# 学 位 論 文 審 査 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)

Materials encompassing contrast agents, targeting molecules, molecular sensors, and drugs become promising tools for cancer management by the detection and targeted therapy. The flexible chemistry and biocompatibility of silica particles and excellent fluorescence properties of organic dyes and semiconductor quantum dots (QDs) attracted the candidate to develop and test silica particle-based bioconjugate platforms for cancer management. These included (i) silica microparticles labeled with anticancer antibodies, fluorescent dyes, and QDs for collecting and micro-spectroscopically detecting cancer cells in the blood, and (ii) silica nanoparticles (SNPs) conjugated with photosensitizer (PS) drugs, singlet oxygen ($^1O_2$) sensors, and cell-penetrating peptides for photodynamic cancer therapy. The research results are classified into four chapters, with general conclusions and prospects in the fifth chapter.

In chapter 1, the candidate provided a general introduction to fundamental aspects of cancer management, including various cancer screening and treatment methods. The main focuses of this chapter are (i) the significance of circulating tumor cells (CTCs) to cancer detection by liquid biopsy, (ii) the current technologies for CTC isolation and detection, and (iii) the production, properties, and detection of $^1O_2$, an important intermediate in photodynamic cancer therapy.

Materials, samples, and experimental methods are discussed in Chapter 2. This chapter includes detailed information about preparing CTC-detecting silica microparticles, and $^1O_2$ storing-releasing-sensing molecules and SNPs. The candidate provided the details about blood cells, cancer cells, cell culturing, cell labeling, $^1O_2$ sensors syntheses, and silica particle modifications using antibodies, QDs, $^1O_2$ sensors, and PS drugs. Next, the candidate provided microscopic and spectroscopic techniques for characterizing $^1O_2$ sensors, silica particles, and labeled cells. These techniques included UV-vis absorption spectroscopy, nuclear magnetic resonance spectroscopy, scanning electron microscopy, steady-state and time-resolved fluorescence spectroscopy, single-particle microspectroscopy, and laser scanning confocal microscopy.

In chapter 3, the candidate discussed multimodal fluorescence microspectroscopic detection of cancer

cells in the presence of blood cells. Cancer cells were collected by targeting the cancer-specific, over-expressed antigens such as EFGR, EpCAM, or CD44, using silica microparticles functionalized with the corresponding antibodies. Here, the large sizes of the silica particles helped isolate the silica particle-cell assemblies by gravity separation. The collected cells were distinguished from blood cells by multiple modalities, including three-color imaging, trimodal fluorescence spectra, and triply degenerate fluorescence decay profiles of nucleus staining dyes or QDs. The candidate found EGFR-based cancer cell capturing provides false positive results due to blood cells binding to the anti-EGFR-antibody functionalized silica particles. Conversely, the widely followed EpCAM antigen-targeted cell detection resulted in false negative results because of the low-level expression of EpCAM antigen in certain cancer cells, including cells that have undergone epithelial to mesenchymal transitions. Therefore, CD44 antigen-selective cell capturing was investigated for different cancer cell types in the presence of blood cells, which is based on retention of the stem-cell specific maker CD44 in several cancer cell types and transitioned cancer cells. Therefore, by combining anti-CD44-antibody and fluorescent labels on silica microparticles, the candidate demonstrated an accurate and multimodal fluorescence microspectroscopy method for collecting and characterizing cancer cells in blood samples.

In chapter 4, the candidate focused on synthesizing $^1O_2$ sensors composed of aminomethyl anthracene and coumarin moieties, preparing SNP nanoassemblies with $^1O_2$ sensors and PS drugs, and evaluating the generation, storing, photo-triggered releasing, and sensing $^1O_2$ in solutions, single particles, and cells. The sensor showed an enormous fluorescence enhancement due to the reaction of $^1O_2$ generated by the PS drug. The mechanisms behind $^1O_2$ sensing and releasing were explained in detail in this chapter. The intracellular uptake and $^1O_2$ generation-storing-sensing abilities of the nanoassembly were studied with or without conjugating a cell-penetrating RGD peptide to the assembly. Single-particle and cell imaging revealed continuous $^1O_2$ release and efficient cell death. In addition, the red emission from the PS and the blue emission from the sensor helped the candidate with bimodal cell imaging.

In chapter 5, the candidate provided general conclusions and prospects of silica-based platforms and nanomaterials comprising fluorescent labels, cancer-targeting molecules, $^1O_2$ sensors, and PS drugs for cancer management.

審査員一同は，これらの成果を高く評価し，また研究者として誠実かつ熱心であり，大学院博士課程における研鑽や修得単位などもあわせ，申請者が博士（環境科学）の学位を受けるのに充分な資格を有するものと判定した。