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Strategies for Controlled Delivery of Biologics for Cartilage Repair

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Abstract

The delivery of biologics is an important component in the treatment of osteoarthritis and the functional restoration of articular cartilage. Numerous factors have been implicated in the cartilage repair process, but the uncontrolled delivery of these factors may not only reduce their full reparative potential and can also cause unwanted morphological effects. It is therefore imperative to consider the type of biologic to be delivered, the method of delivery, and the temporal as well as spatial presentation of the biologic to achieve the desired effect in cartilage repair. Additionally, the delivery of a single factor may not be sufficient in guiding neo-tissue formation, motivating recent research towards the delivery of multiple factors. This review will discuss the roles of various biologics involved in cartilage repair and the different methods of delivery for appropriate healing responses. A number of spatiotemporal strategies will then be emphasized for the controlled delivery of single and multiple bioactive factors in both *in vitro* and *in vivo* cartilage tissue engineering applications.

Keywords

Cartilage tissue engineering; controlled release; growth factors; spatiotemporal strategies

1. Introduction

The avascular and relatively acellular nature of articular cartilage complicates its natural capacity for regeneration upon damage. While numerous clinical therapies, such as microfracture, autologous chondrocyte implantation, and osteochondral grafts, have been developed for the treatment of cartilage injuries, they have been hampered by inferior cartilage repair and significant donor site morbidity [1]. Indeed, given the extensive literature presently highlighting the shortcomings of current clinical techniques for the

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management of chondral and osteochondral injuries, it is clear that the field of cartilage repair remains an area in critical need of innovative alternative therapies [2–4]. In light of the disadvantages hindering the efficacy of currently available cartilage treatment options, the discipline of tissue engineering provides promising alternatives. Particularly, the area of osteochondral tissue engineering leverages the controlled combination of carefully engineered scaffolds, progenitor cells, and biochemical cues for replacing or restoring lost articular cartilage and subchondral bone function. While the selection of an optimal cell type and appropriate scaffold is necessary to reconstruct specific tissues with a particular configuration and function, successful regeneration is greatly influenced by the cellular microenvironment in which cells and tissues grow [5]. Inspired by physiological events that occur during fetal development and long bone formation [6–8], the concept of growing articular cartilage has led to the integration of a wide variety of soluble cues in an effort to mimic natural signaling cascades in the wound healing environment.

In cartilage tissue repair, the goal of eliciting the desired phenotypic responses from host and/or co-delivered progenitor cells remains. Yet, techniques for the delivery of soluble cues and other biologics for the treatment of cartilage and osteochondral defects have evolved over the years from simple bolus injections into the defect to more sophisticated and controlled multi-functional delivery systems. These engineered strategies, which permit localized drug delivery with controlled release kinetics, utilize delivery platforms that typically leverage at least one of several main delivery schemes developed for controlled release. The simplest approach involves the direct intra-articular injection of growth factors or palliative agents into the synovial space. While such injections represent an attractive and relevant option due to the lack of surgery, the frequencies of injections required and the supra-physiological dosages employed often complicate therapeutic efficacy. Additionally, the recapitulation of natural signaling cascades for proper healing and regeneration of damaged tissues would be near impossible with single injections [4, 8–11]. Nevertheless, the motivation to recapture the complexity of endogenous healing cascades into simplified and controlled forms has driven the evolution of materials-based delivery systems towards a range of modalities involving drug release from microspheres, bulk scaffolds, or a combination of both. Although the type of modality employed for drug delivery can determine the mechanism of release, the method of agent incorporation also offers a significant form of modulation for controlled release. Conventional strategies for the incorporation of signaling molecules into engineered delivery systems typically involve either the physical entrapment or the chemical immobilization of factors into or onto a polymer matrix. Recent reviews by Mehta et al. and Santo et al. provide excellent summaries of modern controlled release strategies utilizing such means for bone and orthopedic therapies [12, 13]. By carefully combining the physical entrapment of bioactive agents with the chemical conjugation of other bioactive factors, innovative and smart delivery systems can be engineered to sustain the release of single or multiple biologics in a spatiotemporally controlled fashion for effective cartilage therapy.

This review begins with an overview of the different bioactive factors that have been utilized in delivery and controlled release strategies for cartilage repair. The aim is to first briefly enable an understanding of the different biological approaches available for both *in*

vitro and *in vivo* cartilage repair applications. The following sections will discuss the methods of delivery and assess the current state of recent controlled release strategies developed for cartilage tissue engineering and cartilage repair. Special emphasis will be placed on combining materials-driven and biologically-driven strategies for cartilage repair in order to provide an outlook for future developments that are aware of the needs for both.

2. Cartilage Regenerative Factors

The homeostasis and repair of articular cartilage is regulated by a number of growth factors, differentiation factors, systemic factors, and other biologics. The ultimate response from a specific biologic depends on its identity, and so a critical component in designing a controlled delivery system is the selection of an appropriate factor.

2.1 Growth Factors

Growth factors are a group of soluble signaling molecules that can stimulate cellular division, growth, and differentiation through specific binding of transmembrane receptors on target cells [14]. Among the biologics involved in cartilage repair, growth factors remain the most extensively studied due to their powerful proliferative, proanabolic, and/or anticatabolic properties [15]. These include select members of the transforming growth factor-β (TGF-β) superfamily, fibroblast growth factor (FGF) family, and insulin-like growth factor-1 (IGF-1). Another major growth factor, platelet-derived growth factor (PDGF), plays a role in the wound healing of cartilage defects, but the direct effect of PDGF delivered to a cartilage defect has yet to be investigated [16]. It is most often used within platelet-rich plasma (PRP), which will be discussed in a later section.

2.1.1 Transforming Growth Factor-β Superfamily—The TGF-β superfamily includes over 30 structurally related members and serves an important role in regulating embryogenesis as well as adult homeostasis [17]. Among the TGF-β superfamily, the most heavily investigated growth factors for cartilage repair include prototypic members TGF-β1, 2, and 3 and bone morphogenetic proteins (BMP) 2, 4, and 7. Growth differentiation factors (GDFs), particularly GDF-5, have shown chondrogenic potential *in vitro*, but their ability to promote *in vivo* cartilage repair has yet to be evaluated [18–21].

2.1.1.1 Transforming Growth Factor-βs: TGF-β1, 2, and 3 are considered to be potent stimulators of chondrogenesis, inducing Sox9 expression and increasing cartilaginous extracellular matrix (ECM) production in bone marrow-derived mesenchymal stem cells (MSCs) as well as stimulating synthetic activity in chondrocytes [8, 15, 22]. In animal models, TGF-βs are highly expressed during MSC condensation in the growth plate of long bones [8] and are also implicated in the early stages of cartilage repair [6]. As a result, TGF-β isoforms, particularly TGF-β1 and 3 have been used in a number of promising *in vivo* studies exploring the ability of TGF-βs to promote the repair of cartilage defects [23]. In a rabbit full-thickness cartilage defect model, poly(lactic-co-glycolic acid) (PLGA)/fibrin gel scaffolds loaded with MSCs and TGF-β1 resulted in better cartilage restoration than PLGA/fibrin gel scaffolds loaded with MSCs alone [24]. Additionally, TGF-β1 in calcium alginate beads improved osteochondral tissue repair after 12 weeks compared to alginate scaffolds alone [25]. However, the effects of TGF-β1 on cartilage repair *in vivo* are not always

consistent [26]. Guo et al. demonstrated that blank oligo(poly(ethylene glycol) fumarate) (OPF)-based scaffolds in a rabbit osteochondral defect model resulted in cartilage repair that was equal to or improved relative to OPF scaffolds with MSCs or OPF scaffolds with MSCs and TGF-β1 [27]. Intra-articular injections of TGF-β have also caused synovial fibrosis and endochondral ossification [28, 29]. Several reasons for the observed negative effects of TGF-β delivery have been suggested, including the supra-physiological levels of growth factor employed as well as the relatively non-specific effects of TGF-β on MSC differentiation [27]. As a result, it is important to consider the dosage and presentation of TGF-βs when delivered *in vivo*, understanding that TGF-βs are multifunctional and induce gene responses in different cell types for proliferation and ECM synthesis [30].

2.1.1.2 Bone Morphogenetic Proteins: Similar chondrogenic effects of TGF-β have also been observed for several BMP molecules, likely due to the extensive crosstalk between TGF-β and BMP signaling pathways [31]. Different BMPs, particularly BMP-2, 4, 6, 7, and 9, have been shown to stimulate chondrogenic differentiation of MSCs and/or adipose-derived stem cells (ASCs) either individually or with a TGF-β prototypic member [32]. In a comparison between TGF-β1 and BMP-2 delivery from PLGA microspheres in alginate gel, both groups resulted in greater histological scores for osteochondral repair compared to blank scaffolds at 12 and 24 weeks in a rabbit patellar groove defect [33]. BMPs also play a role throughout the MSC chondrogenic differentiation process, from MSC condensation to proliferation, differentiation, maturation, and calcification. In particular, BMP-2 is expressed throughout the entire chondrogenic process, from proliferation to calcification [8], and hence, a long-term delivery of BMP-2 can be beneficial and has been shown to result in a higher quality repair of cartilage as opposed to short-term delivery [34]. Different BMPs also have different effects on osteochondral repair. In a study delivering BMP-2 or BMP-4 in alginate gels to a rabbit femoral condyle defect, delivering BMP-2 alone resulted in better subchondral bone restoration while BMP-4 alone gave better cartilage tissue repair [35]. BMP-7 has also shown efficacy *in vivo*: BMP-7 delivered from a collagen sponge in conjunction with microfracture led to thicker repair cartilage, superior matrix and superior cell distribution compared to a collagen sponge plus microfracture alone in a rabbit chondral defect [36]. With the ability to induce both cartilage and bone formation, BMPs, particularly BMP-2, are attractive growth factors for the regeneration of the osteochondral tissue unit.

2.1.2 IGF-1—Within articular cartilage, IGF-1 is the main anabolic growth factor and plays a key role in cartilage homeostasis, balancing proteoglycan synthesis and breakdown by chondrocytes [16]. IGF-1 can decrease catabolic responses as well as increase proliferation and cartilaginous ECM production in MSCs and chondrocytes *in vitro* [15, 37]. *In vivo*, IGF-1 has demonstrated the ability to improve filling of chondral defects [38], improve quality of cartilage repair [39, 40], and decrease the postoperative inflammatory response [41]. IGF-1 has also demonstrated an additive effect when combined with other growth factors such as TGF-β1, BMP-2, and BMP-7 [37, 42, 43]. However, achieving similar synergistic results when delivering IGF-1 with other growth factors *in vivo* is an area of much interest. IGF-1 delivered from gelatin microparticles (MPs) in an OPF-based scaffold resulted in higher quality cartilage repair compared to OPF composites alone in a rabbit medial femoral condyle defect at 12 weeks. But the benefits of IGF-1 were not maintained

when co-delivered with TGF-β1 [26]. Similarly, the single delivery of IGF-1 from OPF composites showed an improvement in cartilage morphology over blank scaffolds alone, but the dual delivery of IGF-1 with TGF-β3 did not have a synergistic effect [44]. Further study on combining IGF-1 with other growth factors as well as the appropriate presentation of IGF-1 in a multiple growth factor delivery strategy *in vivo* is needed.

2.1.3 FGF-2—Like IGF-1, the FGF family also plays an important role in the homeostasis of cartilage. In particular, FGF-2, or basic FGF (bFGF), has a potent mitogenic effect on MSCs and chondrocytes [45]. In addition, treatment with FGF-2 increased both the proliferative and chondrogenic potential of MSCs *in vitro* [37, 46, 47]. *In vivo*, the delivery of FGF-2 has been shown to improve both cartilage repair as well as the underlying subchondral bone [48, 49]. However, FGF-2 may have contraindications: FGF-2 induced chondrocyte proliferation in a cartilage explant, but resulted in chondrocyte clonal cluster formation, which is a histopathological feature of osteoarthritis (OA) [50]. Additionally, evidence suggests that FGF-2 may antagonize proteoglycan synthesis and upregulate matrix metalloproteinases (MMPs) [15]. Proper delivery and presentation of FGF-2 may help mitigate potential contraindicatory effects on cartilage repair.

2.2 Anti-Angiogenic Factors

Full-thickness lesions that perforate the subchondral bone and bone-marrow spaces may trigger the onset of angiogenic and osteogenic processes in the cartilage layer, leading to conditions unfavorable for chondrogenesis. As a result, there has been interest in introducing anti-angiogenic factors to inhibit blood vessel growth and restore cartilage tissue to its natural state of avascularity [51]. This class of factors includes endostatin [52–54], suramin [55, 56], Flt-1 [57, 58], and bevacizumab [59]. While these factors demonstrate the ability to block vascularization and inhibit the activity of vascular endothelial growth factors (VEGFs), a clear benefit of this approach in a cartilage defect model remains to be seen.

2.3 Systemic Factors and Notable Pharmaceuticals

The therapeutic role of anti-inflammatory cytokines, chemokines, hormones, and other drugs have also been considered in the repair of cartilage. These biologics do not serve the primary role as a mitogenic or anabolic factor, but mediate the wound healing response through other mechanisms. Stromal cell-derived factor-1 (SDF-1) is a key chemokine in cell trafficking and homing of $CD34^+$ stem cells, particularly MSCs [60], and has the potential to enhance cartilage repair through increased MSC migration to the site of a cartilage defect without the need for additional cell transplantation [61, 62]. In the parathyroid hormone (PTH) family, peptide segments of PTH have been shown to inhibit the progression of OA and advance the repair of shallow chondral defects [63, 64]; and PTH-related proteins (PTHrP) are synthesized by chondrocytes and can suppress induction of hypertrophy [65, 66]. However, the timing of PTH or PTHrP administration to cartilage defects remains an important parameter in affecting treatment outcome. Inhibitory factors on necrosis, apoptosis, MMPs, and aggrecanases have also shown potential in the treatment of cartilage defects [67–70]. Other notable systemic factors and pharmaceuticals are listed in Table 1.

3. Biologic Delivery Methods

Almost as important as the selection of an appropriate biologic is choosing a suitable delivery mechanism to enable an appropriately controlled release and elicit the intended response. The proper delivery of the biologic can affect the dosage as well as the release rate and ultimately determine whether or not a therapeutically effective pharmacokinetic release profile was achieved.

3.1 Injection Delivery

The delivery of a biologic through an intra-articular injection or systemic injection is perhaps the simplest method for minimally invasive administration. The ability to treat not only the articulating cartilage but also the entire joint is a relevant delivery strategy, particularly for the management of pain and degenerative processes in OA. Intra-articular injection of a chondroprotective agent, high-molecular-weight crosslinked hyaluronic acid (HA), was seen to improve joint lubrication and retard the progression of OA in a rabbit anterior cruciate ligament transection model over lower molecular weight HA or a saline solution [71]. The benefit and potential of intra-articular modalities for the presentation of chondroprotective agents has also been demonstrated in other studies [71–76]. In addition to palliative treatments, anti-inflammatory agents have also been delivered through intra-articular means to treat OA inflammation [77–79]. By treating the synovial fluid and lining in addition to articular cartilage, the expression of major pro-inflammatory cytokines such as interleukin (IL)-1 and tumor necrosis factor-α (TNF-α) can be addressed and reduced [80–86]. However, the delivery of chondrogenic growth factors such as TGF-β and IGF-1 in an injection manner can effect unwanted changes in the host tissue due to uncontrolled presentation of these bioactive factors [87–91]. Growth factors have a short half-life *in vivo* and can result in rapid clearance when delivered systemically, hindering their potent mitogenic and/or anabolic effects [92–94]. Additionally, a bolus injection of growth factors gives supra-physiological doses, potentially resulting in pathological and non-specific effects, and an absence of a physiologically effective release profile.

3.2 Bulk Phase Delivery

Although the direct injection of bioactive agents has been used with some success for the treatment of OA, the rapid clearance of such drugs from the synovial capsule generally hinders therapeutic efficacy. Incorporation of biologics within a biomaterial carrier can address this issue by delivering the biologic in a concentrated and controlled fashion. Three-dimensional matrices and porous scaffolds are the most common delivery vehicles, particularly for growth factors. By using a drug-delivering scaffold, focal chondral or osteochondral defects can be treated by releasing the factors to the surrounding tissue or promoting cell infiltration into scaffold. Many different techniques have been developed to regulate the release kinetics of soluble factors as well as retain the molecular bioactivity.

One of the most common approaches for bulk phase delivery is a simple dispersion of the biologic within the matrix. The release of the bioactive factor is then dependent on the interaction between the factor and the matrix, either mediated by encapsulation [24, 48, 95], electrostatic interactions [36, 39, 49, 96–98], immobilization/tethering [99–101], or ECM

affinity [102–104]. Biologics encapsulated or entrapped within bulk matrices often have a large burst release, which can be tuned by scaffold crosslinking density and pore size. However, particular natural materials have innate physical properties that can control the release of growth factors. Yang et al. delivered BMP-2 from fibrin gels and heparin-conjugated fibrin gels in a full-thickness trochlear groove defect in rabbits combined with microfracture [34]. An electrostatic interaction with heparin resulted in a sustained release of BMP-2 (82% over 13 days) compared to a burst release of BMP-2 from normal fibrin gels (88% in first 3 days). This long-term delivery of BMP-2 resulted in greater filling of cartilage as well as a higher quality of cartilage repair as opposed to short-term delivery [34].

While many growth factors can adsorb onto scaffolds made of natural polymers through electrostatic interactions, hydrogen bonding, and/or van der Waals forces, non-covalent binding methods may not enable a long-term sustained delivery. Fan and colleagues achieved stable localization of growth factors by crosslinking TGF-β3 onto PLGA/gelatin/chondroitin sulfate/HA scaffolds through a condensation reaction between the carboxyl group of the hybrid scaffold and amine group of TGF-β3 [99]. Results indicated that the cumulative release of TGF-β3 was reduced to 29.5% over 28 days and that TGF-β3-immobilized scaffolds could induce similar levels of chondrogenic differentiation of seeded MSCs and *in vivo* cartilage repair compared to non-immobilized scaffolds cultured in medium with TGF-β3. However, caution should be taken when tethering factors to monolithic scaffolds. Kopesky and colleagues demonstrated that adsorption of TGF-β1 onto self-assembling peptide hydrogels stimulated chondrogenesis of seeded MSCs *in vitro* whereas biotin-streptavidin tethered TGF-β1 hydrogels did not [105]. The authors suggested that the biotin-streptavidin affinity may exceed the strength of some covalent bonds and that tethering TGF-β1 may have prevented internalization of the receptor-ligand complex [100, 101].

The release of growth factors can also be controlled through their innate affinity to certain ECM epitopes [102]. The use of an alginate-sulfate scaffold enhanced TGF-β1 attachment to the scaffold via heparin-like affinity interactions and resulted in a more sustained release of TGF-β1 over 7 days as opposed to a >90% burst release from regular alginate scaffolds after 1 day [103]. Implantation of similar scaffolds with affinity bound TGF-β1 and BMP-4 demonstrated the ability to induce endogenous regeneration of the osteochondral unit [104].

3.3 Microparticle and Nanoparticle Delivery

Another method of delivery is releasing the selected biologic from micro- or nano-sized carriers. MPs and nanoparticles (NPs) are attractive drug delivery vehicles due to their small dimension, high surface area to volume ratio, high drug loading efficiency, and the ability to quickly respond to environmental stimuli such as temperature, pH, magnetic fields, or ultrasounds [8, 106, 107]. While MPs have been investigated for several decades, NPs as delivery vehicles for cartilage repair have been gaining interest [108–112]. NPs can be endocytosed by cells, allowing for the accumulation of NP-encapsulated drugs, and their exceptionally high surface area to volume ratio enhances their affinity to therapeutic drugs and external stimuli [113]. However, a high surface area to volume ratio may also reduce the

stability of the nano-sized delivery vehicle, and the tendency for certain NPs to aggregate into microscale particles may mitigate the advantages of a NP system [113].

MPs have traditionally been used as a delivery vehicle, either by dispersing the MPs in a continuous phase or using the MPs as building blocks without a surrounding matrix to form macroscopic scaffolds [8]. In particular, introducing MPs in a bulk scaffold, particularly hydrogels, adds a level of complexity and allows greater control over the growth factor release profile, spatial delivery, and leads to greater stability and bioactivity of encapsulated proteins [114, 115].

Similar to bulk phase delivery, biologics can also be incorporated in MPs and NPs through entrapment, ionic interactions, or a combination of both. Ultimately, the subsequent release depends on the composition of the carrier as well as the factor incorporation method. One popular material for the construction of MPs is synthetic PLGA due to its tunable degradation into lactic and glycolic acid [116], its ease of fabrication, and its established safety in other FDA-approved applications [117]. Several research groups have employed PLGA MPs for the delivery of anabolic growth factors to stimulate chondrogenesis/cartilage repair either in a hydrogel [115, 118–120] or as a building block to make PLGA MP plugs [121–123]. By dispersing growth factor-delivering PLGA MPs in a hydrogel, near zero-order release kinetics of TGF-β1 and BMP-2 were observed *in vivo* [118, 120]. However, despite the appeal of PLGA microcarriers for controlled drug release, the use of such hydrophobic materials can adversely affect the bioactivity of encapsulated factors [108, 124]. Growth factor delivery particulates have also been fabricated with other synthetic polymers, including heparin-poly(L-lysine) NPs loaded with TGF-β3 [110], Pluronic F68/heparin NPs for TGF-β2 immobilization [125], and poly(*N*-isopropylacrylamide) NPs for TGF-β1 release [111]. Yet, different carrier matrices may be better suited for different biologics. Wang et al. demonstrated that PLGA MPs were better for releasing IGF-1 while silk MPs were more efficient in delivering BMP-2 [126].

Many natural polymers, notably gelatin [107, 127–136], alginate [137], HA [137–140], chondroitin sulfate [109], and silk [126], have been used to create growth factor delivering MPs. Gelatin is a natural polymer derived from collagen, and by subjecting collagen precursors to either alkaline or acidic processing during the production of gelatin, basic or acidic gelatin with different isoelectric points can be obtained [127, 134]. Resultant gelatin carrier matrices can then achieve either a net negative or positive charge at physiological pH depending on the isoelectric point, allowing for the ionic complexation of various growth factors during drug loading. Acidic gelatin MPs, which can be complexed with positively charged growth factors, were previously used for the controlled delivery of TGF-β1 [131, 133], TGF-β3 [129], IGF-1 [132], and BMP-2 [134] for cartilage and bone tissue engineering applications. However, since natural materials like gelatin and alginate are not native to articular cartilage, new ventures are exploring the use of more chondromimetic carriers with the aim of leveraging potential synergistic effects between growth factors and orthotopically relevant materials.

Ansboro and colleagues recently described a layer-by-layer approach for the fabrication of hollow HA microspheres as carriers of TGF-β3 [140]. In their work, the authors reported

that the delivery of TGF-β3 from HA microspheres to human MSC pellets enhanced the expression of chondrogenic genes (type II collagen and aggrecan) while inhibiting the expression of hypertrophic markers (type X collagen) *in vitro*. Lim and colleagues reported the development of nanoscale and microscale particles using chondroitin sulfate, a negatively charged glycosaminoglycan (GAG) found in articular cartilage [109], where they were able to show the controlled release of TGF-β1 from these particles. An interesting study by Bajpayee and colleagues modeled the effect of NP charge and size on particle uptake and binding in articular cartilage [141]. Using Avidin, a highly glycosylated protein with a high positive charge and a diameter of ~7 nm, as a model protein for the development of NPs as drug carriers, the authors demonstrated that while particles less than 10 nm in diameter were able to penetrate through the full-thickness of bovine cartilage explants, particles that were 15 nm in diameter were confined only to the superficial cartilage layer. Of note, the presence of a positive fixed charge density facilitated the rapid uptake of Avidin via electrostatic partitioning within 24 hrs when compared to neutrally charged NeutrAvidin and enhanced Avidin retention time to over 15 days [141]. Such results indicate that charge properties can be leveraged in innovative nanoparticle designs for the rapid uptake and retention of nanocarriers throughout the entire cartilage layer. Within the context of anti-inflammatory strategies for cartilage repair, the use MP carriers as the controlled delivery vehicles for small anti-inflammatory agents is also a subject of great interest [86, 108, 142–144].

4. Strategies for Controlling Delivery of Biologics

The plethora of studies on single growth factor delivery to induce chondrogenesis and/or facilitate neo-cartilage growth have been indispensible in showing the complexity of the cartilage wound healing environment. The current research on biologics for cartilage repair sees a trend towards multiple growth factor delivery to mimic the numerous signaling cascades involved. Yet the lack of an overwhelming improvement for multiple growth factor delivery over single growth factor delivery exemplifies the need to modulate the temporal expression and spatial distribution of biologics for not only cartilage regeneration, but for the repair of the osteochondral unit as a whole. The following sections will discuss the recent progress in the delivery of multiple biologics and spatiotemporally controlled delivery strategies for cartilage repair applications, and will highlight several advanced options for the design of biologic delivery platforms with potential for precise spatial and/or temporal control over the release of bioactive factors.

4.1 Multiple Biologics Delivery

Musculoskeletal development is an intricate process governed precisely by the activation and interplay of numerous biochemical cell-signaling pathways. In particular, the process of chondrogenesis alone involves the activation of chondrogenic adhesion molecules (integrins) and the up-regulation of chondrogenic growth factors (TGF-β superfamily) and their signal regulators, followed by the up-regulation of anabolic factors (bFGF, IGF-1, VEGF) during hypertrophy [145]. While technically simple, it is extremely unlikely that the delivery of a single factor can stimulate the recapitulation of these signaling pathways for cartilage regeneration. As a result, strides have been made towards the development of

controlled release systems for the delivery of multiple growth factors for cartilage repair in the hopes of increasing therapeutic potency. Since the work of Richardson and colleagues [146], which successfully demonstrated the controlled dual release of both VEGF and PDGF for the rapid formation of a mature vascular network, the field of controlled release has seen the advent of many sophisticated delivery systems for multiple bioactive agents. Indeed, by regulating the delivery of several bioactive factors, the induction of various biological responses in a fashion that promotes optimal cartilage repair becomes theoretically possible.

A common chondrogenic strategy involves the sequential release of TGF-β1 or TGF-β3 to first chondrogenically stimulate a synthetic response by host/delivered progenitor cells, followed by the release of an anabolic or maturation factor like IGF-1 to encourage cartilage matrix production. Our laboratory, as well as others, has previously established that the dual delivery of TGF-β1 and IGF-1 can be achieved with distinct release kinetics [132, 147], and that the released growth factors stimulate the chondrogenic gene expression of MSCs *in vitro* [136]. In the case of bulk hydrogel and NP/MP composite scaffolds, varying the phase of incorporation can modulate the release kinetics of multiple growth factors. By loading TGF-β1 directly into the bulk OPF hydrogel phase while loading IGF-1into highly crosslinked GMPs encapsulated within the OPF hydrogel, Holland and colleagues were able to attain a burst release of the former combined with the simultaneous but sustained release of the latter [132]. Another approach for the sequential delivery of TGF-β1 and IGF-1 involves the use of biodegradable PLGA MPs [148]. Jaklenec and colleagues fused IGF-1 containing and TGF-β1 containing PLGA microspheres using dichloromethane vapor in order to generate three-dimensional drug-eluting scaffolds [147]. Incorporating bovine serum albumin (BSA) into the organic phase during microsphere fabrication improved IGF-1 internalization into the microspheres and hence, delayed the release of IGF-1. The release of TGF-β1 was controlled by either capping or uncapping PLGA with a carboxylic acid chain, where uncapped PLGA led to delayed release of the growth factor due to increased secondary interactions between the PLGA, TGF-β1, and BSA [147]. The development of such a modular design allowed for the combination of PLGA microspheres containing various growth factors or bioactive agents in order to generate custom dual or multiple biologic delivery systems. Indeed, a similar system utilizing PLGA microspheres of different co-polymer ratios and spherical sizes was able to achieve multiple burst releases of encapsulated chondroitin sulfate for potential applications in treating OA [149].

A more recent system leveraged a NP-laden hydrogel composite for the dual delivery of BMP-7 and TGF-β2. TGF-β2-immobilized NPs were created by first mixing Pluronic F68 with heparin to permit hydrogen bonding between the two components [125]. TGF-β2 was subsequently immobilized via ionic complexation with the heparin component. Chitosan and polyvinyl alcohol were then added to promote the formation of individual TGF-β2 immobilized NPs, which were embedded into a BMP-7 immobilized alginate hydrogel bulk phase. The authors were able to show fast release of BMP-7 (up to 80% cumulative release) coupled with the sustained release of TGF-β2 (up to 30% cumulative release) over 21 days *in vitro* [125]. Interestingly, the delivery of both BMP-7 and TGF-β2 from the hydrogel phase, as opposed to delivery from the encapsulated NPs, was slower due to the potential aggregation between growth factors during hydrogel fabrication, highlighting the need to

examine the potential interplay between biologics in multiple growth factor delivery systems. Nevertheless, these systems coupling the delivery of BMPs with TGF-β are especially useful in applications involving MSCs derived from sources other than the bone marrow (i.e., adipose tissue), which require such growth factor combinations for chondrogenesis [150–153]. Despite our ability to tailor the release of multiple therapeutic agents in order to affect a desired cellular responses *in vitro*, one must consider the challenges of accurate preclinical translation *in vivo*. For instance, the dual delivery of TGF-β2 and BMP-7 to trochlear groove defects in rabbits, even with the co-implantation of adipose derived MSCs, failed to elicit any histological improvement in osteochondral tissue repair over controls [98]. Analogously, the co-delivery of TGF-β1 and IGF-1 via OPF/gelatin MP hydrogel composites did not offer any additional benefits over the delivery of IGF-1 alone for osteochondral tissue regeneration *in vivo* [130]. Recently, it was investigated whether the lack of additive or synergistic effects could be due to differences in TGF-β release kinetics between the *in vivo* environment and what was observed *in vitro* [44]. Using a similar OPF composite system, IGF-1 and TGF-β3 were co-delivered to an osteochondral defect site to evaluate tissue repair, where changing the loading phase of TGF-β3 varied its release kinetics. However, the results seemed to confirm a lack of synergy between these two growth factors in affecting a favorable healing response *in vivo* [44].

Bian and colleagues recently described an alternative strategy with high potential for cartilage repair [137]. Their approach entailed the initial transient exposure of MSCs encapsulated in a HA hydrogel to TGF-β3 released from co-encapsulated alginate microspheres to induce chondrogenesis, followed by the exposure to PTHrP to prevent hypertrophy. While the release of TGF-β3 induced the chondrogenesis of MSCs *in vivo* (in a subcutaneous mouse model), the uncontrolled and rapid delivery of PTHrP was unable to inhibit hypertrophic calcification [137]. Another strategy described the combined use of anti-angiogenic agent suramin and TGF-β1 for the generation of hyaline cartilage from periosteal cells on an agarose hydrogel implanted within a subperiosteal space [56]. Together, these studies highlight the complexity of the native joint environment and indicate that caution must be taken when combining multiple growth factors with similar chondrogenic stimulatory effects for controlled delivery *in vivo*. Indeed, it may prove advantageous to instead combine factors with varying anti-inflammatory, anti-angiogenic, chondrogenic, or anabolic biological effects in order to simulate biomimetic cascades and processes for more effective cartilage repair *in vivo*.

Within the context of multiple growth factor delivery, an emerging field of interest comprises the application of PRP. PRP is an enriched blend of growth factors that can be autologously derived through the centrifugation of patient blood. Several protocols exist for the isolation and preparation of PRP for specific applications [13, 154]. In contrast to the rising potential of PRP for cartilage repair, information regarding its composition and mechanisms of action remain relatively scarce. Additionally, it remains unclear how different processing techniques and donor-to-donor variability affect the composition and effectiveness of PRP. Using protein antibody membrane arrays, Krüger and colleagues recently profiled the growth factor composition of human PRP and revealed a plethora of chondrogenic and anabolic growth factors including various BMPs, FGFs, PDGFs, IGFs,

TGF-βs and VEGFs [155]. Indeed, several studies of late have reported the positive effects of PRP on cartilage repair in both diseased and acute defect models [156].

Sundman and coworkers treated osteoarthritic synovium and cartilage explants with PRP *ex vivo* and showed that PRP decreased the gene expression of inflammatory markers including TNF-α and MMP-13 while enhancing endogenous HA production [157]. Raeissadat and colleagues evaluated the effects of PRP injections on functional improvement and quality of life of OA patients in a clinical study and found that even the direct intra-articular administration of PRP ameliorated joint pain and knee stiffness, and improved patients' quality of life in the studied time frame of 6 months [158], which corroborates the results from similar investigations [159–161]. However, the lack of proper controls necessitates further investigations regarding the clinical use of PRP for OA. In an acute femoral defect model, the treatment of osteochondral grafts with PRP prior to implantation actually led to the improvement of graft-host integration when compared to grafts treated with saline solution [162], further indicating that PRP can decrease cartilage degeneration via the inhibition of inflammatory signals and the induction of neo-cartilage integration. Given the multifarious properties of PRP, future combinatory strategies can aim to leverage specific PRP effects through the co-delivery of synergistic or inhibitory factors. One potential strategy could explore the possibility of releasing the anti-angiogenic drug Avastin [163] following the delivery of PRP in order to promote a hypoxic environment, which has been shown to be vital for non-hypertrophic chondrogenesis [164] for articular cartilage repair.

4.2 Spatially Controlled Delivery

It is well known that the extracellular matrix structure of articular cartilage represents an intricate hierarchy of distinct layers that function together to meet the osmotic and viscoelastic demands of the tissue. While advances toward the utilization of multiple bioactive agents are beginning to address some of the complexities of cartilage regeneration *in vivo*, the presence of undefined and potentially negative cross-effects suggests that the method of growth factor presentation to defect sites still requires significant fine-tuning. Indeed, it is recognized that bioactive factors should ideally be delivered in a spatially and temporally controlled fashion in order to elicit maximum therapeutic efficacy. By first reviewing strategies for spatial control followed by strategies for temporal control, this section primarily highlights recent advances made toward the development of technologies or platforms that can allow precise control over spatiotemporal release conditions for cartilage repair.

For cartilage applications, spatial control over drug delivery can be mainly achieved via two distinct approaches: the conditional/permanent immobilization of chondrogenic growth factors to desired regions within a scaffold, or the directional release of chondrogenic growth factors from a reservoir. Within the context of osteochondral tissue repair, growth factor concentration gradients and multiphasic scaffolds are often applied. Out of the strategies available for spatial control, the physical entrapment or chemical conjugation of factors to a scaffold represents the most technically simple approach. Such methods for protein sequestration commonly offer several advantages including localized growth factor presentation, dosage control, and preservation of protein bioactivity. McCall and colleagues

recently investigated a strategy for TGF-β1 immobilization using clinically relevant materials [165]. TGF-β1 was first thiolated by a reaction with 2-iminothiolane (via primary amine groups on the N-terminus), and was subsequently functionalized into poly(ethylene glycol) (PEG)-diacrylate hydrogels using mixed-mode photopolymerization. By changing the initial concentration of thiolated TGF-β1 prior polymerization, it was shown that the growth factor dosage as well as bioactivity could be precisely controlled. Interestingly, co-encapsulated human MSCs, when exposed to the lower dosages of immobilized TGF-β1, actually exhibited equal or greater levels of chondrogenesis when compared to soluble TGF-β1 in culture medium [165].

Implications for the utility of spatially tethered chondrogenic growth factors to act as chondrogenic stimulants or chemoattractants for host progenitor cells in cartilage defects *in vivo* were further provided by Griffin and colleagues, who recently reported the synthesis of photodegradable macromers for the conjugation and release of biologics [166]. In their work, the authors synthesized a class of photosensitive *ortho*-nitrobenzyl (*o*-NB) macromers that could be functionalized with different reactive groups at the benzylic position. These reactive groups, which included alcohols, alkyl halides, amines, carboxylic acids, N-hydroxysuccinyl ester, and biotin, permitted the conjugation of essentially any type of therapeutic agent for cartilage repair. The mechanistic release of such therapeutics could then be externally controlled by light exposure. Additionally, it was previously reported by the same group that *o*-NB macromers could be designed with different photodegradation rates, hence yielding external control over the multistaged release of multiple bioactive factors [167]. As a proof of concept, Griffin and coworkers incorporated *o*-NB macromers that were conjugated with TGF-β1 into PEG hydrogels and demonstrated that photoreleased TGF-β1 maintained high bioactivity and was able to effectively induce the chondrogenic differentiation of human MSCs *in vitro*. The development of such platforms confers researchers with not only options for the precise external control over the spatial presentation of bioactive factors in potential strategies for cartilage repair, but also the temporal patterning of bioactive factor delivery.

While many systems have been designed for the spatially controlled delivery of growth factors to cartilage tissues as a whole, few studies if any have employed spatial gradients with high enough resolution to target the subtle inhomogeneity of the hierarchical articular cartilage makeup. However, as aforementioned, recent innovations using Avidin as a model protein for articular cartilage drug uptake [141] indicate that the particle diameter and fixed charge density of drug-loaded NP systems could be finely tuned to affect NP uptake depth and retention time in order to target distinct zones within the cartilage layer. Currently, spatial concentration gradients usually consist of lower resolution biphasic or multiphasic systems built to stimulate the simultaneous repair of cartilage and subchondral bone in osteochondral composite tissues. In such cases, bilayered composites can easily be used to bias the local delivery of chondrogenic factors to the cartilage layer and osteogenic factors to the subchondral layer in osteochondral defects. As discussed earlier in the section describing the delivery of multiple biologics for cartilage repair, our laboratory has previously evaluated the utility of bilayered OPF composite systems for the spatially

controlled delivery of chondrogenic factors IGF-1 and TGF-β1 or TGF-β3 only to the chondral regions of an osteochondral defect [44, 130].

In line with the need for high spatial resolution scaffolds and interfacial considerations between the cartilage and bone layers of osteochondral composite tissues, Wang and colleagues developed a BMP-2 and IGF-1 gradient biopolymer system with the ability to elicit the corresponding osteogenic and chondrogenic response of MSCs [126]. Using an aqueous-derived silk porous scaffold, silk microspheres loaded with BMP-2 or IGF-1 were differentially mixed together via a gradient maker to generate either single BMP-2/IGF-1 gradients or dual reverse gradients of both growth factors in a single scaffold. However, it was found that only silk scaffolds delivering BMP-2 gradients (i.e., single BMP-2 gradients or reverse BMP-2/IGF-1 gradients) were effective at promoting a gradient response [126], indicating that bioactive agents must be compatible with the delivery platform. More recently, Dormer and coworkers described the creation of a PLGA microsphere-based bioactive plug with a continuous gradient transition between chondrogenic TGF-β1 and osteogenic BMP-2 for osteochondral tissue repair *in vivo* [121]. When implanted into medial femoral defects in rabbits, it was shown that the presence of a reverse continuous gradient of chondrogenic and osteogenic growth factors led to improved histological repair over blank controls. While results highlight the potential of growth factor gradient design for osteochondral tissue regeneration, the lack of controls that test specifically the effect of the gradient on tissue repair necessitates future studies evaluating such an effect.

In contrast to growth factor gradient designs, Li and coworkers developed a unique spatial control strategy based on inhomogeneous bilayered collagen scaffolds that could bias the direction of growth factor release [168]. The described construct comprised a dense collagen layer and a loose collagen layer sandwiching a reservoir of chitosan-heparin NPs loaded with growth factors for the directional release of the anabolic factor bFGF [168]. bFGF was preferentially released through the loose collagen layer, thereby making the loose layer a better cell-adhesive and proliferative substrate with potential for tissue repair *in vivo*. The same release of bFGF was not observed for the dense layer. When this bFGF release platform was utilized for articular cartilage repair in osteochondral defects *in vivo*, the authors showed that the directional release of bFGF toward the subchondral bone (i.e., loose layer facing subchondral bone) stimulated the early up-regulation of endogenous TGF-βs, BMPs, and VEGFs as detected in the synovial fluid [169]. Additionally, the controlled and directional release of bFGF toward the subchondral bone in an osteochondral defect improved histological scores for cartilage repair. Furthermore, such a spatially oriented approach limits the release of growth factors into the synovial space and hence, preserves the supply and longevity of growth factors for optimal therapeutic efficacy.

4.3 Temporally Controlled Delivery

Most, if not all, controlled release strategies enabling spatial control over the delivery of biologics also confer some degree of temporal control. Hence, the following will highlight several recent advances specifically emphasizing precise temporal control for the release of bioactive agents in the context of cartilage-related applications. Accordingly, one class of biomaterials that can offer improved temporal control over traditional delivery systems is

self-assembling peptides. Kopesky and coworkers recently reported the slow and sustained release of TGF-β1 from acellular self-assembled peptide formed from AcN-$(KLDL)_3$-CNH_2 custom peptide sequences [105]. Such KLD peptides were able to efficiently uptake growth factors, which could be loaded into the equilibrium peptide solution before or after self-assembly, with five times greater uptake before self-assembly. This provided the ability to precisely modulate the dosage of growth factors to be released. It was shown that by day 3, only 18% percent of the total TGF-β1 loaded was released, indicating the avoidance of a burst release [105]. By day 21, only 44% of cumulative TGF-β1 was achieved, suggesting the utility of KLD hydrogels for long-term controlled growth factor release and for avoiding the dosage limitations of other single growth factor delivery systems. Furthermore, KLD hydrogels could theoretically maintain growth factors in their bioactive macromolecular form efficiently through designed electrostatic interactions during peptide sequencing. Despite the advantages of such long-term release strategies, the efficacy of delivered peptides and proteins commonly face issues related to short half-lives. To address this problem, Ashley and colleagues developed a generic drug delivery platform based on a tetra-PEG porous hydrogel incorporating β-eliminative linkers that could undergo β-eliminative cleavage in the presence of an electron withdrawing modulator for the release of covalently tethered drugs [170]. The β-eliminative linkers, which were not prone to enzymatic degradation, could be designed to have highly predictable half-lives and could be used to simultaneously conjugate bioactive factors and crosslink PEG hydrogels. The covalent fixation of therapeutics to and their subsequent release from the β-eliminative linkers could then be directly controlled by the half-life of the linker, which can be designed to range from a few hours to over a year [170]. Hence, by combining β-eliminative linkers with short half-lives for drug tethering and those with longer half-lives for hydrogel crosslinking, one can ensure the complete release of growth factors prior to hydrogel degradation. Such a strategy for osteochondral defect repair would allow one to take full biomechanical advantage of having hydrogel structural support while mitigating any form of release kinetics limitations associated with bulk material degradation. Additionally, the ability to fine-tune the temporal release of biologics and to potentially decouple spatial and temporal modulation should inspire future innovations toward the design of growth factor delivery patterns that mimic the cartilage signaling cascades during fetal development in order to stimulate robust cartilage regeneration. Table 2 lists various strategies that have leveraged the use of spatial and/or temporal control for the delivery of biologics for cartilage repair applications.

Other approaches toward achieving temporal control over growth factor presentation typically involve more indirect means via the use of gene therapy. Indeed, many pharmacotherapy strategies that currently exist in the literature describe the use of gene therapy, or the viral/non-viral conditioning of cells into endogenous growth factor depots, for cartilage and osteochondral repair. However, it is not clearly known how the effectiveness of such strategies compares with those that rely on the controlled delivery of exogenous factors. Using bovine articular chondrocytes, Shi and coworkers compared the effectiveness of exogenously and endogenously delivered IGF-1 and TGF-β1 for *in vitro* chondrogenesis [171]. The authors showed no difference between exogenously or endogenously delivered IGF-1, but found that exogenously delivered TGF-β1 elicited

greater chondrogenic gene expression when compared to endogenously delivered TGF-β1. This was confirmed to be due to the non-covalent complexation of a latency-associated peptide with TGF-β1 during endogenous production to form a small latent complex, which was then bound by latent TGF-β1 binding protein to form a large latent complex [171]. While this preserved growth factor bioactivity, the complex was shielded from TGF-β1 receptors on the chondrocyte surfaces. These results suggests that while the endogenous delivery of certain bioactive factors via gene therapy may prove beneficial for cartilage repair, not all factors are suitable with this form of delivery. The current state of gene therapy as a means for temporally controlled chondrogenic growth factor delivery is aptly reflected by the work of Lu and colleagues, who recently reported the utility of a chitosan-based gene-activated matrix encapsulating chitosan/HA NPs carrying plasmids for the prolonged delivery of plasmid genes for transfection [172]. In their application, the authors leveraged a hybrid NP system for the delivery of plasmids encoding TGF-β1, where the presence of HA theoretically improved the transfection efficiency and also provided a substrate with which cells could interact. It was shown that the release of plasmids was sustained for over 120 days *in vitro*, and that the released plasmids stimulated the proliferation of seeded chondrocytes. These results demonstrate that the main advantage of sustained gene therapy when compared to conventional exogenous delivery is the ability to control the timely administration of growth factors in an endogenously relevant fashion, therefore highlighting the potential of such strategies for effective cartilage repair.

5. Conclusion

Despite its perceived simplicity, the consistent repair of articular cartilage still remains a significant clinical challenge. However, the advent of innovative tissue engineering technologies is beginning to address many of the major shortcomings of current clinical approaches. Specifically, tissue engineering approaches leveraging the use of release platforms that offer spatiotemporal control over the delivery of bioactive factors are eliciting favorable outcomes *in vitro* and *in vivo*. In a relatively short time span, release systems for the delivery of biologics have evolved from simple intra-articular modalities into complex multifunctional and modular delivery platforms. While such systems have generally employed the benefits of chondrogenic growth factors in cartilage repair applications, new approaches are now finding utility in the use of biologics with primary effects on progenitor cells other than chondrogenic or anabolic stimulation. As highlighted, therapeutic treatment of cartilage repair could also be garnered from the delivery of various anti-inflammatory, anti-angiogenic, or chondroprotective agents. The delivery of multiple factors simultaneously or in a spatiotemporally designed fashion for cartilage repair applications, as evidenced by the use of biphasic delivery scaffolds or growth factor gradients, is also becoming a popular area of research. Yet, as new findings show, the delivery of more factors may not necessarily elicit additive or synergistic effects in cartilage regeneration. Additionally, studies are now beginning to show that certain bioactive factors may only be compatible with certain delivery platforms. Hence, future efforts should aim to identify these key biologic-to-biologic and biologic-to-platform interactions as such information will be critical to the success of future strategies leveraging the delivery of various biologics for cartilage repair.

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Abbreviations

3,4,6-*O*-Bu$_3$GlcNAc	3,4,6-*O*-tributanoylated-*N*-acetylglucosamine
ASC	Adipose-derived stem cells
bFGF	Basic fibroblast growth factor
BMP-2	Bone morphogenetic protein 2
BMP-4	Bone morphogenetic protein 4
BMP-6	Bone morphogenetic protein 6
BMP-7	Bone morphogenetic protein 7
BMP-9	Bone morphogenetic protein 9
BSA	Bovine serum albumin
ECM	Extracellular matrix
FGF-2	Fibroblast growth factor 2
GAG	Glycosaminoglycan
GDF-5	Growth differentiation factor 5
HA	Hyaluronic acid
IGF-1	Insulin-like growth factor 1
IL-1Ra	Interleukin 1 receptor antagonist
IL-1	Interleukin 1
MMP	Matrix metalloproteinase
MP	Microparticle
MSC	Mesenchymal stem cell
NP	Nanoparticle
***o*-NB**	*ortho*-nitrobenzyl
OA	Osteoarthritis
OPF	Oligo(poly(ethylene glycol) fumarate)
PDGF	Platelet-derived growth factor
PEG	Poly(ethylene glycol)
PLGA	Poly(lactic-co-glycolic acid)
PRP	Platelet-rich plasma
PTH	Parathyroid hormone

PTHrP	Parathyroid hormone related peptide
SDF-1	Stromal-derived factor 1
TGF-β1	Transforming growth factor β1
TGF-β2	Transforming growth factor β2
TGF-β3	Transforming growth factor β3
TNF-α	Tissue necrosis factor α
VEGF	Vascular endothelial growth factor

References

1. Nukavarapu SP, Dorcemus DL. Osteochondral tissue engineering: current strategies and challenges. Biotechnol Adv. 2013; 31:706–721. [PubMed: 23174560]
2. Marcacci M, Filardo G, Kon E. Treatment of cartilage lesions: what works and why? Injury. 2013; 44(Suppl 1):S11–15. [PubMed: 23351863]
3. Mithoefer K. Complex articular cartilage restoration. Sports Med Arthrosc. 2013; 21:31–37. [PubMed: 23314266]
4. Phillips AM. Overview of the fracture healing cascade. Injury. 2005; 36(Suppl 3):S5–7. [PubMed: 16188551]
5. Chen FM, Zhang M, Wu ZF. Toward delivery of multiple growth factors in tissue engineering. Biomaterials. 2010; 31:6279–6308. [PubMed: 20493521]
6. Bos PK, van Osch GJ, Frenz DA, Verhaar JA, Verwoerd-Verhoef HL. Growth factor expression in cartilage wound healing: temporal and spatial immunolocalization in a rabbit auricular cartilage wound model. Osteoarthritis Cartilage. 2001; 9:382–389. [PubMed: 11399103]
7. Brochhausen C, Lehmann M, Halstenberg S, Meurer A, Klaus G, Kirkpatrick CJ. Signalling molecules and growth factors for tissue engineering of cartilage-what can we learn from the growth plate? J Tissue Eng Regen Med. 2009; 3:416–429. [PubMed: 19575393]
8. Santo VE, Gomes ME, Mano JF, Reis RL. Controlled release strategies for bone, cartilage, and osteochondral engineering--Part I: recapitulation of native tissue healing and variables for the design of delivery systems. Tissue Eng Part B Rev. 2013; 19:308–326. [PubMed: 23268651]
9. Chen FS, Frenkel SR, Di Cesare PE. Repair of articular cartilage defects: part I. Basic Science of cartilage healing. Am J Orthop (Belle Mead NJ). 1999; 28:31–33. [PubMed: 10048355]
10. Gaissmaier C, Koh JL, Weise K. Growth and differentiation factors for cartilage healing and repair. Injury. 2008; 39(Suppl 1):S88–96. [PubMed: 18313476]
11. Frisbie DD, Oxford JT, Southwood L, Trotter GW, Rodkey WG, Steadman JR, Goodnight JL, McIlwraith CW. Early events in cartilage repair after subchondral bone microfracture. Clin Orthop Relat Res. 2003:215–227. [PubMed: 12567150]
12. Mehta M, Schmidt-Bleek K, Duda GN, Mooney DJ. Biomaterial delivery of morphogens to mimic the natural healing cascade in bone. Adv Drug Deliv Rev. 2012; 64:1257–1276. [PubMed: 22626978]
13. Santo VE, Gomes ME, Mano JF, Reis RL. Controlled release strategies for bone, cartilage, and osteochondral engineering--Part II: challenges on the evolution from single to multiple bioactive factor delivery. Tissue Eng Part B Rev. 2013; 19:327–352. [PubMed: 23249320]
14. Lee K, Silva EA, Mooney DJ. Growth factor delivery-based tissue engineering: general approaches and a review of recent developments. Journal of the Royal Society Interface. 2011; 8:153–170.
15. Fortier LA, Barker JU, Strauss EJ, McCarrel TM, Cole BJ. The role of growth factors in cartilage repair. Clin Orthop Relat Res. 2011; 469:2706–2715. [PubMed: 21403984]

16. Schmidt MB, Chen EH, Lynch SE. A review of the effects of insulin-like growth factor and platelet derived growth factor on in vivo cartilage healing and repair. Osteoarthritis Cartilage. 2006; 14:403–412. [PubMed: 16413799]
17. Horbelt D, Denkis A, Knaus P. A portrait of Transforming Growth Factor beta superfamily signalling: Background matters. Int J Biochem Cell Biol. 2012; 44:469–474. [PubMed: 22226817]
18. Appel B, Baumer J, Eyrich D, Sarhan H, Toso S, Englert C, Skodacek D, Ratzinger S, Grassel S, Goepferich A, Blunk T. Synergistic effects of growth and differentiation factor-5 (GDF-5) and insulin on expanded chondrocytes in a 3-D environment. Osteoarthritis Cartilage. 2009; 17:1503–1512. [PubMed: 19470416]
19. Coleman CM, Vaughan EE, Browe DC, Mooney E, Howard L, Barry F. Growth differentiation factor-5 enhances in vitro mesenchymal stromal cell chondrogenesis and hypertrophy. Stem Cells Dev. 2013; 22:1968–1976. [PubMed: 23388029]
20. Feng G, Wan Y, Balian G, Laurencin CT, Li X. Adenovirus-mediated expression of growth and differentiation factor-5 promotes chondrogenesis of adipose stem cells. Growth Factors. 2008; 26:132–142. [PubMed: 18569021]
21. Zhang B, Yang S, Sun Z, Zhang Y, Xia T, Xu W, Ye S. Human mesenchymal stem cells induced by growth differentiation factor 5: an improved self-assembly tissue engineering method for cartilage repair. Tissue Eng Part C Methods. 2011; 17:1189–1199. [PubMed: 21875359]
22. Cals FL, Hellingman CA, Koevoet W, Baatenburg de Jong RJ, van Osch GJ. Effects of transforming growth factor-beta subtypes on in vitro cartilage production and mineralization of human bone marrow stromal-derived mesenchymal stem cells. J Tissue Eng Regen Med. 2012; 6:68–76. [PubMed: 21305699]
23. Fan H, Tao H, Wu Y, Hu Y, Yan Y, Luo Z. TGF-beta3 immobilized PLGA-gelatin/chondroitin sulfate/hyaluronic acid hybrid scaffold for cartilage regeneration. J Biomed Mater Res A. 2010; 95:982–992. [PubMed: 20872747]
24. Wang W, Li B, Yang J, Xin L, Li Y, Yin H, Qi Y, Jiang Y, Ouyang H, Gao C. The restoration of full-thickness cartilage defects with BMSCs and TGF-beta 1 loaded PLGA/fibrin gel constructs. Biomaterials. 2010; 31:8964–8973. [PubMed: 20822812]
25. Mierisch CM, Cohen SB, Jordan LC, Robertson PG, Balian G, Diduch DR. Transforming growth factor-beta in calcium alginate beads for the treatment of articular cartilage defects in the rabbit. Arthroscopy. 2002; 18:892–900. [PubMed: 12368788]
26. Holland TA, Bodde EW, Cuijpers VM, Baggett LS, Tabata Y, Mikos AG, Jansen JA. Degradable hydrogel scaffolds for in vivo delivery of single and dual growth factors in cartilage repair. Osteoarthritis Cartilage. 2007; 15:187–197. [PubMed: 16965923]
27. Guo X, Park H, Young S, Kretlow JD, van den Beucken JJ, Baggett LS, Tabata Y, Kasper FK, Mikos AG, Jansen JA. Repair of osteochondral defects with biodegradable hydrogel composites encapsulating marrow mesenchymal stem cells in a rabbit model. Acta Biomater. 2010; 6:39–47. [PubMed: 19660580]
28. Bakker AC, van de Loo FAJ, van Beuningen HM, Sime P, van Lent PLEM, van der Kraan PM, Richards CD, van den Berg WB. Overexpression of active TGF-beta-1 in the murine knee joint: evidence for synovial-layer-dependent chondro-osteophyte formation. Osteoarthritis and Cartilage. 2001; 9:128–136. [PubMed: 11237660]
29. van Beuningen HM, Glansbeek HL, van der Kraan PM, van den Berg WB. Osteoarthritis-like changes in the murine knee joint resulting from intra-articular transforming growth factor-beta injections. Osteoarthritis and Cartilage. 2000; 8:25–33. [PubMed: 10607496]
30. Patil AS, Sable RB, Kothari RM. An update on transforming growth factor-beta (TGF-beta): sources, types, functions and clinical applicability for cartilage/bone healing. J Cell Physiol. 2011; 226:3094–3103. [PubMed: 21344394]
31. Quintana L, zur Nieden NI, Semino CE. Morphogenetic and regulatory mechanisms during developmental chondrogenesis: new paradigms for cartilage tissue engineering. Tissue Eng Part B Rev. 2009; 15:29–41. [PubMed: 19063663]
32. Puetzer JL, Petitte JN, Loboa EG. Comparative review of growth factors for induction of three-dimensional in vitro chondrogenesis in human mesenchymal stem cells isolated from bone marrow and adipose tissue. Tissue Eng Part B Rev. 2010; 16:435–444. [PubMed: 20196646]

33. Reyes R, Delgado A, Sanchez E, Fernandez A, Hernandez A, Evora C. Repair of an osteochondral defect by sustained delivery of BMP-2 or TGFbeta1 from a bilayered alginate-PLGA scaffold. J Tissue Eng Regen Med. 2012
34. Yang HS, La WG, Bhang SH, Kim HJ, Im GI, Lee H, Park JH, Kim BS. Hyaline cartilage regeneration by combined therapy of microfracture and long-term bone morphogenetic protein-2 delivery. Tissue Eng Part A. 2011; 17:1809–1818. [PubMed: 21366427]
35. Lopiz-Morales Y, Abarrategi A, Ramos V, Moreno-Vicente C, Lopez-Duran L, Lopez-Lacomba JL, Marco F. In vivo comparison of the effects of rhBMP-2 and rhBMP-4 in osteochondral tissue regeneration. Eur Cell Mater. 2010; 20:367–378. [PubMed: 21154243]
36. Kuo AC, Rodrigo JJ, Reddi AH, Curtiss S, Grotkopp E, Chiu M. Microfracture and bone morphogenetic protein 7 (BMP-7) synergistically stimulate articular cartilage repair. Osteoarthritis Cartilage. 2006; 14:1126–1135. [PubMed: 16765606]
37. Danisovic L, Varga I, Polak S. Growth factors and chondrogenic differentiation of mesenchymal stem cells. Tissue Cell. 2012; 44:69–73. [PubMed: 22185680]
38. Nixon AJ, Fortier LA, Williams J, Mohammed H. Enhanced repair of extensive articular defects by insulin-like growth factor-I-laden fibrin composites. J Orthop Res. 1999; 17:475–487. [PubMed: 10459752]
39. Tuncel M, Halici M, Canoz O, Yildirim Turk C, Oner M, Ozturk F, Kabak S. Role of insulin like growth factor-I in repair response in immature cartilage. Knee. 2005; 12:113–119. [PubMed: 15749446]
40. Tiwary R, Amarpal, Aithal HP, Kinjavdekar P, Pawde AM, Singh R. Effect of IGF-1 and Uncultured Autologous Bone-Marrow-Derived Mononuclear Cells on Repair of Osteochondral Defect in Rabbits. Cartilage. 2014; 5:43–54.
41. Fortier LA, Mohammed HO, Lust G, Nixon AJ. Insulin-like growth factor-I enhances cell-based repair of articular cartilage. J Bone Joint Surg Br. 2002; 84:276–288. [PubMed: 11922373]
42. Elder BD, Athanasiou KA. Systematic assessment of growth factor treatment on biochemical and biomechanical properties of engineered articular cartilage constructs. Osteoarthritis Cartilage. 2009; 17:114–123. [PubMed: 18571441]
43. Li WJ, Jiang YJ, Tuan RS. Cell-nanofiber-based cartilage tissue engineering using improved cell seeding, growth factor, and bioreactor technologies. Tissue Eng Part A. 2008; 14:639–648. [PubMed: 18419231]
44. Kim K, Lam J, Lu S, Spicer PP, Lueckgen A, Tabata Y, Wong ME, Jansen JA, Mikos AG, Kasper FK. Osteochondral tissue regeneration using a bilayered composite hydrogel with modulating dual growth factor release kinetics in a rabbit model. Journal of Controlled Release. 2013; 168:166–178. [PubMed: 23541928]
45. Ellman MB, An HS, Muddasani P, Im HJ. Biological impact of the fibroblast growth factor family on articular cartilage and intervertebral disc homeostasis. Gene. 2008; 420:82–89. [PubMed: 18565695]
46. Hellingman CA, Koevoet W, Kops N, Farrell E, Jahr H, Liu W, de Jong RJB, Frenz DA, van Osch GJVM. Fibroblast Growth Factor Receptors in In Vitro and In Vivo Chondrogenesis: Relating Tissue Engineering Using Adult Mesenchymal Stem Cells to Embryonic Development. Tissue Engineering Part A. 2010; 16:545–556. [PubMed: 19728793]
47. Solchaga LA, Penick K, Goldberg VM, Caplan AI, Welter JF. Fibroblast Growth Factor-2 Enhances Proliferation and Delays Loss of Chondrogenic Potential in Human Adult Bone-Marrow-Derived Mesenchymal Stem Cells. Tissue Engineering Part A. 2010; 16:1009–1019. [PubMed: 19842915]
48. Ishii I, Mizuta H, Sei A, Hirose J, Kudo S, Hiraki Y. Healing of full-thickness defects of the articular cartilage in rabbits using fibroblast growth factor-2 and a fibrin sealant. Journal of Bone and Joint Surgery-British Volume. 2007; 89B:693–700.
49. Maehara H, Sotome S, Yoshii T, Torigoe I, Kawasaki Y, Sugata Y, Yuasa M, Hirano M, Mochizuki N, Kikuchi M, Shinomiya K, Okawa A. Repair of Large Osteochondral Defects in Rabbits Using Porous Hydroxyapatite/Collagen (HAp/Col) and Fibroblast Growth Factor-2 (FGF-2). Journal of Orthopaedic Research. 2010; 28:677–686. [PubMed: 19918893]

50. Khan IM, Palmer EA, Archer CW. Fibroblast growth factor-2 induced chondrocyte cluster formation in experimentally wounded articular cartilage is blocked by soluble Jagged-1. Osteoarthritis and Cartilage. 2010; 18:208–219. [PubMed: 19748609]
51. Toh WS, Spector M, Lee EH, Cao T. Biomaterial-mediated delivery of microenvironmental cues for repair and regeneration of articular cartilage. Mol Pharm. 2011; 8:994–1001. [PubMed: 21500855]
52. Sun XD, Jeng L, Bolliet C, Olsen BR, Spector M. Non-viral endostatin plasmid transfection of mesenchymal stem cells via collagen scaffolds. Biomaterials. 2009; 30:1222–1231. [PubMed: 19059640]
53. Jeng L, Olsen BR, Spector M. Engineering endostatin-expressing cartilaginous constructs using injectable biopolymer hydrogels. Acta Biomater. 2012; 8:2203–2212. [PubMed: 22370447]
54. Jeng L, Olsen BR, Spector M. Engineering endostatin-producing cartilaginous constructs for cartilage repair using nonviral transfection of chondrocyte-seeded and mesenchymal-stem-cell-seeded collagen scaffolds. Tissue Eng Part A. 2010; 16:3011–3021. [PubMed: 20545556]
55. Hunziker EB, Driesang IM. Functional barrier principle for growth-factor-based articular cartilage repair. Osteoarthritis Cartilage. 2003; 11:320–327. [PubMed: 12744937]
56. Emans PJ, van Rhijn LW, Welting TJ, Cremers A, Wijnands N, Spaapen F, Voncken JW, Shastri VP. Autologous engineering of cartilage. Proc Natl Acad Sci U S A. 2010; 107:3418–3423. [PubMed: 20133690]
57. Matsumoto T, Cooper GM, Gharaibeh B, Meszaros LB, Li G, Usas A, Fu FH, Huard J. Cartilage repair in a rat model of osteoarthritis through intraarticular transplantation of muscle-derived stem cells expressing bone morphogenetic protein 4 and soluble Flt-1. Arthritis Rheum. 2009; 60:1390–1405. [PubMed: 19404941]
58. Kubo S, Cooper GM, Matsumoto T, Phillippi JA, Corsi KA, Usas A, Li G, Fu FH, Huard J. Blocking vascular endothelial growth factor with soluble Flt-1 improves the chondrogenic potential of mouse skeletal muscle-derived stem cells. Arthritis Rheum. 2009; 60:155–165. [PubMed: 19116905]
59. Nagai T, Sato M, Kutsuna T, Kokubo M, Ebihara G, Ohta N, Mochida J. Intravenous administration of anti-vascular endothelial growth factor humanized monoclonal antibody bevacizumab improves articular cartilage repair. Arthritis Res Ther. 2010; 12:R178. [PubMed: 20868495]
60. Lau TT, Wang DA. Stromal cell-derived factor-1 (SDF-1): homing factor for engineered regenerative medicine. Expert Opinion on Biological Therapy. 2011; 11:189–197. [PubMed: 21219236]
61. Sukegawa A, Iwasaki N, Kasahara Y, Onodera T, Igarashi T, Minami A. Repair of Rabbit Osteochondral Defects by an Acellular Technique with an Ultrapurified Alginate Gel Containing Stromal Cell-Derived Factor-1. Tissue Engineering Part A. 2012; 18:934–945. [PubMed: 22097896]
62. Zhang F, Leong WY, Su K, Fang Y, Wang DA. A Transduced Living Hyaline Cartilage Graft Releasing Transgenic Stromal Cell-Derived Factor-1 Inducing Endogenous Stem Cell Homing In Vivo. Tissue Engineering Part A. 2013; 19:1091–1099. [PubMed: 23167782]
63. Orth P, Cucchiarini M, Zurakowski D, Menger MD, Kohn DM, Madry H. Parathyroid hormone [1–34] improves articular cartilage surface architecture and integration and subchondral bone reconstitution in osteochondral defects in vivo. Osteoarthritis and Cartilage. 2013; 21:614–624. [PubMed: 23353669]
64. Kudo S, Mizuta H, Takagi K, Hiraki Y. Cartilaginous repair of full-thickness articular cartilage defects is induced by the intermittent activation of PTH/PTHrP signaling. Osteoarthritis and Cartilage. 2011; 19:886–894. [PubMed: 21571083]
65. Zhang W, Chen JL, Tao JD, Hu CC, Chen LK, Zhao HS, Xu GW, Heng BC, Ouyang HW. The promotion of osteochondral repair by combined intra-articular injection of parathyroid hormone-related protein and implantation of a bi-layer collagen-silk scaffold. Biomaterials. 2013; 34:6046–6057. [PubMed: 23702148]

66. Freyria AM, Mallein-Gerin F. Chondrocytes or adult stem cells for cartilage repair: The indisputable role of growth factors. Injury-International Journal of the Care of the Injured. 2012; 43:259–265.

67. Dang AC, Warren AP, Kim HT. Beneficial effects of intra-articular caspase inhibition therapy following osteochondral injury. Osteoarthritis and Cartilage. 2006; 14:526–532. [PubMed: 16483806]

68. Gilbert SJ, Singhrao SK, Khan IM, Gonzalez LG, Thomson BM, Burdon D, Duance VC, Archer CW. Enhanced Tissue Integration During Cartilage Repair In Vitro Can Be Achieved by Inhibiting Chondrocyte Death at the Wound Edge. Tissue Engineering Part A. 2009; 15:1739–1749. [PubMed: 19119922]

69. Yamamoto A, Warren AP, Kim HT. Minocycline reduces articular cartilage damage following osteochondral injury. Knee. 2012; 19:680–683. [PubMed: 22297212]

70. Chen P, Zhu S, Wang Y, Mu Q, Wu Y, Xia Q, Zhang X, Sun H, Tao J, Hu H, Lu P, Ouyang H. The amelioration of cartilage degeneration by ADAMTS-5 inhibitor delivered in a hyaluronic acid hydrogel. Biomaterials. 2014; 35:2827–2836. [PubMed: 24424207]

71. Elmorsy S, Funakoshi T, Sasazawa F, Todoh M, Tadano S, Iwasaki N. Chondroprotective effects of high-molecular-weight cross-linked hyaluronic acid in a rabbit knee osteoarthritis model. Osteoarthritis Cartilage. 2014; 22:121–127. [PubMed: 24185110]

72. Kwiecinski JJ, Dorosz SG, Ludwig TE, Abubacker S, Cowman MK, Schmidt TA. The effect of molecular weight on hyaluronan's cartilage boundary lubricating ability--alone and in combination with proteoglycan 4. Osteoarthritis Cartilage. 2011; 19:1356–1362. [PubMed: 21872669]

73. Li J, Gorski DJ, Anemaet W, Velasco J, Takeuchi J, Sandy JD, Plaas A. Hyaluronan injection in murine osteoarthritis prevents TGFbeta 1-induced synovial neovascularization and fibrosis and maintains articular cartilage integrity by a CD44-dependent mechanism. Arthritis Res Ther. 2012; 14:R151. [PubMed: 22721434]

74. Li P, Raitcheva D, Hawes M, Moran N, Yu X, Wang F, Matthews GL. Hylan G-F 20 maintains cartilage integrity and decreases osteophyte formation in osteoarthritis through both anabolic and anti-catabolic mechanisms. Osteoarthritis Cartilage. 2012; 20:1336–1346. [PubMed: 22809835]

75. Roth A, Mollenhauer J, Wagner A, Fuhrmann R, Straub A, Venbrocks RA, Petrow P, Braeuer R, Schubert H, Ozegowski J, Peschel G, Muller PJ, Kinne RW. Intra-articular injections of high-molecular-weight hyaluronic acid have biphasic effects on joint inflammation and destruction in rat antigen-induced arthritis. Arthritis Research & Therapy. 2005; 7:R677–R686. [PubMed: 15899053]

76. Seror J, Merkher Y, Kampf N, Collinson L, Day AJ, Maroudas A, Klein J. Articular cartilage proteoglycans as boundary lubricants: structure and frictional interaction of surface-attached hyaluronan and hyaluronan--aggrecan complexes. Biomacromolecules. 2011; 12:3432–3443. [PubMed: 21823600]

77. Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier JP, Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. Nature Reviews Rheumatology. 2011; 7:33–42.

78. Bannuru RR, Natov NS, Obadan IE, Price LL, Schmid CH, McAlindon TE. Therapeutic Trajectory of Hyaluronic Acid Versus Corticosteroids in the Treatment of Knee Osteoarthritis: A Systematic Review and Meta-Analysis. Arthritis & Rheumatism-Arthritis Care & Research. 2009; 61:1704–1711. [PubMed: 19950318]

79. Allen KD, Adams SB, Setton LA. Evaluating Intra-Articular Drug Delivery for the Treatment of Osteoarthritis in a Rat Model. Tissue Engineering Part B-Reviews. 2010; 16:81–92. [PubMed: 19943805]

80. Akasaki Y, Matsuda S, Nakayama K, Fukagawa S, Miura H, Iwamoto Y. Mevastatin reduces cartilage degradation in rabbit experimental osteoarthritis through inhibition of synovial inflammation. Osteoarthritis and Cartilage. 2009; 17:235–243. [PubMed: 18672387]

81. Joosten LAB, Leoni F, Meghji S, Mascagni P. Inhibition of HDAC Activity by ITF2357 Ameliorates Joint Inflammation and Prevents Cartilage and Bone Destruction in Experimental Arthritis. Molecular Medicine. 2011; 17:391–396. [PubMed: 21327299]

82. Bedouet L, Pascale F, Bonneau M, Laurent A. In vitro evaluation of S-(+)-ibuprofen as drug candidate for intra-articular drug delivery system. Drug Dev Ind Pharm. 2013

83. Brochhausen C, Sanchez N, Halstenberg S, Zehbe R, Watzer B, Schmitt VH, Hofmann A, Meurer A, Unger RE, Kirkpatrick CJ. Phenotypic redifferentiation and cell cluster formation of cultured human articular chondrocytes in a three-dimensional oriented gelatin scaffold in the presence of PGE2--first results of a pilot study. J Biomed Mater Res A. 2013; 101:2374–2382. [PubMed: 23377957]
84. Brochhausen C, Zehbe R, Gross U, Libera J, Schubert H, Nusing RM, Klaus G, Kirkpatrick CJ. Cyclooxygenases (COX-1 and COX-2) for tissue engineering of articular cartilage--from a developmental model to first results of tissue and scaffold expression. Biomed Mater Eng. 2008; 18:15–23. [PubMed: 18198403]
85. Watzer B, Zehbe R, Halstenberg S, James Kirkpatrick C, Brochhausen C. Stability of prostaglandin E(2) (PGE (2)) embedded in poly-D,L: -lactide-co-glycolide microspheres: a preconditioning approach for tissue engineering applications. J Mater Sci Mater Med. 2009; 20:1357–1365. [PubMed: 19160024]
86. Ko JY, Choi YJ, Jeong GJ, Im GI. Sulforaphane-PLGA microspheres for the intra-articular treatment of osteoarthritis. Biomaterials. 2013; 34:5359–5368. [PubMed: 23601658]
87. Madry H, Rey-Rico A, Venkatesan JK, Johnstone B, Cucchiarini M. Transforming Growth Factor Beta-Releasing Scaffolds for Cartilage Tissue Engineering. Tissue Eng Part B Rev. 2013
88. Elford PR, Graeber M, Ohtsu H, Aeberhard M, Legendre B, Wishart WL, MacKenzie AR. Induction of swelling, synovial hyperplasia and cartilage proteoglycan loss upon intra-articular injection of transforming growth factor beta-2 in the rabbit. Cytokine. 1992; 4:232–238. [PubMed: 1498258]
89. Mi Z, Ghivizzani SC, Lechman E, Glorioso JC, Evans CH, Robbins PD. Adverse effects of adenovirus-mediated gene transfer of human transforming growth factor beta 1 into rabbit knees. Arthritis Res Ther. 2003; 5:R132–139. [PubMed: 12723985]
90. Davidson ENB, Vitters EL, van den Berg WB, van der Kraan PM. TGF beta-induced cartilage repair is maintained but fibrosis is blocked in the presence of Smad7. Arthritis Research & Therapy. 2006; 8
91. Schmidt MB, Chen EH, Lynch SE. A review of the effects of insulin-like growth factor and platelet derived growth factor on in vivo cartilage healing and repair. Osteoarthritis and Cartilage. 2006; 14:403–412. [PubMed: 16413799]
92. Vo TN, Kasper FK, Mikos AG. Strategies for controlled delivery of growth factors and cells for bone regeneration. Advanced Drug Delivery Reviews. 2012; 64:1292–1309. [PubMed: 22342771]
93. Miller RE, Grodzinsky AJ, Cummings K, Plaas AHK, Cole AA, Lee RT, Patwari P. Intraarticular Injection of Heparin-Binding Insulin-like Growth Factor 1 Sustains Delivery of Insulin-like Growth Factor 1 to Cartilage Through Binding to Chondroitin Sulfate. Arthritis and Rheumatism. 2010; 62:3686–3694. [PubMed: 20722014]
94. Loffredo FS, Pancoast JR, Cai L, Vannelli T, Dong J, Lee RT, Patwari P. Targeted delivery to cartilage is critical for in vivo efficacy of insulin-like growth factor-1. Arthritis Rheumatol. 2014
95. Lopiz-Morales Y, Abarrategi A, Ramos V, Moreno-Vicente C, Lopez-Duran L, Lopez-Lacomba JL, Marco F. In Vivo Comparison of the Effects of Rhbmp-2 and Rhbmp-4 in Osteochondral Tissue Regeneration. European Cells & Materials. 2010; 20:367–378. [PubMed: 21154243]
96. Jiang Y, Chen LK, Zhu DC, Zhang GR, Guo C, Qi YY, Ouyang HW. The inductive effect of bone morphogenetic protein-4 on chondral-lineage differentiation and in situ cartilage repair. Tissue Eng Part A. 2010; 16:1621–1632. [PubMed: 20001220]
97. Mullen LM, Best SM, Brooks RA, Ghose S, Gwynne JH, Wardale J, Rushton N, Cameron RE. Binding and Release Characteristics of Insulin-Like Growth Factor-1 from a Collagen-Glycosaminoglycan Scaffold. Tissue Engineering Part C-Methods. 2010; 16:1439–1448. [PubMed: 20388039]
98. Im GI, Lee JH. Repair of Osteochondral Defects With Adipose Stem Cells and a Dual Growth Factor-Releasing Scaffold in Rabbits. Journal of Biomedical Materials Research Part B-Applied Biomaterials. 2010; 92B:552–560.
99. Fan HB, Tao HR, Wu YN, Hu YY, Yan YN, Luo ZJ. TGF-beta 3 immobilized PLGA-gelatin/chondroitin sulfate/hyaluronic acid hybrid scaffold for cartilage regeneration. Journal of Biomedical Materials Research Part A. 2010; 95A:982–992. [PubMed: 20872747]

100. Kopesky PW, Vanderploeg EJ, Kisiday JD, Frisbie DD, Sandy JD, Grodzinsky AJ. Controlled Delivery of Transforming Growth Factor beta 1 by Self-Assembling Peptide Hydrogels Induces Chondrogenesis of Bone Marrow Stromal Cells and Modulates Smad2/3 Signaling. Tissue Engineering Part A. 2011; 17:83–92. [PubMed: 20672992]

101. Miller RE, Kopesky PW, Grodzinsky AJ. Growth Factor Delivery Through Self-assembling Peptide Scaffolds. Clinical Orthopaedics and Related Research. 2011; 469:2716–2724. [PubMed: 21503788]

102. Shah RN, Shah NA, Lim MMD, Hsieh C, Nuber G, Stupp SI. Supramolecular design of self-assembling nanofibers for cartilage regeneration. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107:3293–3298. [PubMed: 20133666]

103. Re'em T, Kaminer-Israeli Y, Ruvinov E, Cohen S. Chondrogenesis of hMSC in affinity-bound TGF-beta scaffolds. Biomaterials. 2012; 33:751–761. [PubMed: 22019120]

104. Re'em T, Witte F, Willbold E, Ruvinov E, Cohen S. Simultaneous regeneration of articular cartilage and subchondral bone induced by spatially presented TGF-beta and BMP-4 in a bilayer affinity binding system. Acta Biomaterialia. 2012; 8:3283–3293. [PubMed: 22617742]

105. Kopesky PW, Vanderploeg EJ, Kisiday JD, Frisbie DD, Sandy JD, Grodzinsky AJ. Controlled delivery of transforming growth factor beta1 by self-assembling peptide hydrogels induces chondrogenesis of bone marrow stromal cells and modulates Smad2/3 signaling. Tissue Eng Part A. 2011; 17:83–92. [PubMed: 20672992]

106. Butoescu N, Seemayer CA, Foti M, Jordan O, Doelker E. Dexamethasone-containing PLGA superparamagnetic microparticles as carriers for the local treatment of arthritis. Biomaterials. 2009; 30:1772–1780. [PubMed: 19135244]

107. Horisawa E, Kubota K, Tuboi I, Sato K, Yamamoto H, Takeuchi H, Kawashima Y. Size-dependency of DL-lactide/glycolide copolymer particulates for intra-articular delivery system on phagocytosis in rat synovium. Pharm Res. 2002; 19:132–139. [PubMed: 11883639]

108. Whitmire RE, Wilson DS, Singh A, Levenston ME, Murthy N, Garcia AJ. Self-assembling nanoparticles for intra-articular delivery of anti-inflammatory proteins. Biomaterials. 2012; 33:7665–7675. [PubMed: 22818981]

109. Lim JJ, Hammoudi TM, Bratt-Leal AM, Hamilton SK, Kepple KL, Bloodworth NC, McDevitt TC, Temenoff JS. Development of nano- and microscale chondroitin sulfate particles for controlled growth factor delivery. Acta Biomater. 2011; 7:986–995. [PubMed: 20965281]

110. 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 Engineering Part A. 2009; 15:2163–2175. [PubMed: 19413492]

111. Ertan AB, Yilgor P, Bayyurt B, Calikoglu AC, Kaspar C, Kok FN, Kose GT, Hasirci V. Effect of double growth factor release on cartilage tissue engineering. Journal of Tissue Engineering and Regenerative Medicine. 2013; 7:149–160. [PubMed: 22081628]

112. Siu RK, Zara JN, Hou YP, James AW, Kwak J, Zhang XL, Ting K, Wu BM, Soo C, Lee M. NELL-1 Promotes Cartilage Regeneration in an In Vivo Rabbit Model. Tissue Engineering Part A. 2012; 18:252–261. [PubMed: 21902605]

113. Santo VE, Gomes ME, Mano JF, Reis RL. From nano- to macro-scale: nanotechnology approaches for spatially controlled delivery of bioactive factors for bone and cartilage engineering. Nanomedicine (Lond). 2012; 7:1045–1066. [PubMed: 22846091]

114. Wang HA, Leeuwenburgh SCG, Li YB, Jansen JA. The Use of Micro- and Nanospheres as Functional Components for Bone Tissue Regeneration. Tissue Engineering Part B-Reviews. 2012; 18:24–39. [PubMed: 21806489]

115. Spiller KL, Liu Y, Holloway JL, Maher SA, Cao YL, Liu W, Zhou GD, Lowman AM. A novel method for the direct fabrication of growth factor-loaded microspheres within porous nondegradable hydrogels: Controlled release for cartilage tissue engineering. Journal of Controlled Release. 2012; 157:39–45. [PubMed: 21930167]

116. Sheridan MH, Shea LD, Peters MC, Mooney DJ. Bioabsorbable polymer scaffolds for tissue engineering capable of sustained growth factor delivery. J Control Release. 2000; 64:91–102. [PubMed: 10640648]

117. Gilding, DK. Biocompatibility of Clinical Implant Materials. In: Williams, DF., editor. Biodegradable Polymers. CRC Press; Boca Raton, FL: 1981.
118. Reyes R, Delgado A, Solis R, Sánchez E, Hernández A, San Román J, Evora C. Cartilage repair by local delivery of TGF-β1 or BMP-2 from a novel, segmented polyurethane/polylactic-co-glycolic bilayered scaffold. Journal of Biomedical Materials Research Part A. n/a-n/a.
119. Reyes R, Pec MK, Sanchez E, del Rosario C, Delgado A, Evora C. Comparative, Osteochondral Defect Repair: Stem Cells Versus Chondrocytes Versus Bone Morphogenetic Protein-2, Solely or in Combination. European Cells & Materials. 2013; 25:351–365. [PubMed: 23832688]
120. Reyes R, Delgado A, Sanchez E, Fernandez A, Hernandez A, Evora C. Repair of an osteochondral defect by sustained delivery of BMP-2 or TGFbeta1 from a bilayered alginate-PLGA scaffold. J Tissue Eng Regen Med.
121. Dormer NH, Singh M, Zhao L, Mohan N, Berkland CJ, Detamore MS. Osteochondral interface regeneration of the rabbit knee with macroscopic gradients of bioactive signals. J Biomed Mater Res A. 2012; 100:162–170. [PubMed: 22009693]
122. Mohan N, Dormer NH, Caldwell KL, Key VH, Berkland CJ, Detamore MS. Continuous Gradients of Material Composition and Growth Factors for Effective Regeneration of the Osteochondral Interface. Tissue Engineering Part A. 2011; 17:2845–2855. [PubMed: 21815822]
123. Dormer NH, Singh M, Wang LM, Berkland CJ, Detamore MS. Osteochondral Interface Tissue Engineering Using Macroscopic Gradients of Bioactive Signals. Annals of Biomedical Engineering. 2010; 38:2167–2182. [PubMed: 20379780]
124. Giteau A, Venier-Julienne MC, Aubert-Pouessel A, Benoit JP. How to achieve sustained and complete protein release from PLGA-based microparticles? International Journal of Pharmaceutics. 2008; 350:14–26. [PubMed: 18162341]
125. Lim SM, Oh SH, Lee HH, Yuk SH, Im GI, Lee JH. Dual growth factor-releasing nanoparticle/hydrogel system for cartilage tissue engineering. J Mater Sci Mater Med. 2010; 21:2593–2600. [PubMed: 20577785]
126. Wang X, Wenk E, Zhang X, Meinel L, Vunjak-Novakovic G, Kaplan DL. Growth factor gradients via microsphere delivery in biopolymer scaffolds for osteochondral tissue engineering. J Control Release. 2009; 134:81–90. [PubMed: 19071168]
127. Young S, Wong M, Tabata Y, Mikos AG. Gelatin as a delivery vehicle for the controlled release of bioactive molecules. Journal of Controlled Release. 2005; 109:256–274. [PubMed: 16266768]
128. Solorio LD, Vieregge EL, Dhami CD, Dang PN, Alsberg E. Engineered cartilage via self-assembled hMSC sheets with incorporated biodegradable gelatin microspheres releasing transforming growth factor-beta 1. Journal of Controlled Release. 2012; 158:224–232. [PubMed: 22100386]
129. Guo X, Liao J, Park H, Saraf A, Raphael RM, Tabata Y, Kasper FK, Mikos AG. Effects of TGF-beta 3 and preculture period of osteogenic cells on the chondrogenic differentiation of rabbit marrow mesenchymal stem cells encapsulated in a bilayered hydrogel composite. Acta Biomaterialia. 2010; 6:2920–2931. [PubMed: 20197126]
130. Holland TA, Bodde EWH, Cuijpers VMJI, Baggett LS, Tabata Y, Mikos AG, Jansen JA. Degradable hydrogel scaffolds for in vivo delivery of single and dual growth factors in cartilage repair. Osteoarthritis and Cartilage. 2007; 15:187–197. [PubMed: 16965923]
131. Holland TA, Tabata Y, Mikos AG. In vitro release of transforming growth factor-beta 1 from gelatin microparticles encapsulated in biodegradable, injectable oligo(poly(ethylene glycol) fumarate) hydrogels. Journal of Controlled Release. 2003; 91:299–313. [PubMed: 12932709]
132. Holland TA, Tabata Y, Mikos AG. Dual growth factor delivery from degradable oligo(poly(ethylene glycol) fumarate) hydrogel scaffolds for cartilage tissue engineering. J Control Release. 2005; 101:111–125. [PubMed: 15588898]
133. Holland TA, Tessmar JKV, Tabata Y, Mikos AG. Transforming growth factor-beta 1 release from oligo(poly(ethylene glycol) fumarate) hydrogels in conditions that model the cartilage wound healing environment. Journal of Controlled Release. 2004; 94:101–114. [PubMed: 14684275]

134. Kinard LA, Chu C, Kasper FK, Mikos AG. Controlled delivery of rhBMP-2 from OPF hydrogel composites for bone tissue engineering. Journal of Tissue Engineering and Regenerative Medicine. 2012; 6:321–321.

135. Kinard LA, Kasper FK, Mikos AG. Synthesis of oligo(poly(ethylene glycol) fumarate). Nat Protoc. 2012; 7:1219–1227. [PubMed: 22653160]

136. Park H, Temenoff JS, Tabata Y, Caplan AI, Raphael RM, Jansen JA, Mikos AG. Effect of dual growth factor delivery on chondrogenic differentiation of rabbit marrow mesenchymal stem cells encapsulated in injectable hydrogel composites. J Biomed Mater Res A. 2009; 88:889–897. [PubMed: 18381637]

137. Bian L, Zhai DY, Tous E, Rai R, Mauck RL, Burdick JA. Enhanced MSC chondrogenesis following delivery of TGF-beta3 from alginate microspheres within hyaluronic acid hydrogels in vitro and in vivo. Biomaterials. 2011; 32:6425–6434. [PubMed: 21652067]

138. Kim IL, Khetan S, Baker BM, Chen CS, Burdick JA. Fibrous hyaluronic acid hydrogels that direct MSC chondrogenesis through mechanical and adhesive cues. Biomaterials. 2013; 34:5571–5580. [PubMed: 23623322]

139. Kim IL, Mauck RL, Burdick JA. Hydrogel design for cartilage tissue engineering: a case study with hyaluronic acid. Biomaterials. 2011; 32:8771–8782. [PubMed: 21903262]

140. Ansboro S, Hayes JS, Barron V, Browne S, Howard L, Greiser U, Lalor P, Shannon F, Barry FP, Pandit A, Murphy JM. A chondromimetic microsphere for in situ spatially controlled chondrogenic differentiation of human mesenchymal stem cells. J Control Release. 2014

141. Bajpayee AG, Wong CR, Bawendi MG, Frank EH, Grodzinsky AJ. Avidin as a model for charge driven transport into cartilage and drug delivery for treating early stage post-traumatic osteoarthritis. Biomaterials. 2014; 35:538–549. [PubMed: 24120044]

142. Bedouet L, Moine L, Pascale F, Nguyen VN, Labarre D, Laurent A. Synthesis of hydrophilic intra-articular microspheres conjugated to ibuprofen and evaluation of anti-inflammatory activity on articular explants. Int J Pharm. 2014; 459:51–61. [PubMed: 24231051]

143. Hofkens W, Storm G, van den Berg WB, van Lent PL. Liposomal targeting of glucocorticoids to the inflamed synovium inhibits cartilage matrix destruction during murine antigen-induced arthritis. International Journal of Pharmaceutics. 2011; 416:486–492. [PubMed: 21382458]

144. Samad A, Sultana Y, Aqil M. Liposomal drug delivery systems: an update review. Curr Drug Deliv. 2007; 4:297–305. [PubMed: 17979650]

145. Chen WH, Lai MT, Wu AT, Wu CC, Gelovani JG, Lin CT, Hung SC, Chiu WT, Deng WP. In vitro stage-specific chondrogenesis of mesenchymal stem cells committed to chondrocytes. Arthritis Rheum. 2009; 60:450–459. [PubMed: 19180515]

146. Richardson TP, Peters MC, Ennett AB, Mooney DJ. Polymeric system for dual growth factor delivery. Nat Biotechnol. 2001; 19:1029–1034. [PubMed: 11689847]

147. Jaklenec A, Hinckfuss A, Bilgen B, Ciombor DM, Aaron R, Mathiowitz E. Sequential release of bioactive IGF-I and TGF-beta 1 from PLGA microsphere-based scaffolds. Biomaterials. 2008; 29:1518–1525. [PubMed: 18166223]

148. Jaklenec A, Wan E, Murray ME, Mathiowitz E. Novel scaffolds fabricated from protein-loaded microspheres for tissue engineering. Biomaterials. 2008; 29:185–192. [PubMed: 17950842]

149. Jiang T, Petersen RR, Call G, Ofek G, Gao J, Yao JQ. Development of chondroitin sulfate encapsulated PLGA microsphere delivery systems with controllable multiple burst releases for treating osteoarthritis. J Biomed Mater Res B Appl Biomater. 2011; 97:355–363. [PubMed: 21442745]

150. Gimble JM, Guilak F. Differentiation potential of adipose derived adult stem (ADAS) cells. Curr Top Dev Biol. 2003; 58:137–160. [PubMed: 14711015]

151. Hennig T, Lorenz H, Thiel A, Goetzke K, Dickhut A, Geiger F, Richter W. Reduced chondrogenic potential of adipose tissue derived stromal cells correlates with an altered TGFbeta receptor and BMP profile and is overcome by BMP-6. J Cell Physiol. 2007; 211:682–691. [PubMed: 17238135]

152. Kim HJ, Im GI. Chondrogenic differentiation of adipose tissue-derived mesenchymal stem cells: greater doses of growth factor are necessary. J Orthop Res. 2009; 27:612–619. [PubMed: 18985688]

153. Winter A, Breit S, Parsch D, Benz K, Steck E, Hauner H, Weber RM, Ewerbeck V, Richter W. Cartilage-like gene expression in differentiated human stem cell spheroids: a comparison of bone marrow-derived and adipose tissue-derived stromal cells. Arthritis Rheum. 2003; 48:418–429. [PubMed: 12571852]

154. Salvade A, Della Mina P, Gaddi D, Gatto F, Villa A, Bigoni M, Perseghin P, Serafini M, Zatti G, Biondi A, Biagi E. Characterization of platelet lysate cultured mesenchymal stromal cells and their potential use in tissue-engineered osteogenic devices for the treatment of bone defects. Tissue Eng Part C Methods. 2010; 16:201–214. [PubMed: 19469694]

155. Kruger JP, Freymannx U, Vetterlein S, Neumann K, Endres M, Kaps C. Bioactive factors in platelet-rich plasma obtained by apheresis. Transfus Med Hemother. 2013; 40:432–440. [PubMed: 24474894]

156. Zhu Y, Yuan M, Meng HY, Wang AY, Guo QY, Wang Y, Peng J. Basic science and clinical application of platelet-rich plasma for cartilage defects and osteoarthritis: a review. Osteoarthritis Cartilage. 2013; 21:1627–1637. [PubMed: 23933379]

157. Sundman EA, Cole BJ, Karas V, Della Valle C, Tetreault MW, Mohammed HO, Fortier LA. The anti-inflammatory and matrix restorative mechanisms of platelet-rich plasma in osteoarthritis. Am J Sports Med. 2014; 42:35–41. [PubMed: 24192391]

158. Raeissadat SA, Rayegani SM, Babaee M, Ghorbani E. The effect of platelet-rich plasma on pain, function, and quality of life of patients with knee osteoarthritis. Pain Res Treat. 2013; 2013:165967. [PubMed: 24386565]

159. Filardo G, Kon E, Buda R, Timoncini A, Di Martino A, Cenacchi A, Fornasari PM, Giannini S, Marcacci M. Platelet-rich plasma intra-articular knee injections for the treatment of degenerative cartilage lesions and osteoarthritis. Knee Surg Sports Traumatol Arthrosc. 2011; 19:528–535. [PubMed: 20740273]

160. Kon E, Buda R, Filardo G, Di Martino A, Timoncini A, Cenacchi A, Fornasari PM, Giannini S, Marcacci M. Platelet-rich plasma: intra-articular knee injections produced favorable results on degenerative cartilage lesions. Knee Surg Sports Traumatol Arthrosc. 2010; 18:472–479. [PubMed: 19838676]

161. Wang-Saegusa A, Cugat R, Ares O, Seijas R, Cusco X, Garcia-Balletbo M. Infiltration of plasma rich in growth factors for osteoarthritis of the knee short-term effects on function and quality of life. Arch Orthop Trauma Surg. 2011; 131:311–317. [PubMed: 20714903]

162. Smyth NA, Haleem AM, Murawski CD, Do HT, Deland JT, Kennedy JG. The effect of platelet-rich plasma on autologous osteochondral transplantation: an in vivo rabbit model. J Bone Joint Surg Am. 2013; 95:2185–2193. [PubMed: 24352772]

163. Centola M, Abbruzzese F, Scotti C, Barbero A, Vadala G, Denaro V, Martin I, Trombetta M, Rainer A, Marsano A. Scaffold-based delivery of a clinically relevant anti-angiogenic drug promotes the formation of in vivo stable cartilage. Tissue Eng Part A. 2013; 19:1960–1971. [PubMed: 23611597]

164. Leijten JC, Moreira Teixeira LS, Landman EB, van Blitterswijk CA, Karperien M. Hypoxia inhibits hypertrophic differentiation and endochondral ossification in explanted tibiae. PLoS One. 2012; 7:e49896. [PubMed: 23185479]

165. McCall JD, Luoma JE, Anseth KS. Covalently tethered transforming growth factor beta in PEG hydrogels promotes chondrogenic differentiation of encapsulated human mesenchymal stem cells. Drug Deliv Transl Res. 2012; 2:305–312. [PubMed: 23019539]

166. Griffin DR, Schlosser JL, Lam SF, Nguyen TH, Maynard HD, Kasko AM. Synthesis of photodegradable macromers for conjugation and release of bioactive molecules. Biomacromolecules. 2013; 14:1199–1207. [PubMed: 23506440]

167. Griffin DR, Kasko AM. Photodegradable macromers and hydrogels for live cell encapsulation and release. J Am Chem Soc. 2012; 134:13103–13107. [PubMed: 22765384]

168. Li X, Wang J, Su G, Zhou Z, Shi J, Liu L, Guan M, Zhang Q. Spatiotemporal control over growth factor delivery from collagen-based membrane. J Biomed Mater Res A. 2011

169. Li X, Su G, Wang J, Zhou Z, Li L, Liu L, Guan M, Zhang Q, Wang H. Exogenous bFGF promotes articular cartilage repair via up-regulation of multiple growth factors. Osteoarthritis Cartilage. 2013; 21:1567–1575. [PubMed: 23792272]

170. Ashley GW, Henise J, Reid R, Santi DV. Hydrogel drug delivery system with predictable and tunable drug release and degradation rates. Proc Natl Acad Sci U S A. 2013; 110:2318–2323. [PubMed: 23345437]

171. Shi S, Chan AG, Mercer S, Eckert GJ, Trippel SB. Endogenous versus exogenous growth factor regulation of articular chondrocytes. J Orthop Res. 2014; 32:54–60. [PubMed: 24105960]

172. 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:e69950. [PubMed: 23894564]

173. Coburn JM, Wo L, Bernstein N, Bhattacharya R, Aich U, Bingham CO 3rd, Yarema KJ, Elisseeff JH. Short-chain fatty acid-modified hexosamine for tissue-engineering osteoarthritic cartilage. Tissue Eng Part A. 2013; 19:2035–2044. [PubMed: 23638885]

174. Chim H, Miller E, Gliniak C, Alsberg E. Stromal-cell-derived factor (SDF) 1-alpha in combination with BMP-2 and TGF-beta1 induces site-directed cell homing and osteogenic and chondrogenic differentiation for tissue engineering without the requirement for cell seeding. Cell Tissue Res. 2012; 350:89–94. [PubMed: 22684849]

175. Ludwig TE, McAllister JR, Lun V, Wiley JP, Schmidt TA. Diminished cartilage-lubricating ability of human osteoarthritic synovial fluid deficient in proteoglycan 4: Restoration through proteoglycan 4 supplementation. Arthritis Rheum. 2012; 64:3963–3971. [PubMed: 22933061]

Table 1

Biologics delivered for *in vitro* and *in vivo* cartilage repair applications

Biologics	Biological Effect	Biologic Type	*In vitro*/*In vivo* Model	Comments	References
PTHrP	Anti-Hypertrophy	Growth Factor	*In vitro* cultures; *In vivo* OC defect model	Inhibited hypertrophy of chondrocytes and MSCs during differentiation *in vitro*; Reduced, but did not prevent, calcification *in vivo*	[137]
S-(+)-ibuprofen	Anti-inflammatory	Small Molecule	*Ex vivo* OA model	Reduced prostaglandins synthesis at 50 μM. When released from PLGA-PEG microspheres, reduced cartilage degradation at 1 mM *ex vivo*	[82, 142]
Sulforaphane	Anti-inflammatory	Small Molecule	*In vitro* cultures; *In vivo* OA model	Inhibited inflammatory markers including COX-2, ADAMTS-5, and MMP-2 *in vitro*; Delayed progression of OA *in vivo*	[86]
IL-1Ra	Anti-inflammatory	Antigen	*In vitro* cultures; *In vivo* OA model	Bound to IL-1 surface receptors of synoviocytes *in vitro*; Retained in joint without inducing degenerative changes *in vivo*; Specific chondroprotective effects need to be evaluated	[108]
3,4,6-O-Bu3GlcNAc	Anti-inflammatory/Chondroprotective	Small Molecule	*In vitro* cultures	Decreased the expression of IL-1β-stimulated NFkB target genes and increased GAG production and chondrogenic gene expression of IL-1β challenged chondrocytes	[173]
SDF-1	Cell Homing	Chemokine	*In vitro* cultures; *In vivo* OC model and intraperitoneal cell migration model	Did not influence proliferation/chondrogenesis of MSCs in OC defect but resulted in ectopic cartilage formation when delivered with TGF-β1 at 4 weeks *in vivo*	[61, 174]
PRP	Chondrogenic/Anabolic/Anti-inflammatory	Growth Factor Cocktail	*In vitro* and *ex vivo* cultures; *In vivo* OA and OC defect models	Decreased the expression of inflammatory markers including TNF-α and MMP-13 and enhanced endogenous HA production *ex vivo*; OC graft pre-treatment with PRP enhanced graft-host integration *in vivo*	[157, 162]
HA	Chondroprotective	Viscosupplement	*In vitro* cultures; *In vivo* OA models	High molecular weight HA resulted in improved histological scores and lower cartilage friction coefficients when compared to low molecular weight HA *in vivo*	[71]
Proteoglycan 4	Chondroprotective	Viscosupplement	*In vitro* cultures	Supplementation of OA synovial fluid with proteoglycan 4 restored lubricating ability by reducing friction coefficient in cartilage-on-cartilage tests	[175]
Prostaglandins E2	Inflammatory/Anabolic	Small Molecule	*In vitro* cultures	Low concentrations (10^{-9} M to 10^{-6} M) stimulated increased chondrogenic gene expression of articular chondrocytes in 3D	[83]

Table 2

Selected spatiotemporally controlled delivery strategies for *in vitro* and *in vivo* cartilage repair applications

Biologics Delivered	Delivery Platform	*In vitro*/*In vivo* Model	Spatial Strategy	Temporal Strategy	Reference
TGF-β1, IGF-1	Gelatin microparticles encapsulated in bilayered OPF hydrogels	Rabbit femoral medial condyle osteochondral defect	Growth factors delivered from the chondral layer of bilayered OPF construct	Burst release of TGF-β1 with sustained release of IGF-1	[26]
TGF-β3, IGF-1	Gelatin microparticles encapsulated in bilayered OPF hydrogels	Rabbit femoral medial condyle osteochondral defect	Growth factors delivered from chondral layer of bilayered OPF construct	Burst release or sustained release of TGF-β3 with sustained release of IGF-1	[44]
TGF-β1, IGF-1	PLGA microsphere-fused 3D scaffold	*In vitro*	N.A.	Burst or sustained release of TGF-β1; Burst or sustained release of IGF-1	[147]
BMP-7, TGF-β2	Pluronic F68-heparin-chitosan nanoparticles encapsulated in alginate hydrogel	Rabbit trochlear groove osteochondral defect	N.A.	Burst release of BMP-2 with sustained release of TGF-β2	[125]
TGF-β3, PTHrP	Alginate microspheres encapsulated in HA hydrogel	Mouse subcutaneous model	N.A.	Burst release of TGF-β3 with simultaneous release of PTHrP	[137]
BMP-2, IGF-1	Silk microspheres incorporated into aqueous-derived silk porous scaffold	*In vitro*	Single BMP-2 gradient; Single IGF-1 gradient; Continuous BMP-2/ IGF-1 transitional reverse gradient	N.A.	[126]
BMP-2, TGF-β1	PLGA microsphere-based bioactive plug	Rabbit femoral condyle osteochondral defect model	Continuous BMP-2/TGF-β1 transitional reverse gradient	N.A.	[121]
bFGF	Chitosan-heparin nanoparticles in loose/dense bilayered collagen scaffold	Rabbit trochlear groove osteochondral defect model	Directional bFGF release via the loose collagen layer - Compared release toward or away from subchondral bone	Burst release followed by sustained release of bFGF	[168, 169]
BMP-2, microfracture	Heparin-conjugated fibrin (long-term delivery) or fibrin (short-term delivery)	Rabbit trochlear groove osteochondral defect model	N.A.	Burst or sustained release of BMP-2 with bone marrow exposure	[34]
TGF-β1, BMP-4	Affinity binding bilayered alginate sulfate	Rabbit trochlear groove osteochondral defect model	TGF-β1 affinity-bound into chondral layer; BMP-4 affinity-bound into subchondral layer	N.A.	[103]
BMP-2 or TGF-β1	PLGA microspheres encapsulated in alginate matrix overlaid on porous PLGA cylinder	Rabbit trochlear groove osteochondral defect model	TGF-β1, low dose (2.5 μg) BMP-2, or high dose (5 μg) BMP-2 delivered from PLGA in chondral layer	Burst release followed by sustained release of TGF-β1 or BMP-2	[33]