the FT-IR spectra significantly increased with the plasma treatment. Taken together, our results and those reported in the reference (28) suggest that the plasma treatment increased the number of hydroxyl groups and siloxane groups to form a siliceous layer on the outermost surfaces of the PDMS film. The end result was generation of hydrophilic groups on the PDMS (Fig. 5A). Five days after plasma treatment, the PDMS surface recovered some of its original hydrophobicity, as indicated by the water contact angle, although the FT-IR data did not show recovery of hydrophobicity. Hydrophilized PDMS surfaces have been reported to quickly recover their hydrophobicity owing to migration of unreacted oligomers to the surface (31,32). However, this migration might occur only on the outermost surface, at a depth that is shallower than that reached by FT-IR measurement. Thus, one possible explanation for our results is that adsorption of the coating protein on the outermost surface anchored the transformed functional groups, preventing their migration and thus suppressing recovery of hydrophobicity (Fig. 5B, C).

Because many adhesive cells adhered to the plasma-treated polystyrene dish but did not adhere to an untreated polystyrene dish, we first hypothesized that vitronectin was adsorbed only by the plasma-treated PDMS surface, on which the hiPSCs subsequently adhered. However, we found that vitronectin was adsorbed by both plasma-treated and untreated PDMS surfaces. Coating PDMS with a mixture of vitronectin and γ-globulin generated a concentration difference between the two proteins. The affinity of the untreated hydrophobic PDMS surface for γ-globulin might be larger than that for vitronectin because γ-globulin has a hydrophobic Fc region. Moreover, an increase in the concentration of one protein may have suppressed adsorption of the other by means of competitive adsorption (33–35). As a result, addition of γ-globulin apparently blocked the adsorption of vitronectin and thus blocked subsequent adhesion of hiPSCs to the untreated PDMS surface.

Compared to many previously reported cell-patterning methods (5,10,36–39), our method offers three advantages. The first advantage is that the pattern is long-lasting. Although there have been reports of successful patterning of PSCs (37,39), the cells have been found to escape from the pattern within a few days. Long-lasting patterning is important because hPSC differentiation takes at least a few days (11). The use of serum-supplemented culture medium and the presence of feeder cells can cause cell escape because serum and feeder cells supply ECM, which facilitates cell adhesion (15). In this study, we used our serum- and feeder-free culture medium, which contains no ECM and may have supported long-lasting cell patterning. Moreover, we added γ-globulin to block vitronectin adhesion, and thus the cells could not have escaped even if hPSCs had produced small amounts of ECM by themselves.

The second advantage is that the patterned PDMS sheets can be stored for at least 6 days before the cells are plated. We previously described micropatterned plasma treatment using a physical mask (10). However, PDMS surfaces treated with plasma generally regain their hydrophobicity within a few days (31,32). In contrast, the protein coating used in this study might have prevented migration of unreacted oligomers to the outermost surface and enabled retention of the pattern for at least 7 days. Storability enables transportation of patterned PDMS sheets to a remote location and permits quality control because many patterned PDMS sheets of the same quality can be prepared in one batch.

The third advantage is that the patterned surfaces are easy to prepare; the PDMS is covered with a sterilized perforated mask, treated with plasma, and coated with a mixture of γ-globulin and vitronectin. In contrast, microcontact printing, which is one of the easiest conventional methods of patterning (36), requires more preparation steps including fabrication of microstamps, surface modification with alkanethiols, uniform inking on microstamps, and pressure-controlled printing. Both γ-globulin and vitronectin are readily available and non-toxic. The protocol is simple enough for use in a standard cell biology laboratory. Thus, our patterning method is practical for routine cell biology experiments.

Generally, hPSCs form flat colonies on a dish, as do patterned colonies, but the colony sizes are not well controlled, because pipetting is used to dissociate large colonies into small colonies during passaging (1,2,26). Combination of our patterning method with single-cell dissociation (12) may permit equalization of colony size and thus control of self-renewal and differentiation of hPSCs. Moreover, our method may be applicable to other types of ECM components and cells, because hiPSCs also adhere to ECM components such as fibronectin and laminin, which can be coated on glass and on plasma-treated polystyrene culture dishes, and many type of cells adhere to these ECM components (11,18,40).

In summary, our serum- and feeder-free culture medium allowed us to elucidate the relationships between plasma treatment, protein adsorption, and cell adhesion. We found that γ-globulin prevented hiPSC adhesion to untreated PDMS surfaces by blocking vitronectin adsorption. We used our findings to develop a practical cell-patterning method that has three major advantages over previously reported methods. We believe that the fundamental information about hiPSC adhesion to PDMS revealed by this study will serve as a platform for the development of technology to control the microenvironment of hPSCs.

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References

1. **Thomson, J. A., Itskovitz-Eldor, J., Shapiro, S. S., Waknitz, M. A., Swiergiel, J. J., Marshall, V. S., and Jones, J. M.:** Embryonic stem cell lines derived from human blastocysts, Science, **282**, 1145–1147 (1998).
2. **Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., and Yamanaka, S.:** Induction of pluripotent stem cells from adult human fibroblasts by defined factors, Cell, **131**, 861–872 (2007).
3. **Tachibana, M., Amato, P., Sparman, M., Gutierrez, N. M., Tippner-Hedges, R., Ma, H., Kang, E., Fulati, A., Lee, H. S., Sritanaudomchai, H., and other 13 authors:** Human embryonic stem cells derived by somatic cell nuclear transfer, Cell, **153**, 1228–1238 (2013).
4. **El-Ali, J., Sorger, P. K., and Jensen, K. F.:** Cells on chips, Nature, **442**, 403–411 (2006).
5. **Gupta, K., Kim, D.-H., Ellison, D., Smith, C., Kundu, A., Tuan, J., Suh, K.-Y., and Levchenko, A.:** Lab-on-a-chip devices as an emerging platform for stem cell biology, Lab. Chip, **10**, 2019–2031 (2010).
6. **Duffy, D. C., McDonald, J. C., Schueller, O. J. A., and Whitesides, G. M.:** Rapid prototyping of microfluidic systems in poly(dimethylsiloxane), Anal. Chem., **70**, 4974–4984 (1998).
7. **Zhou, J., Ellis, A. V., and Voelcker, N. H.:** Recent developments in PDMS surface modification for microfluidic devices, Electrophoresis, **31**, 2–16 (2010).
8. **Jo, B. H., Van Lerberghe, L. M., Motsegood, K. M., and Beebe, D. J.:** Three-dimensional micro-channel fabrication in polydimethylsiloxane (PDMS) elastomer, J. Microelectromech. Syst., **9**, 76–81 (2000).
9. **McDonald, J., Duffy, D., Anderson, J., Chiu, D., Wu, H., Schueller, O., and Whitesides, G.:** Fabrication of microfluidic systems in poly (dimethylsiloxane), Electrophoresis, **21**, 27–40 (2000).
10. **Hattori, K., Yoshimitsu, R., Sugiura, S., Maruyama, A., Ohnuma, K., and Kanamori, T.:** Masked plasma oxidation: simple micropatterning of extracellular matrix in a closed microchamber array, RSC Adv., **3**, 17749–17754 (2013).

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11. **Yoshimitsu, R., Hattori, K., Sugiura, S., Kondo, Y., Yamada, R., Tachikawa, S., Satoh, T., Kurisaki, A., Ohnuma, K., Asashima, M., and Kanamori, T.:** Microfluidic perfusion culture of human induced pluripotent stem cells under Q2 fully defined culture conditions, Biotechnol. Bioeng., http://dx.doi.org/10.1002/bit.25150 (published online November 13, 2013).
12. **Ohgushi, M., Matsumura, M., Eiraku, M., Murakami, K., Aramaki, T., Nishiyama, A., Muguruma, K., Nakano, T., Suga, H., Ueno, M., and other 5 authors:** Molecular pathway and cell state responsible for dissociation-induced apoptosis in human pluripotent stem cells, Cell Stem Cell, **7**, 225–239 (2010).
13. **Horbett, T. A.:** Mass action effects on competitive adsorption of fibrinogen from hemoglobin solutions and from plasma, Thromb. Haemost., **51**, 174–181 (1984).
14. **Tagaya, M., Ikoma, T., Hanagata, N., and Tanaka, J.:** Analytical investigation of protein mediation between biomaterials and cells, Mater. Express, **2**, 1–22 (2012).
15. **Davis, J. M.:** Basic cell culture, Practical approach, 2nd ed. Oxford University Press, Oxford, U.K (2002).
16. **Hayashi, Y., Furue, M. K., Okamoto, T., Ohnuma, K., Myoishi, Y., Fukuhara, Y., Abe, T., Sato, J. D., Hata, R., and Asashima, M.:** Integrins regulate mouse embryonic stem cell self-renewal, Stem Cells, **25**, 3005–3015 (2007).
17. **Hughes, C. S., Postovit, L. M., and Lajoie, G. A.:** Matrigel: a complex protein mixture required for optimal growth of cell culture, Proteomics, **10**, 1886–1890 (2010).
18. **Braam, S. R., Zeinstra, L., Litjens, S., Ward-van Oostwaard, D., van den Brink, S., van Laake, L., Lebrin, F., Kats, P., Hochstenbach, R., and Passier, R.:** Recombinant vitronectin is a functionally defined substrate that supports human embryonic stem cell self-renewal via αvβ5 integrin, Stem Cells, **26**, 2257–2265 (2008).
19. **Rowland, T. J., Miller, L. M., Blaschke, A. J., Doss, E. L., Bonham, A. J., Hikita, S. T., Johnson, L. V., and Clegg, D. O.:** Roles of integrins in human induced pluripotent stem cell growth on matrigel and vitronectin, Stem Cells Dev., **19**, 1231–1240 (2009).
20. **Hayman, E. G., Pierschbacher, M. D., Ohgren, Y., and Ruoslahti, E.:** Serum spreading factor (vitronectin) is present at the cell surface and in tissues, Proc. Natl. Acad. Sci. USA, **80**, 4003–4007 (1983).
21. **Bale, M. D., Wohlfahrt, L. A., Mosher, D. F., Tomasini, B., and Sutton, R. C.:** Identification of vitronectin as a major plasma protein adsorbed on polymer surfaces of different copolymer composition, Blood, **74**, 2698–2706 (1989).
22. **Nagaoka, M., Si-Tayeb, K., Akaike, T., and Duncan, S. A.:** Culture of human pluripotent stem cells using completely defined conditions on a recombinant e-cadherin substratum, BMC Dev. Biol., **10**, 60 (2010).
23. **Klim, J. R., Li, L., Wrighton, P. J., Piekarczyk, M. S., and Kiessling, L. L.:** A defined glycosaminoglycan-binding substratum for human pluripotent stem cells, Nat. Methods, **7**, 989–994 (2010).
24. **Parker, C. W. and Osterland, C. K.:** Hydrophobic binding sites on immunoglobulins, Biochemistry, **9**, 1074–1082 (1970).
25. **Furue, M. K., Na, J., Jackson, J. P., Okamoto, T., Jones, M., Baker, D., Hata, R., Moore, H. D., Sato, J. D., and Andrews, P. W.:** Heparin promotes the growth of human embryonic stem cells in a defined serum-free medium, Proc. Natl. Acad. Sci. USA, **105**, 13409–13414 (2008).
26. **Hayashi, Y., Chan, T., Warashina, M., Fukuda, M., Ariizumi, T., Okabayashi, K., Takayama, N., Otsu, M., Eto, K., Furue, M. K., and other 4 authors:** Reduction of N-glycolylneuraminic acid in human induced pluripotent stem cells generated or cultured under feeder- and serum-free defined conditions, PLoS One, **5**, e14099 (2010).
27. **Nakagawa, M., Koyanagi, M., Tanabe, K., Takahashi, K., Ichisaka, T., Aoi, T., Okita, K., Mochiduki, Y., Takizawa, N., and Yamanaka, S.:** Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts, Nat. Biotechnol., **26**, 101–106 (2008).
28. **Tagaya, M., Komura, M., Iyoda, T., and Nakagawa, M.:** Printing of Au nanoparticles by Vuv-exposed patterned surfaces of a poly (dimethylsiloxane) film, Trans. Mater. Res. Soc. Jpn., **31**, 273–276 (2006).
29. **Tagaya, M., Yamazaki, T., Tsuya, D., Sugimoto, Y., Hanagata, N., and Ikoma, T.:** Nano/microstructural effect of hydroxyapatite nanocrystals on hepatocyte cell aggregation and adhesion, Macromol. Biosci., **11**, 1586–1593 (2011).
30. **Phely-Bobin, T. S., Muisener, R. J., Koberstein, J. T., and Papadimitrakopoulos, F.:** Preferential self-assembly of surface-modified si/siox nanoparticles on UV/ozone micropatterned poly(dimethylsiloxane) films, Adv. Mater., **12**, 1257–1261 (2000).
31. **Kim, J., Chaudhury, M. K., Owen, M. J., and Orbeck, T.:** The mechanisms of hydrophobic recovery of polydimethylsiloxane elastomers exposed to partial electrical discharges, J. Colloid Interface Sci., **244**, 200–207 (2001).
32. **Vickers, J. A., Caulum, M. M., and Henry, C. S.:** Generation of hydrophilic poly (dimethylsiloxane) for high-performance microchip electrophoresis, Anal. Chem., **78**, 7446–7452 (2006).
33. **Bale, M. D., Mosher, D. F., Wolfarht, L., and Sutton, R. C.:** Competitive adsorption of fibronectin, fibrinogen, immunoglobulin, albumin, and bulk plasma proteins on polystyrene latex, J. Colloid Interface Sci., **125**, 516–525 (1988).
34. **Holmberg, M., Stibius, K. B., Larsen, N. B., and Hou, X.:** Competitive protein adsorption to polymer surfaces from human serum, J. Mater. Sci. Mater. Med., **19**, 2179–2185 (2008).
35. **Tagaya, M., Ikoma, T., Hanagata, N., Yoshioka, T., and Tanaka, J.:** Competitive adsorption of fibronectin and albumin on hydroxyapatite nanocrystals, Sci. Technol. Adv. Mater., **12**, 034411 (2011).
36. **Mrksich, M. and Whitesides, G. M.:** Patterning self-assembled monolayers using microcontact printing: a new technology for biosensors? Trends Biotech., **13**, 228–235 (1995).
37. **Park, J., Cho, C. H., Parashurama, N., Li, Y., Berthiaume, F., Toner, M., Tilles, A. W., and Yarmush, M. L.:** Microfabrication-based modulation of embryonic stem cell differentiation, Lab Chip, **7**, 1018–1028 (2007).
38. **Saha, K., Mei, Y., Reisterer, C. M., Pyzocha, N. K., Yang, J., Muffat, J., Davies, M. C., Alexander, M. R., Langer, R., Anderson, D. G., and Jaenisch, R.:** Surface-engineered substrates for improved human pluripotent stem cell culture under fully defined conditions, Proc. Natl. Acad. Sci. USA, **108**, 18714–18719 (2011).
39. **Paik, I., Scurr, D. J., Morris, B., Hall, G., Denning, C., Alexander, M. R., Shakesheff, K. M., and Dixon, J. E.:** Rapid micropatterning of cell lines and human pluripotent stem cells on elastomeric membranes, Biotechnol. Bioeng., **109**, 2630–2641 (2012).
40. **Barczyk, M., Carracedo, S., and Gullberg, D.:** Integrins, Cell Tissue Res., **339**, 269–280 (2010).

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