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Research article

Modification of bacterial cellulose to scaffold-like structures applied in process engineering

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Abstract: *Scaffolds are three-dimensional structures which provides necessary support for different cells' vital functions. Although they are widely produced from different materials, most of them are not biodegradable. Bacterial bionanocellulose (BNC) has this property and additionally, has similar features to natural, extracellular matrixes. Unfortunately, natural channels which are in BNC's structure have not sufficient diameter to colonize them with, for example, mammalian cells. Some experiments for pores enlargement in cellulose structure have been conducted. Multiple frosting and defrosting of properly prepared BNC's samples has produced some positive results. Application the mixture of sterile vegetable oil and ethanol at the cultured layers of bionanocellulose gave expected results – diameter of the channels and chambers is enough to colonize them with viable cells. The results of described experiments give hope that bacterial bionanocellulose, because of its transformation's simplicity, could be an alternative material for bioplastics productions.*

Keywords: *scaffolds, bionanocellulose, Gluconacetobacter xylinus.*

Introduction

Scaffold is a three-dimensional structure, which provides the necessary support for cells to grow, proliferate and differentiate [1]. It helps them to fulfill their functions which are the most similar to the one fulfilled by natural, extracellular matrix [2, 3]. In case of injuries and organdisorders, scaffolds should protect tissues from further damage. It is a theory that when cells were cultured on scaffolds, there would be a possibility to apply them into human's body and lead to regeneration of damage tissue [1, 3-6]. Scaffold designed for cells' culture and for tissue engineering should have several features [2, 7]. First of all, they should have a three-dimensional structure, which not only allows for cells cultivation but also allows for metabolic processes and migration [2, 5]. The diameter of pores should be sufficient to colonize them with cells [2]. An extracellular matrix should have a proper mechanical strength. It should not cause immune response and

should not be toxic for cells and tissues [2, 3, 7]. Nowadays, scaffolds are produced from many different materials, but unfortunately, most of them are not biodegradable, therefore their insertion into to human body is connected with reoperation to remove the scaffold. [1, 3, 7]. Bacterial bionanocellulose (BNC) synthesized, for example, by strain *Gluconacetobacter xylinus,* has unique properties and enables to using it in biomedical engineering and regenerative medicine [8]. From the chemical point of view, cellulose is a polymer of D-glucose linked with β-1,4 glucosidic bonds. It is a structural component of plants' cell walls [8]. Bacterial BNC has all properties which scaffolds should have. It has appropriate strength, number of channels and chambers. Bionanocellulose is easy to produce, production cost are low. BNC is also biodegradable, which is the most desirable property for medical applications [7-9]. Bacterial cellulose is now widely used in medicine as main material for producing wound dressings, vascular grafts. Some experiments for using BNC to cartilage repair and bone healing are also done and the results are promising [7, 9]. Bionanocelluse, can be widely used not only in medicine and biomedical engineering, but also in bioplastics' production [10, 11, 13]. Increasing production of plastics have negative influence on natural environment. While in 1950, 1 500 000 tons of plastics were produced, in 2012 this number grew to 288 000 000 tons [10]. For this reason, scientists have been looking for alternative methods for plastics productions, which would be save for the environment. The best possibility would be transformation of food industry wastes, other natural wastes or easily culturable materials like bacterial bionanocellulose, to biodegradable bioplastics [10, 11]. Such actions would not only be cheap and save for natural environment, but also easy to produce. Nowadays, some part of food industrial wastes is dissolved in trifluoroacetic acid (TFA) and then is transformed into bioplastics, which are characterized by good mechanical strength and similarity to non-biodegradable plastics [10]. It is also a possibility to obtain bioplastics like polylactic acid (PLA), polyhydroxyalkanoate (PHA) and polybutylene succinate (PBS), with the use of the natural and recombinant host organisms used for fermentative production of these monomers [12].

Experimental

All experiments, which are mentioned in this article, were done at the Institute of Technical Biochemistry, Faculty of Biotechnology and Food Sciences and at Interdepartmental Institute of Radiation Technology, Lodz University of Technology.

Materials

For biosynthesis of bionanocellulose (BNC) bacterial strain *Gluconacetobacter xylinus* was used. For the cultivation of bacteria, a nutrient medium Schramm- Hestrin was used. It was composed of 20 g of glucose, 5 g of yeast extract, 5 g of aminobac, 1.15 g of citric acid, 2.7 g of Na_2HPO_4 and 0.5 g of $MgSO_4$ per 1000 ml of water. In each case, the medium was inoculated with a pre-culture of *Gluconacetobacter xylinus*. The BNC was cultivated in many different cultured plates – squared and round shape.

Methods

The aim of all mentioned experiments were cultivation and then transformation bacterial bionanocellulose into a scaffold-like material, which could be cultivated by different cells and in some cases could replace transplantation process. The first cultivation of *Gluconacetobacter xylinus* was continued, in a proper conditions, for eight days. After finishing, precise rinse process was executed. The membranes were rinsed in water, then in 1% NaOH, again in water, then in 1% CH_3COOH and process was finished by rinsing in the water. Each step took 24 hours. Received bionanocelluse, after precise rinse process, was cut into small squares which were frozen and thawed from 1 to 5 times in 24 hour period times. Defrosting was always done with boiling water. Some pieces of BNC were dried instead of frosting and defrosting. The next several cultures were spotted by sterilized solution of vegetable oil and ethanol (volume ratio 1:1, the oil used in the experiment was regular cooking one). The mixture was applied at the surface of bionanocellulose also in 24 hour period time, starting from the second day of cultivation process. Each cultivation lasted between 7 to 17 days. After finishing, BNCs were rinsed, sliced and then analyzed by electron microscope TM-1000 (Hitachi).

Results and Discussion

It was observed that weight of bionanocellulose, which was frozen and thawed many times, decreased with number of defrosting. It suggested that BNC took less amount of water because of chambers which occurred in its structure during experiments. Microscopic analysis showed that frosting and defrosting confirmed the success of the method. In figure 1, natural structure of bionanacellulose after finishing cultivation process,is shown. BNC's fibers were arranged close to each other. Small channels and chambers were observed, which allows for water accumulation. Unfortunately, spaces between fibers are definitely smaller than bacteria diameteres and cells cultivation on BNC would be imposibble.

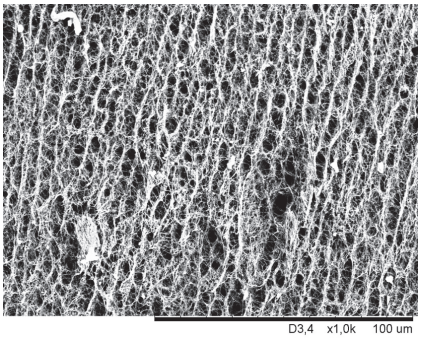

Figure 1. The structure of bionanacellulose after finishing cultivation process

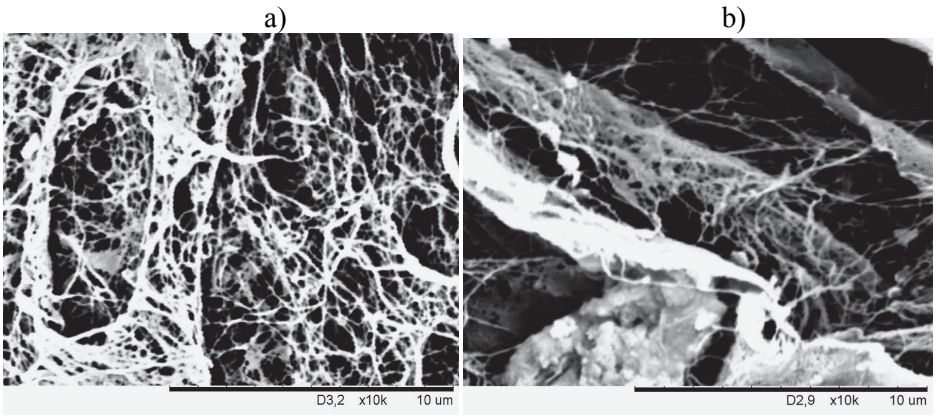

Figure 2. The structures of bionanacellulose which were defrosted four (a) and five (b) times

Figure 2 shows structures of bionanacellulose which were defrost four (a) and five (b) times. Compared to diameters of natural BNC structures gaps, they were much bigger than the ones observed in bacterial bionanocellulose structures, which was not subjected to the process of multiple frosting and thawing. During frosting and defrosting processes, freezing water, whose volume increased by 10% each time, ripped the cellulose structure. Although the amount of space in BNC's structure increased, there was not enough space to culture viable cells and allow them to grow and proliferate. What is more, chambers did not create channels, which are also crucial for cells cultivation. Bacterial bionanocellulose, which vegetable oil was sprayed on during cultivation, showed another properties. An emulsion, which was applied on the membrane during its formation, caused that more pores in BNC structure have been found. The formation of number of channels was observed. Microscopic analysis showed that pores were big enough to colonize them with cells.

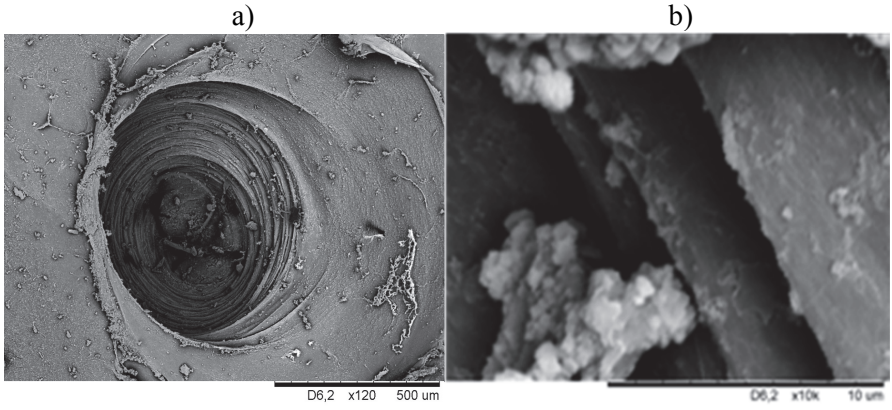

Figure 3. The channel in BNC structure created after regular vegetable oil aplication: a) above and b) side view

Figure 3 presents two different views of the channel in bionanocellulose structures, which was created as a result of regular, vegetable oil aplication. Channel was characterized by 'stepped' structure, which would be conductive for viable cells' cultivation. It was also observed that because of the obtained shape, channel structure would probably be helpful in medicine, in production of artificial meniscus. In figure 4 number of channels and chambers formed after oil application is visible. The fact that bionanocellulose, with its unique properties is easily to transform into different forms, gives hopes for using it in different industrial ways.

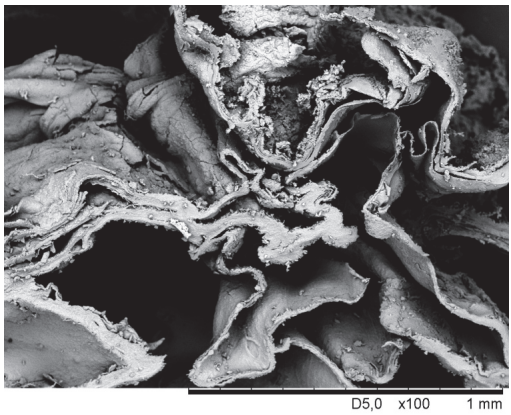

Figure 4. A part of bionanocellulose with number of channles, formed after regular vegetable oil application

Further analysis showed that the diameter of chambers and channles are adequate to colonize them with cells. Presumably, the drop of apllied oil is moving up during culture process, at the same time inner layer of BNC is moving down into medium, making a channel in the structure. In figure 3b, some of *Gluconacetobacter xylinus* are visible. Single bacterium formed single fibers as a result of metabolic processes. The fibers formed layers and their number grows during cultivation process.

Conclusion

Application of sterile vegetable oil during cultivation process showed better results than multiple frosting and defrosting. First method allowed to received chambers, which were not sufficient for culture viable cells. Oil application caused obtaining properties, which theoreticallyallow the scaffolds to comply with all conditions. Unique properties of bionanocellulose caused that it would be a perfect material for producing scaffolds. For that reason scientists should investigate BNC and look for different possibilities of using it not only in medicine and medical enginnering. It is a high probability that BNC could be

a proper material for bioplastics' production. Probably both, bionanocellulose after and before experiments would be suitable for its dissolving in trifluoroacetic acid (TFA) and then for transforming into bioplastics, which would be similar to non-biodegradable plastics, but friendly for natural environment.

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