

Optimizing PEG Length for Enhanced *In Vivo* Pharmacokinetics of a Micellar siRNA Carrier

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Statement of Purpose: RNA interference (RNAi) by small interfering RNA (siRNA) has the potential to be applied therapeutically in the treatment of cancer or other pathologies whose etiology is related to aberrant gene overexpression¹. Due to the poor pharmacokinetic properties of siRNA, its use *in vivo* requires a carrier that provides effective stability in the blood, increased blood circulation time necessary for improved tumor accumulation, and mechanisms for endosomal escape into the cellular cytoplasm for intracellular delivery. Previous studies have established the *in vitro* efficacy of a pH-responsive diblock copolymer-based micelle for the delivery of siRNA². The polymer consists of a pH-responsive, endosomolytic block composed of butyl methacrylate (BMA), propyl acrylic acid (PAA), and dimethylaminoethyl methacrylate (DMAEMA) that is hydrophobic and drives micelle self-assembly and forms the micelle core. The second polymer block consists of a corona-forming poly(DMAEMA) block, which is positively charged at pH 7.4 and condenses anionic siRNA. However, these micellar nanoparticles have a high zeta potential (~17 mV), which causes blood cell aggregation and pulmonary toxicity when administered intravenously. In order to overcome this limitation, we have designed a mixed micelle approach that leverages the same endosomolytic terpolymer core but has a mixed corona containing varied densities and lengths of poly(ethylene glycol) (PEG). Our preliminary studies identified a lead candidate formulation based on the mixed micelles, and the current studies have focused on optimizing the length of the PEG in the corona in order to create a stable, long-circulating, and effective siRNA carrier that can be used to leverage the enhanced permeability and retention (EPR) effect for enhanced target gene silencing within different types of highly-vascularized tumors.

Methods: A library of diblock polymers was synthesized by RAFT polymerization and then utilized to form mixed micelles using solvent exchange from ethanol to PBS. Micelle hydrodynamic diameter and zeta potential were determined using a Malvern zetasizer Nano-ZS. Gene silencing and cytocompatibility were screened in MDA-MB-231 and NIH-3T3 cells, respectively. For circulation half-life and tissue biodistribution studies, BALB/c mice were injected in the tail vein with IRDye800-labeled siRNA loaded into 50/50 mixed micelles with 5 (50D/5k PEG), 10 (50D/10k PEG), or 20 (50D/20k PEG) kDa PEG in the corona. Blood was collected at 5, 10, and 20 min post-injection, and organs were excised at 20 min. The siRNA concentration in the blood samples and organs was then fluorescently measured. All animal experiments were approved by IACUC.

Results: Four polymers were synthesized by RAFT polymerization, all featuring (DMAEMA-co-BMA-co-PAA) as the endosomolytic, micelle core-forming block. The first blocks of these polymers comprised 5k PEG,

10k PEG, 20k PEG, or 12k DMAEMA. The DMAEMA polymer and the PEG polymers were mixed at a 50/50 molar ratio to create 50D/5k PEG, 50D/10k PEG, and 50D/20k PEG micelles. As the length of the PEG was increased, the micelle size increased (35, 45, 65 nm), and the zeta potential decreased (4.6, 2.2, 0.5 mV), for 5k, 10k, and 20k PEG respectively. Reduced zeta potential decreased the rate of cell uptake *in vitro*, but all formulations were cytocompatible and achieved knockdown of 86% or greater under optimal conditions (data not shown). For *in vivo* studies, blood siRNA concentration values were fit to a blood circulation half-life model, which yielded significantly different blood circulation half-life values of 4.6 minutes for 50D/5k PEG, 7.5 minutes for 50D/10k PEG, and 17.7 minutes for 50D/20k PEG (Figure 1A). Fluorescent imaging of organs (Figure 1B) confirmed micelle delivery to the liver. The 50D/5k PEG formulation had significantly more distribution to the lungs relative to 50D/10k PEG (2-fold) and 50D/20k PEG (4 fold). Similarly, the 50D/5k PEG formulation had significantly higher distribution to the kidneys relative to 50D/10k PEG and the 50D/20k PEG micelles.

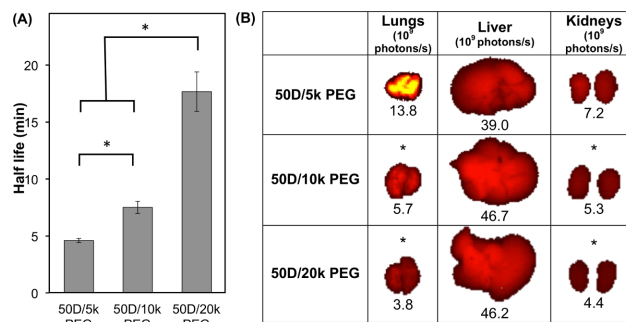


Figure 1. *In vivo* performance of 50D siRNA micelle-plexes formulated with 50% 5, 10, or 20 kDa PEG in the corona. (A) Blood circulation half-lives and (B) Biodistribution of mixed micelles. * < 0.05.

Conclusions: Mixed micelles containing varied molar percentages and molecular weight of PEG in the corona have been synthesized and characterized *in vitro* and *in vivo*. Increasing PEG length in the corona of micelles reduces *in vivo* biodistribution in the kidneys and lungs, which suggests a significant improvement in stability and decreased blood cell aggregation, respectively. The 50D/20k PEG micelles result in a 17.7 minute blood circulation half-life value that can be utilized for delivery to highly-vascularized tumors via the EPR effect. This platform demonstrates the potential for biocompatible siRNA delivery, and ongoing studies will assess the 50D/20k formulation for siRNA delivery to orthotopic breast cancer tumors.

References: 1. G. Meister et al. *Nature*. 2008;431:343–349. 2. A. J. Convertine et al. *J. Control. Release*. 2009; 133:221–229.