Conjugation of Palmitic Acid Improves Potency and Longevity of siRNA Delivered via Endosomolytic Polymer Nanoparticles

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Statement of Purpose: We recently reported biomaterial scaffold-based delivery of siRNA using pH-responsive, endosomolytic polymeric nanoparticles (NPs) for local gene silencing to promote tissue regeneration[1]. The current work is aimed at increasing the potency and longevity of gene silencing using this promising delivery platform. Because siRNA molecule hydrophobization has been demonstrated to generally enhance carrier stability and transfection efficiency[2], the current study was designed to assess the effect of siRNA conjugation to hydrophobic palmitic acid (PA) on target gene silencing in cells relevant to wound regeneration.

Methods: Unmodified siRNA or siRNA-PA was loaded into endosomolytic polymer nanoparticles (NPs). The loading efficiency was characterized by gel electrophoresis. Cellular uptake and retention for these formulations was quantified by flow cytometry. IC_{50} of gene silencing was calculated for knockdown of the model gene luciferase in fibroblasts and mesenchymal stem cells (MSCs). IC_{50} of gene silencing of prolyl hydroxylase 2 (PHD2; a pro-angiogenic gene target) in fibroblasts was measured with qPCR.

Results: The conjugation of siRNA to PA improved NP loading efficiency, with siRNA completely complexed at a lower polymer:siRNA ratio relative to free siRNA (Figure 1). PA conjugation also increased intracellular uptake of the nucleic acid cargo by 35-fold and produced a 3.1-fold increase in intracellular half-life (Figure 2).

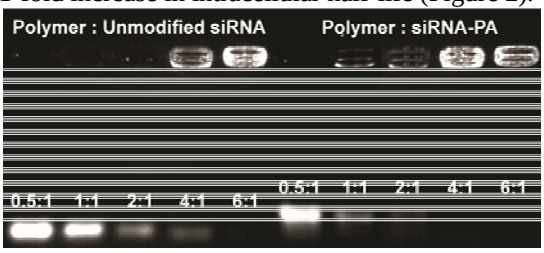

Figure 1. siRNA-PA is more efficiently packaged into NPs at lower N:P ratios.

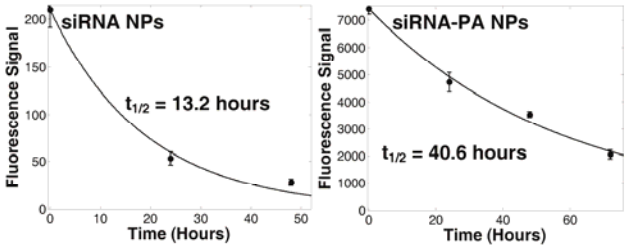

Figure 2. Intracellular delivery and temporal retention were higher for siRNA-PA NPs vs. siRNA NPs.

The higher uptake and improved retention of siRNA-PA NPs correlated to enhanced gene silencing potency (2- to 3-fold improvement in comparison to siRNA NPs) that was consistent across multiple cell types (fibroblasts and MSCs; only MSC data shown here) and genetic targets (luciferase and PHD2) (Figures 3 and 4). siRNA-PA NPs also increased longevity of silencing activity relative to siRNA NPs, with silencing half-life extended from 24 to 186 hours. The siRNA-PA NPs were also effective at gene silencing at a lower ratio of polymer:siRNA in the NP formulations, which is relevant to minimizing nonspecific cytotoxicity.

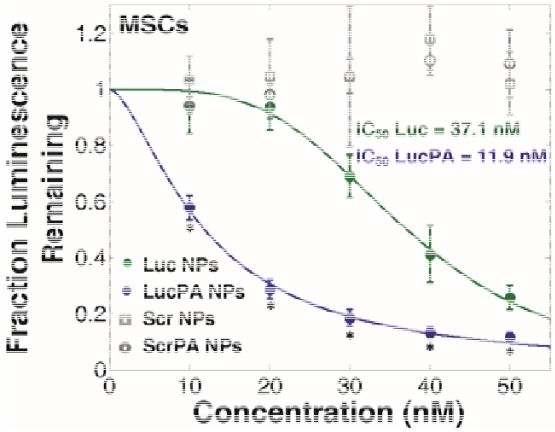

Figure 3. siRNA-PA NPs exhibited superior luciferase silencing vs. siRNA NPs in MSCs.

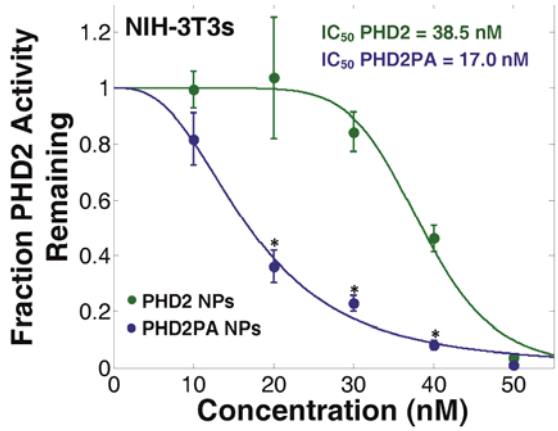

Figure 4. siRNA-PA NPs exhibited significantly more potent PHD2 silencing vs. siRNA NPs in fibroblasts.

Conclusions: siRNA conjugation to PA acts synergistically with endosomolytic polymer NP formulations. PA conjugation reduced the required ratio of polymer:siRNA and improved silencing potency across multiple genes, including therapeutically relevant PHD2, and cell types relevant to tissue regeneration. Conjugation of PA to siRNA significantly reduces the siRNA/polymer dose necessary to effectively silence genes of interest and increases longevity of silencing. Therefore, this conjugation strategy has the potential to enhance functional efficacy for tissue regenerative applications.

References: 1. Nelson CE, et. al. *Adv. Mater.*, 2014, *26*, 607-614. 2. Kubo, T, et. al. *Bioconj Chem.*, 2012, *23*, 164-173.