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Self-assembly of poly(ethylene glycol)-based block copolymers for biomedical applications

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Abstract

Nanostructure fabrication from block copolymers is discussed in this review paper. Particularly, novel approaches for the construction of functionalized poly(ethylene glycol) (PEG) layers on surfaces were focused to attain the specific adsorption of a target protein through PEG-conjugated ligands with a minimal non-specific adsorption of other proteins. Furthermore, surface organization of block copolymer micelles with cross-linking cores was described from the standpoint of preparation of a new functional surface-coating with a unique macromolecular architecture. The micelle-attached surface and the thin hydrogel layer made by layered micelles exhibited non-fouling properties and worked as a reservoir for hydrophobic reagents. These PEG-functionalized surface in brush form or in micelle form can be used in diverse fields of medicine and biology to construct high-performance medical devices including scaffolds for tissue engineering and matrices for drug delivery systems.

Keywords: Heterobifunctional block copolymer; Poly(ethylene glycol); Poly (lactide); Surface modification; Polymeric micelle; Bio-specific recognition

1. Introduction

Block copolymers in a selective solvent have a tendency to self-assemble at surfaces and into micelles [1–4]. At an aqueous interface, the amphiphilic property of block copolymers composed of hydrophilic and hydrophobic segments can cause the distal end of the hydrophilic chain to extend into the bulk aqueous solution, anchoring the hydrophilic block to the substrate surface through hydrophobic segments [1,2]. In an aqueous solution, micelles with core-shell structure are formed through the segregation of insoluble blocks into the core, which is surrounded by hydrophilic shell composed of hydrophilic blocks [3,4]. This interfacial activity of amphiphilic block copolymers provides their high utility in the biomedical field as colloidal dispersants, surface modifiers and drug carriers, prompting many studies of block copolymer adsorption on solid surfaces [5–8], force measurements between tethered layers [9–11] and the characterization of micelle properties. [12–14]. This review describes the recent progress in the field of block copolymer assembly on the surface and in the solution, focusing on the biological and biomedical application of poly(ethylene glycol) (PEG)-based block copolymers. PEG chains tethered on a surface or forming the corona of nanometer-scaled micelle exhibit the ability to sterically exclude other macromolecules and particles (steric stabilization) related to high flexibility and the large exclusion volume of PEG strands in water, and this is particularly useful for preventing the adsorption of proteins and adhesion of

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cells. In this regard, these supramolecular structures involving PEG-based block copolymers should be of a substantial importance for the development of blood-contacting biomaterials which are expected to play a key role in such fields as cell and tissue engineering, bio-sensing and drug delivery systems (DDS).

2. Construction of a PEG-brushed layer using block copolymers

PEG coatings have been used to minimize non-specific fouling of the surface of materials with biocomponents, particularly plasma proteins. For example, a PEGylated surface, which means the surface is covered with tethered chains of PEG using the functionality of PEG end groups, extremely reduces protein adsorption [15,16] resulting in a high blood compatibility [17,18]. PEG-coating can be performed using various methods such as covalent grafting of PEG which has reactive chain-ends to the surface [19,20], graft copolymerization of PEG macromonomer onto the surface [21,22], and direct adsorption of PEG onto surfaces in the form of a surfactant or a block copolymer in which one of the blocks is a PEG [23]. Adsorption of amphiphilic PEG-containing block copolymers on the surface allows temporary passivation of the surfaces towards cell and protein adsorption, but such coatings are not stable and the density of PEG achievable on the surface is typically lower than that required for complete passivation. Further, most of the PEG-coated surfaces possess no reactive group on the PEG chain end. To provide additional functionality on the PEG-coated surface, we designed PEG/polylactide (PLA) block copolymers (PEG–PLA) having an end-functionalized PEG (α-acetal-PEG) segment.

Recently, we have developed a facile and quantitative synthetic method for heterobifunctional PEG [24–28], which denotes PEG having different functional groups at each of both chain ends. When one of the functional end-groups in the heterobifunctional PEG selectivity initiates the polymerization of a hydrophobic monomer, a new heterobifunctional AB block copolymer can be prepared, keeping the other functional group at the PEG chain end available [29,30]. Particularly, lactide was chosen as the hydrophobic segment because PLAs are biodegradable and non-toxic polymers, which are widely utilized as implant materials or tissue engineering scaffolds. Moreover, both PEG and PLA were approved for clinical use by the Food and Drug Administration (FDA).

Reactive block copolymers of α-acetal-PEG–PLA can be utilized as surface modifiers of biodegradable PLA to provide the reactive sites on a PEGylated surface (Fig. 1) [31–33]. α-Acetal-PEG–PLA can be incorporated into the surface layer of PLA using a common solvent for PEG–PLA and the substrate PLA, allowing formation of a dense and stable PEG layer on PLA matrix to inhibit cell adhesion even in the presence of serum. Stability of incorporated block copolymers is enhanced compared with adsorbed molecules because the copolymer molecules become physically entangled with substrate PLA strands and the cohesive force between the PLA segments contributes to prevent the copolymer molecules from leaching out into the aqueous medium. The most serious problem encountered with surfaces designed for the purpose of bio-specific recognition such as the antigen–antibody and the sugar–lectin interactions is the non-specific adsorption of other proteins. In this regard, the non-fouling property provided by PEG coating is certainly a great advantage. As demonstrated in Fig. 2, inhibition of protein (bovine serum albumin) adsorption was achieved on PLA surfaces modified with α-acetal-PEG–PLA copolymers, depending on the PEG molecular weight [33]. Furthermore, ligands including proteins, peptides and sugars can be immobilized to the distal end of these PEG chains utilizing aldehyde functionality, which converted from α-acetal groups, constructing substrates that recognize a specific molecule with a least non-specific adsorption of other components. Actually, the presence as well as the reactivity of the aldehyde group on the surface was experimentally confirmed by employing a model reaction of an aldehyde group with an ESR probe, 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) derivative (Fig. 3) [33]. We further showed that α-acetal-PEG–PLA modified surfaces, which are not adhesive for hepatocytes, can be converted into the selective substratum for hepatocytes by covalent linkage of a carbohydrate ligand specific for the hepatocyte asialoglycoprotein receptor to the distal end of PEG chains [34]. This approach may be generally useful for developing regionally selective microarchitectured scaffolds fabricated from biodegradable polymers for spatial organization of co-cultured cells with different functions. The similar surface engineering was performed by Langer et al., who carried out the synthesis of a PEG–PLA block coplolymer with a biotinylated PEG end group which can be spin-coated on the substrate [35]. This polymer is biodegradable and resistant to non-specific protein adsorption, and the biotin moiety at the distal end of the PEG segment allows surface to be biospecific by binding avidin-conjugated ligands. Cannizzaro et al. also reported the development of a novel biodegradable polymer matrix designed to present bioactive motifs on the surface using a block copolymer of biotinylated PEG with PLA [36]. Surface engineering is achieved using avidin as a bridge between the

biotinylated polymer matrix and biotinylated ligand molecules. In this way, biotinylated peptides (GRGDS) were immobilized on the biotinylated PEGylated surface through the avidin bridge. These GRGDS-immobilized surfaces undergo an appreciable interaction with cells expressing integrin receptor and provoke desired cellular responses including spreading of endothelial cells [36].

Hubbell et al. investigated the new lactide-based PEG polymer networks (GL–PEGs) prepared by UV photopolymerization of two nontoxic macromers, triacrylated lactic acid oligomer emanating from a glycerol center (GL) and monoacrylated PEG [37,38]. By derivatizing the terminal hydroxyl function of the incorporated PEG with a bioactive peptide, these degradable networks become useful as polymer scaffolds for tissue engineering.

The assembly of the polymer film onto the surface based on the electrostatic interaction of the positively charged polymer backbone and the negatively charged metal oxide surfaces was reported using poly(L-lysine)-g-PEG, consisting of a poly(L-lysine) (PLL) backbone with PEG side chains [39,40]. Since this immobilization is based on electrostatic interactions, pH is clearly an important parameter for the tolerability of such systems. The resultant surfaces were found to exhibit drastic reduce of protein adsorption.

Whitesides et al. have used self-assembled functionalized monolayers (SAMs) of ethylene glycol oligomers ($-EG_nOH$, $n = 2-6$ and $-EG_6OCH_3$) with an alkanethiolate tail on gold substrate for the biospecific adsorption of a particular protein with a minimal non-specific adsorption of other proteins [41,42]. It is not clear whether the mechanism for the protein repellent property of SAMs having short oligo(ethylene glycol) chains is similar to that for high molecular weight PEG. The extensive solvation as well as large exclusion volume of PEG in water seems to play a

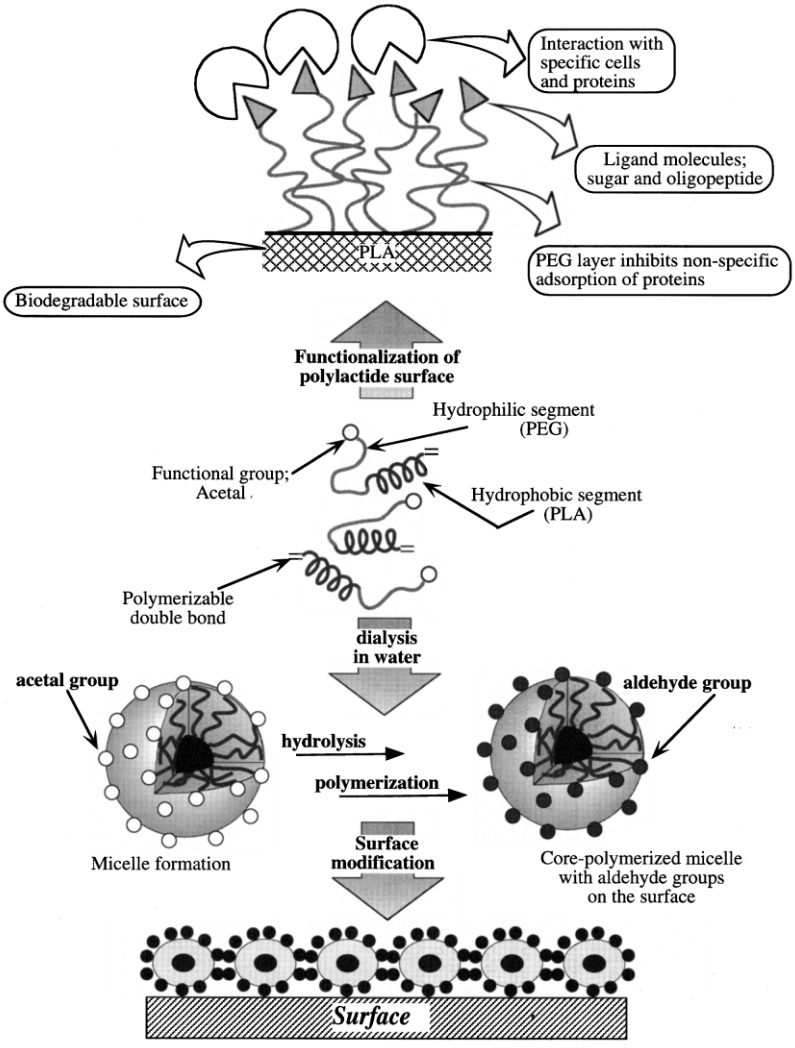

Fig. 1. Schematic representation of the application of heterobifunctional PEG–PLA block copolymer for the construction of functional PEG layer at the materials interface.

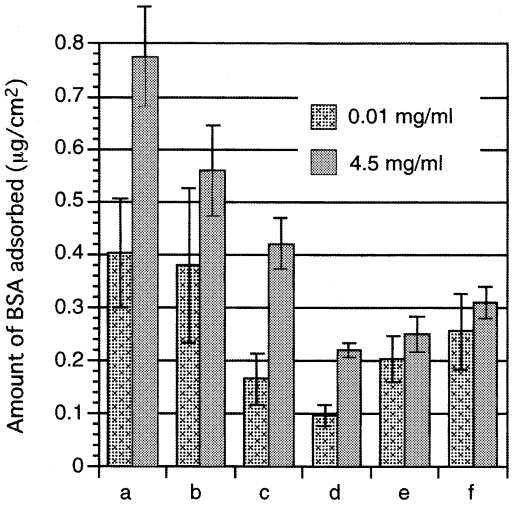

Fig. 2. BSA adsorption from Dulbecco PBS (−) solution on PLA and various α-acetal-PEG–PLA surfaces at room temperature after incubation for 90 min: PLA homopolymer (a), and α-acetal-PEG–PLA block copolymers of different PEG–PLA segments (b–f); (b) PEG–PLA (0.65/11.5), (c) PEG–PLA(1.8/7.0), (d) PEG–PLA (3.3/5.4), (e) PEG–PLA (5.0/4.6), (f) PEG–PLA (8.7/6.9), where the numbers in parentheses denote the molecular weight of the PEG segments and PLA segments in kg/mol, respectively. On a PLA surface, BSA was significantly adsorbed, while on PEG-coated surfaces BSA adsorption clearly decreased. The less-adsorptive character against BSA was more pronounced at the region with higher PEG molecular weight, especially at the PEG/PLA (3.3–5.4) surface, showing minimum adsorption of BSA.

critical role in inhibiting protein adsorption for the high molecular weight PEG, yet SAMs having dense packed oligo(ethylene glycol) groups probably have only insufficient volume to accommodate extensive solvation. They have used SAMs having both tri(ethylene glycol) groups and those with benzensulfonamide groups as a model substrate to study the bio-specific adsorption of carbonic anhydrase (CA), which is a well-characterized monomeric protein (MW = 30 000) that binds para-substituted benzenesulfonamide ligands with equilibrium dissociation constants (K_d) of approximately 10^{-6}–10^{-9} M [43]. When a complex mixture containing nine different protein species with no specific binding property to benzenesulfonamide group (2 mg/ml total concentration) was introduced into the flow cell equipped with surface plasmon resonance (SPR) detector, there was almost no change in the resonance indicating essentially no protein adsorption; however, when CA was present in this protein mixture, SPR detected the binding of CA with no disturbance due to the other proteins. This system thus may provide a convenient method for biophysical studies of biointerfacial recognition.

3. Surface modification with block copolymer micelles

Amphiphilic block copolymers consisting of PEG and PLA are known to assemble spontaneously into the micelle in an aqueous milieu. PEG–PLA micelles were investigated intensively aiming to apply in the field of modulated drug delivery due to the advantageous property provided by the hydrophobic core which can act as a reservoir for drugs [44] and the hydrophilic PEG shell to lend steric stabilization effect. Indeed, drugs can be loaded into the core by physical entrapment or by covalent linkage to the side-chain functional groups of the core-forming segment directly or via spacer molecule [44,45]. Also, the extended plasma half-life of the micelle was confirmed avoiding the recognition by reticulo-endthelial systems (RES) [46] due to the steric stabilization effect of PEG shell. If the layer of polymeric micelle can be immobilized on the surface with maintaining its core-shell architecture, the advantages of micelles in the solution including protein-repelling and drug-releasing properties should also be expected for this micelle-immobilized surface. Webber et al. reported that polystyrene-b-poly(methacrylic acid) micelles were able to be chemically attached to an activated silicon nitride surface using 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide methiodide as a coupling reagent [47,48]. The number-averaged distribution of the size based on the SEM and AFM data for dried micelles fixed on the surface correlated reasonably well with the size distribution data based on the light scattering data of the micelle in solution. However, physical coagulation forces may not always be stable enough to maintain the micelle structure in the process of surface fixation. A disruption of the micelle upon attachment to the surface is reported both experimentally and theoretically [49–51]. Johner and Joanny [51] used scaling arguments to show the disruption of the micelles during their adsorption process. This disruption results in the formation of loops and trains on the surface, and consequently, a densely packed orientation of the micelles may become difficult. In this regard, a method to improve the structural stability of the fixed micelle layer is required.

We recently reported a novel process of surface coating with polymeric micelles from amphiphilic block copolymers of PEG–PLA bearing an acetal group at the PEG end and a methacyloyl group at the PLA end [52,53]. The micelle prepared by dialysis was stabilized by polymerizing the methacryloyl group in the core and made reactive by the hydrolysis of the acetal group on the surface of the micelle into the aldehyde group. The resultant aldehyde-bearing micelle of 30–35 nm in diameter was coated on the aminated surface in the presence of $NaCNBH_3$. Since the micelle bears aldehyde groups on its surface, it reacts with amino groups on the substrate to form Schiff base that can be reduced into a stable secondary amine by $NaCNBH_3$. Due to the polymeriza-

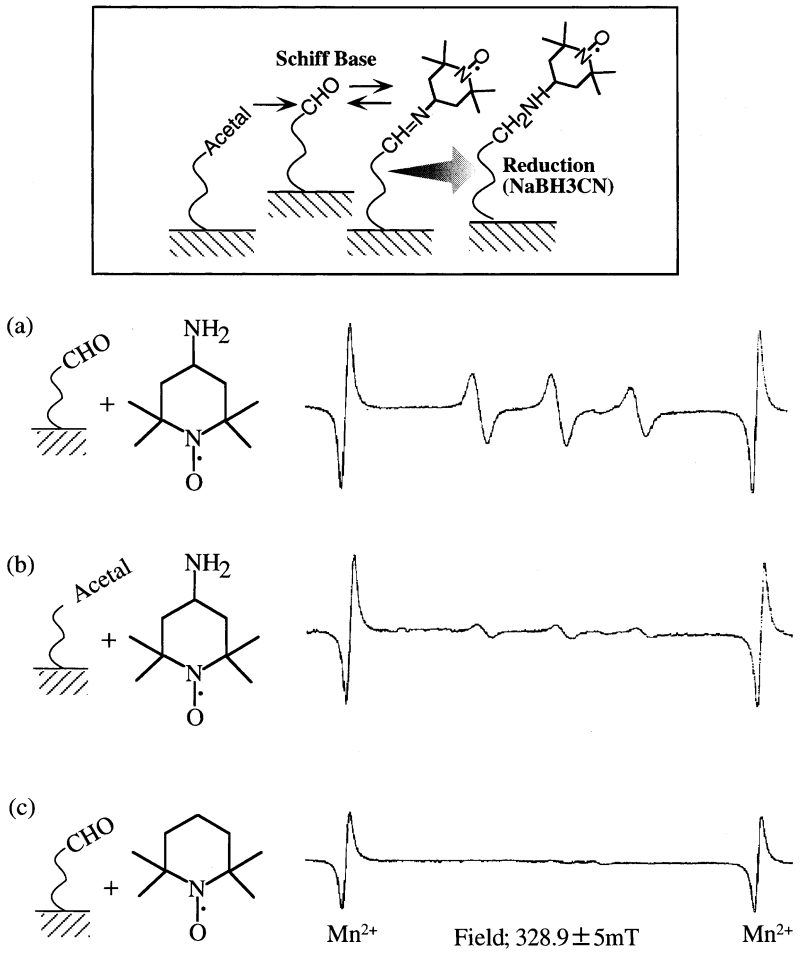

Fig. 3. ESR spectra after the reaction between the PEG-PLA surface and TEMPO derivatives. Three typical signals were clearly observed when 4-amino-TEMPO was used as the surface modification reagent of the aldehyde surface, indicating the effective covalent-conjugation of 4-amino-TEMPO with the aldehyde group at the end of PEG on the surface (a). When the acetal surface was treated with 4-amino-TEMPO as a control experiment, only a slight signal was observed probably due to the physical adsorption of 4-amino-TEMPO on the surface (b). When the aldehyde surface was treated with TEMPO having no functional (amino) group, no ESR signal was observed (c).

tion of the PLA-end located in the core, the core-shell structure was maintained even after the chemical fixation to the substrate, while non-polymerized micelles disrupted when they were attached to the substrates. The protein-repelling property of the micelle-coated surface was found to be comparable with that of the surface with dense coating of PEG, although the micelle coating was able to be performed under moderate conditions, i.e. from low concentration and at ambient temperature [53]. The ligands and a variety of biomolecules can be tethered on the surface via the micelle as a linker to attain specific ligand-receptor interaction on the non-fouling surface. The micelle tethered on a surface may also hold hydrophobic drugs in its core and release them in a controlled manner. In this regard, the surface density of the tethered micelle needs to be high enough to control the loading capacity and release rate of drugs. A multilayer structure was then formed by the alternate coating of polyallylamine (PAlAm) and micelles [53]. After the formation of a single layer of the micelle on the substrate, amino groups were then introduced on the top of the micellar layer by tethering PAlAm followed by the additional attachment of the second layer of the micelles. By repeating these alternate coatings of micelles and PAlAm in the presence of $NaCNBH_3$, micellar multilayers were formed on the substrate. The resultant multilayer is a thin hydrogel possessing layer-by-layer structure, and the thickness of the layer can be controlled by the number of coatings, which is peculiar to this method [53]. This surface hydrogel was found to appreciably prevent adsorption of protein and to release hydrophobic reagents in a controlled manner.

The cross-linking of the micellar core was reported by Liu et al. [54,55]. They used poly(2-cinnamoylethyl methycrylate) as the core-forming block, and carried out its photo-cross-linking to form covalently stabilized micellar architectures. Bates et al. reported on the synthesis of cross-linked poly(butadiene)-b-PEG di-

block copolymers in aqueous solution and obtained cylindrical structures [56]. The another way to achieve micelle stabilization is a cross-linking of the shell of amphiphilic block copolymer micelles presented by Wooley and co-workers [57,58]: they developed a way to prepare shell-cross-linked knedel-like structures (SCKs) of polystyrene-b-poly(acrylic acid) in which poly(acrylic acid) as shell-forming block was cross-linked by amidation with di- and multifunctional amino linkers. The non-cross-linked polymer micelles deformed substantially upon adsorption onto mica and became ellipsoidal upon drying on a carbon surface, whereas the SCKs remained as stable spherical structures under harsh conditions. Recently, they reported that the cross-linked shell of SCKs constitutes a stable network that expands after removal of the degradable core to give hollow nanocages, although the organized assembly of polymer micelles is destroyed upon removal of the nucleating core domain [59,60]. These nanoarchitectures are of a particular interest due to their potential for encapsulation of large quantities of guest molecules, including biologically active components, within the empty core domain.

4. Conclusions

Recently, the tremendous progress have been attained in the characterization and application of nanostructured materials using block copolymers. Nanostructure fabrication from block copolymers involves polymer design, synthesis, self-assembly and derivatization. Block copolymers self-assembled into micelles or adsorbed on the surface both in brush and micelle form afford a powerful means of manipulating the characteristics of surfaces and interfaces, and therefore, are expected to have novel applications. Particularly, biomedical applications have been explored in a variety of research areas from the physico-chemical as well as biological point of views. In this review, we highlighted how the amphiphilic block copolymer having self-assembling properties to form brushes on the surface and to form micelles in the solution. These micelles can further be utilized for surface modification by their adsorption and/or covalent attachment. Novel approaches for the construction of functionalized PEG layers on surfaces were discussed to achieve the bio-specific adsorption of a target protein through an appropriate ligand tethered on PEG layers without non-specific adsorption of other proteins. Further, the intramicellar cross-linking of the core- or shell-forming block of the micelles has led to the preparation of new materials possessing unique macromolecular architectures. The thin hydrogel made by layered structure of core-polymerized micelles exhibited non-fouling properties and worked as the reservoir for hydrophobic reagents. These surfaces functionalized in brush or in micelle form can be used in diverse fields of medicine and biology to construct high-performance medical devices including scaffolds for tissue engineering and matrices for drug delivery systems.

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