Guided Cell Growth Through Surface Treatments

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This paper presents easy methods for guidance of animal cell growth *in vitro*. The surfaces of silicon wafers were treated using simple MEMS techniques in order for the formation of differentiated surfaces. Human epithelial cells incubated on these surfaces showed ordered growth and division patterns along the original design. These methods would be a useful basis in cell-based high throughput screenings and artificial skin.

Key Words : Cell Culture, Cell Patterning

1. Introduction

There has been a big progress in the development of biological research methods during the passed decades. Culture of animal cells, especially, shows outstanding technical progresses recently. Culture of animal cells is important not only for the identification of cellular physiological characteristics but also for the screening of novel drugs. It is well known that many candidate molecules which show target activity in test tube levels do not have any activity in cell-based assays. This phenomenon resulted in the prominent importance of cell-based assays in the middle of drug development. However, it is still a difficult hurdle to integrate cell culture systems into mass HTS (high throughput screening).

Fibroblasts and epithelial cells must be cultured on solid matrices to attach on which they grow and divide. By the way, the growth and division direction of cultured cells are totally random as shown in Fig. 1. It is important to control cell proliferation within limited area or to guide cell proliferation resulting in the patterned growth for cell-based assays as well as cell-based sensors and artificial skin. There have been variety of reports including topographical, physicochemical, and electrical modifications of culture surfaces (reviewed in Jung et al., 2001).

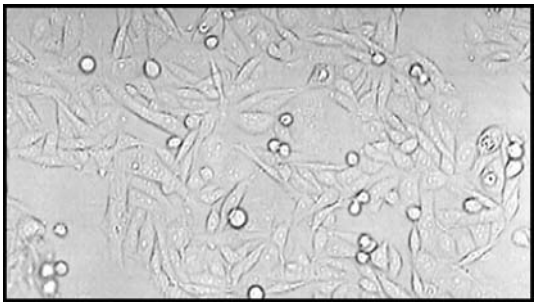

Fig. 1 Hela cells cultured on a commercially available conventional culture dish. Totally random proliferation pattern can be seen

Micro structures or differentiated electrical area can be easily formed on a solid surface by means of photolithography. Simple photolithographic methods other than previous complex or multi-step ones are combined to guide ordered cell growth in this report.

2. Materials and Methods

2.1 Fabrication

Fabrication methods are described briefly in Fig. 2. Fig. 2A shows the method which used

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poly-L-lysine. If a surface is treated with poly-L-lysine, the surface is positively charged because poly-L-lysine bears strong positive charge. Consequently, animal cells with cellular membrane of negative charge can attach on the positively charged surface (Harlow and Lane, 1988). AZ 5214 (Clariant), one of the positive photoresists, was spin-coated on a silicon wafer and baked at 90℃. This wafer was treated with 0.5mg/ml of poly-L-lysine (Sigma) for 20 min at room temperature and washed with distilled water several times to remove unbound poly-L-lysine. After UV-photolithography with a mask, poly-L-lysine was patterned by removing underlaid AZ 5214 with developing solution (Clariant).

Fabrication noted in Fig. 2(B) is the formation of differentiated area of silicon and silicon dioxide. 0.7 μm of silicon oxide layer was made on a silicon wafer by successive dry, wet, and dry oxidations. AZ5214 was spin-coated on this wafer and patterned by UV-photolithography. The exposed silicon oxide area was selectively etched by BHF (buffered hydrofluoric acid). The fabricated matrix was directly used or was treated with 1 mg/ml of poly-L-lysine for 5 min at room temperature just before use.

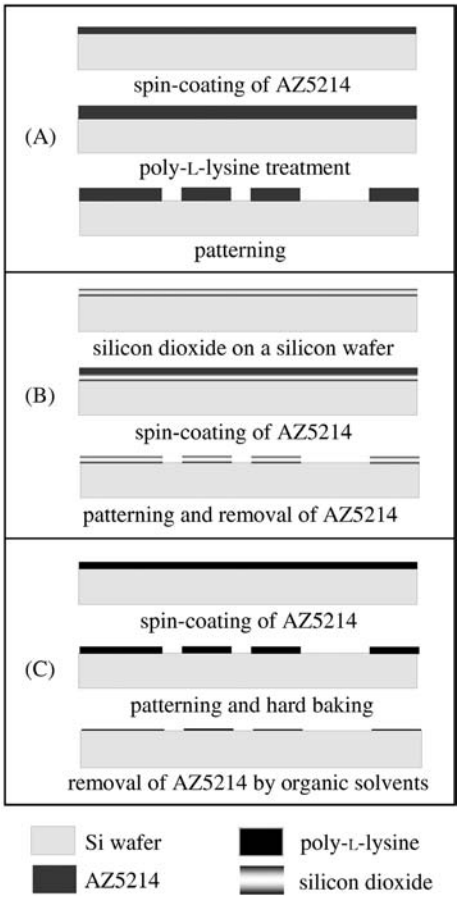

Fig. 2 Schematic diagram of fabrication processes

Several photoresists which are hard-baked after UV-exposure tend to leave traces of their patterns on silicon wafer even after being removed by organic solvents such as acetone and methanol. This phenomenon was adopted as one of the fabrication method, which is depicted in Fig. 2C. AZ5214 was spin-coated on a silicon wafer and patterned by UV-photolithography. This wafer was hard-baked at 120℃ for 5 min and soaked in acetone and methanol successively to remove the photoresist layer.

Mask patterns which were used in photolithography are shown in Fig. 3 with simplification. Each diagram is formed by concentric array, and

Fig. 3 Simplified view of the original patterns on the mask

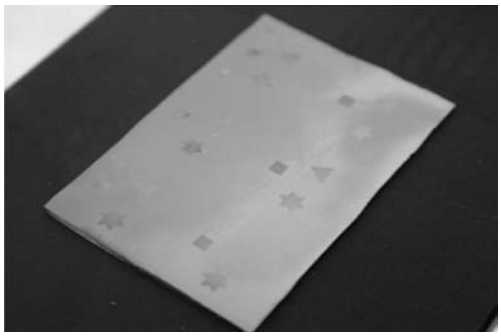

Fig. 4 One of the fabricated matrices. Six groups with different line widths were fabricated on a matrix. Each group is composed of three different diagrams with the same line width

of which the line width is 5, 10, 15, 20, 25, or 30 μm. The space between lines is the same of the line width. One of the fabricated devices is shown in Fig. 4. All of the patterns in a device were fabricated by the same method. Six groups of diagrams mean six different line widths of diagrams. Three different diagrams in a group have the same line width. The different diagrams were used for the observation of cell growth in different curvatures.

2.2 Cell Culture

Hela cells, which had been originated from human cervical carcinoma, were used to test the property of the completed matrices. Wafers were disinfected by treatment with 70% of ethanol for 5 min at room temperature just before use. After being washed with sterile water, the wafers were laid on cell culture dishes (Falcon), and cultured Hela cells were poured with DMEM (Dulbecco's Modified Eagle's Medium, JBI) which was supplemented with 10% of FBS (fetal bovine serum, JBI). After incubation in a humidified CO_2 incubator at 37℃ for 2 or 4 days, the cell growth patterns were observed (Fresheny, 1994).

3. Results and Discussion

Cell growth patterns on the fourth day after the initiation of incubation on a silicon wafer which was treated as Fig. 2(A) is shown in Fig. 5. There were some ordered cell growth patterns on the second day (data not shown). However, crowded cell population ran over the matrix patterns on the fourth day.

This phenomenon was also observed on the matrix fabricated as Fig. 2(B) without poly-L-lysine treatment. Ordered cell growth patterns could be detected on matrices with variable pat tern sizes on the second day (Fig. 6). Especially, more ordered growth pattern was observed on larger pattern (Fig. 6(A) to 6(D)). However, ordered growth pattern dimmed on the fourth day as in the case of Fig. 5. Fig. 7 shows random growth pattern on the fourth day on a silicon wafer which was treated as Fig. 2(B).

The dimness of growth patterns could be prevented by additional treatment with poly-L-lysine. The silicon wafer which was fabricated as Fig. 2(B) was treated with poly-L-lysine just before pouring cultured cells on to the silicon wafer. The cell growth patterns were clearly maintained even on the fourth day of incubation as shown in Fig. 8. These phenomena were detected on surfaces with various sizes of patterns. Fig. 8(A) to 8(C) were photographed with the same magnification. It is clear that cells grow

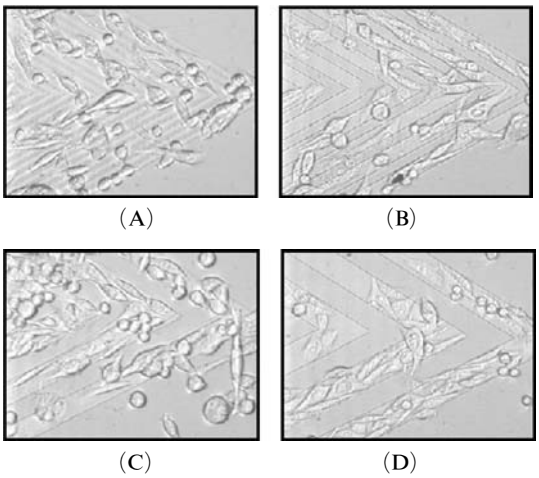

Fig. 6 Cell growth patterns on the second day on a Si wafer which was treated as Fig. 2B without poly-L-lysine

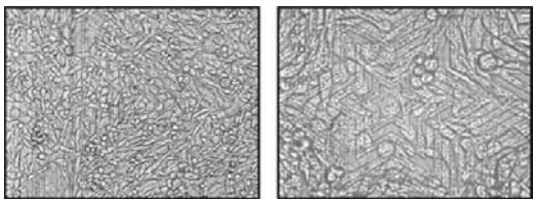

Fig. 5 Cell growth patterns on the fourth day on a Si wafer which was treated as Fig. 2(A)

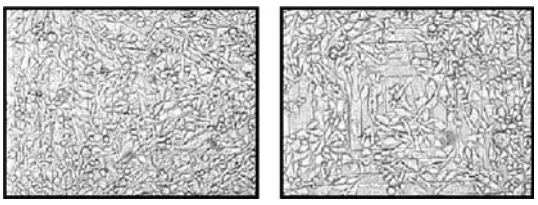

Fig. 7 Cell growth patterns on the fourth day of that of Fig. 6. Cells were incubated two more days

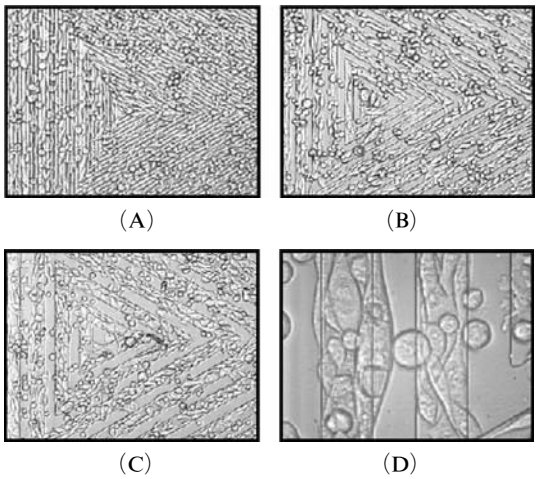

Fig. 8 Cell growth patterns on the fourth day on a Si wafer which was treated as Fig. 2(B) in addition to poly-L-lysine treatment

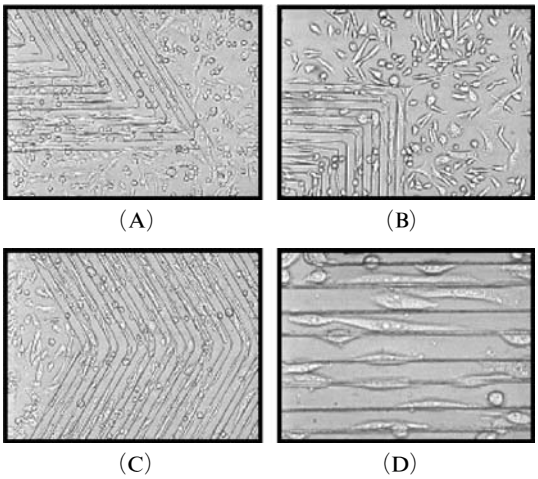

Fig. 9 Cell growth patterns on the second day on a Si wafer which was treated as Fig. 2(C)

only along the poly-L-lysine/silicon dioxide area as shown in Fig. 8(D). It is notable that cells which were repelled out of the poly-L-lysine/silicon dioxide area during the division seemed not to be able to attach on the surface and consequently were dying forming round shapes. Taken together, combination of the two methods in Fig. 2(A) and Fig. 2(B), that is, generation of hydrophilic environment by silicon dioxide and additional charge supply seems to be the most effective method for cells to grow on limited area. This method is also regarded as the advantageous one for long-term incubation. Silicon wafer which was fabricated as in Fig. 2(A) is same as in Fig. 2(B) with additional treatment with poly-L-lysine in appearance. It is possible that the poly-L-lysine layer was damaged during the removal process of AZ5214. The effect of photolithographic processes on poly-L-lysine should be examined by succeeding study.

Cells on wafers which were fabricated as in Fig. 2(C) showed clearly ordered growth patterns on the second day as shown in Fig. 9. Wafers with different diagrams were photographed with the same magnification from Fig. 9(A) to Fig. 9(C). It is peculiar that cells attached preferably on the boundary lines between naked silicon and AZ5214 traces other than on any specified area as in the case of Fig. 8. This result is clear when Fig. 9(D) is compared with Fig. 8(D). There were residual traces of AZ5214 on the silicon wafer which could be detected by naked eyes after removal by acetone and methanol. Cells showed proliferation along these patterns. It is not certain that these phenomena were resulted from the minute topographical differences or chemical differences between the two areas. We also observed similar phenomena from which the surface was hot-embossed in brief (Son et al., 2004).

Compared with Fig. 9, it is notable that there is almost no attachment or growth of cells on areas without patterns in Fig. 6. On the other hand, many proliferating cells can be detected on areas without patterns in Fig. 9. These phenomena seem to be resulted from the different fabrication processes. Hydrophobic bare silicon is protected from direct contact with AZ5214 by silicon dioxide layer in Fig. 2(B) method. However, bare silicon is exposed to AZ5214 in Fig. 2(C) method. During this contact process, therefore, it is possible that the surface property of silicon was affected resulting in the change of innate strong hydrophobicity. The areas without patterns and cells in Fig. 6 and Fig. 8 correspond to the areas from which AZ5214 was removed before the etching of silicon dioxide. This means that the hydrophobicity of silicon may be affected

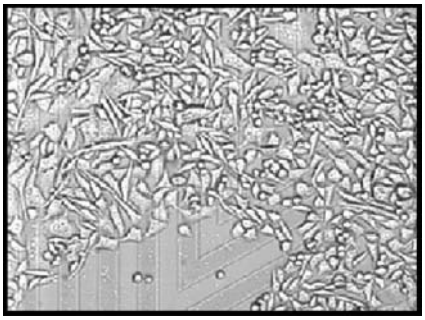

Fig. 10 Cell growth patterns on fourth day of that of Fig. 9. Cells were incubated two more days

Table 1 Comparison of the treatment methods

fabrication method	A	B	B+Lys*	C
incubation				
2 days	+	++	+++	+++
4 days	−	+	+++	−

* treated with poly-L-lysine before culture

through simple contacts with photoresists. The ordered cell growth patterns detected in Fig. 9 totally disappeared on the fourth day of incubation (Fig. 10). The results are summarized in Table 1 with arbitrary points.

4. Conclusion

We described several methods which can control the random growth of cells through the simple combinations of easy photolithographic processes. These include the differential formation of hydrophilic and hydrophobic regions in addition to charge attachment. The effect of the residual traces of photoresist was also examined. These methods are very simple and cheap to realize. The methods reported here may substitute previous methods or be combined with them.

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