Nucleic Acid Delivery from a Bioactive Silk Platform for Tissue Repair

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Statement of Purpose. Dermal wound healing is a major medical burden. Dysregulation of wound healing processes can lead to chronic wounds and increased morbidity and mortality in associated comorbidities, such as diabetes. Localized and prolonged therapeutics release from an effective bioactive platform can accelerate healing mechanisms at the tissue damage site. We hypothesized that the use of a silk-based polymer-nucleic acid delivery platform can facilitate the prolonged delivery of nucleic acids while preserving the bioactivity of payloads for accelerating skin tissue repair following wounding.

Methods. Silkworm silk fibroin ("silk"; 2.5 mg/ml and 10 mg/ml) was evaluated for polymer -DNA complex (polyplex) delivery and transient gene expression in vitro. The linear polyethyleneimine (PEI) polymer was mixed with 1 µg of luciferase encoding plasmid DNA (pDNA) to form the polyplexes. The polyplexes were lyophilized and resuspended in silk. The transfection efficacy of PEI-polyplexes loaded in silk fibroin solution was evaluated on NIH3T3 cells. The final concentration of silk solution in each well was maintained at 2.5 mg/ml and 10 mg/ml with the initial stock solution of 1% (w/v) and 4% (w/v), respectively. The transfection efficacy was determined by recording the luciferase luminescence signal on days 2, 3 and 6 using a microplate reader. Different amounts (31.25 ng - 1.0 µg) of luciferase protein-encoding plasmid DNA (pDNA) were also complexed with linear PEI and formulated together with silk. The PEI:pDNA loading was kept constant (Nitrogen/Phostapte=8) in all cases.

Results. In our preliminary study *in vitro*, silk formulation facilitated high loading of PEI-pDNA polyplex without cellular toxicity. The reduced cellular toxicity resulted in prolonged transient gene expression with a high plasmid DNA payload. Figure 1 (a) shows that with 10 mg/ml of silk formulation, PEI-pDNA polymer DNA complex with an increasing amount of pDNA (31.25 ng- 1µg, constant N/P=8), resulted in enhanced luciferase gene transfection in NIH3T3 fibroblasts till day 6 whereas aqueous formulation of PEI-pDNA complex showed a reduction in the luciferase signal with higher amount of pDNA. Figure 1 (b) shows that with 1 ug of pDNA

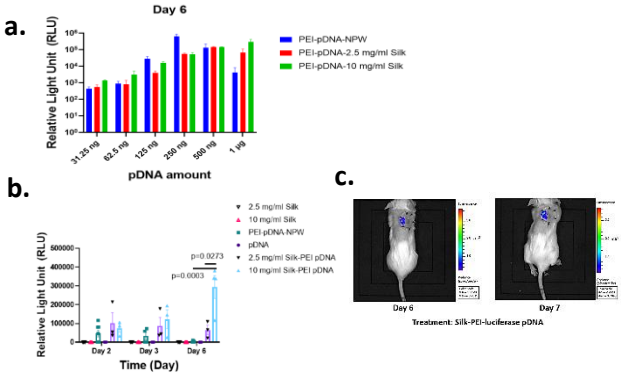

Figure 1. *Enhanced and prolonged transgene expression was seen up till day 6 using silk-PEI-pDNA complex formulation(a). Prolonged and sustained gene delivery efficacy of PEI-pDNA complex in 10 mg/ml silk formulation compared to 2.5 mg/ml formulation or bolus delivery of PEI-pDNA in aqueous formulation 1000 ng pDNA was used in the later in vitro study (b). Silk-PEI-pDNA induced transient gene expression at day 6 and 7. 165 µg of pDNA was used in the in vivo study (c). NPW: nanopure water*

loading at N/P:8, 10 mg/ml silk solution (▲) showed sustained and enhanced transient gene expression compared to 2.5 mg/ml silk formulation (▼) (p=0.0273). On day 6, transfection efficacy was significantly reduced with bolus PEI-pDNA polyplex delivery with aqueous formulation (■) compared to 10 mg/ml silk solution (p=0.0003). We found that PEI-pDNA containing 165 µg of pDNA maintained the transient gene expression *in vivo* for seven days (Fig 1c) delivered in silk substrate Overall, the study suggests that silk formulation can be an effective injectable substrate material for non-viral gene delivery by facilitating higher therapeutic payload, reduced toxicity due to cytotoxic polymeric vehicle and sustained release of therapeutic genes at the wound site. Moreover, it will be interesting to investigate the intrinsic therapeutic efficacy of silk during the healing process that can potentially synergize with the therapeutic outcomes of the delivered nucleic acid resulting in accelerated dermal wound healing.

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