INNOVATIVE CHEMICAL SENSOR TECHNOLOGY BASEDN ON INTEGRATION OF NANOTECHNOLOGY AND BIOTECHNOLOGY

Masuo Aizawa
Tokyo Institute of Technology
Ookayama, Meguro-ku, Tokyo 152-8550, Japan

INTRODUCTION: An explosive progress has been made on chemical sensors and sensing systems these two decades, resulting in successful commercialization of various gas sensors, biosensors, and ion sensors. It should be pointed that increasing social needs of chemical sensors have recently arisen, and that enormous advanced technologies have emerged for development of chemical sensors.

Current social needs of chemical sensors and sensing systems may be exemplified as follows;

1) Environmental monitoring
2) Environmental assessment
3) Safety assessment of chemicals
4) Safety check of foods
5) Security check
6) High-throughput analyses in drug discovery
7) High-throughput analyses in combinatorial chemistry
8) Genome analyses
9) Point-of-care clinical test
10) Home health care test
11) Animal test alternatives
12) Artificial nose and tongue
13) Chemical sensing under extreme conditions
14) Single molecule detection
15) Others

Researchers are encouraged to focus the target to these social needs to develop chemical sensors by integrating advanced technologies. A special attention should be paid on emerging advanced technologies including nanotechnology, nano-biotechnology, genome technology, information technology, bio-informatics, and others. Most of these social needs are concerned with sensing the molecular interactions with either such information molecule as gene and receptor, or a living cell containing a whole set of molecular information networks. A novel category of biosensors and biosensing systems are described.

NEURORECEPTOR-DISPLAYED CELLS ON MICROARRAY ELECTRODES FOR SCREENING PHARMACEUTICALS: A neuroreceptor at the synapse of a neuron junction may recognize the corresponding neurotransmitter, and open the ion channel, which is followed by activating neural information propagation. Neuropharmaceuticals such as narcotic agents modulate the neural information propagation by interacting with neuroreceptors. For screening the neuropharmaceuticals in drug discovery, a high throughput analytical system is essential in assessing the neural effects.

A novel assessing system has been developed by implementing neuroreceptor-displayed cells on microarray electrodes. The details are described for a gene engineered glutamate receptor displayed on the surface of an insect cell.

NON-DESTRUCTIVE SENSING OF CELLULAR STRESS RESPONSES: The physiological status of living cells has been evaluated by measuring respiratory rate, substrate uptake rate and production rate as extracellular variables. In E.coli cells RNA polymerase sigma factors are synthesized and activated to protect the cells from environmental stresses. One of the sigma factors, RpoS, is triggered to be expressed when the cells enter into the stationary phase, which is followed by the expression of genes required for cell survival. RpoS may also regulate the expression of various genes in the cellular response to such stresses as starvation, osmotic stress, and acid shock, heat shock, and oxidative DNA damage.

Non-destructive monitoring system has been developed for evaluating cellular physiological status with the RpoS promoter as stress marker. A reporter plasmid is constructed by inserting green fluorescent protein (GFP) gene under the rpoS promoter and used to transform E.coli cells. The fluorescence of the GFP is measured in intact cells in a non-destructive manner for evaluating the cellular physiological status.

LIVING CELL-BASED SENSING SYSTEM AS ANIMAL TEST ALTERNATIVE: Biological effects of chemicals have been assessed in general by animal tests prior to clinical tests. Increasing efforts, however, have been concentrated on the development of animal test alternatives due to public acceptance. Bioassays based on cultured cells seem promising, though they suffer from long and tedious procedures. To overcome the drawbacks of the conventional cell culture bioassays, we have developed a novel method for assessing chemicals with a cellular biosensing system, in which the cell responses to chemicals are directly transduced to the sensor output.

A living cell contains a whole set of intracellular information networks including gene information networks and signal transduction pathways. Molecular messengers such as hormones, cytokines and neurotransmitters are recognized by receptors embedded on the cell membrane surface, resulting in activation of the intracellular information networks mostly to modulate the gene information networks. The intracellular information networks might be perturbed by foreign chemical substances, which results in cellular responses. The effects of chemicals on the intracellular information networks may thus be assessed by measuring the cellular responses.

Two types of cellular sensing systems have been developed by implementing either endothelial cell, or macrophage-like cell. A family of nitrogen oxide synthases, cNOS and iNOS, catalyze NO generation from L-arginine to L-citrulline. The constitutive isoform of cNOS is steadily expressed in neural and endothelial cells. In contrast, the inducible isoform of iNOS is temporarily expressed, in macrophages, microglia, and astrocytes, in response to various stimulations.

The endothelial cell-based sensing system with cNOS is effective on assessing pharmaceuticals which concern with vascular relaxation. The cultured cells are layered on a polyion complex film under which a NO detecting electrode is fixed.

In a similar fashion, an iNOS-based cellular biosensing system is constructed with macrophage-like cells for assessing immunomodulating pharmaceuticals.

ACKNOWLEDGEMENT: The research has been accomplished by collaboration with Dr.E.Kobatake, Dr. T.Haruyama, Dr. Y.Yanagida, Dr. M.Mie, Dr. Funabashi, Mr. K.Kamei, and Mr. P.Sakunan.