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Electrostatic Immobilization of Cetylpyridinium Chloride to Poly(vinyl alcohol) Hydrogels for the Simple Fabrication of Wound Dressings with the Suppressed Release of Antibacterial Agents

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ABSTRACT: Polymeric systems for antibacterial wound dressings require chemical reactions or syntheses for attaching or incorporating antibacterial moieties into polymer backbones. However, these materials often fail to satisfy the basic requirements, such as easy and inexpensive synthesis. We speculated that a positively charged organic antibacterial agent would be attracted to the polar groups of poly(vinyl alcohol) (PVA) hydrogels and would show suppressed release. PVA hydrogels containing cetylpyridinium chloride (CPC) were prepared by γ irradiation. CPC was barely released from the hydrogels, probably because of electrostatic interactions, and was stable upon γ irradiation. The suppressed release of CPC conferred antibacterial activity against *Escherichia coli* to the surface of the hydrogels, whereas no inhibition zone was observed around the hydrogels. The CPC-containing PVA hydrogels were easy to prepare and contained known and safe materials. The simplicity and safety of this procedure for achieving the suppressed release of antibacterial agents were advantages of these CPC-containing PVA hydrogels. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40456.

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INTRODUCTION

Wound dressings that create and maintain a moist environment are considered to provide optimal wound healing.[1] Wet dressings are classified as modern wound dressings.[2] Wet environmental conditions are effective for skin regeneration without the formation of eschars during healing.[3] Various physical forms, such as films, gels, foams, and ointments, have been developed for use as wound dressings. Among these wound dressings, hydrogels have received special attention because of their unique properties. Hydrogels have most of the desirable characteristics for ideal dressings; these characteristics involve debridement, the maintenance of a moist environment, the absorption of excess exudates, and the prevention of infections.[4]

Antibacterial wound dressings have recently been developed with the consideration of the fact that moist, warm, and nutritious environments in wound beds provide ideal conditions for microbial growth.[5,6] The contamination of both surgical and chronic wounds to some extent with bacteria is almost inevitable.[7] The release of antibiotics should be regulated because the antibacterial activity and cytotoxicity are predominantly affected by the release characteristics. Antibiotic release from polymeric hydrogels can be influenced by one or more physical processes involving the diffusion of fluids and antibiotics.[8,9] However, simple formulations involving hydrogel encapsulation show a rapid release of antibiotics.[10] The suppressed release of antibiotics is required to achieve sufficient antibacterial activity and to lower the potential cytotoxicity of the antibiotics.

A promising approach for the suppression of the release of antibiotics is to attach or incorporate antibacterial components to polymers by chemical reactions.[11] For example, polymethacrylate, which contains pendant biguanide groups and polyvinylbenzyl ammonium chloride, has been proven to have a high biocidal activity against *Staphylococcus aureus* and *Escherichia coli*.[12,13] Because the cationic compound biguanide shows antibacterial activity, poly(hexamethylene biguanide) was developed as the first antibacterial polymer. Quaternary ammonium compounds, such as polyvinylbenzyl ammonium chloride, are traditional organic antibacterial agents. Covalently attached antibacterial moieties have also been used to create antifouling or antibacterial polymer surfaces.[14] These systems use chemical reactions or syntheses to attach or incorporate antibacterial

Table I. Compositions of the PVA/PVP Hydrogels Containing CPC

Composition in the hydrogel			
PVA (%)	PVP (%)	CPC (%)	CPC/PVA-OH molar ratio
18	2	0	0
18	2	0.08	0.58×10^{-3}
18	2	0.16	1.2×10^{-3}
18	2	0.24	1.7×10^{-3}

moieties to polymer backbones. However, their syntheses are often difficult and expensive.[15] Commercially available antibacterial wound dressings are simple composites of polymer substrates and antibiotics, such as poly(hexamethylene biguanide),[16] silver,[17] or iodine.[3]

We have focused on poly(vinyl alcohol) (PVA) as a polymer substrate for antibacterial wound dressings. PVA has hydroxyl groups along the chain, which act as adsorption sites for charged compounds. The adsorption of albumin onto PVA has been well demonstrated.[18,19] PVA is a hydrophilic polymer with good biocompatibility. It has been used in numerous biomedical applications, including implants,[20] contact lenses,[21] and wound dressings.[22,23] We speculated that a positively charged organic antibacterial agent would be attracted by the polar groups of PVA and, thereby, suppress its release. This process would enable the simple manufacture of an antibacterial hydrogel wound dressing without the use of chemical reactions and, thus, ensure biological safety.

In this study, we prepared PVA hydrogels containing cetylpyridinium chloride (CPC), a quaternary ammonium salt, by a well-known crosslinking technique using γ irradiation.[23,24] CPC is a common cationic detergent, which exhibits antibiotic activity against various microorganisms.[25,26] The interaction between CPC and PVA, the mechanical and structural properties of the hydrogels, and the release characteristics of CPC were evaluated. In addition, the antibacterial activity of the hydrogel was evaluated by an agar diffusion test. The results show that CPC was almost retained in the hydrogels; this was probably due to electrostatic interaction between CPC and PVA. The CPC-containing PVA hydrogels showed antibacterial activity on the surface, whereas no inhibition zone was observed in the agar diffusion tests. Thus, we developed a simple manufacturing process without chemical reactions for creating antibacterial hydrogel wound dressings that showed less or no release of the antibacterial agent.

EXPERIMENTAL

Materials

PVA (degree of saponification = 87–89% and viscosity of a 4% solution at 20°C = 23–27 mPa s) and poly(vinyl pyrrolidone) (PVP; PVP K90, viscosity of 5% solution at 20°C = 50–90 mPa s) were purchased from Wako Pure Chemical Industries (Japan). CPC and deuterium oxide (D_2O) were obtained from Sigma-Aldrich (St. Louis, MO).

Adsorption Test for CPC

The adsorption of CPC onto PVA was investigated under aqueous conditions, and the estimation of the adsorption rate was based on the CPC content in the filtrate with an ultrafilter. CPC solution (2 mL) in saline (20–5120 μg/mL) was added to an equal volume of PVA solution in saline (8 mg/mL) in a 15-mL polypropylene tube and immediately vortexed. After intervals of 12 h at room temperature, the mixed solution was filtered with a centrifugal filter device (Amicon Ultra Ultracel-50K; molecular weight cutoff = 50,000, Merck Millipore, Billerica, MA). The absorbance of the filtrates thus obtained was measured with a spectrophotometer (UV-3100S, Shimadzu, Japan). The CPC contents in the filtrates were determined with the UV absorbance at 260 nm.

γ Irradiation Test for CPC

The stability of CPC against γ irradiation was investigated under aqueous conditions. CPC (20 mg/mL) and a CPC/PVA mixed solution in saline (both 10 mg/mL) were prepared, and 1 mL of each solution was added to 2-mL microtubes. The microtubes were sealed in a hermetic bag containing an oxygen absorber (oxygen absorber set A-500-50S, ISO, Japan). The bag was irradiated with ^{137}Cs-generated γ rays (PS-3000SB, Pony, Japan). A dose rate of 705 Gy/h was used to achieve a dose of 50 kGy.

The γ-irradiated solutions (800 μL) were mixed with D_2O (200 μL) and loaded into NMR tubes (diameter = 4 mm). ^{13}C-NMR measurements were performed on a JEOL JNM-ECA600 spectrometer at a ^{13}C frequency of 150 MHz. All of the ^{13}C spectra were obtained with the ^{1}H decoupling mode. Moreover, the γ-irradiated solutions were spectrophotometrically analyzed. The solutions were diluted to achieve a concentration of 40 μg/mL, and the UV spectra (200–300 nm) were measured.

Preparation of the CPC-Containing PVA Hydrogels

The hydrogels were prepared from PVA in a saline solution by γ irradiation. The compositions of the hydrogels are listed in Table I. PVP (2%, PVA/PVP = 9:1) was added to an 18% PVA solution to increase the water-binding capacity.[27] The mixed solution (10.5–11.5 g) of PVA/PVP with concentrations of 18 and 2%, respectively, was poured into a poly(ethylene terephthalate) bag and sealed to achieve an approximate dimension of $2 \times 60 \times 90$ mm^3. Different solutions, each containing 0.08, 0.16, or 0.24% CPC, were γ-irradiated at a dose of 50 kGy (^{60}Co-generated γ irradiation, Koga Isotope, Japan). A hydrogel containing no CPC was also prepared and used as the control. Four hydrogel sheets were used in the following experiments.

To measure the IR spectra, 18% PVA hydrogels containing 5% CPC were prepared by γ irradiation. CPC concentrations of less than 0.24% were too low to be detected by IR measurements. Hydrogels containing no CPC and 5% CPC were air-dried to obtain films for the IR measurements. The 18% PVA solutions containing no CPC and 5% CPC were also air-dried to obtain films as nonirradiated samples.

Characterization of the CPC-Containing PVA Hydrogels

The mechanical properties of the hydrogels were assessed by dynamic viscoelastic measurements with a parallel-plate rheometer (Haake MARS III, Thermo Fisher Scientific, Franklin, MA). Hydrogel sheets with an approximate thickness of 2 mm were cut into discs (diameter = 22 mm) and put on the bottom plate of the rheometer at 37°C. The upper plate (diameter = 20 mm) was moved and allowed to come in contact with the specimens

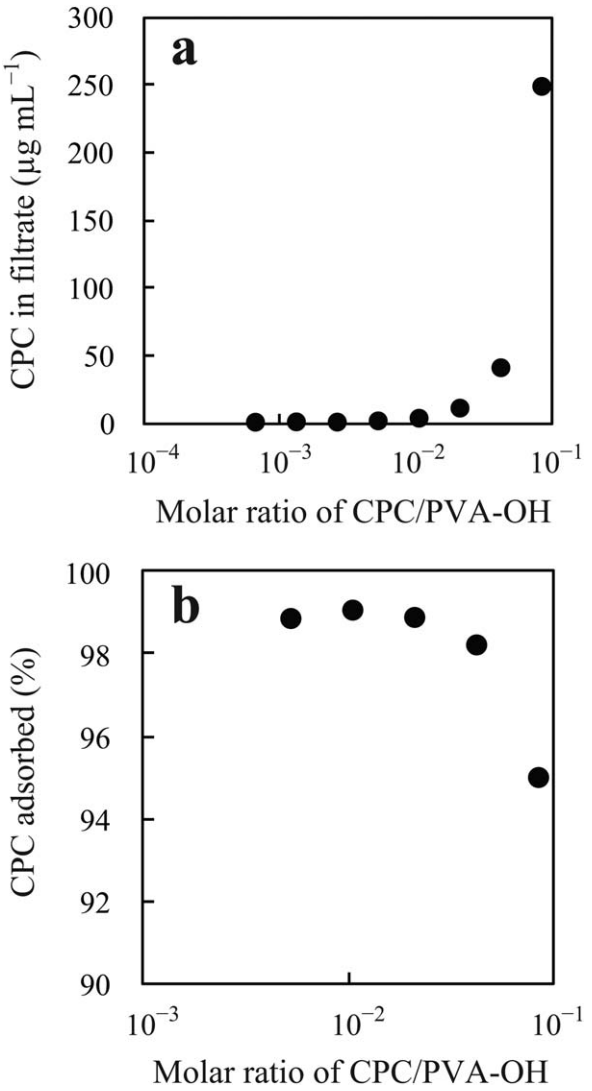

Figure 1. Adsorption of CPC onto PVA under aqueous conditions. CPC/PVA mixed solutions were filtered with an ultrafilter (molecular weight cutoff = 50,000), and the CPC contents in the filtrates were determined by UV spectrophotometry: (a) CPC concentrations in the filtrates as a function of the CPC/PVA-OH molar ratios and (b) CPC concentrations converted to the adsorbed rates of CPC to PVA (%).

to achieve a normal force of 1.5–3.0 N. The oscillation at a constant shear stress (50 Pa) was initiated under a frequency sweep mode (0.1–10 Hz), and the storage modulus (G') was determined at a frequency of 1 Hz.

Thermogravimetric analyses (TGA) of the PVA hydrogels were carried out on a Shimadzu DTG-60/60H thermal analyzer (Japan). The hydrogels were air-dried at 37°C in a drying chamber and additionally dried with a vacuum pump. The dried sample (10–13 mg) was put in an aluminum pan, and the analyses were performed under a nitrogen flow rate of 50 cm^3/min and a heating rate of 10°C/min.

The films obtained from the PVA hydrogels and solutions were subjected to Fourier transform infrared–attenuated total reflection (ATR) spectroscopy. A Nicolet 6700 Smart Orbit Diamond ATR instrument (Thermo Scientific, Franklin, MA) was used. The spectra were recorded in the 4000 to 400-cm^{-1} region with a resolution of 1 cm^{-1}.

CPC Release from the Hydrogels

The release characteristics of CPC from the hydrogels were assessed with a saline solution as the releasing medium. The specimens (ca. 2 × 15 × 15 mm^3) cut from the hydrogel sheets were precisely weighed and immersed in 20 mL of the releasing medium at 37°C. The triplicate specimens were collected at immersion periods of 6, 24, and 72 h. The microresidues in the releasing medium were removed with a syringe filter (pore size = 0.45 μm). The CPC content in the releasing medium was spectrophotometrically determined with the UV absorbance at 260 nm. The releasing rates of CPC against the initial amounts in the specimens (as a percentage) were calculated from the amounts in the releasing medium and the weights of the specimens.

To observe the CPC remaining in the specimens, the UV spectrum (200–300 nm) of the hydrogel sheet containing 0.08% CPC was also measured. After the releasing test, the specimens were set in a quartz cuvette (optical path length = 10 mm), through which the UV light passed vertically.

Antibacterial Evaluation

The antibacterial activities of the hydrogels were evaluated by the agar diffusion test described in the JIS L 1902 standard,[28] which showed the diffusion of the antimicrobial agent from the specimens, followed by the counting of the bacteria, which quantified the antimicrobial activity of the specimens. The Gram-negative bacterium *E. coli* (ATCC 33456), which is commonly found in infected wounds,[29] was used as the test microorganism. The test bacteria were cultured during shaking in lysogeny broth (LB) medium overnight and used for the following tests.

Agar Diffusion Test. The inoculum was spread onto LB agar plates to achieve 10^7 colony forming units (cfu)/cm^2. The disc specimen (diameter = 20 mm, thickness = 1 mm) was placed on the center of the agar plate and then incubated at 37°C for 18 h. When it reached inhibitory concentrations, a clear zone (i.e., an inhibition zone) without colonies was observed around the disc specimens. The width of the inhibition zone was measured.

Counting of the Bacteria. The agar gel in contact with the specimen in the agar diffusion test was cut from the plate, and the bacterial colonies were visually counted with a microscope. The results are indicated as colony forming units per square centimeter.

Statistical Analyses

The data of the antibacterial tests are expressed as means plus or minus the standard deviation (SD; $n = 3$), and statistical analysis was performed with the Dunnett's t test. A p value of less than 0.05 was considered significant compared with the data of the neat hydrogel.

RESULTS

Adsorption of CPC to PVA

CPC was adsorbed onto PVA under aqueous conditions. Figure 1(a) shows the CPC concentrations in the filtrates as a

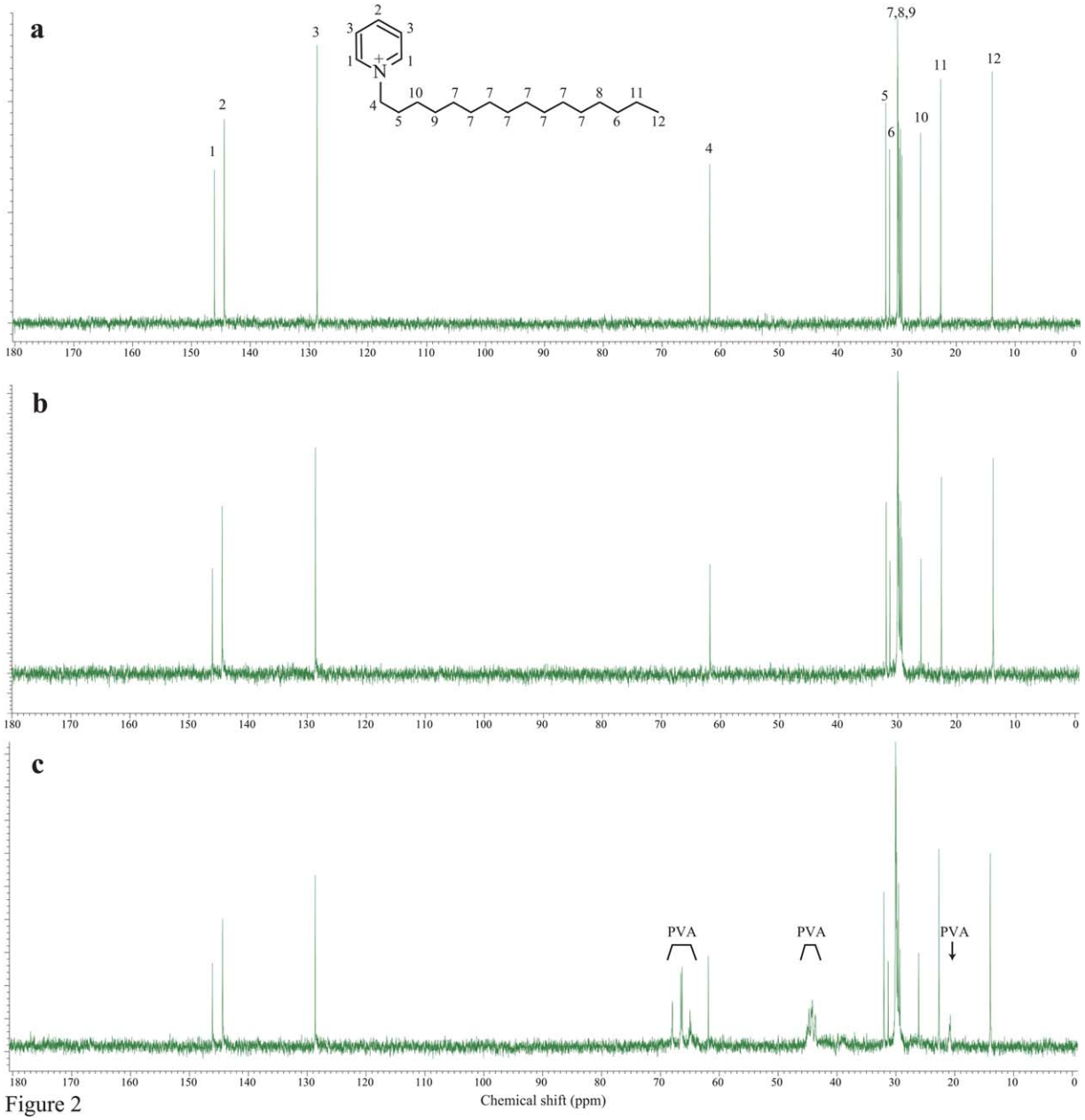

Figure 2

Figure 2. ^{13}C-NMR spectra of the (a) nonirradiated CPC solution, (b) 50-kGy-irradiated CPC solution, and (c) 50-kGy-irradiated CPC/PVA mixed solution. The assignments of the peaks of CPC are shown in part a. The peaks of PVA are indicated in part c. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

function of the molar ratios of CPC to the hydroxyl groups of poly(vinyl alcohol) (PVA-OH) in the mixed solutions. CPC was slightly detected (2.2–2.4 μg/mL) in the filtrates when the CPC/PVA-OH molar ratios were set at 6.5×10^{-4} to 2.6×10^{-3} (initial CPC concentration = 40–160 μg/mL in the adsorption tests). The small amount of CPC in the filtrates was probably due to the passage of low-molecular-weight PVA species, which adsorbed CPC through the filter. When the CPC/PVA-OH molar ratios were above 5.2×10^{-3} (CPC concentration = 320 μg/mL), the CPC concentration in the filtrate increased as the CPC/PVA-OH molar ratio increased. The threshold concentration of CPC corresponded to 0.72% CPC in the 18% PVA hydrogel; this was much higher than those in the PVA hydrogels prepared in this study (Table I).

The CPC concentrations were converted to the adsorbed rates of CPC to PVA [%; Figure 1(b)]. Almost all of CPC (>98%) was adsorbed onto PVA when the CPC/PVA-OH molar ratios were less than 4.1×10^{-2} (corresponding to <5.8% CPC in the 18% PVA hydrogel). A slight decrease in the adsorbed CPC (95%) was observed when the CPC/PVA-OH molar ratio reached 8.3×10^{-2} (corresponding to 12% CPC in the 18% PVA hydrogel). In Figure 1(b), the data for the CPC/PVA-OH molar ratios below

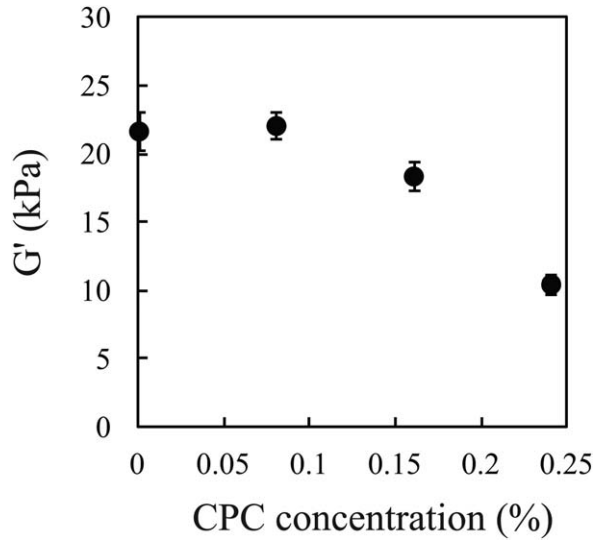

Figure 3. Influence of the addition of CPC on the G' values of the PVA hydrogels. The data are presented as the mean plus or minus SD ($n = 3$).

2.6×10^{-3} (initial CPC concentration < 160 μg/mL) were not used because the influence of CPC passing through the filter (2.2–2.4 μg/mL of CPC) could not be neglected.

Stability of CPC against γ Irradiation

The structural change of CPC upon γ irradiation was evaluated by ^{13}C-NMR and UV spectrophotometry. The ^{13}C-NMR spectrum of the 50-kGy-irradiated CPC [Figure 2(a)] was identical to that of the nonirradiated CPC [Figure 2(b)], where no changes in the chemical shifts were observed. The presence of PVA did not alter the spectrum of the 50-kGy-irradiated CPC [Figure 2(c)]: the spectrum was a summation of those of the PVA and 50-kGy-irradiated CPC. Similar results were obtained by spectrophotometry; the UV spectrum of the 50-kGy-irradiated CPC was almost identical to that of the nonirradiated CPC (data not shown). The decrease in the optical density at 260 nm by irradiation was less than 5%.

Effects of CPC on the Mechanical and Structural Characteristics of the PVA Hydrogels

Figure 3 shows the G' values of the hydrogels as a function of the CPC concentration in the 18% PVA hydrogels. G' decreased as the concentration of CPC increased. At a CPC concentration of 0.24%, G' was 10.5 ± 0.7 kPa.

Figure 4 shows the results of TGA of the hydrogels containing various concentrations of CPC. The weight losses were initiated at about 100°C in all of the hydrogels. When the temperature reached 280°C, the weight losses became more rapid as the CPC concentrations increased. The thermogravimetry curve of the hydrogel containing 0.08% was similar to that with no CPC.

Figure 5 shows the IR spectra of the nonirradiated and 50-kGy-irradiated 18% PVA hydrogels containing 0 or 5% CPC. The spectrum of the 50-kGy-irradiated PVA containing no CPC was almost identical to that of the nonirradiated sample [Figure 5(a)]. The spectra of the 50-kGy-irradiated PVA containing no CPC and 5% CPC were also similar: the peaks of C=C (1471, 1488, and 1507 cm^{-1}) and C=N stretching vibrations of the CPC pyridine ring (1636 cm^{-1}) showed no change with γ irradiation [Figures 5(b,c)]. The C—N stretching vibration of methylene (2847 and 2912 cm^{-1}) of CPC showed no evidence of a peak shift [Figure 5(d)].

Release Characteristics of CPC

The release test revealed that CPC was barely released from the hydrogels. A clear absorbance of the CPC solution at 260 nm (Figure 6) was not clear in the UV spectra of the releasing media even when the immersion of the hydrogels was continued for 72 h [Figures 7(a)]. The net absorbances of the releasing media obtained from the hydrogels containing 0.08, 0.16, and 0.24% CPC were 0.011 ± 0.005, 0.022 ± 0.004, and 0.023 ± 0.006 [Mean ± SD, $n = 9$ ($n = 3$ for each immersion period of 6, 24, and 72 h)], respectively, where the increase in absorbance was almost saturated in 6 h. The optical density at 260 nm (OD_{260}), which linearly correlated with the CPC concentration, could be used to determine the CPC concentration in the releasing medium, and a calibration curve [CPC concentration (μg/mL) = $81.4 \times OD_{260} - 0.03$] was established. The linearity of the calibration curve was lost at CPC concentrations of less than 0.8 μg/mL ($OD_{260} < 0.01$); this indicated that the net absorbance was around the lower limitation of detection. In particular, in 0.08% CPC, four pieces of nine data were below the limitation. The CPC concentrations in the release media were at least less than 1.08 μg/mL for 0.08% CPC, less than 1.35 μg/mL for 0.16% CPC, and less than 2.44 μg/mL for 0.24% CPC [Figure 7(b)]. The releasing rates were calculated to be less than 6.3% for 0.08% CPC, less than 3.9% for 0.16% CPC, and less than 4.1% for 0.24% CPC.

To observe CPC in the hydrogels after the releasing test, the UV spectra of the hydrogels containing 0 and 0.08% CPC were measured (Figure 6). A broad absorbance in the wavelength range 240–280 nm was observed in the hydrogel containing

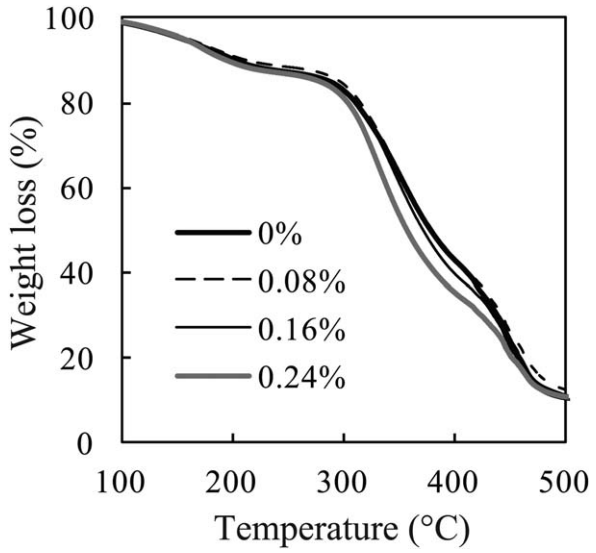

Figure 4. TGA curves of the PVA hydrogels containing various concentrations of CPC. The hydrogels were dried *in vacuo* before the measurements. The numbers in the figure indicate the concentrations of CPC in the hydrogels.

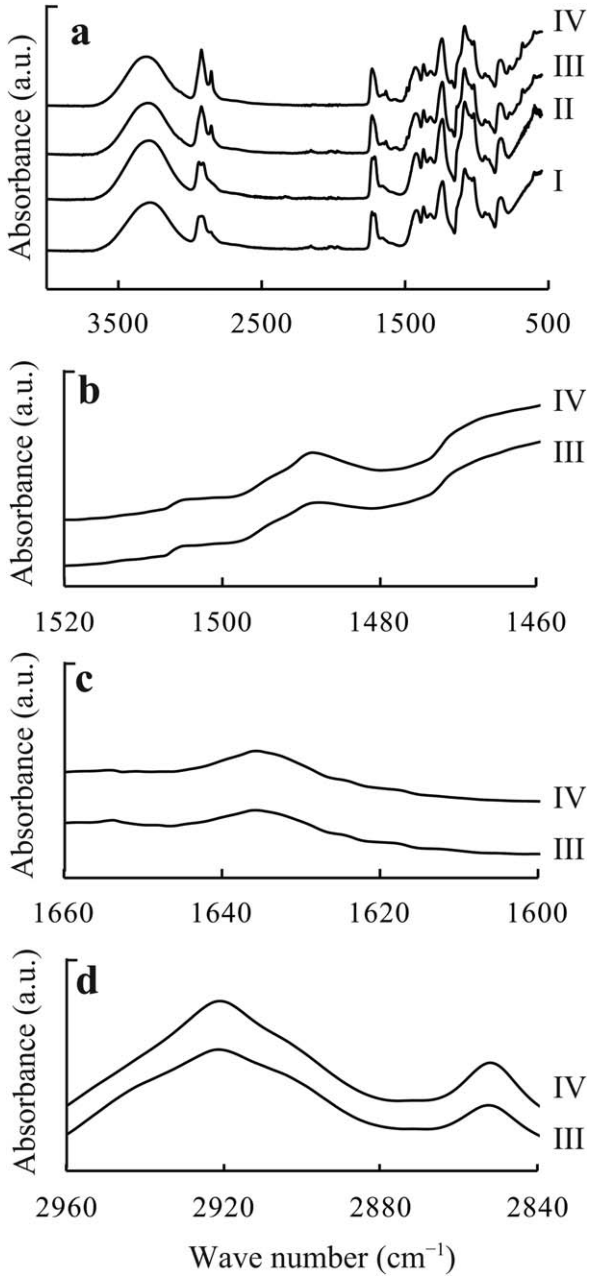

Figure 5. ATR spectra of the dried PVA solutions and hydrogels: (I) a film prepared from the nonirradiated 18% PVA solution containing no CPC, (II) a film prepared from the γ-crosslinked 18% PVA hydrogel containing no CPC, (III) a film prepared from the nonirradiated 18% PVA solution containing 5% CPC, and (IV) a film prepared from the γ-crosslinked 18% PVA hydrogel containing 5% CPC. The figures in parts b–d are magnifications of part a. The peak assignments are as follows: C=C stretching vibrations of the CPC pyridine ring (1471, 1488, and 1507 cm^{-1}), C=N stretching vibrations of the CPC pyridine ring (1636 cm^{-1}), and C—N stretching vibrations of the methylene of CPC (2847 and 2912 cm^{-1}).

0.08% CPC; this suggested the presence of CPC in the hydrogel. There was a strong UV absorption at wavelengths below 240 nm, probably because of PVA and PVP. The UV spectra of the hydrogels containing CPC had higher baselines at wavelengths above 280 nm compared to that of the hydrogels containing no CPC. The hydrogels containing CPC were colored yellow by γ irradiation, which probably elevated the baseline of the wavelength in the UV region.

Antibacterial Activities

The antibacterial activities of the CPC-containing hydrogels were evaluated according to the standard test method JIS L 1902, which covers measurements of the inhibition zones around the specimens and the counting of the number of viable bacteria absorbed in the specimens. The CPC-containing hydrogels showed no inhibition zones; this was similar to the neat hydrogels (Figure 8).

Figure 9 shows the decrease in the number of viable bacteria after contact with the hydrogels. The number of viable cells decreased as the CPC concentration increased. When the CPC concentration reached 0.16%, the number of viable bacteria was significantly lower than that of the hydrogel containing no CPC ($p < 0.05$). The number of viable bacteria in contact with the control hydrogel was similar to the initial value [log(cfu)/cm^2 = 7]; this indicated that the polymers in the hydrogel showed no antibacterial activity against *E. coli*.

DISCUSSION

We successfully fabricated an antibacterial hydrogel wound dressing with suppressed release of the antibacterial agent by a simple and safe process: γ irradiation on a PVA/PVP/CPC mixed solution. The simplicity and safety of the method are advantageous in the biomedical field, where devices with novel materials or chemical reactions are urgently required to ensure biological safety. Synthesized polymers are regarded as new materials in drug approvals, even if the polymer backbones have

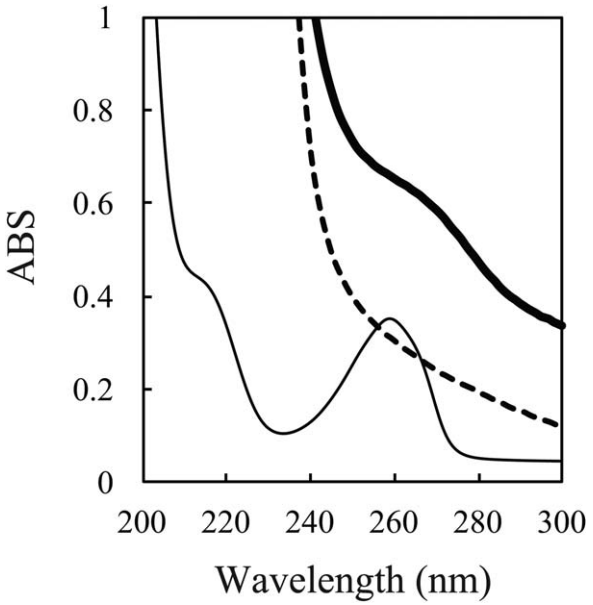

Figure 6. Typical UV spectra of the PVA hydrogel containing 0.08% CPC after the releasing test (bold solid line), the hydrogel containing no CPC (dotted line), and the CPC standard solution at a concentration of 25 μg/mL (narrow solid line).

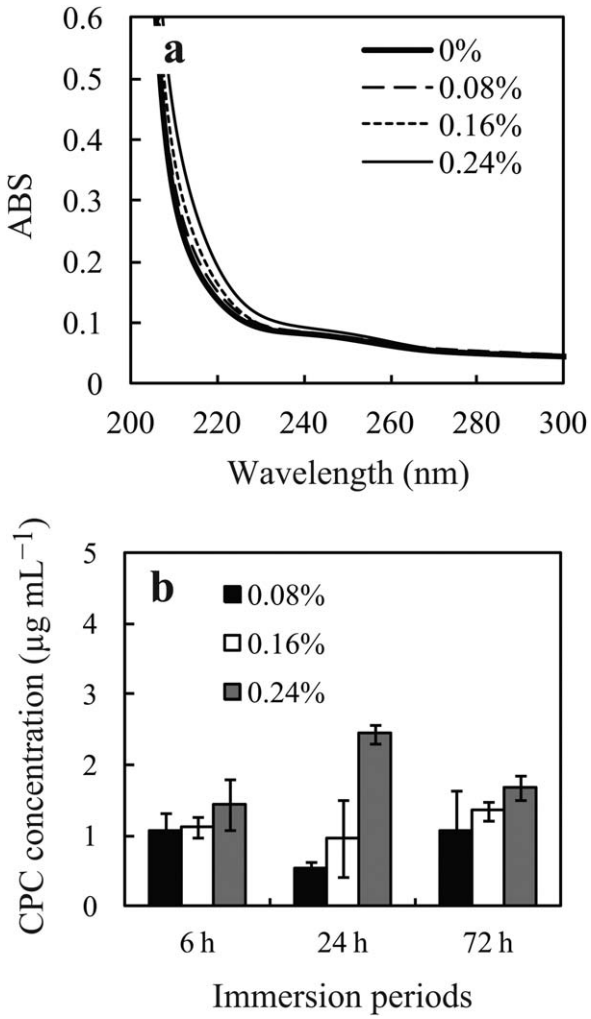

Figure 7. Release of CPC from the PVA hydrogels in the releasing medium: (a) the representative UV spectra of the releasing medium obtained from the PVA hydrogels containing 0–0.24% CPC in 72 h of immersion and (b) the releasing rates of CPC determined by UV absorbance in the releasing media. The numbers in the figure indicate the concentrations of CPC in the hydrogels. The data are presented as the mean plus or minus SD ($n = 3$).

been clinically used and proven to be safe. Kenawy et al.[15] described that easy and inexpensive synthesis is one of the basic requirements for antibacterial polymers. Our process used PVA, PVP, and CPC as components of a wound dressing and γ irradiation as a crosslinking method. PVA and PVP have been clinically used for wound dressings. CPC has been approved as an antibiotic for oral care, although CPC-containing wound dressings have not been developed. γ irradiation is commonly used for sterilizing biomedical devices. Our method for preparing hydrogel wound dressings involved γ irradiation at a dose of 50 kGy and offered simultaneous sterilization. The simplicity and safety of the procedure for achieving the suppressed release of antibacterial agents should accelerate the clinical uses of CPC-containing PVA hydrogels.

The suppressed release of an antibacterial agent is beneficial in reducing a patient's exposure to excessive drugs beyond the required amounts[30] and allows us to minimize dressing change by prolonged activity.[30] If the hydrogel used is a simple polymeric formulation, there is no interaction between the drugs and polymers. This results in rapid drug release through the swelling of the hydrogel and subsequent diffusion of the drug through the swollen hydrogel.[2] Rapid-release antibacterial agents have the limitation of residual cytotoxicity even when suitable amounts of the agent are added.[15] The CPC-containing hydrogel prepared in this study is a potential antibacterial wound dressing material because it suppresses the release of antibacterial agents.

The suppression of CPC release could be explained by the findings that CPC was almost completely adsorbed on PVA by electrostatic interactions and was stable against γ irradiation. The capacity of CPC adsorption occurred at a 4.1×10^{-2} CPC/PVA-OH molar ratio or greater; this suggested that CPC was completely adsorbed on PVA in the hydrogel (0.58×10^{-3} to 1.7×10^{-3} of CPC/PVA-OH molar ratios). γ irradiation for crosslinking of the CPC/PVA/PVP mixed solutions caused the crosslinking of PVA and PVP while maintaining the adsorption of CPC to PVA (and probably to PVP). The small release of CPC detected in the release tests was probably due to noncrosslinked PVA adsorbing CPC. Released fragments of PVA/PVP were detected in the UV spectrum of the releasing medium after the soaking of the hydrogels. In addition to chemical attachment or the incorporation of antibacterial moieties to polymers, our findings revealed an alternative process for achieving the suppressed release of drugs in a polymeric formulation.

The other interactions or chemical bonds were unlikely to act on the suppressed release of CPC. PVA is a highly hydrophilic polymer containing a hydroxyl group in every monomer unit, and the hydrophobic interactions between PVA and CPC could have been negligible. The NMR, IR, and UV data showed no evidence for covalent bonds between PVA and CPC formed by γ irradiation. Furthermore, our preliminary study showed that the CPC molecules were adsorbed completely by the γ-crosslinked PVA hydrogels when the hydrogels were soaked in CPC solutions. The suppressed release of CPC was predominantly due to electrostatic interactions.

The antibacterial activity on the surface of the hydrogels suggested that the structural characteristics of CPC were maintained in the hydrogels. CPC is a quaternary ammonium salt, whose antibacterial activity against Gram-negative bacteria was due to its surfactant properties and high binding affinity for the bacterial cell membrane.[11] We believe that the quaternary ammonium ion of CPC was the adsorption site for the polar PVA-OH. In this case, its surfactant properties could be retained because the long alkyl chains of CPC were solvent-exposed. On the other hand, the cationic charge of the quaternary ammonium ion may have been neutralized and showed no binding affinity. The detailed mechanism of the antibacterial activity of the CPC-containing hydrogels was beyond the scope of this study, but we are investigating the antibacterial activity of other quaternary ammonium compounds to clarify the effects of the alkyl chains and quaternary ammonium ions on the antibacterial activity of PVA hydrogels.

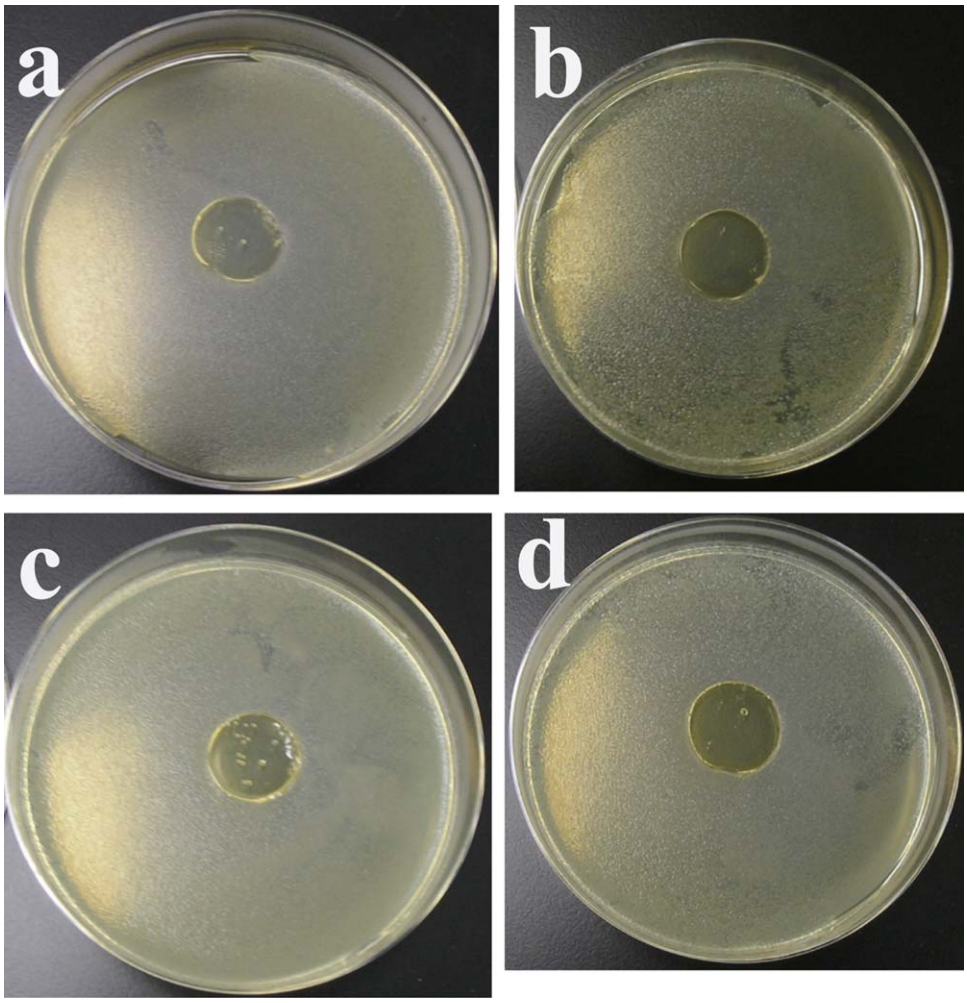

Figure 8. Representative appearances of the disc diffusion test for the CPC-containing PVA hydrogels: (a) 0, (b) 0.08, (c) 0.16, and (d) 0.24% CPC. All of the hydrogels containing CPC showed no inhibition zone similar to the neat hydrogel. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The mechanical properties are also important for hydrogel wound dressings. From our experiments, the PVA hydrogels with G' values below 7 kPa could not be used for wound dressings because of their softness, strong adhesiveness, and very large swelling in water. The decrease in the mechanical properties with the addition of CPC was due to the inhibitory effect of CPC on the γ crosslinking of PVA. This decrease in the crosslinking was supported by TGA. A further increase in the CPC concentration to achieve a higher antibacterial activity would decrease the mechanical properties of the hydrogels. In this case, increasing the γ dose could be a possible solution for maintaining the mechanical properties because the γ crosslinking of PVA proceeds at doses greater than 50 kGy.[24]

A limitation of this study was the lack of cytocompatibility tests. A complex mechanism seemed to underlie the cytocompatibility of the CPC-containing PVA hydrogels. As shown in the UV spectra of the releasing media, the PVA hydrogels released uncrosslinked or degraded PVA molecules; this posed the UV absorbance at a wavelength of less than 220 nm. It has been reported that a γ-crosslinked PVA hydrogel exhibited toxic effects on L929 fibroblasts.[31] The prewashing of the hydrogels may be required to remove toxic components generated by γ irradiation. A simple cytocompatibility test on the CPC-containing PVA hydrogels could not determine the cytotoxicity of CPC and PVA individually. To test the cytocompatibility of the CPC-containing hydrogels, cytocompatibility tests for each component (PVA, degraded PVA, and CPC molecules) are required in addition to the tests for the extracts of the hydrogels. A series of cytocompatibility tests are now under investigation.

CONCLUSIONS

We developed a simple manufacturing process without chemical reactions or syntheses for creating antibacterial hydrogel wound dressings whose antibacterial agents showed suppressed release. The suppressed release of CPC conferred antibacterial activity against *E. coli* to the surface of hydrogels. The method for preparing CPC-containing PVA hydrogels was simple, and the hydrogels contained known and safe materials. The simplicity and safety of the procedure for achieving the suppressed release

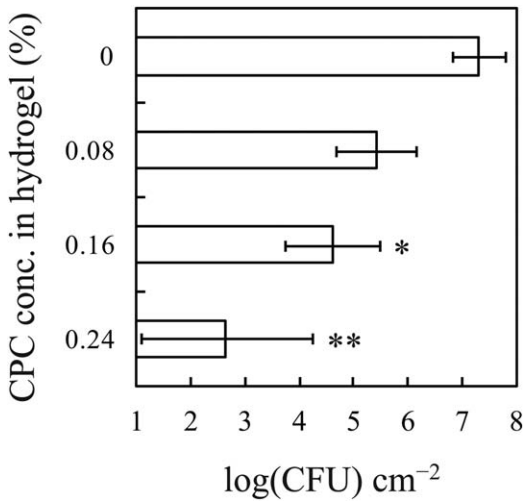

Figure 9. cfu of *E. coli* for the PVA hydrogels containing CPC. We measured the bacterial activity in the area where the hydrogel was in contact with the agar plate by counting the viable bacteria. The initial number of bacterial colonies on the agar plate was set to 7 [log(cfu)/cm^2]. The data are presented as mean plus or minus SD ($n = 3$). *$p < 0.05$. **$p < 0.01$.

of the antibacterial agent were the advantages of these CPC-containing PVA hydrogels.

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