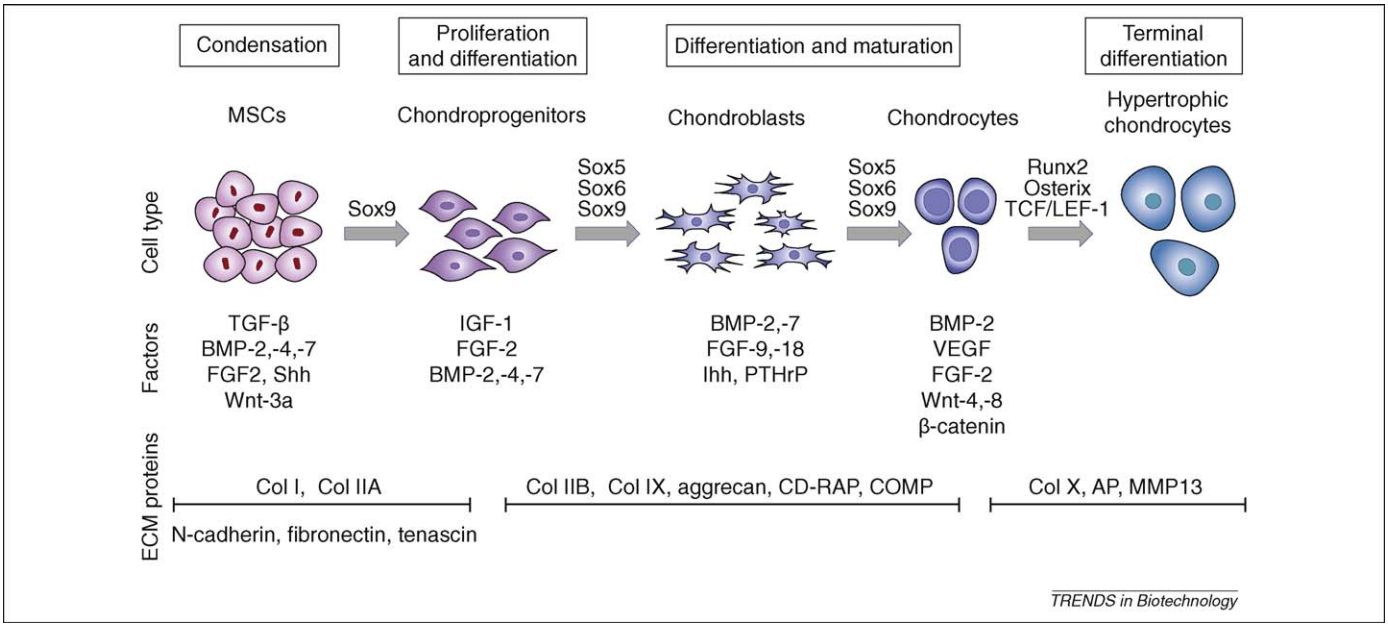


**Figure 2**. Sequence of events leading to the differentiation of mesenchymal stem cells (MSCs) towards chondrocytes. The different stages of chondrogenesis are schematically represented. The temporal expression profiles of the different growth and differentiation factors are shown below and the main transcription factors involved in each step are indicated. Proteins that are characteristic of the extracellular matrix (ECM) for the various stages are also highlighted in the lower part of the figure. Abbreviations: AP, alkaline phosphatase; CD-RAP, cartilage-derived retinoic acid-sensitive protein; Col, collagen; COMP, cartilage oligomeric protein; MMP, matrix metalloprotease; VEGF, vascular endothelial growth factor.

BMPs constitute a large sub-class of 20 polypeptides that have essential roles in chondrogenesis and osteogenesis during skeletal development. Several BMPs, including BMP-2, -4, -6, -7, -13 and -14, can stimulate the chondrogenic differentiation of MSCs [43] and enhance the synthesis of collagen II and aggrecan by chondrocytes *in vitro* [44]. *In vivo*, healing of full-thickness cartilage defects in rabbits was improved when microfracture and recombinant BMP-7 [45] were combined or when a type I collagen sponge containing plasmid DNA for BMP-2 expression was implanted [46]. The use of muscle-derived stem cells that had been retrovirally transduced *ex vivo* to express BMP-4 enhanced chondrogenesis and significantly improved articular cartilage repair in rats [47]. However, when implanted in ectopic localizations, BMPs led to bone formation, suggesting that for an optimal tissue engineering strategy, BMPs must also be regulated. The use of BMP-2, -4 and -7 has been approved for some clinical applications [48], but their potential to enhance cartilage repair still needs to be validated in humans.

### *Fibroblast growth factor family*

In vertebrates, the fibroblast growth factor (FGF) family comprises 22 structurally related proteins that bind one of four FGF receptors (FGFRs). Most FGFs are secreted, with the exceptions of FGF1 and -2 and FGF11–14. The FGFR contains two or three immunoglobulin-like domains and a heparin-binding sequence (HS) necessary for its activation [49]. The interaction between FGF and FGFR-HS stabilizes FGFs and activates multiple signal-transduction pathways. The best characterized are the Ras–mitogen-activated protein kinase pathway (which includes the extracellular-related kinase 1 and 2 [ERK1/2], p38 and c-Jun N-terminal kinase [JNK] kinases), the phosphoinositide-3-OH kinase (PI3K)–protein kinase B (Akt) pathway and the phospholipase C (PLC)γ pathway [50].

The importance of FGF signalling in skeletal development is highlighted by the number of dysplasias that have been attributed to specific mutations in the genes encoding the FGFR1, -2 and -3. Genetic studies have also identified defects in chondrogenesis in mice lacking FGF18 and in the skeletons of *FGF9*$^{-/-}$ mice, which are slightly smaller than those of wild-type littermates [51]. In adult cells, the chondrogenic effect of FGF has been confirmed in only very few studies. The forced expression of *FGFR3*, one receptor of FGFs, in the murine C3H10T1/2 MSC line was shown to be sufficient for chondrogenic differentiation [52], and FGF18, a ligand of FGFR3, promoted the differentiation of limb bud mesenchymal cells to produce cartilage matrix [53]. One study also reported that FGF18 might stimulate repair of damaged cartilage [54]. In cultured chicken chondrocytes, FGF9, another ligand for FGFR3, rapidly induced the upregulation of osteopontin, a marker of hypertrophic and osteoarthritic chondrocytes and osteoblasts but, surprisingly, this was accompanied by inhibition of differentiation and increased proliferation [55]. In adult chondrocytes, FGF2 is mainly mitogenic, whereas MSCs that had been expanded in FGF2-supplemented medium proliferated more rapidly and subsequent chondrogenic differentiation was increased [56]. Furthermore, in a rabbit model, FGF2 stimulated articular cartilage restoration in temporomandibular or articular cartilage defects [57]. The contradictory results for the potential role of various FGFs in chondrogenesis highlight the need for a better characterization of the signalling pathways that are activated by FGFs to be able to fully understand how they affect FGF activity.

### *Insulin-like growth factor family*

The insulin-like growth factor (IGF) family comprises the ligands IGF-1 and IGF-2, the receptors IGF1R and IGF2R, at least six different IGF-binding proteins (IGFBPs) and

multiple IGFBP proteases, which regulate IGF activity. IGF-2 mainly has a role in embryonic and foetal development, whereas IGF-1 is more relevant for cartilage repair. The receptor of both IGF isoforms is the tyrosine kinase receptor IGF1R [58]. The binding of IGF-1 to IGF1R results in activation of its intrinsic tyrosine kinase activity, which leads to the phosphorylation of different intracellular substrates, including those of the PI3K–phosphoinositide-dependent kinase-1 (PDK-1)–Akt pathway and the Ras–ERK pathway [59].

In embryonic development, mice with *IGF-1*$^{-/-}$ mutations display severe growth retardation and have developmental defects in various organs. In adults, IGF-1 and IGF1R are expressed by chondrocytes, osteoblasts and osteoclasts [58]. IGF-1 is considered an essential mediator of cartilage homeostasis through its capacity to stimulate proteoglycan synthesis and to promote chondrocyte survival and proliferation [60]. IGF-1 also induces the differentiation of MSCs towards the chondrocytic phenotype [61]. In a horse model for cartilage defects encompassing sub-chondral bone, IGF-1 alone was able to induce migration of chondrocytes and, moreover, the combined use of chondrocytes and IGF-1 seemed to improve the overall consistency of the repair tissue [58]. However, the anabolic action of IGFs could be counteracted by IGFBPs, and this might account for some of the variable results reported for *in vivo* cartilage repair [62].

### *Wingless family*

In vertebrates, the Wingless (Wnt) family contains more than 20 members that exhibit distinct functions in development. Wnts bind the receptors Frizzled (Fzd) and cooperate with the transmembrane molecules low density lipoprotein (LDL)-receptor-related protein 5 (LRP5) and LRP6 [63]. Most of the Wnt proteins induce the canonical β-catenin-dependent pathway. Indeed, in the absence of Wnt, casein kinase 1α (CK1α) and glycogen-synthase kinase-3β (GSK-3β) phosphorylate β-catenin, which is subsequently degraded by the proteasome. In the presence of Wnts, Fzd phosphorylates Dishevelled, which stabilizes β-catenin. In turn, β-catenin translocates into the nucleus, binds to the transcription factors T-cell factor (TCF) and lymphoid enhancer factor (LEF) and stimulates the expression of its various target genes. Some Wnts can activate a β-catenin-independent pathway via at least three different mechanisms: (i) via calcium/calmodulin-dependent protein kinase II and protein kinase C (PKC) [64]; (ii) via the activation of PLC; and (iii) via the JNK pathway. Wnt signalling is also regulated by inhibitors, including Dickkopfs (Dkks) and secreted Fzd-related proteins (SFRPs).

Various Wnt members are involved in both early and late skeletal development and have a role in the control of chondrogenesis. Wnt-1, Wnt-4, Wnt-7a and Wnt-8 block chondrogenic differentiation but display different effects on hypertrophy. By contrast, Wnt-5a, Wnt-5b and Wnt-11 regulate chondrocyte proliferation and hypertrophic maturation in the embryonic and postnatal growth plates [65]. The role of Wnt-3a on chondrogenesis is more controversial. Wnt members that activate canonical Wnt signalling lead to enhanced ossification and suppression of chondrogenesis [66]. Consistently, *in vitro* loss-of-function analyses revealed that β-catenin activity is necessary and sufficient to repress the differentiation of mesenchymal cells into Runx2-positive skeletal precursors. However, β-catenin was recently shown to be required for both osteogenesis and chondrogenesis in adult mature tissues [67]. Overall, it seems that the Wnt network has dual roles in cartilage; (i) it is an important regulator of chondrocyte development and (ii) deregulation of Wnt signalling might lead to disease, in particular to osteoarthritis.

### *Hedgehog family*

In mammals, the Hedgehog (Hh) family comprises three members, Sonic hedgehog (Shh), Indian hedgehog (Ihh) and Desert hedgehog (Dhh). Hh proteins signal upon binding to Patched (Ptc), a constitutive repressor of the activator Smoothened (Smo). Binding of Hh to Ptc activates Smo, a master regulator of downstream signalling events, which leads to the activation of the nuclear transcription factors Gli1, Gli2 and Gli3, which regulate downstream target genes.

In concert with other signalling molecules, Ihh has been found to function as a central regulator of endochondral ossification, coordinating chondrocyte proliferation, differentiation and ossification of the perichondrium. Expression of Ihh induces the upregulation of parathyroid hormone-related protein (PTHrP), which is expressed in distal chondrocytes of the skeletal elements. PTHrP in turn signals back to the proliferating chondrocytes and prevents them from differentiating into prehypertrophic cells. In agreement with this function, analysis of an *Ihh*-null mutant revealed a markedly reduced chondrocyte proliferation, maturation at an inappropriate position and a failure of osteoblast development in endochondral bones [68]. Consistently, mice overexpressing *Ihh* or a constitutively activated allele of *Smo* displayed increased chondrocyte proliferation [69]. *In vitro*, Shh improved the differentiation of MSCs to the osteoblast lineage [70], upregulated expression of the cartilage markers [71] and impaired adipogenesis of MSCs [72]. Only very few *in vivo* studies are available, but a noteworthy report showed that Shh delivery to bone defects resulted in significant bone regeneration [73]. Like other growth factors, Hh interacts with several signalling pathways, including those in which FGFs, Wnts and BMPs are involved.

## Major obstacles for persistent regeneration of cartilage

Various promising cartilage-engineering strategies that involve the delivery of biomaterials seeded with chondrogenic cells and growth factors have produced encouraging *in vitro* data; however, thus far, no approach has led to the generation of long-term hyaline cartilage replacement tissue *in vivo*. Several different and diverse reasons for the lack of stable functional tissue are possible and are discussed below.

### *Loss of regenerative cells through cell death after transplantation*

In cartilage defects, cells can be lost from the site of injury by leakage of the cell suspension, by apoptosis and by necrosis [74]. Necrosis or apoptosis can be induced by

several factors, including inflammatory cytokines, metalloproteinases, nitric oxide, serum deprivation or mechanical forces. Leakage of the cell suspension is the most likely cause of decreased viability of implanted chondrocytes [75]. However, the loss of chondrogenic cells can also arise from the death of native chondrocytes at the interface between host and repair tissue, which is consistent with observations for experimental wounding of the same tissue. This effect is likely to hinder the full integration of the neotissue into the existing cartilage but can be at least partly rescued by the delivery of anti-apoptotic factors. Indeed, the preservation of an intact tissue at the edges of the lesion is considered necessary to guarantee a favourable environment for the integration of chondrogenic cells [76].

### *Insufficient capacity of chondrogenic cells to integrate within surrounding tissue*

Chondrocytes seem to have a poor capacity to infiltrate existing cartilage tissue. This lack of integration has been attributed to an insufficient secretion of matrix proteins by implanted cells. Indeed, cartilage integration might be enhanced by pretreatment of devitalized cartilage with isolated chondrocytes. Integration might also be affected by the composition and structure of the adjacent native tissue [77]. The complexicity of the cartilaginous tissue, which is composed of various layers that are in direct contact with the sub-chondral bone, further complicates incorporation of implanted cells into a functional tissue. Although the generation of neocartilage with an appropriate zonal organization has been attempted, an exact reproduction of the various layers has not yet been achieved [78].

### *Induction of dedifferentiated chondrogenic cells by inflammatory stress*

Various cells have been used for cartilage repair, including chondrocytes, perichondral or periosteal cells and MSCs. Implantation of cells in conjunction with scaffolds has been shown to improve cartilage repair, but in most cases the defects had been filled with a mix of fibrous and cartilaginous tissues, suggesting that dedifferentiation of the chondrogenic cells to fibroblast-like cells had taken place over time [79]. To avoid such dedifferentiation, one possibility is to implant progenitor cells that have been fully differentiated *in vitro*, which would allow a better control of the differentiation process. An alternative approach consists of implanting undifferentiated cells in the hope of a better integration of the *in situ* differentiated cells. This therapeutic option has already been applied in humans and significantly improved patient outcome, as assessed by the International Knee Documentation Committee (IKDC) score. Improved outcome or therapeutic benefit could be observed over one to five years but, nevertheless, fibrocartilaginous tissue had filled the defects in the scaffold tissue [80]. In this setting, either incomplete differentiation of implanted cells or instability of the chondrocytic phenotype might explain the insufficient regenerative potential of MSCs.

## Conclusions

Recent advances in cell biology and material sciences have contributed to tissue engineering becoming a promising therapeutic modality for the treatment of osteoarticular disorders. Cell-based strategies have not only proved the feasibility of such approaches for cartilage repair but have also provided acceptable clinical results. However, the available protocols are still far from being able to generate a tissue that is comparable to native cartilage with respect to quality and stability. Nevertheless, more-sophisticated approaches, which will combine the delivery of chondrogenic progenitors, in particular MSCs and bioactive growth factors, together with a chondro-conductive scaffold, will be required to achieve a complete healing of cartilage lesions (Figure 3). The success of these strategies will rely on a

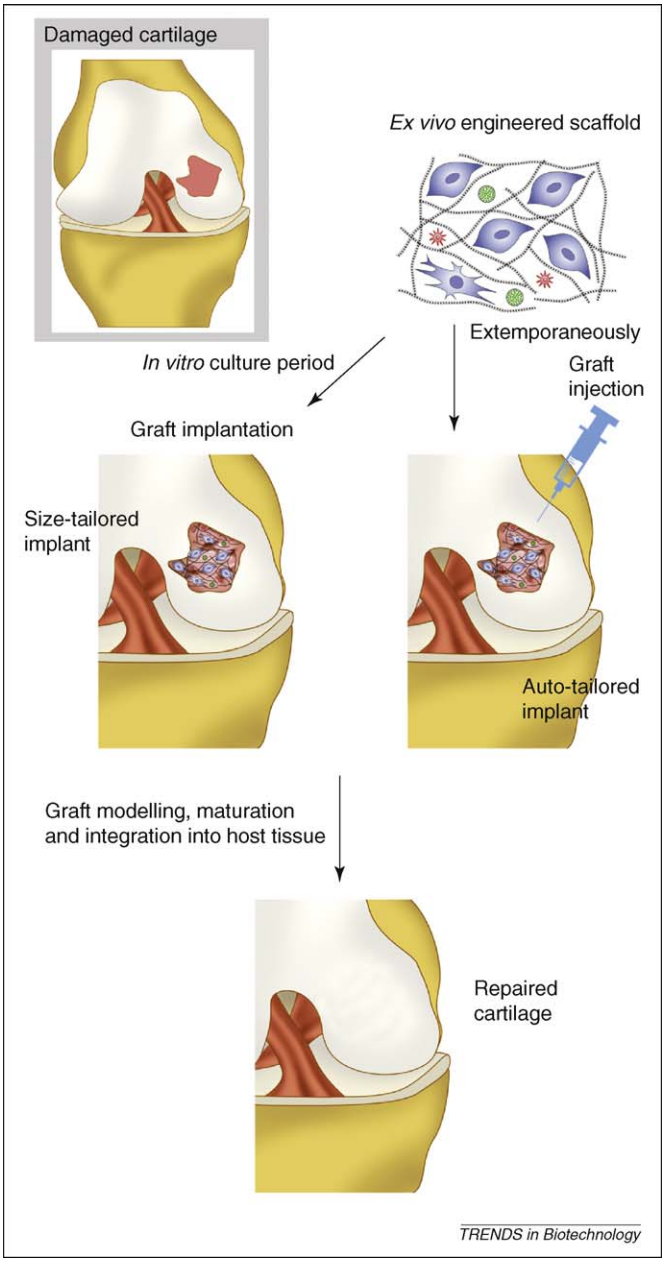


**Figure 3.** Schematic representation of the implantation of engineered scaffolds. Cartilage damage is illustrated in the upper insert. Two options exist for applying *ex vivo*-engineered scaffolds containing chondrogenic cells (see Figure 1). In the first option (shown on the right), injectable scaffolds are prepared at the time of transplantation and are directly injected into the cartilage defect, where they will form a so-called auto-tailored implant. In the second approach (shown on the left), engineered scaffolds are obtained *ex vivo* after a period of culture and can thus be adapted to the size of the lesion before their implantation (size-tailored implant). After subsequent graft maturation and integration into the host tissue, both options should in theory result in the restoration of intact cartilage.

better understanding of the complex molecular events that are involved in induction of chondrogenesis and in maintenance of the chondrocyte phenotype because these events, which take place during embryogenesis, will have to be reproduced in adult tissue repair. This will lead to the identification of the exact factors needed for hyaline cartilage repair, including their bioactive levels and kinetics of application. Moreover, because most of these factors have short half-lifes as recombinant proteins, gene transfer techniques could be adopted to achieve the desired results. Finally, cartilage repair will also require a complete integration of the neocartilage and reconstitution of an appropriate zonal organization for successful cartilage patterning.

## Disclosure statement

The authors have no conflicts of interest to declare.

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