Conjugation of polyethylene glycol to polyamidoamine dendrimer through bis-aryl hydrazone linkage for enhanced gene delivery

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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-acryl 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 hydrozone 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 ^{1}H-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-PEG$_{42}$ was chosen for the gene transfection assay to deliver a green fluorescent protein (GFP) expressing plasmid into HN12 cells and 293T cells. PAMAM G4.0, TransIT keratinocyte transfection reagent (Mirus), and PEI 25K (branched) were used as control groups. GFP expression was observed by fluorescence microscopy after 48h incubation. Transfection efficiency was further quantified by using flow cytometry and western blot assay.

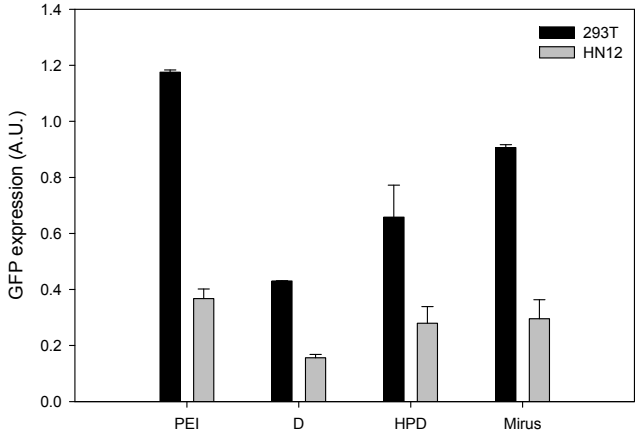
Results/Discussion: The GFP plasmid showed completely retardation at a weight ratio of 10 with G4.0-BAH-PEG$_3$ and a weight ratio of 20 with G4.0-BAH-PEG$_{42}$, indicating that the materials can combine tightly with the DNA plasmid at this weight ratio, which can be used as a reference for gene transfection 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-PEG$_3$ or G4.0-BAH-PEG$_{42}$ induced a modest cytotoxic response. At 20μM, G4.0-BAH-PEG$_3$ still induced a modest cytotoxic response. The cytotoxicity of G4.0-BAH-PEG$_3$ 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-PEG$_{42}$ 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-PEG$_{42}$ 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-PEG$_{42}$ 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-PEG$_{42}$ showed nearly 2 times as that of native PAMAM G4.0. The transfection efficiency of G4.0-BAH-PEG$_{42}$ 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-PEG$_{42}$ has shown some interesting characteristics for future gene transfection assay study.

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