Acta Biomaterialia 8 (2012) 4224–4232

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Polysaccharide gene transfection agents ☆

Wahid Khan [a], Hossein Hosseinkhani [b], Diana Ickowicz [a], Po-Da Hong [b], Da-Shyong Yu [c], Abraham J. Domb [a,*]

[a] Institute of Drug Research, School of Pharmacy, Faculty of Medicine, Center for Nanoscience and Nanotechnology and The Alex Grass Center for Drug Design and Synthesis, The Hebrew University of Jerusalem 91120, Israel
[b] Graduate Institute of Biomedical Engineering, National Taiwan University of Science and Technology (TAIWAN TECH), Taipei 10607, Taiwan
[c] Nanomedicine Research Center, National Defense Medical Center, Taipei 10607, Taiwan

ARTICLE INFO

Article history:
Available online 25 September 2012

Keywords:
Gene therapy
Delivery agents
Polysaccharides
siRNA
Plasmid DNA

ABSTRACT

Gene delivery is a promising technique that involves in vitro or in vivo introduction of exogenous genes into cells for experimental and therapeutic purposes. Successful gene delivery depends on the development of effective and safe delivery vectors. Two main delivery systems, viral and non-viral gene carriers, are currently deployed for gene therapy. While most current gene therapy clinical trials are based on viral approaches, non-viral gene medicines have also emerged as potentially safe and effective for the treatment of a wide variety of genetic and acquired diseases. Non-viral technologies consist of plasmid-based expression systems containing a gene associated with the synthetic gene delivery vector. Polysaccharides compile a large family of heterogenic sequences of monomers with various applications and several advantages as gene delivery agents. This chapter, compiles the recent progress in polysaccharide based gene delivery, it also provides an overview and recent developments of polysaccharide employed for in vitro and in vivo delivery of therapeutically important nucleotides, e.g. plasmid DNA and small interfering RNA.

1. Introduction

Gene therapy involves the insertion of a therapeutic gene into cells, followed by expression and the production of the required proteins. It is a promising approach for the treatment or prevention of a wide range of diseases associated with defective gene expression [1,2]. The fundamental idea is to deliver the gene to cells or tissues. This may be by activation, silencing, introduction or gene knock out and knock down, both in vitro and in vivo [3]. Successful gene therapies depend on the efficient delivery of the genetic material into the cell nucleus and its effective expression within these cells. DNA can be delivered into the cell nucleus either using physical means or by specific carriers that carry the genes into the cells. A number of techniques have been developed for DNA delivery, including direct introduction of the transgene using cell electroporation, microinjection of DNA and incorporation of the gene by vectors [4]. Successful gene therapy depends on the development of effective and secure delivery vectors [5]. The genetic material involves DNA, RNA, antisense oligonucleotide, decoy DNA and/or ribozymes. The idea underlying gene therapy is that human disease can be treated by the transfer of genetic material into specific cells of a patient rather than by conventional drugs; however, it has yet to make its mark in medicine. Successful implementation of gene transfer in the clinic will require the coordinated development of a variety of new technologies and the establishment of unique interactions between investigators from divergent medical and basic science disciplines.

Vectors for delivering genes can be divided into two main groups: (a) viral carriers, where the DNA to be delivered is inserted into a virus, and (b) cationic molecular carriers, which form electrostatic interactions with DNA for delivering gene to cells, and include polymers and lipids [6]. Viral vectors include retroviruses, adenoviruses and adeno-associated viruses. These are effectively used for introducing genetic material into host cells, but immunogenicity, inflammatory effects and safety concerns with the use of such viruses restrict their usefulness [7]. Non-viral vectors have several advantages over viral vectors since they are chemically based materials. They do not integrate into chromosomes, have low immunogenicity, the ability to deliver large genes and no infective risk, are less expensive and easy to handle, and, most importantly, have the potential for large-scale production at a reasonable cost [8].

Recently, significant concerns have focused on non-viral vectors. Such vectors must overcome many barriers, such as low efficiency in delivery to target cells, escape from endosomes,

☆ Part of the Special Issue "Advanced Functional Polymers in Medicine (AFPM)", guest editors: Professors Luigi Ambrosio, Dirk W. Grijpma and Andreas Lendlein.

* Corresponding author. Tel.: +972 26757573; fax: +972 26757076.
E-mail address: avid@ekmd.huji.ac.il (A.J. Domb).

1742-7061/$ - see front matter © 2012 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.actbio.2012.09.022

internalization into the nucleus, and transcription and translation of DNA [5]. The ideal vector must be stable in the systemic circulation, escape the reticuloendothelial system, be able to extravasate tissues and enter the target cell, escape lysosomal degradation and transport DNA to the nucleus to be transcribed [9].

Several non-viral modalities are reported to transfer foreign genetic material into cells; among these, cationic polymers constitute the most promising approach. For this reason, various cationic polymers are rapidly emerging as systems of choice. However, most polycations are toxic to cells and are non-biodegradable. The in vivo application of polyethylenimine (PEI), for example, was shown to be limited by cytotoxicity caused by an excess of positive charge, non-specific interactions and aggregation in the blood [5], while polymers based on amino acids such as poly(lysine) are immunogenic [10]. Thus, various approaches to maximize gene expression are under investigation [11], and a number of cationic polymers, i.e. block co-polymers and dendrimers, have been rationally designed to optimize gene delivery. Among these, polysaccharide has a very important place.

Polysaccharides compile a large variable family of heterogenic sequences of monomers due to the multiple valencies of monomers and the stereospecificity of the glycosidic bonds. The use of natural biopolymers like polysaccharides for various applications has several advantages, such as availability from replenishable agricultural or marine food resources, biocompatibility and biodegradability. Polysaccharides differ not only in the nature of their component monosaccharides, but also in the length of their chains and in the amount of chain branching. This ability to form branched structures distinguishes polysaccharides from proteins and nucleic acids, which occur only as linear polymers. Polysaccharides have the tendency to be extremely bioactive and can offer unique biochemical functions based on their nanoscale organization. They can also provided a stable drug and gene delivery platform [12,13].

Cationic polysaccharides are non-toxic, biodegradable and biocompatible materials. They are especially suitable for transfection and biological applications because they are water soluble and can be readily transported to cells in vivo, and thus act as effective vehicles for transporting agents complexed with them [14].

The major cationic polysaccharides used for gene delivery purposes are either natural or semisynthetic in origin. Semisynthetic cationic polysaccharides are synthesized by the conjugation of various oligoamines to oxidized polysaccharides. Polycations of dextran, pullulan and arabinogalactan grafted with oligoamines of 2–4 amino groups were also investigated and were found to be effective in gene delivery [15]. One of the most relevant characteristics of this kind of carrier is that the polysaccharide hydroxyl groups can be easily modified. It is also possible that sugar-recognition receptors on the cell surface can help internalization [16].

Several reviews of gene therapy research have been published [17–21], a few if which have focused on the technical issues that continue to impede the translation of preclinical studies of gene therapy into effective clinical protocols. This paper provides a critical review of gene therapy, focusing on polysaccharide transfection agent technologies.

2. Therapeutic nucleotides

Gene therapy shows much promise in therapies for various genetic diseases, cancers, viral infections and cardiovascular disorders. Gene delivery includes both the delivery of plasmid DNA (pDNA) encoding therapeutic proteins and RNA interference (RNAi). There are two types of small RNA molecules – microRNA (miRNA) and small interfering RNA (siRNA) or silencing RNA – that are central to RNAi [22]. RNAi is a well-recognized pathway involved in cellular defense against viral invasion, transposon expansion and post-transcriptional regulation. It has rapidly emerged as a promising new strategy for drug target validation and the study of functional genomics, and is currently being evaluated in clinical trials as a potential therapy for diseases of a genetic etiology [23–25]. The principle of RNAi is sequence-specific degradation of mRNA induced by a double-stranded RNA homologous to the target sequence [26–28].

It has already been demonstrated that synthetic siRNA is able to induce RNAi in mammalian cells [29]. Regulation of gene expression using siRNA is a promising strategy for research and treatment of numerous diseases. Over the last few years synthetic siRNA – which performs as a sequence-specific post-transcriptional gene silencing mechanism – has been considered as a new class of nucleic acid therapeutics for the treatment of various infections, genetic disorders and cancers. Despite the high therapeutic potential of siRNA, its application in clinical settings is limited [28], primarily because, when delivered into the bloodstream, naked siRNAs, even when chemically modified, have extremely short half-lives of a few seconds to a few minutes [30]. The delivery of therapeutic siRNA to specific tissues is also a major challenge for systemic siRNA delivery; one reason for this is that the backbone of RNA contains ribose, which has a hydroxyl (OH) group in the 2′position of the pentose ring instead of a hydrogen (H) atom. This extra hydroxyl group makes the RNA backbone more sensitive to hydrolysis [31]. Encapsulation in the delivery system generally provides much better protection of siRNAs against serum degradation. Thus, one of the most challenging tasks to turn siRNAs into clinically acceptable therapeutic drugs is to deliver it using an efficient delivery system [32].

3. Polysaccharide vectors for gene delivery

Polysaccharides are considered to be promising candidates for non-viral gene delivery because of their molecular diversity, which can be modified to fine-tune their physicochemical properties [33,34]. Polysaccharides are able to condense large genes into compact structures and mask the negative DNA charges – necessities for transfecting most types of cell. The mechanism of polymer–DNA complex (polyplex) formation includes electrostatic interaction of polymers with anionic DNA to form polyplexes [35,36]. When mixed with a positive-charged non-viral vector, the molecular size of the pDNA decreases by molecular condensation to form a nanoparticle (Fig. 1). This condensed DNA–vector nanoparticle, with a slightly positive charge, can interact electrostatically with the cell membrane and can thereby be internalized (Fig. 1). After cellular entry, these polyplexes undergo dissociation via endosomal escape to release nucleic acid into the nucleus for gene expression [37]. Further, the cationic nature of polymers allows strong electrostatic interactions with negatively charged DNA and protects the DNA from nuclease degradation [2]. Major cationic polysaccharides used in gene delivery purposes are either natural (chitosan, cyclodextrin and dextran) or semisynthetic derivatives (dextran-spermine), as shown in Fig. 2.

3.1. Chitosan

Chitosan is a cationic linear polymer obtained from chitin that comprises copolymers of β(1-4)-glucosamine and randomly located *N*-acetyl-D-glucosamine (Fig. 2). It has been proved to be a biodegradable, biocompatible, non-antigenic, non-toxic and biofunctional polymer [38]. The main sources of chitin are two marine crustaceans, shrimp and crab [12]. It can be fully or partially deacetylated form of chitin; the degree of deacetylation of typical commercial chitosan is usually between 70% and 95%, and the molecular weight ranges between 10 and 1000 kDa.

ID	Title	Pages
336	Polysaccharide gene transfection agents ☆	9