doi:10.1246/cl.2010.1164

Published on the web October 5, 2010

Human Mesenchymal Stem Cell Behavior on Concentrated Polymer Brushes Presenting Different Surface Stiffness

Kwangwoo Nam,[1,2] Rie Fukaya,[1] Yoshihide Hashimoto,[1] Yukiko Ito,[1,2] Tsuyoshi Kimura,[1,2] and Akio Kishida*[1,2]
[1]*Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, 2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062*
[2]*Japan Science and Technology Agency, CREST, 5 Sanbancho, Chiyoda-ku, Tokyo 102-0075*

(Received August 18, 2010; CL-100715; E-mail: kishida.fm@tmd.ac.jp)

The graft density of poly[poly(ethylene glycol) methacrylate] (PPEGMA) was controlled using atom transfer radical polymerization to observe the change in the differentiation behavior of stem cells because the alteration in the graft density leads to the altered surface elasticity. However, since the cells do not adhere on the high graft density polymer brush, it is impossible to observe such behavior directly. We therefore immobilized collagen onto polymer brushs with controlled graft density to obtain a surface which is effective for cell adhesion and maintains its different surface matrix elasticity. For the cell adhesion, the human mesenchymal stem cell (hMSC) adhesion and differentiation was investigated on the surface of collagen-immobilized polymer brush. It was shown that the hMSC spreading tendency was altered by the surface elasticity implying that the behavior of hMSC would be altered by the stiffness of the polymer brush.

One ultimate goal in regenerative medicine and tissue engineering is to control cell functions such as adhesion, proliferation, migration, cell-to-cell communication, differentiation, and programmable apoptosis in a desired manner.[1,2] Given the development of stem cell techniques on growth factors and cell resources, designable materials are becoming a more important research drive in effective cell production/culturing. Designable materials are key players in the process of tissue formation and regeneration as a scaffold that can direct cell behaviors and functions.[3,4] With a better understanding of the cell–material interactions, scaffolds for cell culture can achieve a greater control over cell behavior, function, and fate.[3,5] Many studies on biocompatible synthetic materials have been done, including combination of chemical characteristics and physically appropriate mechanical properties which are expected to expand the possibilities of designs as implants and as substrates for tissue engineering.[6]

Much research on cell behavior and surface properties of materials has shown that variations in surface properties such as chemistry, topography, surface energy, or stiffness affect cell–material interactions, potentially affecting cell functions.[7] However, a significant issue is that variations in matrix stiffness induce change to molecular or network structure, implying that differentiation of human mesenchymal stem cell (hMSC) had been also affected by chemical or physical differences.[6] Furthermore, it is difficult to change just one physical or chemical parameter while maintaining other parameters. This makes it difficult to control the fate of the cell on the materials surface. That is, if a surface with the same chemical component with different physical properties, it would be possible to investigate the change in the hMSC differentiation behavior more precisely.

For this, we have focused on the creation of a surface which has the same chemical component but different physical properties using atom transfer radical polymerization (ATRP). Using surface-initiated ATRP, it is possible to create a surface with controlled polymer brush density, uniform chain length, and fully extended chain conformation. Furthermore, it is possible to change the density of the polymer brush by controlling the density of the initiator by mixing with a chemical substance that has similar structure with that to the initiator, leading to the alteration of the surface topography and morphology, surface stiffness, and cell adhesion.[8,9] So, controlling the density of the polymer brush would bring an anisotropic model surface with different stiffness without changing other surface properties.

We obtained a high density grafted polymer brush ($M_{n,conv} \approx 6.11 \times 10^4$, PDI ≈ 1.2) on a silicone substrate with a graft density of 0.23 chains/nm^2 using silane coupling agent [6-(2-bromoisobutyryloxy)hexyltriethoxysilane] as initiator. Since the PEG segment of the PPEGMA is large, the graft density is relatively small compared to that to poly(2-hydroxyethyl methacrylate) or poly(methyl methacrylate) which show approximately 0.7 and 0.65 chains/nm^2, respectively.[9,10] But the PPEGMA polymer brush would still exist in a high density polymer brush region (>0.1 chains/nm^2). When the mol % of initiator against halogen-absent silane coupling agent decreases, linear decrease in the dry thickness along with graft density is shown very clearly while molecular weight and static contact angle remains unchanged (Table 1). Considering the fact the M_n was similar for all samples, the length of the fully extended chain in aqueous conditions would be almost the same. The surface stiffness measured with atomic force microscope decreased along with graft density (Supporting Information (SI), Figure 1a[12]). Interestingly, the graft density showed that the polymer brush would still exist in high density polymer brush region until the initiator density decreases to 1%. This implies

Table 1. Characteristics of PPEGMA brush grafted on silicone substrate grafted according to the initiator density

Initiator density[a]	$M_{n,conv}$[b]	σ[c]	M_w/M_n[d]	L/nm[e]	θ/degree[f]
100	6.11×10^4	0.23	1.20	23.4	39
20	5.99×10^4	0.17	1.18	16.7	41
1	5.46×10^4	0.14	1.22	12.7	41
0.1	5.88×10^4	0.01	1.28	1.4	39

[a]Conversion molecular weight %. [b]Calculated by NMR. [c]Graft density (chains/nm^2). [d]Measured by GPC. [e]Dry thickness measured by ellipsometry (nm) ±0.5. [f]Static contact angle (±3), $n = 5$.

that cells or protein would not adhere on the surface of the polymer brush due to size-exclusion when the polymer brush is prepared.[10] This makes it difficult to use this surface for the investigation of the hMSC differentiation behavior.

In order to adopt surface which is effective for cell adhesion and evaluation, chemical modification of the surface with collagen using sulfo-SANPAH by UV irradiation was executed. This is based on click chemistry, where the bromine terminal atom is substituted by the azide function.[11] Then by UV irradiation, the lysine group of the collagen reacts with nitrogen forming a new –NH–NH– bond between collagen and the PPEGMA (SI, Figure 2[12]). The combination of ATRP and click chemistry is important in the aspect that the bromine atom which is left behind and after the ATRP is eliminated and can be functionalized by some other chemical compounds. Furthermore, the collagen immobilization can bring two important functions: first the surface stiffness should be altered according to the graft density of the polymer brush and second, the cells would adhere on the surface of the collagen-immobilized polymer brush.

For this purpose, the collagen was immobilized on the polymer brush with initiator density of 100%, 1%, and 0.1% to make cP100, cP1, and cP01, respectively. As shown in SI, Figure 2b;[12] the decrease in the surface stiffness together with the decrease in the gradient density of the polymer brush is shown. On the other hand, the static contact angle remain approximately 44° for the cP100 and cP1 (SI, Table 1[12]). This indicates the change in the surface stiffness while keeping its other physical, chemical, or geographical parameters.

This resulted in the adhesion of the hMSC on the surface of the collagen-immobilized polymer brush. The number of cells adhered on the surface of the collagen-immobilized polymer brush slightly decreased compared to that to the tissue culture polystyrene (TCPS) as shown in Figure 1a. It should be noted again that there is no cell adhesion on the surface of PPEGMA even in the middle-density graft region (SI, Figure 3[12]). The immobilization of collagen to the PPEGMA brush promoted the cell adhesion compared to that to the PPEGMA brush. The number of cells adhered to the cP100 and cP1 was almost the same. On the other hand, the cell spreading tendency which is a precursor for the cell differentiation behavior showed that the cell spreading was suppressed for the cP1 compared to that to the cP100, where the surface stiffness was lower (SI, Figure 1b[12] and Figure 1b). It is not certain whether the cell differentiation would actually be affected, but it can be concluded that the cell behavior is being affected by the surface stiffness. In the case of cP01, both cell adhesion and spreading was promoted compared to that to the cP100 and cP1 although the surface stiffness was similar to cP1 as shown in SI, Figure 1b.[12] This is thought to be due to the increased amount of collagen immobilization which formed much thicker collagen layer (SI, Table 1[12]).

In conclusion, we successfully prepared anisotropic model surface with different stiffness without changing other surface properties. The results of cell behavior indicate that hMSCs responded differently between highly concentrated and moderately concentrated PPEGMA brushes coated with collagen (cP100 and cP1). Detailed investigation on the influence of surface stiffness on cell functions by using the concentrated polymer brush would lead to the advance in the design of biomaterial that can be applied for regenerative medicine and tissue engineering.

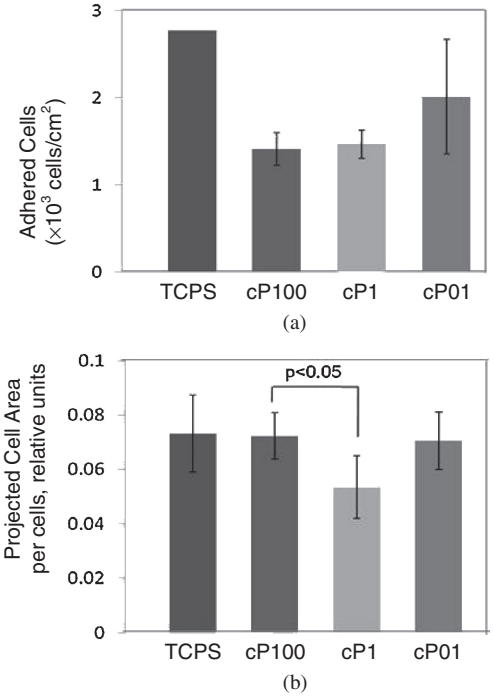

Figure 1. (a) Adhesion of the hMSC on the respective surfaces and (b) projected cell area. The higher value of projected cell area implies that the cell spread was much promoted.

The author would like to express her appreciation to Professor Yoshinobu Tsujii (Institute for Chemical Research, Kyoto University) for his technical advice and discussion on AFM measurement. This work was partly supported by Core Research for Evolutional Science and Technology (CREST) of the Japan Science and Technology Agency (JST).

References and Notes

1 A. J. Engler, S. Sen, H. L. Sweeney, D. E. Discher, *Cell* **2006**, *126*, 677.
2 a) J. Y. Lim, H. J. Donahue, *Tissue Eng.* **2007**, *13*, 1879. b) R. Langer, J. P. Vacanti, *Science* **1993**, *260*, 920.
3 R. W. Sands, D. Mooney, *Curr. Opin. Biotechnol.* **2007**, *18*, 448.
4 M. P. Lutolf, J. A. Hubbell, *Nat. Biotechnol.* **2005**, *23*, 47.
5 Y. Tamada, Y. Ikada, *J. Biomed. Mater. Res.* **1994**, *28*, 783.
6 I. Levental, P. C. Georges, P. A. Janmey, *Soft Matter* **2007**, *3*, 299.
7 J. Y. Lim, J. C. Hansen, C. A. Siedlecki, J. Runt, H. J. Donahue, *J. R. Soc., Interface* **2005**, *2*, 97.
8 a) D. M. Jones, J. R. Smith, W. T. S. Huck, C. Alexander, *Adv. Mater.* **2002**, *14*, 1130. b) S. Edmondson, V. L. Osborne, W. T. S. Huck, *Chem. Soc. Rev.* **2004**, *33*, 14.
9 a) C. Yoshikawa, A. Goto, Y. Tsujii, T. Fukuda, T. Kimura, K. Yamamoto, A. Kishida, *Macromolecules* **2006**, *39*, 2284. b) C. Yoshikawa, Y. Hashimoto, S. Hattori, T. Honda, K. Zhang, D. Terada, A. Kishida, Y. Tsujii, H. Kobayashi, *Chem. Lett.* **2010**, *39*, 142.
10 S. Yamamoto, M. Ejaz, Y. Tsujii, T. Fukuda, *Macromolecules* **2000**, *33*, 5608.
11 J.-F. Lutz, H. G. Börner, K. Weichenhan, *Macromolecules* **2006**, *39*, 6376.
12 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

