Conjugation of polyethylene glycol to polyamidoamine dendrimer through bis-aryl hydrazone linkage for enhanced gene delivery
Quan Yuan¹, W. Andrew Yeudall²,³, Hu Yang¹
¹ Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA 23298
² Philips Institute of Oral & Craniofacial Molecular Biology, ³Massey Cancer Center, Virginia Commonwealth University, Richmond, VA 23298

Statement of Purpose: Dendrimers have been recognized as the most versatile compositionally and structurally controlled nanoscale building blocks. Amine-group-terminated polyamidoamine (PAMAM) dendrimers have been most used for gene delivery. In this work, we designed a new dendrimer vector conjugated with poly (ethylene glycol) (PEG) chains through bis-aryl hydrazone linkages (BAHs) in order to enhance the buffering capacity of the vector.

Methods: The synthesis of PAMAM G4.0-BAH-PEG was based on conjugation of poly(ethylene glycol) (PEG, MW=5000 dalton) to the surface of polyamidoamine (PAMAM) dendrimer G4.0 by a bis-aryl hydrazone linkage, which was introduced by using two heterobifunctional reagents succinimidyl 4-hydrazinonicotinate acetone hydrazone (SANH) and succinimidyl 4-formylbenzoate (SFB).

Their molecular structures were detected by ¹H-NMR. The vector/DNA plasmid complex formation at various weight ratios was analyzed using agarose gel electrophoresis studies. The cytotoxicity of the materials was evaluated by trypan blue assay using HN12 and 293T cells. The high PEGylated dendrimer G4.0-BAH-PEG42 was chosen for the gene transfection assay to deliver a green fluorescent protein (GFP) expressing plasmid into HN12 cells and 293T cells. The high PEGylated dendrimer G4.0-BAH-PEG42 was chosen for the gene transfection assay to deliver a green fluorescent protein (GFP) expressing plasmid into HN12 cells and 293T cells. The high PEGylated dendrimer G4.0-BAH-PEG42 was chosen for the gene transfection assay to deliver a green fluorescent protein (GFP) expressing plasmid into HN12 cells and 293T cells.

Results/Discussion: The GFP plasmid showed completely retardation at a weight ratio of 10 with G4.0-BAH-PEG3 and a weight ratio of 20 with G4.0-BAH-PEG42, indicating that the materials can combine tightly with the DNA plasmid at this weight ratio, which can be used as a reference for gene transfection assay.

At 0.2µM, none of the synthesized vectors induced an obvious cytotoxic response in tumor cells over 48 h. Over the same period of time, 2 µM PAMAM G4.0, PAMAM G4.0, G4.0-BAH-PEG3 or G4.0-BAH-PEG42 induced a modest cytotoxic response. At 20µM, G4.0-BAH-PEG3 still induced a modest cytotoxic response. The cytotoxicity of G4.0-BAH-PEG3 was not tested above 20µM because of its high molecular weight. At 20µM or higher concentration, all the other materials showed considerable cytotoxicity to the tumor cells.

For fluorescence microscopy observation, in 293T cells and HN12 cells, the transfection efficiency of PEI was highest among the materials, but it has much higher cytotoxicity to the cells which corresponded with the result of cytotoxicity. G4.0-BAH-PEG42 showed much higher transfection efficiency as that of native PAMAM G4 and was similar to Mirus in both cells.

In HN12 cells, the number of transfected cells of G4.0-BAH-PEG42 was higher than that of native PAMAM G4.0 and similar to Mirus. The number of transfected cells in PEI 25K was higher than that of the other materials. In 293T cells, the number of transfected cells in PEI 25K was very high. The number of transfected cells by G4.0-BAH-PEG42 was higher than that of native PAMAM G4.0 and similar to Mirus.

In 293T cells and HN12 cells, the transfection efficiency of G4.0-BAH-PEG42 showed nearly 2 times as that of native PAMAM G4.0. The transfection efficiency of G4.0-BAH-PEG42 was comparable to Mirus in both cells and a little lower compared to PEI 25K at the optimal conditions. The transfection efficiency of native PAMAM (G4) was nearly 3 times less than that of PEI 25K in both cells.

Conclusions: G4.0-BAH-PEG42 has shown some interesting characteristics for future gene transfection assay study.

Acknowledgment: The Jeffress Memorial Trust (J-873) and the National Institutes of Health (R21NS063200).