Culture of Myoblast on Gold Film Sputtered on Polydimethylsiloxane Disk

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ABSTRACT

A transparent thin gold film has been designed to culture myoblasts on the surface. The thin gold film was formed on the surface of the polydimethylsiloxane disk by vacuum deposition. The thickness of the film is 30 nm estimated with the current during sputtering. C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse) was cultured for one week on the film. The proliferation of cells was observed with an inverted phase contrast microscope every day. The experimental results show that the proliferation of the cell on the gold film is able to be observed through the transparent gold thin film with the microscope. The water contact angle shows hydrophilic surface of the gold film, which accelerate adhesion and proliferation of cells.

Keywords: Biomedical Engineering, C2C12, Film Electrode, Gold, Sputtering and Polydimethylsiloxane.

1. INTRODUCTION

Cell culture technique has been developed and several methodologies might clinically be applied to regenerative medicine [1]. C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse) adheres to the scaffold, proliferates and differentiates to myotubes *in vitro* [2]. These behaviors of the cell depend on the micro property of the surface.

The photolithography technique enables design of micro-structure of the solid surface for the scaffold of cell culture. The contact angle is one of the parameters for the physical property of the surface. When the water contact angle in the air is smaller than 1.6 rad on a surface, the surface is hydrophilic. The biological cell adheres to the hydrophilic surface. The oxygen plasma ashing is one of the procedures to make the surface hydrophilic. Polydimethylsiloxane (PDMS) is used frequently in the photolithography process. The surface of PDMS is hydrophobic. The surface of the metal, on the other hand, is hydrophilic.

The effect of the surface of the scaffold on cell culture has been studied in the previous studies [3-6]. Several micro-fabrication processes have been designed to control adhesion of biological cells *in vitro* [4-6].

In the present study, gold has been sputtered to make a transparent thin film of gold on polydimethylsiloxane disk, and the behavior of myoblast on the surface has been observed microscopically.

2. METHODS

Film Electrode

A transparent thin gold film has been designed to culture myoblasts on the film. The thin gold film is formed on the surface of the polydimethylsiloxane (PDMS) disk

A silicon wafer (Type N, Matsuzaki Seisakusyo, Co., Ltd.,

Tokyo, Japan) is used for a surface mold for the disk. The diameter and the thickness of the wafer are 50 mm and 0.30 mm, respectively. The surface of the wafer was coated with 0.001 mm thickness of Parylene (Specialty Coating Systems, Inc., IN, USA). After the wafer is enclosed with a peripheral wall of polyimide, PDMS (Dow Corning Corporation, MI, USA) is poured with the curing agent on the wafer. After being degased, PDMS is baked at 383 K for one hour in an oven. The diameter of PDMS disks is 51 mm. The thicknesses of the two kinds of PDMS disks are 2 mm and 5 mm.

To trace the area of the film, a mask of aluminum disk (52 mm diameter, 0.2 mm thickness) has been manufactured (Fig. 1). The mask has a pair of void of 15 mm square at the counter ends for gold deposition. Both surfaces of the disk of PDMS and of the mask of aluminum were cleaned in the etching system (FA-1, Samco Inc., Kyoto), before the sputtering process.

The thin gold film was formed on the surface of the PDMS disk of 2 mm thickness by vacuum deposition in the sputtering equipment (SC-70AT, Sanyu-Electron Co., Ltd., Tokyo, Japan) (Fig. 2). The thickness of the deposited film on the PDMS disk is controlled with the electric current of the equipment: 30 nm with 300 mA in the present study.

Another PDMS disk of 5 mm thickness, which has a hole of 32 mm diameter, is attached on the PDMS disk with electrode to make a rim along the circle (Figs. 3&4). Two PDMS disks are fixed each other with PDMS as adhesive by the following process. The liquid of PDMS was painted on the surface of the two disks. Two disks were attached together, degassed, and baked at 383 K.

After sterilized in the autoclave, the assembled disks are exposed to the oxygen gas in a reactive ion etching system (FA-1, Samco Inc., Kyoto) to be characterized as hydrophilic (oxygen plasma ashing). The disks are placed in polystyrene dish of 70 mm diameter, and used as the culture dish (Fig. 5).

Contact Angle

For comparison, gold was deposited on the half of the surface of PDMS disk (Fig. 6). Variation was made on the process of electric current at spattering: 300 mA, 200 mA followed by 100 mA, and 100 mA followed by 200 mA. Water contact angles were measured by the contact angle analyzer (Phoenix-300, Meiwafosis Co., Ltd., Tokyo, Japan).

The surface of the disks was exposed to the oxygen gas in a reactive ion etching system (FA-1, Samco Inc., Kyoto) to be characterized as hydrophilic (oxygen plasma ashing). The contact angles after five minutes from exposure were compared with that before exposure.

Cell Culture

C2C12 (Mouse myoblast cell line originated with cross-striated muscle of C3H mouse) of thirteenth passage was used for the cell culture. D-MEM (Dulbecco's Modified Eagle Medium) containing 10% FBS (Fetal Bovine Serum) and 1% penicillin/ streptomycin was used for the medium. The cells were seeded on the dish of PDMS with the density of 1000 cells/cm^3. Cells were cultured in the incubator at 310 K with 5% CO_2 for one week. The medium were refreshed every two days. Cells were observed with the inverted phase-contrast microscope (IX71, Olympus Co., Ltd., Tokyo) every day.

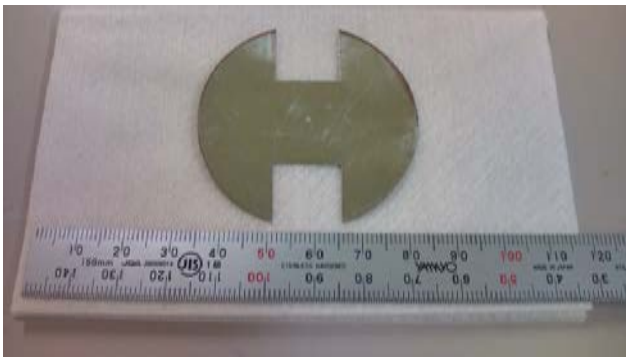

Fig. 1: Mask of aluminum for deposition.

Fig. 2: Deposited gold film on the surface of PDMS.

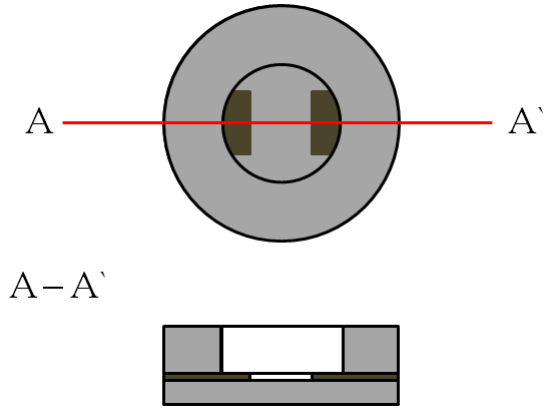

Fig. 3: Design of culture dish of PDMS. Cross-section at line of A-A' (bottom).

Fig. 4: Culture dish of PDMS.

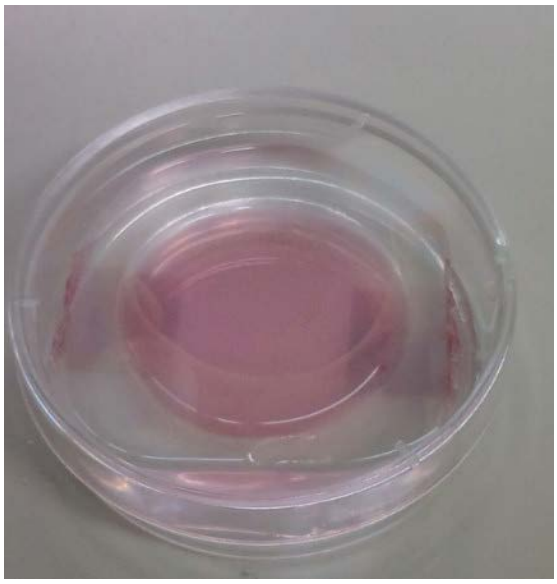

Fig. 5: Culture dish of PDMS placed in polystyrene dish of 70 mm diameter.

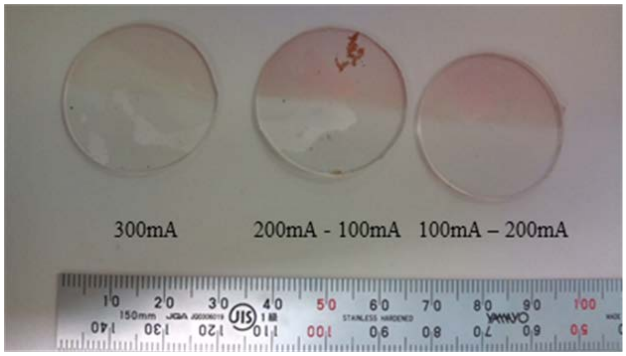

Fig. 6: Gold was deposited on the half of the surface of PDMS disk (upper half in the figure) by three kinds of sputtering process. 300 mA (left), 200 mA followed by 100 mA (middle), and 100 mA followed by 200 mA (right).

3. RESULTS

The color of 100 mA followed by 200 mA is darker than that of 300 mA (Fig. 6), which shows that the thickness of the deposited gold film might increase by sputtering two times. Figs. 7-11 show the water contact angle on the surface of PDMS and gold in the air. Fig. 7 shows the water contact angle bigger than 1.6 rad, which reveals that the surface of PDMS is hydrophobic. Fig. 8 shows the angle smaller than 1.6 rad, which reveals that the surface of the gold film is hydrophilic.

The water contact angle decreases after sputtering, so that the oxygen plasma ashing was effective for the surface to be hydrophilic (Figs. 9&10).

Fig. 11 shows smaller angle than Fig. 8, which shows the process of the spattering two times is better to make the hydrophilic surface.

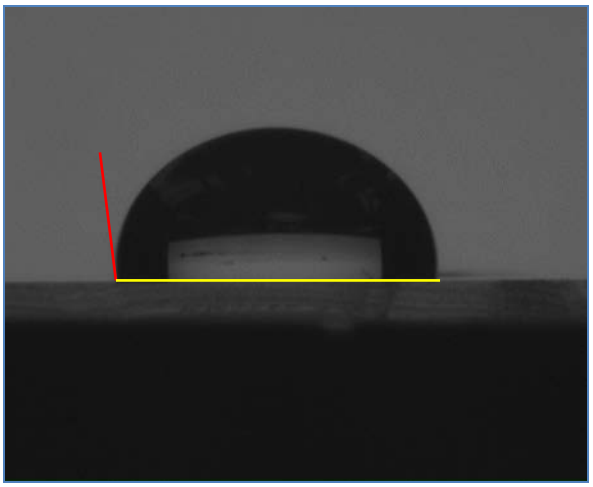

Fig. 7: Contact angle (1.7 rad) on the surface of PDMS before oxygen plasma ashing.

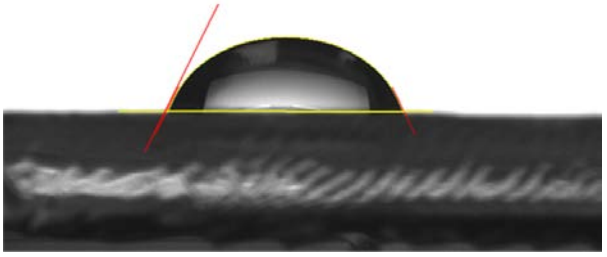

Fig. 8: Contact angle (1.1 rad) on the surface of gold sputtered (300mA) area on PDMS before oxygen plasma ashing.

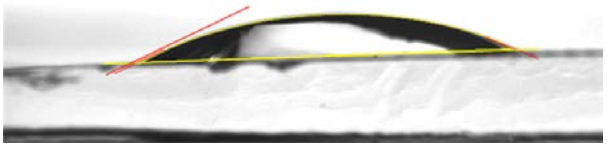

Fig. 9: Contact angle (0.45 rad) on the surface of PDMS after oxygen plasma ashing.

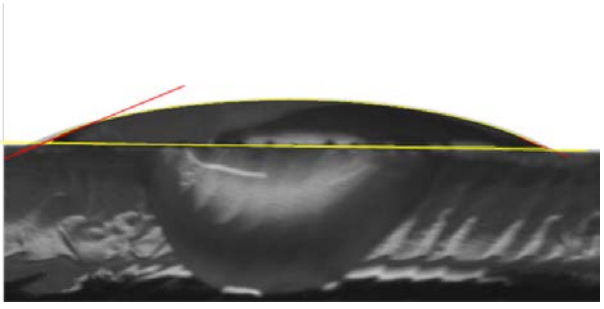

Fig. 10: Contact angle (0.38 rad) on the surface of gold sputtered area on PDMS after oxygen plasma ashing.

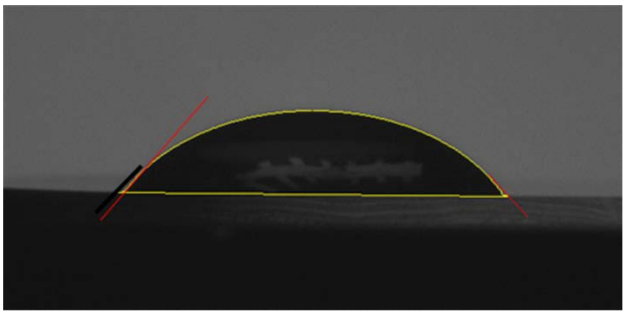

Fig. 11: Contact angle (0.80 rad) on the surface of gold sputtered (100mA & 200 mA) area on PDMS before oxygen plasma ashing.

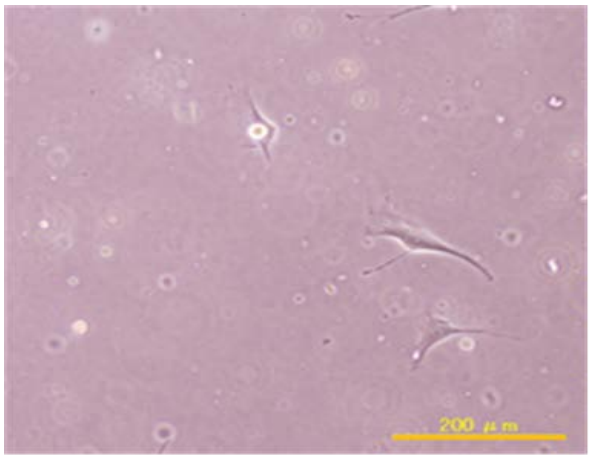

Fig. 12: C2C12 on PDMS after 24 hours. Dimension from left to right is 1 mm.

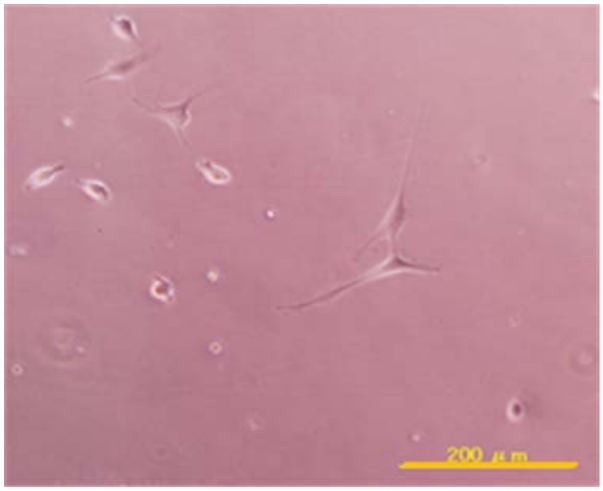

Fig. 13: C2C12 on gold film after 24 hours. Dimension from left to right is 1 mm.

The cells are easily observed with the microscope through the transparent thin film of gold on the PDMS surface (Figs. 13, 15, 16). The Cells adhere, extend pseudo, and proliferate both on PDMS and on gold film (Figs. 12-15). Proliferation of C2C12 tends to be faster on the gold film than on the PDMS even after oxygen plasma ashing.

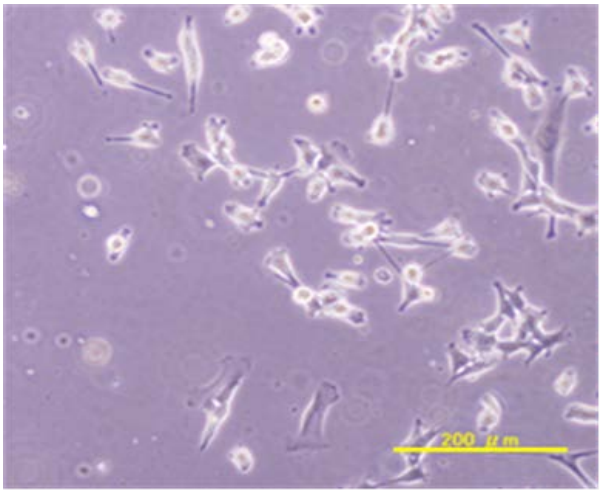

Fig. 14: C2C12 on PDMS after 6 days. Dimension from left to right is 1 mm.

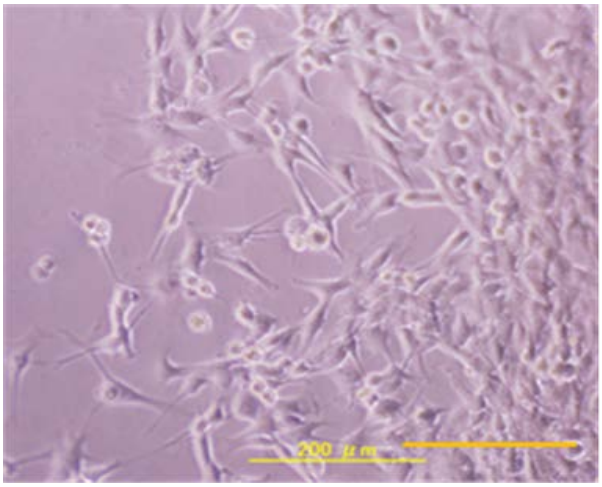

Fig. 15: C2C12 on gold after 6 days. Dimension from left to right is 1 mm.

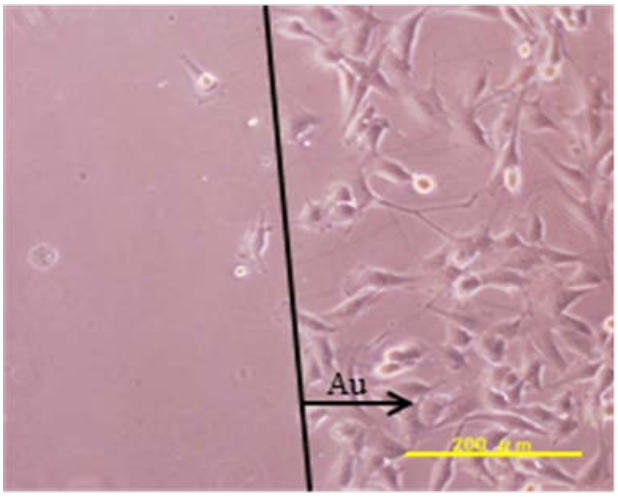

Fig. 16: More cells adhere in the gold film area (right half) on the surface of PDMS. Dimension from left to right is 1 mm.

4. DISCUSSION

The behavior of a biological cell depends on several factors: electrical [7], magnetic [8], and mechanical factors [9-12]. The gold film has a potential to be used as an electrode to control the electrical factors.

The electrical resistance (R) between the ends of the film is calculated by Eq. 1.

$$R = k\,L\,/\,(d\,b) \qquad (1)$$

In Eq. 1, k is resistivity [S^{-1} m], L is length [m], d is thickness [m], and b is width [m].

The resistivity of gold is 2.21×10^{-8} m/S at 293.15 K. The electrical resistance (R) between the ends of the film is estimated 2 S^{-1}, when $k = 2.21 \times 10^{-8}$ m/S, L = 15 mm, d = 0.00001 mm, and b = 15 mm.

To avoid cells get into the space between the PDMS disk and the culture dish, the rim was formed around the PDMS disk, and the suspension of the cells was poured on the PDMS disk.

The contact angle on the gold film deposited on PDMS depends on the process of deposition.

Several factors might govern the adhesiveness of the cell: the micromorphology of the deposited gold film, and the chemical characteristics of the surface. The contact angle is one of the parameters for the physical property of the surface. Proliferation of cells also might depend on these factors.

The photolithography technique has been applied to fabricate the micro channel [13]. The microfluidic system has been applied to sort biological cells [14, 15], and to trap biological cells [16, 17]. The system also used to study local environment around the cultured cell [3, 6]. The micro pattern of the surface has been applied to study the surface effect of adhesion of cells [4, 5, 18-20].

The micro-fabrication technique has also been applied to design microfluidic systems *in vitro* [21-24]. The technique will also be applied to handle cells in diagnostics *in vitro*. The difference of the affinity between the scaffold and the cell might control adhesion of cells *in vitro*. The methodology was developed to estimate adhesiveness of myoblast [25]. The microstructure was designed to capture biological cells [26].

5. CONCLUSION

A transparent thin gold film on the surface of the polydimethylsiloxane disk has been designed to culture myoblasts on the surface. The experimental results show that the proliferation of the cell on the gold film is able to be observed through the transparent gold thin film with the microscope. Proliferation of myoblast tends to be faster on the gold film than on the PDMS even after oxygen plasma ashing.

6. ACKNOWLEDGMENT

This work was supported by a Grant-in-Aid for Strategic Research Foundation at Private Universities from the Japanese Ministry of Education, Culture, Sports and Technology.

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