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Review

Localized, non-viral delivery of nucleic acids: Opportunities, challenges and current strategies

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ABSTRACT

Localized delivery of drugs is an emerging field both with regards to drug delivery during disease as well as in tissue engineering. Despite significant achievements made in the last decades, the efficient delivery of proteins and peptides remains challenging, especially in cases requiring long-term release of proteins after application. The localized delivery of nucleic acids (NA) represents an interesting alternative due to higher physicochemical stability of NA, increased efficiency by harnessing cells as bioreactors for the production of required proteins and improved versatility with regards to expression of specific proteins through plasmid DNA or repression of gene products through siRNA. However, unlike most proteins and peptides, NA must be delivered to the cytoplasm or nucleus to be efficacious, resulting in significant delivery challenges. We herein describe frequently used non-viral vectors for the delivery of NA including polyplexes, lipoplexes and lipopolyplexes and summarize recent developments in the field of nucleic acid delivery systems for local application based on hydrogels, solid scaffolds and physical delivery methods. The challenges associated with the different approaches are identified and options to address these challenges are discussed.

1. Introduction

The efficient delivery of nucleic acids (NA) is one of the most exquisite challenges to formulation scientists nowadays. Unlike small chemical drugs and most biologic drugs, NA must be delivered into the cytoplasm or into the nucleus to be effective. Small interfering RNA (siRNA) and antisense oligonucleotides interact with mRNA and therefore must be transported to the cytoplasm. However, transport of NA across the cell membrane is challenging due to its negative charge and high molecular weight [1]. Plasmid DNA (pDNA) additionally requires translocation into the nucleus where it is transcribed into mRNA, eventually resulting in protein synthesis.

From a physicochemical and formulation point of view NA are almost ideal drug molecules. They possess high stability against both chemical and physical degradation, which is

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exemplified by the possibility to extract and sequence DNA from fossils of extinct species and the remarkable half-life of DNA of 521 years [2,3]. It is therefore not surprising that several studies investigating the stability of formulated NA yielded promising results, especially when nucleic acids were protected from hydrolytic and enzymatic degradation, e.g. through freeze-drying [4–6]. It is also important to note that NA potentially require significantly less formulation development work once an optimal formulation has been found compared to other macromolecular drugs such as proteins and peptides. The relatively simple structure of NA results in minimal changes of physicochemical properties of NA when changing the sequence. In contrast, exchange of a single amino acid may result in significantly changed structure and physicochemical properties in the case of proteins and peptides [7,8]. Therefore, it is reasonable to assume that reformulation efforts to fulfill the needs of NA with a new sequence will be minimal. Furthermore, NA, especially siRNA and antisense oligonucleotides can be simply produced by chemical synthesis with good yield and in high purity. In contrast, production of proteins such as monoclonal antibodies or growth factors requires a cellular expression system and extensive purification and/or refolding [9]. Finally, NA possess high specificity, resulting in expression of a specific protein (plasmid DNA) or inhibition of a specific target gene (siRNA, antisense oligonucleotides), potentially maximizing the ratio of desired to undesired effects compared to alternative therapies [10].

Apart from the numerous advantageous properties of NA, the requirement for delivery into the cytoplasm or nucleus represents a significant challenge. This challenge is addressed using attenuated viral vectors as delivery systems or non-viral delivery strategies. A discussion of the general advantages and disadvantages of viral versus non-viral vectors is beyond the scope of this review and the reader is referred to the review by Guo et al. for detailed information on this topic [11]. We herein focus on non-viral delivery strategies, which are often preferred because of safety concerns associate with viral vectors and versatility and ease of modification of non-viral vectors [11].

In most studies, systemic administration of NA delivery systems through the parenteral route is investigated, primarily because of its versatility, e.g. with regards to treatment of distant or disseminated cells, mostly in the context of tumor therapy. However, achieving efficient delivery of NA to target cells after parenteral application requires overcoming numerous extracellular and intracellular barriers. Stability and compatibility of the carrier system in the blood stream must be maintained to achieve prolonged circulation, allowing the delivery system to reach its target site. This entails surface modification with hydrophilic polymers to avoid uptake of the delivery system by the reticulo-endothelial system (RES), crosslinking to prevent premature release of the NA cargo and control of delivery system size to avoid blocking of capillaries and to allow extravasation at the target site, e.g. in the tumor or liver [12,13]. Additionally, active or passive targeting strategies may facilitate the efficient extravasation and cellular uptake at the site of action. In recent years, stimuli-responsive systems have been developed, further improving accumulation of the delivery system at the site of action. These systems change their physicochemical properties in response to extracellular stimuli such as changes of the pH value, enzymatic environment etc. [14,15]. It is therefore obvious that the successful development of NA delivery systems for systemic administration requires an optimal combination of different properties within the delivery system, representing a significant formulation challenge. Furthermore, the clinical application of these delivery systems has been significantly limited because of concerns with regards to toxicity of the vehicle and unsatisfactory delivery efficiency.

While the majority of studies has focused on improving the efficiency, stability and compatibility of delivery systems for systemic application, localized delivery of NA represents an interesting but largely untapped alternative, worthy of increased exploration. While it is certainly not possible to achieve the versatility of systemic application, e.g. regarding transfection of distant or disseminated cells, localized delivery avoids several major barriers associated with systemic delivery and is therefore more likely to result in safe and efficacious therapy. Achieving sufficiently high drug concentrations at the target site is a common challenge in drug therapy. In the case of NA delivery this general challenge is aggravated by the necessity of intracellular delivery of NA [9]. Because of the sensitivity of NA towards enzymatic degradation, encapsulation is often required but on the other hand efficient release must be maintained [1]. These requirements increase the complexity of delivery systems, especially in the case of systemic delivery.

Within this review, we seek to give an overview of the current status of localized delivery of NA using non-viral delivery systems. The discussion is however focused to topical delivery in the broadest sense, excluding oral, nasal and pulmonary delivery as these delivery routes have additional special requirements to the delivery system. We shortly portray non-viral vectors that are employed for localized delivery of NA and review recent systems for localized NA delivery based on various matrices. Our aim is to highlight challenges but also opportunities associated with this approach to NA delivery and discuss potential fields of application.

2. Non-viral nucleic acid delivery systems employed for localized delivery

The high molecular weight and negative charge of NA represents a major hurdle to efficient cellular uptake. For most cell types, the size requirement for particle uptake is in the rage of up to 200 nm, significantly smaller than the hydrodynamic diameter of DNA of a few thousand base pairs [16]. Furthermore, negatively charged moieties anchored to the cell membrane result in electrostatic repulsion of negatively charged NA. Non-viral delivery agents are designed to improve cellular uptake efficiency either through complexation and charge reversal of NA or through physical methods allowing NA to directly enter the cell. However, the challenge with such delivery systems is on the one hand to prevent the NA degradation within the micro- or nanoparticles and on the other hand to achieve efficient intracellular release [1].