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Editor's Choice

Suppression of Cell Adhesion on Well-defined Concentrated Polymer Brushes of Hydrophilic Polymers

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The cell adhesion was investigated on polymer brushes of poly(2-hydroxyethyl methacrylate) (PHEMA), poly(2-hydroxyethyl acrylate) (PHEA), and poly[poly(ethylene glycol) methyl ether methacrylate] (PPEGMA) with different graft densities (in semidilute and concentrated regimes) and thicknesses. The concentrated polymer brushes (CPBs) of all the three, regardless of differences in chemical structure and hydrophilicity, almost completely suppressed the adhesion of L929 fibroblast cell in contrast to the corresponding semidilute polymer brushes (SDPBs). Along with our previous study on excellent protein repellency of CPB owing to its unique size-exclusion effect (of entropic origin), we concluded that such bioinertness should be commonly found in CPB of neutral and hydrophilic polymers.

Surface-initiated living radical polymerization (SI-LRP) is one of the most promising methods to densely graft well-defined polymers and then to afford a well-defined CPB on various surfaces.[1,2] CPB is characterized by highly stretched conformation of graft chains, nearly to their full length in a good solvent, strong resistance against compression, and super lubrication between swollen brushes. These characteristic structure and properties, quite different from those of SDPB, are well understood to be of entropic origin; the CPB has a large elastic stress (by the conformational entropy) balanced with a huge osmotic pressure (by the mixing entropy) in a solvent. As one of such entropically driven features, we previously demonstrated that CPB of PHEMA showed excellent protein repellency because of its unique size-exclusion effect;[3] proteins, in most cases sufficiently large compared with the distance between the nearest-neighbor graft points, are physically excluded from the CPB layer and hardly adsorbed on it because of effective suppression of nonspecific interaction. This notable result led us to investigate the interaction between cells and CPBs, since nonspecific adsorption of proteins often triggers cell adhesion. The cell-adhesion behavior has been so far reported on some hydrophilic polymer brushes including PHEMA, PPEGMA, and poly(2-methacryloyloxyethyl phosphorylcholin),[4] but the feature of CPB has not been clearly demonstrated presumably because the samples were prepared by SI-LRP but not well characterized. It should be noted that SI-LRP could not provide a well-defined CPB without polymerization conditions optimized.

In order to provide a comprehensive view on this issue, we systematically performed the cell-adhesion test on well-defined polymer brushes of hydrophilic polymers including PHEMA, PHEA, and PPEGMA. PHEMA was previously used to demonstrate the protein repellency on its CPB as mentioned above. PHEA is more hydrophilic than PHEMA; PHEA is water-soluble, while PHEMA especially of a high molecular weight is not completely soluble but swollen in water. Note that there has been no report of successful synthesis of PHEA brushes by SI-LRP. PPEGMA is one of the most extensively studied polymers for biointerfaces. The CPB samples were prepared on silicon wafers by surface-initiated atom transfer radical polymerization (ATRP) (see Supporting Information).[5] The samples with different thicknesses L were obtained by varying the polymerization time. The SDPB samples were prepared by the grafting-to method;[5] the end-functionalized polymer with an alkoxysilyl group was synthesized by ATRP with (2-bromo-2-isobutoxy)propyltriethoxysilane and immobilized on a silicon wafer. Table 1 summarizes the characteristics of studied samples. M_n and M_w/M_n are the GPC-determined values of the free polymers simultaneously produced at polymerization as sufficiently accurate indices for those of the graft polymers,[2] where M_n and M_w are the number- and weight-average molecular weights, respectively. The M_w/M_n was 1.1–1.3 in all cases, suggesting that the brush samples have relatively narrow distribution in chain length. As an absolute value of molecular weight, the theoretical value $M_{n,conv}$ (see the footnote in Table 1) was used to estimate graft density σ; the validity of this assumption was confirmed for some samples by using a GPC equipped with a multiangle laser light-scattering detector. In order to categorize polymer brushes composing different kinds of polymers (with different size of monomers), the dimensionless graft density σ^* (see the caption in Table 1) was estimated. Very recently, we successfully determined, by a combinatorial approach, the crossover density between SDPB and CPB to be around 0.1 in σ^* using the scaling theoretical arguments.[2] According to this criterion, the samples were categorized as CPB or SDPB as indicated in Table 1. The contact angle θ was measured in air at 25 °C by using a water-droplet technique. The θ value for each polymer was almost the same independent of σ^* and L, suggesting that the substrate was fully covered with the polymer segments.

The adhesion test of L929 fibroblast was carried out on the brush samples as well as the ATRP-initiator-fixed substrate and the tissue culture polystyrene (TCPS) as references. This cell was selected as a model fibroblast often used for toxicity test. The samples were incubated with L929 fibroblasts in Dulbecco's modified Eagle medium for 24 h, washed 3 times with fresh

Table 1. Characteristics of studied polymer brushes of PHEMA, PHEA, and PPEGMA

Samples		$M_{n,conv}$[a]	M_n	M_w/M_n	L/nm[d]	σ/chains cm^{-2} [e]	σ^*[f]	θ/degree[g]
(a)	CPB of PHEMA	16900	15700	1.28[b]	19	0.8	0.6	46
(b)	SDPB of PHEMA	18900	15000	1.28[b]	2	0.07	0.1	45
(c)	CPB of PHEA	3700	1400[b]	1.14[c]	5	0.8	0.6	33
(d)	CPB of PHEA	12800	4600[b]	1.11[c]	15	0.7	0.5	31
(e)	SDPB of PHEA	11900	4900[b]	1.27[c]	2	0.09	0.07	32
(f)	CPB of PPEGMA	9000	15000[c]	1.14[b]	4	0.3	≈1	41
(g)	CPB of PPEGMA	39700	45600[c]	1.19[b]	18	0.3	≈1	42
(h)	SDPB of PPEGMA	41600	44500[c]	1.23[b]	1	0.02	≈0.06	42

[a]Calculated according to $M_{n,conv} = [monomer]_0/[EBIB]_0 \times MW \times$ Converstion, where MW is the molecular weight of monomer unit. [b]Estimated by PMMA-calibrated GPC. [c]Estimated by PEG-calibrated GPC. [d]Film thickness in dry state; the error is within 10%. [e]Graft density calculated with L and $M_{n,conv}$. [f]Dimensionless graft density defined as $\sigma^* = a^2\sigma$, where a^2 is the cross sectional area per monomer unit; the σ^* for PPEGMA cannot be accurately determined because of a rough value of average M_n (≈475) of PEGMA monomer. [g]Contact angle estimated by water-droplet method; the error is within 2 degrees.

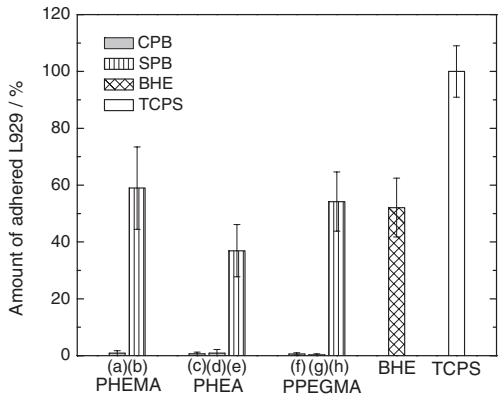

Figure 1. Amount of adhered L929 cells on the brush samples (a)–(h) (Table 1) as well as the ATRP-initiator-fixed and TCPS substrates. Incubation time = 24 h. $[L929]_0 = 5.0 \times 10^4$ cells/cm^2. The vertical axis was normalized with the number of adherent cells on TCPS.

phosphate buffer saline, and observed with a fluorescent microscope to count the number of cells remaining on the surface.[5] Three experiments were performed for each substrate, and at least 4 spots on a substrate were observed to get the mean values. Figure 1 shows the amount of adherent L929 cells on polymer brushes of PHEMA, PHEA, and PPEGMA at 24 h. Regardless of differences in chemical structure and hydrophilicity, all the CPB samples highly suppressed the cell adhesion to almost undetectable level, while the corresponding SDPBs exhibited the cell adhesion similarly to the reference substrates. These results are reasonably understood as follows; the preadsorption of proteins triggering cell adhesion is effectively inhibited on these CPB surfaces. Until now, the protein repellency was demonstrated only on the CPB of PHEMA, but this would be the case with other CPB samples because this property comes from the entropically driven size-exclusion effect unique to CPBs sufficiently swollen in a medium (water for biointerface).

In conclusion, the physical structure (possibly including chain dynamics) of graft chains on the surface play an essential role in preventing protein adsorption and cell adhesion, and CPB of neutral and hydrophilic polymers should be commonly endowed with such entropically-driven bioinertness. Further investigation with other hydrophilic polymers is now in progress.

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5 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

