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Microparticulate poly(vinyl alcohol) hydrogel formulations for embedding and controlled release of polyethylenimine (PEI)-based nanoparticles

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ABSTRACT

Nucleic acid-based therapeutics offer enormous potential in the treatment of various pathologies. For DNA or RNA delivery, nanoparticle systems based on lipids or polymers have been developed. However, colloidal instability in solution, poor storage properties especially as dry powder and little stability against an aggressive environment, e.g. after oral application, are major issues. Furthermore, there is an urgent need for sustained release systems that allow for fine-tuned, long-term temporal and spatial nanoparticle release.

In this paper, we describe the embedding of polymeric nanoparticles for gene delivery, namely polyethylenimine (PEI)-based polyplexes and their corresponding liposome-modified analogues (lipopolyplexes), into microparticulate poly (vinyl alcohol) (PVA) hydrogels. Various parameters are modified, and major differences are found. PVA is explored with two different molecular weights and at different concentrations, furthermore different emulsifiers and acetone for extraction are employed, and the protocol is performed with or without repetitive freeze/thaw cycles for physical PVA crosslinking.

We thereby establish Nanoparticles-in-Microparticle Delivery Systems (NiMDS) that are extensively characterized and shown to allow prolonged storage as easy-to-handle formulation (dry powder) without loss of nanoparticle activity. Unexpectedly, the nanoparticles' PVA encapsulation/release alters important physicochemical nanoparticle properties and biological activities in a favourable way.

Furthermore, we also demonstrate the nanoparticle release to be dependent on the microstructure of the PVA matrix, which is determined by the degree of physical crosslinking through a defined number of freeze/thaw cycles. We show that these defined physically crosslinked PVA hydrogels thus represent sustained release devices for fine-tuned, long-term nanoparticle release in possible therapeutic applications.

Statement of Significance

The present paper for the first time describes the embedding of polymeric PEI-based polyplexes and lipopolyplexes into poly(vinyl alcohol) (PVA) hydrogels, to establish novel Nanoparticles-in-Microparticle Delivery Systems (NiMDS). Through modification of various parameters including different PVA molecular weights and concentrations, different emulsifiers and defined numbers of freeze-thaw cycles for physical PVA crosslinking, sustained release devices are also obtained. Beyond favourable alterations of important physicochemical/biological nanoparticle properties and the possibility for prolonged storage as easy-to-handle formulation (dry powder), we show that these NiMDS also allow the tailor-made, fine-tuned, long-term release of fully active nanoparticles in possible therapeutic applications.

1. Introduction

The approach of using nucleic acids for the treatment of cancer or genetic disorders is a promising strategy, especially with regard to targets otherwise considered as undruggable. However, several

Abbreviations: Gluc, glucose; HN, HEPES-NaCl buffer; Luc, luciferase; LPP, lipopolyplexes; NiMDS, Nanoparticles-in-Microparticle Delivery Systems; PEI, polyethylenimine; PP, polyplexes; PVA, poly(vinyl alcohol).

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physiological hurdles make the delivery of DNA or RNA a major challenge in gene therapy, and many nanoparticle systems based on lipids or polymers have been developed to overcome this [1]. Among those, polyethylenimine (PEI) is a well-known and promising representative that has been extensively explored for DNA and siRNA delivery in vitro and in vivo [2–6]. One major issue, however, is the low colloidal stability of PEI-based complexes ('polyplexes') that tend to aggregate quickly, preventing them from being kept at ambient temperatures. The combination of PEI complexes with liposomes, combining the favourable properties of both, has been explored as well [7,8]. While some of these lipopolyplexes show somewhat enhanced colloidal stability [8], they cannot be stored as dry powder or for a prolonged time in solution. Furthermore, some routes of administration would require protection of the nanoparticles, polyplexes or lipopolyplexes, against an aggressive environment (e.g., after oral application). Finally, in several cases therapeutic applicability and efficiencies would benefit from a sustained nanoparticle release from their site of application over a longer time period. This would allow switching from repeated injection, which is often performed, but rather complicated and accompanied by reduced patient comfort, towards alternative approaches like oral, pulmonary or transdermal application, or the reduction of injection frequencies. Microparticles or macroscopic systems are explored in order to protect a nanoparticle against the environment and allow for its fine-tuned temporal and spatial release. The encapsulation of nanoparticles into a protective matrix, for instance Nanoparticles-in-Microparticle Delivery Systems (NiMDS), is a suitable strategy. Previously, the hydrophobic poly (ε-caprolactone) (PCL) and type-B gelatin have been described as NiMDS for the oral delivery of plasmid DNA [9–12] or siRNA [13] (see [14] for review).

In contrast, in our study we rather chose to generate a NiMDS based on a hydrophilic polymer as carrier system. Poly (vinyl alcohol) (PVA) is a linear, synthetic polymer traditionally used in pharmaceutical science. Its ability to form films or to stabilize suspensions by increasing their viscosity, its bioadhesiveness, its low toxicity as well as many other favourable properties make it one of the best known and most commonly used excipient in pharmaceutical sciences and pharmaceutical industry. PVA-based hydrogels display superior mechanical properties and high biocompatibility [15]. A procedure to generate PVA hydrogels with high mechanical stability, while avoiding the use of crosslinking reagents, is based on repetitive freezing and thawing. This process causes the formation of crystallites, physically crosslinking the polymer strands [16–18]. The resulting PVA hydrogels have been well characterized with regard to their physical properties, depending on the molecular weight of the PVA, the concentration of the PVA solution and the number of freeze/thaw cycles [19]. Studies also showed the suitability of PVA hydrogels as mucoadhesive delivery devices [20] and artificial tissues [21–23]. Moreover, PVA matrices can be produced in various shapes, e.g. as microparticles [17], nanoparticles [24], nanofibers [25] or films [26]. While the feasibility of using PVA hydrogels as a controlled release device for proteins or low molecular weight compounds has been well established [16,17,27], the incorporation of nanoparticulate devices has been limited so far to silver nanoparticles for antibacterial activity [28,29], insulin-loaded PLGA nanoparticles [30] or Doxorubicin-loaded micelles [31]. In particular, their potential as a carrier system for nanoparticles for gene delivery, allowing prolonged nanoparticle storage, protection and controlled release with subsequent nucleic acid delivery has to the best of our knowledge not been explored so far and is established in this study.

2. Materials and methods

2.1. Materials

Branched polyethylenimine 10 kDa was purchased from Polyscience (Eppelheim, Germany) and rhodamine labeled branched 25 kDa PEI was from Surflay Nanotec (Berlin, Germany). 1,2-Dipalmitoyl-sn-glyero-3-phosphocholine (DPPC) was obtained from Avanti Polar Lipids (Alabaster, AL). Poly(vinyl alcohol) 30–70 kDa (87–90% hydrolyzed) and Span® 80 were purchased from Sigma Aldrich (Taufkirchen, Germany), and PVA 190 kDa (Mowiol® 55-96) was obtained from Kuraray (Hattersheim, Germany). Miglyol® 812 was purchased from Sasol (Hamburg, Germany). Labrafil® M CS1944 was kindly provided by Gattefossé (Bad Krozingen, Germany).

The luciferase pGL3 (Promega, Mannheim, Germany) and pEGFP-N1 (CLONTECH, Saint-Germain-en-Laye, France) expression plasmids were propagated in E. coli (DH5α) prior to purification with the MAXI KIT from Macherey and Nagel (Düren, Germany) according to the manufacturer's protocol. Zefluor® PTFE membrane filters with a pore size of 0.5 μm were purchased from VWR. The cytotoxicity detection kit (LDH) and the cell proliferation reagent WST-1 were obtained from Roche (Mannheim, Germany).

2.2. Liposome preparation

DPPC was dissolved in a chloroform/methanol mixture. A lipid film was prepared in a round-bottom flask by removing the solvent in a rotary evaporator. DPPC-liposomes were prepared by hydration of the lipid film in sterile, deionized water above the phase transition temperature in an ultrasonic bath. Liposomes were extruded 23× through a 200 nm polycarbonate membrane in a preheated Mini-Extruder (Avanti Polar Lipids, Alabaster, AL).

2.3. Preparation of PEI polyplexes and lipopolyplexes

PEI polyplexes were prepared as described previously at a PEI/DNA mass ratio 7.5 [32]. Briefly, DNA was dissolved in 5% glucose or in HN-buffer (10 mM HEPES, 150 mM NaCl, pH 7.4) in one vial while the corresponding amount of PEI was dissolved in a second vial of the same buffer. The DNA solution was added to the PEI solution, vortexed briefly and incubated for 30 min at room temperature. For lipopolyplex formation [8], resulting polyplexes were mixed with appropriate amounts of preformed liposomes (based on a lipid/PEI mass ratio of 5), vortexed thoroughly and incubated for 60 minutes at room temperature. For preparing rhodamine labeled nanoparticles, 10 kDa PEI was mixed 10:1 with rhodamine labeled 25 kDa PEI and processed as described above.

2.4. PVA microparticle preparation

Hydrogel-forming PVA microparticles were essentially prepared as described previously [17], with some modifications. PVA was dissolved in an appropriate amount of deionized water to yield a 10% or 20% (w/w) solution, by keeping at ~90 °C for at least 4 hours. The solution was autoclaved and stored at room temperature. PEI polyplexes or lipopolyplexes, prepared as described above and corresponding to 100 μg DNA, were suspended in 4 g PVA solution and stirred for 10 min using a magnetic stirrer. Upon addition of 10 g Span® 80, the mixture was stirred again for 5 min, prior to adding 30 g Miglyol® 812 and stirring for another 15 min. This mixture was added dropwise to 500 ml acetone under continuous stirring. The resulting suspension was vacuum-filtered through a Teflon® membrane (Zefluor® PTFE membrane filters, pore size of