Review Article

Advances in gene therapy for cartilage repair

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Abstract: Articular cartilage defects are a common problem in joints. As articular cartilage has a limited intrinsic capability for self-repair, their clinical treatment remains problematic as none of the current therapeutic options can regenerate adult cartilage and prevent the development of osteoarthritis (OA) over the long term. Treatments based on therapeutic gene vectors typically involve approaches based on the intraarticular delivery of gene vectors or of genetically modified cells, sometimes via biocompatible materials. This review highlights the current state of gene therapy strategies for cartilage repair.

Keywords: Cartilage repair; gene therapy; tissue engineering

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Articular cartilage defects: pathophysiology and current clinical options

The clinical treatment of articular cartilage defects remains problematic and their association with osteoarthritis (OA) is long recognized (1). Articular cartilage has a limited intrinsic capability for self-healing and none of the current therapeutic options can securely and durably regenerate the wounded tissue in its original structure and function in sites of injury thus representing a burden that affects millions of individuals worldwide.

Articular cartilage defects disturb the cartilage surface, either as chondral defects restricted to the articular cartilage or as osteochondral defects extending into the subchondral bone (2). They are mainly caused by acute or repetitive trauma, OA, osteochondritis dissecans, and osteonecrosis. As chondral defects do not have a connection to the subchondral bone, access to pluripotent stem cells is reduced, originating mainly from the synovial lining and the Hoffa fat pad, as the chondrocytes adjacent to the defects do not significantly contribute to the repopulation of the defect (3). Osteochondral defects disrupt the integrity of the osteochondral unit (4). They are, in contrast to chondral defects, spontaneously more efficiently repaired because mesenchymal stem cells (MSCs) from the bone marrow compartment (BM-MSCs) initiate a localized chondro-/osteogenic differentiation within the defect.

The unsolved clinical question of cartilage repair is reflected in a multitude of surgical interventions either replacing the damaged (osteo)chondral tissue or aiming to induce repair. Replacement strategies include the transplantation of autologous or allogeneic osteochondral grafts or focal metallic resurfacing implants. Cartilage repair can chiefly be induced by marrow stimulation techniques such as microfracture (indicated for small symptomatic defects) and autologous chondrocyte implantation (ACI) (indicated for larger defects). Marrow stimulation techniques rely on the chondrogenesis within the chondral defect originating from MSCs mobilized by surgically penetrating the subchondral bone plate. ACI is a two-part surgical procedure (5). First, during arthroscopy and following an evaluation of the defect, (osteo)chondral biopsies are removed from which the chondrocytes are

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Table 1 Gene vehicles for human gene therapy

Type	Advantages	Limitations	References
Non-viral vectors	Not toxic; not immunogenic	Low efficiency; non-integrative; short-term expression	(14)
Adenoviral vectors	High efficiency; no need for cell division	Toxic; immunogenic; non-integrative; short-term expression	(15)
HSV vectors	High efficiency; no need for cell division	Toxic; immunogenic; non-integrative; short-term expression	(16)
Retroviral vectors	Integrative; long-term expression	Risk of insertional mutagenesis; low efficiency; need for cell division	(17)
Lentiviral vectors	Integrative; long-term expression; no need for cell division	HIV-associated material; risk of insertional mutagenesis; low efficiency	(17)
rAAV vectors	High efficiency; stable non-integrative; long-term expression; no need for cell division	Pre-existing humoral responses	(18)

HSV, herpes simplex virus; rAAV, recombinant adeno-associated virus.

subsequently isolated and cultivated under laboratory conditions. After some weeks *in vitro*, the chondrocytes are implanted in a second intervention into the defect. While historically the cells were injected in the defect that was sealed with a periosteal flap, current techniques often attach the chondrocytes to a biodegradable solid matrix, a procedure termed matrix-assisted ACI (MACI). Long-term data from a randomized multicenter trial comparing periosteal flap-based ACI with microfracture showed no superiority of either technique for mid-size defects at ~15 years (6). Overall, such findings show the clear need for novel, more effective approaches for improved cartilage repair.

Principles of gene therapy

Gene therapy may provide powerful tools to enhance cartilage repair. Gene therapy is the concept of delivering genetic material in target cells using a gene transfer vector as a means to support the expression of therapeutic factors over prolonged periods of time relative to the application of recombinant molecules that have a short half-life (minutes to hours) (7,8). To date, about 2,600 gene therapy trials have been initiated worldwide to treat a variety of human disorders (9). Applications include gene addition, gene correction, gene silencing, and gene editing (10) via direct gene delivery in a recipient (*in vivo* approach) or upon indirect administration of genetically modified cells (*ex vivo* approach) (8,11). Gene transfer vectors include non-viral and viral-based constructs, both showing advantages and limitations for gene therapy (12,13) (*Table 1*).

Non-viral vectors (14) have no size limitations and they are easy to generate, being safe and non-immunogenic and avoiding the risk of replication competence. Nevertheless, such vectors promote low gene transfer efficiencies (20–40%) only for short periods of time (some days to week), making them better suited for *ex vivo* approaches. Various types of viral vectors are available for gene therapy, including systems based on adenoviruses, herpes simplex virus (HSV), retro-/lentiviruses, and adeno-associated virus (AAV). Adenoviral and HSV vectors (15,16) can target dividing and non-dividing cells at high efficiencies (~100%), allowing for *in vivo* approaches, but mediating short-term transgene expression (some days to 1–2 weeks) as a result of their maintenance as unstable episomes, and can raise deleterious immune responses. Retroviral vectors (17) stably integrate in the host genome, thus promoting persistent transgene expression (months to years). Still, they are not adapted for direct approaches as they are much less efficient than other vectors (<20% efficiencies) while requiring cell division for integration and potentially initiating insertional mutagenesis and oncogene activation. Lentiviral vectors (17) can integrate in the genome of both dividing and non-dividing cells but they have the potential to carry material derived from the human immunodeficiency virus (HIV). Replication-defective recombinant adeno-associated virus (rAAV) vectors (18) can target dividing and non-dividing cells at high efficiencies (~100%) over prolonged periods of time (months to years) due to their maintenance as mostly stable episomes, thus enabling *in vivo* approaches.

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Table 2 Gene therapy for cartilage repair

Approaches	Vectors	Targets	Genes	Models	References
Direct	Adenoviral vectors	–	IGF-I/IL-1Ra	CD (horse)	(24)
			BMP-2, -6	OCD (pony)	(25)
	rAAV vectors	–	FGF-2	OCD (rabbit)	(26)
				CD (rabbit)	(27)
			IGF-I	CD (rabbit)	(28)
			TGF-β	OCD (minipig)	(29)
			SOX9	OCD (rabbit)	(30)
Indirect	Adenoviral vectors	BMA	BMP-2, Ihh	OCD (rabbit)	(31)
			TGF-β	CD (sheep)	(32)
		Fat/muscle grafts	BMP-2	OCD (rabbit)	(33)
	Lentiviral vectors	BM-MSCs	ZNF145	OCD (rabbit)	(34)

rAAV, recombinant adeno-associated virus; BMA, bone marrow aspirate; BM-MSCs, bone marrow mesenchymal stem cells; IGF-I, insulin-like growth factor I; IL-1Ra, interleukin-1 receptor antagonist; BMP, bone morphogenetic protein; FGF-2, basic fibroblast growth factor; TGF-β, transforming growth factor beta; SOX9, sex-determining region Y-type high mobility group box 9; Ihh, Indian hedgehog; ZNF145, zinc-finger 145 protein; CD, chondral defect; OCD, osteochondral defect.

While a low risk for insertional mutagenesis has been evoked with rAAV (19), no causative effects could be reported regarding the occurrence of pathological events in the human population (20). However, the presence of neutralizing antibodies against the AAV capsid has been evidenced in individuals, including in the joints (21,22), a potential issue for effective translational human gene therapy (23).

Classical gene therapy for cartilage repair

Gene therapy to treat focal cartilage defects has been reported in experimental animal models *in vivo* using direct and indirect gene transfer approaches (*Table 2*). Enhanced repair has been reported by direct administration of adenoviral (24,25) and rAAV vectors (26-30) using the basic fibroblast growth factor (FGF-2) (26,27), insulin-like growth factor I (IGF-I) alone (28) or combined with an interleukin-1 receptor antagonist (IL-1Ra) (24), transforming growth factor beta (TGF-β) (29), bone morphogenetic proteins (BMP-2, -6) (25), and the sex-determining region Y-type high mobility group box 9 (SOX9) transcription factor (30) in chondral (24,27) and osteochondral defects (25,26,28-30) in rabbits (26-28,30), minipigs (29), and horses/ponies (24,25) for periods ranging from 3 to 52 weeks. Improved repair was also evidenced upon implantation of genetically modified BM-MSCs (34), bone marrow aspirates (31,32), and fat or muscle tissue grafts (33) using adenoviral (31-33) and lentiviral vectors (34) coding for TGF-β (32), BMP-2 (31,33), the zinc-finger protein 145 (ZNF145) transcription factor (34), and the Indian hedgehog (Ihh) signalling molecule (31) in chondral (32) and osteochondral defects (31,33,34) in rats (34), rabbits (31,33), and sheep (32) for up to 24 weeks.

Despite promising results, many obstacles remain for the effective gene-based therapy of cartilage defects, especially in the view of clinical translation in patients. They include a number of barriers that may impair gene transfer and expression *in vivo* like the joint environment (synovial fluid, dense extracellular matrices, dissemination) and the presence of agents that may interfere with gene vector adsorption on a cell targets (clinically used heparin, neutralizing antibodies against viral capsid or envelope proteins, helper $CD4^+$ and cytotoxic $CD8^+$ T cell responses against transgene-expressing modified cells) (11,21-23,35-37). While diverse approaches have been developed to tackle these issues (alternative routes of vector administration, use of permissive clinical compounds such as hirudin, administration of transient immunosuppressors, plasmapheresis, use of vector decoys, vector engineering) (38-42), none have been satisfactorily capable of addressing the problem of

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Table 3 Delivery of genetically modified cells seeded on biomaterials for cartilage repair

Vectors	Targets	Genes	Biomaterials	Models	References
Non-viral vectors	Articular chondrocytes	FGF-2, IGF-I, BMP-7	alginate, collagen	OCD (rabbit)	(52-54)
	BM-MSCs	CDMP-1	Collagen		(55)
	Perichondrial stem cells	TGF-β	PLA		(56)
Adenoviral vectors	BM-MSCs	TGF-β, CTGF, SOX9	PGA, CS/PVA, PLGA	OCD (rabbit)	(57-59)
	Periosteal stem cells	BMP-2	PGA	CD (pig)	(60)
Retroviral vectors	Periosteal stem cells	BMP-7, Shh	PGA	OCD (rabbit)	(61)
rAAV vectors	Articular chondrocytes	FGF-2	collagen	OCD (rabbit)	(62)

rAAV, recombinant adeno-associated virus; BM-MSCs, bone marrow mesenchymal stem cells; FGF-2, basic fibroblast growth factor; IGF-I, insulin-like growth factor I; BMP, bone morphogenetic protein; CDMP-1, cartilage-derived morphogenetic protein 1; TGF-β, transforming growth factor beta; CTGF, connective tissue growth factor; SOX9, sex-determining region Y-type high mobility group box 9; Shh, sonic hedgehog; PLA, polylactic acid; PGA, polyglycolic acid; CS, chitosan; PVA, poly(vinyl alcohol); PLGA, poly(lactic-co-glycolic) acid; OCD, osteochondral defect; CD, chondral defect.

adapted gene therapy for cartilage repair, showing the crucial need for novel, more effective systems.

Scaffold-mediated gene therapy for cartilage repair

The manipulation of biomaterials employed in tissue engineering strategies, especially those already in clinical use (43-45), may provide powerful tools to improve the current gene therapy procedures for cartilage repair. Such scaffold-mediated gene transfer systems would be the basis to generate off-the-shelf products as cartilage supportive matrices and cargos for the effective, spatiotemporal delivery and expression of candidate genes in sites of cartilage injury (44,46). Optimally, the scaffolds would be biodegradable, support cell homeostasis, survival, and/or differentiation, display adapted biomechanical properties, and be capable of integration with the adjacent tissues. Biomaterials used to treat cartilage lesions include solid scaffolds and hydrogels based on natural and synthetic compounds. Solid scaffolds [polylactic acid (PLA), polyglycolic acid (PGA), natural type-I/III collagen membrane] (47,48) may be provided by arthrotomy while hydrogels (alginate, collagen, hyaluronic acid) (49,50) can be prepared as injectable formulations compatible with arthroscopic procedures. Nevertheless, none of these systems provide sufficient biologically active cues to promote adapted cartilage repair, an issue that may be addressed by supplementing their use with gene therapy approaches. On the other side, combining the current gene transfer procedures with the use of a scaffold may overcome the remaining barriers to effective gene therapy (51). Thus far, such a concept has been largely reported based on the delivery of genetically modified cells seeded on biomaterials in sites of cartilage lesions (*Table 3*). Improved cartilage repair has been achieved by administration of cells (chondrocytes, progenitor cells) genetically modified with nonviral (52-56), adenoviral (57-60), retroviral (61), and rAAV vectors (62) and next seeded on scaffolds made of PLA (56), PGA (57,60,61), poly(lactic-co-glycolic) acid (PLGA) (59), chitosan (58), collagen (54,55,62), and alginate (52,53). Gene transfer was performed to overexpress FGF-2 (53,62), IGF-I (52), TGF-β (56,58), BMPs (54,60,61), the cartilage-derived morphogenetic protein 1 (CDMP-1) (55), the connective tissue growth factor (CTGF) (59), SOX9 (57), and the sonic hedgehog (Shh) signalling molecule (61) in osteochondral defects in rabbits (52-59,61,62) and pigs (60), with enhanced repair noted for up to 26 weeks.

Nevertheless, such approaches require the complex and invasive preparation, genetic modification, and subsequently seeding of cells on a biomaterial, while direct coating or encapsulation of gene transfer vectors in a scaffold may provide less demanding procedures that may be well suited to promote cartilage repair (11,63-66) (*Figure 1*). Controlled release of gene transfer vectors from biomaterials in strategies that aim at enhancing the processes of cartilage repair have been reported using solid, hydrogel, and hybrid compounds (*Table 4*). The Guilak's group for instance has pioneered the use of scaffold-guided delivery of lentiviral

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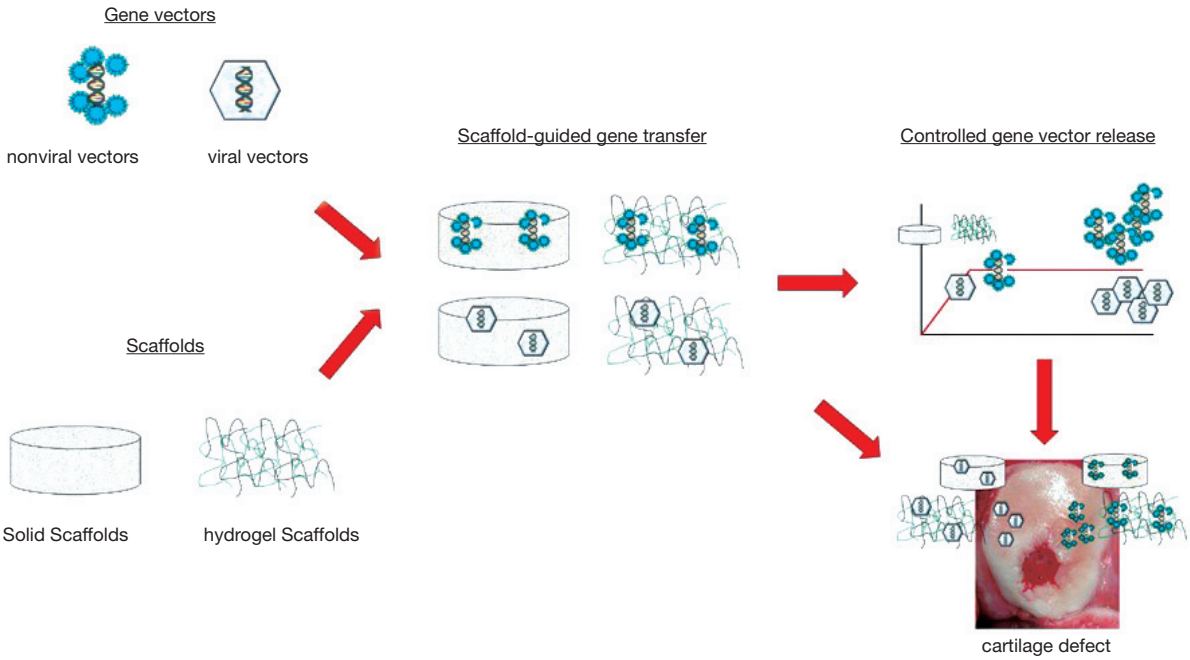

Figure 1 Scaffold-mediated gene therapy for cartilage repair. Gene vectors may be coated onto or encapsulated within solid or hydrogel scaffolds to allow for scaffold-guided gene transfer and controlled gene vector release for an enhanced spatiotemporal effect of the therapeutic gene product being delivered while supporting cartilage repair and overcoming the physiological barriers to effective cartilage gene therapy in lesions (here: full-thickness chondral defect of the patella in a 36-year old woman, treated with PRIDIE drilling during an INSALL proximal realignment of the patella to treat the underlying patellar subluxation).

Table 4 Scaffold-mediated gene therapy for cartilage repair

Vectors	Genes	Biomaterials	Models	References
Non-viral vectors	TGF-β	fibrin/PLGA/PEO/PLL	OCD (rabbit)	(67-69)
Lentiviral vectors	TGF-β, IL-1Ra	PCL	MSC chondrogenesis, cartilage formation, protection against inflammation, protection against cartilage degradation	(70-72)
rAAV vectors	TGF-β	Fibrin	MSC chondrogenesis	(73)
	lacZ, RFP	Alginate, self-assembling peptides, poloxamers and poloxamines	MSC and OA phenotype maintenance	(74-80)
	TGF-β, SOX9	Poloxamers and poloxamines	Experimental human OCD remodelling	(79,80)

rAAV, recombinant adeno-associated virus; TGF-β, transforming growth factor beta; IL-1Ra, interleukin-1 receptor antagonist; lacZ, E. coliβ-galactosidase; RFP, red fluorescent protein; SOX9, sex-determining region Y-type high mobility group box 9; PLGA, poly(lactic-co-glycolic) acid; PEO, poly(ethylene oxide); PLL, poly (L-lysine); PCL; poly(ε-caprolactone); OCD, osteochondral defect; MSC, mesenchymal stem cells.

vectors to target MSCs via three-dimensional (3D) woven poly(ε-caprolactone) (PCL) scaffolds as a means to enhance the commitment of the cells towards chondrogenesis and support the formation of mechanically functional cartilage (TGF-β gene transfer) (70) and to protect them from inflammation and degradation in conditions of joint resurfacing (IL-1Ra gene transfer) (71,72). Hydrogels and micellar systems based on fibrin (73), alginate (74), self-assembling peptides (75), and poloxamers or poloxamines (74,76-80) have been also manipulated to deliver rAAV

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vectors. These systems were developed to target MSCs (79,80) and enhance their chondrogenic potential (TGF-β gene transfer) (73) and to modify (77,79,80) and remodel (TGF-β and SOX9 gene transfer) (79,80) experimental human osteochondral defects in their natural 3D environment. Hybrid scaffolds were also created to transfer non-viral vectors using systems based on fibrin, PLGA, and poly(ethylene oxide)-*b*-poly (L-lysine) (PEO-*b*-PLL) to deliver TGF-β in rabbit osteochondral defects (67-69) as a means to increase cartilage repair and integration with the surrounding knee joint cartilage.

Conclusions

Controlled release of gene therapy vectors from biomaterials is a relatively novel field of research that may provide powerful, non-invasive tools to enhance the processes of cartilage repair in sites of injury. Such scaffold-guided gene transfer systems may also allow to overcome the existing physiological barriers to effective cell and tissue modification *in vivo* (joint environment such as the synovial fluid and extracellular matrices, immune responses, etc.). Even though relatively few data are available in clinically relevant models of cartilage defects *in vivo*, the growing body of evidence showing the benefits of such approaches *in vitro* and *in situ* suggests that controlled scaffold-mediated gene transfer will open new avenues of research to treat articular cartilage lesions in patients in the future, especially when combining this concept with classical clinical procedures like marrow stimulation techniques.

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Footnote

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