Transformation of Cationic Materials into Neutral Biocompatible Systems for siRNA Delivery: Property and Function Characterization

Jie Liu[1,2,3], Jihan Zhou[4], Ying Luo[1]

[1] Department of Biomedical Engineering, College of Engineering, Peking University [2] Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology [3]The Wallace H. Coulter Department of Biomedical Engineering, Emory University [4]Beijing National Laboratory for Molecular Sciences and the Key Laboratory of Polymer Chemistry and Physics of Ministry of Education, College of Chemistry and Molecular Engineering, Peking University

Statement of Purpose Utilization of cationic materials provides the main approach for development of delivery systems for siRNA and other types of nucleic acid-based therapeutics in general. However, mainly because of cationic charges, cationic materials may show severe toxicities that prevents them from clinical applications [1]. The nanoparticles formed from electrostatic interactions may also have stability issues in terms of maintaining particle size/structure in *in vivo* environment [2]. To address the inherent limitations of cationic delivery materials, we aim to developing a new strategy that can avoid the presence of cations under the physiological condition, while preserve the material's capability of forming nanoparticles with nucleic acids through electrostatic complexation. In particular, two types of cationic materials that have been commonly studied for gene delivery, polyamidoamine (PAMAM) and polyethyleneimide (PEI) were chemically modified by converting side chain- or peripheral amines into hydrazides. The resulting new materials, which were neutral at physiological pH, are able to absorb protons and become charged to complex with siRNA at acidic conditions. Property and function characterizations were carried out to understand the stability, safety and effectiveness of the new systems for targeted delivery of siRNA therapeutics.

Methods 1. Synthesis of neutralized dendrimers and polymers: G4.0 PAMAM or 25K PEI was reacted with excess methyl acrylate, hydrazine hydrate, and saccharide ligands, sequentially, and purified by extensive dialysis. 2. Preparation of crosslinked particles: siRNA, selected materials and glutaraldehyde were separately dissolved in PBS at pH 5.0 and mixed at 1:1:1 volume ratio. After the ternary blends were incubated at 37°C for 1 h, an excessive amount of adipic acid dihydrazide (ADH) was added to terminate the crosslinking reaction, and the pH was adjusted to 7. Crosslinked samples were dialyzed before used in characterization and cell experiments. 3. Characterization and transfection: siRNA encapsulation efficiency and siRNA release were determined by electrophoresis, particle size was detected by dynamic light scattering, and cytotoxicity was assessed by MTS assay. Transfection experiments were conducted in HepG2 cells through delivering siRNA against luciferase, and the luciferase expression level was determined by a luciferase assay system normalized to total protein.

Results Upon conversion of amines to hydrazides, the modified PAMAM (PAMAM-HYD) and its GalNAc derivation (GPH) became neutral, showing no positive zeta-potential at pH7. However, under acidic pH 5, the hydrazide-modified materials were positively charged probably due to protonation of tertiary amines (Figure 1). PAMAM-HYD (Figure 2) and PEI-HYD both showed minimal effect to cell viability, in contrast to the original PAMAM or PEI that exerted significant cytotoxicity within the tested concentrations (Figure 2). PAMAM-HYD was modified with GalNAc saccharide ligands for targeting to hepatocyte cells. Stable nanopraticles with controllable sizes were obtained via crosslinking. Materials of high levels of ligand modification, GPH-23 and GPH-40 have diameters around 200 nm (Figure 3), and they remained stable in PBS within 24 h. In contrast, particles formed from unmodified cationic PAMAM showed large shift both in size and distribution (Figure 3). siRNA remained encapsulated in crosslinked system under physiological environment, but was observed released under acidic environment with similar pH as endosomes. Among all the crosslinked particles, GPH-23 system showed over 50% knockdown of luciferase, better than commercial Lipofectamine, in serum-containing media (Figure 4).

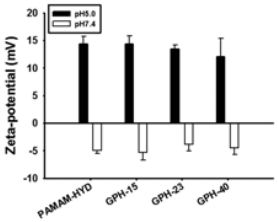

Figure 1. Zeta-potential of siRNA complexation with PAMAM-HYD or GPH at pH 5.0 and pH 7.4

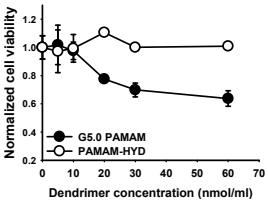

Figure 2. Cytotoxicity of PAMAM G5.0 and PAMAM-HYD

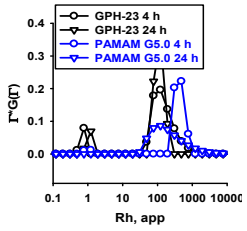

Figure 3. Size distribution of siRNA loaded GPH-23 or PAMAM G5.0 particles within 24 h

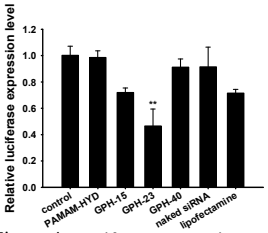

Figure 4. Luciferase expression in HepG2 cells after siRNA delivery

Discussion &Conclusion The novel neutral crosslinked system provides safety, stability, and controllability, and it delivers siRNA efficiently with appropriate ligand modification. This design has shown potential to be extended to other cationic materials to dissolve safety concerns, such as PEI.

The concept of neutral crosslinked systems opens the door for designing biocompatible non-viral gene delivery systems for *in vivo* applications.

Reference

[1] Jain K et al. Int J Pharm. 2010;394:122-42.

[2] Liu J et al.Interface Focus. 2012;2:18.