**Antoni Niekraszewicz,
Magdalena Kucharska,
Iwona Kardas,
Maria Wiśniewska-Wrona,
*Roman Kustosz,
*Adam Jarosz**

Institute of Biopolymers and Chemical Fibres,
ul. M. Skłodowskiej-Curie 19/27, 90-570 Łódź, Poland,
E-mail: sekretarz@ibwch.lodz.pl

***Foundation of Cardiac Surgery Development,**
Zabrze, Poland

Chitosan Coatings to Seal Cardiovascular Prostheses

Abstract
Presented herein is a material and method to seal prostheses of specific cardiovascular prostheses - outlet heart canulaes. Microcrystalline chitosan was chosen as the basic constituent for the coating preparation. The coat was deposited by combined dipping and spraying on prostheses composed of synthetic fibres. Testing was carried out to assess the properties of the sealed prostheses: mechanical features, water and blood permeability, fastness and biodegradability. The research was done as part of a complex programme: Polish Artificial Heart 2007-2011.

Key words: *vascular prostheses, chitosan, sealing, chitosan.*

Introduction

Heart diseases are a serious social problem worldwide, with the incidence and death rates of which belonging to the highest. Statistics published by the American Heart Association (AHA) estimate that heart disease- related mortality surpasses that of cancer by 60%, which is 8 fold that of traffic accidents and 14 fold that of Alzheimer's disease. Estimates point to between 800 000 and 1 million Poles being afflicted with cardiac insufficiency, two third of which are under 65 years of age [1].

To effectively treat patients suffering from acute heart failure in the entire clinical range, a wide variety of prostheses is needed, from short term extracorporeal devices to permanent implants.The development and implementation in clinical practice of a Polish family of heart prostheses is the goal of the far reaching governmental programme named 'Polish Artificial Heart 2007-2011'. The programme includes devices varying with respect to the level of technology, and the time and degree of implantation adjunction, with the ultimate aim of obtaining a wholly permanent implantable heart prosthesis (artificial heart). Over twenty, mainly Polish, R&D centers and several Polish clinics are participating in the programme, coordinated by one of the main participants: the Foundation of Cardiosurgery Development (FCD) [2].

The Institute of Biopolymers and Chemical Fibres (IBWCh) is a partner in the programme, with the task of developing a sealing for specific polyester blood vessel prostheses: canulaes that connect the heart prosthesis to arterial vessels. Commercially available on the market for medical devices are blood vessel prostheses sealed with biopolymers, including protein-based ones like collagen, gelatin, and albumin. Investigations are underway to use resorbable synthetic polymers for this purpose [3]. IBWCh has gained rich experience and knowledge in the use of chitosan for the construction of various biomaterials, hence it was agreed with the coordinator to harness a polymer for the specific function in question. Gel-like microcrystalline chitosan (MCCh), earlier elaborated at IBWCh [4], was the form of polymer selected for the sealing of the prostheses. MCCh, retaining all the merits of virgin chitosan, offers much higher bioactivity as well as a higher ability of biodegradation and resorption.I Its ability to form film directly from an aqueous suspension is also substantial, as well as its thrombogenicity. All these are essential features in the sealing of blood vessel prostheses [5 - 11]. The sealing applied to prostheses ought to be biocompatible, susceptible to step-wise degradation and provide a stable flow of blood (athrombogenicty). Also expected is a lack of permeability through the walls, and a preservation of the implant's elasticity. Within this project, works are being carried out at several R&D centres aimed at the sealing of prostheses with outlet canulaes of an artificial heart chamber using various polymer materials. The selection of the best variant will be made after biological and complex comparative functional testing under dynamic conditions at FCD.

Materials

Prostheses

Vascular prostheses were used of the BARD 004187 style 6010 type (BARD Co) with a diameter of 16 mm

Microcrystalline chitosan (MCCh)

A gel-like form of MCCh was used with a suitable pH. It was prepared from virgin chitosan supplied by Vanson Co:

$\bar{M}_v$ =287 kD,
DD = 80%,
polymer content = 0.5 – 1.0%,
pH = 6.9 – 7.1,
heavy metal content - absence.

Plasticiser

Glycerol, medical grade from Fluka Co, density 1.26 g/ cm^3.

Lysozyme

Lysozyme was supplied by Merck Co, with an activity of 50 000 U/mg, prepared from chicken egg protein. Lysozyme with a concentration of 100 & 200 μm/cm^3 was used in the investigation.

Phosphate-citrate buffer

Phosphate-citrate buffer of pH = 7.23 was used, prepared according to Standard PN-81 C-06504 'Chemical analysis-preparation of buffer solutions'.

Methodology

Method of sealing vascular prostheses with MCCh

Depending upon the phase of the research, the polyester vascular prostheses were sealed by dipping or spraying or by combining the two procedures [12]. The first layer was deposited by dipping the prosthesis in a MCCh blend of 0.5% weight with an addition of 0.7:1 of glyc-

Niekraszewicz A., Kucharska M., Kardas I., Wiśniewska-Wrona M., Kustosz R., Jarosz A.; Chitosan Coatings to Seal Cardiovascular Prostheses.
FIBRES & TEXTILES in Eastern Europe 2011, Vol. 19, No. 3 (86) pp. 106-111.

erol (on dry chitosan). Six consecutive layers were applied by spraying the prosthesis with a MCCh blend of 1% weight with an addition of 0.7:1 of glycerol (on dry chitosan). After the deposition of each singular layer, the prostheses were dried.

Preparation of the sealing blend

The components of adequate proportions were vigorously stirred for about 20 minutes in an Ultra-Turrax T50 homogeniser, IKA-WERKE Co, equipped with a G 45G tool.

Method of preparing a film of the sealing blend.

An aqueous gel-like suspension of MCCh with a 1% weight concentration of the polymer andaddition of a plasticiser was cast upon Teflon plates and dried at 18 °C

Testing of the sealing coating's fastness in wet conditions

The testing included measurements of water permeability and assessment of the structure of the coating settled on the prostheses after they had been kept in water at 37 °C for 12 h, 24 h, 48 h and 5 days in static conditions.

Testing of mechanical properties

The testing was carried out according to the following standards:

- PN-ISO 4593:1999 - Plastics – Film and plates – Estimation of thickness by mechanical scanning.
- PN-EN ISO 527-3:1998 - Plastics – Estimation of mechanical properties at static extension- conditions for the testing of film and plates.
- ISO 7198:1998: "Cardiovascular implants - Tubular vascular prostheses"

Estimation of the biodegradability of chitosan film

The testing was carried out at 37 °C for 180 days in a phosphate-citrate buffer at pH = 7.2 with the addition of lysozyme at a concentration of 10 000 U/cm^3, and at a bath module of 1:250. The film samples were removed from the bath after 30, 90, 180 days of testing and rinsed with distilled water at 50 ^{o}C. 70% ethanol was poured over the samples to deactivate the enzyme. After 10 minutes, the alcohol was filtered off and the samples were dried at 105^{o}C. The following were estimated: the mass loss, amount of aminosaccharides delivered, and the average molecular mass of MCCh by the GPC method.

Assessment of the structure of the modified prostheses

This was done by means of a scanning electron microscope - Quanta 200 firmy FEI Co., USA

Testing of water permeability

The testing was carried out at IBWCh according to Standard PN-79/P-04884.03 "Textile testing methods, Knitted medical articles - prostheses of blood vessels. Estimation of water permeability"

Testing of water permeability under static conditions

The testing was carried out at FCD with fixed length prostheses in a dry and wet state under static conditions and at a pressure of 133 hPa (100 mm Hg). An experimental stand was used for the purpose, made up of a prosthesis with a drain (diameter - 0.5 cm, length -130 cm) connecting it to a water container (vol -650 ml). The other end of the prosthesis was blinded. Prior to measurements, the system was deaerated. The volume of the transuding water and the time were measured.

Testing of the permeability to blood of the prostheses under static conditions

The testing was carried out at FCD in a similar fashion to that for water permeability, in which CPDA anti-coagulated pig blood was applied for a dry state only. The volume of permeated blood and the time were measured.

Analytical methods

Estimation of the average molecular mass (M_v) – the Viscometric method

The average viscometric molecular mass of chitosan was calculated from the limiting viscosity number [η]. Measurements were carried out with the use of a dilution viscometer with capillary No 1, constant K ≈ 0.01 at 25 ± 0.1 °C. An aqueous solution containing 0.1 M of sodium chloride, 4 M urea and 0.2 M of acetic acid in 1 dm^3 served as a solvent. The average molecular mass, c, was calculated from the Mark-Houwink equation [13].

Estimation of the average molecular mass (M_w) - the Gel chromatography method

Measurements were made with gel chromatography apparatus HP-1050 (Hewlett – Pacard), equipped with a HP 1047A refractometric detector and a set of columns with pores of 300 A and 4000 A, filled up with a hard silica gel. The chromatography parameters of the chitosan analysis were as follows:

- columns: GFC-4000 A, GFC–300 A (Polymer Laboratories Ltd.)
- flow speed: 1 ml/mm
- injection volume: 20 μl,
- sample concentration - 0.20%
- continuous phase – acetate buffer 2.33 M.

Results of the investigation

Impact of chitosan molecular mass upon the fastness of the sealing coat

Prostheses of the Bard 004187 style 6010 type with a diameter of 16 mm were sealed with 1.0% aqueous dispersion of MCCh at pH = 7.3, deacetylation degree DG = 80% and varied molecular mass :

1. MCCh V-171: $\bar{M}_v$ = 287 kD,
2. MCCh V-171/10: $\bar{M}_v$ = 133 kD,
3. MCCh V-171/20: $\bar{M}_v$ = 97 kD.

The sealed prostheses were, before and after ethylene oxide sterilisation, subjected to testing of fastness. The impact of the residence time of the modified prostheses in normal saline was observed over time intervals of up to 30 days. The testing was done under static conditions, the results of which are compiled in ***Table 1*** and shown in ***Figures 1*** & ***2***.

Table 1. *Impact of sterilisation and the residence time in normal saline upon the water permeability of prostheses modified with MCCh V-171 after degradation with doses of 10 and 20 kGy; *sterilised with etylene oxides.*

Kind of prosthesis		Coating degree, %	Water permeability, ml/cm^2/min					
			0 h	12 h	24 h	48 h	5 days	30 days
Prosthesis modified with	MCCh V-171	6.26	5.10	6.10	6.10	6.20	8.40	8.4
	MCCh V-171/EO*		5.30	5.90	6.00	6.50	8.90	9.0
	MCCh V-171/10	9.05	6.60	7.10	7.90	7.90	8.00	12.2
	MCCh V-171/10/EO*		6.30	6.90	7.00	7.50	8.90	15.1
	MCCh V-171/20	8.20	6.10	6.73	6.78	6.78	6.76	47.2
	MCCh V-171/20/EO*		6.62	6.65	6.67	6.96	7.24	50.9

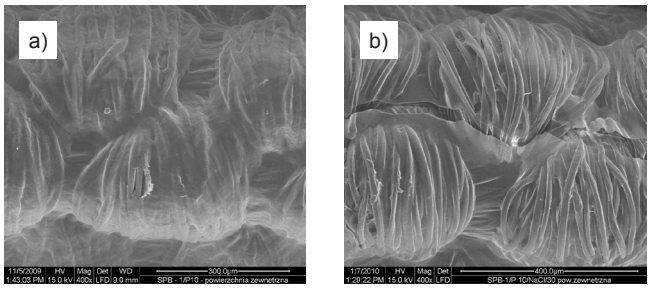

Figure 1. *Appearence of a prosthesis surface modified with MCCh V-171 EO - sterilised: a) before, and b) after 30 days residence in normal saline (magnification 400×).*

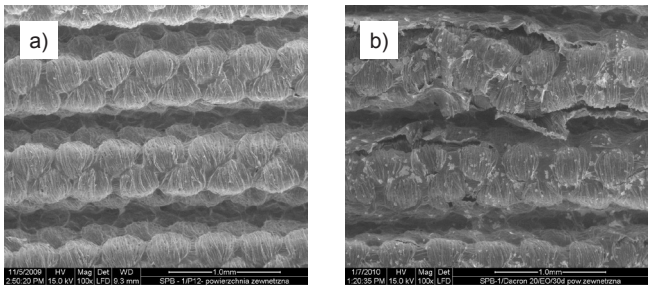

Figure 2. *SEM images of a prosthesis surface modified with MCCh-V/20/EO: a) before, and b) after 30 days residence in normal saline (magnification 100×).*

It was found that water permeability increases with time, during which the prostheses stayed in normal saline. The intensity of the change depends upon the molecular mass of chitosan used for the sealing of the prostheses. The lower the molecular mass, the more intense the changes are. The lowered tightness is caused by cracking and partial crumbling away of the coat. The coat loses elasticity as result of the plasticizer being washing off, thus contributing to the cracking. With chitosan of the lowest molecular weight (MCCh-V-171/20/EO), water permeability after 50 days increased from 6.6 to 50.9 ml/cm^2/min. The change is less significant with prostheses modified with chitosan of the highest molecular weight (MCCh V-171). Sterilisation with ethylene oxide only slightly increases the permeability. In the SEM microphotos in ***Figures 1*** and ***2***, defects are clearly discernible in the form of cracks in the sealing coat on the outer surface of the prosthesis, particularly after 30 days in normal saline.

Testing of mechanical parameters

MCCh with the addition of glycerol as a plasticiser (0.7 weight parts per 1 weight part of dry chitosan) was used in the investigation. The good ability of such a blend to form a coating have been confirmed earlier. In the investigation, it was intended to modify the coating liquid by changing the pH in order to provide optimal elasticity. Mechanical properties were measured of films prepared from MCCh compositions with an addition of glycerol, in which the pH was varied in the range of 6.9 to 7.1:

1. MCCh V171/7.1/07G – pH = 7.1
2. MCCh V171/6.96/07G - pH = 6.96
3. MCCh V171/6.9/07G - pH = 6.90.

In the graphs below, results are presented of measurements of the elongation at break, elasticity and tensile strength of the conditioned and wet films - see ***Figures 3 - 5***.

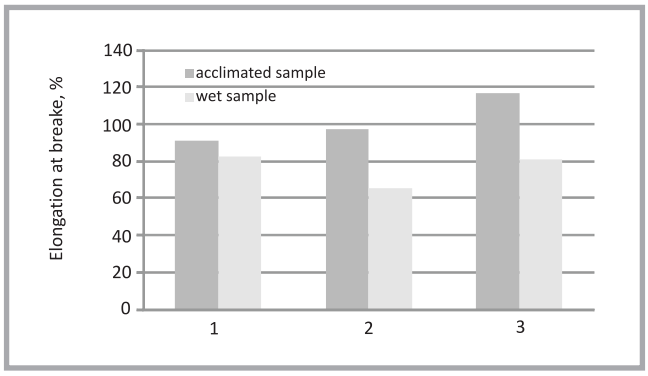

Figure 3. *Elongation at break of MCCh/plasticiser films; 1 - MCCh V171/7.1/07G, 2 - MCCh V171/6.96/07G, 3 - MCCh V171/6.9/07G.*

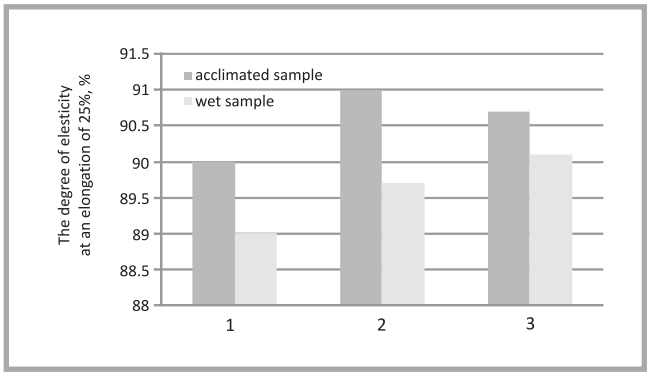

Figure 4. *Degree of elasticity of MCCh/plasticiser films; 1 - MCCh V171/7.1/07G, 2 - MCCh V171/6.96/07G, 3 - MCCh V171/6.9/07G.*

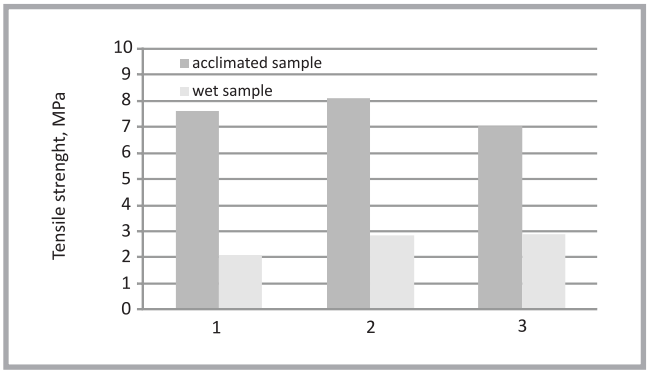

Figure 5. *Tensile strength of MCCh/plasticiser films; 1 - MCCh V171/7.1/07G, 2 - MCCh V171/6.96/07G, 3 - MCCh V171/6.9/07G.*

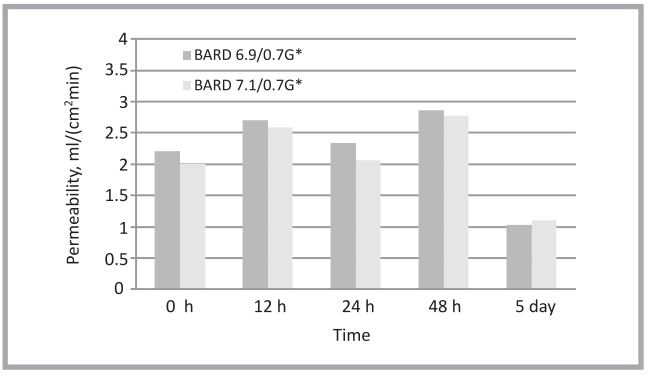

Figure 6. *Impact of residence time in normal saline upon the water permeability of MCCh-modified chitosan prostheses.*

The mechanical properties of dry conditioned films surpass those of the wet films by far. The impact of the pH of coating blends is not univocal. The highest elasticity (about 91%) and tensile strength (7.03 MPa) were revealed by films made of MCCh at pH = 6.96, while elongation was highest (117%) for films of MCCh at pH = 6.90. In wet conditions, the tensile strength and elasticity increased with a decrease in the pH of the MCCh and were best at pH = 6.9. The elongation at break was in fact independent of pH. It may be expected that a decrease in the mechanical strength of the wet film by up to 70% along with a moderate decrease in elasticity should not profoundly affect the mechanical properties of the prostheses themselves.

Mechanical parameters measured in wet conditions better reflect the conditions under which the prostheses are going to function; on that basis MCCh with the lowest pH i.e. 6.9 was selected for further investigation on the sealing, as well as MCCh at pH = 7.1 for comparison.

Susceptibility of the MCCh films to biodegradation

Before the degradation tests, the preparations were steam- sterilised at 120 °C for 20 minutes. The susceptibility to biodegradation was assessed in terms of the mass loss of the samples, the change in the average molecular mass, and the amount of reductive aminosaccharides delivered. The tests were carried out in a buffer at pH = 7.2, and 37 °C in the presence of lysozyme, according to the method described in Methodology, the results of which are compiled in ***Table 2*** and presented as chromatographs in ***Figure 7***.

The examination confirmed the susceptibility of the films to biodegradation under the influence of lysozyme. The mass loss reached about 14% and over 24% after 90 and 180 days, respectively, accompanied by a saccharification of the chitosan, evidenced by the liberation of water-soluble aminosugars. After 90 days, the weight molecular mass dropped by over 84%. With prolonged degradation time (90 days), the curves of the molecular weight distribution changed their shape - from bimodal to monomodal. At the same time, an increase could be seen in the content of the low molecular fraction, as well as a shift in the respective peak toward declining values. It was also found that with the proceeding degradation

Table 2. *Results of the enzymatic degradation of films of MCCh in a lysozyme medium at a concentration of 10 000 U/cm³.*

Parameter	Time, days			
	0	30	90	180
pH	7,22	7,22	7,18	7,29
Mass loss,%	0	6,6	13,9	24,1
Concentration of aminosugars, mg/cm³	0	0,45	0,73	0,53
M_w, g/mol	108 700	54 150	16 780	-
M_w/M_n	4.0	5.5	3.3	-

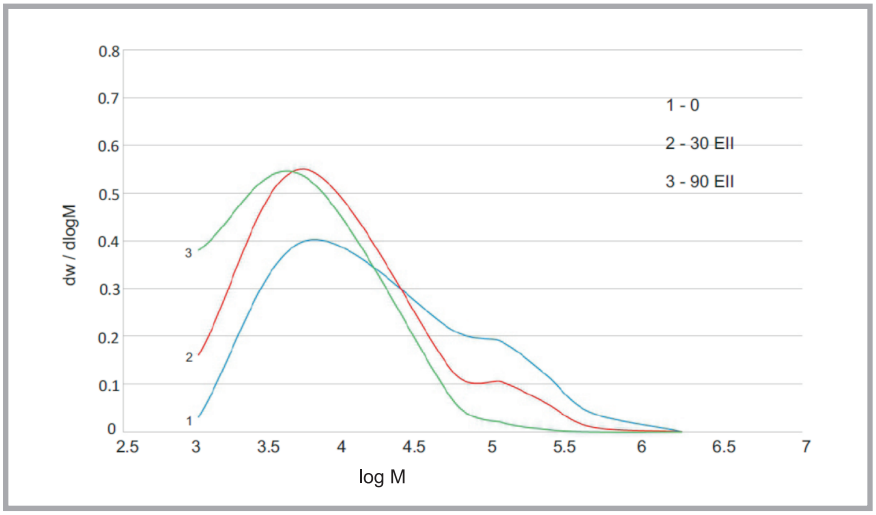

Figure 7. *Molecular mass distribution of MCCh in film form in a lysozyme medium with concentration of 10 000 U/cm³.*

of the MCCh films, the polydispersity M_w/M_n coefficient value changed from 4.0 over 5.5 (after 30 days) to 3.3 (after 90 days). After 180 days of degradation, the molecular mass of the samples was not estimated due to low dissolvability (65% was an insoluble portion).

Fastness of the sealing coat

The fastness of the coat was assessed based on the time-dependent water permeability and observation of the proceeding changes in the surface structure. Tests were performed on BARD prostheses sealed with MCCh at pH = 6.9 and pH = 7.1 with deposited chitosan in the amount of 7.4% weight. The prostheses were sealed by consecutive dipping and spraying, as described in Methodology. The results of permeability as a function of time are shown in ***Figure 6.*** Microscopic images of the outer and inner surface of the prostheses are presented in ***Figures 8*** and ***9*** (see page 110).

It was found that the water permeability of MCCh –sealed prostheses does not depend upon pH in the range of 6.9 - 7.1. It could also be documented that the permeability of prostheses kept in normal saline for 48 hours practically did not change. Longer residence (up to 5 days) improves the prostheses' tightness, which can be explained as a result of the swelling of the chitosan coat and, hence, a better filling of the inter- fibre voids.

Inspection by means of a scanning electron microscope showed that for MCCh with a higher pH of 7.1, the sealing coat is clearly discernible on both the outer and inner surface of the prosthesis, entirely covering the textile structure and filling the inter-fibre voids. For MCCh at a lower pH, the images show a complete coat on the outer surface of the knitwear, with chitosan not penetrating through the wall, nor does a discernible coat appear on the inner surface. This can be explained by the higher dynamic viscosity of MCCh at pH=6.9 and, hence, by the lower permeability of the coating blend applied by spraying upon the outer surface.

After five days residence in normal saline, distinct defects could not be found in the structure of the coat on the surface of the prostheses (coats of MCCh with varied pH), confirming the good fastness of the sealing.

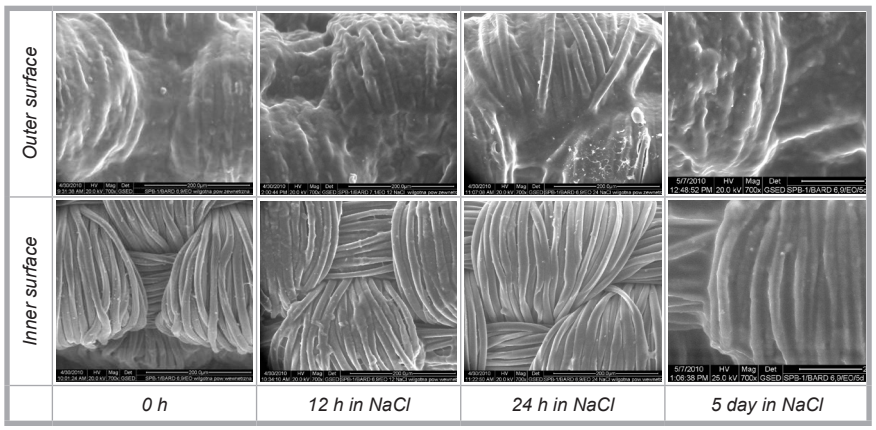

Figure 8. *SEM photos of the outer and inner surface of prostheses MKCh V-171/6.9/0.7/EO modified with MCCh at pH = 6.9.*

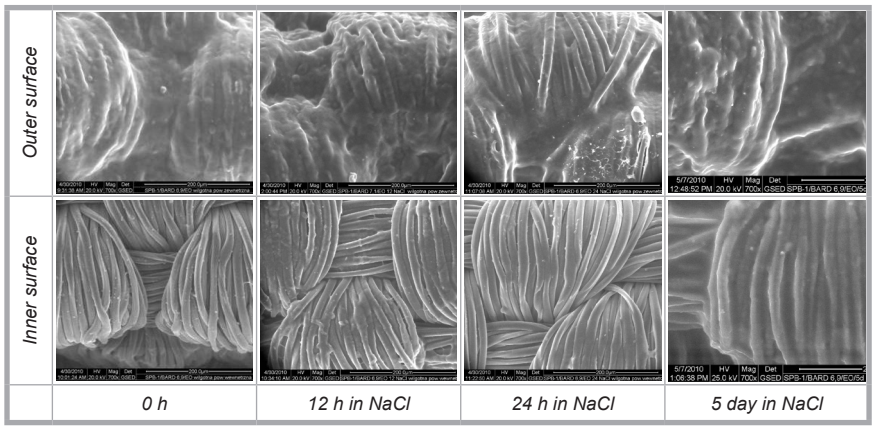

Figure 9. *SEM photos of the outer and inner surface of prostheses MKCh V-171/7.1/0.7/EO modified with MCCh at pH = 7.1.*

Blood and water permeability of the modified prostheses

A static examination of the blood and water permeability of the MCCh - modified prostheses was made at FCD in Zabrze. The same BARD prostheses were tested i.e. sealed with MCCh at a pH of 6.9 and 7.1, with a deposition degree of 7.4% weight. The sealing was accomplished by combined dipping and spraying. The results obtained are presented in ***Figures 10***.

The examination showed that all MCCh-sealed prostheses are characterised (when dry) by at least a twofold lower permeability than the reference prosthesis. Prostheses modified with MCCh at a lower pH of 6.9 (marked BARD 6.9/1-2) reveal a slightly lower permeability than the other (marked BARD 7.1/1-2). The average permeability for this type of sealing exceeded values of 79 ml/min/cm^2 and 112 ml/min/cm^2 in dry and wet conditions, respectively.

In the examination of blood permeability, the prostheses modified with MCCh at pH = 6.9 and those modified with MCCh at pH = 7.1 revealed a permeability of over 40% (average 1.9 ml/min/cm^2) and 65% (average 1.1 ml/min/cm^2), respectively, lower than the unmodified ones (reference). It may be stated that the results correspond with the observation under a scanning electron microscope, which revealed that the sealing coat is formed on both surfaces (inner/outer) when MCCH at pH = 7.1 is used.

Summary

1. Through testing it was confirmed that microcrystalline chitosan (MCCh) in a gel-like suspension form is a suitable material to produce film with good tenacity and elasticity.
2. MCCh of the highest molecular weight was selected for the sealing. Lower values of the parameter (a range of 287 kD to 97 kD was investigated) yield sealing coats that are less durable under the action of fluids (normal saline), showing a higher water permeability.
3. Films of MCCh undergo enzymatic biodegradation. After 90 days, $\overline{M}_w$ was down by over 84%, and the molecular weight dispersion curves changed their shape - from bimodal to monomodal (for a lysozyme concentration of 200 μM/cm^3).
4. Scanning electron microscope inspection documented that MCCh at pH = 7.1 applied to the outer prosthesis surface by spraying penetrates through the knitwear structure and produces a sealing coat on the inner surface as well.

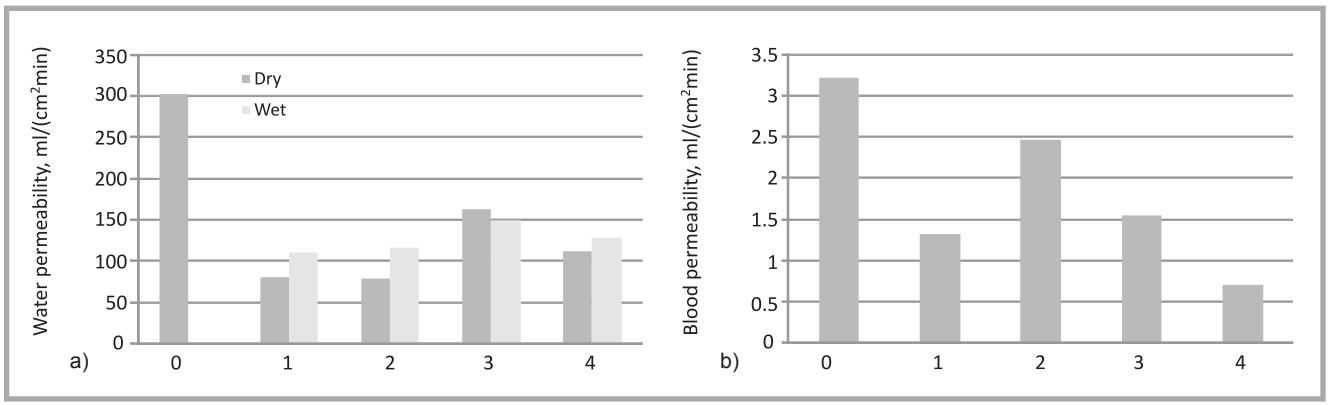

Figure 10. *Water permeability(a) and blood permeability (b) of the modified prostheses under static conditions; 0) reference, 1 - 2) BARD 6.9/1-2 modified with MCCh at pH 6.9, 3 - 4) BARD 7.1/1-2 modified with MCCh at pH 7.1.*

5. Under static conditions, blood vessel prostheses modified with MCCh at a pH of 6.9 and 7.1 showed a water permeability three times lower than that of the non modified reference prostheses.
6. Blood vessel prostheses modified with MCCh at a pH of 6.9 and 7.1 revealed very good blood tightness, characterised by a permeability of 1.9 and 1.1 ml/min/cm^2, respectively.

References

1. www.swiat-zdrowia.pl.
2. http://www.pwpss.pl/teksty/celeprogramu/celeprogramu.html.
3. Niekraszewicz A., Kucharska M., Kardas I., Szadkowski M.; "Resorbable Tightening of Blood Vessel Prostheses Prepared from Synthetic Polymers", Fibres and Textiles In Eastern Europe, vol. 17, No 6(77), 2009 pp. 93-98
4. Polish Patent PL 281975 (1989).
5. Struszczyk H., Urbanowski A., Niekraszewicz A.; "Chitosan - New Forms and Uses", Textile Month, 1, 30 (1993), Textile Asia, 24 (7), 1993 p. 80.
6. Struszczyk H., Kivekäs O., Niekraszewicz A.; „Some Modern Applications of New Chitosan Forms", in A. Domard, Ch.Jeunieux, R. Muzzarelli, G. Roberts, ed., „Advances in Chitin Science", vol. 1, J. Andre Publ., Lyon, France 1996, pp. 482-489.
7. Kucharska M., Niekraszewicz A., Struszczyk H., Bursig H.; „Modified Choriospon Wound Dressings with Chitosan", in H. Struszczyk, ed. „Progress on Chemistry and Applications of Chitin and its Derivatives", Polish Chitin Society, vol. III, 1997 pp. 141-153.
8. Watała C., Kucharska M., Golański J., Więcławska B., Niekraszewicz A., Struszczyk H.; „Studies on Effects of Microcrystalline Chitosan on the Activation of Plateles in Whole Blood", in H. Struszczyk, ed. „Progress on Chemistry and Applications of Chitin and its Derivatives", Polish Chitin Society, vol. IV, 1998 pp. 125-133, .
9. Niekraszewicz A.; Chitozan Medical Dressings, Fibres & Textiles in Eastern Europe, vol. 13, No 6 (54), 2005 pp. 16-18.
10. Applications of Chitin and Chitosan, ed. By Goosen M. F. A.: Technomic Publishing, Lancaster, PA (1997)
11. Polish Patent PL 193846 (1999)
12. Polish Pat. Appl. P-390866.(2010)
13. Domszy J. G., Roberst G.; Macromol. Chemie, Vol. 186, 1985 p. 1671.

Received 21.12.2010 Reviewed 03.02.2011