称号及び氏名	博士（工学）　李　小杰
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論文審査委員	主査　河野　健司 副査　長岡　勉 副査　松本　章一

論文要旨

Dendrimers are a family of synthetic polymers with a regularly branched tree-like structure. The globular shape given to these dendrimers by such a highly branched backbone provides various surface properties and an interior capable of encapsulating guest materials. Furthermore, because molecular chains of dendrimers are grown stepwise by repeatedly introducing branch structures to their chain ends, their molecular sizes and structures can be controlled precisely. The uniformity at molecular level and well-defined composition of dendrimers make them highly attractive as biomedicine with controllable *in vivo* behaviors.

For biomedical applications, bio-related functions are essential for dendrimers to increase their efficacy. Because of their globular structure, the surface region of dendrimer presents particularly attractive moieties for functionalization. For example, polyethylene glycol (PEG) chains have been attached to chain terminals of dendrimers to provide a biocompatible surface. In addition, various surface alkylamide-terminated dendrimers were prepared to provide dendrimers temperature- responsive functions for stimuli-responsive drug delivery. However, toxicity derived from the terminal alkylamide groups limits their biomedical

applications. To overcome this shortcoming, in this thesis, oligo(ethylene glycol) was used for surface modification of PAMAM dendrimers, providing both temperature-sensitive properties and nontoxic characteristics to the dendrimer surface simultaneously.

The interior region of dendrimers is another site for their functionalization. Various kinds of functional molecules and nanoparticles were shown to be encapsulated in the dendrimer interior, thereby providing various functions to dendrimers. Dendrimers with photoactivable metal nanoparticles (e.g. gold nanoparticle) core have been explored for potential bio-imaging and photothermal therapy applications. Furthermore, when dendrimers have functions such as stimulus-sensitivity, the hybrid dendrimers are expected to exhibit multiple functions derived from both interior encapsulated nanoparticle and dendrimer itself. In this thesis, oligo(ethylene glycol)- modified PAMAM dendrimers encapsulating gold nanoparticle were prepared as dually functionalized dendrimers for temperature-responsive cancer photothermal therapy.

Dendrimers with gold nanoparticle core are one of the promising candidates for potential cancer photothermal therapy. However, spherical gold nanoparticle exhibit surface plasmon resonance with visible light around 520 nm that can't penetrate into the body deeply. For a better performance *in vivo*, in this thesis, gold nanorod-core dendrimers having SPR property with NIR light was developed for effective cancer photothermal therapy applications, due to the fact that NIR light is minimally absorbed by skin and tissues and it can penetrate tissues noninvasively and more deeply.

Dendrimers have been investigated intensively as a versatile platform to form organic–inorganic hybrid nanomaterials for various potential biomedical applications. However, few reports describe the use of dendrimer-based hybrid nanomaterials for drug delivery applications. In this thesis, a novel dendrimer-modified gold nanorod platform with drug-covalent conjugation to dendrimer layer as a smart drug delivery system for synergistic cancer photothermal-chemotherapy was developed.

This thesis consists of 6 chapters, and the details of each chapter are shown as follows:

In Chapter 1, the background, the objectives, and the contents of this thesis were described.

In Chapter 2, a new type of temperature-sensitive dendrimer was synthesized using one-step terminal modification of polyamidoamine dendrimers (PAMAM) with various alkoxy diethylene glycols such as methoxy diethylene glycol, ethoxy diethylene glycol, and

propoxy diethylene glycol. The obtained dendrimers exhibited tunable lower critical solution temperature (LCST), depending on PAMAM dendrimer generations and terminal alkoxy groups. Cellular uptake of the dendrimers was enhanced by increasing their incubation temperature above the LCST. In addition, the in vitro cytotoxicity of temperature-sensitive dendrimers at incubation temperatures below and above LCST was much lower than that of their parent PAMAM dendrimers. Results indicate that the dendrimers with oxyethylene unit-enriched surface might be promising to construct intelligent drug delivery systems for cancer therapy.

In Chapter 3, temperature-sensitive and photoactivable dendrimers were prepared by synthesis gold nanoparticles (AuNP) in the interior of propoxy diethylene glycol (PDEG)-modified PAMAM dendrimers. Compared with empty PDEG-G5 PAMAM dendrimers, loading of AuNP caused the elevation of the cloud point of hybrid PDEG-G5-AuNP dendrimers. Photothermal study indicated that the AuNP-loaded dendrimers have photoactivable heat-generating ability under laser irradiation. Furthermore, light irradiation triggered temperature-sensitive phase transfer behavior of hybrid dendrimers was observed, showing the dually temperature and photo responsive properties of hybrid dendrimers. *In vitro* cell-killing experiments showed a huge advantage of temperature-sensitive hybrid dendrimers for photothermal therapy against Hela cells comparing with temperature-insensitive controls, indicating the potential biomedical applications of this dually functionalized dendrimers.

In Chapter 4, a new type of hybrid dendrimer consisting of a gold nanorod (AuNR) core and PEG-modified PAMAM (PEG-PAMAM) dendrons was synthesized by adding PEG-PAMAM G2–G4 dendrimers with a cystamine core at various timing during the AuNR growing reaction. Hybrids of the dendrimers and AuNR exhibiting surface plasmon resonance in the near infrared region were obtained. Whereas PEG-PAMAM G4 dendrimer-AuNR hybrid formed aggregate in an aqueous solution, PEG-PAMAM G2 and G3 dendrimers respectively gave AuNR hybrids with average diameters of 24 nm and 31 nm. Especially, the spherical PEG-PAMAM G3 dendrimer-AuNR hybrid might be regarded as an AuNR-core PEG-PAMAM dendrimer in which the AuNR core was well stabilized by highly hydrated PEG-PAMAM G3 dendrons. The AuNR-core PEG-PAMAM G3 dendrimer exhibited excellent heat-generation capability under near-infrared light irradiation. Incubation with the

AuNR-core PEG-PAMAM G3 dendrimer showed no damage to HeLa cells. However, dendrimer-treated cells were killed effectively by near-infrared laser irradiation, indicating excellent photothermal capability of the AuNR-core PEG-PAMAM G3 dendrimers. Furthermore, AuNR-core PEG-PAMAM G3 dendrimers injected into mice tumor tissues significantly increased the temperature of the tumor when irradiated with near infrared light, resulting in decreased tumor volume. Results demonstrate that AuNR-core PEG-PAMAM G3 dendrimers might be a new nanomaterial for biomedical applications such as cancer photothermal therapy.

In Chapter 5, pH-sensitive drug-dendrimer conjugate-hybridized gold nanorod was prepared as a promising platform for combined cancer photothermal-chemotherapy under *in vitro* and *in vivo* conditions. Poly(ethylene glycol)-attached PAMAM G4 dendrimers (PEG-PAMAM) were first covalently linked onto the surface of mercaptohexadecanoic acid-functionalized gold nanorod (MHA-AuNR), with subsequent conjugation of anti-cancer drug doxorubicin (DOX) to dendrimer layer using an acid-labile-hydrazone linkage to afford PEG-DOX-PAMAM-AuNR particles. The particles with a high PEG-PAMAM dendrimer coverage density (0.28 per nm2 AuNR) showed uniform sizes and excellent colloidal stability. In vitro drug release studies demonstrated that DOX released from PEG-DOX-PAMAM-AuNR was negligible under normal physiological pH, but it was enhanced significantly at weakly acidic pH. The efficient intracellular acid-triggered DOX release inside of lysosomes was confirmed using confocal laser scanning microscopy (CLSM) analysis. Furthermore, the combined photothermal-chemo treatment of cancer cells using PEG-DOX-PAMAM-AuNR for synergistic hyperthermia ablation and chemotherapy was demonstrated both in vitro and in vivo to exhibit higher therapeutic efficacy than either single treatment alone, underscoring the great potential of PEG-DOX- PAMAM-AuNR particles for cancer therapy.

In Chapter 6, results and conclusions of this thesis were summarized.

審査結果の要旨

本論文は、非侵襲的な手段で効果的にがん治療を行うために、樹状高分子デンドリマーを用いて様々なバイオ機能をもつナノマテリアルの開発に関する研究成果をまとめたものであり、次のような成果を得ている。

（１） ポリアミドアミンデンドリマーに種々の構造のオリゴエチレングリコール鎖を導入することで、特定の温度において親水性から疎水性に特性変化する温度応答性デンドリマーを開発し、このデンドリマーが標的病巣選択的なキャリアとして有用であることを示した。

（２） 温度応答性デンドリマーに金ナノ粒子を内包させることで、温度と光に二重応答するデュアルシグナル応答型ハイブリッドデンドリマーを開発した。このハイブリッドデンドリマーのがん細胞殺傷能の発現を温度と光でデュアル制御できることを明らかにし、がん病巣選択的なホトサーマル治療を実現するためのナノ治療デバイスとして有用であることを示した。

（３） ポリエチレングリコールを表面に結合したシスタミンコアデンドリマーの共存下、金ナノロッドを成長させることで金ナノロッドをコアとする生体適合性デンドリマーを開発した。このデンドリマーが、生体浸透性に優れる近赤外光の照射によって強く発熱して高い腫瘍抑制効果を示すことを明らかにし、ホトサーマル治療を実現するためのナノ治療デバイスとして有用であることを示した。

（４） 抗癌がん剤ドキソルビシンとポリエチレングリコールを結合したポリアミドアミンデンドリマーを金ナノロッドに結合させることでデンドリマー金ナノロッドハイブリッドを開発した。このハイブリッドが、抗がん剤による化学効果と近赤外光照射下での発熱効果の相乗作用によって強いがん細胞殺傷能を発現して高い抗腫瘍効果を示すことを明らかにし、ホトサーマルケモセラピーという新しいナノ治療技術の開拓に寄与することを示した。

以上の諸成果は、がん治療用デバイス開発のためのベースマテリアルとしてのデンドリマーの有用性を明らかにするものであり、先進がん治療技術の発展に必要とされる生医学材料開発の技術基盤の構築に貢献するところ大である。また、申請者が自立して研究活動を行うのに必要な能力と学識を有することを証したものである。