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Review article

Hydrosoluble polymers for muscular gene delivery

Caroline Roques [a,b,c,d], Yves Fromes [c,d], Elias Fattal [a,b,*]

[a] Université Paris-Sud, Châtenay-Malabry, France
[b] CNRS, Châtenay-Malabry, France
[c] Inserm, Institut de Myologie, Paris, France
[d] UPMC Université Paris 06, Paris, France

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ABSTRACT

Striated muscle tissue is an attractive target for gene delivery as it can be easily reached and can express exogenous proteins. However, administration of naked DNA results in low transfection levels, and the design and development of safe and efficient gene delivery systems are thus required.

This review is focusing on the characteristics of the striated muscle tissue with regards to features possibly affecting gene transfer, as well as the different soluble polymers that have been evaluated as gene carriers. The described formulations are ranging from polymers displaying a high density of positive charges to non-ionic molecules. Nevertheless, polymers exhibiting few or no positive charges appear to our opinion as the most promising approach to achieve both safe and efficient transfection of the striated muscles.

1. Introduction

Using nucleic acids as a drug is an interesting but still challenging therapeutic approach. Naked plasmid DNA is a large, quickly degraded and negatively charged macromolecule, thus encountering numerous hurdles to enter most cell types and traffic to their nucleus. However, striated muscle cells have demonstrated their capacity to express exogenous proteins to some extent, after direct administration of naked DNA [1]. Additionally, striated muscles correspond to the most abundant tissue of our organism (about 40% of the total body weight), and most skeletal muscles can be readily reached with minimally invasive administration procedures. Healthy, as well as pathological striated muscles might therefore serve as an interesting target for the expression of both constitutive and secreted proteins. After direct intramuscular injection, transfection efficiency of naked DNA remains yet relatively low and areas expressing the transgene are restricted to regions around the needle path. Hence, design and development of gene carriers meeting safety concerns while providing increased efficiency are required to shift from the gene-transfer technique to appropriate treatments. This review will focus on the use of polymers soluble in aqueous, physiological media as potential transfecting agents for both skeletal and cardiac muscles.

2. Anatomical and histological features of striated muscles

The term striated muscle is actually used to describe muscle tissues displaying a wide variety of structures, shapes, sizes and localizations. The two main subtypes of striated muscles are skeletal and cardiac muscles. Here, we will briefly discuss the major differences and similarities between these two subsets of muscles with regards to parameters possibly affecting the gene transfer.

2.1. Skeletal muscles

Skeletal muscle system has a complex organization in terms of shape and distribution of the various muscles in the organism as well as in an individual muscle. Muscle fiber is the structural and functional unit of the skeletal muscles. Myofibers are multinucleated cells resulting from the fusion of mononucleated myocytes [2]. During maturation of the myofibers, nuclei move from their central location to the periphery of the muscle fibers (subsarcolemmal position) [2]. Each fiber is delimitated by a plasma membrane called sarcolemma, which regularly invaginates to form T tubules deeply penetrating the myofibers [2]. In skeletal muscles, myofibers are usually arranged in fascicles of parallel fibers, although various muscle architectures have been described [3].

Myofibers are surrounded by three different levels of connective tissue giving a supracellular organization to the whole muscle [4]. Each fiber is surrounded by an endomysium sheath. Adult muscle fibers are organized as clusters or muscle bundles bordered by a perimysium layer. Finally, a continuous sheath of collagenous epimysium surrounds each entire muscle. The extracellular matrix

* Corresponding author. UMR CNRS 8612, Centre d'Etudes Pharmaceutiques, Université Paris-Sud 11, 5, rue Jean-Baptiste Clément, F-92290 Châtenay-Malabry, France. Tel.: +33 1 46 83 55 68; fax: +33 1 46 61 93 34.
E-mail address: elias.fattal@u-psud.fr (E. Fattal).

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of muscle fibers mainly consists in collagens, glycoproteins (as laminin or fibronectin) and glycosaminoglycans (such as hyaluronic acid, chondroitin sulfate and heparan sulfate) [4].

Besides these adult myofibers, a population of mononucleated stem cells, referred to as satellite cells, accounts for the major source of cells for muscle regeneration [5]. Terminally differentiated skeletal muscles retain capacity to renew and regenerate over time and should be considered as a dynamic tissue, even though physiological regeneration remains a rather slow process. Satellite cells are located at the surface of muscle fibers, and both cell types are tightly bound together via the extracellular matrix [5].

Skeletal muscle can be affected by various alterations (see Fig. 1). Impairments of the muscle tissue may consist in myofibers necrosis or degeneration with eventually a certain degree of regeneration. This regenerative process can lead to restoration of the myofibers or to their atrophy [6]. Necrotic processes might lead to the replacement of myofibers by non-contractile fibrous scar tissue or fat. Muscle tissue can also be the site of inflammation and edema leading to the destruction of the contractile units. In muscular dystrophies, one can frequently observe increased variability of the diameter of muscle fibers [6]. During regeneration of the myofibers, nuclei display a central location in the muscle fibers [7].

These various patho-physiological processes might be observed in a broad spectrum of acquired or inherited pathologies known as myopathies. In muscular dystrophies, impairments of the striated muscle are generally heterogeneous [8], and this pattern must be taken into account when designing therapies. Moreover, evolution of the pathology is not synchronized in every muscle, even for genetically determined myopathies.

2.2. Cardiac muscle

Though heart is formed by striated muscle cells, its anatomical and histological structures are distinct from the skeletal muscles. Cardiac muscle forms four cavities: two thin-walled atria and two thick-walled ventricles. About 30% of the cardiac cells consist in myocytes although they represent about 70% of the heart volume [9,10]. Contrarily to the skeletal myofibers, myocardial cells are usually mononucleated cells arranged as a branched network [9], with a centrally positioned nucleus. Adjacent myocytes are connected by intercalated discs that function as cell–cell junctions. So, cardiac muscle can be considered as a functional syncytium. These intercalated discs are in particular including gap junctions allowing the diffusion of small molecules between two contiguous cells. Most of the volume of the cardiomyocytes is formed by mitochondria and contractile proteins [9]. Thus, diffusion of large molecules might be difficult.

Myocardial cells display a specific arrangement: in the ventricles, myocytes are organized as overlapping layers of muscle bundles following spiral paths [9]. At the epicardial side of the left ventricle, e.g. the outer surface of the heart, orientation of the myocardial cells is preferentially perpendicular to the great axis. At the inner endocardial layer, a more circumferential orientation is observed. Ventricles thus function as a succession of concentric shells [9].

At the tissue level, myocardial cells are embedded within a complex extracellular matrix (ECM) mainly composed of collagen, elastin, glycoproteins and glycosaminoglycans. In addition to ECM, connective tissue contains also non-muscle cells, predominantly fibroblasts [11].

As for skeletal muscles, connective tissue is classified as the endomysium, displaying a pericellular location, the perimysium, surrounding a bundle of myocytes and the epimysium, surrounding the whole muscle [12].

Pathological processes affecting the heart might be acute or progressive and of acquired or inherited origin. Unlike skeletal myofibers, adult cardiac myocytes are terminally differentiated cells, which practically lack the capacity to re-enter the cell cycle [13]. Loss of cardiomyocytes being irreversible, many injuries of the heart lead to a decrease in its contractile capacity referred to

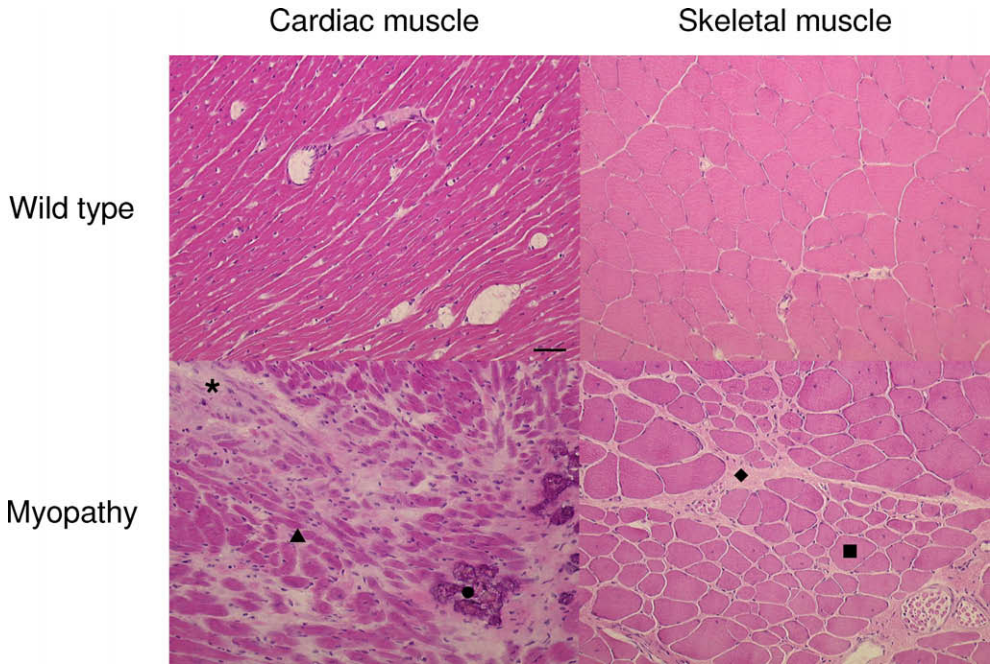

Fig. 1. Typical alterations of the cardiac and skeletal muscles in myopathies. With reference to normal muscle, dystrophic cardiac muscle displays some characteristic features like cardiomyocytes loss replaced by fibrous scar tissue (∗). At the tissue level, some disarray (▲) can be observed, leading to contractile dysfunction. Less frequently, abnormal calcifications can be observed (●). At the level of skeletal striated muscles, similar characteristics can be observed with some specific displays. Interstitial fibrosis (♦) is found in dystrophic skeletal muscles. The process of degeneration and regeneration leads to the heterogeneity of the diameter of the muscle fibers (■). Furthermore, dystrophic muscle fibers usually exhibit nuclei in a central position, while normal matured myofibers have peripheral localized nuclei. Magnification 20×; scale bar, 50 μm.

as heart failure. This syndrome remains a life-threatening condition exhibiting bad prognosis: 5 years survival is less than 50% for patients suffering from symptomatic heart failure [14]. During chronic heart failure, progressive changes in ventricular size and shape occur [14]. Moreover, proliferation of connective tissue can be responsible for conduction defects as well as for impairments of myocardial contractile function [14,15].

3. Therapeutic approaches in myopathies and cardiomyopathies

3.1. Therapeutic targets

Striated muscles can efficiently express proteins, which are usually not synthesized by this tissue, this capacity being first observed with reporter genes (see Fig. 2). Striated muscles thus can be used as a "protein factory" for various therapeutic purposes [16]. Skeletal muscles are highly perfused tissues and consequently can efficiently release secreted proteins to the systemic circulation. Normal skeletal muscles have been proposed to secrete a great diversity of therapeutic proteins, including insulin, clotting factors, hematopoietic factors, angiogenic proteins or antiangiogenic factors [17–21]. Gene delivery to the muscle tissue is also raising interest in the field of vaccination to produce various competent antigens [22–26].

Limb muscles are the most widely used sites to transfer and express exogenous proteins due to their accessibility. Conversely, less accessible muscles, e.g. diaphragm or heart may have to be targeted in a pathological context. Gene delivery may also constitute an interesting approach to treat diseased striated muscles. No curative treatments are currently available for most inherited myopathies. For recessive monogenic disorders, the introduction of a normal copy of the defective gene might revert the phenotype. Notably, administration of plasmid DNA coding for the dystrophin protein has been extensively studied in animal models of Duchenne or Becker muscular dystrophy and even evaluated in Phase 1 clinical trial [27,28]. Evaluations performed on biopsies of the patients included in the dystrophin clinical trial showed no adverse events after administration of naked DNA and some expression of the transgene. However, low levels of expression of the dystrophin were recorded.

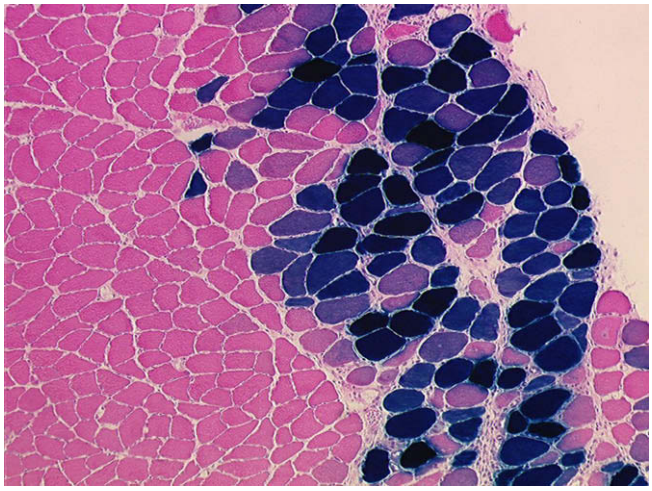

Fig. 2. Striated muscles expressing exogenous gene products. Administration in the quadriceps of a wild-type Syrian hamster of a plasmid (20 μg) coding for the bacterial β-galactosidase associated with tetronic 304 at 5% (w/v) leads to the efficient transcription and translation of the transgene into a functional protein. The enzyme produced can thus efficiently convert one of its substrates into a blue-colored product, thus allowing easy detection of the myofibers that have been efficiently transfected. One can observe that the amounts of protein greatly vary from one transfected fiber to another.

Plasmid coding for angiogenic factors such as vascular endothelial growth factor (VEGF) or urokinase plasminogen activator (uPA) can be of interest in an ischemic context, for both skeletal and cardiac muscles [29]. When considering the cardiac muscle, gene therapy may also provide interesting outcomes for new therapeutic strategies, such as creating a biological pacemaker [30,31] or preserving the contractile properties of the cardiac muscle. As fibrosis may hamper electrical conduction as well as contractile function of the cardiac muscle, inhibition of collagen synthesis or enhancement of collagen degradation has been proposed [32]. However, at late stages of heart failure or after myocardial infarction, reducing collagen content without replacing it by functional muscle tissue may induce severe and even lethal side effects [33].

A more physiological approach might be to delay the progression of the cardiac impairments. Evolution of dilated cardiomyopathy was thus postponed through administration of insulin-like growth factor-1 (IGF-1) [34]. Intracardiac administration of a therapeutic gene coding for IGF-1 demonstrated significant structural and functional benefits in a hamster model of limb-girdle muscular dystrophy [34]. Moreover, short-term treatment with recombinant IGF-1 allowed a significant increase in the lifespan of the diseased hamsters [35].

3.2. Gene delivery to the striated muscles

In 1990, Wolff and coworkers established that naked plasmids coding for reporter genes could efficiently transfect skeletal muscles after direct intramuscular injection [1]. Afterwards, similar experiments were successfully performed in the myocardium and with therapeutic proteins in skeletal muscles [36,37]. Moreover, expression of the transgene could be detected over several months [38]. In mice, about 80% of the muscle fibers were in contact with the injected plasmids, but DNA fails in crossing the epimysium sheath [39]. Transmission electron microscopy studies provide indications that DNA might enter the myofibers through T tubules and caveolae [39].

However, efficient transgene expression was mainly restricted to the area surrounding the injection site [40] and transfection of few myofibers required administration of large amounts of DNA [36]. Moreover, transfection was shown to decrease when increasing the size of the animal [41], possibly because of the thicker perimysium in non-human primates compared to rodents. Striated muscles are highly dense tissues, and their extracellular matrix may represent a physical barrier to an efficient gene delivery to the mature myofibers. This was demonstrated with various viral vectors *in vitro* on fully differentiated myofibers [42,43] and *in vivo* after transfection of the myocardium [44].

To increase the area transfected, multiple sites of injection could be performed, though practical realization would be impossible for the whole body. As striated muscles are highly vascularized tissues, increased diffusion of the plasmid might be obtained from intravascular injections. However, intravenous administration of plasmid DNA does not achieve significant levels of transfection of the skeletal muscles. Hence, hydrodynamic delivery methods have been recently developed and demonstrated efficient gene delivery in the skeletal muscles [45,46]. This procedure consists in the rapid injection of large volumes of an aqueous solution of nucleic acids inducing an increase of the hydrodynamic pressure in the capillaries. Consequently, the permeability of the endothelium is enhanced, thus allowing nucleic acids to reach the target tissue.

When targeting the myocardium, one must take into account the vital function of this organ, and consequently administration should not interfere with it. Due to the high density of capillaries in the cardiac muscle, intravascular delivery appears as an interest-

ing alternative to direct injections [47]. Nevertheless, this method allows only a very short retention time after injection due to high flow. Therefore, some authors have suggested the use of vascular cross-clamping during injections [48,49]. Another interesting approach resides in the use of the pericardial sac as a retention compartment [44]. Additionally, we demonstrated in a previous work that retention of the gene delivery system might be further enhanced by including DNA in a thermosensitive gel [50]. However, this administration route might limit diffusion of the systems to the myocardium due to the thick and collagenous epicardial layer.

The main hurdle to attain clinically relevant treatments consists in designing gene carriers providing safe and efficient plasmid delivery. The last part of this review will focus on the polymeric systems that have been evaluated in the muscle tissue.

4. Soluble polymers for muscular delivery

Most of the non-viral vectors currently developed are based on polycationic molecules, which form interpolyelectrolyte complexes with the polyanionic DNA. Nevertheless, numerous polymers displaying few or no charges appear as particularly interesting to transfect efficiently and safely the striated muscles.

A general description of the several steps encountered from preparation of the gene carriers to expression of the transgene is provided in Fig. 3. Moreover, an overview of the hydrosoluble polymers that have been evaluated as gene carriers in striated muscles is provided in Table 1.

4.1. Cationic polymers

4.1.1. Polyethyleneimine

Since the first publication by Boussif et al. [51], polyethyleneimine (PEI) has remained one of the most widely used cationic vectors to transfer DNA both *in vitro* and *in vivo*. PEI actually corresponds to a class of highly soluble polymers existing either as linear or as branched molecules and exhibiting a large variety of molecular weights (from 0.8 to 800 kDa) and polydispersities. However, the most extensively used PEIs are linear and branched PEI polymers displaying molecular weights (MWs) ranging from 22 to 25 kDa.

Every third atom of PEI is a nitrogen atom and branched PEIs carries primary, secondary and tertiary amino groups (only secondary ones for linear PEI). Moreover, 15–60% of nitrogen atoms of the branched PEI can be protonated under physiological conditions [51–54], while linear PEI displays about 90% protonation at physiological pH [55]. pK_a of PEI molecules varies as a function of the MW, but seems not to depend on the structure of the PEI considered (pK_a ~9 for 2 kDa PEI, 8.5 for branched and linear 25 kDa PEI and 8.3 for 750 kDa PEI) [56].

Due to the presence of numerous positive charges, PEI can efficiently condense DNA through electrostatic interactions with DNA's negatively charged phosphate residues [56]. Complexation between PEI and DNA appears to be essentially entropy driven and does corresponds to the release of counterions. However, other interactions such as hydrogen bonding, van der Waals forces as well as removal of water molecules might also contribute to the polyplexes formation [57]. PEI/DNA complexes are often referred to as polyplexes (complexes between cationic polymers and DNA). Upon complexation with PEI, DNA remains in its B-form, this arrangement being independent of the amount, MW and structure of the PEI used [56].

Complete condensation of DNA through PEI complexation is, dependent on the PEI, usually reached for Nitrogen to Phosphate (N/P) ratios above 2. Complexation leads to the formation of small nanoparticles usually exhibiting sizes ranging from 50 to 100 nm in optimized conditions, i.e. low ionic strength, low DNA concentration and complete condensation of the DNA, as well as a strongly positive zeta potential [53]. Polyplexes display toroidal, globular or rod-like morphology, as assessed by AFM and Trans-

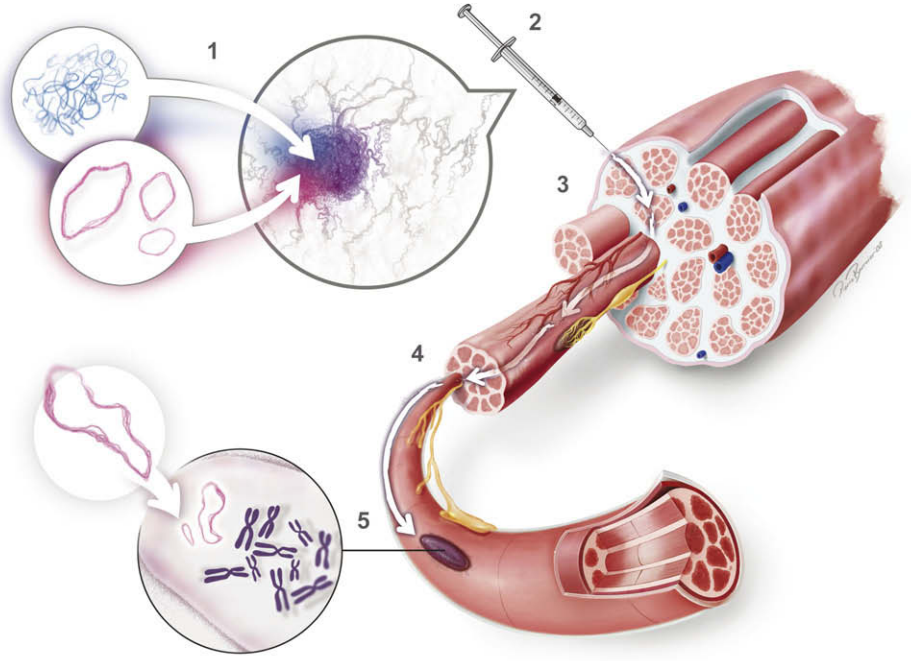

Fig. 3. General representation of gene delivery for intramuscular administration. (1) Formulation of the gene carrier is performed by simple mixing of solutions of the hydrosoluble polymer with the plasmid DNA. (2) Thereafter, administration of the gene delivery system is achieved through direct intramuscular injection. (3) Once administered, the vectors should diffuse within the muscle tissue to allow transfection of a broad area. (4) DNA, with or without the polymer, must cross the sarcolemma and enter the fiber. Cell entry relies strongly on the type of polymer used to perform vectorisation. (5) Finally, plasmid DNA must reach the nucleus to allow transcription of the transgene leading to protein production. When administered through synthetic vectors, plasmid DNA remains in an episomal state, and thus does not integrate into the genome of the transfected cells. Drawing was done by Pierre Bourcier.

Table 1
Overview of the toxicity and efficiency of the different polymers used for muscle transfection (a) charged polymers.

	Polymer	Ad. route	Muscle targeted	Toxicity	Efficiency	Reference
PEI	Branched, 25 kDa	IV	Heart	No increase in expression levels of chemokine receptors No histological evaluation on the myocardium	No detectable levels of the transgene 3 days post-injection No transfection efficiency evaluated	[83]
		IMc	Heart	Not assessed *in vivo*	Decreased, with reference to naked DNA	[85]
		IPc	Heart	Severe lethal effects	Toxicity hampers evaluation of the efficiency	[50]
		IM	Tibialis anterior	Not assessed *in vivo*	Decreased, with reference to naked DNA, independently of the N/P ratio	[86]
		IM	Skeletal muscle	Not assessed *in vivo*	Increased and prolonged expression of the transgene	[89]
		IM	Tongue	Not assessed on muscle tissue	Not assessed on the muscle; efficient transfection of brain stem	[91]
	Branched, 2 kDa	IV	Heart	No increase in expression levels of chemokine receptors No histological evaluation on the myocardium	No detectable levels of the transgene 3 days post-injection No transfection efficiency evaluated	[83]
	Branched, 1.8 kDa	IMc	Heart	Not assessed *in vivo*	Decreased, with reference to naked DNA	[85]
	Linear, 25 kDa	IV	Heart	No increase in expression levels of chemokine receptors No histological evaluation on the myocardium	No detectable levels of the transgene 3 days post-injection No transfection efficiency evaluated	[83]
	Linear, 22 kDa	IM	Hind leg muscle	Damaged myofibers (calcification and atrophy)	Decreased, with reference to naked DNA	[84]
	Linear, MW not specified	IM	Tibialis anterior	Not assessed *in vivo*	Prolonged and 10-fold increased antibody response with reference to naked DNA	[90]
Lipopolymer	PEI 1.8 kDa + Cholesterol	IMc	Heart	Not assessed *in vivo*	Slight increase in transgene expression compared to naked DNA (1.8-fold); prolonged expression Efficient expression of VEGF in ischemic myocardium	[85,104]
Biodegradable cationic polymers	SS-PAED cationic polymers (polyamidoethylenediamine	IMc	Heart	Not assessed *in vivo*	Four to 5-fold increase with reference to naked DNA and 2-fold increase compared to the lipopolymer	[108]
	Dextran-spermine	IM	Left femoral muscle	Not assessed *in vivo*	Slight increase in transgene expression and relatively rapid decrease of transgene expression	[105]
	PPE-EA (polyphosphoester)	IM	Tibialis anterior	Focal inflammation with polymer alone; no evaluation for polymer/DNA complexes	Increased transfection efficiency with reference to naked DNA, but only at low DNA amounts	[106]
Tetronic	304	IMc	Heart	Not assessed	Improved transfection efficiency with reference to naked DNA	[120]
		IM	Tibialis anterior	Not assessed	Up to 30-fold increased efficiency compared to naked DNA in normal muscle; slight increase in dystrophic muscle	[120,121]
PVP	10 kDa	IM	Tibialis anterior	Not assessed *in vivo*	Twofold less efficient than 50 kDa PVP	[123]
	50 kDa	IM	Tibialis anterior	No lesions observed on muscle sections in rats Slight histopathological changes with high amounts of PVP after subcutaneous injections in cynomolgus monkeys	Up to 10-fold increase of gene expression compared to naked DNA Capable to efficiently mediate expression of therapeutic proteins (hIGF-1, hGH, hF. IX, vasostatin, endostatin)	[21,123,124,126–131,133]
PVA	18 kDa	IM	Tibialis anterior	Not assessed *in vivo*	Up to 10-fold increase of gene expression compared to naked DNA; similar transgene expression than with PVP but with a lower dose of DNA	[123,124]
	40 kDa	IM	Tibialis anterior	Not assessed *in vivo*	Levels of transfection similar to naked DNA	[123]
Pluronics	SP1017 (=L61+F127)	IM	Tibialis anterior	No lesions observed on muscle sections presented	Up to 10-fold increase of gene expression with reference to naked DNA; significantly more efficient that 50 kDa PVP; slightly more efficient than 10 kDa PVP Able to efficiently deliver plasmid DNA coding for α-galactosidase A	[144,157,158]
	P85	IM	Tibialis anterior	No lesions observed on muscle sections presented	Up to 20-fold increased efficiency with reference to naked DNA; enhanced gene expression compared to SP1017 carrier	[146]
	L64	IMc	Heart	Not evaluated	Increased transfection efficiency with reference to naked DNA	[145]
		IM	Tibialis anterior	No lesions at the concentrations used to ensure maximal efficiency	Up to 30-fold increased efficiency compared to naked DNA; prolonged expression in rats tolerant to the reporter gene; mediate efficient and long-term expression of inducible EPO	[145,149]

(*continued on next page*)

Table 1 (*continued*)

	Polymer	Ad. route	Muscle targeted	Toxicity	Efficiency	Reference
	Lutrol	IM	Tibialis anterior	Not evaluated	Up to 28-fold increase of transfection efficiency in wild-type mice and 2-fold increase in *mdx* mice with reference to naked DNA; efficient delivery of dystrophin-coding transgene	[121]
Polymers derived from pluronics	PMeOxz	IM	Tibialis anterior	Not evaluated *in vivo*	Up to 18-fold increased efficiency compared to naked DNA; mediate similar transfection efficiency than pluronic L64	[159]
	PEO13–PLGA10–PEO13	IM	Tibialis anterior	Not evaluated *in vivo*	3-fold increase of transfection efficiency with reference to naked DNA at day 5 post-injection; 10 days after administration = no significant difference between naked and formulated DNA	[86]

PEI, polyethyleneimine; IV, intravenous; IM, intramuscular; IMc, intramyocardial; IPc, intrapericardial; MW, molecular weight; N/P, nitrogen to phosphate; PVP, poly vinyl pyrrolidone; PVA, polyvinyl alcohol; IM, intramuscular; IMc, intramyocardial.

mission Electron Microscopy [53,58]. Similar morphologies were obtained with various cationic polymeric carriers when associated to plasmid DNA [53,59].

Small PEI/DNA complexes have a tendency to aggregate notably when increasing ionic strength of the medium, a behavior that is even more pronounced with linear PEI [60–62]. Aggregation can be prevented by dilution of the complexes, decreasing the pH and by increasing the viscosity. Aggregation of the polyplexes is primarily driven by hydrophobic interactions [62].

Due to their overall positive charge, cationic polymer-based carriers interact non-specifically with negatively charged glycoproteins, proteoglycans and sulfated proteoglycans [63–66] located on the plasma membrane. Intracellular entry is then performed through adsorptive [67] or fluid-phase endocytosis [68].

Different pathways have been identified *in vitro* for the cell entry of PEI/DNA complexes [69]. Depending on the cell type, the structure of PEI and the size of the complexes, uptake can be mediated mainly by clathrin- or lipid-raft-dependent routes [70].

After cell entry, polyplexes must escape from the endosomal compartment in order to reach the nucleus and possibly express the transgene. Transfection efficiency of PEI/DNA complexes is in particular due to the so-called proton-sponge effect, that is the capacity of PEI to buffer the acidic pH of the endo-lysosomal compartment through the protonation of amine functions remained uncharged [51,71,72]. This characteristic triggers protection of plasmid DNA against degradation by inhibiting lysosomal nucleases. It also induces swelling and finally rupture of the vesicle mainly through increase in the osmotic pressure within the endo-lysosomes, thus providing efficient release of the DNA into the cytoplasm [73–75]. However, it remains controversial whether branched and linear PEIs exhibit similar buffering capacities [56,76].

Once released in the cytosol, DNA must reach the nucleus through the small pores (~10 nm) of the nuclear envelope [77], which is a major hurdle against its entry due to their small size compared to exogenous DNA. Nuclear entry is supposed to be facilitated by cell division, but many of the *in vivo* applications concern non-dividing cells. Linear PEI-based polyplexes have been demonstrated to be less dependent on the cell cycle than their branched counterpart [78], although no clear mechanism of nuclear import has been identified so far.

Due to these attractive features for the condensation of plasmid DNA in addition to intrinsic endosomolytic capacity, PEIs are extensively investigated for both *in vitro* and *in vivo* applications. Transfection efficiency has been described to depend on the molecular weight and the structure of the PEI (linear vs. branched) as well as on the type of cells transfected [56]. Transgene expression increases with the N/P ratio (in the range of 5–20), independently of the structure and the molecular weight of the PEI considered [79]. Low molecular weight PEI displays increased hydrodynamic diameter, reduced cytotoxic effects and increased transfection ability *in vitro* with reference to 25 kDa branched PEI [80,81], although very low molecular weight PEI has been shown to trigger low transfection efficiency *in vitro* [82]. Furthermore, 25 kDa PEIs (linear or branched) seem to be the most effective *in vivo* compared to their 2 and 750 kDa counterparts [56]. DNA form also remains an important parameter, as circular DNA is more effective for transgene expression with reference to linear DNA [59].

Main applications of PEI-based formulations are directed towards central nervous system, lung and tumors, and are achieved via local or systemic delivery [55,83] with only few papers focusing on the use of such systems to target muscle tissue.

Striated muscles can be reached through several routes of administration and are actually highly perfused tissues, especially the heart. Nevertheless, low levels of plasmid DNA were recorded in the myocardium after intravenous administration of the polyplexes, independently of the molecular weight of the PEI used [84]. Linear PEI mediated higher levels of DNA in the heart 15 min after injection compared to its branched counterpart. However, the transgene was rapidly cleared as no detectable levels were recorded three days after injection, independently of the PEI used [84].

As far as a specific tissue is targeted, local administrations can be performed. Nevertheless PEI/DNA complexes have been demonstrated to be less efficient than naked DNA to transfect cells after direct intramuscular injection in both skeletal [85] and cardiac muscles [86]. Both 25 and 1.8 kDa branched PEI display a similar low transfection efficiency towards the cardiac muscle [86]. Moreover, we demonstrated that intrapericardial administration resulted in severe toxic effects, hampering efficient transgene expression [50].

There are evidences that PEI/DNA complexes are mostly restricted to the extracellular matrix after intramuscular injection, and this may at least partially explain decreased transgene expression [87]. Interactions between cationic delivery systems and glycosaminoglycans (GAGs) have been suggested to hinder efficient transfection [88]. Sulfated GAGs in particular were shown to bind PEI and induce release of the DNA from the polyplexes [88]. Furthermore, both hyaluronic acid and heparan sulfate decreased cellular uptake as well as transgene expression [89]. Turning to linear PEI is not of interest as this carrier displays a significant toxicity and a poor efficacy to deliver DNA *in vivo* after intramuscular injections [85].

Only few approaches have demonstrated some potentialities for polyplexes in the muscle tissue. Intramuscular injections of DNA associated with both branched and linear PEI for vaccination allow obtaining increased and prolonged expression of the antigen [90,91]. For a vaccination purpose, retention of the complexes at the site of injection may not hamper efficient vaccination and PEI might serve as an adjuvant to promote antigenicity of the therapeutic protein. However, potential toxic effects of the polyplexes have not been addressed in these studies. Another application for PEI/DNA complexes consists in targeting brain stem through intramuscular injections in the tongue [92]. Branched PEI/DNA com-

plexes utilized at an N/P ratio of 17:1 were demonstrated to efficiently migrate to the brain via retrograde axonal transport.

The strong capacity of PEIs to condense DNA may also constitute a limitation to these carriers as DNA dissociation from the cationic polymer represents an important issue for efficient gene delivery [93,94]. Transcription activity is thus dramatically decreased at N/P ratios above 1:1 [95]. This result suggests that tightly compacted complexes hamper transcription of the transgene. Moreover, DNA release from linear PEI/DNA complexes can be mediated by soluble proteins present in the cytosol, but this occurs at low N/P ratios (up to 2.5) [96].

One major issue to get PEI-based systems to clinical applications is their potentially severe side effects. Increased cytotoxicity is observed when increasing the molecular weight and/or the branching of PEI [81,97]. After intravenous administration, linear PEI/DNA complexes achieve efficient transfection primarily to the lungs, with other organs exhibiting low transgene expression [98]. Although linear PEI is generally considered as less toxic than its branched counterpart, Chollet and coworkers [99] have demonstrated that efficiency and lethality are closely related when considering linear PEI-based formulations. Efficient transgene expression in the lung was associated to the formation of polyplexes/blood components aggregates. Small thrombi allowed retaining the polyplexes in the lung capillaries and thus promoted transgene expression. But it has also triggered liver necrosis and lung endothelium activation finally leading to the death of numerous treated animals.

At the cellular level, toxic effects induced by PEI rely on two different mechanisms leading to apoptosis. The early stage consists in membrane alterations and is followed by induction of a "mitochondrially mediated apoptotic program" [100]. These cytotoxic effects can be observed with both branched and linear PEI, although experiments were only conducted with high molecular weight linear PEI [100]. Alterations of membrane permeability have also been observed with branched PEI [101].

At nitrogen to phosphate (N/P) ratios usually used for transfection (between 6 and 10), around 80% of PEI remains in its free form [102]. Cytotoxic side effects encountered after polyplexes administration seem to be mediated by free PEI molecules, and thereafter several methods have been tested to remove at least partially these compounds. Ultrafiltration can be used to retain free PEI in the range of 30–60% [61,102]. To achieve complete purification of the polyplexes, size exclusion chromatography (SEC) has also been evaluated [60]. A purification method based on the electrophoresis has been developed as well to remove high rates of unbound PEI molecules independently of the size of the polyplexes [103]. This approach allows removal of around 95% of free PEI, while recovering almost 70% of the PEI/DNA complexes in optimal conditions. However, this method leads to pronounced aggregation of linear PEI carriers, contrarily to the branched PEI-based formulations.

Intravenous administration of the purified polyplexes elicited reduced toxicity, though body weight loss was observed in all animals with lowered activity for few mice [103]. Distribution profiles of complexes purified by SEC or electrophoresis were found to be slightly different [103], thus suggesting modifications of the complexes organization even though no alteration of the mean hydrodynamic diameter or the zeta potential was observed.

Although elimination of free PEI is leading to reduced toxic effects, it also triggers decreased transfection efficiency *in vivo*. Free PEI molecules exert significant effects on the transfection efficiency, most probably during steps following the intracellular entry [60].

4.1.2. PEI derived carriers

Numbers of PEI derivatives have been synthesized and, in particular, several methods have been developed to associate PEI to PEG, primarily to shield the surface charges and thus prevent toxic side effects [55,104].

However, very few PEI derivatives have been investigated to transfect muscle tissue. A water-soluble lipopolymer composed of low molecular weight PEI (1800 Da) conjugated with cholesterol was evaluated. This carrier displayed significantly prolonged and more efficient transgene expression compared to naked DNA and PEI/DNA complexes in myocardial cells [86]. Moreover, cytotoxicity was significantly reduced, and the lipopolymer complexes were demonstrated to efficiently express vascular endothelial growth factor (VEGF) in ischemic myocardium [105]. The carriers were shown to enter myocardial cells via the cholesterol uptake pathway [86].

4.1.3. Biodegradable cationic polymers

Most cationic vehicles appear to be relatively inefficient in promoting transgene expression in the muscle tissue. However, biodegradable cationic carriers are currently thought to be an interesting option for intramuscular delivery of plasmid DNA as they are expected to facilitate polymer/DNA dissociation. Several biodegradable cationic polymers have been developed and tested on either skeletal or cardiac muscles. Dextran-spermine polycations were thus demonstrated to transiently increase reporter gene expression after direct intramuscular injection in the skeletal muscles with maximum efficiency observed for a polymer:DNA weight ratio of 5 [106]. A water-soluble, biodegradable polyphosphoester exhibiting positively charged side chains was also reported to enhance reporter gene expression in Tibialis anterior muscle of mice as well as yielding reduced cytoxicity with reference to branched PEI [107]. *In vivo*, increase in the transfection efficiency was the highest when administering low DNA amounts (2 μg), while benefits of the cationic polymer over naked DNA were progressively lost when raising the dose of plasmids (25 μg).

Few biodegradable cationic polymers have also been evaluated for cardiomyocytes transfection. A cationic poly-β-amino ester (PDMA) displaying a degradable backbone along with cleavable pendant tertiary amine groups has been used to complex DNA [108]. Resulting polyplexes exhibit similar size and morphology than PEI/DNA complexes, but *in vitro* assay performed on PDMA revealed a significantly lower toxicity than branched PEI. Efficiency studies carried out on neonatal mouse cardiac myocytes displayed an increased transfection efficiency as compared to naked DNA. However, no experiments were conducted *in vivo* with these systems to our knowledge.

In vivo delivery to the myocardium was performed with poly(-amido ethyleneamine) carrying reducible disulfide bonds (SS-PAED) [109]. Experiments were carried out on an ischemic rabbit model and yielded 4-fold increased expression of VEGF plasmid driven by a hypoxia inducible promoter compared to the control group. Additionally, SS-PAED carrier was significantly more effective than the above-described lipopolymer.

Polycationic carriers are displaying several interesting features such as condensation and protection of plasmid DNA against degradation as well as attractive endosomolytic activity for PEIs. However, cytotoxicity remains a major barrier to the clinical development of such vectors. So far in striated muscle, PEI-based carriers failed to demonstrate an increased *in vivo* transfection efficiency with reference to naked DNA. Biodegradable cationic polymers might represent an interesting alternative to their non-biodegradable counterparts to reduce toxic side effects as well as enhance transfection efficiency through facilitated release of the DNA from the complexes.

In the past years, several attractive gene carriers displaying few or no positive charges have been developed and evaluated *in vivo* on striated muscles.

4.2. Cationic polymers displaying few charges

4.2.1. Tetronics

Tetronics, also known as poloxamines or synperonics T, are amphiphilic block copolymers displaying a four-branched star structure. Each branch is composed of hydrophilic poly(ethyleneoxyde) (PEO) and more hydrophobic poly(propyleneoxyde) (PPO) blocks fixed on an ethylene diamine central group. Molecular weight and hydrophilic–lipophilic balance (HLB) of these copolymers are depending on the relative amounts and the length of each block. These molecules are often referred to as non-ionic surfactant although they are exhibiting one charge at physiological pH. pK_a of the two tertiary amines was determined to be 7.9–8.0 and 3.8–4.0, respectively [110,111]. In addition, these pK_a values seem not to depend on the length of the PEO–PPO blocks [111]. As tetronic copolymers are displaying amphiphilic properties and protonable amines, the self-association of the unimers (individualized copolymer molecules) to form micelles is strongly depending on the concentration and the composition of the copolymer, but also the temperature, the pH and the ionic strength of the aqueous solution [110,112]. At high temperatures or concentration, these micelles can further rearrange to form lyotropic liquid crystalline phases with increased viscosity [113,114].

Due to these characteristics, tetronic copolymers exhibit various potential uses in the pharmaceutical field, such as matrices for tissue engineering [115], topical and transdermal delivery systems [116,117] or surface modification of nanoparticles [118,119]. First experiments conducted *in vivo* on tetronic copolymers as adjuvants for gene delivery were performed by Prokop et al. [120] on subcutaneous tissue. Tetronic 304, the molecule of the tetronic family displaying the lowest molecular weight, was further demonstrated to increase the gene expression *in vivo* after direct intramuscular injection to both healthy and pathological muscles [121,122]. However, the mechanism by which these carriers are promoting efficient transfection of plasmid DNA *in vivo* is yet to be understood. Tetronic 304 is achieving partial condensation of the DNA in a medium mimicking physiological fluid, resulting in the formation of condensation nodes surrounded by unfolded loops of plasmid DNA (Roques et al., submitted). The morphology of such systems, as observed by electron microscopy, is closely related to non-stoichiometric complexes of DNA with poly-L-lysine [123]. Tetronic/DNA complexes are destabilized at physiological pH, and are easily releasing DNA as demonstrated by gel retardation assay (Roques et al., submitted). Compared to other amphiphilic copolymers, limitations of tetronic-based systems could reside in the high amounts of copolymers requested to ensure maximal efficiency of transfection to the healthy striated muscles *in vivo* (5–10% w/v) and their limited efficiency on pathological muscles [121,122].

4.3. Non-charged polymers

4.3.1. Polyvinyl pyrrolidone and polyvinyl alcohol

Polyvinyl pyrrolidone (PVP) and polyvinyl alcohol (PVA) were the first polymers displaying no condensation of plasmid DNA to be evaluated *in vivo* on striated muscles [124]. Preliminary studies were conducted with PVP and PVA of various molecular weights, 10 and 50 kDa, 18 and 40 kDa, respectively. In addition, various concentrations of both polymers were investigated [124]. PVA 18 kDa at 2% (w/v) and PVP 50 kDa at 5% (w/v) were the most potent to mediate efficient gene transfer after direct intramuscular injection [124]. However, further increase in the concentration of PVP resulted in a reduced transfection efficiency and finally to the inhibition of the transgene expression for PVP concentrations above 20% [124]. At optimal concentrations, gene expression could be enhanced up to 10-fold with reference to naked DNA, with PVA promoting the highest increase in protein expression [124–126]. Moreover, the reporter gene expression increased linearly with DNA amounts injected up to 150 μg when associated to PVP, while saturation was attained at 100 μg with naked DNA [124]. PVP-based formulations also mediated sustained reporter gene expression for at least three weeks [124].

Characteristics of these carriers for gene delivery are completely different from those observed with polycationic vectors. No evidence of condensation of DNA could be observed with these polymers [125]. However, PVP can provide efficient protection against the degradation of DNA by nucleases [124]. At low pH, PVP displays a slightly positive zeta potential thus allowing electrostatic interactions with plasmid DNA [125]. Moreover, PVP and PVA can interact with plasmid DNA through hydrophobic and/or hydrogen bonds [124,125]. PVP is a hydrogen-bond acceptor and PVA a hydrogen-bond donor, while DNA can exert both functions. Molecular modeling studies performed on PVP/DNA systems suggest that PVP provides a more hydrophobic surface on DNA, this result being confirmed by zeta potential analysis of the carrier. Indeed, adding increasing amounts of PVP to plasmid DNA is significantly raising its zeta potential [125]. Due to these characteristics, PVP and PVA formulations are also called protective, interactive, non-condensing (PINC) systems.

Hydrogen-bond formation seems to be a critical parameter for these formulations to promote gene expression after *in vivo* administration [125]. There is a direct correlation between vinyl pyrrolidone monomer content, e.g. the ability of the polymer to form hydrogen bonds with plasmid DNA, and the transfection efficiency [125]. PVP/DNA complexes exhibit a maximum efficiency at pH around 4 and high ionic strength (NaCl 500 mM) [125].

No lesions were observed *in vivo* after direct intramuscular injections of PVP-based formulations in rats [124,125]. After subcutaneous injection into non-human primates, high amounts of PVP led to relatively minor side effects resulting in a slight decrease in globulin and cholesterol concentrations as well as few histological modifications of the liver, lymph nodes and injection sites [127].

Due to its relative innocuity and potential efficiency to deliver genes *in vivo* to skeletal muscles, several evaluations have been performed with therapeutic genes. For most studies, a peak is observed at day 7, and is followed by a decrease in the expression levels of the transgene [21,128,129]. Secretion of human insulin-like growth factor-1 (hIGF-1) or human growth hormone (hGH) could be observed during 21 to 28 days after administration [130,131]. For hIGF-1, the expression of the transgene was localized in the injected muscles, and the secretion was too low to exert any systemic effect. Several other studies allowed detecting systemically secreted proteins [130]. Intramuscular administration of genes coding for antiangiogenic proteins led to systemic expression of these proteins [21,129,132]. Furthermore, administration of PVP/vasostatin coding plasmid resulted in reduced choroidal neovascularization lesions in a rat model, while no adverse events were detected [129]. Endostatin gene formulated with PVP was capable to delay the evolution of metastatic tumors [132] and when associated with the angiostatin coding plasmid in conjunction with removal of the tumor, significantly prolonged survival of the mice was observed [21]. PVP carriers have also been evaluated in association to electroporation to deliver the human factor IX (hFIX) in mice and dogs. Long-term expression was recorded in immunodeficient mice, whereas transient response was observed in dogs due to the development of antibodies against the secreted protein [128].

For a vaccination purpose, PVP by itself can stimulate T cells [133], and PVP-based formulations showed an increased transgene expression as well as antibody responses after direct intramuscular injection in dogs [134].

4.3.2. Pluronics

Pluronics, also known as poloxamers or synperonics, are non-ionic surfactants consisting in linear, synthetic triblock copolymers displaying an A–B–A structure. The A blocks are composed of hydrophilic ethyleneoxyde (EO) units, while the B block corresponds to the more hydrophobic core of propyleneoxyde units (PO), this resulting in amphiphilic properties. Modifying the number of EO and PO units is leading to alterations of the molecular weight as well as the hydrophilic/lipophilic balance of the molecules. These amphiphilic copolymers are usually highly soluble in water and can exist in aqueous solutions as unimers or self-assemble as micelles. Polymer aggregation process is related to an increase in the hydrophobicity of the PO block, hence decreasing its solubility in water [135]. Micelles thus display a hydrophobic PO core surrounded by a hydrophilic corona composed of the EO blocks. Formation of micelles is observed at concentrations of the copolymer above its critical micelle concentration (CMC), this CMC being highly dependent on the temperature and the ionic strength of the medium used [136–141]. These micelles can exhibit various shapes, depending on the concentration of the copolymer, the length of the EO and PO blocks and the temperature [135,140,142,143].

Due to their solubilization properties, their biocompatibility and their ability to form micellar nanocarriers, pluronics are widely used adjuvants in the pharmaceutical industry [144]. More recently, the capacity of such polymers to enhance gene-transfer efficiency *in vivo,* especially in the striated muscles, has drawn growing attention on their potentialities as gene carriers [145–147]. Diverse pluronics exhibiting a wide range of EO/PO ratios and molecular weights have thus been tested *in vitro* [148] and pluronics P85, L64, Lutrol and combination of L61 and F127 (also known as SP1017) have been evaluated *in vivo* [122,145–147].

Although these amphiphilic copolymers generally fail in promoting gene transfer *in vitro*, they have demonstrated their ability to promote expression of reporter and therapeutic genes on both normal and dystrophic muscles after direct intramuscular injections [122,145,146,149,150]. Moreover, SP1017 was demonstrated to be significantly more efficient *in vivo* than polyvinyl pyrrolidone (PVP) to transfect mice tibialis anterior muscles, especially at low DNA amounts, while used at concentrations 500-fold lower than PVP [145]. Typically pluronic block copolymers exhibit maximum efficiency at relatively low concentrations ranging from 0.01% (w/v) to 0.5% (w/v) [145,146] thus providing relatively safe formulations. Toxic side effects have been reported to be correlated to the lipophilicity of the copolymers and to occur at concentrations of pluronics far above those described for applications in gene delivery [151]. In addition, macroscopic lesions were observed on skeletal muscle for concentrations of pluronic L64 above 1% (w/v), these concentrations also correspond to a decrease in the transfection efficiency [146].

While cationic polymers are interacting with and condensing plasmid DNA into relatively small globular or toroidal particles [53], no evidence of interactions could be recorded with most of the pluronic copolymers tested, independently of the method of evaluation (ethidium bromide displacement, gel retardation assay, dynamic light scattering or small-angle neutron scattering) [147,148,152]. Condensation of DNA could only be observed with Lutrol at high copolymer concentrations corresponding to systems exhibiting decreased transfection efficiency [153]. One should also notice that DNA can be condensed into toroids or spheroids in the presence of elevated concentrations of PEO homopolymers and NaCl [154,155]. At the concentrations of pluronics used to efficiently deliver DNA *in vivo*, pluronics fail in protecting plasmids against DNase degradation [147].

The mechanism by which pluronics promote gene expression *in vivo* appears as markedly different from polycationic systems but remains unclear. Pluronic-based systems usually fail in enhancing gene expression *in vitro* on various cell lines [146,156], therefore elucidating their mechanisms of action turns out to be more challenging. Nonetheless it becomes crucial to deepen our understanding of such carriers. Some authors suggested that pluronic copolymers might enhance transfection efficiency through improved diffusion of the DNA in the muscle tissue [145,153], this hypothesis being based on the diffusion of rhodamine-labeled plasmids or the transfected area. Tissue distribution assessed by injection of rhodamine-labeled plasmids is not totally convincing, since the diffusion observed could be greatly modified by slight changes in the angle of injection at the magnification presented. Moreover, area of diffusion of the plasmids is indeed at least equivalent to the area expressing the transgene. However, naked DNA might diffuse into the muscle in a much broader region without being able to efficiently transfect the myofibers. Wolff et al. [39] demonstrated that after direct intramuscular injection of naked DNA in quadriceps muscle of mice, the plasmids were present in the whole muscle and about 80% of the myofibers were in contact with the DNA.

Other possible mechanism is an improved cellular entry due to pluronic interactions with biological membranes [157] and possible transient formation of pores [152]. Moreover, pluronic copolymers may increase nuclear import of DNA from the cytoplasm as suggested by experiments carried out by Pitard and coworkers [146]. Several studies also revealed that pluronic block copolymers could act as biological response modifying agents as they enhance expression of plasmid driven by selected promoters. Pluronics may furthermore activate signaling pathways involving NF-κB [148,158].

Whether pluronics should be co-injected with plasmid DNA or not remains controversial. Experiments performed *in vivo* by several teams [145–147] demonstrated that maximal transgene expression was observed when pluronics L64, SP1017 and P85 were simultaneously injected with the DNA. Conversely, Lavigne et al. [159] observed that SP1017 administered 24 h after plasmid DNA was still capable to enhance therapeutic gene expression, with no statistical difference between delayed and co-injections.

SP1017 and P85 block copolymers are thought to exhibit maximum efficiency for concentrations above their CMC [147]. More extensive studies should be conducted on the organization/micellization of the various pluronic copolymers used in gene delivery protocols and possibly correlated to *in vivo* efficacy of such carriers.

One important feature for pluronic-based formulations relies on the necessary adaptation of the polymer concentration depending on the type of striated muscle transfected (skeletal vs. cardiac) [146]. The differences noticed between skeletal and cardiac muscles could be related to the dissimilarity of structure between those two types of striated muscles. Skeletal myofibers are long, polynucleated cells resulting from the fusion of numerous myocytes, whereas cardiomyocytes consist mainly in mononucleated cells. Sarcolemmal membranes display thus a greater barrier in cardiomyocytes to achieve efficient gene expression.

4.3.3. Polymers derived from pluronic block copolymers

Recently, interest raised by amphiphilic block copolymers for *in vivo* gene delivery has prompted the development of derivatives of these molecules. Two approaches have currently arisen and been tested *in vivo* on striated muscles.

The first option consist in replacing the PEO blocks by more hydrophilic blocks of poly(methyloxazoline) (PMeOxz) [160]. Several block copolymers all displaying a central block composed of 34 propyleneoxyde units were generated. As the majority of pluronics, these compounds seemed not to interact with plasmid DNA, even at high concentration of polymer, and also failed in promoting gene transfer in vitro on HEK293 cells [160]. Moreover, after administration *in vivo* these copolymers were found to increase gene expres-

sion similar to pluronic L64 with reference to naked DNA. These experiments indicate that the chemical composition of the hydrophilic blocks can be altered without modifying the efficiency of such carriers *in vivo*, and thus strongly suggest that the organization and the properties of amphiphilic copolymers are more important than their composition. Further evaluation of the supramolecular assembly of these copolymers, such as establishing the CMC and the CMT, would interestingly improve our understanding of these amphiphilic compounds.

The second approach consists in replacing the PPO block by another hydrophobic backbone. Chang et al. evaluated PEO_{13}–$PLGA_{10}$–PEO_{13} as non-ionic amphiphilic carriers for gene transfer to the skeletal muscle [87]. PLGA segment confers interesting properties of biodegradation to these copolymers. Characterization by AFM and zeta potential measurements indicated that the copolymer interacts and slightly condenses plasmid DNA but only for high concentrations of the copolymer (weight ratio of PEO_{13}–$PLGA_{10}$–PEO_{13}/DNA $\geqslant$ 25:1) [87]. However, similar to pluronics, PEO_{13}–$PLGA_{10}$–PEO_{13} did not change the electrophoretic mobility of the DNA on an agarose gel [87]. After *in vivo* administration to Tibialis anterior muscles of Sprague–Dawley rats, transient expression of the transgene was observed: ten days after administration of the PEO_{13}–$PLGA_{10}$–PEO_{13}/DNA, levels of reporter or therapeutic genes were comparable to those obtained with the naked DNA [87].

5. Conclusion

Gene carriers based on soluble polymers appear as promising tools to deliver plasmid DNA to the striated muscles. Synthetic vectors are highly versatile compounds, allowing numerous modifications of their structure, composition and/or molecular weight in order to adapt the formulation to the target tissue as well as the administration route. Thus, a broad spectrum of pathological conditions might be accessible for gene transfer based on synthetic carriers.

Since conventional polycationic compounds are mediating important toxic side effects, their potential clinical applications seem to be rather limited. More recently developed biodegradable cationic polymers may be an interesting alternative as they might provide increased safety and efficiency towards muscle tissue. Furthermore, more potent and harmless formulations are likely to consist in polymers displaying few or no charges. In particular, the amphiphilic compounds display remarkable inocuity and efficiency *in vivo*, while exhibiting unusual biophysical features when associated with DNA. The major challenge with these carriers currently resides in improving our understanding of their mechanism of action. Progression of our knowledge could subsequently allow further increase in their transfection efficiency.

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