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Modification of Antibacterial Copolymers on the Surface of PVA-Based Microfibers via Thermal Cross-Linking and Their Antibacterial Properties

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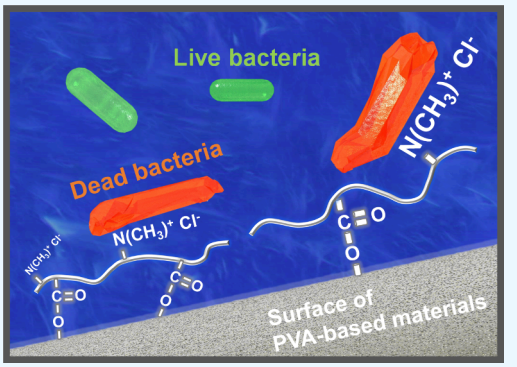
ABSTRACT: Bacterial infections on material surfaces are a serious public health concern worldwide. Although poly(vinyl alcohol) (PVA)-based materials have great potential as medical devices, they lack antibacterial properties on their surfaces and pose bacterial infection risks during implantation surgery. Copolymers containing antibacterial [2-(methacryloyloxy)ethyl]-trimethylammonium chloride (METAC) units were used to modify the surfaces of chemically cross-linked water-insoluble PVA-based microfibers. The copolymers also had carboxy units that were used to react with the hydroxy group of the PVA-based microfibers via a simple thermal treatment at 135 °C. PVA-based materials containing METAC units exhibit significant swelling due to electrostatic repulsions. Because the copolymers were modified on the extreme surface of the microfibers, no difference in the diameters between unmodified microfibers (PM-fiber) and copolymers with METAC unit-modified microfibers (PM-METAC-fiber), in both the dry and swollen states, was observed. The viable bacterial cell numbers, which were evaluated by colony counting, decreased by exposure to the poly(METAC-*co*-methacrylic acid (MAAc)) aqueous solution or PM-METAC-fibers. The value of CFU/mL decreased to 0.1% (against *B. subtilis*) and 3.9% (against *E. coli*) after contact with the PM-METAC-fibers compared to the PM-fibers. The percentage of hemolysis against rabbit red blood cells was equivalent to that of the negative control, suggesting that PM-METAC-fibers can selectively exhibit antibacterial properties. This modification method can be applied to various PVA-based materials if hydroxy groups are present on their surface. This study provides a facile, cost-effective, and promising strategy to impart antibacterial properties to the surface of PVA-based materials without significantly affecting their physicochemical properties.



1. INTRODUCTION

Infections caused by the bacterial contamination of material surfaces are a major public health concern worldwide, posing significant risks to food packaging and storage, water purification systems, household sanitation, agriculture, and medical fields.[1] Coating antibacterial drugs onto material surfaces has received considerable attention as a solution to this problem. In fact, the global antimicrobial coating market size in 2020 was valued at $8.1 billion and is predicted to continue growing at a compound annual growth rate of 13.1% from 2021 to 2028.[2] However, the use of antibiotics as antibacterial drugs for surface coating raises concerns about the emergence of drug-resistant bacteria. The antibacterial properties of silver ions are associated with health problems due to the use of silver, such as metal allergies, cell toxicity, and the release of effluent from the coating surface into the environment.[3,4] Additionally, peptides,[5−7] polymeric materials,[8−10] quorum-sensing inhibitors,[11,12] and surfactants[13,14] have been studied as potential antibacterial drugs. Quaternary ammonium compounds (QACs) exhibit antibacterial activity against a broad range of bacteria at low concentrations. They are characterized by relatively low toxicity and can be used in contact with the human skin.[2] The antibacterial properties of materials coated with QACs have been investigated; however, in many cases, QACs leak from materials, leading to reduced antibacterial activities and environmental spills. To address this problem, QACs are chemically modified on material surfaces via covalent bonding.

Zhao et al. prepared poly(*N*-isopropylacrylamide) microgels with QACs that provided antibacterial properties by coating them onto metal, plastic, and elastomer substrates.[15] Otoni et al. modified the surface of nanocellulose using QAC and

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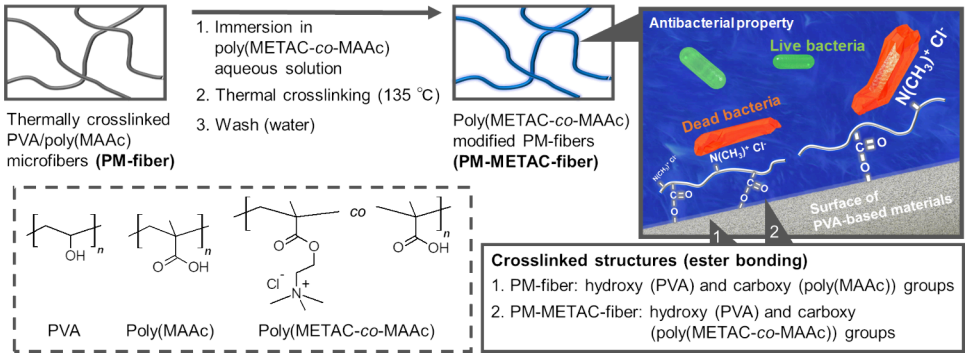

Figure 1. Schematic representation of the modification of antibacterial poly(METAC-*co*-MAAc) on the surface of PVA/poly(MAAc) microfibers via thermal cross-linking.

fabricated a sponge material with pores using a cryotemplating method.[16] The controlled pore size and surface area of the sponges enhanced their antibacterial activity. A QAC-modified chitosan was also developed to enhance its antibacterial properties.[17] In this study, [2-(methacryloyloxy)ethyl]-trimethylammonium chloride (METAC), a QAC, was used as the monomer. Poly(METAC) exhibits antibacterial activity against a wide range of bacterial species, including *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), and *Pseudomonas aeruginosa* (*P. aeruginosa*), and is also effective against drug-resistant bacteria, such as methicillin-resistant *S. aureus* (MRSA).[18−20] In addition, antibacterial polymers are less likely to cause the development of resistant bacteria in the polymers. *E. coli* and *Bacillus subtilis* (*B. subtilis*) did not develop resistance against quinine-based copolymers with METAC units even after the 16th passage.[20] Additionally, METAC can be polymerized with other monomers, such as (meth)acrylate and (meth)acrylamide.

To chemically modify poly(METAC)-based copolymers into poly(vinyl alcohol) (PVA) materials, poly(METAC-*co*-methacrylic acid (MAAc)) with carboxylic acid units was polymerized. PVA is a water-soluble polymer that is commonly used as an additive and stabilizer in paints and other emulsions.[21] In addition, PVA has been approved by the U.S. Food and Drug Administration (FDA)[22] and has been applied to various types of medical devices.[23,24] Owing to their hydrophilic properties, cross-linked PVA materials can contain water, similar to many other biological tissues. Hao et al. fabricated a tough hydrogel consisting of a mixture of PVA, hyaluronic acid, collagen, and poly(ethylene glycol) for use as artificial cartilage.[25] Fan et al. fabricated a microneedle patch consisting of a vascular endothelial growth factor (VEGF)-loaded PVA and graphene oxide to restore cardiac function.[26] The folded microneedle patch returned to its original shape upon irradiation with near-infrared light and punctured the heart for controllable and sustainable release of VEGF. Although these PVA-based materials have significant potential as medical devices, they have no surface antibacterial properties and are associated with the risk of bacterial infection during implantation surgery.

In this study, antibacterial METAC-based copolymers were modified on the surfaces of chemically cross-linked, water-insoluble PVA-based microfibers (Figure 1). Compounds such as glutaraldehyde were used to introduce covalent bonds into PVA-based materials; however, there are concerns about the biotoxicities caused by their unintended cross-linked structures in biomolecules, including proteins, as the reaction proceeds even at room temperature. On the other hand, the introduction of covalent bonds to PVA materials via thermal cross-linking using the carboxy groups of poly(MAAc) requires a relatively high temperature of approximately 135 °C.[27−30] This thermal cross-linking process did not proceed easily at room temperature. Therefore, the formation of unintended cross-linked structures against biomolecules is difficult.

In our previous study, water-insoluble PVA-based microfibers were fabricated by thermal cross-linking of poly(MAAc) and copolymers with MAAc units.[31] By mixing copolymers containing MAAc and METAC units with PVA, microfibers containing METAC units can also be fabricated via thermal cross-linking. When copolymers with METAC units were introduced into the PVA-based microfibers, the microfibers exhibited significant swelling owing to the electrostatic repulsion and osmotic pressure of the METAC units. In fact, cross-linked microfibers consisting of PVA (90 wt %) and poly(MAAc) (10 wt %) showed an expansion ratio of 1.50 ± 0.19 after water immersion at 25 °C for 24 h, whereas cross-linked PVA microfibers with copolymers having MAAc and METAC units showed an expansion ratio of 2.93 ± 0.98 at the same conditions. In addition, owing to its mechanical strength, the mixing ratio of the copolymer in the PVA-based microfibers was 10−25 wt %, and it was difficult to incorporate a large number of METAC units with antibacterial properties. Furthermore, the antibacterial properties of PVA-based microfibers have not yet been evaluated. Therefore, in this study, water-insoluble PVA-based microfibers with antibacterial properties and low expansion ratios were fabricated by chemically modifying the surfaces of PVA/poly(MAAc) microfibers with poly(METAC-*co*-MAAc). The MAAc units of poly(METAC-*co*-MAAc) act as reaction sites for covalent bonding with the hydroxy groups on the PVA/poly(MAAc) microfibers. Microfibers modified with poly(METAC-*co*-MAAc) exhibited antibacterial properties against *E. coli* (Gram-negative bacteria) and *B. subtilis* (Gram-positive bacteria). To the best of our knowledge, this is the first report on the chemical modification of poly(METAC-*co*-MAAc) on the surface of PVA-based materials to enhance their antibacterial properties. This method can be applied to all PVA-based materials, including molded PVA products. The physicochemical and antibacterial properties of the fabricated microfibers were also evaluated.

2. EXPERIMENTAL SECTION

2.1. Materials. MAAc was purchased from Tokyo Chemical Industry Co., Ltd., and purified by passing it

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through a basic alumina column. METAC solution (75 wt % in H_2O) was purchased from Sigma-Aldrich (MO, USA). All other chemicals and solvents were used as received.

2.2. Preparation of Thermally Cross-Linked PVA/Poly(MAAc) Fibers (PM-Fiber). Poly(MAAc) was synthesized according to a previously described protocol.[31] MAAc (7.75 g, 90.0 mmol) and 2,2′-azobis(isobutyronitrile) (AIBN) (73.9 mg, 4.50×10^{-1} mmol) ($[MAAc]_0/[AIBN]_0 = 200/1$) were dissolved in 60 mL of *N*,*N*-dimethylformamide (DMF). After degassing with nitrogen for 60 min, the solution was polymerized for 20 h at 70 °C. The resulting poly(MAAc) was purified by dialysis, first against ethanol/water (1/1 v/v) and then against water, and finally dried by lyophilization. Poly(MAAc) was obtained as a white powder in an 83% yield. The PVA/poly(MAAc) fibers were prepared by NITIVY Co., Ltd. (Tokyo, Japan). B-17 PVA (degree of polymerization 1700, 87−89 hydrolysis; Denka Co., Ltd., Tokyo, Japan) was used as the fiber. The fibers were prepared using a poly(MAAc) mixture ratio of 10 wt %. All fibers were thermally cross-linked by heating in an oven at 135 °C for 24 h. The thermally cross-linked PVA/poly(MAAc) fibers are called PM-fibers.

2.3. Modification of Poly(METAC-*co*-MAAc) on PM-Fibers. Poly(METAC-*co*-MAAc) was synthesized as follows: METAC (75 wt % in H_2O) (18.69 g (METAC: 14.02 g, 67.5 mmol)), MAAc (1.94 g, 22.5 mmol), and AIBN (73.9 mg, 4.50×10^{-1} mmol) ($[Momoers]_0/[AIBN]_0 = 200/1$) were dissolved in 60 mL of *N*,*N*-dimethylformamide (DMF). After degassing with nitrogen for 60 min, the solution was polymerized for 20 h at 70 °C. The resulting poly(METAC-*co*-MAAc) was purified by dialysis, first against ethanol/water (1/1 v/v) and then against water, and finally dried by lyophilization. Poly(METAC-*co*-MAAc) was obtained as a white powder in a 38% yield. The PM-fibers were immersed in a poly(METAC-*co*-MAAc) aqueous solution (4 mg/mL) at 25 °C overnight. The immersed fibers were dried under reduced pressure and thermally cross-linked by heating in an oven at 135 °C for 24 h to form the covalent bond between MAAc units of the poly(METAC-*co*-MAAc) and the hydroxy group on PM-fibers. After modification, the fibers were immersed in surplus water at 25 °C overnight to remove adsorbed (non-cross-linked) poly(METAC-*co*-MAAc). After being immersed, the fibers were washed with water and dried under reduced pressure. The PVA/poly(MAAc) fibers modified with poly(METAC-*co*-MAAc) are called PM-METAC-fibers.

2.4. Swelling Tests of PM-Fibers and PM-METAC-Fibers in Water. The PM-fibers and PM-METAC-fibers were immersed in a surplus of water at 25 °C for 24 h. The swelling ratios were calculated using the following numerical expression: average diameter of swelling fibers (μm)/average diameter of dried fibers (μm). The diameters were measured by using a digital microscope (VHX-7000; Keyence Co., Osaka, Japan).

2.5. Antimicrobial Properties of PM-Fibers and PM-METAC-Fibers. Three single colonies of *E. coli* JCM 20135 (= K-12) (or *B. subtilis* NBRC 13719^T (= Marburg)) on LB agar were collected and dispersed in 3 mL of the LB medium. The bacterial suspension was incubated overnight at 37 °C with shaking. This bacterial suspension (50 μL) was added to 3 mL of the LB medium and incubated at 37 °C with shaking until the turbidity reached approximately 0.5 (wavelength: 565 ± 15 nm). After incubation, the bacterial suspension was diluted 100 times with sterile water and used for antibacterial tests. Ten milligrams of each PM-fiber and PM-METAC-fiber ($N = 3$) was placed in a plastic tube, and 450 μL of sterile water was added. To allow the dry microfibers to swell sufficiently, the tubes were left overnight. Fifty microliters of the diluted bacterial suspension was added to each plastic tube and brought into contact with the microfiber for 1 h at 25 °C. Subsequently, 50 μL of the supernatants was collected and plated onto the LB agar medium using a bacteria spreader. The samples were incubated overnight at 37 °C, and the number of colonies in each sample was counted.

2.6. Hemolysis Tests of PM-Fibers and PM-METAC-Fibers. Hemolysis tests were performed for the PM-fibers and PM-METAC-fibers by monitoring hemoglobin leakage from rabbit red blood cells (RBCs) using an Infinite M1000-SSY microplate reader (Tecan Japan Co., Kanagawa, Japan) according to a previously described protocol.[31−33] First, 1.5 mL of RBCs was washed with 10 mL of phosphate-buffered saline (PBS) and concentrated by centrifugation at 3000 rpm for 5 min. The concentrated RBCs were redispersed in 30 mL of PBS to a final concentration of 5%. Ten (or 20) milligrams of each PM-fiber and PM-METAC-fiber ($N = 3$) was swollen in a significant amount of PBS overnight at 25 °C. The swollen microfibers were placed in a plastic tube, and 800 μL of PBS and 800 μL of RBC suspension were added. The RBCs in PBS were used as a negative control (800 μL of PBS and 800 μL of the RBC suspension). As a positive control (100% hemolysis), the RBCs were treated with 0.2 wt % (final concentration) Triton-X (800 μL of Triton-X PBS (0.4 wt %) and 800 μL of the RBC suspension). After 1 h, the suspensions were added to 2.4 mL of PBS and centrifuged at 3000 rpm for 5 min. The supernatant (150 μL × 3) was transferred to a 96-well microplate. Hemoglobin release was measured at 540 nm by using a microplate reader. The percentage of hemolysis was calculated using the following formula:

$$\begin{aligned}\text{hemolysis (\%)} = ((&\text{OD of sample} \\ &- \text{OD of negative control}) \\ &/(\text{OD of positive control} \\ &- \text{OD of negative control})) \times 100\end{aligned}$$

After the hemolysis tests, the immersed fibers were washed with surplus water and immersed in a large volume of aqueous formaldehyde solution. The fibers were gradually dried by using water/ethanol solutions (30, 50, 70, 90, and 100% ethanol).

2.7. Characterizations. The ^{1}H NMR spectra of the copolymers were obtained by using a JNM-GSX300 spectrometer operating at 300 MHz (JEOL, Tokyo, Japan) to determine the chemical compositions of the synthesized copolymers.

The molecular weight and polydispersity of the synthesized copolymers were determined by gel permeation chromatography (GPC) at 40 °C (0.2 M phosphate buffer at pH 8, 0.7 mL/min) with Shodex SB-802.5 HQ and Shodex SB-804 HQ columns (Showa Denko K.K., Tokyo, Japan) connected to an RID-20A refractive index detector (Shimadzu Co., Kyoto, Japan). Poly(ethylene oxide)/poly(ethylene glycol) was used as the standard to construct a calibration curve.

The morphologies of the fibers were observed by scanning electron microscopy (SEM) (JSM-IT 100, JEOL, Tokyo, Japan) at an accelerating voltage of 10 kV. In addition, the

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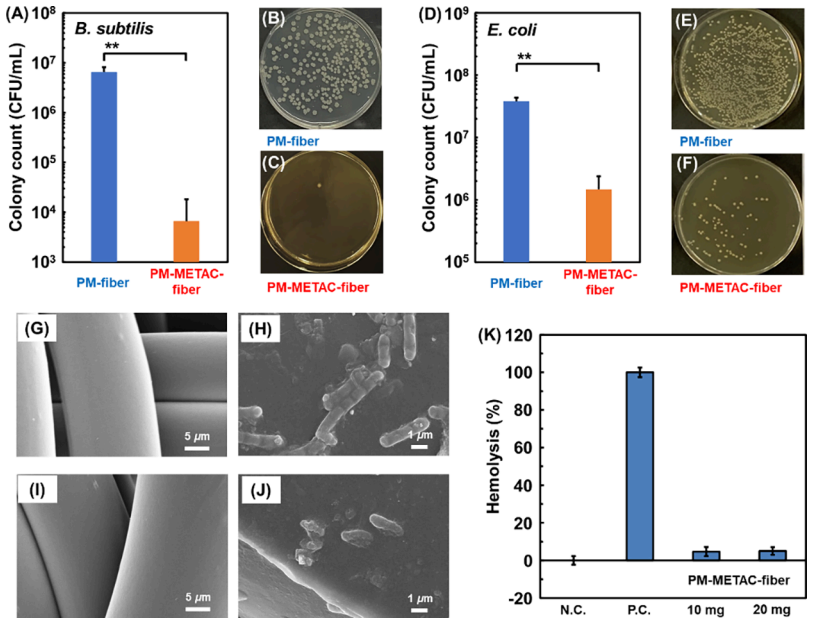

Figure 2. (A−C) Colony counts of PM-fibers and PM-METAC-fibers against *B. subtilis* (**: $p < 0.01$). (D−F) Colony counts of PM-fibers and PM-METAC-fibers against *E. coli* (**: $p < 0.01$). SEM images of (G) PM-fibers and (H) PM-METAC-fibers after contact with a suspension of *B. subtilis*. SEM images of (I) PM-fibers and (J) PM-METAC-fibers after contact with a suspension of *E. coli*. (K) Hemolysis tests of PM-METAC-fibers (N.C.: negative control, P.C.: positive control).

distribution of the C- and Cl-specific signals was observed by energy dispersive spectrometry (EDS) mapping.

Thermogravimetric analysis (TGA) was performed by using a TG-DTA2020S/MS (Bruker, Massachusetts, USA) analyzer between 25 and 500 °C in N_2 at a heating rate of 10 °C/min. The temperature at 10% weight loss ($T_{d,10\%}$) from 150 °C was calculated from the TGA results.

The thermal properties of the fibers were measured by using differential scanning calorimetry (DSC) (DSC-60Plus, Shimadzu Co., Kyoto, Japan). Each sample was analyzed at a heating rate of 10 °C/min. A second scan was performed to determine their thermal properties.

2.8. Statistical Analysis. All data were presented using Student's *t* test as mean ± standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1. Antimicrobial Properties of PM-METAC-Fibers. Poly(MAAc) and poly(METAC-*co*-MAAc) were synthesized by free radical polymerization. The molecular weight (M_n) and molecular weight distribution (M_w/M_n) of each polymer were M_n = 43,100 and M_w/M_n = 1.62 for poly(MAAc) and M_n = 24,200 and M_w/M_n = 3.00 for poly(METAC-*co*-MAAc). The composition of poly(METAC-*co*-MAAc) was calculated using the molar ratios of METAC and MAAc units, determined by ^{1}H NMR spectroscopy. The calculated molar percentages of poly(METAC-*co*-MAAc) were 74.4 mol % METAC and 25.6 mol % MAAc. In our previous study, thermally cross-linked PVA/poly(MAAc) microfibers (poly(MAAc) content: 10 and 25 wt %) exhibited almost 100% residual weight even when immersed in water at 80 °C.[31] This is because the carboxy group of poly(MAAc) and the hydroxy group of PVA form ester bonds due to thermal cross-linking, resulting in water insolubility. In this study, water-insoluble cross-linked PVA/poly(MAAc) microfibers with 10 wt % poly(MAAc) were selected for the modification of antibacterial poly(METAC-*co*-MAAc). The hydroxy groups present on the fiber were used as reaction sites with the carboxy groups of poly(METAC-*co*-MAAc) to enhance the antibacterial properties of the cationic METAC units. When METAC units are introduced inside PVA/poly(MAAc) fibers, the electrostatic repulsion and osmotic pressure of the METAC units cause the fibers to have significant swelling ratios and to change their physicochemical properties. The swelling ratio of the thermally cross-linked PVA/poly(MAAc) microfiber (poly(MAAc) content: 10 wt %) was 1.50 ± 0.19 after immersion in water at 25 °C for 24 h. In contrast, the swelling ratio of the microfibers fabricated similarly using the copolymer containing approximately 48−49 mol % of METAC showed a larger value of 2.93 ± 0.98.[31] There is also room for improvement because the relative amount of the antibacterial component in the METAC units decreases owing to the mixing of PVA. Therefore, in this study, synthesized poly(METAC-*co*-MAAc) was chemically modified on the extreme surface of the PM-fiber, which did not have a significant effect on the swelling ratios and physicochemical properties of the PM-fibers. It was also expected that the antibacterial units of METAC would be present at high densities on the surfaces of the fibers.

Figure 2 shows the antibacterial properties of the PM-fibers and PM-METAC-fibers against *E. coli* (Gram-negative bacterium) and *B. subtilis* (Gram-positive bacterium). The fibers were immersed in each bacterial suspension, the suspensions were spread on agar plates, and the number of colonies was counted after incubation. For *B. subtilis*, the colony count in unmodified PM-fibers was 6.6 × 10^6 ± 1.6 × 10^6 CFU/mL, whereas that in PM-METAC-fiber decreased to 6.7 × 10^3 ± 1.2 × 10^4 CFU/mL (Figure 2A−C). For *E. coli*, the colony count in PM-fibers was 3.8 × 10^7 ± 5.5 × 10^6 CFU/mL, and the colony count in PM-METAC-fiber decreased to 1.5 × 10^6 ± 9.1 × 10^5 CFU/mL (Figure 2D−F). These results indicate that the PM-METAC-fibers exhibit antibacterial properties against *E. coli* and *B. subtilis*. METAC

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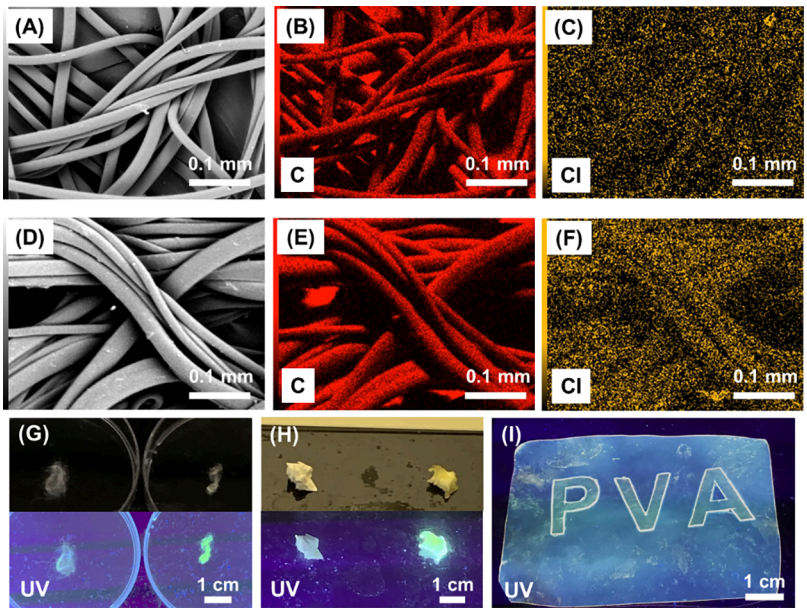

Figure 3. SEM images and EDS mappings (C- and Cl-elements) of (A−C) PM-fibers and (D−F) PM-METAC-fibers. Photos of modified PVA/poly(MAAc)-based materials of (G) microfibers, (H) sponges, and (I) a part of the flat film (Figure 3I) with poly(MAAc-*co*-fluorescein *O*-methacrylate (FMA)) containing fluorescein units.

units exhibited antibacterial properties against a wide spectrum of bacteria, including MRSA and *P. aeruginosa* (as well as *E. coli* and *B. subtilis*),[18−20] and these antibacterial abilities remained even when modified on the surface of microfibers.

In the SEM images of PM-fibers after immersion in the bacterial suspension, the adsorption of bacteria to the PM-fibers was prevented (Figures 2G,H). This is because the PM-fiber was composed of PVA, preventing nonspecific adsorption, and the PM-fiber surface was negatively charged due to the carboxy group of poly(MAAc), resulting in electrostatic repulsion due to bacteria having a negative surface. In fact, the zeta potential of the PVA/poly(MAAc) (poly(MAAc) content: 25 wt %) flat film was −7.7 ± 1.9 mV.[30] In the SEM images in Figure 2I,J, the morphology of adsorbed bacteria on the PM-METAC-fiber exhibited changes in the bacterial cell shape compared with the adsorbed bacteria on flat PVA/poly(MAAc) films (poly(MAAc): 10 wt %) (Figure S1). These results demonstrate the antibacterial properties of the PM-METAC-fiber. Similar changes in the bacterial shape, suggesting antibacterial properties, have been observed for other antibacterial polymers.[17,34,35] The mechanism of the antibacterial properties is as follows: The positively charged METAC units can adsorb onto negatively charged bacterial cell membranes by electrostatic interactions, and hydrophobic parts, such as the main chain and copolymerized hydrophobic units of the polymers, adhere to the bacterial membrane, resulting in the leakage of substances within the cytoplasm.[18] Another reported mechanism is the phospholipid sponge effect, in which positively charged units modified on the surface pull out negatively charged phospholipids, leading to the formation of holes in the bacterial membrane.[36] In addition, it must be considered that although increasing the ratio of hydrophobic units increases antibacterial properties, it also increases cytotoxicity.[37−42]

To examine the antibacterial properties of poly(METAC-*co*-MAAc) in detail, the antibacterial activities of the poly(METAC-*co*-MAAc) aqueous solution were measured at different polymeric concentrations (the total concentration of poly(METAC-*co*-MAAc) was 14−225 μg/mL) (Table S1). The number of bacterial colonies exposed to poly(METAC-*co*-MAAc) aqueous solutions at various concentrations for 1 h decreased for both *E. coli* and *B. subtilis* compared to that of the control. For *E. coli*, the number of colonies was 412 ± 120 in the control, whereas the number of colonies was 2 ± 2 and 0 ± 1 at polymeric concentrations of 14 and 28 μg/mL, respectively, and no colony was observed at more than 56 μg/mL. For *B. subtilis*, the number of colonies was 121 ± 50 in the control, and the numbers of colonies were 414 ± 27, 1 ± 1, 1 ± 2, 0 ± 1, and 0 ± 1 at polymeric concentrations of 14, 28, 56, 113, and 225 μg/mL, respectively. Moreover, when the contact time of the bacteria and polymer solution was 5 min, the number of colonies increased compared to that at 1 h, but a similar tendency for bacterial properties was observed (Table S2). These results suggest that the METAC units exhibited antibacterial properties within a short period of time after contact with the bacteria.

Next, a hemolysis test was performed on PM-METAC-fibers by using RBCs (Figure 2K). The percentage of hemolysis was equivalent to that of the negative control, suggesting that the PM-METAC-fibers maintained high blood compatibility. Asha et al. modified glass substrates by using copolymers containing METAC units.[43] The modified substrates exhibited antibacterial properties against *S. aureus* and *E. coli* but showed low cytotoxicity against normal human lung fibroblast cells. Zhou et al. modified silicon catheter surfaces using polymerized cationic (3-acrylamidopropyl)trimethylammonium chloride and cross-linking agents.[44] These modified catheters showed antibacterial activity against MRSA infection in an in vivo experiment using a murine catheter-associated urinary tract infection (CAUTI) model. These results suggest that PM-METAC-fibers can selectively exhibit antibacterial properties.

3.2. Characterization of Modified Poly(METAC-*co*-MAAc) on PM-Fibers. Figure 3A−F shows the elemental analysis by SEM. In the PM-fiber, carbon was detected in the PM-fibers; however, no chlorine was observed. In contrast, in PM-METAC-fibers, chlorine was observed, along with carbon.

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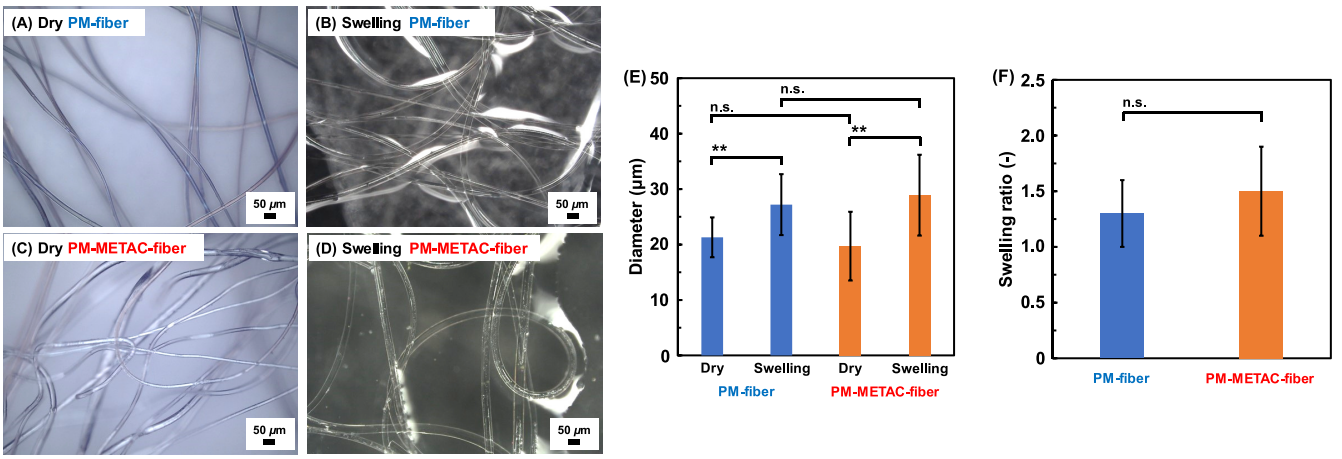

Figure 4. Microscopic images of (A) dry PM-fiber, (B) swelling PM-fiber, (C) dry PM-METAC-fiber, and (D) swelling PM-METAC-fiber. (E) Diameters of PM-fibers and PM-METAC-fibers before and after immersion in water (N = 20). (F) Swelling ratios of PM-fibers and PM-METAC-fibers (**: p < 0.01, n.s.: not significant).

This was due to the presence of chlorine in the METAC units of poly(METAC-*co*-MAAc), which chemically modified the surface of the microfibers. Xiao et al. evaluated poly(*N*-hydroxyethyl acrylamide (HEAA)-*co*-METAC) modified on film substrates using EDS mapping and observed a signal derived from chlorine in the METAC units.[45] In principle, our modification method of poly(METAC-*co*-MAAc) to PM-fibers in this study can be applied to various PVA-based materials as long as there are hydroxy groups on the surface. Thermally cross-linked, water-insoluble PVA/poly(MAAc)-based materials, including microfibers (Figure 3G), sponges (Figure 3H), and a part of the flat film (Figure 3I), were modified with poly(MAAc-*co*-fluorescein *O*-methacrylate (FMA)) containing fluorescein units using the same modification method as poly(METAC-*co*-MAAc). The amount of FMA in poly(MAAc-*co*-FMA) was less than 1 mol % and did not significantly affect the properties of poly(MAAc). Upon UV irradiation, the modified PVA-based materials exhibited FMA-derived fluorescence.

Recently, PVA-based materials with excellent mechanical properties, such as artificial cartilages, have been reported as artificial implants; however, these materials have no antibacterial properties.[25,46−51] Implantable PVA-based materials modified with copolymers containing METAC units are expected to have long-term antibacterial properties because of the low outflow of antibacterial components and the quick removal of killed bacteria by the immune system. In addition, if the METAC units of copolymers are modified on the surface of PVA-based dressings containing antibacterial compounds, such as silver ions and antibiotics, then bacterial infections can be prevented through a combination of two types of antibacterial mechanisms. Dai et al. fabricated a hydrogel containing two antibacterial components: cationic dendrimers and silver nanoparticles.[52] The hydrogels are degradable because of the acids produced by bacterial growth, resulting in the release of antibacterial components. Owing to the synergistic effect of the two antibacterial components, superior antibacterial effects were observed against *S. aureus* in an in vivo test compared to those of the antibacterial components alone. The modification method used in this study is expected to significantly affect the antibacterial properties of various PVA-based materials and products.

3.3. Comparison of Physicochemical Properties between PM-Fibers and PM-METAC-Fibers. Next, the diameters and swelling ratios of PM-fibers and PM-METAC-fibers were measured. Figure 4A−D shows images of PM-fibers and PM-METAC-fibers in the dry and swollen states measured using a microscope. Each fiber was immersed in a large amount of water at 25 °C overnight. PM-fibers without thermal cross-linking were easily dissolved in water under the same immersion conditions. In our previous study, thermally cross-linked PVA microfibers containing 10 and 25 wt % of poly(MAAc) had almost 100% residual weights, maintaining their shapes even after immersion in water at 80 °C for 30 min.[31] The diameters of the PM-fiber and PM-METAC-fiber in the dry state were 21.3 ± 3.6 and 19.7 ± 6.2 μm, respectively. The diameters of the PM-fiber and PM-METAC-fiber in the swollen state were 27.2 ± 5.5 and 28.9 ± 7.3 μm, respectively (Figure 4E). The swelling ratios (diameter of swollen fibers/diameter of dry fibers) were 1.3 ± 0.3 (PM-fiber) and 1.5 ± 0.4 (PM-METAC-fiber) (Figure 4F). There was no difference in the diameters of PM-fibers and PM-METAC-fibers in both the dry and the swollen states.

In this study, the METAC units of poly(METAC-*co*-MAAc), which are antibacterial components, were modified on the surfaces of PM-fibers. This modification method is classified as the grafting-to method, and the carboxy groups of the MAAc units in the copolymers may bond with the hydroxy groups on the fibers at multiple points. In our previous study, as a grafting-to method, polymers with a thiol group at the polymeric chain end were attached to the surface of silica nanoparticles with ene groups using thiol−ene click chemistry.[53] The polymer modified into silica nanoparticles was a pH-responsive cationic poly(2-(diethylamino)ethyl methacrylate (DEAEMA)) with a M_n of 5200. Dynamic light scattering (DLS) measurements showed that the modified polymeric layer on the silica nanoparticles was approximately 40 nm. Boyer et al. modified poly(*N*,*N*-dimethylaminoethyl acrylate) with an M_n of 10500 on the surface of 20 nm gold nanoparticles (AuNPs) via the grafting-to method.[54] Using DLS, the thickness of the polymeric layer on the AuNPs was calculated to be approximately 45 nm. These polymers have a reactive site at the chain end and are connected to each nanoparticle at one point. However, poly(METAC-*co*-MAAc) may be bonded at multiple points, and the polymeric layer

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Table 1. Thermodynamic Properties of Poly(METAC-*co*-MAAc), PM-Fiber, and PM-METAC-Fiber (N.D., Not Determined)

code	T_g (°C)	T_m (°C)	$-\Delta H_m$ (J/g)	$T_{d,10\%}$ (°C)
poly(METAC-*co* -MAAc)	N.D.	N.D.	N.D.	215.2 ± 1.1
PM-fiber	67.2 ± 0.4	164.5 ± 0.5	6.0 ± 0.6	295.0 ± 1.0
PM-METAC-fiber	67.1 ± 0.4	167.4 ± 0.2	7.5 ± 0.3	288.8 ± 3.8

becomes thinner than when bonded at one point on the polymeric chain end. The modified poly(METAC-*co*-MAAc) layer is sufficiently small compared to the diameter of PM-fibers (21.3 μm), and no difference was observed between the diameters before and after modification by the microscope measurement. These results suggest that the PM-fibers and PM-METAC-fibers have similar diameters in the dry and swollen states.

Next, the thermodynamic properties of PM-fibers and PM-METAC-fibers were compared. Table 1 shows the glass transition temperature (T_g), melting point (T_m), enthalpy change (ΔH_m), and temperature at 10% weight loss ($T_{d,10\%}$) of PM-fibers and PM-METAC-fibers (Figures S2 and S3). The peaks (T_g, T_m, and ΔH_m) of poly(METAC-*co*-MAAc) were too weak to analyze by DSC. The values of T_g and $T_{d,10\%}$ between PM-fibers and PM-METAC-fibers were similar to the values of the *t* test ($p > 0.05$). On the other hand, the values of T_m and ΔH_m were different between PM-fibers and PM-METAC-fibers ($p < 0.05$). Compared with the PM-fibers, the PM-METAC-fibers were subjected to additional thermal cross-linking for 24 h to modify the poly(METAC-*co*-MAAc). This additional thermal cross-linking increases the number of cross-linked structures within the PM-fiber and may affect the thermodynamic properties of the entire microfiber. The thermodynamic properties of the flat films were affected before and after thermal cross-linking.[29] Slight differences in the fiber properties can be reduced by optimizing the thermal cross-linking time. These results suggest that the antibacterial PM-METAC-fibers have physicochemical properties similar to those of the PM-fibers.

4. CONCLUSIONS

Poly(METAC-*co*-MAAc) was chemically modified on the surface of PVA-based microfibers by a simple thermal treatment at 135 °C. The poly(METAC-*co*-MAAc) present on the extreme surface did not affect the diameter or swelling ratio of the microfibers. The modified microfibers showed antibacterial properties against *B. subtilis* (Gram-positive bacterium) and *E. coli* (Gram-negative bacterium), and the percentage of hemolysis against rabbit RBCs was equivalent to that of the negative control. These results suggest that the modified microfibers exhibit selective antibacterial properties. The prepared microfibers are expected to be useful in antibacterial sutures and woven fabrics. In addition, we modified the fluorescent copolymers into PVA-based sponges and parts of the PVA-based films using a similar protocol. This modification method can be applied to various PVA-based materials and imparts antibacterial properties without significantly affecting their physicochemical properties at a low cost.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c05637.

The number of bacterial colonies exposed to poly(METAC-*co*-MAAc) aqueous solutions at various concentrations for 1 h and 5 min (Tables S1 and S2); SEM images of *E. coli* and *B. subtilis* on flat PVA/poly(MAAc) films (Figure S1); and DSC and TGA curves of poly(METAC-*co*-MAAc) and fibers (Figure S2 and S3) (PDF)

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Notes

The authors declare no competing financial interest.

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