

## **489g Stability of High Density Pei/Vegf DNA Nanocomplexes in Physiological Fluids**

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Non-viral gene delivery is an attractive approach for gene therapy; however, success has been limited due to poor efficiency compared with viral delivery systems. We have shown that formulations yielding high-density nanocomplexes provide comparatively high transfection efficiency if all other properties are similar. Aggregation of non-viral gene delivery systems following exposure to an environment rich in negatively charged proteins is speculated to decrease transfection efficiency. Aggregated nanocomplexes may be too large to enter target cells and may be rapidly removed by cells of the reticuloendothelial system (RES).

In this work, nanocomplexes formed of DNA and the cationic polymer, polyethylenimine (PEI), were added to solutions of negatively charged proteins associated with cell culture media (DMEM containing FBS) or in vivo experiments (normal human serum, NHS) in order to study the extent of nanocomplex aggregation, protein binding, and long-term stability in these solutions using time resolved multi-angle laser light scattering (TR-MALLS). TR-MALLS is a technique capable of measuring both the radius of gyration and the molar mass of the nanocomplexes.

Negatively charged nanocomplexes ( $N/P = 2$ ) did not change in size appreciably in the presence of serum proteins, but were less stable than positively charged nanocomplexes, (molar mass decreased over time). Most positively charged nanocomplexes did not change in size appreciably upon addition to DMEM or DMEM containing FBS. However, positively charged nanocomplexes experienced a sharp increase in molar mass and density following incubation in protein-containing media or serum. The molar mass of nanocomplexes made at  $N/P = 20$  initially increased slightly upon addition to DMEM/FBS, and then remained stable in size and molar mass. The same nanocomplexes added to DMEM alone (no serum proteins) or to PBS dissociated rapidly, suggesting serum proteins bind to and stabilize nanocomplexes, whereas in the absence of serum, ions and small molecules present in DMEM may promote nanocomplex dissociation.

Positively charged nanocomplexes did not increase in size upon incubation in NHS, but nearly doubled in molar mass. Albumin and components of the complement system are likely to interact with intravenously administered positively charged nanocomplexes, resulting in nanocomplex opsonization. Protein binding may impair nanocomplex interactions with the target cell surface and possibly interfere with target cell internalization of the DNA complexes. PEI/DNA nanocomplexes exhibited higher protein adsorption in cell culture medium as the  $N/P$  ratio increased, but there was no evidence that nanocomplexes aggregated in any of the solutions studied. Nanocomplexes were more stable in the presence of serum than in serum-free media, and the presence of serum proteins resulted in long-term stability of the nanocomplexes.

Due to the improved stability of PEI/DNA nanocomplexes incubated with both protein-containing media and serum, we are currently studying their use following modification with cell uptake ligands for delivery of the CFTR gene to mouse airways.