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Recombinant adeno-associated virus-based gene therapy combined with tissue engineering for musculoskeletal regenerative medicine

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Key Words:
gene therapy; musculoskeletal regeneration; rAAV; stem cell; tissue engineering

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ABSTRACT

Recombinant adeno-associated viral (rAAV) vector-mediated gene delivery is a novel molecular therapeutic approach for musculoskeletal disorders which achieves tissue regeneration by delivering a transgene to the impaired tissue. In recent years, substantial scientific progress in rAAV gene therapy has led to several clinical trials for human musculoskeletal diseases. Nevertheless, there are still limitations in developing an optimal gene therapy model due to the low transduction efficiency and fast degradation of the gene vectors. To overcome the challenges of rAAV gene therapy, tissue engineering combined with gene therapy has emerged as a more promising alternative. An rAAV viral vector incorporated into a biomaterial has a more controlled gene expression, lower immune response, and higher efficiency. A number of biomaterials and architectures have been combined with rAAV viral vectors, each having its own advantages and limitations. This review aims to give a broad introduction to combinatorial therapy and the recent progress this new technology has offered.

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Introduction

Musculoskeletal disorders (MSDs) are conditions caused by injuries or diseases affecting the human musculoskeletal system. They range from genetic muscle diseases such as Duchenne muscular dystrophy (DMD) to bone disorders such as osteoporosis, as well as disorders of the joints, including osteoarthritis and rheumatoid arthritis. MSDs are common diseases affecting people worldwide that often cause inflammation, pain, and disabilities in millions of patients.[1] Current clinical treatments focus more on stopping the progression of the symptoms such as swelling and pain in order to restore the functions of impaired musculoskeletal systems; however, current approaches to regeneration in those affected by MSDs have met with limited success clinically.

As a multidisciplinary field which combines engineering and life sciences to improve or replace biological tissues, tissue engineering is dedicated to restoring, maintaining or improving tissue functions.[2] Traditional tissue engineering aims to combine cells or bioactive molecules with biomaterials, resulting in new tissue formation within the host environment. Approaches to tissue engineering often involve using stem cells that have regenerative properties in combination with biomaterials, which have the correct scaffolding geometry to provide mechanical support and modulate cellular activity.[3] In recent years, gene therapy delivering transgenes to impaired tissue has provided another promising treatment for tissue regeneration in MSDs. Recombinant adeno-associated virus (rAAV) serving as a gene therapy vector has been used extensively in the treatment of MSDs and injuries.[4] As shown in **Figure 1**, the rAAV-based gene therapy approach to tissue regeneration has two routes: (1) viral particles carrying a therapeutic gene can be injected directly into the site of injury *(in vivo)*; (2) viral particles can be used to infect cells, which will later be introduced to the injury site (*ex vivo*).

Since the above approaches for gene transfer are

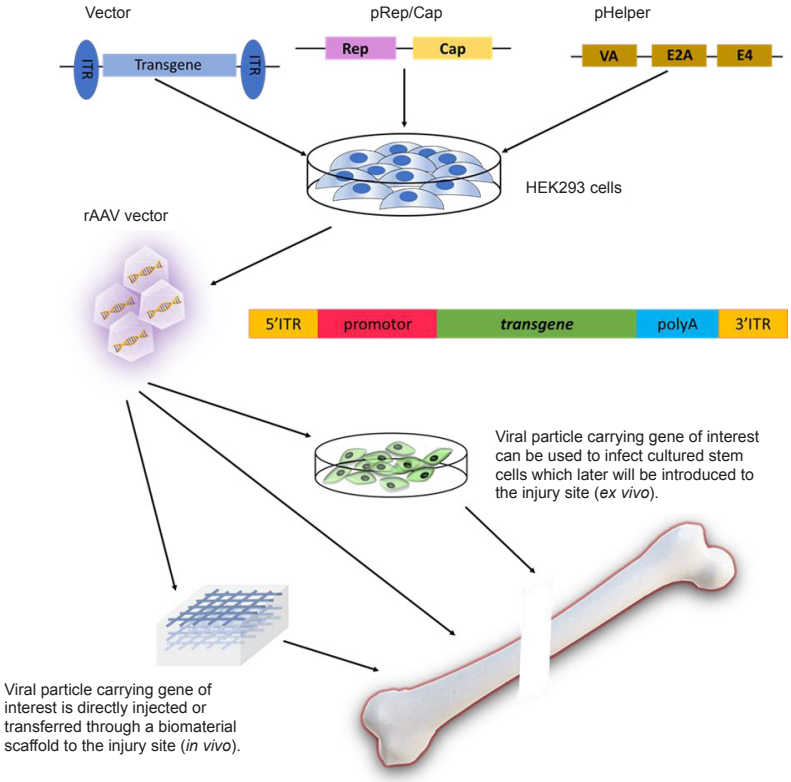

Figure 1. Schematic diagram of rAAV-based gene and cell therapy for bone defect repair. rAAV: recombinant adeno-associated virus.

designed to treat MSDs, recently, the combination of gene therapy with tissue engineering has shown great potential in tissue regeneration.[5] To be more specific, rAAV vectors carrying therapeutic genes can be loaded onto appropriate biomaterials to provide stable, dose- and time-dependent gene expression.[6] Stem cells engineered by *ex vivo* gene transfer can also be loaded onto biomaterials and implanted into the site of injured tissue.[7]

In this review, we will discuss recent progress in tissue engineering, particularly the combination of rAAV-based gene therapy and tissue engineering for the regeneration of musculoskeletal tissues, such as bone, cartilage, muscles and joints. We will introduce advances in rAAV-mediated gene therapy and regeneration in tissue engineering. Requirements for appropriate biomaterials that optimize outcomes will also be discussed. The goal of this review is to introduce rAAV-mediated gene therapy, tissue engineering, and their application in MSDs.

Development of Recombinant Adeno-Associated Virus Vectors

An rAAV is produced by transfecting mammalian cells with several plasmids carrying the therapeutic genes and other components needed for viral assembly. In most scenarios, HEK 293 cells (expressing E1A and E1B) are transfected together with three additional plasmids: the vector that carries the gene of interest flanked by two internal terminal repeats, the RepCap plasmid that carries *Rep* and *Cap* genes, and the pHelper plasmid which provides other genes that are necessary for the replication process (**Figure 1**). As an efficient gene vector, the rAAV has been used extensively in the treatment of musculoskeletal diseases and injuries.[8] Many favourable factors contribute to the preference for selecting rAAV vectors from among all the other viral particles available as gene delivery vehicles. To begin with, rAAVs are capable of infecting a large variety of host cells, including both dividing and quiescent cells.[9] In addition, rAAVs produce long-term transgene expression, a low immune response after infection, and lack toxicity in humans.[10] These unique advantages make rAAV-mediated gene therapy a promising strategy for injured tissue that is not able to undergo rapid regeneration. The use of rAAVs has been approved by the U.S. Food and Drug Administration for over 300 clinical studies on human subjects due to their good safety record and high efficiency.[11] However, there are some limitations associated with the use of rAAV vectors. For example, the low capacity of the gene expression cassette restricts the choice of transgenes. The wild-type AAV consists of a regular icosahedral particle with a small size (diameter = 20 nm) and short viral genome, usually around 4.7 kb; thus, the genes of interest cannot exceed this specific length (< 5 kb).[12] Owing to its ability to infect a large range of host cells, eliciting non-specific gene expression can also be

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a potential danger for the host organisms. Hence, the design of the tissue- or cell-specific rAAV gene delivery system is an important safety issue in clinical trials.[11, 13]

Based on their biological characteristics, several strategies have been used to enhance the application of rAAV vectors in gene therapy. A number of serotypes have been identified since the discovery of AAV (AAV1–12).[14] Previous studies demonstrated that each serotype of rAAV has specific cellular transduction characteristics in different cell types due to its unique tissue tropism.[15, 16] Therefore, increased efficiency in the delivery of a transgene can be achieved by selecting appropriate serotypes for different tissues (**Table 1**).[11, 14, 16-43] Serotypes 6 and 9, for example, show the highest transduction level in myocardium.[17, 18] The correct administration approach also increases transduction efficiency; for instance, serotypes 1 and 2 have the highest efficiency for local delivery into muscles; while serotypes 6, 8 and 9 are more attuned for systemic gene delivery to the entire body. It should be noted that the same serotype of rAAV shows different affinities in the same tissues of different animal models.

Table 1. Common rAAV serotypes for gene delivery

Serotype	Primary target tissues	Host tested	References
rAAV1	Central nervous system, liver	Mouse	16, 19, 20
	Muscle, diaphragm	Human	21, 22
rAAV2	Joints, liver, brain	Mouse	23, 24
	Brain, liver, muscle	Human	11, 14, 25-28
rAAV5	Brain, lung, eye	Mouse	29, 30
	Joints	Monkey	31
	Lung, brain, eye	Human	11, 14
rAAV6	Heart	Mouse	18
	Liver	Human	32, 33
rAAV6.2	Liver	Mouse	34
rAAV7	Brain, central nervous system	Mouse	35
	Brain, eye	Monkey	11, 14
	Liver	Human	36
rAAV8	Kidney, brain, liver, lung	Mouse	34, 37
	Liver, eye	Human	38, 39
rAAV9	Heart, liver, skeletal muscle	Mouse	16, 17, 40
	Heart, liver, muscle, brain, central nervous system, lung, eye	Human	11, 14, 41
rAAVrh.10	Brain, liver	Human	42, 43

Note: rAAV: recombinant adeno-associated virus.

Besides using appropriate serotypes of rAAV, applying tissue-specific promotors can control the expression of the target gene and enhance transduction into the host cells.[11] In addition, using universal promotors such as the promoters of cytomegalovirus, chicken beta-actin, and elongation factor 1-alpha are more prone to inactivation, building up toxicity, and non-specific transgene expression, while tissue-specific promotors increase safety and allow specific gene expression within different tissues. The muscle creatine kinase promoter has specificity for and activation in muscles, allowing the gene of interest to be expressed only in mature muscle cells and muscle fibres such as cardiac and skeletal muscles.[44] Taken together, these findings demonstrate that selection of tissue-specific promoters along with the application of different serotypes can increase the specificity and efficiency of gene therapy.

Tissue Engineering and Regenerative Medicine

Stem cell-based therapy

The paradigm of tissue engineering is composed of cells, signalling molecules and scaffolds.[2] Many researchers choose stem cells, such as mesenchymal stem cells (MSCs), as the best candidate for tissue regeneration because of their unique properties, including clonogenicity and self-renewal.[45] Under different signalling pathways, they have the potential to differentiate into specialized cells. This pluripotentiality or multipotentiality gives rise to new opportunities for tissue repair, by delivering stem cells into the site of injury and controlling their fate by directing their differentiation into desired phenotypes.[46] Bone marrow-derived MSCs (BMSCs) possess the potential to differentiate into bone, cartilage, tendon and connective tissues.[47, 48] Owing to additional advantages such as their ready availability, ease of isolation and avoidance of allogeneic responses,[48] MSCs have been used extensively in animal models for MSDs, including cartilage repair,[49, 50] bone formation,[51] and epidermal healing.[52, 53]

Ex vivo gene therapy and tissue engineering

Growth factors play crucial roles in the proliferation and differentiation of stem cells. In contrast to embryonic stem cells, adult stem cells can only be found in certain parts of the body compartment, and have limited proliferation and differentiation capacities.[46] To overcome this restraint, genetic modification of stem cells via gene transfer enables a better therapeutic approach that promotes tissue repair through

overexpression of growth factors. Osteoinductive proteins such as bone morphogenetic proteins (BMPs) stimulate the osteogenic differentiation of MSCs into bone-forming cells.[54, 55] Viral vectors have been used extensively in preclinical studies to deliver osteoinductive *BMP* genes, since long-term expression of growth factors is required for successful bone formation.[56, 57] Lin et al.[58] transduced human MSCs (hMSCs) with the bone morphogenetic protein 2 (*BMP-2*) gene using a lentiviral vector. Lenti-BMP-2-transduced hMSCs were introduced into hydrogel scaffolds, which were capable of fitting different shapes of defects and were transplanted into severe combined immunodeficient (SCID) mice. Sustained higher expression of osteogenic genes was detected in the Lenti-BMP2 gene group compared with controls. Microcomputed tomographic imaging indicated bone formation as early as 14 days after implantation.[58]

In summary, efficient *in vivo* bone formation can be achieved by encapsulating hMSCs that express the BMP-2 gene in a projection stereolithographically-fabricated hydrogel scaffold.

In vivo gene therapy and tissue engineering

In addition to genetically modifying stem cells, viral vectors containing the gene of interest can be applied to the damaged site via direct injection. rAAV vectors are commonly used in intra-articular administration to block articular inflammation, promoting anabolic activities via the introduction of growth and critical factors.[59] rAAV vectors remain a superior gene delivery strategy *in vivo* because of long-term expression and absence of an immune response.[60] Whether blocking inflammation or delivering growth factors, administration of any agents via rAAV raises potential safety concerns which should be noted.[61] Viral vectors should be controlled temporally and spatially, otherwise, uncontrolled prolonged expression of vector DNA might interfere with the cells' normal function, even leading to a harmful immune response elicited by the host. Therefore, it is particularly important to develop a regulated rAAV expression system to avoid potential side effects. This requirement can be achieved by selecting appropriate serotypes, tissue-specific promotors, administration approaches, and adapted biomaterials.[61, 62]

Controlling the release of rAAV gene delivery by biomaterials

As the third component of tissue engineering, appropriate scaffolds provide the structural and biophysical support for cell growth and tissue regeneration.[7, 63] While the chemical composition determines mechanical maintenance, more advanced scaffold architecture should be able to mimic the natural extracellular matrix of the tissue, providing the optimal biochemical environment for cell infiltration and for the developing functional tissues.[63, 64]

A wide variety of polymers, both natural and synthetic based, has been used in the field of tissue engineering. Several characteristics of the scaffold are necessary, and are shared among all of the material choices: biodegradability, biocompatibility, mechanical properties similar to the site of impairment, and ease of manufacture.[63, 65] In the last decades, gene-activated matrix technology, which combines gene therapy and tissue engineering, has emerged as a novel therapeutic approach. Specifically, growth factors and signalling molecules are incorporated into the biomaterial scaffolds in the form of plasmid DNA instead of proteins. The genes of interest are then transcribed and translated within the endogenous damaged cells, in such a way as to achieve sustained gene expression and promote regeneration; however the efficiency of gene transfer is low.[66] Given the benefits of viral vector-mediated gene therapy, incorporating viral vectors into an engineered biomaterial can potentiate the effect of therapeutic genes further. Such a gene- or cell-activated biomaterial is able to provide a more promising alternative to the traditional gene-activated matrix technology. When applying gene therapy to tissue engineering, additional requirements need to be met to effectively mediate gene vehicle-based gene transfer. Biomaterials that act to control gene expression should maintain a high and prolonged concentration of transgene at the site of interest while minimizing the dose needed for gene transfer. Two common strategies of biomaterial-mediated gene transfer are achieved by encapsulating viral vector during the fabrication process of the biomaterials or incorporating vectors within the preformed construct. Various biomaterial scaffolds are utilized in combination with gene therapy. **Table 2** summarizes the differences in composition and architecture along with the benefits and disadvantages each possesses.[67-79] **Figure 2** covers approaches of incorporating rAAV vectors into different scaffolds.

Collagen is the most abundant protein in the body; and it is also one of the most studied natural polymers due to its natural properties.[67-69] It functions similarly to the extracellular matrix, which provides structural support while improving cell growth and tissue repair simultaneously by modulating cell adhesion, proliferation, and differentiation. Natural polymers form microenvironments that promote tissue regeneration; at the same time, porous-based scaffolds provide a large surface area-to-volume ratio for cell infiltration and nutrient delivery. However, the lack of mechanical strength, in particular, low bearing capacity is one of the biggest challenges in applying them to functional scaffolds.[64, 71] Synthetic polymers, on the other hand, provide stronger mechanical support with versatile structures, toughness, and stabilities. In the study conducted by Gu et al.[40] an rAAV encoding a transgene (rAAV-CMV-GFP or rAAV-CMV-VEGF) was encapsulated into fibrous scaffolds composed of polyester urethane urea and polyester ether urethane urea to form an elastic epicardial patch. Cells seeded onto the rAAV-containing scaffolds showed higher and more sustained transgene expression compared with the direct rAAV injection group; thus demonstrating that controlled release of rAAV vectors using biomaterials could achieve a more localized and efficient gene delivery system, with the synthetic polymers providing structural and mechanical improvement in ischemic cardiomyopathy using an rAAV-CMV-VEGF gene construct. The polyester urethane urea matrix not only had good mechanical properties, but also served as a therapeutic gene delivery system, indicating that

Table 2. Commonly-used gene- and cell-activated biomaterials

Polymer category	Type of scaffold	Source	Advantages	Disadvantages	References
Natural	Porous-based scaffolds	Gelatine, collagen, polysaccharides	1. Biocompatible, biodegradable 2. Low toxicity and inflammation 3. Functionally similar to extracellular matrix	1. Low bearing capacity	67-71
	Hydrogel-based scaffolds	Fibrin glue, fibrin sealant, collagen, gelatine, hyaluronic acid	1. Biodegradable 2. Water-soluble 3. Easily controlled architecture 4. Functionally-similar to extracellular matrix	1. Poor mechanical properties	58, 72-75
Synthetic	Porous-based scaffolds	Polyester urethane urea, polyester ether urethane urea, polycaprolactone, poly-L-lactic acid	1. Strong mechanical properties 2. Easily manipulated 3. Versatile shape, toughness, and stability	1. Low bioactivity 2. Slow degradation 3. Contain acid by-products	40, 65, 76-78
	Hydrogel-based scaffolds	Poly(ethylene oxide), poly(propylene oxide)	1. Water-soluble 2. Better mechanical strength	1. Slow degradation 2. Compromised flexibility	79

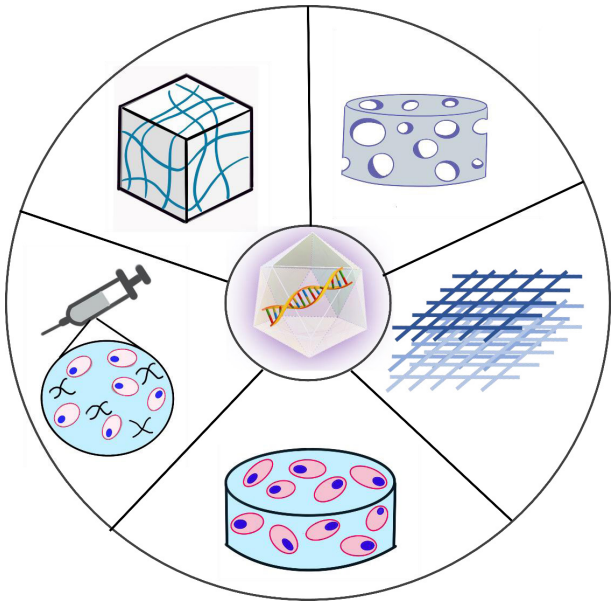

Figure 2. Schematic representation of gene-activated biomaterial scaffolds for delivering rAAV vectors. Starting from the top left in a counter-clockwise order: rAAV vectors can be incorporated into preformed hydrogel-based scaffold,[88] or as a mixture containing cells, polymers, and viral particles for direct injection,[70] or by transfecting stem cells which have been incorporated into a scaffold.[58] An rAAV vector can also be incorporated into a porous or fibrous-based scaffold individually or with stem cells.[40, 78, 89] rAAV: recombinant adeno-associated virus.

such a strategy could also be applied to repair of tendon and ligament post traumatic injury.

Due to their unique properties, hydrogel-based scaffolds have gained extensive attention in the field of tissue engineering over past decades.[80-83] Hydrogels, formed by crosslinking natural or synthetic polymers with liquid, have a high water content which increases hydrophilicity and stability.[84, 85] Holding a large amount of water in the structure increases their resemblance to the natural extracellular matrix, while at the same time, their highly hydrophilic nature makes hydrogels suitable for drug and gene vector delivery; it also facilitates movement of viral vectors encapsulated in hydrogels via diffusion.[72, 79, 86] Rey-Rico et al.[87] explained the many additional benefits and strategies of using hydrogel to deliver gene vectors in their recently-published review. As shown in **Figure 2**, an rAAV could be loaded onto a preformed hydrogel construct by incubation;[88] or by transfecting stem cells which would then be incorporated into the construct.[58, 89] An injectable solution containing cells, hydrogel polymers and virus particles is also a promising approach to delivery of stem cells and viral particles together.[70]

Application of Gene-Activated Biomaterials for Musculoskeletal Regeneration

Gene-activated biomaterials for bone healing

Over recent decades substantial research has shown positive therapeutic effects of cell- or gene-based therapy in promoting bone healing.[4, 90, 91] Successful conversion of osteogenic progenitor cells into osteoblastic cells has been achieved by the overexpression of osteo-inductive genes. Current biomaterial-guided gene transfer provides a new promising approach to increasing gene transfer efficiency. Dupont et al.[92] implanted a self-complementary rAAV (scrAAV) vector-coated poly(ε-caprolactone) scaffold carrying the *BMP-2* gene into immunocompromised rats with femoral defects. The results demonstrated that defects treated with scrAAV2.5-BMP delivery *in vivo* showed increased production of BMP and higher mineral formation.

In *ex vivo* gene transfer therapy, hMSCs transduced with an rAAV-BMP construct are seeded within biomaterials to stimulate cell proliferation. In a study conducted by Sun et al.[70] a gene-activated hydrogel scaffold carrying both rAAV vector and hBMSC simultaneously was developed, such that hBMSCs were transduced with rAAV6-BMP-2 *in vivo* after transplantation to obtain a temporally- and spatially-controlled release of rAAV particles. The results showed that the concentration of rAAV particles encapsulated within scaffolds decreased significantly more slowly compared with direct viral injection, indicating that the hydrogel had an extended release effect in delivering the rAAV vector. Analysis via microcomputed tomographic images, measurement of new bone volume, and bone mineral density demonstrated increased osteogenic capacity of the hBMSCs encapsulated in *BMP-2* gene-activated scaffolds; higher levels of bone formation were observed as early as 6 weeks post implantation. It should be noted that the experimental group treated with hydrogel loaded with both the rAAV vector and hBMSCs had a higher expression level of *BMP-2* and osteogenic-related genes (*OCN* and *ALP*) compared with the control (hydrogel loaded with modified hBMSCs *in vitro*). Taken together, these results suggest that a scaffold fabricated with an rAAV gene vector is an effective gene delivery system in bone tissue engineering that is able to control expression of the *BMP-2* gene and acts to provide sustained and localized signals needed by hBMSCs for proliferation and differentiation into osteogenic cells, enhancing bone formation.

Gene-Activated Biomaterials for Cartilage Regeneration

Rheumatoid arthritis is an autoinflammatory disease affecting a large number of people worldwide. Osteoarthritis is another type of joint disease caused by cartilage degeneration. Early treatments for both rheumatoid arthritis and osteoarthritis include the administration of glucocorticoids to reduce pain and inflammation; however, no effective treatments have been developed to reverse their pathogenesis and progression. Gene therapy provides a potential therapeutic option for arthritis. By modifying gene expression, gene therapy could decrease chronic inflammation via the administration of inhibitors of proinflammatory cytokines, such as interleukin-1 receptor antagonist, TNF-α inhibitor and anti-inflammatory cytokines (interleukin-4, interleukin-10).[93-96] As a treatment for osteoarthritis, gene transfer of growth factors could reduce cartilage degeneration and improve chondrocyte proliferation, which is needed for cartilage repair.[59, 97, 98] rAAV-mediated gene therapy in combination with tissue engineering offers a more powerful therapeutic approach to delivery that achieves sustained overexpression of growth factors, and circumvents their characteristic of rapid degradation.[99, 100] In 2017, Rey-Rico et al.[101] applied a poly (ethylene oxide) (PEO) and poly (propylene oxide) (PPO) copolymer solution with rAAV vectors carrying the transforming growth factor-β (*TGF-β*) gene to human OA chondrocytes. Administration of rAAV-hTGF-β in combination with polymers led to increased expression of TGF-β and a higher level of type-II collagen deposition. The same research group also modified expression of SRY-box transcription factor 9 (SOX9), a DNA binding protein known to regulate skeletal and cartilage production, via PEO-PPO-PEO polymeric micelles coated with rAAV-FLAG-hSOX9. rAAV-mediated *SOX9* gene expression was demonstrated to produce an increase in cell proliferation and improved cartilage remodelling ability.[102] Under a similar approach, Venkatesan et al.[103] coated rAAV-FLAG-hSOX9 onto pNaSS-grafted poly(ε-caprolactone) films. In the study, rAAV-mediated overexpression of the *SOX9* gene via pNass-grated poly(ε-caprolactone) film induced type-II collagen formation and promoted more pronounced chondrogenic differentiation activities compared with any other treatments tested.

Gene-Activated Biomaterials for Skeletal Muscle Regeneration

Even though muscle has an inherent regenerative capacity, many conditions can prevent cells from achieving a full functional recovery. Therefore, studies have attempted to improve muscular growth and prevent formation of fibrous scar tissue which is known to hinder normal function. Critical factors supporting myogenesis have been incorporated into biomaterials to increase the half-life of proteins, in an attempt to increase regeneration of skeletal muscle.[104] However, the delivery of genetically-engineered myoblasts via biomaterials offers a more promising effect on muscle regeneration. In a study performed by Blumenthal et al.[105] myoblasts overexpressing growth factors were seeded onto polyurethane scaffolds and then transplanted onto damaged myocardium; a successful angiogenic effect was observed, indicating that biomaterial-mediated *ex vivo* gene therapy could be a potential strategy for cardiac tissue regeneration. The application of biomaterials could offer protection to the rAAV gene transfer system and ultimately enhance muscle repair. To achieve a more efficient and prolonged effect, research conducted by Moimas et al.[106] used rAAV-mediated gene transfer in addition to a tissue scaffold. In this study, an rAAV vector encoding vascular endothelial growth factor in combination with a collagen-glycosaminoglycan template was applied onto a pectineus muscle flap to induce angiogenesis and muscle formation. The result indicated that rAAV-based gene therapy in combination with biomaterials is a promising

tool to enhance muscle formation.

Muscular dystrophy is a large family of heterogeneous disorders caused by genetic defects in genes encoding muscle cells, preventing muscle from functioning properly. DMD is one of the most prevalent yet lethal muscular dystrophies, affecting 1 in every 3500 live male births. DMD is caused by X-linked genetic mutations of the dystrophin gene, resulting in the losses of structural and functional integrity of cardiac and skeletal muscle cells. Gene therapy offers a promising treatment, such as delivery of the functional micro (or mini)-dystrophin gene by rAAV vectors. In recent decades, our lab has dedicated to developing rAAV-based mini-dystrophin gene replacement therapy to ameliorate the pathology and restore muscle functions through intramuscular or systemic administration.[107, 108] After intraperitoneal injection of rAAV-mini-dystrophin into a severe DMD murine model–10-day-old dystrophin/utrophin double knockout mice–strong mini-dystrophin expression was observed, with restored muscle structural integrity in major skeletal muscles, extending the life-span of treated mice. However, one of the biggest challenges of gene therapy, the host immune response against an rAAV vector, results in diminished transfer efficiency and curtailed transgene expression that is associated with chronic inflammation in dystrophic muscle. Therefore, an rAAV-based gene transfer approach has also been applied to reducing inflammation through inhibition of nuclear factor-κB in DMD animal models, improving muscle pathologies and physiological function.[109-111] This combined gene therapy approach, with gene replacement and anti-inflammatory agents, may achieve a synergistic effect on the treatment of genetic muscle disorders. We expect that using a biomaterial scaffold to control the release of viral vector may potentially be beneficial in treating DMD, but more preclinical research will be needed in the near future. **Table 3** summarises recent progress in combining AAV gene therapy with biomaterials to treat musculoskeletal disorders.[40, 70, 78, 92, 101-103, 106]

Table 3. Biomaterial-mediated AAV gene delivery for musculoskeletal tissue repair

Gene	AAV serotype	Scaffold	Biomaterial source	Clinical application	Reference
BMP-2	AAV6	Hydrogel	Gelatine	Cranial bone formation	70
	AAV6	Porous	PLLA	Bone formation	78
	AAV2.5	Porous	PCL	Femoral bone formation	92
SOX9	AAV2	Micelles	PEO-PPO-PEO copolymer	Cartilage repair	102
	AAV2	Films	PCL	Cartilage repair	103
TGF-β	AAV2	Micelles	PEO-PPO copolymer	Cartilage repair	101
VEGF	AAV2 & AAV9	Fibrous	PEUU & PEEUU	Cardiac tissue regeneration	40
	AAV2	Matrix	Collagen & glycosaminoglycan	Muscle regeneration	106

Note: BMP-2: bone morphogenetic protein 2; PCL: poly(ε-caprolactone); PEEUU: polyester ether urethane urea; PEO: poly(ethylene oxide); PEUU: polyester urethane urea; PLLA: poly-L-lactic acid; PPO: poly(propylene oxide); rAAV: recombinant adeno-associated virus; SOX9: SRY-box transcription factor 9; TGF-β: transforming growth factor-β; VEGF: vascular endothelial growth factor.

Conclusion and Perspectives

By introducing therapeutic genes through *in vivo* or *ex vivo* routes, rAAV-based gene therapy is a promising strategy for treating musculoskeletal diseases and promoting tissue regeneration; yet regulating AAV expression both temporally and spatially is particularly important to avoid an unwanted immune response and achieve high efficiency. Tissue engineering is a field that combines stem cells, bioactive molecules, and scaffolding materials to improve or replace biological tissues. Long-term expression of growth or critical factors is often required for optimal repair and functional restoration. Recent studies have shown that the combination of gene therapy and tissue engineering could circumvent the barriers to both therapies. To be more specific, the viral delivery system could enhance the expression of bioactive factors; in addition, scaffold-mediated gene delivery increases the duration and localization of the transgene that achieves an in situ therapeutic effect. Choosing the best serotypes and promotors would overcome some current obstacles such as low efficiency of transgene expression; at the same time, more suitable designs of scaffold architecture and biomaterial chemical composition will increase the complementarity between gene and tissue-engineering therapy. In future, we expect to see more multidisciplinary translational research leading to potential application in clinical settings.

Author contributions
YW wrote and edited the manuscript; BW and XC supervised and helped to draft the manuscript. BW designed the structure of the review. All authors read and approved the final manuscript.
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