In vitro biodegradation of bacterial nanocellulose under conditions simulating human plasma in the presence of selected pathogenic microorganisms

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Abstract: The biodegradability of bacterial nanocellulose (BNC) was assessed based on a change in its properties under conditions simulating human plasma in the presence or absence of the pathogens *Staphylococcus aureus*, *Candida albicans* and *Aspergillus fumigatus*. It was shown that the dry mass of BNC did not change during 6 months of incubation, except for samples stored in the presence of moulds, where the dry mass reduced by 40 %. The wet mass of all BNC samples increased after 2 months of incubation. Under these conditions, the population number of microorganisms grew about 2 log cycles during the first month and maintained this level for 6 months of storage. After 1 month of storage in sterile fluids, and in the presence of bacteria or fungi, the tensile strength of BNC decreased by 60 % or 70 %, respectively.

Keywords: *in vitro* biodegradation, bacterial nanocellulose, pathogenic microorganisms.

Biodegradacja nanocelulozy bakteryjnej w warunkach *in vitro* symulujących osocze ludzkie w obecności wybranych mikroorganizmów chorobotwórczych

Streszczenie: Oceniano podatność na biodegradację nanocelulozy bakteryjnej (BNC) na podstawie zmiany jej właściwości w warunkach symulujących osocze ludzkie w obecności lub nieobecności patogenów *Staphylococcus aureus, Candida albicans* i *Aspergillus fumigatus.* Wykazano, że sucha masa BNC nie zmieniła się w ciągu 6 miesięcy inkubacji. Wyjątek stanowiły próbki przechowywane w obecności pleśni – ich sucha masa zmniejszyła się o 40 %. Mokra masa wszystkich próbek BNC zwiększyła się już po 2 miesiącach inkubacji. W tych warunkach liczba drobnoustrojów wzrosła o ok. 2 rzędy wielkości w pierwszym miesiącu i utrzymywała się na tym poziomie przez 6 miesięcy inkubacji. Po 1 miesiącu przechowywania, zarówno w jałowych płynach, jak i w obecności bakterii lub grzybów, wytrzymałość mechaniczna BNC zmniejszyła się, odpowiednio, o 60 % i 70 %.

Słowa kluczowe: biodegradacja *in vitro*, nanoceluloza bakteryjna, mikroorganizmy chorobotwórcze.

The materials used for the production of cardiovascular implants must be biocompatible with the human organism and cannot demonstrate toxic, mutagenic or teratogenic effects. They should be neutral to the immunological system and not contribute to blood clot formation. Moreover, these materials must have mechanical and technological properties appropriate for specific applications (especially related to sterilization processes), as well as must be resistant to biodegradation in the human body. These materials should be readily accessible and they should not generate a high cost of implant production [1].

Nowadays, biological tissues, synthetic polymers and combinations of them are most often used in the production of cardiological implants [2]. Unfortunately, none of these materials meets all the above-mentioned requirements. The best commercially available implant is chosen for transplantation, taking into account the age and health condition of the patient. Implants produced from biological tissue have shorter lifespans in comparison with those from synthetic polymers, and sometimes they require re-implantation. Additionally, the patient must take immunosuppressant drugs after transplantation. On the other hand, patients with synthetic polymer implants

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requires lifelong anticoagulant therapy due to a tendency for thrombus formation by these implants [2–4].

Bacterial nanocellulose (BNC) seems to be an interesting alternative for currently used biomaterials. It is a polysaccharide produced by some species of *Gluconacetobacter.* The most efficient producer is *Gluconacetobacter xylinus* [5]. This species produces cellulose in the form of white membranes. Production is relatively inexpensive and the material is more readily available than biological tissues [6]. Therefore, the cost of implant manufacturing can be reduced. Additionally, BNC complies with most of the requirements for biomaterials used for the production of cardiovascular implants. However, in the available literature there are only fragmentary data on the susceptibility of BNC to biodegradation under human conditions. It is the most important problem concerning all materials used as internal implants, especially cardiovascular implants that should be non-biodegradable.

The main aim of this study was to determine changes in the BNC properties occurring during incubation under the conditions simulating human plasma in the presence or absence of pathogens. An additional objective was selection of a method for the quickest evaluation of changes leading to the degradation of BNC.

EXPERIMENTAL PART

Materials

The BNC produced by BOWIL Biotech Ltd., according to the procedure described in the PL 171952, PL 212003 patents, was used in experiments.

Culture and growth conditions

The BNC susceptibility to degradation by microorganisms was carried out in the presence of bacteria *Staphylococcus aureus* PCM2054 from The Polish Collection of Microorganisms in the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences in Wroclaw, as well as yeast *Candida albicans* ATCC10231 and mould *Aspergillus fumigatus var. fumigatus* ATCC96918 from the American Type Culture Collection.

Cultures were prepared by inoculating 100 cm^3 of Tryptic Soy Broth, pH 7.0 (*S. aureus)*, or 100 cm^3 of Maltose Soy Broth, pH 5.6 (*C. albicans* and *A. fumigatus*) with 0.1 cm^3 of liquid culture (at stationary phase of growth) and incubating it with shaking at 37 °C for 24 h (bacteria and yeast) or 72 h (mould).

Preparation of simulated body and phosphate buffered saline fluids

A phosphate buffered saline (PBS, No. 524650 Merck Ltd.) was prepared in accordance with the producer's instructions and sterilized in an autoclave at 115 °C for 20 min. A simulated body fluid (SBF) was prepared by dissolving the mineral components in distilled water according to [7]. The resulting solution was adjusted to pH 7.4 with 6 M HCl and then filtered through the filters with a 45 μm pore size using a Millipore vacuum filtration kit. After filtration, it was subjected to tyndallization in order to obtain a sterile SBF fluid.

Preparation of BNC samples for incubation

Sterile, native BNC membranes cut into squares (25 × 25 mm) – for the determination of wet and dry mass, or into rectangles (15 × 100 mm) – for the determination of mechanical properties, were immersed in 62.5 and 150 cm^3 of sterile SBF fluid or PBS, respectively. Cultures of *S. aureus*, *C. albicans* and *A. fumigatus*, in stationary phase of growth, were added to SBF, with or without BNC, to a final concentration about 10^3 CFU/cm^3 (CFU – colony-forming unit). A high concentration of the microorganisms was used in order to accelerate their effect on the examined material. All samples were incubated at 37 °C for 6 months. At appropriate intervals, samples were taken and their biodegradation degree was determined.

Methods of testing

Enumeration of viable cells

Microbiological enumerations were performed after the appropriate time of storage at 37 °C. Post-incubation SBF was serially diluted with buffered saline (pH 7.0). Dilutions were plated on trypticase soy agar (TSA) (bacteria) or on *Sabouraud agar* at pH 5.6 (fungi*)* and the plates were incubated for 48 or 72 h at 37 °C.

Determination of wet and dry BNC mass changes

BNC samples were removed from solutions after incubation and washed by shaking (70 rpm) in 100 cm^3 of distilled water for 2 hours, to eliminate the PBS or SBF buffer components, which could be adsorbed into the BNC structure and interfere with the measurements of its wet and dry mass changes. The water was changed every 15 minutes. The samples were centrifuged for 10 min at 240 *g* at 20 °C and weighed to determine the wet mass content. To determine the dry mass, the samples were prepared analogically. After weighing, the wet samples were dried to constant mass at 105 °C. The results presented in the figures are averages from three replications ± standard deviation.

Susceptibility of BNC on biofilm formation by microorganisms

The presence of microorganisms growing in the form of biofilms on the BNC surface was determined using MTT [3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide]. Both washed and unwashed BNC sam-

ples (as described earlier) were tested. The process was carried out in sterile working conditions. The MTT solution (Sigma Aldrich, No. M2003) (1 mg/cm^3), supplemented with 10 mM menadione solution (Sigma Aldrich, No. 5625) in the volume ratio 1 : 10 000, was introduced into the wells of 24-well sterile plates in an amount that ensured complete coverage of the BNC membranes. Subsequently, a square of 1 × 1 cm of BNC sample was aseptically transferred to each of them. After 3 hours incubation at 37 °C, a color change of the BNC samples from yellow to black was visually evaluated. The intensity of the color was proportional to the amount of bacterial biofilm growing on the surface of BNC fibers.

Determination of saccharides by thin layer chromatography (TLC)

The post-incubation fluids were purified by thermal precipitation of proteins (110 °C), centrifugation at 9000 *g* for 15 min at 20 °C, filtration using 0.45 μm Syringe Filter PTFE (Cronus), and freeze-drying (94 Pa). 0.01 cm^3 of 20-fold concentrated samples were subjected to thin-layer chromatography on a plate (10 × 20 cm) of silica gel G-60 (Merck, No. 1055530001). The chromatograms were developed two times in a mobile phase system composed of 1-butanol, pyridine and water (6 : 4 : 3, v/v/v). The sugar spots were visualized by spraying with a reagent consisting of sulfuric acid and methanol (1 : 9, v/v), followed by drying. As standards, 0.1 % solutions of D(+)-glucose (Sigma Aldrich, No. 49139) and cellobiose (Merck, No. 219458) were used. The retention factors (R_f) values for the samples were calculated.

Determination of mechanical properties

The mechanical properties of BNC – tensile strength (σ) and elongation at break (ε) – were determined according to modified ASTM D882-00 and PN-81/C-89034 standards, using an Instron 5543 Universal Testing Machine (Instron C., Canton, MA, USA). Initial grip separation was 50 ± 5 mm, and cross-head speed was 10 mm/min. Tensile strength was calculated by dividing the maximum load by the initial cross-sectional area of the sample and expressed in MPa. Elongation at break was calculated as a ratio of the elongation at the point of sample rupture to the initial length of a sample as a percentage. The results presented in the tables are the averages of at least 8 separate replications ± standard deviation. Measurements were also made for non-incubated BNC, which was a control sample.

Statistical analysis

Experimental data were processed using SigmaPlot 11.0 (Softonic International S.L) statistical program with one-way ANOVA for a significance level $p < 0.05$.

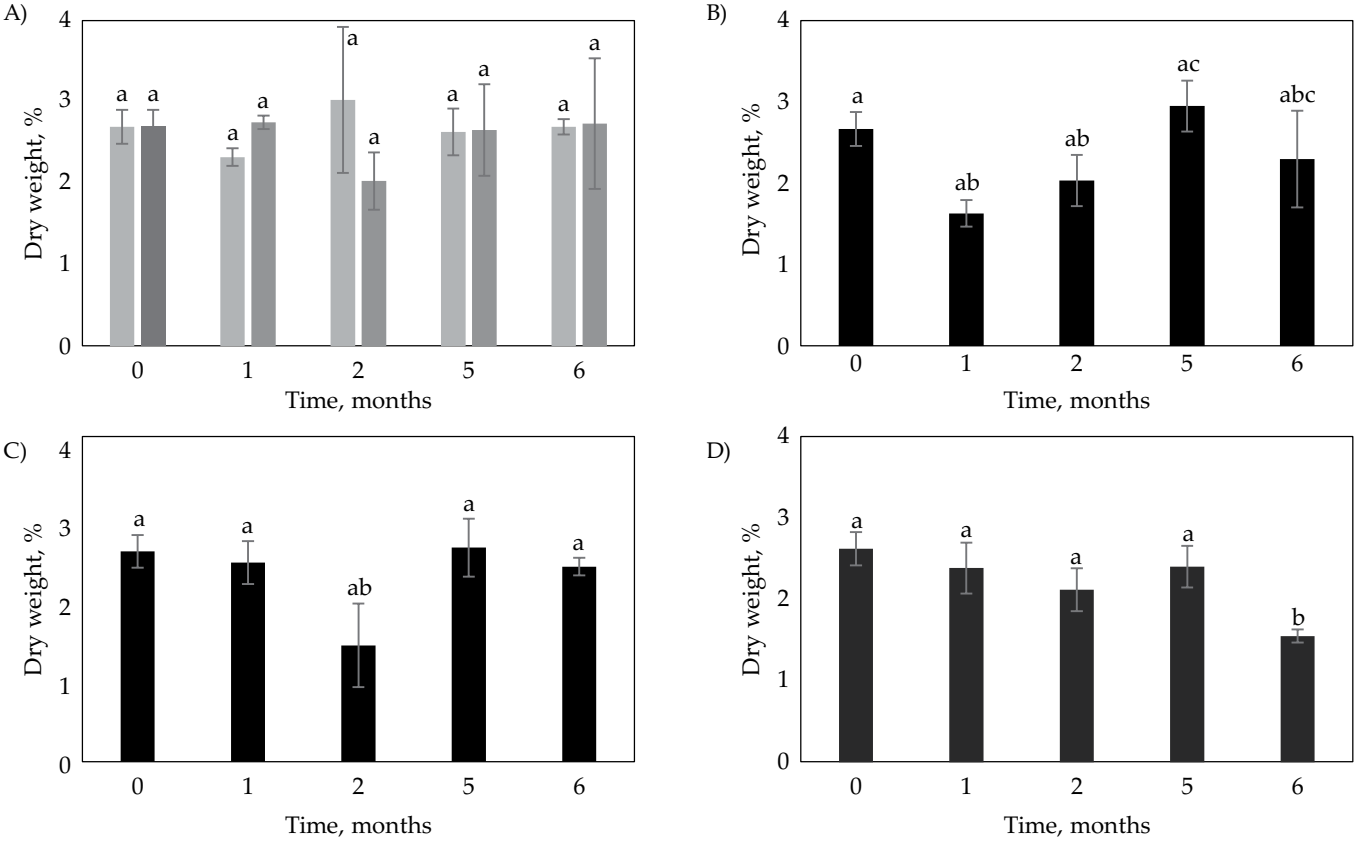

Fig. 1. Changes in dry mass of BNC samples incubated for 6 months in: A) sterile PBS (■), SBF fluids (■), B) SBF fluid in the presence of *S. aureus*, C) SBF fluid in the presence of *C. albicans*, D) SBF fluid in the presence of *A. fumigatus*; a–c values followed by different letters differ significantly ($p < 0.05$)

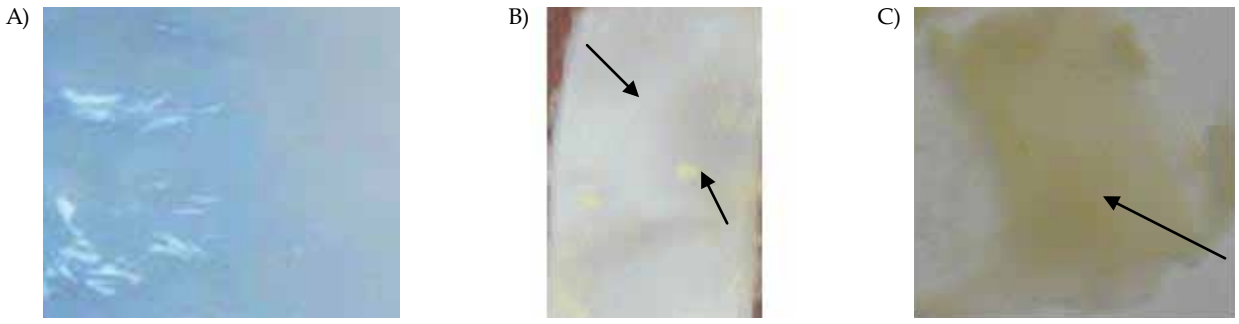

Fig. 2. Pictures of BNC samples: A) not incubated, B) incubated for 2 months, C) incubated for 3 months; arrows show brighter regions in the membrane that indicate a reduction of its thickness due to degradation

RESULTS AND DISCUSSION

The biodegradation of polymeric materials is a complex process and changes of polymer properties can occur with varied intensity. To determine the applicability of BNC as a material for cardiological implant production, several methods were used to observe the degradation processes of a polysaccharide. The effect of the selected pathogenic bacteria, yeast, and mould on BNC biodegradation was investigated under *in vitro* conditions simulating human plasma (PBS and SBF fluids). The SBF fluid is poor in nutrients, therefore, any potential microbial growth in this solution can indicate their ability to hydrolyze polysaccharide and use the products of this reaction as a carbon source, essential for their growth. As a result, this can be observed not only in BNC's dry mass loss and an increase the number of detected microorganisms but also this can be a rise in the wet mass of BNC because the degraded product absorbs more water.

Dry and wet mass changes

Figure 1A shows the changes of BNC dry mass occurring as a result of sample incubation for a period of 6 months (184 days) at 37 °C in sterile PBS buffer or SBF fluid. It was found that the dry mass of BNC samples practically did not change during that time. No changes in the cellulose's dry mass after 41 days of incubation in sterile PBS buffer and SBF fluid were also noted by Li *et al*. [8]. On the other hand, Shi *et al*. [9] and Peng *et al*. [10] demonstrated that the dry mass of BNC is reduced by about 10 %, after 30 and 60 days of exposure in PBS buffer, respectively. The loss of the cellulose's dry mass greater than 10 % was not observed by Chen *et. al*. [11] even after 231 days of BNC incubation in PBS.

The dry mass of the samples did not change significantly during incubation of BNC in SBF fluid in the presence of the *S. aureus* or *C. albicans* (Figs. 1B, 1C) while a significant decrease in BNC dry mass of about 40 % in the samples incubated for 6 months in the presence of the *A. fumigatus* was observed (Fig. 1D).

Degradation of BNC in the presence of these microorganisms was macroscopically visible as brighter regions on the surface of the samples after 2 months of storage (Fig. 2). The obtained results lead to the conclusion that determination of dry mass content of BNC is not a good method for the study of the degradation rate of the material.

A more sensitive method for the detection of degradation in BNC is the determination of changes in its wet mass. The wet mass of all BNC samples increased after 2 months incubation (Fig. 3). A hundred percent increase in the wet mass of the samples incubated for 5 months in the presence of *A. fumigatus* was observed (Fig. 3E). According to Shi *et al*. [9] storage of BNC in humid environment leads to breaking of the hydrogen bonds between hydroxyl groups in polymer chains and the formation of new ones, between hydroxyl groups and water molecules. The result of this process is swelling of the material, and consequently, an increase in its wet mass. The changes of wet mass described above develop during long-term storage of the samples, and according to Chen *et al*. [11], may lead to the destruction of the ordered structure of the BNC. Shi *et al*. [9] showed that the swelling of the material in humid environments is the first step of the degradation process, which facilitates fragmentation of cellulose chains and leads to the formation of cellobiose or even glucose in the second stage of degradation, which manifests itself as a reduction of dry mass of BNC. Therefore, losses of BNC dry mass, during incubation in the SBF fluid in the presence of *A. fumigatus,* indicate an initialization of the second stage of biodegradation consisting of BNC fragmentation. In contrast, 6-months storage of this material in sterile PBS and SBF buffers, as well as in SBF fluid in the presence of *S. aureus* and *C. albicans*, reveals that only the first stage of degradation takes place and this probably leads to the destruction of an ordered structure of this polysaccharide.

Microbial growth and ability to form biofilms on BNC surfaces

The incubation of *S. aureus*, *C. albicans* and *A. fumigatus* was carried out in the SBF fluid in the presence or absence of BNC. The number of cells in populations of *S. aureus* (Fig. 4A) and *A. fumigatus* (Fig. 4C) did not change during 8 weeks of storage in SBF fluid without the BNC, while the population of *C. albicans* decreased about 1 log cycle after 4 weeks of incubation (Fig. 4B). In the presence of

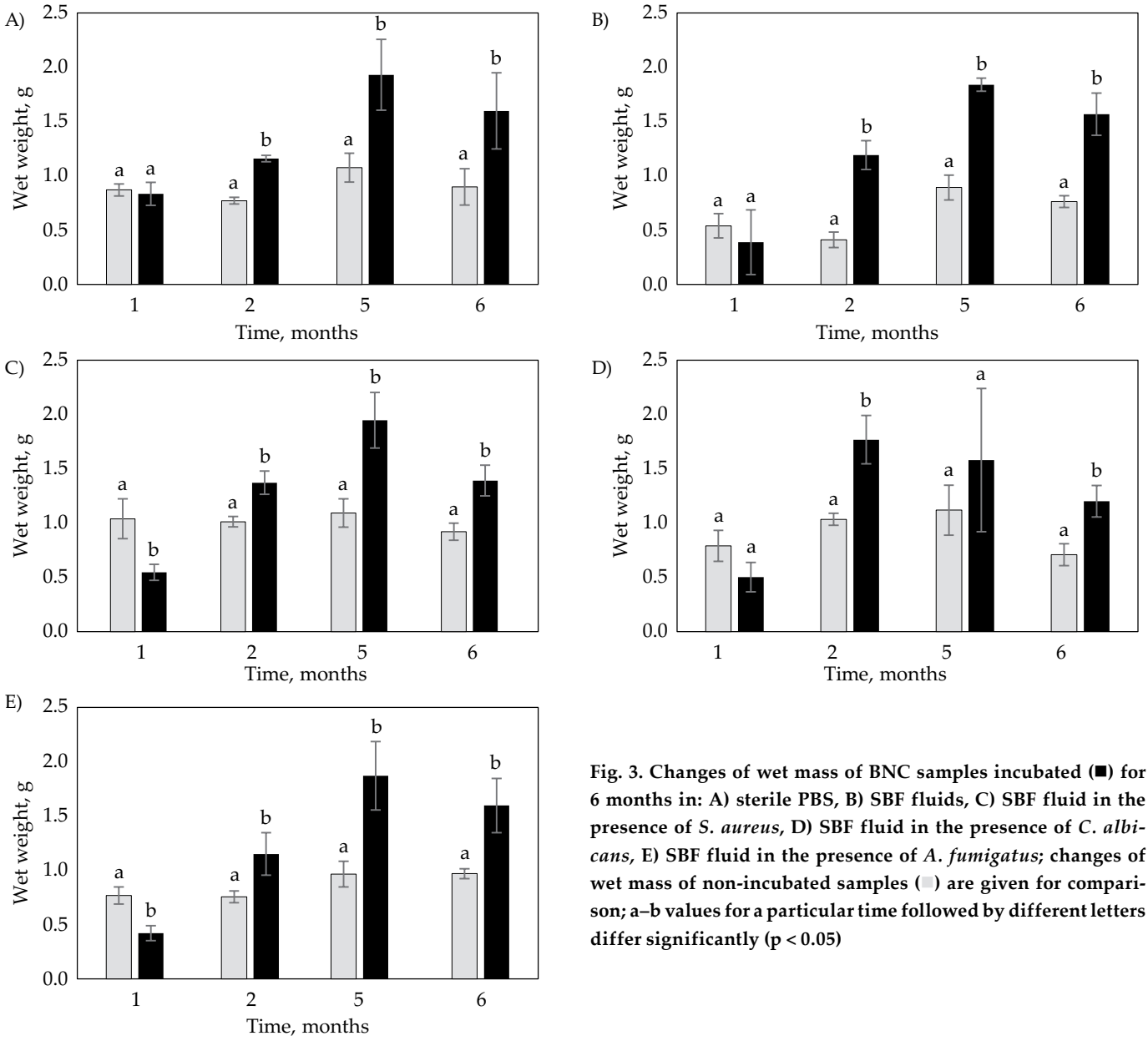

Fig. 3. Changes of wet mass of BNC samples incubated (■) for 6 months in: A) sterile PBS, B) SBF fluids, C) SBF fluid in the presence of *S. aureus*, D) SBF fluid in the presence of *C. albicans*, E) SBF fluid in the presence of *A. fumigatus*; changes of wet mass of non-incubated samples (■) are given for comparison; a–b values for a particular time followed by different letters differ significantly ($p < 0.05$)

BNC, the number of all microorganisms increased from about 3 log CFU/cm^3 to about 5 log CFU/cm^3 during the first month, and maintained this level for 6 months of storage (Fig. 5). Increasing the number of cells in microbial populations confirms their ability to hydrolyze BNC and use its degradation products as nutrients.

The ability of microorganisms to produce cellulases has been demonstrated in several papers [12–14]. Pourramezan *et al.* [15] isolated strains of *S. aureus* from the gastrointestinal tract of snails (*Archachatina marginata),* which showed the ability to decompose cellulose. However, this activity was lower compared to that of other types of bacteria such as *Bacillus* sp. or *Streptococcus* sp. [15, 16]. The cellulolytic properties of different yeast species, *i.e.*, from the genus *Candida* sp. were also observed [17, 18]. Moulds are well-known producers of cellulases that are used in the food industry to obtain fermented saccharides from cellulose [13, 19]. The high activity of cellulases from *A. fumigatus* is described in many papers [20–22].

S. aureus and *C. albicans* are able to metabolize cellulose but to a lesser degree than *A. fumigatus.* Amorphous cellulose regions are hydrolyzed faster than crystalline regions [23, 24]. The BNC has a high degree of crystallinity [25] and probably bacteria and yeasts slowly degrade the amorphous part, whereas moulds degrade both amorphous and crystalline regions. The presence of endo-1,4-β-glucanase is essential for the hydrolysis of cellulose's amorphous regions. The poorly-developed enzymatic system of *S. aureus* and the yeast *C. albicans* allows them only to biodegrade the amorphous form of BNC. In this case, a small amount of degradation products allows for the microorganisms growth, but the dry mass losses of BNC were not observed. Hydrolysis of crystalline regions requires the presence of both endo- and exo-1,4-β-glucanase [26]. Unlike bacteria and yeasts, moulds have a well-developed enzymatic system allowing for the decomposition of both forms of cellulose [22]. Therefore, the dry mass losses are visible in the case of the mould's activity.

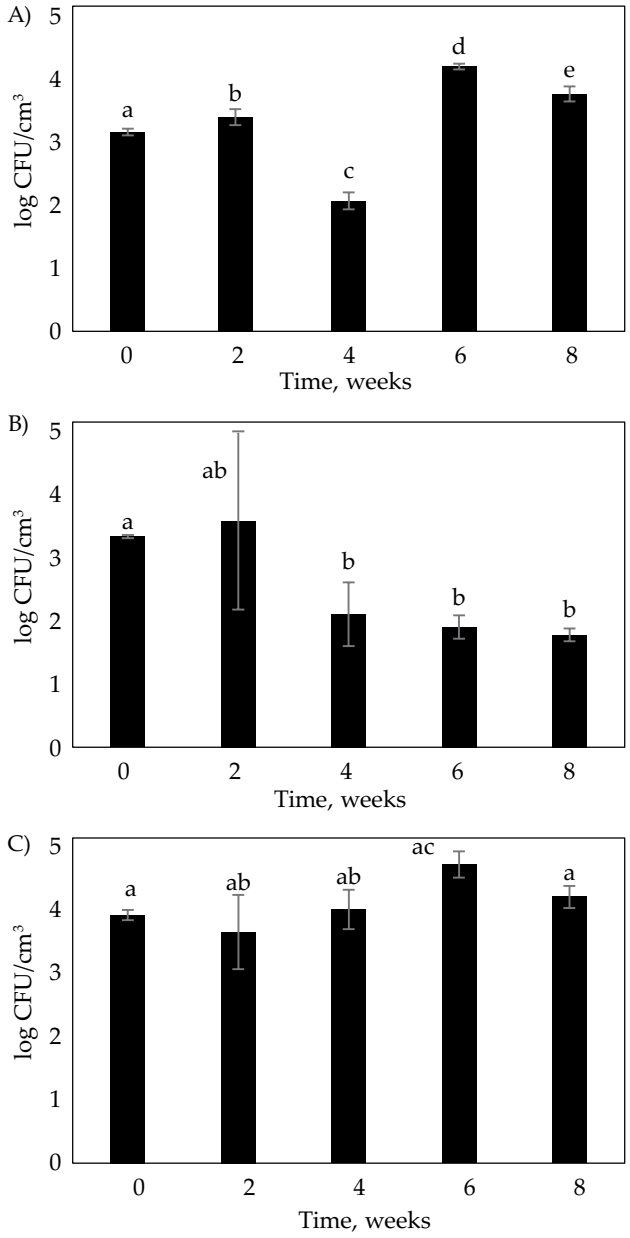

Fig. 4. Survival of: A) *S. aureus*, B) *C. albicans*, C) *A. fumigatus* during incubation for 8 weeks in SBF fluid without BNC; a–d values followed by different letters differ significantly ($p < 0.05$)

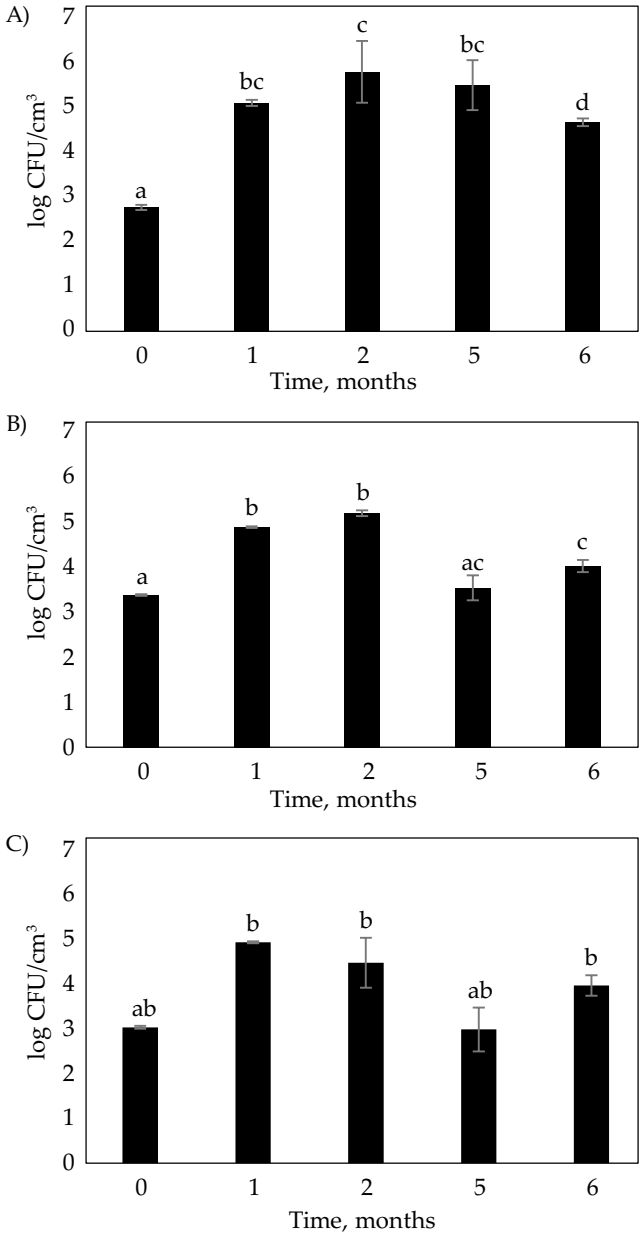

Fig. 5. Survival of: A) *S. aureus*, B) *C. albicans*, C) *A. fumigatus* during incubation for 6 months in SBF fluid in the presence of BNC; a–c values followed by different letters differ significantly ($p < 0.05$)

S. aureus, *C. albicans*, and *A. fumigatus* possess an ability to form biofilms on different surfaces [27–29]. However, it can be seen in Table 1 that bacteria and yeasts, in contrast to moulds, do not form a permanent biofilm on the BNC surface. Kaur and Singh [28] demonstrated that *A. fumigatus* extracellularly excretes a number of metabolites, like proteases, phospholipases and mycotoxins, which facilitate its colonization of different tissues. Nonetheless, exoglucanases are probably the most responsible for colonization of BNC [22]. These enzymes are not produced by bacteria and yeasts, but only by moulds.

T a b l e 1. Biofilm formation on BNC surfaces by selected microorganisms during incubation at 37 °C in SBF fluid for 6 months

Microorganism	Storage time, months			
	1	2	5	6
S. aureus	-	-	-	-
C. albicans	-	-	-	-
A. fumigatus	+	+	+	+

(+) – biofilm presence, (-) – biofilm absence.

The presence of saccharides in the SBF fluid after BNC incubation

No bands were found on the chromatograms obtained from the all analyzed post-incubation fluids, indicating the lack of BNC degradation products (data not shown). This may be caused by either a low sensitivity of the method or by consumption of the BNC hydrolysis products by growing microorganisms (Fig. 5). Therefore, all of the examined post-incubation fluids were concentrated 20-fold. The bands were visible only on the chromatograms of concentrated post-incubation fluids in which moulds *A. fumigatus* were present – indicating the occurrence of BNC degradation processes. The R_f values for these samples were lower than those corresponding to glucose and cellobiose standards, which probably reveals the presence of oligosaccharides in the tested samples, rather than mono- and disaccharides (Fig. 6). No products of BNC hydrolysis were detected in the other concentrated samples (data not shown).

The determination of the presence of BNC hydrolysis products in the post-incubation fluids is not a good method for checking the degree of polymer biodegradation, especially in the presence of microorganisms, due to their ability to metabolize the mono- and disaccharides produced during this process. Hu and Catchmark [30] using high performance liquid chromatography (HPLC), demonstrated the presence of glucose and cello-oligosaccharides in SBF fluid and PBS buffer after storage of BNC in the presence of cellulases. They carried out controlled BNC degradation by the addition of specified cellulases to incubation fluids, but in this study the cellulases were produced by the microorganisms. The determination of the type and concentration of enzymes produced in this way is not possible, due to the different metabolisms of the microorganisms.

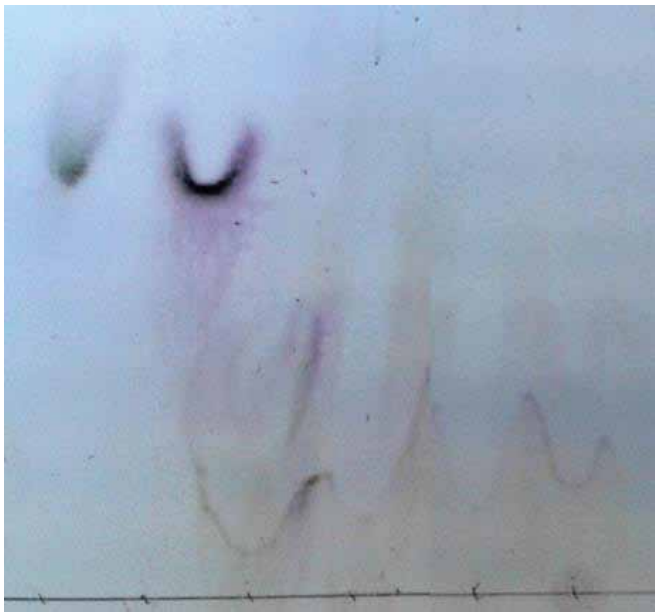

Fig. 6. A chromatogram of a 20-fold concentrated SBF fluid after BNC incubation in the presence of *A. fumigatus* for: 3 – 1 month, 4 – 2 months, 5 – 5 months, 6 – 6 months; 1 – glucose reference, 2 – cellobiose reference

Mechanical properties of BNC

Biomaterials dedicated to cardiovascular implants should have the appropriate mechanical properties. Hence, the changes in mechanical properties of the BNC, resulting from biodegradation, were determined. The tensile strength (σ) of the native BNC samples was *ca.* 5 MPa, and elongation at break (ε) – *ca.* 18 %. McKenna *et al.* [31] showed that BNC had a lower tensile strength value, equal to 1.5 MPa, and comparable elongation at break value, about 20 %. However, Backdahl *et al.* [32] demonstrated that the tensile strength and elongation at break of bacterial cellulose varied, depending on the culture conditions, moisture content of samples and the way of BNC preparation for measurements.

The mechanical properties of the native BNC changed after 1 month of storage (Table 2). The tensile strength of the samples incubated in sterile PBS and SBF fluids, as well as in SBF fluid in the presence *S. aureus*, decreased by *ca.* 60 %, and the tensile strength of the samples incubated

T a b l e 2. Changes in the tensile strength (σ) and elongation at break (ε) values of the BNC samples incubated for 6 months

Sample	1 month		2 months		5 months		6 months	
	σ, MPa	ε, %	σ, MPa	ε, %	σ, MPa	ε, %	σ, MPa	ε, %
Not stored	5.1 ± 1.20^{a}	18.2 ± 6.82^{ab}	5.1 ± 1.20^{a}	18.2 ± 6.82^{a}	4.1 ± 0.35^{a}	15.2 ± 3.80^{a}	5.1 ± 1.20^{a}	18.2 ± 6.82^{a}
Stored in:								
Sterile PBS	2.2 ± 0.49^{bd}	20.4 ± 3.28^{a}	2.5 ± 0.57^{b}	20.4 ± 3.28^{a}	2.4 ± 0.30^{b}	14.6 ± 3.87^{a}	2.8 ± 0.47^{b}	25.4 ± 5.39^{b}
Sterile SBF	2.3 ± 0.39^{bc}	21.4 ± 11.22^{a}	2.6 ± 0.27^{b}	22.8 ± 2.79^{a}	2.1 ± 0.28^{bc}	17.9 ± 2.80^{a}	2.0 ± 0.30^{bc}	19.9 ± 2.74^{ab}
Presence of *S. aureus*	2.9 ± 0.81^{b}	18.2 ± 2.33^{ab}	1.6 ± 0.44^{c}	22.6 ± 2.87^{a}	2.0 ± 0.25^{c}	14.6 ± 5.88^{a}	2.2 ± 0.51^{bc}	18.5 ± 3.64^{a}
Presence of *C. albicans*	1.5 ± 0.18^{cd}	17.6 ± 1.58^{ab}	1.5 ± 0.17^{c}	17.3 ± 2.03^{a}	2.0 ± 0.22^{c}	19.1 ± 5.09^{a}	1.7 ± 0.28^{c}	18.5 ± 2.50^{a}
Presence of *A. fumigatus*	1.3 ± 0.29^{d}	14.5 ± 1.76^{b}	nd	nd	nd	nd	nd	nd

nd – not determined,

[a–d] – values for a particular column followed by different letters differ significantly ($p < 0.05$).

in the presence of *C. albicans* and *A. fumigatus* by more than 70 %. In most cases, changes of elongation at break were statistically insignificant. Prolonged incubation of the samples resulted in no significant changes of both parameters, except for samples stored in the presence of *A. fumigatus*. These samples already disintegrated during their preparation for the determination of mechanical properties (Table 2).

Despite the deterioration of the mechanical properties during the incubation of samples in sterile PBS and SBF fluids for 6 months, the tensile strength of BNC was *ca*. 2 MPa. This value is similar to the mechanical strength of the natural human heart valves [33]. Therefore, despite the degradative changes of BNC occurring under conditions simulating human plasma, the BNC seems to be an appropriate material for cardiovascular implants.

CONCLUSIONS

During 6 months of incubation at 37 °C in sterile PBS and SBF fluids at pH 7.4, degradative changes in BNC did not occur. However, pathogenic microorganisms, *i.e*., *S. aureus*, *C. albicans* and *A. fumigatus*, can grow in SBF fluid during the incubation with BNC samples, which indicates their ability to hydrolyze cellulose and utilize the degradation products as a carbon source. Among the tested pathogenic microorganisms, the greatest cellulolytic, and simultaneously degradative, ability belonged to *A. fumigatus*. Additionally, these microorganisms form permanent biofilms on the BNC surface during the 6-month incubation. It was noted, that the most sensitive method of detection of BNC degradation, among all used, was the study of the mechanical properties of the samples. Less sensitive methods were the determination of wet mass of BNC and number of microorganisms in post-incubation fluids. Changes of BNC dry mass, estimation of saccharides by thin layer chromatography in post-incubation fluids and biofilm formation by microorganisms allow only the detection of late stages of biodegradation. In order to complete a full evaluation of the degradation changes occurring in the BNC under conditions simulating human plasma, it is necessary to study the structural properties, surface morphology and thermal stability of this polymer. The results of structural (X-ray diffraction, Fourier-transform infrared spectroscopy, scanning electron microscope) and thermal (thermogravimetric analysis) studies are currently being analyzed and will be discussed in a separate paper.

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