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Development And Formulation Of Drug-Loaded Hydrogel For Cartilage Regenerative Potential

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Abstract

Cartilage damage and degeneration pose significant challenges in orthopedic medicine, innovative approaches for regenerative therapies. The hydrogel is designed to provide a supportive matrix for cell growth and differentiation while delivering therapeutic agents to promote tissue repair. Drug-loaded hydrogels have been extensively studied for cartilage regenerative medicine, offering promising results for tissue engineering and drug delivery applications. These hydrogels, composed of homopolymers and copolymers, can absorb water and create an appropriate microenvironment similar to the extracellular matrix (ECM). Various polymers and crosslinking methods are explored to optimize the hydrogel's mechanical properties, biocompatibility, and drug release kinetics. Additionally, the study investigates the efficacy of different drugs, growth factors, and bioactive molecules for enhancing chondrogenesis and cartilage regeneration within the hydrogel scaffold. The developed drug-loaded hydrogel holds great promise for addressing the unmet clinical need for effective cartilage regenerative therapies, offering potential advancements in orthopedic medicine.

Keywords: Cartilage, Hydrogel, Extracellular Matrix (ECM), Scaffolds, Tissue Engineering.

INTRODUCTION:

Cartilage is a connective tissue structure that supports body movement by creating low-friction surfaces between bony joints. Cartilage does not contain vascular and neural elements, which severely limits its ability to regenerate after injury. Microfracture and autograft techniques are the current medical standards for repairing cartilage defects. However, these methods cannot completely restore the structural and functional integrity of cartilage tissue, which requires the development of alternative methods [1]. The extracellular matrix (ECM) is responsible for the lubricating functions of cartilage. Therefore, smart and tunable ECM-mimicking structures can act as temporary cartilage substitutes by providing the necessary environmental conditions for regeneration. However, these scaffolds must be biocompatible and biodegradable, minimize immunogenic reactions, stimulate cell proliferation and integrate effectively with surrounding tissues. In addition, they must have a porous structure that facilitates cell migration and communication, and their mechanical properties match the

original cartilage tissue, support newly formed tissues, and provide the necessary signals for cell recruitment and differentiation [2]. The ability of cartilage to regenerate is impaired by its inherent limited vascularity, which leads to poor proliferative capacity of chondrocytes, the main cell type of cartilage. Current methods for treatment of well-defined osteochondral lesions include drilling, autologous chondrocyte implantation, and osteochondral allografting. These treatment options led to the formation of fibers containing both type I and type II collagen, which have reduced strength and elasticity compared to cartilage [3]. Cartilage scar tissue also has a higher coefficient of friction than cartilage, which can impede movement relative to the smooth surface of cartilage and lead to earlier degeneration. Degenerated joints with greater cartilage damage or loss, such as osteoarthritis, are often ultimately treated by replacing the joint entirely with metal implants. Although these current treatments reduce pain and increase mobility, there is a growing need for treatment options that restore the original biological properties of cartilage. The regenerative capacity of damaged cartilage is limited and the potential morbidity associated with bone and cartilage grafting or grafting, cartilage . regeneration is an attractive choice. The field of cartilage tissue engineering is being developed to create biologically compatible synthetic cartilage structures. These constructs consist of appropriate cell types seeded on biomaterial scaffolds to create a durable tissue repair system that can potentially be implanted in a single step.

CARTILAGE TISSUE ENGINEERING:

Tissue engineering is the art of using (biological) material to create new tissue, which replaces worn out or lost original tissue that mimics its original function. Plastic joint cartilage cannot adequately repair itself when damaged. This causes cartilage degeneration and inevitably joint damage. The need to treat this progressive degeneration and effectively repair or replace damaged cartilage has created the field of cartilage tissue engineering. The main goal of cartilage engineering is to repair a joint or articular cartilage. Similar to epiphyseal growth disc cartilage, articular cartilage is a hyaline cartilage whose ECM contains abundant glycosaminoglycans and type 2 collagen as the most abundant protein [4]. Unlike articular cartilage, there is no clear clinical need for epiphyseal cartilage tissue engineering. When we talk about cartilage tissue engineering, we are talking specifically about articular cartilage. cartilage development, focusing on the development of long bones, growth plate and especially articular cartilage. Many of the molecular mechanisms identified in long bone development can be applied to tissue engineering strategies. In addition to biological knowledge, it is important to understand the history of clinical applications of articular cartilage repair and how this led to the tissue engineering strategies used today. Another important element to create an optimal and effective treatment is the source of the cells, if the cells are used. Here we focus on historically used cell sources, current trends, and future treatment options. Currently available commercial biomaterials and their use in cartilage tissue engineering are highlighted, as well as emerging smart materials tailored to specific selected requirements.

HYDROGEL:

Three-dimensional cross-linked polymer networks known as hydrogels are intelligent enough to respond to changes in environmental stimuli, such as ionic strength, pH, temperature, the presence of an enzyme, and an electric field, and to expand or shrink appropriately. They are springy and squishy when swollen, similar to real tissue and displaying good biocompatibility [7]. Medical science discovers new therapeutic moieties with particular carriers for the transport of medications into specific locations of the body. Hydrogels can transport genetically altered pharmaceuticals, such as proteins and peptides, and increase the therapeutic efficacy of medications with the aid of conventional means [8]. The hydrogels may consist of homo-polymeric, co-polymeric, semi-interpenetrating, or interpenetrating polymer networks, depending on the preparation techniques used. Thermoplastic co-polymeric biodegradable hydrogels with the best mechanical strength have recently been developed for biomedical applications. Because hydrogels are easy to make and apply, they are frequently utilized as medication carriers. Hydrogels can be produced using numerous "classical" chemical methods. These comprise both multi-step processes involving the synthesis of polymer molecules with reactive groups and their subsequent cross-linking, as well as one-step processes like polymerization and parallel cross-linking of multifunctional monomers by reacting polymers with appropriate cross-linking agents.

HYDROGEL PROPERTIES:

The application of hydrogels, or hydrophilic gels, in pharmaceutical and biomedical engineering is gaining extensive interest.

SWELLING PROPERTIES:

Hydrogel can undergo quick, reversible changes in response to even slight environmental changes. The hydrogel's physical texture may change in response to changes in environmental factors such as temperature, pH, electric signal, and the presence of other ionic species or enzymes [9].

MECHANICAL PROPERTIES:

The desired mechanical property of the hydrogel could be achieved by changing the degree of Crosslinking and by increasing the degree of crosslinking a stronger hydrogel could be achieved though the higher degree of crosslinking decreases the % elongation of the hydrogels creates a more brittle structure [9].

HYDROGEL TYPES:

Hydrogels can be classified as polymers according to the type of cross-linking forces between chains, into chemical hydrogels, physical hydrogels, and a combination of both. simultaneous cross-linking.

PHYSICALLY CROSS-LINKED HYDROGEL:

Polymer networks must meet the following conditions to form hydrogels: (a) strong interchain interaction to form a stable coupling into a molecular network; and (b) must promote the penetration of water into the polymer network and in the hydrogel stay. Hydrogels that meet these requirements can be prepared using non-covalent methods such as electrostatic, hydrogen bonding, and hydrophobic forces between polymer chains. The hydrogels formed as a result of these interactions are exclusively physical gels and exhibit high water sensitivity and thermal reversibility. The lifetime of such a hydrogel in a physiological environment is short, ranging from a few days to a maximum of a month. Thus, physical gels are useful and are used when a short-term release of the drug is required. This type of hydrogel is safe to use for clinical purposes because no toxic covalent cross-linking molecules are required for gelation.

CHEMICAL CROSS-LINKED HYDROGEL:

The formation of physical gels through clusters of molecules causes them to form free chain loops and consequent inhomogeneity, which is related to short-lived network imperfections. Chemical cross-linked hydrogel networks are easy to control compared to physical hydrogels because their synthesis and applications do not depend only on pH. Chemical cross-linking can be used to modify the physical properties of hydrogels. In general, swelling behavior, biodegradability and mechanical strength are modulated by covalent cross-linking. Covalent cross-linking can be done in many different ways.

IMPROVEMENTS IN HYDROGEL STRUCTURE:

The mechanical integrity of hydrogels is important for cartilage regeneration. Conventional hydrogel designs that rely on a single polymer network typically produce hydrogel structures with mechanical qualities that are significantly less than those of natural cartilage. Focus is moving from conventional hydrogels, which use a single polymer for hydrogel fabrication, to more complex hydrogel systems with mixtures of multiple polymers, often including two or more independent networks, in an effort to increase the mechanical properties of hydrogels to approaches those of hyaline cartilage. These systems may show better integration with surrounding tissue in vivo, in addition to frequently achieving stronger mechanical properties than networks of individual polymers. In this section we examine recent advances within the framework of different network types.

INTERPENETRATING NETWORKS:

Hydrogels utilizing interpenetrating networks (IPNs) consist of two or more distinct crosslinked networks that are not covalently bonded to one another, but rather partially entangled to the point where dissociating the network's constituent parts requires breaking chemical bonds [10] [11]. As a result, IPN hydrogels have more mechanical qualities than hydrogels made of individual component networks, which is why cartilage tissue engineering applications find them interesting [12] [13]. In order to better replicate the physical characteristics

of native tissue or to replicate the presentation of bioactive cues accessible to cells within the hydrogel structures, designers of IPNs for hydrogel creation have recently begun to incorporate two or more extra networks into their designs [14] [15].

SEMI-INTERPENETRATING NETWORKS:

Semi-Ipns Are Made Up of a Crosslinked Network That Has Branched or Linear Polymers Embedded in It. Therefore, it is theoretically possible to separate the polymers and network without disrupting chemical connections [16] [17]. HA Is One of The Most Often Occurring Macromolecules Dispersed Throughout These Networks. Utilizing semi-ipns' capacity to gradually leach low-molecular-weight macromolecules is another way to take advantage of their features [18].

DOUBLE NETWORKS:

A double network is made up of two networks that are crosslinked together yet have distinctly different mechanical characteristics. Because the network can yield under mechanical load, the first network often offers a rigid structure, and the second network is ductile, resulting in more toughness than the corresponding single networks alone would have produced [19]. Due to their improved mechanical qualities over typical hydrogels, particularly their ability to approximate the mechanics of native hyaline cartilage, these kinds of networks have attracted interest in cartilage tissue engineering [20]. Since cartilage and other bone tissues naturally contain double networks in their extracellular matrix (ECM) to obtain their strong mechanical characteristics, double networks are also conceptually interesting Combinations of two acrylamide polymers, poly (2-acrylamido-2-methylpropanesulfonic acid) (PAMPs), poly(acrylamide) (PAAM), and/or poly (N, N-dimethylacrylamide) (PDMAAM), are often used double networks for cartilage tissue engineering Utilize these hydrogels with double networks [21] [22] [23]. Double networks are frequently made of non-degradable polymers, and while their durability makes them potentially valuable materials for mechanical support in tissue defect, by encapsulating human chondrocytes, this approach increased the synthesis of cartilage matrix components and compressive modulus by utilizing the comparatively higher reactivity of methacrylate groups to form a double network. Hydrogels utilizing this kind of network are constantly being created; one such hydrogel is an injectable, cytocompatibility double network[25] [26].

DUAL NETWORKS:

Dual networks consist of two materials crosslinked into the same network by comparable crosslinking methods [27]. Although two networks aren't as robust as double networks, each element in a two-network could give the hydrogel more advantages. For instance, one material might help the hydrogel integrate with the surrounding tissue more effectively, while another might attract cells to it and encourage their migration [28]. use a dual network hydrogel of heparin and tyramine to enclose bovine chondrocytes in vitro, they observed enhanced cell survival and proliferation. Moreover, compared to cells in a single-component dextran-tyramine hydrogel, they observed increased deposition of collagen and chondroitin sulfate [29].

SCAFFOLDS:

Tissue engineering approaches tissue repair and regeneration by using scaffolds and growth factors separately or in combination [5]. A scaffold is a porous, three-dimensional template that facilitates the necessary creation of tissue both physiologically and physically. When it comes to cartilage, it is anticipated that the scaffolds created will be pliable and supple, much like native cartilage. Hydrogels, decellularized extracellular matrix, and nanofiber matrices are a few of the scaffold types that have been thoroughly studied for cartilage regeneration. Unlike autografts and allografts, there is no supply constraint on scaffolds used in cartilage repair. These structures act as templates for the development of tissues and are specifically designed from biomaterials. Scaffolds must therefore meet the necessary requirements, which may include having the right architecture, being biocompatible, degradable, or having particular chemical and physical qualities [30]. This can be achieved by choosing suitable materials, additives such as pore fillers, and production methods. Three main groups of scaffolds can be used in cartilage tissue engineering: (1) protein-based polymers; (2) carbohydrate-based polymers; (3) synthetic polymers.

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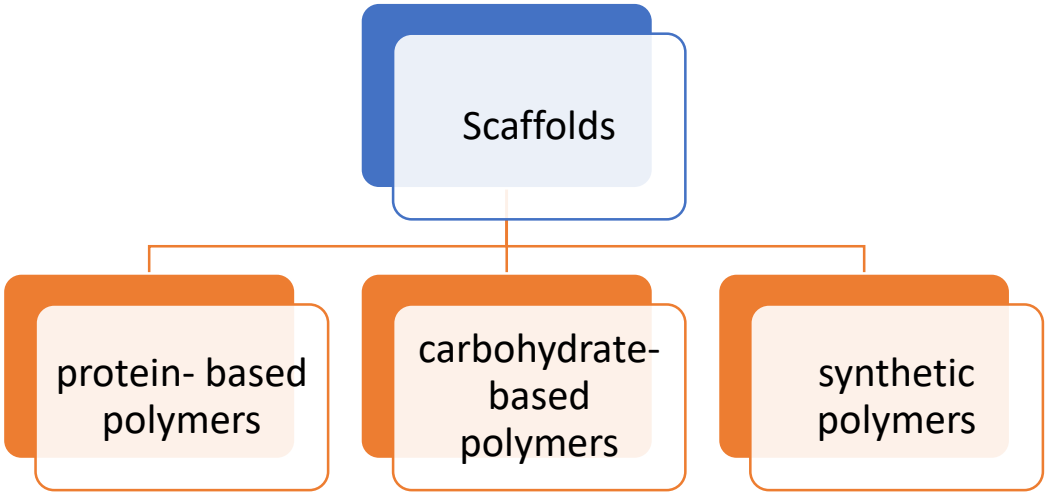
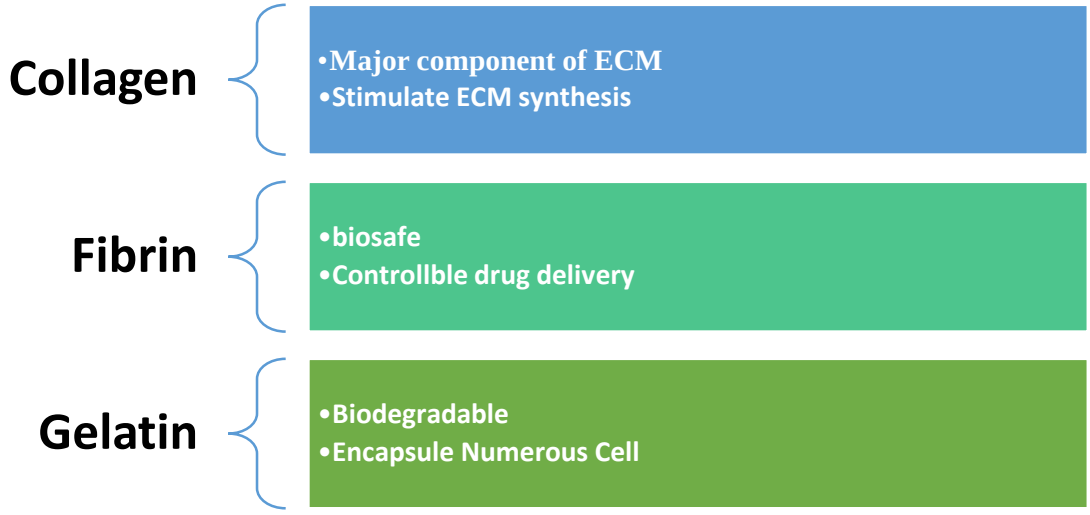


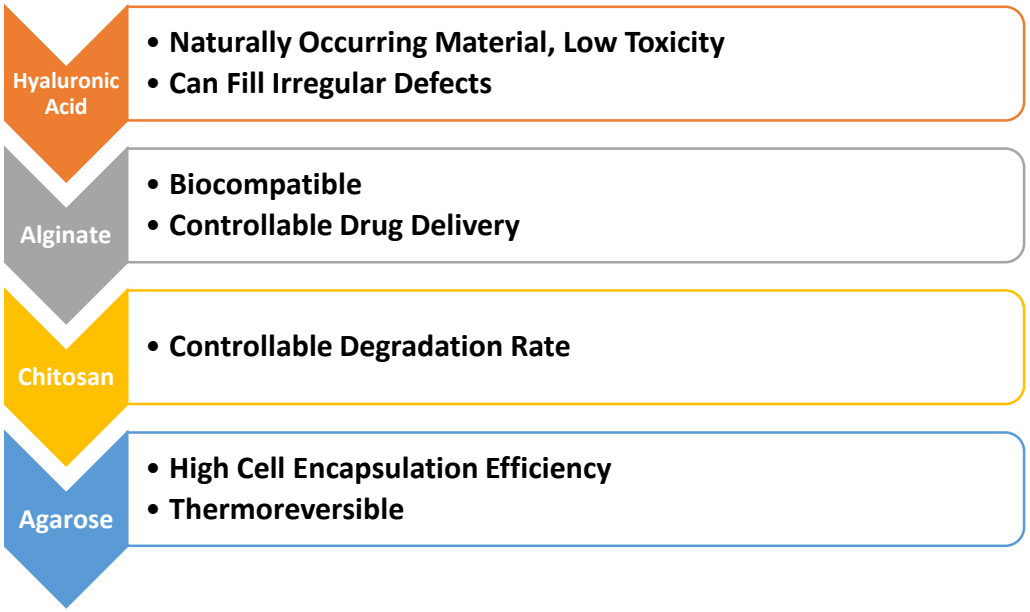
PROTIEN-BASED POLYMERS:

Protein-based polymers such as collagen, gelatin, and fibrin are utilized in bioengineered scaffolds. Gelatin is formed from denatured collagen and can bind proteins, peptides, and growth factors and allow for cell adhesions. Fibrin, a protein matrix derived from fibrinogen, is a crucial component of blood clots. Collagen's function as a scaffolding material allows cells to maintain their phenotypes since it is the primary structural component of the extracellular matrix(ECM) [31]. Integrin binding controls cellular events. Regarding clinical research, 21 patients with Grade III chondral defects in the distal femur underwent implantation of a collagen type I scaffold seeded with autologous chondrocytes. A random comparison was made between these patients and nine other patients who had the same lesion treated with microfracture. When the patients were followed up after two years, they reported improved function, increased motion, and significantly lower pain scores than they had before surgery [32].



CARBOHYDRATE-BASED POLYMERS:

Hydrogel scaffolds have also included the use of carbohydrates, such as hyaluronic acid, alginate, chitosan, and agarose [33]. These scaffolds have characteristics similar to the extracellular matrix of cartilage in that they are made of crosslinked polymers that absorb a lot of water. They are also effective in encapsulating cells and preserving the spherical morphology of the chondrocytes within the scaffold [34]. Hydrogel scaffolds can be altered by adjusting their gelation mechanism, adding synthetic materials, or including growth factors to promote chondrogenesis [35] [36].



SYNTHETIC POLYMERS:

Polylactic acid (PLA), polyglycolic acid (PGA), polycaprolactone (PCL), and polylactic-co-glycolic acid (PLGA) are the most often used materials in synthetic polymer-based scaffolds [37]. These materials can be spun into nanofibers or woven. Patients with defects in the patellofemoral cartilage had surgery and were monitored for up to two years afterward using a synthetic scaffold that contained calcium sulfate, PGA, and PLGA [38]. Compared to other scaffolds, the study's results showed improved short-term outcomes; nevertheless, the restoration of subchondral bone with hyaline cartilage was not possible with other synthetic scaffolding materials, such as carbon fiber, polybutyric acid, Dacron®, and Teflon®. Ceramics: tricalcium phosphate, hydroxyapatite (Ha), and bioactive glass are also taken into account while creating scaffolds for cartilage replacement, as these ceramic materials encourage the formation of an apatite layer that resembles bone, which serves to anchor the cartilage scaffold that is placed on top of the osteochondral defect to its original bed. A 3D scaffold was used to evaluate the treatment of osteochondral or chondral defects in the knee in a recent study. Using Ha nanoparticles and layered collagen type I fibrils to create a synthetic scaffold for the bones [39] [40].

Synthetic Polymers	Advantages
Polyactic acid (PLA)	High tensile strength. High modulus-able to bear loads..
Polyglycolic acid (PGA)	Good mechanical strength. High modulus. Natural degradation product (glycolic acid).
Polycaprolactone (PCL)	Good osteoinductive potential. Non-toxic degradation products. Good mechanical properties.
Polyactic-co-glycolic acid (PLGA)	Enhanced mechanical strength compared to PLA or PGA alone. Biodegradable and Biocompatible. Resistance to hydrolysis.

GROWTH FACTORS:

Growth factors are physiologically active polypeptides that are endogenous chemicals that can be used to stimulate cell development, accelerate chondrogenesis, and improve the treatment of cartilage abnormalities. Cells and supporting structures form a network through which cartilage heals.

TRANSFORMING GROWTH FACTOR (TGF):

The TGF-β superfamily is commonly used to stimulate cartilage repair, including TGF-β1, BMP-2 and BMP-7, TGF-β3, and existing cartilage-derived morphogenetic proteins (CDMP-1 and CDMP-2). induce chondrogenic differentiation and stimulate cartilage extracellular matrix production, which is the most commonly used growth factor for chondrogenesis, decreases or eliminates IL-1 catabolism by stimulating extracellular matrix (ECM) synthesis, synovial membrane, and chondrogenesis development of BMSCs. BMP-2 has been used in other orthopedic applications to stimulate bone growth in either fracture healing or fusion mass formation, but has the potential to stimulate matrix synthesis and reverse chondrocyte differentiation [41]. BMP-7 supports the synthesis of cartilage matrix, coordinates with anabolic growth factors, and inhibits certain catabolism-related genes such as MMP-1, MLP-13, and IL-8.

FIBROBLAST GROWTH FACTOR (FGR):

The fibroblast growth factor (FGF) family, especially FGF-2 (basic FGF, bFGF), and FGF-18, act by binding to cell surface receptors, promoting anabolic pathways, and reducing. catabolic aggrecan's enzyme activity [42]. In mice, subcutaneous administration of FGF-2 prevented OA, while FGF-2 knockout mice were found to accelerate OA. However, FGF-2 should be used with caution, as higher doses of FGF-2 may promote increased inflammation by antagonizing insulin-like growth factor (IGF)-1 and regulating MMPs.

INSULIN-DEFICIENT GROWTH FACTOR (IGF):

IGF-1 is another growth factor that helps maintain the integrity of articular cartilage and induces anabolic effects on cartilage healing by reducing catabolic effects. IGF-1 works better with other growth factors, such as TGF-β and BMP-7. Mice with chronic IGF-1 deficiency are more likely to have joint cartilage damage, and increased IGF-1 increases joint protection [43].

PLATELET-DERIVED GROWTH FACTOR (PDGF):

Increases proteoglycan synthesis and promotes cell proliferation through PDGF, a chemotactic factor. Mesenchymal cells have been shown to accelerate cartilage formation and wound healing. By regulating NF-κB signaling, PDGF has also been shown to reverse the effect of IL-1β on cartilage degeneration [44].

PLATELET-RICH PLASMA (PRP):

PRP is also considered a potential source of growth factors based on its role. wound healing and treatment of other musculoskeletal disorders. Clinical trials have evaluated the role of intra-articular PRP injections in the treatment of OA [45]. showed that knee OA patients who received three intra-articular injections of PRP had improved clinical function and decreased pain compared with knee OA patients who received three intra-articular injections of hyaluronic acid. Treatment of hip OA patients with ultrasound-guided PRP injection into the affected hip showed improved patient assessment scores (WOMAC and Harris Hip scores) and decreased pain at six-month follow-up [46].

COMMON GROWTH FACTORS USED IN CARTILAGE REGENERATION:

BMP-2	Stimulates ECM production. Increases ECM turnover. Increases aggrecan degradation.
BMP-7	Stimulates ECM production. Inhibits cartilage degradation by decreasing ILs and MMPs.
FGF-2	Increases aggrecan degradation. Inhibits proteoglycan synthesis. Upregulates MMPs.
IGF-1	Stimulates ECM production. Decreases ECM catabolism.

TGF-β1	Stimulates ECM production. Inhibits cartilage degradation by decreasing ILs and MMPs.
PRP	Biologic cocktail of multiple growth factors and cytokines.
PDGF	Chemotactic factor for mesenchymal cells Suppresses IL-1–induced cartilage degradation
FGF-18	Stimulates ECM production in injured joints Increases chondrocyte proliferation

CONCLUSION:

In summary, drug-loaded hydrogels present a viable approach for improving cartilage regeneration and may even prevent the development of osteoarthritis in cartilage regenerative medicine. With their capacity to target certain regions, they function as an efficient drug delivery system that offers a platform for controlled drug release to promote tissue regeneration. All things considered, drug-loaded hydrogels are a useful strategy in the field of cartilage tissue engineering, providing hope for better therapies for disorders linked to cartilage defects such as osteoarthritis.

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