Surface Immobilization of Plasmid DNA with a Cell-Responsive Tether for Substrate-Mediated Gene Delivery

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Statement of Purpose: Gene delivery enables mammalian cell manipulation necessary for disease treatment, tissue development, and comprehension of biochemical functions and cellular response. Currently, most non-viral gene delivery is conducted as a bolus delivery leading to inefficiencies in gene transfer due to mass transport issues. Degradation, aggregation, and clearance of the DNA containing particles from the system may be prevented by surface immobilization of the vectors. Many methods of plasmid immobilization require careful modification to allow the binding of the vector to the substrate yet enable cellular internalization of the plasmid. As an alternative to current immobilization approaches, we chemically bind plasmid DNA to a substrate via an enzymatically-labile peptide sequence, allowing for cell-responsive gene delivery. In our design, the DNA is functionalized using a peptide nucleic acid (PNA) clamp. Coupling peptides that include a matrix metalloproteinase-1 (MMP-1) degradable sequence are attached to this conjugate to form DNA-PNA-peptide (DPP) conjugates. In this work, we demonstrate DPP formation, immobilization to a model gold surface, and the ability to promote gene delivery in a cell-responsive manner.

Methods: DPP conjugates were formed utilizing the gWiz vector (Genlantis, San Diego, CA), which encodes for green fluorescent protein (GFP) and contains PNA-binding sites. An overnight incubation of gWiz with maleimide-functionalized bis-PNA (Panagene, Daejeon, Korea) was followed by another overnight incubation with either an MMP-labile peptide or a non-MMP-labile peptide (Pi Proteomics, Huntsville, AL) to create DPP or sDPP conjugates, respectively. A self-assembled monolayer (SAM) was formed on Au-coated substrates (500 Å Au-coated mica – Asylum Research, Santa Barbara, CA or 100 Å Au-coated glass discs – Platypus Technologies, Madison, WI) by treatment with mercaptoundecanoic acid. Following filtration and concentration, DPP conjugates were attached to SAM-modified substrates in the presence of N-(3-dimethylpropyl)-N-ethylcarbodiimide hydrochloride and N-hydroxysulfo-succinimide sodium salt. Surface topography was observed by imaging of 25 kDa polyethylenimine (PEI)-complexed surfaces with a NanoScope IIIa multimode AFM (Digital Instruments, Santa Barbara, CA). YOYO®-1 Iodide (Molecular Probes, Eugene, OR) stained samples were PEI-complexed and imaged with a Bioscope II AFM (Veeco, Santa Barbara, CA) and an LSM 510 NLO Multiphoton Microscope (Carl Zeiss, Inc., Thornwood, NY), enabling verification that the features observed in AFM contained DNA. The amount of DNA bound to the DPP-modified surfaces was determined by analysis of Quant-iT™ PicoGreen® (Molecular Probes) stained samples on BioTek Synergy 2 Multi-Mode Microplate Reader (Winooski, VT). NIH/3T3 cells were plated on DPP-modified samples and imaged 24 h post-transfection using a Leica AF6000 Microscope (Bannockburn, IL). GFP expression was quantified using a Becton Dickinson FACSCalibur Flow Cytometer (Franklin Lakes, NJ).

Results: AFM imaging confirmed the successful immobilization of the conjugates to the model substrate. Because surfaces treated with unmodified DNA showed no surface features, the DPP binding was found to be specific. The presence of DNA bound to the surface *via* the conjugate tethering mechanism was verified using AFM-confocal overlay, in which the PicoGreen-stained fluorescent features observed in the confocal image correlated well with the features observed in the AFM image. Analysis of the DPP-treated surfaces with a fluorescent plate reader (Figure 1A) demonstrated that the DPP-treated surface had 42±16 ng of DNA/cm^2, while there were 4.2±2.1 ng of DNA/cm^2 on the surfaces treated with unfunctionalized plasmid DNA. Fluorescence microscopy and flow cytometry (Figure 1B) revealed that the DPP surfaces transfected a significantly higher percentage of live cells relative to the sDPP surfaces ($p=0.033$). This result indicated that the peptide must be cleaved to release the DNA, and thus gave evidence that the system promoted cell-responsive transfection.

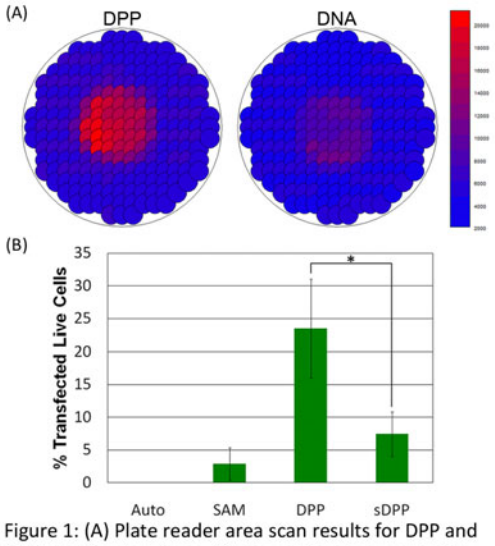

Figure 1: (A) Plate reader area scan results for DPP and DNA modified surfaces (B) Flow cytometry results for NIH/3T3 cells plated on modified surfaces. The asterisk denotes a statistically significant difference between the indicated values ($p=0.033$).

Conclusions: This work details the formation of plasmid conjugates that are covalently tethered to a substrate. Gene delivery in response to a cellular enzyme has been accomplished using this system. The relatively low transfection efficiency observed may be due to the multiple tethers that are likely to exist between the substrate and the DPP conjugates. Thus, we are presently exploring the effects of a reduction in tether number. In the future, the tunability of this design will enable localized gene delivery with highly specific spatial and temporal control of the plasmid release.