Carbohydrate Polymers 83 (2011) 346–353

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Synthesis, characterization and antimicrobial activity of modified cellulose-graft-polyacrylonitrile with some aromatic aldehyde derivatives

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ARTICLE INFO

Article history:
Received 25 January 2010
Received in revised form 12 July 2010
Accepted 22 July 2010
Available online 30 July 2010

Keywords:
Aminated grafted cellulose
Solid state NMR
Antimicrobial activity
Bactericidal effect

ABSTRACT

Antimicrobial grafted cellulose materials have been elaborated by the grafting of acrylonitrile onto cellulose by the use of $KMnO_4$/citric acid initiation method followed by the amination reaction with ethylenediamine. The aminated grafted cellulose was reacted with some aromatic aldehyde derivatives. The grafted cellulose, aminated grafted cellulose and its derivatives were characterized by FT-IR and solid state NMR spectra. These modified cellulose materials displayed antimicrobial activity against representative some strains of bacteria and fungi. In the presence of aminated grafted cellulose or its derivatives, the growth inhibition reached almost 100% with the fungal species. The modified grafted cellulose derivatives (EDA MC-g-PAN II and EDA MC-g-PAN III) were challenged with *Bacillus subtilis* as a function of contact time. The biocidal results showed that these derivatives have bactericidal effect against the bacterial species.

1. Introduction

With the growing of public health awareness of disease transmissions and cross-infection caused by the microorganisms, use of antimicrobial materials has been increased in many application areas like medical devices, drugs, health care products and hygienic applications, water purification systems, hospital furniture, dental surgery equipment, textiles, food packaging, and food storage (Gottenbos, Van der Mei, Klatterm, Nieuwenhuis, & Busscher, 2002; Kenawy, Worley, & Broughton, 2007; Sun & Sun, 2001a), protective clothing for medical and chemical works, other health related products (Duran, Marcato, De Souza, Alves, & Esposito, 2007; Margaret, Sau, Vincent, Iva, & Andrew, 2006). Antibacterial packaging material that can improve product quality and keep it free from microbial adhesion (Park & Zhao, 2004), have been developed as new ecological functional materials to meet the challenges. There have been many studies on antibacterial plastics, antibacterial fibers (Kenawy & Abdel-Fattah, 2002; Kenawy, Bowlin, et al., 2002) and antibacterial ceramics (LaCoste, Schaich, Zumbrunnen, & Yam, 2005). The continuous search for potential antimicrobial agents has lead to identification of antimicrobial biomaterials that are based on polymers or their composites.

In recent years, antibacterial textile fibers have gained an increasing attention because they offer several interesting properties. It could be either bactericidal (to kill bacteria) or bacteriostatic (to prevent the bacterial proliferation) and in the two cases it protects the human body (Bourgeois, 2000). Cellulose, which is a naturally occurring complex polysaccharide, is biodegradable and the most abundant renewable organic raw material at low costs in the world. Its derivatives have many important applications in the fiber, paper, and packaging industries. There is a need to make the material antimicrobial, especially for products requiring a high degree of safety for the general population.

Modification of cellulose by graft copolymerization techniques allows one to chemically change the cellulose chain by introducing functional polymeric chains, which leads to new cellulose products with new properties (Abu-Laiwi, Ahmed, & Ibrahim, 2003). The graft copolymerization of monomers onto cellulosic fiber has been carried out by different techniques, such as reversible addition-fragmentation chain transfer (RAFT) polymerization (Roy, Knapp, & Guthrie, 2008), atom transfer radical polymerization (ATRP) (Huang, Murata, Koepsel, Tussell, & Matyjaszewski, 2007; Lee et al., 2004), thiocarbonate-H_2O_2 chemical initiation methods (Hebeish, Waly, & Abdel-Mohdy, 1997), and ceric (IV) ion chemical initiation method (Macdowall, Gupta, & Stammett, 1984; Nonaka, Noda, & Kurihara, 2000).

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0144-8617/$ – see front matter.
doi:10.1016/j.carbpol.2010.07.047

Antibacterial surface of fabrics and polymers have been developed by the introducing or grafting quaternary ammonium salts (Cen, Neoh, & Kang, 2003; Kilibanov, 2007; Kurt, Wood, Ohman, & Wynne, 2007; Kurt, Gamble, & Wynne, 2008; Lewis & Kilibanov, 2006; Murata, Keopsel, Matyjaszewski, & Russell, 2007; Park, Wang, & Klibanov, 2006; Sauvet, Fortuniak, Kazmierski, & Chojnowski, 2003; Son, Kim, Ravikumar, & Lee, 2005; Waschinski, Herdes, Schueler, & Tiller, 2005; Waschinski & Tiller, 2005; Waschinski, Barnert, et al., 2008; Waschinski, Zimmermann, et al., 2008), phosphonium salts (Kenawy, Abdel-Hay, El-Shanshoury, & El-Newehy, 2002; Kenawy & Mahmoud, 2003; Kenawy, Abdel-Hay, El-Magd, & Mahmoud, 2006), molecularly engineering peptides (Jayaraman, Yarmush, & Roth, 2002), N-halamines (Kou et al., 2006; Liang et al., 2007; Liu & Sun, 2008; Ren, Kou, et al., 2008; Ren, Liang, Worley, Tzou, & Hung, 2008; Sun & Xu, 1999; Sun & Sun, 2001b; Sun & Sun, 2002) or compounds releasing bacterial moieties such as metal ions (Estevào, Mendonça-Hagler, & Nascimento, 2003). Among these, aromatic derivatives have been extensively studied due to their stabilities and efficacies in inactivating bacteria. The infection of hospital personal and patients with pathogenic microorganisms could be minimized if they were using protective clothing containing antimicrobial materials.

In this work, we report the modification of cellulose-graft-polyacrylonitrile, prepared by using $KMnO_4$/citric acid as discussed in our previous paper (El-Khouly et al., 2010), with diamine followed by the reaction with aromatic aldehyde derivatives to prepare new cellulose materials with antimicrobial properties. The antimicrobial activities of the resulting cellulose derivatives were studied. Such studies are believed to be important in the endeavour to synthesis antimicrobial cellulose fiber.

2. Experimental

2.1. Materials

Cellulose-graft-polyacrylonitrile (C-g-PAN) was prepared from Egyptian cotton cellulose samples (Un-C) (El-Mahalla Company for Spinning and Weaving, Egypt) and acrylonitrile (Aldrich) using $KMnO_4$/citric acid redox system by the most efficient condition reported in a previous paper (El-Khouly et al., 2010). Ethylenediamine, p-hydroxybezaldehyde, p-chlorobenzaldehyde, vanillin (4-hydrox-3-methoxy-benzaldehyle), and anizaldehyde were supplied by Wako Pure Chemical Industries, Ltd., Japan and used without further purification. The following reagent grade chemicals were purchased from Kishida Chemical Co. Ltd., Japan and were used as received; glacial acetic acid, absolute ethanol, methanol, and dimethylformamide (DMF).

2.2. Instruments

FT-IR spectra were recorded with Bio-Rad FTS 6000 spectrometer with 32 scans at the highest resolution of 2 cm^{-1}. Approximately 1.0 mg of sample was pressed onto potassium bromide as a thin film (ca. 10 μm). Solid state NMR spectra were performed by using Delta2-NMR spectrometer (B0 = 9.4 T) with resonance frequency of 100.52 MHz at Kyushu University, Japan. Zirconia rotors (ϕ 6 mm) with Aurum tube cape were used to hold samples. 5 kHz spinning speed and decoupling with TPPM at γB1/2P = 100 kHz were utilized. For CP/MAS measurements, a 1H 90° pulse length of 5.25 μs and a contact time of 0.5 ms were applied.

2.3. Modification of cellulose-graft-polyacrylonitrile (C-g-PAN)

2.3.1. Modification of (C-g-PAN) with ethylenediamine (EDA)

To a 500-ml two-neck flask, equipped with nitrogen inlet, and reflux condenser, was charged 5 g of cellulose-graft-PAN in DMF solvent (10% by weight). After heating to 90 °C, an appropriate amount of ethylenediamine was added with vigorous stirring, and the reaction continued for 24 h under nitrogen at 120 °C. The modified EDA-cellulose-graft-PAN (EDA MC-g-PAN) was precipitated in warm water, washed three times with distilled water and finally with methanol, and then dried under vacuum at 60 °C for 24 h. The structure of aminated grafted cellulose was confirmed by FT-IR and solid state NMR spectroscopy.

2.3.2. Modification of the EDA MC-g-PAN with some aromatic aldehyde derivatives

To a solution of aromatic aldehyde (vanillin, p-hydroxybenzaldehyde, p-chlorobenzaldehyde, anisaldehyde) (2 mmol) in 20 ml of absolute ethanol was added with stirring (1 mmol) of EDA MC-g-PAN and 1 ml glacial acetic acid. The reaction was stirred at room temperature for 48 h and then the system was fitted to reflux at 85 °C for 24 h. The product was filtered and washed with methanol to remove the excess aldehyde and acetic acid. The products (EDA MC-g-PAN I–IV) were collected and dried in vacuum oven at 40 °C for 24 h. The percent yield (PY %) was evaluated and the product was characterized by FT-IR and solid state NMR spectroscopy (cf. Scheme 1)

2.4. Antimicrobial assessment

2.4.1. Microorganisms

These included the Gram negative bacteria (*Escherichia coli* JM 109, *Pseudomonas putida* ATCC 12633^T), Gram positive bacteria (*Bacillus subtilis* subsp. *subtilis* JCM1465T, *Staphylococcus aureus* subsp. *aureus* ATCC 12600^T, *Enterococcus faecalis* JCM 5803^T, and *Listeria innocua* ATCC 33090^T), and the yeast (*Candida guilliermondii* JCM 1539^T and *Saccharomyces cervisiae* JCM 7255^T).

2.4.2. Media

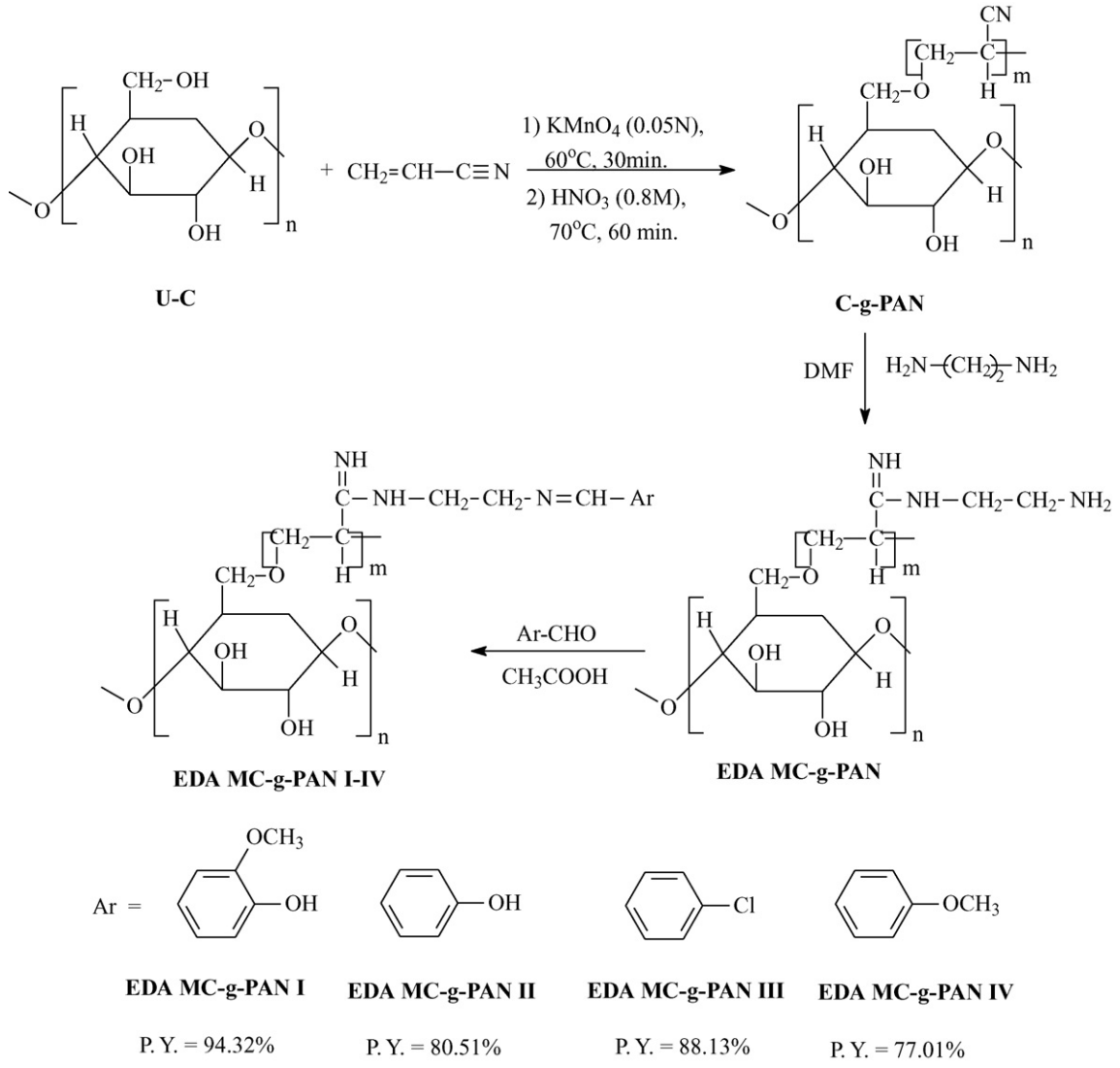
Tryptic Soy Agar and Tryptic Soy Broth were used to grow the bacterial cultures. On the other hand, Sabouraud Agar (which contains per one litre 20 g glucose + 10 g peptone + 15 g agar) and Sabouraud Broth (which contains per one litre 20 g glucose + 10 g peptone) were used to grow the *C. guilliermondii* and *S. cervisiae*.

2.4.3. Antimicrobial activity test

The antimicrobial spectra of the modified cellulose samples bearing an active functional group were determined against the tested organisms on solid samples on the solid media. 20 mg of powder modified cellulose samples (as disks with 60 mg of KBr) were put on the agar plates inoculated with the tested bacteria or yeast. After incubated for 24 h, the agar plates were checked for the diameter of inhibition zones. The modified cellulose samples, which produced inhibition zones, were further assayed at different concentrations in order to quantify their inhibitory effects.

2.4.4. Quantitative assay of the antimicrobial activity

These tests were performed by the dilution method (5 ml of liquid media in test tube); in order to establish the minimal inhibitory concentration (MIC) of modified cellulose against the tested microorganisms. The media used were Tryptic Soy Broth medium with 0.6% yeast extract for bacteria and Sabouraud Broth medium for the yeasts. Each culture medium (5 ml) containing was enriched with different concentrations (1, 2 and 4 mg/ml) of the modified cellulose samples was inoculated with each test microorganisms with shaking. After 24 h incubation and shaking for the tested bacteria and yeasts, different dilutions (10^{-1} to 10^{-8}) were made by successively adding 1 ml culture into 9 ml suitable culture media. Then, 100 μl of this culture was seeded on an agar plate. The plates were put into an incubator at culture temperature (30 or 37 °C according to the type of microorganism) for over 24 h. The



Scheme 1. Modification of cellulose-graft-polyacrylonitrile.

Table 1
Characteristic IR absorption data (wavenumber/cm^{-1}) for cellulose bands.

Bond assignments	UN-C	C-g-PAN	EDA MC-g-PAN	EDA MC-g-PAN I	EDA MC-g-PAN II	EDA MC-g-PAN III	EDA MC-g-PAN IV
OH stretching vibration	3436	3383	3353	3418	3356	3363	3350
C–H str. vibration	2901	2905	2909	2903	2903	2903	2903
C–C (ring) stretching vibration and OH in plane binding	1641	1645	1652	1645	1652	1664	1670
CH_2– bending	1431	1452	1434	1434	1440	1434	1428
CH– bending	1369	1367	1391	1360	1366	1372	1372
CH rocking in ring	1324	1324	1323	1329	1323	1329	1335
	1277	1281	1292	1273	1280	1273	1295
CO stretching	1243	1238	1249	1224	1224	1236	1249
Ring frequency	1163	1160	1162	1162	1162	1162	1162
Association bond C–O str./C–C str.	1118	1110	1106	1112	1111	1113	1108
C–O–C vibration	1055	1061	1062	1062	1062	1061	1057
C1 group frequency	889	889	895	895	895	895	889

Table 2
IR analysis (wavenumber/cm^{-1}) of modified cellulose with some aromatic aldehydes.

Bond assignments	UN-C	C-g-PAN	EDA MC-g-PAN	EDA MC-g-PAN I	EDA MC-g-PAN II	EDA MC-g-PAN III	EDA MC-g-PAN IV
C≡N	–	2243	–	–	–	–	–
–C–N–	–	–	1554	1553	1553	1546	1559
C=N	–	–	1652	1515	1602	1528	1670
OH aromatic	–	–	–	–	3241	–	–
C–H in CH_3O–	–	–	–	2742	–	–	2751
CH aliph.	2901	2905	2909	2903	2903	2903	2903
C–Cl	–	–	–	–	–	778	–
Ar–H	–	–	–	846	840	840	845

number of colonies was countered and three repeats were needed for each sample. The numbers of living colonies or colony forming unit (CFU)/ml were counted and the inhibition of cell growth can be quantified as follows:

$$\text{Number of surviving of cell}\,(\%) = \frac{A-B}{A}$$

where A and B are the number of the colonies detected from the negative control and treated samples, respectively.

2.4.5. Antibacterial mode of action

A desired amount of the modified cellulose samples was placed in the culture media, and then 50 μl of the bacterial suspension was added. The flask was shaken at 37 °C for the prescribed different times (2–48 h). After contacting the modified grafted cellulose derivatives with the bacteria for different times, 100 μl of the bacteria was pipetted out from the flask and diluted several time as discussed before and 100 μl of the diluted suspension was spread on an agar plate. The plate was kept at 37 °C for 24–48 h and the numbers of viable cells were determined by counting those colonies formed on the plate.

3. Results and discussion

In the pervious paper (El-Khouly et al., 2010), we achieved the best conditions for the grafting of acrylonitrile onto cellulose by using $KMnO_4$/citric acid redox system. Also the characterization and the thermal stability for the grafted cellulose were studied comparing with the unreacted cellulose and the PAN polymer. The modification of cellulose-graft-polyacrylonitrile was carried out by introducing the active amino groups into the polymer chain by reacting it with EDA. This aminated product is expected to be more active than the cellulose-graft-PAN.

The amination reaction takes place in DMF as a solvent. The product was washed with hot distilled water, followed by methanol to remove unreacted diamine. The reaction yield was 90% and the aminated grafted cellulose was collected as yellow powder and was dried under vacuum at 40 °C for 24 h (cf. Scheme 1) The resulting modified cellulose is expected to be important for many applications due to the presence of different functional groups, e.g. (OH, NH_2, NH). The reactivity of the aminated grafted cellulose (EDA MC-g-PAN) towards the aromatic aldehydes was found be higher than that of the grafted cellulose itself.

The untreated cellulose (U-C), C-g-PAN and EDA MC-g-PAN were characterized by solid state NMR and FT-IR. The IR spectral data of all samples are listed in Tables 1 and 2. In Fig. 1, IR spectrum of C-g-PAN showed strong peak at 2242 cm^{-1} for C≡N group. This band disappeared after the modification of cellulose-graft-PAN with ethylenediamine as shown in the IR spectrum of the EDA MC-g-PAN, which showed strong band appeared at 1554 cm^{-1} region for C–N in (–C–NH), 2909 cm^{-1} for C–H aliphatic, the absorption band appearance at 1654 cm^{-1} for C=N, and peaks at 3365 cm^{-1} for (NH_2). The FT-IR results showed the modification of C-g-PAN with the ethylenediamine.

In order to verify the structure of the obtained grafted and modified products by methods other than elemental analysis ^{13}C CP MAS NMR spectroscopy was performed. ^{13}C CP MAS NMR spectroscopy has been used extensively in the field of cellulose chemistry (Horii, Yamamoto, Hirai, & Kitamaru, 1989; Hoshino, Takai, Imura, & Hayashi, 1989; Pawlowski, Sankar, Gilbert, & Fornes, 1987; Stephenson, 1985) yielding detailed information on cellulose structure and substituent distribution. Fig. 2 shows the ^{13}C Cp MAS NMR of cellulose (U-C), C-g-PAN and EDA MC-g-PAN. The spectrum of the pure cotton cellulose is typical of cellulose (Masuda et al., 2003). It consists of the following resonances: 105 (C-1), 89 (crystalline C-4), 84 (amorphous C-4). 75, 72 (C-2, C-3, C-5), and 64 (C-6) ppm. In the cellulose-graft polyacrylonitrile spectrum, additional PAN signals, that is, an intense and clear distinguishable peak at 125 ppm due to the nitrile carbons and the broad resonance centered around 40 ppm due to the carbon resonance of the PAN backbone are observed. Solid state NMR spectrum of EDA MC-g-PAN is characterized by disappearance of the resonance at 125 ppm which is related to nitrile group. In NMR spectrum of EDA MC-g-PAN, signal resonated in the 42–48 ppm is due to carbon resonance of the diamine (CH_2 groups) and the signal resonated in 172.9 ppm is due to carbon resonance in NH–C=NH group. Other peaks are related to the cellulose and the grafted polymer.

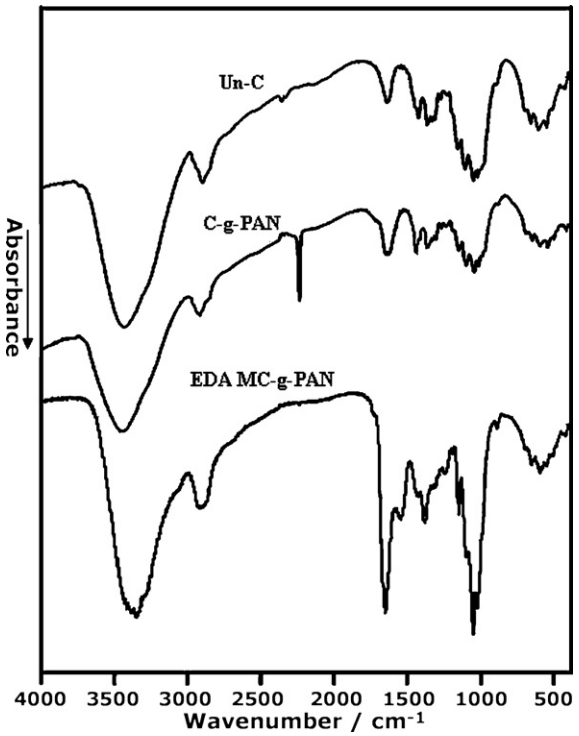


Fig. 1. FT-IR spectra of cellulose (Un-C), cellulose-graft-polyacrylonitrile (C-g-PAN), and aminated cellulose-graft-polyacrylonitile (EDA MC-g-PAN).

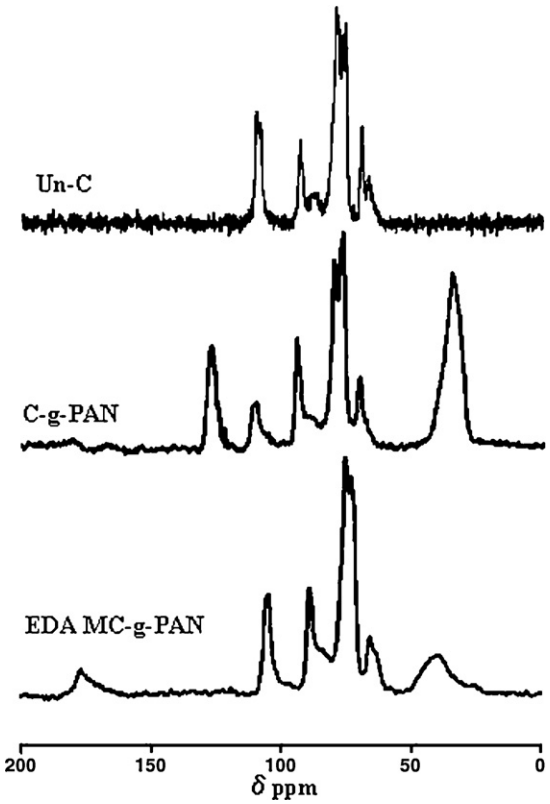


Fig. 2. ^{13}C CP MAS NMR spectra of Un-C, C-g-PAN, and EDA MC-g-PAN.

3.1. The modification of EDA MC-g-PAN with aromatic aldehyde derivatives

The Schiff base formation between aminated grafted cellulose and various aldehydes was carried out in absolute ethanol. Series of four different aminated cellulose-graft-PAN was prepared by condensation of (vanillin, p-hydroxybenzaldehyde, p-chlorobenzaldehyde and p-methoxybenzaldehyde) in the presence of glacial acetic acid as catalyst in an oil bath at 85 °C with stirring. Excess amounts of aldehydes were used to ensure complete condensation reaction has occurred.

The Schiff bases were formed at room temperature, but heating was used to ensure the complete condensation and to increase the reaction yield (Scheme 1).

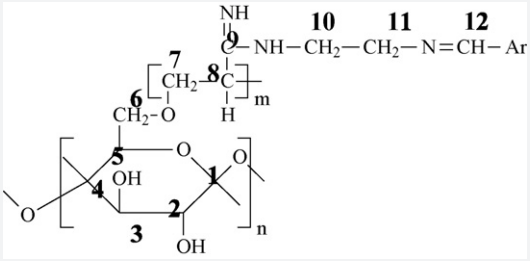
IR spectra of the modified EDA MC-g-PAN I–IV compounds showed broad band at 3350–3418 cm^{-1} due to (NH of secondary amine) and at 1515–1670 cm^{-1} due to C=N group. The IR spectra of EDA MC-g-PAN I and EDA MC-g-PAN IV showed absorption bands at 2742 and 2751 cm^{-1} region, respectively due to the methoxy group (OCH_3). EDA MC-g-PAN III showed strong band at 778 cm^{-1} region due to the C–Cl group as listed in Table 2.

All the modified EDA MC-g-PAN derivatives were characterized by solid state ^{13}C NMR spectroscopy. In Table 3, the resonated peaks related to the aldehyde carbons are listed. The resonated peaks related cellulose carbons were found and no substantial modifications of cellulose resonances in the 60–110 ppm region can be observed for all modified EDA MC-g-PAN compounds, indicating that the modification process does not remarkably change the chemical structure of the cellulose matrix.

3.2. Antimicrobial activity of the modified celluloses

The antimicrobial activities of modified celluloses against *E. coli*, *P. putida*, *B. subtilis*, *S. aureus*, *E. faecalis*, *L. innocua*, *C. guilliermondii*, and *S. cervisiae* were examined using the plate disk diffusion essay method and viable cell counting methods. As shown in Table 4, the inhibition zone diameter that varied according to the active group on the cellulose and the testing microorganism. The results showed that the untreated and the grafted cellulose were not active against the selected microorganisms while the EDA MC-g-PAN and its derivatives showed activity against the same microorganisms.

Table 3
Solid state ^{13}C NMR data (δ, ppm) of the modified of EDA MC-g-PAN.



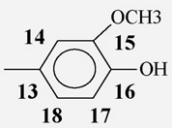
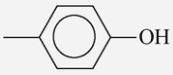
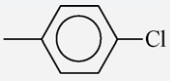
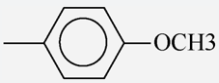
Ar	C1–C6	C7–C8	C9	C10–C11	C12	C13	C14	C15	C16	C17	C18	OCH3
Vanillin 	60–110	38–46	175	38–46	164	129.9	116.8	149.2	145	116.8	125	56.3
p-Hydroxybenzaldehyde 	60–110	38–46	175	38–46	164	129.9	130.6	116	160.3	116	130.6	–
p-Chlorobenzaldehyde 	60–110	38–46	175	38–36	164	134	130.6	127.8	136	127.8	130.6	–
Anisaldehyde 	60–110	38–46	175	38–46	164	129	130.6	121.2	164.3	121.2	130.6	175

Table 4
Diameters of inhibition zones (mm) produced by cellulose-graft-PAN and modified cellulose samples against various tested bacteria and a yeast.

Test organisms	Inhibition zone diameter (mm)						
	Cellulose and modified cellulose derivatives						
	Un-C	C-g-PAN	EDA MC-g-PAN	EDA MC-g-PAN I	EDA MC-g-PAN II	EDA MC-g-PAN III	EDA MC-g-PAN IV
E. coli	0.0	0.0	25.0	23.0	22.0	23.0	21.0
B. subtilis	0.0	0.0	16.0	23.0	21.0	19.0	18.0
S. cervisiae	0.0	0.0	31.0	22.0	25.0	24.0	20.0

However, EDA MC-g-PAN showed high antimicrobial activity. This high antimicrobial activity of EDA MC-g-PAN might be due to primary amino group (NH_2).

The growth inhibiting effect was quantitatively determined by calculating the ratio of surviving cell number (M/C). As shown in Fig. 3, the growth inhibitory effect of compound EDA MC-g-PAN differed among the bacteria and the fungi species. The inhibition becomes stronger in the order *C. guilliermondii* = *S. cervisiae* > *B-subtilis* > *E. faecalis* ≥ *L. innocua* = *E. coli* > *P. putida* > *S. aureus*. The results also show that the inhibitory effect is increased with an increase in the concentration of the compound.

The antimicrobial activity of EDA MC-g-PAN against the selected microorganisms was high. It killed 47–100% of the microorganisms at a concentration of 2 mg ml^{-1}. However, concentration of 2 and 4 mg ml^{-1} of the compound killed 99.9–100% of the tested fungi. In the case of *S. aureus*, the compound EDA MC-g-PAN killed 57% at a concentration of 4 mg ml^{-1}.

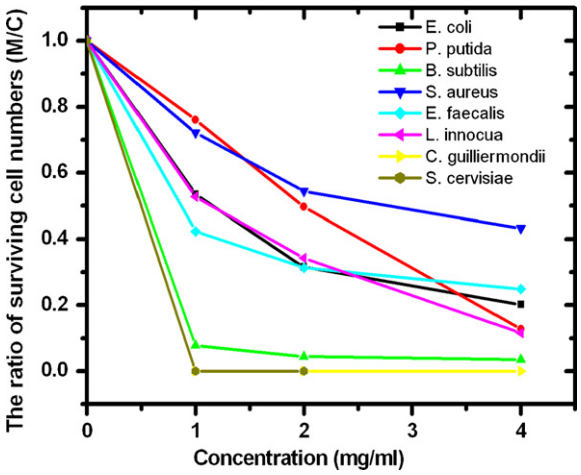


Fig. 3. Growth inhibition of different concentrations of compound EDA MC-g-PAN.

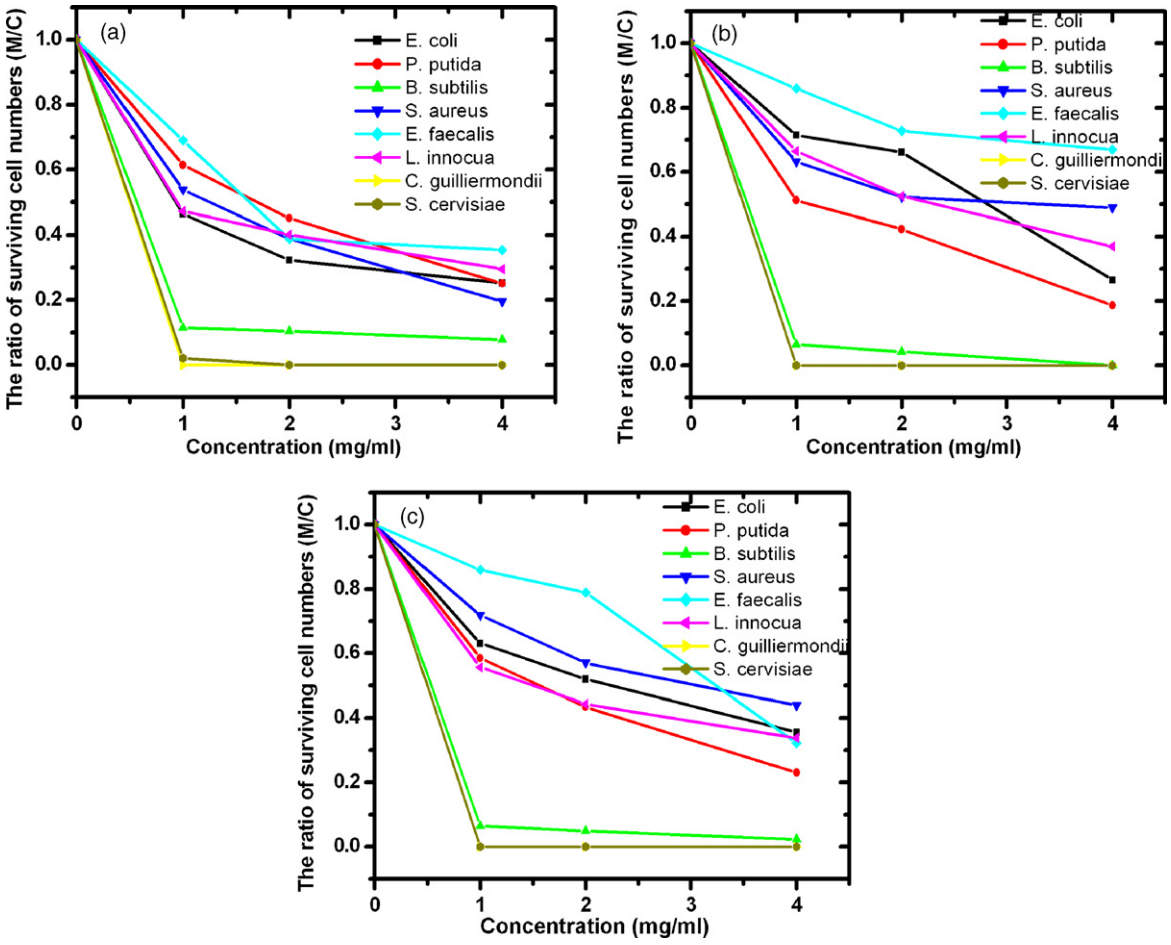


Fig. 4. (a) Growth inhibition of different concentrations of compound EDA MC-g-PAN I. (b) Growth inhibition of different concentrations of compound EDA MC-g-PAN II. (c) Growth inhibition of different concentrations of compound EDA MC-g-PAN III.

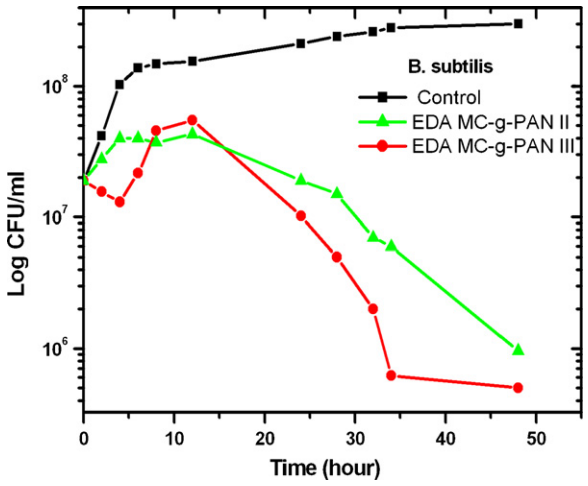


Fig. 5. Bacteriostatic or bacteriocidal nature of EDA MC-g-PAN II and EDA MC-g-PAN III against *B. subtilis* specie.

The shaking method tends to be more suitable for evaluating the antimicrobial activity of the substances with relatively large entities including the samples address in the current work. The effects of the concentrations of EDA MC-g-PAN I–III compounds are shown in Fig. 4(a–c), respectively. The growth inhibition increases with increasing the polymer concentration. The growth inhibition reached to 100% with the fungi species, indicating almost all fungi were killed.

Fig. 4a–c shows the effect of concentration of the modified EDA MC-g-PAN I–III, respectively on the bacteria and the fungi species. As shown in Fig. 4a, the inhibition becomes stronger in the order *C. guilliermondii* = *S. cervisiae* > *B-subtilis* > *S. aureus* > *E. coli* ≥ *L. innocua* = *P. Putida* > *E. faecalis* with increasing the concentration of EDA MC-g-PAN I. The concentration of 2 mg ml^{-1} killed 61–99.99% of the tested microorganism. However, increasing the concentration to 4 mg ml^{-1} killed 75–100%. The modified cellulose compound has little effect on E. faecalis with increasing the concentration. In the case of modified compound EDA MC-g-PAN II, Fig. 4b, the order of the inhibition growth is *C. guilliermondii* = *S. cervisiae* > *B-subtilis* > *P. Putida* > *E. coli* ≥ *L. innocua* > *S. aureus* > *E. faecalis* with killing range of 73–100% of the tested microorganism. The concentration of 2 mg ml^{-1} killed 100% of the fungi species. EDA MC-g-PAN II has little effect on the *S. aureus* and *E. faecalis* with increasing the concentration. The concentration 2 mg ml^{-1} of EDA MC-g-PAN III, Fig. 4c, killed 65–100% of the tested microorganism. The inhibition and the sensitivity of the microorganisms were in the order *C. guilliermondii* = *S. cervisiae* > *B. subtilis* > *P. Putida* > *L. innocua* > *E. coli* > *S. aureus* > *E. faecalis*.

Based on the above results, all the modified cellulose derivatives were found to be active against the tested microorganisms. They were found to be more active against the fungus species. Also, these derivatives were found to be more active with *B. subtilis*, and EDA MC-g-PAN I was found to be more active against *S. aureus* with concentration of 4 mg ml^{-1} (inhibitory effect = 81%).

Generally, the potency of inhibition varied according to the cellulose derivative and the test strain. Antimicrobial activity of the materials tested was selective toward certain microorganisms, obviously resulting from the structural affinity between the wall of the microorganism and the tested materials.

3.3. Bacterial mode of action

The antibacterial activity of EDA MC-g-PAN II and EDA MC-g-PAN III was evaluated on the basis of its bacteriostatic and bactericidal mode of action as shown in Fig. 5. The effect of EDA MC-g-PAN II and EDA MC-g-PAN III with concentration of 2 mg ml^{-1} on *B. subtilis* growth was reported as a function with the incubation time. The results showed a sharp decline in the viable cell counts in comparison with the initial control one, indicating that the EDA MC-g-PAN II and EDA MC-g-PAN III has bactericidal mode of action.

Generally, it was observe that the mode of action of cellulose biocides is interpreted in terms of the following sequence of elementary processes (Franklin & Snow, 1981; Hungo & Longworth, 1964; Hungo & Longworth, 1966; Kanazawa, Ikeda, & Endo, 1993): (a) adsorption onto the bacterial cell surface; (b) diffusion through the cell wall; (c) binding to the cytoplasmic membrane; (d) disruption of the cytoplasmic membrane; (e) release of the cytoplasmic constituents; and (f) the death of the cell.

4. Conclusion

Aminated grafted cellulose was prepared by the grafting of acrylonitrile onto cellulose by the use of $KMnO_4$/citric acid followed by the amination reaction with ethylenediamine. The aminated grafted cellulose was characterized by FT-IR and solid state NMR spectra. Then, the aminated grafted cellulose was reacted with some selected aromatic aldehyde derivatives to introduce biologically active functional groups on the cellulose fiber. The modified aminated grafted celluloses were characterized by FT-IR and solid state NMR spectra. The aminated grafted cellulose and its derivatives were tested against the bacterial species (*E. coli*, *P. putida*, *B. subtilis*, *S. aureus*, *E. faecalis*, and *L. innocua)* and the fungal species (*C. guilliermondii*, and *S. cervisiae*). The modified grafted cellulose materials exhibited high antimicrobial activities against *B. subtilis*, *C. guilliermondii*, and *S. cervisiae*. Also, with low concentration of the modified grafted cellulose materials, the growth inhibition against the all fungal species reached to 100% and almost 60% of the bacterial species. Moreover, by increasing the concentration EDA MC-g-PAN I, it can kill more than 80% of *S. aureus*. EDA MC-g-PAN II and EDA MC-g-PAN III have bactericidal effect on *B. subtilis*, indicating that most of these compounds have bactericidal effect on the microorganisms. It can be concluded that these new cellulosic materials have good antimicrobial properties and can be used in many application areas especially textiles, health care products and hygienic applications.

Acknowledgments

The authors would like to thank Mr. Mohamed A. Abd El-Rahman and Mr. Said E. Desouky, Division of Microbial Science and Technology, Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, for their excellent technical assistance during the antimicrobial activity testing of the polymers.

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