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主　論　文　の　要　旨

論文題目　Fluorescence-based Multi Responsive Micro-sensor for Local Single Cell Analysis
(局所細胞計測のためのマルチ蛍光センサ)

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論　文　内　容　の　要　旨

In this thesis, multi-fluorescence beads sensor which can respond to both pH and temperature were proposed for single cell measurement. Local vibration stimulus driven by optical tweezers was applied on a single sensor for rapid injection into cytoplasm. Multi-fluorescence pillar sensors were also proposed for simultaneous sensing of calcium, pH and temperature during octacalcium phosphate (OCP) conversion.

Measurements of cellular temperature and pH can provide critical information on cell activities. The development of micro- and bio-compatible sensors that can reveal temperature and pH changes in cells has become an urgent demand. But very few fluorescent micro-sensors that can simultaneously detect pH and temperature changes in their surroundings have been reported. The interference between different indicators is also another difficulty in sensor fabrication. On the other hand, an effective method of injecting a certain amount of sensors (as small as a single sensor) into a target cell is also necessary for intracellular measurements. Unfortunately, the conventional methods for intracellular measurements are either damaging the cell easily or requiring long injection time. Consequently, in this study, we propose a synthesis method of a novel multi-fluorescent micro-sensor for simultaneous measurements of temperature and pH and also a rapid injection method of a single sensor into a target cell by applying local vibration stimulus on the sensor using optical tweezers.

Firstly, we proposed a synthesis method of a novel multi-fluorescent micro-sensor based on polymeric microbeads which can respond to both pH and temperature change of the surroundings. Two different kinds of fluorescent dyes (Rhodamine B and FITC) are introduced to a single microbead simultaneously, but the positions of FITC and Rhodamine B are different. So any interference from each fluorescent dye is expected to be negligible by this method. Fluorescence microscopy is used to monitor fluorescent probes. Fluorescence responses of Rhodamine B and FITC to both temperature and pH were studied. The results showed that Rhodamine B demonstrated an excellent linear relationship between relative fluorescence intensity and temperature, while it was found to be independent on pH. The calibrated sensitivity of Rhodamine B is −3.4%/◦C, with a temperature accuracy of 0.1 ◦C. On the other hand, FITC is found to be sensitive to both pH and temperature. We propose a temperature compensation method for pH calibration. After temperature compensation, the pH accuracy calibrated based on the pH sensitivity of FITC improves from 1.5 to 0.2.

Then we proposed to use the multi fluorescent micro sensor which can respond to pH and temperature changes to measure the temperature and pH changes of influenza virus-infected and uninfected cells on the surface. After the virus solution was added into the cell dish, the fluorescent sensor was attached to virus-bound and virus- unbound cell surfaces using optical tweezers. The temperatures and pH in virus-bound cell and virus-unbound cell were determined using a fluorescence microscope by monitoring the changes in the fluorescence intensity of the sensor. We found that influenza virus multiplication increased the temperature of cells by approximately 4–5°C and decreased the pH of cells by approximately 0.5-0.6.

In order to realize selective adhesion and quantitative injection of a single sensor on cell surface, we introduced liposome layers containing photochromic material (spiropyran) on the surface of the micro-sensor. Zeta potential of the liposome layers can be switched between negative and positive by photoisomerization of spiropyran. A single sensor can be manipulated by opticaltweezers and transferred to a cell surface, thereafter adhering selectively to the cell surface under UV illumination without excess sensor adhesion. Then we proposed rapid injection of fluorescence sensors into a target cell by applying local vibration stimulus circularly near the sensor using optical

tweezers. The results showed that the vibration applied on the sensor could push down the sensor, inducing a downward displacement. This displacement caused a corresponding deformation of the cell membrane, which increased the contact area between the sensor and the cell membrane. Without vibration, the sensor was injected into the cytoplasm in 5 h by lipofection at an injection rate of 40%. By applying the vibration stimulus, we succeeded in the rapid injection of the sensor in 30 min at an injection rate of 80%.

At last, we proposed the design of OCP analysis chip and also the fabrication of multi fluorescence micro sensor pillars with different sizes for calcium, pH and temperature sensing respectively in microfluidic chip. The chip allows the co-culture of OCP and osteocyte and the sensors in the middle channel can respond to the changes of surroundings in the chip. PEG-DA mixed with different fluorescent dyes (Fluo-3, FITC, Lumidot 480 for calcium, pH and temperature sensing, respectively) was used as the material of sensor pillar. All of the sensor pillars can be excited by same laser wavelength and recognized with each other by their different size (calcium sensor: Φ20μm, pH sensor: Φ15μm, temperature sensor: Φ10μm). Their fluorescence responses to calcium, pH and temperature changes were detected. Then after delivery of OCP and injection of fluoride solution in the chip, the fluorescence changes of different sensor pillars were observed for 1h by fluorescence microscopy. Calcium, pH and temperature changes of surroundings in the chip during OCP-HA conversion can be calculated based on the fluorescence changes of the sensor pillars. The results showed that calcium ion concentration in the surroundings decreased for 50 nMol while pH value in the surroundings decreased for 0.4 unit in the chip during OCP-HA conversion.

As a conclusion, multi-sensing of more than two parameters were realized by introducing several indicators to micro-beads or micro-pillars. Rapid injection of a single particle sensor without damage the cell was also realized by applying vibration stimulus using optical tweezers. For the microbeads which possess high accuracy, it can be manipulated easily by optical tweezers and have a great potential in single cell measurements. For the pillar sensors, they show potentials in the measurements of microenvironments in microfluidic chips. These applications are quite important in future biomedical fields.