# Application of Inorganic Particles Modified with Polyvinylamine to Produce Antibacterial Paper

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Antibacterial activity is one of the desired functionalities in paper and board grades, especially for packaging. This study designed a contact-active antibacterial surface using polyvinylamine (PVAm) bonded onto inorganic particles (kaolin), and investigated appropriate ways to utilize the treated inorganic particles as antibacterial carriers to produce antibacterial paper. Antibacterial inorganic particles were prepared by modifying the surface of kaolin through a polyelectrolytes multilayering (PEM) technique with a PVAm and polyacrylic acid system. The pH control during the PEM process affected the adsorption amount of PVAm and dispersion stability of PEM-treated kaolin. The PEM-treated kaolin was applied to prepared handsheets *via* two ways, internal addition or surface treatment. Only the surface-treated handsheets had a noticeably reduced bacteria ratio. Antibacterial activity was > 99.9% for *Escherichia coli* and > 99% for *Listeria monocytogenes*. The inactivation of bacteria with damaging membranes was confirmed by a dual staining method. The surface coverage of the PEM kaolin on the handsheets was an important factor for inactivation of the bacteria. As a result, the surface treatment of antibacterial inorganic particles was determined to be the proper strategy to produce antibacterial paper.

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## INTRODUCTION

Since the discovery of chemical antibiotics, such as penicillin in 1928, the development of antibiotics and antibacterial products have worked towards human life being free from pathogenic bacteria. Antibiotic treatments generally work by a biocides-leaching mechanism, in which the biocidal agents are released from the surface of their bound substrate into their surroundings, where they counteract the bacteria (Illergård *et al.* 2012). In contrast, surfaces modified by organic compounds, including quaternary ammonium, kill only bacteria physically interacting with the surface (Isquith *et al.* 1972; Tiller *et al.* 2001). These surfaces, referred to as contact-active surfaces, are fabricated with an irreversible adsorption of antibiotics onto the surface (Isquith *et al.* 1972; Illergård *et al.* 2012, 2015). The advantages of contact-active surfaces are that they do not pollute the environment due to their non-leaching character (Illergård *et al.* 2012, 2015), they can be used permanently (Klibanov 2007; Illergård *et al.* 2012), and they can destroy bacterial cell walls no matter the type or species of bacteria (Milovic *et al.* 2005; Illergård *et al.* 2015).

There have been many studies concerning antibacterial paper. These have dealt with aspects such as surface modification of fibers (Varaprasad *et al.* 2016) and surface coating of paper (Amini *et al.* 2016; Rehim *et al.* 2016; El-Samahy *et al.* 2017). Polyvinylamine (PVAm) is one of the cationic polyelectrolytes that has been found to exhibit antibacterial activity (Westman *et al.* 2009a, b; Illergård *et al.* 2010). It has also been demonstrated that cellulose fibers modified with PVAm (Illergård *et al.* 2012, 2015) or other cationic polyelectrolytes, such as chitosan (Imani *et al.* 2011), show antibacterial activity. These antibacterial cellulose fibers have been modified by a polyelectrolyte multilayering (PEM) technique (Illergård *et al.* 2012, 2015). The PEM technique is used to form fuzzy layers of macromolecules onto substrates with repetitive adsorption of charged compounds (Decher and Hong 1991; Decher *et al.* 1992; Decher 1997). Desired properties of multilayers can be constructed with controlling the types of polyelectrolytes, number of layers, salt concentration, and pH (Schönhoff 2003). It has been suggested that PEM treatment on cellulose fibers can contribute to improved paper strength (Wågberg *et al.* 2002; Ryu *et al.* 2011; Chin *et al.* 2012) and changes the wettability of paper (Lingström *et al.* 2006).

Because PVAm can also improve adhesion between fibers (Pelton and Hong 2002; Pelton 2014), paper with PEM-treated cellulose fibers using PVAm could have great potential to show not only antibacterial activity but also high strength. However, fabrication of antibacterial paper using the treated cellulose fibers may lead to reduced antibacterial activity when inorganic particles are used to make paper such as filled paper and coated paper. Another way to apply the PEM technology to the papermaking process is surface modification of inorganic particles. Inorganic particles, such as ground or precipitated calcium carbonate, clay, and talc, are frequently used as filler and coating pigment in the papermaking industry. The PEM treatment of ground calcium carbonate particles to improve the strength of filled paper and filler retention efficiency has already been studied (Ahn *et al.* 2012; Lee *et al.* 2013a, b). If PEM treatment using PVAm on inorganic particles is possible to provide antibacterial properties, it is expected that application of antibacterial paper can be extended to various paper grades including filled paper and coated paper grades. Because PVAm is a weak polyelectrolyte, the adsorbed amount and charge properties of PVAm can be controlled by adjusting the pH. Among inorganic particles, kaolin is stable over the whole pH range, in contrast with calcium carbonate. Therefore, the present study investigates PEM treatment using PVAm on kaolin particles and suggests an appropriate strategy to apply PEM-treated inorganic particles to fabricate antibacterial paper.

## EXPERIMENTAL

### Materials

Kaolin, as an inorganic particle, was purchased from Sigma Aldrich (Milwaukee, WI, USA).

The mixed hardwood bleached pulp fibers (Hw-BKP) were supplied by Moorim P&P in Ulsan, Korea. The Hw-BKP was beaten to 400 mL of Canadian Standard Freeness (TAPPI T227 om-99 (1999)) using a laboratory beater (Daeil Machinery Co., Daejeon, Korea).

Cationic polyvinylamine (PVAm, Xerolex 1300, BASF, Ansan, Korea) and

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anionic polyacrylic acid (PAA, $M_w$ to 100 kDa, Sigma Aldrich, Steinheim, Germany) were used for polyelectrolytes multilayering. Cationic polyacrylamide (C-PAM, Percol 63, BASF, Bradford, UK) and anionic micropolymer (Telioform M300, BASF, Suffolk, VA, USA) were used as retention aids, and alkyl ketene dimer (AKD, Ashland Inc., Wilmington, DE, USA) was used as the internal-sizing agent. Polyvinyl alcohol (PVOH, $M_w$ 13,000 to 23,000 g/mol, 98% hydrolyzed, Sigma Aldrich, Milwaukee, WI, USA) was used as the surface-sizing agent. Sodium chloride (NaCl) was used to control the salt concentration. Hydrochloric acid (HCl) and sodium hydroxide (NaOH) were used to adjust pH.

Strains of *Escherichia coli* (*E. coli*) O157:H7 (ATCC 35150, ATCC 43889, and ATCC 43890) and *Listeria monocytogenes (L. monocytogenes)* (ATCC 15313, ATCC 19111, and ATCC 19115) were obtained from the bacterial culture collection of Seoul National University in Seoul, Korea and used in this experiment. Stock cultures were stored at -80 °C in tryptic soy broth (TSB, Difco, Becton Dickinson, Sparks, Detroit, MI, USA) supplemented with 15% glycerol. Working cultures were streaked onto tryptic soy agar (TSA, Difco, Detroit, MI, USA) plate, incubated at 37 °C for 24 h, and stored at 4 °C. Each strain of *E. coli* O157:H7 and *L. monocytogenes* was grown in 5 mL of TSB at 37 °C for 24 h. Cells of each strain were collected by centrifugation at 4000 rpm at 4 °C for 20 min and washed two times with 0.2% peptone water (PW, Bacto, Sparks, MD, USA). The final pellets were re-suspended in sterile PW, corresponding to approximately $10^8$ CFU/ mL to $10^9$ colony-forming units (CFU)/mL for fraction of *E. coli* O157:H7 and $10^7$ CFU/mL to $10^8$ CFU/mL for fraction of *L. monocytogenes*.

## Methods

*Formation of polyelectrolytes multilayer on kaolin particles*

Kaolin particles were dispersed to 30 wt.% using a 0.01 M NaCl aqueous solution. The PVAm and PAA were diluted to 50 g/L using 0.01 M NaCl solution. A polyelectrolyte layer was formed through one adsorption step and two washing steps. In the adsorption step, polyelectrolyte was added to the kaolin suspension and the suspension was agitated for 5 min. In the washing step, excess polyelectrolytes that were not adsorbed by kaolin particles were removed by centrifugation at 3000 G for 20 min and decantation of the supernatant containing the excess polyelectrolytes. After dilution to 30 wt.% using a 0.01 M NaCl solution, the suspension was re-dispersed by using an ultrasonicator for 3 min (VCX 750, Sonics, Newtown, CT, USA). This washing step was performed 2 times. The addition levels of PVAm and PAA for the PEM treatment were 3 wt.% based on the oven-dried kaolin weight. The first layer on a negatively charged surface of kaolin particles was formed by adding cationic PVAm, and then anionic PAA was added to form the second layer. Finally, a PVAm/PAA multilayer on kaolin particles was formed with the repetitive laying process until seven layers were present. The pH during polyelectrolytes multilayering (PEM) treatment was adjusted at two conditions: one was a pH 9.0/3.5 condition in which a PVAm layer was formed at a pH of 9.0 and PAA layer was formed at a pH of 3.5, and the other was pH 9.0/9.0 in which both PVAm and PAA were adsorbed at a pH of 9.0. The pH was adjusted during the washing step.

The zeta potential and particle size of the PEM kaolin were measured by using a Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK), and Malvern Mastersizer 2000 (Malvern Instruments Ltd., Malvern, UK), respectively. In the measurement, the

PEM kaolin suspension was diluted to 0.1 g/L using a 0.01 M NaCl solution, and the pH was adjusted to a pH of 3.5 for the odd layers of the PEM kaolin in the case of the pH 9.0/3.5 condition and a pH of 9 for the others. The particle size was represented as volumetric average particle size. The adsorbed amount of PVAm on kaolin particles was evaluated by an analysis of nitrogen content using a Kjeldahl Protein/Nitrogen Analyzer (Kjeltec Auto 1035/1038 System, Tecator AB, Hilleröd, Denmark). Because only the PVAm contained nitrogen in this system, the adsorbed amount of PVAm was calculated using a PVAm concentration-nitrogen content calibration line.

*Preparation of antibacterial paper*

Filled handsheets were prepared using a handsheet former (Daeil Machinery Co., Daejeon, Korea). The target grammage of filled handsheets was 150 g/m$^2$, and the target filler content was 20%. Fillers used in this experiment were untreated kaolin, three layers of PEM kaolin using a pH 9.0/3.5 system, and seven layers of PEM kaolin using a pH 9.0/3.5 system. The retention system was a micropolymer system using C-PAM and a micropolymer, and AKD was added as an internal sizing agent. The addition levels of C-PAM, micropolymer, and AKD were 0.03%, 0.05%, and 0.1% based on dried fibers and fillers in the stock.

The surface treatment of PEM kaolin on the filled handsheets was conducted through a pigmentizing technique. The pigmentizing agent was prepared by mixing the three layers PEM-treated kaolin suspension using a pH 9.0/3.5 system and the PVOH solution with a 50:50 weight ratio. The solids content of the pigmentizing agent was 24%. There were two types of base paper: one was handsheets with untreated kaolin, and the other was handsheets filled with three layers PEM kaolin using a pH 9.0/3.5 system. The pigmentizing agent was coated on both sides of the base paper using a laboratory rod coater (GIST Co., Ltd., Daejeon, Korea), and the surface-treated handsheets were dried at 120 °C in a hot-air dryer (Han Baek Scientific Co., Seoul, Korea). The coat weight of the mixture of the PEM kaolin and PVOH was 14.1 g/m$^2$ ± 1.3 g/m$^2$ for the low coat weight condition and 27.5 g/m$^2$ ± 0.4 g/m$^2$ for the high coat weight condition. Surface images of the filled handsheets and the surface-treated handsheets were obtained from a field emission scanning electron microscope (FE-SEM, SUPRA 55VP, Carl Zeiss, Oberkochen, Germany). Kaolin content in the filled handsheets and surface-treated handsheets was evaluated *via* the measurement of ash content based on the TAPPI T211 om-93 (1993) test method.

*Evaluation of antibacterial performance*

Antibacterial performance of the filled handsheets and surface-treated handsheets was evaluated by measuring the ratio of bacteria reduction in the bacteria suspension in which the handsheets were soaked (Ramos *et al*. 2012). The types of handsheets used for the antibacterial performance evaluation are described in Table 1. There were two controls: a bacterial suspension without handsheets (blank) and handsheets made of only fibers (pulp); three filled handsheets: handsheets filled with untreated kaolin (FU), handsheets filled with three layers PEM kaolin (F3L), and handsheets filled with seven layers PEM kaolin (F7L); and three surface-treated handsheets: low coat weight on F3L (PL_F3L), high coat weight on F3L (PH_F3L), and high coat weight on FU (PH_FU). Each handsheet was cut into approximately 0.5 cm × 0.5 cm square pieces. The accurately weighed 0.4 g of the handsheets specimens were soaked in 10 mL of deionized water, and then 100 $\mu$L of

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the mixed culture of *E. coli* O157:H7 and *L. monocytogenes* was added. The initial bacteria fraction in the bacteria-added suspension was $10^6$ CFU/mL to $10^7$ CFU/mL for *E. coli* O157:H7 and $10^5$ CFU/mL to $10^6$ CFU/mL for *L. monocytogenes*. The suspension was incubated under slow shaking conditions for 18 h at 23 °C. After 18 h of incubation, 100 $\mu$L of the suspension was spread-plated onto selective media. Sorbitol MacConkey agar (SMAC; Difco, Detroit, MI, USA) and Oxford agar base (OAB; Difco, Detroit, MI, USA) with antimicrobial supplement (Bacto Oxford antimicrobial supplement; Difco, Detroit, MI, USA) were used as the selective media for enumeration of *E. coli* O157:H7 and *L. monocytogenes*, respectively. All plates were incubated at 37 °C for 24 h to 48 h before counting the colonies characteristic of the respective pathogens.

**Table 1.** Condition of Antibacterial Tests

| Group | Symbol | Condition | Ash Content (%) |
|---|---|---|---|
| Control | Blank | A bacteria suspension without handsheets | - |
| | Pulp | Handsheets prepared made of only fibers | - |
| Filled Handsheets | FU | Handsheet with untreated kaolin | 20.4 |
| | F3L | Handsheet with 3 layers PEM kaolin | 22.3 |
| | F7L | Handsheet with 7 layers PEM kaolin | 20.1 |
| Surface-treated Handsheets | PL_F3L | Low coat weight on F3L | 23.8 |
| | PH_F3L | High coat weight on F3L | 25.7 |
| | PH_FU | High coat weight on FU | 24.8 |

*Evaluation of cell membrane integrity after exposure onto antibacterial paper*

A square handsheet of 1 cm × 1 cm was fixed on a glass slide using double-sided adhesive tape, and then 10 $\mu$L of *E. coli* O157:H7 culture ($10^7$ CFU/mL to $10^8$ CFU/mL) was dropped onto the handsheet. This sample was placed in a closed chamber at 37 °C and 99% relative humidity for 2 h. Thereafter, 10 $\mu$L of a bacteria Live/Dead bacterial viability kit (L-7012, Molecular Probes, Eugene, OR, USA) solution was applied to the sample, and the sample was stained for 30 min in the dark. The stained images were obtained using a confocal microscope (SP8 X, Leica, Wetzlar, Germany). Emission light from viable cells and damaged cells was observed in the green and red range, respectively.

## RESULTS AND DISCUSSION

PVAm has antibacterial activity (Westman *et al.* 2009a, b; Illergård *et al.* 2010), and previous studies have proved that PVAm can form contact-active surfaces on substrates such as cellulose fibers (Illergård *et al.* 2012). In those references, the higher adsorption of PVAm onto the substrate improves antibacterial activity (Illergård *et al.* 2015). This study attempted to find a PEM treatment condition for high adsorption amounts of PVAm, and to apply the antibacterial kaolin to paper for great antibacterial performance.

The formation of a PVAm/PAA multilayer on the surfaces of kaolin was confirmed by the zeta potential of the PEM kaolin (Fig. 1) and the cumulative adsorbed
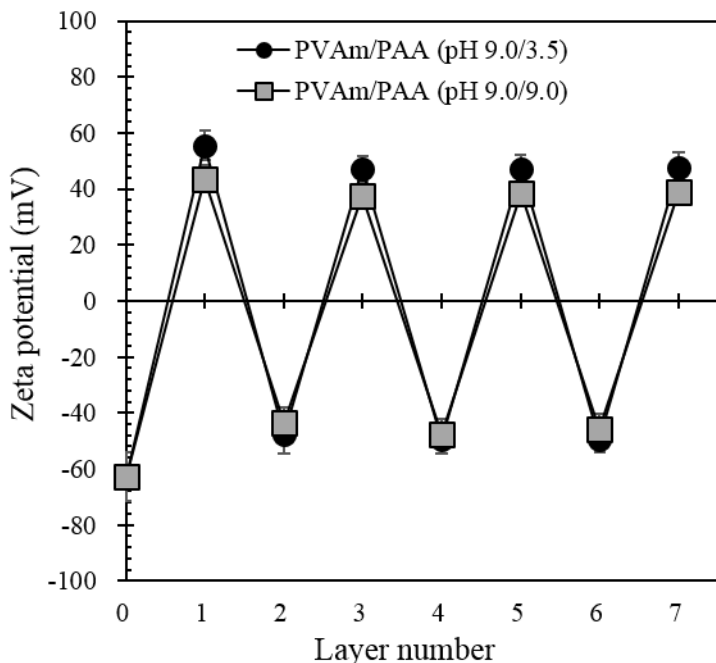
amount of PVAm (Fig. 2).



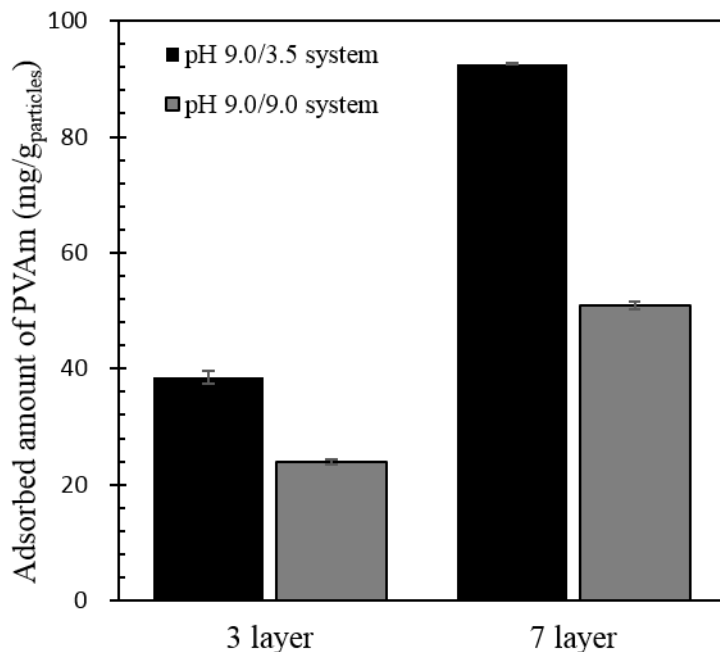**Fig. 1.** Zeta potential of PEM kaolin



**Fig. 2.** Cumulative adsorbed amount of PVAm on kaolin

The zeta potential of the PEM kaolin showed a positive value at odd layers, for which cationic PVAm was in the outermost layer, and a negative potential at even layers

that held the anionic PAA in the outermost layer. This repetitive pattern indicated an alternate formation of PVAm and PAA on the kaolin surface. The cumulative adsorbed amount of PVAm at seven layers was higher than that at three layers. This result was also evidence for growth of the multilayer. As a result, the surface of kaolin particles was successfully modified with a PVAm/PAA multilayer.

The change in pH conditions during the formation of multilayers affected the adsorbed amount of PVAm and zeta potential of PEM kaolin. In the multilayer system at a pH of 9.0/3.5, PVAm was adsorbed at a pH of 9.0 and PAA was adsorbed at a pH of 3.5. In this system, the polyelectrolyte at the outermost layer was highly dissociated, but the added polyelectrolyte for formation of a new layer was less dissociated. This pH condition was favorable for a high adsorption amount of both PVAm and PAA. In contrast, in a multilayer system at a pH of 9.0/9.0, PVAm was less ionized but PAA was fully ionized. In a system with a pH of 9.0/9.0, it was likely that the highly ionized PAA showed a smaller adsorption amount than the less ionized PAA in the system with a pH of 9.0/3.5. The low adsorption amount of PAA in the system with a pH of 9.0/9.0 negatively affected the adsorption of PVAm in the subsequent step. Therefore, a system with a pH of 9.0/3.5 was the better condition for a high adsorption amount of PVAm as well as a high charge of PEM kaolin compared to a system with a pH of 9.0/9.0.

Figures 3 and 4 represent the dispersion stability of the PEM kaolin suspension of 0.1 g/L as a function of time and the average particle size of the PEM kaolin that was measured immediately after preparation (0 min in Fig. 3), respectively. The PEM kaolin particles in a system with a pH of 9.0/3.5 showed no flocculation and good stability (Fig. 3(a)), while the PEM kaolin particles in a system with a pH of 9.0/9.0 were flocculated and showed poor stability at the odd layers (Fig. 3(b)).
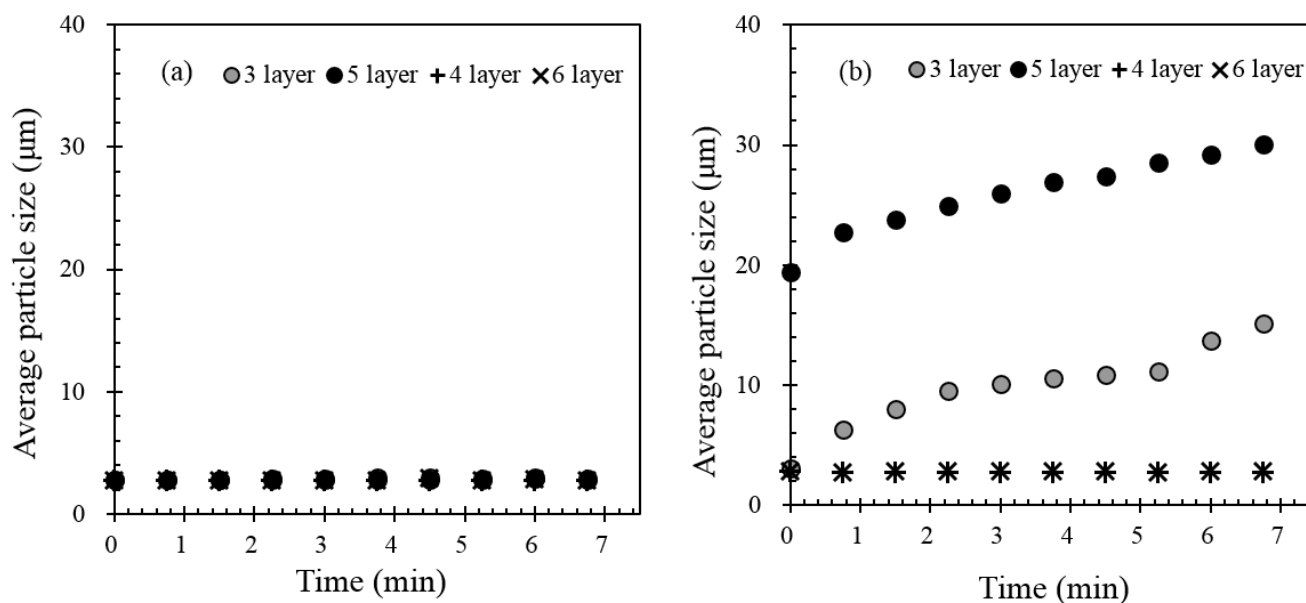


**Fig. 3.** Dispersion stability as a function of time of (a) PEM kaolin in a system with pH of 9.0/3.5 and (b) PEM kaolin in a system with pH of 9.0/9.0

It was likely that the flocculation of the odd layers of the PEM kaolin in a system with a pH of 9.0/9.0 was related to low ionization of PVAm in the outermost layer, which

had a weaker repulsive force than PVAm in the outermost layer in a system with a pH of 9.0/3.5. In other words, a system with a pH of 9.0/3.5 led the PEM kaolin to show a relatively large specific area without flocculation as well as a relatively high charge and adsorbed amount of PVAm. It is known that PVAm inactivates bacteria through a contact-active mechanism that destroys bacteria cells directly on the surface (Illergård *et al.* 2015). That is, the specific surface area of PVAm-modified kaolin is important for antibacterial activity. Accordingly, the PEM kaolin prepared in a system with a pH of 9.0/3.5 was more appropriate as antibacterial inorganic particles than the PEM kaolin prepared at a pH of 9.0/9.0.
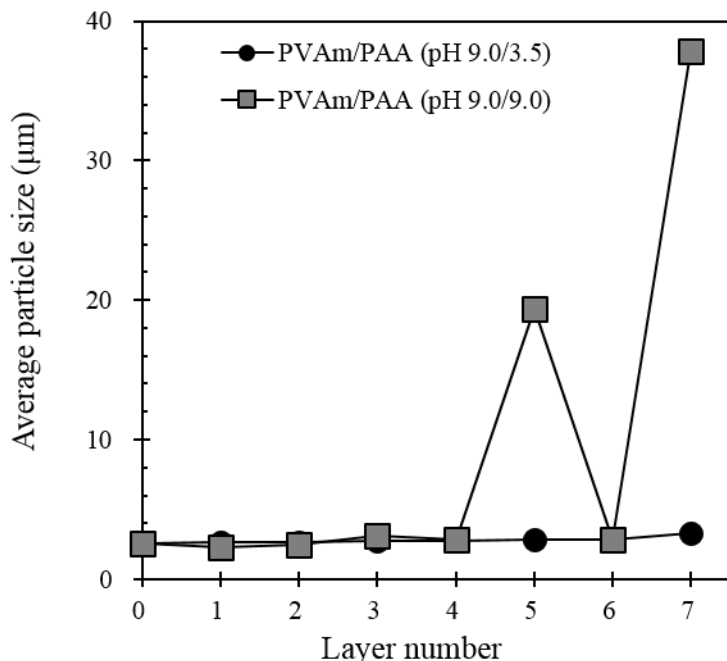


**Fig. 4.** Average particle size of PEM kaolin; these values are measured at 0 min in Fig. 3

Figure 5 shows the surface images of the filled handsheets with untreated kaolin, three layers PEM kaolin and seven layers PEM kaolin prepared in a system with a  pH of 9.0/3.5, and the surface-treated handsheets. Untreated kaolin, three layers PEM kaolin, and seven layers PEM kaolin particles were observed at the surface of filled handsheets (Figs. 5(a), (b), and (c), respectively).

Although those filled handsheets contained approximately 20% to 22% kaolin, little of the PEM kaolin was actually present on the surface of the filled handsheets. In contrast, the surfaces of the surface-treated handsheets were almost fully covered by three layers of PEM kaolin (Figs. 5(d), (e), and (f)). The increments of kaolin content after pigmentizing were 1.5%, 3.4%, and 4.4% for PL_F3L, PH_F3L, and PH_FU, respectively. In other words, the coverage of PEM kaolin on the sheet was much better in the cases of surface-treated handsheets despite the small incremental increase of kaolin content.
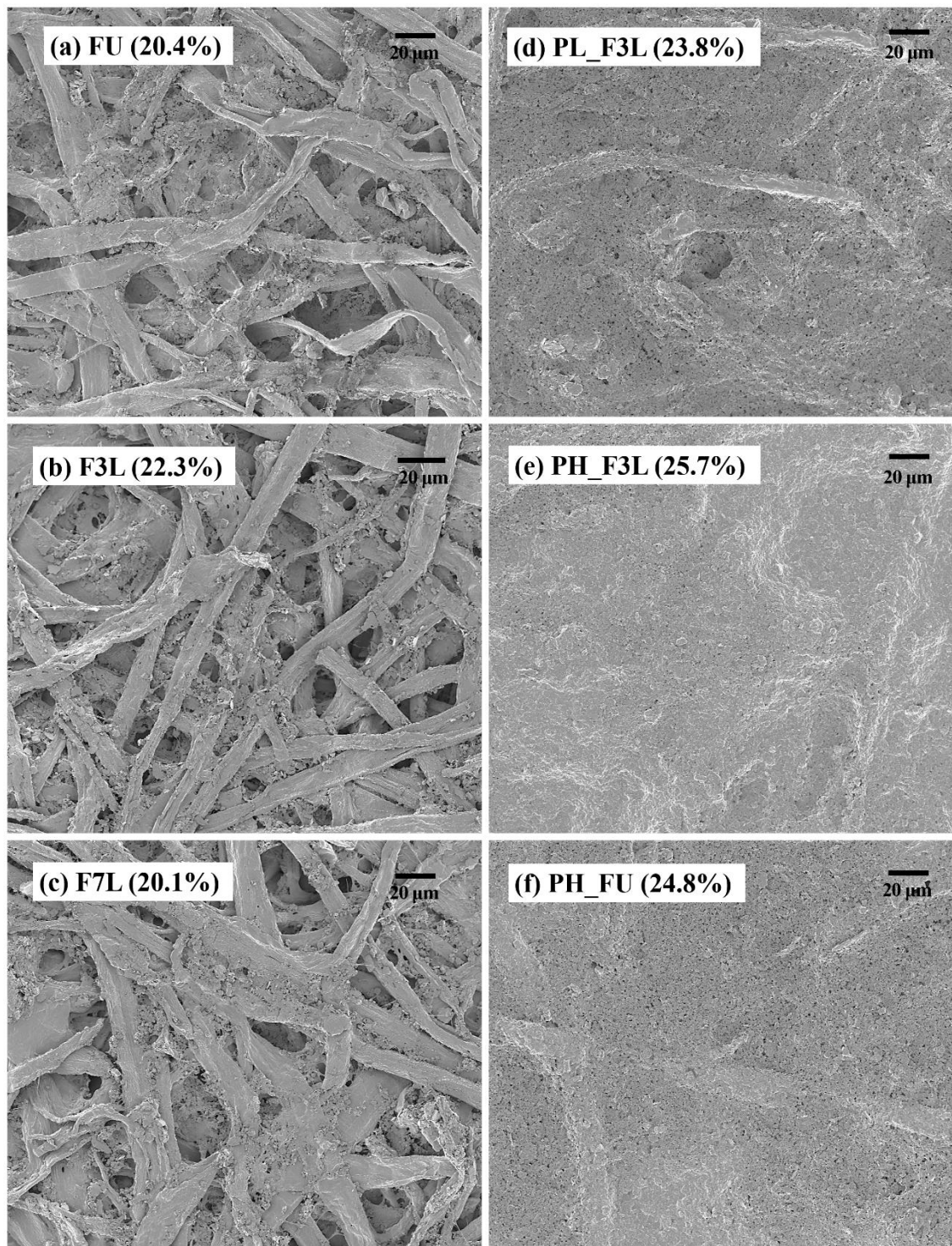
**Fig. 5.** Surface images of (a) FU, (b) F3L, (c) F7L, (d) PL_F3L, (e) PH_F3L, and (f) PH_FU; Parenthesis refers to kaolin content (Scale bar : 20 μm)

Figure 6 depicts the concentration of live *E. coli* (Fig. 6(a)) and *L. monocytogenes* (Fig. 6(b)) in bacteria only suspension (blank condition) and handsheets-soaked bacteria suspension (other conditions) without nutrients after incubation for 18 h. The blank condition maintained its initial bacteria concentration ($10^6$ CFU/mL to $10^7$ CFU/mL for *E.*

*coli* O157:H7 and $10^5$ CFU/mL to $10^6$ CFU/mL for *L. monocytogenes*). Both the *E. coli* and *L. monocytogenes* were still alive after an 18 h incubation without nutrients. The bacteria concentration in "PULP" and filled handsheets conditions (PU, F3L, and F7L) decreased, but the reduction was small. A majority of bacteria were still alive in the conditions of F3L and F7L despite the exposure of handsheets filled with three or seven layers PEM kaolin. In contrast, the surface-treated handsheets decreased bacteria concentration. The reduced population of bacteria was $> 10^6$ CFU/mL for *E. coli* O157:H7 and $> 10^5$ CFU/mL for *L. monocytogenes*. In addition, PH_F3L and PH_FU (high coat weight condition) decreased bacterial concentrations more than PL_F3L (low coat weight condition) regardless of the base handsheets. As a result, only surface-treated handsheets showed a reduction in the bacteria population.
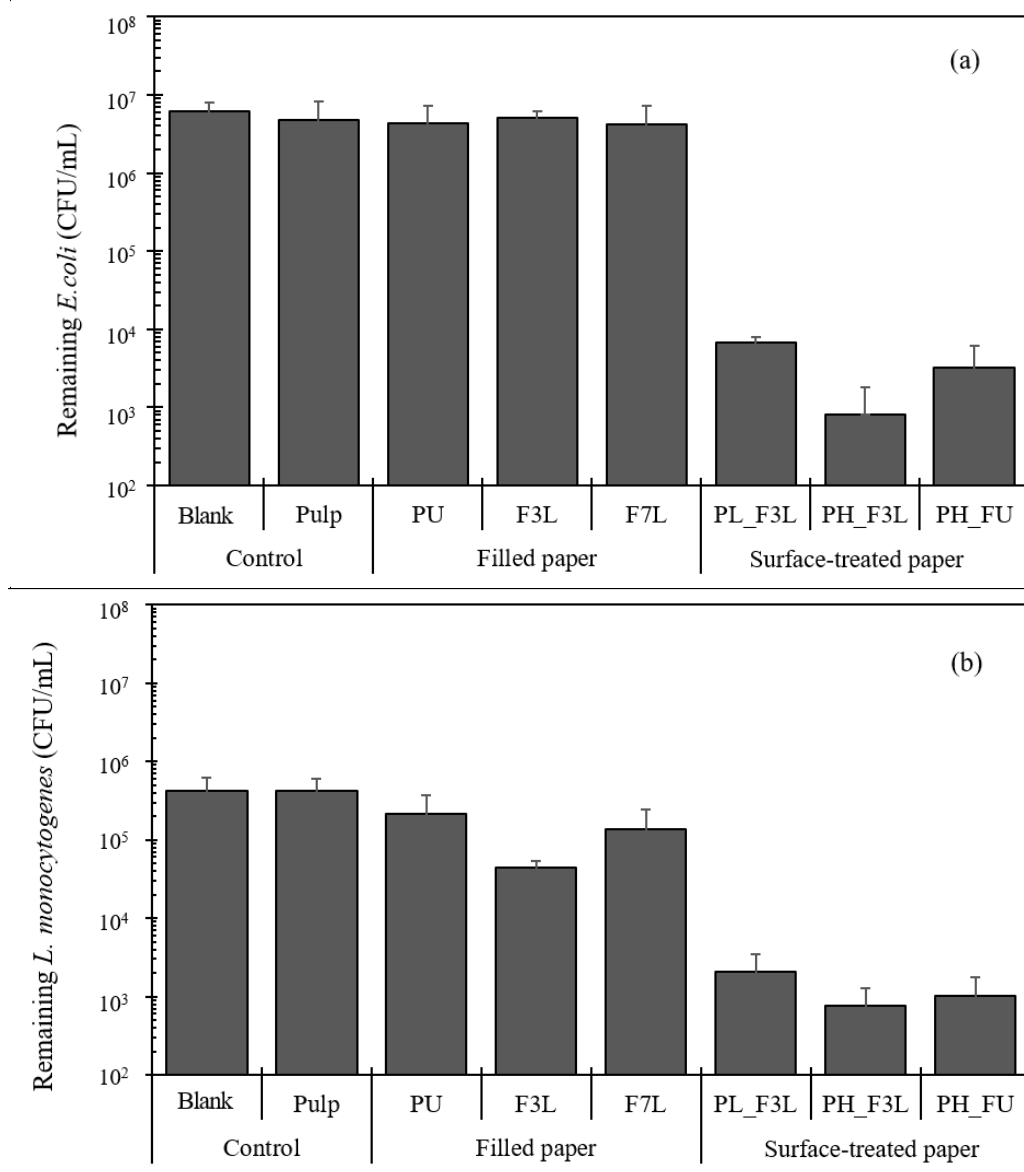


**Fig. 6.** Alive bacteria population for (a) *E. coli* O157:H7 and (b) *L. monocytogenes* in handsheets-soaked bacteria suspension after 18 h incubation

Table 2 shows the antibacterial activity of the filled handsheets and surface-treated handsheets as a reduction ratio of bacteria population compared to the remaining bacteria population in the blank condition. As shown in Fig. 6, the filled handsheets with three layers of PEM kaolin or seven layers of PEM kaolin had poor antibacterial activity, whereas the surface-treated handsheets with the surface treatment of three layers PEM kaolin showed strong antibacterial activity, with more than a 99.9% reduction in remaining CFUs.

**Table 2.** Antibacterial Activity Described as Reduction Ratio of Bacteria Population Compared to Remaining Bacteria Population of Blank Condition

| Groups | Symbols | Condition | Performance | |
|---|---|---|---|---|
| | | | *E. coli* | *L. monocytogenes* |
| Control | Pulp | Only pulp (without fillers) | - | - |
| | FU | Untreated kaolin | - | - |
| Filled Handsheets | F3L | 3 layers PEM kaolin | - | + |
| | F7L | 7 layers PEM kaolin | - | - |
| Surface-treated Handsheets | PL_F3L | Low coat weight on F3L | +++ | ++ |
| | PH_F3L | High coat weight on F3L | +++ | ++ |
| | PH_PU | High coat weight on FU | +++ | ++ |

Note: -, +, ++, and +++ mean < 90%, 90% to 99%, 99% to 99.9%, and > 99.9%, respectively

The antibacterial mechanism of PVAm is a contact-active mechanism, in which a contact active surface kills bacteria by physical interaction (Illergård *et al.* 2011, 2012). Therefore, low antibacterial activity for the filled handsheets was likely due to poor coverage of PEM kaolin on the surface of the handsheet. The surface-treated handsheets that were fully covered with PEM kaolin had great performance on the inactivation of bacteria. It is known that bacteria have amphoteric charge properties due to either carboxyl, phosphate, or amino groups, and the isoelectric point of gram-positive bacteria is a weakly acidic condition around a pH of 3 to 4 (Van der Wal *et al.* 1997). The charge of bacteria in this antibacterial test would be negative because the pH of the bacterial suspension used in this study was neutral. Therefore, the positive surface of the surface-treated handsheet with the three layers of PEM kaolin provided more immobilization of bacteria, which resulted in a greater chance to inactivate bacteria. In contrast, PEM kaolin was partially covered on the surface of the filled handsheets, although the filled handsheets contained > 20% of PEM kaolin. This indicated that bacteria had a low possibility to encounter the PEM kaolin, even if all of the bacteria immobilized on the surface of the filled handsheets. The reduction ratio of bacteria was approximately 30% for *E. coli* and 90% for *L. monocytogenes* in the case of the filled handsheets with three layers of PEM kaolin. Consequently, the surface treatment of PEM kaolin on the paper sheet was much more effective for strong antibacterial activity than an internal addition of PEM kaolin.

The decrease in bacteria population might have been due to the immobilization of the bacteria on the surface, not the inactivation of bacteria on the surface-treated

handsheets. Thus, it was necessary to confirm the inactivation of the immobilized bacteria on the surface of the handsheet. Figure 7 shows the stained *E. coli* O157:H7 immobilized onto the surface of the filled handsheets with untreated kaolin ((a) and (b)) and the surface-treated handsheets with three layers of PEM kaolin ((c) and (d)) using a bacteria Live/Dead bacterial viability kit. This staining kit is a mixture of SYTO9 and propidium iodide (PI). The SYTO9 (green color) labels all bacteria with intact membranes or with damaged membranes due to the diffusion of SYTO9 into the membrane, whereas PI (red color) can penetrate only damaged membranes and label DNA inside bacteria membranes.
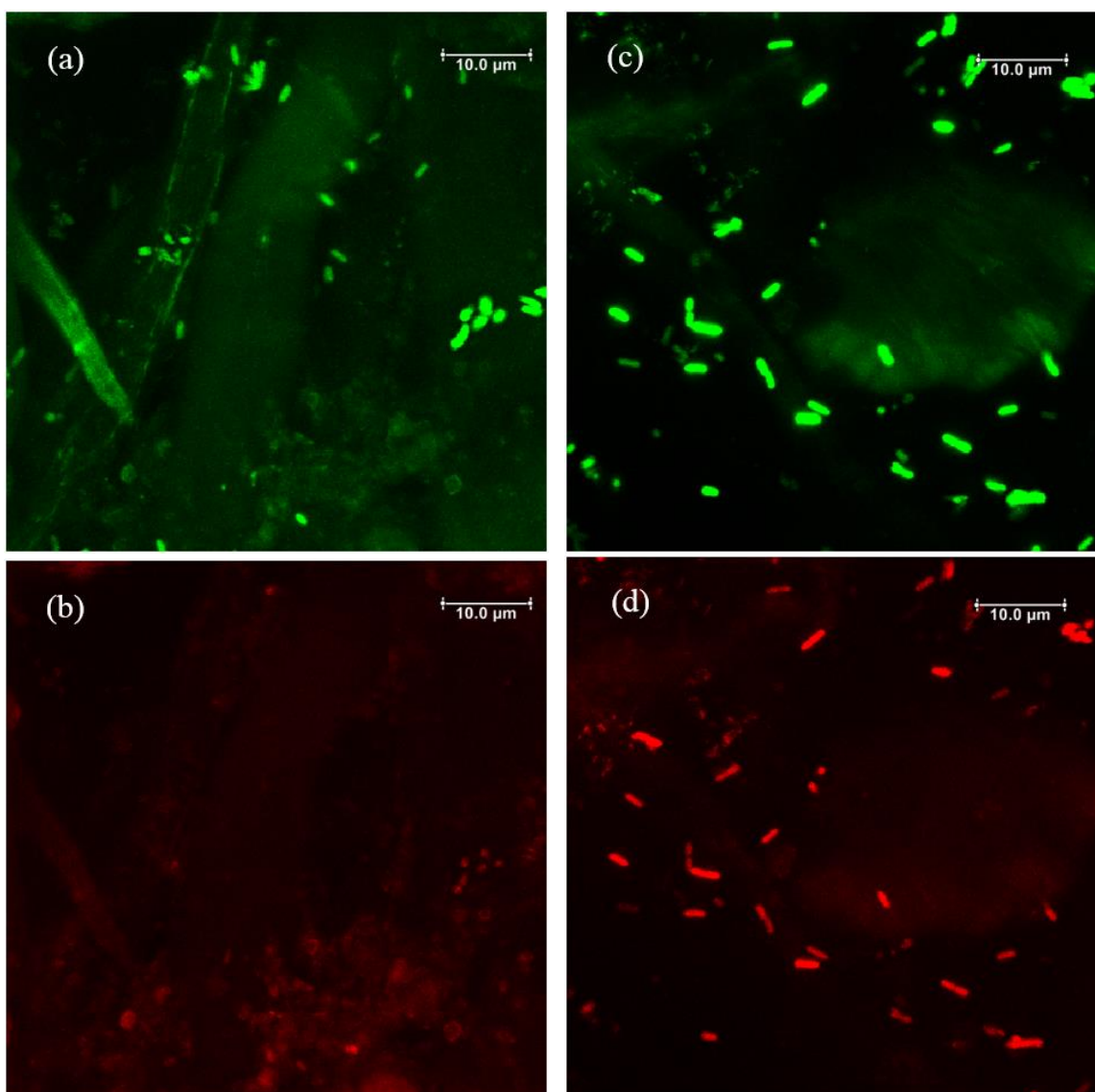


**Fig. 7.** Bacteria membrane integrity of *E. coli* O157:H7 on filled handsheets with untreated kaolin ((a) and (b)) and surface-treated handsheets treated with three layers of PEM kaolin ((c) and (d)) after 2 h exposure

The *E. coli* O157:H7 immobilized onto the surface of the filled handsheets with untreated kaolin was stained by only SYTO9 (green color, Fig. 7(a)). This meant that all *E. coli* O157:H7 were alive. In contrast, *E. coli* O157:H7 on the surface-treated handsheets

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with three layers of PEM kaolin was stained by both SYTO9 (Fig. 7(c)) and PI (red color, Fig. 7(d)). This indicated that the membranes of the *E. coli* O157:H7 on the surface-treated handsheets were damaged, and the bacteria were inactivated (Avalos Vizcarra *et al*. 2013). Therefore, this demonstrated that surface-treated handsheets inactivated the pathogens and the antimicrobial mechanism was responsible for the membrane damage.

## CONCLUSIONS

1. A polyvinyl amine/polyacrylic acid (PVAm/PAA) multilayer was formed on the surface of kaolin particles. The PVAm adsorption and the dispersion stability of the modified particles were affected by the pH condition during the polyelectrolyte multilayering (PEM) process. The pH system of 9.0/3.5 (indicating the pH values during treatments with PVAm and PAA, respectively) was more favorable for a high adsorption amount of PVAm and better dispersion stability of PEM kaolin particles.

2. The surface treatment of PEM kaolin on paper was more effective for antibacterial activity than the internal addition of PEM kaolin to paper. It seemed that the surface coverage of PEM kaolin on paper was an important factor for antibacterial performance.

3. The results of a bacterial-staining assay demonstrated that bacteria immobilized onto the surface of PEM kaolin-treated paper were inactivated by damaging the membrane.

## ACKNOWLEDGMENTS

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