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TECHNICAL NOTE

Patterning of 293T fibroblasts on a mica surface

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Abstract Controllable cell growth on the defined areas of surfaces is important for potential applications in biosensor fabrication and tissue engineering. In this study, controllable cell growth was achieved by culturing 293 T fibroblast cells on a mica surface which had been patterned with collagen strips by a microcontact printing (μCP) technique. The collagen area was designed to support cell adhesion and the native mica surface was designed to repel cell adhesion. Consequently, the resulting cell patterns should follow the micro-patterns of the collagen. X-ray photoelectron spectroscopy (XPS), water contact angle (WCA) measurement, atomic-force microscope (AFM) observation, and force-curve measurement were used to monitor property changes before and after the collagen adsorption process. Further data showed that the patterned cells were of good viability and able to perform a gene-transfection experiment in vitro. This technique should be of potential applications in the fields of biosensor fabrication and tissue engineering.

Keywords Cell adhesion · Microcontact printing · Collagen · Gene transfection · Mica

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Introduction

Controlling cell positioning and adhesion on a surface is important for its potential applications in biosensor fabrication for drug toxicity and environment monitoring [1], tissue engineering [2], bioelectronics research [3, 4] and basic studies of biology, where the roles of cell adhesion, shape, and proliferation are investigated as a function of cell-cell and cell–extracellular matrix interactions [5]. Several methods have been proved to enable efficient control of cell adhesion on the defined areas of some substrates, for example microcontact printing (μCP) of polymers [6], soft lithography [7–10], and three-dimensional microfluidic systems [11, 12]. However, current methods for controlling cell adhesion are limited to a few substrates and the materials used for controlling cell adhesion are still scarce. It is interesting and also challenging to find new substrates and new materials for controlling cell adhesion to surfaces.

Among the various techniques for patterning cells, μCP is extensively used to transport materials to surfaces [13]. In a common procedure of this technique, molecules that can support cell adhesion are adsorbed on the bottom surface of a PDMS stamp and then transferred to the substrate surfaces by direct contact. The vacant areas either have the function of repelling cell adhesion [12] or are endowed with the functions of repelling cell adhesion by further treatment [14]. Consequently, the cells can only adhere to the areas defined by μCP. In contrast, it is also feasible to transfer molecules that can repel cell adhesion to defined areas on the surface by μCP, leaving the vacant areas for cell adhesion.

As far as the substrate is concerned, mica is a promising material for patterning applications which has a surface of atomic-scale flatness [15, 16]. It has been reported that the

mica can be used as the substrate to pattern proteins for immunoassay [17]. Although we believe it will be meaningful to fabricate cell micro-patterns on a mica surface, no such research has yet been performed.

Among the various materials for controlling cell adhesion [6, 14], biomaterials should be an ideal choice because of their good biocompatibility and low toxicity. Collagen is an important biomaterial which has been extensively used for tissue engineering because of its excellent promotion of cell growth [18]. We have reported that collagen can be used to guide cell adhesion on PDMS surface in the presence of hydrophobins [19]. However, whether collagen can be used to guide cell growth on a mica surface is still unclear. Many factors, for example the feasibility of immobilizing collagen on the mica surface by μCP and the stability of the collagen patterns in the cell culture medium, will substantially affect the resulting cell patterns. We can only obtain good cell patterns when the collagen is well transferred on to the mica surface and retains its patterns. Experimental data are needed to validate the strategy.

In this study, we developed a new strategy for controlling cell adhesion on the surface by using mica as the substrate and using collagen as the material to control cell adhesion. The procedure is shown in Scheme 1. Briefly, the collagen micro-patterns were constructed on the mica surface by μCP and then 293 T fibroblast cells were seeded on the collagen-patterned mica surface in serum free Dulbecco′s modified Eagle medium (DMEM). After 4 h the cells were cultured in DMEM with 10% serum for another 48 h. Because the collagen promoted cell adhesion and the native mica surface repelled cell adhesion, the cells could only adhere to the collagen areas and grow. A gene-transfection experiment was carried out to investigate the viability of the cells. We found that the micro-patterns of 293 T fibroblast cells could be obtained, the patterns of which followed the patterns of collagen. What is more, the patterned cells had good viability and expressed fluorescent proteins in vitro. Multiple experiments, for example XPS, WCA measurement, and AFM observation, were also carried out to monitor the surface-modification process.

Materials and methods

General

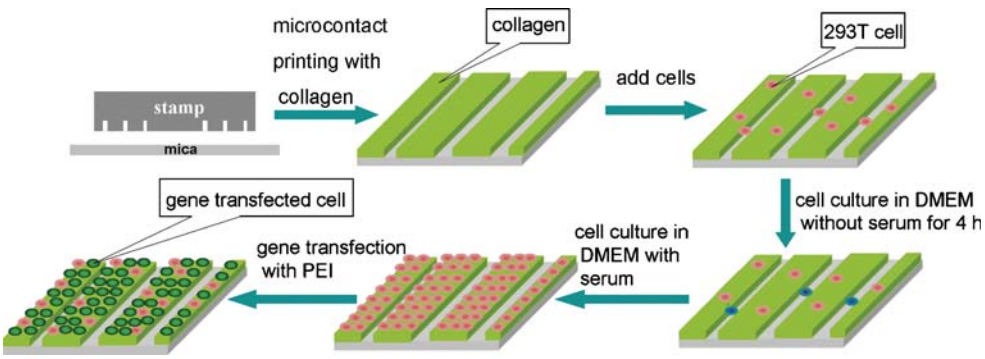
Rat-tail type I collagen, the PDMS silicone elastomer kit (Sylgard 184 Dow Corning), bovine serum albumin (BSA), and polyethylenimine (PEI; *MW*25,000) were purchased from Sigma. The concentration of collagen was 1 mg mL^{-1}, the pH7.5. DMEM was purchased from Invitrogen. Fetal bovine serum was purchased from Tianjin Shengda. Plasmid DNA pEGFP-C2 (4.7 kb) was purified from *E.coli* DH5α and was stored in Tris–EDTA buffer (10 mmol L^{-1} Tris-HCl, 1 mmol L^{-1} EDTA, pH 8.0) at –20°C. The concentration of plasmid DNA was determined by spectrophotometric analysis using a DU-7 Spectrophotometer (Beckman). Aqueous solutions were prepared with redistilled water.

XPS measurements

Collagen was transferred on to the mica surface by μCP using a featureless stamp. The chemical composition of the mica surface was measured by XPS (PHI-5300; Phi, USA) employing a monochromatic Mg Kα radiation source (hv= 1253.6 eV). The energy range was from 0 to 1100 eV and the electron take-off angle was fixed at 45°. The energy resolution of the analyzer was 0.8 eV. The sensitivity was 80–1600 KCPS. The peaks in the elemental core-level spectra were fitted using UNIX on an Apollo Domain series 3500.

WCA measurements

Mica surfaces were modified with collagen by coating with collagen solution for 30 min. The wettability of the native mica surface and the collagen-modified mica surface was investigated by measuring their static WCAs. WCAs were



Scheme 1 Strategy for micro-patterning of cells

measured by an optical contact angle meter (Kruss DSA100) according to the manufacturer's instructions. The WCA values were averaged from ten measurements. All the data were within 3° of the average value.

AFM observation

Mica surfaces modified by collagen were prepared in the same way as described above. AFM observation was performed using a Nanoman D3100 (Veeco Metrology, USA) in tapping mode in an ambient environment. Commercial Si tips were used and the spring constant was 20 Nm^{-1}. The drive frequency was 290 kHz. The scan speed was 1 Hz.

Force curve measurement

Au-coated Si_3N_4 tips (k=0.12 Nm^{-1}; Veeco Metrology, USA) were immersed in an aqueous solution of collagen (1 mg mL^{-1}) for 12 h. The force curves between the collagen-coated tips and the native mica surface were measured on a multimode AFM (Nanoman D3100; Veeco Metrology) in aqueous solution.

Fabrication of collagen micro-patterns on the mica surface

The PDMS stamp was prepared by curing a mixture of liquid PDMS and cross-linker (10:1 *w*/*w*) on the silicon master at 60°C overnight. The silicon master was then peeled off and the PDMS stamp was obtained. The PDMS stamp had a broad surface with embedded long strips. Three nearby embedded strips were in a group. In a group, the width of the strips was 30 μm, 50 μm, and 50 μm, respectively.

The PDMS stamp was washed with 70% alcohol before μCP. The PDMS stamp was inked with the collagen solution for 2 min and the collagen solution was transferred on to the mica surface by direct contact. The contact time of μCP was 1 h. Then the stamp was peeled off. The patterned mica surface was carefully washed with distilled water and dried under a stream of nitrogen gas. The collagen micro-patterns on the mica surface were observed by use of a scanning electronic microscope (SHI MADZU SS-550) equipped with SS-550 Software (Main) Version 2.05.

Fabrication of cell micro-patterns on the mica surface and gene transfection

293 T fibroblast cells (a human embryonic kidney cell line) were routinely cultured in DMEM supplemented with 10% (*v*/*v*) fetal bovine serum. The cells were rinsed with phosphate-buffered saline (PBS), treated with trypsin-EDTA (0.25%), and incubated for 10 min (37°C) in a 5% CO_2 incubator to detach the cells. The cell suspension was then supplemented with the same volume of DMEM containing 10% (*v*/*v*) serum to stop the reaction of trypsin. After centrifugation for 5 min at 1000 rpm, the cells were suspended in 1 mL DMEM without serum.

In cell-patterning experiments the mica slices with collagen micro-patterns were carefully placed into a 24-well plate. The cell suspension was dripped into the wells and the cells were incubated in a 5% CO_2 incubator at 37°C for 4 h. The cells were then cultured in DMEM containing 10% serum. At the same time, PEI was used to deliver DNA into 293 T fibroblast cells in this study. The

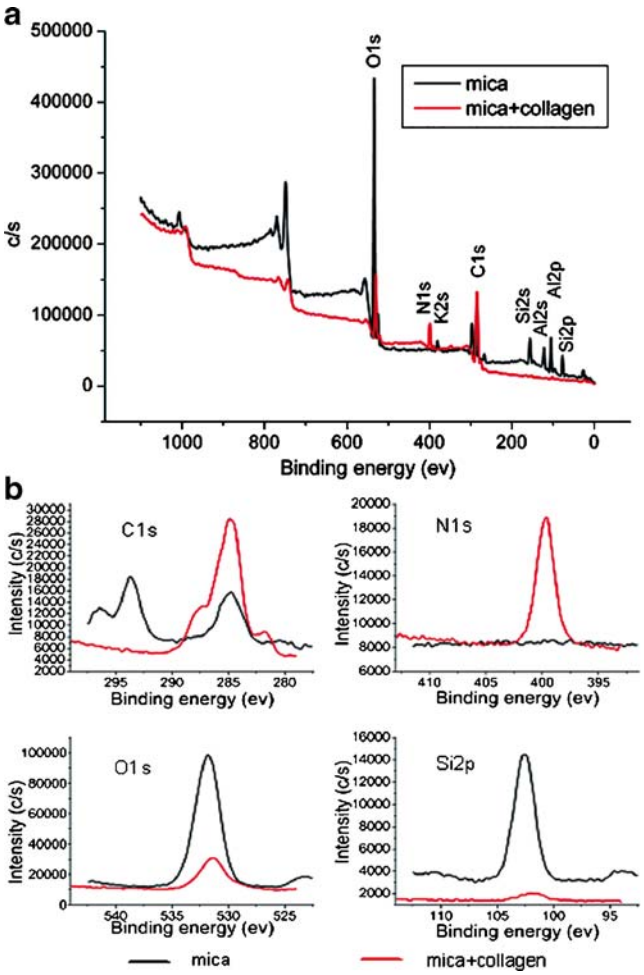


Fig. 1 XPS spectra of the mica surface before and after modification with collagen. (**a**) The full spectra. (**b**) The spectra of N 1 s, C 1 s, O 1 s and Si 2p

Table 1 The relative atomic composition of the native mica surface and the mica surface modified by collagen

Sample	C 1s	N 1s	O 1s	Al 2p	Si 2p
Mica	10.99	0.04	56.98	12.86	14.50
Mica+collagen	65.84	10.68	20.88	0.39	1.45

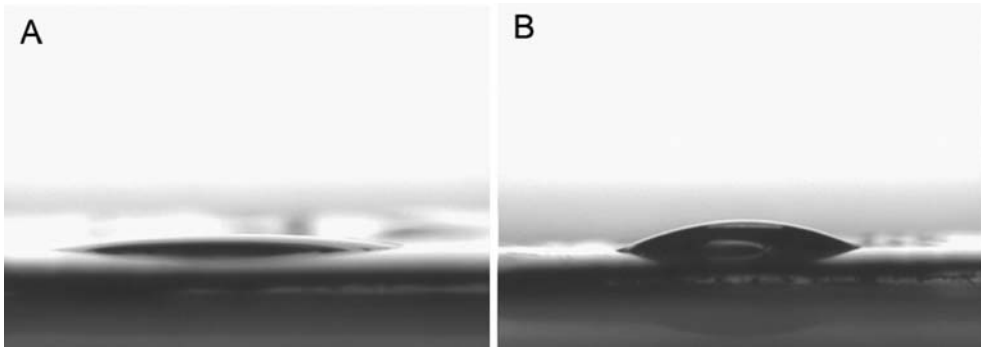


Fig. 2 WCAs of (**a**) a mica surface and (**b**) a mica surface modified by collagen

complexes containing PEI and plasmid DNA were prepared in 100 μL PBS solution. DNA used in each well was 2 μg and the PEI used in each well was 1.33 μL (1 mg mL^{-1}). The mixture was incubated at room temperature for 30 min. Once the cells were cultured in DMEM containing 10% serum, the mixture of PEI and DNA was directly dripped into the cell-culture medium, and 48 h later the cells were observed under a TE 2000-U (Nikon, Japan) fluorescence microscope equipped with Spot software.

Results

XPS measurement

XPS was used to monitor changes of surface chemical composition during the modification process (Fig. 1). Collagen was transferred on to the mica surface by using a featureless PDMS stamp. The native mica surface exhibited its unique XPS spectrum, which was shown as a higher intensity of O 1 s (534 eV) signal associated with lower intensity of Si 2 s (157 eV), Si 2p (106 eV), Al 2 s (123 eV), and Al 2p (78 eV) signals. After modification of the mica surface by collagen, an N 1 s peak appeared at 400 eV. The nitrogen arises from the collagen and is not present in native mica. Consequently, this result indicated that collagen had been successfully immobilized on the mica surface by μCP.

The relative atomic composition of the surface is shown in Table 1. After modification, the signal of N 1 s increased from 0.04 to 10.68. Meanwhile, the signal intensity of Al 2p decreased from 12.86 to 0.39 and the signal intensity of Si 2p decreased from 14.5 to 1.45. The result quantitatively showed that the collagen had covered the mica surface to a large extent.

WCA measurement

WCA measurement was used to investigate the surface wettability shift induced by the modification process (Fig. 2). The native mica surface had a WCA of about 0.3°, which showed that the mica surface was extremely hydrophilic. After modification by collagen, the WCA increased to around 17.3°. The changes of WCA were related to the difference between the wettability of mica and that of collagen. Surface modification with collagen did not obviously change the hydrophilicity of the mica surface. The hydrophilicity of the modified mica surface would facilitate the adsorption of collagen and should be beneficial to the formation of cell patterns on the mica surface according to the collagen patterns.

AFM measurement

Stable adsorption of collagen on the mica surface is important for the formation of the cell patterns on the mica surface. The adsorption of collagen on the mica surface was investigated by AFM. As shown in Fig. 3, the native mica surface is of atomic-scale flatness, which is the same as reported previously [15, 16]. After modification with collagen, the surface became rough. This roughness was caused by collagen adsorption on the mica surface.

The interaction between collagen and the mica surface was also measured by AFM (Fig. 4). The adhesion force between collagen and the mica surface was approximately 10^4 pN. Our previous research showed that the adhesion force between BSA and mica was only approximately 10^2 pN [20]. It is obvious that the interaction between collagen and mica is much stronger. The strong interaction is thought to be related to the electrostatic property of the collagen. The collagen is positively charged and the mica is negatively charged. There is a strong electrostatic

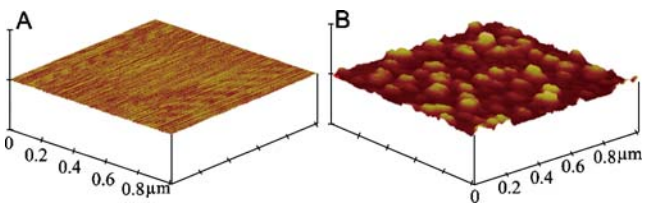


Fig. 3 AFM images of (**a**) a native mica surface and (**b**) a mica surface modified by collagen

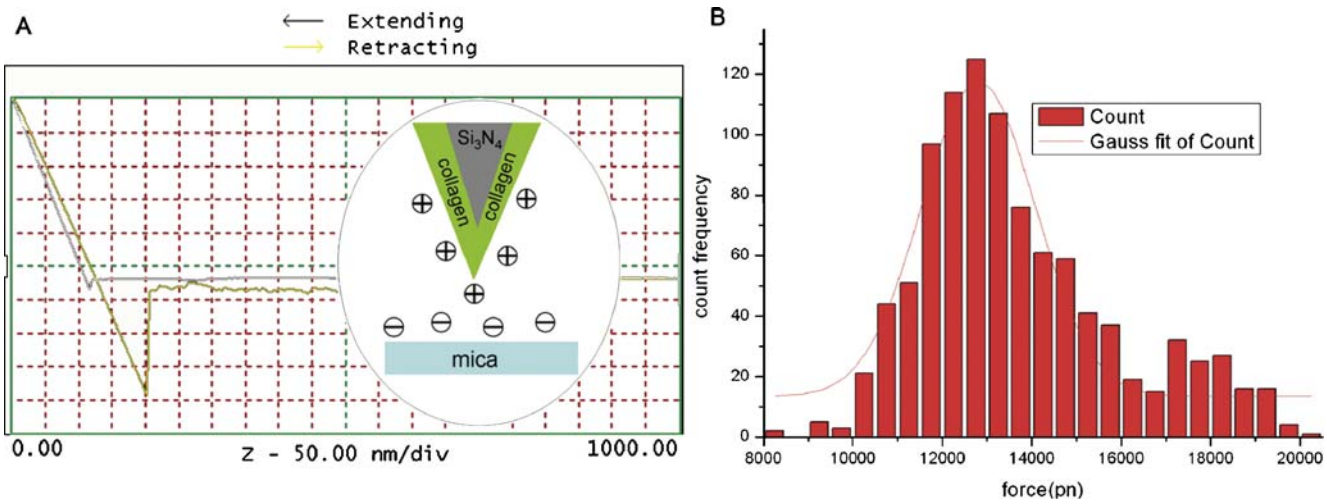


Fig. 4 Adhesion force measurement between mica and collagen: (**a**) a typical force curve and (**b**) the distribution of adhesion forces. An AFM tip coated with collagen was used in the measurements

interaction between collagen and mica. This strong interaction will result in good stability of immobilization of collagen on the mica surface.

Collagen micro-contact printing on mica surface

The collagen micro-patterns on the mica surface were made by μCP using a PDMS stamp [17]. The bottom surface of the PDMS stamp had embedded long stripes of 30 μm, 50 μm, and 50 μm, respectively. Figure 5 shows that the collagen formed a layer on the mica surface after μCP. The layer was cut into several panels by long strips, the widths of which were 30 μm, 50 μm, and 50 μm, respectively. Compared with the PDMS stamp surface, it was confirmed that the collagen micro-patterns on the mica surface were consistent with the shape of the PDMS stamp surface. The border effects and possible differences in the interfacial tension in the vicinity of the patterned structures are common problems for successful patterning. Judging by Fig. 5, these effects did not have an obvious influence on the resulting pattern in the experiment. That means collagen could be well transferred on to the mica surface according to the pattern of the stamp by μCP.

Cell viability on the collagen coated mica surface and the native mica surface

In this study, the collagen area was designed to support cell adhesion and the native mica surface was designed to repel cell adhesion. Consequently, the cell viability on the two surfaces was important for formation of cell patterns on the mica surface.

We cultured 293 T fibroblast cells on the native mica surface and on the mica surface coated with collagen in serum-free DMEM for 4 h and observed the cell viability on both surfaces by use of an optical microscope. As shown in Fig. 6, the cells on the collagen-coated mica surface grew well and had the shapes of typical 293 T fibroblast cells. However, the cells on the native mica surface were globular and did not grow normally. These cells could not adhere to the surfaces and were easily removed by replacing the cell culture medium. This different cell viability on the two

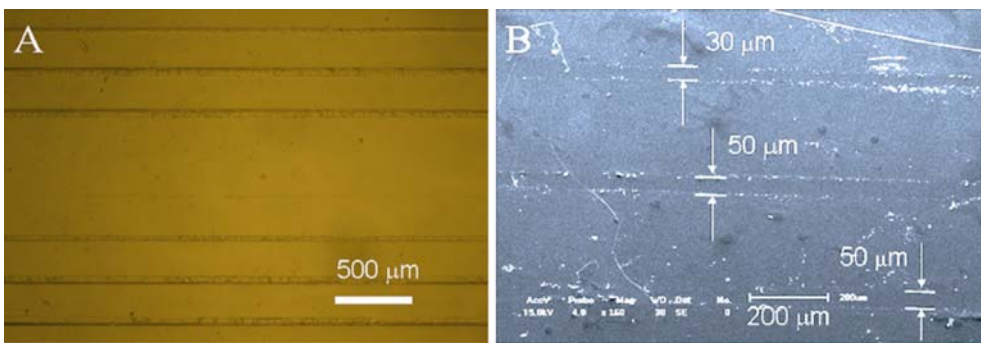


Fig. 5 (**a**) Optical microscopic image of the PDMS stamp surface. (**b**) SEM image of the collagen micro-patterns on the mica surface

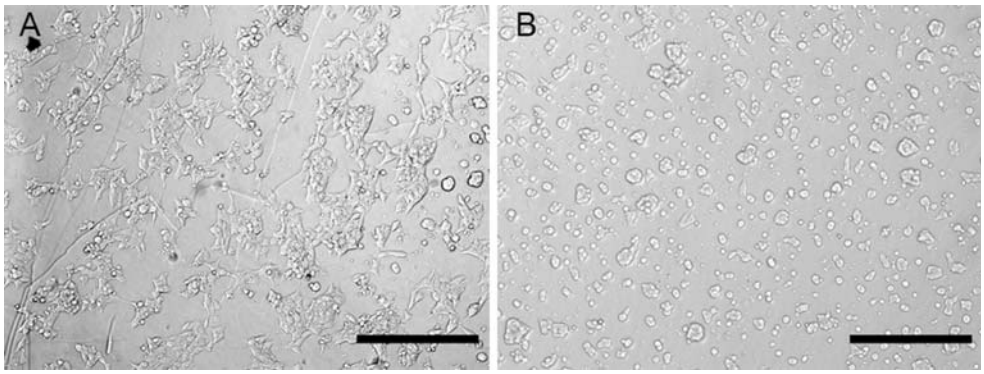


Fig. 6 Optical microscopic images of the cells on (**a**) a collagen-coated mica surface and (**b**) a mica surface after 4 h in serum-free DMEM. The length of the *scale bar* is 200 μm

surfaces established a basis of control of cell growth on the mica surface by use of collagen patterns.

Cell micro-patterns on the mica surface

Cell micro-patterns were achieved by directly culturing 293 T fibroblast cells on a mica surface with collagen patterns in serum-free DMEM for 4 h and then in DMEM containing 10% serum for another 48 h. Meanwhile, in order to examine the viability of the cell patterns, a gene-transfection experiment was performed at the same time with a typical non-viral gene-delivery vector PEI [21, 22]. After 48 h in DMEM containing serum, the 293 T fibroblast cells formed a pattern with the appearance of large panels separated by long parallel strips (Fig. 7). The dimensions of the cell patterns were approximately the same as those of the collagen patterns on the mica surface (Fig. 5), which indicated that our method could efficiently control the cell patterns on the mica surface. We found that the cell patterns could be stable for at least 4 days. Although this was not the maximum duration, these data show that the cell patterns can be stable for a period of time sufficient for most biological researches, for example gene transfection.

Control of cell adhesion by the collagen patterns was believed to play an important role in the formation of the cell patterns. The collagen areas could promote cell adhesion and the mica areas lacked such a function. Cells would preferentially adhere to the collagen areas when they were seeded on the substrate. This process established the basic feasibility of formation of controllable cell patterns according to the collagen patterns.

When the cells were cultured in DMEM with serum, they would proliferate and grow. With the number of cells becoming larger and larger, they might migrate to the nearby areas. If the cells migrated to the native mica surface, the cell patterns would inevitably be destroyed. Consequently, control of cell migration by collagen is also very important for formation of the cell patterns. In this study, the cells preferentially grew on the collagen areas and cell migration seemed to stop at the edge of the mica areas (Fig. 7). The reason for cells′ preferential growth on the collagen areas was considered to be related to the fact that the collagen could promote cell adhesion and growth better than the native mica surface. Obviously, this option would benefit the growth of the cells. However, the detailed mechanism for the "wisdom" of the cells is unclear.

Under the fluorescent microscope, a large number of the patterned cells expressed green fluorescence protein, i.e. these cells were green in appearance (Fig. 7b). Gene-transfection experiments can only be performed with cells of good viability. Dead cells, which lost their functions, could not express green fluorescent proteins in vitro. This

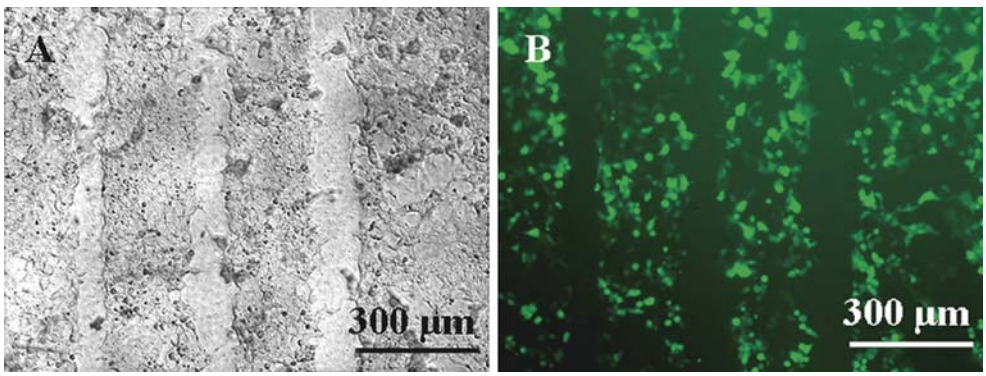


Fig. 7 (**a**) Optical microscopic image of the cell micro-patterns on the mica surface with collagen patterns. (**b**) Fluorescent microscopic image of the gene transfection result on the cell pattern observed under a fluorescence microscope

result indicated that the cell patterns were of good viability and should be of potential application in tissue engineering and biosensor fabrication.

In previous research, a common strategy was to reduce the serum in order to obtain good cell patterns [12]. The serum in the cell-culture medium contains many proteins and growth factors with functions similar to that of collagen. If these proteins and growth factors were adsorbed by the native mica surface, they could support cell growth, which would then destroy the cell patterns. In this study, we also removed serum from the DMEM when the cells were seeded on to the mica surface. It should also be noteworthy that the cells were cultured in serum-free DMEM for 4 h and then in DMEM containing 10% serum. The purpose of the serum supplement was to improve the cells′ viability [23]. Long-term cell culture without serum might be good for maintaining the micro-patterns of cells, but would be bad for maintaining their viability. It is crucial to choose a proper time period to culture the cells without serum balancing the two contradictory requirements. After a series of experiments, 4 h was selected as the serum-free time. From the experimental results we can see that supplementing with serum after 4 h did not obviously affect the cell patterns and the cells maintained their viability.

Compared with our previous work on cell patterning on a PDMS surface [19], cell patterning on the mica surface has many advantages. The mica surface is of atomic-scale flatness and proper rigidity. Cell patterns can easily be obtained by μCP on the mica surface, which requires a flat and rigid surface. However, the PDMS surface is soft. We cannot use μCP to fabricate cell patterns on a PDMS surface. Instead, we have to rely on a copper mask. Furthermore, the mica surface is hydrophilic, which will facilitate adsorption of proteins on it. The PDMS surface is hydrophobic and we have to make it wettable by treatment with hydrophobin before adsorption of proteins.

In this paper we have presented a method for fabrication of cell patterns on a mica surface. The cell patterns on the surface are very useful. For instance, the patterned cells can be used as biosensor systems to monitor the spreading speed of cells [11] or to detect the interactions between two types of cells [24]. It has also been well documented that the cell patterns have great potential in tissue engineering [25, 26]. Therefore, our method is expected to be of great applications in the fields of tissue engineering and biosensor fabrication.

Conclusion

In summary, we developed a method for fabrication of cell micro-patterns on a mica surface by using collagen as the material to guide cell adhesion. Electrostatic interaction between the positively charged collagen and the negatively charged mica surface made the micro-patterns of collagen very stable, which facilitated further cell culture. The cells were well confined on the mica surface that was defined by collagen. A gene-transfection experiment indicated that the patterned cells were of good viability and should be extensively used in the field of tissue engineering and biosensor fabrication.

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