Danuta Ciechańska

Institute of Chemical Fibres
ul. M. Sklodowskiej-Curie 19/27,
90-570 Łódź, Poland
E-mail: dciechan@iwch.lodz.pl

Multifunctional Bacterial Cellulose/ Chitosan Composite Materials for Medical Applications

Abstract
This paper presents some results concerning the characterisation of the structural parameters of bacterial cellulose/chitosan composite materials with regard to their medical application. Some chemical-physical, mechanical and biological properties of modified bacterial cellulose are presented. The innovatively-modified bacterial cellulose characterised by the combined properties of both cellulose and chitosan (especially high susceptibility for enzymatic degradation and bioactivity) is produced by the Acetobacter xylinum strain adapted to a medium containing polyaminosaccharide modifiers.

Key words: *modified bacterial cellulose, hydrogel dressings, biomaterials, bacterial cellulose/chitosan composites.*

Introduction

If a wound is to heal effectively, it must be maintained in a wet condition, such as is often provided by modern wound dressings. However, the best dressing is the patient's own skin, which is permeable to vapour and protects the deeper layer tissue against mechanical injuries and infection. For many years, with the exception of transplanting the patient's own skin, biological dressings of pig skin or human cadaver skin have been applied. The disadvantage of such dressings is their antigen properties, which limits the span of the application. Moreover, this method is very expensive, and so is rarely applied.

The scientific basics of moist wound therapy were created by G.D. Winter [1]. His pioneering research initiated the concept of active wound dressing, which creates and maintains the optimum conditions required for the regeneration of broken tissue. Occlusive wound dressings may come in form of foam, gel, hydrogel and aerosol [2,3]. They maintain the proper moisture level and constant temperature of the wound bed, accelerate healing, activate autolytic debridement of the wound, protect newly-formed cells, facilitates angiogenesis and reepithelisation, alleviate pain, and protect the wound against bacteria and contamination [4,5].

Bacterial cellulose is a natural polymer whose properties are similar to the hydrogels produced from synthetic polymers; for example, it displays high water content (98-99%), good sorption of liquids, is non-allergenic and can be safely sterilised without any change to its characteristics. Being similar to human skin, bacterial cellulose can be applied as skin substitute in treating extensive burns 6,7].

Bacterial cellulose is synthesised by the acetic bacterium *Acetobacter xylinum* [8-10]. The fibrous structure of bacterial cellulose consists of a three-dimensional network of microfibrils containing glucan chains bound together by hydrogen bonds.

In the Institute of Chemical Fibres (IWCh), Poland, an ecological method has been developed for manufacturing of bacterial cellulose/chitosan composite materials suitable for medical applications [11]. Modified bacterial cellulose combines the properties of both cellulose and chitosan. The modification of the bacterial cellulose already occurs during microbiological synthesis by introducing selected bioactive polysaccharides, such as various chitosan forms and their derivatives, into the culture medium. It has been found that glucosamine and N-acetylglucosamine units are incorporated in the cellulose chain [12]. Such composite materials can be applied in treating burns, bedsores, skin ulcers, hard-to-heal wounds and wounds requiring frequent changes of dressing.

The aim of this paper is to present some results concerning an estimation of the molecular, chemical and morphological structure of bacterial cellulose modified by chitosan. Some of the physical-mechanical and biological properties of modified bacterial cellulose with regard to theirs medical application are presented.

Materials and Methods

Modified bacterial cellulose (MBC) in a form of hydrogel has been obtained during microbial synthesis under static conditions with the use of the acetic bacterium *Acetobacter xylinum* (ŁOCK 0805) from the Pure Culture Collection of the Institute of Microbiology and Fermentation, Technical University of Łódź, Poland. The biosynthesis proceeded over 7 days, at a temperature of 30°C in a standard Hestrin-Schramm culture medium with the addition of two different chitosan forms [13]. The chitosan forms used for the modification of the bacterial cellulose were prepared in the Department of Biomaterials, the Institute of Chemical Fibres, Łódź, Poland.

Analytical Methods

The average polymerisation degree ($\overline{DP}$) and molecular weight distribution were determined by gel permeation chromatography [14]. The structural analyses of MBC were performed using Fourier Transformed Infrared spectrometry (FTIR) and scanning electron microscopy (SEM). The water release rate was determined by the method described in [15].

The amount of aminosaccharides released from dressings under lysozyme degradation was estimated colorimetrically by the DNS method [16]. Antibacterial activity tests were performed by the quantitative method against *Escherichia coli* (ATCC 11229) and *Staphyloccocus aureus* (ATCC 6538) according to Polish and Japanese standards [17,18].

Results and Discussion

Characteristic of bacterial cellulose/ chitosan materials

Bacterial cellulose is produced in the form of a pellicle of the desired size and shape on the surface of a culture medium in static conditions, or in the form of a gel-like pulp in dynamic conditions. The method of culture and biosynthesis conditions strongly in uences the physicochemical and utility properties of bacterial cellulose [19-21]. It is possible to modify bacterial cellulose during microbial synthesis through the addition of a suitable modifier to the culture medium [11,13]. The modification of bacterial cellulose carried out using a nutrient medium composed of different chitosan forms seems to be the most promising method for producing valuable material for medical application. An estimation of the optimal parameters of biosynthesis enables us to obtain modified cellulose with the sdesired molecular, morphological and chemical structure [22]. The chemical modification of bacterial cellulose consists in introducing the glucosamine and N-acetylglucosamine units into the cellulose chain, in consequence of the degradation of chitosan modifier present in nutrient medium within the biosynthesis process [12]. The elucidation of the incorporation mechanism and the composition of the resulting cellulose/chitosan copolymer should be the subject of further study.

Two types of hydrogels of bacterial cellulose modified by chitosan acetate (MBC/O) and chitosan lactate (MBC/M), characterised by a water content of 99.0-99.5% and a polymer content of 0.5-1.0% (cellulose 90-93% dry weight and chitosan 7-10% dry weight) were obtained directly in a static culture.

Table 1. Molecular weight distribution of modified bacterial cellulose (cellulose fraction).

Symbol of sample	Molecular characteristic of cellulose						
	$\bar{M}n$, kD	$\bar{M}w$, kD	$\bar{D}Pw$	$\bar{M}w$ / $\bar{M}n$	DP fraction, %		
					DP<200	200<DP<550	DP>550
MBC/O	134.7	255.1	1575	1.89	3	13	84
MBC/M	143.4	257.5	1590	1.80	2	12	86

Table 2. Molecular weight distribution of modified bacterial cellulose (chitosan fraction)

Symbol of sample	Molecular characteristic of chitosan										
	$\bar{M}p$, kD	$\bar{M}n$, kD	$\bar{M}w$, kD	$\bar{M}w$ / $\bar{M}n$	$Mw \times 10^3$ fraction, %						
					<5	5-50	50-100	100-200	200-400	400-800	>800
MBC/O	12.0	11.4	29.8	2.62	12	74	9	4	1	0	0
MBC/M	15.8	13.3	24.8	1.86	7	84	8	1	0	0	0

The molecular structure analyses of hydrogels by gel permeation chromatography (GPC) were performed separately for both chitosan and cellulose fractions. The chitosan fraction was extracted from cellulose/chitosan composite with an acetate buffer (pH 4.5) [22]. The results are presented in Tables 1 and 2 and in Figures 1 and 2.

As can be seen from Table 1 and Figure 1, the molecular characteristic of cellulose is similar, regardless of the chitosan form used for modification. The values of average molecular weight $\bar{M}n$, $\bar{M}w$, average polymerisation degree $\bar{D}Pw$, polydispersion $\bar{M}w/\bar{M}n$ and the participation of individual DP fractions are at the same level.

The results presented in Table 2, and the molecular weight distribution curves of the chitosan fraction of MBC (Figure 2), show some differences in the molecular characteristics of both types of chitosan modifiers.

Samples of unmodified and modified bacterial cellulose hydrogels were analysed by FTIR spectroscopy. The FTIR spectra are presented in Figure 3.

Comparing the FTIR spectra, the peaks for modified bacterial cellulose were found at 1650 cm^{-1} (amide I) and 1560 cm^{-1} (amide II), attributed to the amide groups characteristic for chitosan.

The morphological structure of MBC was analysed by scanning electron microscopy. SEM photographs of unmodified and modified bacterial cellulose obtained in static culture are presented in Figure 4.

When analysing the SEM photographs, it can be seen that the modified bacterial cellulose consists of microfibres with diameters in the order of tenths of a micrometer, which form a three-dimensional network.

The physico-mechanical properties of bacterial cellulose from the static culture were estimated in a wet state. The results are presented in Table 3. A comparison of

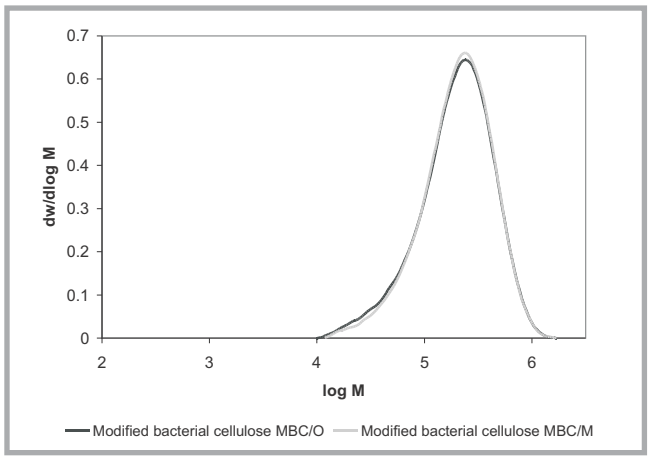

Figure 1. Molecular weight distribution curves of cellulose fraction obtained from modified bacterial cellulose.

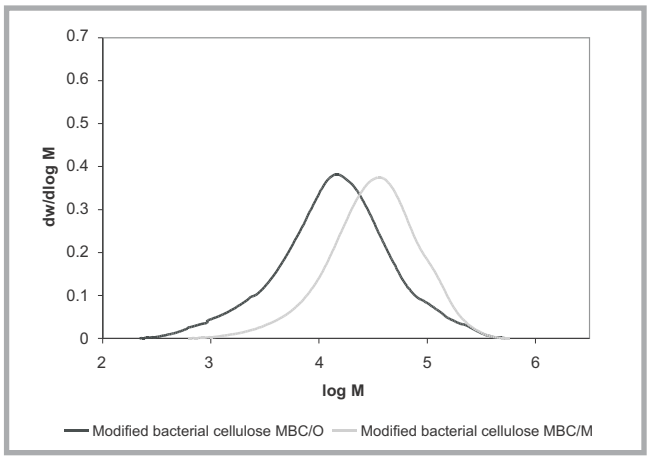

Figure 2. Molecular weight distribution curves of chitosan fraction obtained from modified bacterial cellulose.

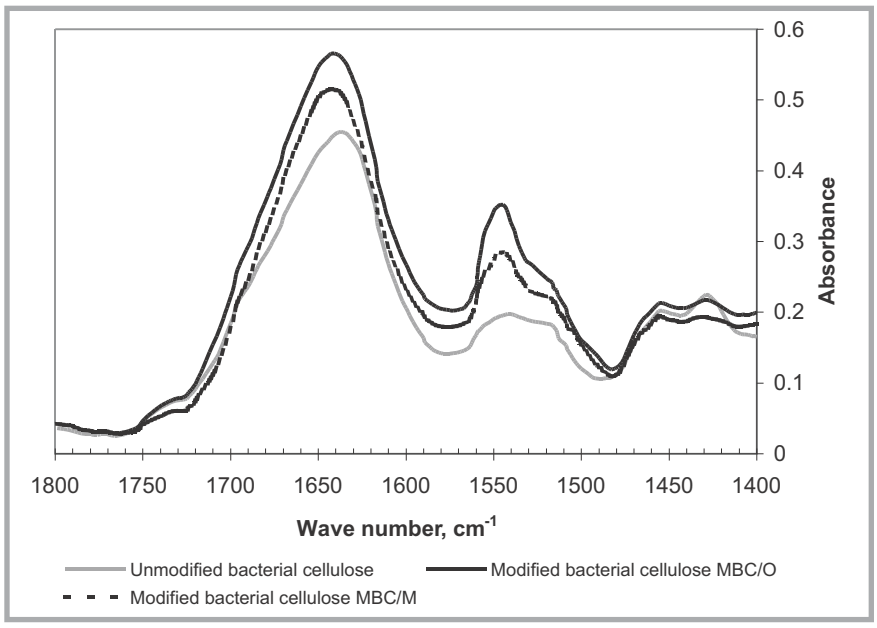

Figure 3. *FTIR spectra of unmodified and modified bacterial cellulose (static culture).*

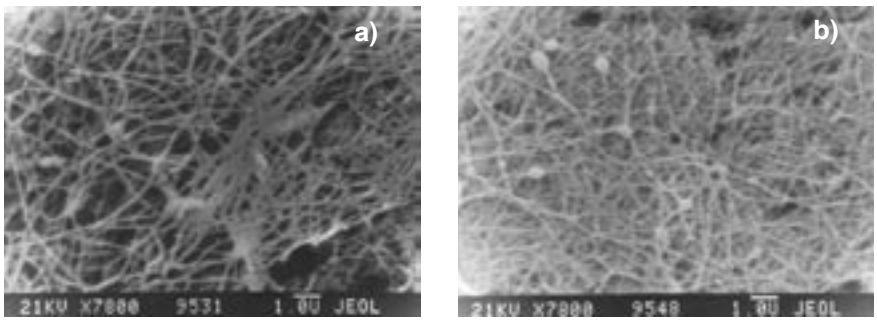

Figure 4. *SEM photographs of modified bacterial cellulose: a - sample MBC/O, and b - sample MBC/M.*

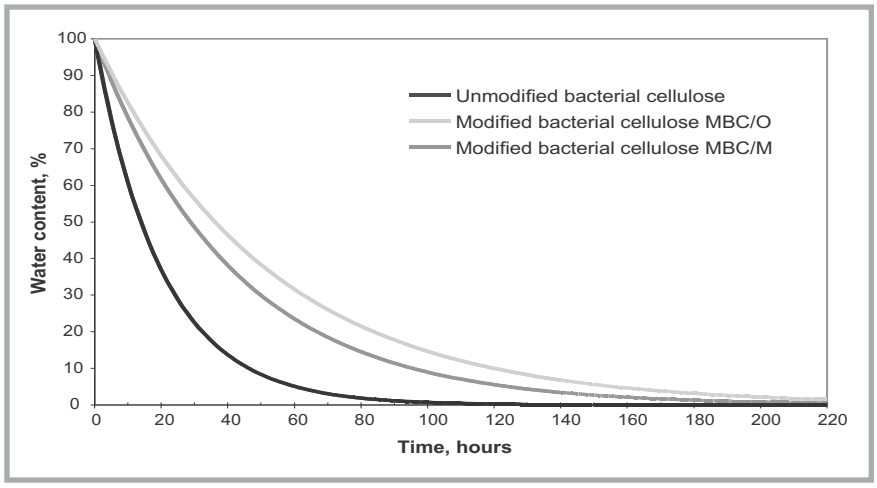

Figure 5. *Water release rate for unmodified and modified bacterial cellulose.*

Table 3. *Physico-mechanical properties of modified bacterial cellulose.*

Symbol of sample	Physico-mechanical properties			
	Thickness, mm	Max. breaking load, N	Breaking stress, MPa	Elongation at break, %
Unmodified bacterial cellulose	2.45	8.2	0.22	38
MBC/O	0.98	13.6	0.93	36
MBC/M	0.92	14.2	1.02	39

the results in Table 3 reveals that chitosan has a favourable impact on the mechanical properties of modified bacterial cellulose. High elongation at break indicates good elasticity of the bacterial cellulose, which is very important from the medical point of view. An elastic dressing which fits the wound site well is good protection against external infection.

In modern therapy, maintaining the proper wound moisture is very important. A moist environment facilitates the penetration of active substances into the wound, and enables easy and painless dressing change without damage to the newly formed skin.

Within the confines of the research, the sorption/desorption properties of unmodified and modified bacterial cellulose were determined in order to estimate its usefulness in the treatment of wounds requiring wet conditions and frequent dressing change. For the equation:

$$m_t = m_\alpha (100 - e^{-kt})$$

where:

m_t - the amount of water evaporated from a sample after time t, %

m_α - the total amount of water in a sample, %,

describing the rate of water desorption, parameter k is calculated. Using this equation, the period of water half-release was determined. The results are presented in Figure 5 and Table 4.

As can be seen from Table 4, the higher the parameter k, the shorter the water half-release period. In the case of modified bacterial cellulose, the water half-release period is significantly longer than in that of the unmodified bacterial cellulose. From the graph presented in Figure 5, it can be concluded that the water release process is slower for modified bacterial cellulose, and all the water evaporates from the MBC after 8-9 days, compared to about 4 days in the case of unmodified bacterial cellulose. Therefore it seems reasonable to use modified bacterial cellulose as dressings for wounds requiring wet healing conditions.

Biological properties of bacterial cellulose/chitosan materials

One of the features of chitosan is its susceptibility to specific hydrolytic enzymes. During the enzymatic degradation of chitosan, bioactive mono- and oligosaccharides are being released, which stimulate angiogenesis and tissue regeneration.

Table 4. *Parameter k and period of water half-release for unmodified and modified bacterial cellulose.*

Symbol of sample	Parameter k	Period of water half-release, h
Unmodified bacterial cellulose	0.0497	14
Modified bacterial cellulose MBC/O	0.0192	36
Modified bacterial cellulose MBC/M	0.0241	29

Table 5. *Susceptibility of MBC to enzymatic degradation by lysozyme.*

Time of enzymatic degradation, days	Amount of aminosaccharides released from modified bacterial cellulose			
	MBC/M, mg/cm^3		MBC/O, mg/cm^3	
	Lysozyme concentration, µg/cm^3			
	40	80	40	80
0	0.000	0.000	0.000	0.000
1	0.034	0.081	0.053	0.106
3	0.064	0.113	0.064	0.113
6	0.073	0.133	0.073	0.113

Table 6. *Bioactivity tests for modified bacterial cellulose (bacteria count after 24h incubation)*

Symbol of sample	*Escherichia coli* (ATCC 11229)			*Staphylococcus aureus* (ATCC 6538)		
	Total bacteria number, CFU	Bacterio-static activity	Bactericidal activity	Total bacteria number, CFU	Bacterio-static activity	Bactericidal activity
Unmodified bacterial cellulose	5.2×10^8	-	-	3.2×10^8	-	-
MBC/O	$< 2.0 \times 10^1$	8.7	3.4	2.9×10^6	2.0	-1.8
MBC/M	$< 2.0 \times 10^1$	7.4	3.7	1.3×10^7	1.4	-2.4

Lysozyme, the enzyme that can be found in human body uids, has the capability to degrade chitosan. The enzymatic degradation of modified bacterial cellulose was carried out in a lysozyme solution of two different concentrations. The results are presented in Table 5.

From Table 5, it can be seen that both the concentration and time of the enzymatic reaction in uence the amount of degradation products (aminosugars) released from MBC. The higher the lysozyme concentration (for a specified time), the greater is the amount of aminosugars released from both types of modified bacterial cellulose. The results obtained demonstrate that modified bacterial cellulose is susceptible to lysozyme degradation.

One of the factors affecting the wound healing process is infection caused by microorganisms present in the environment. The application of wound dressing materials which possess good antibacterial and barrier properties against microorganisms may be a recommended solution to this problem.

The bacteriostatic and bactericidal activity of modified bacterial cellulose against *Escherichia coli* Gram (-) and *Staphylococcus aureus* Gram (+) bacteria was determined. The results are presented in Table 6.

Bioactivity tests (Table 6) revealed that modified bacterial cellulose is characterised by bacteriostatic activity against both *Escherichia coli* and *Staphylococcus aureus*. However, the inhibition of bacteria growth is clearly higher in the case of *Escherichia coli.* Both types of modified bacterial cellulose show some bactericidal activity against *Escherichia coli.*

Conclusions

Modifying bacterial cellulose with chitosan during its biosynthesis results in a composite material with glucosamine and N-acetylglucosamine units incorporated into the cellulose chain, which is characterised by a number of valuable features:

- good mechanical properties in wet state,
- high moisture-keeping properties,
- release of mono- and oligosaccharides under lysozyme action,
- bacteriostatic activity against Gram (-) and Gram (+) bacteria and bactericidal activity against Gram (+) bacteria.

These features make modified bacterial cellulose an excellent dressing material for treating different kinds of wounds, burns and ulcers.

Acknowledgement

This work was carried out as part of research project No 4T09B04222 financially supported by the Polish Ministry of Science.
The author would like to thank Mr Janusz Kazimierczak and Ms Monika Czapnik from Institute of Chemical Fibres, Poland for their co-operation in work concerning the preparation of bacterial cellulose/chitosan composite materials.

References

1. *G.D. Winter, Nature, 1962, 193, pp. 293-294.*
2. *G.A. Kannon, A.B. Garrett, Dermatologic Surgery, Vol. 21, Issue 7, July 1995, pp. 583-590.*
3. *S. Misterka, 'Rodzaje materiałów opatrunkowych' in: M. Nałęcz, 'Problemy Biocybernetyki i Inżynierii Biomedycznej', T. 4 'Biomateriały', red. M. Kuś, Wydawnictwa Komunikacji i Łączności, p. 288 (1990).*
4. *D.T. Turner, Pharm. J., 222, p. 421-422 (1979).*
5. *C.K. Field, M.D. Kerstein, Am. J. Surg., Vol. 167, Issue 1, Supplement 1, January 1994, pp. S2-S6.*
6. *Patent US 4,788,146.*
7. *Patent EP-28993 (1998).*
8. *S. Hestrin, M. Schramm, Biochem. J., 58, 345 (1954).*
9. *F.C. Lui, R.M. Brown, J.B. Copper, Science, 230, 822 (1985).*
10. *K. Wrześniewska-Tosik, D. Ciechańska, E. Wesołowska, in: 'Cellulose and Cellulose Derivatives', ed. J. Kennedy, Woodhead Publishing Ltd. England, p. 15 (1995).*
11. *Pat Appl. P-328529 (1998).*
12. *D. Ciechańska, S. Bahrke, S. Haebel, H. Struszczyk, M.G. Peter, 'Incorporation of Glucose into Chitosan by Acetobacter xylinum', EUCHIS'04, 6th International Conference of the European Chitin Society, Poznań, 31.08-03.09. 2004.*
13. *D. Ciechańska, H. Struszczyk, K. Guzińska, Fibres & Textiles in Eastern Europe, Vol. 6, No. 4 (23), 61 (1998).*
14. *K. Edelman, E. Horn, Faserforschung Textiltechn., 7, 153, 1953.*
15. *K.H. Bodek, 'Estimation of microcrystalline chitosan usability as a polymer carrier of drugs', D.Sc. Dissertation, Medical Academy of Łódź (2002), p. 86 (in Polish).*
16. *Standard Procedure SPR/BBP/5, GLP No G-016 (IWCh).*
17. *PN-ISO 7218:1998.*
18. *JIS L.1902.1998.*
19. *S. Yamanaka, Proc. ISF'94, p. 445, Japan (1994).*
20. *D. Ciechańska, H. Struszczyk, K. Guzińska, Fibres & Textiles in Eastern Europe, Vol. 7, No. 3 (26), 49 (1999).*
21. *S. Yamanaka, K. Watanabe, N. Kitamura, M. Iguchi, J. Mater. Sci. 24, 3141 (1998).*
22. *D. Ciechańska, 'Biological dressing materials from modified cellulose', Research project report No 4T09B 042 22, Institute of Chemical Fibres, Łódź, 2004 (in Polish).*

Received 17.09.2004 Reviewed 19.11.2004