Innovations in Bio-Analytical Chemistry and Biomedical Engineering by Nanobiodevices

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The development of biomolecular analytical technology for omics research is highly required to realize a healthy and long-lived society and additionally we need to develop the analytical technologies for cells, bacteria, viruses, exosomes, organs, small animals, and humans.

We succeeded in developing nanostructures of about 1 nm to several tens of nm that can be applied to bio-analytical chemistry. In particular, we constructed a new nanostructure that can experimentally achieve the nonequilibrium transfer and entropy barrier transfer of biomolecules in nanostructures, which was theoretically predicted to be essential for ultrahigh speed biomolecule analysis. By using these nanostructures, the fastest DNA analysis was achieved in 1 to 100 milliseconds and millions to 10 million times faster than before. Furthermore, we developed three-dimensional nanowires for ultrafast separation of not only DNA but also RNA and protein molecules.

We have developed nanobiodevices that can analyze cells, bacteria, viruses, exosomes, and bioaerosols as well. With the development of micro- and nanopores, millisecond level analysis for a single virus, bacterium, and cell was realized by measuring the pA level ultrasmall current when a single virus, bacterium, and cell passes through the micro- and nanopores. Furthermore, we developed separation technologies for cancer cells, bacteria, viruses and exosomes from body fluids (blood, urine, saliva, etc.) with ultra-high efficiency by precisely controlling and constructing the nanowire structure.

We succeeded in developing a nanowire structure that can interact with exosome surface membranes with high efficiency in order to realize early-stage diagnosis of diseases. About several hundred million to one billion nanowires were fabricated on a small chip and adsorbed 10 to 100 exosomes on each nanowire. With this nanowire device, 99% or more of 1 billion to 100 billion exosomes present in 1 mL of body fluid such as urine can be isolated, and all about 2,500 types of human miRNA contained in exosomes can be detected with high sensitivity. In addition, clinical studies conducted large-scale analysis of urinary exosome miRNA in hundreds of healthy individuals and patients with cancer and lifestyle-related diseases. Big data analysis by machine learning resulted in minimally invasive early-stage diagnosis of 6 types of cancer, diabetes, dementia, and arrhythmia.

We have developed a microfluidic bridge circuit that can measure extremely small currents to identify pathogens and drug-resistant bacteria by micro- and nanopores. This circuit efficiently reduced background noise to about 1/10,000 of the conventional level and

realized all detection with the same device, from viruses of about 100 nm to bacteria cells of about 10 μm. Furthermore, we machine-learned clinical strains owned by Nagoya University School of Medicine as teacher data, and demonstrated that pathogens can be identified with an accuracy of 90% or more. By applying an electric field to the micro- and nanopores, the cell wall of the bacterium is punctured, and the ions inside the bacterium are released into the pores, resulting in changes in the measured current values. We have demonstrated that even drug-resistant bacteria, which were extremely difficult to identify in the past, can be identified with high accuracy.

We have developed a new nanoparticle synthesis technology for quantum dots and quantum sensors, which have high cell safety for iPS cells and other types of cells, in order to realize iPS cell regenerative medicine. These nanoparticles have extremely high light transmission in the living body, since they emit fluorescence in the near-infrared light region. We have also developed a new method for introducing these nanoparticles into cells with high efficiency and safety. With these quantum dots and quantum sensors, we have succeeded in labeling stem cells with high efficiency and in real-time *in vivo* imaging of single cells in live animal organs using a multi-photon microscope. Furthermore, we were the first in the world to succeed in imaging the regenerative ability of stem cells using a quantum sensor.

In addition to *in vivo* imaging of human iPS cells, we realized *in vivo* imaging of iPS cell differentiated cells, such as nerve cells for Parkinson's disease treatment, pituitary hormone-producing cells, corneal endothelial/corneal epithelial cells for ophthalmic treatment, knee joint chondrocytes, and lung cells for bioengineered lung. This technology is accelerating the practical application of regenerative medicine by succeeding in developing an *in vivo* safety evaluation technology for iPS cell differentiated cells, which is indispensable for obtaining approval for regenerative medicine.

In collaboration with NIH and Nagoya University School of Medicine, we have developed a fusion technology of quantum materials and photoimmunotherapy in order to improve the effect of cancer photoimmunotherapy. This technique was applied to photoimmunotherapy for small cell lung cancer and malignant pleural mesothelioma.

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