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Fabrication of Patterned Thermoresponsive Microgel Strips on Cell-adherent Background and Their Application for Cell Sheet Recovery

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Abstract

Interfaces between materials and cells play a critical role in cell biomedical applications. Here, a simple, robust and cost-effective method is developed to fabricate patterned thermoresponsive poly(N-isopropylacrylamide-co-styrene) (pNIPAAmSt) microgel strips on a PEI-precoated, non-thermoresponsive cell-adherent glass coverslip. The aim is to investigate whether cell sheets could be harvested from these cell-adherent surfaces patterned with thermoresponsive strips comprised of the microgels. We hypothesize that if the cell-to-cell interaction is strong enough to retain the whole cell sheet from disintegration, the cell segments growing on the thermoresponsive strips may drag the cell segments growing on the cell-adherent gaps to detach, ending up with a whole freestanding and transferable cell sheet. Critical value concerning the width of the thermoresponsive strip and its ratio to the non-thermoresponsive gap may exist for cell sheet recovery from this type of surface pattern. To obtain this critical value, a series of strip patterns with various widths of thermoresponsive strip and non-thermoresponsive gap were prepared using negative microcontact printing technology, with COS7 fibroblast cells being used to test the growth and detachment. The results unraveled that COS7 cells preferentially attached and proliferated on the cell-adherent, non-thermoresponsive gaps to form patterned cell layers and that they subsequently proliferated to cover the microgel strips to form a confluent cell layer. Intact COS7 cell sheets could be recovered when the width of the thermoresponsive strip is no smaller than that of the non-thermoresponsive gap. Other cells such as HeLa, NIH3T3, 293E and L929 could grow similarly, that is, they showed initial preference to the non-thermoresponsive gaps and then migrated to cover the entire patterned surface. However, it was difficult to detach them as cell sheets due to the weak interactions within the cell layers formed. In contrast, when COS7 and HeLa cells were cultured successively, they formed the co-cultured cell layer that could be detached together. These freestanding patterned cell sheets could lead to the development of more elaborate tumor models for drug targeting and interrogation.

Keywords

Biointerface, Functional interface, Micropatterning, Thermoresponsive microgels, Cell sheet harvesting, microgel strips, Thermoresponsive cell removal.
1. Introduction

Interfaces dictate cell performance and play a critical role in many cell biomedical applications by controlling processes such as cell attachment, migration, orientation, critical adhesion and differentiation through delicate surface engineering.\textsuperscript{1-5} Among various engineered interfaces, the patterned surfaces in which adhesive islands located on a non-adhesive background have attracted much interest in recent years. Ingber et al pioneered cell spreading confinement on micropatterned surfaces and demonstrated the geometrical control of cell fates.\textsuperscript{6} They showed that adherent cells could be switched from growth to apoptosis by using micropatterned substrates containing extracellular matrix protein coated islands of decreasing size to restrict cell extension. They also demonstrated that cell spreading could be varied whilst keeping the total cell-matrix contact area constant by changing the gap distance between multiple focal adhesion-sized islands. By topographical modifications to the substrate surfaces, the shapes individual cells adopt could be manipulated, thereby resulting in fatal consequences of growth or death. Patterns on polymeric surfaces can be fabricated by soft lithography, photolithography and electron beam lithography. With well-defined surface patterns, the choice of polymers and surface film coating approaches have also enabled elaborate studies of the effects of surface chemical nature and morphological structures on the interactions between cells and the substrate during dynamic processes of cell adhesion, spreading and growth.\textsuperscript{7-9} Intact cell sheets could non-invasively detach from thermoresponsive platforms and maintain their cell-to-cell connections. These cell sheets could be used as 2D or 3D biomedical constructs or for tissue damage repair.\textsuperscript{10-14} Patterned thermosensitive coatings have recently been developed for cell detachment control, with several recent studies reporting their attractive benefits for cell sheet harvesting.\textsuperscript{15-18} Okano group prepared a micropatterned thermoresponsive surface which consisted of strip-like micropatterns of
poly(N-isopropylacrylamide) (pNIPAAm) brush domains and poly(N-isopropylacrylamide)-b-poly(N-acryloylmorpholine) domains using RAFT-mediated block copolymerization and photolithography. They showed that aligned cell clusters could be harvested as a tissue-like cellular monolayer from these patterned surfaces.\textsuperscript{15} The same group also prepared polyacrylamide (pAAm) patterns on a thermoresponsive pNIPAAm surface fabricated on a cell-repellent background, and the detachment of the adhered cells could be more rapidly achieved from the patterned surface after reducing temperature.\textsuperscript{18}

Microcontact printing (µCP) has been demonstrated as a technique for the delivery of micro-nanometer materials such as proteins,\textsuperscript{19} DNA,\textsuperscript{20} bacteria,\textsuperscript{21} colloids and others,\textsuperscript{22, 23} as surface patterns onto a target substrate. A stamp (which is usually made of poly(dimethylsiloxane) (PDMS)) inked with the materials of interest is used to print onto the substrate surface, with the desired pattern formed. Compared with other microfabrication techniques such as photolithography and soft lithography, both of which are more elaborate to operate, it is easy to use µCP to fabricate micropatterned surfaces onto various backgrounds.

In this work, we aim to examine how the critical width of the thermoresponsive strip micropatterned on a cell-adherent non-thermoresponsive background affects the cell sheet to detach from the entire surface using the strategy of µCP. To achieve this aim, a series of micropatterned surfaces with various widths of thermoresponsive strip and cell-adherent non-thermoresponsive background gap were prepared through µCP, and COS7 cells were cultured on those patterned surfaces. Once confluent, their detachment behavior was examined by reducing temperature. Results showed that COS7 cells preferentially adhered within the cell-adherent gaps to form cell patterns. After these gaps were occupied, cells would migrate onto the microgel strips to form a confluent cell layer, but the cell sheet could then be harvested by thermally stimulating the expansion of the underlying thermoresponsive microgels without disrupting their patterns. Whether an intact cell sheet could be recovered or not depended not only on the design of the patterns, but also on cell types. COS7 cell sheets could be recovered when the width of the thermoresponsive strip is
equal to or larger than that of the cell-adherent gap, but NIH3T3, HeLa and L929 cell sheets could not be recovered under the same conditions because these cells could not hold to each other tightly enough. However, when HeLa and COS7 cells were seeded on a patterned surface successively, a co-cultured cell sheet could be recovered due to the integrity of the COS7 cells in the patterned layer. This approach may provide a useful platform for harvesting patterned cell sheets for building elaborate tissue constructs, e.g., as in vitro tumor models.

2. Experimental and materials

2.1. Materials

All the chemicals were obtained from Sigma-Aldrich. N-isopropyl acrylamide (NIPAAm) was purified by recrystallization from a toluene/hexane mixture (1:3) and dried in vacuum. Styrene (St) was purified by distillation under reduced pressure, ammonium persulfate (APS) was purified by recrystallization from water. N, N’-methylene bisacrylamide (MBA) was used as received. The molecular weight of PEI is 750 kDa. All water used in this experiment was processed by Milli-Q system (Milli-Q Advantage A10 Water System Production Unit). Cell culture plates (6-well plates, from Corning) were used as received. Glass coverslips (20 × 20 mm²) were immersed into piranha solution (H₂O₂/H₂SO₄ = 1:3 by volume) at 90 °C for 1 h to remove organic impurities, followed by abundantly rinsing with tap water and UHQ water, and dried by nitrogen purging. They were then immersed in 1 wt % PEI solution (pH=5) before use.

PDMS stamps were commissioned from CapitalBio Corporation (China) and the smooth surface bear ridges with depth about 10 µm, and the widths of the ridges and gaps between them are 50, 100, 200 and 500 µm, respectively.
2.2. pNIPAAmSt microgel preparation

PNIPAAmSt microgels were prepared by surfactant-free precipitation polymerization as described in our previous work. After 1.50 g of NIPAAm, 0.04 g of MBA, 0.50 g of styrene and 0.05 g of fluorescein-5-isothiocyanate (FITC-Na) were added in 190 mL of water, the reaction mixture was transferred to a four-necked round-bottom flask equipped with a condenser and a nitrogen inlet, and then heated to 70 °C under a gentle stream of nitrogen. After 1 h, 0.12 g of an initiator (APS) was dissolved in 10 mL of water (oxygen free) and added to the flask to initiate polymerization. The reaction was continued for 4 h while keeping the reaction in a nitrogen environment by continuous N$_2$ purging. Following the synthesis, the microgels were purified by three centrifugation cycles at 10,000 rpm for 60 min, with removal of the supernatant and redispersion between each cycle.

**Microgel characterization.** The thermoresponse behavior of the resulting pNIPAAmSt microgels was characterized by dynamic light scattering (DLS, Zetasizer Nano instrument from Malvern Instruments Ltd, with the detector positioned at the scattering angle of 173°) in the temperature range of 20-50 °C. The microgel dispersion was heated steadily and the microgel size determined every 2 °C by letting the microgel dispersion equilibrate at each temperature for 10 min. The $\zeta$-potential of the pNIPAAmSt microgels was measured as an indicator of microgel charges using the same Malvern Zetasizer instrument. The sample was prepared in the same way as prepared for size analysis and each mobility value was the average of 100 runs. Transmission Electron Microscopy (TEM, JEM-2100UHR, JEOL) was also used to characterize the dimensions of the microgels. Scanning Electron Microscopy (SEM, S-4800, Hitachi) was used to help examine the microgel patterned surface, with sample surfaces coated with a thin Au layer to increase the contrast and quality of the images.
2.3. Patterned pNIPAAmSt microgel strips on cell-adherent surface via negative μCP

**Direct utilization of PDMS stamps.** The coverslips immersed in PEI solution were rinsed with UHQ water to remove any excess PEI and then dried with nitrogen. The thickness of the PEI polymer layer was 13±3 nm, determined by a variable angle spectroscopic ellipsometry (Jobin-Yvon UVISEL). The microgel monolayer was obtained by spin-coating (1000 rpm) 1.0 wt % microgel dispersion onto the PEI-precoated glass coverslips. The fabricated PDMS stamp was first treated with plasma to make the surface hydrophilic, and then put on a cup of hot water to make a thin layer of water to condense on the stamp surface. When the water layer began to evaporate from the edge of the surface, the PDMS stamp was immediately used to contact the microgel film for a few seconds, then the stamp was carefully peeled away, with the patterned strips formed on the coverslip surface.

**Indirect utilization of PDMS stamps.** The boiling agarose solution (3.5 wt %) was sonicated to release gas bubbles, and then poured carefully onto the surface of a PDMS stamp. After the cooled agarose hydrogel was peeled away from the PDMS stamp, the agarose stamp with the complementary pattern of the PDMS stamp was formed. It was then dried with N₂ to remove any excess moisture on the surface, and then used to make the pNIPAAmSt pattern, with the procedure being just kept the same as that of the PDMS stamp. When the agarose stamp was peeled away, the pNIPAAmSt pattern complementary to that of the PDMS stamp was formed on the coverslip.

The patterns were then immersed in deionized water for 48 h to release superfluous fluorescent dye, and then the patterns were heated to 120 °C for 2 h for sterilization. The morphologies of the patterned surfaces were monitored by inverted fluorescence microscope (Leica, DMI3000, Germany) and scanning electron microscopy (SEM, S-4800, Hitachi). Patterns were named as a-b, with “a” denoting the gap width between the thermoresponsive strips (µm) and “b” denoting the width of the microgel strips (µm).
2.4. Cell culture

After the glass coverslips with the pNIPAAmSt microgel patterning were annealed at 120 °C for 2 h, they were transferred into six-well tissue culture plates under sterile conditions for subsequent use. Cells (2 × 10^4/mL) were seeded uniformly on the coverslips with patterned pNIPAAmSt microgels and cultivated in DMEM medium containing 10 % FBS at 37 °C and under 5 % CO₂. Unless specified, cell lines used in this work were cultured under the same processes and conditions. All the experiments were undertaken in triplicates and fresh and warm culture medium (preheated to 37 °C to avoid any possible cell detachment due to temperature drop) was used to replace the old medium every other day. After confluent cell layer formed (3~4 days), cold fresh DMEM was added and the cell detachment process was then observed at ambient temperature (about 20 °C) in real time.

3. Results and discussion

3.1 Characterization of pNIPAAmSt microgels

The pNIPAAmSt microgels produced have a rather compact structural feature with dry size about 200 nm from TEM imaging (Supporting Information, Figure S1a). The temperature-dependent hydrodiameter of the pNIPAAmSt microgels was analyzed using dynamic light scattering (DLS) (Figure S1b). The polydispersity of the particles was consistently around 0.022 at 20 °C, indicating the narrow particle size distribution achieved using the precipitation polymerization approach. The particles displayed a temperature-dependent size, with an average diameter of ~600 nm at 20 °C decreasing to ~270 nm at 38 °C. The volume phase transition temperature (VPTT) of the pNIPAAmSt microgels was 28 °C, which is lower than that of the pNIPAAm microgels (31~32 °C) as a result of copolymerization with the hydrophobic monomer styrene. With APS used as initiator, the microgels bear negative charges ~ -13.3 mV (Figure S1b).
3.2. Preparation of patterns

Microcontact printing (μCP) is a typical means of soft lithography and has been successfully applied to patterning various materials ranging from organic molecules, polymers, proteins to nanoparticles and colloids on different substrates. Although μCP has been used to fabricate colloidal microsphere patterns, the physical pressure and temperature between PDMS stamps and substrates are always introduced in the process of μCP. In this work, the thermoresponsive microgel patterns were prepared by the negative μCP method based on our previous work. A monolayer of thermoresponsive microgels was first spin coated on the PEI-precoated glass coverslip. Before use, the PDMS stamp was treated with plasma to make the surface hydrophilic. It was then put over a cup of hot water to condense a thin layer of water on the surface. When the surface was saturated with moisture, the stamp was immediately put in direct contact with the microgel film under the fingertip. After a few seconds, the stamp was carefully peeled away so that the microgels in direct contact with the stamp surface could be lifted off with it. Uncontacted microgels were left to form patterns on the substrate (Figure 1, up line). Note that some boundary areas and patterned objects might not have the exact widths, showing the limitation of this approach. It is however easy to manipulate and reproduce. On the other hand, the complementary pattern could also be prepared using the corresponding agarose stamp (Figure 1, low line). These patterns could be formed in areas about 1 cm², which are large enough for us to study cell growth and detachment behavior.

SEM images (Figure S2) clearly show that the patterns were composed of microgels. The microgels are not very close-packed, but the gaps between the microgels are similar to what was observed in our previous work and thus have no obvious effect on cell growth and detachment. Therefore, for all patterns formed, the gaps between the microgels would not be considered. It should be noted that if the microgel patterns were formed on the bare glass coverslip (without PEI), patterns would be lost in PBS for no longer than 3 days. Thus it is unsuitable for observing cell behavior. With being PEI precoated on the glass coverslip, the
electrostatic interaction and chain entanglement between microgels and PEI helped anchor the microgels and stabilized the patterns. These patterns could be stable in PBS for at least 7 days (the longest period assessed), though their fluorescent intensity faded away to some extent (Figure S3). This confirms that the pNIPAAmSt microgels deposited on the PEI-precoated coverslip were robust and would not drop off in cell culture media.

### 3.3. Cell culture on patterned substrates

Figure 2 shows an exemplar set of observations of cell attachment and proliferation during the first 3 days after seeding. COS7 cells were first seeded on the 100-100 (denoting gap and microgel strip widths) pNIPAAmSt microgel strips at a density of $2.0 \times 10^4$ cells/mL. The microscopic images of COS7 cells cultured on the microgel patterned coverslip were recorded at different time points in cell culture medium. After 3 h seeding of the dispersed COS7 cells, cells settled and attached randomly on the whole surface. After 12 h, more cells attached in non-thermoreponsive gaps than on microgel strips. When cultured for 24 h, cells preferentially proliferated within the gaps and cell patterns formed. Then, the COS7 cells proliferated further, forming more clearly patterned cell layers. With culturing time increasing, cells migrated from gaps onto the microgel strips and eventually formed a confluent cell layer on the whole surface (Figure 2).

Figure 3 further shows that COS7 cells could form patterned cell layers after 2 days on surface patterns of 50-100, 100-50 and 200-100, with the first number denoting the gap width in µm and the second number denoting strip width also in µm. In our previous work, we showed that NIH3T3 fibroblast cells could be cultured onto the same microgel monolayer to obtain harvestable cell sheets.\(^{22}\) However, in this work, we found that COS7 cells preferentially adhered and proliferated on the cell-adherent non-thermoreponsive gaps first to generate patterned cell layers before they start to move onto the thermoresponsive microgel
strips to form the complete cell monolayer. Thus, cells prefer to adhere and grow on cell-adherent gaps if choices are available. This phenomenon was also found to be true for other cell types such as NIH3T3, 293E, HeLa and L929 cells, as they all preferred to adhere on the PEI-precoated gaps to form patterned cell layers during the early stage of culturing, just as shown in Figure S4.

It is now widely known that cell adhesion relies on the properties of the surface such as hydrophobicity, chemical composition and topography. To investigate whether difference in hydrophobicity between the spacing gap and the strip is responsible for the observed preference in cell adhesion, the strip and gap were further characterized with water contact angle measurements. Since the dimensions of patterned pNIPAAmSt microgel strips and gaps are in micrometer range, then contact angles of the spin coated pNIPAAmSt microgel monolayer and PEI-precoated coverslip were measured to represent those of the patterned pNIPAAmSt microgel strips and gaps. Relative to the PEI-precoated glass coverslip (contact angle lower than 20°), pNIPAAmSt microgels are less hydrophilic both above and below VPTT (32.3±2.1° at 20 °C; 68.7±6.2° at 38 °C). This means that cells prefer to attach and grow on the hydrophilic PEI surface at the early stage.

Tsai et al used pNIPAAm microgels to form patterns on PS substrate via dip coating. In their work, dense pNIPAAm microgel layers are more hydrophilic than sparsely pNIPAAm microgel coated PS substrates, but they found that NIH3T3 cells would preferably adhere onto the latter. These authors consider that the surface hydrophobicity varied via the pNIPAAm density is likely to be responsible for generating cell micropatterns on dip-coated pNIPAAm microgel substrates, where preferential cell adhesion is observed only on hydrophobic areas immediately after seeding. Yet in this work, COS7 cells preferentially adhered to and spread on hydrophilic PEI coated gaps on glass coverslip. Ishizaki et al. have shown that cells could immediately adhere to the superhydrophilic surfaces in a selective manner after seeding, whereas cells needed 24 - 72 h after seeding to adhere to the superhydrophobic surfaces. The difference in cell
attachment was attributed to the difference in protein adsorption between the two surfaces, for more time was needed for the cells to produce their own extracellular matrix (ECM) molecules and form a protein layer suitable for cell attachment on the superhydrophobic surface.

The sensitivity of cells to the difference between microgels and PEI-precoated coverslip is so high that cells would even stretch to get into a narrow scratch on the microgel strips (as the red arrow indicated in Figure 3).

Taken together, surface hydrophobicity may well be an important factor, but other interactions such as chemical nature and surface topology must also play roles in the dynamic process of cell adhesion. Whilst cells clearly prefer to adhere to the hydrophilic surface, increase in surface density of the microgels appears to deter them. In addition, the more hydrophobic microgel surface is not preferred due to the time lapse needed to produce ECM layer to mediate cell attachment.

Figure 3 also showed clearly that COS7 cells adopted an orientation on the 50-100 patterned surface (the white arrow) where it can be seen that the cells elongated along the direction of the microgel patterns and formed alignment. To quantify the orientation of the spread COS7 cells to the direction of the pattern, the angle between the cell spread direction and the pattern direction was analyzed. The orientation angle was measured as illustrated in Figure 4a. It can range from 0° to 90°, where 0° means that the long axis of the cell is parallel to the direction of the pattern and that 90° represents the orientation that is perpendicular to the pattern direction. Figure 4b shows that in the range of about 10°, the cells in the gaps of 50 and 100 µm have almost the same alignment orientation angles (69 % and 63 %, respectively). They are higher than that of only 36 %, in the gap of 200 µm. These values mean that cells would adopt orientations largely alongside the directions of patterns if the gap widths are not larger than 100 µm.

### 3.4. Cell sheet harvesting by reducing temperature

After COS7 cells formed a confluent layer on the pNIPAAmSt microgel patterned surface, the detachment
of the cell sheet was examined against temperature reduction. The cell layer on the patterned surface was first separated from the cells attached on the non-patterned region using a blade. Then, the culture plate was placed under room temperature (about 20 °C) for observation without disturbance. Results showed that cell layers could detach from the patterns of 50-100, 50-50, 100-100 and 50-200. However, cell layers could scarcely detach from the patterns of 100-50, 200-50, 200-100 and 500-500 (Figure 5). Live/dead staining assay using calcein-AM and propidium iodide (PI) showed that above 95% COS7 cells remained alive in the peeled cell sheet (Figure S5a). To further examine the viability, the harvested cell sheet was transferred to a standard TCPS well and cultured. After 24 h, cells could be observed to spread out from the cell sheet (Figure S5b).

Thus, for cells to detach, the width of the thermoresponsive microgel strip must be the same or greater than that of the non-thermoresponsive cell-adherent gap. Provided the cell-cell interaction between COS7 cells is strong enough, the adhesive force between the cell layers and the non-thermoresponsive gaps could be conquered by the detachment force from the thermoresponsive strips. This works well for patterns with strips and gaps below 200. For the pattern of 500-500, the cell layer could not detach spontaneously. It was noted that longer incubation time was required for COS7 cells to get confluent on such a patterned surface. With the widths becoming large, the patterning effects arising from the two opposite surfaces would work differently. To further investigate whether these cell layers could detach from pattern of 200-100 by external force or not, a 100 μL pipette tip was used to pipette the culture medium and inject gently against the surface confined cell sheet. Results showed that only the cell layer regions on microgel strips could be detached, whilst the cell layer regions growing in the PEI gaps still remained (Figure S6).

The same set of surface patterns with strips and gaps under 200 μm was also used to culture HeLa, NIH3T3 and L929 cells. As described previously, these cells would prefer to proliferate on the gaps, then on the whole surfaces to become confluent, but they could not detach as intact cell sheets by temperature stimuli
from either thermoresponsive or non-thermoresponsive surface regions spontaneously, though individual cells on the thermoresponsive strips became contracted (Figure 6).

The difference may suggest that COS7 cells have the strongest cell-cell interaction amongst all cell types studied and that this might play an important role in cell sheet recovery process. Cell-cell interactions are clearly important in keeping the integrity of the cell sheet during detachment. In the case of detachment of the co-cultured cell sheet, the strong interacting COS7 cell layer must provide a scaffolding to support the weakly interacting HeLa cell layer so that they can detach simultaneously. To test this assumption, HeLa cells (stained red with DiD) were first seeded on the pattern 100-100. Once HeLa cells formed patterned cell layers in the gaps, COS7 cells (stained green with DiO) were seeded as the second type of cells. When confluent cell layers were formed on the whole surface, warm culture medium was replaced by cold culture medium to stimulate detachment. Results showed that the entire cell sheet containing both COS7 and HeLa cell sheet could detach from the surface as a whole entity (Figure 7). Thus, by harnessing the strong cell-cell interactions, patterned cell sheets may be recovered using this platform through manipulating the repulsive and attractive interactions against the substrates via the width of the patterned strips. Patterned cell sheets can be used as building units for constructing cell spheroids as tumor tissue models for testing the efficacy of anti-cancer drugs.

4. Conclusion and outlook

In this study, we developed a simple microcontact printing technique to fabricate patterned thermoresponsive microgel strips with various widths and gaps on non-thermoresponsive cell-adherent surface. These patterned surfaces were then used to investigate how the critical widths of strip and gap affected COS7 fibroblast cell sheet recovery. It was found that COS7 cells could preferentially attach and proliferate in the gaps between the microgel strips to form patterned cell layers during the early stage of
culturing. The cells then migrated from the PEI-coated gaps to the microgel strips to form a confluent cell layer. These COS7 cell sheets could be recovered when the width of the thermoresponsive strip is not narrower than that of the non-thermoresponsive cell-adherent gap, and the detachment did not appear to damage the cells and patterns. Other cells such as 3T3 fibroblast, HeLa and L929 cells grew on these patterned surfaces similarly, but cell sheets could not be harvested due to the lack of strength within them. However, co-culturing of COS7 and HeLa cells could lead to the successful detachment again due to the strength of the COS7 cells in the patterned cell layer. Thus, these surface patterns can work as a platform for harvesting co-cultured cell sheets, useful for building functional tissue constructs such as tumor models.

**Associated content**

The Supporting Information including the TEM image of the microgel, SEM image of pattern of 50-50, Fluorescent and bright-field images of patterns, Different cell types adhered on patterns, Live/dead fluorescent staining of a freshly detached COS7 cell sheet from pattern 100-100 and COS7 cell layer formed on pattern 200-100 is available free of charge via the Internet at http://pubs.acs.org.

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References


Figure captions

Figure 1. Fluorescent and bright-field images of representative patterns of 50-50, 100-50, 100-100, 200-100, 200-50 and 500-500 prepared by PDMS stamps (up line); patterns of 50-50, 50-100, 100-100, 100-200, 50-200 and 500-500 prepared by agarose stamps (low line). The first number means the width of the cell-adhesion region and the second number means the width of the thermoresponsive microgel region.

Figure 2. Time-lapse microscopic images of adhesion, migration and proliferation of COS7 cells on pattern 100-100 with different incubation times. The scale bars is 100 µm.

Figure 3. Microscopic images illustrate that on patterns of 50-100, 100-50 and 200-100, COS7 cells preferred to adhere and proliferate inside the gaps. The scale bar is 100 µm.

Figure 4. (a) Schematic to show the measurements of the cell orientation angles; (b) Orientation angle frequency distribution of cells cultured on patterns of 50-100, 100-50 and 200-100 after 48 h. 100 cells in total were measured on each pattern.

Figure 5. Detachment behavior of COS7 cell layers on different patterns. The cell culture medium was first replaced with cold fresh medium (4 °C), and then placed at room temperature (about 20 °C) for observation without disturbance. Cell layer detached from patterns of 50-100, 50-50, 100-100 and 50-200, but could not detach from patterns of 100-50, 200-50, 200-100 and 500-500.

Figure 6. Detachment behavior from other types of cell layers on 100-100 pattern. The cell culture medium was first replaced with cold fresh medium (4 °C), and then placed at room temperature (about 20 °C) for
observation without disturbance. (a) NIH3T3 cell layer; (b) L929 cell layer and (c) HeLa cell layer. The scale bar is 100 µm.

Figure 7. The procedure to grow and harvest patterned HeLa (red) and COS7 (green) cell sheets by sequential co-culturing from 100-100 pNIPAAmSt microgel strips. The scale bar is 100 µm.
Figure 1
Figure 3

Patterns of microgel

Cell culture on surface

Overlay
Figure 4
Figure 5
Figure 7

HeLa cells first seeded

COS7 cells second seeded

Patterned cell layers

Patterned cell sheets