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Patterning of cells on functionalized poly(dimethylsiloxane) surface prepared by hydrophobin and collagen modification

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ABSTRACT

Controllable cell growth on poly(dimethylsiloxzne) (PDMS) surface is important for its potential applications in biodevices. Herein, we developed a fully biocompatible approach for patterning of cells on the PDMS surface by hydrophobin (HFBI) and collagen modification. HFBI and collagen were immobilized on the PDMS surface one after another by using copper grids as a mask. HFBI self-assembly on PDMS surface converted the PDMS surface from hydrophobic to hydrophilic, which facilitated the following immobilization of collagen. Collagen had admirable ability to support cell adhesion and growth. Consequently, the HFBI/collagen-modified PDMS surface could promote cell adhesion and growth. What is more, the native PDMS surface did not support cell adhesion and growth. Patterning of cells was achieved by directly culturing 293T cells (the human embryonic kidney cell line) on the PDMS surface patterned with HFBI/collagen. Further studies by means of gene transfection experiment *in vitro* showed that the patterned cells were of good bioactivities. Herein, the biocompatible preparation of cell patterns on the PDMS surface could be of many applications in biosensor device fabrication.

1. Introduction

Controllable cell positioning on the surface is important in biosensor fabricating (Gross et al., 1995), tissue engineering (Langer and Vacanti, 1993), bioelectronics (Fromherz et al., 1991) and basic biology studies (Chen et al., 1997). For instance, the patterned cells can be used as biosensor systems to monitor the spreading speed of cells by using two fluorescent dyes (Chiu et al., 2000) and to detect the interactions between two types of cells (Li et al., 2007). PDMS is a cheap, transparent, flexible material that has been used extensively in medical implants and biomedical devices because of its biocompatibility (Park et al., 1999), low toxicity (Ertel et al., 1994) and high oxidative and thermal stability (Dahrouch et al., 2003). Controllable cell growth on PDMS surface will be attractive for its potential applications in cell biology study and biosensor device fabrication. However, the PDMS surface is hydrophobic, which makes the immobilization of biomaterials difficult. For instance, the protein and cell solutions may be distributed poorly on the surface by the coating method. Therefore, it is important to improve the hydrophilicity of the PDMS surface before applying it as the substrates for protein adsorption and cell adhesion. A number of methods have been reported to be efficient to improve the wettability of the PDMS surface, such as oxidation of the surface by ultraviolet (Genzer and Effimenko, 2000), plasma treatment (Xia and Whitesides, 1998), CO_2 pulsed laser (Khorasani et al., 1999), polymer grafting on PDMS (Okaniwa and Ohta, 1997), etc. Meanwhile, these reports also reveal that additional efforts have to be done to overcome certain disadvantages, such as the recovery of the hydrophobicity (Kim et al., 2006) and the physical damage to the surface (Diaz-Quijada and Wayner, 2004).

Recently, a biological method to improve the wettability of PDMS surface by using hydrophobins has been reported (Qin et al., 2007; Wang et al., 2007). Hydrophobins are surface-active proteins and first discovered in 1991 in fungi (Kong et al., 2002), which can be divided into two classes. Class I hydrophobins often form random patterns on the surface, which are mainly nanoscopic rodlets and bundles. Class II hydrophobins often form highly ordered structures on the surfaces, which are mainly monolayer films and crystalline fibrils. Both classes of hydrophobins are small molecules (7–15 kDa). Their amino acid sequences include very specific hydrophobic and hydrophilic regions. When they self-assemble on a hydrophobic surface, the hydrophobic regions of hydrophobins are attached to the hydrophobic surface and the hydrophilic regions of hydrophobins are exposed to the outside. This process converts the surface to a hydrophilic one (Jung et al., 2001). Although differences in the biophysical properties of

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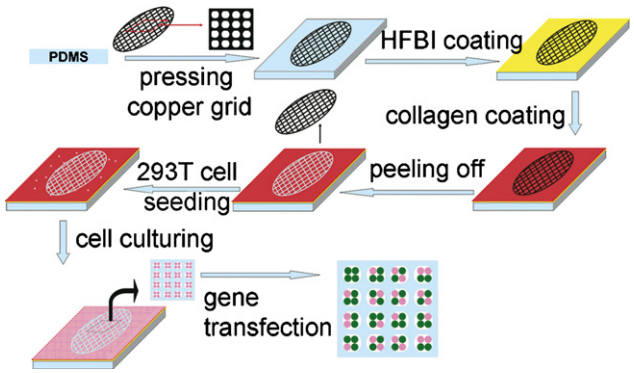

Scheme 1. Schematic depicting patterning of cells on HFBI and collagen-patterned PDMS surface.

hydrophobins have not yet been related to differences in primary structure, it has been established that the N-terminal part, at least partly, determines wettability of the hydrophilic side of the assemblage. Due to their amphiphilic nature and self-assembly property, hydrophobins have been widely applied. For instance, they can improve the biocompatibility of biomaterials (Janssen et al., 2004), purify target proteins (Collen et al., 2002), reduce the nanoscale relative surface of friction for biomedical application (Misra et al., 2006), etc.

As far as cell culture is concerned, collagen is often used as a material for supporting cell growth because of its good property to promote cell adhesion (Themistocleous et al., 2004). However, collagen cannot be well distributed on the PDMS by means of directly coating because of the hydrophobicity of the PDMS surface.

Herein, we developed a novel method to pattern cells on the PDMS surface via hydrophobins and collagen modification. HFBI, a hydrophobin II protein, was patterned onto the PDMS surface using the copper TEM grids as a mask, converting the PDMS surface to a hydrophilic one. The collagen was then coated on the HFBI areas on the PDMS surface. After the copper grids were peeled off, 293T cells were seeded on the substrate. Since the native PDMS surface repelled cell adhesion and growth while the modified PDMS surface supported cell growth very well, the cells only grew on the HFBI/collagen areas on the PDMS surface. Further gene transfection experiment conducted on the cell patterns demonstrated that the patterned cells are of admirable bioactivities (Scheme 1).

2. Materials and methods

2.1. General

PDMS elastomer kid (Sylgard 184 Dow Corning), rat-tail type I collagen and bovine serum albumin (BSA) were purchased from Sigma. Polyethylenimine (PEI MW 25000) was supplied by Prof. Yun-Qi Geng in Lifescience Department of Nankai University. Dulbecco's Modified Eagle Medium (DMEM) was purchased from Invitrogen Corp. Fetal bovine serum was purchased from Tianjin Shengda Corp. Collagen was solved in water to 1 mg/mL pH 7.5. Plasmid DNA pEGFP-C2 (4.7 kb) was donated by Prof. Xiao-Dong Zhang in Lifescience Department of Nankai University. The plasmid was purified from *Escherichia coli* DH5α and stored in TE buffer (10 mmol/L Tris–HCl, 1 mmol/L EDTA, pH 8.0). The concentration of plasmid DNA was measured by means of spectrophotometric analysis using a DU-7 Spectrophotometer (Beckman). Hydrophobin II HFBI was supplied by Prof. Min-Qiang Qiao in Lifescience Department of Nankai University and was diluted to 100 μg/mL with water.

2.2. Coating HFBI and collagen on PDMS substrate

Silicon elastomer and the curing agent (10:1, w/w) were mixed and poured onto the glass substrate. This substrate was incubated at 60 °C overnight and then the glass substrate was peeled off. The PDMS was rinsed with water for several times, ultrasonicated in water for 10 min, washed with 75% ethanol solution, dried with a nitrogen stream and finally cut into 1 cm^2 squares. HFBI coating on PDMS was achieved by incubating PDMS in the solution of HFBI for 20 min at 20 °C, followed by blowing off excess solution and dried with a nitrogen stream. PDMS surface directly coated with collagen was prepared in the same way. PDMS surface coated with HFBI/collagen was prepared in the same way.

2.3. XPS measurements

The modification of PDMS by HFBI and collagen was confirmed by XPS (PHI-5300, Phi, USA) employing a monochromatic Mg Kα radiation source ($h\nu$ = 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 and the sensitivity was 80–1600 KCPS. The peaks in the elemental core-level spectra were fitted using UNIX on an Apollo Domain series 3500.

2.4. Water contact angle (WCA) measurements

WCAs were measured by using a 2 μL water droplet on the surfaces under ambient conditions with an optical contact angle meter (Kruss, DSA100). The WCA values were averaged values from ten individual measurements at different locations.

2.5. HFBI and collagen patterns on the PDMS substrate

Copper TEM grids were pressed onto the PDMS surface. Then the HFBI solution is dripped onto the PDMS surface. The surface was incubated at 20 °C for 1 h and then blown off excess solution with a nitrogen stream. Collagen solution was dripped onto this substrate and dried in the same way. Finally, the copper TEM grids were carefully peeled off.

2.6. Cell culture

293T cells were routinely cultured in DMEM supplemented with 10% (v/v) heat inactivated fetal bovine serum. Before patterning, the cells were rinsed with phosphate buffer solution (PBS pH 7.4), treated with trypsin–EDTA (0.25%) for 5 min and incubated at 37 °C for 10 min to be detached from the well plates. The cell suspension was then supplemented with the same volume of DMEM with 10% (v/v) serum to stop the reaction of trypsin. After being centrifuged for 5 min at 1000 rpm, the cells were resuspended in 1 mL DMEM supplemented with 2% fetal bovine serum. The cell suspension solution was dripped onto the HFBI/collagen-patterned PDMS surface, which had been placed in the wells of a 24-well plate. The cells were then incubated in a 5% CO_2 incubator at 37 °C for cell culture. The result was observed by a fluorescence microscope TE 2000-U (Nikon Japan) equipped with Spot software.

2.7. Gene transfection using the cell patterns

293T cells were cultured on the HFBI/collagen-patterned PDMS surface according to the method above. PEI and plasmid DNA were mixed in 100 μL PBS solution. The mixture was incubated in room temperature for 30 min and then directly dripped into the wells of the plate. DNA in each well was 2 μg and the PEI in each well was 1.33 μL (1 mg/mL). The cells were cultured at 37 °C for 48 h. Gene

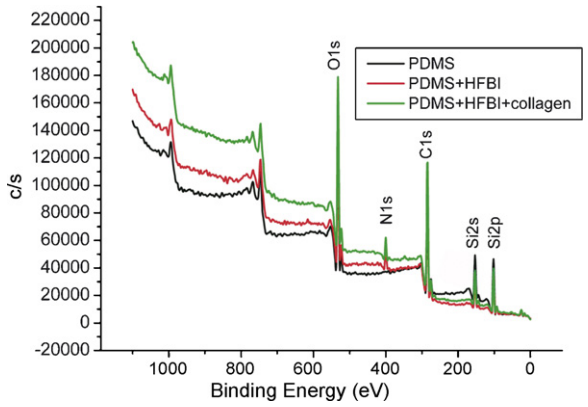

Fig. 1. XPS spectra of PDMS surface before and after modification.

Table 1
XPS determination of the relative atomic compositions on the PDMS surface, HFBI-modified PDMS surface and collagen-coated HFBI-modified PDMS surface

Spectra sample	C 1s (%)	N 1s (%)	O 1s (%)	Si 2p (%)
PDMS	50.09	0.00	27.61	22.30
PDMS + HFBI	51.87	5.80	28.68	13.48
PDMS + HFBI + collagen	56.30	4.47	24.76	13.86

transfection efficiency was observed by detecting the expression of green fluorescence proteins by using a TE 2000-U fluorescence microscope (Nikon Japan) equipped with Spot software.

3. Results and discussion

3.1. XPS measurements

Protein immobilization on the PDMS surface altered the surface chemical composition of PDMS. Consequently, the immobilization of HFBI and collagen on the PDMS surface was investigated by XPS measurement. The native PDMS surface exhibits its typical XPS spectra, which is characterized by a high intensity of O 1s and C 1s signals and a low intensity of Si 2s and Si 2p signals (Fig. 1). No N 1s signal is detected in the PDMS spectra. After HFBI and collagen modification, a high intensity of N 1s signal at 400 eV and a decrease of Si 2p signal appeared.

Table 1 quantitatively showed the relative atomic composition of PDMS surface before and after modification. The HFBI modification changed the chemical composition of PDMS surface significantly. The intensity of N 1s signal increased from 0% to 5.8% and the intensity of Si 2p signal decreased from 22.3% to 13.48%. N belongs to the amino group in HFBI and collagen. Si belongs to PDMS. The appearance of N signal and the decrease of Si signal indicated that HFBI had covered on the PDMS surface to a great extent. Collagen modification changed the chemical composition of the PDMS surface once more. However, the signal intensity did not change significantly after collagen modification. This can be ascribed to the similar chemical composition of HFBI and collagen. Because both of HFBI and collagen are composed of amino groups, it is reasonable to deduce that the chemical composition of HFBI is similar to that of collagen. Therefore, collagen coating did not change the surface composition very much.

3.2. WCA measurements

The changes of the wettability caused by the HFBI/collagen modification on the PDMS surface were investigated by WCA measurement (Fig. 2). The WCA of the native PDMS surface was 117°; while after HFBI modification, the WCA of the PDMS surface decreased to 56.3°. This result indicated that the PDMS surface was converted from hydrophobic to hydrophilic. The secondary structure of HFBI includes hydrophobic β sheets and hydrophilic α helixes. When HFBI was coated on the PDMS surface, the hydrophobic β sheet of HFBI was supposed to be attached to the hydrophobic PDMS surface and the hydrophilic α helix of HFBI was supposed to be exposed to the outside (Wang et al., 2007). This highly ordered self-assembly process grafted hydrophilic chemical groups onto the outside of hydrophobic PDMS surface and converts the PDMS surface from hydrophobic to hydrophilic. After collagen modification, the WCA of the PDMS surface was 92°. The change of WCA was probably due to that the WCA of the collagen layer was different from the WCA of the HFBI layer. This result indicated that collagen could be efficiently immobilized on the PDMS surface via HFBI.

3.3. Cell culture on multiple surfaces

Most techniques concerning cell patterning typically require the functionalization of surfaces with different types of molecules that either promote or inhibit cell adhesion and growth. In the experiment, the PDMS surface was designed to repel cell growth and the HFBI/collagen-modified PDMS surface was designed to promote cell growth. Therefore, the cell living status on PDMS surface and on HFBI/collagen-modified PDMS surface is the key factor that determines whether these cells can grow on the PDMS surface according to the HFBI/collagen patterns.

293T cells were cultured on the native PDMS surface and the HFBI/collagen-modified PDMS surface, respectively. After 16 h cell culture, the cell living status on both surfaces was observed under a microscope (Fig. 3A and B). The cells grew poorly on the native PDMS surface. The number of the cells on the native PDMS surface was much smaller than that on the HFBI/collagen-coated PDMS surface. In addition, these cells did not fully adhere to the PDMS surface. Some of them just adhered to other cells without contacting the substrate surface. The result indicated that the native PDMS surface was not a suitable substrate for cell adhesion and growth. Therefore, it could be used as the substrate to repel cell growth. On the HFBI/collagen-modified PDMS surface, the cells grew very well with a cell confluence level of about 100%. These cells were closely attached to the substrate. They had the shape of normally

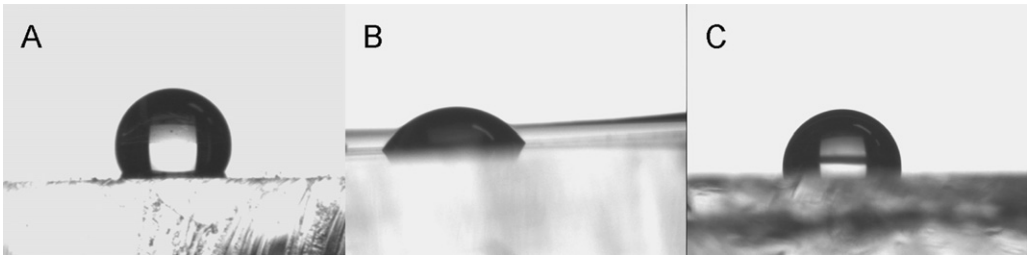

Fig. 2. WCA on (A) a PDMS surface, (B) a HFBI-modified PDMS surface and (C) a HFBI/collagen-modified PDMS surface.

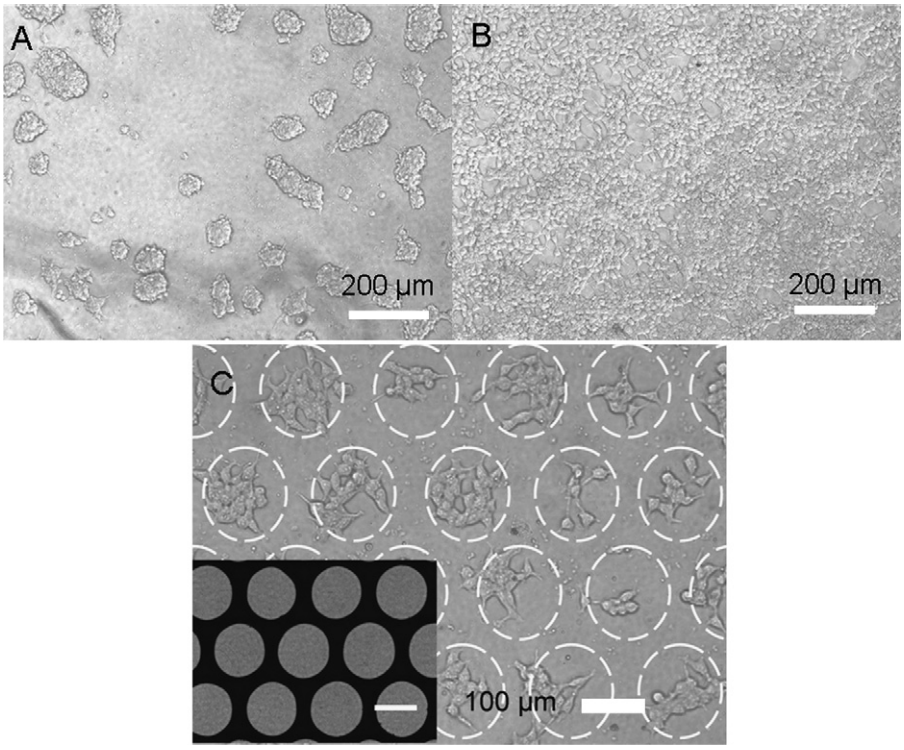

Fig. 3. Cells living status on (A) a native PDMS surface and (B) a HFBI/collagen-modified PDMS surface. The cells have been cultured in DMEM supplemented with 2% fetal bovine serum for 16 h. (C) Templated cell growth on HFBI/collagen-patterned PDMS surface. The inner image was copper TEM grids.

cultured cells. Therefore, the HFBI/collagen-modified PDMS is suitable for cell growth and can be used as the surface for promoting cell growth.

Routinely, 293T cells were cultured in DMEM that was supplemented with 10% fetal bovine serum. However, the serum contained some proteins that have similar functions as collagen. Once these proteins were fixed on the native PDMS surface, the cells would grow on these places. Herein, the cells were cultured in DMEM supplemented with 2% serum in this work. In such a medium, the cells could not grow in the normal environment. As a result, they grew poorly on the native PDMS surface. Meanwhile, the collagen on HFBI/collagen-modified PDMS surface could promote cell adhesion. Consequently, the cells grew very well on the HFBI/collagen-modified PDMS surface.

3.4. Templated cell growth on the HFBI/collagen-patterned PDMS surface

The cell living status on the native PDMS surface was different from that on the HFBI/collagen-modified PDMS surface. This difference established the possibility to achieve templated cell growth on the PDMS surface by controlling the HFBI/collagen patterns: the native PDMS surface was used to inhibit cell growth and the HFBI/collagen-modified PDMS surface was used to support cell growth.

The HFBI/collagen micropatterns on the PDMS surface were fabricated by using the copper TEM grids. Briefly, the copper grids were pressed on the PDMS surface. The HFBI solution was coated on the substrate. Then the collagen solution was coated on the substrate in the same way. Finally, the copper grids were peeled off and the HFBI/collagen micropatterns were achieved.

The copper grids have parallel vacant ellipses (Fig. 3C inner image). When pressed on the PDMS surface, the copper grids shielded parts of the PDMS surface and only left the ellipses parts exposed to the outside. Therefore, HFBI and collagen could only be immobilized on the ellipse parts. In this process, the HFBI modification increased the hydrophilicity of the PDMS surface. Because the collagen was solved in water solution, the HFBI modification facilitated the following collagen coating.

Cell patterns are fabricated by directly culturing 293T cells on the HFBI/collagen-patterned PDMS surface (Fig. 3C). Almost all the cells preferentially grew on the HFBI/collagen areas and their patterns resembled the pattern of copper grids. However, nearly no cell grew on the native PDMS surface. The cell number was different in each ellipse on the PDMS surface. In some ellipses, the cell almost covered the entire surface; while in other ellipses, there were only a few cells. The number of cells in an ellipse part was randomly determined when they were deposited from the cell suspension. There would be more cells in an ellipse after cell culture, if there were more cells distributed in the ellipse previously. The cell culture time was another factor that determined the cell confluence level in an ellipse. There would be more cells in the same ellipse when the cells were cultured for a longer time.

Further cell culture showed that the cell patterns on the HFBI/collagen-patterned PDMS surface were of good stability. They were confined in the HFBI/collagen surface even if when the cells have already been very crowded (Fig. S1). When there was not enough living space for cell growth, the cells underwent the cell apoptosis process (Fig. S2). The stability of cell patterns is influenced by many factors such as the cell type, the serum, the type of non-adhesive and the design of patterned substrate. Even apparently unimportant factors can significantly influence the stability of the cell patterns, such as the frequency at which the cell culture medium is changed (Mrksich et al., 1997). In this work, the cell patterns can be well confined for at least 4 days. Although this time is not the longest one that has ever been obtained to confine cell patterns (25 days for 3T3 fibroblasts using mannitol-terminated SAMs on Au (Luk et al., 2000); 60 days

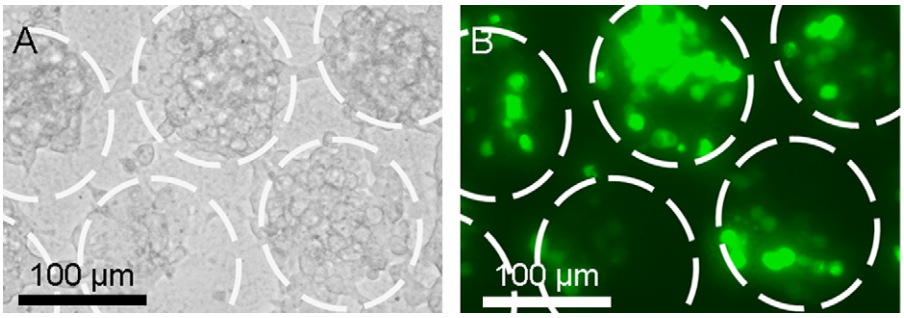

Fig. 4. Gene trasfection on the cell patterns. (A) Phase contrast image of templated cells after gene transfection. (B) Correspondent fluorescence microscope image of (A).

for primary bone cells on interpenetrating polymer networks as non-adhesive surface (Thomas et al., 1999)), it is still comparable to the time in some previous researches (Rozkiewicz et al., 2006). Consequently, the cell patterns are of enough stability for most experiments.

3.5. Gene transfection on the cell patterns

The bioactivity of the resulting cell patterns was investigated by gene transfection experiment with PEI. PEI has very high gene transfection efficiency with 293T cells. The cells of good bioactivity can express green fluorescence proteins after successful gene transfection. Whereas, the cells that lose their bioactivity cannot express these proteins. Consequently, we can use the number of gene-transfected cells to determine the bioactivity of the cell patterns. Fig. 4 shows the fluorescence microscopic image of the cells patterns after gene transfection and the corresponding phase contrast images. A number of cells were green under the fluorescence microscope, which indicated that the patterned cells had quite high gene transfection efficiency. The result also revealed that the cell patterns were of admirable bioactivity and should be useful in cell biology study and biosensor device fabrication. The technique presented herein will be useful for a number of biosensor systems, such as in the studies of cell migration or invasion by using fluorescent dyes, in detecting neuronal development, in the control of tumor growth, etc.

4. Conclusions

We have demonstrated a bioactive approach to patterning cells on the PDMS surface that combines hydrophobin HFBI for surface wettability conversion and collagen for supporting cell growth. HFBI modification converted the hydrophobic PDMS surface to a hydrophilic one and thus promoted the following collagen immobilization on the PDMS surface. Collagen had admirable ability to support cell adhesion and growth. Consequently, the HFBI/collagen-modified PDMS surface could support cell adhesion and growth; while, the native PDMS surface could not. Cell patterns were achieved by directly culturing cells on the HFBI/collagen-patterned PDMS surface. The resulting cell patterns showed high bioactivities in gene transfection experiment. This bioactive method for cell patterning should be of extensive applications in cell biology study and biodevice fabrication fields.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2008.07.045.

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