Nanoskin Bacterial cellulose structured- Towards the Development of regenerative medicine

Olyveira, G.M*, Costa, L.M.M*, Basmaji, Pierre**

*Centro de Ciências Naturais e Humanas CCNH-UFABC, Rua Santa Adélia, 166. Santo André-SP, Brazil, gmolyveira@yahoo.com.br
*Innovatec's - Biotechnology Research and Development-Brazil, pierre@innovatecsco.com

ABSTRACT

Bacterial cellulose (BC) has established to be a remarkably versatile biomaterial and can be used in wide variety of applied scientific endeavors, especially for medical devices. In fact, biomedical devices recently have gained a significant amount of attention because of increased interesting tissue-engineered products for both wound care and the regeneration of damaged or diseased organs. The architecture of BC materials can be engineered over length scales ranging from nano to macro by controlling the biofabrication process. It has unique properties that make it an exciting candidate as a vascular graft material: strength, good integration into host tissue, and flexibility of production in various shapes and sizes. In this article, the structural features of microbial cellulose and its properties are discussed with AFM and SEM and novel bionanocomposites from BC/collagen and BC/chitosan are presented for artificial blood vessels by modifying culture medium of bacterial cellulose.

Keywords Nanoskin®, bacterial cellulose, artificial blood vessels

1 INTRODUCTION

Nanocellulose, such as that produced by the bacteria Gluconacetobacter xylinus (bacterial cellulose, BC), is an emerging biomaterial with great potential in several applications. The performance of bacterial cellulose stems from its high purity, ultra-fine network structure and high mechanical properties in dry state[1] . These features allow its applications in scaffold for tissue regeneration, medical applications and nanocomposites. A few studies have used bacterial cellulose mats to reinforce polymeric matrices and scaffolds with wound healing properties. These advances are reviewed and prospects with future development in these areas are proposed.

BC is purity cellulose made by bacterial fabrication via biochemical steps and self-assembling of the secreted cellulose fibrils in the medium. Shaping of BC materials in the culture medium can be controlled by the type of cultivation that changes chain sizes, origin of strains that produced different proportion of crystalline phase of BC and kind of bioreactor. Then it obtained BC hydrogel or BC in dry state by methods like freeze-drying.[1]

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[2,3].

The structural features of microbial cellulose, its properties and compatibility of the biomaterial for regenerative medicine can be changed modifying its culture medium[4,5] or surface modification by physical[6,7] and chemical methods[8,9] to obtain a biomaterial with less rejection with celular contact and blood contact cells interation. For example morphological changes in bacterial cellulose were obtained with differents culture medium, Bodin et al. obtained BC tubes with different shapes and sizes depending on the product requirements, which has made BC interesting to be explored for use in other biomedical applications such as bone graft material and a scaffold for tissue engineering of cartilage and blood vessels [10].

Beyond controlling the size and shape of the BC, the microscopic morphology can also be changed in several ways. A high oxygen ratio during static cultivation has been shown by both Watanabe and Yamanaka[11] and Hult et al. [12] to increase the density and subsequently the toughness of the BC membrane.

Shaking velocities and the addition of dyes has further been shown to have a profound effect on the aggregating of the sub-elementary microfibrils, thereby reducing the crystallinity of the BC network[13,14]. Different structures of BC obviously affect its mechanical properties as well as cell attachment into the material.

Surface modification of biomaterials is becoming an relevant method to improve the multifunctionality of biomedical devices, as well its biocompatibility with low costs and long time required to develop new materials. The adhesion of human endothelial cells to polytetrafluoroethylene surfaces, used in vascular prostheses, was improved when the material was treated with nitrogen and oxygen plasma[15]. The effect of oxygen plasma on the surface modification of different starch based biomaterials (SBB) and on modulating bone–cells behaviour was described by Alves and colleagues[16]. The

authors observed that the adhesion and proliferation of osteoblast-like cells were enhanced by the plasma treatment on ethylene vinyl alcohol and polycaprolactone materials[17].

To be used in biomedical applications, improved cellulose integration with host tissue is required. Chemical surface modifications and incorporation of bioactive molecules are examples of what can be done to make BC an ideal material for reparative tissue engineering. Andrade et al.[18,19] analyzed Chimeric proteins containing a cellulose-binding module (CBM) and an adhesion peptide (RGD) to improve the adhesion of human microvascular endothelial cells (HMEC) to bacterial cellulose (BC). The results obtained demonstrated that recombinant proteins containing adhesion sequences were able to significantly increase the attachment of HMEC to BC surfaces. The results also showed that RGD decreased the in-growth of HMEC cells through the BC and stimulated the formation of cord-like structures by these endothelial cells. Thus, the use of recombinant proteins containing a CBM domain, allows control of the interaction of this material with cells.

It is the intention of this work to broaden knowledge in this subject area and stimulate the practical application of nanocelluloses in medicine with new materials obtained with its surface modification of bacterial cellulose, etiher by chemical and physical modification or its culture medium.

2 EXPERIMENTAL DETAILS

2.1 Materials

Bacterial cellulose membranes, ~500 mm thick, were supplied from Innovatecs-Produtos Biotecnológicos Ltda, Brazil; Chitosan medium molecular weight and Collagen from calf skin were supplied by Sigma AldrichFonts and Spacing.

2.2 Synthesis of bacterial cellulose

Nanoskin® bacterial cellulose (BC) produced by Gram-negative acetic acid bacteria Gluconacetobacter xylinus can be obtained from the culture medium in the pure 3-D structure consisting of an ultra fine network of cellulose nanofibres (3–8 nm), highly hydrated (99% in weight), and displaying higher molecular weight, higher cellulose crystallinity (60-90%), enormous mechanical strength and full biocompatibility.

2.3 Bionanocomposite preparation

In the present study, we have explored a novel biomaterial, and prepare different bacterial cellulose nanocomposites(BC) ; a) Pure BC, b) BC with collagen and c) BC/chitosan. Bacterial cellulose nanocomposite was obtained by immersion collagen/chitosan into bacterial cellulose medium and posterior soft drying at 50°C by 12 hours.

2.4 Bionanocomposite 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.

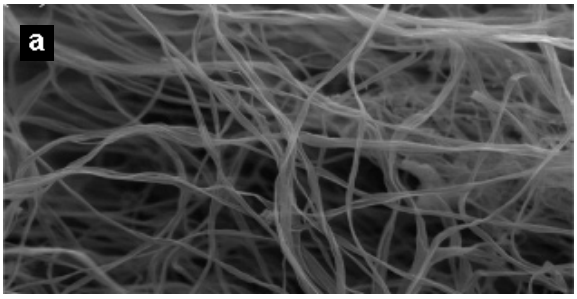
The AFM observation was conducted using Scanning Probe Microscope AJ-III system with a triangular microfabricated cantilever (Mikro Masch Co., Russia).

Transmission infrared spectroscopy (FTIR, Perkin Elmer Spectrum 1000)- Influences of collagen and nanotholits 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 Scanning Electronic Microscopy (SEM) and Atomic Force Microscopy (AFM)

Nanoskin® mats were characterized by SEM and TEM. Fig. 1(a,b) shows respectively, as an example, SEM and AFM image of Nanoskin® surface morphology formation.



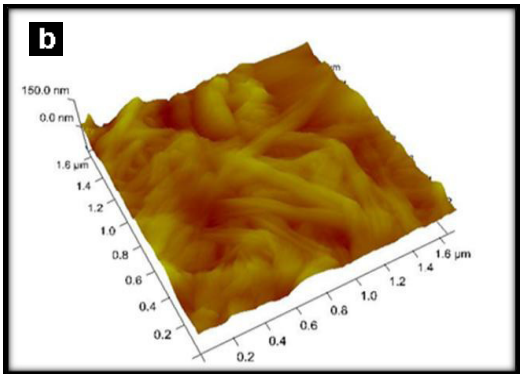


Fig.1. a) Nanoskin® Scanning electron microscopy (SEM); b) Nanoskin® Atomic force microscopy (AFM) images .

Microbial cellulose possesses high crystallinity, high tensile strength, and extreme insolubility in most of the solvents, moldability and high degree of polymerization. The thickness of cellulose fibrils is generally 0.1–10 mm, one hundred times thinner than that of cellulose fibrils obtained from plants with good shape retention [20]

The biopolymer degrades at higher temperatures (>300 °C), although the alkali-treated cellulose membraneis more stable (between 343 and 370 °C). Composites prepared by adding bacterial cellulose increase mechanical properties like bending strength and Young modulus [21,22]. The mechanical properties of cellulose are due to the uniqueness of uniform nanoscale network structure, which is oriented bi-dimensionally when compressed.

In figure 2 and 3 it can be observed differents morphological surface of BC/chitosan and BC/collagen obtained from change medium culture of cellulose bacterial. With FTIR analysis in figure 4, it can be observed that chitosan biocomposites presents more changes in bacterial cellulose structure.

The main features of the bacterial cellulose are: 3500 cm^{-1}: OH stretching, 2900 cm^{-1}: CH stretching of alkane and asymmetric CH2 stretching, 2700 cm^{-1}: CH2 symmetric stretching, 1640 cm^{-1}: OH deformation, 1400 cm^{-1} : CH2 deformation, 1370 cm^{-1}: CH3 deformation, 1340 cm^{-1}: OH deformation and 1320-1030 cm^{-1}: CO deformation.

With chitosan biocomposite, it obtained more OH stretching (at 2900 cm^{-1}) mainly because of NH_2 interaction with hydroxyl groups of bacterial cellulose and with this interaction it was obtained porous structure in chitosan/bc biocomposite which is better for artificial blood vessel application. Strength and flexibility analysis of both biocomposites will be done in future work.

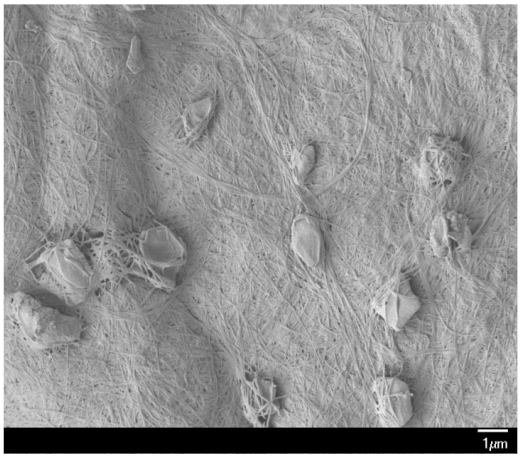


Fig. 2.-Bacterial Cellulose /chitosan surface morphology

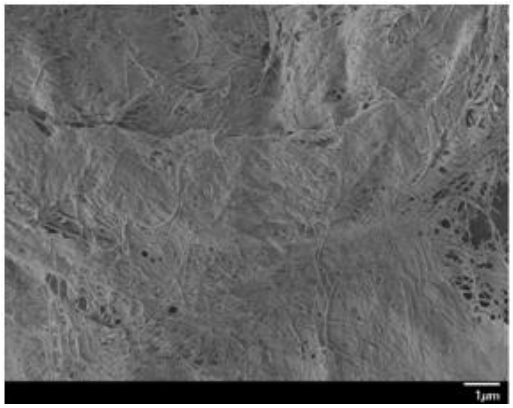


Fig. 3.-Bacterial Cellulose /collagen surface morphology

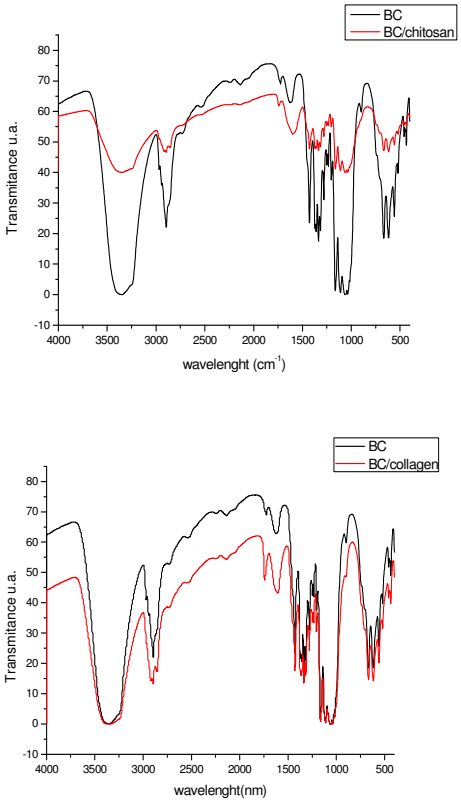


Fig. 4.-FTIR spectra of Bacterial Cellulose /chitosan and collagen biocomposites.

4 CONCLUSION

Nanoskin® has been used in different areas of medicine as: substitute of blood vessels and linfatics, on lesions of

tegument (serious burns, skin graft in the donor and receiving areas), facial peeling, infectious dermolysis, abrasion of tattoos chronic ulcers, Hanseníase of the distal members.

Experimental studies for clinical application have been accomplished with Nanoskin® membrane in several conditions as: protective cover for reconstruction of nerves; duraplasty; healing of epithelial lesions of cornea; healing of duodenal lesions; substitute of blood vessels; cuffs for of reconstruction of micronerves; reconstruction of the retroperitoneum; and technical training in microsurgery.

In this work, it was report two initial reports in biocomposites with bacterial cellulose with promising characteristics to artificial blood vessel applications, chitosan has better interaction with bacterial cellulose . Strenght and flexilbility analysis and incorporation of biomolecules in the system will be done in future work

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