In situ modeling of biosynthesis and physico-mechanical properties of bacterial nanocellulose

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Abstract

Cellulose is the most widespread biopolymer in nature, and its rich source is primarily plant tissues, where it occurs in complex with lignins, hemicellulose and other components. The process of its purification is associated with the use of environmentally unfriendly chemical compounds. An alternative is bacterial nanocellulose (BNC), produced by microorganisms in a chemically pure form. Many bacteria have the ability to biosynthesise it, with the most efficient producer being bacteria of the *Komagataeibacter* genus. BNC has the same chemical structure as plant-derived cellulose; however, it exhibits different properties. The fibres of bacterial nanocellulose reach widths in the range of 25 to 100 nm, form a three-dimensional network through numerous intermolecular and intramolecular hydrogen bonds and van der Waals interactions. Bacterial cellulose is a material with high crystallinity, porosity, high mechanical strength and elasticity as well as high water-holding capacity. In addition, it is biocompatible; the spatial and highly hydrated structure successfully mimics the natural extracellular matrix. These properties account for BNC's broad application potential not only in the medical field, but also in the food industry, electronics and environmental protection.

Despite a number of advantages of bacterial cellulose, its commercialisation has been hampered, mainly due to the high cost and low efficiency of the manufacturing process. The yield of cellulose production, as well as its final properties, is strongly dependent on culture conditions, such as the composition of the culture medium, the type of culture, environmental conditions, and *in situ* modifications. Substrate additives that increase the efficiency of biosynthesis or modulate the properties of BNC can be divided into two groups: compounds that affect bacterial metabolism and substances that incorporate into the three-dimensional structure of cellulose. In addition, a very important factor for the quality of BNC is the selection of the production strain and its genetic modifications. Thus, by controlling the culture parameters of bacteria of the genus *Komagataeibacter*, it is possible to modulate the mechanical strength, porosity, absorption capacity and other physical and chemical properties of bacterial cellulose.

Improving the yield and properties of bacterial cellulose is an important issue for its applicability and subsequent commercialisation. Its biodegradability, ease of obtaining it, specific modification procedures for applications and low emissions during production will be key in the decision to replace bacterial cellulose with synthetic materials in many industries.

The purpose of this study was to determine the effects of selected *in situ* modifications of bacterial cultures of the genus *Komagataeibacter* on the yield and properties of bacterial cellulose, such as mechanical strength, degree of crystallinity, fibre thickness and alignment, as well as the ability to hold and retain water.

The first stage of the study examined the effect of modifying the medium with κ-carrageenan in double helices matrix at a concentration of 0.2%-0.8% and in the form of hydrated aggregates at a concentration of 2% to 4%. The first variant yielded membranes with relaxed

structure and thicker fibres, while the second variant yielded membranes with high porosity and thinner fibres. All cellulose composites with κ-carrageenan were characterised by reduced water holding capacity and swelling, as well as a reduced degree of crystallinity. On the other hand, water retention time, tensile and compressive strength were improved. In the culture of mouse ATDC5 chondrocytes on the obtained composites, the effect of *in situ* modification of bacterial cellulose with κ-carrageenan on the survival and proper differentiation of cells was evaluated.

In the second stage of the study, the effects of i*n situ* modification of the medium using carboxymethylcellulose (CMC) and hydroxyethylcellulose (HEC) on biosynthesis yield, cellulose film structure and fibre thickness, and crystallinity were evaluated. Medium modification with HEC did not affect biosynthesis yield, while it caused a slight reduction in cellulose production with CMC. The structure of the composites changed, being looser in the case of CMC, or consisting of thinner fibres in the presence of HEC. In both variants, the crystallinity of cellulose was lower than that of native cellulose. The composites and native bacterial nanocellulose were then modified with glycerol from a concentration range of 0.1% to 10%. It was found that soaking the membranes in a 2.5% glycerol solution allowed obtaining membranes with the highest free swell absorptive capacity of the artificial exudate. In turn, rehydratation capacity was highest for membranes modified with 10% glycerol. In all plasticiser concentration variants, BNC composites showed higher absorptive capacity and rehydratation capacity than native cellulose, with the highest values obtained for composites with CMC. A similar trend was observed for the mechanical strength of the modified membranes. The safety of the composites was evaluated using the human keratinocyte cell line HaCeT. A protective effect of cellulose and its composites on the tested cells in the presence of different concentrations of glycerol was observed. Moreover, for all variants of BNC and composites impregnated with 2.5% glycerol, the survival of HaCeT cells remained constant or increased compared to controls.

The next stage of the research was focused on the influence of culture conditions on the yield and strength parameters of bacterial cellulose. As part of the implementation of the work, a surface airflow bioreactor (SAF) was designed with the ability to introduce air parallel to the growing cellulose film. Using the Plackett-Burman Design, the significance of the effects of bioreactor size, culture time, age and volume of inoculum, s/v ratio, glucose concentration, pH, air-flow rate and humidity on dry weight of cellulose, the glucose conversion into BNC and dry weight per surface unit were evaluated. The obtained results showed that most of the studied input factors significantly affect BNC yield. Further research showed that among the many factors that have a positive effect on simultaneous yield and mechanical strength, glucose concentration, culture time and air flow rate are the most important.

As part of the next stage of work, the effect of simultaneous modification of the medium with ethanol and lactic acid on the yield and properties of bacterial cellulose was determined, yet undescribed. The most beneficial concentrations using a single compound

were determined at 1% for ethanol and 0.6% for lactic acid. Optimisation of the process in terms of co-supplementation of the tested compounds allowed to select the optimal medium at pH 6.25, containing 0.73% lactic acid and 0.34% ethanol. The yield of BNC biosynthesis in this medium was increased several times (4.36 g/l), compared to the efficiency on unmodified SH medium (0.77 g/l). The study analysed the changes occurring in media supplemented with one or both modifying compounds. Co-supplementation with ethanol and lactic acid allowed biosynthesis high porosity membranes with significantly increased water-holding capacity. Regardless of the type of medium modification, the degree of crystallinity and average crystallite size were reduced for all variants. Strength parameters such as stress, Young's modulus, tensile strength at break and toughness were significantly improved.

In the final stage of the work, a new strain of cellulose-producing bacteria was isolated. It was identified as *Komagataeibacter hansenii* and named SI1. The cellulose produced by this strain shows high porosity and susceptibility to stretching. *K. hansenii* SI1 was shown to exhibit rapid growth and synthesize cellulose by day 4 of culture. From the tested carbon sources, the highest biosynthesis yield was obtained in glycerol medium. It should be noted that the membrane retained the ability to plastic deformation only when produced in a medium containing glucose. The study also showed that a complex organic nitrogen source was necessary for maximum process efficiency. In contrast, the effect of pH on BNC biosynthesis and membrane mechanical properties was dependent on the used source of carbon. The effect of medium modification with ethanol, lactic acid and vitamin C was negligible or negative on the yield, while significant changes in structure and mechanical strength were observed. In particular, vitamin C enhanced the unique characteristics of BNC produced by *K. hansenii* SI1. An increase in the porosity of the cellulose and its ability to stretching was observed as the concentration of vitamin C in the medium increased. In addition, these membranes were characterised by higher crystallinity than unmodified BNC.

The results of the study in the presented work confirm the beneficial effect of *in situ* modification of bacterial cellulose using large- and low-molecular-weight compounds on the biosynthetic yield and/or its physical and mechanical properties. In addition, it is shown that the appropriate selection of process parameters will allow increasing not only the efficiency of the process, but also the quality of the produced cellulose films. The SAF bioreactor developed as part of the research makes it possible to simultaneously increase the productivity and yield of the process, as well as the strength parameters of the BNC. In turn, the method of obtaining unique films with increased porosity and ability to stretch using a newly isolated strain of *K. hansenii* SI1 has been patented (Pat.241158).