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Template assisted synthesis of porous nanofibrous cellulose membranes for tissue engineering

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Abstract

Porous, nanofibrous bacterial cellulose (BC) membranes were produced by the bacterium *Acetobacter xylinum*. The bacterium was cultivated in an appropriate culture medium under static conditions. *In situ* pore formation was attained through the use of pin templates with diameters varying from 60 to 300 μm composed of polyestirene (ϕ=300 μm) or optical fibers (ϕ=60 μm), which were placed on culture medium with the pins immersed in the liquid. Cellulose biosynthesis occurred around the pins leaving tiny pores on the cellulose membrane. After removal of the template the biofilm was dried at 50 °C/24 h. Physico-chemical properties of BC membranes, like degree of crystallinity, swelling and tensile strength were not significantly altered after pore formation. Microstructure evaluation revealed that the film matrix is composed of long nanofibers isotropically distributed on its surface. Round-shaped pores with diameters varying between 60 and 300 μm, depending on the pin template used, were formed in the cellulose membranes. These pores exhibited no border failures that could start crack propagation along the film surface. Microporous membranes could be useful for applications in repairing tissues, which require high oxygenation rates or wound contracture delay.

Keywords: Tissue engineering; Porous membranes; Scaffolds; Bacterial cellulose

1. Introduction

Tissue engineering is an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve tissue or a whole organ function. Typical application examples are implants of scaffolds composed of biodegradable polymers or inert materials coated with bioactive biomaterials that allow growth of new tissues of particular types of cells [1,2]. After the formation of the new tissue, polymeric scaffolds are gradually degraded into small molecular weight compounds, which can be absorbed by the body or excluded out of the body [3].

In recent years the search for new classes of biopolymers, with specific properties to be used as scaffolds in tissue engineering, has attained great interest, like cellulose, polyhydroxyalkanoates, polylactates and blends of these materials [4–9].

In natural environments biofilms are associated with some microbial styles of life [10] and constitute a kind of self-protection that allows microorganisms to survive in adverse conditions [11]. Bacterial cellulose (BC) is a kind of extra cellular polysaccharide present in the biofilm produced by several bacteria, notably by *Acetobacter xylinum*, as long nanofibers. This polymer is highly crystalline and its degree of crystallinity varies depending on the origin and mode of chemical treatment [12]. BC is composed by glucose molecules joined by β(1→4)-glycosidic bonds forming branchless linear chains [13,14]. Cellulose biosynthesis is a process involving several steps regulated by some individual

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and well characterized complex enzymes and proteins. This process includes the synthesis of uridine diphosphate glucose (UDP-glucose), which is the precursor of cellulose biosynthesis, followed by glucose polymerization in β(1→4)-glucan chains by complex proteins [13,14]. Bacterial cellulose fibers exhibit a wide range of dimensions ranging from 1 to 25 nm in width, which corresponds to 10–250 polyglucan chains and from 1 to 9 μm in length (formed by 2000–18,000 glucose residues). Among six known polymorphs of cellulose, two common crystalline forms are of interest: cellulose I and II [15]. It is known that in the cellulose I, which is synthesized by plants and also by *A. xylinum* in static culture, parallel β(1→4)-glucan chains are uniaxially disposed, whereas β(1→4)-glucan chains of cellulose II are randomly arranged. Furthermore, crystalline cellulose I exhibits two main unit cell structures called cellulose I_α and I_β, which consist of one chain triclinic and two chain monoclinic unit cells, respectively [16,17], which are difficult to distinguish by X-ray diffractometry. The cellulose synthesized by *A. xylinum* is known to be composed by cellulose I_α as majority phase and cellulose I_β in smaller fraction [18–20]. The degree of crystallinity of cellulose influences some physico-chemical properties of cellulose like swelling and water binding [21].

Bacterial cellulose is used in a wide range of applications, from the food industry to electroacustic devices, as phone diaphragms. Bacterial cellulose is also potentially suitable for applications as scaffolds in tissue engineering due to its unique properties, including a high water retention capability (hydrophilic), a fine fibrous network, which allows cell growth and proliferation, high tensile strength, *in situ* moldability and low production cost [22–26]. In medicine, non-porous cellulose membranes are used as stent coatings, for dura-mater substitution in tumor or trauma cases or as skin protection in cases of burn and deep wounds. In odontology, cellulose films are applied for periodontal tissue recovering [27,28].

Despite the studies concerning bacterial cellulose membranes in medical applications as substitute for cartilaginous and skin tissues, production of porous bacterial cellulose films were reported only recently by Siqueira and Moreschi [29] and are not widely used in tissue engineering. Porous membranes consisting of large pores are usually indicated for preventive and healing treatments of wounds, in particular those in which exudation and oxygenation are necessary [30]. Among the possibilities of using porous membranes, a particular case is of great interest in tissue engineering: wound healing mechanism. Porous membranes composed of collagen I and chondroitin-6-sulfates are able to delay wound contracture and promote dermal regeneration. The optimal pore size lies between 20 and 120 μm, which promote the delay of fibroblast migration and avoids wound contraction and scar formation, allowing time for wound healing [31]. Fig. 1 shows the range of possible applications of porous cellulose membranes as a function of the pore diameter.

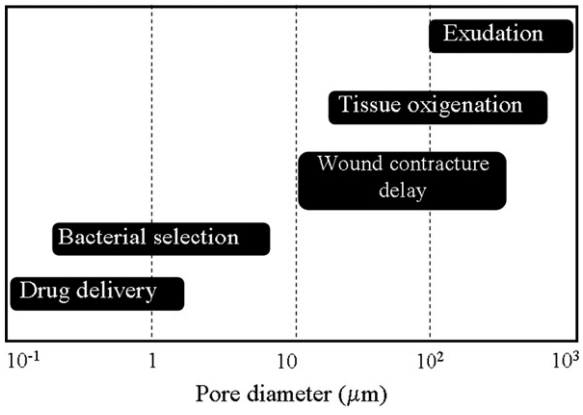

Fig. 1. Schematic diagram of the several applications of porous BC membranes in function of the pore diameter.

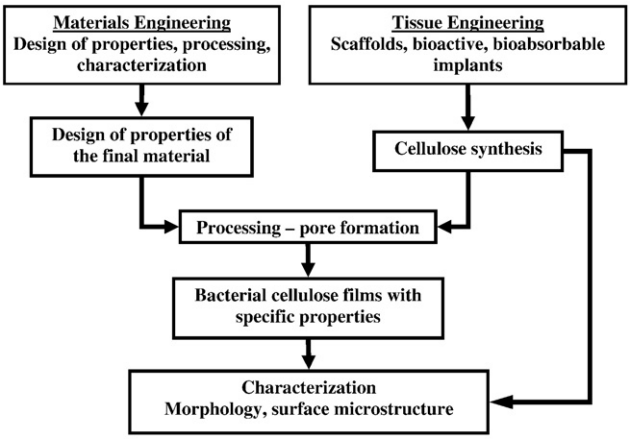

Fig. 2. Schematic flow chart for the production of porous bacterial cellulose membranes.

This work reports the biosynthesis of nanofibrous bacterial cellulose films containing *in situ* produced micropores. An innovative design of features (porosity, pore size distribution and pore geometry) of the cellulose membrane was developed to modify/improve a determined property for a specific application, which in turn intrinsically depends on these features. The flow chart shown in Fig. 2 illustrates schematically the multidisciplinary approach for the manufacturing of the porous cellulose membranes.

2. Experimental

2.1. Synthesis

Bacteria *A. xylinum* ATCC 23769, acquired from "Collection of Tropical Culture (CCT)" (André Tosello Foundation) was used for the cellulose production.

A. xylinum inoculum was prepared by its cultivation under static conditions, at 30 °C, in a 125 mL Erlenmeyer flasks, containing 25 mL of Hestrin & Schramm medium [32]. Cellulose production was carried out by the cultivation of the bacterium for 7 days, under static conditions at 30 °C, in 250 mL Erlenmeyer flasks containing 50 mL of modified Hestrin & Schramm medium, pH 6.6, containing (per liter): 5.0 g peptone, 5.0 g yeast extract, 1.15 g citric acid, 2.27 g Na_2HPO_4 and 20 g glucose. In order to produce porous