Novel Approaches for The Construction of Cell Specific Surfaces using Heterobifunctional Poly(ethylene glycol) (PEG)

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1. Introduction

Modification with hydrophilic polymers is the most common way to improve the surface properties of devices used for biological and biomedical applications [1]. In particular, poly(ethylene glycol) (PEG) coating has most widely been used to minimize non-specific fouling of devices surface with biocomponents, including plasma proteins. For example, a PEGylated surface, which means the surface attached with the tethered chains of poly(ethylene glycol) using the functionality of PEG end groups, is reported to extremely lessen protein adsorption [2], resulting in the acquisition of high blood compatibility [3]. PEG-coating can be performed in a variety of methods such as covalent tethering of PEG with reactive chain-end, graft copolymerization of PEG macromonomer onto the surface [4], and physical adsorption of PEG surfactants and block copolymers composed of PEG segments [5]. However, most of the PEG-coated surfaces thus far reported possess no reactive group on the free-end of tethered PEG chain. To provide the further functionality on the PEG-coated surface, heterobifunctional poly(ethylene glycol), which denotes PEG having different functionalities at each of both chain ends can be utilized as surface modifiers in two ways, to provide the reactive sites on PEGylated surface. First approach is the modification of poly(vinyl chloride) surface by surface plasma polymerization of heterobifunctional PEG macromonomers. The systematic research on

the synthesis of heteroPEGs, such as $\text{NH}_2\text{-PEG-OH}$ and CHO-PEG-OH [6, 7], can be applied to new heteroPEG macromonomer synthesis, having a polymerizable end along with a functional group at the other end [8]. Surface plasma polymerization of heteroPEG macromonomer would give tree-like tethered chain on the surface (Fig. 1). Another approach is the construction of functionalized PEG-brushed layer on biodegradable polylactide (PLA) surface through a simple coating with reactive block copolymer of end-functionalized PEG/PLA [9–11]. This surface-engineering is shown schematically in Figure 1.

Figure 1. Schematic representation of the application to the construction of a PEG layer using a HeteroPEG macromonomer (a) and a PEG/PLA block copolymer (b).

The resultant interfacial structure composed of

brush-like or diffused layers of PEG-chains appears to minimize protein-surface interaction. It should be noted that this end-functionalized PEG/PLA is a major outcome of our systematic research on the synthesis of heteroPEG [6, 7]. When one of the functional end-groups in the heteroPEG selectively initiates the polymerization of a hydrophobic monomer, a new heterobifunctional AB block copolymer can be created, keeping the other functional group at the PEG chain end available. An acetal group is installed at the α -chain end of both PEG macromonomers and PEG/PLAs which is able to be delivertized to an aldehyde group by the moderate acid treatment. Apparently, in both approaches, a reactive group located on the PEG chain end provided the site for chemical immobilization of functional molecules such as proteins and peptides. In this way, PEG layer with terminal aldehyde group was readily prepared which may have both non-fouling and ligand binding properties. Phenylboronic acid was introduced using aldehyde group for the separation of blood-cell population such as lymphocyte through a recognition of sugar residues existing on the surface of lymphocytes [12]. As a binding site for sugar residues of lymphocytes, a focus was given on phenylboronic acid groups in this study. This paper deals with a study done to evaluate a selective retention of lymphocyte subpopulation on PEGylated surfaces with phenylboronic acid moieties which may be useful to construct a novel cell separator for lymphocyte subpopulations. The accurate analysis of the surface properties of these PEGylated surfaces from physicochemical (contact angle, ζ potential) as well as biological (selective adhesion of rat lymphocyte subpopulation) points of view is reported, as a basis for the further application of this strategy in the biomedical field.

2. Experimental Section

2.1 Materials

Commercial tetrahydrofuran (THF), 3,3-diethoxy-1-propanol (Aldrich), methacryloyl anhydride (Aldrich), 3-aminophenylboronic acid (Aldrich), ethylene oxide (EO) (Saisan) and L-lactide(LA) (Aldrich) were purified by conventional method [13]. EO was dried over calcium hydride and distilled under an argon atmosphere.

2.2 Synthesis of PEG Macromonomers having

Phenylboronic Acid End Group and Acetal-PEG/PLA Block Copolymers. The detailed procedure for the synthesis of these polymers were described elsewhere [9, 10, 14, 15], and these polymers were synthesized using the procedure.

2.3 Polymer Characterization. The molecular weight of PEG segment was determined by both gel permeation chromatography (GPC) and MALDI-TOF-MS measurements at the end of EO polymerization. The molecular weight of the PLA segment was determined using ^1H NMR spectrum by estimating the ratio of methine protons in the PLA segment and methylene protons in PEG segment based on the number-averaged molecular weight (M_n) of PEG determined from the MALDI-TOF-MS results.

2.4 Surface Plasma Polymerization of HeteroPEG Macromonomers. After poly(vinyl chloride) (PVC) sample was treated by oxygen plasma (0.15 Torr, Fwd=95W, Ref=20-16W, 1min), the sample was soaked in hydrochloric acid (0.1 mM) for 15 min at ambient temperature, then dried in vacuo for 1 day. After 1 wt% of heteroPEG macromonomer solution was degassed by argon bubbling, the peroxidized PVC was soaked and reacted at 80 °C for 5 h.

2.5 Spin Coating of α -Acetal-PEG/PLA/ Conversion of Acetal End into Aldehyde Group/ Functionalization with Phenylboronic Acid Group [9-11]. The glass substrates, which were cleaned by a Piranha etch, were placed in 2 % (v/v) solution of 3-(Trimethoxysilyl)propyl methacrylate/ethanol. The glass substrates were dried at 160 °C for 24 h under vacuum. The PEG-brushed layer was constructed on this silanized glass surface by the spin coating of toluene solution of PLA(4 % (w/v)), followed by the acetal-PEG/PLA(2 % (w/v)). The PEGylated glass substrate was immersed into aqueous media adjusted to pH 2 to transform an acetal group at the PEG-chain-end into an aldehyde end group. The resulting substrate was immersed into PBS solution of the m-aminophenylboronic acid, then subjected to sodium cyanoborohydride in a similar condition mentioned above.

2.6 Characterization of the PEGylated Surfaces. Contact Angle [10, 11]. Static contact angle measurements were carried out by both a sessile droplet technique for the water-in-air system and a captive bubble technique for the air-in-water system. ζ -potential [9,10]. The ζ -potential over a pH range of 2-11 at 25 °C was

measured by LEZA-600(Otsuka Electric Co., Osaka, Japan) in 10 mM NaCl solution, and the pH was adjusted with 10 mM HCl or NaOH.

2.7 Evaluation of the selective lymphocyte adhesion on PEGylated surfaces. In order to carry out a quantitative assay of cell/material interaction, a flat ribbon-like chamber with a plane-parallel-type channel [hybrid field-flow fractionation/adhesion chromatography (FFF/AC)] was used in this study. By FFF/AC method, the time course of cell/material interaction can be followed by controlling the contacting time-period of cells with the channel wall [16].

3. Results and Discussion

3.1 Synthesis of HeteroPEG Macromonomers and PEG/PLA Block Copolymers. On the synthesis of hetero-PEG macromonomers, the acetal end group can be converted to aldehyde group by an acid treatment. Thus, *m*-aminophenylboronic acid, which is a binding site for sugar residues on the surface of lymphocytes, was utilized for the delivatization because it is known that tetrahedral-formed boronic acid can form stable and reversible covalent bonds with polyol compounds including sugars.

Table 1. Molecular weights of PEG macromonomer and PEG/PLA block copolymers

sample ^a	PEG ^b			PLA
	Mn	Mw	Mw/Mn	Mn
1 Boron-PEG (2.7)	2720	2840	1.04	—
2 Acetal-PEG/PLA(5.6/8.1)	5600	5840	1.04	8100
3 MeO-PEG/PLA(5.5/7.0)	5470	5800	1.06	7000

^a Molecular weights (MW) of PEG and each segments of PEG/PLA were abbreviated as follows: PEG (2.4) and PEG/PLA(5.6/8.1, 2.8/7.1, 5.5/7.0) where the numbers in parenthesis denote the MW $\times 10^{-3}$ of the PEG segments and PLA segments in kg/mol, respectively.

^b Values determined from GPC results.

Detailed characterization of this polymer (Boron-PEG) was reported in the previous paper using ¹H NMR, GPC, and MALDI-TOF-MS measurements [14]. After the EO polymerization, a PEG/PLA block copolymer with an acetal group at the PEG chain end can be prepared, because potassium alkoxide has the ability to initiate LA polymerization. A PEG/PLA with a methoxy moiety at the α -terminus is also synthesized using 2-methoxyethanol as an initiator. The molecular weight of PEG and each segment of PEG/PLA is summarized in Table 1.

3.2 Static Contact Angle Measurements in water. Heterobifunctional PEG macromonomers thus obtained were utilized for the surface plasma polymerization. After the PVC surface was coated with Boron-PEGs using plasma polymerization, the wettability significantly increased. Since the contact angle of PVC substrates was less than 0.5, the value of 0.86 on surfaces modified by Boron-PEGs indicates the effective surface modification by PEG macromonomer. The static wettability of the PLA surface covered with acetal-PEG/PLA was also estimated using air-in-water system. The relatively high values of $\cos \theta$ on the surface are most likely attributed to an appreciable coverage of the upper most surface by the PEG chains. It is interesting to note that the wettability on the surface modified by PEG macromonomer was apparently higher than that for PEG/PLA, suggesting the superior coverage of PEG by the plasma polymerization.

3.3 ζ Potential Measurements. Figure 2 provides the pH-dependent ζ potential profiles of Boron-PEG modified PVC substrates (Fig. 2(a)) and MeO-PEG/PLA coated or Boron-PEG/PLA coated glass substrates (Fig. 2(b)), respectively.

Figure 2. ζ potential variation of HeteroPEG (a) and PEG/PLA (b) coated substrates as a function of pH in 10mM-NaCl solution; (▲) Boron-PEG(2.7) modified PVC, (■) Boron-PEG/PLA(5.6/8.1) coated glass, (●) MeO-PEG/PLA(5.6/8.1) coated glass.

With increasing pH of the medium (6 to 8), ζ potential of Boron-PEG modified PVC slightly decreased, due to an actual increase in the fraction of boronate anions at higher pH. On the other hand, on Boron-PEG/PLA coated surface, the increasing pH greatly decreased the ζ potential. The difference in change of ζ potential is presumably due to the difference of surface arrangement between the surfaces modified with

HeteroPEG macromonomer and coated with Boron-PEG/PLA block copolymer. It should be noted that a considerable fraction of the surface boronic acid could be in a tetrahedral form of negatively charged boronate anions at the physiological pH7.4 on both surfaces (Fig. 2(a) and (b)).

3.4 Evaluation of the Selective Adhesion of Lymphocyte Subpopulation. The PEG layer possessing functional group at each free end thus prepared showed high utility as a novel interface such as surface used for the cell separation. The recovery of two major subpopulations of lymphocytes, B and T cells, from Boron-PEG macromonomer modified PVC channels were evaluated at pH7.4 and 4°C at varying stop-flow times, and the results are summarized in Figure 3. The prolonged stop-flow time, the settling time of lymphocytes in the channel, decreased the recovery of both T and B cells, reflecting the strength of cell/polymer interaction. Further worthy to mention is a substantial difference in the recovery of lymphocyte subpopulation from the chamber. The selective recovery of T cells was obvious, clearly demonstrating that B cells show higher affinity than T cells to Boron-PEG macromonomer modified PVC surfaces. An interaction between PEG/PLA coated surface and lymphocytes will be published elsewhere.

Figure 3. Effects of stop-flow time on the recovery of lymphocyte subpopulations from Boron-PEG macromonomer modified PVC chambers at flow velocity of 2.0 cm/sec. Data are expressed as the mean of four experiments with S.E.M.

4. Conclusion

Heterobifunctional PEG macromonomers and α -acetal-PEG/PLAs were utilized as surface modifier on the surfaces of PVC and polylactide to functionalize those surfaces. These polymers are useful as surface modifier in biomedical field, having non-fouling and ligand-immobilizable character. The surface with phenylboronic acid at the PEG chain end thus prepared can be applied

to cell separation surfaces demonstrated by a highly selective separation of lymphocyte subpopulations, B and T cells.

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