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Aryl Azide Based, Photochemical Patterning of Cyclic Olefin Copolymer Surfaces with Non-Biofouling Poly[(3-(methacryloylamino)propyl)dimethyl(3-sulfopropyl)ammonium hydroxide]

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Dedicated to Professor Eiichi Nakamura on the occasion of his 60th birthday

Copolymers of ethylene and a cyclic olefin, such as norbornene or cyclopentene, are a new class of thermoplastics commonly known as cyclic olefin copolymers (COCs). They possess properties desirable for the fabrication of microfluidic devices, biosensors, medical devices, and diagnostic disposables, including optical clarity, low autofluorescence, low birefringence, moldability, high glass-transition temperature (70 to 160 °C), low water uptake, and resistance to common organic solvents.[1–3] In spite of these favorable properties, the use of COC as a material for the aforementioned applications has been limited owing to its hydrophobicity and the lack of functionalizability. In this respect, some methods for modifying/functionalizing the COC surface have recently been reported.[4–6] Especially, functionalization of the inner surface of channels has been attempted to minimize the hydrophobic interactions between the hydrophobic COC surface and biomolecules, leading to the undesired adsorption of biomolecules inside of the channels, and to improve the efficiency of COC-based microfluidic devices.[4,6a]

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Although most polymeric materials, such as polycarbonate, poly(methyl methacrylate), and polydimethylsiloxane, have been functionalized by simple chemical reactions,[7] these methods are not applicable directly to the functionalization of COCs because of its saturated hydrocarbon structure which has only C–C and C–H bonds; hence, physical grafting[4] and chemical oxidation[5] have generally been used. The chemical oxidation, such as ozone[5a] and oxygen plasma treatments,[5b–e] generates oxygen-containing functional groups, such as epoxide, ketone, alcohol, and carboxylic acid, which could be subsequently used for the attachment of molecules of interest. On the other hand, photografting[6] is an attractive alternative to physical grafting and chemical oxidation because it makes the strong covalent attachment of molecules onto surfaces and allows for easy fabrication of polymeric surfaces. In addition to the possibility of pattern generation by photografting,[6a] the UV transparency of COCs would, in principle, make it possible to selectively functionalize the inner surface of the COC-based channels.

Nonspecific adsorption of bioentities occurs commonly in contact with artificial surfaces and causes the deteriorated performance of microfluidic channels as well as many undesirable biological responses, such as thrombus formation and bacterial infection.[8] The minimization of biofouling in microfluidic devices has been investigated based on polymer coating. Ethylene glycol based compounds have generally been used for non-biofouling coating, and zwitterionic polymers, such as phosphorylcholine, sulfobetaine, and carboxybetaine, have recently been suggested as a new type of non-biofouling materials.[9] For example, our group reported that poly[(3-(methacryloylamino)propyl)dimethyl(3-sulfopropyl)-ammonium hydroxide] [poly(MPDSAH)] was highly efficient in minimizing the nonspecific adsorption of proteins (Figure 1 a).[9a]

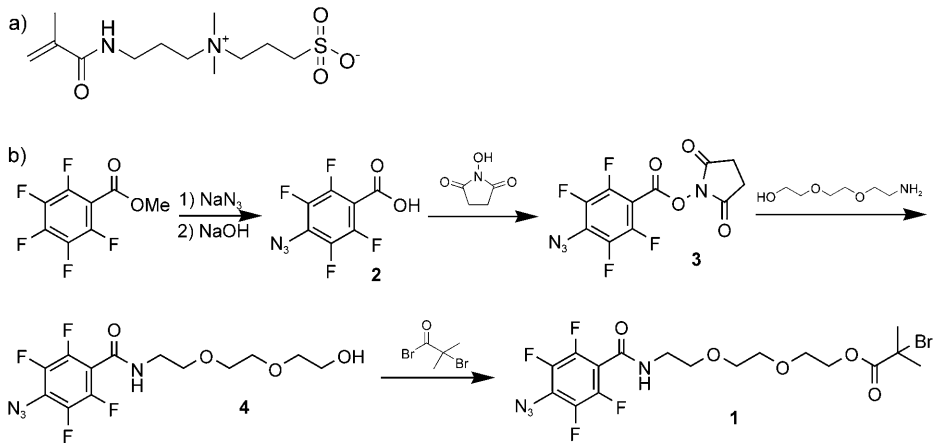

Figure 1. a) Structure of MPDSAH. b) Synthesis of aryl azide based, atom-transfer radical polymerization (ATRP) initiator (**1**).

Aryl azide compounds—widely used in biology—have been applied for photografting of carbon-based materials, such as polymers[10a–c] and carbon nanotubes.[10d] Photolysis of aryl azide yields a highly reactive nitrene, which inserts into C–H and C–C bonds as one of the possible reaction pathways.[11] An aryl azide containing aldehyde or quaternary amine has recently been patterned on the COC surface.[12] In this work, we designed and synthesized an aryl azide containing atom-transfer radical polymerization (ATRP) initiator from methyl pentafluorobenzoate and used it as a photografting reagent for patterned, surface-initiated ATRP (SI-ATRP)[13] of MPDSAH on the COC surface (Figure 1b; see the Experimental Section for the synthesis). We found that the photografting successfully occurred with a COC slab as a physical barrier because of the optical transparency of COC, which showed the possibility in functionalizations of the inner surface of COC channels. Patterns of cells were also generated on the COC surface by using the non-biofouling property of poly(MPDSAH).

To investigate the photochemical attachment of the initiator (**1**) onto a COC surface, we first performed the photoreaction in the absence of a photomask. The initiator was spin-coated on a COC surface, followed by 100 s UV irradiation (254 nm). Another COC slab was placed on the COC substrate after spin-coating, for the confirmation of the possibility of indirect patterning of the initiator. The SI-ATRP of MPDSAH was subsequently performed by following our reported procedures:[9a] Briefly, copper(II) bromide, 2,2′-dipyridyl, MPDSAH, water, and methanol were added to the Schlenk flask. The resulting solution was deoxygenated by passing a continuous stream of dry argon, and to the solution was added copper(I) bromide. The initiator-coated COC substrate was placed in another Schlenk flask, and then the Schlenk flask was degassed under vacuum and purged with dry argon. The reactant solution was transferred to the Schlenk flask containing the initiator-coated COC substrate, and the polymerization was carried out for 14 h at room temperature.

After photoreaction, the water contact angle of the substrate was changed to 46.8° from 94.5°, indicative of the successful attachment of the initiator, **1**, onto the COC surface, by UV irradiation through the COC slab (Figure 2a). The SI-ATRP of MPDSAH further decreased the contact angle to 5.5°.[9a] The attachment was further confirmed by X-ray photoelectron spectroscopy (XPS, Figure 2b). In the XPS spectrum of the initiator-attached substrate we observed characteristic peaks of the initiator at

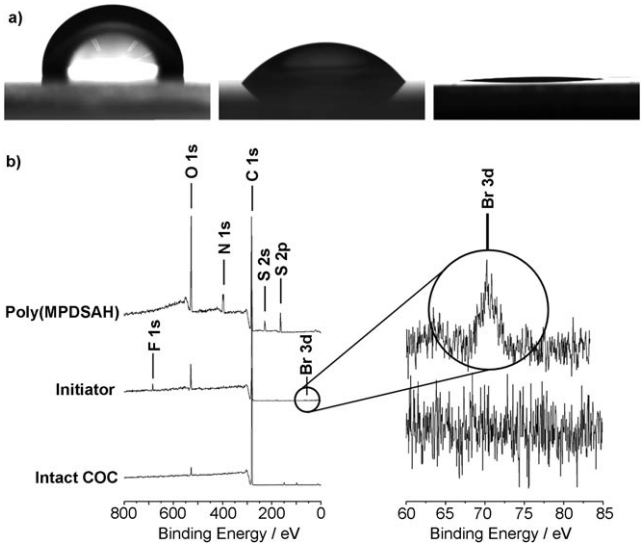

Figure 2. a) Static water contact angles of COC substrates: intact, initiator-attached, and poly(MPDSAH)-coated COC. b) XPS spectra of COC substrates.

687.66 (F 1s), 532.63 (O 1s), 70.23 (Br 3d), and 393.93 eV (N 1s), while the spectrum of the intact COC was composed of only the carbon peak. After the formation of poly(MPDSAH) films, the XPS spectrum showed peaks at 530.72 (O 1s), 399.5 (N 1s), 284.92 (C 1s), and 167.41 eV (S 2p). In particular, the S peak at 167.41 eV appeared in the spectrum owing to poly(MPDSAH). Taken together, these results clearly showed that the aryl azide based ATRP initiator was attached onto the COC substrate by photoreaction, and the photoreaction could be achieved indirectly with a COC slab with a barrier. In addition, as one of potential ATRP monomers, the non-biofouling MPDSAH was successfully polymerized from the initiator-attached COC surface.

Photoreactions have advantages for the pattern generation over other surface-based reactions, because they can be

seamlessly combined with conventional photolithographic techniques.[14] In this work, we generated the micropatterns of poly(MPDSAH) and used them as a platform for protein and cell patterns by utilizing the non-biofouling property of poly(MPDSAH). The procedure for the pattern generation of the poly(MPDSAH) film is depicted in Figure 3. The initiator, **1**, was spin-coated on a COC surface, followed by placing a TEM grid as a model photomask. The sample was then covered with another COC slab, and the UV light was irradiated through the upper COC slab for 1 h. After 14 h SI-ATRP, the poly(MPDSAH) pattern was visualized by TRITC-streptavidin (Figure 4a): The relatively bright red color was observed as a grid pattern that corresponded to the area unexposed to the UV light. In other words, streptavidin was adsorbed only onto the nonfunctionalized COC surface, and poly(MPDSAH) precluded the adsorption of streptavidin effectively. After confirming the feasibility of pattern generation by using the indirect photopatterning of the compound **1** and SI-ATRP of MPDSAH, we initiated the generation of cell patterns by the site-selective adsorption of fibronectin, which is known to enhance the attachment of cells, on the poly(MPDSAH)-patterned COC substrate. The protein, fibronectin, was adsorbed only onto the areas of intact COC. NIH 3T3 fibroblast cells (5×10^5 cells) were then transferred to a dish containing the COC substrate and incubated at 37 °C for 24 h. The optical micrograph showed a grid pattern of cells (Figure 4b). As a control, we also incubated the cells on an intact, nonpatterned COC substrate, and did not observe any patterns but did observe the nonspecific adsorption of the cells (see the Supporting Information). Further experiments on the stability of the formed poly(MPDSAH) films showed that the exposure of the poly(MPDSAH) films to the air did not deteriorate their non-biofouling property for at least three days (see the Supporting Information).

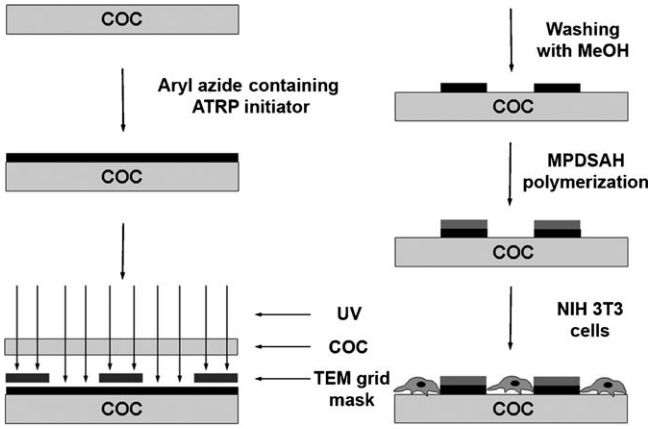

Figure 3. Procedure for pattern generation of poly(MPDSAH) and NIH 3T3 fibroblast cells on COC surfaces by indirect photopatterning.

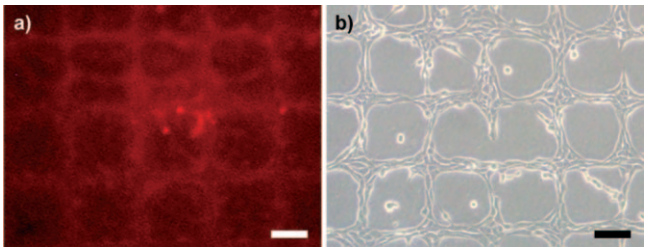

Figure 4. Pattern generation on COC surfaces: a) TRITC-streptavidin and b) NIH 3T3 fibroblast cells. The scale bar is 100 μm.

In summary, we suggested a photoreaction-based functionalization of COC, based on aryl azide chemistry. The possibility of functionalizing the inner surface of COC-based channels was investigated by indirect UV irradiation, and, as one of the applications, the non-biofouling coating of poly(MPDSAH) was demonstrated. In addition, the pattern generation of NIH 3T3 fibroblast cells was achieved by using the non-biofouling property of poly(MPDSAH). We believe that the patternability of the inner surface of COC-based microchannels would widen the applications of COC in microfluidic devices and related areas.

Experimental Section

Experimental details, including the pattern generation of TRITC-SA and cells, are described in the Supporting Information.

Synthesis of the ATRP initiator **1**:

a) 4-Azido-2,3,5,6-tetrafluorobenzoic acid (**2**): To a stirred solution of methyl pentafluorobenzoate (500 mg, 2.21 mmol, 1 equiv) in acetone (30 mL) and water (10 mL) was added sodium azide (138 mg, 2.12 mmol, 0.96 equiv) at ambient temperature, and the reaction mixture was heated at reflux for 8 h. The crude mixture was cooled down to room temperature, diluted with water (20 mL), and extracted with ethyl acetate (3× 50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated to give 650 mg of yellow oil (4-azido-2,3,5,6-tetrafluorobenzoic acid methyl ester). To a stirred solution of 4-azido-2,3,5,6-tetrafluorobenzoic acid methyl ester (650 mg, 2.61 mmol) in methanol (10 mL) and water (1 mL) was added aqueous NaOH solution (~1 mL, 20%), and the reaction mixture was stirred at ambient temperature overnight. The reaction mixture was evaporated under reduced pressure and then acidified with diluted aqueous HCl solution. The crude product was extracted with dichloromethane (3×50 mL), dried over $MgSO_4$, filtered, and concentrated to give 554 mg of yellow solid in 90% yield. MS: calcd m/z for $[M+H]$: 236.01; found $[M+H]$: 236.0.

b) 4-Azido-2,3,5,6-tetrafluorobenzoic acid 2,5-dioxo-pyrrolidin-1-yl ester (**3**): To a stirred solution of compound **2** (554 mg, 2.36 mmol) in 10 mL dichloromethane were added *N*-hydroxysuccinimide (NHS; 272 mg, 2.36 mmol, 1 equiv) and dicyclohexylcarbodiimide (497 mg, 2.41 mmol, 1.02 equiv) at ambient temperature. The reaction mixture was stirred at ambient temperature overnight, and the resulting suspension was filtered and washed with dichloromethane. The filtrate was evaporated under reduced pressure and purified by flash column chromatography (hexane/ethyl acetate = 5:1) to give 645 mg of white solid in 82% yield. ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 2.92$ ppm (s, 4H).

c) 4-Azido-2,3,5,6-tetrafluoro-*N*-(2-(2-(2-hydroxyethoxy)ethoxy)ethyl)-benzamide (**4**): To a stirred solution of compound **3** (200 mg, 0.602 mmol, 1 equiv) and 2-(2-(2-aminoethoxy)ethoxy)ethanol (108 mg, 0.723 mmol, 1.2 equiv) in acetonitrile (30 mL) was added triethylamine (91 mg, 0.903 mmol, 1.5 equiv) at ambient temperature, and the reaction mixture was stirred for 3 days. The crude mixture was diluted with water (30 mL) and extracted with dichloromethane (2×50 mL). The combined organic

layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting oil was purified by flash column chromatography (hexane/ethyl acetate = 1:1) to afford a white oil (220 mg, 0.602 mmol, quantitative). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.59–3.68 (m, 12H), 2.01 ppm (s, 1H).

d) 2-(2-(2-(-4-Azido-2,3,5,6-tetrafluorobenzamido)ethoxy)ethoxy)ethyl 2-bromo-2-methylpropanoate (**1**): Compound **4** (130 mg, 0.355 mmol, 1 equiv) was dissolved in dichloromethane (30 mL) and pyridine (29 mg, 0.366 mmol, 1.03 equiv). To this solution was added a solution of 2-bromo-2-methylpropanoyl bromide (90 mg, 0.390 mmol, 1.1 equiv) in dichloromethane (5 mL) dropwise at ambient temperature. The reaction mixture was stirred for 5 h and then quenched with water (10 mL). The organic layer was separated, and the aqueous layer was further extracted with diethyl ether (2 × 30 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting oil was purified by flash column chromatography (hexane/ethyl acetate = 3:1 to 1:1) to afford a brown oil (**1**, 129 mg, 0.250 mmol, yield = 70.5%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.29 (t, 2H), 3.65–3.74 (m, 10H), 1.92 ppm (s, 6H).

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Keywords: azides · atom-transfer radical polymerization · cyclic olefin copolymers · MPDSAH · photoreactions

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