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Research review paper

Functional and biodegradable dendritic macromolecules with controlled architectures as nontoxic and efficient nanoscale gene vectors

Kui Luo [a], Bin He [a], Yao Wu [a], Youqing Shen [b,c,*], Zhongwei Gu [a,**]

[a] National Engineering Research Center for Biomaterials, Sichuan University, Chengdu 610064, China
[b] Key Laboratory of Biomass Chemical Engineering of Ministry of Education, Zhejiang University, Hangzhou 310027, China
[c] Center for Bionanoengineering, Zhejiang University, Hangzhou 310027, China

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ABSTRACT

Gene therapy has provided great potential to revolutionize the treatment of many diseases. This therapy is strongly relied on whether a delivery vector efficiently and safely directs the therapeutic genes into the target tissue/cells. Nonviral gene delivery vectors have been emerging as a realistic alternative to the use of viral analogs with the potential of a clinically relevant output. Dendritic polymers were employed as nonviral vectors due to their branched and layered architectures, globular shape and multivalent groups on their surface, showing promise in gene delivery. In the present review, we try to bring out the recent trend of studies on functional and biodegradable dendritic polymers as nontoxic and efficient gene delivery vectors. By regulating dendritic polymer design and preparation, together with recent progress in the design of biodegradable polymers, it is possible to precisely manipulate their architectures, molecular weight and chemical composition, resulting in predictable tuning of their biocompatibility as well as gene transfection activities. The multifunctional and biodegradable dendritic polymers possessing the desirable characteristics are expected to overcome extra- and intracellular obstacles, and as efficient and nontoxic gene delivery vectors to move into the clinical arena.

Contents

* Corresponding author. Tel./fax: +86 571 87953993.
** Corresponding author. Tel.: +86 28 85410336; fax: +86 28 85410653.
E-mail addresses: shenyq@zju.edu.cn (Y. Shen), zwgu@scu.edu.cn (Z. Gu).

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1. Introduction

Gene therapy, by introducing exogenous gene, gene segments or oligonucleotides into specific cells of the patient (Mhashilkar et al., 2001; Wong and Chiu, 2010), has attracted significant attention over the past two decades as a potential therapeutic modality for treating a variety of both inherited and acquired diseases. Gene therapy has also been investigated as an alternative strategy to traditional radiotherapy and chemotherapy for cancer treatment (El-Aneed, 2004; Fu et al., 2012; Gurnani et al., 1999; Kang et al., 2010). Hundreds of clinical trials of gene therapy have been carried out, and some breakthroughs have been achieved at the beginning of the 21st century (Huang and Kamihira, 2013). However, no single successful outcome was reported. One of the reasons is that the plasmid DNA is easily degraded by serum nucleases in the bloodstream (Niven et al., 1998). To address this challenge, it is proposed to protect plasmid DNA using tools called gene vector and specifically deliver to targeted tissue/cells (Li and Huang, 2000; Verma and Somia, 1997). Currently, however, the main obstacle for human gene therapy is the lack of safe, efficient and controllable methods for gene delivery (Pack et al., 2005).

To design and prepare biocompatible and efficient gene vectors, it requires complete understanding of the interaction mechanism between the target cell and delivery system (Felgner et al., 1994; Hatakeyama et al., 2011; Radwan Almofti et al., 2003), as well as the intercellular traffic and targeting mechanism (McLendon et al., 2010; Tan et al., 2013). Some barriers in each step of the gene delivery procedure have to be overcome, including targeting delivery the plasmid DNA to target tissues/cells, the cellular uptake of the complexes by endocytosis, cellular release taken place to initiate DNA transcription and translation (Al-Dosari and Gao, 2009). Currently, gene vectors can be divided into two categories: viral vectors and nonviral vectors, each category has its own advantages and disadvantages (Mintzer and Simanek, 2009; Seow and Wood, 2009). Viral gene vectors, such as retrovirus, adeno-associated virus, lentiviruses, adenoviruses, herpes simplex virus and pox virus (Chen et al., 2011; McTaggart and Al-Rubeai, 2002; Williams et al., 2009), consist of viruses to efficiently carry their genome from one host cell to another, which can deliver the genes into the cells for expression (Huang and Kamihira, 2013) and have demonstrated some advantages, such as high gene transfection efficacy, constant expression and expression of therapeutic genes (Hewinson et al., 2013). However, the concerns along with viral vectors are the limitations in target-cell specificity, immunogenicity, toxicity, resistance to repeated preparation and the high costs of manufacturing, which forced researchers to design other vectors (Thomas et al., 2003).

Nonviral vectors offer one alternative to overcome this dilemma, showing opportunities for improving biosafety, greater chemical function flexibility and large-scale production (J. Guo et al., 2011; Pathak et al., 2009; van Gaal et al., 2011; Viola et al., 2010). In general, nonviral vectors, including synthetic vectors, natural materials and functional natural products, are materials that can electrostatically bind and condense DNA or RNA into particles with diameter of one to several hundred nanometres, navigate the plasmid DNA to cellular entry (Han et al., 2012). Various synthetic vectors, such as nanoparticles (Gajbhiye and Gong, 2013; Guo et al., 2010; Kumar et al., 2013; Xu et al., 2008), lipids (L. Li et al., 2012), cationic polymers (Dai et al., 2011; Eltoukhy et al., 2012; Q. Hu et al., 2012; Sun and Zhang, 2010; Venkataraman et al., 2011), micelles (Tian et al., 2005; Zhu et al., 2008), peptides (Gong et al., 2012; Han et al., 2013; Saccardo et al., 2009), polypeptide (He et al., 2012; Yen et al., 2013) and dendrimers (Mehrabadi et al., 2012; Šebestík et al., 2012), offer potential routes for compacting plasmid DNA, siRNA duplex, microRNA and ODN for systemic delivery. However, compared to viral analogs, the main limitation of nonviral vectors is the low *in vitro* and *in vivo* gene transfection efficacy as they are hindered by numerous extra- and intracellular obstacles, and in some cases of toxicity and *in vivo* instability. Different strategies have been carried out to improve the drawbacks, and the efforts are still ongoing (Wang et al., 2012; Yang et al., 2012).

Various macromolecules including natural materials and synthetic polymers have been employed as gene delivery vectors and extensively studied (Canine and Hatefi, 2010; De Smedt et al., 2000; Merdan et al., 2002; Sun and Zhang, 2010; Xu et al., 2009), showing great potential applications (Lv et al., 2006). The polymers with high molecular weight (HMW) as nanoscale gene vectors demonstrated high gene transfection efficacy, due to the improvement of DNA stability and uptake (Ward et al., 2001). However, there are still some risks as toxicity resulted in the slow degradation *in vivo* and cytotoxicity derived from potential adverse interactions with membranes (Fischer et al., 2003; Kunath et al., 2003; van de Wetering et al., 1997). The polymers with low molecular weight (LMW) demonstrated good biosafety due to the possible elimination from the kidneys *in vivo*. However, compared to HMW counterparts, the LMW polymeric vectors exhibit reduced DNA condensation abilities due to their lower electrostatic interactions, lower stability of DNA/vector complexes and much lower transfection efficacy. As an alternation to cationic polymeric vectors, biodegradable polymeric systems with HMW and low charge density are designed and exhibit high efficient gene delivery (S. Guo et al., 2011), some of which have surpassed the efficiency of the commercial available transfection reagents polyethylenimine (PEI) and Lipofectamine 2000 both *in vitro* and *in vivo* (Ahn et al., 2002; Ding et al., 2012; J.-H. Kim et al., 2011; Luten et al., 2008; Zhong et al., 2005).

Dendritic macromolecules, including dendrimers, dendrons, hyperbranched polymers and their relative hybrids (Gao and Yan, 2004), have been studied as gene delivery vectors both *in vitro* and *in vivo* (Dufès et al., 2005; Fu et al., 2007; Guillot-Nieckowski et al., 2007; Paleos et al., 2007, 2009; Shen et al., 2010). Compared to linear cationic polymeric analogous, the highly branched, globular architecture of these macromolecules give rise to a number of interesting properties, such as increased solubility, very low intrinsic viscosities and nanoscale size (Dufès et al., 2005; Kadlecova et al., 2013). Meanwhile, the similar properties of HMW counterparts along with high gene transfection, as well as potential toxicity, were observed by dendritic polymeric vectors as nanoscale gene delivery vectors (Luo et al., 2012). Therefore, the biodegradable dendritic polymeric vectors have been focused on gene delivery (Wu et al., 2005, 2006). Compared to the non-degradable dendritic vectors, the potential advantage of biodegradable counterparts is their reduced toxicity and the avoidance of accumulation of the vectors in the cells after repeated administration, since the degradation enhances the cleanse of vectors from cells and bodies once it ends its task as carriers (Cheng et al., 2012; He et al., 2012; Son et al., 2010), as shown in Fig. 1. Additionally, the degradable features of the vectors can increase the release of DNA from the complexes into the cytosol. In this review, the recent researches of functional and degradable dendritic systems as efficient and nontoxic gene vectors will be summarized and discussed. The challenges and prospect of functional and biodegradable dendritic macromolecules as nanoscale gene delivery vectors are also outlined.

2. The design of functional nonviral vectors for gene delivery

The transfection process of DNA/vector complexes includes several steps and parameters (Mulligan, 1993; Wang et al., 2012): i) the formation of compact DNA/vector complexes, ii) overcoming several physiological barriers, such as evade uptake by macrophages, the clearance by renal filtration and degradation by endogenous nuclease, and delivery DNA/vector complexes to target tissues/cells, iii) the endocytosis of DNA/vector complexes, iv) lysosome escape, v) nucleus transfection. Additionally, low side-effects, both toxicity/pathogenicity of the delivery vehicle and immune responses to the gene therapy, should be considered. Fully understanding the desirable characteristics effecting the transfection will be beneficial for the design of efficient and safe nonviral gene vectors with the ability to overcoming delivery