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Surface Biomodification of Surgical Meshes Intended for Hernia Repair

Abstract
The article presents a method of producing a composite surgical mesh. A synthetic, macroporous mesh with mechanical properties similar to that of the abdominal wall and with reduced surface density compared to conventional surgical meshes was made of polypropylene (PP) monofilament. The mesh was used as a substrate for a layer of modified bacterial cellulose (MBC) produced by a microbial synthesis with the use of the Acetobacter xylinum strain. The PP mesh was coated with the MBC layer directly in the process of biosynthesis in liquid culture medium with the addition of chitosan modifier. During the resorption process, under the action of body enzymes, amino sugars are released from the MBC layer, which have the ability to stimulate tissue granulation and accelerate the wound healing process, while preventing formation of scars.

Key words: *hernia, lightweight surgical mesh, semi-resorbable mesh, modified bacterial cellulose.*

Introduction

A hernia is an abnormal protrusion of an organ or its part outside the body cavity which normally contains it. The opening through which organs bulge outside the body cavity is called the hernia gate. Most frequently, hernias occur in the groin or the abdomen, and statistically in four cases of inguinal hernia there is one case of abdominal hernia

The only effective treatment of a hernia is a surgery. Nowadays, the methods, by which hernia repairs are performed can be divided into two general categories:

- Tension methods, which involve an approximation of tissue edges and stitching them together to close the hernia gate. In this type of repair, stitches or sutures exert tension on tissue on each side of the hernia gate in order to keep it closed.
- Tension-free methods, which involve an implantation of a prosthesis, which is to complement the defect in the fascia and close the hernia gate without causing a tension in the surrounding tissue.

The use of plastic surgical meshes, most often made of polyester, polypropylene or expanded PTFE, enables a significant decrease in the recurrence rate, even below 10%, while the tension operations pose a risk of recurrence of up to 50% [1].

The disadvantage of tension-free methods, especially for large abdominal hernias, is the patient's discomfort occurring a long time after implantation, which is associated with stiffening of the implant due to the ingrowth of a fibrous tissue. Another shortcoming is an introduction of a certain amount of alloplastic material into the patient's body, which is necessary to obtain sufficient strength of the implant, but which also prolongs the healing process and may trigger a foreign body reaction.

Among the most serious complications resulting from the use of surgical meshes are infections. When the pores in the mesh are smaller than 10 microns, bacterial cells with an average size of 1 micron can freely enter and colonize the mesh. On the other hand, neutrophilic granulocytes, and macrophages, because of their large size, cannot penetrate the pores and destroy the bacteria. This may be a potential source of secondary infection [2]. In such implants also histiocytes may accumulate and contribute to the rejection of the implant as a foreign body [3].

Macroporous implants facilitate direct contact of macrophages with bacteria to prevent the build-up of bacteria in the pores as well as promote rapid processes of fibroplasia and angiogenesis, which significantly reduces the infiltration and growth of bacteria [4].

A new, third generation of medical devices used for hernia repair are partially resorbable meshes [5, 6]. Their use results in a significant reduction in an intra-abdominal pressure and improves blood circulation in the tissues adjacent to the implant (mainly the peritoneum and the kidneys) [7, 8]. Non-resorbable meshes introduce a high risk of adhesions and fistulae when they are in direct contact with the abdominal organs (in 40-80% of cases), while in the case of partially or completely absorbable meshes those complications are rare, which is associated with a reduced risk of adhesion to the internal organs [2, 3]. The introduction of a completely resorbable meshes, unfortunately, contributed to an increased risk of hernia recurrence, which makes it necessary to carry out subsequent operation, this time with the use of non-resorbable implant.

Bacterial cellulose is an interesting, modern material widely applied in medicine. It is produced by bacteria, such as of the *Acetobacter* genus. It is secreted outside the cells in the form of fibrils, which are combined in ribbon-shaped nano-fibres forming a three-dimensional network. Bacterial cellulose in contrast to the plant cellulose is free from lignin, pectin and hemicellulose. An important characteris-

Table 1. *Characteristic of chitosan oligomers (manufacturer data).*

Percentage of fraction	
monomer	0
dimer	2.40
trimer	12.49
tetramer	17.90
pentamer	15.55
hexamer	9.78
heptamer	3.73
octamer	1.54
nanomer	1.43

Table 2. *Physical-mechanical parameters of Dallop M/MN mesh.*

Parameter	Value
Surface density, g/m^2	53.1 ± 1.4
Puncture resistance, N	1486 ± 77
Suture pull out strength, N	33.8 ± 7.8
Breaking force in the longitudinal direction, N	360 ± 25
Breaking force in the transverse direction, N	82 ± 5

tic of the bacterial cellulose is its ability to be shaped in the place of its formation (in situ).

Bacterial cellulose may be used as a component of dietary foods [9], as membranes for electro-acoustic transducers [10], as a filtration material, extremely strong paper [11], or in the form of a suspension having coating, binding and thickening properties [12].

A number of applications of bacterial cellulose in medicine and veterinary medicine have also been reported [13 - 16]. Many advantages of bacterial cellulose have been observed, such as: fast pain relief, good adaptation to the injured place, less discomfort for patient after surgery, low risk of secondary infection, ease of observation of the healing progress due to its transparency, acceleration of the healing process, high ability to absorb exudates, easy dressing removal after regeneration of epithelium cells, reduction in treatment time and cost.

The results of previous work carried out at the Institute of Biopolymers and Chemical Fibres [17, 18] and literature data [19], indicate that the bacterial cellulose modified by chitosan or its oligomers, is a biocompatible biomaterial, which is not cytotoxic or genotoxic, causing no irritant and allergic reaction, exhibiting no acute toxicity and integrates well with the living tissue. Based on studies [18], the ability has been shown to produce vascular prostheses of bacterial cellulose modified directly during its synthesis as well as to carry out the synthesis of cellulose on a pleated synthetic carrier or on a commercial unsealed vascular prosthesis produced by knitting. Controlling the amount of glucosamine and N-acetylglucosamine in bacterial cellulose it is possible to prepare biomaterials with new properties. For example, a suitable amount of N-acetylglucosamine in the bacterial cellulose makes it susceptible to hydrolytic action of lysozyme in the body and results in a biodegradable material [20].

The aim of the study was to develop a new type of composite hernia mesh combining several advantages. In order to minimize the amount of alloplastic material introduced into the patient's body and to reduce the risk of secondary infection, a non-resorbable, macroporous, light (weighing less than 80 g/m^2) polypropylene knitted mesh was applied as a base material. The use of a layer of modified bacterial cellulose (MBC) synthesized on a polypropylene mesh was assumed in order to reduce the risk of adhesion to the internal organs, and the formation of fistulas and scar tissue in the implant.

Materials

Bacterial strain

In the study the *Acetobacter xylinum* strain (CCM 2360) obtained from the Czech Collection of Microorganisms was used. The strain was stored in a liquid nutrient medium containing 3% yeast extract, 0.3% calcium carbonate, 3% ethanol.

Culture medium

In the biosynthesis process of modified bacterial cellulose the Hestrin-Schramm (HS) culture medium [21] was used, which contained (in 1000 cm^3):

- glucose (Hasco-Lek S.A., Poland) 20.0 g,
- yeast extract (Becton Dickinson, USA) 5.0 g
- Bacto™ Soytone (Becton Dickinson, USA) 5.0 g
- disodium phosphate, anhydrous (POCh S.A., Poland) 2.7 g
- citric acid 1-hydrate (POCh S.A., Poland) 1.2 g
- 96% ethanol (PPS Polmos Warszawa S.A., Poland) 20.0 cm^3

Chitosan modifier

The culture medium was modified by 2 - 60 g per 1000 cm^3 of Chito Oligo-100 chitosan oligomers (Amicogen, Korea) with the characteristic presented in ***Table 1***. The chitosan modifier was selected on the basis of previous studies [15, 16].

Synthetic carrier

Prototype surgical mesh Dallop M/MN produced by Tricomed S.A. Lodz, Poland was used in the study as a carrier for a bacterial cellulose layer. The mesh was made of polypropylene (PP) monofilament using a knitting technique. It was characterized by a high macro-porosity and a low surface density. According to the declaration of the manufacturer the mesh meets the chemical purity requirements for surgical meshes. ***Table 2*** shows the physical-mechanical parameters of the mesh, as declared by the manufacturer.

Methods

Microbial synthesis of MBC

Biosynthesis of modified bacterial cellulose was carried out in sterile flat-bottomed glass vessels in a HS culture medium modified by Chito Oligo-100 chitosan oligomers, in amounts of 2 to 60 g per 1000 cm^3. The culture was carried out for 2 to 7 days at 30 °C in an incubator. The obtained pellicles were washed with distilled water until the complete removal of culture medium components (water conductivity after washing <20 μS). Next, the modified cellulose pellicles were soaked in 1% NaOH and autoclaved at 121 °C for 15 min. After that the pellicles were washed with distilled water until neutral pH and conductivity <20 μS.

Microbial synthesis of MBC on a synthetic carrier

Biosynthesis of modified bacterial cellulose on DallopM/MN PP mesh was carried out in sterile flat-bottomed glass vessels in a HS culture medium modified by Chito Oligo-100 chitosan oligomers in the amount of 60 g per 1000 cm^3. PP mesh was sterilized in 70% ethanol, placed at the bottom of the vessel and a liquid culture medium was added. The culture was carried out for 2 to 5 days at 30 °C in an incubator, either on one or on both sides of the mesh. The PP car-

rier pre-treatment was carried out using three methods: UV-C irradiation by a wavelength of 366 nm during 6 hours before biosynthesis, autoclaving at 121 °C for 15 minutes with direct contact with steam and low-temperature plasma treatment - one day before biosynthesis. Surface modification of the PP mesh by low-temperature plasma was carried out at the Department of Mechanical Engineering, Lodz University of Technology, under the following conditions: air flow of 5.7 cm^3/min, pressure of 15 Pa, discharge power of 20 W and frequency of 14 MHz. After separation from the culture medium the meshes covered in MBC were purified by the same method as used for the purification of MBC pellicles.

Assessment of MBC susceptibility to hydrolytic and enzymatic degradation

Susceptibility of MBC to degradation was evaluated at in vitro conditions, using as a medium a phosphate buffer (pH 7.23) - in the case of hydrolytic degradation, and a solution of lysozyme in phosphate buffer - in the case of enzymatic degradation. Prior to degradation test the samples in phosphate buffer at a ratio of 0.1 g/100 mL were steam sterilized (121 °C, 15 min). A solution of lysozyme at a concentration of 4400 U/ml was added under sterile conditions to the samples intended for enzymatic degradation. The degradation progress was assessed by the amount of amino sugars released after 14 days of incubation at 37 °C. Amino sugars content in the buffer solution and in the enzyme solution was determined by a colorimetric method with 3,5-dinitrosalicylic acid (DNS) using a calibration curve prepared for amino glucose in the assay conditions [22].

Analytical methods

Evaluation of structure and properties of MBC

The α-cellulose content in MBC samples was determined according to the Polish Standard PN-50099-62P.

GPC analysis was carried out according to the Turbak [23, 24] procedure using HP 1050 (Hewlett Packard, USA) apparatus equipped with a HP 1047A refractometric detector and a set of columns with a rigid hydrophilic packing material based on a polymeric gel. Samples were prepared for the analysis according to the modified Ekmanis method [25].

The analysis of the crystal structure of MBC was performed by wide-angle X-ray scattering using URD-6 diffractometer (Seifert) in a reflection mode. MBC pellicle was placed in a holder enabling the appropriate thickness of the sample. In the case of the composite MBC/PP surgical mesh only a cellulose layer was examined. WAXS measurements were made in the angular range of 4 - 60 degrees, in 0.10 degrees steps and counting time of 15 seconds. Diffraction curves were separated into amorphous and crystalline components. The degree of crystallinity was calculated using the modified Hindelech-Johnson method. Also the crystallite sizes were calculated.

Morphological structure analysis of MBC and composite MBC/PP surgical mesh was performed by scanning electron microscopy (SEM) using a JSM-5500LV microscope (Jeol, Japan), equipped with a tungsten electron source. Test samples were coated with a layer of gold using a JFC 1200 sputter coater (Jeol, Japan). Sputter coating operation was carried out under a vacuum of 0.003 Pa. Microscopic observations were carried out with the accelerating voltage of 15 kV. Observations were made at various magnifications depending on the nature of the sample, from the magnification of 100×, in the case of a PP mesh to the magnification of 20 000× in the case of a well-defined MBC.

Physical and mechanical properties of composite surgical meshes were evaluated according to the relevant standards:

- Width and length - PN EN 1773:2000
- Surface density - PN EN 12127:2000
- Puncture resistance - PN-EN ISO 12236:2007
- Suture pull out strength - ISO 7198:1998
- Breaking force, elongation at max. force in the transverse and longitudinal directions - PN-EN ISO 13934-1:2002

Results and discussion

Studies on the biosynthesis of MBC in a culture medium with chitosan oligomers

In the studies related to the biosynthesis of MBC, the Acetobacter CCM 2360 strain was used. In the first stage of research, the cultures were performed un-

Table 3. *Biosynthesis yield of 7-day culture and selected properties of bacterial cellulose modified by chitosan oligomers with different concentrations.*

Concentration of chitosan oligomers in the culture medium, %	Biosynthesis yield, g/dm³	Alpha-cellulose content, %	Surface density, g/m²
0.2	5.45	98.32	24.22
0.5	4.89	96.11	21.73
1.0	4.68	94.94	20.80
2.0	4.60	93.12	20.44
6.0	4.27	89.66	18.98
9.0	1.15	87.80	5.11

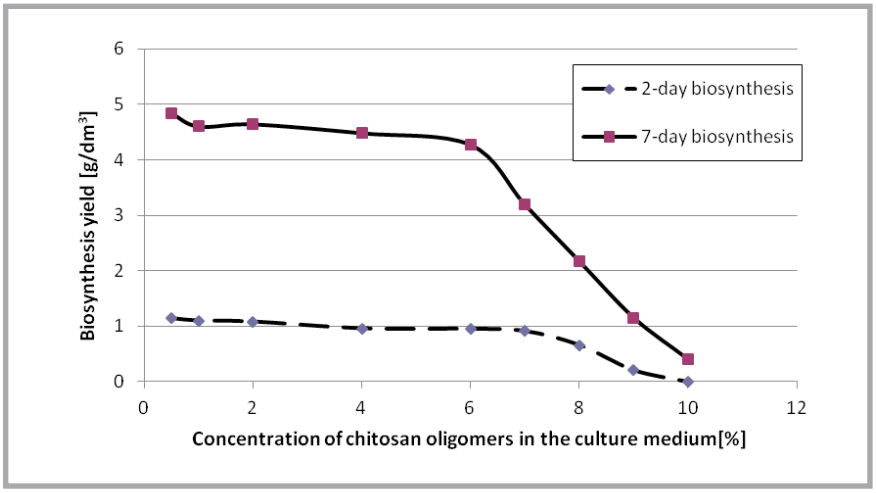

Figure 1*. Influence of chitosan oligomers concentration in the culture medium and the time of culture on the yield of MBC biosynthesis.*

Table 4. *Influence of chitosan oligomers concentration in the culture medium on hydrolytic and enzymatic degradation of MBC.*

Concentration of chitosan oligomers in the culture medium, %	Hydrolytic degradation	Enzymatic degradation
	Released amino sugars, mg/g MBC	
0.2	0	7.7
0.5	0	9.0
1.0	0	10.6
3.0	0	12.3
6.0	0	13.9
9.0	0	15.2

Table 5. *Molecular characteristic of unmodified bacterial cellulose and MBC modified with chitosan oligomers at a concentration of 0.2 wt% and 6 wt% in the culture medium.*

Concentration of chitosan oligomers in the culture medium, wt%	$\bar{M}n$, g/mol	$\bar{M}w$, g/mol	$\bar{M}w / \bar{M}n$	$\bar{D}Pw$	Percentage of DP fraction, %		
					DP<200	200<DP<550	DP>550
0	173000	344000	2.0	2.12	1	9	90
0.2	163000	321000	2.0	1.98	2	10	88
6.0	161000	305000	1.9	1.88	2	10	88

Table 6. *Crystallinity degree and crystallite size, defined by WAXS, for unmodified bacterial cellulose and for MBC modified with chitosan oligomers at a concentration of 6wt% in the culture medium.*

Sample	Crystallinity degree, %	Crystallite size, nm	
		D1	D2
Unmodified bacterial cellulose	74.6	6.15	6.12
MBC modified with chitosan oligomers at a concentration of 6wt% in the culture medium	72.7	6.22	6.15

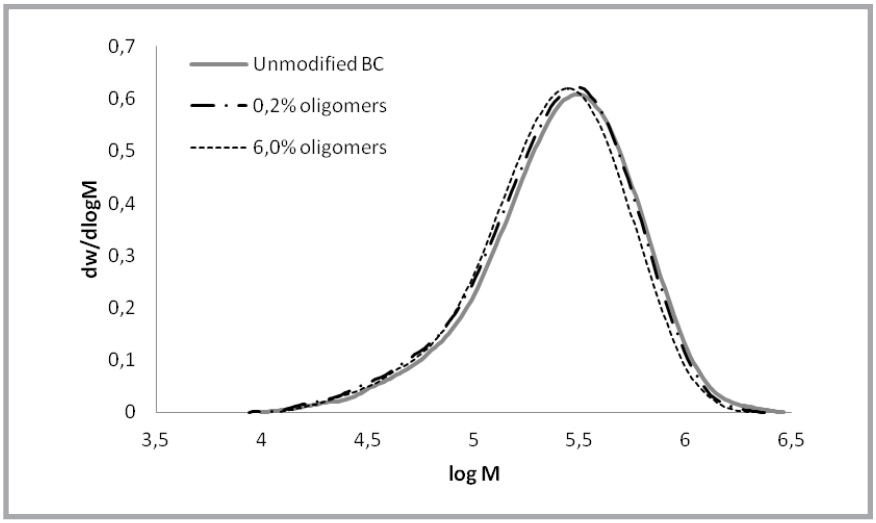

Figure 2. *Molecular weight distribution curves of unmodified bacterial cellulose and MBC modified with chitosan oligomers at a concentration of 0.2 wt% and 6 wt% in the culture medium.*

der stationary conditions for 7 days at 30 °C. Chito Oligo-100 chitosan oligomers were used as a modifier at concentrations of 0.2 to 9.0 wt%. The results are shown in ***Table 3.***

In the next stage, the impact of chitosan oligomers concentration in the culture medium and the cultivation time on the yield of MBC biosynthesis process was studied.

The use of a biopolymer with the structure required to maintain the quality parameters of partially resorbable mesh was assumed in the study on the composite surgical mesh. the biopolymer layer should provide good adhesion to the synthetic carrier and maintain a low surface density that is characteristic for "light" meshes. Therefore, the cultivation time was reduced to two days (***Figure 1***). It was found that the amount of MBC obtained in a 2-day culture is sufficient to uniformly cover the polypropylene mesh.

Samples of bacterial cellulose modified by chitosan oligomers at a concentration in the culture medium of 0.2 – 6 wt%, were subjected to hydrolytic and enzymatic degradation in order to determine the amount of the released amino sugars (biologically active substances). MBC samples were subjected to 14-day tests of: hydrolytic degradation (in a phosphate buffer) and an enzymatic degradation (in a lysozyme solution of activity corresponding to 4400 U/cm^3). The results of degradation as measured by concentration of released amino sugars are shown in ***Table 4***.

Chitosan oligomers at a concentration of 6 wt% in the culture medium were chosen as a modifier in the further experiments. It was the highest concentration to achieve both the high yield of MBC biosynthesis and the high susceptibility to enzymatic degradation.

To evaluate the morphology and structure of MBC, gel permeation chromatography (GPC), Wide-Angle X-ray Scattering (WAXS) and scanning electron microscopy (SEM) were used. This study was to compare the molecular structure of the MBC obtained with the use of chitosan oligomers in the culture medium:

- at a concentration of 0.2 wt% - the highest biosynthesis yield,
- at a concentration of 6% wt% - the highest concentration of the modifier in the culture medium, for which both the highest biosynthesis yield of 4.3 g/dm^3 and a high content of chitosan modifier in cellulose was obtained.

Figure 2 shows differential curves of the molecular weight distribution (MWD) for the following samples: unmodified bacterial cellulose and MBC modified with chitosan oligomers at a concentration of 0.2 wt% and 6wt% in the culture medium. All the curves have similar shapes. A slight reduction in the average molecular weight with the higher concentration of chitosan modifier was observed. The weight-average molecular weight Mw of unmodified bacterial cellulose was approximately 344000 g/mol, while of MBC modified with 6 wt%. oligomers in the culture medium was 305000 g/mol. Differential MWD curves of the MBC samples modified with 0.2 wt% and 6 wt% chitosan oligomers in the culture

medium are slightly shifted in the direction of lower average molecular weights in relation to the unmodified bacterial cellulose. All differential MWD functions have a uniform molecular weight distribution, as evidenced by the polydispersity ratio Mw/Mn of ≅ 2.0.

X-ray scattering curve recorded for samples of MBC modified with chitosan oligomers at a concentration of 6 wt% in the culture medium is shown in ***Figure 3***. Calculated values of the degree of crystallinity and crystallite sizes are presented in ***Table 6***.

Diffractograms recorded for samples of unmodified bacterial cellulose and MBC modified with chitosan oligomers at a concentration of 6 wt% in the culture medium show patterns typical for cellulose I, with a crystallinity degree above 70%. Diffraction curves are dominated by two strong peaks, whose angle positions are approximately at 15 and 23 degrees (***Figure 3***). For all tested samples, these peaks have the greatest intensity of the peaks characteristic for cellulose I. Therefore, the crystallite sizes were determined from the analysis of both peaks. In ***Table 6***, they are marked respectively as D1 and D2.

SEM photographs of MBC modified with chitosan oligomers at a concentration of 6 wt% in the culture medium are shown in ***Figure 4***.

Gradually increased magnification allows observing morphological details of the sample. At a magnification of 2500×, the surface of the sample appears to be quite smooth with few visible fibres (***Figure 4.a***). At a magnification of 5000× (***Figure 4.b***) the layout of individual nanofibres of uniform thickness can be seen in the order of several hundreds of nanometres, isotropically arranged relative to each other to form a mesh. Occasionally, increased fibre density at the surface and adhesion of adjacent elementary fibres was observed. Further magnification of 10000× and 20000× (***Figures 4.c*** and ***4.d***) made it possible to estimate the thickness of the filaments at approximately 100 nm.

Studies on the biosynthesis of the modified bacterial cellulose on a fibrous polypropylene carrier

The aim of this study was to evaluate the possibility to modify the surface of the

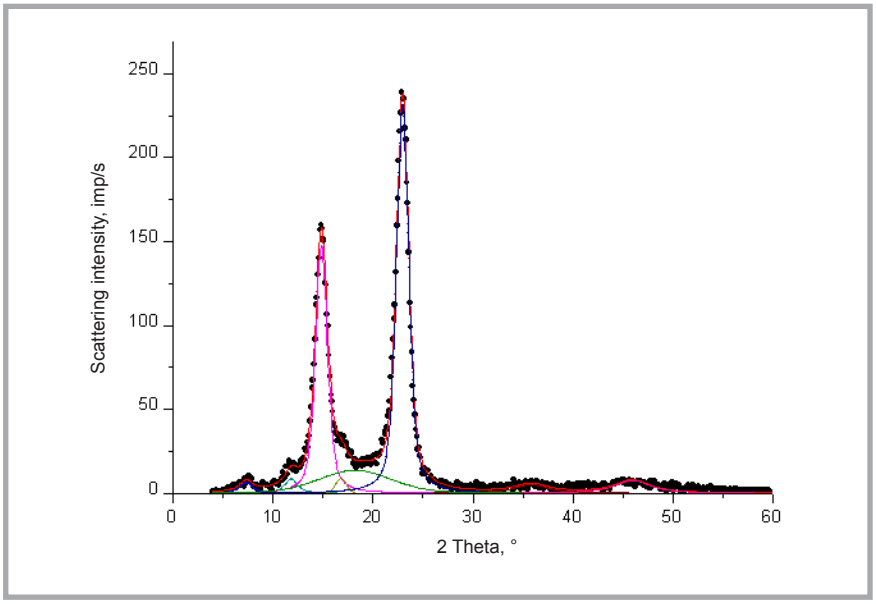

Figure 3. *WAXS curve for a sample of the MBC modified with chitosan oligomers at a concentration of 6 wt% in the culture medium (the curve is also representative for samples of unmodified bacterial cellulose).*

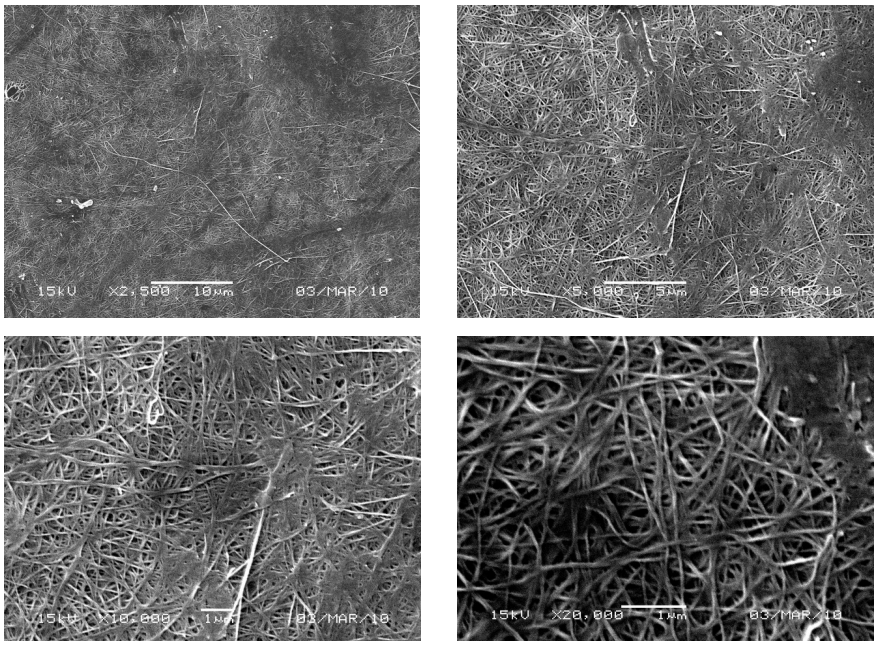

Figure 4. *SEM photographs of modified bacterial cellulose at magnification of: a) 2500×, b) 5000×, c) 10000×, d) 20000×.*

synthetic surgical mesh Dallop M/MN during the biosynthesis of MBC. The biosynthesis was carried out for 2 days at 30 °C, with the use of the *A. xylinum* CCM 2360 strain in HS culture medium containing 6 wt% of chitosan oligomers. The study was performed taking into account the standard conditions that have been developed in previous research on a biosynthesis of modified bacterial cellulose. One of the parameters affecting the biosynthesis process was the amount of the culture medium. The amount of the culture medium was in the range of 0.7 - 2.2 dm^3 per 1m^2 of the mesh area. The obtained results are shown in ***Figure 5***.

The biosynthesis yield and MBC surface density are directly proportional to the amount of the culture medium (***Figure 5***). Extension of biosynthesis period does not significantly influence the yield of the process due to the utilization of the whole culture medium by the microorganisms during the process. In the case of experiments with the use of culture medium in the range of 0.7 - 0.9 dm^3 per 1 m^2

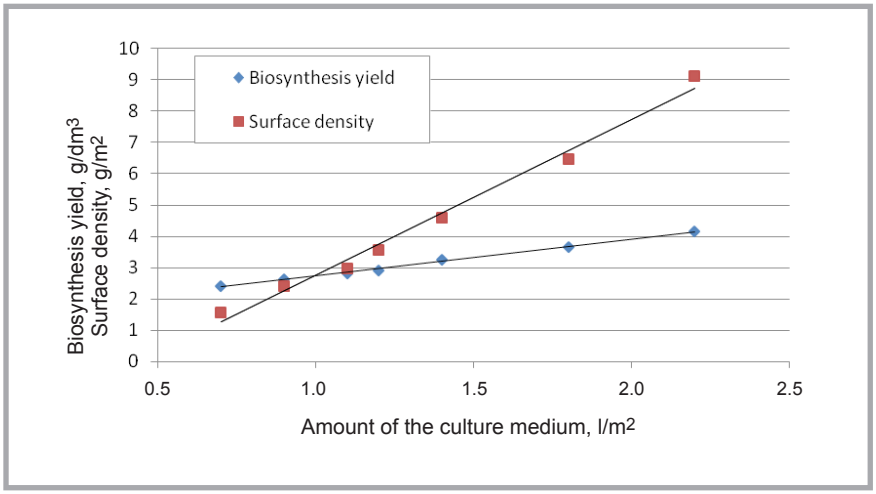

***Figure 5**. Influence of the depth of the culture medium in a biosynthesis vessel on MBC yield and surface density.*

***Table 7.** Biosynthesis yield and the surface density of MBC layer.*

Mesh covered	Biosynthesis yield, g/dm^3	Surface density, g/m^2
on one side	3.67	6.47
on both sides	3.01	5.31

***Table 8.** Peel off strength of the composite MBC/PP mesh.*

Peel off strength	Unmodified PP mesh	Pre-treated PP mesh		
		UV	121 ºC	Cold plasma
in the longitudinal direction, %	100	195.07	154.93	85.92
in the transverse direction, %	100	197.87	136.17	118.09

***Table 9.** Selected strength properties of the PP mesh.*

Parameter	Before UV treatment	After UV treatment
Puncture resistance, N	1426	1420
Breaking force in the longitudinal direction, N	370	360
Breaking force in the transverse direction, N	80.2	87.4

of the mesh, a poor growth of MBC was observed resulting in an uneven MBC layer covering the mesh. Increasing the amount of the culture medium to 1.8 dm^3 per 1m^2 of the mesh resulted in an even growth of MBC on the whole surface of the mesh, and the MBC film well adhered to the polypropylene carrier. The use of larger amounts of the culture medium (2.0 - 2.2 dm^3/m^2) also resulted in a uniform growth of MBC on the mesh, but in 10% of experiments with 2.0 dm^3/m^2 and 30% of experiments with 2.2 dm^3/m^2 the MBC layer had poor adhesion to the PP carrier.

Also, the experiment was performed using the culture medium in an amount of 1.8 dm^3/m^2 and the biosynthesis period of 1 day, then the mesh was turned upside down and the process continued for another 1 day, in order to cover the other side of the mesh. A mesh coated on both sides was obtained with the surface density of the MBC layer about 18% less compared with the mesh coated on one side. The results are shown in ***Table 7***.

Some problems observed during the study and associated with the adhesion of MBC to the PP mesh probably arose due to the fact of insufficient adhesion of microbial cells, and as consequence, the MBC layer to the PP substrate. Attempts have been made to improve the adhesion by surface modification of the mesh before the biosynthesis process. Three different methods were applied: UV treatment, heat treatment at 121 °C in a steam autoclave and low-temperature plasma treatment.

All the methods of treatment affect the changes in the structure of the surface layer of the PP mesh, by creating macroradicals that under the influence of atmospheric oxygen gives rise to peroxide, carboxylic, ketone and hydroxyl groups [26, 27]. This increases the contact angle of the material, but does not affect its structure or strength parameters. In most cases, these processes are reversible, so it is important that the treatment should be performed directly before the process of biosynthesis. In most cases, MBC layers formed during biosynthesis on the pretreated meshes exhibited higher adhesion to the PP substrate. The increase in adhesion was assessed by measurement of the peel off strength of MBC layer. For the measurement tests, the composite surgical meshes were used, which were obtained in a 2-day biosynthesis of MBC using the culture medium in an amount of 1.8 dm^3 per 1m^2 of the mesh, at a temperature of 30 °C. The results are shown in ***Table 8.***

The best method of surface modification of PP mesh was UV irradiation. The results of the measurements of selected strength parameters before and after UV treatment are shown in ***Table 9***.

The tests showed that UV radiation does not adversely affect the strength parameters of the PP mesh, which fall within the range declared by the manufacturer and are comparable to the values measured prior to UV treatment.

Based on the study, chitosan oligomers at a concentration of 6 wt% in the culture medium were selected as the optimal modifier, while a 2-day biosynthesis carried out at 30°C using culture medium in amount of 1.8 dm^3 per 1m^2 of the mesh was considered as the optimum conditions of the process.

Biomodified surgical mesh obtained in this study comprised PP mesh and attached to it the MBC layer, in which the moisture content, although dependent on the pressing degree of MBC, could be as high as 99%. This form, however, is not favorable in terms of its medical application due to limited surgical handiness, large changes in weight, depending on the hydration degree of the MBC layer, and problems with choosing a proper packaging for the sterilization process. On the other hand, the MBC layer of the composite mesh cannot be completely dry, since in the drying process an excessive shrinkage and separation from the PP mesh takes place. Dry MBC layer is also less flexible and more susceptible to damage. Attempts were made to modify

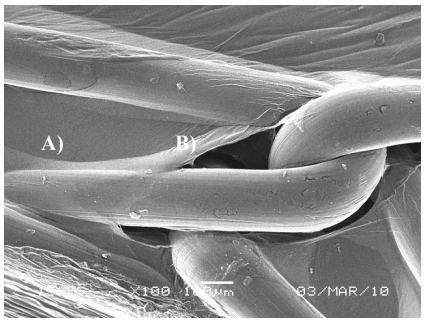

Figure 6. *View of a stitch of the PP monofilament (100×) coated with MBC layer; A) - boundary area exposed by sample cutting, B) MBC layer covering the mesh.*

the MBC layer by immersing the composite mesh for 24 hours in 10 or 15% glycerol solution and drying at 40 °C. Composite mesh immersed in a 10% glycerol solution remained flexible after drying and showed good surgical handiness. The sample immersed in 15% glycerol solution showed a much greater surface density and gave the feeling of too high a stickiness.

The surface density of composite meshes immersed in 10% glycerol was determined. For the chosen biosynthesis variant (using 1.8 dm^3 per 1 m^2 of the mesh), the final surface density of the composite mesh was about 170 g/m^2. They were also the percentage of the individual components of the composite. The PP substrate accounted for 31.5 wt%, water for 10 wt% and glycerol for nearly 50 wt% of the total weight of the composite mesh.

Mechanical properties and performance of the selected composite surgical mesh were also assessed. On the basis of the results obtained it was found that covering the PP mesh with an MBC layer does not significantly change the implant strength parameters. Despite the introduction of MBC material, elasticity of the composite mesh did not decrease and allowed proper fit to hernia repair. Elasticity of the meshes was evaluated by measuring their flexural rigidity, which, in the longitudinal direction was 0.052 mN/m for PP mesh, and 0.096 mN/m for composite mesh, while in the transverse direction it was identical for both types of meshes and amounted to 0.048 mN/m

Figures 6 - 9 show SEM photographs of the composite MBC/PP surgical mesh.

General view (***Figure 6***) shows a fragment of polypropylene knitted mesh coated with an MBC layer. In the places where stitches of PP monofilament interlace (***Figure 7***), a minor discontinuity of the MBC layer was observed. Apart from these points the MBC layer covers both the PP mesh surface and the spaces between the PP filaments. In ***Figures 8*** & ***9*** elementary nanofibres of modified bacterial cellulose can be seen.

Conclusions

1. A method was developed for the biosynthesis of modified bacterial cellulose susceptible to partial enzymatic degradation by lysozyme - an enzyme found in body fluids. Based on the study, as a modifier with the best ability to degrade, chitosan oligomers were selected in a concentration of 60 g per 1 dm^3 of HS culture medium.
2. An improved degradation of modified bacterial cellulose obtained was possible by increasing the content of chitosan oligomers and due to a lower crystallinity and polymerization degree, compared to the unmodified bacterial cellulose or MBC synthesized in a medium with a lower content of oligomers.
3. A method for bio-modification of PP surgical mesh with a layer of MBC directly in the biosynthesis process was developed. One- or two-side coated composite surgical meshes were obtained with different surface density depending on the amount of the culture medium used.
4. In order to maintain sufficient elasticity of the MBC layer and functional properties of surgical meshes obtained, they were immersed in 10 wt% glycerol and dried at 40 °C for 4 hours. Surface density of the composite surgical mesh obtained in selected conditions was approximately 170 g/m^2 (including MBC layer, water and glycerol). Studies on sterilization methods, biocompatibility and biomedical research of developed

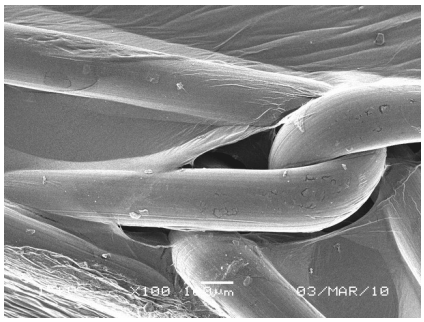

Figure 7. *View of composite mesh (100×). Around interlacing PP monofilaments a discontinuity of the MBC layer is visible.*

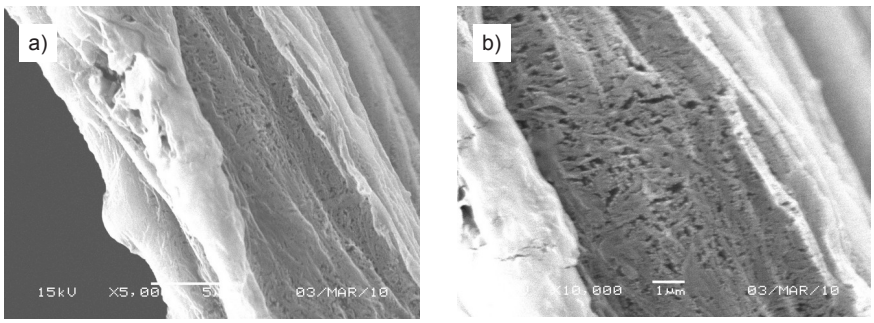

Figure 8. *View of the 'A' area of MBC layer; a) magnification 5000×, b) magnification 10000×.*

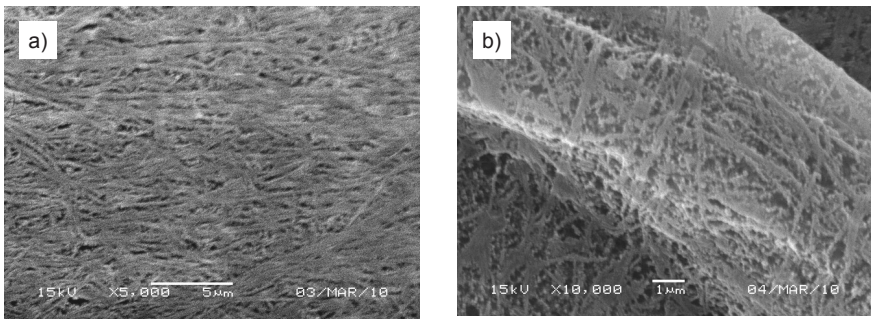

Figure 9. *View of the 'B' area of MBC layer. a) magnification 5000×, b) magnification 10000×.*

composite hernia meshes shall be the subject of another paper.

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