Tailoring carriers for specific applications by unveiling the role of individual components in pBAE/polynucleotide polyplexes: on road to rational formulations

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Introduction

Polynucleotide-based therapies steadily grow into transforming the whole (bio)medicine field as they show tremendous potential for prevention or treatment for a wide range of infectious diseases or genetic disorders.[1] Currently there are more than 2.400 clinical trials comprising gene therapies.[2] Remarkably, the first treatment applied worldwide, SARS-CoV-2 prophylactic vaccines, has only given a glimpse of the involved intricacies and revealed the untapped potential of polynucleotide-based therapies.[3,4] Despite their theoretical full potential, these therapies still find unsatisfactory clinical outcomes which is mainly related to the low chemical stability and poor bioavailability of polynucleotides in-vivo.[5]

Overcoming these drawbacks is accomplished by compartmentalizing the polynucleotides into designed nanocarriers (***Figure 1A***) programmed to deliver them into the specific tissue and intracellular compartment.[6] Genetically engineering viral vector emerged as a natural carrier for gene therapies as their ubiquitous ability to infect and transport the therapeutic nucleic acids across cellular barriers.[7] However, viral vectors are concerned in different aspects, such as safety, immunogenicity, insertional mutagenesis, low loading capacity and high production costs. These drawbacks triggered the development of nanotechnology-based non-viral delivery systems (***Figure 1B***), which on the one hand are modular to meet application requirements and on the other hand their production is cost-effective.[9]

Among non-viral carriers, poly(β-amino ester)s (pBAE) are an outstanding option as a carrier, due to their high building blocks versatility, high transfection efficacy, low toxicity and excellent biocompatibility and biodegradation profile[10]. Upon contact with polynucleotides, pBAEs condense into polymeric nanoparticles, (polyplexes), that protects the fragile cargo and transports it into the targeted tissue[11]. Additionally, pBAE building blocks contain hexyl monomers (C6) that provide certain hydrophobicity to the polymer, that allows freeze-drying of the polyplexes, a remarkable property for achieving long-term storage and ameliorated logistics of distribution.[9,12] Further functionalization of the pBAE vectors with short cationic oligopeptides (OM-pBAE), such as lysine (K), Arginine (R), and Histidine (H) (C6CK3, C6CR3 and C6CH3 respectively) are designed not only to increase their buffering capacity and ability to compact nucleic acids due to electrostatic interactions, but also to promote cellular internalization and nucleic acid transfection.[9]

As we previously described, C6CK3 promotes the plasmatic membrane crossing, C6CH3 assists in the endosomal scape development and C6CR3 gives an advantage to nuclear subcellular localization. Cationic peptides, however, increase the polyplex positive surface charge resulting in transfection promiscuity. Consequently, these pBAE-based polyplexes have low selectivity. Decreasing the surface charge and thus promiscuity is achieved by coating the polyplexes with Aspartic acid (D) functionalized pBAEs. In this sense, it is clear that careful combination of different OM-pBAEs is thus crucial for achieving an optimal functionality.[9,13]
Indeed, various OM-pBAEs combination have achieved optimal results *in-vitro* and *in-vivo*.[11] Despite these outstanding outcomes, the different pBAEs structure in the polyplexes and therefore their influence in the final properties is still unclear. These unknown characteristics involve a time-consuming experiments without specific and clear application due to the uncertainty of their target and uptake specificity, which are needful characteristics in order to achieve the medical needs.

Recently, we demonstrated the microstructure of C6CR3/pDNA polyplexes and an oligopeptide-dependence of time evolution of the cationic polyplexes using high resolution optical microscopy.[14,15] We aimed to understand the microstructure of polyplexes composed by mixtures of OM-pBAE and determine the role of individual components in the final formulation and create a library of OM- pBAE carriers based on a rational selection in function of the chased properties for an specific application.
For this purpose, we study six polyplex constructions with different compositions: KH, KHD, RH, RHD, RK and RKD, combining different OM-pBAEs (***Figure 1C***). We reveal the microstructure and physicochemical properties of the different polyplexes. Our results allow the rational

engineering of carriers for any required application and allows for the further development of pBAEs building blocks for the optimal delivery of polynucleotides.

Experimental methods

Being assisted with advanced techniques such as Fluorescence Resonance Energy transfer (FRET), enhanced dark field spectral microscopy, Atomic Force Microscopy (AFM) and microscale thermophoresis (MST), we have been able to understand and therefore tailor the OM-pBAE carriers, by selecting the most accurate oligopeptide combination, based on the needs or interests requested for the specific application goal. Therefore, we have been able create a wide OM-pBAEs library for different application goals.

Conclusions

Thanks to the mechanical and physicochemical properties given by each oligopeptide combination (***Table 1***), an OM-pBAE NPs library has been created. Thus, for the first time, we reveal properties that will allow tailoring the carriers by selecting the most accurate oligopeptide combination[16], based on the needs or interests requested for the specific application goal. Consequently, our results will boost future research on OM-pBAE to facilitate their transfer to clinical applications.

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Figures

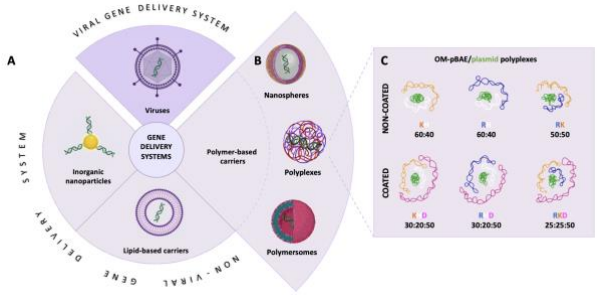

Figure 1. **Gene delivery systems scheme.** A) Schematic classification of viral and non-viral gene delivery systems. B) Polymer-based nanocarriers subtypes. C) Poly (b aminoester) - pBAE/plasmid polyplexes constructions and its polymer/plasmid ratio used.

Table 1. Scheme of the different NPs constructs properties and their possible applications. In where "*" mean lowest values and "***" higher values.

NPs Formulation	Z - potential	Size	EE	Adhesion	Binding Affinity	Stability	Applications
KH	***	*	***	**	***	*	Vaccination purposes, immunotherapy[15]
KHD	*	**	**	**	**	**	Inhaled delivery and muscle regeneration purposes
RH	***	*	*	***	*	**	Chronic diseases (cardiovascular, artheosclerosis, tumour treatment...)
RHD	*	***	*	***	**	**	Muscle regeneration purposes and inhaled delivery
RK	**	*	*	**	*	**	Chronic autoimmune disease (diabetes)
RKD	*	**	**	***	***	**	Dermo-cosmetic aims (retarding ageing)