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Shape Transformation Following Reduction-Sensitive PEG Cleavage of Polymer/DNA Nanoparticles

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Abstract

PEGylated polycation/DNA micellar nanoparticles have been developed that can undergo shape transformation upon cleavage of the PEG grafts in response to an environmental cue. As a proof-of-principle, DNA nanoparticles with higher PEG grafting density adopting long, worm- and rod-like morphologies, transition to more condensed nanoparticles with spherical and short-rod morphologies upon cleavage of a fraction of the PEG grafts from the copolymer. This shape transformation leads to increased surface charges, correlating with improved transfection efficiency.

The development of improved nonviral DNA delivery systems holds great promise for a number of disease applications including cancer, immune deficiencies, and metabolic disorders.^{1–3} Nanoparticles are an attractive nonviral delivery vehicle, owing to their ability to protect DNA from degradation, target specific cells and tissues, and improve the cellular binding and uptake.^{1, 4–8} In addition, recent studies have reported the ability to prepare nanoparticles with different shapes, significantly affecting the biological response on a systemic, tissue, and cellular level.^{9–14} For example, filamentous nanoparticles, after intravenous administration, have been shown to circulate significantly longer than their spherical counterparts in both rats and mice.¹⁵ Conversely, spherical gold nanoparticles showed higher cellular uptake compared to rod-shaped particles *in vitro*.¹⁶

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Electronic Supplementary Information (ESI) available: experimental methods, characterization of nanoparticle aspect ratio, TEM images of non-cleavable /PEI-g-PEG/DNA nanoparticles following DTT treatment, gel electrophoresis before and after PEG cleavage, zeta potential measurements of non-cleavable /PEI-g-PEG/DNA nanoparticles after DTT treatment, transfection efficiency of non-cleavable /PEI-g-PEG/DNA nanoparticles after DTT treatment, and nanoparticle stability in serum and salt. See DOI: 10.1039/

Generating a DNA nanoparticle delivery system that combines enhanced circulation stability and improved cell uptake through transformation of nanoparticle shape offers significant potential to improve nonviral gene delivery *in vivo*. The concept of shape-switching polymeric nanoparticles has been reported previously using a poly(lactide-co-glycolide)-based system, where nanoparticle shape switching from rod-like to spherical could be obtained using pH, temperature, or chemical cues over timescales ranging from minutes to hours.¹⁷ However, this study, and the aforementioned studies on nanoparticle shape, has not reported the ability to control the shape of plasmid DNA-containing nanoparticles for nonviral gene therapy applications. Previously, we have reported the ability to tune the shape of polymer/plasmid DNA nanoparticles through modulation of solvent polarity during nanoparticle assembly, generating a series of shapes ranging from spherical, rod-like, and worm-like.¹⁸ Furthermore, additional studies have also shown that polymer/DNA nanoparticle shape can influence circulation time as well as uptake and intracellular trafficking.^{19, 20} However, no study has reported the ability to transform the shape of DNA-containing nanoparticles in response to an external stimulus.

In this proof-of-concept study, we have generated a polymer/DNA nanoparticle system that can rapidly transform its shape from worm-like nanoparticles to condensed, spherical and short rod-like particles in a reducing environment. The system utilizes a linear polyethylenimine-*graft*-polyethylene glycol (*I*PEI-*g*-PEG) copolymer to condense plasmid DNA. *I*PEI is a widely used polymer for efficient nonviral gene delivery *in vitro* and *in vivo*, also showing reduced toxicity compared to branched PEI-based systems.^{21–25} PEG is often conjugated to *I*PEI and other polycation-based systems due to its ability to improve particle stability and reduce aggregation in physiological media.^{26, 27}

Fig. 1A shows a schematic representation of the shape transformation system. PEG has been linked to the *I*PEI backbone through a disulfide linkage, which can be

To demonstrate the reduction-sensitivity of the polymer, we first characterized the fraction of degradation of *I*PEI-*g*-PEG using gel permeation chromatography (GPC). As shown in Fig. 1B–C, the polymer degraded significantly by varying both the concentration of DTT and the time of incubation in reducing environments. From this study, we have chosen 5 mM DTT as the reducing condition for all subsequent shape transformation studies.

Three different *I*PEI-*g*-PEG copolymers were produced with increasing PEG grafting degrees, 0.5%, 1%, and 2%. Transmission electron microscope (TEM) images of nanoparticles prepared following polymer/DNA self-assembly showed that particles formed shapes ranging from long rods at 0.5% PEG grafting to worm-like shapes, with a small fraction of ring shapes, at 1% and 2% PEG grafting (Fig. 2A–C). The ring and worm-like shapes exist simultaneously, as we have shown previously in both experiments and computational modelling,¹⁸ reflecting the similar degrees of compactness of the complexes and free energy levels between the two shapes. Upon incubation with 5 mM DTT for 2 h, however, particles displayed a drastic transformation in morphology. At 0.5% PEG grafting, nanoparticles exhibited a spherical morphology with a diameter of 100 – 150 nm (Fig 2D). At higher grafting degrees, nanoparticles transitioned to short, rod-like shapes, with sizes ranging from 140 nm (length) × 80 nm (diameter) to 140 nm × 60 nm (Fig 2E–F and Fig

S1A, ESI). The presence of both ring-like and worm-like shapes does not appear to affect shape transformation, as the ring-like shapes were no longer evident after DTT treatment. Furthermore, after DTT treatment, particles displayed similar shapes to /PEI/DNA nanoparticles prepared without PEG (Fig. S2, ESI). Aspect ratios of the nanoparticles were measured from the TEM images to further confirm the shift in nanoparticle shape. Prior to PEG cleavage, aspect ratios were 5, 14, and 26 for 0.5%, 1% and 2% PEG grafting, respectively. However, after removal of PEG, aspect ratios dropped significantly to 1.4, 1.7, and 2.3 (Fig. S1B, ESI). As a control, we also prepared an /PEI-g-PEG/DNA nanoparticle where PEG was linked to the /PEI backbone through a non-cleavable linker. When these nanoparticles were exposed to 5 mM DTT treatment, no shape transformation was observed (Fig. S3, ESI).

In addition to TEM image analysis, nanoparticle surface charge was characterized with zeta potential measurements to confirm that PEG cleavage occurred simultaneously with shape transformation (Fig 2G). Zeta potential characterization is a common, effective method to confirm PEG shedding from a nanoparticle surface.^{28–30} Prior to PEG cleavage, all particles displayed near-neutral zeta potentials of +7 mV. After PEG removal, zeta potential rose significantly for all nanoparticles to 26 mV. While it is not possible to confirm that all PEG has been removed, a significant fraction has been cleaved, as the zeta potential reached levels similar to /PEI/DNA control nanoparticles. Once again for control, we tested the zeta potential of the non-cleavable /PEI-g-PEG/DNA nanoparticles and showed that DTT treatment did not lead to a zeta potential increase (Fig. S4, ESI). It is important to note that the particle shape transformation process did not lead to any detectable DNA release, as characterized by gel electrophoresis before and after PEG cleavage (Fig S5, ESI). The mechanism for shape transformation in this system appears to be driven solely by the removal of PEG from the nanoparticle surface.

We next investigated the kinetics of shape transformation over the course of 2 h through TEM image analysis. Nanoparticles were incubated with 5 mM DTT for times ranging from 5 min to 2 h, after which the DTT was quenched with a 2-fold molar excess of maleimide to stop the shape transformation. As shown in Fig. 3, nanoparticle shape exhibited a gradual transformation over the course of the 2 h incubation. At initial time points of 5 min and 15 min, a fraction of rod-like nanoparticles began to appear. Intermediate time points of 30 min and 60 min displayed a significant fraction of smaller, rod-like particles much shorter than the initial worm-like nanoparticles. By 2 h, particles transitioned completely to condensed, short-rod morphologies.

Finally, we tested the transfection efficiency of our nanoparticles before and after shape transformation using a luciferase reporter assay in HeLa cells. For this study, nanoparticles were pre-incubated with or without 5 mM DTT for 2 h, and then added to cells at a 2- μ g DNA dose. After 48 h, luciferase expression was assessed. Particles without DTT incubation showed very low luciferase expression (Fig. 4). However, upon PEG cleavage and shape transformation, luciferase expression increased significantly for all groups to a level similar to that of /PEI/DNA control nanoparticles. Control nanoparticles prepared with a non-cleavable linker did not show any increase in transfection efficiency following 5 mM DTT treatment (Fig. S6, ESI). Using TEM image analysis, we also confirmed that nanoparticle

shape did not change upon incubation with 150 mM sodium chloride or 10% (v/v) FBS (Fig. S7, ESI). The increase in transfection efficiency is primarily due to the PEG cleavage and shape transformation. It is important to note that, because shape transformation occurs concurrently with the removal of the PEG layer, the increase in transfection efficiency cannot be solely attributed to the shape, and more likely the combined result of surface charge and shape. It is worth noting that the shape transformation occurred as a result of PEG chain cleavage, a phenomenon that should be examined for additional PEGylated gene carriers. Considering the transfection efficiency of the spherical particles rising to the level similar to /PEI control particles, and the superior stability of the worm-like particles¹⁵, the polymer/DNA nanoparticle shape transformation scheme may lead to a useful strategy for nonviral gene delivery. This post-assembly shape transformation indicates the dynamic nature of the condensed DNA micelles. We are currently investigating additional environmentally-sensitive linkages that can allow us to investigate the effect of nanoparticle shape transformation *in vivo* to best harness the potential benefits of improved nanoparticle circulation stability and improved cell uptake and transfection efficiency in a single delivery system.

Conclusions

In conclusion, we have developed an /PEI-g-PEG/DNA nanoparticle delivery system that can transform its shape from long, worm-like to more condensed spherical and short-rod nanoparticles upon cleavage of the PEG grafts. The cleavage of PEG grafts also corresponds to a significant increase in nanoparticle surface charge, leading to a marked increase in transfection efficiency *in vitro*. Our novel, DNA shape-transformation system may assist the development of improved nonviral gene carriers for future *in vivo* applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

(A) Schematic representation of /PEI-g-PEG/DNA nanoparticle shape transformation under reducing conditions, where /PEI is grafted to PEG through a disulfide (d) linkage, which can be cleaved in the presence of a reducing agent, e.g. glutathione, DTT, etc. The /PEI-g-PEG carrier with higher PEG grafting degree condenses plasmid DNA into worm-like micelles, which transforms into spherical and rod-like micelles upon cleavage of the PEG grafts. As a result, the spherical micelles exhibit higher surface charge (zeta potential). The spherical and rod-like micelles also reflect more condensed morphology formed by plasmid DNA and /PEI-g-PEG with low grafting degrees (dotted line); (B) /PEI-g-PEG copolymer degradation after 1 h over varying DTT concentrations as characterized by GPC; (C) /PEI-g-PEG copolymer degradation in 1 mM DTT over varying incubation times as characterized by GPC.

Fig. 2. TEM images of /PEI-g-PEG/DNA nanoparticles (A–C) before and (D–F) after 2 h treatment with 5 mM DTT for 0.5% (A, D), 1% (B, E), and 2% (C, F) PEG grafting degrees, respectively. All scale bars represent 500 nm. (G) Average zeta potential of nanoparticles in water before and after PEG cleavage. Each bar represents mean $\pm$ standard deviation ($n = 3$).

Fig. 3. TEM images of the kinetics of /PEI-*g*-PEG (1%)/DNA nanoparticle shape transformation following 5 mM DTT treatment for (A) 0 min, (B) 5 min, (C) 15 min, (D) 30 min, (E) 60 min, and (F) 120 min. All scale bars represent 500 nm.

Fig. 4. Transfection efficiency of */PEI-g-PEG/DNA* nanoparticles before and after PEG cleavage compared to */PEI/DNA* control nanoparticles in HeLa cells. Each bar represents mean $\pm$ standard deviation ($n = 4$).