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主　論　文　の　要　旨

論文題目　Development of Multifunctional Nanomaterials for *In vivo* Imaging and Novel Therapy of Tumors
(多機能ナノマテリアルによる腫瘍 *in vivo* イメージングと新規治療法の開発)

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論　文　内　容　の　要　旨

Nowadays, advanced nanoprobes are being widely developed for novel biomedical imaging that can realize the diagnosis of diseased tissues in the early stage without surgery or biopsy. In relative to conventional anatomy-based biomedical imaging, novel biomedical imaging is noninvasive, safe and real-time, which shows great potential in clinical applications. In Chapter 1, we first review biomedical imaging modality based on nanoprobes. Optical imaging (OI) includes fluorescence imaging (FI), bioluminescence imaging (BLI), chemiluminescence imaging (CLI) et al., which has high sensitivity and is equipped with multicolor for imaging. However, OI suffers from low penetration depth, so it is usually applied to the analysis of shallow tissues in living body. Magnetic resonance imaging (MRI) is able to analyze deep tissues with high spatial resolution, but low sensitivity and high cost are inevitable. Computed tomography (CT), which scans the tissue via X-rays, shows high spatial resolution and unlimited tissue-penetration depth as MRI. But it involves high cost and radiation risk. Positron emission tomography (PET) possesses good penetrability, high intensity and specificity in spite of low spatial resolution, high cost and radiation risk. Photoacoustic imaging (PAI) combines high sensitivity from OI and good penetrability from ultrasound imaging (USI), which has great prospects for application in clinical diagnosis. Due to the inherent limitations of each imaging modality for in vivo analysis, it is difficult for mono-modal imaging to simultaneously meet the requirements of

sensitivity, spatial resolution and tissue penetration depth. Therefore, the design of multimodal imaging probes that integrates two or more imaging modalities is beneficial to obtaining accurate information, and will become the trend of the development of imaging CAs for precise diagnostics in the future, which is also reviewed in this chapter.

At present, cancer therapies remain a vital challenge in the clinical application because conventional therapeutic strategies including surgery, chemotherapy and radiotherapy, involve inevitable weaknesses. They are invasive, poorly specific and lowly efficient. Besides, they cause substantial damage to normal cells and immune cells, leading to adverse side effects towards main organs, which becomes counterproductive to recovery and ultimately results in the overall debilitation of the patient. By virtue of the development of various NPs with unique physicochemical properties, novel therapies based on nanomedicines have provided hopeful opportunities for cancer treatment, which are summarized in the later Chapter 1. Magnetic hyperthermia therapy and photothermal therapy (PTT) are able to heat and thermally ablate tumor tissues by virtue of external alternating magnetic field or laser irradiation. Photodynamic therapy (PDT), a type of light-triggered therapy, can convert oxygen into reactive oxygen species (ROS) and causes oxidative damage to tumor cells with the help of photosensitizer. Sonodynamic therapy (SDT) activates sonosensitizers to produce excessive ROS via certain acoustic cavitation effects under ultrasound (US), resulting in cancer cell apoptosis. Likewise, chemodynamic therapy (CDT) is a continuous chemical process that converts H_2O_2 in the TME into toxic ROS, generally based on Fenton or Fenton-like reactions. In the actual application, single modality of therapy maybe not thorough enough to eliminate tumors by administering a low dose of nanomaterials. Taking biocompatibility into consideration, it's unlikely to use drugs with extremely high dose in spite of the complete curative efficiency. Hence, synergetic therapies have been attracted much attention to promote the therapeutic effect, which are also summarized in this chapter.

In Chapter 2, we report a fluorescent and magnetic nanoprobe (QMNP-RGD) and successfully demonstrate that QMNP-RGD can be efficiently delivered into U87MG cells and used for fluorescence/magnetic resonance (MR) bimodal imaging. The electrostatic assembly of QMNP-RGD included the preparation of TMADM and modification of QDs with RGD peptide. First, commercial QDs were selected as the fluorophore due to their high fluorescence intensity and remarkable stability. Since being verified as a type of tumor-targeting ligand for active recognition towards $\alpha_v\beta_3$ integrin, which is overexpressed on the membrane of many kinds of tumor cells, RGD peptide has been used to decorate QDs (denoted as QD-RGD) to promote delivery into

tumors. As QDs are stabilized by negatively charged carboxylic groups, it is necessary to create a positively charged magnetic nanomaterial for electrostatic binding with QD-RGD. We therefore introduced a cationic polysaccharide to encapsulate SPIO NPs to form fluorescent and magnetic nanoprobes (QMNP-RGD) based on the electrostatic force. We examined the integration of QMNP-RGD by the size and zeta potential detection. The cytotoxicity was also evaluated. Finally, we proved that QMNP-RGD could be used for *in vitro* fluorescence/MR bimodal imaging of U87MG tumor cells via FI of cells, flow cytometric analysis and bimodal imaging of cell pellets.

In Chapter 3, we report a simultaneous production of versatile magnetic gadolinium oxide (Gd_2O_3) and CuS NPs through a simple one-pot synthesis method at room temperature and further decorate them into a fluorescent and tumor-targeting nanoprobe (BCGCR), which integrates NIR fluorescence/T_1-weighted MR bimodal imaging and NIR light-triggered PTT and enhanced CDT of tumors. The assembly of versatile BCGCR includes the preparation of BSA-capped CuS/Gd_2O_3 NPs (denoted as BCG) and subsequent decoration. BCG is produced using BSA as a template and stabilizer via a biomimetic mineralization according to the methods of previous reports, with some modification. Cu^{2+} and Gd^{3+} are anchored in BSA to form a Cu^{2+}-BSA-Gd^{3+} complex based on the affinity between amino acid residues and metal ions. Then, OH^- is introduced to adjust the pH (approaching 12), which induces BSA to extend into a hollow structure. Afterwards, CuS nanocrystals form and gradually grow following the addition of S^{2-}, while Gd^{3+} reacts with OH^- to generate Gd_2O_3 NPs. The obtained BCG is further conjugated with a Cy5.5 fluorophore for NIR fluorescence with diminished scattering, absorption and autofluorescence from the living organism, which is suitable for highly sensitive *in vivo* FI. Assisted by magnetic Gd_2O_3 NPs for MRI, which provides high spatial resolution and unlimited penetrability, this nanoprobe can be applied for dual modality imaging to guide cancer treatment using an NIR laser. Then sulfo-NHS-acetate was introduced to block -NH_2 of BSA to prevent self-polymerization in the subsequent peptide conjugation. The assembly of RGD peptide facilitates active transport towards tumors, since it has been accepted as a valid tumor-targeting ligand for selective recognition towards $\alpha_v\beta_3$ integrin overexpressed on the membrane of various tumor cells. Benefiting from the enhanced permeability and retention (EPR) effect, as well as $\alpha_v\beta_3$ integrin-mediated active recognition, BCGCR can partially arrive at tumors after intravenous administration, and preferentially accumulate into lysosomes by endocytosis. The process of BCGCR accumulation can be monitored in real time using both NIR FI from Cy5.5 fluorophores with an intense signal and tissue-penetrable MRI owing to Gd element. Guided by the dual-mode imaging, the

tumor site is exposed under NIR laser irradiation for treatment. The hyperthermia produced by internal CuS with high PCE ablates tumors and simultaneously accelerates the ionization of CuS. Then the generation of ·OH is promoted by both hyperthermia and released Cu^{2+}, leading to the disintegration of cellular proteins and DNA. As a consequence, the synergistic PTT and enhanced CDT of tumors can be realized under guidance by bimodal imaging. We first examined and ascertained the components of BCG, and the characteristics of BCGCR. Then, BCGCR was applied to *in vitro* U87MG tumor cells for bimodal imaging and photo-assisted therapy of tumors. Finally, we demonstrated that BCGCR the feasibility of fluorescence/MR bimodal imaging-guided synergetic PTT and intensified CDT of tumors in living mice.

In Chapter 4, summary and perspectives were focused on. We believe that the strategy of integrating different components with respective function, such as electrostatic binding, encapsulation and covalent modification, will be beneficial to exploring other advanced nanomaterials for the clinical application of multimodal imaging-guided synergetic cancer therapies.