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Bioactive Factors for Cartilage Repair and Regeneration: Improving Delivery, Retention, and Activity☆

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Abstract

Articular cartilage is a remarkable tissue whose sophisticated composition and architecture allow it to withstand complex stresses within the joint. Once injured, cartilage lacks the capacity to self-repair, and injuries often progress to joint wide osteoarthritis (OA) resulting in debilitating pain and loss of mobility. Current palliative and surgical management provides short-term symptom relief, but almost always progresses to further deterioration in the long term. A number of bioactive factors, including drugs, corticosteroids, and growth factors, have been utilized in the clinic, in clinical trials, or in emerging research studies to stabilize the inflamed joint environment or to promote new cartilage tissue formation. However, these therapies remain limited in their duration and effectiveness. For this reason, current efforts are focused on improving the localization, retention, and activity of these bioactive factors. The purpose of this review is to highlight recent advances in drug delivery for the treatment of damaged or degenerated cartilage. First, we summarize material and modification techniques to improve the delivery of these factors to damaged tissue and enhance their retention and action within the joint environment. Second, we synthesize recent studies using novel methods to promote new cartilage formation via biofactor delivery, that have potential for improving future long-term clinical outcomes. Lastly, we review the emerging field of orthobiologics, using delivered and endogenous cells as drug-delivering "factories" to preserve and restore joint health. Enhancing drug delivery systems can improve both restorative and regenerative treatments for damaged cartilage.

Graphical Abstract

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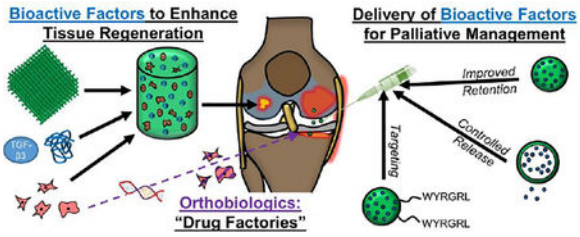

Keywords

Cartilage; drug delivery; osteoarthritis; scaffolds; orthobiologics

1. Introduction

Articular cartilage is a durable tissue that enables the load transmission and articulation of joints [1]. The dense extracellular matrix (ECM) of cartilage is composed of a zonally-organized network of type II collagen fibers [1], allowing the tissue to resist complex loading patterns, including compression, tension, shear, and friction [2,3]. The ECM also contains a high concentration of proteoglycans (PG), forming negatively-charged aggregates that promote fluid pressurization upon loading [4–6]. Combined, these ECM components (Fig 1A) generate a unique viscoelastic material that is optimized to bear load over a lifetime of use [7]. However, articular cartilage is also frequently injured, both traumatically and with aging and disease. Due to the relative avascular nature of the tissue and the limited ability of cells to migrate to the tissue for repair, cartilage lacks an intrinsic healing capacity [8]; once injured, the tissue loses many of its load-bearing traits, making the adjacent cartilage more vulnerable to wear [9]. Furthermore, injury can elicit an inflammatory response throughout the joint, with increased levels of synovial cytokines that elicit further degradation and damage to the tissue. Ultimately, with these accompanying chemo-mechanical insults, injuries often progress and conclude in joint-wide osteoarthritis (OA; Fig 1B), a debilitating disease that affects nearly 50 million people in the United States alone [10].

Common surgical interventions to treat cartilage injuries include chondroplasty [11,12] (removal of torn tissue) and microfracture [13,14] (marrow stimulation), however both provide only short-term symptomatic relief and do not prevent OA progression. More recently, chondrocyte implantation techniques [15,16] have shown superiority to microfracture for up to 5 years [17], but their ability to produce hyaline cartilage and establish long-term functionality have not yet been verified [13,14]. Due to this eventual onset of degeneration, clinicians and scientists have sought to deliver bioactive factors, consisting of proteins, nucleic acids, carbohydrates, or molecules (synthetic or biologic) that elicit a response in host tissues or cells [18–20]. Clinically speaking, most drugs used to address cartilage injury are intended for palliative OA treatment, and can provide some measure of pain and symptom relief. Nonsteroidal anti-inflammatory drugs (NSAIDs), such as Ibuprofen and Diclofenac, have shown some benefits over placebo treatment [21–23], but both oral and topical administration results in inefficient delivery of the drug to the joint. The higher doses required to provide pain relief may also cause harmful consequences throughout the rest of the body (e.g. cardiovascular) [24,25]. Additional clinically-used

bioactive factor treatments include intra-articular corticosteroid and hyaluronan injections; the former provides both pain/inflammatory relief and anti-catabolic activity [26–28], while the latter can, to some extent, restore joint lubrication and provide some chondro-protective capacity [29,30]. However, retention of these bioactive factors within the joint is limited, as these agents are readily cleared due to their relatively small molecular size [31,32], and therefore only provide short-term benefits [28]. Management of cartilage injuries and osteoarthritis would be greatly improved with localized delivery and retention of these factors.

At the other end of the spectrum, tissue engineers are developing new strategies to replace the damaged cartilage, either in small defects or after joint-wide degeneration. Unfortunately, current “replacements” do not generally result in long-term hyaline cartilage; nevertheless, there is potential in utilizing bioactive factors to induce the formation of functional tissue. For example, *in vitro* culture of hydrogel constructs with chemically defined medium containing the growth factor TGF-β3 promotes the chondrogenesis of mesenchymal stem cells (MSCs) [33,34], indicated by increased type II collagen and PG deposition. Furthermore, a plethora of other growth factors (BMP-2, BMP-7, IGF-1, FGF-2 [19,35]) have been used to promote neocartilage tissue formation. However, scaffolds that simply encapsulate these factors alone often release them within a matter of hours [36,37], and so cannot guide chondrogenesis and tissue formation (which occurs over the course of weeks to months). Additionally, while certainly chondrogenic, these factors can elicit negative responses, such as synovial fibrosis [38] or ossification [39], if introduced to off-target cells and tissues within the joint. Therefore, prolonged delivery without continual supplementation and precise localization to the desired cells are paramount in engineering functional cartilage tissue following injury.

With this lack of effective long-term therapeutics and treatments, novel techniques have emerged to promote repair and regeneration, via the combination of materials, cells, and bioactive factors to replace, rejuvenate, or protect the native cartilage matrix (Fig 1C). One general approach involves reprogramming the joint environment and/or wound interface with bioactive factors (Fig 1D-left), typically to restore the behavior of chondrocytes, calm the inflammatory milieu, or simply relieve pain. Another involves incorporating chondrogenic factors into scaffolds, with the intention of guiding cells to produce new cartilaginous matrix to replace the damaged or missing tissue (Fig 1D-right). Regardless of the application, drugs and growth factors can have a significant impact on these processes.

In this review, we focus on advances in drug delivery systems for cartilage treatment applications. First, we discuss innovative approaches that specifically address the inflamed joint environment and the damaged tissue interface, to improve retention and localization of palliative therapeutics. Next, we describe novel scaffold fabrication techniques that better control the release and activity of these bioactive factors in order to enhance neocartilage formation for tissue replacement. Finally, this review examines the emerging field of orthobiologics, utilizing cells as “biological producers” of bioactive factors, and discusses the strengths and current limitations of these approaches.

2. Enhanced Delivery and Retention of Bioactive Factors for Joint Preservation

2.1. Current Drug Usage for Joint Pain is Relatively Ineffective

Oral delivery of NSAIDs (Fig 2A) remains a common mode of treatment for both focally injured and degenerated cartilage, as these drugs inhibit cyclooxygenase enzymes, reducing the production of biological mediators involved in inflammation (e.g. prostaglandin E2). While pain and inflammation relief are provided to some extent, daily usage at higher doses [40] is required in order to provide a noticeable benefit. Moreover, these larger doses can lead to gastrointestinal [41–43] and cardiovascular [44,45] complications, and thus topical administration has better indications for OA management with NSAIDs [46,47]. Topical NSAIDS (Fig 2B) are applied via cream, gel, or patch 1–3 times daily, and have shown a 10- to 20-fold increase in NSAID concentration in synovial tissues over serum and plasma levels [48,49], indicating a more targeted delivery. However, the use of topical NSAIDs can be a burden on patients, resulting in skin irritations and withdrawal issues in patients [50,51]. Perhaps of greatest significance, for both oral and topical delivery, is that pharmacokinetic analyses have determined a drug half-life of 1–7 hours within the joint [52,53].

Intra-articular injections of bioactive factors (Fig 2C), as is typically done with corticosteroids (e.g. triamcinolone acetonide, dexamethasone) and hyaluronic acid (HA), can improve efficiency of delivery. For example, injected corticosteroids have shown slightly favorable outcomes to oral NSAIDs for up to 4 weeks with regards to patient-reported pain relief [54]. Similarly, HA injections show slightly improved results over NSAIDs, and significantly lower the incidence of adverse events and withdrawal [55]. However, residence time of these molecules is a concern, as corticosteroids and HA concentrations diminish rapidly post-injection [56,57], with a residence time of only 6–25 [58,59] and 22–56 days [56,60], respectively. For this reason, intra-articular delivery to maintain therapeutic levels consists of continuous monthly injections [55,61], creating a greater burden on patients with regards to comfort and finances. Moreover, even precise image-guided injection to the site of injury did not improve patient-reported pain outcomes [62], likely due to poor retention. This indicates that both localization and retention are required to improve the efficacy and longevity of bioactive corticosteroids. Lastly, while corticosteroids can provide pain and inflammatory relief for a few weeks, short-term high doses of these corticosteroids are detrimental to cartilage health (volumetric cartilage loss and tissue atrophy). The long-term release of lower concentrations of these factors could greatly improve their impact while preserving cartilage tissue structure and function. The following sections detail novel methods to improve the delivery, localization, retention, and release of these and other bioactive factors into the joint environment (Fig 2D) to provide patients with longer-term symptomatic relief.

2.2. Carriers for Enhanced Drug Delivery and Retention

The rapid clearance of NSAIDs and corticosteroids is related to their relatively low molecular weight (<1kDa), which allows the lymphatic vessels in the synovium and joint capsule to clear these small molecules. Even larger hyaluronic acid (6–100MDa) molecules are either enzymatically digested and/or cleared from the joint within weeks [60,63]. Other

small molecules, including monoclonal inflammatory antibodies (e.g., TNF inhibitors), receptor antagonists (e.g., IL-1Ra), and protease inhibitors (e.g., MMP inhibitors, cathepsins), can serve as disease-modifying osteoarthritis drugs (DMOADS) and are under development, but are similarly cleared from the joint soon after injection in the absence of a delivery system [64]. To address this event, a number of new materials (microcarriers, nanocarriers, liposomes, hydrogels) have been developed to encapsulate bioactive factors and control their release from larger particles that are not as easily cleared from the joint.

Many micro-carrier – drug combinations are currently under development for OA treatment [64]. Perhaps the most advanced among these combinations (already under testing in a Phase III trial) take advantage of poly-lactic-glycolic acid (PLGA) microcarriers (FX006, Flexion Therapeutics, 35–55 microns in diameter) [65–67]. This proposed treatment consists of carriers that contain crystals (<5 microns) of the corticosteroid triamcinolone acetonide. Encapsulation results in a triphasic pharmacokinetic release profile that provided patients with pain relief lasting 12 weeks longer than placebo treatment. This release and activity profile is consistent with prior degradation profiles of the PLGA polymer [68], and can be elongated by increasing the ratio of lactide (slower degradation) to glycolide (faster degradation) groups. However, the FX0006 carrier only provided slight improvement in OA pain outcomes over intra-articular administration of the drug itself [65], leaving lots of room for clinically-relevant improvement. Other materials have been utilized for both micro- and nano-carrier development (Fig 3A), including gelatin, chitosan, polycaprolactone (PCL), polyanhydrides, and polyester amides (PEA) [69,70]. When produced at a small length scale, nanoparticles provide a greater surface-to-mass ratio than microparticles, and can be more easily taken up by the desired cells through endocytotic mechanisms in order to control cell response and fate [71,72]. For example, Kang et al [73] determined that *in vitro* culture of MSCs with kartogenin-loaded nano-particles (150nm) resulted in improved chondrogenesis compared to micro-particles (1.8μm) loaded similarly. However, the smaller size of nano-particles also increases the likelihood of phagocytosis and degradation by macrophages [74], as well as clearance from the joint. This phenomenon likely explains the finding that *in vivo* retention of kartogenin was superior when delivered from micro-particles. Many of these drug carriers can also carry multiple factors, with a differential release profile for each factor. For example, Kang et al [75] fabricated chitosan nanospheres containing diclofenac, allowing for immediate anti-inflammatory benefits, while covalently conjugating kartogenin to the particle, promoting longer-term chondrogenic enhancement [75]. This concept of polymer functionalization with bioactive factors, especially to carriers, may enhance retention and prolong release, as it creates covalent linkages via one of many chemistries (e.g. activated esters, thiol-ene processes, imine formation, Michael addition) [76,77]. Lastly, both micro- and nano-particles are typically fabricated as solid spheres with embedded bioactive factors. Alternatively, capsules can be fabricated with solid biomaterial outer shells and aqueous cores, with the release of bioactive factors regulated via permeation through the polymer shell (Fig 3A).

Drug delivery via liposomes is similar in many ways to delivery from polymeric capsules. Composed of a lipid bilayer, liposomes have high loading efficiencies for hydrophilic drugs within their cores, are well tolerated, and are tunable with respect to size (3–500nm; Fig 3A) [78,79]. Taking advantage of the fine control of flow rates afforded by microfluidic

assembly, reproducible and consistent liposomes can be fabricated. Likewise loading of hydrophobic factors within the bilayer system can permit the dual delivery of multiple biofactors [79,80]. Concerns over liposomal integrity do exist, especially in the high-stress joint environment, but these are mitigated by the development of fortified liposomal fabrication, primarily through PEGylation [81]. Furthermore, liposomes can be easily functionalized for enhanced retention and targeting [82,83]. These technologies have advanced considerably for cancer and tumor targeting applications, but examples of these modifications for the treatment of cartilage injuries and osteoarthritis are limited at present [84,85]. Other drug delivery systems similar to liposomes include micelles (lipid monolayer for hydrophobic molecule delivery) and dendrimers (branched polymer system) [86,87].

The final major category of drug delivery systems is hydrogel encapsulation. These hydrogels, including hyaluronic acid or chitosan, are modified polymers that can be crosslinked to encapsulate bioactive factors and then control their delivery within the joint. Taking advantage of thermo-sensitive crosslinking behavior [88], factors can be incorporated in an aqueous solution that then crosslinks into a hydrogel when at body temperature post-injection [89,90]. Such hydrogels can encapsulate DMOADs without affecting factor bioactivity, a potential concern with synthetic carriers that require solvents or heat for fabrication. Other controlled drug release “depots” (Fig 3B) include elastin-like polypeptides (ELP), thermo-sensitive polymers that are aqueous below body temperature, but become insoluble at body temperature [91–93]. Application of this delivery system allowed for sustained release of the IL-1 receptor antagonist (IL-1Ra, anakinra), significantly reducing cartilage degeneration and synovitis in a mouse OA model [92].

Modifications can be made to natural and synthetic polymers in order to tune their responsiveness in the intended joint application, allowing bioactive factors to become available when needed. For example, enzyme cleavable particles and hydrogels are of significant interest [94], as enzyme activity is elevated in the degenerated or diseased setting. Under these conditions, bioactive factors would be released to quell inflammation and pain during periods of high cytokine activity. For instance, Joshi et al [95] developed a triglycerol monostearate hydrogel system that degrades in response to an “arthritis flare” *in vivo*; the incorporation of triamcinolone acetonide into these hydrogels allowed for release of the corticosteroid in the presence of elevated enzymes found in joint inflammation. Furthermore, recent studies have fabricated MMP-cleavable hydrogels that similarly degrade and release bioactive factors upon demand [96–98]. Other drug delivery systems are designed to be pH- or temperature-responsive [99–102]; a “flaring” inflamed joint is typically accompanied by a drop in pH [103] and increase in temperature [104]. Finally, an emerging arena of drug delivery relates to controlling release with external stimuli such as ultraviolet or infrared light, magnetic or electrical fields, or ultrasound [105–108]. Refining and expanding the chemical and biophysical cues that instigate biofactor delivery will increase the range of applications that can be addressed to slow or reverse joint degeneration.

2.3. Targeting the Damaged or Inflamed Cartilage Environment

Even when delivered to the joint environment using one of the carriers described above, bioactive factors are often not active at the desired site of injury or inflammation. For this

reason, bioactive factors are now being targeted (Fig 3C) to inflammatory cells or the damaged cartilage interface to either quell the inflamed environment, prevent further cartilage deterioration, or even promote regeneration at the site of injury. Targeting of damaged or degenerated cartilage can be achieved via modification of drug delivery agents with a type II collagen binding peptide (WYRGRL) [109]. The peptide can be covalently linked to a multitude of biological (hyaluronan, chondroitin sulfate) and synthetic (PEG, PLGA, DOTAM) polymers (Table 1) that carry bioactive factors. Cartilage defects and degeneration expose the underlying cartilage matrix, uncovering the type II collagen-rich ECM (Fig 1A) and promote attachment of peptide-conjugated carriers. Jiang et al utilized maleimide-PEG-PLGA nanoparticles to couple WYRGRL peptides that promoted nanoparticle binding to damaged cartilage [110]. Other groups have used the WYRGRL peptide to localize pepstatin-A [111] or hyaluronic acid [112] to damaged regions of cartilage in order to promote anti-inflammatory activity or improve lubrication. Additional binding motifs on the damaged cartilage have been explored, such as hyaluronan-binding peptides [113], heparin binding peptides [114,115], β-cyclodextrin [116], aldehyde amine interactions [117–119], and even type II collagen monoclonal antibodies [120]. All of these cartilage-targeting mechanisms can enhance the delivery and retention of bioactive factors to the damaged cartilage interface.

Technologies have also been developed to improve penetration of delivery agents into the tissue (Fig 3D). In order to easily penetrate through the small pores of cartilaginous matrix, drug delivery agents need to be less than 5nm. However, this size restriction greatly decreases the residence time of such delivery systems within the synovial joint. In order to better promote the movement of delivered factors into the cartilage matrix, large cationic particles have shown some utility [121,122]. For example, a study by Bajpayee and colleagues determined that the charged molecule avidin (~7nm) infiltrated into cartilage explants more than 10 times further than its neutral counterpart (neutravidin). This penetration was due to electrostatic interactions [123], and this enhanced capacity for penetration was recently confirmed *in vivo* in a rat model of OA [124,125], and was ultimately used to deliver dexamethasone [126]. Other groups have utilized positively-charged particles in order to enhance bioactive factor delivery within the cartilage tissue [87,127,128]. For instance, Brown et al developed PLGA nanoparticles with quaternary ammonium cations that penetrated into cartilage *ex vivo* [129], allowing for improved delivery into the tissue prior to clearance from the joint. Improved penetration of bioactive factors can be achieved through other physical mechanisms; magnetic nanoparticles coupled with an external magnetic field can be “pushed” through the cartilage tissue at a nearly 50-fold increase over static conditions [130].

Beyond targeting moieties in the tissue itself, cells themselves offer another opportunity for biofactor localization based on surface antigens expressed on a target cell type (Fig 3E). For instance, a number of studies have utilized a chondrocyte-affinity peptide (CAP, DWRVIIPPRPSA) [131,132]. One recent study noted reductions in inflammation following injection of CAP-bound anti-Hif-2α siRNA [133], with delivery of the anti-inflammatory siRNA directly to chondrocytes in cartilage. By providing close contact between the delivery vehicle and chondrocytes, such affinity peptides likely enable efficient endocytosis of factors through clathrin and caveolin-mediated pathways [132]. Chondrocytes are not the only cells

available for targeting, numerous groups have targeted both inflammatory cells and synoviocytes in the joint space. Chiesa et al [134] utilized a GE11 peptide that targets epidermal growth factor in synovial fibroblasts. This peptide was conjugated to PLGA nanocarriers containing dexamethasone, reducing expression of inflammatory markers in synovial fibroblasts. Similarly, Vanniasinghe et al [85] utilized liposomes conjugated with a Cys-HAP-1 peptide in order to target fibroblastic synoviocytes, delivering the steroid prednisolone. By specifically targeting inflammatory cells in the synovial environment, efficient drug delivery can reduce inflammatory cytokine levels and promote a return to tissue homeostasis.

A number of disease modifying OA drugs (DMOAD) under clinical investigation target TNF-α (infliximab, etanercept, adalimubab) or IL-1 (anakinra, canakinumab). While these agents can effectively abrogate signaling through these pathways, only limited short-term symptom relief has been observed in animal studies [135] and clinical trials [136,137]. To improve on these efforts, targeting of angiogenic markers, which are prevalent in arthritic regions of the joint, has been used to locally reduce inflammatory activity at these sites. Several groups have tested a fusion protein system (F8) that utilizes a human monoclonal antibody that recognizes the extra-domain A of fibronectin [138], an angiogenic marker. By conjugating recombinant IL-4 or IL-10 (anti-inflammatory cytokines) to the F8 antibody, targeted anti-inflammatory benefits were achieved [139,140]. Similar targeting antibodies for angiogenesis (L19 for extra-domain B of fibronectin [141], F16 for extra-domain A1 of tenascin C [142], and G11 for extra-domain C of tenascin C [143]) have been utilized for cancer therapeutics, and may find application for the delivery of anti-inflammatory cytokines in cartilage applications [141].

3. Scaffold Delivery Systems to Tune Growth Factor Release

Even with the improved delivery, retention, and targeting of NSAIDs, corticosteroids, hyaluronic acid, antibodies, antagonists, cytokines and siRNA, almost all outcomes have been measured relative to placebo controls. Moreover, these therapies simply act as a palliative treatment in patients, given that cartilage tissue is not replaced, but rather protected from further degenerative processes. For longer-term replacement of damaged tissue, current clinical techniques include microfracture and autologous chondrocyte implantation (ACI). Microfracture involves penetrating the subchondral bone to recruit marrow elements, most notably MSCs, in order to fill the defected tissue void space with a marrow-clot. While the short-term clinical outcomes of microfracture show moderate defect fill and pain relief [144,145], both biopsy and preclinical animal studies (Yucatan minipig) demonstrate inferior fibrocartilaginous tissue formation [146,147]. This failure in functional restoration engenders elevated stresses in the surrounding tissue, leading to long-term wear, defect expansion, and OA progression. To improve the quality of formed cartilage tissue, ACI and its subsequent iterations (matrix-induced autologous chondrocyte implantation; MACI) use chondrocytes without and with scaffold carriers to fill cartilage defects, and are governed by the principle that native cartilage cells may better reproduce cartilage matrix [148,149]. With these matrix-assisted and augmented procedures, long-term clinical pain scores show some improvement over microfracture. However, long-term quality of life measures and magnetic resonance imaging (MRI) evaluation of formed tissue showed little difference compared to

microfracture [150], calling into question the durability and cost effectiveness of these procedures [16,151]. Cartilage tissue engineering aims to improve on patient-reported outcomes by utilizing scaffolds to provide a template for functional neocartilage tissue formation. Indeed, the utilization of bioactive factors within these scaffolds may better direct cells, both marrow-derived MSCs and articular chondrocytes, to regenerate cartilage tissue.

3.1. Drug Delivery to Promote Chondrogenesis

For decades, cell-seeded hydrogel constructs have been cultured in chemically-defined media containing a variety of growth factors to promote cartilage tissue formation. While these cultured constructs have gained traction as cartilage replacements [152], and are being tested pre-clinically in animal models (e.g. rabbit, sheep) [153,154], the manufacturing variability and costs associated with their production may complicate translation and regulatory approvals. Furthermore, bioactive factors are not available *in vivo* at concentrations (5–100ng/mL) applied during *in vitro* culture, and cultured constructs often require weeks to months to mature. For this reason, numerous acellular cartilage scaffolds have incorporated bioactive factors during fabrication, with the aim of guiding endogenous cells to regenerate cartilage. A systematic literature search (Pubmed: “cartilage scaffold drug delivery”: September 2013-September 2018) determined that TGF-β1 and TGF-β3 are, by far, the most-widely used factors (18 of 44 studies), followed by BMPs (BMP-2, BMP-7), IGF-1, and FGF (Fig 4A). Other common factors, including drugs and biocompounds, include insulin [155], dexamethasone [36,156], and kartogenin [157]. When such factors are simply incorporated in a soluble form in hydrogels, the majority are released in the first week (Fig 4B – red lines), and do not substantially improve the in vivo formation of cartilage tissue that occurs over weeks to months [158]. Thus, prolonged release and activity of these factors from scaffolds is required for the long-term promotion of cartilage tissue formation.

Similar to drugs for the treatment of OA symptoms, micro- and nano-carriers can enable the prolonged release of bioactive factors from scaffolds to enhance chondrogenesis and tissue formation. For instance, Han et al [159] utilized PLGA microspheres to prolong the release of TGF-β1 in a chitosan-gelatin hydrogel, showing a 50% release of the growth factor by 14 days, a significant improvement from direct incorporation of TGF-β1 into HA hydrogels [37], where >90% release occurred in the first 7 days. Similarly, Deepthi et al [160] fabricated polyelectrolyte (cross-linked chondroitin sulfate) nanoparticles containing TGF-β; these delivery agents increased MSC proliferation and proteoglycan deposition in culture. Furthermore, similar to carriers discussed earlier that respond to inflammatory cues, Mohanraj et al [161] developed mechanically-activated capsules that, under loading, rupture and release bioactive factors. This technology provides on-demand release of factors to enhance matrix formation and organization in order to combat these loads. An alternative approach for promoting sustained release is the incorporation of the bioactive factors into electrospun nanofibers [162,163] or 3D-printed microfibers [164]. Compared to hydrogels, from which growth factors can diffuse, encapsulation of factors into fibrous matrices can slow or stop release until fiber degradation has occurred. Given that a variety of polymers (collagen, PLLA, PLGA, PCL; Table 1) with distinct degradation profiles can be utilized, bioactive factor release can be achieved for >50 days [163]. Additionally, functionalizing

scaffolds with growth factors, similar to drug delivering carriers [76,77], can further enhance retention and activity throughout the scaffold. One such modality utilizes heparin binding domains to sequester growth factors, improving retention and prolonging release for over 28 days [165,166]. These approaches (Fig 4B – green lines), where bioavailability of factors can be achieved over longer time windows, can support the long-term maturation of cartilage-like tissue. Finally, the controlled release of growth factors from these scaffolds has also been shown to augment microfracture procedures [167,168], directing hyaline cartilage formation over fibrocartilage formation.

3.2. Spatiotemporal Control for Complex Tissues (e.g. Osteochondral Unit)

Several tissues in the body exhibit spatially varying regions of cartilaginous tissue and interfaces [174], and so require spatial control of cell phenotype and behavior that may be achieved through localized and differential biofactor release. Most notably, cartilage is part of an osteochondral unit, consisting of a gradient interface between cartilage and the underlying subchondral bone (Fig 4C). Other examples of cartilage-containing tissues include the fibro-cartilaginous meniscus, intervertebral disc (IVD), and temporomandibular joint (TMJ) tissues, as well as tendon-to-bone interfaces. Often, techniques to promote chondrogenesis must be combined with techniques to regenerate other tissues, with spatial release of factors to induce the formation of each individual tissue. Biphasic scaffolds that reconstitute the osteochondral unit typically consist of a softer hydrogel or fibrous top layer with incorporated chondrogenic factors (e.g., TGF-β), and a stiffer bottom layer that contains osteogenic factors (e.g., BMP-2) [159,175–177]. While these biphasic scaffolds have promise, stress concentrations can occur at the interface between scaffold components, rendering the construct susceptible to delamination or rupture.

For this reason, gradient scaffolds have been developed and allow for a more gradual transition between layers (Fig 4C). Di Luca et al [178] fabricated a 3D-printed PCL scaffold with a gradient of PEG-functionalized TGF-β3 and BMP-2. Brush functionalization allowed for the TGF-β3 to have its highest concentration in the top layer and gradually decline with depth, whereas the BMP-2 was concentrated in the bottom bone layer and gradually decreased over the transition to the upper layer. Similarly, Dormer et al [179] achieved gradient spatial control with TGF-β1 and BMP-2 loaded PLGA microspheres using a programmable syringe pump system to partially mix two separate dispersions carrying each growth factor. Following sintering of the microspheres, gradient scaffolds enabled spatial tissue growth in a rabbit femoral condyle model, providing the essential architecture for an anatomic osteochondral unit. Since the subchondral bone provides load support to healthy articular cartilage, the discussed techniques may improve the probability of success for cartilage constructs by also regenerating the bone underneath, while simultaneously creating a smooth transition between the two units. Ultimately, these techniques would be critical for patients with full-thickness cartilage injuries, or those with compromised subchondral bone due to prior marrow recruitment procedures (e.g. microfracture).

3.3. Improving Cell Infiltration and Integration of Cartilage Scaffolds

One additional concern for cartilage and osteochondral scaffolds, especially those that do not allow for cell encapsulation, is a limited migration into the center of scaffold. For

example, electrospun nanofibrous scaffolds can be quite dense, and so cell infiltration is limited due to the small pore size [180]. Similarly, cartilage derived matrices containing chondroitin sulfate can present issues for both MSC (from bone marrow) and chondrocyte (from neighboring cartilage, possible in injury or mechanically perturbed scenarios) migration [181–183]. Aside from changing scaffold structure or porosity to better allow cell infiltration (which may compromise mechanics and other properties), the incorporation of chemotactic growth factors (e.g. PDGF [184,185], FGF [186,187], SDF-1α [188,189]) can enhance cellular recruitment and migration within scaffolds (Figure 4D). For example, Liebesny et al [184] utilized PDGF in a lysine-leucine-aspartic acid based hydrogel (AcN-KLDLKLDLKLDL-CNH2; KLD) hydrogel to improve the migration of MSCs from bone marrow, thus augmenting the microfracture procedure. Furthermore, these cell-recruitment approaches could be combined with pro-chondrogenic factors (e.g. TGF-β) to promote neo-cartilage formation by cells recruited during microfracture [168]. Similar techniques can be applied to induce chondrocyte migration from neighboring tissue [187], in the case of partial-thickness cartilage defects. Dual-delivery approaches are especially useful for these applications, in that a short burst of chemotactic factors can promote early cell migration, followed by a longer-term release of chondrogenic factors to induce the recruited cells to produce cartilage matrix [184,188].

Even if scaffolds can recruit cells and promote chondrogenesis, one potential issue that still remains is integration with the surrounding tissue. During normal load bearing, mechanical stresses at this interface can be quite significant if the scaffold is not well integrated with the surrounding healthy tissue [190]. In a recent study in meniscus, Qu et al [185] used PDGF to enhance migration from native tissue, while simultaneously providing small doses of collagenase to loosen the native matrix in order to improve integration. These findings could be directly applied to cartilage tissue engineering, using small amounts of degradation to create a more seamless border between the healthy neighboring tissue and the scaffold's neo-tissue (Figure 4D) [191–193]. Alternatively, Allon et al [194] used a collagen adhesion protein in order to improve day zero integration mechanically, though the lack of retention of the protein may have caused a loss in these mechanics by Day 21. This retention, as noted previously, could certainly be improved; kartogenin release from scaffolds through PLGA microspheres improved integration between scaffolds and the surrounding tissue in a rabbit model [195]. Likewise, TGF-β3, with its strong matrix forming stimulus, improved the integration strength between core-ring constructs [196]. Thus both cell migration and mechanical integration can be enhanced via drug delivery approaches, increasing the likelihood of success in cartilage tissue engineering. By combining techniques to promote chondrogenesis with both spatiotemporal release and that promote integration, scaffolds are now better suited to replace injured cartilage in all shapes and sizes.

4. Orthobiologics: Delivering Cell-based Local "Drug Factories"

While many (if not most) drug delivery systems rely on a purified agent with a defined delivery profile, this need not be the case. Indeed, many factors that improve cartilage formation are produced by healthy cells, both in the joint and in other autologous cell sources. For this reason, cellular therapeutics have become increasingly popular. For instance, mesenchymal stem cell (MSC) and platelet-rich plasma (PRP) injections have

become increasingly popular, as these cells produce anti-inflammatory proteins and growth factors that can quell an inflamed joint and promote tissue regeneration. Recent work has further tailored this behavior and the inherent production capabilities of cells both *in vitro* and *in vivo*, turning cells into "drug factories" that produce the desired bioactive factors or ECM components in a localized fashion after implantation. This final section will review the use of such cell based orthobiologics as potential localized drug delivery agents.

4.1. MSC and PRP Injections to Localize Cell-based "Drug Factories"

MSCs, also known as multipotent mesenchymal stromal cells, and more recently, "medicinal signaling cells" [197], represent a heterogeneous population of stromal derivatives [198,199]. Traditionally, these cells were of great interest to the musculoskeletal field given their ability to differentiate into functionalized cells resembling osteoblasts, chondrocytes, and adipocytes. MSCs have been applied in the context of regenerative medicine, where controlled differentiation of MSCs can be used to form native tissue-like constructs [200–202]. More recently, however, others have begun to consider the immuno-modulatory capacity of MSCs on their own [203,204]. In vivo, MSCs appear to migrate to sites of injury from perivascular niches and are purported to release factors that regulate tissue inflammation and recruit additional tissue reparative cells (Fig 5A). In this sense, MSCs can act as both the carrier and the producer of a potential therapeutic, "living drug producers".

In the context of cartilage, intra-articular MSC injections have gained traction as a potential treatment for osteoarthritis (OA). These injections typically involve an aspiration (bone marrow or adipose) weeks beforehand, followed by expansion to obtain a high dose of cells, and ultimately a second "procedure" for cell application [205,206]. To avoid this second procedure, point-of-care systems have also been developed to concentrate bone marrow or adipose for same-day application [207–209], yet the long-term benefits of these therapies are not fully proven. Although there is no clear consensus on the direct chondro-regenerative effect of MSC injections, many studies have reported improved pain scores in patients with OA [210,211]. Lamo-Espinosa et al [212] showed that a high dose (100×10^6) of bone marrow-derived MSCs and hyaluronic acid delivered intra-articularly improved WOMAC scores by 16.5 points after 12 months compared to hyaluronic acid injections alone. Additionally, Soler et al [213] showed not only decreased pain and improved physical functioning, but also signs of regeneration via MRI T2-mapping, 12 months following intra-articular injection. While compelling, the exact mechanism by which MSCs are modulating the articular microenvironment has yet to be elucidated. Furthermore, the MSCs can be retained within the joint for long time periods (>10–12 weeks, [214,215]), as their size limits clearance through the lymphatic vessels. It should be noted that the majority of these cells localize to the synovial lining and fat pad [216,217], and may act to quench the activities of inflammatory cells in these tissues within the joint [218].

In addition to MSCs, other cell and cell product-based orthobiologics have become commonly used in clinical practice. For example, platelet-rich plasma (PRP) offers an unmodified biological additive that can be isolated at the time of surgery/intervention and has a much simpler regulatory pathway than other engineered delivery systems. PRP is isolated from autologous whole blood, through one or two centrifugation and isolation steps,

separating plasma from red blood cells and concentrating the platelets within the plasma. Once activated by calcium chloride or other mechanisms (e.g. contact with collagen), PRP delivers a myriad of growth factors to the injection site, most notably TGF-β, PDGF, FGF, VEGF, and IGF [219,220]. The delivery of these growth factors to the joint can have a positive anti-inflammatory benefit [221,222], and may provide a cost-effective and regulatory-sensitive treatment pathway compared to delivery of specific growth factors. Furthermore, many of the factors found in PRP can increase matrix production and promote chondrogenesis. More recently, to combine the combinatorial factors of PRP with controlled delivery systems, several groups have incorporated activated-PRP into scaffolds [223–225], and have reported improved cartilage regeneration using this approach. While ease of use and low cost make PRP an intriguing option, perhaps the greatest drawback is the high variability of its components between patients, and within the same patient at different times [226,227].

4.2. Exosomes as Therapeutic Delivery Vehicles for Cartilage Repair and Regeneration

Cell-to-cell communication through soluble factors (endocrine, paracrine and autocrine signaling) is fundamental to the development of organisms and the specialization of musculoskeletal tissues. Both platelets and MSCs can release soluble factors to improve regeneration, and many have attempted to harness this soluble factor signaling between cell populations using either indirect ‘conditioned media’ [228,229] or direct ‘co-cultures’ [230,231] contexts. For instance, mixtures of chondrocytes and MSCs result in improved MSC chondrogenesis either in pellet culture [232,233] or within 3D scaffold environments [234,235]. Our understanding of the mechanisms of this cell-to-cell signaling has been further expanded by the emergence of extracellular vesicles (EVs) and other mechanisms by which cells convey information to each other [236].

Recent evidence points to a role for exosome and EV secretion as a primary mediator of the therapeutic effects exerted by MSCs [237–239]. Exosomes are naturally occurring, cell-produced nanoparticles ranging from 40–100 nm. Exosomes are formed by the folding-in of the membrane of multi-vesicular bodies, and can be released into the environment by fusion with the cell membrane (Fig 5B). MSC-generated exosomes contain a range of protein [240] and miRNA [241] products that target various cell immune and repair pathways [240,242,243]. Due to their likely role in the immunomodulatory capacity of MSCs, exosomes present an attractive therapeutic opportunity. If MSCs act as “living drug carriers”, then exosomes may represent “drug deliverables” that can be “manufactured” outside of the body and subsequently injected into damaged environments. The most common method for exosome isolation from MSCs is ultracentrifugation [244]. Once isolated, they can be delivered intra-articularly, allowing for the therapeutic effects of MSCs that can be applied in a more precise and controlled manner.

Several groups have investigated the therapeutic potential of exosome delivery. Vonk et al [245] tested the effect of bone-marrow MSC derived exosomes on OA chondrocytes cultured in the presence of TNF-α, as a model for OA cartilage inflammation. They showed that exosome delivery decreased expression of COX2 and pro-inflammatory interleukins, and reduced collagenase activity, indicating an anti-inflammatory benefit. Furthermore,

increased production of proteoglycans and type II collagen was observed, implying a potential for exosomes in promoting cartilage regeneration. Similarly, Tofiño-Vian et al [246] treated OA chondrocytes with interleukin(IL)-1β to provoke an inflammatory response, and evaluated the effect of exosomes on the production of inflammatory mediators by these cells. Exosome treatment decreased production of TNF-α, IL-6, PGE_2, and NO. Additionally, they noted decreased metalloproteinase activity and increased anti-inflammatory IL-10 and type II collagen expression. These studies demonstrate that exosomes may be an ideal and natural drug delivery system that can be utilized and tuned for chondro-protective and chondro-regenerative applications.

4.3. Local Biofactor Delivery via Genetic Modification

To further enhance cell-derived delivery strategies, techniques in gene therapy are being utilized as a means to reprogram cell behavior for therapeutic action [247–249]. A benefit of this approach is that one can more precisely control and tune the advantageous activity of the delivered cells. In the context of MSC injections, gene therapy can be applied to enhance their inherent immunomodulatory capacity by fine-tuning their ability to reach inflamed areas or by upregulating the expression of anti-inflammatory proteins (Fig 5C - left). For example, Shen et al [250] determined that MSCs modified to overexpress CXCR2 were more able to target inflamed mucosa in a mouse model of oral mucositis. While not intended for the knee joint environment, this approach could be adapted to target the inflamed synovium in order to protect cartilage. Furthermore, Xia et al [251] genetically engineered MSCs to express a TNFα blocker, Atsttrin, and so suppressed matrix proteases and inflammatory factors and halted the degenerative progression in a mouse model of OA.

Viral transduction is an efficient method for genetic modification of cell behavior and commonly includes the use of lenti-, retro-, and adeno-associated viral vectors [252]. Rowland et al [253] made use of scaffold-mediated lentiviral gene delivery system to induce a single MSC population towards site-specific chondrogenic and osteogenic phenotypes in osteochondral constructs. In this instance, the lentiviral vectors enabled the efficient transduction of genetic material so that MSCs overexpressed either TGF-β3 or BMP-2 in defined regions, without the antagonistic consequences of both growth factors expressed simultaneously in the same cells at the same location. Venkatesan et al [254] developed a recombinant adeno-associated vector (rAAV) genetically encoding SOX9, an early prochondrogenic transcription factor. The rAAV-SOX9 vector was applied to MSCs to induce chondrogenesis upon seeding of 3D-woven PCL scaffolds. This resulted in a prolonged period of SOX9 expression (>21 days) and protected against hypertrophic differentiation of cells in the construct. Even more sophisticated systems have been developed using CRISPR based gene editing methods [255], where the vectors reprogram host cells to produce anti-inflammatory factors when inflammation spikes. Much like the bio-response material delivery systems, these advanced gene editing and gene therapy methods can reprogram endogenous cells to act in a bio-responsive and autonomous manner to quench inflammation when it arises [256]. Ultimately, these approaches offer the potential of direct joint injection, allowing for local reprogramming of chondrocytes and synovial fibroblasts in a degenerative joint (Fig 5C – right). Such an approach could provide both long-lasting biofactor delivery, in a physiologic context, and in an on-demand manner. Large

animal (equine model) and human clinical trials in this arena are now underway, with early findings showing great promise [257–260]. Several potential limitations and concerns with these approaches do however decrease enthusiasm. These relate to vector retention within the joint, the possibility of off-target transduction, and vector immunogenicity [261–263]. These attributes will certainly need to be addressed as these therapies move through clinical trials.

5. Conclusions

The use of bioactive factors in cartilage injury management clinically has progressed in recent years. Innovative biological and engineering technologies have significantly improved the efficacy and efficiency of these factors. Emerging techniques and methods in material synthesis, polymer modification and functionalization, carrier development, and scaffold fabrication, have improved: 1) delivery to the joint environment, 2) localization at damaged, inflamed, or regenerated tissue, and 3) retention and activity of therapeutic factors in the joint. Additionally, as the field of orthobiologics continues to grow, cell-based, cell-product, and cell reprogramming-based therapeutic delivery mechanisms will offer an alternative to conventional delivery approaches. Serving as endogenous “drug factories”, these cells, sometimes enhanced by gene therapy, offer the potential for durable and efficient bioactive production that might not only stabilize joints, but also enhance tissue regeneration. Each of these delivery strategies require additional evaluation, specifically with regards to safety (novel polymer systems, cell sources, gene therapy), consistency (biological variability), and clinical efficacy (significant improvement in patient-reported outcomes) over the long term. Furthermore, these clinical outcomes are often the result of numerous complex variables; for example, even structural restoration of cartilage tissue may not fully quell patient-reported pain or discomfort. Thus, new drug delivery cartilage therapies must aim to address both local functional (i.e., tissue regeneration) and joint-wide issues (i.e., inflammation, pain), and establish predictive relationships between these objective tissue-level metrics and clinical outcomes. That said, delivery of bioactive factors for both palliative OA management and cartilage regeneration has already improved preclinical outcomes and will likely see a marked increase in clinical implementation in the coming years, as the versatility and potency of these systems is increasingly validated in large animals and human clinical trials.

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Abbreviations

ECM	extracellular matrix
PG	proteoglycans
OA	osteoarthritis
NSAID	nonsteroidal anti-inflammatory drug

TGF	transforming growth factor
MSC	mesenchymal stem cell
BMP	bone morphogenetic protein
IGF	insulin-like growth factor
FGF	fibroblast growth factor
HA	hyaluronic acid
kDa	kilodaltons
MDa	megadaltons
TNF	tumor necrosis factor
IL	interleukin
MMP	matrix metalloproteinase
DMOAD	disease-modifying osteoarthritis drug
PLGA	poly-lactic-glycolic acid
PCL	polycaprolactone
PEA	polyester amides
nm	nanometer
PEG	polyethylene glycol
ELP	elastin-like polypeptide
CAP	chondrocyte-affinity peptide
ACI	autologous chondrocyte implantation
MACI	matrix-induced autologous chondrocyte implantation
SDF	stromal cell-derived factor
PRP	platelet-rich plasma
MRI	magnetic resonance imaging
WOMAC	Western Ontario and McMaster Universities Arthritis Index
PDGF	platelet-derived growth factor
VEGF	vascular endothelial growth factor
EV	extracellular vesicle
PGE	prostaglandin

NO	nitric oxide
rAAV	recombinant adeno-associated vector

7. References

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STATEMENT OF SIGNIFICANCE

Articular cartilage is a remarkable and sophisticated tissue that tolerates complex stress within the joint. When injured, cartilage cannot self-repair, and these injuries often progress to joint-wide osteoarthritis, causing patients debilitating pain and loss of mobility. Current palliative and surgical treatments only provide short-term symptomatic relief and are limited with regards to efficiency and efficacy. Bioactive factors, such as drugs and growth factors, can improve outcomes to either stabilize the degenerated environment or regenerate replacement tissue. This review highlights recent advances and novel techniques to enhance the delivery, localization, retention, and activity of these factors, providing a synthesis of the cartilage drug delivery field that can guide future research in restorative and regenerative treatments for damaged cartilage.

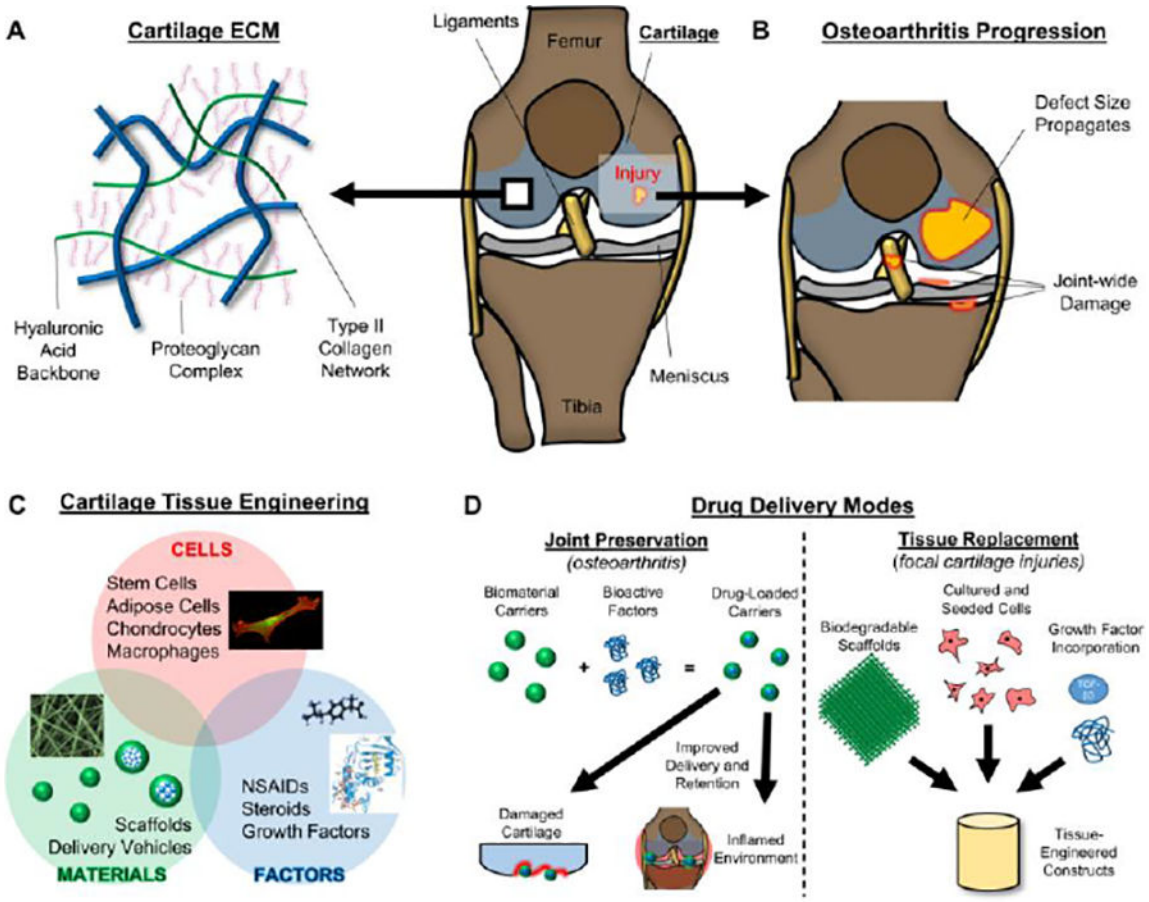


Figure 1.

Cartilage Composition, Disease Progression, Tissue Engineering, and Treatment Modalities. [A] Cartilage is composed of a type II collagen network (blue), interwoven with long hyaluronan chains (green) and their associated proteoglycan complexes. [B] Cartilage injuries often increase in size and can result in joint-wide degenerative changes. [C] Cartilage tissue engineering combines cells, materials, and factors in order to treat damaged cartilage. [D] Two distinct modes of biofactor delivery for cartilage applications. (Left) Joint preservation via bioactive factor incorporation into biomaterial carriers to target cells within the joint. (Right) Tissue replacement via cell-based scaffolds that are supplemented with growth factors.

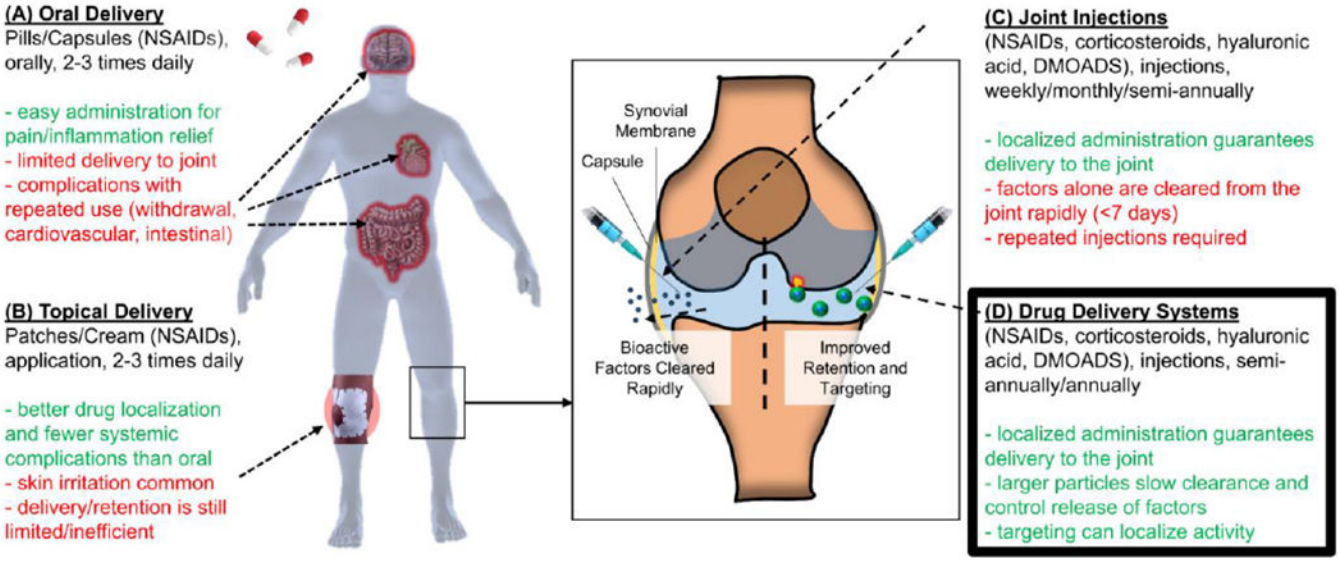


Figure 2.

Drug Delivery Approaches. Benefits (green) and drawbacks (red) of [A] oral delivery, [B] topical delivery, and [C] joint injection of bioactive factors, highlighting the need for [D] drug delivery systems to enhance delivery and retention of these factors.

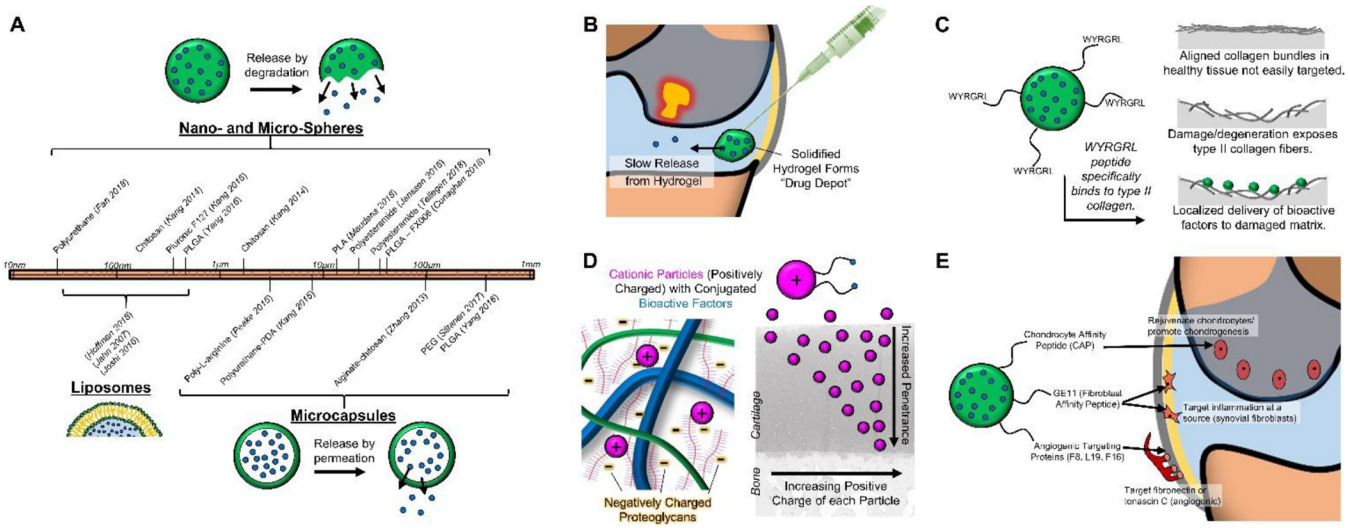


Figure 3.

Intra-articular Drug Delivery Systems. [A] Materials used for nano- and micro-spheres, liposomes, and microcapsules span a wide range of sizes (10nm – 1mm). [B] Hydrogel "drug depots" can extend drug release within the joint. [C] Targeting molecules (e.g. WYRGRL) can focus delivery to the damaged cartilage interface. [D] Cationic particles can increase penetrance for improved drug delivery within cartilage tissue. [E] Drug delivery vehicles can target specific cells and structures (e.g. chondrocytes, synovial fibroblasts, angiogenic regions).

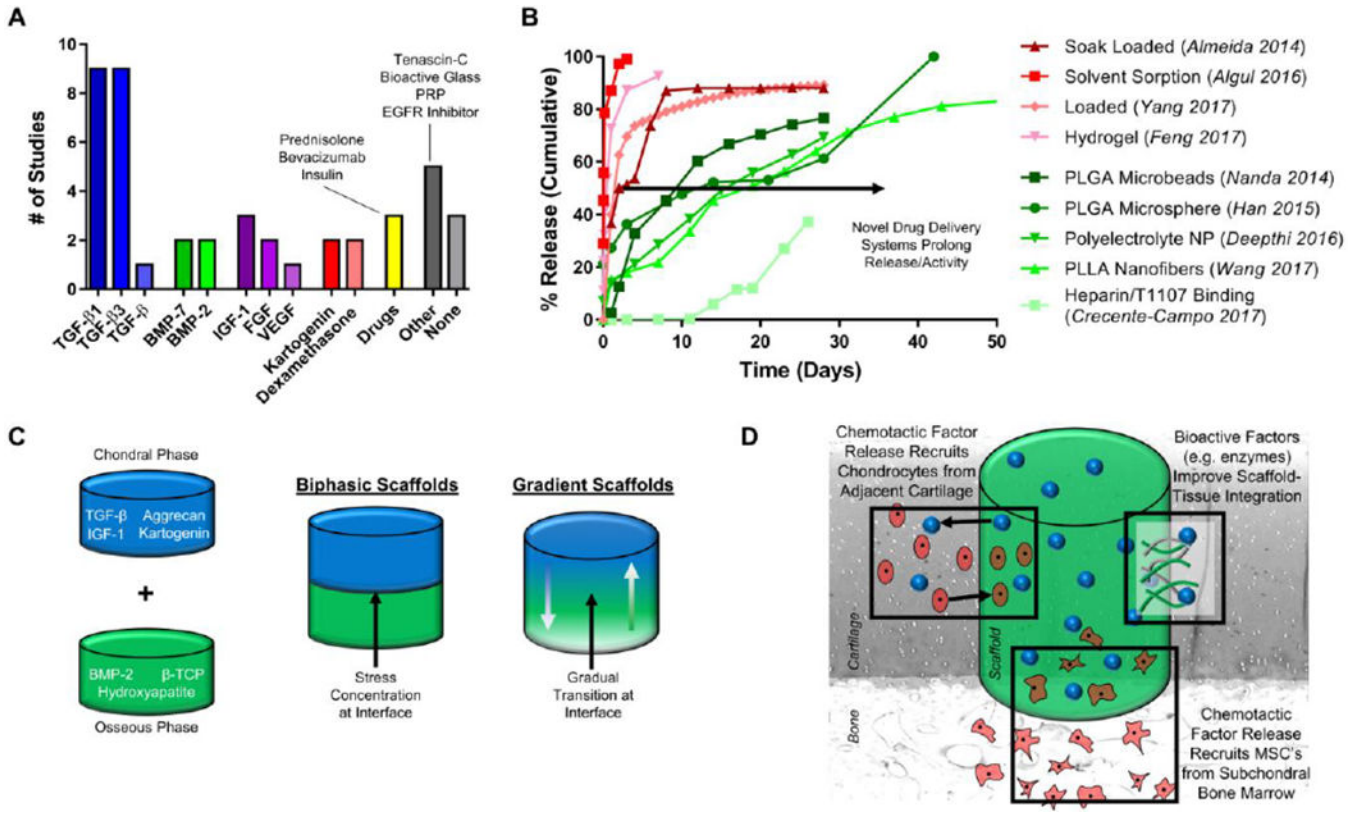


Figure 4.

[A] Systematic literature search (Pubmed: cartilage scaffold drug delivery) revealed trends in growth factor release for promoting chondrogenesis (query window: Sep 2013-Sep 2018). [B] Characteristic drug release profiles of factors encapsulated or soak-loaded in hydrogels/scaffolds (red) or within particles/more elaborate systems (green). Values replotted from literature sources [36,37,159,163,169–173]. [C] Depiction of chondral and osseous scaffolds for spatiotemporal control, combined into biphasic and gradient scaffolds. [D] Mechanisms to improve cartilage repair by increased chondrocyte migration from adjacent cartilage, increased MSC recruitment from the subchondral bone, and improved integration between the scaffold and surrounding tissue.

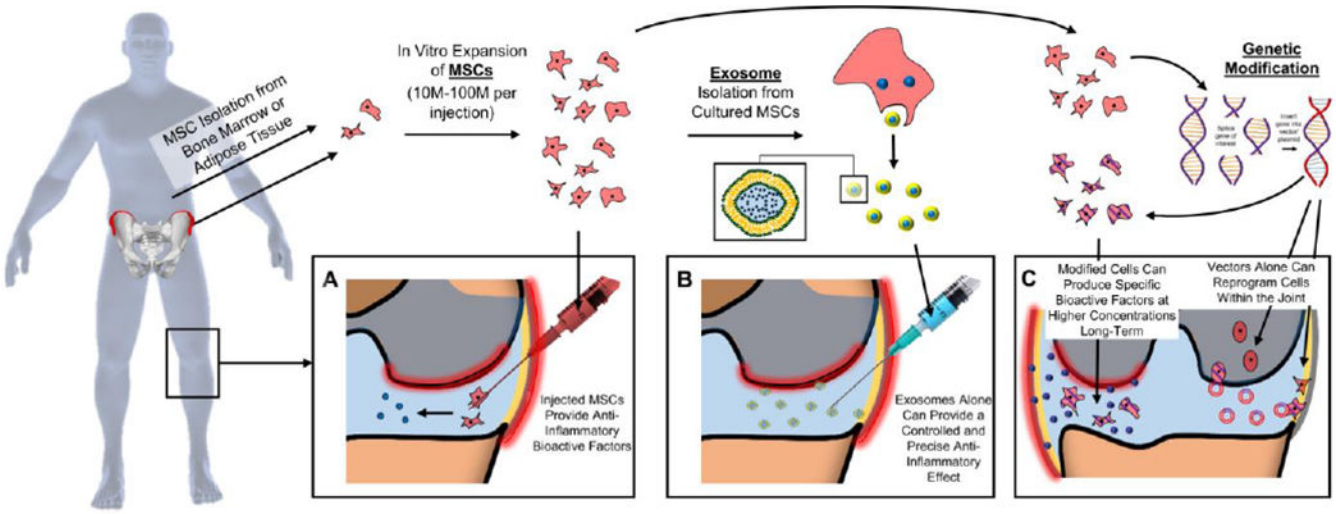
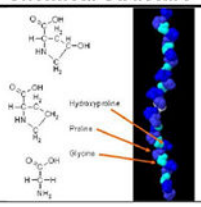
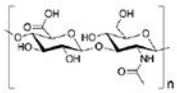
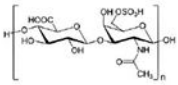
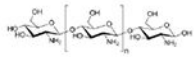
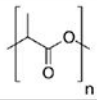
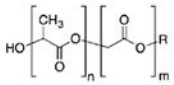
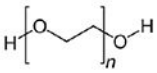
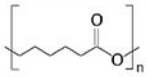


Figure 5.

Cells and Cell-based Products as Drug-Delivery Mechanisms. MSCs are isolated from either bone marrow or adipose tissue, expanded, and [A] injected into the joint to deliver bioactive factors. Cultured MSCs can also be used to isolate the therapeutic exosomes for [B] more controlled and precise delivery of factors. [C] Genetic modification of cultured cells or direct delivery of the vector into the joint can modify cells to provide local and sustained production of therapeutic factors.

Table 1.

Common polymer types (natural and synthetic) used as drug-delivering carriers and drug-releasing scaffolds. Chemical structure, advantages, drawbacks, and cartilage applications provided.

	Polymer Name	Chemical Structure	Advantages	Drawbacks	Uses
Natural	Collagen (type I/II)		Excellent biocompatibility, Bioresorbable, Natural component of ECM, Already used clinically	Type II collagen (cartilage-specific) can be immunogenic, relatively low biomechanics	Tissue engineering scaffolds and gels, Used for MACI and other clinical cartilage restoration techniques
	Hyaluronic Acid **[HA]**		Chemically modifiable, natural GAG in cartilage matrix	Requires modification to form 3D structures	Tissue engineering hydrogels / scaffolds, drug depots, intra-articular injection
	Chondroitin Sulfate **[CS]**		Chemically modifiable, native to cartilage tissue	Low molecular weight	Hydrogels for tissue engineering, nanoparticle carriers
	Chitosan		Biocompatible, non-cytotoxic, contains cartilage components	Slow gelation for in situ applications	Microsphere carrier, tissue engineering scaffold
Synthetic	Polylactic acid **[PLA]**		Easily processed, elongated degradation, high strength	Acidic byproducts, auto-catalytic degradation	Tissue engineering scaffolds, drug carriers
	Poly(lactic-co-glycolic acid) **[PLGA]**		Selectable degradation based on copolymer ratio	Acidic byproducts, poor long-term stability	Nano-carriers (e.g. FX006), micro-carriers, tissue engineering scaffolds
	Polyethylene glycol **[PEG]**		Easily functionalized	Non-degradability, no inherent biologic impact	Microcapsules, drug depots, hydrogels, liposomal fortification
	Polycaprolactone **[PCL]**		Easily processed and manufactured	Slow degradation, intracellular resorption	manufactured scaffolds (e.g. 3D-printing), drug delivery