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Bioinspired Antimicrobial and Biocompatible Bacterial Cellulose Membranes Obtained by Surface Functionalization with Aminoalkyl Groups

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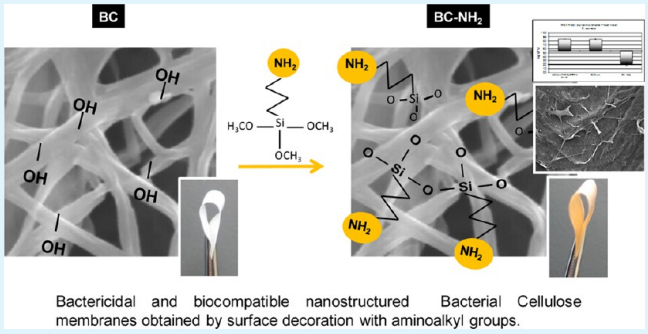
Supporting Information

ABSTRACT: There has been a great deal of interest in the use of nanostructured bacterial cellulose membranes for biomedical applications, including tissue implants, wound healing, and drug delivery. However, as bacterial cellulose does not intrinsically present antimicrobial properties, in the present study, antimicrobial bacterial cellulose membranes were obtained by chemical grafting of aminoalkyl groups onto the surface of its nanofibrillar network. This approach intends to mimic intrinsic antimicrobial properties of chitosan. Interestingly, these novel grafted bacterial cellulose membranes (BC-NH_2) are simultaneously lethal against *S. aureus* and *E. coli* and nontoxic to human adipose-derived mesenchymal stem cells (ADSCs) and thus may be useful for biomedical applications. In addition to these biological properties, the bioactive nanostructured BC-NH_2 membranes also present improved mechanical and thermal properties.



Bactericidal and biocompatible nanostructured Bacterial Cellulose membranes obtained by surface decoration with aminoalkyl groups.

KEYWORDS: *bacterial cellulose, aminoalkyl groups, antimicrobial, biocompatibility*

INTRODUCTION

The contamination by microorganisms is an issue of enormous concern particularly for the biomedical and food industries.[1] In this context, a broad variety of antimicrobial materials, including polymers and biopolymers, molecular compounds, and nanoparticles, have been developed in recent years for application in these fields.[2−4]

Biopolymers have attracted increasing interest because of their renewable character, intrinsic biocompatibility, biodegradability, and specific features. However, although biopolymers, such as chitosan[5,6] and some peptides,[7] exhibit intrinsic antimicrobial properties, most of them, like cellulose, are not natural biocides. Cellulose, because of its abundance and widespread utilization (e.g., paper, textiles, and composites) is among the most studied biopolymers. Most available cellulose is produced by plants, however, some bacteria, belonging mainly to the *Gluconacetobacter* genus, are also able to produce an unique extracellular form of this polysaccharide, known as bacterial cellulose (BC), that is particularly interesting to be exploited in these research areas. In fact, this highly pure form of cellulose is obtained as a swollen membrane with ∼99% of water,[8−10] and the physical and mechanical properties, because of its peculiar tridimensional micro- and nanofibrillar structure, along with its inherent biocompatibility,[10] have generated considerable interest in this biopolymer for use in the biomedical area, e.g., as a substitute for skin, as surgical implants, and as support for drug delivery and wound healing membranes.[11,12]

Considering the enormous potential of BC, its functionalization with groups able to impart antimicrobial properties is of great interest to develop novel BC-based functional biomaterials. For example, antimicrobial BC-based materials have been obtained by incorporation of silver and silver chloride nanoparticles,[13,14] sorbic acid,[15] benzalkonium chloride,[16] and potassium sorbate,[17] into the 3D network of the membrane.

However, for these type of materials, the potential leaching of the biocidal agents represents a major drawback that results in a decrease in the antimicrobial effectiveness for medium-long-

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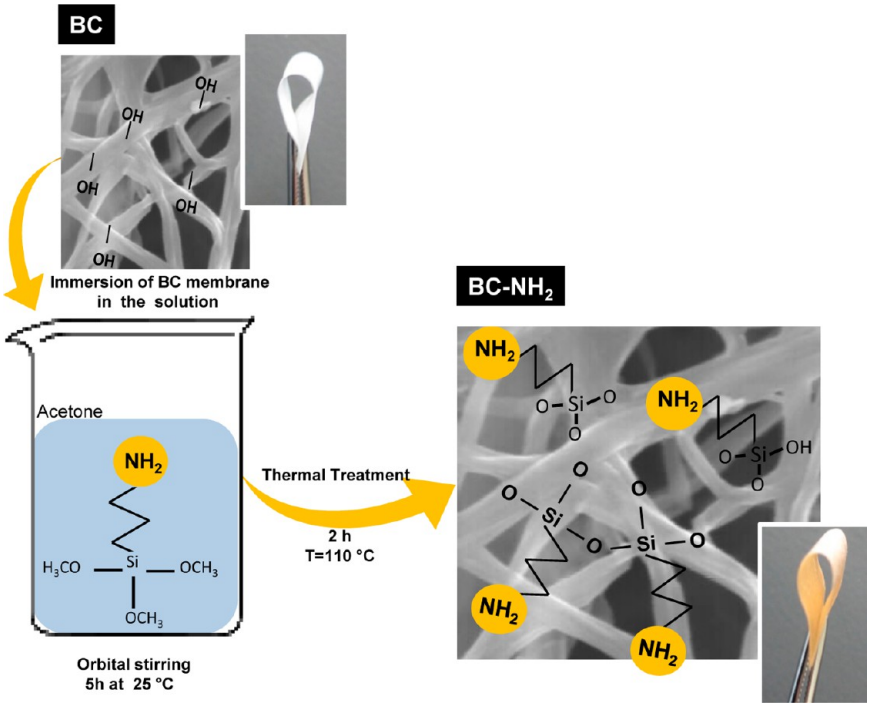
Scheme 1. General Procedure for Silane-Bacterial Cellulose Chemical Grafting Using APS as Active Reagent to Prepare BC Membranes with Antimicrobial Properties; Images of BC Mats and Its Flexibility before (BC) and after (BC-NH_2) Chemical Grafting with APS



term applications and in environmental problems associated with the residual toxicity of some conventional antimicrobial agents.[1] Therefore, the creation of surfaces with permanent biocide activity via chemical (or enzymatic) grafting of bioactive moieties onto the biopolymer surface constitutes an interesting strategy to overcome these problems. Several studies on the covalent surface functionalization of cellulosic substrates, mainly vegetal cellulose fibers, have already been described. For example, Feese et al. prepared photobactericidal cellulose nanocrystals by appending cationic porphyrins onto the cellulose surface.[3] Antibacterial cellulose fibers were also prepared by RAFT surface graft polymerization with 2-(dimethylamino)ethyl methacrylate.[18]

In this vein and inspired by the intrinsic bactericidal activity of chitosan, which is associated with the presence of free amino groups along the polymeric chain,[5,19,20] we have, in the present work, chemically grafted aminoalkyl groups at the surface of BC nanofibrills using 3-aminopropyltrimethoxysilane in order to mimic chitosan antimicrobial properties. Several silane derivatives have already been used to functionalize cellulosic substrates for distinct applications such as the preparation of reinforcing elements for composites or for catalytic applications.[21−24] However, the present study goes a step further since our purpose is to apply this chemical grafting approach to produce new nanostructured cellulose based antimicrobial and biocompatible biomaterials, under environmentally friendly conditions.

In recent years, we have been studying BC in different perspectives, ranging from its production[25] to the development of new nanocomposite materials[26−31] and drug delivery systems.[32] In the present work, the aminoalkyl-grafted BC membranes were characterized in terms of antimicrobial activity and in terms of cell response studies with adipose-derived stem cells (ADSCs), analyzing the citotoxicity, proliferation, and cell adhesion properties. Finally, BC-NH_2 membranes were also thoroughly characterized in terms of chemical composition, morphology, thermal stability, and mechanical properties.

EXPERIMENTAL SECTION

Materials. Bacterial cellulose (BC) membranes were produced in our laboratory using *Gluconacetobacter xylinum*[29] bacterial strain and Hestrin−Schramm culture medium.[33]

3-Aminopropyltrimethoxysilane (APS, 97%) and acetone were purchased from Sigma-Aldrich and used without further purification.

Synthesis of BC-NH_2 Membranes. Five wet BC membranes (5 × 5 cm^2, ∼220 mg of dry cellulose) were immersed in a previously prepared solution of 3-aminopropyltrimethoxysilane (APS) (10 mL) in acetone (100 mL) and maintained in this solution for 5h with orbital stirring at 25 °C. The BC membranes were then washed with acetone, to remove the unreacted APS and other impurities and then dried at room temperature. Finally, dry BC membranes were heated at 110 °C for 2 h (Scheme 1). The cured BC-NH_2 samples were submitted to a Soxhlet extraction with acetone for 10 h and the resulting membranes were dried at room temperature in a dissector.

Characterization of the BC-NH_2 Membranes. The functionalization of the BC membranes was confirmed by attenuated total reflectance fourier transform infrared (ATR-FTIR) spectroscopy and solid state ^{13}C and ^{29}Si nuclear magnetic resonance (NMR) spectroscopy. ATR-FTIR spectra were obtained using a Nicolet Nexus 670 FT-IR spectrophotometer equipped with a KRS-5 crystal of refractive index 2.4 with an incidence angle of 45°. The spectra were taken in transmittance mode over the wavenumber range of 600−4000 cm^{-1}, with a resolution of 4 cm^{-1} and after 128 scans. ^{13}C and ^{29}Si solid-state NMR spectra were obtained using a Bruker 400 WB Plus spectrometer. Spectra were collected by using a 4 mm CP-MAS probe with a sample spinning rate of 10000 Hz. ^{13}C CP-MAS spectra at 100.6 MHz of the solid samples were obtained using 12h spectral accumulation time, a time domain of 2K points, a spectral width of 29 kHz, a contact time of 1.5 ms and an interpulse delay of 5 s. ^{29}Si NMR spectra at 79.5 MHz were also recorded over 12 h using a standard

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pulse sequence, a time domain of 1K points, a spectral width of 55 kHz, a contact time of 2 ms, and an interpulse delay of 5s.

The N content of the BC membranes was determined by CHN elemental analysis using a Eurovector EA 3000 elemental analyzer. The Si content of the samples was determined by Inductively Coupled Plasma (ICP), using a Jobin Yvon 70 Plus spectrophotometer.

The surface morphology of BC and BC-NH_2 membranes was assessed by scanning electron microscopy (SEM) using a Hitachi SU-70 microscope. Samples were deposited on a glass plate and coated with carbon.

The thermal stability of the BC and BC-NH_2 membranes was determined by thermogravimetric analysis (TGA) using a TGA/SDTA 851 Mettler Toledo instrument, at a scanning rate of 10 °C/min, from room temperature to 900 °C under a nitrogen atmosphere.

The thickness of the BC and BC-NH_2 membranes was measured using a digimatic MDE-25TJ micrometer. An average thickness was calculated from ten measurements taken at different points of the sample.

The mechanical performance of the materials was determined using a MTS Insight 10 equipment with a load cell of 250 N connected to an extensometer. Tests were performed at 50 mm/min. An average value of at least five replicates for each sample was obtained. Results were expressed as mean ± standard deviation.

Quantitative Assessment of Antimicrobial Properties. The bactericidal activity of functionalized BC mats was tested using *Staphylococcus aureus* and *Escherichia coli* bacteria using the standard Dynamic Shake Flask Method.

The bacterial strains were provided by DSMZ, Deutsche Sammlung von Mikroorganismen and Zellkulturen GmbH (German Collection of Microorganisms and Cell Cultures). *Staphylococcus aureus* ATCC 6538 (Gram +) and *Escherichia coli* ATCC 10536 (Gram −) were maintained frozen (−80 °C) and transferred monthly on TSA (Tryptone Soya Agar) made of 15 g/L tryptone; 5 g/L soya peptone; 5 g/L NaCl and 15 g/L neutralized bacteriological agar.

The bacterial preinoculum cultures were grown overnight at 37 °C in 20 mL of nutrient broth (made of 1 g/L beef extract; 5 g/L neutralized peptone; 2 g/L yeast extract; 5 g/L NaCl) subjected to horizontal shaking at 100 rpm. The samples were placed in contact with a bacterial liquid suspension and subjected to vigorous shaking for the duration of the test to ensure the best contact between bacteria and sample. At 0 and 24 h contact times, the bacterial concentration (CFU/mL) of the microbial suspension was determined by plating serial dilutions on Plate Count Agar to obtain the overall number of bacteria (CFU- Colony Forming Units). The specific conditions for these tests were as follows: 10 mg of BC-NH_2 membranes were placed in flasks containing 5 mL of phosphate buffer +5% nutrient broth and inoculated with a bacteria inoculum of 1×10^5 UFC/mL. The samples were incubated for 24 h at room temperature under vigorous shaking. BC membranes without functionalization were used as a reference and flasks containing only the inoculated broth media were used as an internal reference for bacterial growth. All samples were analyzed in duplicate and have been previously subjected to sterilization in an autoclave. The antibacterial activity, i.e., bacteria log reduction, of the samples was calculated as follows: log reduction = log CFU T_{24} control sample − log CFU T_{24}. In the standard dynamic shake flask method, at least a 1 log reduction in bacteria load is required to claim antibacterial properties.

In Vitro Cell Response. A short-term cytotoxicity evaluation of BC and BC-NH_2 membranes was carried out using adipose derived stem cells (ADSCs).[34]

Here, membranes with an area 6 cm^2 (thickness ≤35 μm), were rinsed with Milli-Q water and sterilized with 70% ethanol for 1h. To prepare extracts of test materials, sterilized samples were incubated in Dulbecco's modified Eagle's medium (DMEM, Sigma Chemicals Co, USA), supplemented with Glutamax (Sigma) and 10% fetal bovine serum (SFB, Gibco), at 37 °C for 24 h.

In the in vitro cytotoxicity assays, ADSCs were seeded and allowed to grow for 24 h in 96-well microplates at a density of 4×10^3 cells/well in the presence of standard culture medium. The cultures were then treated for 24, 48, and 72 h with the extracted media. As controls, standard culture medium (control), high-density polyethylene (negative control, USP Rockville, USA), and polyvinyl chloride (positive control, Portex, UK) were also used. The metabolic activity of viable cells was determined by colorimetric assay (Cell Proliferation Kit I MTT, Roche). Briefly, viable cells could reduce MTT to formazan pigment, which is then dissolved in 1 mL of dimethylsulfoxide (DMSO) after removal of the culture medium. The cell number per well is proportional to the recorded absorbance at 550 nm using an ELISA microplate reader. Cell viability was expressed as the ratio of the amount of formazan crystals formed by control and sample cells. All assays were conducted in triplicate. Mean values and their standard deviations were calculated.

Scanning electron microscopy (SEM) studies were carried out on cultured human ADSC on BC, BC-NH_2 mats and control surfaces to observe the adhesion and morphology of the cells in the surface of the mats. Aliquots containing 5×10^4 cells were seeded onto the BC and BC-NH_2 mats in 24-well flat-bottomed culture plates (Costar). After 24 h, samples were fixed with 2% glutaraldehyde in a cacodylate buffer (0.1 M, pH 7.4) and post fixed using OsO_4 for 1 h, washed in phosphate buffer solution, and dehydrated using series of graded ethanol solutions. Samples were dried through CO_2 critical point, gold sputtered, and analyzed in a SA-3400N Hitachi microscope.

RESULTS AND DISCUSSION

The chemical grafting of aminoalkyl groups onto cellulose surfaces (Scheme 1) normally involves three steps:[21] (i) the hydrolysis of the silane derivative (APS) to give the corresponding silanol derivative; (ii) the adsorption of the hydrolyzed species onto BC nanofibrills through hydrogen bonding between silanol and cellulosic OH; and (iii) chemical condensation leading to siloxane bridges (Si−O−Si) and to grafting onto cellulose nanofibrills surface through Si−O−C bonds. The siloxane bridges resulting from self-condensation contribute to the formation of a polysiloxane network on the BC nanofibers surface as will be discussed below.[21]

Table 1 shows the thickness and the N and Si contents of the pristine bacterial cellulose (BC) and the chemically grafted BC mats (BC-NH_2) studied here. The effectiveness of the BC modification using this approach was initially confirmed by the increase in dry weight (about 55%) and thickness (about 87%) of the BC mats after reaction, as well as by their color change from white to yellowish (Scheme 1). The N (3.4 wt %) and Si (7.3 wt %) contents (Table 1), as determined by elemental analysis and ICP respectively, are also a clear indication of the successful modification with the APS silane derivative. Moreover, these values are in excellent agreement with the structure of this silane moiety, where the N:Si molar ratio showed to be 1. The N and Si contents (Table 1) point out to an APS:anhydroglucose ratio slightly below 1, which means that a high degree of modification/coverage of BC nanofibers has taken place, as also confirmed by the SEM images shown in Figure 4.

Table 1. Physicochemical Characterization of BC and BC-NH_2 membranes

sample	dried weight (mg)	thickness (μm)	N content (%)	Si content (%)
BC	221 ± 4	110 ± 12		
BC-NH_2	342 ± 6	206 ± 9	3.4 ± 0.02	7.3 ± 0.9

Structural Characterization of BC-NH_2. Apart from the typical absorption bands of cellulose,[35] at around 3300 cm^{-1}, corresponding to the OH stretching vibrations; at 1250−1460 and 2850−2980 cm^{-1} corresponding to CH and CH_2 stretching vibrations; and at 1170−1050 cm^{-1} assigned to the

vibrations of the C−O−C bonds of glycosidic bridges, the spectrum of the BC-NH_2 sample displays a band at 1580 cm^{-1}, attributed to the bending of NH_2 groups and a increment of the band at 2880 cm^{-1} associated with the CH_2 vibrations of the propyl moiety of the silane moiety (Figure 1). The presence of

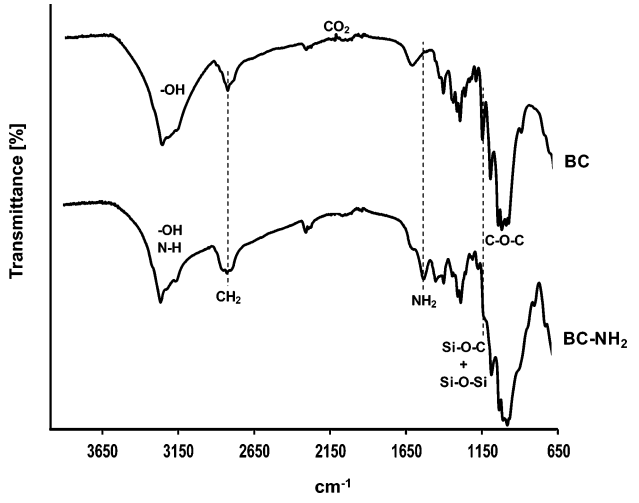

Figure 1. ATR-FTIR spectra of bacterial cellulose (BC) and bacterial cellulose after treatment with APS (BC-NH_2).

Si−O−cellulose and −Si−O−Si− bridges, that correspond to the condensation of the hydroxyl groups of the silane derivative with the hydroxyl groups of cellulose and of those silanols that react with themselves, respectively, are not easily seen by FTIR, since the typical vibrations of these moieties occur around 1150 and 1135 cm^{-1}, and are masked by the large and intense cellulose C−O−C vibration bands.[36]

The solid-state ^{13}C NMR spectra of BC and BC-NH_2 are displayed in Figure 2. The typical spectrum of BC shows peaks in the 105−60 ppm range:[37,38] from the anomeric C1 ($\delta \approx 105$ ppm); C4 (86−92 ppm crystalline and 79−86 ppm

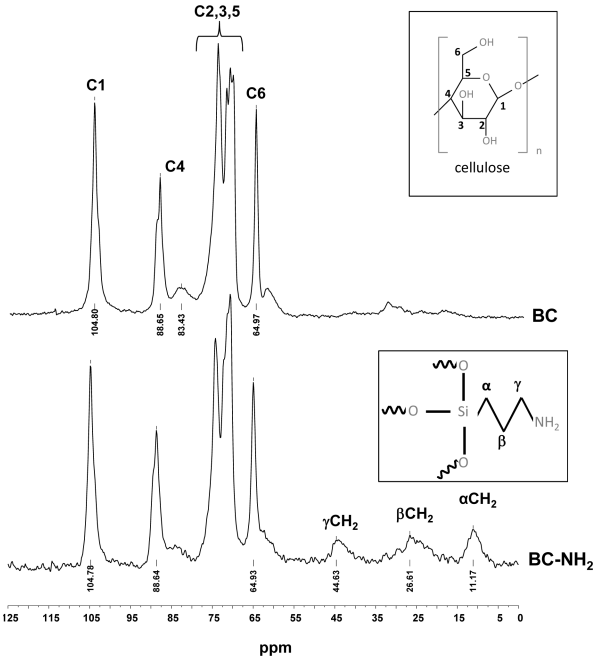

Figure 2. Solid-state ^{13} C NMR spectra of BC and BC-NH_2.

amorphous); C2, C3, and C5 (72−79 ppm); and finally the C6 carbon ($\delta \approx 64$ ppm).

In addition to the carbon resonances of the cellulose chain, the spectrum of BC-NH_2 displays three new peaks at around 11, 27, and 45 ppm assigned to the αCH_2, βCH_2, and γCH_2 resonances of the aminopropyl groups of the silane coupling agent grafted onto the BC nanofibrills (Figure 2).[22]

Solid-state ^{29}Si NMR spectroscopy allowed additional information about the structure of BC-NH_2 mats to be acquired, namely the nature of the silicon atoms environments i.e., the number of siloxane bridges attached to a silicon atom. As already mentioned, the self-condensation of the silanol groups can occur, to give dimeric, linear oligomeric and or three-dimensional structures.[39−42] Figure 3 shows the ^{29}Si

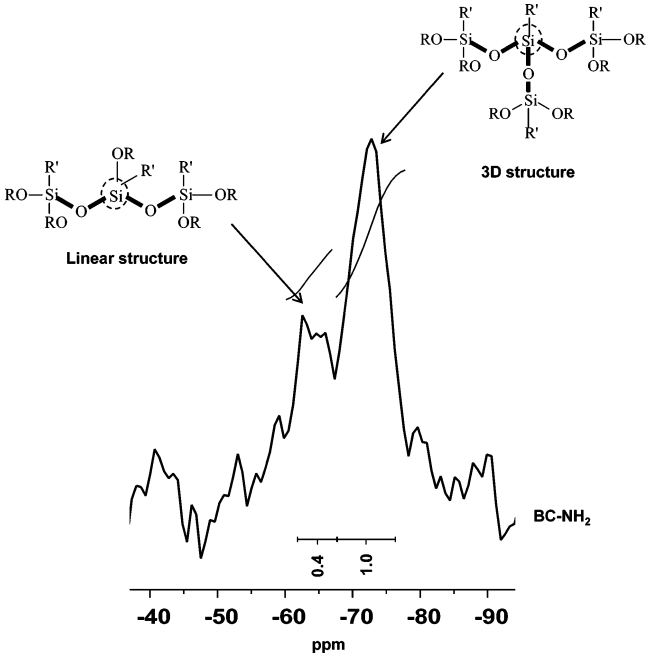

Figure 3. Solid-state ^{29}Si RMN spectra of BC-NH_2.

solid-state NMR spectrum of a BC-NH_2 membrane, where it is possible to observe the presence of two dominant ^{29}Si environments associated with the homopolycondensation structures grafted to cellulose, i.e., the linear (40%) and three-dimensional (60%) structures. Similar ^{29}Si spectra and similar percentages have been found by Salon et al. in a study of the interaction between silane coupling agents and vegetal cellulose fibers.[41]

Morphology of BC-NH_2. SEM analysis (Figure 4) was carried out in order to study the effect of the introduction of aminoalkyl silane moieties onto the surface of the nanofibrills on the morphology of the membranes. The BC-NH_2 membranes show a typical 3D network structure of BC; however, the nanofibrills are thicker because of the coverage with a 3D silane structure. This morphology is clearly associated with the formation of Si−O−Si bridges as indicated by the ^{29}Si NMR spectrum.

Thermal and Mechanical Properties of BC-NH_2. The thermal stability and degradation profile of the unmodified BC and BC-NH_2 mats were assessed by thermogravimetry (Figure 5). The unmodified BC exhibited the typical thermal degradation profile of cellulosic substrates, with a maximum decomposition rate around 349 °C. However, the BC-NH_2

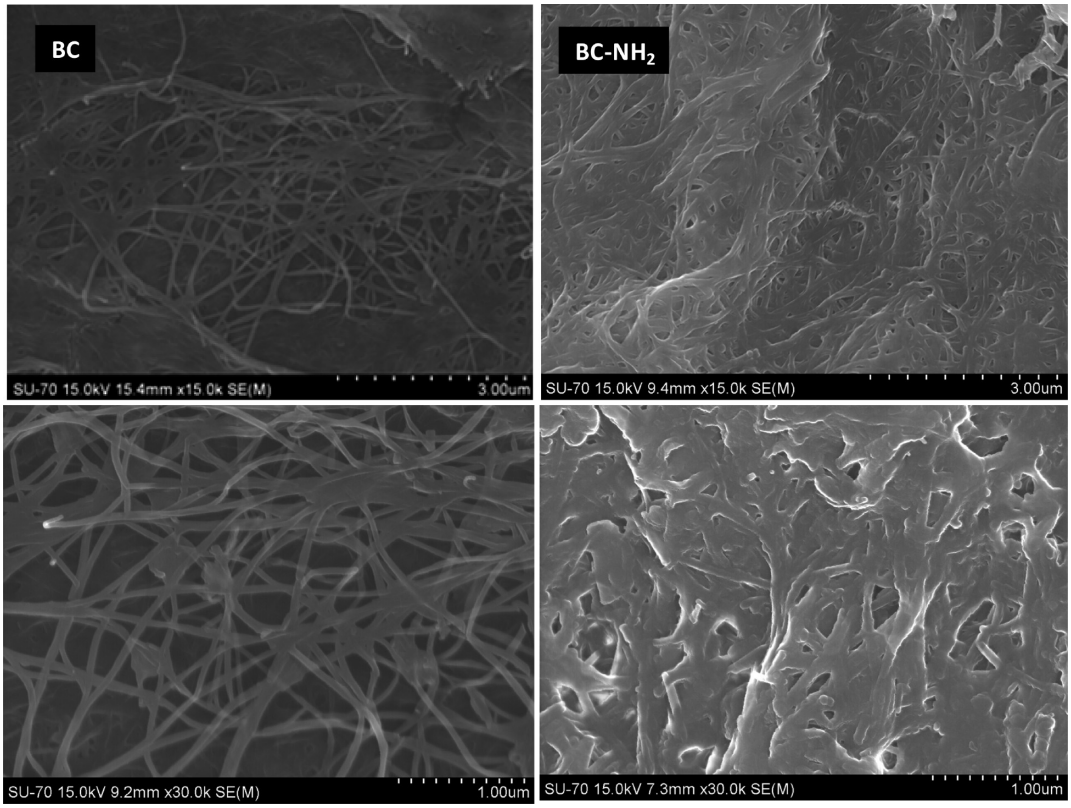

Figure 4. SEM images of the surface of BC and BC-NH_2 mats with two different magnifications (15.0 and 30.0 k).

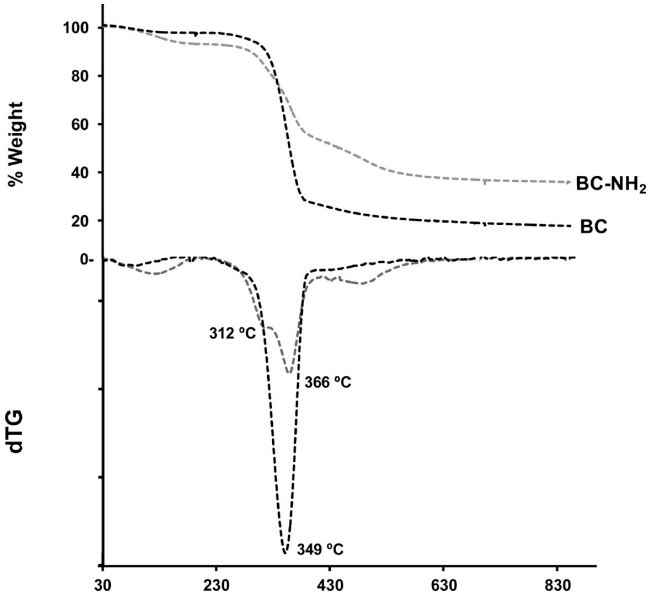

Figure 5. TGA and DTG of BC and BC-NH_2.

mats presented a double step weight loss profile with maximum degradation temperatures at around 312 °C (weight loss of 30%) and 366 °C (weight loss of 50%). The significant increase in the inorganic residue observed at ∼900 °C for the BC-NH_2 mats (of about 16%, considering that the unmodified BC have already 20% of residue) further supports the chemical modification of BC with the silane coupling agent. Moreover, this result is in agreement with the Si content found by ICP, considering that the increment in the inorganic residue is mainly due to SiO_2. Finally the water loss observed in the thermograms at ∼100 °C for BC and BC-NH_2 is in the same range (∼10%) demonstrating that functionalization with APS did not significantly affect the water affinity of the mat.

Table 2 lists the mechanical properties namely Young's modulus, tensile strength and elongation at break for the BC and BC-NH_2 mats. It can be seen that the physical strength, i.e., Young's modulus and tensile strength of the BC mats increased after functionalization with the silane derivative, certainly due to the formation of a rigid polysiloxane layer on the nanofibrills surface as observed by SEM. These mechanical properties could also be in part due to the establishment of strong hydrogen bonds between the amino groups of the appended silane moieties and the hydroxyl groups of the cellulose backbone.[23,24,43] The presence of a rigid polysiloxane layer on the surface of the nanofibrills also justifies the decrease (57%) in the elongation at break after chemical modification. However, the BC-NH_2 membranes are still flexible, as shown in Scheme 1, and therefore suitable for several applications including wound healing membranes and drug delivery systems.

Table 2. Mechanical Properties of BC and BC-NH_2

sample	Young's modulus (MPa)	tensile strength (MPa)	strain (%)
BC	3067 ± 55	5.7 ± 0.1	2.8 ± 0.2
BC-NH_2	3559 ± 72	6.0 ± 0.1	1.2 ± 0.2

Bacterial Activity of BC-NH_2. The effect of the introduction of aminoalkyl groups onto the BC nanofibrills on bacteria growth was assessed by inoculating BC-NH_2 mats with Gram negative (*E. coli*) and Gram positive (*S. aureus*) bacteria. BC mats without functionalization were used as reference, while an experimental control was produced by inoculating both bacteria in media without samples. For the

culture containing the starting BC membranes, there was no reduction in bacterial cells; in fact, there was a 2−3 log increase in growth of bacteria for *E. coli* and *S. aureus* (Figure 6).

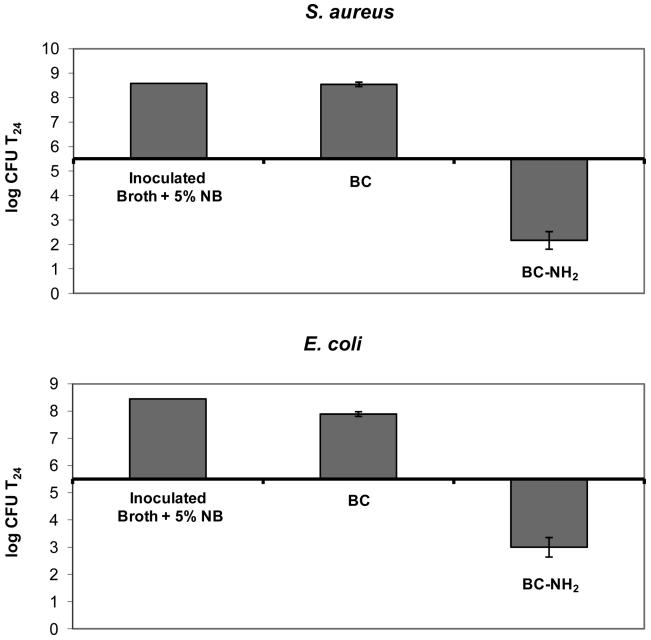

Figure 6. Antimicrobial activity of BC-NH_2 membranes toward *S. aureus* and *E. coli*. The results obtained are compared with reference sample (unmodified BC membranes) as well as inoculated broth added with 5% of nutrient broth (NB).

Nonetheless, BC-NH_2 membranes showed a significant reduction in bacterial viability for both *E. coli* and *S. aureus* bacterial strains after 24 h. The initial bacterial concentration of *E. coli* and *S. aureus* was 12×10^5 and 50×10^4 UFC/mL and the final concentration in the presence of BC-NH_2 was 25×10^2 and 34×10^1 UFC/mL, respectively (Figure 6). In terms of the reduction in bacteria load, both bacterial strains were inhibited by BC-NH_2 mats, with 3.1 and 3.5 log reduction of the initial inoculated *E. coli* and *S. aureus*, respectively.

Thus, the bactericidal activity of BC-NH_2 is due to the introduction of bioactive aminoalkyl groups onto the BC nanofibrills, that provided a polycationic nature to BC mats.[44−46] In addition to the presence of NH_2 groups, the antimicrobial activity of BC-NH_2 can also be due to the length of the corresponding alkyl chain, since it has been demonstrated[1,47] (for N-alkyl chitosan derivatives) that antimicrobial properties increase with increasing alkyl chain length due to the resulting lipophilic properties of the material, which affect the mode of interaction with the cytoplasmic membrane of the bacteria.[48]

In Vitro Cell Response to BC and BC-NH_2 Membranes.

The effect of the introduction of 3-aminopropyltrimethoxysilane moieties onto the BC membrane network, on the viability, proliferation and cell adhesion of human ADSCs was studied. A wide variety of cell lines have been recently used to determine citotoxicity and biocompatibility of novel materials based on bacterial cellulose (BC), including osteoprogenitor cells,[49] chinese hamster ovary cells (CHO-K1),[50] human umbilical vein endotelial cells (HUVECs),[51] SH-SY5Y human neuroblasts, N1 $\times$ 10^{-115} rat neuroblasts, rat pheochromocytoma (PC12), and in particular, adipose derived stem cells (ADSCs).[52] ADSCs have emerged as important tools for cell therapy because they exhibit the capacity to differentiate into multiple mesodermal cell lineages, mainly bone, cartilage, and adipocytes.[53,54] Moreover, ADSCs can be isolated from numerous tissues, because they reside within the connective tissue of most organs, and specifically in adipose tissue.[55,56] In addition, they express all components of the bone morphogenetic protein (BMP)/BMP receptor signaling pathway.[57]

Because of our interest in developing a BC-NH_2-based scaffold for the autologous reconstruction of bone, we decided to select this cell type because of its potential to differentiate easily in osteoprogenitor cells under specific conditions, maintaining at the same time its proliferative activity.

As expected standard growth values were obtained with the negative control (high-density polyethylene, HDPE) and a dramatic reduction of cell number was found with the positive control (polyvinyl chloride, PVC), as shown in Figure 7. The

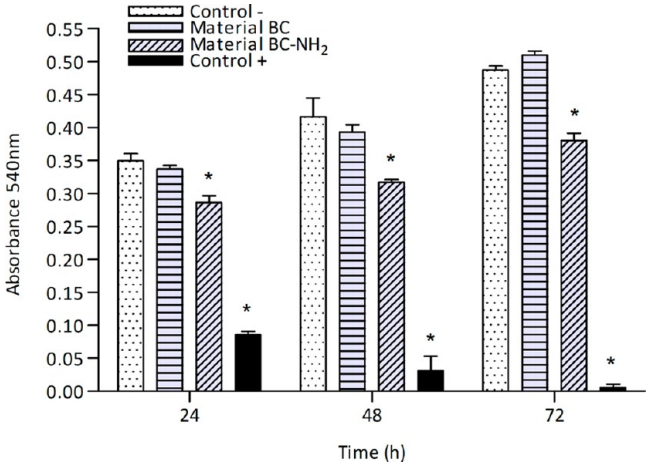

Figure 7. ADSCs proliferation on contact with BC and BC-NH_2 membranes, of positive and negative control during 24, 48, and 72 h. Data are presented as mean ± standard deviation of three independent experiments (*$p < 0.05$).

comparison of proliferation rates between cells cultured in media with BC or BC-NH_2 and in control media indicated that no significant differences were found in growth rate between cells cultured in the presence of BC membranes extracts and that of the negative control, and that BC material is noncytotoxic.[58,59] In the case of cells cultured in the presence of BC-NH_2 membranes extracts, a significantly lower growth rate was seen when compared with the negative control ($p < 0.01$). However, considering that the viability and proliferation rates above 70% of the control were observed here, and that according to EN ISO 10993−5:2009[60] a material is considered cytotoxic if cell viability is reduced by more than 30%, we can state that BC-NH_2 mats are not cytotoxic for ADSCs.

With respect to the ADSC seeding assessment, SEM analysis was conducted in order to determine cell morphology, spreading, and adhesion onto BC and BC-NH_2 membranes. Figure 8 shows the micrographs of ADSCs taken after 72 h of seeding on the BC and BC-NH_2 membranes. These images showed ADSCs in contact with the surfaces of BC and BC-NH_2. For the BC membranes, ADSCs were well-spread, adhered correctly, and proliferated to form a continuous layer of cells fully covering the cellulose membranes. In the case of BC-NH_2 membranes, a lower degree of cell adhesion, spread, and proliferation were observed. These results were in good

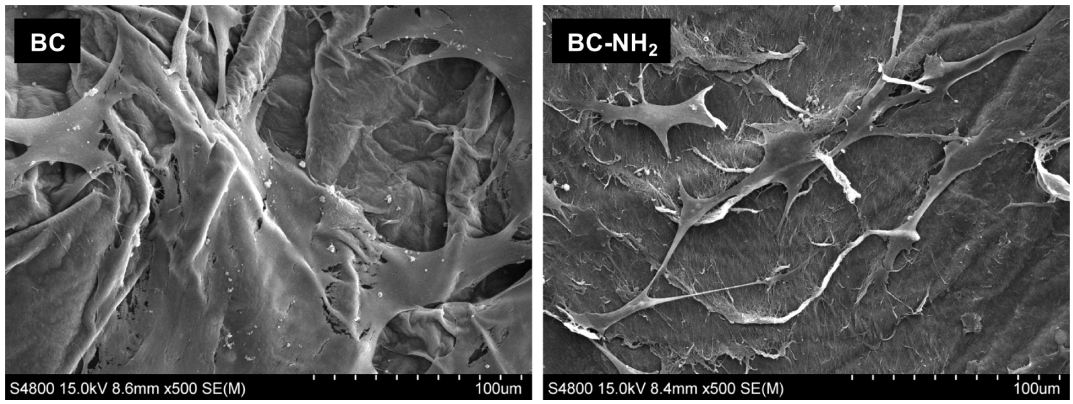

Figure 8. SEM images showing morphology of ADSCs at 72 h after seeding on the surface of: BC and BC-NH_2 membranes.

agreement with the relatively lower growth rate obtained with cells cultured in extracted medium from BC-NH_2.

These in vitro studies showed that, although we observed slight differences in proliferation rate and adhesion of ADSCs before and after chemical modification of BC, there is no cytotoxicity in any case and BC-NH_2 membranes are suitable for biomedical applications. These differences could be somehow associated with the change of the surface nanoscale structure of BC (see high magnification of BC and BC-NH_2 in Figure 4). In fact, as previously demonstrated, the porous network and natural nanofibers structure of BC are ideal for harboring cell growth.[61] In this sense, the formation of a 3D silane structure onto the BC nanofibrills, which reduces the membranes porosity, together with its surface properties, could be one possible reason for the relatively lower cell adhesion and proliferation.

CONCLUSION

In this study, it has been shown that the introduction of aminoalkyl groups onto BC nanofibrills using a silane chemical grafting approach produces a BC membrane with antimicrobial activity while maintaining intrinsic biocompatibility. This strategy will enable the loss of antimicrobial activity associated with the leaching of bioactive components that normally occurs when they are simply blended with the polymeric matrices to be reduced. Moreover, this chemical modification had a low impact on the thermal and mechanical performance of the nanofibrillar structure of BC. These novel BC-NH_2 membranes can therefore be seen as affective antimicrobial candidates in tissue engineering, tissue implants, wound healing therapy, and drug delivery applications.

ASSOCIATED CONTENT

Supporting Information

This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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DEDICATION

This article is dedicated to Prof. Iñaki Mondragon, passed away on 13th February 2012.

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