Journal of Drug Delivery Science and Technology 42 (2017) 18–37

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

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

Review article

Molecular nanoparticle-based gene delivery systems

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ARTICLE INFO

Article history:
Received 19 January 2017
Received in revised form
15 March 2017
Accepted 15 March 2017
Available online 18 March 2017

Keywords:
Cyclodextrins
Calixarenes
Fullerenes
Pillarenes
Cyclopeptides
Cyclotrehalans

ABSTRACT

The last years have witnessed an impressive development of material sciences following the burst of nanotechnology, which has greatly contributed to the development of nonviral gene delivery systems with improved properties for the efficient transport of nucleic acid payloads for gene therapy purposes. In spite of intense efforts, emulating the transfecting capabilities of viruses with synthetic systems represents an extraordinary challenge still far from accomplishment. Achieving this goal requires general and flexible approaches allowing the construction of appropriate carriers and the elucidation of the key rules governing their capacity to supramolecularly interact with nucleic acids and mediate its active transport/delivery through biological membranes. Molecular nano-objects exhibiting persistent shape- and volume, termed "molecular nanoparticles (MNPs)", in combination with diversity-oriented precise macromolecular chemistry, offers excellent opportunities towards these goals by enabling the pre-organization of functional elements, the generation of libraries of discrete architectures and the implementation of structure/activity relationship studies. The body of work on cyclodextrin, calixarene, pillarene, fullerene, macrocyclic peptide and cyclotrehalan platforms here reviewed illustrates the potential of the MNP concept to produce single isomer constructs that can be programmed to mediate the safe delivery of nucleic acids to the target cells.

Contents

1. Introduction

The prospects of gene therapy as a strategy for the treatment, cure or prevention of human disorders are strongly associated to

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http://dx.doi.org/10.1016/j.jddst.2017.03.012
1773-2247/

our capacity to develop safe and efficient nucleic acid carriers (vectors) that can provide protection to exogenous gene material (DNA or RNA) and promote the anticipated beneficial effect in the target cells or tissues [1]. Viruses are able to perform this task in a very competent manner, and a number of viral vectors have successfully progressed to clinical trials and, in a few cases, to the market [2,3]. Nevertheless, the concerns associated to their intrinsic immunogenicity, high production costs and limitations regarding the size of the polynucleotide that can be transported jeopardize translation from bench to bedside [4]. Nonviral vectors provide opportunities to overcome these problems, but the efforts to emulate virus levels of performance have met limited success [5]. Despite the broad battery of systems developed along the years of research, including cationic polymers, dendrimers, lipids and nanoparticles, there is no consensus up to date on the physicochemical properties of the carrier that empower efficacious DNA or RNA transport across the cellular barriers and the final manifestation of the expected biological function. A main reason is that most of the nonviral vectors reported so far lack accurate structural and/or conformational definition, which represents a major limitation for conducting structure-activity relationship (SAR) studies in view of vector optimization. Thus, although living/controlled polymerization currently allows accessing a variety of cationic polymers with a high degree of homogeneity, as far as polydispersity and molecular weight is concerned [6], it remains a considerable defy to precisely control the sequences of individual oligomer units with a definite size and in a specific topology [7,8]. Cationic lipids, on their side, are generally used in multicomponent formulations that interact with nucleic acids as liposome entities, not as individual molecules; they must therefore be considered as a type of nanovector, with the intrinsic incertitude in surface composition associated to supramolecular soft materials [9,10]. Differently from classical polymers, dendrimers can be manufactured as precise macromolecules; yet, they lack the ability to maintain a well-defined three-dimensional topology, and their conformation and structure are highly dependent upon external parameters not always amenable to control [11,12]. Consequently, an urgent need remains in the field for molecularly well-defined vectors susceptible of systematic chemical modifications through procedures warranting diastereomeric purity and well-suited for diversity-oriented schemes.

Precision macromolecular synthesis, relying in highly efficient regioselective transformations, represents an appealing concept towards the above channels. Monodisperse architectures with accurately controlled arrangements of chemical bonds and functional groups and covering a broad range of sizes and shapes become accessible through these postulates, offering considerable potential for the encapsulation, delivery and controlled release of (bio)pharmaceuticals, including nucleic acids [13,14]. The inherent characteristics of macromolecules, especially the large number of functional groups, the increasingly complicated purification steps, with the subsequent penalty in the overall isolated yield, and the high degree of conformational freedom make it difficult to guarantee a defined primary structure even if relatively simple reaction sequences are implemented. Such limitation can be overcome to a great extent by linking precisely defined subunits using “click” chemistry and other efficient chemical transformations [15]. In order to achieve further control on the hierarchical organization of the resulting “nanomolecules”, particularly in the presence of nucleic acids, the use of rigid platforms with definite arrangements of functional groups is advantageous. Nanometric molecular systems exhibiting persistent topology (shape and volume), either of natural/technological origin or accessed through precision macromolecular synthesis, have been named “molecular nanoparticles” (MNPs). Note that this notion, as advanced by Cheng and coworkers [13], differs from the more extended use of the MNP term to designate aggregated nanoparticles assembled from small molecules through noncovalent interactions. In the context of this review the first sense has been adopted: hereinafter MNP refers to nanosize molecular entities well-defined in chemical structure. To fit in this definition, their elaboration must warrant strict monodispersity regarding stereochemistry, molecular weight, size, functionality, symmetry or any other property ascribable to a single isomer entity. The installation of cationic functional groups on the periphery of MNPs can then promote attractive coulombic interactions with the polyphosphate backbone of nucleic acids, whereas the overall molecular shape shall impose packing constraints to the self-assembly. The presence of additional functionalities may further promote collective secondary interactions, eventually imparting transfection capabilities to the resulting MNP/nucleic acid nanocomplexes. The possibility of correlating specific chemical modifications of the MNP to measurable effects on the sequence of events leading to successful delivery of the DNA or RNA cargo enables unprecedented opportunities for breaking the current boundaries in nonviral gene delivery.

2. Molecular nanoparticles in gene delivery

Several cage molecules and rigid macrocycles have been already used as scaffolds for the construction of MNP vector prototypes [16]. Cyclodextrins and calixarenes have pioneered the field, which has been further enriched with newcomers such as pillarenes, fullerenes, macrocyclic peptides and cyclotrehalans. This review will discuss the advances carried out in the last years in the design of well-defined MNPs for nucleic acid delivery, with emphasis in their potential for providing information on the molecular determinants governing efficient transfection. MNP-based aggregates (e.g. resorcarene-based micelles or nanofibers assembled from cyclic peptides) [17,18], star polymers constructed on MNP cores or polymers/dendrimers having pendant or backbone-embedded MNP building blocks (e.g. cyclodextrin polymers or fullerene-centered dendrimers) [19,20] do not conform to this definition and will not be discussed here. Approaches based on the use of cage molecules, such as cyclodextrins or cucurbiturils, to modulate the nucleic acid complexing properties of cationic polymers or dendrimers through rotaxanation strategies are also out of scope; the reader is eagerly addressed to excellent reviews and recent contributions dealing with these architectures to expand the information [21,22].

2.1. Cyclodextrins

Cyclodextrins (CDs) are cyclic oligosaccharides formed by six (αCD), seven (βCD) or eight (γCD) α(1 → 4)-linked D-glucopyranosyl units that feature a truncated cone-shaped toroidal macroring, with a hydrophilic exterior and a relatively hydrophobic inner surface (Fig. 1). In aqueous media, CDs are able to host hydrophobic molecules in the cavity to form inclusion complexes, thereby increasing the solubility of the guest and protecting it from the environment. These remarkable properties have found broad application in drug formulation. However, the straightjacket imposed by the rigid cup-like CD architecture critically limits the size and geometry of the potential guests [23–25]. The dimension barrier can be broken by creating multicavity systems that expose several CD moieties with an overall geometry matching that of the guest molecule of interest [26]. Alternatively, chemical functionalization has been put forward to impart self-assembling capabilities to the CDs. Nanospheres, nanocapsules or nanovesicles with a much higher loading capacity that the individual CD derivatives have been devised in this manner [27–30], paving the way for