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Non-viral polyplexes: Scaffold mediated delivery for gene therapy

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ABSTRACT

Non-viral gene delivery is emerging as a realistic alternative to the use of viral vectors with the potential to have a significant impact on clinical therapies. The documented dangers of using the efficient recombinant viruses as carriers have led many to explore the possible advantages of using polymer-based non-viral vectors. To date there is no gene delivery vehicle that contains all the desirable characteristics but they do exist individually in a variety of non-viral carriers, e.g. degradable, low toxicity, cell specific, relatively efficient and capable of delivering multiple genes. Polymers may not be as effective as the viral vehicles; however, the continued focus and growth of knowledge in this field has already resulted in improved delivery. Over the past 10 years, significant progress has been made through the design of specific polymers for this application. Another interesting development in this field is the influx of research on combination approaches to non-viral gene delivery. Scaffolds made of both natural and synthetic materials are being utilized to aid in sustained delivery of the polymer vectors. While the non-viral gene therapy field is currently receiving a large degree of dedicated research there is now the realistic potential of a clinically relevant output. This review presents a summary of combinatorial delivery systems of non-viral polyplexes delivered via tissue engineered scaffolds. For polyplexes to move into the clinical arena, it is important that we uncover and understand the technical hurdles that need to be overcome so that the efficacy of this promising technology can be established.

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1. Introduction

Polymeric systems have only recently become the backbone of gene delivery vectors. This is because most viral therapies have proven to be efficient but risky to the patient [1–4]. Genetic mutations are the root cause of several underlying diseases and pathologies. The only hope for many of these patients is a successful and safe gene therapy treatment. Although there have been reports on the use of polyplexes, efficient delivery systems have yet to be designed for these vector systems. Many challenges stand in the way of effective non-viral gene therapy, including (but not limited to) sustained long-term transgene expression, spatial and temporal control of that expression, targeting a specific tissue, and cellular infiltration leading to the delivery of DNA to the nucleus [5–9].

The aim of this review is to capture recent developments in combinatorial polymer-based systems that have been used as non-viral carriers for complexing the plasmid DNA and also as delivery vehicles for controlled release (Fig. 1).

2. Non-viral gene delivery

Non-viral gene delivery lacks the efficiency of viral mediated gene transfer but poses a safer method of gene transfection. As the delivery of naked plasmid is an inefficient method to mediate gene transfection, non-viral vectors are used [10]. Cationic polymers and liposomes are used to enhance gene transfection by protecting the plasmid from nuclease degradation and enabling its passage through the cell membrane. As the vectors are cationic they are positively charged, hence they have a natural affinity for the negatively charged plasmid DNA and cell membrane [10–13].

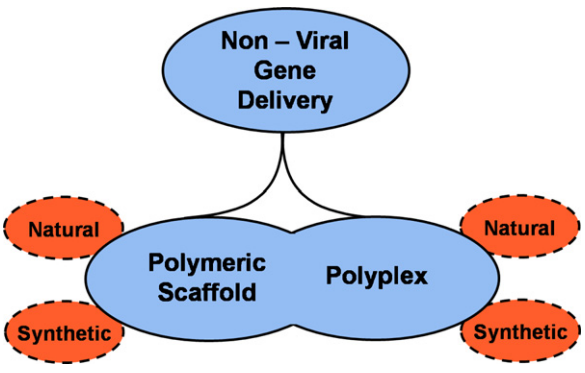

Fig. 1. Concept diagram of the combinatorial system of polyplexes and scaffold mediated delivery for gene therapy.

2.1. Polyplexes

A polyplex is formed as a result of electrostatic interactions between the cationic amine groups of the polymer and the anionic phosphate groups of DNA. In this form the DNA is condensed but remains active and is capable of transfecting cells. The effective diameters of polyplexes have been reported to range from 30 to 500 nm [8,14]. The polyplex is responsible for protecting the plasmid from enzymatic degradation. The characteristics of the polyplex can be controlled through the choice of polymer and through the N/P ratio. The N/P ratio is a measure of the number of nitrogen atoms on the polycation to the number of phosphate groups associated with the DNA. For the most part a surplus of positive charge or polymer is added to the polyplex. There are two main reasons for this: the excess positive charge results in a final positive zeta potential for the polyplex and the plasmid is protected by the polymer. An overall positive charge is important as it is well documented that positively charged particles are taken up by cells faster than negatively or neutrally charged particles [8,10–14].

The role of the polyplex is to effectively deliver the DNA into the nucleus of a cell. There are many unknowns with regard to this transfer and the mechanisms involved. Firstly the polyplex must be internalized by the cell membrane. There are many different possible routes including receptor mediated endocytosis, pinocytosis and phagocytosis [6,8,11]. Receptor mediated endocytosis or clathrin-dependent endocytosis is the most common method of internalization as ligands may be attached to the polyplex to facilitate this process [6,15,16]. The addition of ligands is discussed further in a subsequent section. Pinocytosis is the process by which cells internalize liquids which contain suspended or soluble particles [16]. Untargeted polyplexes that form an electrostatic relationship with the cell membrane may be internalized in this way [8]. Phagocytosis is another possible method of internalization. It involves the ingestion of particles larger than 0.5 μm in diameter [16]. Targeting and internalization of microspheres to phagocytic cells *in vivo* can be achieved through size exclusion. It is important to note that internalization mechanisms may also be largely dependent on the cell type, the polymeric vector used, and the process parameters of that polyplex [6].

After internalization occurs, the polyplex is believed to be most commonly contained within an endosytic vesicle. It is then transferred to late endosomes and lysosomes. In these compartments the pH rapidly changes to the range of 4.5–6. There are also many degradative enzymes in these vesicles. In order for transfection to occur, the DNA must