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Antimicrobial and Thermal-Responsive Layer-by-Layer Assembly Based on Ionic-Modified Guanidine Polymer and PVA

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Summary

Water-soluble polymers, cationic polyhexamethylene guanidine hydrochloride (CPHGH) with antimicrobial property and acetalyzed poly(vinyl alcohol)/sodium acrylate (APVA-co-AANa) with temperature-responsive property were synthesized, and used as cationic and anionic polymer respectively to assemble multilayers to attempt to obtain a unique antimicrobial polymer system via the technique of layer by layer (LbL) assembly. The measurement of turbidity change for APVA-co-AANa aqueous solution under different temperatures revealed that the lower critical solution temperature (LCST) of APVA-co-AANa can be tailored by controlling the degrees of acetalysis (DA). Lower LCSTs were observed for the APVA-co-AANa with a higher DA. AFM images reveal that the particles on the surface of (CPHGH/APVA-co-AANa) and (PDADMAC/APVA-co-AANa) multilayers became larger after the material was treated at 60 °C; while the roughness of the surfaces was increased as the layer number increase and then decrease. Moreover, antimicrobial test also proved that cellulose fiber assembled with (CPHGH/APVA-co-AANa) multilayers exhibited higher antimicrobial activity against coliform and staphylococcus aureus.

Introduction

With increasing public health awareness about the effects of bacteria and micro-organisms, developing antibacterial or antimicrobial materials has attracted substantial interest. Antimicrobial properties, which destroy or inhibit the growth of microorganisms and make commonly-used materials bioactive, are extremely important to our society. The modern antimicrobial materials have come into practical application in the form of antibiotic fiber products since 1960s [1], and have been used in antimicrobial plastics since 1980s [2]. Since then they have experienced fast development with their application penetrating into various areas, such as chemical industry, fibers, food, electrical appliances and cement, covering almost all major kinds of fibers and plastics products, such as dacron, polypropylene fibres, acrylon, PP, ABS, PE, and PVC, etc [3-6].

Cellulose is a naturally occurring polysaccharide and the most abundant renewable organic raw material in the world. Its derivatives have many important applications in

fiber, paper and packaging industries. To render this material with antimicrobial property is a current need of society, especially for the products used in the occasions that need a high degree of safety for the civilian population.
Organonitrogen-sulfur compounds, organohalide compounds, methylenebis(thiocyanate), dithiocarbamates, quaternary ammonium surfactants and organosulfur compounds are conventional biocides. While many of these are quite efficient in killing microorganisms or inhibiting their growth, they also tend to be toxic or harmful to human beings, animals, or other non-target organisms; as well as expensive. Another concern is the leaching or migration of the biocides with low molecular weight, thus lowering their long-term effectiveness.
Since a series of substituted guanidines were reported to have antimicrobial properties in the 1930s, the guanidine derivates with antibacterial and antifungal activity have been investigated as medical, crop protection agents and antiseptics for industry products, food and other goods for daily use [7]. They are soluble in water and have broad-spectrum antimicrobial property, which is environmentally friendly and convenient for use.
The technique of layer by layer (LbL) assembly, which is based on the electrostatic attraction of oppositely-charged polyions was proposed originally by Iler [8] and extended recently by Decher [9-10]. According to these studies, alternating adsorption of anionic and cationic polyelectrolytes leads to formation of regular multilayer assemblies. This technique has been extensively applied to fabricate various advanced materials [11-13], such as thin polymer films, biomaterials, and microcapsules. Recently, the LbL technique has been adopted to enhance inter-fiber bonding, which strengthens the paper by forming a polyelectrolyte multi-layer through spontaneous sequential adsorption of oppositely charged polyelectrolytes from diluted aqueous solution into pulp fibers [14].
In this paper, water-soluble polymers, cationic polyhexamethylene guanidine hydrochloride (CPHGH) with antimicrobial property and acetalyzed poly(vinyl alcohol)/sodium acrylate (APVA-co-AA-Na) with temperature-responsive property were synthesized, and used as cationic and anionic polymer respectively to assemble multilayers. The polymer-based antimicrobial agent eliminates the leaching problems encountered by traditional biocides; whereas the thermal-responsive property allows the antimicrobial polymer to be exposed under a controllable manner. Apart from the attempt to obtain a unique antimicrobial polymer system via LbL, the effect of temperature on the roughness and morphology of the films on wafer surface was determined by AFM.

Experimental

Materials

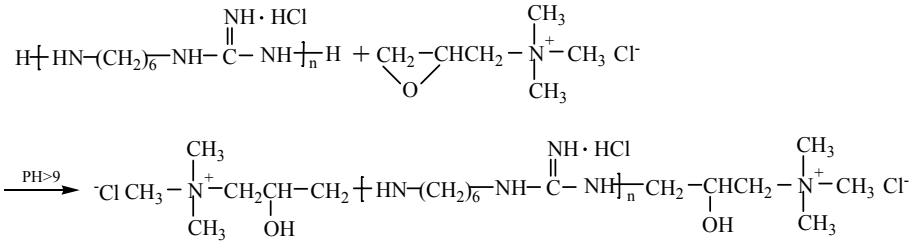
Vinyl acetate (VAc) and acrylic acid (AA) were obtained from Shanghai Puqiao Chemical Tech. Co. and purified via distillation. Initiator 2,2-Azoisobutyronitrile (AIBN) was recrystalized twice with ethanol prior to use. Glycidyltrimethylammonium chloride (GTMAC) and poly-diallyldimethyl- ammoniumchloride (PDADMAC) were obtained from Fluka Co. Polyhexamethylene guanidine hydrochloride (PHGH) was prepared by the condensation polymerization of hexamethylene diamine and guanidine hydrochloride. Other chemicals were analytical grade and used without further purification.

Characterization Techniques

The NMR spectra were recorded in DMSO-d6 at 25°C using a Bruker 400MHz spectrometer operating at 300.13 and 75.5 MHz for 1H and 13C nuclei, respectively. Turbidity of APVA-co-AANa was obtained using a HACH 2100AN turbidimeter. Molecular weight of PVA-co-AANa was determined by 1515 Breeze gel permeation chromatography (GPC) with water elution phase. Atomic force microscope (AFM) images were obtained using a MultiMode Scanning Probe Microscope (Nanoscope IIIa) with a J-scanner (maximum scan area is 125×125μm2) (Veeco Instrument).

Synthesis of cationic PHGH

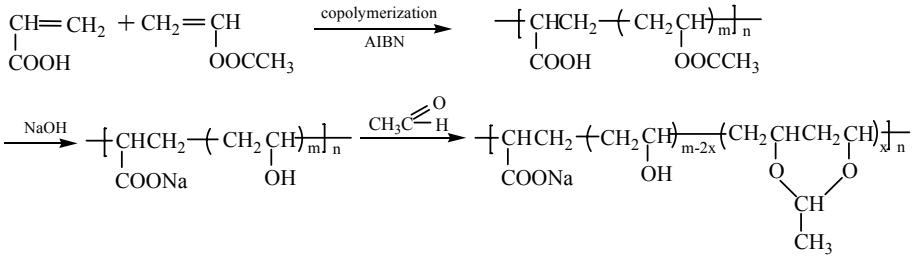
Cationic PHGH (CPHGH) was prepared by the condensation polymerization of hexamethylene diamine and guanidine hydrochloride, followed by the reaction with GTMAC in a basic solution at 80°C for 1 hour to introduce the ended quarternary ammonium groups. The reactive mixture was dialyzed for 24 hours to separate any unreacted monomers. The synthesis is schematically shown in Scheme 1:



Scheme 1. Scheme of CPHGH synthesis

Synthesis of acetalyzed PVA-co-AANa(APVA-co-AANa)

PVA-co-AANa was synthesized by a free-radical polymerization of VAc and AA in methanol, followed by hydrolysis in a NaOH methanol solution. A chosen amount of HCl was added into PVA-co-AANa solution and then acetaldehyde was added dropwise at 15°C over 30 mins. After the completion of acetaldehyde feeding, the mixture was kept at 15°C for 1 h and 28°C for 24 h. The reaction was stopped by neutralization with 2N NaOH aqueous solution. The solution obtained was dialyzed for 24 hours with a dialysis membrane of molecular weight cut-off 10000. The synthesis is schematically shown in Scheme 2:



Scheme 2. Scheme of APVA-co-AANa synthesis

Multilayer buildup

APVA-co-AANa, CPHGH and PDADMAC were used to prepare polyelectrolyte solutions. All solutions were made using deionized and distilled (DD) water containing 10mM NaCl. The concentrations of APVA-co-AANa, CPHGH and PDADMAC solutions were 0.044, 0.1 and 0.2 wt.-%, respectively.
The silicon wafer with a 100 nm oxidized layer and rayon fiber (from MiniFiber Inc.) were used as substrates. Substrates were activated following a procedure reported previously [15]. The substrate was alternately immersed into the CPHGH, PDADMAC and APVA-co-AANa solutions for 10min each. Following each dip coating, the substrate was rinsed with 10mM NaCl aqueous solution for three times and multilayers were assembled on the substrates. The multilayers were heated in NaCl solution bath at 60°C for 10min and then rinsed by super pure water, dried at 60°C.
Cellulose fibers were used as substrates to be built up one or three layers, which following the same procedure for the wafer assemble. Because APVA-co-AANa, the homopolymer of CPHGH and PDADMAC are water soluble, they can be removed by washing the treated fibers thoroughly with DD water for 3 times. The assembled fibres were dried at 25 or 60°C.

Turbidity measurement

A HACH 2100AN turbidimeter was employed for the turbidity measurements. Samples were solved into deionized water or NaCl solution at various concentrations and then placed into a water bath with temperature control. The samples were heated from 20°C at a step of 2.5°C. The samples were kept in the water bath for 10 minutes at each temperature then taken out and dried up quickly before inserted into the turbidimeter. The turbidity was then recorded at each temperature.

AFM characterization

A MultiMode Scanning Probe Microscope (Nanoscope IIIa) with a J-scanner (maximum scan area is $125\times125\mu m^2$) (Veeco Instrument) was operated in contact mode to record the images of the PVA-co-AANa and APVA-co-AANa film using a contact tip (NP-20 Oxide-Sharpened Silicon Nitride Probes from Veeco Nano-ProbeTM). The AFM images were flattened by applying a 1^{st} order polynomial fit to remove defects from the image, due to vertical (Z) scanner drift. Due to the difficulty in identifying the morphology changes for the fibres, we use silica wafer as a model substrate in the current work to facilitate the operation of AFM. So far, we only studied the surface roughness under dry condition (i.e. after polymers were dried on wafer).

Antimicrobial test

The antimicrobial tests were carried out at the Guangzhou Microbe Institute (Guangzhou, China). A shake-flask method was used to quantify the antibacterial activity of cellulose fibers and the modified fibers against coliform (ATCC8739) and staphylococcus aureus (ATCC6538). The modified fibers were mixed with the cultivation for 1 h.

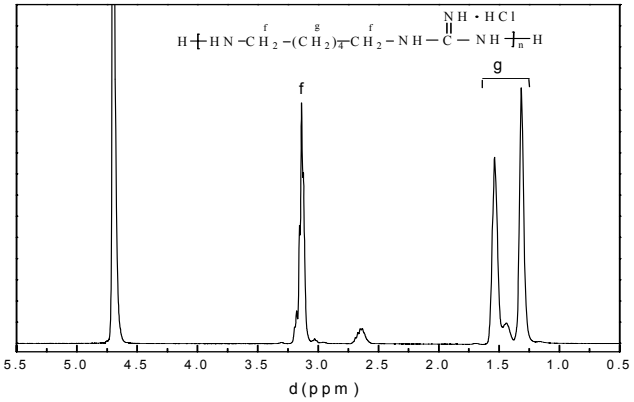
Results and Discussion

Characterization of CPHGH

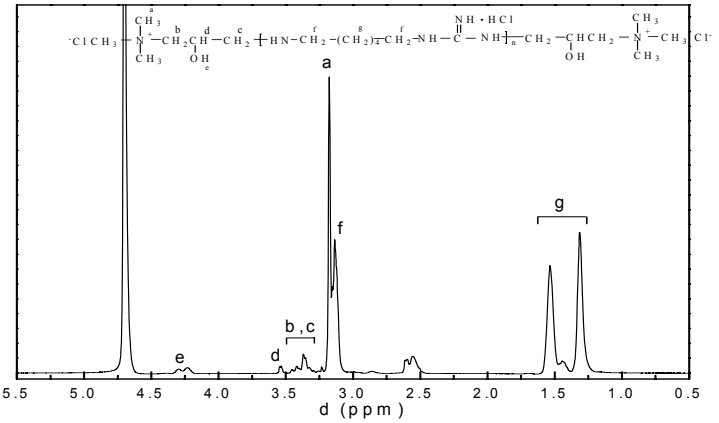
Figure 1 shows the ^{1}H-NMR spectra of PHGH and CPHGH sample. The peaks for PHGH are methylene protons g, 1.22-1.61ppm, methylene protons f, 3.03-3.22ppm. Compare to ^{1}H-NMR spectrum of PHGH, there are new peaks at 3.18, 3.26-3.47, and 3.47-3.56 and 4.20-4.30ppm in CPHGH , which correspond to the three methylic protons a, the methylene protons b, c, methine proton d and hydroxyl protons e on ended GTMAC groups, respectively. The results confirmed that the grafting of GTMAC on PHGH. The graft percentage of CPHGH (GP) can be calculated based on the integrated areas from ^{1}H-NMR spectra as follows:

$$GP = S_{3.26\text{-}3.47} / (S_{1.22\text{-}1.61} + S_{3.03\text{-}3.22}) \times 100 \quad (\mathbf{1})$$

where $S_{3.26\text{-}3.47}$ is the integrated area of CH_2 protons on the GTMAC side chain (protons b and c); and $S_{1.22\text{-}1.61}$ and $S_{3.03\text{-}3.22}$ are the integrated area of CH_2 protons on the PHGH backbone (protons f and g).



(a)



(b)

Figure 1. ^{1}H-NMR of PHGH (a) and CPHGH (b)

Effect of reaction time and temperature on grafting percentage of GTMAC

To optimize the reaction time and temperature for better grafting reaction, the effects of time and temperature on the GP were investigated at 4 different temperatures: 60, 70, 80 and 90°C; and reaction time varied from 20 to 120 min. The results are presented in Figure 2. Clearly, both reaction time and temperature are of vital importance in determining the GP. For the current systems, the GP increased with the increase of reaction time and temperature at first, then decreased, which may be related to the decomposition of quaternary ammonium salt. The maximum grafting percentage (i.e., 3.25 mol %) was observed at 80°C and 60 min reaction time.

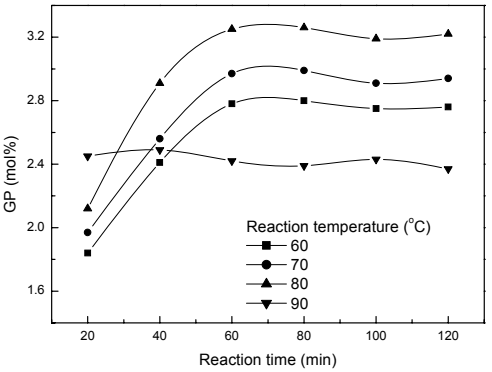


Figure 2. Effect of temperature and time on the grafting percentage of GMTAC

Characterization of APVA-co-AANa

^{1}H spectra of APVA-co-AANa are shown in Figure 3. The peaks for PVA-co-AANa are CH_2, 1.2-1.6ppm, and CH, 3.7-4.0ppm. The methylene proton resonances were rather broad due to the combination of spin-spin coupling and configurational splittings. Three distinct resonance signals of the hydroxyl proton appear at 4.65, 4.45 and 4.20ppm, which are attributed to the syndiotactic (mm), heterotactic (mr) and

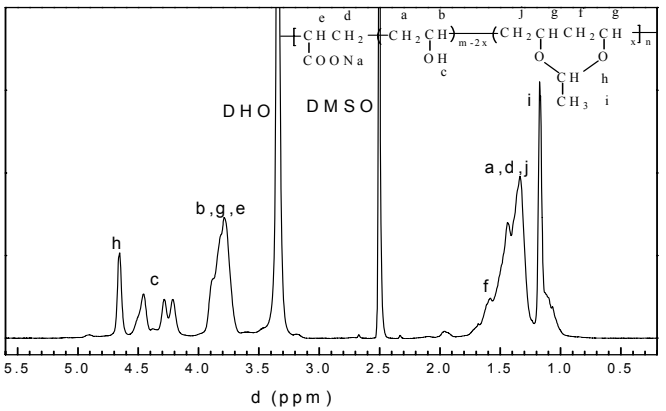


Figure 3. ^{1}H-NMR of APVA-co-AANa (Mw=67,459)

isotactic (rr) triad sequences, respectively [16-17]. The peaks at 2.5 and 3.3ppm are the resonance peaks of remnant protons from DMSO and water.
The two resonance peaks appearing at 1.0-1.2ppm and 4.65ppm correspond to the acetal ring of APVA-co-AANa. The peaks at 1.0-1.2ppm result from the methyl group of the acetal ring. The O-CH-O protons of the acetal ring appear at 4.65ppm. They shifted to the lowest field due to the influence or coupling effect of the two adjacent oxygen atoms. The above results confirmed that the acetalation reaction did occur between PVA-co-AANa and acetaldehyde.

Thermo-sensitivity of APVA-co-AANa

A series of APVA-co-AANa with different DA were prepared and their phase transition temperatures were investigated by turbidity. Turbidity curves are used to characterize the cloud point of APVA aqueous solution.

Influence of DA on LCST of APVA-co-AANa

The turbidity curves of aqueous solution of APVA-co-AANa with different DA (10mmol/L, VAc:HAA=95:5, M_w=67,459) at various temperatures are plotted in Figure 4. In our research, the cloud point or LCST is defined as the intersection of two straight lines drawn through the curves of turbidity at low and high temperatures, respectively. When the temperature was lower than LCST, all of the three samples were transparent solutions and their turbidities were close to 0, indicating that the turbidity was independent of temperature. However, after being heated to a certain temperature near the LCST, the transparent solutions of APVA-co-AANa started to become cloudy, followed by the rapid increase in turbidity. On the other hand, the PVA-co-AANa solution kept clear and the turbidity was close to 0 during heating. The phase transition is the result of partial acetalization of the hydroxyl group on PVA-co-AANa. The acetal rings bring the hydrophobic group in PVA-co-AANa chains. At low temperatures, the remaining hydroxyl groups on APVA-co-AANa form inter-molecular hydrogen bonds with water and/or the cage-like structures surrounding the acetal rings to make the APVA-co-AANa dissolve in water. Heating of the solution causes the destruction of the hydrogen bonds, and the exposure of hydrophobic acetal

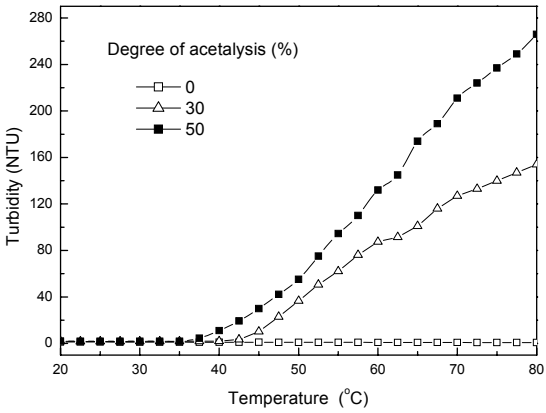


Figure 4. Turbidity of aqueous solution containing PVA-co-AANa or APVA-co-AANa at different temperature (10mmol/L, VAc:HAA=95:5, M_w=67,459)

rings, leading to the formation of hydrophobic aggregates or hydrophobic association, a phase-separated state. This is corresponding to a morphological change from the random coils of APVA-co-AANa to the intermolecular hydrophobic association [18-19]. Figure 4 shows that the LCSTs decrease continuously with the increases of DA of APVA-co-AANa. The hydrophobic aggregates appear at lower temperature, so that partial acetalization renders APVA-co-AANa thermal sensitive. The phase transition temperature of APVA-co-AANa can be readily tailored by controlling the DA.
Moreover, the acetal rings and carboxyl group of APVA-co-AANa chains facilitated the dissolution of PVA in cold water (e.g., 15°C). For a highly hydrolyzed PVA, a preparation temperature of above 80°C is required for complete dissolution in water in an acceptable time. With APVA-co-AANa, the acetal rings disrupt inter and intra chain hydrogen bonding and prevent APVA-co-AANa from forming crystals, i.e., acetal rings and carboxyl group increase the water solubility of the APVA-co-AANa. Consequently, the APVA-co-AANa can be dissolved in water at low and room temperatures easily.

Layer-by-layer assemble of CPHGH / APVA-co-AANa and PDADMAC/ APVA-co-AANa on cellulose fiber and silicon wafer

APVA-co-AANa and CPHGH are thermo sensitive and antimicrobial water-soluble ionic polymers. The thermal sensitivity makes it attractive in the applications that demand "smart" material response to environmental stimuli whereas antimicrobial properties prevent material from being attached by bacteria or fungi. The current work is attempted to assemble the APVA-co-AANa and CPHGH, APVA-co-AANa and PDADMAC multilayers on cellulose fibers to render the surface of the fibres temperature responsive and antimicrobial as well. Due to the difficulty in identifying the morphology changes for the fibres, we use silica wafer as a model substrate in the current work to facilitate the operation of AFM. As shown in Table 1, APVA-co-AANa has the highest negative charges in basic solution while the CPHGHs exhibit similar positive charges in the solution around pH 3-10. Both of them can be used to modify the cellulose fibers via L-b-L assembly.

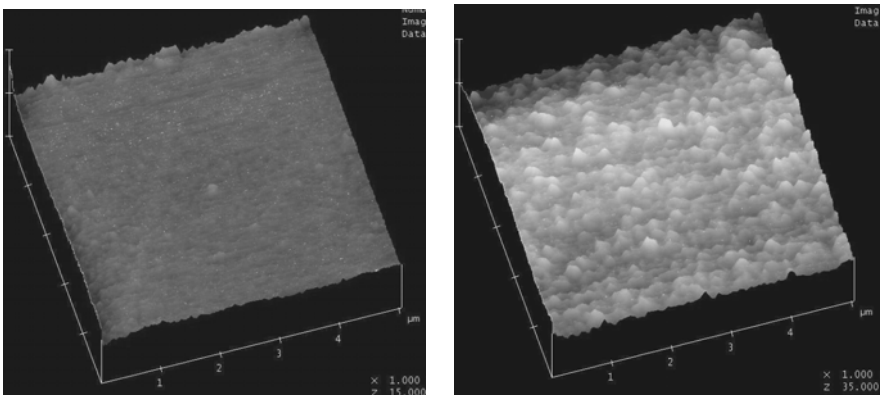
Table 1. Charge density of APVA and CPHGH

APVA-co-AANa[1]		CPHGH[2]	
pH	Charge density meq/g	pH	Charge density meq/g
4.87	-0.25	3.74	0.91
6.03	-0.32	4.78	0.83
7.52	-0.36	6.45	0.77
8.26	-0.38	7.60	0.76
9.38	-0.39	8.77	0.81
10.02	-0.37	9.90	0.69

Note 1: Measured with the solution of [APVA-co-AANa]=0.044 wt.-%, [NaCl]=10mM
2: Measured with the solution of [CPHGH]=0.1 wt.-%, [NaCl]=10mM

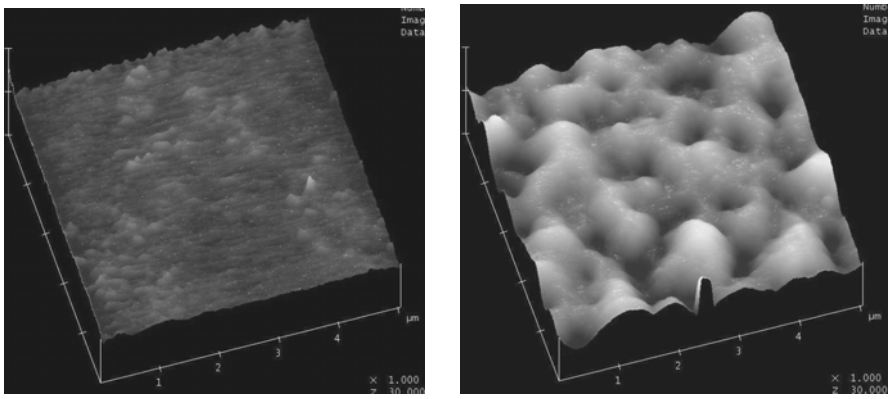
APVA-co-AANa and CPHGH, APVA-co-AANa and PDADMAC multilayers were assembled on cellulose fiber in 0.044wt.-% APVA and 0.1wt.-% CPHGH, or 0.044wt.-% APVA and 0.2wt.-% PDADMAC solution with 0.01M NaCl solution and pH=9 at room temperature. The same assembly was also performed on silicon wafer for the purpose of comparison. The modified substrates were immersed into DD water at 60°C for 20min

and dried in oven at 60°C for 20min to observe the thermo sensitivity of the films. The AFM images of the wafer surface are shown in Figures 5 and 6. Changes in surface morphology are visible. The size and the height of particles on the heat-treated surfaces are much larger than those on the heat-untreated surfaces. After exposed to heat, the surfaces became rougher than the original one. The roughness, listed in Table 2, of heat-treated and heat-untreated surfaces with different multilayers further illustrated the behaviors, i.e., the roughness of heat-treated surface increased considerably as the increase of layer number. However, when the layer number reached higher, the roughness of heat-treated surface decrease instead owning to the surface has being covered by an even polymer film that tends to be flattened. In contrast, the roughness of control samples without L-b-L assembly remained the same or similar. The increment in roughness potentially benefits the strength development of fibre products as well as the retention of functional fillers or additives at the wet-end of conventional or specialty papermaking processes whenever the system temperature is above LCST.



(a) Non heat-treated wafer (b) Heat-treated wafer

Figure 5. Flattened image of non heat-treated and heat-treated (CPHGH / APVA-co-AANa $)_{17}$ assembled multilayers on wafer



(a) Non heat-treated wafer (b) Heat-treated wafer

Figure 6. Flattened image of non heat-treated and heat-treated (PDADMAC / APVA-co-AANa$)_{17}$ assembled multilayers (17 layers) on wafer

Table 2. Roughness (Rq) of multilayers by AFM contact mode (500nm scale)

	Suface of wafer	CPHGH / APVA-co-AANa						PDADMAC / APVA-co-AANa					
Number of layers	0	5	9	12	13	17	21	5	9	12	13	17	21
Rq/ nm 25°C	0.11	0.48	0.75	0.89	0.93	0.95	0.91	0.56	0.71	0.95	1.20	1.16	1.01
60°C	0.12	0.91	1.24	1.57	1.68	1.74	1.10	1.13	1.57	2.3	3.57	1.67	1.59

Antimicrobial test

The antimicrobial activities of the modified cellulose fibers assembled with CPHGH, PDADMAC or APVA-co-AANa against E. coli and S. aureus are listed in Table 3. As can be seen, with one layer CPHGH and three layers (CPHGH/APVA-co-AANa) on fibers, an excellent antimicrobial activity (100% inhibition with coliform and staphylococcus aureus) was achieved. In contrast, three layers consisting of (PDADMAC/APVA-co-AANa) possessed relatively poor antimicrobial activity (51.38% inhibition against E. coli and 99.99% against staphylococcus aureus). In the current test, the modified fibers were mixed with the cultivation only for 1 h, which also indicated that the effective inhibition has been rapidly reached. It is well known that quaternary ammonium groups in PDADMAC are effective in inhibiting bacterial growth. However, overall performance of guanidine polymers appeared to be much better.

Table 3. Antimicrobial activity of modified fibers against coliform and staphylococcus aureus

Sample assembled on cellulose fibers[1]	Antimicrobial activity / %	
	E. coli (ATCC8739)	staphylococcus aureus (ATCC6538)
$(CPHGH)_1$	100	100
$(CPHGH / APVA\text{-}co\text{-}AANa)_3$[2]	100	100
$(PDADMAC / APVA\text{-}co\text{-}AANa)_3$	51.38	99.99

* Note 1. The solution of [APVA-co-AANa]=0.044 wt.-%, [CPHGH]=0.1 wt.-%, [PDADMAC]=0.2 wt.-%, [NaCl]=10mM.
2. Number "3" represents the fiber was assembled with three-layer film with the top layer consisting of cationic polymer.

Conclusions

The novel antimicrobial cationic PHGH had been successfully synthesized and the hydrophobic acetal rings were introduced into APVA-co-AANa chain after the partial acetalation reaction happened, which rendered the hydrophilic PVA-co-AANa partially hydrophobic or amphiphatic. LCST decrease continuously with the increases of DA of PVA-co-AANa. The phase transition is the result of partial acetalization of the hydroxyl group on PVA-co-AANa.

(CPHGH/APVA-co-AANa) and (PDADMAC/APVA-co-AANa) multilayers were assembled on cellulose fiber and silicon wafer to render the surfaces of substrates thermo-sensitivity. AFM images of silicon surface revealed that the particles on the surface became larger after the material was exposed at 60°C; and the roughness of the surfaces was increased as the layer number increase and then decrease, thus

providing direct evidence for the thermo-sensitivity of the substrates assembled with (CPHGH/APVA-co-AANa) and (PDADMAC/APVA-co-AANa) multilayers.
Cellulose fiber assembled with (CPHGH/APVA-co-AANa) multilayers exhibited higher antimicrobial activity against E. coli and staphylococcus aureus. The growth inhibition reached 100%.
The temperature sensitivity depends on the number of layers of modified PVA whereas the antibacterial activity relies on the cationic PHGH which must locate on the top layer of LbL assembly.

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References

1. Tollar M, Štol M, Kliment K (1969) J. Biomed. Mater. Res. 3(2): 305
2. Karen JQ, James MC (1988) British Polymer Journal 20(1): 25
3. Wang XH, Du YM, Fan LH, Liu H, Hu Y (2005) Polym. Bull. 55: 105
4. Tansir A, Vikrant K, Nahid N (2006) Polym. Int. 55: 1398
5. Jadwiga B (1996) J. Appl. Polym. Sci. 61(3): 567
6. Radhesh K, Helmut M (2005) Polym. Int. 54: 1180
7. Zhang YM, Jiang JM, Chen YM (1999) Polymer 40: 6189
8. Iler RK (1966) J. Colloid Interface Sci. 21: 569
9. Decher G, Hong JD, Schmitt J (1992) Thin Solid Films 210: 211
10. Decher G, Lvov Y, Schmitt J (1994) Thin Solid Films 244: 772
11. Choi K, Kwak J, Lee C, Kim H, Char K, Kim DY, Zentel R (2008) Polym. Bull. 59: 795
12. Gao YY, Chen XR, Liao B, Ding XB, Zheng ZH, Cheng X, Pang H, Peng YX (2006) Polym. Bull. 56: 305
13. Tuo XL, Chen D, Cheng H, Wang XG (2005) Polym. Bull. 54: 427
14. Wagberg L, Forsberg S, Johansson A (2002) J. Pulp and Paper Sci. 28: 222
15. Ram MK, Salerno M, Adami M, Faraci F, Nicolini C (1999) Langmuir 15: 1252
16. Velden G, Beulen J (1982) Macromolecules 15: 1071
17. Moritani T, Kuruma I, Shibatani K, Fujiwara Y (1972) Macromolecules 5: 577
18. Li L, Shan H, Yue C, Lam Y, Tam K (2002) Langmuir 18(20): 7291
19. Li L (2002) Macromolecules 35(15): 5990