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Historical Perspective

Functionalized hydrogels as smart gene delivery systems to treat musculoskeletal disorders

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ABSTRACT

Despite critical advances in regenerative medicine, the generation of definitive, reliable treatments for musculoskeletal diseases remains challenging. Gene therapy based on the delivery of therapeutic genetic sequences has strong value to offer effective, durable options to decisively manage such disorders. Furthermore, scaffold-mediated gene therapy provides powerful alternatives to overcome hurdles associated with classical gene therapy, allowing for the spatiotemporal delivery of candidate genes to sites of injury. Among the many scaffolds for musculoskeletal research, hydrogels raised increasing attention in addition to other potent systems (solid, hybrid scaffolds) due to their versatility and competence as drug and cell carriers in tissue engineering and wound dressing. Attractive functionalities of hydrogels for musculoskeletal therapy include their injectability, stimuli-responsiveness, self-healing, and nanocomposition that may further allow to upgrade of them as "intelligently" efficient and mechanically strong platforms, rather than as just inert vehicles. Such functionalized hydrogels may also be tuned to successfully transfer therapeutic genes in a minimally invasive manner in order to protect their cargos and allow for their long-term effects. In light of such features, this review focuses on functionalized hydrogels and demonstrates their competence for the treatment of musculoskeletal disorders using gene therapy procedures, from gene therapy principles to hydrogel functionalization methods and applications of hydrogel-mediated gene therapy for musculoskeletal disorders, while remaining challenges are being discussed in the perspective of translation in patients.

Statement of significance: Despite advances in regenerative medicine, the generation of definitive, reliable treatments for musculoskeletal diseases remains challenging. Gene therapy has strong value in offering effective, durable options to decisively manage such disorders. Scaffold-mediated gene therapy provides powerful alternatives to overcome hurdles associated with classical gene therapy. Among many scaffolds for musculoskeletal research, hydrogels raised increasing attention. Functionalities including injectability, stimuli-responsiveness, and self-healing, tune them as "intelligently" efficient and mechanically strong platforms, rather than as just inert vehicles. This review introduces functionalized hydrogels for musculoskeletal disorder treatment using gene therapy procedures, from gene therapy principles to functionalized hydrogels and applications of hydrogel-mediated gene therapy for musculoskeletal disorders, while remaining challenges are discussed from the perspective of translation in patients.

1. Introduction

Disorders of the musculoskeletal system, *i.e.* the cartilage, bone, meniscus, ligaments, and tendons, affect millions of people worldwide annually [1–5] and the situation is getting more serious as the world population is aging. Of critical importance, none of the current clinical strategies has been able thus far to durably and reliably regenerate the injured tissues, neither in their native organization nor in their mechanical functions [6–9]. Interestingly, treatments based on the delivery of therapeutic gene products in sites of musculoskeletal injury, referred to as gene therapy, have provided certain optimism to manage these disorders [10,11].

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Principally, in gene therapy, the aim is to smartly and locally deliver gene-based candidate sequences in various targets including cells, tissues, and live organisms [12,13]. In this way, compared with the direct application of the therapeutic product itself displaying a short half-life [14], gene therapy utilizes powerful tools called gene vehicles or vectors, including nonviral and viral vectors, to afford long-term therapeutic effects of the gene products [12,15]. During a relatively long history, gene therapy experienced ups and downs and thus far, large efforts explored the clinical and laboratory potential of this procedure for the treatment of various disorders such as neurodegenerative diseases [16,17], autoimmune disorders [18,19], cancer [20,21], and also musculoskeletal diseases [22,23].

Classical gene therapy, consisting of direct (*in vivo*) delivery of gene vectors or indirect (*ex vivo*) delivery of genetically modified cells [24], has a number of physical and biological impediments like non-target-dissemination and toxic, immune, and inflammatory responses [14,25–27]. Although a plethora of approaches have been developed to overcome these obstacles such as alternative routes of vector administration, the use of permissive clinical components, *e.g.* hirudin, and of immunosuppressive agents, plasmapheresis, the manipulation of vector decoys and engineered vectors, there is still a serious need for versatile strategies to definitely tackle such challenges for clinical gene therapy [28–34]. Inspired by tissue engineering, scaffold-assisted gene therapy has attracted strong, recent attention, based on the administration of vectors/cells inserted in a bio-framework to allow for their controlled release while reducing the biological impediments noted when rather than delivering gene vectors or genetically modified cells into their targets.

Scaffolds commonly employed in tissue engineering strategies are classified into solid (fibrous, porous), hydrogel, and hybrid systems fabricated from natural or synthetic biomaterials [35–39], all amenable to gene therapy [22,24], where each category has its own advantages and limitations. Considering any type of tissue engineering application, intended scaffolds possess common features including biodegradability, biocompatibility, nontoxicity, three-dimensional (3D) porous environment, and target-tissue biomechanical consistency [40–43]. Still, important aspects to consider when generating adapted gene therapy-based treatments for musculoskeletal disorders include the question of injectability (solid - fibrous and porous - scaffolds are not adapted, in contrast to hydrogels that may be tailored as injectable systems for the minimally invasive delivery of gene vectors) and the issue of the mechanical properties of the system (here, solid scaffolds are superior to hydrogels) [44]. Hydrogels exhibit further attractive features in particular their ability to mimic musculoskeletal tissues and to self-heal and their ease of manipulation, tailoring, and production.

The development of nanocomposite hydrogels, 3D physically- or chemically-crosslinked networks reinforced with nanomaterials, however, introduced mechanically stronger frameworks which gained attention in diverse applications such as biomedicine [45–48], electronics [49–52], and sensors [53–56]. Even though nanocomposite hydrogels have been extensively utilized in tissue engineering and drug delivery applications [57–61], this concept remains still new in the field of vector gene delivery. Different attractive features of nanomaterials such as mechanical properties, size, and shape in combination with elastic and high water-contained hydrogel networks may create functionalized structures for gene therapy applications. Furthermore, other advanced functionalities in hydrogels such as self-healing and shear-thinning abilities [62–65], exploited by tissue engineering and drug delivery, may also make more progress for the delivery of therapeutic gene vectors. These functionalities combine injectability (due to shear thinning) and strong structure (due to self-healing) in a hydrogel [66,67].

To date, while there are a number of studies and general reports on scaffold-mediated vector gene therapy [68–77], most of them focus on cellular and biological evaluations, *e.g.* transgene expression and activities, without an insightful view of the value of functionalized scaffolds from a structural, chemical, and mechanical point of view specifically for viral gene delivery. Also, although some reviews described the value of scaffolds to mediate gene delivery [78–81], none thoroughly explained the importance and role of "smart" hydrogels and of incorporating (multi)functionalities. In reality, a functionalized hydrogel is not only a physical substrate or coverage for vectors but it is purposefully engineered toward an intelligent and multifunction structure. Hence, this review aims to introduce functionalized hydrogels as promising novel platforms for gene therapy, focusing mostly on gene delivery systems. We first discuss the recent advances in hydrogel-mediated gene delivery for musculoskeletal regeneration. We then describe functionalized hydrogels with a concentration on injectability, nanocomposite, and self-healing hydrogels. Finally, we introduce functionalized hydrogels as a promising platform with great potential to address the current challenges in treating musculoskeletal disorders *via* gene therapy. This review may be of significant value to readers investigating efficient gene delivery strategies for patients suffering from musculoskeletal diseases.

2. Musculoskeletal tissues

The stability and mobility of the body depend on the tissues that form the musculoskeletal system [82]. Musculoskeletal damages and related conditions are the global founding cause of physical disability [83]. Upon damage, musculoskeletal tissues exhibit diverse healing profiles and clinically applied methods lack a full ability to restore the functionality of injured tissues.

Bone tissue, a hierarchically organized structure, consists of organic (type-I collagen fibers) and mineral (hydroxyapatite - HAp) phases, tolerates the body weight, and plays a decisive role in movement [84,85]. While the bone is intrinsically capable of healing, there are still critical issues impeding complete full bone regeneration with the occurrence of bulky lesions and subsequent consequences of trauma, cancer, or congenital diseases, even following accepted clinical approaches based on autografts showing graft source restrictions and a risk of infection or on allografts resulting from insufficient tissue integration [86].

The articular cartilage is a strong, flexible connective tissue that protects the body's joints and bones. This white gliding tissue, owing to its avascularity and lack of connection with the subchondral bone, does not adeptly host reparative cells, hence, upon injury, cartilage has a restricted ability for self-healing, potentially leading to osteoarthritis (OA) if left untreated [6,87,88]. Furthermore, in spite of classical surgeries such as stimulation techniques and autologous chondrocyte implantation, these techniques may not be reliable for a durable treatment of cartilage lesions, rather resulting in the formation of an imperfect, mechanically weak, fibrocartilaginous tissue mainly composed of type-I collagen instead of mechanically strong, hyaline cartilage made of type-II collagen and proteoglycans [89].

Tendon and ligament injuries include approximately 30% of musculoskeletal disorders [90]. The tendons that join the muscles to the bones play a role in joint motion by transmitting forces from muscles to the bones [91–93]. The ligaments are also connective tissues that connect bones, providing joint stability [94,95]. Having similarities in their structure and extracellular matrix (ECM) contents, both tissues have moderate intrinsic capabilities of healing and the current clinical approaches, *e.g.* autografts, allografts, xenografts, prostheses, and sutures, normally lead to non-standard tissues with high rates of re-rupture [95,96].

As another connective joint tissue, the meniscus plays essential roles in the knee in distributing loads, absorbing shocks, supporting stability, lubricating the cartilage, and facilitating cartilage nutrition [97–99]. In spite of such significant functions, the meniscus is prone to injuries and is unable to fully regenerate even upon clinical procedures such as meniscectomy, sutures, and grafting, with diverse restrictions that may lead to OA [100–103].

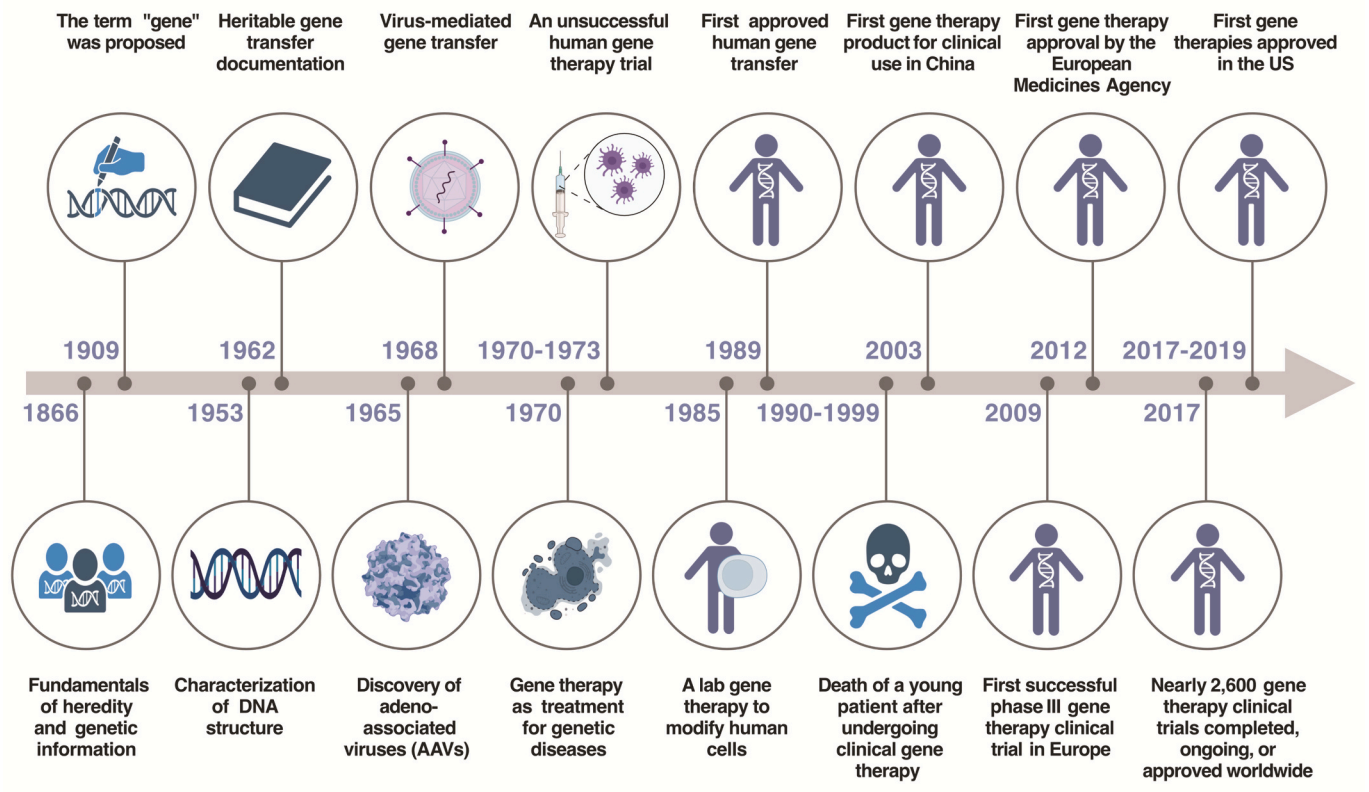


Fig. 1. History of gene therapy (created with Biorender).

In this scheme, the emergence of gene therapy demonstrated its potential as a powerful tool to enhance the regeneration of musculoskeletal tissue damage *via* temporal and spatial delivery of therapeutic candidate gene sequences.

3. Gene therapy

3.1. Principles

As a simple definition, gene therapy is classically the introduction of a gene in a cell *via* a vehicle as a means to transfer or express a selected therapeutic product (protein, nucleic acid) which is carried out through transcription and translation (protein) or may be directly used (nucleic acid: DNA or RNA) [104,105]. This concept has been explored for decades as a way to manage genetic diseases [106–108] (Fig. 1).

Based on the type of disorder, *e.g.* inherited monogenic, cancer, infection, *etc.*, the utilized gene therapy methods can vary including gene correction or replacement, gene augmentation or addition, gene alteration or silencing, gene marking, gene editing or reprogramming [12,109–113]. Besides genes involved in inherited disorders, the most regularly delivered sequences in human gene therapy experiments are tumor suppressors, suicide genes and enzymes, antigens, cytokines, silencers, and replication inhibitors to fight cancer, as well as growth factors/receptors and signaling molecules for regenerative medicine, depending on the specific aim/target that needs to be achieved [17,114,115]. From another perspective, gene therapy can be viewed as a drug delivery system, which enables the transfer of an agent at predetermined sites in a controlled release manner.

The transfer of gene vehicles (vectors) is classically divided into the direct delivery of gene sequences to the targets (*in vivo* gene therapy) and the indirect delivery of gene sequences to cells/tissues (adult differentiated cells such as articular chondrocytes, bone cells, tendon/ligament fibroblasts, and meniscal fibrochondrocytes; adult progenitor mesenchymal stromal cells - MSCs, embryonic stem cells - ESCs, and induced pluripotent stem cells - iPSCs) prior to re-administration in the recipient (*ex vivo* gene therapy) [74] (Fig. 2).

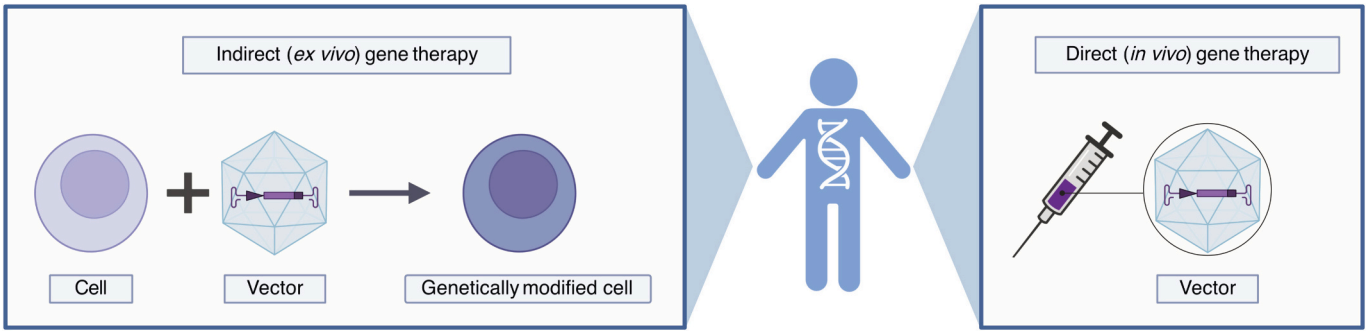
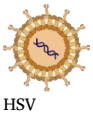


Fig. 2. Classical gene therapy. The application of gene vectors may be performed *via* indirect (*ex vivo*) delivery of genetically modified cells/tissues in the recipient (left) or *via* direct (*in vivo*) delivery of the gene vector to the target (right) (created with Biorender).

Table 1
Nonviral and viral vectors in gene therapy.

Vector Class	Type	Advantages	Shortcomings
nonviral vectors	- naked DNA or RNA - DNA-protein conjugation - physical carriers - chemical carriers	- no toxicity - no immunogenicity - large gene capacity - inexpensive, simple production	- low transfer efficiency - dividing cells - short-term gene expression - high degradation rate
viral vectors	 adenoviral vectors	- large gene capacity - high transfer efficiency - dividing/nondividing cells	- toxicity - immunogenicity - short-term gene expression - unstable episomes
	 HSV vectors	- large gene capacity - high transfer efficiency - dividing/nondividing cells	- toxicity - relative immunogenicity - short-term gene expression - unstable episomes
	 retroviral vectors	- average gene capacity - long-term gene expression - stable integrants	- relative toxicity - relative immunogenicity - limited transfer efficiency - limited tropism - dividing cells - insertional mutagenesis - relative toxicity
	 lentiviral vectors	- average gene capacity - dividing/nondividing cells - long-term gene expression - stable integrants	- relative immunogenicity - limited transfer efficiency - limited tropism - insertional mutagenesis - HIV material
	 rAAV vectors	- high transfer efficiency - dividing/nondividing cells - long-term gene expression - stable episomes	- relative toxicity - relative immunogenicity - limited gene capacity

Abbreviations: HSV, herpes simplex virus; rAAV, recombinant adeno-associated virus; HIV, human immunodeficiency virus. Created with Biorender.

The techniques to transfer candidate genes mostly rely on the use of nonviral vectors (transfection) or viral vectors (transduction), each one with specific advantages and shortcomings [14,22] (Table 1). Upon transfer, foreign genes enter the cells to reach the nucleus and subsequently either merge with the host genome or are maintained as an episome that supports the transient transgene expression [116].

3.2. Nonviral vectors

Nonviral vectors are theoretically simple, yet complicated in practice. These delivery systems have been developed to conveniently and safely transfer nucleic acids (small and large DNA, RNA) [117–119] in target locations using naked DNA, DNA-protein conjugation, physical procedures (needles, ballistic DNA, electroporation, sonoporation, photoporation, magnetofection, hydroporation, mechanical massage) [120–126], and chemical compounds (inorganic particles, calcium phosphate, silica, gold, synthetic/natural biodegradable materials including lipid nanoemulsions, solid lipid nanoparticles [127], peptide beads [128,129], and polymer-based vectors) [125,130–139] (Table 1).

Nonviral vectors have several advantages regarding their safety such as a lack of replication competence and the absence of toxic and immune responses in the host following their administration [136,140]. In contrast, their major drawbacks include their low transfection rates (20–40%), short-term gene expression (only a few days), the necessity for target cell division, and an elevated risk of degradation during transport to the lysosomal compartment, restricting their effective application *in vivo* and making them better suited for *ex vivo* gene therapy [141].

3.3. Viral vectors

Due to the natural aptitude of viruses to penetrate diverse cell populations, a variety of them have been manipulated to meet the requirements needed for gene therapy applications. The most well-known viral vectors derived from viruses include adenoviral vectors, herpes simplex viral (HSV) vectors, retroviral/lentiviral vectors, and recombinant adeno-associated virus (rAAV) vectors, each one again with specific advantages and shortcomings [14,142] (Table 1). The most significant concerns related to the use of viral vectors are associated with safety issues including the occurrence of toxic and immune responses in the host following their administration, their potential dissemination to non-target sites, and risk for insertional mutagenesis upon integration in the host genome [24,143,144].

Adenoviral vectors are highly immunogenic and, while highly effective (up to 100% transduction rates) in both dividing and nondividing cells for suitable *in vivo* gene therapy, they only support short-term gene expression (up to 14 days) as they are kept under unstable episomal forms [27,145,146], as reported with HSV vectors [146].

Retroviral vectors instead promote long-term gene expression (months to years) as a result of their stable integration in the host genome but therefore carry a risk for insertional mutagenesis while they have a limited tropism, requiring cell division for gene expression, making them also better suited for *ex vivo* gene therapy [27,147]. As an alternative, lentiviral vectors share the properties of retroviral vectors but they can target either dividing or nondividing cells, although they carry the further risk or introducing HIV-based material in the recipient [148].

To improve issues related to the immunogenicity and toxicity of the

Table 2
Barriers to effective gene transfer in musculoskeletal tissues and current approaches to overcome them.

Barriers	Approaches
physical and biological barriers	- alternative routes of vector administration - alternative clinical compounds - alternative vector serotypes - cloning of tissue-specific or disease-inducible promoters - reduced vector doses
pre-existing host immune responses	- alternative routes of vector administration - transient host immunosuppression - plasmapheresis, saline flushing - alternative vector serotypes - application of proteasome inhibitors
cell-specific and cell-associated rate-limiting steps for efficient transgene expression	- use of scAAV - use of hybrid vectors - introduction of nuclear localization signals - application of proteasome inhibitors - cloning of tissue-specific or disease-inducible promoters - use of trans-splicing vectors, hybrid vectors
specific features and potentially deleterious effects of the vectors and/or treatments	- use of non-infectious VLPs, replication-defective vectors - use of vexosomes or exosomes - use of chromatin insulators - use of artificial chromosomes, integration-deficient vectors

Abbreviations: scAAV, self-complementary AAV; VLPs, virus-like particles.

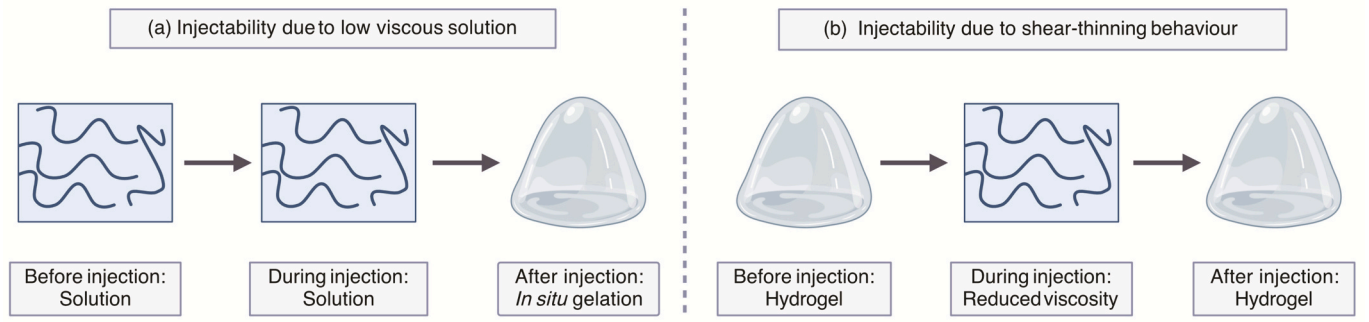


Fig. 3. Hydrogel injectability. Injectability arises from (**a**) a low-viscosity solution (precursor) or from (**b**) a shear-thinning hydrogel (created with Biorender).

abovementioned gene vehicles, rAAV vectors have been introduced to gene therapy, where the recombinant genome of AAV has been fully removed from its viral coding sequences [149,150]. rAAV vectors afford high transduction rates (up to 100%) in both dividing and nondividing cells, allowing for long-term gene expression (months to years) as they are kept under stable episomal forms and making them suitable for *in vivo* gene therapy. Yet, these vehicles have a relatively small gene packaging capacity (~ 4 kb), even though trans-splicing vectors have been created to further enhance the size of the gene being carried, and they may elicit humoral immune responses in the host by pre-existing neutralizing antibodies against the viral capsid proteins [149,150]. Nevertheless, rAAV vectors remain overall particularly competent systems for musculoskeletal gene therapy [27,151,152].

3.4. Current challenges in gene therapy and options to address them

Despite the availability of a variety of gene vectors, a number of critical barriers still preclude their effective use in clinical applications targeting musculoskeletal tissues, including the presence of physical and biological barriers (dense ECM, heparin, pH and enzymatic environment, blood-binding factors, solid tumors, body fluids and secretions, stratified and tight target, and non-target tissues), host immune responses, cell-specific and cell-associated rate-limiting steps (vector uptake, internalization, transport, and processing), and specific features and potentially deleterious effects of the vectors and/or treatments [153] (Table 2).

While a number of approaches have been developed to tackle these hurdles, including the use of alternative routes of vector administration, of clinical compounds (hirudin), or of lower vector doses, transient host immunosuppression, plasmapheresis, or saline flushing, the application of proteasome inhibitors, and the manipulation of the vectors themselves by engineering (alternative serotypes, replication-defective and non-infectious vectors, hybrid vectors, alternative promoters, and compartment-specific localization signals, insulators, artificial chromosomes, integration-deficient constructs, (v)exosomes) (Table 2), such strategies remain very complex and/or time-consuming for translational gene therapy [32,33,154,155].

In this regard, the idea of exploiting biomaterials employed in tissue engineering procedures has raised increased attention to conveniently overcome such barriers, opening new avenues of research in musculoskeletal gene therapy [22]. The following sections will most particularly focus on introducing the use of functionalized hydrogels for musculoskeletal gene therapy among the many biomaterials currently available, due to their possible minimal invasive delivery, ability to fill hardly accessible and irregular target sites, high formulation capacity, hydrophilic nature, and their aptitude to physically mimic body tissues [156–159].

4. Functionalized hydrogels

As a general definition, hydrogels are 3D polymeric networks capable of maintaining a large amount of water (even >90%) within their structure [160–162]. The 3D structure of hydrogels arises from crosslinks (physical, chemical, or both) formed between polymeric chains. Due to the aqueous medium and to their swellability, hydrophilicity, permeability, and potential for injectability and encapsulation, hydrogels well resemble the ECM of human tissues, particularly of soft tissues, and are also attractive candidates for delivery purposes [158,159,163,164].

Both synthetic and naturally occurring biopolymers and a combination of both can be used to manufacture hydrogels. Synthetic biopolymers are generally stronger and, upon precise control during the production process, they may exhibit predictable behaviors such as biodegradability and mechanical stability. On the other hand, natural biopolymers, due to their biological origins, while weaker in their mechanical properties, often display better matrix-cell interactions, but may trigger host immune responses. Polyvinyl alcohol (PVA) [165–167], poly(ethylene) glycol (PEG) [168], poly(lactic acid) (PLA) [169], poly(lactic-co-glycolic acid) (PLGA) [170,171], and PEG-PPO-PEG copolymers [172] are typical examples of synthetic biopolymers used to prepare hydrogels. Regarding biopolymers of natural sources, hyaluronic acid (HA) [173], collagen [174,175], alginate [176], peptides [177], gelatin [178,179], and fibrin [180,181] are frequently employed in hydrogel fabrication. Special features such as the chemistry of the applied polymers, the type and density of crosslinks, the ease of modification, and the nature of the hydrogel (*e.g.* high water content and hydrophilicity) allow for the inclusion of various functionalities in the hydrogel to improve its performance including its mechanical properties, loading capacity, release profiles, degradation rate, biocompatibility, and environmental responsiveness.

4.1. Injectable hydrogels

Injectability is a functional feature that allows for the transfer of a hydrogel containing for example cells, drugs, or therapeutic factors to the tissue of interest in the patient's body in a minimally invasive fashion, eliminating the difficulties associated with non-injectable hydrogels such as complex, arduous surgeries with high costs and long-term healing times [182–185]. Injectability allows the hydrogel to completely occupy a defect space and reach inaccessible injured sites. The injectable hydrogel has to be in a liquid form with appropriate viscosity in order to be injected using a tool like a syringe for conversion into a gel form *in vivo* (sol-gel transition) [167,186–188]. Injectability can arise from either low-viscosity fluids (solutions, suspensions) where the hydrogel precursor easily flows through the needle of a syringe upon pressure, or from the shear-thinning behavior of the hydrogel in which its viscosity, due to shear loads at injection *e.g.* in a needle, decreases to easily flow through the needle and, upon load removal, can restore its initial viscosity (the latter one being termed "thixotropy" from a rheological perspective) [189,190] (Fig. 3).

Upon injection in a site of tissue damage, it is necessary that the hydrogel undergoes a crosslinking process, *i.e.* gelation, to optimally

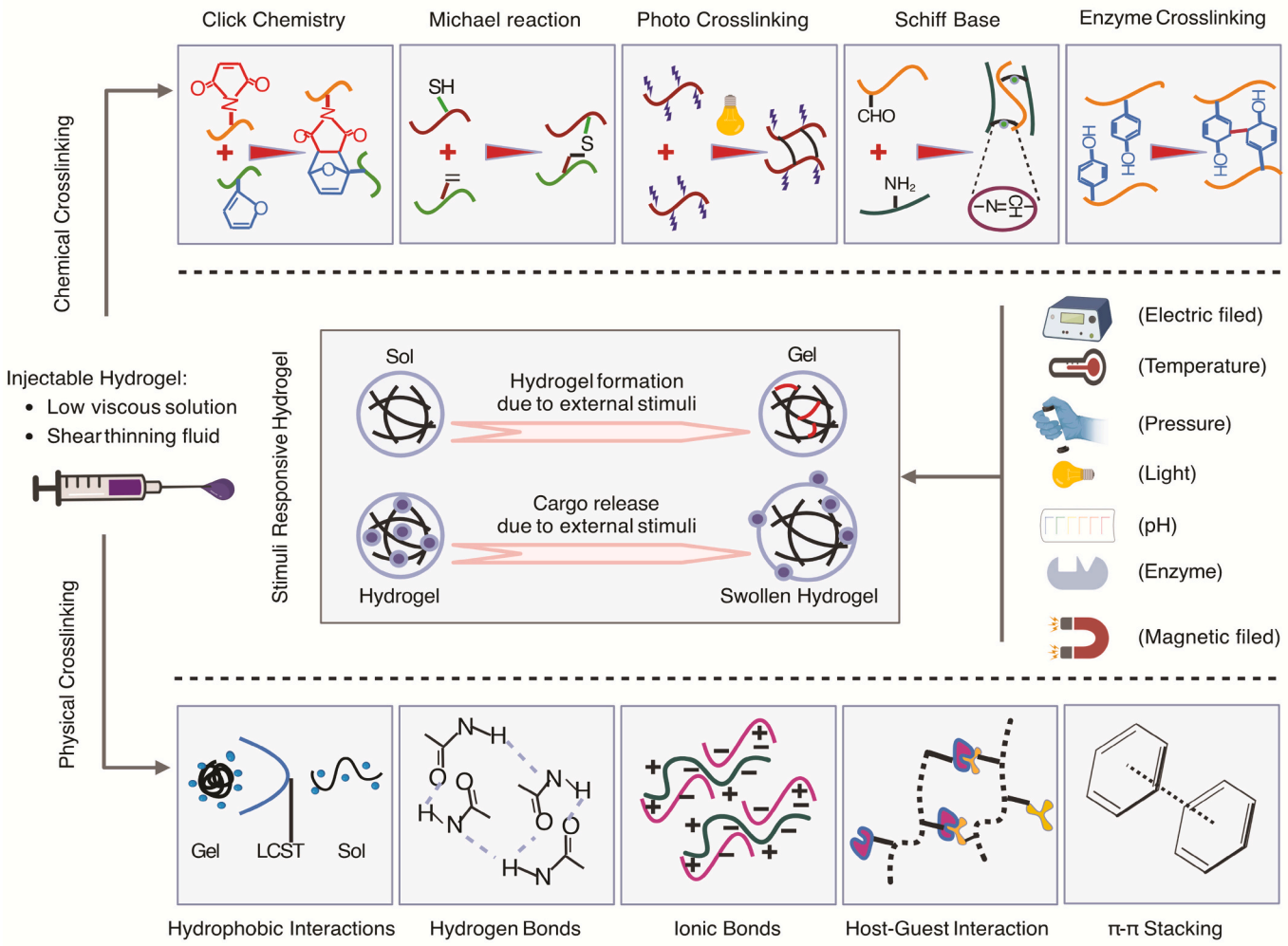


Fig. 4. Chemically- and physically-crosslinked injectable hydrogels and stimulus-responsive hydrogels with hydrogel formation and cargo release upon external stimuli (electric field, temperature, pressure, light, pH, enzyme, magnetic field) (central panel). Chemical crosslinking (top panel) includes click chemistry, Michael addition (reaction), photo-crosslinking, Schiff base reaction, and enzyme crosslinking while physical crosslinking (bottom panel) includes hydrophobic interactions, hydrogen and ionic bonds, host-guest interactions, and π-π stacking (created with Biorender).

occur. The gelation kinetics (rate and time) is, therefore, one of the crucial features of injectable hydrogels since too slow gelation rates can lead to an uneven dispersion of the cargos and the rapid, undesirable loss of encapsulated materials, while too rapid gelation rates may clog the injection tool or prevent the hydrogel to sufficiently fill the target site [190]. It is also important that the crosslinking process does not lead to the generation of toxic by-products but rather provides a biocompatible, porous, and physiologically stable platform that allows for a timely release of the cargos upon safe degradation, resulting in the effective treatment of injured tissues or for the neoformation of new, adapted tissue [190,191].

The process of hydrogel crosslinking, mostly referred to as *in situ* gel formation occurs *via* intermolecular interactions (physical crosslinking) or upon covalent interactions (chemical crosslinking), where both may be influenced by diverse external stimuli such as temperature, pH, light, ultrasounds, electric/magnetic fields, and biomolecular compounds (*e.g.* enzymes) that may also have an impact on the release of the cargos [192–195] (Fig. 4). The stimulus-responsiveness of injectable hydrogels is a concept that may be used either for the formation of a hydrogel network (stimulus-induced hydrogelation) by hydrogel crosslinking upon external stimulation or for the controlled release of the hydrogel cargo upon such external stimulation that alters the structure and shape of the hydrogel [196,197].

During physical crosslinking, a series of weak bonds are formed among polymeric chains of the hydrogel due to various interactions, including electrostatic interactions (hydrogen and ionic bonds) [197,198], van der Waals forces (dipole-dipole interactions and London dispersion forces) [199,200], π-π stacking [201], host-guest [202–204], and hydrophobic interactions [205–207] prone to environmental changes and easily reversible upon stimuli such as temperature, pressure, pH, light, and enzymes [208]. Physically-crosslinked hydrogels are therefore relatively weak structures from a mechanical point of view, but cell- and tissue-friendly since no chemical crosslinking agents are used. Chemically-crosslinked hydrogels consist of a polymeric network of more durable covalent linkages with higher resistance to the physiological environment, arising from various reactions such as the Schiff base reaction [209], Michael addition [210], click chemistry [210,211], photopolymerization [212], and enzyme-assisted reactions [213,214]. Chemically-crosslinked hydrogels are thus stronger and more stable structures from a mechanical point of view, with a good resistance against physiological conditions making them adapted for *in vitro* and *in vivo* applications and with higher controlled release profiles and degradation rates, but they carry a risk for potential toxicity due to the presence of chemical crosslinking agents. It is noteworthy that dual- or multicrosslinking mechanisms, *e.g.* physical/physical, chemical/chemical, or physical/chemical, may be more attractive to increase the hydrogel injectability, mechanical properties, release profiles, and degradation rates [215–218].

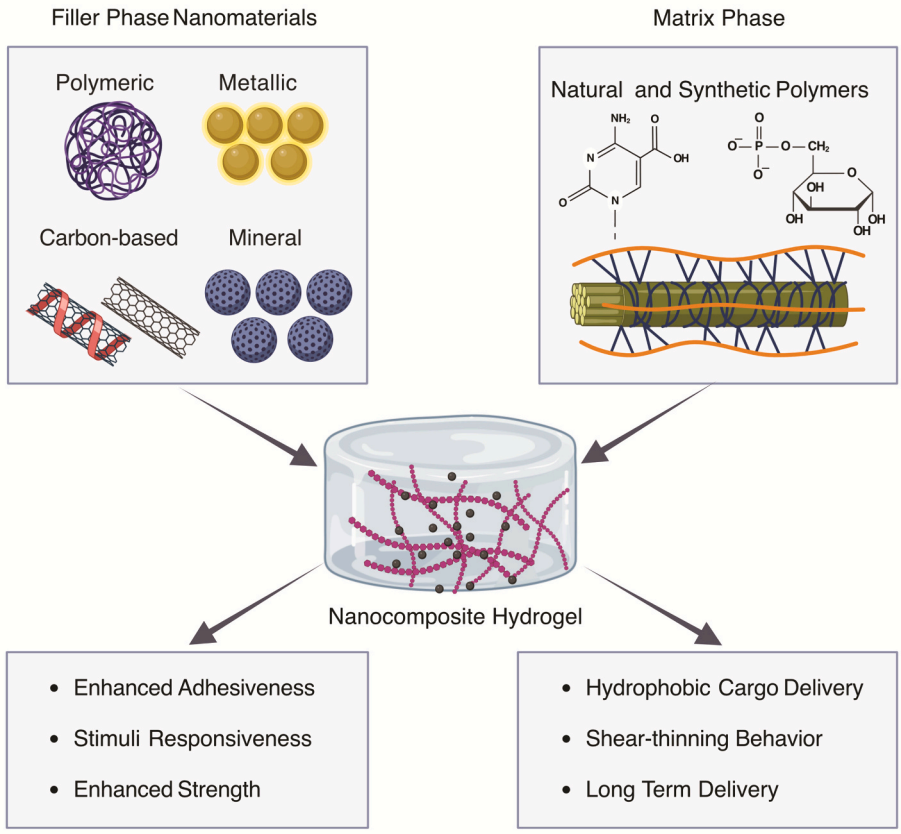


Fig. 5. Nanocomposite hydrogels and potential benefits. Incorporation of nanoscaled filler phase such as polymeric, metallic, mineral, and carbon-based nanomaterials in the polymeric (natural, synthetic) matrix phase may lead to the formation of nanocomposite hydrogels with enhanced features beneficial to delivery applications (created with Biorender).

4.1.1. *Physically-crosslinked injectable hydrogels*

The injectability of physically-crosslinked injectable hydrogels formed by hydrogen bonding is facilitated through the dissociation of weak hydrogen bonds at injection time due to applied shear forces that may subsequently be reformed upon force removal [219]. The necessity for hydrogen bond formation is the existence of hydrogen atoms and electronegative atoms such as nitrogen, oxygen, and fluorine that may exist in pure polymers, the combination of polymers, polymer/additive, and modified polymers. Systems based on PVA [220], poly(N-acryloyl glycinamide) [221,222], methylcellulose/HA [223], gelatin/agar [224], starch/carboxymethyl cellulose [225], and ureidopyrimidone-modified PEG [226] are some examples of injectable hydrogels prepared through hydrogen bonding.

Another type of electrostatic interaction that contributes to physical crosslinking is ionic linkages based on the electrostatic attraction between two groups of opposite charges. The most frequently used polymer capable of forming injectable hydrogels *via* such interactions is alginate which crosslinks in the presence of cations such as Ca^{2+} [227]. In addition, combinations of polymers or polymer/nanomaterials with opposite charges termed polyelectrolyte complexes such as chitosan-based hydrogels [228–230], alginate/positively charge modified organosilica [230], and HA/pectin/multivalent ions (Fe^{3+}) have also been reported as injectable hydrogels crosslinked upon electrostatic interactions [231].

Hydrophobic interactions present in amphiphilic molecules have further been attributed to physically-crosslinked injectable hydrogels. Among them, temperature-induced hydrophobic associations have been reported where sol-gel transition may be achieved by altering the temperature of the medium. In this scenario, a hydrogel network (amphiphilic polymer) that is soluble (sol) below the lower critical solution temperature (LCST) may gel above this temperature [232,233]. Polymeric systems such as poly (N-isopropyl acrylamide), poloxamers, poly (vinyl ether), poly (N-vinyl caprolactam), and modified chitosan have been described to behave in such a fashion [234,235].

This type of hydrogel can also be prepared by host-guest associations, where one molecule containing a cavity acts as the host while the other molecule behaves as the guest. The size of both the host cavity and the guest, as well as hydrophobic interactions, are critical parameters for the effective formation of these particular bonds. Macromolecules like cyclodextrin and its derivatives are well-known hosts capable of interacting with guest molecules such as PEG, azobenzene, and adamantine [236–238].

4.1.2. *Chemically-crosslinked injectable hydrogels*

The crucial key related to chemically-induced injectable hydrogels is that crosslinking has to occur after injection under physiological conditions.

The Schiff base reaction is the most commonly used chemistry to generate injectable hydrogels by the formation of imine bonds between materials containing amine and carbonyl groups, ranging from small to macromolecules [238]. Systems generated *via* this reaction include glutaraldehyde/chitosan [239,240], gelatin/glutaraldehyde/chitosan [240], HA/tyramine [241], oxidized alginate/gelatin [242], oxidized alginate/carboxymethyl chitosan [243], and poly(oligo(ethylene glycol) methyl ether metacrylate)/cellulose nanocrystals [244].

The Michael addition that belongs to the larger class of conjugate additions is another extensively used chemistry to create a covalent network in hydrogels, occurring between materials containing electrophilic (*e.g.* amine or thiol) and nucleophilic (*e.g.* vinyl) groups [245,246]. Vinyl sulfone-modified dextran/thiolated PEG [247], and

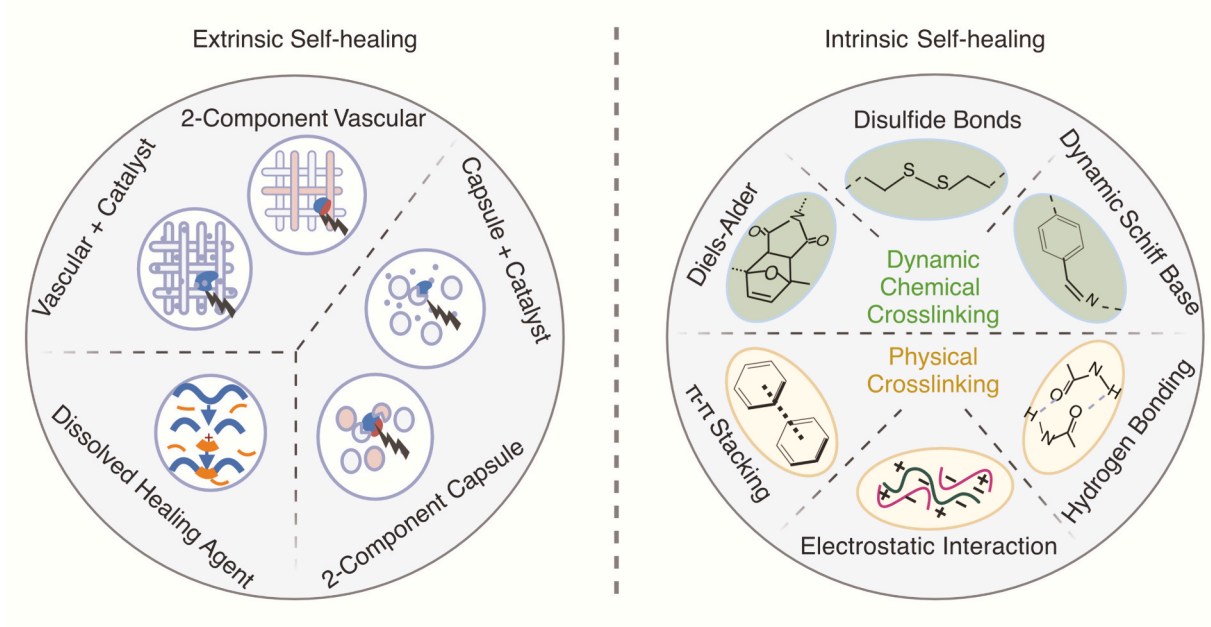


Fig. 6. Extrinsic and intrinsic self-healing. Extrinsic self-healing mechanisms (left panel) include the release of encapsulated healing agents from capsules and from vascular systems and the dissolution of the healing agents while intrinsic self-healing mechanisms (right panel) are based on dynamic chemical bonds (disulfide, Schiff base, Diels-Alder bonds) and on physical interactions (hydrogen bonds, electrostatic interactions, π-π stacking) (created with Biorender).

thiol-modified polymers [248] are examples of hydrogel systems crosslinked *via* such as reaction.

Click chemistry is another approach to create covalent linkages in injectable hydrogels, with a number of advantages like a rapid reaction rate, simplicity, the almost lack of by-products, and a high chemoselectivity efficiency [249], although the need for harmful chemicals such as azides and metal catalysts may restrict its biomedical applications. The click-type Diels-Alder reaction, however, is a promising candidate for bioapplication as it may overcome such constraints [249,250].

Crosslinking *via* photopolymerization using ultraviolet (UV) or visible light is another strategy to generate injectable hydrogels in a rapid, easy, and cost-effective manner. The gelation process begins with light irradiation of the hydrogel containing photoinitiator molecules and photosensitive polymers followed by the formation of free radicals that subsequently attack the double bonds of the polymeric chains and by the completion of the chemical crosslinking of the hydrogel [251]. Still, this method has some drawbacks including the use of toxic photoinitiators, uneven crosslinks, and unreacted double bonds particularly when using UV light that does not diffuse well throughout the hydrogel. Aqueous mixtures of gelatin/gelatin methacryloyl (GelMA) [252], HA loaded with silicon (Si)-based nickel oxide (NiO) nanoflowers [253], and methacrylate modified *O*-acetyl-galactoglucomannan/thiolated cellulose nanocrystal [254] are the most recent examples of photo-crosslinkable injectable hydrogels.

Crosslinking using enzyme-assisted reactions is a new approach to generate injectable hydrogels with a clear benefit for biomedical applications due to mild reaction requirements (neutral pH, moderate temperature, aqueous medium) that reduce the cytotoxicity of the method. Enzymes that are most commonly used to catalyze the crosslinking process include transglutaminase, horseradish peroxidase (HRP), phosphopantetheinyl transferase, tyrosinase, and lysyl oxidases. For instance, an injectable hydrogel based on tyramine-modified gelatin was generated by crosslinking using a combination of HRP and hydrogen peroxide catalysis [255–257].

4.2. Nanocomposite hydrogels

While hydrogels, as mentioned above, have many benefits, one of the most important drawbacks associated with their application in tissue engineering and delivery purposes, particularly in musculoskeletal tissues which play a role in body weight toleration and transforming loads into the muscles, is their weak mechanical properties (especially upon dehydration). This feature makes them difficult to handle and when inserted in body tissues, they may easily be degraded upon small stresses and may therefore not perform their defined aims. To prevail these restrictions, combining hydrogels (matrix phase) with nanomaterials (filler or hard phase) as nanocomposite hydrogels (Fig. 5) led to the emergence of novel types of functional hydrogels with improved mechanical properties [258–260]. This advantage arises from the extraordinarily small size of nanomaterials (1–100 nm) with large surface-to-volume and aspect ratios, which cause high matrix-filler interactions with enhanced mechanical strength [261]. Besides mechanical improvement, nanomaterials can add further value to hydrogels such as optical, electrically conductive, magnetic, and biological features depending on the type of nanomaterial and intended application [262,263].

From another perspective, nanocomposite hydrogels are hybrid (organic/inorganic) nanoscaled structures and include particles, laminates, fibers, *etc.* with an organic matrix (natural or synthetic polymers) capable of forming hydrogel *via* physical or chemical crosslinking, as discussed in the previous section. More interestingly, nanomaterials may participate in the hydrogel crosslinking process, either physically or chemically or both, provided that they possess appropriate functional groups on their surfaces naturally, for example, hydroxyl groups in nanosilica particles, or previously modified with suitable functional groups [264–267]. In this case, nanomaterials can be referred to as multi-purpose materials which not only enhance the quality of the hydrogel, *e.g.* mechanically or biologically, but also take part in the crosslinking process. The final properties of a nanocomposite hydrogel are a mixture of individual characteristics of a hydrogel and incorporated nanofiller as well as synergies arising from interactions between these materials. One of the obstacles in delivering therapeutic factors using conventional hydrogels is the burst release of the encapsulated

Table 3
Hydrogel-mediated gene vector delivery to treat musculoskeletal disorders.

Vector Class	Type	Gene	Material	Target Tissue	Features, Study Systems, Duration of Effects	Refs.
nonviral vectors	pDNA	BMP-2	RGD-alginate	bone	- *in vitro* release (MC3T3-E1 cells) - osteogenesis (78 days), ectopic bone (mouse)	[308]
			alginate, alginate/CaP	bone	- *in vitro* release (MSCs) - osteogenesis (16 weeks), ectopic bone (goat)	[309]
		BMP-2/TGF-β3	alginate/nHAp	bone, cartilage	- *in vitro* release (MSCs) - osteo−/chondrogenesis (28 days)	[310]
	mRNA	SOX9/MYOD	fibrin	cartilage	- *in vitro* release (MSCs) - chondro−/myogenesis (3 weeks)	[311]
	RNAi	COX-1/−2 miRNAs	HA/PLGA	tendon	- *in vitro* (tenocytes)/*in vivo* (chicken) release - reduced adhesions (chicken) (6 weeks)	[312]
		antimiR-221	fibrin/HA	cartilage	- *in vitro* (MSCs) and *in vivo* (mouse) release - ectopic cartilage (mouse) (4 weeks)	[313]
		agomiR-29b-5p	SAP	cartilage	- *in vivo* release (OA rat) - cartilage repair (10 weeks)	[314]
		Wwp1 siRNA	PEG/PLA	bone	- *in vivo* release (mouse) - bone repair (3 weeks)	[315]
		noggin siRNA	glycol chitosan/DBM	bone	- *in vitro* release (MSCs) - osteogenesis (2 weeks)	[316]
		miRNA-20a/ noggin siRNA	PEG	bone	- *in vitro* release (MSCs) - osteogenesis (3 weeks)	[317,318]
		TGF-β1	fibrin	cartilage	- *in vitro* release (MSCs) - chondrogenesis (3 days)	[318]
viral vectors	rAAV vectors	SOX9	PEO-PPO-PEO	cartilage	- *in vivo* release (minipig) - cartilage repair (4 weeks)	[172]
		IGF-I	alginate	cartilage	- *in vivo* release (minipig) - cartilage repair, reduced OA (1 year)	[319]

Abbreviations: pDNA, plasmid DNA; mRNA, messenger RNA; RNAi, RNA interference; rAAV, recombinant adeno-associated virus; BMP-2, bone morphogenetic protein 2; TGF-β, transforming growth factor beta; SOX9, sex-determining region Y-related high-mobility group 9; MYOD, myoblast determination; COX, cyclooxygenase; miRNA (miR), microRNA; Wwp1, WW domain-containing E3 ubiquitin protein ligase 1; siRNA, small interfering RNA; IGF—I, insulin-like growth factor I; RGD, Arg-Gly-Asp; CaP, calcium phosphate; nHAp, nanohydroxyapatite; HA, hyaluronic acid; PLGA, poly (lactic-*co*-glycolic acid); SAP, self-assembling peptide; PEG, poly (ethylene) glycol; PLA, poly(lactic acid); DBM, demineralized bone matrix; PEO, poly(ethylene oxide); PPO, poly(propylene oxide); MSCs, mesenchymal stromal cells; OA, osteoarthritis.

cargos. The possible interactions between incorporated nanoparticles and therapeutic factors may inhibit burst release [268]. Furthermore, due to the hydrophilic nature of the hydrogels, hydrophobic cargos can not be efficiently loaded in them and tend to be quickly released under biological conditions. Therefore, nanomaterial incorporation in a hydrogel can facilitate the encapsulation of hydrophobic agents and provide long-term delivery [269,270]. Compared with conventional hydrogels, nanomaterial incorporation may induce enhanced tunable behaviors such as swelling/deswelling, absorption/expulsion, diffusion, dissolution, and degradation in the hydrogel that can provide conditions for a controlled release of the encapsulated cargos [271–273]. The modification of nanomaterials with appropriate motifs can also lead to stimuli-responsive hydrogels, making them suitable choices for delivery purposes. It was also reported that the addition of a nanomaterial for instance bacterial cellulose [274] or of silicate nanoplatelets [275] can enhance the shear-thinning behavior of a hydrogel, hence allowing to simply produce injectable hydrogels. One of the other benefits of nanocomposite hydrogels, especially for tendon/ligament repair, may be their enhanced adhesion to tissue surfaces due to the nanocomposite surface roughness and irregularities due to the presence of the nanomaterial [276].

So far, nanomaterials incorporated into hydrogels were mostly based on mineral nanoparticles (HAp, silica, silicates, calcium phosphate), natural and synthetic polymeric nanoparticles (cellulose, polyesters, cyclodextrins), metallic nanoparticles (gold, silver, iron oxide), and carbon-based nanomaterials (carbon nanotubes - CNTs, graphene) [258,277–279] (Fig. 5). These combinations can create intelligent hydrogels with custom-made functionalities, tune their physical and mechanical properties, trigger the cargo release upon external stimuli, facilitate the embedding of hydrophobic cargos, and generate multi-responsive networks, effects that can be hardly found in a hydrogel alone [280]. Briefly, nanocomposite hydrogels may display a larger capacity for drug/vector encapsulation and transformation, durability, and delivery efficiency compared with unfilled hydrogels.

4.3. Shear-thinning and self-healing hydrogels

In tissue engineering and delivery systems (genes, drugs, *etc.*), the designed hydrogel has to ideally maintain its integrity and mechanical properties during regeneration of the damaged tissue, or else it may rapidly disintegrate, leading to a burst release of the embedded cargos. This issue is particularly important for hydrogels used in musculoskeletal tissues that are continuously exposed to static and dynamic loads to retain their stability within a long-term treatment. Self-healing as a functionality may add benefits to hydrogels to avoid their rapid destruction, provide conditions for a long-term controlled delivery, and degrade at a rate consistent with the rate of ingrowing tissue to ensure adequate mechanical protection [189,281–283]. Self-healing, inspired by biological organisms, is a process defined as the ability of a material to restore its initial structure and mechanical properties upon damage. Traditionally, self-healing mechanisms are categorized into extrinsic and intrinsic self-healing [284–287] (Fig. 6).

In extrinsic mechanisms, the healing agent is encapsulated in hollow structures (micro/nanocapsules, micro/nanofibers, tubes, *etc.*), released upon defect, and polymerized in the site of defect to repair the damage site through an irreversible, one-cycle chemical reaction. This type of self-healing mechanism may not lead to the restoration of the original material structure. Other limitations such as irreversibility (one-time healing cycle), the use of toxic solvents and healing agents, environmental sensitivity (*e.g.* moisture), high costs, and difficulties in the encapsulation process, restrict this mechanism for biomedical applications [285,288,289].

Intrinsic self-healing instead does not involve the use of healing agents and relies on the nature of materials, for example, functional groups of polymer or additives. Here, the healing process can proceed rapidly at ambient temperature (autonomous self-healing) or may need external stimulation to induce healing (non-autonomous or stimulus self-healing) [179,290]. The advantages of this process include its rapidity, its ability to restore the original shape, the absence of external

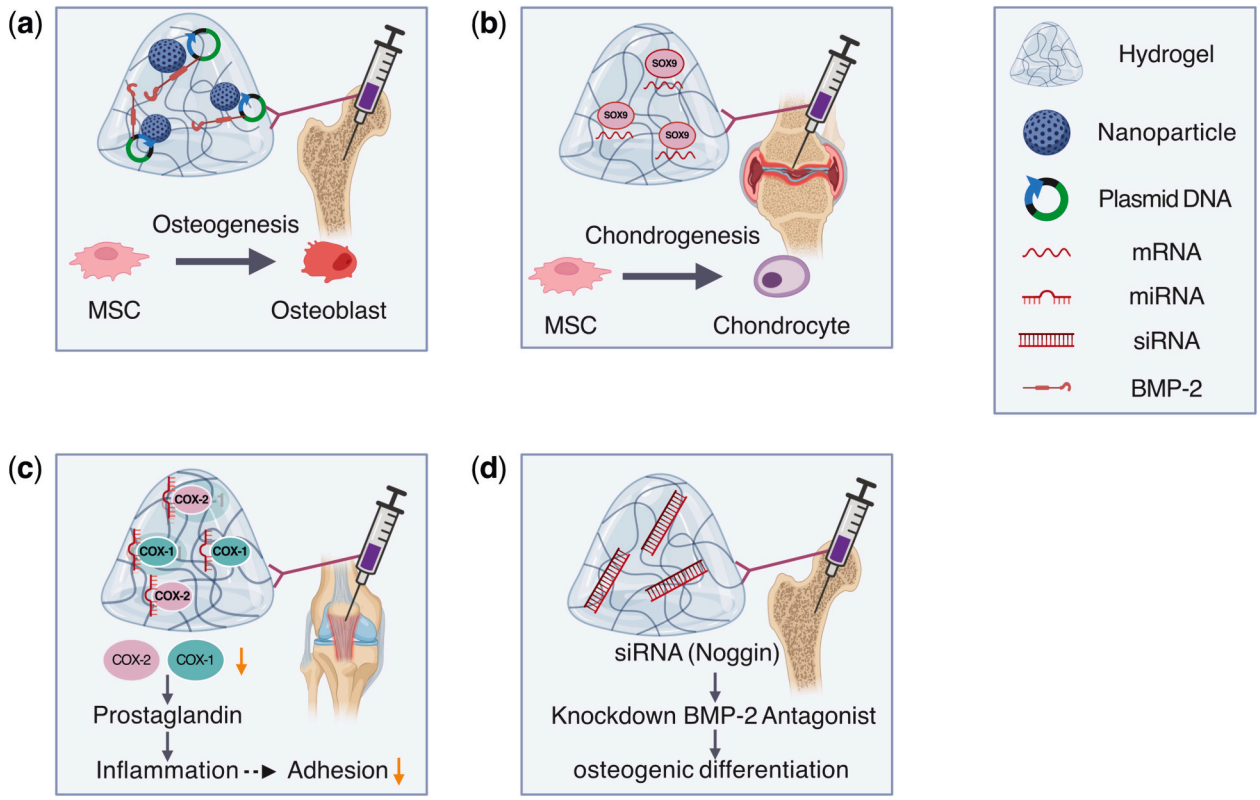


Fig. 7. Concept of hydrogel-mediated delivery of nonviral vectors. (**a**) Application of a plasmid DNA (pDNA) coding for BMP-2 *via* an injectable RGD-alginate hydrogel for bone formation [308]. (**b**) Administration of an mRNA coding for SOX9 *via* an injectable fibrin hydrogel to enhance the chondrogenic differentiation of MSCs [311]. (**c**) Delivery of COX-1 and COX-2 miRNAs *via* an injectable HA/PLGA nanocomposite hydrogel to reduce tendon adhesion [312]. (**d**) Transfer of an siRNA targeting noggin, a BMP antagonist, *via* an injectable glycol chitosan/DBM nanocomposite hydrogel to enhance the osteogenic differentiation of MSCs [316] (created with Biorender).

healing agents or catalysts, and multiple healing cycles (reversibility), making intrinsic self-healing an appropriate candidate for delivery systems [190,285].

Similar to the crosslinking processes (physical or chemical) described previously to form hydrogel networks, self-healing may occur due to various chemistries including dynamic (reversible) non-covalent bonding (physical), dynamic (reversible) covalent bonding (chemical), or a combination of both. Although, from a chemical perspective, hydrogel network formation and self-healing seem to be the same, the former may be static and irreversible, and upon damage, the hydrogel may not restore its original structure and mechanical properties. However, in the case of self-healing hydrogels, the key point is that the chemical or physical associations must be able to repeatedly reform after rupture. Hydrogen bonds [291,292], ionic associations [293], π-π stacking [294,295], guest-house interactions [296], metal-ligand coordination [297], electrostatics [298], and hydrophobicity [299,300] are frequent examples of non-covalent interactions applied in self-healing hydrogels. Chemistries mostly applied in the design of self-healing hydrogels include acylhydrazone bonds [301,302], Diels-Alder [303], Schiff base (imine bond) [304,305], and thiol-disulfide reactions [306,307]. In recent years, there has been also great attention given to the development of shear-thinning (injectable) and self-healing hydrogels, proposed by Bertsch et al. as third-generation self-healing hydrogels [190].

5. Hydrogel-mediated gene therapy for musculoskeletal disorders

A variety of hydrogels have been manipulated for the innovative delivery of therapeutic gene vectors to repair musculoskeletal disorders, including nonviral and viral vectors (Table 3).

5.1. Hydrogel-mediated delivery of nonviral vectors

Hydrogels have been used as platforms to deliver nonviral vectors, including DNA (plasmid DNA - pDNA) [308–310] and RNA [80] such as messenger RNAs (mRNAs) [311] or interfering RNAs, a strategy to repair musculoskeletal disorders by knocking down disease-associated genes *via* microRNAs (miRNAs, miRs) and/or small interfering RNAs (siRNAs) [312,313,315,317,318,320].

5.1.1. Hydrogel-mediated delivery of plasmid DNA

To address the limitations associated with the use of recombinant bone morphogenetic proteins (BMPs) for bone repair (*e.g.* costs, rapid *in vivo* protein degradation, difficulty in protein retention, side effects at high BMP-2 doses) [321], Krebs et al. [308] reported the benefits of the effective release of a pDNA coding for BMP-2 from a cost-effective injectable alginate hydrogel modified with adhesion ligands containing the Arg-Gly-Asp (RGD) peptide sequence for cell attachment, allowing for the durable (78 days) osteogenic differentiation of pre-osteoblastic cells (MC3T3-E1) and for ectopic bone formation (2 weeks) in the back of injected mice (Fig. 7a).

Wegman et al. [309] used a similar approach, showing the value of providing a pDNA for BMP-2 *via* controlled release using an injectable alginate nanocomposite (calcium phosphate ceramic) hydrogel for MSC osteogenesis and ectopic bone formation (16 weeks) in goats. Gonzales-Fernandez et al. [310] further demonstrated that co-delivery and release of pDNAs for BMP-2 and for the transforming growth factor beta 3 (TGF-β3) using an injectable alginate nanocomposite (nanoHAp - nHAp) promoted the sustained (28 days) osteo−/chondrogenic differentiation

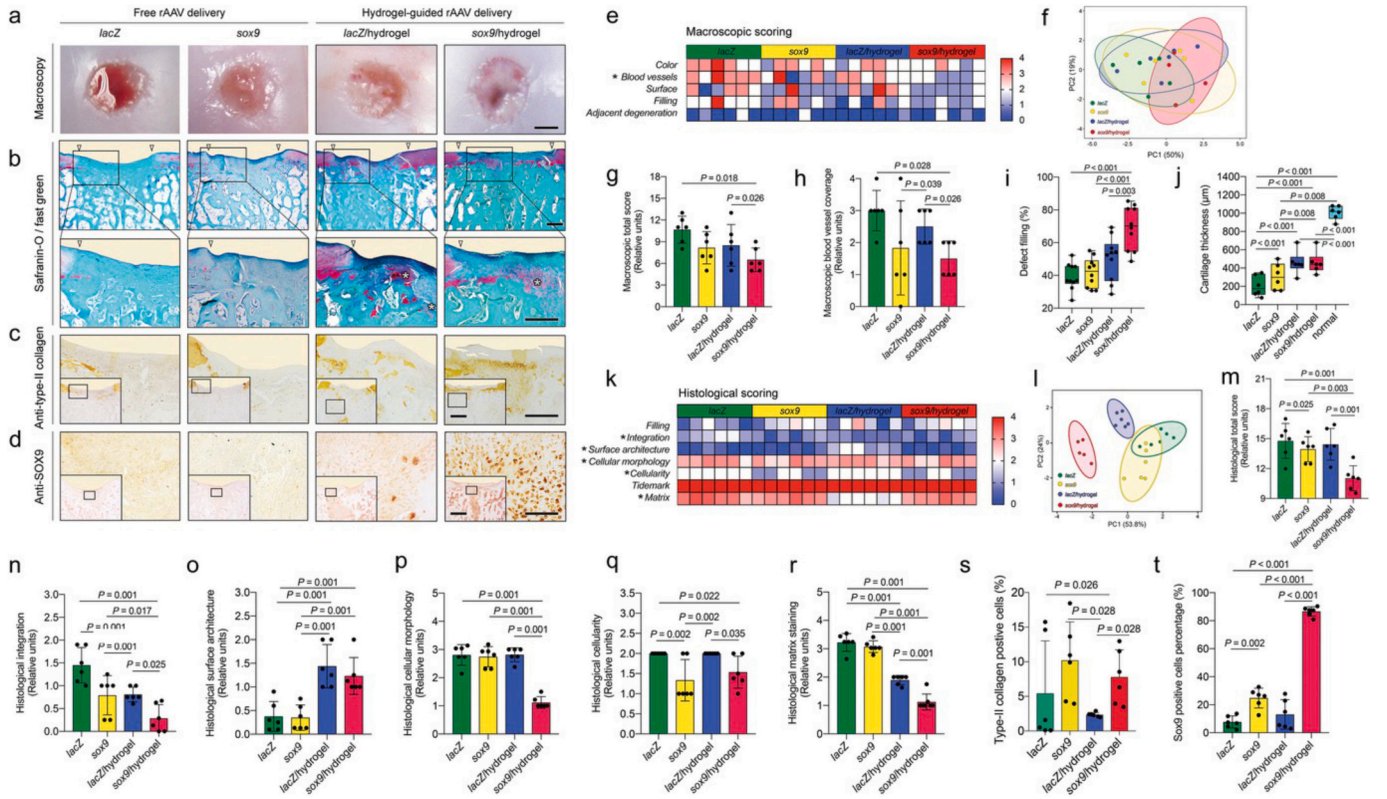


Fig. 8. Concept of hydrogel-mediated delivery of viral vectors. Administration of an rAAV SOX9 vector *via* an injectable thermoresponsive PF127 hydrogel for cartilage repair in minipig full-thickness chondral defects over a period of 4 weeks *in vivo*. Macroscopic (**a**), histological (**b**), and immuno-histochemical analyses (**c, d**) of cartilage repair with heat maps (**e, k**), principal component analyses (**f, l**), repair scores (**g, h**: macroscopic scores; **i, j, m-r**: microscopic scores), and immuno-histomorphometric analyses (**s**: type-II collagen; **t**: SOX9) [172] (reproduced with permission from the Journal).

of MSCs (TGF-β3/BMP-2) *in vitro*.

5.1.2. Hydrogel-mediated delivery of mRNAs

An interesting approach was developed by Ledo et al. [311] who used the high gene transfer capacity and specificity of RNAs to create an injectable fibrin hydrogel carrying mRNAs coding for the cartilage-specific transcription factor sex-determining region Y-related high-mobility group 9 (SOX9) and for the muscle-specific transcription factor myoblast determination (MYOD) (Fig. 7b). The authors reported the persistent (3 weeks) chondro−/myogenic differentiation of MSCs (SOX9/MYOD) *in vitro* upon therapeutic controlled release.

5.1.3. Hydrogel-mediated delivery of interfering RNAs

The RNA interference (RNAi) technology has been also successfully employed by different groups for a variety of musculoskeletal applications.

The use of miRNAs has been reported for its ability to prevent deleterious effects of some disease-associated genes and agents in musculoskeletal disorders. To tackle the problem of pathological adhesions in injured tendons due to the local production of pro-inflammatory mediators (cyclooxygenases 1 and 2 - COX-1, COX-2), Zhou et al. [312] proposed an approach based on the controlled delivery of COX-1 and COX-2 miRNAs *via* an injectable HA nanocomposite (poly (lactic-*co*-glycolic acid)) (PLGA) hydrogel (Fig. 7c). The system, allowing for durable gene silencing *in vitro* (21 days in tenocytes) and *in vivo*, was capable of reducing adhesions in the tendons of chicken *in vivo* for 6 weeks. Next, injectable fibrin/HA hydrogels were prepared by Lolli et al. [313] as carriers of antimiR-221, a locked nucleic acid miRNA inhibitor targeting the anti-chondrogenic miR-221. Subcutaneous implantation of osteochondral biopsies treated with such a controlled release hydrogel system in mice enhanced cartilage repair over a period of 4 weeks *in vivo*. Recently, Zhu et al. [320] applied injectable hydrogels generated from the self-assembling peptide (SAP) $RADA_4$ to provide agomir-29b-5p, a cholesterol-modified miRNA mimic that alleviates cell senescence and attenuates OA progression, effectively regenerating chondrocytes and repairing the cartilage in an *in vivo* OA model in rats for 10 weeks.

Similarly, siRNAs have been utilized to counteract the undesirable influence of a number of pathological processes involved in musculoskeletal diseases. For instance, Wang et al. [315] created an injectable poly(ethylene) glycol (PEG) nanocomposite (poly(lactic acid) - PLA) hydrogel carrying an siRNA against the negative regulator of bone formation WW domain-containing E3 ubiquitin protein ligase 1 (Wwp1), enhancing bone formation in murine mid-diaphyseal femur fractures *in vivo* in a controlled manner for 3 weeks. In addition, an injectable glycol chitosan nanocomposite (demineralized bone matrix - DBM) hydrogel was proposed by Kim et al. [316] for the controlled delivery of an siRNA targeting noggin, a BMP antagonist, leading to increased osteogenic differentiation of MSCs for 3 weeks *in vitro* (Fig. 7d).

Of further interest, the combined use of miRNAs and siRNAs has also been described by Huynh et al. [317,318] who generated injectable photodegradable PEG-based hydrogels for the active light-triggered controlled release of both an siRNA targeting noggin and the pro-osteogenic miRNA-20a, promoting the osteogenic differentiation of MSCs for 3 weeks *in vitro*.

5.2. Hydrogel-mediated delivery of viral vectors

Hydrogels have also been manipulated to formulate and release viral vectors as off-the-shelf, controlled treatments of musculoskeletal disorders, with a particular focus on the delivery of rAAV vectors [74,172,318,319]. In particular, Lee et al. [318] employed a fibrin

hydrogel to deliver an rAAV TGF-β1 vector in MSCs, reporting the effective upregulation of cartilage-specific gene expression in MSCs *in vitro* for 3 days. Our group also generated injectable hydrogels as controlled release carriers of rAAV vectors, including a thermoresponsive PEO-PPO-PEO (PF127) hydrogel based on poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) poloxamers for the delivery of an rAAV SOX9 construct [172] and an alginate (AlgPH155) hydrogel for that of an rAAV IGF-I vector [319]. Specifically, the PF127/rAAV SOX9 *in situ* gelling system allowed for the safe improvement of cartilage repair in clinically relevant full-thickness chondral defects in minipigs for 4 weeks *in vivo* without any detrimental or immune responses *via* an effective controlled release of the vectors [172] (Fig. 8). Most strikingly, the AlgPH155/rAAV IGF-I system was capable of both safely promoting cartilage repair and reducing perifocal OA and inflammation in a similar large animal model for one year *in vivo*, again without any detrimental or immune responses [319], supporting the concept of injectable, minimally invasive hydrogel-guided controlled gene delivery for long-term cartilage repair and OA protection.

6. Conclusions and perspectives

Scaffold-mediated gene therapy based on the manipulation of various biocompatible materials (solid, hydrogel, or hybrid scaffolds of natural or synthetic origin) acting as gene-activated matrices has a strong potential to provide convenient and durable off-the-shelf, patient-independent treatments for widespread use to manage human musculoskeletal disorders [22,24,71,78,322–325]. Solid scaffolds have been reported to offer viable solutions for musculoskeletal gene therapy, including systems derived from collagen [326–338], gelatin [339], silk fibroin [340], HA [341], PEG [342], HAp [332,334,337,343], PLGA [344–346], poly(ε-caprolactone) (PCL) [69,347–350], and silicon [351] for the goal of bone formation and repair [326–340,342–347] and for cartilage resurfacing and osteochondral repair [69,341,348–351]. On the other hand, hydrogels display a number of attractive features for the goal of musculoskeletal therapy, including their ability to physically mimic musculoskeletal tissues and to be functionalized as “smart” (tissue- and disease-targeted) materials by adjusting (i) their injectability for a minimally invasive application, (ii) their stimulus responsiveness, and (iii) their self-healing ability to provide optimal conditions in translational approaches. Due to their versatility, hydrogels (as well as nanocomposite hydrogels) have long been employed to carry cells and drugs [9,36,40,44,46,157–159,164,174,175,186,193,227,270] but more creatively, they have now been reported as novel, highly potent gene delivery platforms to treat musculoskeletal disorders as demonstated here [172,308–319]. Hydrogels with a mechanical strength compatible with the host tissue due to *in situ* crosslinking may shield the gene vectors being carried from injection-associated shear forces and protect them from aggregation/degradation in the ECM and from potential host immune responses, gradually and safely releasing them in a controlled and sustained manner over extended periods of time while enhancing the efficacy of target cell transduction and thus the levels and duration of gene expression in a specific site [22–24,27,71,74,78,79,117,143,144,151–153,227,322–325]. Overall, hydrogels are easy to manipulate and tailor and can be produced at reduced costs and in a scalable manner by advancing and leveraging synthesis techniques (including 3D printing and microfluidics) [77,352–355] and optimizing raw material selection for widespread use [356], with the least burdensome option for the patients.

A number of hydrogels are already in clinical use as cost-effective options [357,358] to treat focal articular cartilage lesions and OA or to improve bone repair in patients as components alone (HA, chondroitin sulfate/PEG, PVA) [359–361], in conjunction with cells (chondrocytes, MSCs, whole blood) (HA derivatives, alginate, chitosan, collagen, fibrin) [357,358,360,362–372], or as carriers of recombinant agents (gelatin and HA for basic fibroblast growth factor, *i.e.* FGF-2, and triamcinolone acetonide) [366,373]. Still, while a significant number (> 1500) of clinical trials have involved gene therapy procedures for some decades [374], somewhat few of them aim at treating musculoskeletal disorders *via* scaffold-free (synovial fibroblasts modified with a retrovirus-interleukin 1 receptor antagonist, *i.e.* IL-1Ra, and an rAAV-tumor necrosis factor alpha antagonist vector for rheumatoid arthritis, irradiated chondrocytes modified with a retrovirus-TGF-β1 vector and an rAAV-IL-1Ra for OA) [22,374–388] or scaffold-guided procedures (collagen gel with an adenoviral-platelet-derived growth factor construct for diabetic food ulcer, hyaluronan hydrogel/HAp/beta tricalcium phosphate with nonviral- or adenoviral-IL-10, -TGF-β, -BMP-2 constructs and MSCs for bone and cartilage regeneration, collagen/HAp and octacalcium phosphate with a nonviral vascular endothelial growth factor construct for bone repair) [389–392]. This relative scarcity possibly reflects the elevated costs of preclinical and clinical investigations, the introduction of novel drug treatments as alternatives, and the perception of the risk associated with gene therapy (musculoskeletal disorders are non-lethal conditions) [22,374,382–384].

While data are still scarce for tendon, ligament, and meniscus research, hydrogel-guided nonviral and viral gene delivery has been innovatively developed and successfully reported in some experimental studies *in vitro* and *in vivo* as a means to enhance bone healing and cartilage repair [172,308–319]. Nevertheless, this concept remains in its infancy compared with the large body of literature available in other fields of biomedicine such as tissue engineering and drug delivery [9,36,40,44,46,47,82,157–159,164,175,186,193,196,207,211,227,241,251,252,270,272] and compared with other types of systems (solid scaffolds) [326–351]. This shows the critical, urgent need to thoroughly conduct additional, extensive preclinical work *in vivo*, including in large animal models relevant of the disorders, *via* a combination of gene therapy procedures and the use of hydrogels, precluding at this stage an immediate translation of the systems in patients. Additionally, significant efforts will have to be placed in the design of affordable and scalable, optimized hydrogels supporting both a safe and effective protection and delivery of the vectors, displaying (i) a gradual biodegradability that does not impact the long-term stability and release of the therapeutic genes, (ii) a controlled and on-demand cargo release, (iii) no detrimental inflammatory/immunogenic effects, and (iv) sufficient mechanical strength consistent with the host tissue. This is also true regarding (i) the gene vectors themselves that will have to be carefully designed and manufactured (vector class, dose, method of formulation, *etc.*; choice of genes: growth factors, transcription factors, signaling agents, anti-inflammatory/anti-oxidant compounds, *etc.*; choice of the control element/promoter: high level, tissue-specific, disease-regulatable, *etc.*) and (ii) the route of administration to avoid dissemination of the vectors to non-target locations.

In conclusion, hydrogels have the potential to provide effective, safe, and long-term gene-based delivery, off-the-shelf (patient-independent) tunable compounds where multifunctionalities may be introduced for a minimally invasive (injectable) tissue- and/or disease-specific treatment of musculoskeletal disorders. Directions for future investigation include to optimize both the types and production steps of hydrogels and gene vectors and to investigate the combined systems obtained in relevant animal models *in vivo* for a workable translation in the clinics in patients in a near future.

CRediT authorship contribution statement

Mohammadsaeid Enayati: Writing – review & editing, Writing – original draft, Visualization, Validation, Funding acquisition, Conceptualization. **Wei Liu:** Writing – review & editing, Visualization, Validation, Conceptualization. **Henning Madry:** Writing – review & editing, Visualization, Validation, Funding acquisition. **Rasoul Esmaeely Neisiany:** Writing – review & editing, Visualization, Validation, Conceptualization. **Magali Cucchiarini:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors have no competing interests to declare.

Data availability

Data will be made available on request.

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