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# Designing an interface and cell for cellular biosensing

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**Abstract:**

Cells are very intelligent and smart transducers because they transmit various molecular signals in response to extracellular stimuli, both chemically and physically. This fact suggests that living cells can act as sensors in qualified analysis. Such cellular biosensors for qualified analyses can be employed as smart tool in high-throughput analysis in clinical drug discovery. Clinical drug discovery in most cases begins with molecular screening in order to select a lead substance. This process is a key step in successful drug development. Lead substances are identified on the basis of their ability to affect objective biological properties. In order to judge the molecular efficacy of a lead substance, both animal experiments and cell-based bioassays have been employed. In high-throughput assays, cellular biosensing is one of the “smart” methods. However, it is not easy to perform sensing of cellular signals in cell culture conditions. Here, we present some tactics to perform practical cellular biosensing through designing both a molecular interface on the sensor and the cell itself.

## Introduction

The complete series of procedures of clinical drug discovery can be summed up in 2 phases: in vitro research and clinical application. Drug discovery in most cases starts from in vitro research, which includes the acquisition of lead substances based on molecular assays. The lead substance may condition the result of the drug development. This implies that the in vitro assay process is a key step in successful drug discovery. This process is complicated and takes a vast amount of time. Therefore, high-throughput screening is now in demand as a faster method of identifying lead substances. With regard to the high demand, qualified analysis may provide a smart solution. Qualified analysis is a sensing concept that has been proposed and developed by the author [1]. Sensor tools basically act to determine the concentration of an analyte, thereby providing a quantitative analysis. In contrast, sensors for quantitative determinations have been developed to evaluate the efficacy of a molecule against its target, *e.g.*, human and environmental. Cellular biosensing is a smart tool for qualified analysis because cells represent the minimum functional and integrating communicable unit of living systems. In addition, cultured cells and tissues transduce a variety of chemical and physical signals in response to extracellular stimulation. Such cellular responses may be usefully employed as parameters to obtain chemical information about both pharmaceutical and chemical safety [1]. As illustrated in Fig. 1, cellular biosensing is a smart approach to screen lead substances that can influence cells and organs. In the field of drug discovery, smart assay processes are classified as high-throughput analysis (HTA) methods. The efficiency of HTA is a decisive factor in drug discovery.

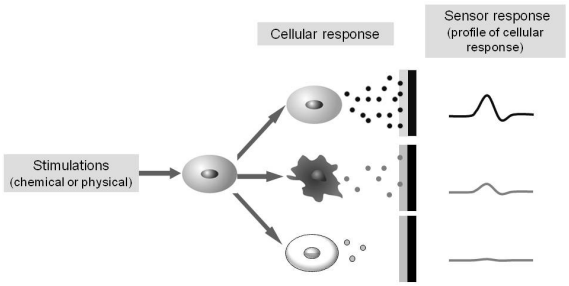


*Figure 1 Conceptual scheme of cellular biosensing*

In this presentation, two different approaches to develop cellular biosensing systems will be explained.

**1. Living cell adhesive and culturable sensor matrix for electrochemical sensing of cellular nitric oxide**

The author has given particular attention to cellular nitric oxide (NO). Endothelial cells produce NO as an intra- and extracellular signal substance to control blood pressure. NO also acts as signal substance among various cells and organs. This suggests that a system constructed from cultured endothelial cells and a NO sensor may provide information about a given chemical on the basis of its efficaciousness in blood pressure control. In order to perform NO sensing under cell culture conditions, a seamless cell-to-sensor system is required [2]. In our past studies, contiguous cellular NO monitoring has been performed successfully. However, both NO selectivity and sensitivity in a cellular NO biosensing system can be further improved. The author explored the scope of these improvements for both NO selectivity and sensitivity and designed and synthesized a novel type of NO sensor matrix. The novel NO selective sensor matrix can capture (and accumulate) NO for electrochemical sensing. The sensor matrix was prepared in the form of a thin membrane on an electrode surface. The membrane surface functions to enable the adhesive activity of mammalian cells. On the basis of these 2 tactics, cells can be cultured directly on the sensor surface and the biosensor can perform contiguous cellular NO monitoring with good NO selectivity.

The designed sensor matrix was synthesized as an $Fe^{2+}$-polymer complex, which is a type of metal coordinated polymer; the coordinated $Fe^{2+}$ immediately forms a complex with NO when the NO is diffused into the polymer. The sensor matrix can then accumulate NO. The accumulation step is important to achieve high sensitivity and selectivity against NO. Accumulated NO in the matrix will be oxidized electrochemically when a potential is applied to the electrode, *i.e.*, double-pulse step potential application. The first applied potential step (700 mV vs. AgAgCl) oxidizes possibly interfering substances that are contained in the cell culture media. In other words, the first applied potential step flushes out any impurities in the sensor matrix that iscoated on the electrode. The second applied potential step (750 mV vs. AgAgCl) oxidizes the $Fe^{2+}$-coordinated NO. As the potential application is performed with a 60-s interval, the sensor matrix is able to accumulate NO in this time period, which is beneficial for good NO sensitivity.

**2. Engineered post-synapse model cells for clinical agents affecting the central nervous system**

The recent developments in genetic and cellular engineering have enabled the fabrication of a designed and engineered model cell. The engineered model cell has been designed to provide appropriate functionality in cell-based assays (qualified analysis), including cellular biosensor application.

Recently, the importance of engineered model cells for living cell-based bioassays has been recognized, especially in cases of receptor-expressing cells because receptors are one of the main targets for neural drug discovery [3, 4]. However, owing to the low level of cellular signals, receptor-expressing cell-based biosensors often have a low signal-to-noise (S/N) ratio. In order to improve the S/N ratio in receptor-expressing cell-based biosensors, it is important to amplify the cellular responses.

In the case of ionotropic receptor-expressing cells (post-synapse model cells), the cellular response, i.e., ion flux in the whole cell, is controlled by the number of receptors on the cell membrane and the ion flux level per individual receptor. The ion flux causes membrane potential but its level is also mainly amenable to the membrane potential [5].

In order to amplify the cellular responses on receptor-expressing cells (post-synapse model cells), we have improved the model through 2 different approaches in cellular engineering. In this presentation, I would like to discuss the new design for post-synapse model cells with which a high S/N ratio can be obtained. The approach involves attempts to increase the ion flux level per individual receptor, which is directly linked to the amplification of cellular responses (i.e., ion influx through the glutamate receptor).

**Conclusion**

The strategic design and construction of both sensor interface and post-synapse model cells for cellular biosensing are potentially useful in “qualified analysis.” Qualified analysis can be employed to evaluate the effectiveness in living systems. The information from the cellular biosensor is very useful in the fields of both drug discovery and chemical/foods safety. In this study, we focused on obtaining a high S/N ratio and selectivity. As described above, there are different approaches either with respect to the sensor interface (molecular interface and material) or to cell engineering (bioengineering and cellular engineering). These approaches

may provide a smart way to develop cellular biosensors for HTA.

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