学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Development of fluorescent molecules and nanobioconjugates for cell imaging and singlet oxygen sensing
（細胞イメージングと一重項酸素センシングに向けた蛍光分子とナノバイオコンジュゲートの開発）

Recently, theranostics, a combination of diagnosis and therapy, has become a key modality in cancer management. Certain intrinsic limitations in conventional cancer diagnosis/therapy strategies lead to the development of nanomaterial-basedtherapeutics. Different nanoparticles have been developed into theranostics by combining multimodal contrast/imaging agents, drugs, and targeting moieties for cancer diagnosis, monitoring, therapy, and treatment follow-up. Theranostics for fluorescence imaging or fluorescence molecular tomography (FMT) using organic dyes and semiconductor nanocrystals with ligands/antibodies against cancer markers receive much attention in basic research and clinical applications. Also, nanomaterials combining FMT with chemotherapy, hyperthermia, or phototherapy enter the clinical stage. This thesis focuses on nanobioconjugates combining fluorescence probes, photosensitizers, fluorogenic sensors, and cancer-targeting biomolecules.

I use fluorescence probes such as semiconductor quantum dots (QDs), a coumarin derivative, and a porphyrin to detect or image cancer cells. Also, QDs and porphyrins generate singlet oxygen ($^1O_2$), an essential reactive oxygen species (ROS) in photodynamic therapy (PDT), which is detected using an electron donor-acceptor (D-A) fluorogenic sensor molecule. The cancer-targeting biomolecules include anticancer antibodies and a peptide. Rationally designed nanobioconjugates using the above components help me enrich and efficiently detect circulating tumor cells (CTCs) in blood samples, and produce, detect, store and release $^1O_2$ in a solution or cells.

Chapter 1 of the thesis provides a general introduction to fundamental aspects of cancer management. Next, I discuss the significance of CTC-based liquid biopsy and the current detection technologies for CTC isolation and enrichment. The importance of nanomaterials-based immunocapture and optical detection based on their fundamental properties are also discussed. Next, I discuss the role of $^1O_2$ in cancer therapeutics due to its cytotoxic effect on various biological substrates. I also discuss a few biological and chemical processes involved in the generation of $^1O_2$ followed by its detection using fluorescent molecular probes. Chapter 2 provides the experimental procedures and techniques used throughout the study. I discuss the procedure for the functionalization of silica, attachment of antibodies on functionalized

silica microparticles, QD labeling with the antibodies, and the attachment of QD-antibody conjugate on the functionalized silica particles. I also discuss the synthesis of a $^1O_2$ sensor molecule, preparation of n anoassemblies, and the conjugation of cell-penetrating peptides on the nanoassembly. Next, I discuss the procedure for cell culture and cell labeling. I also discuss time-resolved fluorescence spectroscopy used fo r the characterization of CTCs. Finally, I discuss UV-vis absorption spectroscopy, steady-state and time-r esolved fluorescence spectroscopy, single-particle microspectroscopy, confocal laser microscopy, nuclearmag netic resonance spectroscopy, and scanning electron microscopy used in this thesis. In chapter 3, I discu ss a multimodal fluorescence microspectroscopic detection of the mesenchymal-antigen specific CTCsand their collection and characterization. I use self-segregating immunosilica microparticles to capture the pr e-labeled cellsand the cells are identified from multicolor images, fluorescence spectra, and fluorescence decay profile of dyes or QDs. The large size of silica microparticles avoids endocytosis and externalforce for cell separation, and the CD44 antigen-selective cell capture results in an error-free detection. The C D44-targeted method combined with the above modalities shows a 9-fold detection accuracy for CTCs a mong blood cells. In chapter 4, to increase the efficiency of intracellular $^1O_2$ generation, detection, and r elease, I synthesize a $^1O_2$ sensor composed of an aminomethyl anthracene and a coumarin moieties.I co nstruct a nanoassembly of a sensitizer and the sensor and investigate the ability of the assemblytogene rate, store, sense, and release $^1O_2$ at the ensemble, single-particle, and cell levels. In all cases, the senso r shows anenormous fluorescence enhancement due to $^1O_2$ generation by the photosensitizer. Themechan ism behind the sensing and releasing is explained in detail in this chapter. The intracellular $^1O_2$generat ion and uptake ability of the nanoassembly is studied after conjugating an RGD peptide. The single-par ticle and cell imaging reveal a continuous $^1O_2$ release andefficient cell death. In addition, the fluorescenc e from the photosensitizer and the sensor help colocalized fluorescence cell imaging. Thus, this workhig hlights the utilization of programed nanocarriers for multimodal cancer therapeutic strategies.