Acta Biomaterialia 48 (2017) 378–389

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

Well-defined star polymers for co-delivery of plasmid DNA and imiquimod to dendritic cells

Wenjing Lin [a,b], Samuel Hanson [a], Wenqing Han [a], Xiaofang Zhang [b], Na Yao [b], Hongru Li [a,c], Lijuan Zhang [b,*], Chun Wang [a,*]

[a] Department of Biomedical Engineering, University of Minnesota, 7-105 Hasselmo Hall, 312 Church Street S.E., Minneapolis, MN 55455, USA
[b] School of Chemistry and Chemical Engineering, South China University of Technology, Guangzhou 510640, PR China
[c] College of Pharmacy, State Key Laboratory of Medicinal Chemical Biology and Tianjin Key Laboratory of Molecular Drug Research, Nankai University, Tianjin 300071, PR China

ARTICLE INFO

Article history:
Received 13 August 2016
Received in revised form 8 October 2016
Accepted 26 October 2016
Available online 27 October 2016

Keywords:
Co-delivery
Star copolymer
Micelles
DNA delivery
Imiquimod

ABSTRACT

Co-delivery of antigen-encoding plasmid DNA (pDNA) and immune-modulatory molecules has importance in advancing gene-based immunotherapy and vaccines. Here novel star polymer nanocarriers were synthesized for co-delivery of pDNA and imiquimod (IMQ), a poorly soluble small-molecule adjuvant, to dendritic cells. Computational modeling and experimental results revealed that the polymers formed either multimolecular or unimolecular core-shell-type micelles in water, depending on the nature of the outer hydrophilic shell. Micelles loaded with both IMQ and pDNA were able to release IMQ in response to intracellular pH of the endo-lysosome and transfect mouse dendritic cells (DC2.4 line) *in vitro*. Importantly, IMQ-loaded micelle/pDNA complexes displayed much enhanced transfection efficiency than IMQ-free complexes. These results demonstrate the feasibility of co-delivery of pDNA and IMQ to antigen-presenting cells by multifunctional polymer nanocarriers with potential use in gene-based vaccine approaches.

1. Introduction

The development of nano-scale drug carriers is bringing fundamental changes to clinical medicine. A recent and emerging trend is the focus on singular but multifunctional nanocarriers, capable of simultaneous or temporally coordinated delivery of two or more therapeutic and diagnostic modalities, so as to achieve synergistic biological responses. Such nanocarriers can be based on polymeric materials that accommodate multiple types of cargo (e.g. small-molecule drugs, proteins/peptides, DNA/RNA, inorganic molecules) with very different physico-chemical properties and mechanisms of action [1–6].

Combining gene therapy with traditional small molecule drug therapy is highly attractive in treating complex diseases such as cancer. Nonviral polymeric gene vectors have been designed to carry both plasmid DNA (pDNA) and small molecule drugs and target them to the same group of cells [7–11]. Ideally, polymeric nanocarriers suitable for co-delivery of pDNA and drugs should have the following essential features: (i) condensing pDNA to form stable colloidal particles conducive to efficient cellular uptake; (ii) binding to drugs to enhance solubility and stability; (iii) releasing pDNA intracellularly to achieve transgene expression; (iv) releasing drugs intracellularly to reach their molecular targets; (v) not toxic to cells.

Co-delivery is particularly important in gene-based vaccine approaches. Polymeric carriers for antigen-encoding pDNA can be designed to transfect antigen-presenting cells (APCs) [12–14]. At the same time, immunoregulatory adjuvants, such as Toll-like receptor (TLR) ligands, are needed for stimulating APCs (mainly dendritic cells and macrophages) and inducing antigen-specific humoral and cellular immune responses [15–17]. Evidence suggests that delivering both antigens and adjuvants simultaneously to the same APCs may lead to more potent immune responses than delivering them separately [18–21]. Various formulation strategies have been reported, which combine pDNA with immunostimulatory adjuvants and integrate them into the same polymer platforms, thus maximizing the chance of simultaneous delivery of these molecules to the same target cells. One example is layer-by-layer stacking of pDNA and poly(I:C), a TLR3 ligand, with a cationic biodegradable poly(β-amino ester) through electrostatic interactions, so as to achieve simultaneous delivery from degradable polylactide microneedles [22]. Another example is covalent conjugation of a synthetic TLR7 agonist to the surface of chi-

* Corresponding authors.
E-mail addresses: celjzh@scut.edu.cn (L. Zhang), wangx504@umn.edu (C. Wang).

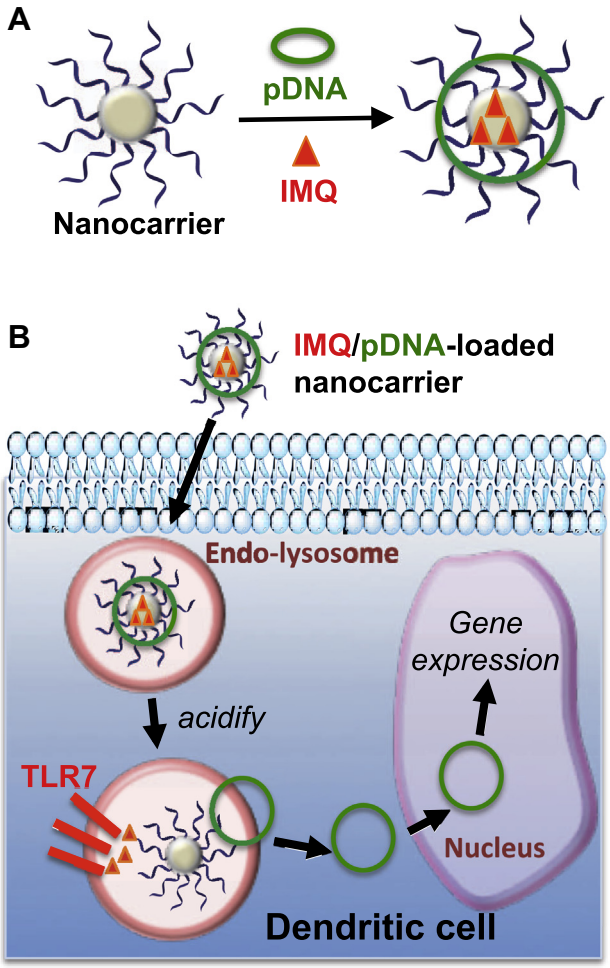
http://dx.doi.org/10.1016/j.actbio.2016.10.038
1742-7061/

tosan/DNA polyplexes via a polyethylene glycol (PEG) linker to accomplish simultaneous transfection and stimulation of macrophages [23].

Imiquimod (IMQ) is a small-molecule immunostimulatory adjuvant with the ability of stimulating APCs through TLR7 [24,25]. IMQ is poorly soluble in water, and the most common formulation, a 5% topical cream, partially addresses this problem [26–28]. In some preclinical and clinical studies involving DNA vaccine, IMQ is administered as injection or through topical application [24,29]. Also considering that its molecular target, TLR7, is located in the endo-lysosome of the cell [30], it is logical to design polymeric particles that encapsulate IMQ with improved solubility and deliver IMQ to the endo-lysosome after entering the cell through endocytosis. Indeed, the group of Trimaille has recently reported core-shell-type polymeric micelles encapsulating the hydrophobic IMQ molecules in the hydrophobic core of polylactide. These IMQ/polymer micelles were able to stimulate dendritic cells *in vitro* more effectively than free nonencapsulated IMQ, presumably due to more efficient cellular uptake and intracellular release [31,32]. In an earlier study, acid-labile microparticles of acetalated dextran loaded with IMQ were shown to release IMQ rapidly in aqueous buffer of pH 5. Improved immunostimulatory effect of macrophages was observed, presumably due to the microparticles releasing IMQ triggered by the slightly acidic environment inside the endo-lysosome [33]. While these previously reported polymer micelles and microparticles have shown promise as carriers for IMQ, they were not designed for co-delivery of both pDNA and IMQ.

The co-delivery approach of pDNA and IMQ to dendritic cells is illustrated in Scheme 1. A polymeric nanocarrier specifically designed for co-delivery should have the dual capacity of binding to both pDNA and IMQ through electrostatic and hydrophobic interactions, respectively. The resulting IMQ/pDNA/nanocarrier complexes may enter a dendritic cell via phagocytosis and be internalized into the endo-lysosome. Subsequent acidification of the endo-lysosome should trigger the release of IMQ, allowing it to engage its molecular target, TLR7, located in the endo-lysosomal membrane. In parallel, the nanocarrier should be able to transport pDNA across intracellular barriers and eventually reach the nucleus, where gene transcription occurs (Scheme 1).

In this study, we aim to develop synthetic polymer nanocarriers for pDNA/IMQ co-delivery. Specifically, we focus on addressing two important issues with the co-delivery scheme: one, how different structural designs of the polymer nanocarrier affect the delivery capacities of pDNA and IMQ, and two, how co-delivery of IMQ influences transfection efficiency of pDNA in dendritic cells. To investigate these questions, we have synthesized and characterized two star polymers with certain common structural features for binding to both pDNA and IMQ (Scheme 2). The star polymers have β-CD as core, which connects to 21 arms of linear polymer chains consisting of several blocks. The inner block connected to β-CD is composed of polycaprolactone (PCL). Its hydrophobicity should enable IMQ loading through hydrophobic interaction. Biodegradability of PCL should ensure low cytotoxicity and may also facilitate IMQ release inside the endo-lysosome. Next to the PCL block is a cationic block of poly(2-aminoethyl methacrylate) (PAEM). Bearing primary amines in its side chains, PAEM is a chemically simple yet effective delivery vehicle for pDNA. It was used previously by our group as a model polymer for structure-function relationship studies on polymer-mediated DNA vaccine delivery to dendritic cells [34–36]. One of the two star polymers, represented as β-CD-(PCL-*b*-PAEM)$_{21}$, contains only the PCL and PAEM blocks in its arms. The second star polymer, named β-CD-(PCL-*b*-PAEM-*b*-PPEGMA)$_{21}$, has an additional outer block of poly (polyethylene glycol methacrylate) (PPEGMA), meant to provide steric stabilization of the star polymer in aqueous solvents and prevent nonspecific interaction with proteins and cells. These star polymers were evaluated for their capacities of loading and releasing IMQ under physiological and endosomal pHs and for binding and condensing pDNA. Cytotoxicity and the impact of IMQ loading on gene transfection efficiency in dendritic cells were also determined. This exploration of structure-property relationship in the context of IMQ/pDNA combined delivery may lead to important insight that informs the design of more effective nanocarriers for gene-based immunotherapy and vaccine approaches.



Scheme 1. Illustration of the general approach of polymeric nanocarrier mediated co-delivery of imiquimod (IMQ) and plasmid DNA (pDNA) to a dendritic cell.

2. Materials and methods

2.1. Materials, cell culture, and plasmid

ε-Caprolactone (ε-CL, 99%, Aldrich, St. Louis, MO, USA) was dried over calcium hydride and distilled under reduced pressure before use. Poly(ethylene glycol) methyl ether methacrylate (PEGMA, M_n = 475 Da, 99%, Aldrich) was purified by passing through a column filled with neutral alumina to remove inhibitor. Tetrahydrofuran (THF) was dried over sodium using benzophenone as a dryness indicator and distilled under nitrogen prior to use. Toluene was distilled over calcium hydride. Imiquimod (98%) was purchased from Sigma Chemical Co. 2-Bromoisobutyryl bromide (98%, Alfa Aesar, Ward Hill, MA, USA), 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA, 99%, Aldrich), paraformaldehyde (99%, Aldrich), *tert*-butyl N-(2-hydroxyethyl)carbamate,