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A Novel Fiber-Optic Biosensor for On-Line Monitoring of Cell Cultivation

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A novel fiber optic biosensor has been developed to monitor the growth of hexapod cells on-line. By measuring the light intensity, the dynamic growth of hexapod cells can be observed continuously. Such a fiber-optic sensor has many advantages, including rapid response, high sensitivity, immunity to electromagnetic interference, small detector size, and facilitated operation. Potential applications exist in drug developing and screening.

1. Introduction

Fiber-optic sensing is a well-developed approach in sensor development.[1,2] It possesses many advantages, including rapid response, high sensitivity, immunity to electromagnetic interference, small detector size, facilitated operation and the possibility of on-line and dynamic measurement. In recent years, fiber optic technology has made extraordinary contributions to research in detection in chemistry and biology. Optical fibers have been increasingly employed as sensors in biochemical,[3–5] biomedical,[6] and environmental areas. Recent developments in optical biosensors have been reviewed by Dinh.[7]

Living cells possess the ability to continuously monitor their microenvironment and respond to local changes. Because of their high sensitivity to the environment, living cells are used as monitoring objects in biosensors are becoming increasingly popular. Many researchers have implemented living cells in bioprocess monitoring by combining living cells with fiber optics to achieve high-precision detection.

R. Walt *et al.*[8] has fabricated a live-cell array biosensor by immobilizing bacterial cells on the face of an optical imaging fiber containing an array of high-density microwells. With the array, a response to 100 nM Hg^{2+} was detected.

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Ogurtsov *et al.*[9] have developed an optical oxygen sensor to monitor the metabolic activity of living cells via their consumption of dissolved oxygen. The optical oxygen sensor has reliably monitored lower numbers of cells than densitometry (10^4–10^5 vs 10^6–10^7 cells/ml).

Due to the impermeability of cells and plasma membranes to light, cells grown in liquid culture reduce the light flux of the culture fluid. Fiber optics can realize on-line measurement of the light intensity change as cells grow. In this study, by converting cellular growth into voltage signals, we have developed a biosensing system based on fiber optics and their on-line monitoring of the growth of hexapod cells.

2. Experimental

2.1 *Hexapod cell culture and experimental methods*

Generally, according to the state of the cell in culture, there are two types of cells: suspended cells and adhered cells. An adhered cell, as its name implies, grows on the bottom of its culture vessel. Hexapod cells belong to the category of adhered cells. The average diameter of a hexapod cell is 10–20 μm, and the time for hexapod cells to double their numbers is 18 h. Hexapod cells must be cultured at a constant temperature of under 28°C.

The vessel used to culture hexapod cells must be sterilized at 120°C for 2 h to eliminate most bacteria. The basis of the culture medium is fetal calf serum. The color of the hexapod cell culture medium is primrose yellow. The organic glass vessel containing the medium appears uniformly transparent. The culture medium not only promotes hexapod cells' growth and propagation, but also shows obvious effects of protecting cells, repairing damaged cells and increasing the adherence of cells to the vessel bottom.

To discover the relationship between the cell growth rate and light transmittance, two groups of experiments under different concentrations of cells and different cultivation environments are carried out, respectively, along with the measurement of voltage corresponding to the light transmittance change. To compare the growth of different numbers of cells in the same volume of medium, we simultaneously monitor the growth of 300,000 cells/ml, 100,000 cells/ml and the nutritive medium alone in the first group of experiments. In the second group of experiments we add viruses and serum to the culture medium to verify whether the experiment is consistent with the principle that viruses handicap the growth of hexapod cells and serum accelerates their growth.

2.2 *Experimental system*

The experimental system must avoid disturbance, noise and vibration from the environment. Continuous measurements are taken in a thermostated container. Three fibers are used in the experiment. To prevent them from interfering with one another, black insulated adhesive tape is used to wrap all the vessels containing cell culture medium.

Red light with $\lambda = 650$ nm is emitted from the optical fiber, the head of which is immersed in the culture medium. The cell density on the surface of the vessel changes as cells grow up from the bottom. Therefore, the light transmittance of the vessel changes. Accordingly, the light intensity received by the photodiode opt101 below the vessel decreases, which induces the voltage to decrease.

The opt101 photodiode fabricated by B-B Corporation (USA) is a monolithic photodiode with an on-chip transimpedance amplifier. The output voltage increases linearly with light intensity. The integrated combination of photodiode and transimpedance amplifier on a single chip eliminates the problems commonly encountered in discrete designs such as leakage current errors, noise pick-up, and gain peaking due to stray capacitance. The noise of opt101 is 100 μVrms, which indicates the detection limit of our setup.

The experimental setup is shown in Fig. 1. The light signal is transformed to a voltage by the amplifier and converting circuits. The voltage is collected in a computer. The voltage change reflects the change in the light transmittance of the culture medium, thus reflecting the growth of the cells.

3. Results and Discussion

When cells begin to adhere to the bottom of the vessel, light transmittance through the culture medium decreases. The more the cells grow, the less light is transmitted through the vessel. The more quickly the cells grow, the faster the voltage drops. In Fig. 2, the slope of the curves represents the growth rate of the hexapod cells. Because of the different positions and depths of the three fibers in the medium, the original voltage values of the three fibers are not at the same level. Therefore, comparison of the slopes of the curves is very significant to the experiments. Note that the zero point on the x axis in Fig. 2 is not the

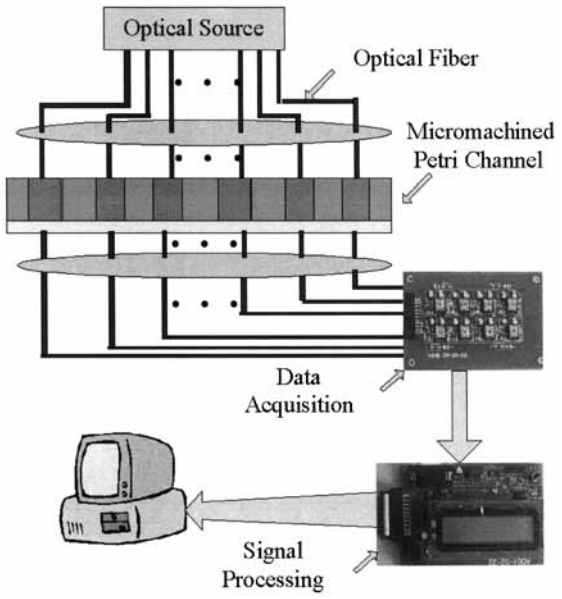


Fig. 1. Schematic of cell-based fiber-optic biosensing system.

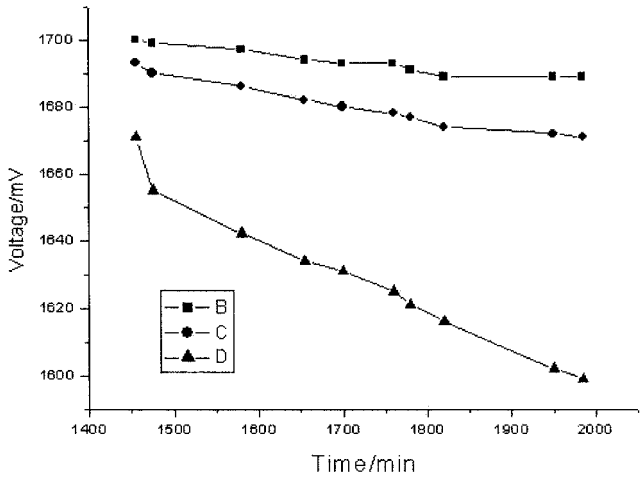


Fig. 2. Fiber-optic sensor detection of different numbers of hexapod cells. B: nutrient medium C: 100,000 cells/ml D: 300,000 cells/ml.

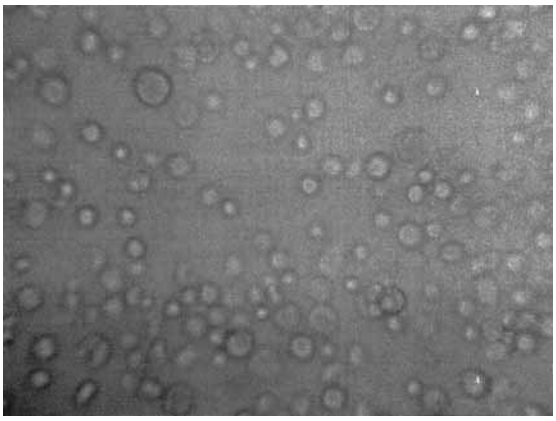
time when the cultivation process begins, but one day later. The entire cultivation process for a hexapod cell takes around three days. However, the growth rate is not uniform for the three days; in the first day and the third day the growth process is very slow. For this reason, we choose the most steady, representative and fastest growth period of the hexapod cells to illustrate the experiment and draw our conclusions.

The abscissa represents time and the ordinate voltage. Figure 2 shows that the delta values of voltage changes are 11 mV, 22 mV and 72 mV under the conditions of culture medium only, medium with 100,000 cells/ml, and medium with 300,000 cells/ml, respectively. The three curves in Fig. 2 are all nearly linear. After a linear fit of the curves in Fig. 2, the slopes of the hexapod cell growth curves are –0.026 mV/min, –0.041 mV/min and –0.122 mV/min, respectively. Obviously, the voltage corresponding to the cell concentration of 300,000/ml changes more and faster than that of 100,000/ml, which illustrates that a faster growth rate and a resulting larger number of cells is achieved from a high original concentration of cells than from a low original concentration of cells. At the same time, we have also observed that, in the nutrient only solution, the curve remains almost constant because no cell growth takes place in it. The small change of 11 mV is because of noise and stochastic drift.

The two pictures in Fig. 3 straightforwardly demonstrate the features of the cells at the end points of the above experiments described in Fig. 2. They were taken using an electron microscope immediately after the experiment for the culture medium with 100,000 cells/ml and 300,000 cells/ml. In Fig. 3(a), cells adhere to only some parts of the vessel bottom, while in Fig. 3(b), because more cells are available, cells cover almost the entire bottom surface of the vessel. As a result, greater and faster change can be observed in the 300,000 cells/ml curve than in the 100,000 cells/ml curve.

Comparison between Fig. 2 and Fig. 3 reveals that using fiber optics is a feasible method for monitoring cell growth.

(a)



(b)

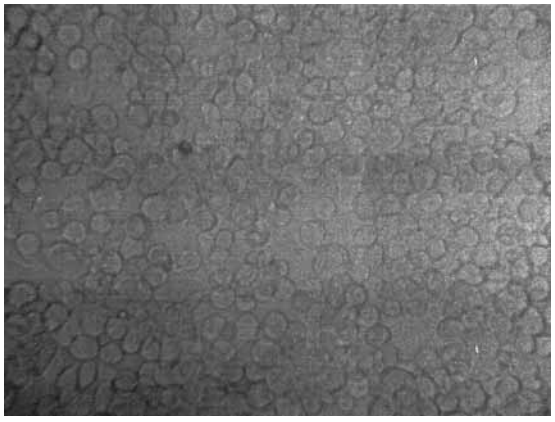


Fig. 3. Micrograph (×200) of hexapod cell growth on the bottom of the vessel. (a) 100,000 cells /ml (b) 300,000 cells/ml.

In Fig. 4, the slope of the curves represents the growth rate of the hexapod cells and the zero point on the x axis in Fig. 4 is one day after cultivation begins, which is the same as in Fig. 2. Figure 4 demonstrates the growth process of the hexapod cells in three different culture environments: normal culture medium, medium with viruses, and medium with serum. The start time is 1500 min after the initiation of the growth process. The three curves recorded separately in Fig. 4 are all nearly linear. After a linear fit of the three curves in Fig. 4, the slopes of hexapod cell growth curves are –0.048 mV/min, –0.031 mV/min, and –0.056 mV/min. In order to clearly illustrate the slopes, a straightforward and amplified figure focused on the ten start points in Fig. 4 is shown in Fig. 5. It was created supposing that the three curves have the same original point, i.e., omitting the different intercepts of the three curves. Thus, we can obviously see the different slopes of the three curves, which represent each of the cell growth rates. The difference of three slopes indicates that viruses suppress the growth rate while serum promotes the growth rate effectively. Such results show the great potential of the fiber-optic sensor for drug screening and development.

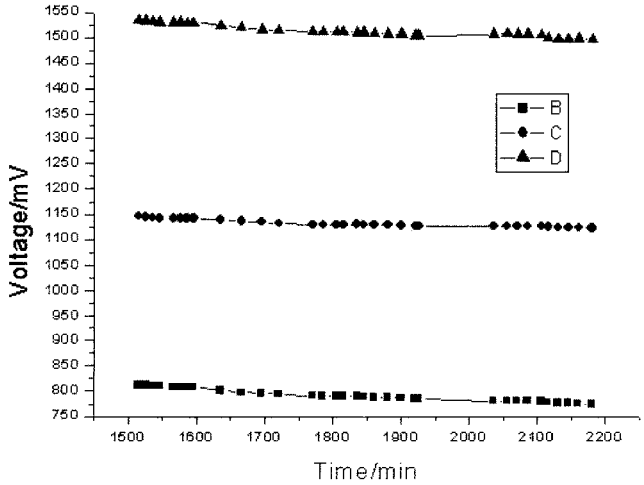


Fig. 4. Growth curves in different culture environments. B: serum added C: virus added D: normal culture medium

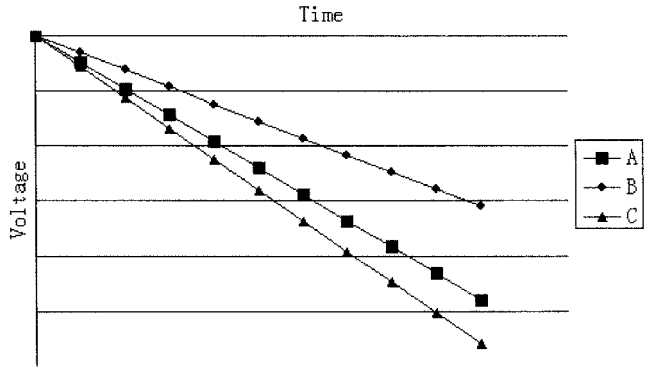


Fig. 5. Slope comparison of Fig. 4. A: normal culture medium B: virus added C: serum added

4 Conclusions

In this study, we used a fiber-optic-based biosensing system to confirm the feasibility for fiber optics to monitor cell growth on-line. A new method measuring light intensity was implemented. The stability of the fiber-optic system is good, and it can guarantee continuous measurement for the entire growth process of cells. Unreasonable values were not observed.

In two experiments, we employed a biosensor based on fiber optics to monitor the growth of hexapod cells under different conditions. By applying fiber-optic sensors to drug screening and development, on-line and noninvasive measurements can be realized, which have potential prospects in medical applications.

5. Further Work

Osteoblast cells have a close relationship to bone loss. Monitoring the growth of osteoblast cells and their responses to different drugs may help in the development of new medicines based on Chinese traditional agents to treat bone loss disease. Such fiber-optic sensors may provide a feasible method for high-throughput drug screening.

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