Nanomedicine: Nanotechnology, Biology, and Medicine
11 (2015) 1575–1584

nanomedicine
Nanotechnology, Biology, and Medicine

nanomedjournal.com

Stimuli-responsive liposomes for the delivery of nucleic acid therapeutics

Fatemeh Movahedi, MS[a], Rebecca G. Hu, PhD[b], David L. Becker, PhD[b], Chenjie Xu, PhD[a,*]

[a]*School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore*
[b]*Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore*

Received 28 November 2014; accepted 11 March 2015

Abstract

Nucleic acid therapeutics (NATs) are valuable tools in the modulation of gene expression in a highly specific manner. So far, NATs have been actively pursued in both pre-clinical and clinical studies to treat diseases such as cancer, infectious and inflammatory diseases. However, the clinical application of NATs remains a considerable challenge owing to their limited cellular uptake, low biological stability, off-target effect, and unfavorable pharmacokinetics. One concept to address these issues is to deliver NATs within stimuli-responsive liposomes, which release their contents of NATs upon encountering environmental changes such as temperature, pH, and ion strength. In this case, before reaching the targeted tissue/organ, NATs are protected from degradation by enzymes and immune system. Once at the area of interest, localized and targeted delivery can be achieved with minimal influence to other parts of the body. Here, we discuss the latest developments and existing challenges in this field.

Key words: Nanomedicine; Nanoparticle; Stimuli-responsive liposome; Controlled drug delivery; Nucleic acid therapeutics

Nucleic acid therapeutics (NATs) are composed of deoxyribonucleic acid (DNA) and/or ribonucleic acid (RNA) based molecules that aim to enhance or eliminate specific gene expression and consequently the levels of the related protein.[1] NATs have attracted much attention over the last decade as they have proven to be useful tools in deciphering complex biological processes, and demonstrated efficacy in ameliorating disease conditions that are related to abnormal expression of specific gene or protein.[2] Unfortunately, most of the work is still confined to basic research in the laboratory and few have been translated into clinical applications.[3] This delay has been attributed to some extracellular and intracellular hurdles faced by NATs such as degradation, non-specificity and systematic toxicity (Figure 1).[4]

The first challenge is the survival of NATs in the blood stream where NATs are exposed to serum nucleases, serum

Abbreviations: DC-Chol, 3β-[N-(N′,N′-dimethylaminoethane)-carbamoyl] cholesterol; DDAB, dimethyldioctadecylammonium bromide; DLPC, dilauroyl phosphatidylcholine; DMRIE, 1,2-dimyristyloxypropyl-3-dimethyl-hydroxy ethyl ammonium bromide; DMG, dimyristoyl-*sn*-glycerol; DOAB, dimethyldioctadecylammonium bromide; DODAP, 1,2-dioleoyl-3-dimethylammonium propane; DOPE, dioleylphosphatidylethanolamine or 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine; DOPG, 1,2-dioleoyl-sn-glycero-3-phospho-(1′-rac-glycerol) (sodium salt); DOTAP, 1,2-dioleoyl-3-trimethylammonium propane or N-[1-(2,3-dioleoyloxy)]-N,N,N-trimethylammonium propane methylsulfate; DSG, distearoyl-*sn*-glycerol; DPPC, dipalmitoylphosphatidylcholine; DPPA, 1,2-dipalmitoyl-*sn*-glycero-3-phosphate acid; DSPC, 1,2-Distearoyl-*sn*-glycero-3-phosphatidylcholine; DSPE, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine; DSPE-PEG, 1,2-distearoyl-*sn*-glycero-3-phosphoetanolamine-N-[amino(polyethylene glycol)]; DSPE-PEG$_{2000}$-NH$_2$, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (ammonium salt); DSTAP, 1,2-distearoyl-3-trimethylammonium-propane; MPB-PE, 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide] (sodium salt); PE, phosphatidylethanolamine; POPE, 1-palmitoyl-2-oleoyl-snglycero-3-phosphoethanolamine; SOPC, 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine; SPION, super paramagnetic iron oxide nanoparticles; TNS, 6-(p-toluidino)-2-naphthalenesulfonic acid; TMAG, N-(α-trimethylammonioacetyl)-didodecyl-D-glutamate chloride.

The XCJ lab is partially supported by the Tier-1 Academic Research Funds by Singapore Ministry of Education (RG 64/12 to XCJ). The DLB lab is supported by the Start-up Funds by Lee Kong Chian School of Medicine, Nanyang Technological University.

*Corresponding author at: School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore, Singapore.
E-mail address: cjxu@ntu.edu.sg (C. Xu).

http://dx.doi.org/10.1016/j.nano.2015.03.006
1549-9634/

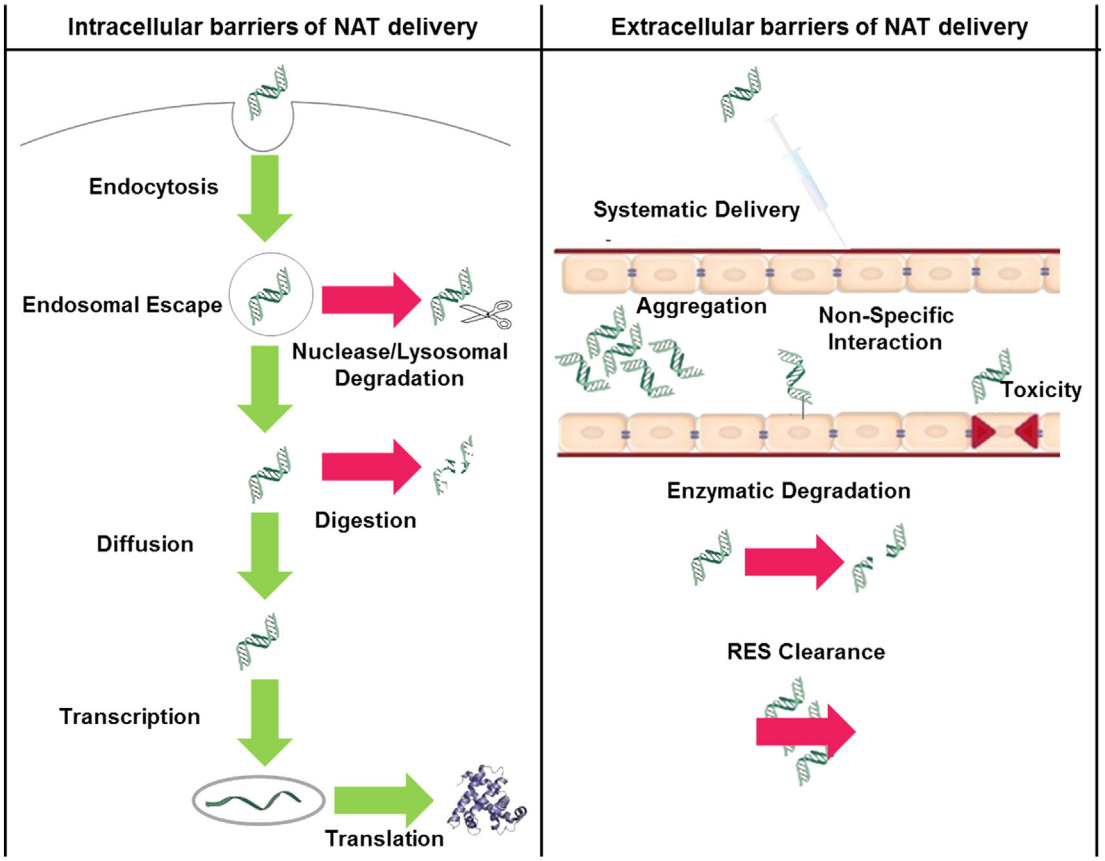

Figure 1. Extracellular and intracellular barriers in the delivery of nucleic acid therapeutics. Adapted with permission from Jones et al.[5] Copyright (2013) American Chemical Society.

proteins and immune cells. Any surviving NAT needs circulate to the target tissue while avoiding filtration by the kidneys. Once at the target tissue NATs face another set of challenges imposed by the vascular endothelial barrier which is tightly controlled, a dense network of extracellular matrix that hinders the diffusion of NATs, and the lipophilic cellular membrane that prevents the uptake of large and charged molecules. Finally, even after successfully entering cells, NATs have to escape the endosomes/lysosomes to reach the cytoplasm and in some cases the nucleus.

One way to overcome these hurdles is to deliver NATs with nanoparticle (NP)-based carriers or nanocarriers.[6] NPs offer several advantages including: (i) NPs with the appropriate surface coating may increase the concentration and efficacy of NATs at the target site by reducing the binding of serum proteins and minimize degradation by serum nucleases[7]; (ii) NPs can be designed to achieve regulated controlled release during transportation or at the site of action[8]; (iii) it is feasible to incorporate both hydrophobic and hydrophilic drugs into NPs and improve bioavailability by enhancing the solubility of hydrophobic drugs[9]; (iv) NPs can be decorated with specific ligands targeting specific receptors that were over-expressed on the diseased cells.[10]

Among the wide range of NP-based platforms, liposomes are one of the most studied nanocarriers.[11] Liposomes consist of a biocompatible phospholipid bilayer and they can protect amphiphilic, hydrophilic, and/or hydrophobic drugs against a variety of threats that lead to immediate dilution and degradation.[12] They enter cells mainly through the endocytosis pathway, in which the liposomes are engulfed by the plasmid membrane and taken into the cytoplasm.[13] Once inside the endosomes/lysosomes, there is an electrostatic interaction between the lipoplex (complex of liposome and nucleic acid) and cytoplasmic-facing side of endosomal membrane. This interaction leads to the formation of neutralized pairs, which disrupt the endosome/lysosome and release NATs to cytoplasm.[14]

The use of liposomes for drug delivery started shortly after their invention by Bangham and coworkers in 1965.[15,16] The first liposome-based drugs (Myocet and Doxil) were approved by USA Food and Drug Administration (FDA) for cancer treatment in 1995.[17] Since then, a wide range of therapeutics including both hydrophobic (e.g. paclitaxel) and hydrophilic (e.g. hydroxyurea) small molecules have been successfully incorporated in liposomes to improve their efficacy and/or efficiency. Today there are about 8 commercialized liposome-based products and another 53 liposomal formulations in different stages of clinical trials.[18,19]

The success of liposomes in optimizing small molecule drugs has driven researchers to explore the possibility of addressing the challenges met by NATs *in vivo*.[20,21] An advantage of using liposomes is their biocompatibility and biodegradability since most are made with naturally occurring lipids.[22-25] In addition, the composition-dependent surface charge of liposomes can help