Reactive Oxygen Species-responsive polyplex micelles as a PEG-detachable platform for plasmid DNA delivery

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Statement of Purpose: Gene delivery is one of most promising therapies to treat genetic diseases[1]. Recently, it has been demonstrated that polyplexes prepared from plasmid DNA (pDNA) and positively charged polymers can initiate therapeutic release in response to a significant change of physiological parameters (e.g., pH, MMPs, etc) during pathogenesis such as tumor progression. Reactive oxygen species (ROS) is suggested as an emerging parameter because its over-production is a hallmark of inflammation, tumor progression, and injury. Although progress has been made[2], much work remains to improve the ROS sensitivity of therapeutic release in pathophysiological conditions. The present study sought to develop polyplex micelles that can detach PEG for plasmid DNA delivery in response to pathophysiological levels of ROS production. Hence, we developed a new class of proline (P) oligomer-linked ROS responsive polymer, PEG_{113}-b-CP_5K-b-$pDMAEMA_{90}$-co-$pBMA_{60}$. This polymer has i) a hydrophilic detachable PEG as corona, ii) ROS cleavable CP_5K peptide as a linker, and iii) plasmid condensing cationic pDMAEMA with core stabilizing hydrophobic pBMA. Its ROS responsiveness, cell toxicity and cellular uptake were characterized.

Methods: The ROS-cleavable polymer was synthesized by combination of bio-conjugation methods and RAFT polymerization. Briefly, the ROS cleavable proline peptide (CP_5K) was conjugated with 5 kDa mPEG-MAL through cysteine end to yield mPEG-CP_5K-NH_2 conjugate, followed by coupling with NHS-ECT to produce PEG-CP_5K-ECT. The macro-CTA was used to polymerize N, N-dimethylaminoethyl methacrylate (DMAEMA) and n-butyl methacrylate (BMA) in a ratio of 60:40 using RAFT polymerization. A CP_5K-free control polymer, PEG_{113}-b-$pDMAEMA_{90}$-co-$pBMA_{60}$ (control polymer) was also synthesized by RAFT polymerization as reported previously[3]. The polyplex micelles were prepared at different n/p ratio (1-30) by mixing appropriate amount of polymer and pDNA (10 ug/ml). The size and zeta potential of polyplex were determined by Dynamic Light Scattering (DLS). The morphology of polyplex was characterized by transmission electron microscopy (TEM). Cell viability of RAW 264.7 macrophages was measured at different n/p ratio by alamar blue assay. Proline polyplex (n/p=5) micelles were prepared from AlexaFluor 488 conjugated dsDNA (50 nm) and its cellular uptake by RAW 264.7 macrophage was compared with the control polyplex micelles using flow cytometry. Macrophages were treated with LPS/IFN-γ to induce ROS overproduction.

Results: When treated with 3-morpholino-sydnonimine hydrochloride (SIN-1)(2 mM), a producer of peroxynitrile, PEG was detached from cationic pDMAEMA-co-pBMA, indicating ROS mediated cleavage of proline micelles (**Fig. 1A**). An increase in ζ-potential and destabilization of proline polyplexes (n/p=30) further supported the SIN-1-mediated destabilization (**Fig. 1B, C**). The polyplexes with n/p 5 or higher demonstrated stable micelle with an average diameter of 150 nm (**Fig. 1B**). Their ζ- potential was close to zero, suggesting shielding of the positively charged core with PEG corona, which was further supported by agarose gel electrophoresis (**Fig. 1D**).

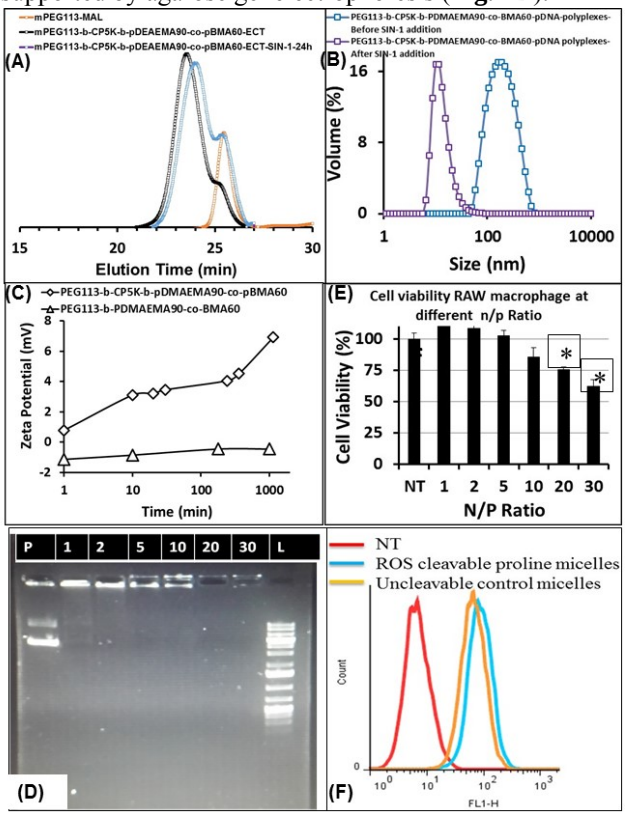

Figure 1. (A) GPC chromatogram shows cleavage of PEG from the proline polymer after 24 h treatment of SIN-1. **(B)** Proline polyplexes at n/p 30 ratio destabilize within 4 h treatment of SIN-1(4 mM). **(C)** The increase in zeta potential of the proline micelles compared to the control after SIN-1 treatment indicates detachment of PEG from proline micelles. **(D)** Agarose gel electrophoresis shows an effective binding of plasmid-polymer at n/p 5 or higher. **(E)** Cell viability of macrophages is maintained when exposed to polyplexes at different n/p ratio. **(F)** Activation of macrophages by treatment with LPS/IFN-γ promotes cell uptake (6h) of proline polyplexes over control polyplexes.

Macrophages maintained over 85% cell viability when treated with polyplexes up to n/p 10 ratio (**Fig. 1E**). Activated macrophages showed higher uptake of proline polyplexes compared to control polyplexes (**Fig. 1F**).

Conclusions: The new class of ROS-cleavable copolymer polyplex micelles demonstrated promising results for therapeutic delivery in physiologically relevant conditions. The ongoing studies to present include hemolysis, polyplex stability, optimized cellular uptake and gene transfection.

References: 1. Liu ZH. Prog Polym Sci. 2010; 35:1144–1162. **2.** Shim MS. Angew Chem Int Ed 2013; 52:1–5. **3.** Nelson CE. ACS Nano, 2013; 7; 8870–8880.