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Biosynthesis of Modified Bacterial Cellulose in a Tubular Form

Abstract
Presented herein is a method to prepare tubes of modified bacterial cellulose immediately in the course of biosynthesis conducted with Acetobacter sp. The biosynthesis was carried out under both static and dynamic conditions. The material of the tubes obtained was tested and assessed with respect to its structural, physical-mechanical and biological properties, as well as its chemical purity. The testing witnessed a high biocompatibility of the modified bacterial cellulose, essential for the material in blood vessel prostheses. It was found that the mechanical properties of the tubes can be improved by applying a polyester plaited carrier in the course of the dynamic synthesis of the modified cellulose.

Key words: *modified cellulose, biosynthesis, vascular prostheses.*

Introduction

Defects of the cardiovascular system which require surgical repair are a serious social problem, affecting about 0.5% of the population. About 20 000 cardiovascular operations a year are carried out in Poland, resulting from atherosclerosis, injuries and aneurysms. Vessel prostheses, made mainly of polyester woven or knitted fabrics, are employed in about 50% of the treatments. The percentage is higher in the US, reaching 75%.

Over the last 50 years, a wide array of synthetic polymers has been harnessed for the manufacture of vessel prostheses: poly-(vinyl alcohol), polyamide, polyacrylonitrile, and polytetrafluoroethylene [1]. American, German and British companies lead the way in the manufacture of woven and knitted vessel prostheses made of poly-(ethylene terephtalate) and polytetrafluoroethylene, offering unsealed and sealed prostheses in which the sealant constitutes either bovine cross-linked gelatine or bovine collagen. Tricomed Co, the sole Polish producer, offers knitted vessel prostheses of poly-(ethylene terephtalate) in unsealed and sealed (porcine gelatin) version. Virtually all manufacturers of the vessels employ animal-derived substances as sealants.
Three kinds of artery prostheses are used in clinical surgery nowadays [2]:

- biological - autogenic (patient's own veins or arteries) and allogenic (e.g. modified umbilical cord veins), Allogenic transplants are only used in extreme cases and after suitable modification (treatment with DMSO and freezing)
- semi-biological – prostheses are built up of a non-resorbable synthetic matrix superficially impregnated with a resorbable polymer like gelatin derivatives, human albumin and collagen
- synthetic - from poly-(ethylene terephtalate) or expanded polytetrafluoroethylene.

High quality demands are placed on vessel prostheses. For many years after the implantation the prosthesis material should resist degradation caused by the environmental conditions of the human body, mainly the temperature and presence of enzymes and body fluids. No genotoxic action of the prosthesis upon the tissue surrounding the implant is allowed nor an allergenic action upon the patient's organism. The material ought to be flexible and elastic, easing adjustment to the vessels repaired. Artificial implants, particularly those which come in direct contact with blood, must be haemocompatible, resistant to internal stress caused by blood pressure, and impermeable to blood elements. The inner surface of a vessel prosthesis cannot cause the adhesion of haemocytes and ought to be covered with endothelium cells, while connecting tissue should cover the outer surface.

Experimental investigations have shown that implants of poly-(ethylene terephtalate) and expanded polytetrafluoroethylene are close to a model solution, yet the prostheses available are encumbered by defects that limit their application. Expanded polytetrafluoroethylene (ePTFE), for example, with its suitable porosity and low thrombogenic action, lends itself for the preparation of low diameter prostheses (below 8 mm). The material is, however, prone to degradation during radiation sterilisation, and its surface binds lipids dissolved in the blood, thus contributing to atherosclerosis. Although porous, the (ePTFE) prostheses are not ingrown with tissue. Moreover, these prostheses carry a high risk of bacterial infection [3]. Other disadvantages are a low elasticity, which makes connecting with the vessel repaired difficult, and bleeding, which may occur at the interconnection. Furthermore, most semi-biological prostheses exhibit a long healing time and run the risk of transmitting animal – derived diseases.

The surface of all the synthetic vessel prostheses produced is haemostatically active, contributing to the forming of clots of varied thickness on the surface

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of the vessel walls. This phenomenon brought about the pre-operation sealing (pre-clotting) of macro-porous prostheses [4]. The method exploits the clotting of blood occurring when the blood comes in contact with a thrombogenic surface, inherent to synthetic prostheses. This instance of the self-sealing of a prosthesis results in acquiring a surgical tightness provided by a fibrin layer containing blood morphotic elements deposited on the inner surface. Unfortunately, this phenomenon also has its hazard – the surface clots formed make the surface highly thrombogenic [5], and an uneven out-pressing of the clots may cause uncontrolled bleeding. The out-pressing does not warrant a complete removal of the clots, thus running the danger of an embolism.

The drawbacks of currently available commercial prostheses have encouraged scientists to investigate new, ever better materials. World-wide intensive research is under way to develop prostheses that would mimic the features of natural blood vessels.

These were the reasons why the authors undertook to investigate the possibility of preparing biological blood prostheses based upon an advanced material: modified bacterial cellulose [6, 7]. The material can be obtained by modification of the nutrient medium in the microbiological synthesis of cellulose. The bacterial synthesis method adopted, the conditions of the biosynthesis and the efficiency of the bacteria strain are all factors influencing not only the output of the process but also the physical-mechanical characteristic and related useful properties of the cellulose prepared [8, 9].

Bacterial cellulose is produced i.e. by bacteria of the *Gluconacetobacter* type. It is secreted from cells in the form of fibrils built up of cellulose chains. Unlike natural plant cellulose, the bacterial kind does not contain lignin, pectin and hemicellulose. Bacterial cellulose is highly water-absorptive and does not cause irritation nor allergic reactions, which make the material suitable for use as artificial skin in wound healing [10]. Thanks to its high chemical purity and outstanding physical-chemical features, it may also be used as an additive in dietary food, in the manufacture of electro-acoustic and filtration membranes, as ultra-strong paper, and, in the form of micro-fibre, as suspension of good covering, binding and thickening properties [11 - 15].

Very positive is the fact that the shape desired can be given to products of bacterial cellulose in the course of its generation (*in situ*) [16]. Patents [17, 18] describe the instant forming of shapes, such as tubes of bacterial cellulose, in the course of biosynthesis suited to replace blood vessels or other organs.

Bacterial cellulose modified with chitooligosaccharides combines the properties of both chitosan and cellulose, leading to an improvement in its biocompatibility and bioactivity. Incorporating chitooligosaccharide segments into the cellulose chain may provide conformity with the properties of natural blood vessels where mucopolysaccharides display an integral multifunctional system, which stands for the elasticity of the blood vessel and the forming of an athrombogenic surface [19].

Polyaminosaccharides and their derivatives enhance wound healing [20] by inducing fibroblasts to deliver interleukin -8, which is responsible for the migration and proliferation of cell blood vessel endothelium [21, 22].

Up to now, several uses of bacterial cellulose are known in human and veterinary medicine. The application of Biofill® and Gengiflex® bacterial cellulose in surgery and as implants in dentistry is described in [23 - 26].

Investigations into the biosynthesis of bacterial cellulose by D. Klemm et. al. [27] confirmed the possibility of forming tubes which were next implanted as prostheses of the carotid artery in micro operations with rats. Based on histological preparations, it was found that four weeks after the operation the inner surface of the implant had been entirely covered by a layer of endothelium cells, while on the outer surface the implant had been tightly covered by connecting tissue.

Also reported is the biocompatibility of bacterial cellulose with live tissue and its impact upon the clotting of blood. It was found that subcutaneous implants in rats did not cause chronic inflammation for 1, 4 and 12 weeks after the operation. The fresh tissue generated growing over the implant revealed the presence of newly formed capillary blood vessels and synthesised collagen [28]. Comparison of the bacterial cellulose with commercial prostheses of poly-(ethylene terephtalate) (Dacron®) and ePTFE (GORE-TEX®), with regard to the interaction with blood shows that amongst the materials examined, bacterial cellulose demonstrated the least intensive and most delayed activation of the clotting cascade [29].

The objective of the research presented herein was the preparation of a method to produce prostheses of blood vessels based on bacterial cellulose modified with chitooligosaccharides. The results of earlier investigations into the synthesis of bacterial cellulose [30, 31] were a starting point for the present research, aimed at the preparation of tubes for implants.

The investigations can be subdivided into the following :

- Biosynthesis of modified bacterial cellulose using selected matrices
- Assessment of the structure, morphology and physical-mechanical properties of prototypes of vessel prostheses of modified bacterial cellulose.
- Assessment of the biological properties of the vessel prostheses obtained.

Materials and methods

An acetic strain of bacteria - *Acetobacter sp.* (CCM 2360) from the Czech Collection of Microorganisms, Masaryk University, Brno, Czech Republic was used in the synthesis of bacterial cellulose.

The nutrition medium Hestrin-Schramm was also used, composed of the following (on 1000 cm^3): glucose 20.0 g, yeast extract 5.0 g, soy peptone 5.0 g. di-sodium phosphate 2.7g, acetic acid 1.2 g, ethanol 20.0 cm^3, and chitosan oligomers 2.0 g.

The characteristic of the chitosan oligomer ChitoOligo-100 (by Amicogen, Korea) given by the manufacturer is shown in ***Table 1***.

Assessment of structural properties

GPC analysis was carried out according to the Turbak procedure [32, 33] using Hewlett Packard HP 1050 apparatus equipped with a RI HP 1047A refractometric detector and set of columns with a rigid hydrophilic filling based on polymeric gel. Samples were prepared for the analysis according to the modified Ekmanis method [34].

Infrared spectrophotometric analysis was made with the use of FTIR Unicam apparatus with WinFIRST software of Mattson Co, within a wave number range of 4000 - 650 cm^{-1}.

Table 1. *Characteristic of chitosan oligomers (from the manufacturer) used in the biosynthesis of modified bacterial cellulose in tubular form.*

Parameter	Value
pH (1% aq.)	4.9
Loss on drying, %	4.58
Residue on ignition, %	0.2
As_2O_3 (as As), ppm	< 1.0
Pb, ppm	< 10.0
Coli-form bacteria	negative
Total aerobic microbial count, cfu/g	200
Fungi	negative
Particle size, μm	< 250
Total glucosamine, %	78.1
Deacetylation, %	92
Oligosaccharide composition, %	
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
dimer-octamer	64.82

Microscopic images were taken with a scanning electron microscope - ESEM type Quanta 200 of FEI Co (USA).
The water retention value (WRV) was measured according to Standard ISO 23714:2007.

Assessment of physical-mechanical and chemical properties

The physical-mechanical testing (wall thickness, peripheral and longitudinal tenacity, and suture retention strength**)** of the prosthesis prototypes was carried out at the Laboratory of Metrology of IBChF

Figure 1. *Culture on the inner surface of the silicone tube.*

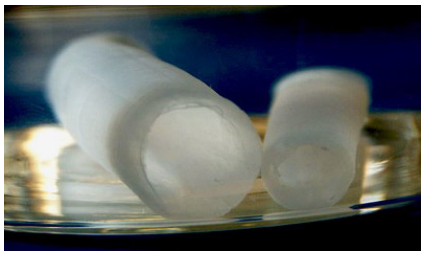

Figure 2. *Prototypes of the vessel prostheses obtained from the culture in the silicone tube.*

(certificate PCA AB 388) according to Standard ISO 7198:1998.

Chemical purity assessment of the prosthesis prototypes was made at Tricomed Co according to procedures applied in the testing of the company's own prostheses. Tested were aqueous extracts of the material assessed, prepared in the following way: mass proportion of the test sample to water - 1 : 10, temperature - 37 °C ± 1 °C, and the extraction time was 24 h. The chemical purity assessment included the following tests:

1. Visual assessment of the aqueous extracts – in accordance with Polish Pharmacopeia VII: 2006.
2. pH of the aqueous extracts - in accordance with Standard PN-EN ISO 3071:2007 - 'Textiles, pH estimation of aqueous extracts'.
3. Permanganate value of the aqueous extracts - in accordance with Standard PN-P-04896:1984 - 'Methods of Chemical Testing. Knitted medical devices. Estimation of the permanganate value of aqueous extracts'.
4. Ultraviolet absorption of the aqueous extracts - in accordance with Standard PN-P-04990:1989 - 'Methods of Chemical Testing. Knitted medical devices. Estimation of the absorbance maximum of ultraviolet radiation'.
5. Content of chloride anions - in accordance with Standard PN-P-04895:1984 - 'Methods of Chemical Testing. Knitted medical devices. Estimation of the content of chloride anions'.
6. Content of ammonia ions - in accordance with Standard PN-P-04992:1989 - 'Methods of textile testing. Textile dressing materials. Estimation of ammonia and ammonia salt content'.
7. Content of sulfate ions - in accordance with Standard PN-P-04781-04:1987 ' Methods of textile testing. Textile dressing materials. Estimation of sulfate content'.
8. Content of surfactants - in accordance with Standard PN-P-04781-14:1989 'Methods of textile testing. Textile dressing materials. Estimation of foaming agents'.
9. Content of heavy metal ions - in accordance with Standard PN-P-04991:1989 - 'Methods of Chemical Testing. Knitted medical devices. Estimation of wash off ions of heavy metals'.
10. Content of substances dissolvable in 2-propanol - in accordance with Standard PN-P-04607:1983 - 'Testing of textile raw materials and yarn. Estimation of a non-fibrous substance'.

Testing of biological properties

Bioactivity

The prosthesis prototypes were subjected to the following tests:

1. *In vitro* testing of cytotoxic action according to Standard PN-EN ISO 10993-5:2001 by a direct contact method at the Dept. of Cell Culture at the Medical University, Wrocław, Poland.
2. *In vitro* assessment of haemocompatibility based upon examination of haemolytic action using erythrocytes and whole blood according to Standards PN-EN ISO 10993-1:2004 and PN-EN ISO 10993-4:2006 at the Dept. of Experimental Surgery and Testing of Biomaterials at the Medical University, Wrocław, Poland.
3. *In vivo* assessment of skin sensitisation using guinea pigs according to Standard PN-EN ISO 10993-10:2007 and OECD directives (OECD Guideline for the Testing of Chemicals), method No 406 (Skin Sensitisation). The testing was carried out at the Laboratory of Medical and Veterinary Products at the Institute of Occupational Medicine, Łódź, Poland.
4. *In vivo* assessment of Intradermal reactivity using rabbits according to Polish Pharmacopeia VIII, European Pharmacopeia Pharmacopeia VI and Standards PN-EN ISO 10993-10:2007 and PN-EN ISO 10993-12:2008. The testing was carried out at the Laboratory of Medical and Veterinary Products at the Institute of Occupational Medicine, Łódź, Poland.
5. Local post-implant effect along with an *in vivo* assessment of histopathology using rats according to Standard PN-EN ISO 10993-6:2007. The testing was carried out at the Laboratory of Medical and Veterinary Products at the Institute of Occupational Medicine, Łódź, Poland. The Laboratory decided on the observation time and exact locality of the implant.

Prior to physical-chemical, chemical purity and biocompatibility testing, all the samples of tubes of bacterial cellulose were sterilised by a fast electron beam at a dose of 28 kGy. The samples were packed in polyethylene film of the type used for the sterilisation of medical de-

Figure 3. Dynamic culture in a reactor with a rotating PTFE shaft and the polyester vein reinforcement applied - BR-1 (Tricomed SA).

Figure 4. Prototype of a prosthesis obtained in a reactor with a rotating shaft and the polyester vein reinforcement applied - BR-1 (Tricomed SA).

Table 2. Selected physical-mechanical and useful properties of tubes from the static culture.

Inner diameter of		Peripheral strength/CV,		Peripheral tenacity,	Longitudinal tenacity/CV,		Suture retention strength/CV,		Water permeability at a pressure of. 120 mm Hg,
the matrix, mm	relaxed tube, mm	cN	%	cN/mm	cN	%	cN	%	ml/min·cm²
10	6.6	290	13.6	14.5	934.0	9.3	51.1	5.5	0.86
16	12.3	240	11.1	12.6	451.0	10.1	40.1	5.4	0.89

Table 3. Selected physical-mechanical and useful properties of the vessel prosthesis prototypes obtained in a reactor with a rotating shaft.

Variant	Inner diameter of relaxed tube,	Peripheral strength/CV,		Peripheral tenacity,	Longitudinal tenacity/CV,		Suture retention strength/CV,		Water permeability at a pressure of 120 mm Hg,
	mm	cN	%	cN/mm	cN	%	cN	%	ml/min·cm²
Without a carrier	8.7	219	15.7	11.0	329.0	12.4	35.4	8.9	0.93
With PET a carrier	8.6	297	7.8	14.9	9090.0	8.2	70.0	6.7	0.89

vices at Tricomed Co. The Unit of Radiation Sterilisation of Medical Devices and Implants at the Institute of Radiation Chemistry and Nuclear Technique in Warsaw carried out the sterilisation.

Experimental

The biosynthesis of the modified bacterial cellulose was conducted in a static culture on the inner surface of a silicone tube to form the tubular shape of a prosthesis. The silicone tube played the role of a bioreactor. Trials were also carried out in a dynamic culture in a bioreactor equipped with a rotating PTFE rod half-immersed in the culture medium. A carrier made of PET was also used in the syntheses.

Once the bacterial cellulose synthesis had been completed, the tubes of modified bacterial cellulose obtained were washed with distilled water to remove components of the culture medium (the conductance of the cellulose after washing was < 20 μS), and then they were submerged in 1% aqueous NaOH and placed in a steam autoclave at 121 ºC for 15 min. Afterwards the tubes were washed with water to a neutral pH and conductance below 20 μS.

Results

Static culture on the inner surface of the silicone tube

The biosynthesis was conducted with the use of silicone tubes as matrix (***Figures 1*** and ***2***). The tubes were 10 and 16 mm in diameter. The culture proceeded in a warming chamber for 7 days with the forced air flow at 30 ºC.

The outcome of the culture were tubular prototypes of vessel prostheses with a surface density of about 10 g/m². Their physical-mechanical and useful properties are shown in ***Table 2***.

Tubes from the static culture prepared on silicone matrices are characterised by a peripheral tenacity in the range of 12 to 14 cN/mm, a longitudinal tenacity from about 450 to approx. 930 cN, a suture retention strength from about 40 to 50 cN, and by a favourable water permeability below 1 ml/min cm² at a pressure of 120 mm Hg.

Dynamic culture in a reactor equipped with a rotating shaft partly immersed in a nutrition medium

A reactor equipped with an 8 mm PTFE shaft was employed in the synthesis of the modified bacterial cellulose in a tube shape. The shaft, half-immersed in a nutrition medium, was driven by an electric motor at 10 r.p.m (see ***Figure 3***). Tubes were prepared with a surface density of about 10 g/m². In another version a pleated polyester vein reinforcement of the BR-1 type (by Tricomed Co) was used to improve the tenacity of the prosthesis prototypes. The vein reinforcement applied on the PTFE shaft (see ***Figure 3***) served as a carrier. Composite prostheses were prepared in which the bacterial cellulose was deposited onto the polyester carrier (see ***Figure 4***). The surface density of such tubes was also about 10 g/m². The modification allowed to prepare tubes which, when taken out of water, maintained their shape, could be freeze-dried without deformation and, after wetting with glycerol, dried without disturbing the inside diameter of the tube. ***Table 3*** shows selected physical-mechanical properties of the prosthesis

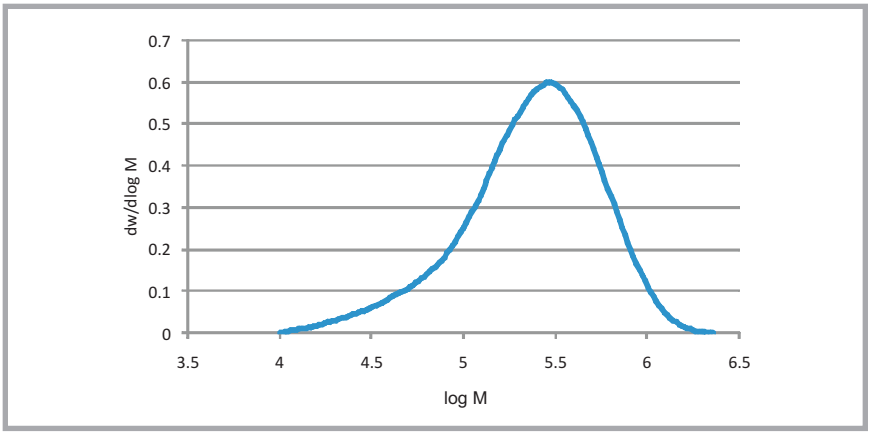

Figure 5. *Molecular mass distribution curve of modified bacterial cellulose produced by the static method.*

Table 4. *Selected molecular parameters of modified bacterial cellulose produced by the static method. GPC measurements.*

$\bar{M}n$, kD	$\bar{M}w$, kD	$\bar{M}w$ / $\bar{M}n$	$\bar{D}Pw$	Percentage of DP fractions %		
				DP<200	200<DP<550	DP>550
150.2	312.0	2.1	1954	5	14	81

prototypes obtained by the method described herein.

It was found that the growth of the modified bacterial cellulose in a tubular form proceeds both with and without a synthetic carrier. Good adhesion is achieved between the carrier and cellulose. The carrier-modified tubes showed much better mechanical properties compared with those without a carrier: the peripheral tenacity increased by 35%; the longitudinal tenacity saw a 27-fold increase, and the suture retention strength increased by 98%. Both kinds of tubes of modified bacterial cellulose are characterized by very low water permeability; a factor favourable in vessel prostheses.

Molecular, structural and morphology characteristic

Gel chromatography (GPC) was employed to analyse the molecular, structural and morphology characteristic of the vessel prostheses made by the static method. Results of the analysis are shown in ***Figure 5*** and ***Table 4***.

The modified bacterial cellulose obtained is characterised by an average numeric molecular mass of 150 kD, an average weight molecular mass of about 310 kD, a polydispersity of 2.1, and an average polymerisation degree equal to 1954 with a high percentage of the DP > 550 fraction.

Samples of the bacterial cellulose modified with chitosan oligomers were analysed by infrared spectroscopy. A wide band within the range 3384 - 3422 cm^{-1}, attributed to the stretching vibration of the OH and/or NH group [35], could be observed in the FTIR spectrum (***Figure 6***). Also detected was the presence of the following bands: 1630 - 1660 cm^{-1}, corresponding to the stretching vibration of the C=O bond in 1st order amides, 1540 - 1570 cm^{-1}, ascribed to the bending vibration of the –NH bond in 2nd order amides, and 1040 - 1150 cm^{-1}, which represents the –C-O-C- group of the glycoside bond [36].

It can be seen in the SEM images (***Figure 7.a*** & ***7.b***) that the modified bacterial cellulose is built up of interlaced nanofibres in a 3D network. SEM measurements indicate single fibre diameters in the range of about 28 to approx. 47 nm (average value - 35.8 nm, SD = 5.04 nm).

In ***Figure 8*** SEM images are shown of vessel prosthesis prototypes with a polyester vein reinforcement prepared in a reactor with a rotating shaft. It may be inferred from the images that the integration of the bacterial cellulose with the carrier was accomplished.

Assessment of chemical purity

Quality parameters concerning the chemical purity of the vessel prosthesis prototypes made by the static method are compiled in ***Table 5***. The examination was carried out at the Tricomed Co. The chemical purity was assessed by comparing the test results with quality standards adopted at Tricomed for synthetic knitted vessel prostheses.

Most of the results measured fall into the allowable limits for synthetic vessel prostheses. There are, however, some deviations resulting from the specific material of the prostheses. The limit-exceeding permanganate value and content of ammonia ions may be caused by the liberation of glucosamine units from the structure of the modified bacterial cellulose in the course of extraction from the sample and by the presence of nutrient medium residues in the sample.

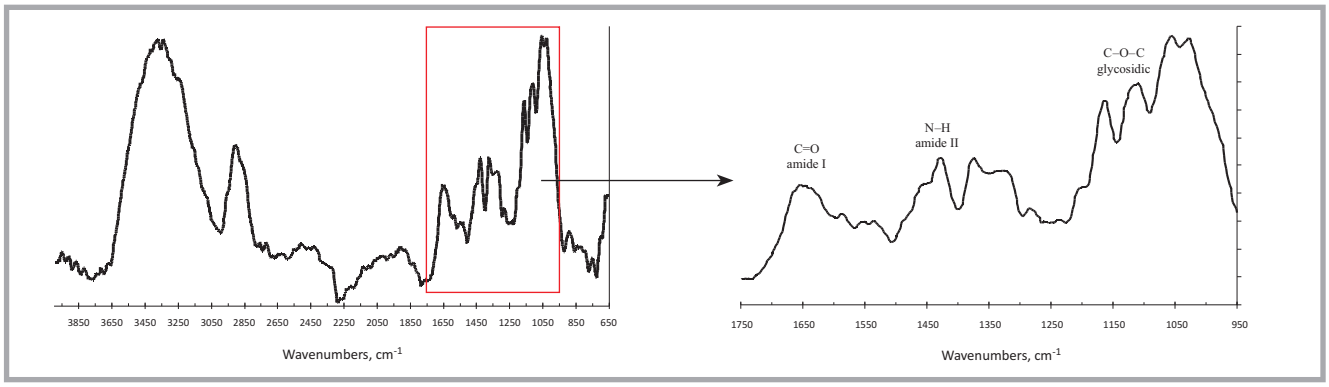

Figure 6. *FTIR spectrum of modified bacterial cellulose produced by the static method.*

Biological testing

Biological testing was carried out with prototypes of prostheses prepared from the modified bacterial cellulose in the course of static biosynthesis in silicone tubes of 10 mm diameter.

Assessment of cytotoxic action.

An *in vitro* assessment was carried out on a reference cell line of mouse fibroblasts of the 3T3/Balb type. It was found that the prototypes did not exert a cytotoxic action in direct contact with 3T3/Balb mouse fibroblasts.

Assessment of haemocompatibility

The testing of haemocompatibility was carried out based upon the examination of the haemolytic action using erythrocytes and whole blood, as well as upon the estimation of the parameters of the plasma clotting system and the morphological observation of blood cells. The examination was made with human blood after contact with a blood vessel prosthesis of modified bacterial cellulose. In tests with concentrated erythrocytes after contact with the test sample and an extract of the sample in physiological solution, the average percent of haemolysis amounted to 0.25 ± 0.06%, while the upper limit allowable, according to the standard, is 3%.In tests with whole blood (CPD) after direct contact with the sample surface, the average percent of haemolysis amounted to 0.14 ± 0.03%. The upper limit allowed by the standards is 1%.

Assessment of sensibilisation action

The sensibilisation effect of an aqueous extract from the vessel prosthesis upon skin was tested *in vivo* on guinea pigs under closed exposure according to the Magnusson and Kligman method. It was found that the prototypes of vessel prostheses do not pose the risk of human sensibilisation

Assessment of intradermal reactivity

The intradermal reactivity caused by extracts from the vessel prostheses in a polar- (0.9% NaCl) and non-polar solution (cotton oil) was tested *in vivo* on rabbits. The testing documented the absence of an irritating action

Assessment of the local post-implant effect and histopathology

The testing was carried out on Imp:WIST rats. It was certified that vessel prostheses of the modified bacterial cellulose when implanted into the vertebral muscles of rats for 1 month do not cause any inflammation or growth of the connecting tissue in the muscles surrounding the implant. The implant was surrounded by a capsule of connecting tissue The growth of blood vessels and fibroblasts was found in the structure of the implants (symptoms of vascularisation and fibrillation). Detailed results of the biological testing will be published separately

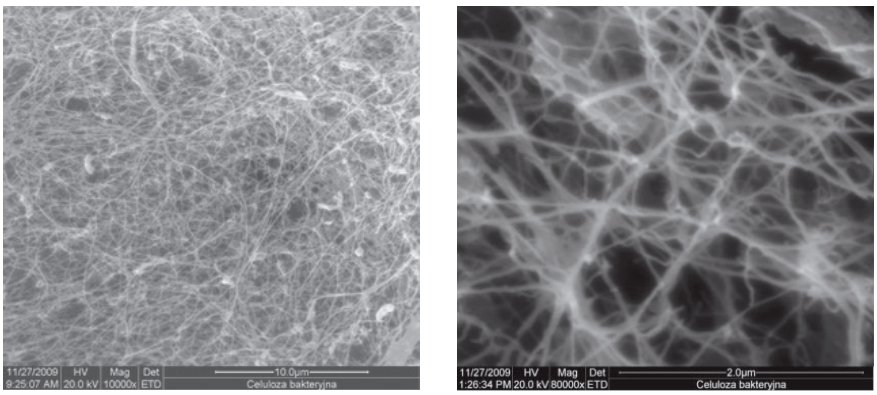

Figure 7. *SEM images presenting the structure of modified bacterial cellulose produced by the static method; a) magnification 10 000× b) magnification 80 000×.*

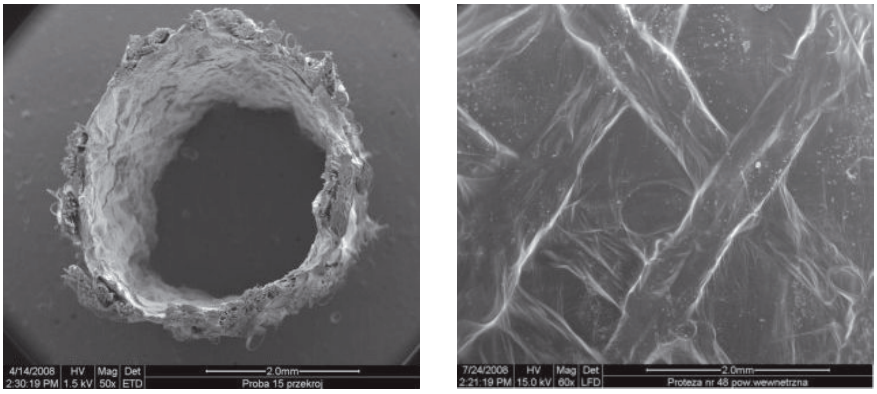

Figure 8. *SEM images of vessel prosthesis prototypes prepared by the dynamic method in a rotating reactor with the use of a polyester vein reinforcement of the BR-1 type as the carrier: a) cross section and b) inner surface.*

Table 5. *Quality parameters characterising the chemical purity of the vessel prosthesis prototypes made by the static method.*

Parameter analysed	Unit	Tricomed quality standard	Test results	
			Repet. 1	Repet. 2
Visual assessment of extract : - transparency - colour	(-)	transparent	transparent	
		colourless	colourless	
pH of sample tested	pH unit	5.5-8.0	6.42	6.19
pH of reference		-	6.31	6.31
Deviation- pH		± 1.0	0.11	0.12
Permanganate value	mg O_2/g	≤ 0.08	1.820	0.744
max. absorbance in ultraviolet at wavelength	A_{max} λ 230 nm	≤ 0.3	0.381	0.354
characteristic absorbance value at wavelength	A λ 245nm	≤ 0.3	0.265	0.232
content of chloride ions	mg Cl^-/g	≤ 0.02	not exceeding 0.02	
content of ammonia ions	mg NH_4^+/g	≤ 0.01	far exceeding 0.01	
content of sulfate ions	mg SO_4^{2-}/g	≤ 0.05	not exceeding 0.05	
content of heavy metals ions	mg Pb^{2+}/g	≤ 0.01	not exceeding 0.01	
foaming agents	foam height (cm)	none	none	
specific conductance	µS/cm	-	24.8	33.9

Summary

A method was elaborated to prepare modified bacterial cellulose formed in a tubular form in the course of biosynthe-

sis. The process is conducted with *Acetobacter sp.* (CCM 2360) bacteria and a chitosan oligomers-modified medium. The biosynthesis was accomplished in two ways: a) dynamic - in a reactor equipped with a rotating shaft partly immersed in the medium, and b) static - in which a silicone tube constitutes the reactor. The mechanical properties of the prostheses, such as the peripheral and longitudinal tenacity, and suture retention strength could be improved by using a plaited polyester carrier in the dynamic version of biosynthesis.

Chromatographic examinations documented the following molecular characteristics of the modified bacterial cellulose: an average numeric molecular mass of 150 kD, an average weight molecular mass of about 310 kD, a polydispersity of 2.1, and an average polymerisation degree of about 2000 with a high percentage of the DP>550 fraction.

In the FTIR spectra of the modified bacterial cellulose, bands could be seen that are characteristic of chitosan. SEM images show that the structure of the modified bacterial cellulose is built up of interlaced nanofibres in a 3D network. The SEM measurements indicate diameters of single fibres in the range of about 28 to approx. 47 nm. The SEM images also reveal a good integration of the modified bacterial cellulose with the synthetic carrier.

The assessment of chemical purity confirmed a far reaching conformity with the standards for synthetic vessel prostheses *In vitro* testing showed that the prototypes do not exert a cytotoxic action in direct contact with mouse fibroblasts of the 3T3/Balb type. The examination made with animals proved that the vessel prostheses do not cause any sensibilisation or irritation (intradermal reactivity).

Based on the results of the assessment of the post-implant local effect, it was concluded that blood vessel prostheses of the modified bacterial cellulose when implanted into rats for 1 month do not cause any inflammation or overgrowth of the connecting tissue in muscles surrounding the implant, which was surrounded by a capsule of connecting tissue. The growth of blood vessels and fibroblasts was found in the structure of the implants (symptoms of vascularisation and fibrillation).

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