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COMMENTARY

Recent tissue engineering-based advances for effective rAAV-mediated gene transfer in the musculoskeletal system

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ABSTRACT

Musculoskeletal tissues are diverse and significantly different in their ability to repair upon injury. Current treatments often fail to reproduce the natural functions of the native tissue, leading to an imperfect healing. Gene therapy might improve the repair of tissues by providing a temporarily and spatially defined expression of the therapeutic gene(s) at the site of the injury. Several gene transfer vehicles have been developed to modify various human cells and tissues from musculoskeletal system among which the non-pathogenic, effective, and relatively safe recombinant adeno-associated viral (rAAV) vectors that have emerged as the preferred gene delivery system to treat human disorders. Adapting tissue engineering platforms to gene transfer approaches mediated by rAAV vectors is an attractive tool to circumvent both the limitations of the current therapeutic options to promote an effective healing of the tissue and the natural obstacles from these clinically adapted vectors to achieve an efficient and durable gene expression of the therapeutic sequences within the lesions.

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Introduction

Musculoskeletal tissues, including the articular cartilage, bone, tendons, and muscles significantly differ in their ability to repair spontaneously upon injury.[1] While the cartilage has a very limited ability to self-repair, most fractures of long bones heal on themselves except for large segmental defects. On the other hand, self-repair of tendons often results in a poor quality tissue.[2] In addition, the intrinsic ability of muscles to heal may be compromised under severe trauma conditions, leading to the formation of fibrous scar tissue.[3] So far, the limitations associated with the current clinical options and the increment of incidences of musculoskeletal injuries have argued for the necessity of looking for alternative therapeutic options tailored to each tissue type that may lead to its regeneration.

In this scenario, gene transfer has emerged as an alternative technology to directly transfer genes encoding for therapeutic factors in sites of injury, resulting in a temporarily and spatially defined delivery of a candidate agent.[4] Diverse nonviral[5] and viral gene vehicles (vectors derived from adenoviruses - AdV, retro/lentiviruses, or herpes simplex viruses - HSV)[6] have been developed to target human cells that may be affected in a variety of musculoskeletal tissues. Most particularly, vehicles based on the non-pathogenic human adeno-associated virus (AAV) have considerable advantages over other, more classical vectors that currently make them preferred systems to treat human disorders.[4]

rAAV-mediated gene transfer for musculoskeletal system repair

Features of rAAV vectors

Recombinant AAV (rAAV) vectors are derived from a non-pathogenic, replication-defective human parvovirus[7] that can be manipulated to produce recombinant viral constructs by removing all AAV coding sequences and replacing them by a transgene cassette,[8,9] making them less immunogenic than AdV and less toxic than HSV[10-12] (Fig. 1A). Most experimental work has been initiated with the serotype 2 of the virus (AAV-2),

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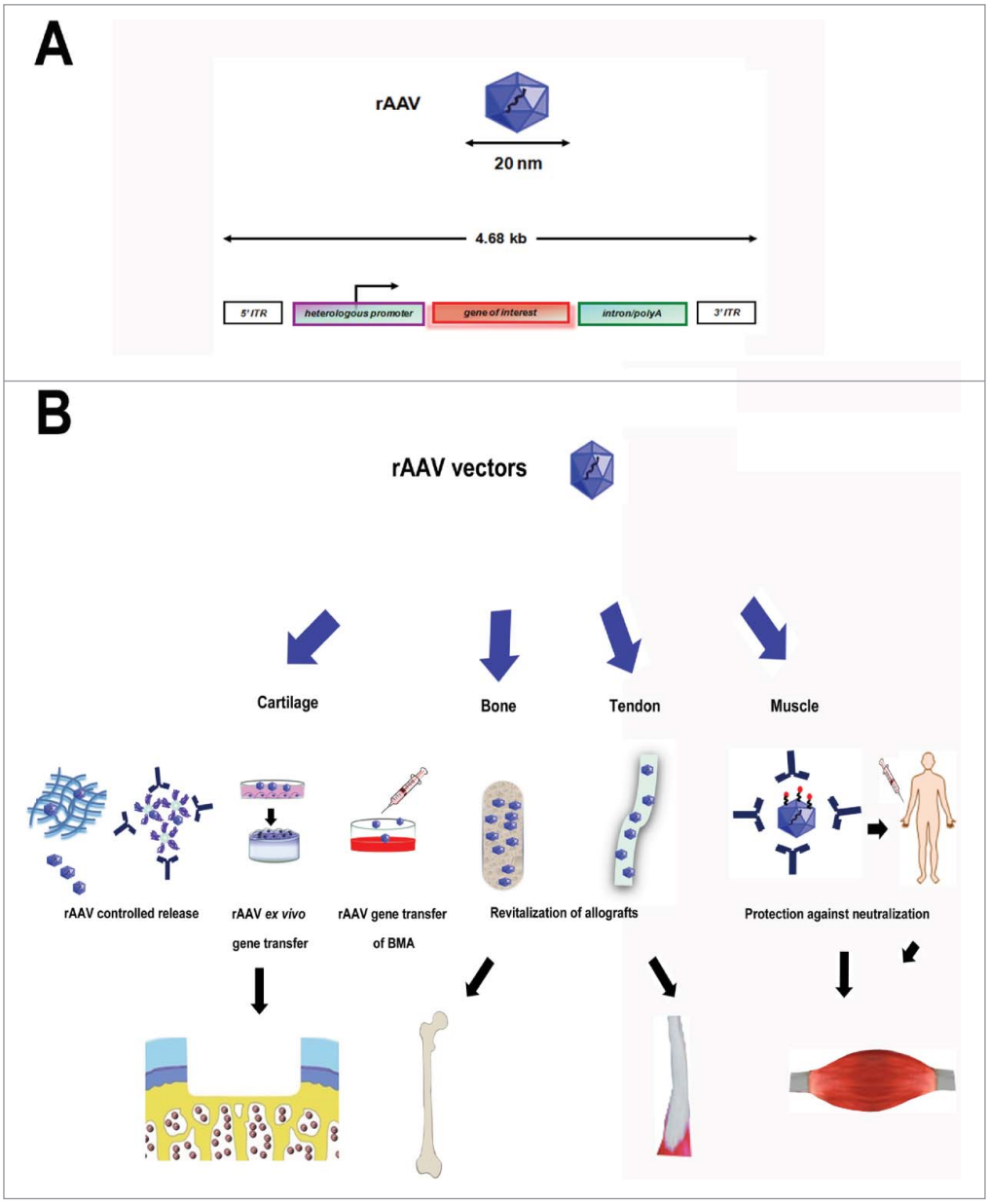

Figure 1. Concepts for rAAV-mediated gene transfer using tissue engineering approaches in the musculoskeletal system. (A) Genomic organization of rAAV vectors. Classical rAAV vector with 2 inverted terminal repeats (ITRs) at either end of a transgene cassette (heterelogous promoter, gene of interest, intron/polyA signal). The arrows show the viral transcription promoter. (B) Principal tissue engineering strategies for rAAV mediated-gene transfer in the musculoskeletal system. rAAV can be encapsulated in different biomaterials such as hydrogels or polymeric micelles to achieve a controlled release profile at the site of injury. The vectors may be delivered *ex vivo* by genetically modification of cells that are subsequently seeded onto a matrix and implanted in the recipient. Different patient-related materials including bone marrow aspirates (BMA) and allografts can be endowed with biological factors enhancing cell/tissue reparative processes via rAAV-mediated gene transfer. Polymers can be used to overcome rAAV physiological barriers when administered through classical routes to achieve an efficient gene transfer in the target location.

including to target tissues of the musculoskeletal system,[4,13] yet other natural serotypes have been identified and tested in diverse cells and tissues (AAV-1 to -12, with a focus on AAV-5 in the orthopaedic field).[4] One remaining, critical issue when using rAAV is the pre-existence of neutralizing antibodies in the human population, mostly directed against the AAV capsid proteins with a higher prevalence for AAV-1 and -2,[14] and possible specific cellular responses for instance by activation of the Toll-like Receptor (TLR) 9/MyD88 and interferon-1 cascade in plasmacytoid dendritic cells by particular serotypes (AAV-1, -2, and -9).[14] Active work is ongoing to overcome such hurdles and controlled delivery approaches based on the use of solid

scaffolds and hydrogels coated with or encapsulating rAAV vectors to mask potentially immunogenic viral epitopes may allow to produce safer systems of gene transfer.[4] rAAV are small (~20 nm) vectors, capable of transducing both dividing and nondividing cells at relatively high gene transfer efficiencies (up to 100%),[4,15] allowing for direct gene transfer approaches *in vivo* through the dense extracellular matrix of targeted tissues.[16] These potential advantages from rAAV make them as the vector of choice to treat human disorders.[4]

rAAV-mediated gene transfer for tissue regeneration: Implications

rAAV vectors can be directly introduced into the body (*in vivo* approach) or indirectly by collection, modification and re-implantation of the patient's cells in sites of injury (*ex vivo* approach).[2] Direct administration of rAAV is a simple and cost-effective gene transfer approach but it requires the availability of a considerable cell population in a damaged tissue susceptible to transduction for expression of the transgene being delivered at appropriate therapeutic levels. Also, direct rAAV gene transfer by intra-articular injection as commonly performed in musculoskeletal translational research may lead to the dissemination of the vectors to non-target tissues early on (liver, kidney, lymph nodes)[17-20] while vector DNA might be rapidly cleared, becoming detectable only at the site of injection at extended periods of time[17,19-31] and leading to prolonged transgene local expression (at least a year, the longest time points examined).[31,32] Another issue is a possible contralateral effect of the gene treatment in nonmodified locations (joints) upon circulation of the therapeutic product and/or by trafficking of vector-modified cells, even though this observation has been mostly reported when using adenoviral and retro-/lentiviral vectors[33-38] and only in rare cases with rAAV.[30] Yet, such effects may be prevented when providing the vector treatment by arthrotomy that allows for a more precise delivery of the vectors in the joint.[39-42] Also, the existence of patient-associated factors and of physiological barriers (pre-existence of neutralizing antibodies in the host against the viral capsid proteins, inhibition of transduction by particular anticoagulants) may interfere with an effective rAAV delivery, processing, and expression of the transgene in the target cells by blocking vector transduction or by redirecting distribution of rAAV to non-target tissues.[4,43] Remarkably, delivery of rAAV via polymeric biomaterials may overcome such limitations by providing a controlled release of the vectors only where necessary.[4]

Even though *ex vivo* delivery may obviate these problems by introducing cells instead of gene products in sites of injury, it remains a costly procedure, requiring more complex and laborious steps of cell harvesting and expansion. The identification of alternative, convenient gene delivery procedures is thus under active investigation, such as options based on the supply of tissue biopsy samples (whole bone marrow aspirates, fat, muscle) instead of isolated progenitor cells.

Combination of convenient tissue engineering strategies with clinically adapted rAAV vectors may improve current therapeutic options while increasing the efficiency of rAAV-mediated gene transfer, leading to the elaboration of safe and effective treatments against tissue injuries in patients.

Exploiting the concept of tissue engineering for an effective rAAV-mediated gene transfer

As no single approach is capable of promoting the regeneration of the different musculoskeletal tissues, tailored strategies based on then optimal combination of a therapeutic factor with a biomaterial acting as a vehicle of the gene vector (direct *in vivo* approach) or as a cell-supportive matrix (indirect *ex vivo* approach) adequate to the properties of each tissue in question are necessary to promote its regeneration. The most advanced synergic technologies for improving both rAAV-mediated gene transfer and current therapeutic options in the different tissues of the musculoskeletal system are presented in the following sections (Fig. 1B).

rAAV gene transfer in cartilage

Articular cartilage is the smooth tissue that covers the ends of bones, allowing for a successful load transmission and mobility within the joints. Due to the lack of access to blood supply, the cartilage has a limited ability to self-healing and full repair of cartilage defects is therefore a major clinical challenge that may progress to osteoarthritis, a critical disorder affecting a large number of patients worldwide.[44,45] Despite the availability of several therapeutic options to repair injured cartilage (marrow-stimulating techniques such as microfacture, transplantation of tissue or cells

including autologous chondrocytes - ACI - or mesenchymal stem cells - MSCs, replacement surgery),[46-48] none of them can reproduce the natural functions of the native, hyaline cartilage (type-II collagen and proteoglycans), rather leading to the formation of a poorly mechanically functional fibrocartilaginous surface (type-I collagen).[49]

Current approaches for improved rAAV-2-mediated gene transfer in the cartilage focus on the incorporation of the vectors into biomaterials in order to achieve a controlled release profile of rAAV in the site of injury.[50-53] These techniques may be combined with bone marrow stimulation for chondral defects and the controlled release of rAAV vectors from the biomaterial could provide a suitable, lasting stimulus to increase the chondrogenic potential of cells that populate the lesion. Hydrogel systems, exhibiting a release pattern via diffusion process, are advantageous materials to achieve this goal as they may be modulated to reduce vector spreading to non-target tissues.[4] Different polymers from both natural[50,53] or synthetic origin[51,53] have been tested to prepare hydrogels as rAAV controlled delivery systems in cartilage regeneration (Table 1). Poly(ethylene oxide) (PEO) and poly (propylene oxide) (PPO) tri-block copolymers (poloxamers and poloxamines), described as "smart" or "intelligent" polymers due to their capacity to form polymeric micelles and to undergo sol-to-gel transition upon heating,[54] have recently showed to be efficient rAAV-mediated delivery systems.[52,53]

Combination of rAAV with tissue engineering approaches has also been exploited to circumvent physiological barriers limiting rAAV-mediated gene transfer such as the neutralization by neutralizing antibodies against the viral capsid proteins present for instance in the synovial fluid from patients with joint diseases[55] or the inhibition of rAAV adsorption at the target cell surface by specific anticoagulants (heparin).[4,56] We recently reported that encapsulation of rAAV in poloxamer PF68 and poloxamine T908 polymeric micelles allowed for an effective, durable, and safe modification of human MSCs (hMSCs) to levels similar to or even higher than those noted upon direct vector application (up to 95% of gene transfer efficiency).[52] Of further note, these copolymers were capable of restoring the transduction of hMSCs with rAAV in conditions of gene transfer inhibition like in the presence of heparin or of a specific antibody directed against the AAV capsid proteins, enabling effective therapeutic delivery of the chondrogenic sex determining region Y-box 9 (*sox9*) sequence leading to an enhanced chondrocyte differentiation of the cells.[52]

Recent gene transfer approaches for cartilage regeneration focused on the use of autologous compounds capable of improving the effectiveness of the microfacture technique. By these methods, a bone marrow aspirate was collected from the patients and transduced *ex vivo* with viral vectors.[57] The resulting clotted bone marrow containing transduced cells and vectors was implanted into cartilage defects.[57] While this approach was initially described for the delivery of AdV,[57] its use with rAAV may appear more advantageous as these vectors allow for more sustained levels of transgene expression and avoid the undesirable immune responses associated with AdV.[58-61] An increase in chondrogenic processes have been described by transfer of transforming growth factor β 1 (TGF-β1),[58,60,61] insulin-like growth factor I (IGF-I),[59,60] and the transcription factor *sox9*[60] to bone marrow aspirates from both human[58,59,61] or minipig origin[60] via rAAV-2.

Treatment of large chondral defects is usually performed by ACI using cells from a lesser-weight bearing part of the joint. Although this technique already showed satisfactory long-term results in patients, with the production of hyaline-like repair tissue following transplantation of chondrocytes into chondral defect,[62] the *ex vivo* modification of cells via rAAV may lead to a better quality of the repaired tissue. Fibrin glue (FG) alone[63] or combined with other polymers[64,65] has been also used for the *ex vivo* delivery of rAAV by encapsulation of genetically modified cells such as chondrocytes[63] and periosteal cells[64,65] to heal full-thickness chondral defects.[63-65] Both IGF-I[63-65] and the bone morphogenetic protein 2 (BMP-2)[64,65] incorporated in rAAV-5[63] or -2[64,65]) have been reported to be potent factors that increase chondrogenesis of encapsulated cells when implanting in chondral defects from horse[63] and minipig models.[64,65]

rAAV gene transfer in bone

Bone tissue has a highly hierarchical structure based on type-I collagen fibers and nanohydroxyapatite matrix, making it a tissue with unique mechanical properties. The bone has an intrinsic ability to self-repair that may nevertheless be exceeded when the

Table 1. Tissue engineering approaches for rAAV gene transfer in articular cartilage.

Materials	Genes	Strategy	Outcomes	Efficacy	Systems	Refs
FG	GFP, TGF-β1	encapsulation in FG gel	controlled rAAV-2 delivery for cartilage repair	high efficiency at low FG concentration, decline after 8 days	hMSCs	[50]
RAD16-I	*lacZ*, RFP	encapsulation in RAD16-I pure or combined with HA	controlled rAAV-2 delivery for cartilage repair	80% transduction efficiency with spheres at 0.4%, time-course decline of expression	hMSCs	[51]
poloxamer F68, poloxamine 908	*lacZ*, *sox9*	encapsulation in micelles	controlled rAAV-2 delivery for cartilage repair	enhanced expression, restoration in conditions of gene transfer inhibition	hMSCs	[52]
alginate poloxamer	*lacZ*, RFP	encapsulation in alginate/poloxamer composite systems	controlled rAAV-2 delivery for cartilage repair	AlgPH155+PF127 [C]) leaded to a most controlled release profile, higher transduction efficiency with AlgPH155+PF127 [H]	hMSCs	[53]
BMA	*lacZ*, TGF-β	mixing with rAAV-2	direct gene transfer of BMA for cartilage repair	enhanced chondrogenesis and reduced hypertrophy with TGF-β	hBMA	[58,61]
	lacZ, TGF-β, *sox9*, IGF-I	mixing with rAAV-2	direct gene transfer of BMA for cartilage repair	enhanced chondrogenesis and reduced hypertrophy with *sox9*	minipig BMA	[60]
	lacZ, IGF-I	mixing with rAAV-2	direct gene transfer of BMA for cartilage repair	enhanced chondrogenesis, hypertrophy, and osteogenesis with IGF-I	hBMA	[59]
FG	IGF-I, GFP	rAAV-5-transduced chondrocytes embedded in FG gel	cartilage healing in full-thickness chondral defects	enhanced chondrocyte predominance and type-II collagen expression	chondrocyte implantation in chondral defect (horse)	[63]
FG/PGA matrix	IGF-I, BMP-2	rAAV-2-transduced periosteal cells seeded in FG/PGA matrix	cartilage healing in full-thickness chondral defects	enhanced chondrocyte-like morphology with BMP-2	periosteal cell implantation in chondral defect (minipig)	[65]
				dedifferentiation of cells from superficial zones in contrast to those from deeper zones (chondrocytes morphology)	periosteal cell implantation in chondral defect (minipig)	[64]

Abbreviations: GFP, green fluorescent protein; TGF-β1, transforming growth factor β 1; *lacZ*, E. coli β-galactosidase; *sox9*, sex determining region Y-box 9; RFP, red fluorescent protein; IGF-I, insulin-like growth factor I; BMP-2, bone morphogenetic protein 2; hMSCs, human mesenchymal stem cells; FG, fibrin glue; RAD16-I, $(RADA)_4$ peptide; HA, hyaluronic acid; AlgPH155+PF127 [C], alginate/poloxamer complex systems crosslinked at room temperature; AlgPH155+PF127 [H], alginate/poloxamer complex systems crosslinked at high temperature (50°C); hBMA, human bone marrow aspirates; PGA, poly(glycolic acid).

fracture gap is too big or unstable.[66] Other critical issues include the rate of morbidity during tissue graft harvesting and the high prevalence of pseudoarthrosis associated with lumbar spine fusion.[67] Therefore, complete regeneration of bone tissue remains a challenging issue.

Although autografts are considered the gold standard to treat large bone defects, their use is still restricted by the limited graft availability and by donor site morbidity.[68] Even though devitalized cadaveric allograft tissue may help to overcome these issues, its use is hindered by a limited integration with the host bone.[68] Revitalization of allografts by rAAV-mediated gene transfer of therapeutic, osteogenic factors is an advantageous approach to increase allograft integration,[69-72] based on the administration of morphogens like the BMPs[71,72] and of angiogenic factors such as the vascular endothelial growth factor (VEGF)[69] (Table 2).

An innovative strategy involves the dual immobilization of rAAV carrying VEGF and the receptor activator of nuclear factor kappa-B ligand (RANKL) on the cortical surface of allografts to modulate angiogenesis and bone resorption,[69] showing marked remodelization and vascularization that led to a new bone collar around the graft. A limitation for rAAV immobilization on allografts is the limited porosity of the material that compromises the possibility of obtaining a uniform and reproducible coating.[73] To overcome this limitation, a demineralization method to increase surface absorbance while retaining the structural integrity of the allograft was further developed.[73] Demineralized bone wafers (DBW) obtained by this procedure showed an increased absorbance for uniform rAAV coating, without difference in transduction efficacy when implanted in mice *in vivo* compared with mineralized allografts.[73] The use of self-complementary AAV (scAAV) that bypass the need for second-strand synthesis into the host cells also allowed to increase the transduction efficacy in the hematoma of healing allografts.[42,43]

An rAAV coating strategy has been also involved for biological activation of bone-related biomaterials.[74,75] Nasu et al.[74] lyophilized rAAV-*lacZ* and rAAV-BMP-2 (serotype 2) in hydroxyapatite, β-tricalcium phosphate (β-TCP), and titanium (Ti) alloy. When implanted in rat muscles, a higher β-galactosidase activity and significant induction of bone formation were observed when rAAV-*lacZ* and rAAV-BMP-2 were immobilized into hydroxyapatite scaffolds.

rAAV-mediated gene transfer in tendons

Tendons are unique connective tissue structures that connect and transmit forces from the muscle to the bone, storing elastic energy and withstanding high tensile forces necessary for locomotion.[76,77] Tendon injuries are common pathologies presenting a clinical challenge due to the poor responses of injured tendons to treatments, resulting in a tissue with inappropriate strength or limited mobility.[78] Therapeutic options to repair ruptured tendons include suture, autografts, allografts, and synthetic prostheses yet none of them allowed for the successful, long-term healing, resulting instead in incomplete tendon strength and functionality.[79] Alternative treatments based on the delivery of morphogens may induce tendon and ligament formation from progenitor cells basede on the use of BMP-12 (or growth/differentiation factor 7 - GDF-7), BMP-13 (GDF-6), and BMP-14 (GDF-5)[80,81] for instance (Table 3). Of further note, delivery of morphogens via rAAV has been employed to target synovial tenocytes *in vitro*,[80] being more effective that nonviral and adenoviral vectors without eliciting immune responses.[82]

One of the main limitations of using grafts for tendon reconstruction is the appearance of recurrent adhesions that may result in inflammation, fibrosis, and paucity of tendon differentiation signals during healing limiting joint flexion.[83] To solve these hurdles, Basile et al.[83] loaded rAAV-2/5 vectors expressing GDF-5 in tendon allografts as a means to improve the functional properties and abolish fibrotic adhesions. Coating of freeze-dried allografts with rAAV-GDF-5 resulted in significantly improved metatarsophalangeal joint flexion in a murine model compared with rAAV-*lacZ* controls. More recently, the same authors optimized rAAV-GDF-5 loading in freeze-dried allografts, showing that lower doses of GDF-5 were more effective to suppress adhesions, without adverse effects on the strength of the repair.[84]

rAAV-mediated gene transfer in muscles

Skeletal muscles (40–45% of the adult human body mass) generate forces permitting voluntary movement and locomotion.[3] Despite a strong ability for self-repair, exposition of muscles to compromised conditions such as severe trauma may impair muscle function, leading to contracture and chronic pain.

Table 2. Tissue engineering approaches for rAAV gene transfer in bone.

Materials	Genes	Strategy	Outcomes	Efficacy	Systems	Refs
PCL	BMP-2	coating in porous scaffold	controlled scAAV2.5 delivery for bone repair	peak of BMP-2 expression after 1 week in hMSCs, at 7 weeks in human amniotic fluid-derived stem cells, higher bone ingrowth *in vivo* with acellular scaffolds	hMSCs, human amniotic fluid-derived stem cells, femoral defects (rat)	[75]
hydroxyapatite, β-TCP, Ti	*lacZ*, BMP-2	Coating of rAAV-2 in scaffolds	biological activation of bone-related biomaterials	induction of bone formation when using hydroxyapatite scaffolds	insertion of fibroblasts in back muscle (rat)	[74]
allografts	*lacZ*, caALk2	coating in allograft	revitalization of structural allografts	new bone formation	segmental femoral graft model (mice)	[70]
	RANKL, VEGF	dual coating of allograft	revitalization of structural allografts	marked remodelization and vascularization with gene combination	segmental femoral graft model (mice)	[69]
	BMP-2	coating in allograft	repair of craniofacial bone defects	increased osteogenesis with scAAV *versus* rAAV	cranioplasty (mice)	[72]
			healing of critical defects	reduced resorption with increased graft bone volume compared with autografts (scAAV2.5)	C3H10T1 cell femoral graft model (mice)	[71]
DBW	*luc*	coating of DBW	increase in allografts porosity for uniform rAAV coating	uniform coating with no effect on transduction efficiency	C3H10T1 cell implantation in muscle (mice)	[73]

Abbreviations: BMP-2, bone morphogenetic protein 2; *lacZ*, E. coli β-galactosidase; caALk2, activin receptor-like kinase-2; RANKL, receptor activator of nuclear factor kappa-B ligand; VEGF, vascular endothelial growth factor; *luc*, luciferase; hMSCs, human mesenchymal stem cells; PCL, poly-ε-caprolactone; β-TCP, β-tricalcium phosphate; Ti, titanium; DBW, demineralized bone wafers.

Table 3. Tissue engineering approaches for rAAV gene transfer in tendons and muscles.

Targets	Materials	Genes	Strategy	Outcomes	Efficacy	Systems	Refs
Tendons	allograft	*lacZ*, GDF-5	coating of rAAV2.5 in tendon allograft	flexor tendon reconstruction	peak of expression at 7 days, acceleration of wound healing	NIH3T3 fibroblasts *in vivo* implantation (mice)	83
		luc, GDF-5	Coating of rAAV2.5 in tendon allograft	optimization of vector loading to increase tendon repair	higher efficiency at lower GDF-5 doses	*in vivo* implantation (mice)	84
Muscles	polyinosinic acid, polylysine	mSeAP	systemic administration of rAAV-2	adjuvant effect of polymers to increase efficiency *in vivo*	increased expression, reduced neutralization	i.v. injection (mice)	92
	PEG	*lacZ*	rAAV-2 PEGylation	protection against antibody neutralization	protection against neutralization, drop of infectivity with increase in PEG concentration	HEK293T cells	90
	CCPEG, SSPEG, TMPEG	*lacZ*	rAAV-2 PEGylation	protection against antibody neutralization	best protection against neutralization with higher transgene expression via TMPEG	HeLa cells i.m. and i.v. injection (mice)	91

Abbreviations: GDF-5, growth and differentiation factor 5; *luc*, luciferase; mSeAP, murine secreted alkaline phosphatase; *lacZ*, E. coli β-galactosidase; PEG, polyethylene glycol; CCPEG, PEG activated by cyanuric chloride; SSPEG, PEG activated by succinimidyl succinate; TMPEG, PEG activated by tresyl chloride; i.v., intravenous; i.m., intramuscular.

Current therapeutic approaches to treat these pathologies may not insure the total recover of muscle functionality, often resulting in the formation of dense scar tissue. In this sense, the challenge for muscle repair is to stimulate tissue healing while preventing the fibrosis.

rAAV have been described as potential transfer tools for gene transfer to muscles (Table 3), already involved in several clinical trials for the treatment of different pathologies related with this tissue.[85] Remarkably, rAAV-mediated gene transfer in muscles has been clearly identified as a safer and more effective methodology than nonviral vectors.[86,87] Current routes of administration of rAAV for muscle gene transfer include both localized and systemic gene transfer. One of the challenges limiting rAAV delivery in muscular tissue is the existence of neutralizing antibodies against viral capsid proteins, considerably reducing the efficiency of gene transfer upon intravascular and intravenous injection[88] and in some cases via intramuscular administration.[89]

The use of polymers to coat rAAV as a means to afford protection against neutralization without compromising transduction efficiency is an attractive strategy to overcome these inconveniences. Lee et al.[90] tested the conjugation of rAAV-2 with activated polyethylene glycol chains (PEGylation) to protect gene transfer from neutralizing antibodies. Yet, even though evasion from neutralization was achieved, transduction efficiencies were reduced compared with unmodified vectors.[90] Further modification of rAAV-2 using PEG activated by tresyl chloride (TMPEG) allowed to protect AAV against neutralization *in vitro* and *in vivo* when administered intravenously in mice.[91]

Conclusions and perspectives

Adapting tissue engineering platforms to gene transfer approaches mediated by rAAV vectors is an attractive tool to circumvent not only the current limitations from actual therapeutic options but also the natural obstacles from these clinically adapted vectors to achieve an efficient and durable gene expression in the host individual. A variety of systems (hydrogels, solid matrices, microspheres) of both natural and synthetic origin have been exploited to control the delivery of rAAV in a target tissue, showing promising results in different regenerative medicine approaches to treat disorders of the musculoskeletal system. The use of "smart" polymers may also contribute to achieve a productive rAAV-mediated gene transfer by overcoming natural barriers that preclude the effective vector targeting when administered via classical routes. So far, the manipulation of polymeric scaffolds acting as a supportive matrix for rAAV-genetically modified cells is a valuable strategy to increase the healing potential while providing the mechanical strength necessary for the functionality of the tissue. Incorporation of rAAV vectors in autologous materials harvested from the patients may endow them with adapted biological signals to enhance tissue regenerative processes while minimizing the risk of immunological responses and facilitating the integration of the new tissue.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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