Cellular Interactions of Dendron Micelles Controlled by the Length of Hydrophilic Chains

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ABSTRACT SUMMARY
In this study, we systematically investigate the relationship between end-group charge effects and PEG chain lengths on cellular interactions of dendron micelles (DMs) self-assembled from PEGylated dendron-based copolymers (PDCs). The results reveal that the end-group charge effects on nano-bio interactions are highly dependent upon the chain length of the PEG outer layer, providing a critical design cue for the DMs.

INTRODUCTION
Nanoparticles (NPs) with positively charged surfaces (e.g. –NH₂ termini) are known to non-specifically interact with cells and cause toxicity via membrane destabilization, which can be diminished by charge neutralization or carboxylation. This surface charge effect has been extensively studied on poly(amidoamine) (PAMAM) dendrimers due to their chemically well-defined, hyperbranched structure that magnifies the effect of their surface groups.¹-³ However, the relationship of end-group charge effect and PEG chains surrounding the NPs have not been systematically understood despite their critical roles played in determining cellular interactions of NPs.

We have previously designed and prepared a set of novel dendron-based copolymers with different end groups (–NH₂, -Ac, and –COOH) to investigate effect of the surface charges on their cellular interactions. Surprisingly, all of the end-group modified dendron micelles exhibited low levels of cellular interactions and cytotoxicity.⁴, ⁵ Clearly, the presence of PEG chains is one of the major contributing factors that lead to the absence of charge-dependent cellular interactions of the DMs. We hypothesized that the charge effects on cellular interactions could be modulated via changing the length of surrounding PEG chains. In this study, we therefore synthesized a series of PDC with different lengths of PEG chains (2K and 600 Da) to investigate the relationship between the end-group charge effects and the length of PEG chains of DMs.

EXPERIMENTAL METHODS
End-group modified PEGylated dendron-based copolymers were synthesized using published protocol³, ⁴ and then characterized by ¹H-NMR (DPX-400 NMR spectrometer, Bruker Biospin Co., MA, USA) and gel permeation chromatography (600 HPLC pump, 717plus Autosampler, and 2414 Refractive Index detector (Waters, Milford, MA, USA)) using THF as the mobile phase at 1 mL/min with Waters Styragel® HR2 and HR4E columns at 30 °C.

To formulate rhodamine-labeled DMs, PDC (PDC-NH₂, PDC-COOH, or PDC-Ac) and PDC-Rhodamine (10 wt.%) were premixed and dissolved in DMF and then dialyzed against distilled water for 24 h at room temperature. Particle sizes and zeta potentials of the prepared DMs were measured using a NICOMP 380 Zeta Potential/Particle Sizer (Particle Sizing Systems, Santa Barbara, CA). The morphologies of DMs were analyzed by transmission electron microscopy (TEM, JEM-1220, JEOL Ltd., Japan).

For cellular uptake study, KB cells were incubated with DMs for 2 or 4 hours. After incubation, the cell were washed three times and then fixed using paraformaldehyde. The specimens were visualized using a Carl Zeiss microscope (LSM 710, Carl Zeiss MicroImaging GmbH, Gena, Germany), and images were obtained using a 40X objective (Objective "C-Apochromat" 40x/1,20 W corr, Carl Zeiss MicroImaging GmbH, Gena,
Flow cytometry measurement was also employed to obtain the quantitative results. For this purpose, treated cells were trypsinized before fixation and analyzed on a LSR Fortessa™ flow cytometer (Becton Dickinson, Franklin Lakes, NJ).

For cytotoxicity study, KB cells were treated with different concentrations of DMs ranging from 1-100 μM. Cell viability was assessed using a CellTiter 96 Aqueous One Solution (MTS) Assay (Promega, Madison, WI, USA). The UV absorbance was measured at 490 nm using a Labsystems Multiskan Plus microplate reader (Labsystems, Finland).

RESULTS AND DISCUSSION
The end-group modified PDCs and corresponding DMs were made and fully characterized using above mentioned techniques. In the cell uptake study, while amine-terminated DMs with short PEG600 chains (DM600-NH2) interacted with KB cells, their DM2K counterparts and the other two end-group modified DMs displayed no interaction (Figure 1). In addition, the non-specific cellular interactions were accompanied with higher cytotoxicity (Figure 2). The results indicated that the end-group charge effects are highly related to the length of PEG chains surrounding the DMs.

![Figure 1. Cellular uptake of end-group modified dendron micelles. Confocal images of KB cells treated with rhodamine-labeled DM2K-NH2 (A), and DM600-NH2 (B). (C) Flow cytometry results of the cellular association of end-group modified DMs.](image)

![Figure 2. Cell viability of KB cells after 24 h incubation with amine-terminated DM2K and three different end-group modified DM600. *p<0.05](image)

CONCLUSION
Herein we report that cellular interactions of the PEGylated DMs are dependent on their surface groups and the hydrophilic PEG chain length. This study helps understand the role of surface charge and PEG in tuning biological properties of NPs.

REFERENCES

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