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# Functionalization of Polylactide (PLA) Surface using End-functionalized Block Copolymer of α-acetal-Poly(ethylene glycol) (PEG)/PLA

Hidenori Otsuka [1], Yukio Nagasaki[2], Yasuhisa Sakurai[3], Teruo Okano[3], and Kazunori Kataoka [1*]

Department of Materials Science, Graduate School of Engineering, The University of Tokyo,
Hongo 7-3-1, Tokyo 113-8656, Japan
[1] Fax:03-5841-7139, e-mail:otsuka@bmw.mm.t.u-tokyo.ac.jp/kataoka@bmw.mm.t.u-tokyo.ac.jp
[2] Department of Materials Science and Technology, Science University of Tokyo,
Noda, Chiba 278-8510, Japan
[2]Fax:0471-23-9362, e-mail:nagasaki@rs.noda.sut.ac.jp
[3] *Institute of Biomedical Engineering, Tokyo Women's Medical University*
[3]Fax:03-3359-6046, e-mail:tokano@lab.twmu.ac.jp

This paper deals with novel approaches established by our group for the construction of a functionalized poly(ethylene glycol) (PEG) layer, PEG-brushed layer possessing a reactive group at the free end of tethered PEG chain, on substrates. An AB-type block copolymer composed of α-acetal-poly(ethylene glycol) (PEG) as the hydrophilic segment and polylactide (PLA) as the hydrophobic segment was synthesized, and utilized to construct the functionalized PEG layer on the biodegradable polylactide surface by simple coating. In this way, a PEG-brushed layer with a terminal aldehyde group was readily prepared which may have both non-fouling and ligand-binding properties. Based on the characterization of these PEGylated surfaces from a physicochemical (contact angle, ζ potential, electron spin resonance) as well as biological (protein adsorption) point of view, our strategy to construct a functionalized PEG layer was confirmed. Active functional groups were present at the tethered PEG-chain end, these materials will have a high utility in the biomedical field.
Key words: poly(ethylene glycol) (PEG), polylactide (PLA), aldehyde, surface characterization

## 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, a poly(ethylene glycol) (PEG) coating has most widely been used to minimize non-specific fouling of the device surface with biocomponents, including plasma proteins. For example, a PEGylated surface, which means the surface attached to the tethered chains of poly(ethylene glycol) using the functionality of the PEG end groups, is reported to extremely lessen protein adsorption[2], resulting in the acquisition of high blood compatibility[3]. Although the PEG-coating can be performed by a variety of methods, most of the PEG-coated surfaces so far reported possess no reactive group on the PEG chain end. To provide the further functionality on the PEG-coated surface, we designed a block copolymer having end-functionalized PEG as a hydrophilic segment. Polylactide (PLA) was chosen as the hydrophobic segment because it is biodegradable and non-toxic, and is widely utilized as implant materials. Further, acetal group is installed at the α-chain end of poly(ethylene glycol)/polylactide block copolymers (PEG/PLA) which, apparently, is able to be delivatized to aldehyde group by the moderate acid treatment.

Our strategy is to construct a functionalized PEG layer on a biodegradable PLA surface through a simple coating of a reactive block copolymer of α-acetal-PEG/PLA[4-6]. After the construction of the polymer layer composed of α-acetal-PEG/PLA on a PLA surface, acetal groups at the free end of PEG chains are converted into aldehyde groups. This surface-engineering is shown schematically in Figure 1.

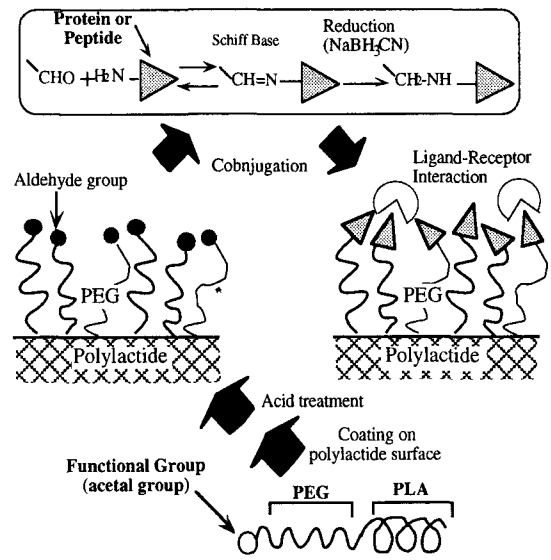


Figure 1. Schematic representation of the application to the construction of a PEG layer using a PEG/PLA block copolymer.

The hydrophobic PLA segments of the block copolymers anchor into the PLA surface, while the water-soluble PEG chains should extend into the bulk

aqueous medium. Various ligands including proteins, peptides, and sugars can be covalently immobilized to a functionalized-PEG coating on a PLA surface. An aldehyde group is very useful for this purpose due to its stability in water and its high reactivity with primary amino groups. The focus of this study was the accurate analysis of the surface properties of α-acetal- (or aldehyde-) PEG/PLA covered on a PLA matrix, as a basis for the further application of this strategy in the biomedical field, including functionalization of PLA-based devices. Particularly, the effect of varying the PEG molecular weight (MW) of the block copolymers was investigated from a physicochemical (ζ potential, static and dynamic wetting) as well as a biological (protein adsorption) point of view.

## 2. EXPERIMENTAL

2.1 Synthesis of Acetal-PEG/PLA Block Copolymers.

α-Acetal-PEG/PLA block copolymers were synthesized by a one-pot anionic ring-opening polymerization of EO followed by LA using potassium 3,3-diethoxypropanolate (PDP) as an initiator at room temperature under argon. The molecular weight of the PEG and PLA segments were determined by GPC, MALDI-TOF-MS, and NMR measurements.

2.2 Preparation of PEGylated surface and conversion of the acetal group into aldehyde group.

The glass substrates, which were cleaned by a Piranha etch (boiling mixture of 50% (v/v) sulfuric acid and 50 % (v/v) hydrogen peroxide), were placed in 2 % (v/v) solution of 3-(Trimethoxysilyl)propyl methacrylate/ethanol. The glass substrates were dried at 160 ℃ 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 string 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 string was immersed into PBS solution of the ESR probe, then subjected to sodium cyanoborohydride ($NaBH_3CN$). The electron spin resonance (ESR) was measured on an ESR spectrometer using $Mn^{2+}$ as the standard signal,

2.3 Characterization of acetal-PEG/PLA surfaces.

***Contact Angle.*** 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. The dynamic advancing ($\theta_{adv}$) and receding ($\theta_{rec}$) angles were obtained by extending and then contracting the volume of the drop at two different rates (1.98 ml/sec:$V_1$, 0.13 ml/sec;$V_2$). ***ζ potential.*** The ζ potential of acetal-ended PEG surfaces with different PEG chain lengths as a function of pH(5.8-8) was measured in phosphate buffer solution(I=0.01).

***Protein adsorption.*** Protein adsorption on the film samples was measured using bovine serum albumin (BSA) in phosphate-buffered saline (Dulbecco PBS (-)) solution (0.15 M, pH7.4), and the adsorbed amount was determined using the protein analysis kit (micro BCA protein assay reagent kit, Pierce, Rockford, IL, USA) based on the bicinchoninic acid (BCA) method.

## 3. RESULTS AND DISCUSSION

3.1 Synthesis of Acetal-PEG/PLA.

One of the objectives in this study is to investigate the effect of the variation in PEG chain length on surface properties. This includes assays on protein adsorption and cellular attachment to get a biochemical insight into the behavior of tethered PEG under biological conditions.

Table1.Molecular weights of PEG/PLA block copolymers

| sample[a] | PEG | | | | | | PLA |
|---|---|---|---|---|---|---|---|
| | Mn | | Mw | | Mw/Mn | | Mn |
| | GPC | MS | GPC | MS | GPC | MS | NMR |
| 1 | 685 | 650 | 770 | 720 | 1.12 | 1.10 | 11470 |
| 2 | 1920 | 1880 | 2110 | 1930 | 1.09 | 1.03 | 7020 |
| 3 | 3570 | 3340 | 3750 | 3470 | 1.05 | 1.04 | 5410 |
| 4 | 5100 | 5050 | 5670 | 5210 | 1.11 | 1.03 | 4640 |
| 5 | 8910 | 8730 | 9080 | 8810 | 1.02 | 1.01 | 6940 |

[a] 1;PEG/PLA(0.65/1.1), 2;PEG/PLA(1.8/7.0), 3;PEG/PLA(3.3/5.4), 4;PEG/PLA(5.0/4.6), 5;PEG/PLA(8.7/6.9).

For this purpose, numerous acetal-PEG/PLAs with different lengths of both PEG and PLA were synthesized. Molecular weights (MW) of PEG/PLA segments were abbreviated as follows (Table 1): PEG/PLA(0.65/11.0, 1.8/7.0, 3.3/5.4, 5.0/4.6, 8.7/6.9) where the numbers in parenthesis denote the MW of the PEG segments and PLA segments in kg/mol, respectively.

3.2 ζ potential measuraments.

Figure 2 provides the ζ potential profiles of acetal-PEG/PLA-coated glass substrates as a function of PEG molecular weight. The ζ potential of a cleaned glass surface showed clearly a negative charge(-50 to -70 mV). Covalent grafting of a silane layer onto the glass surface results in the introduction of methacryloyl groups and eventually increased the ζ potential, yet still showing a negative charge (~-20mV) (data not shown). When PLA homopolymer(M.W.; 20000) was spin-coated on silanized glass surface, the ζ potential was decreased again. This is due to negative charge of PLA polymer itself as reported by Dunn *et al.*[7]. Coating of PEG/PLA block copolymers onto PLA increased the ζ potential, and further a progressive increase is obvious with increasing PEG molecular weight,

indicating a screening of the surface charge. This means that the formation of the PEG brush layer shifts the position of the slipping plane into the solution side. Hydrophilic PEG segments are considered to expand away from the surface due to their strong hydration power.

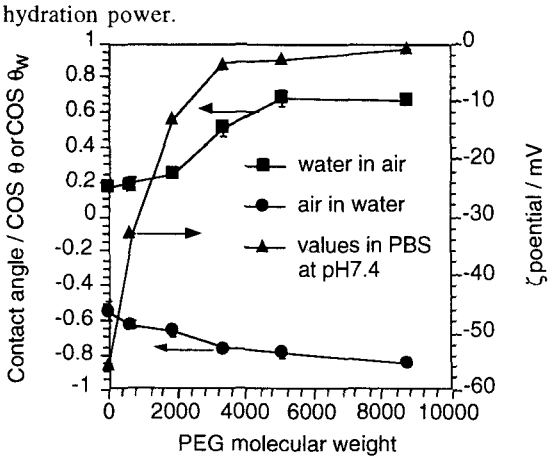


Figure 2. ζ potential variation and static contact angles in both water-in-air and air-in-water systems as a function of PEG M.W. on PEG/PLA coated substrates.

3.3 Static and dynamic contact angle measurements.

The static and dynamic wettability of the surface covered with PEG/PLA was estimated by the contact angle measurement (Figure 2). In both systems (water-in-air and air-in-water), coating of the PEG/PLA block copolymer on the PLA surface progressively increased its wettability with increasing PEG molecular weight, as indicated by a decrease in the static contact angle. One of the most sensitive methods that provides information on the outermost polymer surfaces of a few angstroms is the contact angle measurement. Thus, the relatively high contact angles on the surfaces containing the lower molecular weight PEG are most likely attributed to an incomplete coverage of the uppermost surface by the PEG chains.

The dynamic contact angle was then measured to estimate the dynamics of the uppermost surface. Comparing the dynamic wetting behavior of the PEG/PLA surfaces with different PEG MWs, the most striking finding is the marked decrease in receding contact angle on a PEG/PLA (3.3/5.0) surface resulting in the maximum hysteresis. This effect is further enhanced by employing slower extending/contracting velocity ($V_2$) of a water droplet, because of a longer contact time between the film surface and the water droplet. Hysteresis in the dynamic contact angle may be caused by the hydration of PEG segments. In the dry state, the PEG chain should assume a conformation flat to the surface experienced by the advancing contact line. Upon hydration, however, the PEG chain should extend from the surface due to the hydration of PEG chains. As a result, the receding contact line experiences a more hydrophilic surface than the advancing contact line.

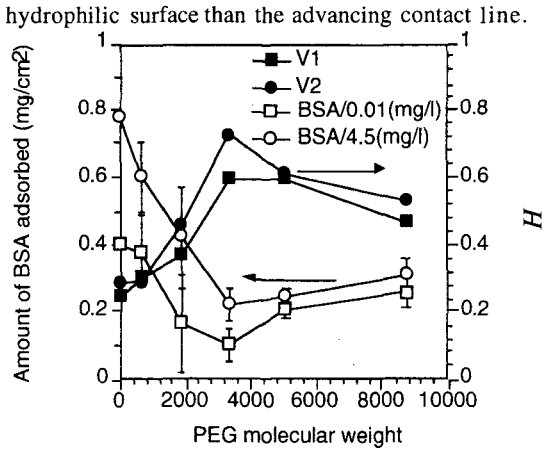


Figure 3. Reduced hysteresis and the adsorbed amount of BSA on PEG/PLA coated substrates as a function of PEG M.W.

In Figure 3, the dimensionless form, referred to as reduced hysteresis $H$, was plotted as a function of PEG MW to investigate the surface reorganization. The $H$ value increases with increasing PEG MW and then shows a maximum at a medium PEG chain length (PEG/PLA(3.3/5.0)), followed by an appreciable decrease. This surface rearrangement and interfacial orientation of PEG/PLA at the water interface occurs due to the high surface tension of water, which provides a strong driving force for polymer strands to orient so as to minimize the interfacial tension. It is worth noting that the maximum value of $H$ observed in PEG/PLA(3.3/5.0) is significantly attributed to the minimum value of $\theta_{rec}$, due to the highest hydration power. A conformational reorientation of the hydrophilic segment, PEG, is greatly influenced by the hydration power in water environment, and the surface property significantly affect the protein adsorption on the surface.

3.4 Protein adsorption.

Protein adsorption on PEG/PLA surfaces was measured using bovine serum albumin (BSA) as a model protein. Figure 3 shows the BSA adsorption from Dulbecco PBS (-) solution on various PEG/PLA surfaces. On a PLA surface, BSA was significantly adsorbed, while on PEG-coated surfaces BSA adsorption clearly decreased. As the PEG MW was increased, the amount of BSA adsorbed on the surface significantly decreased up to a PEG MW of ca. 3300. A further increase in PEG MW resulted in a slight increase in BSA adsorption. Thus, minimum adsorption was obtained at a medium PEG chain length, *i.e.*, PEG/PLA(3.3/5.4), note that this surface revealed the maximum hysteresis and $H$ value in dynamic contact angle measurement. Generally, PEG-coated substrates have shown reduced adsorption of proteins with increasing PEG MW. However, reduction in protein adsorption onto the present surfaces may not depend upon PEG MW but rather upon the high levels

of water that bind to the PEG component to increase PEG-hydration.

3.5 Conversion of an acetal group to an aldehyde group.

A functionalization of the PEG chain end provides the means for attaching ligand molecules for further chemical modulation of the surface. After the construction of the PEG/PLA surface, the acetal groups at the PEG-chain-end were successfully transformed into aldehyde end groups. An aldehyde group reacts smoothly with amino groups forming a Schiff base, a chemical path which can be employed for conjugation of proteins and peptides. To confirm the presence as well as to examine the reactivity of aldehyde group on the surface, model reactions with 2, 2, 6, 6-tetramethyl-1-piperidinyloxy (TEMPO) derivatives as label agents were performed and ESR specta of TEMPO derivatized surfaces were successively recorded (Figure 4)[5]. When the acetal surface was treated with 4-amino-TEMPO, only a slight signal was observed probably due to the physical adsorption of 4-amino-TEMPO on the surface (Fig. 4(b)). When the aldehyde surface was treated with TEMPO having no functional (amino) group, no ESR signal was observed (Fig. 4(c)). Contrary to these control treatments, three typical signals were clearly observed when 4-amino-TEMPO was used as the surface modification reagent, indicating that the effective covalent-conjugation of 4-amino-TEMPO with the aldehyde group at the end of PEG on the surface took place (Fig. 4(a)).

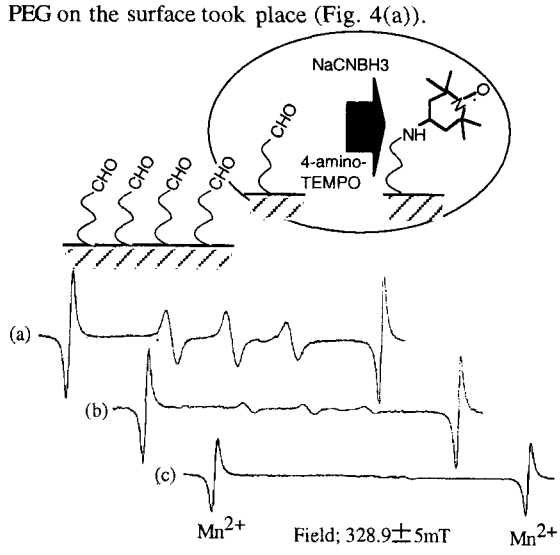


Figure 4. ESR spectra after the reaction between the PEG/PLA surface and TEMPO derivertives; a: aldehyde surface-4-amino-TEMPO, b: acetal surface-4-amino-TEMPO, c: aldehyde surface-TEMPO systems.

These results strongly suggest the high utility of these surfaces in the field of biomaterials; as proteins, sugars, and peptides can be immobilized at the end of PEG moiety by end-group activating PEG chains, the surface will show to support the growth of anchor dependent cells. Carbohydrate-based cell recognition has been applied in tissue engineering. The most extensively studied example is the use of monosaccharide binding to the asialoglycoprotein receptor on hepatocytes. Our surfaces were covalently modified with lactose, and the behavior of hepatocytes on these surfaces are investigated. Primary rat hepatocytes attached to and spread on the lactose-PEG/PLA surfaces but did not attach to control surfaces coated with PEG/PLA having no lactose. The lactose-immobilized PEG/PLA surface was also characterized by staining the surface with a fluorescent affinity label specific for galactose and quantifying the fluorescence. The quantity of fluorescein-labeled galactose-specific lectin, Ricinis communis (RCA 120, vector Labs), binding to surfaces was dependent on PEG molecular weight and therefore lactose surface concentration and/or lactose mobility at the PEG chain end. Polymers with no lactose were also investigated and showed negligible RCA binding, proving the specificity of the ligand-recepter interaction.

## 4. CONCLUSION

Heterobifunctional block copolymers, α-acetal-ω-hydroxy-PEG/PLA, were synthesized and coated on a polylactide surface, followed by the conversion of the acetal group into a aldehyde group. In this way, a PEG-brushed layer with a terminal aldehyde group was readily prepared which has both non-fouling and ligand-binding properties. Furthermore, aldehyde groups were confirmed to be present at the tethered PEG-chain end and can be derivertized with bioactive proteins and peptides with amino or hydrazide functionality. These results highlight the potential of this system to act as an engineered biomaterial as well as tissue engineering scaffold.

## 5. ACKNOWLEDGEMENTS

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