Superhydrophilic anti-fouling electrospun cellulose acetate membranes coated with chitin nanocrystals for water filtration

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Electrospun cellulose acetate (CA) random mats were prepared and surface coated with chitin nanocrystals (ChNC) to obtain water filtration membranes with tailored surface characteristics. Chitin nanocrystals self-assembled on the surface of CA fibers into homogenous nanostructured networks during drying that stabilized via hydrogen bonding and formed webbed film-structures at the junctions of the electrospun fibers. Coating of CA random mats using 5% chitin nanocrystals increased the strength by 131% and stiffness by 340% accompanied by a decrease in strain. The flux through these membranes was as high as 14217 L m−2 h−1 at 0.5 bar. The chitin nanocrystal surface coating significantly impacted the surface properties of the membranes, producing a superhydrophilic membrane (contact angle 0°) from the original hydrophobic CA mats (contact angle 132°). The coated membranes also showed significant reduction in biofouling and biofilm formation as well as demonstrated improved resistance to fouling with bovine serum albumin and humic acid fouling solutions. The current approach opens up an easy, environmental friendly and efficient route to produce highly hydrophilic membranes with high water flux and low fouling for microfiltration water purification process wash water from food industry for biological contaminants.

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1. Introduction

Electrospinning is a century old process patented by Cooley and Morton in 1902 that is used for producing continuous fibers [1,2]. The first patent on industrial electrospinning appeared in 1934, where Formhals disclosed the equipment for commercial production of artificial threads/filaments of cellulose acetate [3]. Electrospinning is a very versatile technique for producing polymeric fibers in nano- to-micron scale from polymeric solutions and has been of great commercial and research interest. More recently, this technology has been investigated by researchers because of the continuing interest in applications in nanoscience and its potential to generate nanofibers [4–6].

Cellulose acetate based membranes are used extensively in industrial scale and have the advantage of being derived from an abundant natural polymer, viz. cellulose. Though cellulose acetate based membranes produced by phase inversion is a popular membrane material, electrospun cellulose acetate membranes materials have several advantages, specifically, the open and interconnected pore structure and the large specific surface area while having shown potential in air and water filtration [6,7]. However, membrane filtration and especially pressure-driven liquid filtration using electrospun membranes are challenging due to limitations related to mechanical strength and chemical and thermal stability [8]. Electrospun random mats usually have poor mechanical strength due to the highly porous non-woven structure and with weak fiber–fiber connection via physical entanglements [9]. Process modifications to increase fiber–fiber interactions and reinforcing of electrospin fibers using nanoparticles are becoming a highly promising route to address this issue [10–14]. In addition, biofouling is a significant and constant problem with membrane filtration and specifically for hydrophobic cellulose acetate membranes [15,16]. Methods to address biofouling can include mechanical or chemical cleaning operations but another area of focus is the manipulation of the surface chemistry of the membranes to create a surface inhospitable for biofilm formation [17,18].

In the quest for developing new advanced materials that utilize natural polymers, biobased nanoparticles from cellulose and chitin have been explored in the last two decades [19–27]. We have...
successfully reinforced biopolymer fibers electrospun with low (> 10 wt%) and high concentrations (50 wt%) of chitin and cellulose nanocrystals [22,25,26]. However, it was noticed that addition of nanoparticles to spinning solutions significantly affected the spinnability and process yield which are both significant challenges hindering the use of reinforced fibers in high volume applications such as water purification [26].

Biofouling refers to the undesirable accumulation of a biotic deposit on a surface. This deposition may be due to both macroscopic and microscopic organisms. In contrast to abiotic kinds of fouling (scaling, organic and particle fouling), biofouling is a special case because the foulant, can grow at the expense of biodegradable substances from the water phase, turning them into metabolic products and biomass. “Biofilm” is an expression for a wide variety of manifestations of microbial aggregates [28]. Biofilms are understood to be mixtures of bacterial cells embedded in an extracellular polymeric matrix (EPS) made up of polysaccharides, proteins and nucleic acids [29]. Biofilm formation is a development process, which initially involves the adhesion of bacterial cells to a surface and production of EPS resulting in more firmly and irreversible bacterial attachment that cover and protect the cells from adverse conditions [30]. The abiotic fouling on the other hand, is the formation of ‘cake layer’ or ‘gel layer’ consisting of rejected materials and in membrane filtrations, NOMs are a major contributor for abiotic fouling.

In this current study, chitin nanocrystals are impregnated through electrospun cellulose acetate (CA) in a process to change the surface chemistry of the electrospun fibers. Chitin, poly(β-(1→4)-N-acetyl-D-glucosamine, acts as the structural polymer in the exoskeletons of arthropods, in the cell walls of fungi and yeast, and in other microorganisms [31]. Chitin nanocrystals, rod-like particles with typical dimensions of 400 nm in length and 30 nm in diameter, can be extracted through acid hydrolysis from the above mentioned sources [32,33]. These nanocrystals have high surface area, good mechanical properties and also possess antifungal and antibacterial properties. In a recent study, chitin nanocrystals were successfully incorporated in a PVDF membrane prepared through phase immersion to enhance the anti-fouling performance [34]. The current approach was aimed at combining the ease of producing CA electropsun membranes and its efficiency in membrane applications with unique surface characteristics of chitin nanocrystals to create a new generation of high flux, super-hydrophilic, anti-fouling composite membranes for microfiltration water purification for food-processing industries. The fiber morphology, mechanical properties, contact angle, water flux and fouling were evaluated and discussed in this context.

2. Experimental section

2.1. Materials

Cellulose acetate (CA), Mₙ 50,000, was purchased from Sigma-Aldrich Chemistry, USA. Acetic acid (96%, EMSURE®), and acetone, analysis grade, were purchased from Merck KGaA (Germany). All chemicals were used as received without further purification.

Chitin nanocrystals (ChNC) were prepared via hydrochloric acid hydrolysis [27,32,33,35]. Deproteinized and bleached chitin flakes (Sigma-Aldrich, Germany) underwent an acid hydrolysis reaction with 3 N hydrochloric acid at 80 °C for 90 min. When the reaction was complete, the resulting suspension was centrifuged to remove the excess acid and subsequently to collect the turbid supernatant containing the chitin nanocrystals. This collected fraction, the chitin nanocrystal suspension, was dialyzed against distilled water to achieve a suspension neutral pH and finally sonicated to ensure separation of the individual nanocrystals from one another prior to storage. The chitin nanocrystals suspension was briefly sonicated prior to impregnation on the electrospun cellulose acetate membranes. Concentration of the initial chitin nanocrystals suspension was 0.53 wt%.

2.2. Preparation of electrospun cellulose acetate membranes

A schematic representation of the processing route used to prepare the membranes is given in Fig. 1. Cellulose acetate, 5.0 g (Mₙ 50,000), was dissolved in a 45 g 1:1 mixture of concentrated acetic acid and acetone and stirred overnight (12 h) to ensure complete dissolution [22]. Electrospinning of the cellulose acetate

![Fig. 1. Scheme showing the methods and materials involved in the membrane processing and functionalization. (i) Electrospinning of CA mats, (ii) impregnation of CA mats and (iii) drying and heating of the impregnated mats are the process steps. The (a) electrospun cellulose acetate (CA) mat, (b) chitin nanocrystals (ChNC) used for impregnation (photo of the ChNC suspension, the AFM image of nanocrystals and the chemical structure of chitin) and (c) the CA-ChNC membrane mat obtained after impregnation are shown.](image-url)
solution (Fig. 1, step i) was undertaken using the 150 mm Laboratory Electrospinning Platform (Electrospinz-ES1a, New Zealand) and a high voltage power supply, with a supplied voltage of 10 kV, 150 mm tip to collector distance, and a flow rate of 0.1 mL h⁻¹. Electrospinning was performed at room temperature.

The chitin nanocrystals, with a diameter of 20 nm ± 10 nm and length of 300 nm ± 100 nm, were used to impregnate the CA electrospun mats (Fig. 1, step ii), as shown in Fig. 1. Impregnated membranes (CA-ChNC) were prepared via Buchner funnel filtration apparatus with the cellulose acetate membrane on a 90 mm diameter glass frit. The chitin nanocrystal suspension of 0.2 g dry weight (see Fig. 1), was drip fed through the electrospun cellulose acetate fibers. This was to allow for maximum exposure time for the chitin to accumulate on the cellulose acetate fibers. The chitin infused cellulose acetate membranes were air dried for 24 h and then heated to 100 °C for 10 min (Fig. 1, step iii) to ensure binding between the chitin and the cellulose acetate fibers [36,37]. Membranes were weighed on an analytical balance before and after impregnation to determine mass of chitin nanocrystals accumulated on the cellulose acetate mat. 5% of the total mass of the CA-ChNC mat is due to the ChNC.

The viscosity of the cellulose acetate electrospinning solution was measured using the 5V-10 VibrOViscometer (A&D Company, Japan) with a glass sample holder. The solution was sampled every 5 s for 2 min at a vibration frequency of 30 Hz. The electrical conductivity of the cellulose acetate electrospinning solution was determined using a SevenEasy™ conductivity meter (METTLER TOLEDO AG, Switzerland).

2.3. Characterization

2.3.1. Membrane porosity and microstructure

Porosity of the scaffolds was evaluated based on the weight and density of the scaffolds. The porosity was defined as the volume fraction of the voids (νₚ) and was calculated using

\[ v = 1 - \frac{\rho_e}{\rho_t} \quad (1) \]

where ρₑ is the experimental density of the scaffold and ρₜ is the theoretical density of a non-porous scaffold. The densities of ChNC and cellulose acetate were taken as 1.46 and 1.3 g/cc, respectively. The experimental density, ρₑ, was determined based on the weight and volume of the samples cut into strips. All reported results are based on the average of three measurements.

The Brunauer–Emmett–Teller (BET) surface area and pore volume of the CA and ChNC-CA membranes were determined by nitrogen adsorption-desorption isotherm measurements at 77 K.

The surface morphology of the electrospun fibers and the membrane were examined using MAGELLAN 400, SEM (FEI Company) or FEG-SEM (Zeiss, Merlin). The fiber samples were placed on conductive tape and sputter coated with tungsten. Images were taken operating at 3 kV and a working distance of 10 mm for MAGELLAN 400, SEM (FEI Company) whereas a 2.5 kV and 8 mm working distance was used in the case of FEG-SEM (Zeiss, Merlin). Post-filtration imaging to observe the continued presence of chitin nanocrystals on the surface of the electrospun CA mats after 5 L distilled water at 0.5 bar pressure was performed with MAGELLAN 400, SEM (FEI Company). The membranes were sputter coated with gold and observed in the SEM at an acceleration voltage of 3 kV.

The chitin nanocrystals as well as CA and CA-ChNC membranes surfaces were imaged using MultiMode 8 AFM (Bruker, Nanoscope controller, Santa Barbara, California, USA). A drop of diluted suspension of each sample was deposited onto freshly cleaned mica and left to dry at room temperature in the case of chitin nanocrystals. In the case of the electrospun membranes, a small piece of the membrane is mounted on the metal stub using double-sided tape. All the samples were imaged in tapping mode. Height, amplitude and phase images were recorded. The instrument was operated at a resonance frequency of 350 kHz and a spring constant of 10–200 nm⁻¹.

2.3.2. Mechanical properties

The tensile tests were performed on the CA and CA-ChNC mats using a universal testing machine, Shimadzu Autograph AG-X (Shimadzu, Japan), with a load cell 500 N. The thickness of the mats were determined using SEM imaging of the cross-section of cryo-fractured films, sputter coated with Au. Test specimens, conditioned at 45% relative humidity for 1 week, with dimensions of 50 x 5 mm were mounted on paper windows for ease of handling and mounting. A preload of 0.1 N was applied and a strain rate of 2 mm/min and gauge length of 20 mm were used. The stress–strain curves were plotted from the measured load and sample extension (measured by video camera).

The stress is defined as

\[ \sigma = \frac{F}{A_0} \quad (2) \]

and the strain as

\[ \varepsilon = \ln(L/L_0) \quad (3) \]

where \( F \) is the force at break, \( A_0 \) is the area of cross-section of the tensile sample, and \( L_0 \) is the initial sample length and \( L \) is the sample length at break. The elastic modulus is calculated from the initial part of the slope from the stress–strain curve. 4–6 test samples were tested for each material and the average values are reported.

2.3.3. Thermal stability

Thermogravimetric analysis was performed using TGA (Q500 TGA, TA Instruments) with 5 mg sample heated to 800 °C at 10 °C min⁻¹ under \( N_2 \) atmosphere. Onset of thermal degradation is the temperature at which 95% of the mass of the original sample remains.

2.3.4. Water flux, permeability and fouling

Flux tests were performed by filtering distilled water through the membranes using a dead-end cell (HP 4750, Sterlitech, USA) with \( N_2 \) gas to maintain constant pressure at desired pressures. The time for 0.3 L of distilled water to pass through the membranes was recorded and used for the flux calculations. Flux, \( J \), was calculated as

\[ J = \frac{Q_p}{A_m} \quad (4) \]

where \( Q_p \) is the filtrate volume through the membrane per time and \( A_m \) is the area of the membrane. \( A_m (14.6 \text{ cm}^2) \) is a constant value provided by Sterlitech. Membranes were compacted at 0.5 bar for 5 min prior to flux experiments. Permeability was calculated from the linear regression slope from plotting the water flux at 0.4–1.2 bar pressure. Correlation factors for both were 0.99.

Anti-fouling capability of the CA and CA-ChNC membranes was determined by measuring the flux decline over time. Bovine serum albumin, fraction V (Merck Millipore, Germany) 2 g L⁻¹ stock solution and humic acid (Alfa Aesar, Germany) 0.5 g L⁻¹ stock solution
solution were prepared by dissolving the foulant in distilled water and used as prepared. Filtration of the foulant solutions through individual membranes in the dead end cell occurred at 0.13 bar pressure via a peristaltic pump (Model 323S, Watson-Marlow, United Kingdom) for 60 min. Every 15 min, the flux at 0.5 bar was measured using the dead end cell with N2 gas applied to maintain pressure. The flux was plotted against time to determine the effect of the chitin nanocrystals on the surface of the cellulose acetate fibers on fouling and cake formation of the CA and CA-ChNC membranes.

### 3. Results and discussion

#### 3.1. Morphology

Cellulose acetate fibers were successfully electrospun under the given conditions to form random mats. The viscosity and conductivity of the cellulose acetate electrospinning solution were 1144 mPa s and 8.67 mS cm⁻¹, respectively. Fig. 2a shows the electrospun CA fibers where randomly aligned fibrous mats are visible. The electrospun fibers had diameters in the range of 0.5–3.3 μm, with the fiber distribution showing most were between 0.5–1.7 μm (Fig. 2b). The individual fibers’ surface morphology showed ridge-like surfaces (Fig. 2c).

The chitin nanocrystals’ dimensions are 20 ± 10 nm diameter and length of 300 ± 100 nm which agrees with out earlier reports [25,35]. Impregnation of the ChNC on to the surface of the electrospun fibers was undertaken to ensure that the surface functionality of the chitin nanocrystals was utilized and readily accessible. The SEM morphology studies show the hierarchical network formation from the microscale (electrospun fiber networks, Fig. 2d–e) to the nanoscale (ChNC networks, Fig. 2f–g). Fig. 2d shows the overall chitin nanocrystal impregnation network on the surface of the CA fiber random mats, which is extensive even with a relatively low load level of 5% of the mass of the cellulose acetate membrane. The ChNC coatings on the CA fibers (Fig. 2f) were highly homogeneous and were considered to be stabilized via H-bonding that was created during the drying step. Ma et al. have used a similar approach to coat TEMPO cellulose nanowhiskers on to electrospun PAN scaffolds on a PET nonwoven substrate [36]. Fig. 2g also shows the build–up and film formation tendency of the chitin nanocrystals in the junctions (crossover) of the electrospun fibers, which reduces the pore sizes after impregnation. This was shown by the decrease in the porosity of the CA-ChNC membranes when compared to CA membranes, from 88.1% to 85.6%. The average pore diameter decreased from 11.02 nm (CA) to 10.07 nm (CA-ChNC) based on BET measurements. The BET surface area was determined to be 2.73 m²/g for CA membranes and increased to 3.709 m²/g with the addition of the 5% ChNC. This increase may be attributed to the nanotexturing of the CA fibers with ChNC.

To further test the robustness of the chitin nanocrystal layer on the cellulose acetate fibers, 5 L of distilled water at 0.5 bar was passed through the membrane in the dead end cell. Fig. 3a–b SEM images show that the chitin crystals’ webbing between the fibers survives as well as the chitin crystals coating the individual fibers. The AFM images of a second CA-ChNC membrane that had 26 L distilled water pass through the membrane (Fig. 3c–d) show the chitin nanocrystals on the fiber surfaces also are retained.

#### 3.2. Mechanical and thermal properties

The stress–strain curves and the tensile data of the electrospun membranes with and without chitin nanocrystal coating are given in Fig. 4 and Table 1. The results show that the impregnation of electrospun cellulose acetate with ChNC has positively influenced the tensile strength and E-modulus of the mats whereas the strain has decreased (Table 1). The tensile strength increased by 131%,
Fig. 2. Electrospun cellulose acetate fibers were imaged with SEM to show fiber mat formation (a), surface morphology (b, c) and to determine fiber size distribution (d). After filtration impregnation, the cellulose acetate mat structure is retained (e, f) and the chitin nanocrystals were present both on the surface of the cellulose acetate fibers (g, h) and also formed web-like structures at fiber junctions (g).
from 1.43 MPa to 3.31 MPa, while the E-modulus by 340%, from 0.34 GPa to 1.16 GPa, with the infusion of 5% of ChNCs. The stress-stain behavior also changed significantly after impregnation with a low amount of ChNCs.

This remarkable shift in mechanical performance can be attributed to the stiffening effect of the ChNCs coated on individual

Fig. 3. The robustness of the membrane shown by (a) fiber structure and (b-d) chitin crystals on the CA fibers after undergoing 5 L water filtration process. SEM images showing (a) fiber structure and (b) the coated surfaces and ChNC web formations. AFM images showing phase images of the chitin nanocrystal coating on the CA fiber at (c) 5 × 5 μm (d) 15 × 1.5 μm.

Fig. 4. The effect of ChNC coating on the mechanical properties and the thermal stability of CA electrospun fibers (a) stress–strain curves and (b) TGA curves are shown.
The effect of chitin nanocrystals on mechanical properties, thermal stability, and water flux of the membranes.

<table>
<thead>
<tr>
<th></th>
<th>Tensile strength, MPa (± SD)</th>
<th>Strain, % (± SD)</th>
<th>Young’s modulus, GPa (± SD)</th>
<th>T onset °C</th>
<th>Flux, l m⁻² h⁻¹ (± SD)</th>
<th>Permeability</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td>1.43 (0.21)</td>
<td>6.23 (1.21)</td>
<td>0.34 (0.02)</td>
<td>304</td>
<td>13400 (700)</td>
<td>13300</td>
</tr>
<tr>
<td>CA-ChNC</td>
<td>3.31 (0.45)</td>
<td>3.42 (0.49)</td>
<td>1.16 (0.05)</td>
<td>293</td>
<td>14000 (300)</td>
<td>14100</td>
</tr>
</tbody>
</table>

The water flux measurements (Table 1) show that the water flux at 0.5 bar and the permeability was not changed by the addition of the chitin nanocrystals. This high flux post-impregnation could be attributed to the hydrophilic nature of the CA-ChNC membrane while the surface coating of the cellulose acetate fibers where the chitin nanocrystals align along the cellulose acetate fibers first and then form webs between the fibers can influence flux and permeability. The high flux for the CA and CA-ChNC membranes is one of the attributes of these membranes that show promise in microfiltration applications, such as ready-to-eat vegetable process water. In comparison, Ma et al. have reported a flux of 5900 L m⁻² h⁻¹ bar⁻¹ for PAN nanofiber mats impregnated with cellulose nanocrystals with a support layer [37] while our CA-ChNC membranes have a flux of 27,900 L m⁻² h⁻¹ bar⁻¹. This could be a result of our membranes having larger pore sizes, not requiring a support layer and the hydrophilicity of the cellulose acetate membrane in comparison to the PAN nanofibers.

3.3. Water flux and permeability

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3.4. Fouling behavior

The flux variations of the membranes were evaluated to confirm the anti-fouling potential of the CA-ChNC membranes and to determine what effect the chitin nanocrystals would have on the abiotic fouling of the membranes. The change in flux was determined over 60 min of dead end cell filtration. As can be seen in Fig. 5, the flux of the CA membranes steadily decreased over time as either 2 g/L bovine serum albumin or 0.5 g/L humic acid solutions were continually passed over the membrane. In comparison, the flux of the CA-ChNC membranes remained constant over the 60 min test period. While both membranes showed a decrease from the pure water flux upon initial contact with the membranes, after that initial contact the flux remained high and steady for the chitin nanocrystals coated membranes. These results indicate a promising potential for this type of membrane for future applications and further evaluation.

As biofouling is always a consideration in membrane applications, the use of chitin nanocrystals as inhibitors of such biofouling on the surface structure of these cellulose acetate fiber mats was considered. Biofilm formation was utilized to evaluate biofouling on chitin nanocrystals impregnated electrospun cellulose acetate. Figs. 6 and 7 shows, respectively, SEM micrographs and Ruby FilmTracer confocal images of mats kept in contact for 18 and 24 h with cultures of E. coli CECT 516. In all cases the cellulose acetate membrane infused with chitin nanocrystals demonstrated significant resistance to be colonized by E. coli (Figs. 6b and 7b and f) in comparison to the electrospun cellulose acetate membranes (Figs. 6a and 7a and e), with a 48% decrease in biofilm formation after 18 h and with a 87.7% decrease after 24 h of incubation, according to the results obtained with FDA (Table 2).

The results of FDA staining showed a much higher enzymatic activity of E. coli on raw CA membranes than on ChNC specimens. The higher number of PI-marked, non-viable cells on ChNC membranes is also apparent when comparing Fig. 7c–h. After 24 in contact with membranes, the viability of bacterial cell became notably reduced with most cells damaged. The differential staining with Ruby FilmTracer revealed the protein network of extracellular substances providing the mechanical stability of biofilms. Furthermore, with increasing incubation time, we observed an increase of extracellular matrix formation which would indicate a biofilm proliferation on CA membranes, while on CA-ChNC this formation was considerably lower, revealing a reduced biofouling (Fig. 7). The results of DAPI/PI double staining is shown in Fig. 8. In this system, all cells exhibit blue (DAPI) fluorescence due to nucleus staining, whereas nonviable bacterial cells display red fluorescence (Propidium iodide, PI) with dye uptake depending on cell membrane integrity and physiological state of the bacterial cells. Again, the antibacterial activity of CA-ChNC is apparent in comparison with non-coated CA.
A possible explanation of this behavior is the antimicrobial activity of chitin. Chitin and derivatives as chitosan have been investigated as an antimicrobial material against a wide range of target organisms like algae, bacteria, yeasts and fungi. Several models have been proposed, the most acceptable being the interaction between positively charged chitin/chitosan molecules.
and negatively charged microbial cell membranes. In this model the interaction was mediated by the electrostatic forces between protonated $-\text{NH}_3^+$ groups and the negative residues, presumably by competing with $\text{Ca}^{2+}$ for electronegative sites on the membrane surface. This electrostatic interaction results in twofold interference: i) by promoting changes in the properties of membrane wall permeability, inducing internal osmotic imbalances and the inhibition of microbial growth and ii) by the hydrolysis of the peptidoglycans in the microorganism wall, leading to the leakage of intracellular electrolytes such as potassium ions and other low molecular weight proteinaceous constituents [41].

The formation of a biofilm includes several steps but a prerequisite is the adhesion of microbial cells to a solid surface. Studies of bacterial adhesive properties have indicated that a number of cell surface physico-chemical factors contribute to the process of adhesion. Such factors include cell surface hydrophobicity, the presence of extracellular polymers and cell surface charge. The latter determines the electrostatic interaction between the cell and the substratum [42].

The value of the contact angle gives the basic information on the hydrophobicity of surfaces. For CA and CA-ChNC membranes water contact angles are given in Table 2. The cellulose acetate membrane had a hydrophobic contact angle of 136.8° while the CA-ChNC membranes demonstrated extreme hydrophilicity with a measured contact angle of 0°, since all the water drop was absorbed by the membrane. The contact angles are dependent upon the chemical composition, porosity, and surface roughness and hydrophilicity increases with the presence of N, O, I, Cl, H, and F. The chemical structure of the chitin nanocrystals on the surface of cellulose acetate (Fig. 1) is contributing to the dramatic reduction in the contact angle [26,34,31]. As a general rule, hydrophobic bacteria adhere on hydrophobic surfaces, whereas hydrophilic microorganisms attach to hydrophilic surfaces. The interaction between two hydrophilic entities (E. coli cells and CA membranes in our case) is favored because they can enter into closer contact through the facilitated “squeezing of water” in between, but the bio-surface interactions are somewhat more complex due to cell appendages, such as pili and flagella that makes direct contact between surfaces quite difficult [43].

The $\zeta$-potential of the membranes is shown in Table 2. All membranes were negatively charged. CA membranes reached a surface potential of $-30.2 \text{ mV}$ whereas the CA-ChNC membranes displayed a $\zeta$-potential of $-4.7 \text{ mV}$ at pH 7.5. As with contact angle, the chemical structure of the chitin nanocrystals on the surface of cellulose acetate (Fig. 1) is changing the surface properties of the CA membrane, making it less negative. Electrostatic repulsion could be expected to play a role in bacterial adhesion, given the negative surface charge of bacterial outer membranes (the $\zeta$-potential of E. coli is aprox. $-30 \text{ mV}$) [44]. The data show, however, that the more negatively charged surfaces were more prone to suffer bacterial colonization as revealed by FDA (Table 2), FilmTracer and Live/Dead (Fig. 6) staining and by SEM imaging (Fig. 5). It has also been shown that some bacteria could interact with negatively charged particles if they bind to cationic sites on the cell surface to form clusters favored by the repulsive interactions with negatively charged domains [45,46].

Summarizing, besides the antimicrobial activity of chitin, the hydrophilic CA-ChNC membranes were much more resistant to

![Fig. 8. DAPI/PI double staining of Escherichia coli CECT 516 on mats of CA (a) and CA-ChNC (b) after 18 h of cultures in contact with mats. Bacterial nucleus were visualized in blue by DAPI. Dead cells were stained in red by PI.](image-url)
bacterial colonization than unmodified CA mats. The possibility to convert highly hydrophobic membrane surfaces into super-hydrophilic surface via surface functionalization with ChNC is also expected to open up new possibilities in membrane technology. The membrane selectivity/rejection based on size exclusion and/or adsorption is of relevance in this context and will be reported in detail in future.

4. Conclusions

Chitin nanocrystals were successfully infused on to the electrospun cellulose acetate fiber networks resulting in a novel and highly efficient surface treatment approach for low-fouling membrane processing. The hierarchical morphology is shown by the membranes where micron scaled electrospun fiber network is surface coated with ChNC networks in nanoscale with pore sizes in the range on 10 nm. The ChNC coating on individual CA fibers that are ‘tied’ together at junction points by chitin nanocrystals webs increased the mechanical strength and modulus of the membranes. Addition of the chitin nanocrystals on the CA membrane surfaces resulted in decreased biofilm formation and abiotic fouling tendency accompanied with a transition from highly hydrophobic to super-hydrophilic surfaces. This is attributable to surface chemistry chitin nanocrystals and surface interactions of cellulose acetate membrane and E. coli cells. Chitin nanocrystals on cellulose acetate mats thus resulted in high flux membrane which shows potential in future water purification of process wash water from food industry containing biological and organic contaminants.

Author contributions

The experimental work was carried out by L. Goetz and B. Jalvo. AP Mathew performed the mechanical testing and R Rosal G significantly contributed to the biofilm characterization. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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