Reviews

Polyethylenimine-based polyplex nanoparticles and features of their behavior in cells and tissues*

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The design of efficient systems for the targeted delivery of nucleic acids into cells is a rapidly developing area of polymer chemistry, molecular biology, and medicine. Complexes between DNA or RNA polyanions and various polycations, which are usually called polyplexes, hold promise as such delivery systems. Polyethylenimines (PEIs) and their derivatives are often used in research for the preparation of such complexes with plasmid DNA, oligonucleotides, and small RNA. Polyplex nanoparticles are employed for the delivery of genetic material into cells in culture and for the development of methods for the treatment of genetic and cancer diseases. The properties of polyplexes depend on the size, dispersity, and hydrophilicity of the used PEI or its derivatives and the ratio of polymers in the complex, which are responsible for the size, surface charge, and hydrophilicity of the resulting nanoparticles. The efficiency of polyplexes is determined by their ability to interact with components of biological systems on the surface and inside the cells, as well as with the blood vascular walls and the extracellular matrix during systemic *in vivo* use.

Key words: polyethylenimine, polyplexes, nucleic acids, block copolymers, polyethylene glycol, nanoparticles, transfection, gene therapy.

An alteration of the regulation of biological systems by introducing nucleic acids into the cells has been one of hot areas of biology, chemistry, and medicine for several past decades. Gene therapy has raised high expectations in the treatment of genetic and cancer diseases. A much broader spectrum of diseases can be cured by modulating the activity of genes using small interfering RNAs or antisense oligonucleotides. The bottleneck of these approaches is the design of a fully controlled, safe, and both efficient

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and rather cost-saving system for the delivery of nucleic acids to cells. In many cases, it is desirable that this delivery system be also specific for the cell type. One of the rational approaches to the design of such systems, as it has long been clear,[1] is based on the use of polycations in order to shield the negative charge of nucleic acid polyanions, which hinders the uptake of DNA or RNA into cells. Polycations can form complexes with nucleic acids, which are called polyplexes.[2] In polyplexes, DNA exists in the tightly packed compact state, and interactions with polycations protect the DNA from hydrolytic enzymes and provide sufficient stability in biological media. Polyplexes are nonpathogenic, many of them being weakly or non-immunogenic and relatively low toxic. The modification of the parent polymers is a quite easy route to prepare particles with diverse physicochemical properties, as well as to attach various components for the interaction with biological structures, resulting in a radical change in the behavior of polyplexes. The production of polyplexes is not expensive, and complexes of DNA with polymeric cations, with attached ligands, which interact with receptors on the surface of a given type of cells, are easily reproduced.[3,4] Polyplexes have already been used for the systemic and local administration in order to develop methods for the treatment of genetic and cancer diseases.[5—11]

The use of polyethylenimine (PEI) is one of the most successful attempts to design an efficient system for the delivery of nucleic acids to cells.[12,13] This approach proved to be much more efficient than most of other methods proposed for the transfection both earlier[14—17] and later.[18—22]

Polyethylenimines and their derivatives

Polyethylenimines with different lengths are produced by the polymerization of ethylenimine (aziridine) or oxazoline, giving rise to branched or linear polymers (Fig. 1, *a*, *b*) with the same repeat unit ($-CH_2-CH_2-NH-$). The presence of amino groups in PEI makes it possible to perform various modifications, resulting in a change in the physicochemical and biological properties of the polymer. The most commonly used modification of PEI is the attachment of polyethylene glycol (PEG),[23—28] a hydrophilic polymer, which can influence the size and surface charge of the resulting polyplexes, as well as the interaction with components of the reticuloendothelial system involved in protecting the body from foreign particles. Polyethylene glycol or, more precisely, its bifunctional derivatives are quite often employed to attach additional components to polycations. As a rule, such components either facilitate the entry into cells[25,29—32] or act as ligands for internalizable receptors on the cell surface[6,15,24,26,33—42] (Fig. 1, *c*). The latter provides the selective penetration into the cells bearing these receptors on their surface.

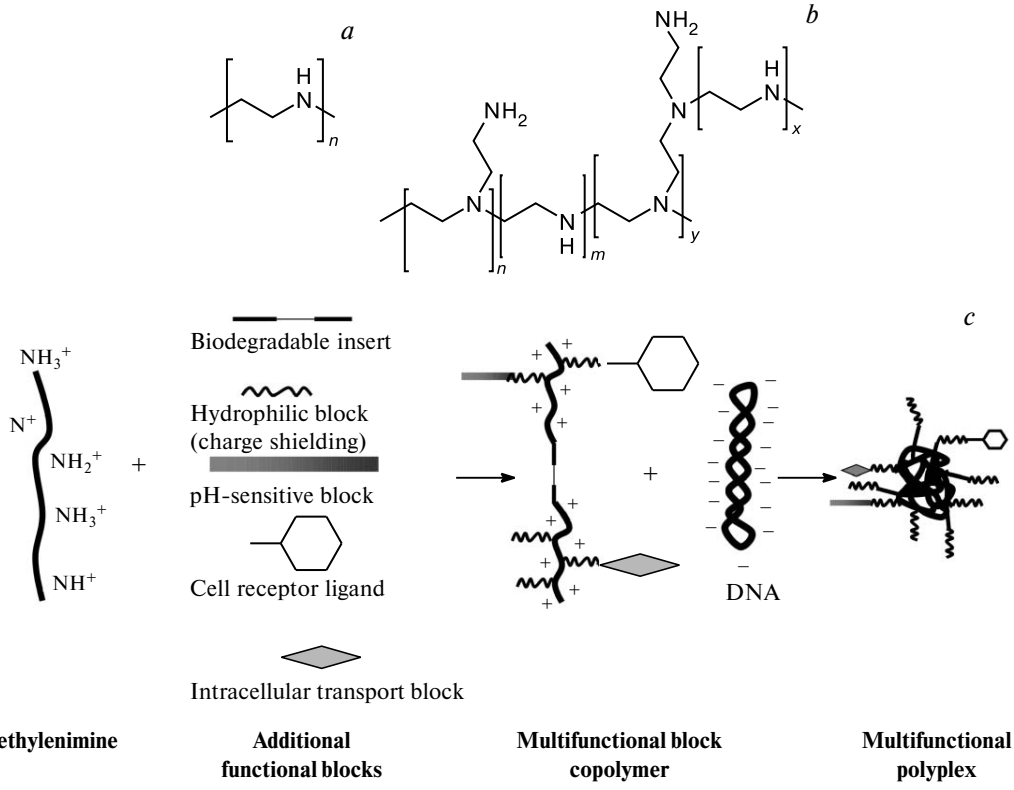

Fig. 1. Linear polyethylenimine (*a*), branched polyethylenimine (*b*), and the flow chart of the design of multifunctional polyplexes (*c*).

Such charged polycations as PEIs can nonspecifically interact with the negatively charged cell surface in the culture or with the vessel walls in the body. At high concentrations, this can cause toxic effects, which are more pronounced in the case of high-molecular-weight polymers[43] primarily due to cell necrosis resulting from the loss of cell membrane integrity.[44,45] This toxicity is primarily due to the interaction of PEI with syndecans on the cell surface.[46] At lower concentrations and longer times, the apoptosis pathway can be activated through the damage of mitochondrial membranes and electron-transport chain and the cytochrome C release.[45,47,48] In the case of systemic administration of PEI involved in complexes with DNA, the former is rapidly (within a few minutes) released from the bloodstream and is accumulated in the liver,[49] where necrotic foci are observed at high PEI concentrations,[50] and, to a lesser extent, in the spleen.[49] In addition, the aggregation of polyplexes caused by their interaction with proteins and blood cells leads, at large doses of polyplexes, to obstruction of pulmonary capillaries accompanied by toxic effects.[50]

A method commonly used for reducing toxic effects is based on the attachment of hydrophilic polymers, for example, of PEG.[51—53] The number of attached residues of PEG rather than its length is essential for a decrease in toxicity.[51,52] The modification of PEI by attaching PEG also increases the residence time in the bloodstream only when employing the multiple modification by short PEG (molecular weight is 550), whereas long PEG (5000—20000) have only a slight effect on the pharmacokinetics of PEIs.[49] Other modifications of linear and branched PEIs also often led to the reduction of cytotoxicity of PEI-based polyplexes. Examples are linear PEI (432 Da) cross-linked with tricyclo[5.2.1.0]decanedimethanol diacrylate, linear PEI (432 Da) in a mixture with branched PEI (1.8 kDa) cross-linked with ethylene glycol diacrylate, permethylated branched PEI (25 kDa); linear PEI (2 kDa) with dodecylated primary amines, *etc*.[54] It should also be noted that, although PEIs are non-immunogenic, they can serve as adjuvants for glycoproteins,[55] which imposes limitations on modifications of PEIs for the delivery of nucleic acids.

Formation of polyplex nanoparticles

The mixing of polycations with DNA in different ratios, which are most often expressed as the ratio of the number of polycation amino groups (N) to the number of phosphate groups of DNA (P), can afford complexes with different charges and sizes. At low N/P ratios, complexes between transgene DNA and PEI cause an insignificant decrease in the hydrodynamic size of DNA and poorly protect DNA from enzymatic degradation in biological fluids, resulting in low efficiency of transfection with such complexes. At an N/P ratio close to unity, neutral complexes prone to aggregation are formed. Stable complexes are produced[56] at N/P ratios not lower than 2—3. However, even at such ratios, these complexes are prone to aggregation in due course *via* hydrophobic interactions and van der Waals forces.[57] The stability of polyplexes increases with increasing N/P ratio due to an increase in the electrostatic repulsion of the complexes, the surface potential of which takes rather high values (up to 30—35 mV) in complexes of DNA with PEI.[43] An increase in the N/P ratio from 2 to 20 leads to a decrease in the size of the complexes[58] from ~1000 to 100—200 nm. At high N/P ratios, a large fraction of PEI exists in the free form.[59] Thus, already at $N/P = 6$, only 58% of 25 kDa PEI is in the DNA-bound state.[60] The purification of suspensions of polyplex nanoparticles from an excess of PEI using different methods led to the reduction of the cytotoxicity but also decreased the efficiency of transfection of cells in culture.[61,62] Apparently, an excess of free PEI in polyplex preparations is necessary for more efficient transfection because this excess precludes undesirable interactions of the complexes with glycosaminoglycans on the surface of transfected cells and hinders the premature unpacking of DNA.[63] The attachment of PEG to PEI decreases the surface potential of the particles that formed and significantly alters the interaction with nucleic acids, resulting in changes both in the average size of the particles and their size distribution. Detailed investigations of the dependence of transfection on the degree of modification of 25 kDa PEI by polyethylene glycol with 24 monomeric units in the chain, as well as on the N/P ratio in the complexes, showed that these dependences are non-monotonic and are similar in shape for all the cells under study.[25] Meanwhile, the absolute values of transfection maxima (in percentage) can differ by more than an order of magnitude. Studies of the size distribution of polyplex particles showed[25] that there is a significant positive correlation between the efficiency of transfection and the fraction of particles with a diameter from 50 to 75 nm for all the cell lines under study. This correlation is revealed only using atomic force microscopy, which provides more accurate data on the particle size distribution compared to dynamic light scattering,[64] in which the data are approximated by a sum of standard Gaussian distributions.

Since a similar effect on the efficiency of transfection was observed for different genetic constructs,[25] the difference in the efficiency of transfection for various cell lines should be sought in the interactions of polyplexes with cells and in further steps of intracellular transport.

Interaction with cells and intracellular transport of polyplexes

It is known that PEIs added into cells first interact with negatively charged heparan sulfate and chondroitin sulfate chains, which are present on cell-surface syndecans.[46,65] The interaction with syndecan-1 can strongly

inhibit the cellular uptake of polyplexes.[65] Apparently, this can be due to unpacking of delivered nucleic acids on the cell surface caused by interactions with negatively charged chains of heparan sulfate.

Since DNA displays its effect only upon entering the cell nucleus, it is among substances that require intracellular delivery.[66] After the interaction with the cell surface, polyplexes enter the cells *via* different endocytosis pathways:[5] clathrin- and raft-mediated endocytosis, macropinocytosis, and phagocytosis. In this case, endocytosed polyplex particles appear in closed membrane vesicles (endosomes), and they should be released from endosomes to the hyaloplasm in order to reach the cell nucleus. The modeling of the uptake and intracellular transport of PEI-based polyplexes by cells demonstrated that the rate of cellular uptake of polyplexes and the absence of unpacking of DNA before the release from endosomes to the hyaloplasm of the cell,[25] which is required for the entry of DNA into the nucleus, are of most importance for transfection. The attachment of the ligand to an internalizable receptor to PEI can alter the endocytosis pathway utilized by polyplexes to enter the cell and, correspondingly, imparts cell specificity to the polyplex with respect to the cells expressing this receptor. For instance, the attachment of a peptide specific for melanocortin type 1 receptors, which are overexpressed in melanoma cells,[67] results in that polyplexes are rapidly drawn into the melanoma cell *via* clathrin-mediated endocytosis[6] rather than *via* the slower raft-mediated pathway, as is the case with unmodified polyplexes. As a result, polyplexes are not stayed on the cell surface, and DNA is unpacked mainly inside the cell, which greatly increases the efficiency of its delivery.

However, the attachment of the ligand may be insufficient for the specific uptake of polyplexes into target cells.[68] In order to reduce the nonspecific penetration of PEI-based polyplexes into non-target cells, PEIs are generally shielded by hydrophilic molecules, for example, by PEG or hydroxypropyl methacrylate. This modification reduces the toxicity of polyplexes and their circulation time in the blood, but it decreases the efficiency of transfection.[68,69] This means that it is necessary to screen different ratios of all the components in the block copolymer and examine all required characteristics (the efficiency of transfection, cytotoxicity, toxicity at the body level, the circulation time, cell specificity, *etc*.) in order to find their optimum ratio.

Polyethylenimines differ from many other polycations capable of forming compact complexes with DNA in that these complexes can provide noticeable, although rather low, efficiency of the transport of delivered nucleic acids from acidified endocytic compartments. The proton sponge hypothesis was proposed to explain this fact. According to this hypothesis, the release of PEI-containing polyplexes from endosomes is attributed to the accumulation of protons and chloride as a counterion due to the buffer properties of PEIs under weakly acidic conditions, resulting in the osmotic disruption of endosomes.[12] This hypothesis was heavily debated, and numerous data were published for and against it. In particular, it has recently been shown that PEI cannot influence the pH of endosomes,[70] as it was assumed by the authors of the hypothesis. Apparently, the transport from endosomes is to the greatest extent determined by the direct action of PEIs and PEI-containing polyplexes on lipid membranes. The efficiency of transfection with PEI-based polyplexes can be increased by using an additional component facilitating the transport from endosomes.[71,72]

It was found that DNA delivered by PEI-based polyplexes enters the nucleus mainly during the mitosis.[26,73,74] Recently, by means of observations of single cells, we obtained direct evidence that a small fraction of cells (about 10%) can be transfected with PEI-containing polyplexes bypassing mitosis.[26] In this case, the transport of DNA can occur *via* nuclear pores or pores in membranes, the formation of which is induced by PEI.[75]

It should be noted that the addition of polyplexes to cells is not indifferent for the latter with respect to the expression of their own genes. Recent studies have demonstrated[76] that the transfection of HEK293T cells with polyplexes bearing the gene for the green fluorescent protein leads to the expression of not only this reporter gene but also of a number of cellular genes, the latter being different, at different times after transfection. It is interesting that the transfection with lipoplexes (liposome-mediated transfection) also triggers the expression of cell genes. It should be noted that the set of genes induced to be expressed within 2 h totally differs from the set of genes that are expressed upon the addition of polyplexes, while the set of genes that are expressed upon the transfection with both polyplexes and lipoplexes was observed after 8 h.

Application of polyplexes for the delivery of nucleic acids in the body

Like other polycations, PEIs protect nucleic acids from enzymes that are present in the blood, primarily from deoxyribonuclease 1.[77–79] The positive surface charge that is formed causes opsonization of polyplexes, aggregation of polyplexes and blood cells,[50] and the interaction of polyplexes with negatively charged components of the extracellular matrix.[80] The modification with PEG or other hydrophilic polymers can substantially suppress these processes and makes the polyplexes more accessible to the cells, into which DNA or RNA has to be delivered.[27,81] Therefore, it is possible to use selective components that determine the predominant interaction with a certain type of cells with expression of particular surface receptors. A great importance is placed on this interaction in the development of the selective action on tumors characterized by overexpression of various receptors.[82] Many dozens of internalizable surface receptors, the overexpression of which

is characteristic of various types of malignant tumors, are known,[83] and the list of such receptors is constantly growing.

The authors of the recent review[84] with the impressive title "Quo vadis polyplex?" mentioned, based on the studies of *in vivo* oligonucleotide delivery by polyplexes, that only 10% of these studies used biodegradable polymers. Nevertheless, the number of such publications is growing every year. The cytotoxicity — another adverse property of PEI — increases in parallel with the efficiency of transfection with genes delivered by PEI-containing polyplexes.[85] This gives rise to, at the first glance, a paradoxical situation. However, despite this seemingly irresolvable paradox, techniques developed in recent years allow one to achieve both the biodegradability of PEI-containing polyplexes and a decrease in their toxicity. One of the main, commonly accepted, approaches involves the synthesis and application of block copolymers, which either contain rather short PEIs alternating with biodegradable components, or, otherwise, are linked to other components by biodegradable spacers. The former approach can be illustrated by the triblock copolymer of biodegradable polycaprolactone, PEG, and branched 25 kDa PEI.[86] In this case, the efficiency of transfection was almost the same as in the case with unmodified PEI, but the cytotoxicity was almost 100-fold lower, the authors obtaining biodegradable polyplexes. A typical example of another approach is PEGylated (using tetraethylene glycol) PEI (~2 kDa), which was assembled, by means of the click reaction, to the 22 kDa block copolymer linked by biodegradable S—S bonds.[87] This block copolymer proved to be 22 times less toxic than 25 kDa PEI, but it ensured 6 times higher efficiency of transfection compared to the starting 2 kDa PEI, which corresponds to 17% efficiency of 25 kDa PEI.

Polyplexes with ligands for receptors, which are, to some extent, specific for cancer cells, can be used in suicide gene therapy with delivery of genes, which express a product that can convert nontoxic precursors to highly toxic metabolites.[88] Yet another factor capable, in some cases, of increasing the specificity and efficiency of polyplex nanoparticles for tumors is the so-called enhanced permeability and retention effect[89] due to the formation of rather large holes in the walls of rapidly growing disordered tumor vessels. This accounts for the efficient polyplex penetration into experimental mouse melanoma tumors, but not into normal tissues.[41] It is interesting that polyplexes both with a peptide ligand for the internalized melanocortin receptor and without the ligand equally efficiently penetrate into the tumor, but the transfection of cells was much more pronounced when genes were delivered by liganded polyplexes.

The available data show that PEI and its relatively simple derivatives provide a promising basis for gene transport systems in cell cultures. These substances have a considerable potential for the design of fully controlled systems for gene therapy. More complex systems having different functions that are often difficult to combine are required for the successful solution of the problem of gene delivery to the target site in the body. For example, it is necessary, on the one hand, to protect the delivered nucleic acid from nucleases both outside and inside the cell, as well as to prevent the unpacking throughout the delivery route, from the administration site to the target site – the nucleus of the required type of cells. On the other hand, the genetic material that appears in the nucleus should be rather easily unpacked to exhibit its action. Therefore, an increasing attention is given to the design of complex multifunctional systems consisting of numerous components,[27,90] which fulfill their functions at different steps of DNA or RNA transport by interacting with various systems in the body or by hindering this interaction. This route holds promise for the design of artificial transport structures as complex and sophisticated as systems possessed by viruses that carry their genetic material into the cell nucleus.

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References

1. J. H. McCutchan, J. S. Pagano, *J. Natl. Cancer Inst.*, 1968, **41**, 351.
2. P. L. Felgner, Y. Barenholz, J. P. Behr, S. H. Cheng, P. Cullis, L. Huang, J. A. Jessee, L. Seymour, F. Szoka, A. R. Thierry, *Human Gene Ther.*, 1997, **8**, 511.
3. H. C. Kang, M. Lee, Y. H. Bae, *Crit. Rev. Eukaryot. Gene Expr.*, 2005, **15**, 317.
4. D. Ibraheem, A. Elaissari, H. Fessi, *Int. J. Pharm.*, 2014, **459**, 70.
5. U. Lungwitz, M. Breunig, T. Blunk, A. Gopferich, *Eur. J. Pharm. Biopharm.*, 2005, **60**, 247.
6. M. O. Durymanov, E. A. Beletkaia, A. V. Ulasov, Y. V. Khramtsov, G. A. Trusov, N. S. Rodichenko, T. A. Slastnikova, T. V. Vinogradova, N. Y. Uspenskaya, E. P. Kopantsev, A. A. Rosenkranz, E. D. Sverdlov, A. S. Sobolev, *J. Control. Release*, 2012, **163**, 211.
7. M. M. Ivanova, A. A. Rosenkranz, O. A. Smirnova, V. A. Nikitin, A. S. Sobolev, V. Landa, B. S. Naroditsky, L. K. Ernst, *Mol. Reprod. Dev.*, 1999, **54**, 112.
8. A. S. Sobolev, A. A. Rosenkranz, O. A. Smirnova, V. A. Nikitin, G. L. Neugodova, B. S. Naroditsky, I. N. Shilov, I. N. Shatski, L. K. Ernst, *J. Biol. Chem.*, 1998, **273**, 7928.
9. S. Patnaik, K. C. Gupta, *Expert. Opin. Drug Deliv.*, 2013, **10**, 215.
10. G. Y. Wu, C. H. Wu, *J. Biol. Chem.*, 1988, **263**, 14621.
11. M. Kullberg, R. McCarthy, T. J. Anchordoquy, *J. Control. Release*, 2013, **172**, 730.
12. O. Boussif, F. Lezoualc´h, M. A. Zanta, M. D. Mergny, D. Scherman, B. Demeneix, J. P. Behr, *Proc. Natl. Acad. Sci. USA*, 1995, **92**, 7297.

13. B. Demeneix, J. P. Behr, *Adv. Genet.*, 2005, **53**, 217.
14. A. A. Rozenkrants, S. V. Iachmenev, A. S. Sobolev, *Dokl. Akad. Nauk SSSR* (*Engl. Transl.*), 1990, **312**, 493 [*Dokl. AN SSSR*, 1990, **312**, 493].
15. E. Wagner, M. Zenke, M. Cotten, H. Beug, M. L. Birnstiel, *Proc. Natl. Acad. Sci. USA*, 1990, **87**, 3410.
16. G. Y. Wu, C. H. Wu, *J. Biol. Chem.*, 1987, **262**, 4429.
17. A. A. Rosenkranz, S. V. Yachmenev, D. A. Jans, N. V. Serebryakova, V. I. Murav´ev, R. Peters, A. S. Sobolev, *Exp. Cell Res.*, 1992, **199**, 323.
18. L. Jin, X. Zeng, M. Liu, Y. Deng, N. He, *Theranostics*, 2014, **4**, 240.
19. D. He, E. Wagner, *Macromol. Biosci.*, 2015, **15**, 600; DOI: 10.1002/mabi.201400524.
20. A. Aied, U. Greiser, A. Pandit, W. Wang, *Drug Discov. Today*, 2013, **18**, 1090.
21. E. Wagner, *Adv. Genetics*, 2014, **88**, 231.
22. D. W. Pack, A. S. Hoffman, S. Pun, P. S. Stayton, *Nature Rev. Drug Discovery*, 2005, **4**, 581.
23. S. Katayose, K. Kataoka, *Bioconjug. Chem.*, 1997, **8**, 702.
24. W. Guo, R. L. Lee, *AAPS. Pharm. Sci.*, 1999, **1**, E19.
25. A. V. Ulasov, Y. V. Khramtsov, G. A. Trusov, A. A. Rosenkranz, E. D. Sverdlov, A. S. Sobolev, *Mol. Ther.*, 2011, **19**, 103.
26. M. O. Durymanov, A. V. Yarutkin, Y. V. Khramtsov, A. A. Rosenkranz, A. S. Sobolev, *J. Control. Release*, 2015, **215**, 73.
27. T. Wang, J. R. Upponi, V. P. Torchilin, *Internat. J. Parm.*, 2012, **427**, 3.
28. G. A. Trusov, A. V. Ulasov, E. A. Beletkaia, Y. V. Khramtsov, M. O. Durymanov, A. A. Rosenkranz, E. D. Sverdlov, A. S. Sobolev, *Dokl. Biochem. Biophys.* (*Engl. Transl.*), 2011, **437**, 77 [*Dokl. AN*, 2011, **437**, 77].
29. E. Kleemann, M. Neu, N. Jekel, L. Fink, T. Schmehl, T. Gessler, W. Seeger, T. Kissel, *J. Control. Release*, 2005, **109**, 299.
30. S. R. Sirsi, R. C. Schray, X. Guan, N. M. Lykens, J. H. Williams, M. L. Erney, G. J. Lutz, *Hum. Gene Ther.*, 2008, **19**, 795.
31. J. Nguyen, X. Xie, M. Neu, R. Dumitrascu, R. Reul, J. Sitterberg, U. Bakowsky, R. Schermuly, L. Fink, T. Schmehl, T. Gessler, W. Seeger, T. Kissel, *J. Gene Med.*, 2008, **10**, 1236.
32. I. V. Alekseenko, E. V. Snezhkov, I. P. Chernov, V. V. Pleshkan, V. K. Potapov, A. V. Sass, G. S. Monastyrskaya, E. P. Kopantzev, T. V. Vinogradova, Y. V. Khramtsov, A. V. Ulasov, A. A. Rosenkranz, A. S. Sobolev, O. A. Bezborodova, A. D. Plyutinskaya, E. R. Nemtsova, R. I. Yakubovskaya, E. D. Sverdlov, *J. Translat. Med.*, 2015, **13**, 78.
33. J. H. Jeong, S. H. Kim, S. W. Kim, T. G. Park, *J. Biomater. Sci. Polym. Ed.*, 2005, **16**, 1409.
34. H. Cheng, J. L. Zhu, X. Zeng, Y. Jing, X. Z. Zhang, R. X. Zhuo, *Bioconjug. Chem.*, 2009, **20**, 481.
35. S. J. Chiu, N. T. Ueno, R. J. Lee, *J. Control. Release*, 2004, **97**, 357.
36. M. Kursa, G. F. Walker, V. Roessler, M. Ogris, W. Roedl, R. Kircheis, E. Wagner, *Bioconjug. Chem.*, 2003, **14**, 222.
37. M. F. Wolschek, C. Thallinger, M. Kursa, V. Rossler, M. Allen, C. Lichtenberger, R. Kircheis, T. Lucas, M. Willheim, W. Reinisch, A. Gangl, E. Wagner, B. Jansen, *Hepatology*, 2002, **36**, 1106.
38. T. Blessing, M. Kursa, R. Holzhauser, R. Kircheis, E. Wagner, *Bioconjug. Chem.*, 2001, **12**, 529.
39. S. H. Kim, J. H. Jeong, S. H. Lee, S. W. Kim, T. G. Park, *Bioconjug. Chem.*, 2008, **19**, 2156.
40. G. Abourbeh, A. Shir, E. Mishani, M. Ogris, W. Rodl, E. Wagner, A. Levitzki, *IUBMB. Life*, 2012, **64**, 324.
41. M. O. Durymanov, T. A. Slastnikova, A. I. Kuzmich, Y. V. Khramtsov, A. V. Ulasov, A. A. Rosenkranz, S. Y. Egorov, E. D. Sverdlov, A. S. Sobolev, *Biomaterials*, 2013, **34**, 10209.
42. C. Rudolph, N. Sieverling, U. Schillinger, E. Lesina, C. Plank, A. F. Thünemann, H. Schönberger, J. Rosenecker, *Biomaterials*, 2007, **28**, 1900.
43. K. Kunath, A. von Harpe, D. Fischer, H. Petersen, U. Bickel, K. Voigt, T. Kissel, *J. Control. Release*, 2003, **89**, 113.
44. D. Fischer, Y. Li, B. Ahlemeyer, J. Krieglstein, T. Kissel, *Biomaterials*, 2003, **24**, 1121.
45. S. M. Moghimi, P. Symonds, J. C. Murray, A. C. Hunter, G. Debska, A. Szewczyk, *Mol. Ther.*, 2005, **11**, 990.
46. H. Zhang, T. Xia, H. Meng, M. Xue, S. George, Z. Ji, X. Wang, R. Liu, M. Wang, B. France, *ACS Nano*, 2011, **5**, 2756.
47. G. Grandinetti, N. P. Ingle, T. M. Reineke, *Mol. Pharm.*, 2011, **8**, 1709.
48. A. Hall, A. K. Larsen, L. Parhamifar, K. D. Meyle, L. P. Wu, S. M. Moghimi, *Biochim. Biophys. Acta (BBA) — Bioenergetics*, 2013, **1827**, 1213.
49. K. Kunath, A. von Harpe, H. Petersen, D. Fischer, K. Voigt, T. Kissel, U. Bickel, *Pharm. Res.*, 2002, **19**, 810.
50. P. Chollet, M. C. Favrot, A. Hurbin, J. Coll, *J. Gene Med.*, 2002, **4**, 84.
51. X. Zhang, S. Pan, H. Hu, G. Wu, M. Feng, W. Zhang, X. Luo, *J. Biomed. Materials Res. Part A*, 2008, **84**, 795.
52. H. Petersen, P. M. Fechner, A. L. Martin, K. Kunath, S. Stolnik, C. J. Roberts, D. Fischer, M. C. Davies, T. Kissel, *Bioconjug. Chem.*, 2002, **13**, 845.
53. Z. Zhong, J. Feijen, M. C. Lok, W. E. Hennink, L. V. Christensen, J. W. Yockman, Y. H. Kim, S. W. Kim, *Biomacromolecules*, 2005, **6**, 3440.
54. L. Parhamifar, A. K. Larsen, A. C. Hunter, T. L. Andresen, S. M. Moghimi, *Soft Matter*, 2010, **6**, 4001.
55. F. Wegmann, K. H. Gartlan, A. M. Harandi, S. A. Brinckmann, M. Coccia, W. R. Hillson, W. L. Kok, S. Cole, L. P. Ho, T. Lambe, *Nature Biotechnology*, 2012, **30**, 883.
56. S. Choosakoonkriang, B. A. Lobo, G. S. Koe, J. G. Koe, C. R. Middaugh, *J. Pharm. Sci.*, 2003, **92**, 1710.
57. M. Ogris, P. Steinlein, M. Kursa, K. Mechtler, R. Kircheis, E. Wagner, *Gene Ther.*, 1998, **5**, 1425.
58. P. Erbacher, T. Bettinger, P. Belguise-Valladier, S. Zou, J. L. Coll, J. P. Behr, J. S. Remy, *J. Gene Med.*, 1999, **1**, 210.
59. J. P. Clamme, J. Azoulay, Y. Mely, *Biophys. J.*, 2003, **84**, 1960.
60. Y. T. Ko, U. Bickel, J. Huang, *Oligonucleotides*, 2011, **21**, 109.
61. S. Boeckle, G. K. von Gersdorff, S. van der Piepen, C. Culmsee, E. Wagner, M. Ogris, *J. Gene Med.*, 2004, **6**, 1102.
62. J. Fahrmeir, M. Gunther, N. Tietze, E. Wagner, M. Ogris, *J. Control. Release*, 2007, **122**, 236.
63. M. Hanzlikova, P. Soininen, P. Lampela, P. T. Mannisto, A. Raasmaja, *Plasmid*, 2009, **61**, 15.
64. C. M. Wiethoff, C. R. Middaugh, in *Nonviral Vectors for Gene Therapy*, Ed. M. A. Findeis, Humana Press, 2001, 349.
65. S. Paris, A. Burlacu, Y. Durocher, *J. Biol. Chem.*, 2008, **283**, 7697.

66. A. A. Rosenkranz, A. V. Ulasov, T. A. Slastnikova, Y. V. Khramtsov, A. S. Sobolev, *Biochemistry* (*Moscow*) (*Engl. Transl.*), 2014, **79**, 928 [*Biokhimiya*, 2014, **79**, 1148].
67. A. A. Rosenkranz, T. A. Slastnikova, M. O. Durymanov, A. S. Sobolev, *Biochemistry (Moscow*) (*Engl. Transl.*), 2013, **78**, 1228 [*Biokhimiya*, 2013, **78**, 1564].
68. E. Wagner, C. Culmsee, S. Boeckle, *Adv. Genetics*, 2005, **53**, 333.
69. E. Wagner, *Pharm. Res.*, 2004, **21**, 8.
70. R. V. Benjaminsen, M. A. Mattebjerg, J. R. Henriksen, S. M. Moghimi, T. L. Andresen, *Mol. Ther.*, 2013, **21**, 149.
71. S. Kakimoto, T. Hamada, Y. Komatsu, M. Takagi, T. Tanabe, H. Azuma, S. Shinkai, T. Nagasaki, *Biomaterials*, 2009, **30**, 402.
72. M. Ogris, R. C. Carlisle, T. Bettinger, L. W. Seymour, *J. Biol. Chem.*, 2001, **276**, 47550.
73. S. Brunner, T. Sauer, S. Carotta, M. Cotten, M. Saltik, E. Wagner, *Gene Ther.*, 2000, **7**, 401.
74. S. Brunner, E. Furtbauer, T. Sauer, M. Kursa, E. Wagner, *Mol. Ther.*, 2002, **5**, 80.
75. S. Hong, P. R. Leroueil, E. K. Janus, J. L. Peters, M. M. Kober, M. T. Islam, B. G. Orr, J. R. Baker, M. M. Banaszak Holl, *Bioconjug. Chem.*, 2006, **17**, 728.
76. T. M. Martin, S. A. Plautz, A. K. Pannier, *J. Gene Med.*, 2015, **17**, 33.
77. N. B. Kurnick, *Arch. Biochem. Biophys.*, 1953, **43**, 97.
78. S. N. Tamkovich, A. V. Cherepanova, E. V. Kolesnikova, E. Y. Rykova, D. V. Pyshnyi, V. V. Vlassov, P. P. Laktionov, *Ann. N.-Y. Acad. Sci.*, 2006, **1075**, 191.
79. D. Nadano, T. Yasuda, K. Kishi, *Clin. Chem.*, 1993, **39**, 448.
80. M. Ruponen, P. Honkakoski, S. Ronkko, J. Pelkonen, M. Tammi, A. Urtti, *J. Control. Release*, 2003, **93**, 213.
81. A. Yousefi, G. Storm, R. Schiffelers, E. Mastrobattista, *J. Control. Release*, 2013, **170**, 209.
82. D. Hanahan, R. A. Weinberg, *Cell*, 2000, **100**, 57.
83. T. A. Slastnikova, A. A. Rosenkranz, M. R. Zalutsky, A. S. Sobolev, *Curr. Pharm. Des*, 2015, **21**, 1227.
84. O. M. Merkel, T. Kissel, *J. Control. Release*, 2014, **190**, 415.
85. Y. Yue, C. Wu, *Biomaterials Sci.*, 2013, **1**, 152.
86. X. Shuai, T. Merdan, F. Unger, T. Kissel, *Bioconjug. Chem.*, 2005, **16**, 322.
87. N. Zhao, S. Roesler, T. Kissel, *Int. J. Pharm.*, 2011, **411**, 197.
88. S. Duarte, G. Carle, H. Faneca, M. C. P. De Lima, V. Pierrefite-Carle, *Cancer Lett.*, 2012, **324**, 160.
89. H. Maeda, *Adv. Drug Delivery Rev.*, 2015, **91**, 3; DOI: 10.1016/j.addr.2015.01.002.
90. V. P. Torchilin, *Adv. Drug Delivery Rev.*, 2012, **64**, 302.

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