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Recent advances in musculoskeletal local drug delivery

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Abstract

Musculoskeletal disorders are a significant burden on the global economy and public health. Advanced drug delivery plays a key role in the musculoskeletal field and holds the promise of enhancing the repair of degenerated and injured musculoskeletal tissues. Ideally, drug delivery should have the ability to directly deliver therapeutic agents to the diseased/injured sites with a desirable drug level over a period of time. Here, we present a mini-review of the current state-of-the-art research associated with local drug delivery and its use for the treatment of musculoskeletal disorders. First, an overview of drug delivery strategies, with a focus on issues related to musculoskeletal pathology, potential therapeutic strategies, conventional and non-conventional drugs, and various delivery systems, is introduced. Then, we highlight recent advances in the emerging fields of musculoskeletal local drug delivery, involving therapeutic drugs (e.g., genes, small molecule therapeutics, and stem cells), novel delivery vehicles (e.g., 3D printing and tissue engineering techniques), and innovative delivery approaches (e.g., multi-drug delivery and smart stimuli-responsive delivery). The review concludes with future perspectives and associated challenges for developing local drug delivery for musculoskeletal applications.

Keywords

musculoskeletal disorder; drug delivery; 3D printing; gene delivery; small molecule therapeutic; smart stimuli-responsive; cell engineering; tissue engineering

1 Introduction

The musculoskeletal system plays a crucial role in providing support, stability, and movement to the body, while musculoskeletal disorders may lead to significant burdens on the global economy and public health [1–3]. Musculoskeletal disorders may affect the

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SZ performed literature review and drafted the manuscript. SZ, MX, and BL discussed the ideas and outlines, and critically reviewed and revised the manuscript. All authors have approved the final version of the manuscript.

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human body's movement or musculoskeletal functions and usually involve various abnormal physiologies in bones (e.g., osteoporosis, trauma), muscles (e.g., sarcopenia), and joints (e.g., osteoarthritis) [4–9]. The World Health Organization has predicted a rapidly growing burden of musculoskeletal disorders, partly due to the increasing age of the global population [10,11]. To prevent and treat musculoskeletal disorders, various treatments like surgery and systemic drug treatment have been developed. Besides systemic drug delivery, local drug delivery has attracted great attention for the treatment of musculoskeletal disorders, mainly because local drug delivery may deliver therapeutic agents to the desired site of action, may provide an optimal drug level for controlled periods of time, and may reduce undesirable side effects or toxicity [12–18]. In this review, we attempt to present a brief overview of recent advances of local drug delivery in the field of musculoskeletal treatment and repair. First, musculoskeletal drug delivery is introduced in the terms of musculoskeletal pathology, therapeutic solutions, various drugs, and carrier systems. Next, we highlight recent advances in the field of musculoskeletal drug delivery, including new therapeutic drugs, novel delivery vehicles, and innovative delivery approaches. Finally, we conclude with challenges and perspectives of future research directions in the development of local drug delivery for musculoskeletal disorders.

2 Drug delivery for musculoskeletal disorders

2.1 Musculoskeletal disorders

Musculoskeletal diseases or injuries represent the chronic or acute pathological conditions that afflict bone, muscle, joint, cartilage, ligament, tendon, or other connective tissues, as illustrated in Figure 1 [4]. Various conditions, including open extremity injuries, osteoarthritis, degenerative disc disease, ligament trauma, bone metastasis, infection, tumor, and cancer are common in daily life; they account for the second greatest cause of disability worldwide. The risk of musculoskeletal diseases usually increases with age and is often associated with various complications, which may cause morbidity and present tremendous impact on quality of life. Musculoskeletal diseases or injuries vary considerably in their forms and may create a significant burden on the global economy and public health. For instance, musculoskeletal disorders account for more than 70 million office visits together with 130 million clinical encounters each year in the US alone, costing the US health care system more than \$150 billion [19].

Prevalent and severe forms of musculoskeletal diseases or injuries may include trauma, arthritis, metabolic disorders, infection, tumor, cancer, etc. [10,11,20]. Arthritis, including osteoarthritis and rheumatoid arthritis, is defined as a degenerative and inflammatory disease that causes pain and loss of mobility, and afflicts 40 million people in the US alone [21]. The musculoskeletal system is also susceptible to pathogenic bacteria or fungi, and prosthetic joint infection, for instance, affects about 2% of arthroplasties and leads to a cost about \$3.2 billion per year [22]. With a rapidly growing demand for arthroplasties, which is expected to exceed 4 million cases in the US by 2030, the complication of infection will only become more common [23]. Malignant tumors, as the leading cause of death worldwide, are also closely associated with musculoskeletal diseases. It is reported that the annual expenses for the productivity costs of cancer mortality is expected to be \$147.6 billion in 2020 [24]. For

instance, bone metastases have become one of the most severely debilitating secondary complications, and occurred in ~70% of patients with breast or prostate cancer and in 15–30% of patients with other soft tissue tumors. Osteosarcoma is another common malignant tumor of bone that most commonly affects children, adolescents, and young adults. It is a very aggressive neoplasm that can only be treated by surgical removal of the primary lesion along with multidrug chemotherapies [25]. Besides these severe musculoskeletal diseases, trauma, osteoporosis, and genetic diseases may result in about 6 million fractures in the US every year [17,21]. Overall, musculoskeletal disorders are characterized by pain and reduced physical function; they may lead to a major burden on public health, pose major threats to healthy aging, and they commonly result in early retirement and reduced financial security.

2.2 Therapeutic strategies

The right choice of therapeutic strategies may allow effective and rapid healing to be achieved in treating various diseases, including musculoskeletal disorders and cardiovascular diseases. There is a growing realization that the safety and effectiveness of therapeutic treatments, besides surgical treatments, must be considered as they may ultimately affect the health and life of patients. Current therapeutic strategies like systemic drug delivery have limited capability in ameliorating musculoskeletal diseases or injuries because most of the drugs are not released in a sustained or on-demand manner, or are not released at the correct injury site [17,26,27]. By contrast, local drug delivery has the ability to precisely control and maintain high concentrations of drug at desired locations while minimizing system toxicity and undesirable side effects [9,12,17,28–33]. By targeting diseased/injured musculoskeletal tissues, local drug delivery may provide an optimal dose of drug and achieve enhanced therapeutic efficacy by circumventing metabolic catabolism and clearance compared to systemic drug delivery. Local drug delivery has been applied to treat musculoskeletal disorders in clinics but may require different delivery routes [34–38]. For instance, to treat osteoarthritis, intraarticular injection of drugs has been widely used. To treat muscle disorders, intramuscular or subcutaneous injection is typically chosen to release drug locally and gradually. To repair bone defect or fracture, implant or three-dimensional (3D) scaffolds coated with drugs may be preferred. Currently, considerable effort has been devoted to developing new drugs, novel delivery vehicles, and innovative delivery approaches. The structures and intermolecular interactions of drugs and delivery vehicles may be tailored to obtain a high drug loading efficiency and a controlled and sustained release; the delivery dynamic may be tuned to achieve an on-demand and targeted delivery. For instance, there are multiple delivery vehicles that can be used to control drug release in common uses: nano/microcapsules, degradation-controlled polymeric vehicles, swelling-controlled hydrogels, and affinity-based delivery vehicles [9,29,30,39–46].

There are still some major challenges related to musculoskeletal local drug delivery. For instance, most of the existing delivery vehicles carry only a single drug, have limited loading capacity, or present short-term release (burst release); they are still far from satisfactory for most practical applications where sustained and on-demand release, more than one drug, or smart-responsive delivery is needed [47–49]. Moreover, limited structural controllability (e.g., uncontrolled degradation), weak mechanical properties (easy to break during implantation), side effects, loss of drug bioactivity, limited drug variety, and burden on drug

storage in vehicles, are seen as obstacles for the clinical applications of most local drug delivery strategies [10,12,13]. Meanwhile, different musculoskeletal disorders may present unique challenges in developing appropriate local drug delivery strategies. For instance, drugs locally delivered to joints may be rapidly cleared or may be difficult to penetrate through the cartilage matrix [27,37,50–52]. Some delivery vehicles (like implant coatings and 3D scaffolds) may suffer from weak mechanical properties, which may reduce the structural function [32,53–55]. Local drug delivery for tendons and ligaments is also limited due to the dense connective tissue structures which contain abundant thick collagen fibers that may inhibit drug penetration and diffusion [56,57]. In addition, muscle tissues from different parts of the body usually show complex physiological environments and metabolic processes, which may greatly affect drug bioactivity, release kinetics, and potential side effects during local drug delivery treatments [20,58,59].

2.3 Drugs for musculoskeletal drug delivery

There is no doubt that therapeutic drugs play a crucial role in treating injuries and reducing or curing illness. In musculoskeletal drug delivery, the term “drug” is not limited to only therapeutic agents such as antibiotics, anti-inflammatory drugs, and anti-cancer agents; its scope has grown sharply over the last few decades to include growth factors, non-viral genes (DNAs, RNAs), and even tissue engineering scaffolds and regenerative cells/tissues [3,13,15,31,60–65]. The conventional drugs commonly used can be categorized into three broad categories, namely, non-steroidal anti-inflammatory drugs (NSAIDs), antimicrobial and antiviral drugs, and anti-cancer drugs.

NSAIDs are one of the most frequently used drugs to reduce inflammation by suppressing cyclooxygenases, primarily for symptoms associated with osteoarthritis and other chronic musculoskeletal disorders. However, oral administration of NSAIDs is limited by their significant toxicity, which may cause various clinical side effects, like gastrointestinal conditions, renal disorders, and other hypersensitivity reactions. Therefore, various delivery vehicles including dendrimers, nano/microcapsules, implant-coatings, hydrogels, etc. have been studied to deliver NSAIDs locally [33,41,66–70]. Local delivery of antibiotics for musculoskeletal infections (like superficial cellulitis, osteomyelitis) has been widely used since such delivery type may offer high local drug concentrations and relatively easy drug diffusion to avascular areas of wounds [71]. However, the increasing antibiotic resistance has led to a severe public health crisis and a notable effort to develop new antibiotics [72]. Ling et al. developed a new antibiotic called teixobactin that was discovered in a screen of uncultured bacteria [68]. The structure of teixobactin is shown in Figure 2a. This antibiotic was found to be effective in killing *Staphylococcus aureus* (*S. aureus*) or *Mycobacterium tuberculosis* by inhibiting their cell wall synthesis. More strikingly, no mutants of these bacteria were observed. Lehar et al. introduced a novel antibody-antibiotic conjugate to eliminate intracellular *S. aureus* [46]. This conjugate consists of an anti-*S. aureus* antibody and a highly efficacious antibiotic (Figure 2b) and was found to be superior to conventional antibiotics like vancomycin. This provides a new strategy to design and develop novel antimicrobial drugs against intracellular *S. aureus*, which is associated with chronic or recurrent musculoskeletal infections. Recently, the delivery of bioactive peptides to combat musculoskeletal infections caused by bacteria, fungi, and viruses has become increasingly

popular. Bormann et al. chose five different synthetic peptides that were nine amino acids long and composed of inexpensive amino acids, and analyzed their antimicrobial effects against various bacterial species, efficacy on biofilms, and effect on osteoblast-like cells [73]. The results demonstrated that these short peptides had high antimicrobial activities even against bacteria in biofilms, suggesting a robust potential to prevent or treat implant associated infections in orthopedics without harming host's osteoblasts. There are also more than 90 antiviral drugs approved for the treatment of virus related infectious diseases [74]. To fight musculoskeletal cancers (e.g., osteosarcoma, rhabdomyosarcoma, soft tissue sarcoma, metastatic carcinoma), local delivery of anti-cancer drugs to tumor is also a promising strategy [75]. Besides the conventional anti-cancer drugs, new anti-cancer agents including small-molecule tyrosine-kinase inhibitors [76,77], drugs targeting epigenetic alterations [78], and immune-checkpoint inhibitors or monoclonal antibodies [79,80] have been developed to treat musculoskeletal cancers; however, most of them exhibit a narrow therapeutic index, have limited and unstable efficacy, and show obvious variations in toxicity among individual patients.

In addition to conventional medications, many drugs including growth factors, genes, and other regenerative drugs have emerged as critical factors to treat musculoskeletal diseases through local delivery. Successful musculoskeletal regeneration usually requires growth factors/bioactive proteins to provide signals at local injury sites to stimulate cells to migrate and to trigger the healing process. To meet this requirement, regenerative strategies to treat musculoskeletal disorders, involving bone defects, osteonecrosis, articular cartilage defects, meniscal tears, and volumetric muscle loss, often employ local delivery of various biological factors, such as growth factors and cytokines [4]. The growth factors used for bone repair are usually soluble signaling proteins secreted from cells and may include transforming growth factor- β s (TGF- β s), bone morphogenetic proteins (BMPs), insulin-like growth factors (IGFs), platelet-derived growth factors (PDGFs), and vascular endothelial growth factors (VEGFs) [81]. Qu et al. found that cell migration could be enhanced under a gradient of PDGF-AB using the knee meniscus as a model system. They created a nanofibrous scaffold that could sequentially release active collagenase and PDGF-AB in a localized and controlled manner [82]. As shown in Figure 2c, water soluble poly(ethylene oxide) (PEO) nanofibers (green) could release a matrix-degrading enzyme (X) in a burst manner over 24 h. Then, controlled release of a chemoattractant (filled black circle) from hyaluronic acid (HA) nanofibers (blue) led to the recruitment of additional meniscal cells to the interface of the scaffold over 5 weeks. Finally, a highly porous scaffold of slow-degrading poly(ϵ -caprolactone) (PCL) nanofibers (red) was prepared by dissolving PEO fibers and degrading HA fibers. This design, based on programmed growth factor delivery, effectively enabled and directed cell migration toward repair and regeneration of dense connective tissues, which usually have limited repair due to the lack of cells at the wound site. This strategy also provides a framework to treat many common musculoskeletal injuries, such as bone defects, osteonecrosis, joint replacement, etc.

To avoid chronic impairment, gene transfer is a promising strategy for treating musculoskeletal disorders and can improve repair and regeneration at injured or diseased sites through local expression of therapeutic nucleic acids. There are two ways to achieve the therapeutic effects of nucleic acids: one comes from the nucleic acid itself (siRNA); the

other is from the expressed proteins encoded by nucleic acids. Parenti et al. found that the transcription factor EB (TFEB) is a viable therapeutic target in Pompe Disease [83,84]. Overexpression of TFEB in cultured myoblasts from a Pompe disease murine model reduced glycogen stores and lysosomal size, facilitated autophagosome processing, and alleviated excessive accumulation of autophagic vacuoles. The results suggested that TFEB modulation of autophagy could effectively improve the functions of skeletal muscle tissues. Malcolm et al. achieved efficient gene silencing and siRNA delivery using cytocompatible nanoparticles for musculoskeletal cell types [85]. The siRNA mediates degradation of complementary cytosolic mRNA via the RNA interference pathway, providing a safe and effective drug delivery system for musculoskeletal disorder treatments. Figure 2d shows the diblock copolymer structure and the self-assembly process of cationic nanoparticles electrostatically complexed with negatively charged siRNA. It was found that the nanoparticle mediated siRNA cytocompatibility was cell type dependent, and that hydrophobicity played a key role in gene silencing for musculoskeletal cell types. Besides the genes, ligand-targeted drugs are emerging to be used to treat musculoskeletal and connective tissue disorders through local delivery [61]. Applying targeting ligands with a weak affinity for normal cells but a strong affinity for pathologic cells is an effective approach to maximize safety and efficacy of therapeutic drugs. Li et al. prepared bivalent biparatopic antibody-drug conjugates targeting human epidermal growth factor receptor 2 (HER2) (Figure 2e) and found that these new conjugates could induce HER2 receptor clustering [31]. By conjugating with tubulysin-based microtubule inhibitor, the antibody-drug conjugates showed strong anti-tumor activity over ado-trastuzumab emtansine in tumor models, implying a good safety profile in non-human primates to support its potential translation into clinical trials for treating soft tissue sarcoma. In addition, novel drugs based on regenerative cells/tissues have been studied for musculoskeletal drug delivery [4,86–89]. Murray et al. reported that mesenchymal cells expressing PDGF receptor beta (PDGFR β) contributed to skeletal muscle and cardiac fibrosis and developed a genetic system in mice to identify the molecular mechanisms [87]. Based on studies using fibrosis models (skeletal muscle laceration, cardiotoxin muscle injury, etc.), they found that PDGFR β -Cre effectively targeted quiescent PDGFR β ⁺ cells and activated myofibroblasts in both skeletal and cardiac muscles. Skeletal muscle stiffness was significantly reduced in mice treated with a small-molecule inhibitor of α_v integrins (CWHM 12) compared to control. The results obtained by collagen staining and digital morphometric assessment (Figure 2f) further showed that PDGFR β -Cre treated mice had significantly higher protection from angiotensin II-induced cardiac fibrosis compared to controls. Therefore, the α_v integrin was depleted in the growth factor receptor beta, which inhibited cardiotoxin and laceration-induced skeletal muscle fibrosis and angiotensin II-induced cardiac fibrosis, thus providing a promising strategy to prevent or treat muscle fibroses.

2.4 Carrier systems for musculoskeletal local drug delivery

Most therapeutic drugs are sensitive to pH, temperature, etc. which can affect their biological activity thereby limiting their clinical efficacy. Meanwhile, systemic administration of drugs usually lead to low drug concentrations at poorly vascularized injury sites and high risks of toxicity to healthy tissues/organs; therefore, local delivery of drugs is often highly desirable for musculoskeletal tissues, especially the ones that can offer drug

concentrations within desired therapeutic windows for a specific period of time. Up to now, to effectively deliver therapeutic drugs, various carrier systems ranging from dendrimers, nano/microparticles, degradable polymeric vehicles, swelling-controllable hydrogels, affinity-based delivery vehicles, and implant coatings, to three-dimensional (3D) scaffolds, have been studied as local delivery vehicles to treat musculoskeletal disorders [20,29,32,44,62,90–98]. Some representative examples of local delivery strategies for musculoskeletal applications are summarized in Table 1.

The delivery vehicles based on molecular level design can be grouped as dendrimers, polymers, oligopeptides, etc. Highly controllable dendritic structures combined with nano-size may allow dendrimers to be a leading carrier in local drug delivery applications [115]. The association of a dendrimer and a drug may occur in different ways, including via covalent or non-covalent interactions. The non-covalent interaction can be achieved by simple encapsulation of drugs inside dendrimers or by electrostatic interactions between dendrimer surfaces and charged drugs. This strategy generally does not require engineering of the drug or the dendrimer, although the interaction is usually relatively weak. Covalent association may occur through stable or cleavable bonds (cleaved only when reaching the target). The covalent associations are typically achieved by engineering of the drug or the dendrimer; however, they may suffer from potential loss of efficacy due to the stable chemical bondings or undesired cleaving before reaching targeted tissues. Hu et al. studied polyethylene glycol (PEG) modified polyamidoamine (PAMAM) dendrimer as carriers to deliver kartogenin (KGN) to regulate chondrogenic differentiation of mesenchymal stem cells (MSCs) to treat osteoarthritis; the construction of this dendrimer is shown in Figure 3a [99]. Zhang et al. described a new method to prepare dendrimer-assisted hydrophilic nanoparticles as sensitive vehicles to deliver epidermal growth factor receptor antibody (anti-EGFR) for rapid recognition and enhanced isolation of target tumor cells (Figure 3b) [100]. These dendrimer-based hybrid systems showed robust delivery performance, providing an emerging treatment platform in the field of musculoskeletal tumors that may extend to muscles, bones, and nerves. Besides dendrimers, many new polymers have also been designed and used as musculoskeletal drug delivery vehicles. Rai et al. used biodegradable PCL as a vehicle to deliver vancomycin for treating chronic osteomyelitis from methicillin-resistant *Staphylococcus aureus* or MRSA [39]. Liu et al. reported the fabrication of novel poly(lactic-co-glycolic acid)-dextran (PLGAD)-based delivery vehicles and used them to deliver anti-cancer drug of cisplatin (CDDP) for osteosarcoma cells [101]. The results showed that PLGAD/CDDP exhibited robust anti-tumor activity and could provide a promising platform in the treatment of osteosarcoma. In addition, Huang et al. developed a novel bone-targeting liposome which contains an oligopeptide of eight aspartate residues, and showed that this delivery system could effectively carry osteogenic phytomolecules to prevent estrogen depletion-induced osteoporosis [102].

Delivery vehicles in the form of nano/microparticles are one of the main categories of drug carriers for the treatment of musculoskeletal diseases, because of their unique properties stemming from their nanostructures [104,116–121]. Besides the common nanoparticles like mesoporous silica particles, polymeric particles, etc., many novel forms including micelles, emulsion particles, magnetic particles, liposomes, and microbubbles have been studied [103,119,120,122]. Li et al. reported an effective method to fabricate lignin-based complex

micelles in an ethanol/water mixture; these micelles were pH-responsive and exhibited controlled release properties for ibuprofen delivery in the treatment of a variety of musculoskeletal disorders and painful conditions [103]. Zhang et al. developed docetaxel emulsion particles which were coated with denatured soy protein isolates using an anti-solvent precipitation-ultrasonication technique [104]. The solubility of docetaxel nanoparticles was improved and led to high internalization into cancer cells, thus achieving enhanced cell cytotoxicity against musculoskeletal cancer cells. Talaie et al. used superparamagnetic iron oxide nanoparticles as vehicles to facilitate the monitoring and control of platelet-rich plasma (PRP) *in vivo* with noninvasive tools, thus achieving site-specific targeting of PRP for the treatment of injured muscle tissues [105]. Ayre et al. developed a novel delivery vehicle based on antibiotic-loaded nano-sized liposomes (Figure 3c), which resulted in a controlled and gradual release of antibiotic over a long period [106]. The results showed that the liposomal drug delivery system provided a potential platform to reduce infections in cemented joint replacements. In addition, a combined ultrasound-and-microbubble-mediated diclofenac gel delivery was developed which showed enhanced efficacy for the treatment of adjuvant-induced rheumatoid arthritis in rats [107].

Musculoskeletal implants may provide mechanical stability and may also need to have the capability to deliver drugs at the site of injury to promote healing and/or to prevent infections. Compared to other methods, implant coating has attracted extensive attention because of its integrated features of simplicity and versatility. A polymeric nanofiber coating with tunable combinatorial antibiotic delivery was developed by Ashbaugh et al. using electrospinning [108]. The coelectrospinning strategy is illustrated in Figure 3d. The antibiotic-loaded poly(lactic-co-glycolic acid) (PLGA) nanofibers and PCL were deposited on implants and generated conformal films after heat treatment. The coatings presented controlled release of antimicrobial agents and could prevent biofilm-associated infection *in vivo*. Finsgar et al. prepared a novel multilayer coating on stainless steel implants using alternating layers of biocompatible chitosan and non-steroidal anti-inflammatory drug diclofenac [109]. The improved corrosion behavior, drug release, and biocompatibility properties of the coatings further indicated potential applications in stainless steel-based hip replacements. Li et al. also developed nanocoatings on implants that could load single or multiple drugs to tune immune responses to reduce implant associated infections [110–112]. Various multifunctional coatings that can simultaneously promote osseointegration and prevent infection of orthopaedic implants have also been investigated. For instance, a mussel-inspired multifunctional hydrogel coating was developed by Cheng et al. (Figure 3e) and showed a controlled release of short cationic antimicrobial peptide and synthetic silicate nanoparticles, suggesting the potential to prevent infections and promote new bone formation in surrounding tissues in the field of bone repair, joint replacement, and osteonecrosis treatment [45].

Three-dimensional porous scaffolds may serve an important role in the guidance of new musculoskeletal tissue regeneration and in repair of bone defects without the need for allografts or autografts. Scaffolds with the capability to incorporate drugs either physically or chemically and to locally deliver drugs at the defect site in a sustained manner are highly desirable for the treatment of musculoskeletal disorders. Nowadays, various 3D scaffolds with drug delivery capability have been created from natural, synthetic, ceramic, and

composite materials including hydrogels, nanofibrous aerogels, microsponges, etc. [60,90,95,113,123–129]. A 3D bioactive hydroxyapatite/PCL composite scaffold was prepared by Bao et al. using a computed tomography-guided fused deposition modeling method, and showed robust delivery of VEGFs and BMPs [90]. The developed biomimetic artificial bone scaffolds exhibited effective healing of large weight-bearing bone defects, revealing a great potential in replacing autogenous bone grafts. Zhao et al. developed polyethylenimine-grafted cellulose nanofibril aerogels as a simple and safe drug delivery vehicle for musculoskeletal tissue treatment and regeneration involving bone defect repair, meniscal tear treatment, muscle loss treatment, and articular cartilage repair [113]. Because of its unique pH- and temperature-responsiveness, the developed aerogel could be a promising candidate as a new generation of delivery vehicles compared with conventional carriers made from synthetic polymers. In addition, a stem cell secretome-rich hydrogel was fabricated by Waters et al. which contained photocrosslinkable micro-porous networks and a nanoclay component to control the release of multiple growth factors in hMSC derived secretome [114]. As shown in Figure 3f, the photo-crosslinkable nanocomposite hydrogels were comprised of silicate nanoplatelets, gelatin methacrylate (GelMA) and hMSC derived growth factors (secretome). The results showed that the encapsulation of stem cell derived secretome in hydrogels provided a valuable therapeutic platform for musculoskeletal repair and regeneration.

3 Emerging strategies and techniques for musculoskeletal local drug delivery

Recent advances in musculoskeletal drug delivery have explored novel drugs, innovative assembly techniques for delivery vehicles, and smart and multifunctional delivery strategies. Examples of emerging strategies and techniques associated with drug delivery for the treatment of musculoskeletal disorders can be grouped into five categories: 3D printed scaffolds, multiple drug delivery, smart stimuli-responsive drug delivery, small molecule therapeutics and gene delivery, and stem cell engineering [4,10,15,20,86,122,130,131]. Many of these strategies and techniques are still new and are being actively explored, with research shedding light on how to achieve effective and targeted delivery, especially for musculoskeletal tumor and cancer treatments.

3.1 3D printed delivery vehicles

Recently, 3D printing technique has provided an effective strategy to control structures (e.g., geometry shape, pore structures), properties (e.g., surface wettability, mechanical properties), and functions (e.g., antibacterial capability, enhanced healing capability) of scaffolds, which are widely used in drug delivery for musculoskeletal disorder treatment and tissue regeneration [60,86,95,123,129,132]. Many building blocks have been used for 3D printing and these include several categories: synthetic/natural materials, extracellular matrix-based biomaterials, stem cells, etc. Meanwhile, various drugs including growth factors have also been added to achieve local drug delivery. A 3D PCL scaffold grafted with BMP-2 attached via polydopamine chemistry was printed by Lee et al. to promote osteogenic differentiation for bone tissue engineering in treating bone defects, osteonecrosis, and joint replacement caused by trauma, tumor, or infection [129]. Figure 4a illustrates the

schematic illustration of hybrid 3D porous PCL scaffold and *in vitro* experiments. The *in vitro* results showed significantly enhanced cell proliferation and osteoconductivity, indicating the promising capability of this scaffold as a useful construct for bone tissue engineering. Trombetta et al. reviewed the preparation of calcium phosphate ceramics using 3D printing for bone drug delivery, and investigated their biocompatibility, bone regenerative potential, and capability of delivering drugs and cells for bone defect repair and regeneration [60]. The major technologies (Figure 4b) used in 3D printing of pure or composite calcium phosphate scaffolds include vat polymerization, powder bed fusion, material extrusion, and binder jetting [60,133–136].

Besides the common construction of scaffolds, vascularization of 3D printed scaffolds is another important topic for musculoskeletal drug delivery. Wang et al. reported a combined set of approaches to identify 3D-printed scaffolds for vascularized bone tissue engineering [95]. Moreover, porous 3D-printed poly(propylene fumarate) scaffolds were designed and evaluated, and showed potential for evaluating printed scaffolds for biological applications, especially for drug delivery and tissue engineering. In addition, using stem cells for 3D printing is an important strategy for musculoskeletal drug delivery. Yu et al. reported a novel microcontact printing (μ CP) method to modulate focal adhesion and hMSCs, and found that differentiation of hMSCs could be induced by controlling the focal adhesion without any biochemical factors [137]. A polyvinyl alcohol (PVA) film was used as trans-print media for this μ CP procedure, and SEM and fluorescent images were performed to evaluate its capacity to pattern collagen type I (COLI) on soft and high stamp-adhesive polydimethylsiloxane (PDMS) substrates (Figure 4c). This study provided a promising chemical/biological-free strategy to engineer hMSCs for clinical skeletal tissue engineering applications. Ong et al. reviewed the current advances and applications of 3D bioprinting for musculoskeletal disease and trauma treatments and discussed their limitations [86]. The 3D bioprinting technologies and the cell types used are illustrated in Figure 4d. Although many 3D printed scaffolds have been fabricated for musculoskeletal drug delivery, achieving a hierarchical structure like the native tissue is still a challenge; for instance, bones are thick and complex and require adequate vascularization to achieve normal functions. Therefore, further studies are needed in creating adequately vascularized musculoskeletal constructs with clinically-relevant thickness and mechanical properties. In addition, work associated with skeletal tissue grafts and functional neuromuscular junctions is also important for musculoskeletal drug delivery and regeneration.

3.2 Multiple drug delivery

Musculoskeletal disorders usually involve several different diseased or injured conditions, and their ideal treatment should be driven by multiple drugs simultaneously. Therefore, drug delivery systems which can load multiple drugs and independently control the release of each drug have drawn wide attention [45,47–49,121,138,139]. Jiang et al. fabricated capsule integrated polypeptide multilayer films using layer-by-layer assembly technology, and these films were capable of loading multiple oppositely charged drugs [47]. As shown in *in situ* confocal laser scanning microscopy (CLSM) images, two oppositely-charged drug molecules could be effectively loaded within one polypeptide multilayer film (Figure 5a). Moreover, this delivery vehicle could load drugs any time after film postpreparation,

providing an effective strategy to prevent infection and to enhance tissue regeneration for the treatment of disorders in the fields of bone defect repair, fracture fixation, joint replacement, and other bone trauma treatments. A multidrug-eluting bi-layered microparticle-mesh scaffold (BMMS) was developed by Chamundeswari et al. using an electrospinning technique [48]. The schematic work flow of this BMMS fabrication process is shown in Figure 5b, and the SEM image of the prepared scaffold is presented in Figure 5c. The multiple drugs were co-encapsulated in the scaffolds and included hydrophobic dexamethasone, which was loaded in mesh, and hydrophilic ascorbic acid and β -glycerophosphate or proline, which were loaded in microparticles. Because of the controlled and sustained release of these drugs, the bi-layered microparticle-mesh scaffolds showed great potential to enhance osteogenic or chondrogenic lineage differentiation from MSCs for musculoskeletal tissue (bone, muscle, tendon, etc.) regeneration. In addition, nanoparticles such as calcium phosphate particles and dendrimer hybrid particles have been incorporated into scaffolds to achieve multiple drug delivery to treat musculoskeletal disorders, such as bone defect, cartilage defect, osteonecrosis, osteoarthritis, and intervertebral disc degeneration [15,93,100,122,140–142]. Extensive research in the design of delivery vehicles for multiple drugs has resulted in a variety of delivery systems for the treatment of various musculoskeletal disorders. However, precisely on-demand delivery, mechanism, *in vivo* toxicity, and challenges in cancer therapies need to be further studied. More studies are also needed to accelerate the translation of these multiple drug delivery approaches into clinical use, which may ultimately lead to improved therapeutic outcomes in musculoskeletal patients.

3.3 Smart stimuli-responsive drug delivery

Stimuli-responsive drug delivery systems have shown promising potential in the treatment of musculoskeletal disorders due to their control capability of on-demand release profiles, which can greatly enhance therapeutic effectiveness and reduce systemic toxicity [103,117,118,143–149]. Recently, musculoskeletal drug delivery systems with smart-responsive capacity have been created and shown to be responsive to specific stimuli, either exogenous (like magnetic field, light, voltage, etc.) or endogenous (such as pH, temperature, enzyme, etc.). Extracorporeal physical stimuli can be applied for musculoskeletal disorders, especially for bone/muscle tissues which can be applied through intramuscular or subcutaneous injection. Although the targeted delivery and controlled drug release can be achieved by magnetically guided or in thermo-, light- or ultrasound-triggered manners, the safety and/or biodegradability of responsive factors (like magnetic, light-responsive nanoparticles) and undesired tissue damage caused by external stimuli still remain [118]. Endogenous stimuli-responsive delivery systems can take advantage of specific microenvironmental changes associated with musculoskeletal diseases as well as pathological situations such as inflammatory diseases or infections, and are widely used for bone defect repair and anti-infection as implant coating or 3D scaffolds. However, endogenous triggers are indeed difficult to control because they may vary from patient to patient. Some examples of stimuli-responsive nanocarriers associated with exogenous factors are presented in Figure 6a: (i) magnetic-responsive vehicles, (ii) light-triggered vehicles, and (iii) voltage-responsive vehicles. The stimuli-responsive nanocarriers associated with endogenous factors like pH and enzyme are illustrated in Figure 6b. A

magnetic stimuli-responsive chitosan-based drug delivery vehicle was developed by Harris et al. by assembling superparamagnetic Fe_3O_4 nanoparticles and the antibiotic vancomycin into chitosan microbeads [143]. The magnetic stimulation resulted in an increase of therapeutic drug release to maintain desirable drug levels, suggesting clinical potential for the prevention or treatment of musculoskeletal infections. Responsive delivery vehicles that could be remotely triggered via micro-shock wave were created by Gnanadhas et al. and were used to resolve *S. aureus* infection and to control blood sugar levels [150]. Besides the exogenous stimuli, several endogenous variations have also been exploited to control the delivery of drugs in musculoskeletal tissues. Luo et al. developed a new redox dual-responsive prodrug-nanosystem using hydrophobic conjugates of paclitaxel and oleic acid [98]. Figure 6c presents the redox dual-responsive drug release of prodrug nanoparticles in the presence of two opposite stimuli within tumor cells. The results showed that this prodrug-nanosystem provided a new approach to assemble redox dual-sensitive conjugates and enhanced their drug loading and release capability for treating musculoskeletal tumors originating in bone or soft tissues. In addition, various other delivery systems that are able to control drug biodistribution in response to pH, temperature, enzymes, light, ultrasound, and electrical stimuli have been designed and showed promise for preventing or treating various musculoskeletal disorders [118,147]. In spite of some disease recognition, less than 5% of the injected dose of most targeted systems could reach diseased or infected sites. Meanwhile, the permeability and retention effect observed in preclinical studies may vary significantly from what may occur clinically. Therefore, in the future, additional focus should be directed to more clinically relevant models/systems in order to translate preclinical outcomes to clinical trials.

3.4 Small molecule therapeutics and gene delivery

Small molecule and gene therapeutics have become promising drug delivery strategies for musculoskeletal tissue repair and regeneration, which can mitigate the limitations associated with growth factors, for instance, protein instability, contamination issues, and immunogenic responses [19,63,151,152]. Due to low toxicity, flexibility, and cost-efficiency, small molecule therapeutics (usually <500 Da) have emerged as potential alternative drugs targeting musculoskeletal disorders. Sacchetti et al. developed a novel intraarticular delivery system assembled with PEG-modified single-walled carbon nanotubes (PEG-SWCNTs), which persisted in the joint cavity, entered the cartilage matrix, and delivered gene inhibitors to treat osteoarthritis [153]. As illustrated in Figure 7a, the SWCNTs modified with PEG chains were loaded with morpholino antisense oligomers (mASOs) against green fluorescence protein (GFP) and intraarticularly injected into the knees of both healthy and arthritic GFP transgenic mice. Moreover, they performed *in vivo* studies of expression of interleukin-1 and tumor necrosis factor α in osteoarthritis and found that there was no protein overexpression in PEG-SWCNT-650-treated knees compared to control knees of osteoarthritis mice. Besides targeting cartilage, various delivery systems based on small molecule therapeutics have been investigated for skeletal muscle regeneration, bone formation, ligament formation, etc. [152]. Although promising, one major issue associated with small molecule therapeutics still remains: due to their small size, small molecule therapeutics may enter non-target cells/tissues and induce unwanted physiological responses. Therefore, more research needs to be done to develop new small molecule therapeutics and

smart delivery vehicles and to examine function mechanisms and dosage requirements prior to clinical applications of small molecule therapeutic delivery.

Besides small molecule therapeutics, gene delivery is another emerging strategy for the treatment of musculoskeletal disorders, where local and controlled expression of proteins and/or therapeutic nucleic acids can be achieved. Bioreducible fluorinated peptide dendrimers (BFPD) were developed by Cai et al. and were capable of circumventing various physiological barriers to achieve highly efficient and safe gene delivery in cases involving intramuscular and intratumoral routes [140]. Figure 7b presents the schematic illustration for preparation of BFPDs and biomedical applications of BFPD polyplexes. Zhao et al. reported an effective strategy to synthesize a polymeric construction of Metformin (PolyMet) based on the conjugation of linear polyethylenimine with dicyandiamide [154]. The schematic illustration and representative transmission electron microscope images of PolyMet nanoparticles are shown in Figure 7c. The PolyMet achieved an enhanced tumor suppressive efficacy and combined carrier and anti-cancer activities for *in vivo* siRNA delivery. Although therapies based on gene delivery are promising for musculoskeletal repair or regeneration, limitations associated with efficacy and safety still remain; future studies should focus on genetic vector design and novel engineering strategies for gene delivery for musculoskeletal tissue repair.

3.5 Stem cell engineering

Using tissue engineering strategies to treat defects after tumor resection is considered to be transformative in clinical musculoskeletal repair and regeneration. In particular, stem cell engineering that can stimulate the healing of diseased and injured tissues has motivated the development of cell-induced interventions in musculoskeletal drug delivery [4,9,86,88]. Quarta et al. reported an artificial niche to maintain muscle stem cells (MuSCs) *in vitro* in a potent, quiescent state, which could lead to sustained quiescence for up to a week of both mouse and human MuSCs [89]. The schematic of the fabrication process of engineered muscle fibers (EMFs) on a microfluidic chip is shown in Figure 8a. A scaffold with parallel nanofibrils and with a shape and geometry similar to those of myofibers was generated from collagen I solution. This strategy of maintaining quiescence showed great potential for engineering stem cells isolated from musculoskeletal tissues, and was studied to treat musculoskeletal disorders involving volumetric muscle loss, muscular dystrophy, and other muscle defects. The living cell factories, consisting of cells and therapeutic agents, have gained wide attention for musculoskeletal tissue regeneration. Electrohydrodynamic (EHD) spraying is capable of creating such living cell factories, and Naqvi et al. reviewed this cell microencapsulation technology and discussed various regulation parameters including voltage applied, biomaterial properties, complex structures, and arrangement of microcapsules and microcarriers [88]. The schematic of EHD technology, Taylor cone formation, and electric field simulation are shown in Figure 8bi. They also described the monodispersed structures, core-shell structures of microcapsules and microcarriers, and their potential applications for musculoskeletal tissue regeneration, as illustrated in Figure 8bii-iv. Currently, cell encapsulation and engineering technologies are still in the early stage. In future studies, more *in vivo* functions, repair evaluation, and animal models are needed to

identify the role of stem cells and to reveal the distinct healing mechanisms behind the therapeutic benefits of using stem cell engineering.

4 Concluding remarks and perspectives

Drug delivery, as an effective therapeutic strategy, has been widely used for the treatment of various musculoskeletal disorders, like infection, tumor, cancer, etc. and has attracted increasing interest. Advanced drug delivery strategies have been developed for various drugs in a variety of vehicle forms, and aimed at treating a variety of musculoskeletal disorders involving bone, cartilage, tendons, ligaments, and skeletal muscle, as discussed in this review. With the development of new drugs (like genes, small molecule therapeutics, stem cells, etc.), innovative tools (such as 3D printing, tissue engineering technique, etc.), and novel delivery strategies (for instance, multiple delivery, smart stimuli-responsive delivery, etc.), many new concepts have been proposed and studied to achieve precisely controlled and on-demand drug delivery for musculoskeletal treatments. The remarkable progress in material chemistry and drug delivery has led to the design of sophisticated delivery systems using well-engineered structures. Studies at the material level and involving *in vitro* performance have been performed using relatively simple and conventional delivery strategies, such as calcium phosphate cements, microcapsules/particles/micelles, biodegradable polymeric scaffolds/hydrogels, and modified implants; the results usually show improved outcomes in treating various musculoskeletal disorders. Although some *in vitro* proof of concept studies have been reported for a number of delivery systems, only a few have been tested in preclinical *in vivo* models, and few have reached the clinical stage, in particular, for the emerging delivery systems. This could be related to the design of the drug delivery systems. To achieve smart-responsive, controlled, and highly efficient delivery, sophisticated designs are usually required; however, such designs may make clinical development more complex, especially in terms of the manufacturing process and quality control. In addition, many clinical trials of the existing musculoskeletal delivery systems have been suspended likely because of potential safety hazard, cost issues, and insufficient efficacy.

Despite major progress in the development of local drug delivery and related musculoskeletal repair and regeneration applications, many challenges associated with emerging delivery techniques, function mechanisms, toxic potential, and especially clinical uses still need further investigation. To address some of the common challenges faced in musculoskeletal drug delivery, as described in this review, further work is required to realize their clinical use through incorporating expertise from the fields of nanotechnology, materials science, genetics and medicine, cell biology, and tissue engineering. Furthermore, the “black box” associated with musculoskeletal drug delivery, focused on what drugs, vehicles, and strategies should be used (the input) to achieve the desired functional delivery and effective therapy for patients (the output), has not yet been unpacked. Up to now, clinical trials of most of the musculoskeletal local drug delivery systems, in particular, the emerging ones, are scant or are in the early stages. In considering the existing novel concepts, perhaps the focus should now shift toward some simpler and easier delivery systems that usually have a better chance of reaching the clinic. In addition, the relationship between pathogenesis of musculoskeletal diseases and functional mechanism of local

delivery systems needs to be further studied so targeted local drug delivery strategies can be developed for specific musculoskeletal tissues (like bone, joint, cartilage, tendons, etc.) and their disorders, to achieve an effective match between therapeutic strategy and injury/diseases, and to maximize practical applications that benefit from their unique designs. In conclusion, current musculoskeletal delivery systems are still in their infancy and need more *in vivo* or clinical investigations, while new systems utilizing novel therapeutic strategies are continually emerging. Given the rapid development in new therapeutic drugs and delivery techniques, and the prospects of advanced drug delivery systems as reviewed here, the study of drug delivery will continue to spark interest in clinical studies for the treatment of both common and rare musculoskeletal disorders.

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Figure 1. Musculoskeletal tissues with a high incidence of injuries and degeneration. The skeleton, joints, cartilage, intervertebral discs, tendons, ligaments, and muscles are part of the musculoskeletal system, which provides stability and motion. Musculoskeletal diseases because of injuries and degeneration are one of the major causes of pain and disability. Reprinted from [4] with permissions from Elsevier.

Figure 2.

(a) Schematic structure and molecular structure of teixobactin. (b) Model of the antibody-antibiotic conjugate (not drawn to scale). (c) Reprogramming the wound interface augments dense connective tissue repair. (d) Diblock copolymers form cationic nanoparticles via self-assembly that can complex with negatively charged small interfering RNA (siRNA). (e) Schematic of the HER2-specific biparatopic antibody and the tubulysin variant AZ13599185. (f) Picrosirius red staining of cardiac tissue 14 days after commencement of AngII treatment in control and Itgavflox/flox; PDGFR β -Cre mice. Scale bars 1 mm in whole heart sections, 70 μ m for magnified fields. Reprinted from [31,46,68,73,81,82] with permissions from Elsevier, Nature Publishing Group, and American Chemical Society.

Figure 3.

(a) Schematic illustrations of construction of KGN-PAMAM conjugates. (b) Illustration of the synthetic procedure for the anti-EGFR functionalized and dendrimer-assisted hydrophilic magnetic nanoparticles and the applications in cell capture. (c) Proposed liposome-pluronic structure. (d) The nanofiber-film coating was prepared by coelectrospinning of antibiotic-loaded PLGA and PCL fibers simultaneously onto the titanium K-wire implants followed by heat treatment to generate a conformal PCL film embedded with PLGA fibers. (e) Schematic illustration of the formation of a highly adhesive, multifunctional hydrogel coating on Ti surfaces. (f) Photo-crosslinkable nanocomposite hydrogels comprised of silicate nanoplatelets, GelMA and hMSC derived growth factors (secretome), and microbioreactor with deep concave wells to harness hMSC secretome. Reprinted from

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Figure 4.

(a) Schematic illustration of hybrid 3D porous PCL scaffold and *in vitro* experiments. (b) Schematic depiction of the major technologies used in 3D printing of pure or composite calcium phosphate scaffolds for bone regeneration and drug delivery. (c) Novel microcontact printing (μ CP) could effectively pattern COLI on soft and high stamp-adhesive PDMS substrates. (i): Schematic diagram of novel μ CP procedure which uses a PVA film as a trans-print media instead of directly printing on the substrate. (ii, iii): Scanning electron micrograph (SEM) validation of stamp features of S3.6 (ii) and L20 (iii) patterns, respectively. The height of the features on the stamps was 5 μ m. (iv, v) Fluorescent images of Cy3-labeled patterned COLI showing novel μ CP method could successfully print protein on 2.1 kPa soft and high stamp-adhesive substrates. Scale bar in (ii) and (iii) shows 10 μ m, in (iv) and (v) shows 50 μ m. (d) 3D bioprinting technologies (top) and cell types used in 3D

bioprinting (bottom). Reprinted from [60,87,129,137] with permissions from Springer, Nature Publishing Group, Elsevier, and Wiley-VCH.

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Figure 5.

(a) *In situ* CLSM images of polypeptide multilayer films after incubating in (i1, ii1) fluorescein isothiocyanate-labeled bovine serum albumin (green) for 15 min, followed by incubating in (i2, ii2) Rhodamine B-gentamicin (red) for 15 min. (i3) Combined pictures of (i1) and (i2). (ii) Z-stack images of two angle views (i.e., 90 and 20°) of polypeptide film in the film thickness direction. (b) Schematic work flow of the BMMS fabrication process. (c) SEM of the BMMS containing the electrosprayed microparticles on top of the electrospun nanofibrous mesh. Reprinted from [47,48] with permissions from American Chemical Society and Royal Society of Chemistry.

Figure 6.

(a) Stimuli-responsive nanocarriers due to exogenous factors. (i) Actuation mechanisms based on the heat generated by an alternating magnetic field (AMF) leading to on-demand pulsatile drug release from mesoporous silica nanoparticles (MSNPs). Capping system based on complementary DNA sequences. (ii) Examples of light-triggered drug delivery. Delivery of doxorubicin through the near-infrared-triggered induction of dehybridization of the DNA conjugated at the surface of gold nanorods. (iii) Voltage-responsive vesicles. Structure of polystyrene-β-cyclodextrin (PS-β-CD) and poly(ethylene oxide)-ferrocene (PEO-Fc), and

representation of the voltage-responsive controlled assembly and disassembly of PS- β -CD-PEO-Fc supramolecular vesicles. **(b)** Stimuli-responsive nanocarriers due to endogenous factors. (i) TAT-peptide-decorated liposomes comprising a hydrolyzable PEG shell allowing improved exposure of the TAT sequence at low pH. (ii) Multifunctional liposomal nanocarrier responsive to matrix metalloproteinases (MMP2) for drug delivery via TAT-mediated internalization. **(c)** Schematic representation of redox dual-responsive drug release of prodrug nanoparticles in the presence of two opposite stimuli within tumor cells. Reprinted from [98,120] with permissions from American Chemical Society and Nature Publishing Group.

Figure 7.

(a) (i) PEG-SWCNTs as chondrocyte-specific drug delivery systems. Morpholino antisense oligomers (mASOs) are composed by about 25 morpholine rings carrying a nitrogenous base and connected through uncharged phosphoroamidate linkages. (ii) *In vivo* effects of the injected PEG-SWCNTs. (b) (A) Chemical structure of polyhedron oligomeric silsesquioxane (POSS) core-based G2 poly(L-lysine) dendrimers (OAS-G2-Lys). (B) Peripheral amino groups of OAS-G2-Lys partially fluorinated by heptafluorobutyric anhydride. (C) Reversible cross-linking of FG2-Lys using different amount of 3,3'-dithiodipropionic acid-di(N-succinimidyl ester) (DSP). (D) BFPD complexation with DNA to form BFPD polyplexes. (E) BFPD polyplexes for intratumoral gene delivery. (F) BFPD polyplexes efficiently transport across the numerous intracellular barriers, including: (i) cellular internalization, (ii) endosomal escape, (iii) cytoplasmic trafficking to the nucleus, (iv) polyplex disassembly and DNA release in the perinuclear regions, and (v) nuclear entry of free DNA. (c) Anionic hyaluronic acid (HA)+siRNA mixture was condensed by cationic PolyMet into a negatively charged PolyMet/(HA+siRNA) complex (i, iii). 1,2-dioleoyl-3-trimethylammonium-propane chloride salt/cholesterol cationic liposomes were added to the complex to form lipid coating, then DSPE-PEG and DSPE-PEG-anisamide were used to liposome by the post-insertion method to form lipid-polycation-hyaluronic acid-PolyMet final nanoparticles (ii, iv). Reprinted from [140,153,154] with permissions from American Chemical Society and Nature Publishing Group.

Figure 8.

(a) (i) Schematic of the fabrication process of EMFs on a microfluidic chip. The design is based on arrays of 20 chambers ($500\ \mu\text{m} \times 300\ \mu\text{m} \times 7\ \text{mm}$), with media inlet and outlet ports for fluidic lines constituted by five parallel channels ($50\ \mu\text{m} \times 50\ \mu\text{m}$) used to exchange solutions and to perform cell seeding through the chambers. Monomers of collagen I were extruded through a nozzle in the chambers to generate EMFs. (ii) Representative immunostaining of a single mouse myofiber (top) and a single EMF (bottom). Scale bar, $50\ \mu\text{m}$. Scanning electron microscopy images (insets) show MuSCs localized on the fibers. Scale bar, $5\ \mu\text{m}$. (b) (i1) Schematic illustrating single needle

arrangement for production of microcapsules and microcarriers of uniform size using EHD technology. (i2) Taylor cone formation with increasing applied voltage illustrating dripping zone, transition zone, and jet forming zone. (i3) Influence of applied voltage and needle diameter on microcapsule size. (ii) Schematic representations of microcapsules and microcarrier morphologies formed with various constituent configurations in monodispersed spraying mode. (iii) Coaxial spraying and different combinations and conformations of core-shell microcapsules. (iv) Potential application areas of microcapsules and microcarriers (iv1) Injectable regenerative therapies (iv2) Remote activation devices (iv3) Controlled spatio-temporal drug or cell release. Reprinted from [88,89] with permissions from Wiley-VCH. and Nature Publishing Group.

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Table 1.

Local drug delivery strategies for musculoskeletal treatments

Carrier system	Delivery vehicle	Property/function	Application	Ref.
Molecular/ polymer	PEGylated polyamidoamine dendrimer	To induce chondrogenic differentiation of mesenchymal stem cells via cytoplasmic delivery of kartogenin	Osteoarthritis treatment	[99]
	Dendrimer-assisted hydrophilic nanocarrier	To improve the hydrophilicity and to accelerate cell capture and antibody conjugation	Rapid targeting and efficient capturing of tumor cells	[100]
	Biodegradable poly(e-caprolactone)	To achieve diffusion-dissolution controlled release of vancomycin	MRSA infection treatment	[39]
	Poly (lactic-co-glycolic acid)-dextran carrier	To enhance the anti-cancer potential of cisplatin in osteosarcoma cells	Osteosarcoma treatment	[101]
	Liposome containing oligopeptide	To deliver osteogenic phytomolecule icaritin	Osteoporosis treatment	[102]
Nano/micro particle	Lignin-based complex micelle	To achieve pH-dependent and controlled release of ibuprofen via self-assembled micelles	Local drug delivery	[103]
	Denatured protein-coated docetaxel nanoparticle	To improve intracellular delivery using denatured soy protein isolate-coated docetaxel nanosuspensions	Cytosolic delivery	[104]
	Superparamagnetic nanoparticle	To target PRP to sites of tissue damage	Muscle injury and disease treatment	[105]
	Liposome	To incorporate antibiotic-loaded nano-sized liposomes into polymethyl methacrylate bone cement	Anti-infection in joint replacements	[106]

Carrier system	Delivery vehicle	Property/function	Application	Ref.
Implant coating	Diclofenac gel	To mediate diclofenac gel delivery via ultrasound and microbubble	Rheumatoid arthritis treatment	[107]
	Combinatorial antibiotic coated polymeric nanofiber	To locally deliver combinatorial antibiotics from implant surfaces via electrospun composite coatings	Biofilm-associated infection treatment	[108]
	Chitosan/diclofenac coating	To deliver drugs in controlled and multi-mechanism manners via multilayers of polysaccharide chitosan and anti-inflammatory drugs	Hip replacement	[109]
	Multilayer polypeptide nanocoating	To deliver interleukin 12 p70 at the implant/tissue interface	Device-associated anti-infection	[110–112]
	Multifunctional hydrogel coating	To deliver cationic antimicrobial peptide and synthetic silicate nanoparticles via adhesive, osteoconductive, and antimicrobial hydrogel coatings	Bone implant surface modification and functionalization	[45]
3D porous scaffold	Bioactive hydroxyapatite/PCL composite scaffold	To spatiotemporally deliver dual-cytokines to promote osteogenesis via drug loaded hydrogels	Large weight-bearing bone defect repair	[90]
	Cellulose nanofibril aerogel	To achieve controlled release of drugs based on pH and temperature changes via polyethylenimine-grafted cellulose nanofibril aerogels	New drug delivery carrier	[113]
	Stem cell secretome-rich nanoclay hydrogel	To deliver growth factor-rich stem cell secretome via nanocomposite hydrogels	Tissue repair and regeneration	[114]