FABRICATION AND CHARACTERIZATION OF SELF-ASSEMBLED MONOLAYERS ON MICROELECTRODE ARRAY FOR CELL CULTURE

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Abstract: This study investigated the chemical covalent bounding and binding efficiency of surface-modified microelectrode arrays (MEA) on the gold-based polyimide (PI) substrate fabricated by using micro-electro-mechanical systems (MEMS) techniques. To increase the biocompatibility, self-assembled monolayers (SAMs) were formed on the MEA to immobilize the poly-D-lysine (PDL) on the electrodes. Spectra of the Fourier transform infrared reflection (FTIR) was applied to characterize the molecular structure of MUA-SAMs and PDL on electrode. The fluorescence of FITC labeled Poly-L-lysine (PLL) further proved the binding efficiency on the MUA-SAMs modified electrode. Furthmore, the impedance of SAMs-modified electrodeonly increased slightly. Our experiment of PC12 cells culturing on the modified electrodes displayed a good affinity for cell growth on the electrode which demonstrated the PDL-MUA-SAMs modification of MEA with can provide a better cell culturing environment for electrophysiological stimulation and recording.

Keywords: Self-assembled monolayers, FTIR, Fluorescent analysis, Impedance measurement, Cell growth.

Introduction

Varied types of cells culturing on MEA has been successfully applied for neuroelectrophysiological studies [1], drug screenings [2], and neuronal prosthesis [3]. Recent studies have been focused on investigating the properties and mechanisms of neurons or cells cultured on MEA [4].

To improve the biocompatibility between cells and electrodes, researchers have designed the MEA with varied materials [4,5]. Others adopted some forms of surface modification techniques in terms of physical or chemical biocompatible modifications to perfect their cell growing environment, such as coating extracellular matrix on the electrode [56]. Among them, the features of SAMs possess two dimensional and highly ordered structures on the specific substrates [7]. Research has utilized SAMs-modified electrode to provide biocompatible and affinitive interface for long-term cell culture [8].

The aim of this study was to describe a surface-modified gold-based MEA which were fabricated by using MEMS techniques for better cell affinity and growth. The biocompatible material PI [9] was chosen to be the electrode substrate which was modified by SAMs with immobilized PDL . The properties and the functional groups of the modified MEA as well as the impedance were quantified. Finally, the PC12 cells were cultured on SAMs-modified MEA to assess the efficiency and cell affinity.

Materials and Methods

The fabrication processes of designed gold-based microelectrode were as follows. First, ptotosensitive PI, Durimide 7320SE, was used as a substrate and processed after exposure (100 mJ/cm^2) and development procedures under the photolithography process. Polycrystalline Au (200nm) films were then prepared by evaporating the metals onto the PI-spun glass (4.5×4.5 mm^2) to form the microelectrode on the substrate. The SAMs were formed by immersing the gold-based microelectrode into ethanolic 1 mM solution of 11-Mercaptoundecanoic acid (MUA, COOH-(CH2)10-SH, Aldrich) for 24 h. Subsequently, the MUA SAMs was immersed into the mixture of 10 mg·ml^{-1} 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, Sigma) and 5 mg·ml^{-1} N-Hydroxysuccinimide (NHS, Fluka) aqueous solutions for 30 min. Water-soluble EDC-NHS was used to activate O=C-OH group on the MUA-SAMs gold microelectrode. Finally, PDL and FITC-PLL were immobilized on the activated MUA-SAMs gold microelectrode.

FTIR was utilized to identify the chemical species binding on the electrode surface. Elemental compositions and chemical boundings were observed in the FTIR spectrums. The FTIR spectra of MUA-SAMs modified electrode and MUA-SAMs-PDL electrode were scanned in incident angle of 75° grazing reflectance mode (Bomem, DA8.3 , Harrick).

The fluorescence of FITC-PLL on the microelectrodes was observed by confocal laser scanning upright microscope (Leica TCS SP2, DMRE).

ISSN: 1727-1983

Equation (1) represents the immobilized efficiency of FITC-PLL by calculating the intensity of green fluorescence of each pixel (Leica Confocal Software, LCS).

$$\mu(I) = \frac{1}{N_{Pixel}} \sum_{Pixel} I_i \qquad (1)$$

where μ stands for the mean of the fluorescent intensity per pixel, and the "N_{Pixel}" and "I_i" are the pixel number and fluorescent intensity, respectively.

The impedance of the modified and unmodified microelectrodes was measured by HP 4284A Precision LCR meter. Analytical results were expressed as mean ± standard deviation. Statistical significance was tested by using an unpaired *t*-test.

The Rat pheochromocytoma PC12 cells were maintained in RPMI1640 medium (Gibco) with 5% horse serum (HS), 10% fetal bovine serum (FBS), 100μg/ml penicillin-G, 100μg/ml streptomycin and 29.2μg/ml glutamine in uncoated culture flask. To test the compatibility of microelectrodes, PC12 were cultured on MEA at a density of 1×10^5 cells/ml per dish and incubated in RPMI1640 at 37°Ç, humidified, 5% CO_2 environment.

Results

To quantify the chemical boundings and elemental components, a series of tests were performed on the our modified MEAs. The FTIR spectra of the gold-MUA and gold-MUA-PDL with the pure gold plate as reference were shown in Figures 1 and 2. In Figure 1, asymmetric and symmetric stretching vibrations of the methylene groups of MUA were also observed at 2922 and 2850 cm^{-1}. The methylene bonding (-CH_2-) which existed inherently in MUA and MUA-PDL molecule was near 2970 cm^{-1}. In addition, the primary amine bonding (-CH_2-NH_2) and amide bonding (-CONH-) exist near 3280 cm^{-1} after immobilized PDL on gold-MUA electrode.

In Figure 2, the FTIR absorption peak at 1700 cm^{-1} (C=O in carboxylic acid) was correlated with the functional group of the MUA-SAMs. The spectra in the range of 1300~ 400 cm^{-1} were called finger-print region and stood for the specific structure of the chemical compounds. The peak close to 720 cm^{-1} indicates that alkane group (-CH_2-CH_2-CH_2-CH_2-) of MUA existed on modified surface. Meanwhile, the sulfur compounds of the MUA-SAMs electrode was observed at 610 cm^{-1}. These two peaks evidenced the formation of MUA-SAMs on the gold surface. We also can observe two peaks of amide I (C=O in O=C-NH, at 1613 cm^{-1}) and II (-NH in O=C-NH, at 1548 cm^{-1}) (Figure 2). These indicated that PDL was immobilized with covalent amide bounding on the MUA-SAMs electrode.

Since PDL and PLL are optical isomers, FITC-PLL can also be immobilized on the gold-MUA substrate. Thus, green fluorescence was utilized to observe the immobilization of FITC-PLL by using the confocal microscope. The averaged length of the FITC-PLL molecule on the gold-MUA electrode was 137.95± 11.50 um (n=20, Figure 4). The effective quantity of the fluorescence of FITC-PLL was measured at 1.42 ± 0.09 /pixel (n=20). Because the molecule of the FITC-PLL was immobilized on the gold-MUA surface by amide I and II bonding [5], these fluorescent measurements imply the binding efficiency of the MUA on the gold electrodes.

The impedance of pure gold and gold-MUA-PDL electrodes measured by LCR meter was shown in Figure 5. From *t*-test, the means of these two impedance measurements are not significantly different ($P<0.05$). The impedance of modified electrodes was found only slighly increase, compared to pure gold electrode. From the cell culturing, we can clearly observe. PC12 cells, indicated in white arrow, can successfully grow on the open area (40μm×40μm) of each microelectrode after MUA-PDL modification in Figure 6. This phenomenon indicated the proper modification would make cell adhere on the electrode more closely.

Discussion

The PDL was coated on the MUA-modified gold electrode which unique molecular bonding was characterized by FTIR spectra. The chemical bonding found in the range of 1300~ 400 cm^{-1}, known as finger-printregion, has bben used for identifying the specific molecule of the SAMs-modified substrate. This evidence proved that the PDL can be immobilized on the MUA-SAMs modified gold electrode by covalent amide bonding. Moreover, the fluorescence of the FITC-PLL provided additional observation on the binding efficiency of MUA on the gold electrodes. After the surface modification process, the impedance of the gold electrode increased slightly compared with the Nam's group [8]. Our cell culture on the SAMs-modified electrode indicated that immobilized PDL on MUA promote better cell adhesion to electrodes. Without significant increase in the impedance of SAMs-modified electrode, better cell affinity on the electrode would provide better recording and stimulation efficiency during electrophysiological studies. In addition, the transparent substrate enable the observation of cell growth on either upright or inverted microscopes.

Conclusions

The MUA-SAMs can efficiently immobilize PDL on the gold electrods by covalent amide bounding which was confirmed in FTIR spectra. The fluorescence of FITC-PLL illustrated the binding between PLL-derives to MUA-modified surface. We have shown that the impedance only increased slightly after surface modification on the elelctrode. However, beter affinity of culturing cells on the electrodes exhibited the effect of the gold-MUA-PDL modified microelelctrode.

ISSN: 1727-1983

These characteristics of MUA-PDL modified electrode are essential for future electrophysiological stimulation and recording processes..

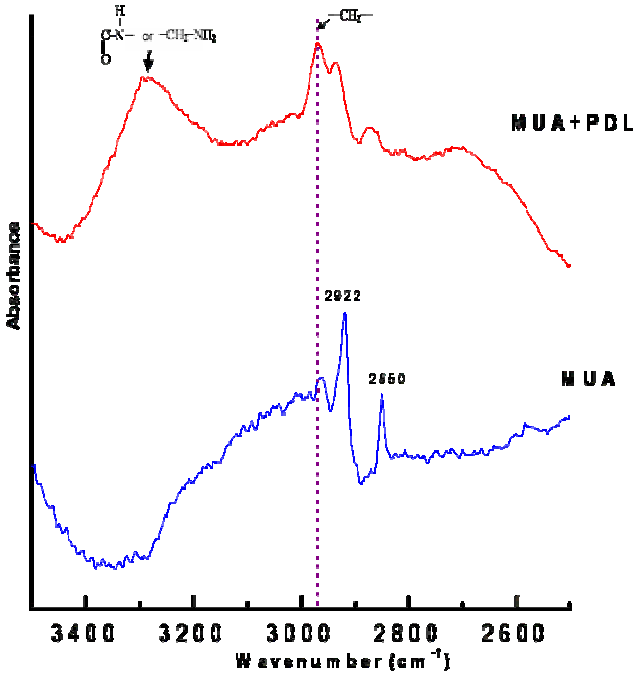

Figure 1: FTIR spectra of gold substrates with MUA and MUA-PDL at 2500~ 3500 cm^{-1}

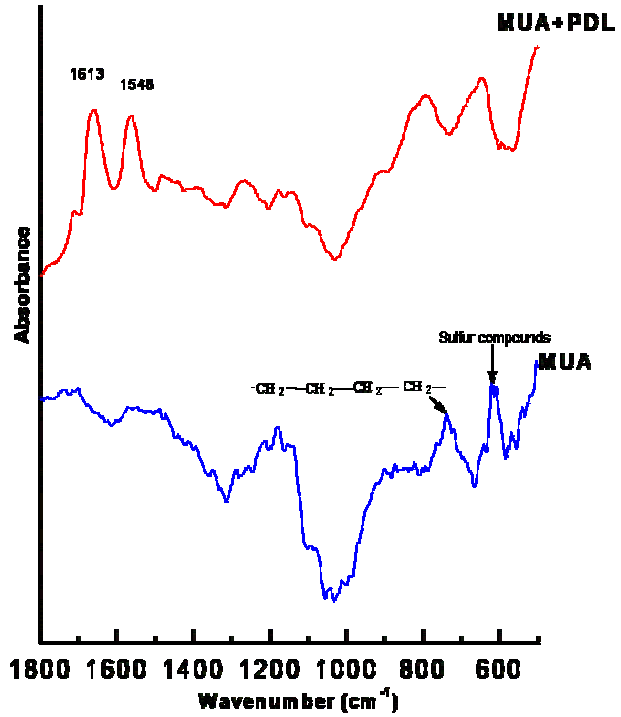

Figure 2: FTIR spectra of gold substrates with MUA and MUA-PDL at 500~ 1800 cm^{-1}

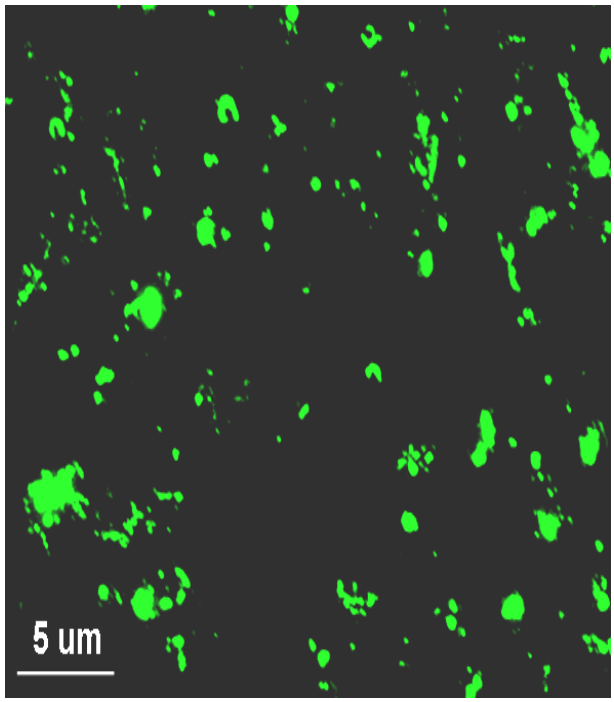

Figure 3: The observation of FITC-PLL fluorescence on gold-MUA electrode

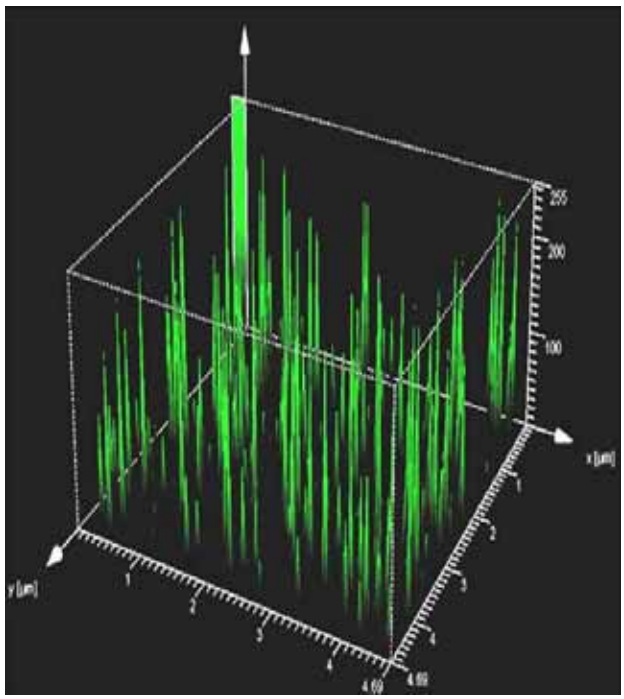

Figure 4: The analysis of FITC-PLL fluorescence on gold-MUA electrode

ISSN: 1727-1983

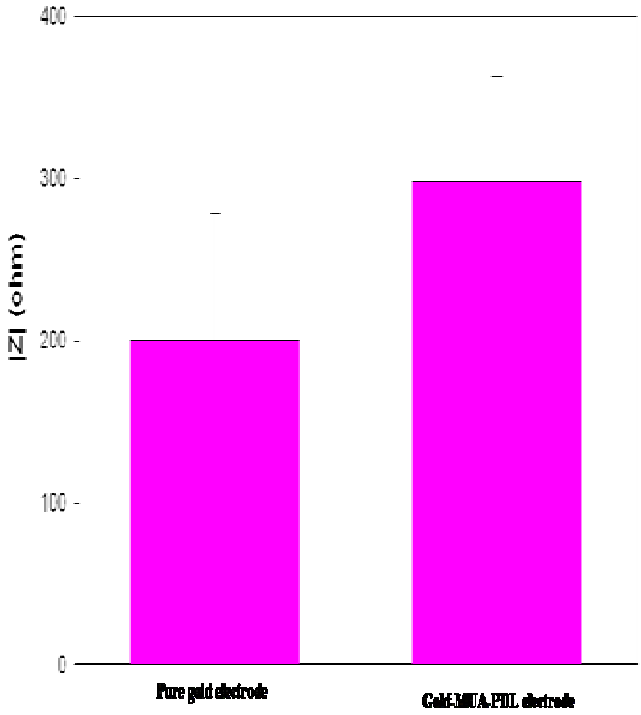

Figure 5: The impedance of pure electrode and gold-MUA-PDL electrode (n=40, *t*-test)

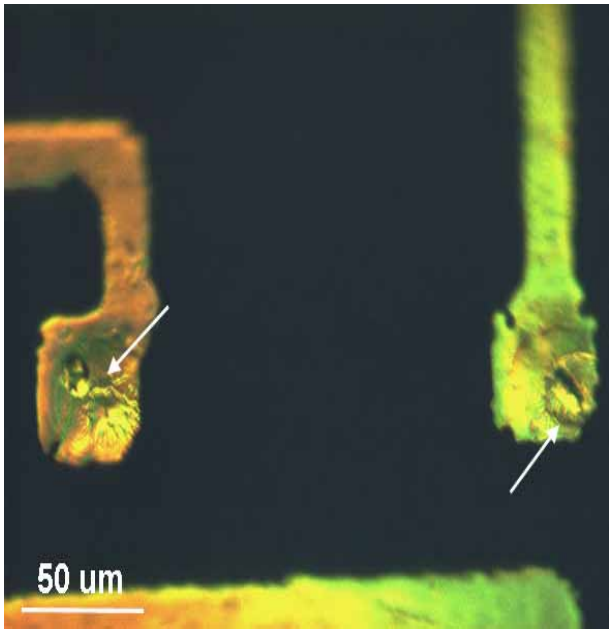

Figure 6: PC12 cells (indicated by arrow) were cultured on the gold-MUA-PDL microelectrodes

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ISSN: 1727-1983