

# Syntheses and Characterizations of Smart and Biodegradable Dendritic Nanoparticles for Controlled Drug Delivery

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## Abstract

In order to transport therapeutic agents across blood brain barrier (BBB) for sustained time in response to biological need, we designed and synthesized novel dendritic nanoparticles with thermoresponsive and biodegradable properties as drug delivery systems. The nano-sized dendrimer was prepared by coupling reaction of poly(L-lysine) (PLL) dendron and poly(N-isopropylacrylamide) (PNIPAAM) grafted with poly(L-lactic acid) (PLLA). The chemical structure and molar mass of the dendrimer was characterized and confirmed by fourier transform infrared spectroscopy (FTIR) and matrix assisted laser desorption/ionization time of flight mass spectroscopy (MALDI-TOF). The dendrimer was responsive to temperature changes by showing lower critical solution temperature (LCST) measured by UV-VIS spectroscopy. The LCST depended on the concentrations of the dendrimer. The dendrimer also demonstrated biodegradable properties with decreasing their molar mass as a function of time.

## Introduction

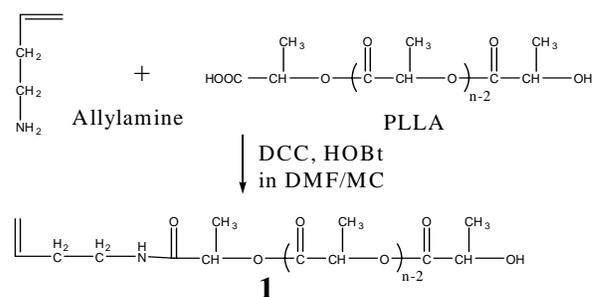
The dendritic polymers including benzyl ether<sup>1</sup>, propyleneimine<sup>2</sup>, amidoamine<sup>3</sup>, L-lysine<sup>4</sup> and carbosilane<sup>5</sup> dendritic segments have been extensively studied for application in drug delivery and other biomedical fields due to their high drug loading capacity, precise control of size, shape and placement of functional groups. In addition, dendrimers are also nanoparticles. *In vitro* and *in vivo* experiments showed that nanoparticles might have long blood circulation times and a low reticuloendothelial system (RES) uptake. They were able to strongly interact with the brain blood vessel endothelial cells of mice, and then be taken up by these cells by endocytosis. Bound drugs as intact molecules were then released and exhibited their pharmacological action on the central nervous system (CNS)<sup>6,7</sup> However, the current available dendrimers exhibit insufficient physicochemical response to stimuli, which include temperature, pH, and electrical fields, and can not achieve sustained drug delivery to meet biological need.

In our studies, we have designed and developed a novel dendrimer which is both biodegradable and responsive to temperature change. The dendrimer consists of poly(L-lactic acid) (PLLA), N-isopropylacrylamide (NIPAAM) and 3-generation poly(L-lysine) (PLL) units. PLLA, a biodegradable hydrophobic polymer, is chosen because of its combination of biodegradability, biocompatibility, hydrophobicity and good mechanical strength<sup>8-10</sup>. Poly(N-isopropylacrylamide) (PNIPAAM) is used due to its unique thermo-sensitive properties in water<sup>11-13</sup>. This “intelligent” polymer exhibits a dramatic solubility transition at the lower critical solution temperature (LCST) in an aqueous solution in the vicinity of 32 °C. It expands and swells when cooled below the LCST, and it shrinks and collapses when heated above the LCST. The LCST as well as the environmental responsive properties of the polymers may be manipulated by changing the polymer compositions<sup>14,15</sup>. PLL is selected owing to its excellent hydrophilic nature and many of cationic polyamine groups on the surface for conjugating targeting moiety<sup>16</sup> and increasing blood-brain barrier (BBB) permeability<sup>17</sup>. In this report, we describe the design and synthesis strategy of the dendrimer, and illustrate its thermo-responsive and biodegradable properties.

## Experimental Methods

### Synthesis of Polymers

PLLA (molar mass 2000 g·mol<sup>-1</sup>) terminated with allyl group was prepared by coupling conjugation of PLLA with allylamine through 1,3-dicyclohexylcarbodiimide (DCC) reaction with *N*-hydroxybenzotriazol (HOBt) in *N,N*-dimethylformamide (DMF) and methylene chloride (MC) mixture at room temperature (Scheme 1). PNIPAAM grafted with PLLA was synthesized through free radical polymerization in DMF under nitrogen

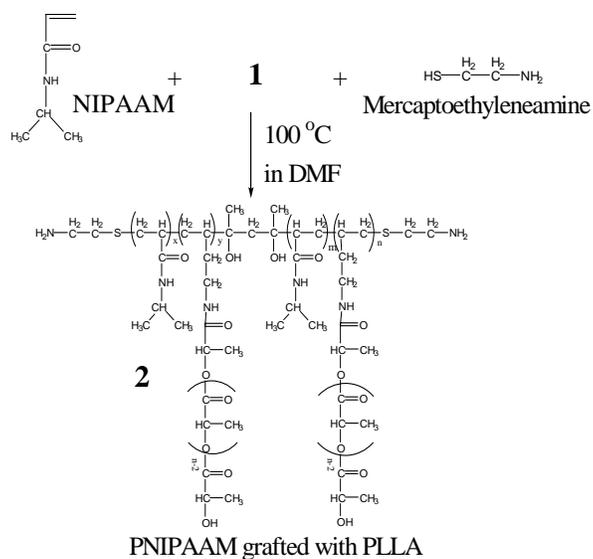


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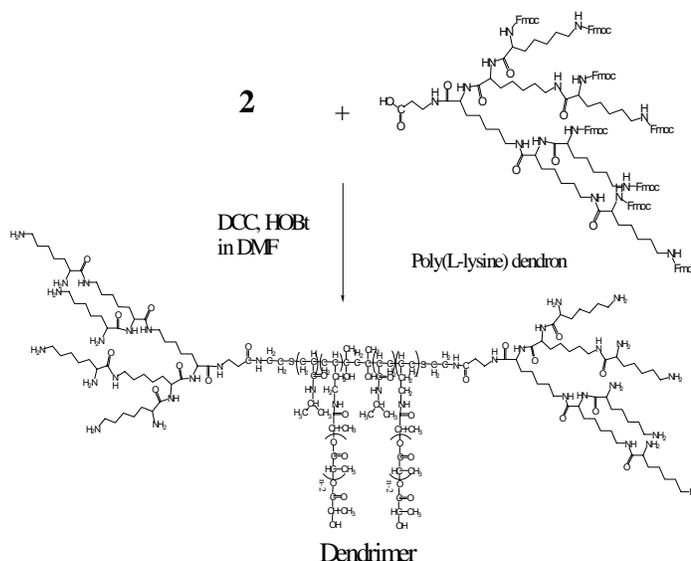
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**Scheme 2.**



**Scheme 3.**

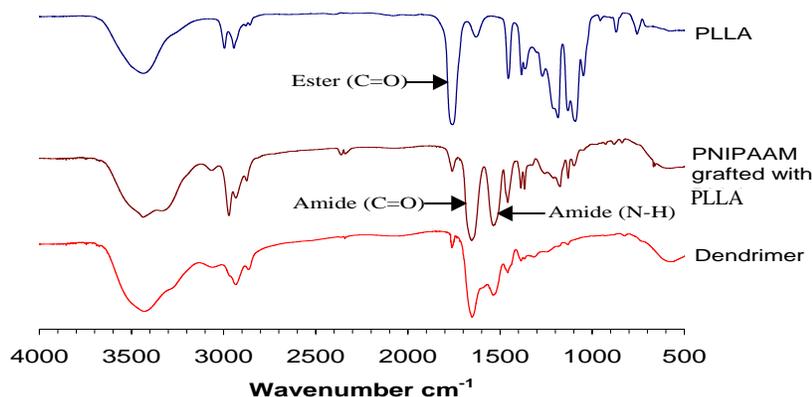
condition at 100 °C for 4 h (Scheme 2) and pentanedione peroxide was used as a catalyst. The dendritic polymer was prepared by DCC coupling reaction of PLL dendron and PNIPAAm grafted with PLLA in DMF at room temperature.

### Characterizations

The molar masses of the polymer samples, PLLA, PNIPAAm grafted with PLLA, and dendrimer, were determined by a MALDI-TOF with a Voyager Biospectrometry Workstation (Perseptive Biosystem, Inc). A N<sub>2</sub> laser radiating at 337 nm wavelength with 3 ns pulses was used and the ions generated by the laser pulses were accelerated to 25 kV energy. The polymer solutions at a concentration of 10 mg/ml were prepared using water as a solvent for the dendrimer and THF as a solvent for the other two polymers. The polymer solutions were then mixed with a matrix solution of 2,5-dihydroxybenzoic acid (Aldrich) at polymer:matrix = 1:9 (v/v) ratio for the MALDI-TOF measurements. The number and weight average molar masses of the polymers were determined in the linear mode. The chemical structures of the polymers were characterized by a FTIR (JASCO 460) under nitrogen environment at a resolution of 4 cm<sup>-1</sup>. The transmittance of the polymers in PBS (pH=7.4) with various concentrations was measured as a function of temperature by PerkinElmer Lambda 25 UV-VIS spectroscopy at 500 nm. At each temperature interval the polymer solutions were equilibrium for 30 min. The LCST of the polymers in PBS was defined as a 95 % of the maximum transmittance.

### Results and Discussion

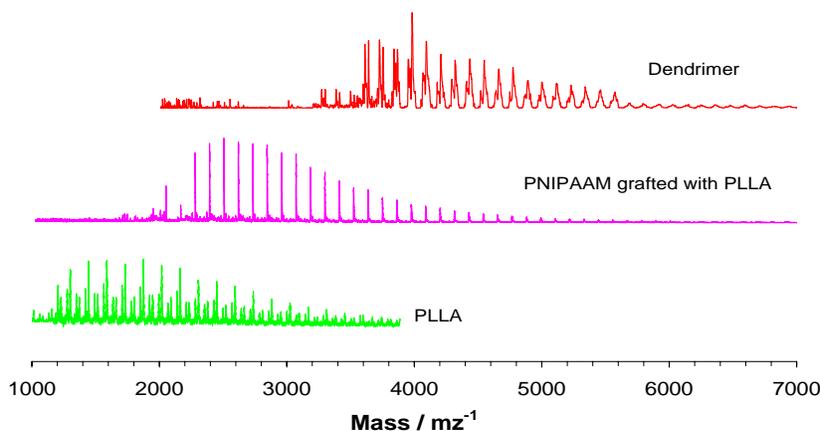
In order to confirm the success of the syntheses, we characterized the chemical structures of the PLLA, PNIPAAm grafted with PLLA and dendrimer through studying their infrared absorption bands which match their natural vibrational modes



**Figure 1.** FTIR spectra of PLLA, PNIPAAm grafted with PLLA and dendrimer.

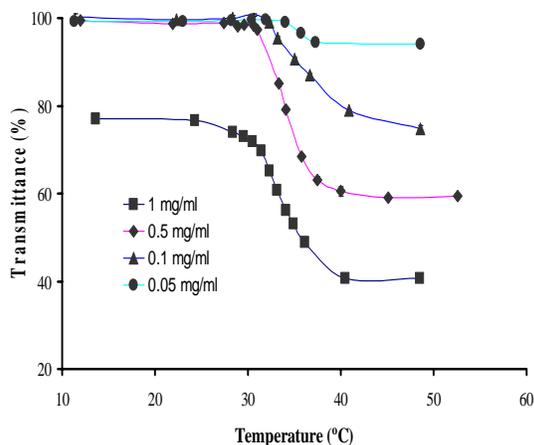
using FTIR (Figure 1). In Figure 1, PLLA shows ester C=O stretching at  $1764\text{ cm}^{-1}$ . After the PLLA is grafted to the PNIPAAm, the following characteristic FTIR bands of the PNIPAAm appear,  $\text{-NH-}$  stretching and bending at  $3080\text{ cm}^{-1}$  and  $1545\text{ cm}^{-1}$ , respectively, and amide C=O stretching PNIPAAm at  $1665\text{ cm}^{-1}$ . In addition, the band at  $2975\text{ cm}^{-1}$  of the PNIPAAm grafted with PLLA is stronger than the band at  $2945\text{ cm}^{-1}$  due to more amount of  $\text{-CH}_3$  present in the polymer. After PLL dendrons are attached to the PNIPAAm grafted with PLLA, the FTIR spectrum shows that the band at  $2945\text{ cm}^{-1}$  becomes stronger than the band at  $2975\text{ cm}^{-1}$  due to a lot of  $\text{-CH}_2\text{-}$  present in the PLL dendrons.

In order to further confirm the success of the dendrimer synthesis, we determined the molar masses and molar mass distributions of the PLLA, PNIPAAm grafted with PLLA and dendrimer by using MALDI-TOF. As shown in Figure 2, the molar masses increase with the step of the syntheses, in the order of PLLA < PNIPAAm grafted with PLLA < dendrimer. The number average and the weight average molar mass of the dendrimer are around  $4200$  and  $5200\text{ g}\cdot\text{mol}^{-1}$ , respectively. The molar mass distribution of the dendrimer is  $1.2$ . In addition, the transmission electronic microscopy (TEM) results (data are not shown here) show that the particle size of the dendrimer in dry state is around  $20\text{-}40\text{ nm}$ .

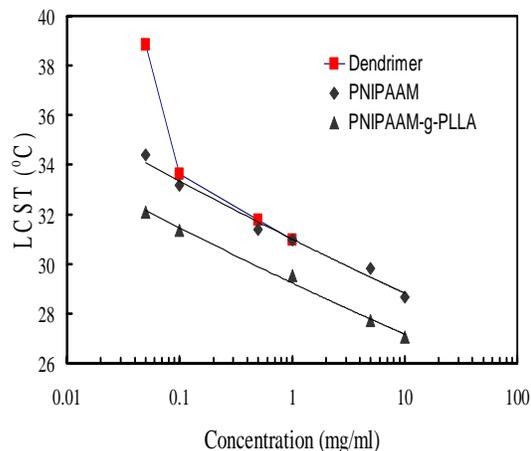


**Figure 2.** The MALDI-TOF spectra of PLLA, PNIPAAm grafted with PLLA and dendrimer.

Figure 3 shows the transmittances of the dendrimer in PBS ( $\text{pH}=7.4$ ) solutions with various concentrations as a function of temperature by using UV-VIS spectroscopy. The results indicate that the dendrimer show the LCST at  $31, 32, 34,$  and  $39\text{ }^\circ\text{C}$  at concentrations of  $1, 0.5, 0.1,$  and  $0.05\text{ mg}\cdot\text{ml}^{-1}$ , respectively, which increase with decreasing the concentration of dendrimer. However, at the lowest concentration of  $0.05\text{ mg}\cdot\text{ml}^{-1}$ , the thermoresponsive property of dendrimer became obscure. These results might be explained as follows: 1) The higher is the concentration, the stronger the dendrimer interacts with each other and the lower is the LCST. 2) Due to the positive charge and hydrophilicity of PLL in the dendrimer, the electrostatic interaction of the dendrimer becomes stronger with decreasing concentration so that the LCST at concentration of  $0.05\text{ mg}\cdot\text{ml}^{-1}$  is obscure.



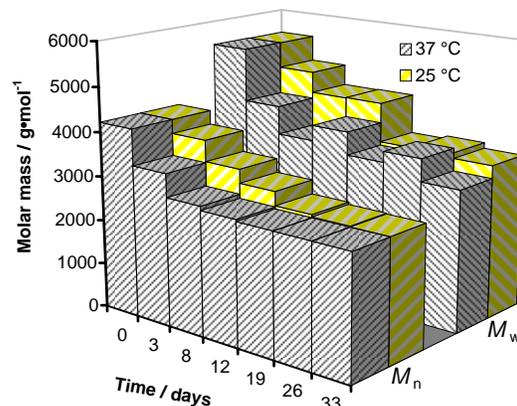
**Figure 3.** UV-VIS spectra of PLLA, PNIPAAm grafted with PLLA and dendrimer.



**Figure 4.** The LCST of PLLA, PNIPAAm grafted with PLLA and dendrimer.

Figure 4 shows the concentration dependence of the LCST of the polymers. The LCST of PNIPAAm alone and PNIPAAm grafted with PLLA decreased linearly with logarithmic concentrations, and the latter is 2 °C lower than the former over the concentrations due to the hydrophobicity of the PLLA. When PLL is conjugated at both ends of PNIPAAm grafted with PLLA, the LCST of the dendrimer shows non-linear relationship and the highest value compared to that of other two types of polymers over concentration  $< 1 \text{ mg}\cdot\text{ml}^{-1}$  due to the hydrophilicity of the PLL.

Figure 5 shows the molar mass of the dendrimer at concentration of  $0.1 \text{ mg}\cdot\text{ml}^{-1}$  with time at temperature below the LCST at 25 °C and above the LCST at 37 °C using MALDI-TOF. The molar mass of the dendrimer decrease with time, indicating that the dendrimer degrades due to the hydrolytically degradable PLLA component. Both the number and weight average molar mass of the dendrimer decreases faster at 37 °C than at 25 °C and reaches a stable value after 12 days. This means that the degradation of the dendrimer may be completed after 12 days since the initial weight average molar mass of the dendrimer is approximately  $5500 \text{ g}\cdot\text{mol}^{-1}$ , that of the PLLA is about  $2000 \text{ g}\cdot\text{mol}^{-1}$ , and the weight average molar mass of the dendrimer minus that of the PLLA is about  $3500 \text{ g}\cdot\text{mol}^{-1}$  which is the stable value after 12 days.



**Figure 5.** Molar mass of the dendrimer as a function of time at temperature 25 and 37 °C.

## Conclusions

We have successfully synthesized a smart and biodegradable dendrimer that contains PLLA as a biodegradable hydrophobic unit, PNIPAAm as a thermo-responsive unit and branched poly(L-lysine) as a dendron. The chemical structure and the molar mass of the dendrimer were confirmed by FTIR and MALDI-TOF. The dendrimer showed thermo-responsive properties with the LCST of 31, 32, 35 and 39 °C at concentration 1, 0.5, 0.1 and 0.05  $\text{mg}\cdot\text{ml}^{-1}$ , respectively, which increased with decreasing concentration. The dendrimer also demonstrated biodegradable property with molar mass decreasing up to 12 days. The degradation of the dendrimer depended on temperature, and above the LCST, the dendrimer was more susceptible to degradation. Further studies on targeted and sustained release of nerve growth factor using the designed dendrimer are being conducted in our laboratory.

## Acknowledgements

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## References

1. Dahan A and Portnoy M, Synthesis of poly(aryl benzyl ether) dendrimers on solid support. *Macromolecules* 2003. 36, 1034-1038.
2. Baille WE, Malveau C, Zhu XX, Kim YH, and Ford WT, Self-diffusion of hydrophilic poly(propyleneimine) dendrimers in poly(vinyl alcohol) solutions and gels by pulsed field gradient NMR spectroscopy. *Macromolecules* 2003. 36, 839-847.
3. Hedden RC and Bauer BJ, Structure and dimensions of PAMAM/PEG dendrimer-star polymers. *Macromolecules* 2003. 36, 1829-1835.
4. Choi JS, MacKay JA, and Szoka FC, Low-pH-sensitive PEG-stabilized plasmid-lipid nanoparticles: Preparation and characterization. *Bioconjugate Chemistry* 2003. 14, 420-429.
5. Dantlgraber G, Baumeister U, Diele S, Kresse H, Luhmann B, Lang H, and Tschierske C, Evidence for a new ferroelectric switching liquid crystalline phase formed by a carbosilane based dendrimer with banana-shaped mesogenic units. *Journal of the American Chemical Society* 2002. 124, 14852-14853.
6. Schroeder U, Sommerfeld P, Ulrich S, and Sabel BA, Nanoparticle technology for delivery of drugs across the blood-brain barrier. *Journal of Pharmaceutical Sciences* 1998. 87, 1305-1307.
7. Kreuter J, Nanoparticulate systems for brain delivery of drugs. *Advanced Drug Delivery Reviews* 2001. 47, 65-81.
8. Cima LG, Vacanti JP, Vacanti C, Ingber D, Mooney D, and Langer R, Tissue engineering by cell transplantation using

- degradable polymer substrates. *Journal of Biomechanical Engineering* 1991. 113, 143-151.
9. Langer R, Biomaterials in drug delivery and tissue engineering: one laboratory's experience. *Accounts of Chemical Research* 2000. 33, 94-101.
  10. Schmidmaier G, Wildemann B, Stemberger A, Haas NP, and Raschke M, Biodegradable poly(D,L-lactide) coating of implants for continuous release of growth factors. *Journal of Biomedical Materials Research* 2001. 58, 449-55.
  11. Okano T, Molecular Design of Temperature-Responsive Polymers as Intelligent Materials. *Advances in Polymer Science* 1993. 110, 179-197.
  12. Hoffman AS, Intelligent Polymers in Medicine and Biotechnology. *Artificial Organs* 1995. 19, 458-467.
  13. Galaev IY and Mattiasson B, 'Smart' polymers and what they could do in biotechnology and medicine. *Trends in Biotechnology* 1999. 17, 335-340.
  14. Lowe TL, Benhaddou M, and Tenhu H, Partially fluorinated thermally responsive latices of linear and crosslinked copolymers. *Journal of Polymer Science Part B-Polymer Physics* 1998. 36, 2141-2152.
  15. Lowe TL, Virtanen J, and Tenhu H, Hydrophobically modified responsive polyelectrolytes. *Langmuir* 1999. 15, 4259-4265.
  16. Park JU, Ishihara T, Kano A, Akaike T, and Maruyama A, Preparation of dendritic graft copolymer consisting of poly(L-lysine) and arabinogalactan as a hepatocyte specific dna carrier. *Preparative Biochemistry & Biotechnology* 1999. 29, 353-370.
  17. Poduslo JF, Wengenack TM, Curran GL, Wisniewski T, Sigurdsson EM, Macura SI, Borowski BJ, and Jack CR, Molecular targeting of Alzheimer's amyloid plaques for contrast-enhanced magnetic resonance imaging. *Neurobiology of Disease* 2002. 11, 315-329.