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REVIEW ARTICLE

Current Trends in Viral Gene Therapy for Human Orthopaedic Regenerative Medicine

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Abstract

BACKGROUND: Viral vector-based therapeutic gene therapy is a potent strategy to enhance the intrinsic reparative abilities of human orthopaedic tissues. However, clinical application of viral gene transfer remains hindered by detrimental responses in the host against such vectors (immunogenic responses, vector dissemination to nontarget locations). Combining viral gene therapy techniques with tissue engineering procedures may offer strong tools to improve the current systems for applications *in vivo*.

METHODS: The goal of this work is to provide an overview of the most recent systems exploiting biomaterial technologies and therapeutic viral gene transfer in human orthopaedic regenerative medicine.

RESULTS: Integration of tissue engineering platforms with viral gene vectors is an active area of research in orthopaedics as a means to overcome the obstacles precluding effective viral gene therapy.

CONCLUSIONS: In light of promising preclinical data that may rapidly expand in a close future, biomaterial-guided viral gene therapy has a strong potential for translation in the field of human orthopaedic regenerative medicine.

Keywords Viral vectors · Biomaterials · Orthopaedic regenerative medicine · Gene therapy

1 Introduction

Regeneration of injured orthopaedic tissues (articular cartilage, bone, meniscus, tendons, ligaments) remains problematic in light of their insufficient or deficient capacity to regenerate at both structural and biomechanical levels.

In absence of vascularization, the articular cartilage that allows for load transmission and mobility of the joints has a poor ability for self-repair and lesions resulting from trauma or generalized osteoarthritis (OA) [1–3] tend to further deteriorate if left untreated, leading to the production of a poor fibrocartilaginous repair tissue made of type-I collagen instead of a hyaline cartilage naturally composed of type-II collagen and proteoglycans, even following surgical treatment (microfacture, autologous chondrocyte implantation—ACI, administration of mesenchymal stem cells—MSCs, replacement surgery) [4, 5].

In contrast, the hierarchical, vascularized bone with type-I collagen fibers and nanohydroxyapatite matrix for skeletal support and mobility has an intrinsic ability to heal. Still, such potential might be constrained when the lesions (or fractures) are too large to fully regenerate [6, 7], even following autografting procedures that are hampered by limited graft availability, donor site morbidity, and graft integration [8].

Injuries to the meniscus, a tissue with loadbearing and stabilization functions that is essentially made of type-I

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collagen and vascularized only in its periphery (10–30% of the structure), poorly heal in the prevalent, central (avascular) region like after trauma or during OA progression [9]. Approaches to treat meniscal lesions include meniscal reconstruction, replacement (allografts, substitutes), and cell application but they do not always restore the meniscus in its full mechanical integrity [10].

Connective tendons that transmit elastic forces between bone and muscles for locomotion and ligaments between bones for stability, both composed of type-I collagen, may be submitted to injury (tears, ruptures, tendinopathy) that poorly heal, forming a scar tissue of lesser quality or with adhesions [11, 12] and none of the current treatments (suture, grafts, synthetic prostheses) promote a long-term reconstruction of functional tissues.

In this regard, gene therapy [13] may provide powerful, clinically adapted tools to express therapeutic candidate sequences in orthopaedic lesions in a temporarily and spatially defined manner relative to the direct administration of recombinant agents that generally display very short pharmacological half-lives (minutes to hours) [14–20]. While nonviral vectors have been long manipulated for human gene therapy [21, 22], viral vectors became the focus of orthopaedic regenerative medicine due to their natural entry pathway in target cells and to their overall higher gene transfer efficiencies [19, 23].

2 Viral gene transfer for orthopaedic tissue repair: classical approaches

2.1 Viral vectors

A variety of viral vectors have been employed to treat orthopaedic lesions including gene vehicles based on adenoviruses [24], herpes simplex viruses (HSV) [25], retro/lentiviruses [26, 27], and on the adeno-associated virus (AAV) [28–30]. Episomal adenoviral and HSV vectors are highly efficient to modify dividing and nondividing cells ($\sim$ 100% transduction efficiencies) but only over limited periods of time (some days to 1–2 weeks) while provoking detrimental host immune responses [31]. Integrative retro/lentiviral vectors persist in the host genome but transduce cells at lower efficiencies ($<$ 20% unless manipulated by cell selection) and having a risk for insertional mutagenesis and tumorigenesis [32]. In addition, retroviral vectors can target only dividing cells while lentiviral vectors may carry human immunodeficiency virus (HIV)-derived genetic material. Gutless rAAV vectors can optimally and durably target dividing and nondividing cells ($\sim$ 100% transduction efficiencies for months to years) [33] as predominant stable episomes, even in the presence of a dense extracellular matrix due to their small size ($\sim$ 20 nm) [34]. The absence of viral coding sequences in the rAAV genome make these vectors much less immunogenic and toxic than adenoviral and HSV vectors and a possible cause-to-effect of rAAV-associated genotoxicity (insertional mutagenesis) [35] has been excluded to date when using a gutless vector [36].

According to the gene delivery efficiency of a vector, direct *in vivo* vector application (adenoviral, HSV, or rAAV vectors) or indirect ex vivo cell-associated gene transfer (retro/lentiviral vectors) may be favorized for orthopaedic applications in a recipient.

2.2 Classical viral gene therapy for orthopaedic tissue repair: direct gene transfer (Table 1)

Direct gene administration has been documented for the treatment of cartilage defects using adenoviral [37] and rAAV vectors [38–42] to deliver the insulin-like growth factor I (IGF-I) [41], transforming growth factor beta (TGF-β) [42], basic fibroblast growth factor (FGF-2) [38, 39], interleukin 10 (IL-10) with an IL-1 receptor antagonist (IL-1Ra) [37], and the sex-determining region Y-type high mobility group box 9 transcription factor (SOX9) [40], leading for instance to enhanced cartilage repair for 16 weeks in rabbits [38, 40].

Direct gene therapy has been also attempted for bone healing using adenoviral [43–49] and retro/lentiviral vectors [50–52] to deliver the bone morphogenetic proteins (BMP-2, -4) [43, 45–50], vascular endothelial growth factor (VEGF) [44], LIM mineralization protein-1 (LMP-1) [52], and cyclooxygenase-2 (COX-2) [51] in bone defects or fractures, leading for instance to improved bone repair for 16 weeks in rabbits [45].

Direct administration of therapeutic genes via rAAV-mediated gene transfer has also been performed in the meniscus [53, 54] and tendons/ligaments [55–57] to promote the healing of human experimental meniscal lesions in situ upon overexpression of FGF-2 [53] and TGF-β [54] for 15 days [54] and the repair of human experimental anterior cruciate ligament (ACL) lesions in situ using FGF-2 [55] or of injured chicken flexor tendons with FGF-2 [56, 57] or VEGF [57] for 6 weeks [57].

2.3 Classical viral gene therapy for orthopaedic tissue repair: indirect gene transfer (Table 2)

Indirect gene transfer approaches in cartilage lesions include the administration of MSCs (bone marrow, perichondrium/periosteum, muscle, adipose tissue, tendons) [58–65], articular chondrocytes [66–68], bone marrow aspirates [69, 70], or tissue grafts (muscle, fat) [71, 72] modified by adenoviral [58, 60, 66, 67, 69–72], retro/lentiviral [59, 62–65], and rAAV vectors [61, 68] to deliver

Table 1 Direct gene transfer in orthopaedic regenerative medicine

Applications	Vectors	Genes	Targets	References
Cartilage repair	Adenoviral vectors	IL-10 + IL-1Ra	Horse CD (16 weeks)	[37]
	rAAV vectors	FGF-2, SOX9, IGF-I, TGF-β	Rabbit OCD (16 weeks)	[38–42]
Bone healing	Adenoviral vectors	BMP-2, VEGF	Rabbit femoral fracture (16 weeks)	[43–49]
	Retro-/lentiviral vectors	BMP-4, LMP-1, COX-2	Rat femoral fracture (28 days)	[50–52]
Meniscus repair	rAAV vectors	FGF-2, TGF-β	Human meniscal lesion (15 days)	[53, 54]
Tendon/ligament healing	rAAV vectors	FGF-2, VEGF	Human ACL and chicken flexor tendon injuries (6 weeks)	[55–57]

rAAV, recombinant adeno-associated virus vector; IL-10, interleukin 10; ; IL-1Ra, interleukin 1 receptor antagonist; FGF-2, basic fibroblast growth factor; SOX9, sex determining region Y-box 9; IGF-I, insulin-like growth factor I; TGF-β, transforming growth factor beta; BMP, bone morphogenetic protein; VEGF, vascular endothelial growth factor; LMP-1, LIM mineralization protein 1; COX-2, cyclooxygenase 2; CD, chondral defect; OCD, osteochondral defect; ACL, anterior cruciate ligament

Table 2 Indirect gene transfer in orthopaedic regenerative medicine

Applications	Cells, tissues	Vectors	Genes	Targets	References
Cartilage repair	MSCs	Adenoviral vectors	IGF-I, BMP-2	Rat CD (8 weeks)	[58, 60]
		Retro-/lentiviral vectors	BMP-4/sFlt-1, ZNF145, SOX trio	Rat OCD and OA (24 and 16 weeks)	[59, 62–65]
		rAAV vectors	TGF-β	Rat OCD (12 weeks)	[61]
	Articular chondrocytes	Adenoviral vectors	BMP-7, IGF-I	Horse CD (8 months)	[66, 67]
		rAAV vectors	IGF-I	Horse CD (8 weeks)	[68]
	Bone marrow	Adenoviral vectors	TGF-β, IHH, BMP-2	Sheep/rabbit CD (24 and 13 weeks)	[69, 70]
	Tissue grafts (muscle, fat)	Adenoviral vectors	BMP-2	Rabbit OCD (13 weeks)	[71, 72]
Bone healing	MSCs	rAAV vectors	BMP-2, VEGF	Mouse tibial defect (16 weeks)	[74]
		Adenoviral vectors	BMP-2	Rat femoral defect (8 weeks)	[73]
	Tissue grafts (muscle, fat)	Adenoviral vectors	BMP-2, -7	Rat femoral defect (12 weeks)	[71, 77, 79–81]
		rAAV vectors	VEGF, RANKL, caAlk2	Mouse femoral defect (6 weeks)	[75, 76, 78]
Tendon healing	MSCs	Adenoviral vectors	TGF-β	Rabbit Achilles tendon injury (8 weeks)	[82]
	Tissue grafts (tendon, fat)	Adenoviral vectors	BMP-12, IGF-I, TGF-β	Rat Achilles tendon and horse FGST injuries (8 weeks)	[84–86]
		Lentiviral vectors	Scx	Rat patellar tendon injury (2 weeks)	[88]
		rAAV vectors	GDF-5	Mouse FDLT injury (3 weeks)	[83, 87]

MSCs, human mesenchymal stem cells; rAAV, recombinant adeno-associated virus vector; IGF-I, insulin-like growth factor I; BMP, bone morphogenetic protein; sFlt-1, a VEGF antagonist; ZNF145, zinc-finger protein 145; SOX trio, sex determining region Y-boxes 5, 6, and 9; TGF-β, transforming growth factor beta; IHH, indian hedgehog; VEGF, vascular endothelial growth factor; RANKL, receptor activator of nuclear factor κB ligand; caAlk2, constitutively active form of the ALK2 receptor; Scx, scleraxis; GDF-5, growth and differentiation factor 5; CD, chondral defect; OCD, osteochondral defect; OA, osteoarthritis; FGST, flexor digitorum superficialis tendon; FDL: flexor digitorum longus tendon

IGF-I [58, 67, 68], BMPs (BMP-2, -4, -7) [59, 60, 63, 66, 70–72], TGF-β [61, 69], sFlt-1 (a VEGF antagonist) [62, 63], the SOX trio (SOX-5, -6, -9) [65], the zinc-finger protein 145 (ZNF145) [64], and indian hedgehog (IHH) [70], leading for instance to enhanced cartilage repair for 8 months in horses [67, 68].

Indirect gene therapy for bone healing has been achieved by applying MSCs (bone marrow) with or without extra bone matrix [73, 74] and tissue grafts (bone, muscle, fat) [71, 75–81] modified by adenoviral [71, 73, 77, 79–81] and rAAV vectors [74–76, 78] to produce BMPs (BMP-2, -7) [71, 73, 74, 77–81], VEGF [75], the receptor activator of nuclear factor κB ligand (RANKL) [75], and the constitutively active form of the ALK2 receptor (caAlk2) [76], promoting for instance bone healing for 16 weeks in mice [74].

Indirect modification of MSCs (bone marrow) [82] and tissue grafts (tendon, muscle) [83–88] via adenoviral [82, 84–86], lentiviral [88], and rAAV vectors [83, 87] for implantation in experimental lesions has also been tested to enhance tendon healing upon gene transfer of the growth and differentiation factor 5 (GDF-5) [83, 87], IGF-I [85], TGF-β [82, 86], BMPs (BMP-12) [84], and scleraxis (Scx) [88] for 8 weeks in rabbits [82] and horses [85].

2.4 Classical gene transfer: limitations

A large number of issues remain regarding the effective use of classical gene transfer strategies. While direct gene transfer is a simple procedure, it may lead to vector dissemination to non-target organs and clearance from the body [89–92]. On the other side, the manipulation of genetically modified cells in indirect gene transfer protocols may allow to control the gene vector cargo and promote cell repopulation in sited of lesions, although being dependent on invasive methods of extraction [93]. Another critical problem is the pre-existing immunity against viral vectors (neutralizing antibodies and cellular immune responses against the viral capsid proteins) [94–98]. Finally, physiological barriers may further impair gene transfer like the presence of patient-associated factors (presence of inhibiting anticoagulants) [99] or viral vector-specific features (rate-limiting steps of viral genome processing for effective transgene expression) [100, 101].

3 Viral gene therapy and tissue engineering for orthopaedic tissue repair

3.1 Concepts

A novel, highly promising strategy to overcome such hurdles is to combine the use of viral gene transfer with existing tissue engineering approaches based on the manipulation of clinically relevant biocompatible and biodegradable materials (hydrogel, solid, and hybrid scaffolds) that can mimic the natural properties of orthopaedic tissues, integrating well with the surrounding tissue while providing a supportive, scaffolding environment for cell division/differentiation and tissue repair [102–113] (Fig. 1).

3.2 Tissue engineering for the delivery of genetically modified cells and tissues

Strategies based on the seeding genetically modified cells onto scaffolds have been developed for implantation in

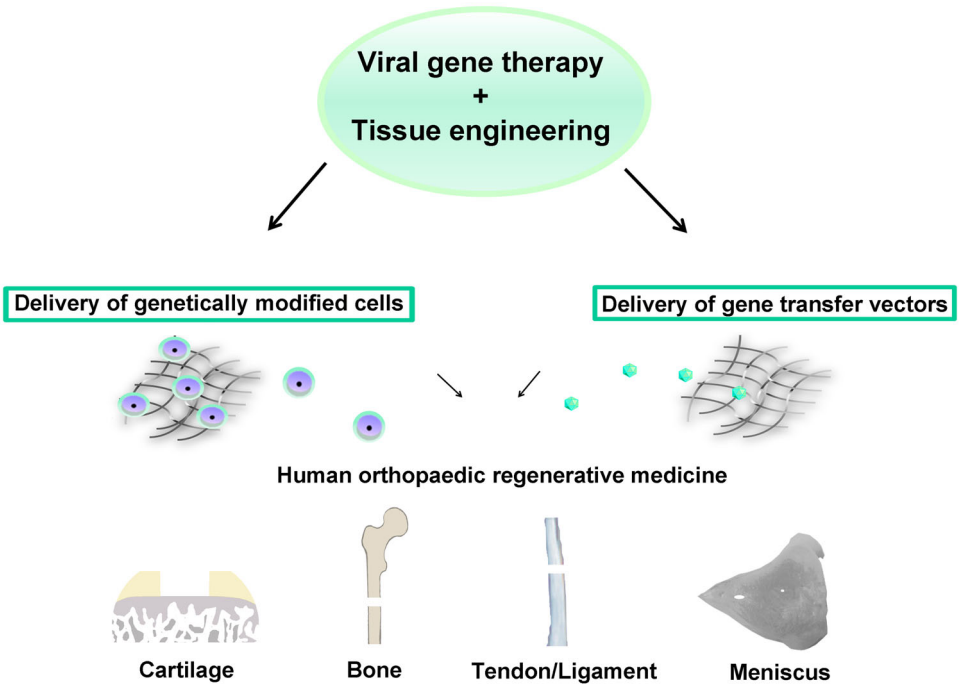

Fig. 1 Current strategies combining viral gene therapy and tissue engineering approaches for human orthopaedic regenerative medicine. Biomaterials may be engineered either to act as scaffolds for genetically modified cells or to behave as a guiding system for optimized viral gene transfer, providing in both cases a supportive environment for improved tissue repair

sites of cartilage injury [114–119], bone defects [120–132], and tendon/ligament lesions [133, 134].

Specifically, MSCs (bone marrow, periosteum, adipose tissue) [114, 115, 117–120, 122–126, 128–133], articular chondrocytes [116], tenocytes [134], muscle cells [120, 121], and fibroblasts (skin) [127] have been first transduced with adenoviral [117, 119, 122, 124, 126–130, 132–134], retro/lentiviral [114, 115, 120, 121, 123–125, 131], and rAAV vectors [116, 118] to overexpress BMPs (BMP-2, -4, -7, -12) [114, 115, 120–124, 127, 130–132, 134], TGF-β [119, 130], FGF-2 [116], VEGF [128], stromal cell-derived factor 1 (SDF-1) [129], connective tissue growth factor (CTGF) [134], SOX9 [117], the runt-related transcription factor 2 (Runx2/Cbfa1) [125, 126], Mohawk homeobox (MKX) transcription factor [133], chondromodulin 1 (Chm-1) [118], and sonic Hedgehog (SHH) [115]. Genetically modified cells were next seeded in scaffolds based on polyglycolic acid (PGA) [114, 115, 117], polycaprolactone (PCL) [125], polylactic acid (PLA)/PCL [126], poly(DL-lactic-co-glycolic acid)/tricalcium phosphate (PLGA/TCP) [128], chitosan/poly(vinyl alcohol) (CS/PVA) [119], and type-I collagen (matrix, sponge, carrier) [116, 118, 120–124, 129–133], or encapsulated in a poly(ethylene glycol) diacrylate (PEGDA) hydrogel [127] or matrigel [134]. The systems were next implanted in sites of tissue injury, leading for instance to cartilage repair in osteochondral defects in rabbits for 26 weeks [115], to bone healing in rats for 1 year [127], and to tendon repair in rats for 8 weeks [134].

3.3 Tissue engineering for the delivery of viral gene vectors

As such approaches remain complex and invasive, strategies based on scaffold-guided viral gene transfer may provide more convenient procedures to offer off-the-shelf therapeutic systems adapted for orthopaedic regenerative medicine, a concept initially employed to deliver nonviral vectors in their targets [19, 135–138]. Immobilization or encapsulation of viral gene vectors in adapted biomaterials may thus allow for a controlled release of the vehicles while protecting them from deleterious host responses [139–147].

Scaffold-guided viral gene transfer for the goal of cartilage repair has been attempted using both hydrogel and micellar systems (alginate, poloxamer PF68 and poloxamine T908 polymeric micelles based on poly(ethylene oxide)—PEO—and poly(propylene oxide)—PPO—triblock copolymers, self-assembling RAD16-I peptide hydrogels, polypseudorotaxane gels) [148–154] and solid scaffolds (PCL) [155–158] carrying lentiviral [155–158]

Table 3 Scaffold-guided approaches for viral gene transfer in orthopaedic regenerative medicine

Applications	Vectors	Scaffolds	Genes	Effects	References
Cartilage repair	rAAV vectors	Self-assembling peptide (RAD16-I, RAD-HA) hydrogels	*lacZ*, RFP	hMSC targeting	[150]
		Poloxamers, poloxamines, micellar systems	*lacZ*, RFP, SOX9	hMSC targeting, chondrogenesis (SOX9), protection from NAbs	[149]
			lacZ	Targeting of cartilage defects in situ, protection from NAbs	[151]
			TGF-β	Remodeling of cartilage defects in situ	[153]
			SOX9		[154]
		Alginate, alginate/poloxamers	*lacZ*	hMSC targeting	[148]
		Polypseudorotaxane gels			[152]
	Lentiviral vectors	PCL	TGF-β	hMSC chondrogenesis	[155]
			IL-1Ra	hMSC chondrogenesis and immunomodulation	[156]
				Joint preservation and resurfacing	[157, 158]
Bone healing	Adenoviral vectors	β-TCP	Runx2	Bone formation (rats)	[159]
	rAAV vectors	β-TCP, hydroxyapatite, Ti	BMP-2		[160]
		PCL			[161]
		PLLA		Bone formation (mice)	[162]

rAAV, recombinant adeno-associated virus vector, RAD16-I, $(RADA)_4$ peptide, HA, hyaluronic acid, PCL, poly-ε-caprolactone, β-TCP, β-tricalcium phosphate, Ti, titanium, PLLA, poly-L-lactide acid, *lacZ*, E. coli β-galactosidase, RFP, red fluorescent protein, SOX9, sex determining region Y-box 9, TGF-β, transforming growth factor beta, IL-1Ra, interleukin 1 receptor antagonist, Runx2, runt-related transcription factor 2, BMP-2, bone morphogenetic protein 2, hMSC, human mesenchymal stem cell, Nabs, neutralizing antibodies

and rAAV vectors [148–154] (Table 3). Such systems were employed to overexpress TGF-β [153, 155], an IL-1Ra [156–158], and SOX9 [149, 154] as a means to safely target hMSCs [148–150, 152] and enhance their potential for chondrogenesis and immunomodulation [155, 156], to remodel experimental models of cartilage defects in situ [151, 153, 154], and to permit biological joint preservation and resurfacing [157, 158].

Similar approaches have been reported for bone healing strategies upon delivery of adenoviral [159] and rAAV vectors [160–162] coated on solid scaffolds (β-TCP, PCL, poly-L-lactide acid—PLLA) [159–162] to overexpress and BMP-2 [160–162] and Runx2/Cbfa1 [159] as a means to promote bone formation and healing in bone defects like for 12 weeks in rats [161] (Table 3).

Thus far, there is no report available showing the benefits of such a strategy for meniscal repair although those were discussed in a recent review of the literature [18]. Regarding the treatment of injured tendons and ligaments, again only a study cited the potential application of scaffold-guided gene transfer for tendon regeneration but using a nonviral vector [137].

4 Conclusions and perspectives

Gene therapy combined with tissue engineering procedures for the controlled delivery of viral gene vectors is a relatively novel but very promising and achievable field of research for the goal of orthopaedic regenerative medicine. Indeed, as stated by Evans et al. [163] "arthritis gene therapy is becoming a reality", a therapeutic concept supported by the growing body of clinical trials in orthopaedics [164, 165] and for the treatment of a wide spectrum of human disorders (monogenic, infectious, cardiovascular, neurological, ocular, and inflammatory diseases, cancer) [166]. With the current use of a variety of biocompatible materials well suited for orthopaedic applications [107], scaffold-guided viral gene transfer may offer off-the-shelf compounds capable of both enhancing the repair of injured orthopaedic tissues while overcoming the remaining barriers to effective viral gene therapy. Interestingly, the first case of gene-activated treatment has been successfully reported by Bozo et al. [167] who employed a collagen-hydroxyapatite sponge coated with a nonviral vector coding for VEGF as a bone substitute to enhance the healing of a mandible bone defect in a patient, overall supporting the concept for translation in orthopaedic patients using viral gene constructs. Nevertheless, additional work is needed in preclinical models to define the optimal viral vector, gene, and scaffold combination *in vivo* prior to receive regulatory approval and testing in patients. Also, the novel three-dimensional bioprinting technology may further provide strong tools for the one-step fabrication of scaffold-vector composites mimicking the structural features of a specific injured tissue [168–170]. Finally, it remains to be seen whether genome editing approaches to directly modulate the host DNA [171] may be also combined with scaffold-guided strategies for orthopaedic applications [172, 173] or whether the use of exosomes may provide additional benefits to gene- and tissue engineering-based concepts [174]. Overall, such approaches have the potential to find their way in the clinics to treat patients with orthopaedic injuries in a close future.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical statement There are no animal experiments carried out for this article.

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