Surface Stability of SU-8 Film for Accurate Biopotential Detection

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Biopotential is important and sensitive information in order to monitor and control growth condition for living cells. For detecting biopotential change accurately, a stable biocompatible polymer film is required. In this study, a stable surface of a SU-8 film is characterized, and it is possible for applying to a cover material of electrode surface or an element of a biological implanted device. Surface potential $V_{\text{SU-8}}$ of a SU-8 film is measured by making a metallic electrode contact. As a standard equilibrium, $V_{\text{SU-8}}$ indicates exponential decreasing to reach at +21mV. Biopotential V_{bio} of plant cells indicates large potential change for 10 to 42mV range. This potential change means an individualistic signal of living cells. By combination of a SU-8 film and living cells, it is capable of obtaining accurate and effective data.

Keywords: SU-8 resist, plant cells, surface potential, biopotential, biocompatible material

1. Introduction

Biopotential is one electrical signal due to living cells. It can indicate sensitive information as related to living cells. In general, it is known that biopotential controls growth condition of cells. In order to analyze this field, a stable standard material is required to detect accurate biopotential change, such as a biocompatible polymer film.

Biocompatible materials have been focused and investigated extensively in various basic bioscience and engineering fields. In particular, polymer materials, such as polydimethylsiloxane (PDMS) and polytetrafluoroethylene (PTFE), have been yielded in practical applications, e.g., an artificial blood vessel, a cell culture substratum [1,2]. With regard to biomaterial, it is critical to have no interaction with a living tissue by contacting a biocompatible material. Interface characterization of a biomaterial has often discussed in terms of surface bonding state and protein adsorption capacity [3,4].

In this study, we focus on a SU-8 film which is epoxy-based negative photoresist as an interface material. In general, a monomer of SU-8 resist has

eight epoxy functionalities; high degree of cross-linking after UV exposure can be obtained [5]. Therefore, a SU-8 film is stable in terms of mechanical and chemical interaction. Moreover, SU-8 has recognized as a candidate of biocompatible material by the Food and Drug Agency (FDA) of the United States [6]. In fact, a drug delivery MEMS (MicroElectroMechanical System) device, a microfluidic device and a probe for neuronal recording applications by SU-8 resist were reported [7-9].

The aim of this study is to characterize a stable surface of a SU-8 film in terms of surface electrical potential. In order to measure $V_{\text{SU-8}}$ as a surface electrical potential of a SU-8 film, a pair of tungsten needles is used. As a standard equilibrium, $V_{\text{SU-8}}$ indicates exponential decreasing, and its value reaches to +22mV. Then, V_{bio} as biopotential of plant cells is also measured by using the measurement system. The value of V_{bio} shows large potential change for 10 to 42mV range just after making a metallic electrode contact. This potential change should indicate an individualistic signal of living cells. In order to evaluate

quantitatively, potentials both $V_{\text{SU-8}}$ and V_{bio} are fitted by an exponential function, and time constant τ is obtained from fitting parameters. Average of τ for $V_{\text{SU-8}}$ is 41s, however τ of V_{bio} changed randomly. According to compare $V_{\text{SU-8}}$ with V_{bio} , a SU-8 film is capable to use as a standard film of surface potential analysis. A SU-8 film is possible for applying to a cover material of electrodes surface or an element of a biological implanted device.

2. Experiment

2.1. Sample Preparation

A double SU-8 layer structure for experimental sample was prepared in order to form a flat and low detect surfaces. Figure 1 shows a sample fabrication process. First, a glass plate (Matsunami Glass, S1111, 0.9mm thickness) was sawn by $26\text{mm} \times 26\text{mm}$ square. Then the glass was ultrasonic cleaned by dipping into acetone, ethanol and deionized water for 5min respectively. Then, the SU-8 3005 photoresist liquid was dropped on the glass surface. The kinetic viscosity of the SU-8 liquid was $65\text{mm}^2/\text{s}$ according to SU-8 3000 series data sheet [10]. The spin rate was ramped in two steps up to 3000 rpm. Prebaking was carried out for 5min at 95°C on a hotplate. Then, the glass was cooled down to room temperature. Then, whole area of the SU-8 film was exposed to 365nm UV light at $6.0\text{mJ}/\text{cm}^2$ for 8s utilizing a UV spot light system (Yamashita denso, Hypercure-200). After the exposure, the SU-8 film was cross-linked by performing a two-step post exposure bake (PEB) process on a 65°C hot plate for 1min and for 4min at 95°C . The SU-8 film was soaked in the SU-8 developer for 5min in similar to the standard SU-8 lithography process. After soaking, hardbaking was carried out at 200°C for 60s. Then another SU-8 film was formed on the bottom SU-8 film with the same procedure parameters.

As shown in Fig.2a, pothos (*Epipremnum aureum*), a kind of foliage plants, was employed as a living tissue because pothos is easy to daily cultivation. The pothos cells were prepared as follows: a leaf of pothos was cut then stored under ambient conditions for 1h before the measurement. Then, a piece ($1\text{cm} \times 1\text{cm}$) was clipped from the leaf, and peeled epidermis to expose mesophyll cells as shown in Fig.2b. Figure 2c shows an optical micrograph of the pothos cells. It was observed that the size of one cell was 40 to $50\mu\text{m}$. The distance of tungsten electrodes was adjusted to be the size of 3 cells as shown in Fig.2d.

Fig.1. SU-8 film fabrication process (cross-section).

Fig.2. Plant sample preparation for this experiment and optical microscope images of pothos cells.

Fig.3. Potential measurement system (cross-section).

2.2. Potential measurement system

A potential measurement system is illustrated in Fig.3. In order to measure surface potential $V_{\text{SU-8}}$ of a SU-8 film and biopotential V_{bio} of pothos cells, we employed a pair of tungsten needle. Curvature radius of the needle was $20\mu\text{m}$ which was smaller than a pothos cell size around $50\mu\text{m}$. Terminal of a digital multimeter (KENWOOD, DL-712, the input impedance was larger than $11\text{M}\Omega$) connected to the electrode needles. A liquid drop of deionized water (below $0.06 \times 10^{-4} \text{ S/cm}$) was put on the sample surface. The electrodes were immersed in deionized water for 60s. Then the electrodes were moved slowly to make a contact with the SU-8 film surface and pothos cells. The distances between two tungsten electrodes were set at $409\mu\text{m}$ for a SU-8 film and $100\mu\text{m}$ for pothos cells. In order to monitor the large variation of in initial stage, the measurement interval was adjusted to be 1s until 60s of contacting time. After 60s, the data were recorded in interval 10s. Three different positions, A, B and C for a SU-8 film, *a*, *b* and *c* for pothos cells were selected.

3. Results

3.1. Surface potential $V_{\text{SU-8}}$ of SU-8 film

Figure 4 shows the dependency of surface potential $V_{\text{SU-8}}$ on the contacting time of the SU-8 film. $V_{\text{SU-8}}$ indicates a maximum value just after making an electrode contact. Then $V_{\text{SU-8}}$ exponentially decreases with the contact time. The potential $V_{\text{SU-8}}$ reaches to the equilibrium value about $+21\text{mV}$.

3.2. Biopotential V_{bio} of plant cells

Figure 5 shows the dependency of biopotential V_{bio} on the contacting time of pothos cells. We focus on that dynamic change of V_{bio} generates during the contacting time of electrodes at position *a* for 6s and position *c* for 32s. V_{bio} also reaches to the equilibrium value in exponential function. The equilibrium value is about -21mV .

4. Discussion

According to comparison $V_{\text{SU-8}}$ with V_{bio} , the potential changes after making an electrode contact are different. It is considered that dependency of $V_{\text{SU-8}}$ on contacting time causes surface charge existence. The surface charge is neutralized on the SU-8 film surface during the measurement, so $V_{\text{SU-8}}$ decreases as the contacting time. The reason of the surface charge movement would be surface bonding structure. The thermal treatment at the end of SU-8 lithography process

Fig.4. Dependency of surface potential $V_{\text{SU-8}}$ on contacting time of a SU-8 film.

(a) time region: 0 to 300s

(b) time region: 0 to 60s

Fig.5. Dependency of biopotential V_{bio} on contact time of 3-plant-cell.

occupies an important role in regard with surface stability. In general, main purpose of hardbaking is conducted to volatilize solvent in a films and to enhance adhesion and durability between a film and a substrates. The cross-linking reaction should act to finish epoxy functionalities through this process. Thus, electrical charges in the SU-8 film surface cannot move to electrodes. In regard with equilibrium value, it is possible that zeta potential between the SU-8 film and electrodes should be reflected.

On the other hand, biopotential V_{bio} of plant cells is generated by several factors. For example,

Table 1. Surface potential characteristics of SU-8 film and plant cells.

Kinds of potential	V _{SU-8}			V _{bio}		
Measurement position	A	B	C	a	b	c
Equilibrium value V _{eq} (mV)	+22	+20	+19	-21	-21	-18
Range of potential change ΔV(mV)	20	21	14	42	15	10
Time constant τ (s)	41.8	44.4	42.7	83.3	76.9	23.6

ion concentration difference inside and outside of a cell is one major factor [11,12]. Therefore, it seems that drastic changes are observed as individualistic biological reaction of living tissues by comparing with a standard equilibrium of a SU-8 film.

In order to evaluate quantitatively potential changes of V_{SU-8} and V_{bio}, we estimate the time constant by fitting to Eq.1,

$$V(t) = V_{eq} + \Delta V \exp\left(-\frac{t}{\tau}\right), \quad (1)$$

where V_{eq} is equilibrium potential values at t=300s and ΔV is absolute range of potential change from t=0s to 300s. The symbol of τ denotes the time constant. Equilibrium value V_{eq} should reflect zeta potential property. ΔV and τ indicate stability of potential change. Table 1 summarizes these fitting parameters of V_{SU-8} and V_{bio}. Equilibrium V_{eq} shows same value for each potential. ΔV of surface potential of a SU-8 film is mostly same among A, B and C. However, the potential range ΔV of V_{bio} indicates quite different value among the measurement position a, b and c. The time constant τ of V_{SU-8} is 41s on average from electrodes contact, on the other hand, τ of V_{bio} is not same value at each point. Therefore, the stability of potential change of a SU-8 film can be evaluated quantitatively. We believe that a SU-8 film is capable to use as a standard film of surface potential through quantitative evaluation.

5. Conclusion

Surface potential V_{SU-8} of the SU-8 and biopotential V_{bio} of plant cells are measured and analyzed. V_{SU-8} decreases exponentially with the contacting time. A SU-8 film indicates stable film property due to hardbaking procedure. Contrariwise, in regard with V_{bio}, dynamic change is observed during the electrodes contact. Dynamic change of V_{bio} is considered as individualistic biological reaction of living tissues. A SU-8 film can use as a standard film of surface potential and be applied to a cover material of electrodes surface

or a biological implanted device.

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