学　位　論　文　内　容　の　要　旨

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Surface-Enhanced Raman Spectroscopy (SERS) for Intracellular pH Monitoring at Individual Single Cells

（単一細胞内 pH 分析を指向した表面増強ラマン分光法）

The protons play a critical role in bio-reactions in the highly compartmentalized cell which is a dynamic and heterogeneous system undergoing various metabolism. Much valuable information of various cellular processes could be reflected through intracellular pH variation. For example, abnormal pH of the lysosomes is often involved in diseases. Furthermore, cancer cells feature a reversed pH gradient, namely intracellular alkalinization and extracellular acidosis. Therefore, studies on the variations of the intracellular pH are crucial for elucidating the interplay with cell functions, gaining insights into both pH-related pathological processes, and developing new therapeutic strategies.

Although several techniques have been proposed for intracellular pH measurements, among them, surface-enhanced Raman spectroscopy (SERS) has been attracted attention increasingly due to the following advantages: (a) no damage for the biological samples; (b) negligible disturbance from the fluorescence of biological matters and the Raman signal of water; (c) no quenching or photobleaching. However, tailored nanosensor highly sensitive for a specific pH range of a biological condition has not been reported. The apparent dissociation constant (pKa) of acid ligands is known to be sensitive to NP curvature. Thus, SERS pH sensitivity should be regulated by NP morphology. However, the dependence of SERS pH sensitivity on NP morphology has been overlooked. Moreover, these NP-based probes are trapped in the endo-lysosomal system, limiting the pH sensing to the inside of these acidic vesicles. And the delivery of the NP remains unspecific. Using SERS-active glass micropipettes or fiber tips have been proposed for pH sensing site-specifically. These techniques, although highly appealing, suffer from the large structure of the probe, which might cause drastic cell deformation and damage. What is more, the pH variations of the nucleus are poorly understood, although the nucleus is the target of most anticancer drugs. In the light of the foregoing, an alternative strategy is required in order to monitor the cytosolic and nuclear pH for a better understanding of the behavior of cells.

To address the first issue, namely developing a method to tailor the pH sensing probe sensitive for a targeted pH range, in this work (chapter 2), we investigated the coupling of the morphology of gold-coated silver nanoparticles to their sensitivity for pH sensing. We synthesized nearly spherical and flower-like silver nanoparticles and coated them with thin gold layers (AuAgNPs and AuAgNFs, respectively) to reduce the cytotoxicity of silver and functionalized them with 4-mercaptobenzonic acid (4-MBA), which is the most commonly used probe molecule. We compared pKa behaviors of 4-MBA fixed on AuAgNPs and AuAgNFs and found that the 4-MBA fixed on AuAgNFs with higher curvatures gave a smaller apparent pKa (6.58) than that on AuAgNPs that have smaller curvatures (7.01). By carefully analyzing the SERS peak of carboxyl, we found that anisotropic AuAgNFs could provide

a more sensitive pH monitoring ability between 5 and 8 compared to nearly spherical AuAgNPs. This result indicated that SERS pH-sensitive range and sensitivity could be controlled by choosing nanoparticles with different curvatures. To exclude any incidence of potential NP toxicity on the pH-sensing results, cytotoxicity tests on A549 cells were also performed, showing that AuAgNFs possessed very low cytotoxicity. By taking advantage of this sensitivity, pH sensing inside lysosomes was successfully performed in A549 cells with and without anticancer drug (cisplatin) treatment. In contrast, pH sensing inside lysosomes performed with 4-MBA modified AuAgNPs in A549 with and without anticancer drug treatment showed large fluctuations, emphasizing the importance of the appropriate selection of the NP.

To address the left issue, in this study (chapter 3), we propose an approach based on gold-deposited silver nanowires endoscopy to study cytosolic and nuclear pH variations with high sensitivity. The sensing probe was fabricated by depositing gold nanostructures on silver nanowires (Au-dep-AgNW) via visible laser light induction to enhance the pH sensing sensitivity and modified the surface with 4-MBA. The pH sensing capability was tested by subjecting the probe to different pH solutions, and the related calibration curve gave a slope of 0.1 for the range of pH values between 5.5 and 7.4 approximately, while the calibration curve from our previously reported gold etched silver nanowires via galvanic replacement reaction (Au-etched-AgNW) gave a slope of 0.05, indicating dramatically improved sensitivity for pH sensing, most likely due to the enhanced curvature of the Au nanostructures deposited on the silver nanowire surface. The as-obtained probe was applied for site-specific sensing of the cytosolic and nuclear pH in living Hela cells, and the estimated pH values turned out to be stable over time with a value of 7.3 both for cytosol and for the nucleus. The same experiments were performed on the hypoxia-mimetic agent cobalt chloride. The trends of pH changes measured by Au-dep AgNW endoscopy are consistent with those monitored by pH-responsive fluorescence dyes. Notably, a large cytosol–nucleus pH gradient was observed in cobalt chloride-treated cells over time, indicating that the presence of the drug affects nuclear pH regulation. These two intracellular measurements confirmed the excellent capability of the probe for pH sensing. The probe was finally used to monitor the pH response of cells upon the anticancer drug cisplatin. Notably, a small cytosol–nucleus pH gradient was observed in cisplatin-treated cells over time, most likely due to the spoiling of the regulation of the nucleus. The potential of our endoscopy technique on the cytosolic and nuclear pH sensing over the conventional NP-based pH sensing was highlighted by the comparison with the AuAgNFs-based probe. The results reported clearly show that the Au-dep-AgNW endoscopy is a promising powerful tool for pH-sensing applications in biological systems.