Recharging a Gene Delivery Vector: Towards Tunable Stability and Biofunctionalization Docking Sites

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Introduction:

Transferring a therapeutic gene to tumor cells is one of the most promising ways of treating cancers. Among non-viral gene delivery vectors, the polymeric-based complexes are one of the leading designs. However, no single multifunctional so-called polyplex has yet been able to go through the numerous locks posted along its systemic and intracellular trafficking. Those bottlenecks include plasma shielding, low immunogenicity, specific cell entry and endolysosomal escape. We have recently established a proof of concept for the use of a heterodimeric coiled-coil tethering system [1] enabling the oriented biofunctionalization of a model polyplex vector [2]. Briefly, a 5-heptad alpha-helical peptide (Kcoil) was covalently grafted onto a polyplex as a docking site for a protein tagged with its complementary peptide (Ecoil). Specific targeting of a polyethylenimine (PEI)/DNA polyplex to the epidermal growth factor receptor (EGFR) of an epidermoid cancer cell line (A431) was achieved through the strong and oriented docking of epidermal growth factor (EGF) onto the polyplex surface. This coiled-coil toolbox proved highly efficient in the fine-tuning of EGF surface density to target a model gene delivery vector towards a cell membrane receptor.

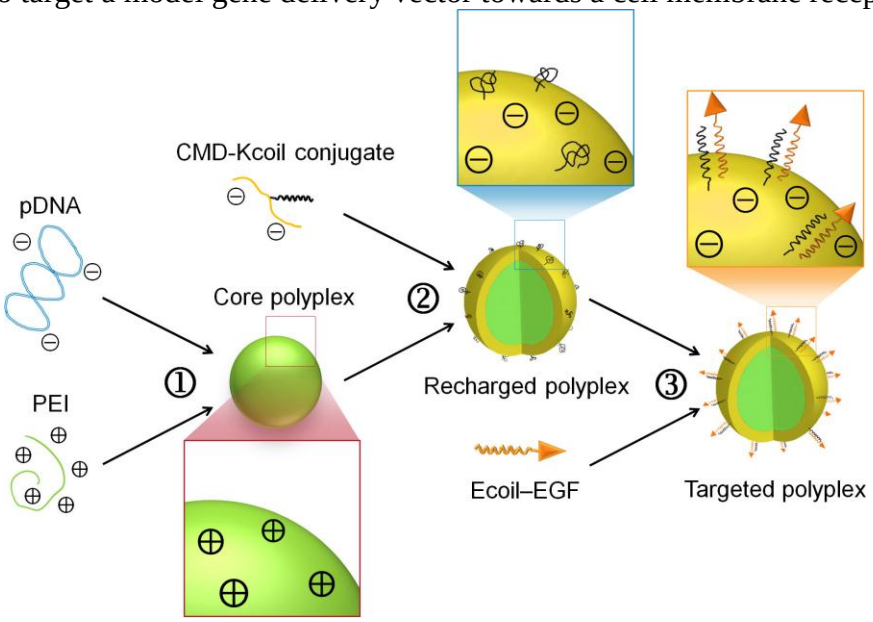

Figure 1. Three-step formation of our recharged and functionalized complexes.

To carry this promising platform a step further, we are now tackling the issues of stability in plasma, low immunogenicity, and endolysosomal escape of our vector. To do so, we designed a biodegradable polyanionic coating for positively charged PEI/DNA vectors based on carboxymethylated dextran (CMD). This layer can host Kcoil docks [3] and we hypothesized two additional features: its ability to loosen the tight PEI/DNA interaction, which is believed to be partly responsible for the limited gene expression of that system [4]; and its ability to shield the polyplex to achieve greater plasma stability by reversing its surface charge [5].

Materials and Methods:

CMDs were synthetized from 10-kDa dextran and chloro-acetic acid, and quantified by ^{1}H-NMR. PEI/DNA loosening was tested on agarose gel electrophoresis with a DNA-specific fluorophore probing. Size and surface charge of complexes were measured by dynamic light scattering (DLS). *In vitro* assays were performed on A431 cells and results were quantified by fluorometry in flow cytometry and live cell confocal microscopy.

Results:

We produced CMDs with various degrees of carboxymethylation (*dcm*) that were tested for polyplex coating and destabilization. Agarose gel electrophoresis indicated that no DNA was released from PEI/DNA complex after CMD addition, but DNA/SYBR Green partial fluorescence recovery suggested DNA loosening triggered by CMD (fig. 2A). The tested formulations yielded well defined stable complexes, as suggested by DLS monitoring (fig. 2B). These complexes displayed a negative surface charge ranging from -11 (mV) to -54 (mV) (fig. 2C). The 10% CMD yielded a quite larger complex, suggesting a successive layer-by-layer coating of free PEI and CMD that was successfully monitored by DLS (fig. 2D).

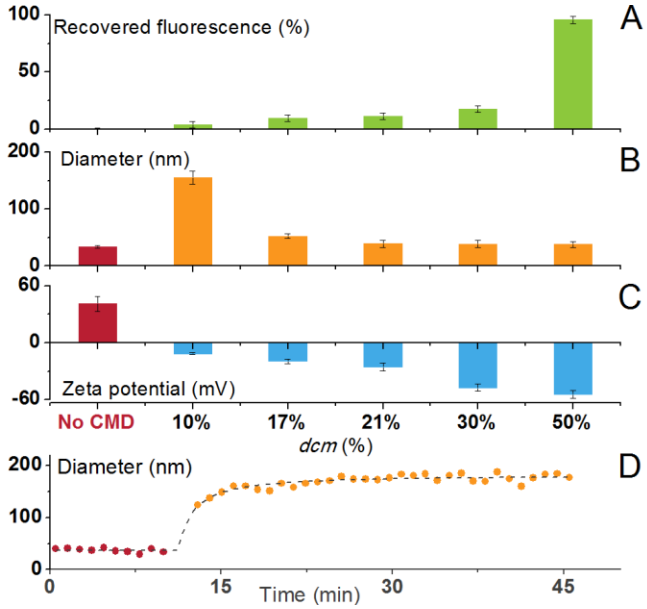

Figure 2. Impact of the *dcm* of dextran on the coating of PEI/DNA complexes: recovered DNA/SYBR Green fluorescence relatively with naked DNA in agarose gel electrophoresis (A); diameter of complexes after coating with CMD (B); surface charge of complexes after coating with CMD (C); coating kinetics with the 10% CMD (D).

Discussion:

We have previously carried out a proof of concept of the use of our coiled-coil tethering system for the surface enhancement of a polyplex gene vector. We then designed and carried out the biophysical characterization of a bio-degradable anionic coating for vector shielding and PEI/DNA interaction loosening. Different *dcm* triggered a tunable charge reversal and DNA loosening on PEI/DNA complexes. We are now focusing on *in vitro* tests to further explore our promising gene delivery vector in terms of surface biofunctionalization and gene transfer.

References:

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