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Nanopores Structure in Electrospun Bacterial Cellulose

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ABSTRACT

Bacterial cellulose (BC) has established to be a remarkably versatile biomaterial and can be used in wide variety of applied scientific endeavours, especially for medical devices, lately, bacterial cellulose mats are used in the treatment of skin conditions such as burns and ulcers, because of the morphology of fibrous biopolymers serving as a support for cell proliferation, its pores allow gas exchange between the organism and the environment. Moreover, the nanostructure and morphological similarities with collagen make BC attractive for cell immobilization and cell support. **In this work, we obtain first electrospun bacterial cellulose mats after chemical treatment and without conductive additives.** With DMA/LiClmechanism dissolution, modified bacterial cellulose was easily electrospun in chloroform/acetone solvents in comparison with BC unmodified. FTIR peaks results are consistent with proposed interactions between cellulose and DMA/LiCl solvent system.

Keywords: Bacterial Cellulose; Electrospinning; Drug and Cell Delivery

1. Introduction

Cellulose is a semicrystalline polymer and its crystallinity depends on the originand on the isolation and processing methods. The complex structural hierarchy of cellulose, due to profuse hydrogen bonding, is manifested by the existence ofseveral polymorphs (crystalline forms). Native cellulose has a polymorph structure of cellulose I that exists in two crystalline forms: Iα (in algae and bacteria) and Iβ (in higher plants) [1,2]. Although chemically identical to plant cellulose, the cellulose synthesized by bacterial has a fibrillar nanostructure which determines its physical and mechanical properties, characteristics which are necessary for modern medicine and biomedical research [3,4]. The structural features of microbial cellulose, its properties and compatibility of the biomaterial for regenerative medicine can be changed by modifying its culture medium [5-7] or surface modification by phyical [8] and chemical methods [9,10] to obtain a biomaterial with less rejection with celular contact and blood contact cells interation. Moreover, the nanostructure and morphological similarities with collagen make BC attractive for cell immobilization and cell support. However, to obtain a biomaterial with less rejection and better celular contact and blood contact cells interation in human and animal medicine, homogeneous size porous is necessary. The chemical groups (OH) found in the structure of nanobiocellulose can serve as support for incorporation of drugs [11,12].

Cellulose chains have a strong tendency to aggregate to form highly ordered structures. The highly regular constitution of the cellulose molecule, the stiffness of the molecular chain and the extensive hydrogen bonding capacity favors molecular alignment and aggregation. In order to dissolve cellulose, one has to find a suitable solvent to break down the prevailing hydrogen bond network, *i.e.*, the initial supramolecular structure of cellulose should be destroyed in order to obtain a homogeneous (one-phase) solution. The two-component DMA/LiCl solvent system is perhaps the most used for homogeneous cellulose modification [13]. The dissolution mechanism with DMA/LiCl takes advantage of the strong intermolecular interaction between the cellulose and the strong dipole such as in the case of DMA carbonyl group. Homogeneous functionalization of DMAc/LiCl provides a fairly inert and thermally stable solvent system for cellulose, which is still an important tool for a homogeneous conversion of the polysaccharide. These media which exemplarely small be treated here in some detail, dissolve even high molecular cellulose (DP > 1000) completely after preactivation at a LiCl concentration of 7% - 9% by weight. Advantages claimed for DMA/LiCl solutions in homogeneous reactions of cellulose are a full availability of hydroxy groups, permitting a minimal chain degradation at a temperature below 100˚C, a high

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versatility with regard to the type of reaction intended and a favorable reagent yield due to rather small consumption for side reactions [14,15].

In this work, bacterial cellulose mats was acetylated and after processing by electrospinning to produce artificial symmetric nanoporous that can be applied in catalysis, drug and cell delivery. An electrospinning system comprises a polymer solution, contained in a syringe with a connected. The polymer solution is usually provided a charge using a high voltage power source. In the process, a high voltage electric field is applied to the tip of the needle connected to the syringe containing the polymer solution. The charged polymer jet is directed to the grounded collector. As the jet travels in air, the solvent evaporates, resulting in formation of polymer fibers, which are collected as a nonwoven fiber mesh on the grounded collector [16,17]. The parameters that affect electrospinning can be classified into three categories: 1) solution parameters such as viscosity, conductivity/polarity, and surface tension; 2) process parameters such as applied electric voltage, tip-to-collector distance, diameter of the needle tip, feed rate, and the hydrostatic pressure applied to the polymer solution; and 3) ambient parameters such as temperature, air velocity, and humidity of the electrospinning chamber [18].

One of the advantages of the e-spinning process over the conventional film-casting technique is the highly porous nature of the electrospun (e-spun) fiber mats which exhibit much greater surface area that assumingly could allow drug molecules to diffuse out from the matrix much more conveniently [19], when these fibrous materials are used as carriers for delivery of drugs. E-spun CA fiber mats have been explored as affinity membranes [20] antimicrobial membranes [21], three-dimensional (3D) structures resembling the urinary bladder matrix (UBM) [22], and drug-delivery membranes [23].

2. Materials and Methods

2.1. Materials

The bacterial cellulose used as raw material here was Nanoskin® provided from Innovatec´s (São Carlos SP, Brazil). Lithium chloride (BioXtra, ≥99.0%-Sigma Aldrich); N,N dimethylacetamide (DMAc, puriss. p.a., ≥99.5%-Sigma Aldrich); acetic anhydride (≥98.0%-Synth-BR); Methanol (anhydrous, >99%-Synth-BR); Chloroform (anhydrous, ≥98%-Synth-BR) were used as received.

2.2. Methods

2.2.1. Cellulose Dissolution and Acetylation

The dissolution and acetylation steps were based on Ass *et al*. with some modifications [24]. A mixture of bacterial cellulose and DMAc (0.05l) was heated to 120˚C and stirred for 2 hours, in a reflux condenser. After that, lithium chloride solution (6 mmol·L^{-1}) was ad- ded and the mixture was heated to 150˚C with stirring for 2 hours. Then, acetic anhydride solution (1 mmol·L^{-1}) was added dropwise. The system was kept at 100˚C for 1 hour and then cooled to room temperature. The product was precipitated with methanol and purified by Soxhlet extraction in methanol. After that, it was dried at 50˚C in a vacuum oven.

2.2.2. Solutions to Be Processed

Solution was prepared at a concentration of 8% w/w of acetylated cellulose in chloroform. The solution was stirred on magnetic stirrer at room temperature for 3 hours. After this period the solution was processed by electrospinning using voltage of the 20 Kw and 12 cm distance from the needle until collector. Nanofibers mats were collected in grounded metal collector.

2.3. Characterization

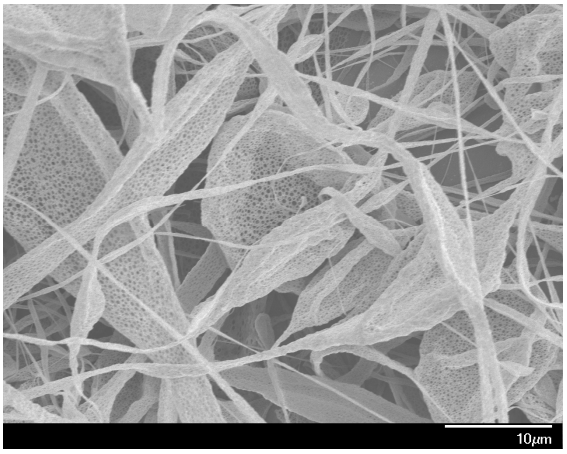
Scanning Electron Microscopy (SEM)—Scanning electronic microscopy images were performed on a PHILIPS XL30 FEG. The samples were covered with gold and silver paint for electrical contact and to perform the necessary images.

Transmission infrared spectroscopy (FTIR, Perkin Elmer Spectrum 1000)—Influences of DMA/LiCl in bacterial cellulose was analyzed in the range between 250 and 4000 cm^{-1} and with resolution of 2 cm^{-1} with samples.

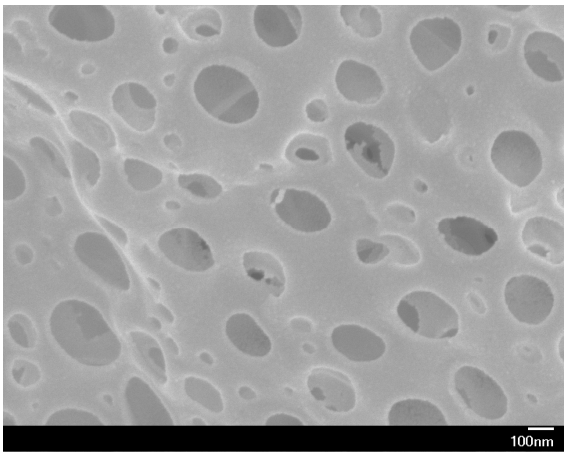
3. Results and Discussion

3.1. SEM

Bacterial cellulose mats were characterized by SEM. **Figures 1(a)** and **(b)** shows, as an example, SEM images of electrospun cellulose membrane samples are shown in **Figure 1**. Pores uniformly distributed throughout the fibers can be observed with a low size dispersion and average size around 20 - 100 nm. As to electrospinning, previous studies have demonstrated that the deposition rate of fibers during electrospinning is in the order of several meters per second, the solution jet was elongated up in less than a second, and the elongation rate can reach up high velocity, which leads to a dramatic increase of the surface-area-to-volume ratio with in milliseconds. The orientation of pores along the longitudinal direction of the electrospun fibers is attributed to the rapid stretching effect during electrospinning [25,26]. In **Figures 1** and **2**, it can be observed different sizes porous formation with electrospinning and casting polymers mats.

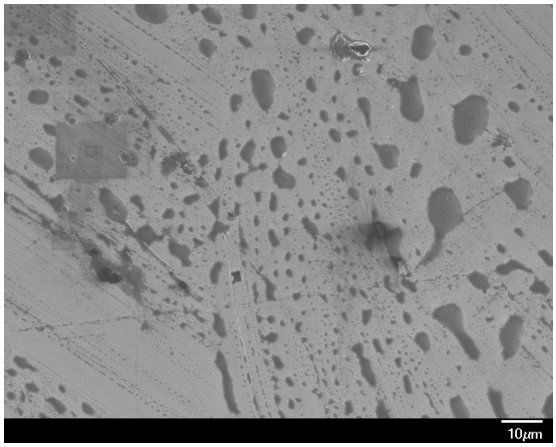


(a)

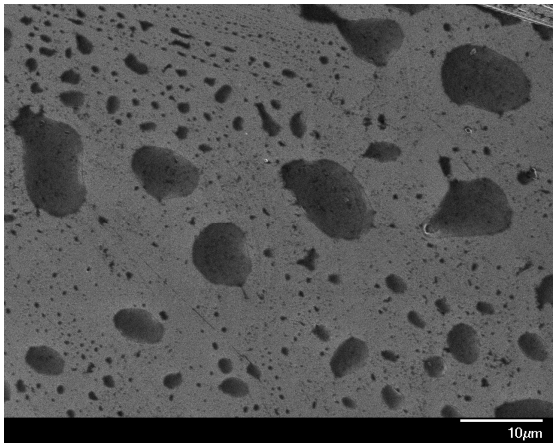


(b)

Figure 1. Micrographs of electrospun mats from BC-acetyl/ chloroform solution with ampliation: (a) 5000×; (b) 8500×.



(a)



(b)

Figure 2. Micrographs of casting mats from a BC-acetyl/ chloroform solution with ampliation: (a) 5000×; (b) 8500×.

Wendorff *et al.* [27] found that when spinning parameters and highly volatile solvents were chosen appropriately, porous electrospun fibers are obtained. So, with these changes is bacterial cellulose mats, modified bacterial cellulose were easily electrospun in chloroform/acetone solvents in comparison with BC unmodified, resulting in symmetric nanopore structure for drug and cell delivery.

3.2. FTIR

Influences of acetylation in bacterial cellulose electrospun mats was analyzed in the range between 250 and 4000 cm^{-1} and with resolution of 2 cm^{-1} with samples. The main features of the bacterial cellulose in infrared spectroscopy is: 3500 cm^{-1}: OH stretching, 2900 cm^{-1}: CH stretching of alkane and asymmetric CH_2 stretching, 2700 cm^{-1}: CH_2 symmetric stretching, 1640 cm^{-1}: OH deformation, 1400 cm^{-1}: CH_2 deformation, 1370 cm^{-1}: CH_3 deformation, 1340 cm^{-1}: OH deformation and 1320 - 1030 cm^{-1}: CO deformation [28]

The mechanism of cellulose dissolution in DMA/LiCl family of solvents is accompanied by the strong intermolecular interaction between cellulose and a strong N-O dipole. The dissolution mechanism in DMA/LiCl family of solvents takes advantage of the strong intermolecular interaction between the cellulose and the strong dipole such as in the case of DMA carbonyl group [29]. It can be observed that acetylation change mainly peaks at 3500, 2900, 1750 cm^{-1} and region between 560 - 740 cm^{-1}. FTIR spectra of the original bacterial membrane indicate a broad hydroxyl peak centering around 3500 - 3600 $cm^{-1}$and CH stretching of alkane at 2930 cm^{-1}. It can be observed in **Figures 3(a)** and **(b)** that these groups of pure bacterial cellulose were changes by chemical treatment resulting in changes in FTIR peaks relative to the carbonyl peak at 1740 cm^{-1} (**Figure 3(b)**) and cloroalkanes peaks at 540 - 760 cm^{-1} (**Figure 3(c)**). It can also be concluded that because of evaporative cooling during electrospinning, the atmospheric water vapor condensed onto the fiber surface formed droplets leading to a porous surface fiber and this evaporation changes peaks intensity

of peaks in electrospinning/casting films (**Figures 3(a)-(c)**).

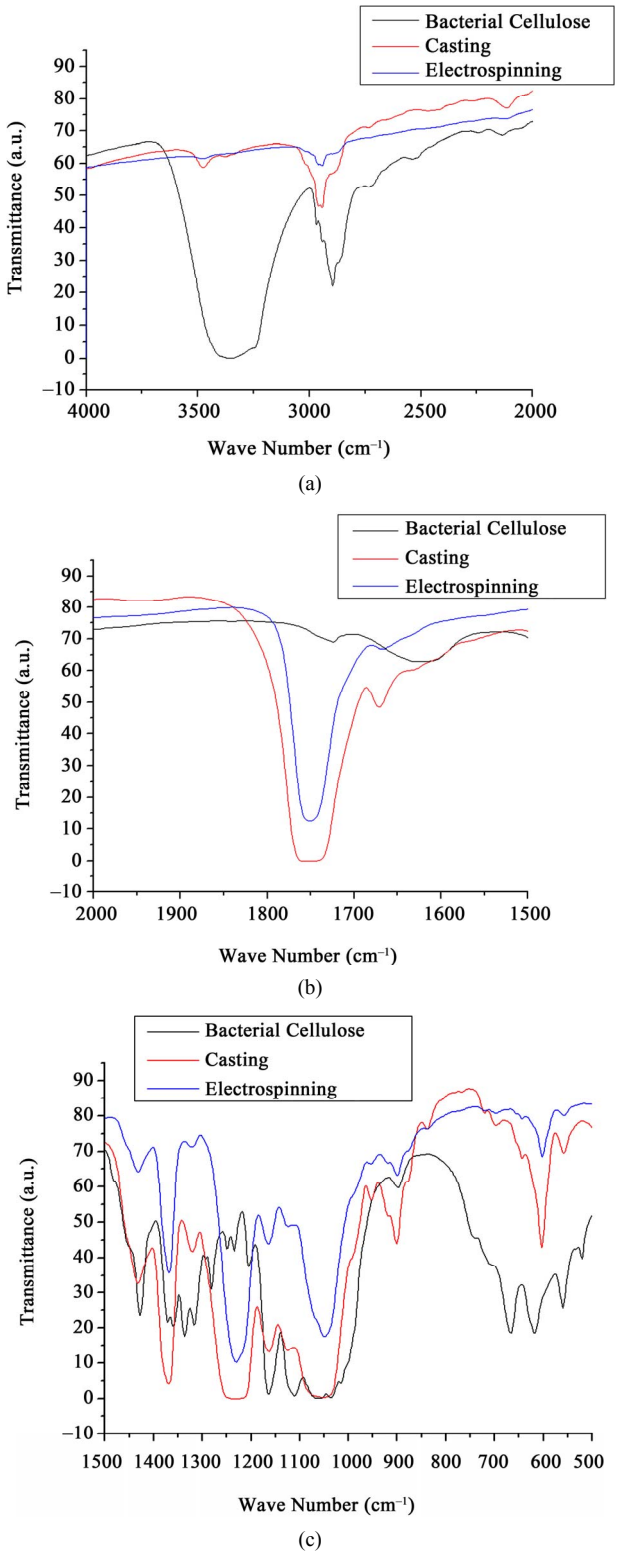


Figure 3. FTIR spectra of the samples BC, Chemical modified BC casting films and chemical modified BC electrospun mats.

4. Conclusions

It was reported a first bacterial cellulose electrospun by chemical modified groups. Electrospun bacterial cellulose mats presents more symmetric nanopore structure in bacterial cellulose than casting films mats observed by SEM images mainly because the orientation of pores along the longitudinal direction of the electrospun fibers is attributed to the rapid stretching effect during electrospinning. With DMA/LiCl mechanism dissolution, modified bacterial cellulose were easily electrospun in chloroform/acetone solvents in comparison with BC unmodified because spinning parameters and highly volatile solvents were chosen appropriately after BC modification. FTIR peaks results are consistent with proposed interactions between cellulose and DMA/LiCl solvent system.

These results confirm that the BC is ideal scaffold requires with porous structure which can provide maximum integration with cells and body fluids, plus have a nanostructure surface which facilitates the adhesion of cells.

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