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Highly Protein-Resistant Coatings and Suspension Cell Culture Thereon from Amphiphilic Block Copolymers Prepared by RAFT Polymerization

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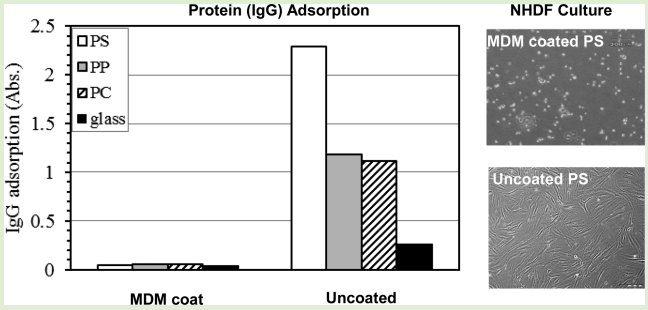
ABSTRACT: Novel amphiphilic block copolymers composed of hydrophobic (poly(2-methoxyethyl acrylate): M) and hydrophilic (poly(*N*,*N*-dimethylacrylamide): D) segments were synthesized by living radical polymerization: a reversible addition−fragmentation chain-transfer polymerization. Two types of amphiphilic block copolymers, triblock (MDM) and 4-arm block ($(MD)_4$) copolymers with specific compositions (D/M = (750−1500)/250), were prepared by a versatile one-pot synthesis. These copolymers show good adhesion to various types of substrates (e.g., polystyrene, polycarbonate, polypropylene, Ti, and glass), and the surface coating showed high protein repellency and a low contact angle for water, regardless of the substrate. The two opposing characteristics of high protein repellency and good substrate adhesion were achieved by the combined effects of the molecular architecture of the block copolymers, the high molecular weight, and the characteristics of each segment, that is, low protein adsorption capability of both segments and low glass transition temperature of the hydrophobic segment. Further, a polystyrene dish coated with the MDM block copolymer could be sterilized by γ-ray irradiation and used as a good substrate for a suspension cell culture that exhibits low cell adhesion and good cell growth.



INTRODUCTION

The inhibition of nonspecific adsorption of biomacromolecules, especially proteins, at solid−liquid interfaces is an important consideration for materials used in many biorelated applications such as biochemical devices, biosensors, microarrays, drug-delivery systems, and controlled cell growth systems, because protein adsorption on the surfaces changes the surface properties and limits the accuracy and precision of analysis. Because protein adsorption is believed to be the first step in the inflammatory response, it may also lead to platelet adhesion, thrombosis, and microbial infections in medical devices and in vivo implanted materials. Among many approaches for imparting high resistance to protein adsorption so far investigated, hydrophilic modification of the surface, using hydrophilic poly(ethylene glycol) (PEG) units, in particular, has been one of the most promising approaches.[1−3] Because of the hydrophobicity of proteins, PEG-coated surfaces show a high resistance toward protein adsorption owing to the low polymer−water interfacial energy and high hydrophilicity. To date, many types of hydrophilic and amphiphilic systems using PEG or PEG-containing polymers such as polymer brushes,[4,5] self-assembled monolayers,[2] amphiphilic networks,[6] graft and comb copolymers,[7,8] and block copolymers[9−11] have been fabricated and used as protein-repellant and antibiofouling materials. However, the development of new effective protein-resistant materials without using PEG is still required, because PEG is known to be unstable in the presence of oxygen and transition metals, thus, reducing the lifetime of the material.[12] It is also desired to fabricate a protein-resistant surface on different types of biomedical substrates by a simple procedure. A polymer coating is the simplest and most versatile method to create a protein-resistant surface. So far, it has been reported that some polymers with specific structures, such as 1,2-propandiol methacrylate copolymers[13] and zwitterionic polymers (e.g., phosphoryl choline-functionalized polymers[14] and sulfobetaine polymers[15]) showed good antifouling properties. However, some difficulty is often encountered in achieving both high protein repellency and good substrate adhesion simultaneously. In the present study, we report novel amphiphilic block polymers that provide both characteristics in their coatings.

Living radical polymerization techniques such as atom-transfer radical polymerization (ATRP),[16−18] nitroxide-mediated radical polymerization (NMP),[19] and reversible addition−fragmentation chain transfer (RAFT) polymerization[20−22] have been widely used for the synthesis of block polymers,[23−30] graft polymers,[31−34] and hyperbranched copolymers[35−38] with well-controlled molecular architectures. In particular, RAFT

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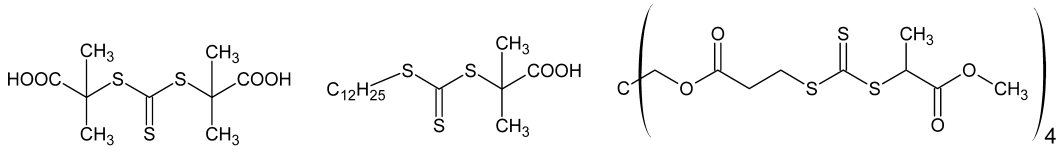
Scheme 1. RAFT (Reversible Addition−Fragmentation Radical-Transfer Polymerization) Agents for Triblock Copolymer (CTA-1), Diblock Copolymer (CTA-2), and 4-Arm Block Copolymer (CTA-3)



CTA-1 CTA-2 CTA-3

polymerization has been used as the simplest method because it requires only the addition of a specific chain-transfer agent in a radical polymerization system.[23−26,28,31−34,36] In the present study, novel amphiphilic diblock, triblock, and 4-arm block copolymers, consisting of hydrophobic poly(2-methoxyethyl acrylate) (PMEA) and hydrophilic poly(*N*,*N*-dimethylacrylamide) (PDMAA) segments were synthesized by RAFT polymerization. The protein adsorption, substrate adhesion, and suspension cell culture were investigated for surfaces coated with these block copolymers.

MATERIALS AND METHODS

Materials. The two monomers, *N*,*N*-dimethylacrylamide (DMAA, Kohjin Co., Japan) and 2-methoxyethyl acrylate (MEA, Toagosei Co. Japan), were purified by passing through a column of activated alumina to remove the inhibitor and stored in the dark at low temperature (4 °C) until use. The initiator 2,2′-azobis(isobutyronitrile) (AIBN, Kanto Chemical Co. Inc. Japan) was recrystallized from methanol and stored under the same dark, low-temperature conditions. 1,4-Dioxane (Wako Pure Chemicals, 99.5%) was used as a solvent after purification by passing through activated alumina. As a poor solvent, diethyl ether (Wako Pure Chemicals, Japan, 99.5%) was used without purification.

RAFT Agents. The chemical structures of the three RAFT agents used in the present study, 2-(1-carboxy-1-methylethylsulfanylthiocarbonylsulfanyl)-2-methylpropionic acid (CTA-1), 2-dodecylsulfanylthiocarbonylsulfanyl-2-methylpropionic acid (CTA-2), and pentaerythritol tetrakis(3-(*S*-(1-methoxycarbonylethyl)trithiocarbonyl)propionate) (CTA-3), are shown in Scheme 1. CTA-1, CTA-2, and CTA-3 were used for the synthesis of the triblock, diblock, and 4-arm block copolymers, respectively. CTA-1 and CTA-2 were prepared according to previously reported procedures.[39,40] Briefly, for CTA-1, a 50% NaOH aqueous solution (10.09 g, 126 mmol) was slowly added (over 40 min) to a solution consisting of carbon disulfide (1.37 g, 18 mmol), chloroform (5.38 g, 45 mmol), tetrabutylammonium hydrogen sulfate (0.12 g, 0.35 mmol), acetone (2.62 g, 45 mmol), and hexane (6 mL) under a nitrogen atmosphere while keeping the temperature between 20 and 24 °C. The reaction mixture was stirred for another 2 h at the same temperature and was left overnight. After water (45 mL) was added to dissolve the resulting solid, 12 N HCl (6 mL) was added, and nitrogen gas was bubbled in order to remove the gas evolved. After filtration and washing with water five times, the sample was dried at room temperature and subsequently recrystallized using 60% aqueous acetone. Thus, CAT-1 was obtained as a yellow powder (0.934 g). The yield was 37% based on carbon disulfide.

For CTA-2, a 50% NaOH aqueous solution (0.84 g, 11 mmol) was slowly added to a solution consisting of 1-dodecanethiol (2.00 g, 10 mmol), tetrabutylammonium hydrogen sulfate (0.27 g, 0.80 mmol), and acetone (6.0 g, 103 mmol) at 4 °C under a nitrogen atmosphere. After stirring for 20 min, carbon disulfide (0.76 g, 10 mmol) in acetone (1.0 g, 17 mmol) was slowly added, followed by the addition of chloroform (1.86 g, 16 mmol) and 50% aqueous NaOH (4.0 g, 50 mmol; over 30 min). The reaction mixture was then stirred under nitrogen at ambient temperature for 7 h and left overnight. Water (25 mL), followed by 12 N HCl (2.5 mL), was added, while nitrogen gas was bubbled in order to remove the gas evolved. After filtration and washing with water five times, the sample was dried at room temperature (3.01 g of yellow powder was obtained). Isopropanol (25 mL) was added to the powder and then stirred. The undissolved solid was filtered off and identified as *S*,*S*′-bis(1-dodecyl)trithiocarbonate (1.09 g). The orange filtrate was evaporated to dryness and subsequently recrystallized from hexane. Thus, CAT-2 was obtained as a yellow powder (1.13 g). The yield was 31% based on 1-dodecanethiol.

The third RAFT agent, CTA-3, was prepared as follows. Triethylamine (2.04 g, 20 mmol) was added dropwise to a stirred solution of pentaerythritol(3-mercaptopropionate) (1.22 g, 2.5 mmol) and carbon disulfide (2.00 g, 26 mmol) in dichloromethane (10 mL) at room temperature. The solution gradually turned deep yellow and turbid during the addition. The solution was allowed to stir for an additional 1 h. Methyl 2-bromopropionate (1.94 g, 12 mmol) was then added dropwise. The resulting solution turned clear and turbid within a few minutes. The solution was stirred for a further 3 h and left overnight. The mixture was washed with 5% $KHSO_4$ aqueous solution and subsequently with water (three times each). The dichloromethane layer was dried over $MgSO_4$ and evaporated to afford a thick orange oil. The oil was purified by silica gel column chromatography using acetone and hexane as the eluents (gradient elution from 1:2 to 1:0) to obtain the target RAFT compound (2.53 g, 88%).

The three RAFT agents obtained were all stored in the dark and at low temperature (4 °C). NMR data are as follows. CTA-1: ^{13}C NMR (in CD_3OD): δ 25.6 ($\underline{C}H_3$), 57.2 ($\underline{C}(CH_3)_2$), 176.1 ($\underline{C}O_2H$), 220.3 ($\underline{C}$═S). CTA-2: ^{1}H NMR (in $CDCl_3$): δ 0.88 (t, J = 8 Hz, 3H, $CH_2C\underline{H}_3$), 1.25−1.30 (m, 16H, $(C\underline{H}_2)_8$), 1.30−1.40 (m, 2H, $SCH_2CH_2C\underline{H}_2$), 1.68 (quintet, J = 8 Hz, 2H, $SCH_2C\underline{H}_2$), 1.73 (s, 6H, $C(C\underline{H}_3)_2$), 3.28 (t, J = 8 Hz, 2H, $SC\underline{H}_2$). CTA-3: ^{1}H NMR (in $CDCl_3$): δ 1.60 (d, J = 8 Hz, 12H, $CHC\underline{H}_3COOCH_3$), 2.80 (t, J = 8 Hz, 8H, $C\underline{H}_2CO$), 3.61 (t, J = 8 Hz, 8H, $C\underline{H}_2S$), 3.74 (s, 12H, $COOC\underline{H}_3$), 4.16 (s, 8H, $C\underline{H}_2O$), 4.81 (q, J = 8 Hz, 4H, $SC\underline{H}CH_3$).

Sample Nomenclature. The block copolymers consisting of PMEA and PDMAA segments, that is, the diblock copolymer (PMEA-*b*-PDMAA), triblock copolymer (PMEA-*b*-PDMAA-*b*-PMEA), and 4-arm block copolymer (PMEA-*b*-PDMAA)$_4$, are represented as MD, MDM, and $(MD)_4$, respectively. Also, the compositions of the block copolymers are denoted using the mole ratio of each monomer relative to CTA (RAFT agent), for example, M(250)−D(250), M(250)−D(1500)−M(250), $(M(250)-D(750))_4$. The random copolymers are similarly represented as D(1500)-*co*-M(500). In the present study, the block copolymers were mainly synthesized by the one-pot procedure described in the next section. Thus, the resulting block copolymer was composed of pure PMEA segments and a PDMAA segment that was slightly copolymerized with MEA. For example, when the conversion of MEA in the first polymerization step was 90 mol %, the residual MEA monomer (10 mol %) was incorporated in the second polymerization (PDMAA). The actual composition of the block copolymer prepared by the one-pot procedure is represented as M(230)−[D(1500)-*co*-M(40)]−M(230) and $(M(230)-[D(750)\text{-}co\text{-}M(20)])_4$.

Syntheses of Polymers. *Preparation of the Triblock MDM Copolymer by a Two-Step Synthesis.* Step 1: Preparation of macro-CTA-PMEA: A solution of MEA (5.84 g, 44.8 mmol), CTA-1 (25.4 mg, 0.090 mmol), AIBN (1.4 mg, 0.008 mmol), and 1,4-dioxane (20 mL) was sealed in a 50 mL flask equipped with a magnetic stir bar and then purged with nitrogen for 90 min. The polymerization was conducted by heating the reaction flask at 70 °C for 18 h. The reaction mixture was quenched by cooling and exposing the solution to air. The

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polymer was precipitated into diethyl ether. The yellowish oily product was collected and washed three times with diethyl ether and then dried in vacuo at 40 °C. The conversion determined by ^{1}H NMR was 92%. Step 2: The PMEA-*b*-PDMAA-*b*-PMEA (MDM) triblock copolymer via RAFT polymerization was synthesized as follows: A solution of the yellowish oil obtained in step 1 (2.67 g), DMAA (6.66 g, 67.2 mmol), AIBN (0.7 mg, 0.004 mmol), and 1,4-dioxane (20 mL) was sealed in a 50 mL flask equipped with a magnetic stir bar and then purged with nitrogen for 90 min. The polymerization was conducted by heating the reaction flask at 70 °C for 24 h. The reaction mixture was quenched by cooling and exposing the solution to air. The polymer was precipitated into diethyl ether. The precipitate (powder) was collected, washed three times with diethyl ether, and then dried in vacuo at 40 °C. The conversion of DMAA, as determined by ^{1}H NMR, was 99.9%.

Preparation of Triblock (MDM) Copolymer by One-Pot Synthesis. A solution of MEA (2.92 g, 22.4 mmol), CTA-1 (12.7 mg, 0.045 mmol), AIBN (0.7 mg, 0.004 mmol), and 1,4-dioxane (10 mL) was sealed in a 50 mL flask equipped with a magnetic stir bar and then purged with nitrogen for 90 min, and the reaction flask was placed in an oil bath (70 °C). After 8 h, a DMAA solution (DMAA (6.66 g, 67.2 mmol) in 1,4-dioxane (10 mL)) purged with nitrogen was added to the hot MEA solution via syringe within 2 min. Heating was continued for another 24 h, and the polymerization was quenched by cooling and exposing the solution to air. The polymer was precipitated into diethyl ether. The precipitate (powder) was collected, washed three times with diethyl ether, and dried in vacuo at 40 °C. The final conversions of MEA and DMAA, as determined by ^{1}H NMR, were 99.9 and 99.5%, respectively.

Preparation of 4-Arm ((MD)$_4$) Block Polymer by One-Pot Synthesis. The 4-arm block copolymer was synthesized with CTA-3 as the chain-transfer agent and AIBN as the initiator in a manner similar to that described for the triblock copolymer. The final conversions of MEA and DMAA, as determined by ^{1}H NMR, were 99.9 and 99.5%, respectively.

Preparation of Diblock (MD) Copolymer by One-Pot Synthesis. The diblock copolymer was synthesized using CTA-2 as the chain-transfer agent and AIBN as the initiator. MD was collected by the same procedure used for the triblock copolymer. The final conversions of MEA and DMAA, as determined by ^{1}H NMR, were 99.9 and 99.5%, respectively.

Preparation of Random (M-co-D) Copolymer. The random copolymer was synthesized using a solution of MEA (2.92 g, 22.4 mmol), DMAA (6.66 g, 67.2 mmol), CTA-1 (12.7 mg, 0.045 mmol), AIBN (0.7 mg, 0.004 mmol), and 1,4-dioxane (20 mL). M-*co*-D was collected by the same procedure used for the triblock copolymer. The conversions of MEA and DMAA, as determined by ^{1}H NMR, were 99.3 and 98.3%, respectively.

Coatings. *Surface Coating.* A uniform aqueous dispersion of copolymer was prepared by mixing water (10 g) and copolymer (0.5 g), followed by the addition of an aqueous solution (150 μL, 20 wt %) of sodium dodecylbenzenesulfonate (SDBS). Here, a very small amount of SDBS surfactant was added to improve the dispersion of the copolymer and its stability and wettability to the substrate. The aqueous dispersion was coated on the surface of a polystyrene (PS) dish (nontreated PS dish: 35 mm, Corning Inc. (NY), No. 430588) by spin-coating with a spin coater (Opticoat, model MS-A150: Mikasa) at 2000 rpm for 20 s. After coating, the PS dish was dried in air at 80 °C for 10 min to fix the copolymer on the surface. The PS dish was then washed by immersion in an excess amount (120 g per dish) of sterile water at 50 °C for 10 min. This was repeated three times to remove surfactant and unfixed polymer. Finally, the PS dish was dried in an incubator at 40 °C for 5 h. For MDM, the coating was also applied to other substrate (plates), that is, polypropylene (PP), polycarbonate (PC), and titanium (Ti) plates, and a glass dish (AS ONE, FS-45), in addition to a PP centrifuge tube (IWAKI, 15 mL centrifuge tube, 2323−015) in a manner similar to that described above. Here, only the PP plate and PP centrifuge tube were pretreated by irradiation of corona (3 × 5 s) at 250 W using a CTW-0212 (Wedge Co, Ltd.) to increase the surface wettability.

Extracts from Coated Surface. During the MDM-coating process, the wash water was concentrated by 42.5× and then subjected to HPLC measurements in order to check the materials detached from the surface. To confirm the adhesion to the substrate, the extraction test was further performed for the MDM-coated PS dish, glass dish, and PP, PC, and Ti plates under the following conditions: 1.54 mL water per dish (plate), 40 °C, 15 h. The extracts from the coated surface were evaluated using HPLC. The extraction test was also carried out using phosphate buffered saline (PBS) for the MDM-coated PS dish. The surface contact angles for water (θ_w) before and after the extraction test were also examined.

Sterilization. The MDM-coated PS dish and PP centrifuge tube were treated by γ-ray irradiation at 10 kGy for sterilization. The effects of the γ-ray irradiation on the protein adsorption and the contact angles were investigated for the MDM-coated PS dish and PP centrifuge tube.

Measurements. *^{1}H NMR Spectroscopy.* ^{1}H NMR measurements were conducted with a JEOL JNM-LA300 spectrometer operating at 300 MHz with $CDCl_3$ as a solvent.

HPLC. The MDM block copolymer detached from the coated surface during the washing and extraction process was measured by HPLC (Alliance System, Waters 2695 Separations Module: Japan Waters Ltd.) under the following conditions: Using a dual λ absorbance detector and inertsil ODS-3 column, 20% acetonitrile aqueous solution, 40 °C.

Molecular Weight. The weight-average and number-average molecular weights (M_w and M_n) and the M_w/M_n ratio of the block copolymers were measured using size exclusion chromatography (SEC): Tosoh HLC-8220GPC equipped with a refractive index detector, a set of two TSK-gel α-M columns (length: 30 cm each), *N*,*N*-dimethylformamide with 0.1-mM LiBr, 40 °C, 1.0 mL/min (flow rate). Calibration was performed with PMMA standards (Shodex Standard M-75).

Differential Scanning Calorimetry (DSC). Measurement of the glass transition temperatures (T_g) of the triblock copolymer, a random copolymer, and two homopolymers were performed using a DSC-7 (PerkinElmer Inc.) under a nitrogen gas atmosphere, heating from −78 to 150 °C at a heating rate of 1 °C min^{-1}. The glass transition temperatures were obtained from the second run after heating up to 150 °C in the first run.

Transparencies. The optical transmittance of an aqueous dispersion of the block copolymer was determined at 600 nm with a UV−visible spectrophotometer (V-530, Jasco Co., Japan) in a cubic PS cuvette (10 × 10 × 30 mm) at 25 °C. Ultrapure water was used as the reference.

Particle Size. The average particle size (median diameter) of an aqueous dispersion of the block copolymer was measured using a dynamic light scattering (LS) particle-size analyzer LB-550 (HORIBA, Ltd.) at 25 °C. The concentration of the block copolymer was 0.5 wt %.

Coating Thickness. The thickness of the coated layer of MDM on the PS dish was measured using an ellipsometer (UVISEL, HORIBA, Ltd.) under the following conditions: Incident angle = 60°, wavelength range = 1.5−6.5 eV (826−191 nm), n = 3, nine points were measured in each sample.

Scanning Electron Microscopy (SEM). The surface of the PS dish coated with the block copolymers was observed using a scanning electron microscope (SEM: SEM007, Nihon Denshi Co.).

Surface Contact Angle. The surface contact angles for water (θ_w) were measured using a surface-contact-angle measuring instrument (WPI-3000: Kyowa Kagaku) by depositing a drop of water (8 μL) on the surfaces of the pristine and coated substrates (n = 9).

Protein Adsorption. The protein adsorption on the surfaces of the various substrates (PS and glass dishes; PP, PC, and Ti plates; PP centrifuge tube) coated with the block and random copolymers were examined using horseradish peroxidase (HRP)-conjugated antimouse immunoglobulin (IgG) antibody (Abbiotec, LLC). All the coated surfaces were immersed in 1 mL of a 0.2 μg/mL antibody solution and then incubated at room temperature for 2 or 24 h. After rinsing with phosphate buffered saline (PBS (−)), 1 mL of 3,3′,5,5′-tetramethylbenzidine (TMB, substrate for HRP) was added, and then the

enzymatic reaction was discontinued by the addition of 1 mL of 1 N HCl. Next, 0.2 mL of this solution was transferred into a 96-well plate, and the absorbance was measured at 450 nm on an MTP-810 Lab microplate reader (Hitachi Corp.; n = 3). The blank test was performed using TMB and HCl. Then, the adsorption of IgG (Ad(IgG)) on the various substrates coated with block copolymers were determined by subtracting the blank value (0.034) from the observed value. The adsorption of bovine serum albumin (BSA) on an MDM-3-coated PS dish was examined by pouring a BSA solution (0.04 mg/mL, 1 mL) consisting of FITC (Fluorescein isothiocyanate: A9771 SIGMA) and PBS buffer on the coated surface at room temperature for 2 h. After rinsing lightly with 3 mL of PBS three times, the BSA adsorbed on the coated surface was measured using a microplate reader (MTP-810Lab, Corona Electric Co. Ltd. Japan) with an excitation at 490 nm and an emission at 530 nm. As a control, the adsorption on the surface of a noncoated PS dish was also measured in a similar manner.

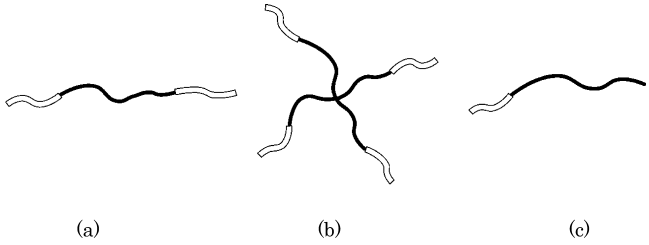
Cell Attachment. Human skin fibroblast cells (NHDF, DS Pharma Biomedical Co., Ltd.) were seeded at 1×10^4 cells/cm^2 in a 35 mm PS dish with an MDM coating. NHDF was cultured with 10% fetal bovine serum (FBS) and Dulbecco's Modified Eagle Medium (DMEM: Gibco) for 3 d. Cell attachment was observed using phase-contrast microscopy (CKX41, Olympus Co., Ltd.). For cell counting, an ATP assay was performed with a cellular ATP kit (Toyo B-net Co., Ltd.). In brief, 1 mL of an ATP solution was added to the dish and incubated for 1 min at room temperature after the rinsing step with PBS (−). Then, 0.2 mL of this solution was transferred into a 96-well plate, and the luminescence was measured on an MTP-810 Lab microplate reader. The cell culture in the 35 mm MDM-coated PS dish was also conducted for Balb3T3 cells (3T3; Human Science Research Resources Bank, Japan) with the culture medium (DMEM) supplement with 10% FBS. The 3T3 cells were seeded on the surface at a density of 1.0×10^4 cells/cm^2 and incubated in 5% CO_2/95% air at 37 °C for 3 d.

Cell Growth. Mouse macrophage-like cells (J774A.1, JCRB9108, The Health Science Research Resources Bank, HSRRB) were seeded at 0.5×10^4 cells/cm^2 in a 35 mm MDM-coated PS dish. J774A.1 was cultured with 10% FBS and DMEM for 6 d. Cell growth was observed using phase-contrast microscopy. The cell number was counted using NucleoCounter (ChemoMetec).

RESULTS AND DISCUSSION

Architecture of Block Polymers. The aim of this study is to develop new coating materials that satisfy the characteristics of good substrate adhesion and ultralow protein adsorption, and in addition, low cell adhesion and high cell growth thereon. We designed triblock and 4-arm block copolymers consisting of PMEA (hydrophobic)−PDMAA (hydrophilic)−PMEA (hydrophobic) sequences, because hydrophobic and flexible PMEA chains at both ends may be favorable for attachment to the substrate, and both the PMEA and PDMAA segments may contribute to high resistance to protein adsorption. Scheme 2a,b shows the structure of the triblock and 4-arm block copolymers consisting of PMEA and PDMAA segments. Here, although PMEA is essentially a hydrophobic polymer, it is quite different from conventional hydrophobic polymers such as PS and poly(methyl methacrylate) (PMMA), which have been widely used as hydrophobic components in amphiphilic block copolymers.[21,25,32,33] In contrast to PS and PMMA, PMEA exhibits high substrate adhesion owing to its low glass-transition temperature (−34 °C) and functional groups. Also, PMEA exhibited unique low protein adsorption despite its high hydrophobicity,[41,42] which was attributed to the water molecules (i.e., freezing bound water) adsorbed on the $-OCH_3$ groups of PMEA.[43] However, because of the intractability of PMEA (it is very sticky and has difficulty in forming a self-standing film), MEA has been used only as an ingredient for copolymers. Recently, two types of soft and mechanically tough PMEA-based materials (films), that is, PMEA−clay nanocomposites (M-NC)[44] and MEA/DMAA copolymer−clay nanocomposite gels (MD-NC gels),[45] were developed using exfoliated inorganic clay through an in situ free radical (co)polymerization in aqueous media. The resulting M-NC film and MD-NC gel film showed superb properties such as high transparency (regardless of the clay concentration), high mechanical properties (e.g., highly stretchable and widely controlled modulus and strength),[44,45] effective cell cultivation, and subsequent thermoresponsive cell harvest without enzymatic treatment.[46,47] Also, the safety of these materials was confirmed by in vitro cytotoxicity tests using V79 cells.[45] In the present study, novel MEA-based amphiphilic block copolymers, that is, triblock MDM and 4-arm block $(MD)_4$ copolymers, were synthesized by living RAFT polymerization, and the biomedical characteristics, such as protein adsorption and cell attachment and growth on their coated surfaces, were investigated and compared with those of diblock and random copolymers.

Scheme 2. Amphiphilic Block Copolymers Consisting of Hydrophobic PMEA (white) and Hydrophilic PDMAA (black) Segments[a]



[a](a) Triblock MDM copolymer, (b) 4-arm $(MD)_4$ block copolymer, and (c) diblock MD copolymer.

Synthesis of Block Copolymers. In the synthesis of the triblock MDM copolymer using CTA-1 (RAFT agent), the time dependence of the MEA conversion during the first step of the living radical polymerization is shown in Figure 1. The

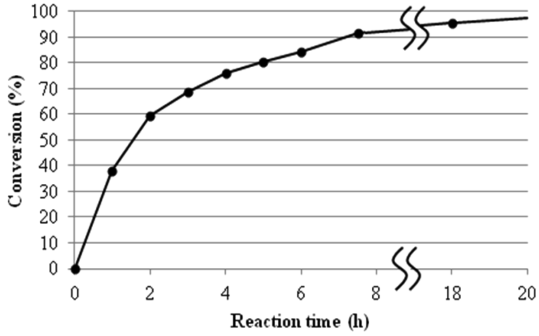


Figure 1. Monomer conversion as a function of polymerization time for reversible addition−fragmentation chain-transfer (RAFT) polymerization of 2-methoxyethyl acrylate (MEA) with CTA-1 at 70 °C.

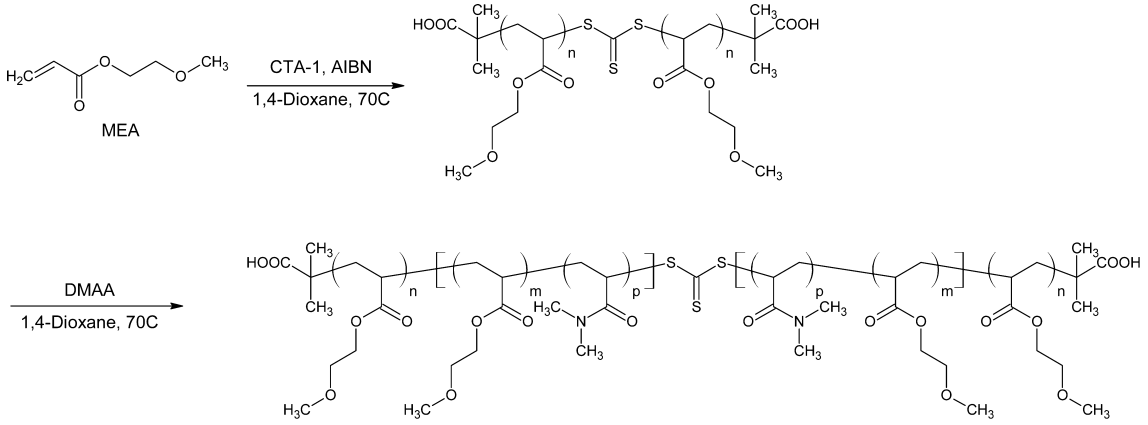
MEA conversion reached 90% within 8 h, and it slowly increased upon further increasing the polymerization time. Therefore, the synthesis of the ideal block copolymer requires two steps. The first step is the synthesis of the PMEA segment attached to the RAFT agent (M-CTA1-M). After the polymerization, M-CTA1-M should be collected by precipitation into a poor solvent, followed by purification and drying

Table 1. Characteristics of Block Copolymers Prepared by Reversible Addition−Fragmentation Chain-Transfer Polymerization of MEA and DMAA with Trithiocarbonate RAFT Agents

			conversion[b] (%)			
block copolymer	composition[a]	CTA	MEA	DMAA	M_w[c] (×10^3; g/mol)	M_w/M_n[c]
MDM-1	M(230)-[D(750)-*co*-M(40)]-M(230)	CTA-1	99.7	96.8	99	1.92
MDM-2	M(230)-[D(1200)-*co*-M(40)]-M(230)	CTA-1	100	98.1	98	2.19
MDM-3	M(230)-[D(1500)-*co*-M(40)]-M(230)	CTA-1	99.9	98.0	141	2.17
MDM-3*	M(230)-D(1500)-M(230)	CTA-1	92.0	99.9	152	1.88
m-RAFT[d]	M(230)-CTA1-M(230)	CTA-1	92.0		58	1.41
MDM-4	M(230)-[D(5000)-*co*-M(40)]-M(230)	CTA-1	99.9	98.6	284	2.66
MDM-5	M(230)-[D(10000)-*co*-M(40)]-M(230)	CTA-1	99.9	99.5	439	2.85
$(MD)_4$	$(M(230)-[D(750)-co-M(20)])_4$	CTA-3	99.9	99.0	206	2.42
MD	M(230)-[D(1500)-*co*-M(20)]	CTA-2	98.9	95.1	169	2.24
M-*co*-D	D(1500)-*co*-M(500)	CTA-1	99.3	98.3	140	2.41

[a]Number in bracket after M (D) represents the mole ratio of MEA (DMAA) relative to CTA. [b]Determined by ^{1}H NMR spectroscopy. [c]Determined by SEC. [d]Macro-RAFT agent for MDM-3*.

Scheme 3. One-Pot Synthesis of Triblock Copolymer Prepared by RAFT Copolymerization of MEA and DMAA with Bifunctional CTA-1



under vacuum, as described in the Materials and Methods. The characterization data of the macro-RAFT agent (M-CTA1-M), such as the conversion (^{1}H NMR), molecular weight (M_w), and M_w/M_n ratio (SEC), are given in Table 1. A second polymerization of DMAA was then started in the presence of M-CTA1-M, which acts as a macro-RAFT agent for DMAA. Thus, triblock MDM copolymers consisting of pure PMEA and pure PDMAA segments were prepared. However, this procedure had several disadvantages in terms of productivity, such as the labor-intensive synthetic process (e.g., the precipitation and purification of the intermediate), amount of waste (e.g., good and poor solvents), and loss of the monomer (MEA). More importantly, M-CTA1-M as a macro-RAFT agent was quite intractable (highly viscous and oily) because of the very low T_g of the PMEA segment, and lengthy periods of time were often needed to collect and purify it. In addition, as discussed in a later section, the triblock copolymers prepared by a one-pot procedure showed almost the same protein resistance and adhesion to substrates as those of the triblock copolymer synthesized in two steps. Thus, in the following work, we mostly adopted the one-pot synthesis, that is, a sequential addition living radical polymerization, for the block copolymers.

In the sequential addition reversible deactivation radical polymerization, the second monomer (DMAA) was subsequently added to the PMEA solution without any separation and purification of the intermediate macro-RAFT agent, and the second polymerization was continued for 24 h. The conversion of both monomers was almost 100% in the final product. Thus, in the present study, different types of block copolymers were prepared in a similar manner except for the use of a different RAFT agent, i.e., CAT-1 for MDM, CAT-2 for MD, and CAT-3 for $(MD)_4$. In these block copolymers, the PDMAA segments were slightly copolymerized with approximately 10 mol % of MEA, which was residual monomer from the first step of the polymerization, as shown in Scheme 3 for the triblock copolymer.

Characterization of Block Copolymers. The progress of the RAFT polymerization was followed by ^{1}H NMR, and the resulting block copolymers were analyzed by ^{13}C NMR and SEC measurements. Figure 2 shows the ^{1}H NMR spectrum for a typical MDM block copolymer (MDM-3 in Table 1) prepared from an initial solution with MEA/DMAA = 1.00/3.00. In the spectrum, the peaks at δ 4.20 and δ 2.90 correspond to the protons of MEA (−COOC$\underline{H}_2$−) and DMAA ((−CON(C$\underline{H}_3$)$_2$), respectively. The conversions of the monomers shown in Table 1 were calculated from the ^{1}H NMR data.

The characterization of the block copolymers, such as conversion (^{1}H NMR), molecular weight (M_w), and M_w/M_n ratio (SEC), are summarized in Table 1 for the triblock copolymers (MDM-1−5) with different compositions and the diblock (MD), 4-arm block $(MD)_4$, and random (M-*co*-D) copolymers. In MDM-1−5, the composition of DMAA was varied between 750 and 10000, while the MEA component was

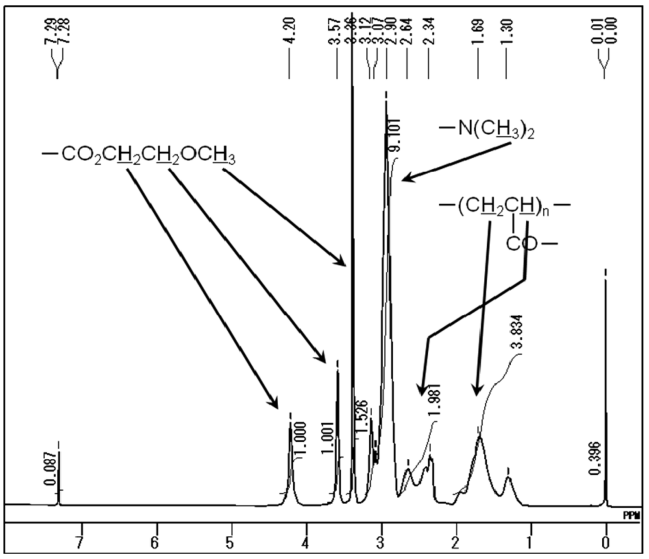


Figure 2. ^{1}H NMR spectrum of MDM triblock copolymer (sample: MDM-3, $CDCl_3$). Molar ratio in the feed was MEA/DMAA = 1.00/3.00.

fixed at 250. MDM with a lower PDMAA length (e.g., 500) was hardly obtained as a uniform sample. On the other hand, MDM-3* was a triblock copolymer with a composition similar to MDM-3, but it consists of pure PMEA and PDMAA segments because it was synthesized in two steps. The SEC chromatograms for these block and random copolymers as well as a macro-RAFT agent listed in Table 1 are shown in Figure 3.

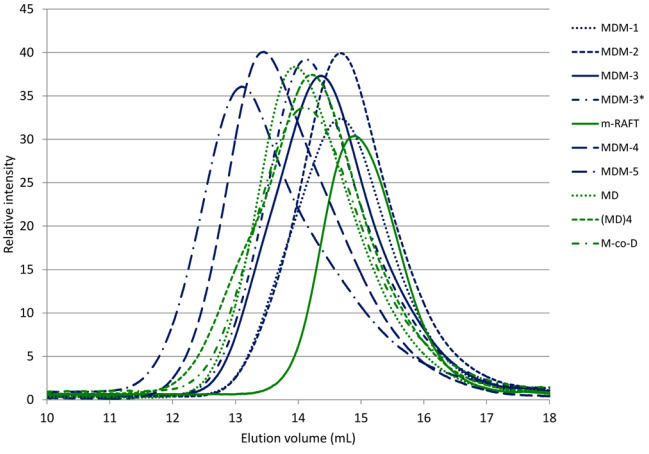


Figure 3. SEC traces as a function of time for the amphiphilic triblock, 4-arm block, diblock, and random copolymers, as well as a macro-RAFT agent listed in Table 1. All the samples were polymerized in 1,4-dioxane at 70 °C in the presence of the RAFT agent (CTA1−CTA3).

All the SEC traces except for that of $(MD)_4$ were unimodal, indicating that the block copolymers do not contain significant amount of low-molecular-weight homopolymers. In the case of $(MD)_4$, a slight shoulder appeared in the range of higher molecular weight, which can be attributed to the product of star polymer coupling reaction as observed in the synthesis of a polystyrene star polymer via RAFT polymerization.[48]

In Table 1, it should be noted that the M_w/M_n values of all the block copolymers, including triblock MDM and 4-arm block $(MD)_4$, prepared in the present study, were always close to or higher than 2. This value is much higher than that ($M_w/M_n \approx 1.2$) of block copolymers (e.g., DEGA-*b*-DMAA[49] and NIPA-*b*-DMAA-*b*-NIPA[50]) prepared by RAFT polymerization in organic solvent so far reported. In these reports, although both block copolymers were prepared in organic solvent (DMF and THF) and at high temperature (70 and 65 °C), similarly to the present study, there were large differences in the molecular weight of the block copolymers and the other synthetic conditions, for example, the monomer concentration in the reaction solution, the mole ratio of RAFT agent/second monomer, and the monomer conversion. In the present study, the MDM and $(MD)_4$ block copolymers were prepared with a high concentration of monomer (4.5 M), a low mole ratio of RAFT agent (1/1540) in the reaction solution, and a high monomer conversion (100%). Consequently, the polymers had high molecular weights (M_w = 150000−400000). In contrast, the diblock and triblock copolymers in the references were prepared using a low monomer concentration (1.7[49] and 1.6 M[50]), relatively high mole ratios of RAFT agent (1/100[49] and 1/400[50]), and low monomer conversions (54[49] and 50−62%[50]) and, consequently, had low molecular weights (M_w = 15000[49] and 24000−36000[50]). Thus, the higher polydispersity of the MDM and $(MD)_4$ block copolymers was attributed to the synthetic conditions and the high molecular weight. The increasing trend of polydispersity index (PDI) with increasing molecular weight was hardly reported because the previous studies mostly focused on block copolymers with a low molecular weight. There are only a few examples in the literature, reporting increasing PDI with increasing molecular weight in RAFT polymerization, for example, in the synthesis of the diblock copolymer of isobutylene with amino acid-based monomers by RAFT polymerization,[51] in which the PDI (M_n) changed from 1.16 (6600) to 1.48 (13500) and 1.34 (11000) to 1.54 (19400), even though the molecular weight was much lower than that of the polymer reported in this study. Even for homopolymers prepared by RAFT polymerization in organic solvent,[52−54] low values of M_w/M_n (1.1) were accompanied by low molecular weights (M_w = 3000−22000). It was very difficult to achieve low M_w/M_n values under the synthetic conditions used in the present study. On the other hand, from the result (M_w/M_n = 1.41) of the macro-RAFT agent (M-CTA1-M) shown in Table 1, it was estimated that the high dispersity of MDM-3 resulted from both processes of the macro-RAFT agent synthesis and the following DMAA polymerization. The reason why we adopted these synthetic conditions, despite the resulting high polydispersity, was to achieve the desired characteristics, that is, strong adhesion to the substrate and high protein repellency in addition to a high polymerization yield, which is important for the future mass production of coating materials.

Figure 4a shows the ^{13}C NMR spectrum for triblock copolymer MDM-3 (Table 1). All the peaks were assigned to the carbons in the PMEA and PDMAA segments. From the ratio of the peak area, the MEA/DMAA ratio was found to be 1.00/3.03, which is very close to the ideal value (1.00/3.00). Figure 4b(1)−(5) shows the carbonyl carbon signals in the ^{13}C NMR spectra (172−177 ppm) for the two block copolymers (MDM-3 and MDM-3*), the random copolymer (M-*co*-D), and the individual polymers PMEA and PDMAA, respectively. In addition to the main peaks observed in the range 173.9−174.4 ppm, which are assigned to the carbons in the PMEA and PDMAA segments, a broad peak at around 175 ppm was observed in M-*co*-D (Figure 4b(3)). This is likely assigned to the random sequence of the MEA and DMAA units.

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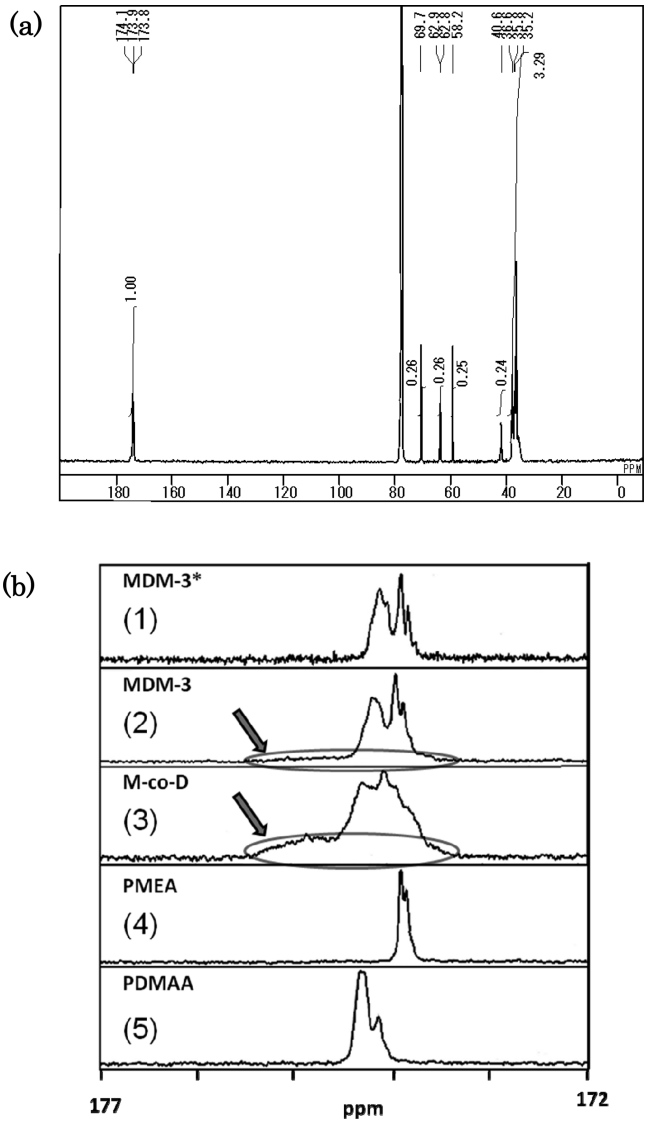

Figure 4. (a) ^{13}C NMR spectrum of MDM triblock copolymer (sample: MDM-3: M(230)-[D(1500)-*co*-M(40)]-M(230)). (b) Comparisons of carbonyl carbon signals. (1) triblock copolymer (MDM-3*), (2) triblock copolymer (MDM-3), (3) random copolymer (M-*co*-D), and pure polymers ((4) PMEA and (5) PDMAA). The deuterated solvent of all the samples was $CDCl_3$.

Hirota et al.[55] also reported a broad peak at around 175 ppm, assigned to the carbonyl carbon in the random units in poly(MEA)-*co*-poly(2-hydroxyethyl methacrylate). A similar but smaller broad peak at around 175 ppm was also observed for MDM-3 (Figure 4b(2)). This is because the MDM-3 sample contained a small amount of the random sequence of MEA and DMAA in the center segment; the composition is M(230)-[D(1500)-*co*-M(40)]-M(230). On the other hand, it was observed that the block copolymer MDM-3*, which was prepared by a two-step synthesis, did not have such a broad peak (Figure 4b(1)). This is consistent with the fact that MDM-3* has no random sequence of MEA and DMAA. Thus, from the ^1H and ^{13}C NMR spectra, it was confirmed that the MDM block polymers prepared by the one-pot synthetic procedure consist of pure PMEA segments and slightly copolymerized PDMAA segments.

DSC measurements were performed for two typical samples, MDM-3 (triblock copolymer) and M-*co*-D (random copolymer), as well as M and D (homopolymers). The results showed that both homopolymers showed distinct values of T_g at −37 °C (M) and 124 °C (D), which are consistent with those reported previously,[44] and that M-*co*-D also showed a single T_g at 50 °C. In contrast, MDM-3 showed two values of T_g at −34 and 112 °C. This indicates that MDM-3 consist of block sequences of M and D. The reason for the slight lower T_g value (112 °C) for the D sequence compared with that of the D homopolymer is attributed to the slight copolymerization of the D sequence in MDM-3, that is, D(1500)-*co*-M(40). These data strongly support the block character of MDM-3, in contrast to the random characteristics of M-*co*-D.

The copolymers synthesized in the present study showed different solubility in water depending on the structure and composition. The optical transmittance of aqueous dispersions of the block and random copolymers (5 wt %) and their particle sizes are given in Table 2. The random copolymer (M-*co*-D) and triblock copolymers with high DMAA content (>5000, MDM-4 and MDM-5) formed a transparent aqueous dispersion, and the dispersion of the diblock MD copolymer was translucent. On the other hand, the other polymers, MDM-1, MDM-3, and $(MD)_4$, formed opaque (white) dispersions with particle sizes in the range 400−500 nm. Because the latter aqueous dispersions were fairly unstable and, more importantly, it was difficult to uniformly apply a thin coat to the hydrophobic substrate because of the relatively high contact angle (e.g., 53° for the MDM-3 aqueous dispersion against a PS dish), a small amount of surfactant (SDBS) was added to the dispersions. The characteristics of the aqueous dispersions with SDBS (0.3 wt %) are also given in Table 2. In all dispersions, the particle size became smaller, for example, approximately 200−350 nm for MDM-1, MDM-3, and $(MD)_4$, and the dispersions became more stable. Further, the contact angles of all the dispersions against a PS dish were largely down to less than 5°.

Table 2. Characteristics of Aqueous Dispersions of Block and Random Copolymers[a]

	aqueous dispersion without SDBS		aqueous dispersion with SDBS[b]	
sample	T[c] (%)	D_{avg} (nm)	T[c] (%)	D_{avg} (nm)
MDM-1 M(230)-[D(750)-*co*-M(40)]-M(230)	0.3	431	0.7	208
MDM-3 M(230)-[D(1500)-*co*-M(40)]-M(230)	0.2	466	0.4	328
MDM-4 M(230)-[D(5000)-*co*-M(40)]-M(230)	93.5	271	99.1	56
MDM-5 M(170)-[D(10000)-*co*-M(160)]-M(170)	94.8	281	98.8	66
$(MD)_4$ (M(230)-[D(750)-*co*-M(20)])$_4$	15.6	479	43.3	348
MD (M(230)-[D(1500)-*co*-M(20)]	68.3	195	97.9	66
M-*co*-D D(1500)-*co*-M(500)	99.6	26	99.0	29

[a]The concentration of the copolymer in the aqueous dispersion was 5 wt % for the measurement of optical transmittance (T) and 0.5 wt % for the measurement of average particle size (median diameter: D_{avg}). [b]The concentration of SDBS was 0.3 wt %. [c]Optical transmittance at 600 nm.

Coatings on PS Dish. The aqueous dispersions (with SDBS) of the block and random copolymers listed in Table 1

were coated on a 35 mm PS dish by spin-coating, followed by drying and washing, as described in the Materials and Methods. The resulting coated surface was transparent and uniform in all cases, as observed by optical microscopy and SEM. The coating thickness of MDM-3 was evaluated with an ellipsometer to be 133 ± 3 nm. The contact angle for water (θ_w) was changed by the coating from 79° for the uncoated PS dish to the values shown in Table 3. In the case of MD, M-*co*-D, and MDM-5, the contact angles on their surface coatings were close to that on the uncoated surface. This indicates that these three copolymers were mostly removed from the surface during the washing process. In fact, the detached polymer was qualitatively observed as a white residue in the freeze-dried wash water of the MD, M-*co*-D, and MDM-5 coatings. The poor adhesion of MD, M-*co*-D, and MDM-5 was mainly due to the molecular architecture and the composition, as discussed later.

Table 3. Contact Angle for Water (θ_w) and Adsorption of IgG (Ad(IgG)) on PS Dish Surfaces Coated with MDM, $(MD)_4$, MD, and M-*co*-D

sample	composition	θ_w	Ad(IgG)
MDM-1	M(230)-[D(750)-*co*-M(40)]-M(230)	22.2	0.024
MDM-2	M(230)-[D(1200)-*co*-M(40)]-M(230)	24.6	0.006
MDM-3	M(230)-[D(1500)-*co*-M(40)]-M(230)	24.8	0.005
MDM-3*	M(230)-D(1500)-M(230)	21.5	0.017
MDM-4	M(230)-[D(5000)-*co*-M(40)]-M(230)	24.8, 46.3	0.012, 0.06
MDM-5	M(170)-[D(10000)-*co*-M(160)]-M(170)	61.6	(1.192)
$(MD)_4$	$(M(230)-[D(750)-co-M(20)]_4$	33	0.021
MD	(M(230)-[D(1500)-*co*-M(20)])	74.3	(0.742)
M-*co*-D	D(1500)-*co*-M(500)	69.8	(0.673)
	uncoated PS dish (Corning)	79.0	2.185
MDM-3 (γ-ray 10kGy)	M(230)-[D(1500)-*co*-M(40)]-M(230)	24.5	0.012

In contrast, the contact angles on the surfaces coated with MDM-1−3, MDM-3*, and $(MD)_4$ were approximately 20−30°, which indicated that the triblock and 4-arm block copolymers are strongly attached (i.e., well coated) on the PS dish. In fact, the detached polymer was hardly observed in the same freeze-drying procedure in the coating process of these polymers. For MDM-3, little detachment observed in the washing process was quantitatively evaluated by HPLC measurements (Figure 5). The detached polymer was slightly but clearly observed in the first and second washings (1st + 2nd), but it was hardly observed in the third washing. From the data shown in Figure 5 and the coating thickness (133 nm), the amount of detached polymer was estimated to be approximately 3.5 wt % of the total MDM-3 coated on the PS dish. Here, the detached polymer was not a homopolymer of PDMAA or PMEA, but probably part of a block copolymer. This was supported by HPLC measurements (Figure 4: the profile of the detached polymer was the same as that of the original MDM-3) and ^{1}H NMR measurements (showing both the MEA and DMAA components) of the detached polymer. The D/M ratio (3.54) is slightly higher than that (3.0) of the original MDM-3. On the other hand, the fluctuating θ_w observed for the MDM-4 coating (Table 3) may be attributed to the partial detachment of the coated polymer because of its high solubility and intermediate DMAA/MEA ratio between MDM-3 and MDM-5. Thus, it was concluded that MDM and $(MD)_4$ with a DMAA content in the range 750−1500 at a fixed MEA content (250) were well coated onto the PS dish, and the coated surfaces exhibited low contact angles for water. In order to confirm the stable adhesion of typical MDM, an extraction tests with warm water and PBS were further conducted for the MDM-3-coated PS dish. Even after extraction with water (40 °C) or PBS (40 °C) for 24 h, the PS dish surface coated with MDM-3 maintained a low θ_w (23°) in both the cases. Further, even after three consecutive extractions, the coated surface still

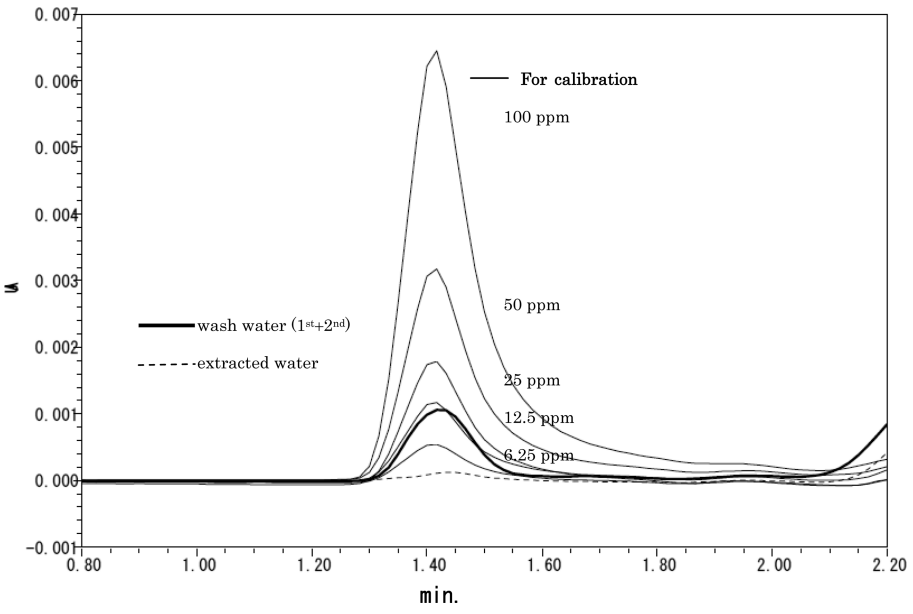

Figure 5. HPLC charts measured for wash water obtained from MDM-3/PS dish-coating process and extracted water obtained from MDM-3-coated PS dish. Both waters were used for measurements after concentrating 42.5 times from the original. Calibration data were obtained for aqueous solutions of MDM-3 block copolymer (6.25−100 ppm).

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exhibited a low θ_w (24°). In addition, almost no detached polymer was observed in the HPLC measurements for the (concentrated) extracted water (Figure 5). These results indicate that MDM-3 adhered strongly to the PS dish.

The poor adhesion of the diblock MD and random M-*co*-D copolymers shown in the above (Table 3) is attributed to the architecture of the copolymers. Although MD and M-*co*-D have the same overall composition as MDM-3, only MDM-3 showed good adhesion to the substrate because of its triblock architecture. In random and diblock architectures, the hydrophobically driven adsorption of the copolymer onto the surface would be decreased. Further, the fact that triblock MDM-4 showed a more stable adhesion than diblock MD, despite MDM-4 having a lower M content (%) than MD, suggests the importance of the triblock architecture consisting of a hydrophobic (M)−hydrophilic (D)−hydrophobic (M) sequence. Thus, the strong adhesion to the substrate and the high protein resistance (shown in the next section) are attributed to the architectures of the triblock MDM and 4-arm block $(MD)_4$ copolymers and their high molecular weights.

Protein Adsorption. Nonspecific protein adsorption on the surface of the PS dishes coated with the block and random copolymers was examined using an HRP-conjugated antimouse IgG antibody. As shown in Table 3, the PS dish surfaces coated with MDM-1−3 showed very low protein adsorption (Ad(IgG) = 0.01 ± 0.01), regardless of the composition, in contrast to the high adsorption observed on the uncoated PS dish surface (Ad(IgG) = 2.19). Also, MDM-3 coated surface exhibited very low protein adsorption (Ad(IgG) = 0.01) even after performing the extraction tests with warm water or PBS. In addition, both PS dish surfaces coated with $(MD)_4$ and MDM-3* also showed very low protein adsorption, similar to MDM-1−3 (Table 3). These results indicate that the 4-arm block copolymer consisting of outer PMEA and inner PDMAA segments can inhibit protein adsorption, as similarly shown by the MDM triblock copolymers. Also, the fact that MDM-3 and MDM-3* showed the same low protein adsorption indicates that the slight modification of the center segment, that is, M(230)-[D(1500)-*co*-M(40)]-M(230), does not decrease the low protein adsorption property of MDM. The low-protein-adsorption data described above changed little even when the adsorption time was increased to 24 h.

On the other hand, for the other coatings with MD, M-*co*-D, and MDM-5, higher protein adsorption was observed, which was probably due to the incomplete coating of the copolymers, as evidenced by the contact angles. Also, the fluctuating protein adsorption observed for MDM-4 was probably due to the partially incomplete coating, as similarly shown by the value of θ_w (Table 3). Thus, it was concluded that the triblock MDM and 4-arm block $(MD)_4$ copolymers with favorable compositions of DMAA and MEA exhibit ultralow protein adsorption, as well as high adhesion to the PS dish surface.

The protein repellency of the MDM-3 coating on a PS dish was also examined using another protein (fluorescence BSA). It was observed that the MDM-3-coated surface exhibited very low protein adsorption; the adsorption of BSA on MDM-3/PS and noncoated PS is 0.161 and 1.045 (unit is fluorescent intensity per 10 cm^2), respectively. It was concluded that the MDM-3 coating also gave low protein repellency for BSA. For extension studies on the protein repellency, the in vivo thrombotic properties of the MDM coating will be reported in a subsequent paper.

The protein adsorption on the surface of the MDM-3-coated PS dish was further examined after γ-ray irradiation, which is important in actual use in biomedical and biochemical applications. It was found that the very low protein adsorption on the MDM-3-coated PS dish was maintained even after γ-ray irradiation of 10 kGy, as shown in Table 3.

MDM Coating on Various Substrates. A typical triblock copolymer, MDM-3, was coated on the surfaces of different types of substrates, that is, PP, PC, and Ti plates and a glass dish, which are useful for biomedical applications, in a manner similar to the PS dish. In the case of PP, the plate was pretreated by irradiation of corona (3 × 5 s) before coating to improve the surface wettability of the substrate. The adhesion of MDM-3 to each substrate was evaluated by measuring θ_w and Ad(IgG) for the coated surfaces. It was observed that all the substrates showed low θ_w after coating and washing, around 25°, as shown in Table 4. This indicates that MDM-3 was well adhered to the various types of substrates, including highly hydrophobic PP, which is normally difficult to be coated with hydrophilic polymer. Also, it was observed that the protein adsorption is largely depressed in all substrates by coating with MDM-3 compared with the uncoated surfaces. The adsorption of IgG on the MDM-3 coated surface was approximately 0.01 ± 0.01 (Table 4), regardless of the type of substrate.

Table 4. Surface Contact Angles for Water (θ_w) and Protein Adsorption (Ad(IgG)) on Surfaces of Various Types of Substrates Coated with MDM-3

		θ_w	Ad(IgG)
	sample	substrate coated	substrate coated
1	MDM-3/PS	79.0 → 24.8	2.19 → 0.005
2	MDM-3/PP	91.1 → 25.9	1.15 → 0.024
3	MDM-3/PC	87.9 → 24.3	1.08 → 0.013
4	MDM-3/Ti	69.1 → 23.2	1.05 → 0.022
5	MDM-3/glass	57.9 → 26.8	0.23 → 0.004
6	MDM-3/PP tube		1.01 → 0.014
7	MDM-3/PP tube γ-ray (10 kGy)		1.01 → 0.013

The protein adsorption on the surface of the four substrates was also investigated using three different types of triblock MDM copolymers, i.e., MDM-1, MDM-2, and MDM-3. The results are summarized in Figure 6. All the coated surfaces showed very low protein adsorption, regardless of the type of

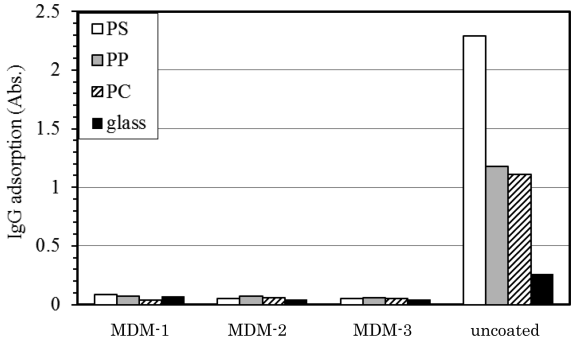

Figure 6. Protein (IgG) adsorption on surfaces coated with MDM triblock copolymers with different compositions. All MDM copolymers were coated on different substrates (PS, PC, PP, and glass). Adsorption of IgG (Ad(IgG)) was evaluated by absorbance measurements at 450 nm.

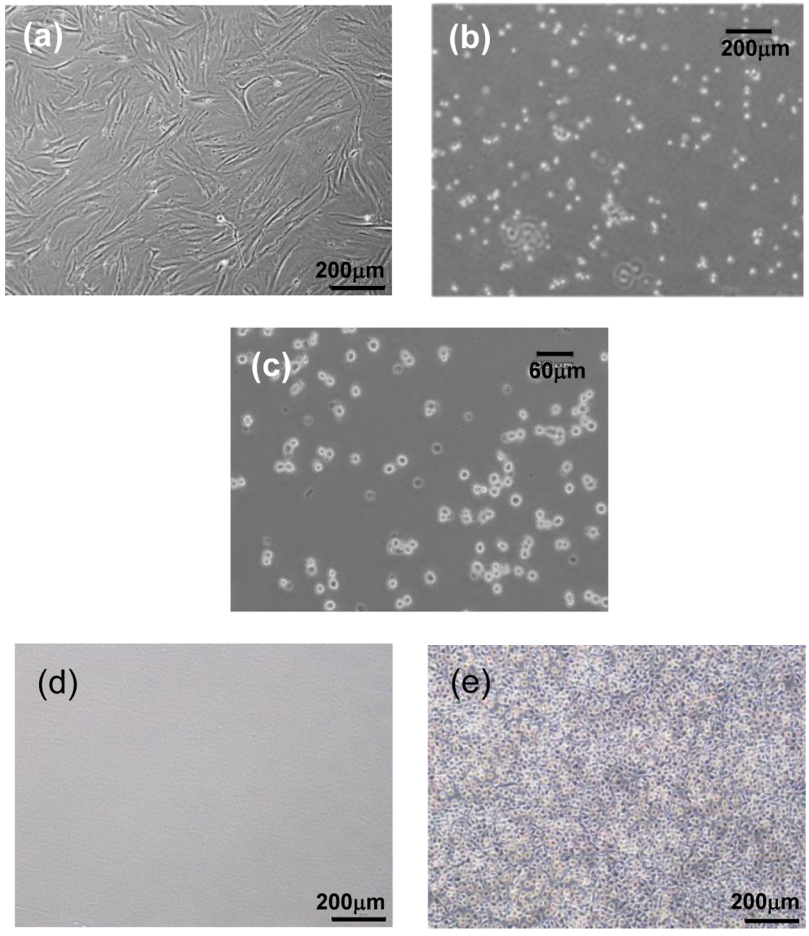

Figure 7. (a, b) Cell attachment of NHDF on a PS dish coated with (a) none, (b) MDM-3 after 3 d culture (initial seeding 1×10^4). (c) Cell culture of suspension cell (J774A.1, initial seeding 2×10^4 cells/dish, 10.4×10^5 cells/dish after 6 d culture). (d, e) PS dish surface coated with (d) MDM-3 and (e) none after a culture of Balb3T3 cells for 3 d, followed by washing with PBS two times.

substrate and the composition of MDM (MDM-1−3). Thus, it was revealed that MDM can be coated on different types of substrate that are useful for biomedical applications and developing highly protein-repellent surfaces.

Further, the coating of MDM-3 was applied to a centrifuge tube made of PP, because it is desirable to develop a PP centrifuge tube with ultralow protein adsorption. It was found that the PP centrifuge tube can be well coated with MDM-3, and the resulting coated surface showed high protein repellency not only for the as-coated surface, but also for the coated surface post-treatment by γ-ray irradiation (10 kGy), as shown in Table 4.

Cell Culture on Surface Coated with MDM. NHDF is a well-known human dermal fibroblast cell and usually shows a spindle shape when cultured on a tissue-culture PS (TCPS) dish, as shown in Figure 7a. When NHDF was seeded on the PS dishes coated with the triblock copolymer MDM-3, it was observed that NHDF hardly attached to the surface coated with MDM-3 (Figure 7b). Thus, the surface of the MDM-3-coated PS dish showed not only low protein adsorption but also low cell attachment. The experiment of NHDF attachment described above does not prove whether the suspensions of NHDF are alive or dead on the surface. J774A.1 (macrophage) is a murine macrophage cell line that can be cultured on TCPS but could proliferate as a suspension cell when cultured on a low-cell-attachment surface. The J774A.1 cultures are then able to reveal the cytotoxicity of the coated surface. The cell number of J774A.1 (initial seeding = 2×10^4 cells/dish) after being cultured for 6 d on the surface coated with the MDM-3 block copolymer (Figure 7c: 10.4×10^5 cells/dish) are almost the same as that on TCPS. This result indicates that the surface coated with the MDM block copolymer was safe for cell culture, especially for suspension cells and that the cells could be cultured on the surface in the suspension state. Furthermore, the low cell attachment on the surface of the MDM-3-coated PS dish was also confirmed by a culture of 3T3 cells. After culturing for 3 days, the surface was washed by PBS two times. As shown in Figure 7d, the cultured 3T3 cells were completely removed by light washing with PBS (no cell attachment was observed); in contrast, 3T3 cells cultured on the normal tissue culture PS (TCPS) dish were strongly attached on the surface even after the washing process (Figure 7e).

■ CONCLUSION

New types of amphiphilic block copolymers, triblock (MDM) and 4-arm block ($(MD)_4$) copolymers, consisting of hydrophobic PMEA and hydrophilic PDMAA segments, were successfully synthesized by RAFT polymerization. The one-pot synthesis was adopted upon considering both the simple synthesis and the high protein repellency points of view. Because of the structure of the MDM sequences, the high molecular weight, and the individual characteristics of PDMAA (hydrophilic, low protein adsorption) and PMEA (hydro-

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phobic, low T_g, low protein adsorption) segments, MDM and $(MD)_4$ exhibited good adhesion to various types of substrates, such as PS, PC, PP, Ti, glass, and a PP centrifuge tube, particularly in a specific composition range (D = 750−1500 and M = 250). All the surfaces coated with the MDM copolymers showed good adhesion and ultralow adsorption of protein (IgG), regardless of the type of substrate. Further, the PS dish coated with MDM-3 (M(230)-[D(1500)-*co*-M(40)]-M(230)) could be sterilized by γ-ray irradiation and used as a good substrate for a suspension cell culture that exhibits low cell adhesion and good cell growth. Thus, it was revealed that the MDM and $(MD)_4$ block copolymers prepared under the conditions in the present study possess the characteristics of both stable adhesion to the substrate and high protein repellency thereon, in addition to the high polymerization yields. The aim of this study, that is, developing practically applicable protein-resistant coating materials, was achieved, and the resulting amphiphilic triblock MDM and 4-arm block $(MD)_4$ copolymers prepared by a sequential addition polymerization will be used as promising coating materials with distinguished biomedical characteristics.

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Notes

The authors declare no competing financial interest.

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