Advanced Drug Delivery Reviews 99 (2016) 28–51

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

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

PEGylation as a strategy for improving nanoparticle-based drug and gene delivery☆

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ARTICLE INFO

Article history:
Received 27 June 2015
Received in revised form 21 September 2015
Accepted 26 September 2015
Available online 9 October 2015

Keywords:
Mucosal delivery
Liposomes
Stealth coatings
Mononuclear phagocyte system (MPS)
Enhanced permeability and retention (EPR) effect

ABSTRACT

Coating the surface of nanoparticles with polyethylene glycol (PEG), or "PEGylation", is a commonly used approach for improving the efficiency of drug and gene delivery to target cells and tissues. Building from the success of PEGylating proteins to improve systemic circulation time and decrease immunogenicity, the impact of PEG coatings on the fate of systemically administered nanoparticle formulations has, and continues to be, widely studied. PEG coatings on nanoparticles shield the surface from aggregation, opsonization, and phagocytosis, prolonging systemic circulation time. Here, we briefly describe the history of the development of PEGylated nanoparticle formulations for systemic administration, including how factors such as PEG molecular weight, PEG surface density, nanoparticle core properties, and repeated administration impact circulation time. A less frequently discussed topic, we then describe how PEG coatings on nanoparticles have also been utilized for overcoming various biological barriers to efficient drug and gene delivery associated with other modes of administration, ranging from gastrointestinal to ocular. Finally, we describe both methods for PEGylating nanoparticles and methods for characterizing PEG surface density, a key factor in the effectiveness of the PEG surface coating for improving drug and gene delivery.

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☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Non-Antigenic Regulators-Maiseyeu".

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http://dx.doi.org/10.1016/j.addr.2015.09.012
0169-409X/

1. Introduction

In order to deliver adequate concentrations of systemically administered therapeutics to target tissues, these materials must circulate in the blood stream for as long as possible. However, proteins and peptides are rapidly degraded and cleared from the blood stream, necessitating approaches for increasing circulation time. One such approach is to coat the surface of the therapeutic with an inert polymer that resists interactions with components of the blood stream, imparting "stealth" properties. Polyethylene glycol (PEG) is the most widely used "stealth" polymer in the drug delivery field, due to its long history of safety in humans and classification as Generally Regarded as Safe (GRAS) by the FDA. Considered the first reports of PEGylation for drug delivery, Davis and Abuchowski described in 1977 the covalent attachment of PEG to bovine serum albumin and liver catalase proteins [1]. They found that by optimizing the PEGylation chemistry and the extent of PEGylation, they could increase the systemic circulation time and decrease the immunogenicity of the proteins without significantly compromising activity. In 1990, the FDA approved the first PEGylated protein product, Adagen®, a PEGylated adenosine deaminase enzyme for severe combined immunodeficiency disease [2]. Since then, 8 other PEGylated protein therapeutics have been FDA approved for treatment of diseases ranging from rheumatoid arthritis to age-related macular degeneration [2].

The success of protein PEGylation as a method for producing longer circulating, and thus, more efficacious intravenous therapies led to investigations of nanoparticle (NP) PEGylation for systemic applications in the early 80s and 90s [3–5]. Recognized as foreign objects, NPs are readily cleared from systemic circulation by the cells of the mononuclear phagocyte system (MPS), precluding accumulation in target cells and tissues. However, similar to what was observed with PEGylated proteins, PEG coatings on NPs shield the surface from aggregation, opsonization, and phagocytosis, thereby prolonging circulation time. The first FDA approval of a PEGylated nanoparticle (NP) product, Doxil®, came in 1995. Doxil "Stealth®" liposomes increased doxorubicin bioavailability nearly 90-fold at 1 week from injection versus free drug, with a drug half-life of 72 h and circulation half-life of 36 h [6–8]. In the years since, PEGylation has become a mainstay in NP formulation. Although much of the initial development of PEGylated NPs focused on systemic administration, in this review we also highlight the benefits of NP PEGylation for overcoming biological barriers to effective delivery associated with numerous modes of delivery, ranging from injection into the eye to topical mucosal applications. Special emphasis is given to studies that directly compare PEGylated to non-PEGylated formulations to specifically demonstrate the benefits of NP PEGylation. Further, we discuss common methods for PEGylating NPs, as well as quantifying a critical parameter that influences the efficiency of delivery, the surface PEG density.

2. Nanoparticle PEGylation for improved systemic delivery

2.1. The potential fates of systemically administered nanoparticles

Systemically administered NPs can potentially reach and deliver therapeutic payloads to every vascularized organ/tissue in our body. Prolonging the retention time in the blood has been accepted as the frontline strategy, since it provides higher probability of circulating NPs to encounter, and partition into, the targets of interest. However, this task has been challenging primarily due to the presence of the MPS. The MPS consists of dendritic cells, blood monocytes, granulocytes, and tissue-resident macrophages in the liver, spleen, and lymph nodes that are responsible for clearing, processing, and degrading exogenous materials in the blood stream [9]. Unlike other organs, endothelia in organs associated with the MPS are often fenestrated, which facilitates screening of circulating entities. NPs as large as 100 nm can passage through the endothelial fenestrae in the liver and spleen, and also through permeable vascular endothelia in lymph nodes [10,11]. Thus, the MPS provides a critical defense mechanism that protects against foreign pathogens, but at the same time, rapidly eliminates therapeutic NPs from the blood stream. NPs circulating in the blood are readily recognized by serum proteins called opsonins, including complement compounds, immunoglobulins, fibronectin and apolipoproteins [12]. Adsorption of opsonins onto NP surfaces (opsonization) renders NPs more susceptible to phagocytosis by cells in the MPS. Opsonized NPs are taken up by MPS cells via numerous types of opsonin-recognizing receptors abundant on the cell surface, including complement, Fc and fibronectin receptors [13,14]. Although opsonin absorption to NPs occurs preferentially via hydrophobic interactions [15,16], electrostatic interactions and hydrogen bonding interactions have also been shown to mediate opsonization [17]. Of note, NPs can also be directly captured by macrophages by opsonin-independent scavenger receptors [18–20] that often recognize repeating patterns [12]. It has been reported that several tens to hundreds of types of serum proteins readily interact with circulating NPs, thereby forming a protein corona on the NP surface [21,22]. The corona formation non-specifically facilitates uptake of NPs by cells encountered during their circulation, including endothelial cells [21,22], similar to the opsonin-dependent MPS cell uptake. Thus, protein absorption not only reduces the circulation time, but also weakens the targeting capabilities of NPs functionalized for targeting specific cells [23].

Aggregation of circulating NPs can also undermine their circulation time, regardless of uptake by MPS or other non-target cells. Uncharged, hydrophobic NPs rapidly aggregate via van der Waals and/or hydrophobic forces in aqueous conditions. In contrast, positively or negatively charged NPs, due to the repulsive forces, generally retain their colloidal stability in aqueous solutions with low ionic strength. However, under high ionic strength, such as in the blood, electrostatic interactions between NPs with counterions neutralize the particle surface charge, thereby rendering the NP surface amenable to aggregation. Prolonged exposure to circulating serum proteins also elevates the chance of NP aggregation [22]. Large aggregates formed by NP–NP interactions and/or protein adsorption are prone to physically block pulmonary capillary beds, providing another mechanism by which NPs are eliminated from the blood circulation. For example, DNA NPs based on numerous cationic polymers or lipids were found destined to the lung rather than the liver [24–26], presumably due to the entrapment of aggregates in narrow capillary beds. Lastly, NPs can be also cleared by renal excretion, but typical NPs designed for drug and gene delivery applications are likely to avoid glomerular filtration due to their relatively large sizes (>10 nm). Overall, conventional NPs are generally cleared from the blood circulation within 10 min following systemic administration, irrespective of the NP composition [9,27].