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# Effect of poly(amidoamine) dendrimers on the structure and activity of immune molecules

Jiansheng Lin [a], Wenxi Hua [a], Yi Zhang [b], Chenghua Li [b], Wei Xue [b], Jian Yin [c], Zonghua Liu [b,*], Xiaozhong Qiu [a,**]

[a] Department of Anatomy, Key Laboratory of Construction and Detection of Guangdong Province, Southern Medical University, Guangzhou 510515, China
[b] Department of Biomedical Engineering, Jinan University, Guangzhou 510632, China
[c] Department of Anatomy, Hunan University of Chinese Medicine, Changsha 410208, China

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ABSTRACT

*Background:* Poly(amidoamine) (PAMAM) dendrimers are widely used biomedical polymers, which are extensively applied in drug delivery, gene delivery, contrast agent, etc. In these biomedical applications, the bio-safety of the PAMAM dendrimers is a critical issue, which affects not only their toxicity to the host but also the expected in vivo biofunctions of the materials. To clarify the bio-safety of PAMAM dendrimers, the effects of generation 5 PAMAM dendrimers with amine, hydroxyl or carboxyl groups on immune molecules were explored in this work.

*Methods:* Specifically, the effect of the PAMAM dendrimers on the secondary structure and conformation of immune molecule γ-globulin was studied by using ultraviolet-visible, fluorescence, and circular dichroism spectroscopies. The effect of the PAMAM dendrimers on complement activation was determined by enzyme-linked immunosorbent assay. Further, the effect of the PAMAM dendrimers on antigen–antibody reaction was studied by using human red blood cell agglutination assay.

*Results:* The results showed that, the PAMAM dendrimers could affect the secondary structure and conformation of γ-globulin, and inhibited complement activation. Generation 5 PAMAM dendrimer with carboxyl group at 10 mg/mL impaired red blood cell (RBC) antigen–antibody reaction.

*Conclusions:* From these results, the effects of the PAMAM dendrimers on immune molecules depend on their bulk structure and surface groups.

*General significance:* This work provides important information for the immunocompatibility evaluation, preclinical design, and clinical applications of PAMAM dendrimers.



## 1. Introduction

Poly(amidoamine) (PAMAM) dendrimers are a type of highly branched polymers that have unique physical and chemical properties, such as three-dimensional globular architecture, and functional groups on their surface. Therefore, PAMAM dendrimers have extensive biomedical applications in drug delivery [1], gene delivery [2], imaging [3], etc. In these biomedical applications, the bio-safety of PAMAM dendrimers is a critical issue, which mainly includes hemocompatibility, tissue-compatibility, and immunocompatibility. Bio-safety of the biomedical materials affects not only their toxicity to the host but also their pre-designed in vivo biofunctions. Based on this perspective, the biocompatibility of PAMAM dendrimers should be comprehensively evaluated. To clarify the bio-safety of PAMAM dendrimers, many researchers have investigated their biological effects on blood tissue, various tissue-cultured cells, etc. For example, some researchers reported the effects of PAMAM dendrimers on the structure and function of key blood components, including aggregation, morphological alteration and hemolysis of human red blood cells (RBCs), structural and conformational change and polymerization function of fibrinogen, and blood coagulation [4–6]. Some researchers found that PAMAM dendrimers promote acute lung injury by inducing autophagic cell death [7].

For any biomedical materials, their effect on host immune system (i.e., immunocompatibility) is also a key aspect of their bio-safety evaluation. Compared to hemocompatibility evaluation, immunocompatibility evaluation of biomedical materials is much less clarified, probably because of the complexity of immune system and the difficulty in immunity-related experimental studies. As for PAMAM dendrimers, some related studies include the mechanism of cell death induced by PAMAM dendrimers in RAW 264.7 murine macrophage-like cells [8], biological responses of J774A.1 murine macrophage-like cells induced by PAMAM dendrimers such as intracellular reactive oxygen species, cytokine production and cytotoxicity [9].

* Correspondence to: Z. Liu, Department of Biomedical Engineering, Jinan University, Guangzhou 510632, China. Tel.: +86 20 85223062.
** Correspondence to: X. Qiu, Department of Anatomy, Key Laboratory of Construction and Detection of Guangdong Province, Southern Medical University, Guangzhou 510515, China. Tel.: +86 20 61647752.
*E-mail addresses:* tliuzonghua@jnu.edu.cn (Z. Liu), qiuxzh@aliyun.com (X. Qiu).

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To explore the potential impact of PAMAM dendrimers on the immune system, this work focuses on the effects of PAMAM dendrimers on immune molecules. Besides, it is reported that the surface group of PAMAM dendrimers plays a key role in their biocompatibility [7, 10–12]. Therefore, PAMAM dendrimers with amine, hydroxyl or carboxyl groups (their chemical structures illustrated in Fig. 1) were used to compare the influence of different surface groups. Specifically, the effect of PAMAM dendrimers on the secondary structure and conformation of immune molecule γ-globulin was studied by using UV–vis, fluorescence, and circular dichroism (CD) spectroscopies. The effect of PAMAM dendrimers on complement activation was determined by enzyme-linked immunosorbent assay (ELISA). Further, the effect of PAMAM dendrimers on antigen–antibody reaction was studied by using human RBC agglutination assay. This work provides important information for the bio-safety evaluation, preclinical design, and clinical application of PAMAM dendrimers.

## 2. Materials and methods

### 2.1. Materials

PAMAM dendrimers (with an ethylenediamine core) with amine (–$NH_2$, generation 5), hydroxyl (–OH, generation 5) or carboxyl (–COOH, generation 4.5) groups were purchased from Weihai CY Dendrimer Technology Co., Ltd (Weihai, China), as abbreviated G5 PAMAM–$NH_2$ (catalog number CYD-150A), G5 PAMAM–OH (catalog number CYD-150H), or G4.5 PAMAM–COOH (catalog number CYD-145C), respectively. The sizes of the PAMAM dendrimers were measured with a Zetasizer Nano-ZS zeta potential analyzer (Malvern Instruments), as shown in the supplementary information. γ-Globulin was purchased from Sigma-Aldrich (catalog number G4386-1G, St. Louis, MO, USA). Commercial C3a ELISA kit was purchased from eBioscience (catalog number 83919001, San Diego, USA). Blood grouping reagents of monoclonal antibodies anti-A and anti-B were purchased from Hemo-Pharmaceutical & Biological Reagent Co., Ltd (catalog number 5012012, Shanghai, China). Fresh whole blood was donated by healthy consenting volunteers and was collected in sodium citrate vacuum tubes. The whole blood was centrifuged at 1000 ×*g* for 5 min, and the supernatant plasma was collected. The remaining RBC pellet was washed with phosphate buffered saline (PBS, pH 7.4). The PAMAM dendrimers and γ-globulin were dissolved in PBS before use.

### 2.2. Effect of PAMAM dendrimers on the structure and conformation of γ-globulin

UV–vis absorption spectra of γ-globulin (0.1 mg/mL) containing different concentrations of PAMAM dendrimers were recorded with a UV-2550 spectrometer (Shimadzu Corporation, Kyoto, Japan). The spectra were recorded from 200 to 400 nm at room temperature in quartz cuvettes of 1 cm optical path length.

Fluorescence emission spectra of γ-globulin (0.1 mg/mL) containing different concentrations of PAMAM dendrimers were recorded with a Hitachi F-7000 fluorescence spectrophotometer (Hitachi High-Technologies Corp., Tokyo, Japan). The spectra were recorded at $\lambda_{exc}$ 280 and $\lambda_{em}$ from 290 to 450 nm at 25 °C. All fluorescent spectra were recorded with 1 × 1 cm path length quartz cells, excitation and emission slit widths of 5 nm, and scan rate of 1200 nm/min.

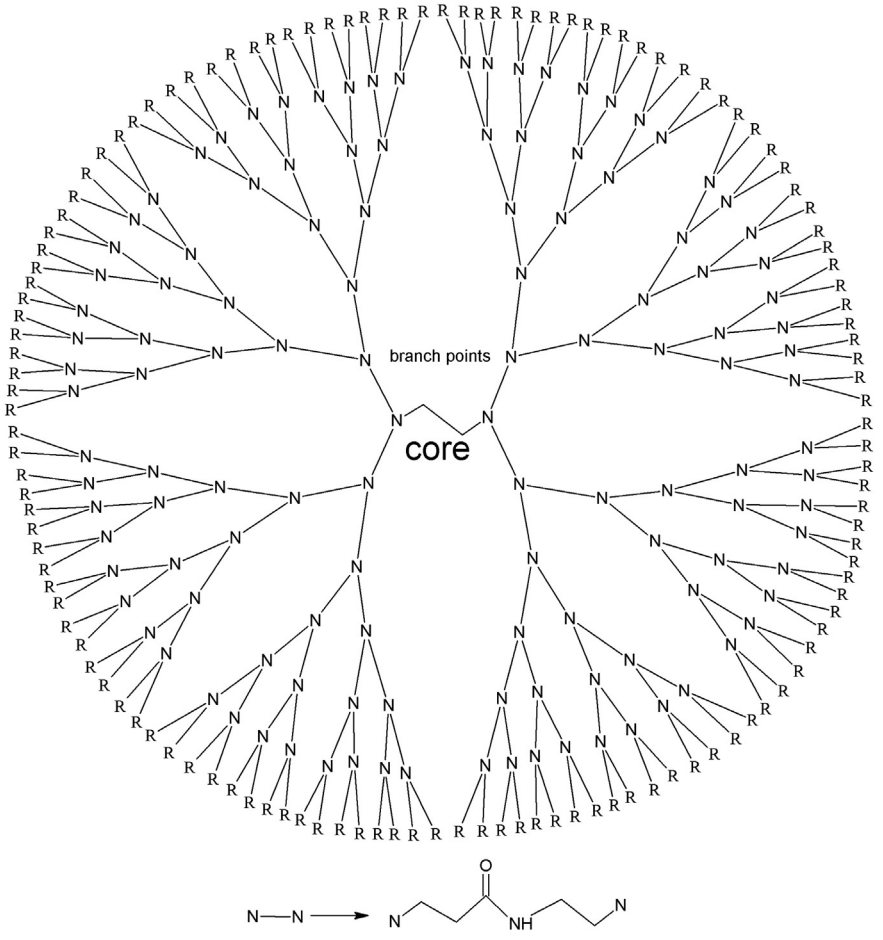


**Fig. 1.** Chemical structure of the PAMAM molecule (G5 PAMAM–$NH_2$ as R═$NH_2$, G4.5 PAMAM–COOH as R═COOH, and G5 PAMAM–OH as R═OH).