Dendritic Polyethylene Glycol–Poly (D,L-Lactide) (PEG-PDLLA) Nanoparticles for Gene Delivery

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Statement of Purpose: Dendrimers have been recognized as the most versatile compositionally and structurally controlled nanoscale building blocks. The synthesis and characterization of novel thermoresponsive highly branched polyamidoamine –polyethylene glycol–poly (D, L-lactide) (PAMAM–PEG–PDLLA) core–shell nanoparticles was described in our previous work.[1] We found that these nanoparticles could self-assemble into sub-micron/micron aggregates and have good cytocompatibility. These nanoparticles were evaluated as potential vectors for gene delivery in this study.

Methods: Dendritic PEG–PDLLA core–shell nanoparticles were synthesized by modification of PAMAM dendrimer G3.0 by PEG diol (1500, 6000 or 12,000 g mol^{-1}) and sequential ring-opening polymerization of DLLA through the hydroxyl groups of tethered PEG chains.[1] Their macromolecular structures were characterized by ^{1}H-NMR. The mean particle sizes of dendritic PEG-PDLLA and dendritic PEG-PDLLA/ GFP plasmid polyplexes at 25 ºC and 37 ºC were measured using dynamic light scattering.

293T cell line was used in this work. Delivery of Green fluorescent protein (GFP) plasmid by dendritic vectors to 293T cells was studied. PAMAM G3.0, PEI 25K (branched) and TransIT keratinocyte transfection reagent (referred to as TransIT) were used as control groups. The cells were incubated for 6 h with dendritic vector/plasmid with N/P ratios of 50 μg/1 μg, 100 μg/1 μg and 200 μg/1 μg, or PEI/plasmid at 20 μg/1 μg, or TransIT/plasmid at 5 μl/1 μg, rinsed, and then cultured for 48h. Transfection efficiency was then determined by using flow cytometry and western blot assay. The cell viability post-gene transfection was evaluated by MTT assay.

Results/Discussion: The structures of dendritic PEG–PDLLA nanoparticles were confirmed by ^{1}H-NMR with expanded characterization in our previous work.[1]

The mean particle sizes of G3-PEG1500-PDLLA, G3-PEG6000-PDLLA and G3-PEG12000-PDLLA increased dramatically as temperature increased 25 ˚C to 37 ˚C. After complexation with plasmid, the mean particle size of the polyplexes still displayed temperature responsiveness and underwent size increase. However, the size of the polyplexes was comparable to that of dendritic vector at the same temperature.

According to qualitative fluorescence microscopy observation, the cells transfected by PEI displayed the strongest fluorescence intensity. The cells transfected by G3-PEG1500-PDLLA showed stronger fluorescence intensity than those treated by PAMAM G3. The cells transfected by G3-PEG6000-PDLLA and G3-PEG12000-PDLLA did not display appreciable fluorescence intensity.

The gene transfection was further quantified using low cytometery analysis. As shown in Figure 1, the number of transfected 293T cells by G3-PEG1500-PDLLA was higher than that of PAMAM G3.0 but similar to TransIT. The number of transfected cells in PEI 25K was higher than that of the other materials. PEG6000-PDLLA and G3-PEG12000-PDLLA did not transfect many cells.

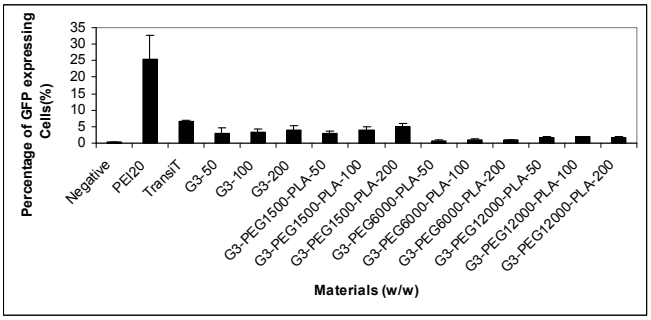

Figure 1. *Flow cytometery analysis of transfected cells*

Analysis of GFP expressed following transfection was done with Western blot. The GFP expression by G3-PEG1500-PDLLA was nearly 2 times that of PAMAM G3.0 (Figure 2).

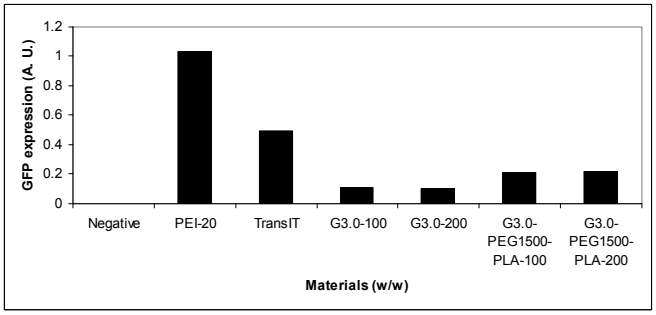

Figure 2. *Western blot assay of GFP expression*

MTT assay showed that at N/P ratios of 50, 100, and 200, none of G3-PEG1500-PDLLA, G3-PEG6000-PDLLA, and G3-PEG12000-PDLLA induced an obvious cytotoxic response in 293T cells following gene transfection. In contrast, G3 showed various extents of cytotoxicity at the selected N/P ratios. PEI induced a very high cytotoxic response at an N/P ratio of 20, while TransIT showed negligible cytotoxicity.

Conclusions: G3-PEG1500-PDLLA is able to transfect significantly more cells and induce a higher level of GFP expression than unmodified G3.0 and doesn't induce cytotoxic response. It can be further functionalized with other functional moieties for more efficient gene delivery.

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1. Kailasan et al. *Acta Biomaterialia* **2010,** 6 (3), 1131-1139.