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Mini Review

The Fabrication of Cell Chips for Use as Bio-sensors

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Abstract

The development of biosensors for the detection of chemicals that are used by cells is of great interest to the pharmaceutical and environmental fields because of the potential for biosensors to measure unknown materials that cannot be detected based on their DNA or protein reactions. However, various problems associated with the introduction of cells to micro-devices, such as low reproducibility and sensitivity, must be overcome before biosensors can be used on a commercial scale. Here, we briefly discuss the cultivation of cells within microchips *in vitro* as well as the biosensor technologies that allow detection of cell properties and subsequent evaluation of specific analytes.

Keywords: Cell-based biosensor, Extracellular matrix, Label-free detection, Peptides

Introduction

Cell-based biosensors can be used to detect materials and measure microbial phenomena that can not be detected by conventional biosensors that are used for the analysis of DNA and proteins. This is because cell-based biosensors directly detect the physiological properties of target materials that are affected by the presence of the cells. Initially, cell-based biosensors consisted of microbial biosensors used in the environmental field to evaluate parameters such as Biological Oxygen Demand (BOD) in water, however, cell-based biosensors that utilize animal cells have since been developed. Currently, a wide range of cell-based biosensors are used to measure the physiological effects of analytes, such as chemicals and drugs, on animal cells[1]. In addition, cell-based biosensors have been combined with surface modifications to allow *in vitro* cell culture within the micro chip and subsequent conversion of the physiological phenomena of these cell cultures to a readable signal.

In this paper, we review two major technologies, *in vitro* cell cultivation and cell-based biosensors, and discuss their applications.

In vitro Cell Cultivation Technologies

In vitro cell cultivation is one of basic processes used in the development of cell-based biosensors, however, the stabilization of cells and tissues in *in vitro* is difficult because the *in vitro* proliferation methods of even commonly used cells and tissues have not been well-established[1]. Therefore, to bolster the existing technologies and to mimic *in vivo* environments, the micro-fabrication of 3-dimensional extracellular matrix (ECM) structures and microfluidic networks has been extensively studied, and these systems have been used to transport soluble materials, such as a nutrients and oxygen, to areas where they are required. These microfluidic methods have the additional advantage of inducing the physical functionality of the cells via alterations in the surface tension within the micro system.

1. Cells and ECMs (Extracellular Matrices)

Nanotechnologies that use advanced surface chemistry induced by nano-patterning technology allow creation of *in vivo*-like environments, which allow stable cell growth. Several nano-patterning technologies that utilize photolithography have been developed for the modification of surface properties, and photo-induced chemistry and soft technologies that contain nano-contact printing and fluidic patterning have also been developed for use in ECMs[2]. These nano-level surface patterning technologies can control the interaction between cells and the ECM because the cell wall is also nano level patterned, as shown in Figure 1. This results in the formation of a cell layer structure along the well defined 3-dimensional structure, which is fabricated using biodegradable materials that are laminated, molded and photo-polymerized.

MEMS is one of the biochemical and mechanical

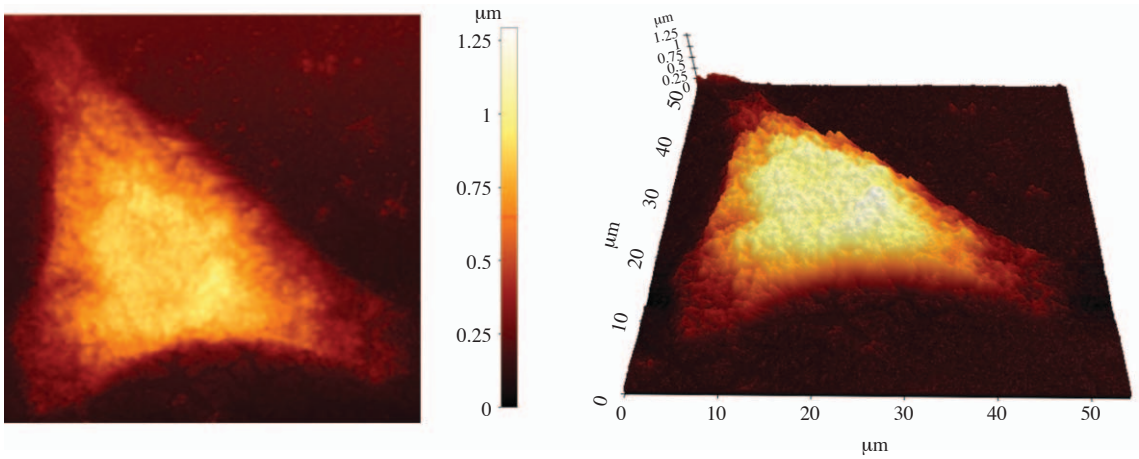

Figure 1. AFM topology of a Hela cell immobilized on a Au surface.

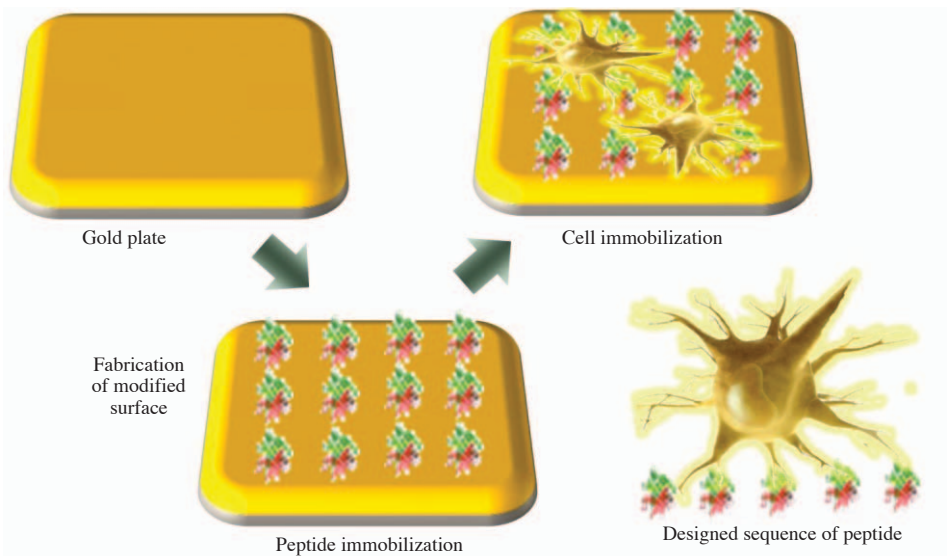

Figure 2. Feeder free cell immobilization system using a modified peptide.

processes by which changes in the properties of the cells, such as cell adhesion and cell morphology, within the micro fabricated chip can be explained[3,4]. For example, the mechanical-properties of the surface to which the cells adhere can be determined by nano-pattern size control of the degree of cell spread, as shown in Figure 2. Increasing the cell density in a limited area can lead to cell death, whereas cells are sustained and grow well when they are able to spread freely. Therefore, the adhesive-properties of the surface to which the cell is integrated to form a scaffold are varied to control the cell density. The portion of the biosensor to which the cells adhere can then be used to control several cell properties when they are in their segmentation status[5]. For example, the elastic micro-pattern can be used to directly measure the adhesive force with which the cells attach to the ECM surface, as well as the interaction force between the cells and the ECM[6]. This method has various advantages with regards to the precision and simplicity of biosensors compared to conventional methods of measurement that merely rely on the strain of the substrate because the physical properties of the surface can be changed by altering the geometrical design of the elastic adhesive posts without any chemical modification. These 3-dimensional ECM technologies can also be enhanced by combining 3-dimensional microfluidic technologies with the surface modifications, which, in turn, causes the induction of a nano-pattern. These multi directional inputs may be one of the best methods for preventing cell death as a result of hypoxia.

2. Proteins and Peptides

Chemists, surface engineers, physicists, biologists, pharmacists and material engineers have extensively studied the specific functionality of the interface between the substrate and the cells. Cell adhesion to proteins, such as fibronectin, collagen, and laminin, that have been coated onto the surface of various matrices has been used to immobilize and stabilize cells *in vitro*[7-13]. However, this method has several disadvantages. For example, although cells adhere to a protein, the orientation of the cell when it is immobilized on the surface cannot be controlled. In addition, proteins must be isolated and purified for the cell immobilization process to be successful. Furthermore, an immune reaction may be induced by the proteins if they are coated on a scaffold[14]. Finally, the maximum binding energy of the protein, which is found on the hydrophobic surface and is exerted by the hydrophobic side chains of the amino acid, may lead to the denaturalization of the protein and deformation of the cell binding motif[15,16]. Introduction of nano-level immobilized peptides as a cell binding and recognition motif may solve several of the aforementioned problems (Figure 3). Peptides are stable in a wide range of pHs and in the presence of many of the chemicals used in the sterilization and storage process, and they are also able to function even after undergoing simple structural changes[14,17-20]. In addition, peptides can be integrated into a system at high densities because of their nano level size, which can increase their ability to bind cells (Figure 4). Furthermore, peptides only bind one receptor corresponding to the target cell because they have only one motif, whereas ECM proteins have multiple motifs that can recognize various cells. Finally, although linear peptides in binding systems may be degraded enzymatically, as occurs in living organisms[21,22], cyclic peptides can be introduced to remove the problems associated with linear peptides[23-27]. Taken together, these nano-level peptide technologies play a major role in the development of novel bio compatible platforms that utilize nano-patterning technologies and nano-fabrication technologies[28-30].

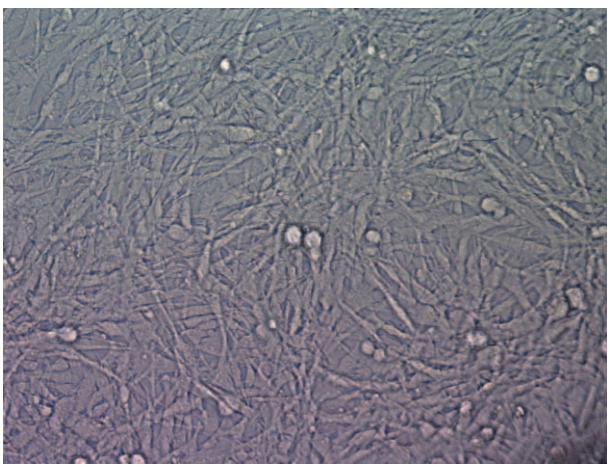

Figure 3. A brain cancer cell (U373MG) on a PMMA.

Analytical Techniques for the Creation of Cell-based Biosensors

Various techniques for the to quantitative detection of the degree of cell lysis that occurs in a system exist. Of these techniques, microsystems that involve electrical separations, such as electrophoresis, make it possible to detect and quantify cell lysis using

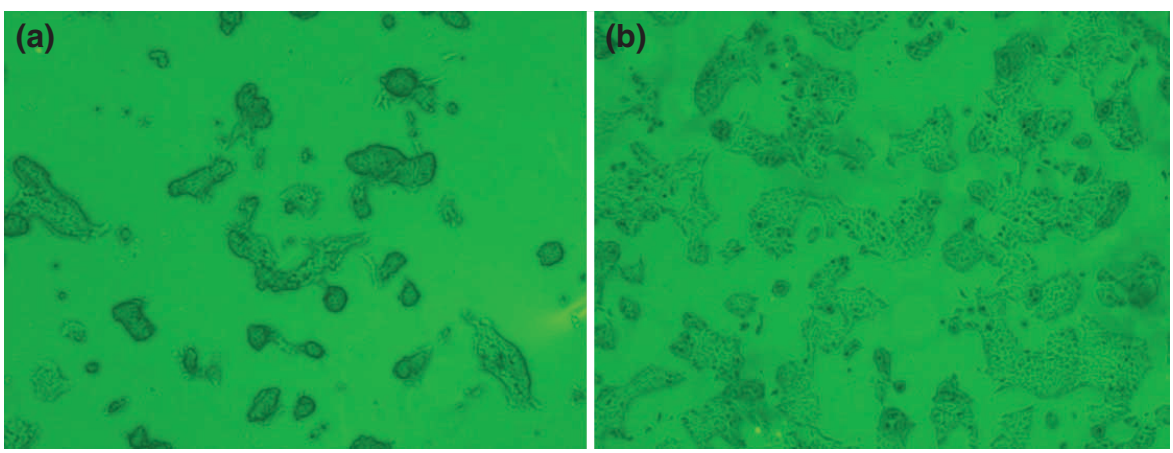

Figure 4. The effect of a designed peptide on a feeder free system for the evaluation of mouse embryonic stem (MES) cells, (a) without peptides and (b) with peptides.

material adhesion techniques and fluorescence markers. In these methods, cells are lysed and the sample is then translocated through an optical detector to measure the lysates. These micro systems can also be combined to confirm that denaturation of the protein occurred after the quantitative measurements and the translation of the protein occurred[31-33]. The capture of proteins using antibodies is also a good quantitative method for the analysis and detection of proteins that does not require a complicated preparation process[32]. In addition, the microfluidic total analytical system, which utilizes DNA hybridization to detect DNA, can be used to detect cell functions by coating solid structures or the inside of the micro channel with antibodies[34-36].

Label-free detection technologies, however, represent an inexpensive, simple alternative method of detection that functions by measuring the charge or current on the surface of a sensor containing an antibody. These methods include the Field Effect Transistor (FET) type electrical sensors, which consist of a carbon nano tube (CNT) or a silicon nano wire (SiNW) with an antibody immobilized on their surface[37,38], as well as mechanical biosensors that use a cantilever to detect proteins based on their mass[39,40]. Cantilever biosensors are particularly sensitive, with some being able to detect as little as a few fM of protein These label-free detection methods can also be combined with sample preparation and separation steps in micro fluidic systems to allow cell viability to be detected.

Cells utilized in cell-based biosensors play a role as target recognition modules, and the primary reason to use a cell as an analyte recognition module is because it provides functional analysis of analytes such as chemicals, pH, and temperature via various highly developed biochemical pathways that are highly sensitive to various biochemical stimuli. In addition, using cell-based biosensors for functional analysis allows the detection of unknown materials that cannot be detected using conventional biosensors because the cell-based biosensor utilizes physiological functions to detect materials. No biosensors that use a binding reaction of DNA and antiboidies can provide a physiological analysis of the analytes.

Cell based biosensors can be classified based on the type of secondary transduction module that is used to monitor the change in the cell reaction. Here we classify and briefly describe several cell based biosensors.

1. Resistance Based Biosensors

Resistance based biosensors utilize the change in resistance that occurs between electrodes as a result of changes in the characteristics of the walls of cells that are adhered onto parallel electrodes. Resistance based biosensors can be used to measure the degree of cell adhesion, cell spread and cell motility. Keese *et al*. described the use of cell based biosensors to indirectly measure the degree of cell movement or cell spread against specific chemicals for their detection. In addition, Ehret *et al*. utilized interdigitated electrodes (Figure 5) to measure the change in resistance that occurred as a result of cell growth[41,42]. The cross resistance is affected by the membrane proteins, and is also related to ion translocation that occurs due to the opening and closing of an ion channel, therefore, the characteristics of a cell membrane can be evaluated by measuring the difference in the cross resistance of the membrane inside and outside of a cell using a patch clamp. Furthermore, the membrane protein can be used to measure chemicals based on the work that occurs in an ion channel as a result of the cell's metabolism[43]. However, in order to measure the resistance of a cell membrane using a micro electrode deposited onto a substrate it is necessary to isolate the actual change in the resistance of a cell membrane from noise that is induced by the movement and protrusion of cells.

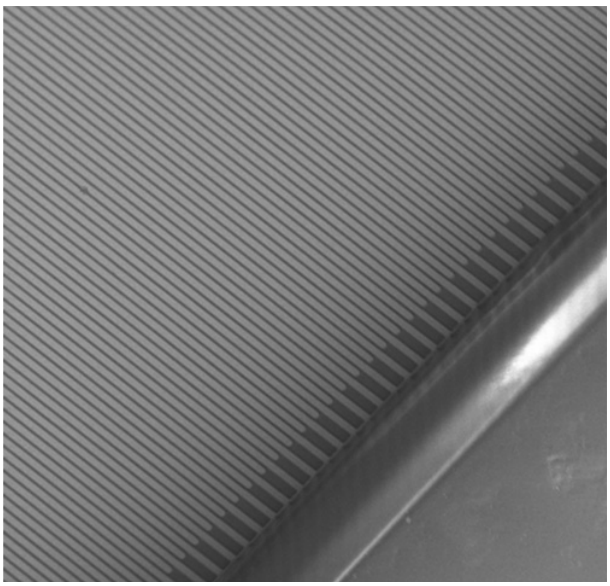

Figure 5. Ultra micro interdigitated electrode array (Au electrodes, 300 nm spacing, 2 μm width).

2. Metabolism Based Biosensors

Measurable secondary metabolytes or related environmental parameters such as pH, temperature, oxygen and carbon dioxide can be used to measure the metabolism of cells in response to specific chemicals and analytes. A typical method used for the detection of cell metabolism is to measure the pH of the solution

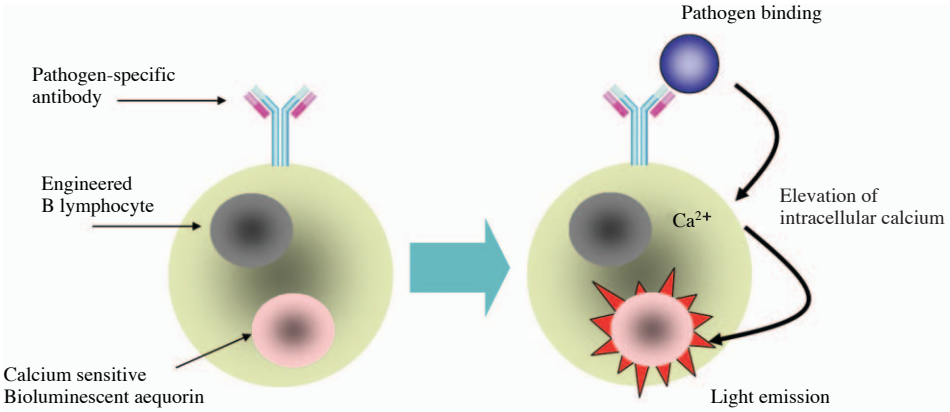

Figure 6. The detection principles employed in CANARY sensors.

near the cell because it makes broth media acidic[44-46]. Metabolism can also be measured by determining the consumption rate of oxygen, the generation rate of carbon dioxide or lactate, and the thermal status by microcalorimetry[47,48]. However, these indirect measurements of cell metabolism by secondary metabolytes or environmental parameters might be difficult to interpret because cell metabolism is affected by various unknown stimuli.

3. Optical Biosensors

The fluorescence detection method has been widely used to measure DNA hybridization, DNA amplification, antibody affinity, and enzyme reactions, as well as cell metabolism. In addition, fluorescence tagging indicators, such as nanoparticles and antibodies, are often used to detect specific cell reactions on the cell membrane or inside of the cell. Currently, however, most cell based biosensors that use fluorescence are designed for use in high throughput screening tools[49,50].

Most optical cell-based biosensors that have been developed to detect toxic materials or bacteria use recombinant cells[51-53]. For example, Rider *et al.* described the use of a cell based biosensor known as CANARY (Cellular Analysis and Notification of Antigen Risks and Yields) to detect pathogens such as SARS and anthrax (Figure 6[51]). This biosensor utilized recombinant B cells, which posses calcium-sensitive bioluminescent aequorin engineered B lymphocytes and target specific antibodies on the membranes of the target pathogens. When pathogens are bound to the antibodies on the membrane, this affinity reaction induces elevation of the intracellular calcium concentration, which results in light emission from calcium-sensitive bioluminescent aequorin in the cells.

The main advantage of the fluorescence detection method is that it enables *in situ* detection of the degrees of various reactions in the cell without causing lysis[54]. In addition, label-free detection technologies such as SPR (Surface Plasmon Resonance) and ellipsometry can be applied to cell based biosensors without the need for a fluorescence signal[55-57].

4. Electrical Biosensors

The action potential of a cell is a useful method for the detection of cell characteristics, however, measuring the cell membrane potential requires sophisticated processes that involve the use of electrodes that are attached via a patch clamp or by piercing the cell, which is not convenient for the development of cell based biosensors. Therefore, various methods involving the use of microelectrodes for the measurement of extracellular action potential have been studied because this method can detect energy outside of the cell without the need for electrodes. The first recording of an action potential was reported by Tomas *et al.* in 1972[58], who described the measurement of the *in situ* extra-potential action potential of a chick myocardial cell cluster using a micro electrode array. This method allowed the extracellular action potentials of neuron cells[59], dissociated neurons[60,61], and cardiac myocytes[62,63] to be measured using a parallel electrode array. Currently, the extracellular action potential of cardiac myocytes and neuron cells derived from stem cells can be measured using a micro electrode array[64,65], and this method represents a typical method for the measurement of extracellular potential in various fields. In addition, commercial systems that have been developed by Plexon Inc. (Dallas, US), Multichannel systems GmbH (Reutilingen, Germany), and Alpha MED Sciences, Co. (Tokyo, Japan) allow extracellular micro electrode arrays to be directly applied to biosensors[66-69]. Furthermore, cultures can be grown directly on the micro electrode assay for biochemical

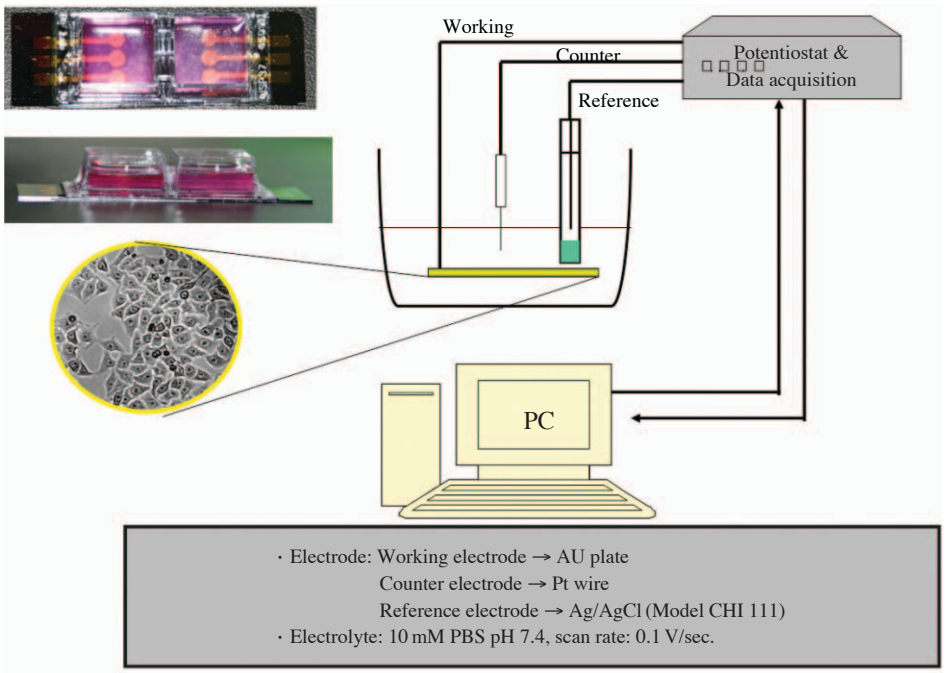

Figure 7. An electrochemical cell-based sensor system that uses cyclic voltammetry.

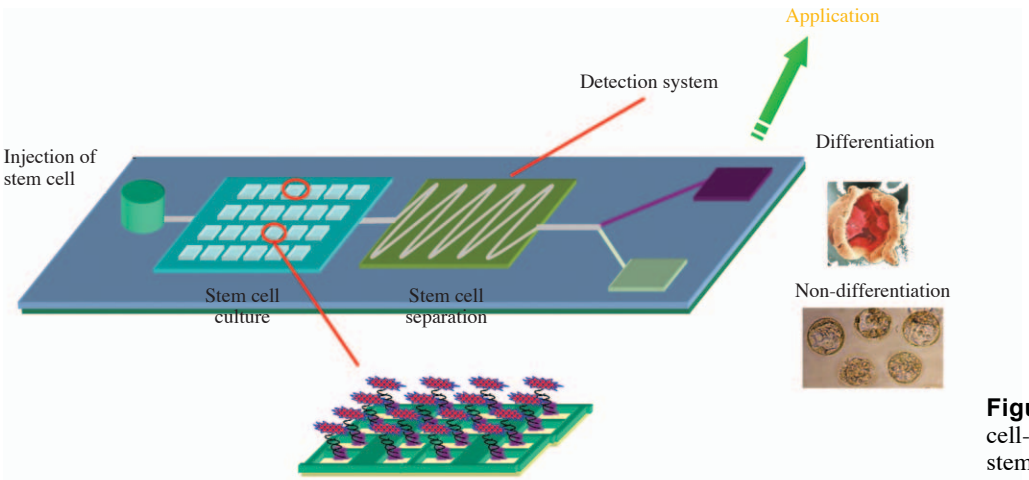

Figure 8. Application of a cell-based sensor system to a stem cell chip.

sensing of neuron cell networks[69] and embryonic chick myocardial cells[43].

The monitoring of extracellular potential for the detection of chemicals can be validated using the various techniques described and shown in Figure 7. However, there are several obstacles to be overcome before electrical cell based biosensors can be used commercially. For example, a reproducible cell based biosensor with a stable Signal to Noise Ratio (SNR) must be developed for use in living cells. To solve this problem, specific characters from cell layers with different densities and various characteristics must be obtained.

Outlook

Although many micro devices that utilize cells and tissues have been developed in academia and commercial fields, most of the devices developed to date have been utilized for simple tests. For cell based biosensors to be useful for larger applications, more efficient sample preparation processes, such as separation and purification, are required. Development of a stable sample preparation process would enable automated total analytical systems with high reproducibility. Ultimately, these processes may lead to the use

of large scale nano-systems in humans, in which cells are conveyed into the system after auto separation and purification and then cultured into a 3-dimensional nano structure that is used to detect various targets and bacteria in the blood. The method used to detect signals from individual cells would include label-free electrical detection to transfer the signal generated inside the system to an outside recording device. In addition, when an *in vivo* like environment can be developed within a micro chip, proliferation and differentiation of stem cells can be controlled by the chip, as shown in Figure 8. Based on the findings of this report, it is anticipated that systems such as these will be in use in the near future after 3 dimensional cultivation techniques for the development of nano systems, automatic cell isolation techniques, and label-free detection technologies are developed and refined.

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