Spider Silk Gene Delivery Systems for Intracellular Cell Targeting

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Statement of Purpose: Intracellular gene delivery by non-viral nano-vehicles is a growing field in gene therapy with the main purpose to deliver a cargo carrying genetic material to a particular site in the cell. Spider silks provide a unique avenue to design highly tailored and well-defined gene carriers with low toxicity and high transfection efficiency. Furthermore, silks are biocompatible and biodegradable and can be modified to target both specific cell types and specialized subcellular subunits. The unique feature of our platform is that all aspects of the delivery system can be precisely designed via genetic engineering, tailored to accommodate carrier size, binding and release kinetics, degradation rates, cell affinity and specificity and carrying capacity for nucleic acids, such as plasmid DNA, siRNA and microRNA. Further, silks generate robust materials, thus generating carriers that can survive handling, injection and in vivo function. We have exploited these features in the design of novel multifunctional nano-scale silk-based ionic complexes to target specific organelles, such as nucleus in mammalian cells. The basic designs include a tetra-block polymer consisting of a spider silk domain to define nanoparticle diameter, a payload sequence to define gene loading capacity, a cell penetrating block and/or nuclear localization sequence to govern intracellular delivery, and a cell affinity domain for cell targeting.

Methods: The spider silk gene delivery systems were engineered in a similar fashion to our previously published procedures, specificity (Numata et al., Bioconjugate Chem. 2011; 22: 1605). The presence of correct inserts in each construct was confirmed by PCR and DNA sequencing and the spider silk-based proteins were expressed and purified as reported previously (Rabotyagova et al., Biomacromolecules. 2009; 10:229). pDNA complexes were prepared at different N/P ratios, where N/P ratio a measure of ionic balance of the DNA-protein complexes. Green Fluorescent Protein (GFP) was used as a reporter gene. To investigate specificity for cell targeting, intracellular delivery, and nuclear import mammalian cell lines were used for transfection experiments. The cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA). Expression of GFP was assessed by fluorescence microscopy and FACS analysis. Immunofluorescence of fixed cells was performed according to standard procedure. Cytotoxicity to the cells was characterized by the standard MTS assay (Promega, Madison, WI).

Results: Two sets of the recombinant spider silk gene delivery systems containing poly(L-lysine) sequence, cell recognition domain, three different cell penetrating blocks, and nuclear localization sequence were cloned, expressed, and purified using metal affinity chromatography. SDS-PAGE analysis indicated successful expression and purification of the silk gene delivery systems. Molecular weights of the spider silk-based proteins were confirmed by MALDI-TOF. Ionic complexes were prepared with pDNA encoding a reporter gene at N/P ratio 5 and were characterized by DLS and Zetasizer Nano. The silk gene delivery systems demonstrated low cytotoxicity against human cells as indicated by MTS assay. To evaluate intracellular delivery and nuclear import four distinct constructs from two different sets were used. As demonstrated by immunofluorescence staining as well as FACS data, only the pDNA/silk-based protein complexes containing both the cell recognition domain and nuclear localization sequence have demonstrated useful transfection efficiency and subsequent expression of a reporter gene (Figure 1). Transfection experiments of GFP encoded plasmid by silk proteins without cell penetrating block exhibited extremely low fluorescence. Additionally, pDNA complexes with silk proteins only, demonstrated no nuclear import and showed no fluorescence signal.

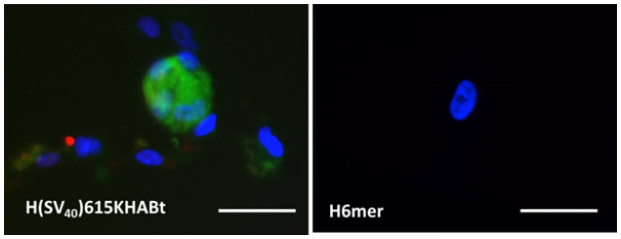

Figure1. Fluorescent microscopy images of human cells transfected with H(SV_{40})615KHABt and silk 6mer only. DAPI (for nuclei staining) is in blue, GFP is in green. Scale bar is 20μm.

Conclusions: The recombinant silk proteins containing the poly (L-lysine) sequence, the cell recognition domain, the nuclear localization sequence and the cell penetrating domains showed enhanced nuclear import to the cells nuclei as demonstrated by expression of GFP. pDNA/silk-based protein complexes without the cell penetrating and nuclear localization sequences did not demonstrate specificity towards the cell nucleus. The silk-based pDNA complexes revealed low cytotoxicity towards the human cells. The present study suggests that the multifunctional bioengineered spider silk-based gene delivery systems containing varied functional peptides are promising candidates for development of tailor gene carriers and can serve as versatile platform for gene delivery needs.