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SHORT COMMUNICATION

Transformation of bacterial cellulose into a scaffold-like material as a new hope for tissue engineering

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ABSTRACT

Scaffolds are three-dimensional structures used in medicine, especially in tissue engineering, for the reconstruction of damaged tissue or organ. They should be constructed in a special way, to provide support for different cells' vital functions. Bacterial cellulose produced by *Gluconacetobacter xylinus* has congruous features to natural occurring extracellular matrixes. Natural pores which occure in cellulose's structure have not adequate diameter to colonize them with viable cells. The authors conducted some experiments in order to enlarge channels in cellulose structure. Repeated frosting and defrosting of accurately prepared cellulose samples has created positive results but application of sterile mixture of vegetable oil during culture process gave expected results – diameter of the channels and chambers was big enough to colonize them with viable cells.

***Keywords*:** scaffold, bacterial cellulose, tissue engineering, *Gluconacetobacter xylinum*

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1. INTRODUCTION

Scaffolds are three-dimensional structures used in medicine, especially in tissue engineering, for the reconstruction of damaged tissue or organ. They can be made from different types of polymers, both natural and synthetic. Scaffold should be constructed in such way that it could be possible to culture them with viable cells [Hutmacher 2000]. Scaffolds should perform functions as close as possible to those performed by naturally occurring extracellular matrices [Hutmacher 2000, Kaźmierczak et. al. 2016]. The idea of creating scaffolds is to culture them with viable cells. Those artificial, extracellular matrices should allow cells to differentiate, proliferate and maintain the proper metabolic and catabolic processes [Kaźmierczak et al. 2016, Chen et al. 2002] The idea is that scaffold cultured with living cells should be placed in the human body, in the place of a damaged or other organ's disorders and cause its regeneration. After its completion, scaffold should be degraded and adsorbed in the tissues, which eliminates the need for its surgical removal [Chen et al. 2008, Ashjaran 2013]. The researchers , by using scaffolds, would like to recover entire limbs in special bioreactors [Peter 2004]. The future ideology of this idea is illustrated as follows, in the Figure 1:

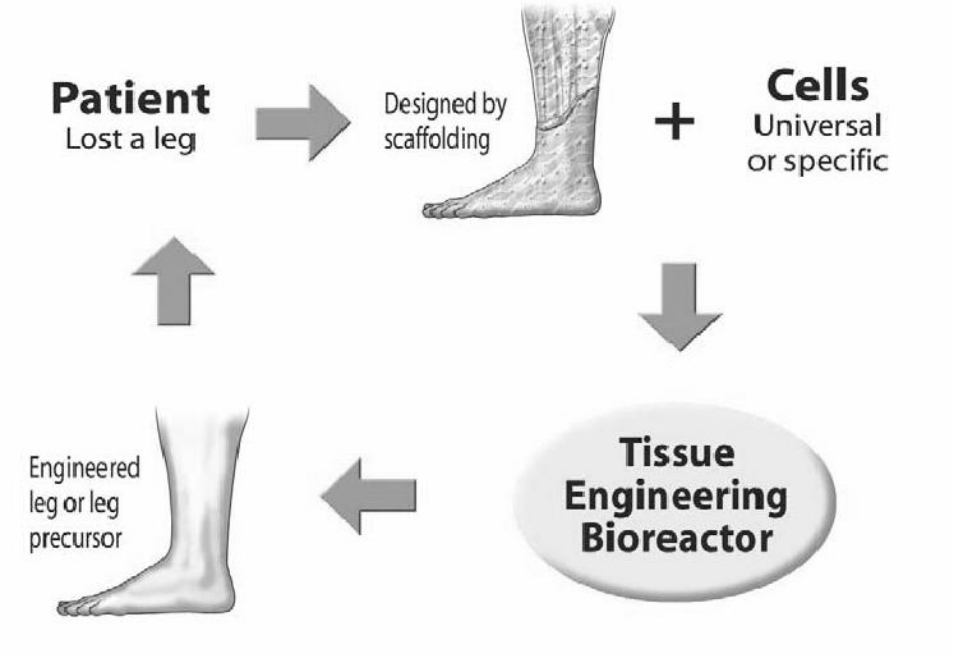

Figure 1. Diagram showing the use of scaffold in case of hypothetical lost of leg [Peter 2004].

Despite the fact that many ways of obtaining scaffolds is known, most of the material using to their production is not biodegradable. Some of production methods are also complicated and very expensive [Kaźmierczak et al. 2016, Ashjaran 2013].

Bacterial cellulose (BC), from the chemical point of view, is a polymer of D-glucose linked with β-1,4-glucosidic bonds. It is synthesized by different types of bacteria stains but

Gluconacetobacter xylinus strain is most commonly used for industrial scale production [Wang et al. 2011]. Bacterial cellulose is widely used in medicine. It is a main material for producing wound dressings, dressings for burns, vascular grafts and also is used for bone healing and cartilage repair. What is more, under scanning electron microscope, it could be observed that BC has structure which is similar to natural occurring extracellular matrices. Bacteria cellulose has all properties which scaffolds should have [Kaźmierczak et al. 2016, Wang 2011]. It has appropriate strength, number of channels and chambers, it is biodegradable, easy to produce. Production cost of BC are low [Mucha et al. 2012]. The only disadvantage is that the channels and chambers are too small to enable culturing it with viable cells [Wang et al. 2011].

2. MATERIALS AND METHODS

Bacterial strain *Gluconacetobacter xylinus* was used for biosynthesis of cellulose. Bacterial strain was taken from an Institute of Technical Biochemistry, Faculty of Biotechnology and Food Sciences, Lodz University of Technology. 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 Schramm- Hestrin medium was inoculated with a pre-culture of *Gluconacetobacter xylinus.*

The aim of all experiments mentioned in this article was cultivation of bacterial cellulose and then transformation it into a scaffold-like material, which next could be cultivated by viable cells and in some cases could be applied into human's body and lead to regeneration of damage tissue. The cultivation of *Gluconacetobacter xylinus* was continued in many cultured plates, in proper conditions of pH and temperature. It lasted until the desired thickness of the sheets of bacterial cellulose was obtained. For several cultures, cooking vegetable oil was used. Selection cultures were spotted by sterilized solution of vegetable oil and ethanol (volume ratio 1:1). Starting from the second day of cultivation process, the mixture was applied at the surface of cellulose in 24 hour period time.

The other cultures were carried out without applying oil mixture. When cultivation processes were finished, specific rinse process was executed. The obtained membranes were rinsed alternately in water, in 1% solution of NaOH and in1% solution of CH_3COOH. Process was finished by rinsing in the water in order to obtain a neutral. Cellulose membranes, which were cultured without applying vegetable oil, after precise rinse process, were cut into small square- shaped pieces which were alternately frozen and thawed from 1 to 5 times in 24 hour period times. Each defrosting was done with boiling water.

3. RESULTS

It was perceived that weight of bacterial cellulose, which was frozen and thawed many times, declined with number of defrosting processes. Microscopic analysis, presented in the Figure 2, showed that frosting and defrosting caused occurring of small channels and chambers but spaces between cellulose fibers were definitely too small to culture bacteria or viable cells on obtained scaffolds.

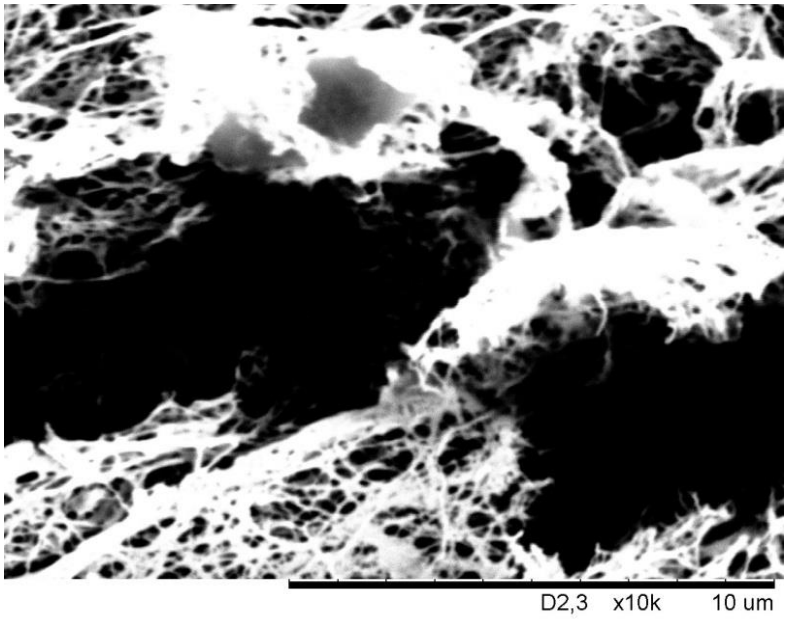

Figure 2. Visible pores in bacterial cellulose's structure after defrosting processes.

Application of sterile mixture of vegetable oil and ethanol during culture process gave expected results – a large number of channels and pores were observed during microscopic analysis, what was presented in Figure 3a) and 3b). What is crucial, diameter of the channels and chambers was big enough to colonize them with viable cells.

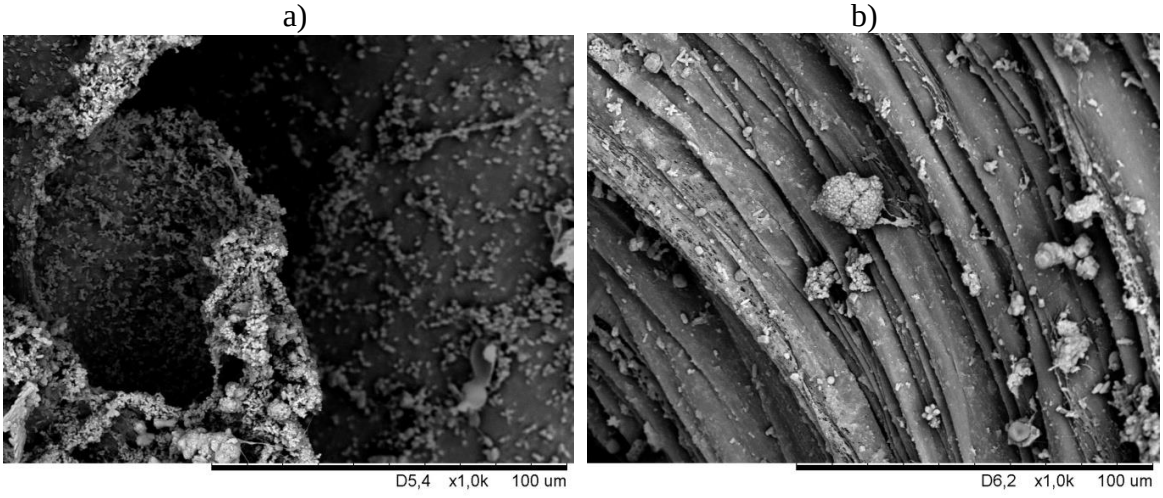

Figure 3. Channels and chambers in cellulose structure after oil application (a), *Gluconacetobacter xylinum* in the channel, which occurred after oil application (b).

4. DISCUSSION AND CONCLUSIONS

Decreasing weight of multiple frosting and defrosting cellulose membranes suggested that bacterial cellulose took less amount of water because of increasing number of pores which occurred in its structure during experiments. During frosting and defrosting processes the volume of freezing water increased roughly by 10% each time. This phenomenon ripped the cellulose structure. Although the amount of pores and channels in cellulose structure increased, there was not enough space to culture viable cells and to provide necessary support for different cells' vital functions. What is more, chambers also did not create channels, which are also essential for cells cultivation. Bacterial cellulose, which vegetable oil was applied on during cultivation, showed another properties. An oil – ethanol mixture applied on the membrane during its formation, induced that more pores in cellulose structure have been found. The formation of various channels and chambers was observed. Further microscopic analysis showed that the diameter of chambers and channels are suitable to colonize them with viable cells.

All experiments were successful. Application of mixture of sterile vegetable oil during cultivation process indicated better results than multiple frosting and defrosting of cellulose membranes. Frosting and the wing in boiling water allowed to received bigger pores but not big enough to provides necessary support for different cells' vital functions. Oil application caused obtaining properties, which theoretically allow the scaffolds to comply with all conditions. Unique properties of bacterial cellulose and low costs of production caused that it would be a perfect material for producing scaffolds.

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