

## BIOACTIVE COMPOSITE MATERIALS

**Magdalena Kucharska, Antoni Niekraszewicz, Jolanta Lebioda,  
Kinga Malczewska-Brzoza, Ewa Wesołowska**

*Institute of Biopolymers and Chemical Fibres,  
ul. Skłodowskiej-Curie 19/27, 90-570 Łódź, Poland*

### 1. Introduction

During recent years there has been a growing demand for biological dressings, which could protect the wound in an individual phases of healing.

The main objective of the investigations is to elaborate new, composite biomaterials for medical applications.

New, composite materials based on the bioactive fibres containing substances with antimicrobial properties as well as various, useful chitosan forms were elaborated. These materials are recommended for construction of wound dressings useful in initial phase of therapy which is often connected with inflammatory state and heavy exudation.

It can be assumed that due to control release of the antibacterial agent the infection will be inhibited and the presence of chitosan will make easier the wound healing process and will shorten therapy. Modified staple polypropylene fibres characterised by antibacterial activity and selected chitosan forms such as: microcrystalline chitosan (MCCh) and chitosan fibrids were used in research. The manufacture process of bioactive composite materials in a form on non-woven prepared using wet or dry method as well as sponges prepared by lyophilization technique was elaborated.

Healing of the wounds is a complicated process and requires application, sometimes, a few kinds of wound care dressings depending on the phase of treatment. The factors that determine the choice of dressing are the type, size and location of wound, intensity of exudation, depth of damaged tissue and stage of healing.

Modern dressings for treatment of wound in the first stage of healing (cleaning phase) should [1]:

- protect wound from losing moisture,
- remain non-adherent to the wound and easy in removal,
- provide protection against infection,
- to be safe in use (non-toxic, non-allergic).

Infection of wounds is, the most often, the result of presence Gram-positive and Gram-negative bacteria such as: *Staphylococcus aureus*, *Pseudomonas aeruginosae*, and *Escherichia coli*. That is why the majority of modern dressings exploit, apart from, constituents to promote healing e.g. hydrocolloids, alginates, also antibacterial agents containing silver compounds. Of significant importance is that no known BACTERIAL RESISTANCE has developed to the silver ion as opposed to current antibiotics [2 - 4].

Overview of silver properties:

- potent anti-microbial agent,
- kills microorganisms instantly,
- blocks respiratory enzymes,
- heals indolent wounds,
- non-toxic to human tissue,
- no bacterial resistance,
- pro-healing properties,
- anti-inflammatory properties.

Silver ion kills micro-organisms instantly by blocking the respiratory enzyme system (energy production) while having no negative effect on human cells. The speed of action is almost instantaneous once the silver reaches the microbe. The efficacy of microbe killing is based not only on the amount of silver ion present, but likely also on the presence of other silver radicals generated by a silver releasing product. Silver exerts its antimicrobial effects by interfering with the respiratory chain at the replication.

Presently a number of companies exploit the silver compounds in new generation dressings e.g. Silveron, Acticoat, Aquacel Ag, Actisorb Silver 220 have various form and contain the different amount of silver compounds calculated to area unit (3 – 500 mg/100 cm<sup>2</sup>).

## 2. Experimental

In the frame of research work realized in IBWCh concerning dressing materials characterized by antimicrobial properties the following products were elaborated:

1. non-woven obtained using wet forming method containing bioactive fibres
2. chitosan sponges containing bioactive fibres or active agents obtained using lyophilization method

### 2. 1. Materials and methods

#### - *microcrystalline chitosan*

polymer obtained according to the method elaborated at IBWCh [5].

Physico-chemical parameters: chitosan with  $\bar{M}_v = 445$  kD, DD = 82%, polymer content in suspension = 2.43, WRV = 700 %

**- chitosan microfibrils**

new, useful chitosan form produced according to the method elaborated in the frame of research project No 3 T08E 012 28 in dynamic conditions with the usage of Dispax Reactor Labourr-Pilot 2000/4.

Physico-chemical parameters of aqueous suspension of microfibrils: chitosan with  $\bar{M}_v = 330$  kD, DD = 80%, polymer content in suspension = 2.0 – 2.4%, WRV = 7000 – 8000%, pH = 7.1 – 7.2; length of fibrils (in wet state) = 100 – 300  $\mu\text{m}$ , width (in wet state) = 5 – 15  $\mu\text{m}$ .

**- Bioactive polypropylene fibres**

fibres manufactured according to the method elaborated at IBWCh containing Irgaguard B 7000 (silica with Ag<sup>+</sup> ions) (Producer–Ciba) [6, 7]

Physico-chemical parameters of fibres:

Titre–2.76 dtex, tenacity–44.3 cN/tex, elongation–46.5%. antibacterial agent content–1 wt%

Microbiological activity:

*E. coli*: bacteriostatic activity – (6.6); bactericidal activity – (3.7)

*S. aureus*: bacteriostatic activity – (5.6); bactericidal activity – (3.6)

Fibres used for study were cut into 3 mm.

**- Antibacterial agents**

preparation M-20 (Produser: Esel Techtra Inc.), aluminosilicate containing Ag<sup>+</sup> i Zn<sup>++</sup> ions.

**- Microbiological activity**

Bioactivity tests were carried out in vitro conditions in Accredited Microbiological Laboratory (Accreditation Certificate number AB 388) according to Standard JIS L 1902 : 2002.

**- Assessment of physico-mechanical properties of dressings**

Physico-mechanical parameters of obtained non-wovens were determined by Accredited Metrological Laboratory (Accreditation Certificate number AB 388) according to Polish Standards:

For products in non-wovens form :

PN-EN ISO 9073-2:2002 (thickness)

PN-EN 29073-1:1994 (basis weight)

PN-EN 29073-3:1994 (breaking force, elongation)

For products in sponge form:

PN-EN ISO 4593: 1999 (thickness)

PN-EN 29073-1:1994 (basis weight)

PN-EN 29073-3 (breaking force)

**- Assessment of water retention value (WRV)**

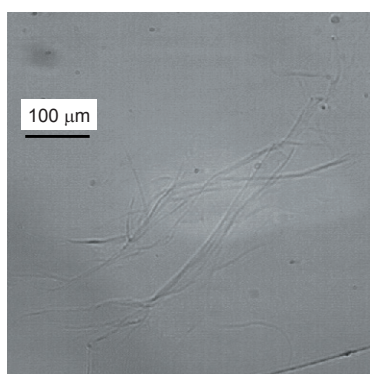
WRV of obtained products was determined according to standard procedure SPR/BPB/14 in Biomaterials Laboratory of IBWCh which possess GLP Certificate.

## 2. 2. Results and discussion

### 2.2.1. Non-woven containing bioactive fibres manufactured by wet forming metod

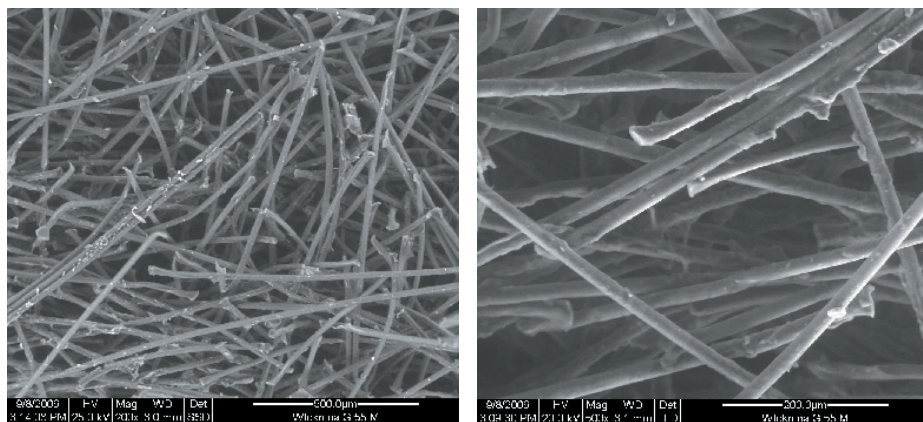
Non-woven containing bioactive fibres were produced using wet method of forming from aqueous mixture of: 4 part by weight of bioactive polypropylene fibres with the addition of Irgaguard B 7000, 1 part by weight of chitosan fibrils with the addition of microcrystalline chitosan (5 wt% calculated on dry polymer content in fibrils) and 5 part by weight plasti-cizer (glycerol). Non-woven were dried at temp. of 20 °C.

For this stage of studies the chitosan fibrils (Figure 1), microcrystalline chitosan and bioac-tive PP fibres characterised by properties described in Chapter 1 were used.



**Figure 1.** Microscopic photo of chitosan microfibrils.

Structure of non-woven was analyzed by SEM (FEI Company QUANTA 200). Surface of these products is shown in Figure 2



**Figure 2.** SEM photo of non-woven surface.

The structure of non-woven is quite close and individual fibres are combined by layer of chitosan fibrils. Physico-mechanical studies confirmed its good elasticity and tenacity (Table 1). There is also very interesting that addition of chitosan fibrils to PP fibres caused increase of WRV over 10 times.

**Table 1.** Some physico-mechanical properties of PP/chitosan non-woven; \* - WRV (water retention value for PP non-woven – 1,6%).

Parametr	Result
Non-woven constitution	PP fibres – 80% chitosan microfibrils – 20%
Basis weight, g/m <sup>2</sup>	219.0
Breaking force, N	0.5
Elongation at break %	8.7
WRV, %	19.0*

PP/chitosan non-woven were tested on bioactivity against Gram-negative bacteria *Escherichia coli* and Gram-positive bacteria *Staphylococcus aureus*. The results of study are presented in Table 2.

**Table 2.** Results of PP/chitosan non-woven bioactivity test.

Bacteria strain	Bacteriostatic activity	Bactericidal activity
<i>E. coli</i> – gram (-)	3.7	2.3
<i>S. aureus</i> - gram (+)	5.6	3.3

According to results presented above, non-woven show good bioactivity, both against Gram (+) and Gram (-) bacteria strains.

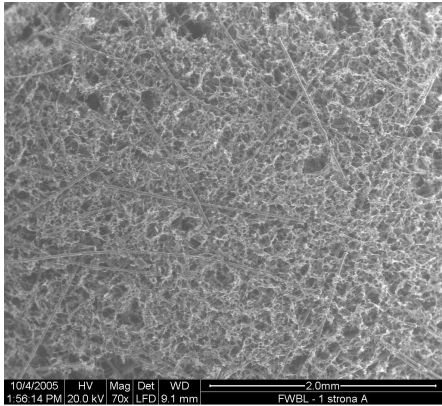
### 2.2.2. Chitosan sponges containing bioactive fibres.

Composite chitosan sponges were prepared using lyophilization metod (freeze drying) of aqueous suspension of chitosan fibrils, bioactive PP fibres containing Irgaguard B 7000 and plasticizer (glycerol).

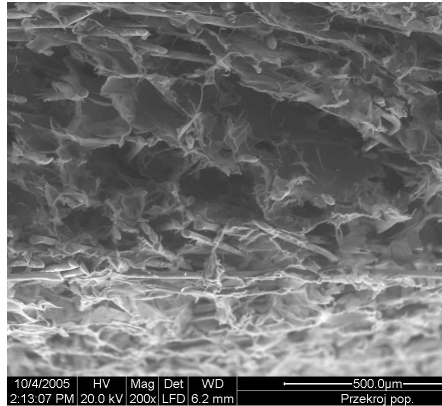
Figure 3 and 4 present the structure of surface as well as cross-section of composite chitosan sponges.

Manufactured composite sponges were tested on bioactivity against gram (-) bacteria *Escherichia coli* and gram (+) bacteria *Staphylococcus aureus*. The results of study are presented in Table 3.

Bioactivity studies proved that sponge containing 20 wt% of bioactive fibres has very good bacteriostatic properties as well as shows a small bactericidal activity. However sponge containing lower amount of PP bioactive fibres (10 wt%) does not show bactericidal properties.



**Figure 3.** SEM photo of sponge surface (Sponge chit. fibrids/PP bioactive fibres)



**Figure 4.** SEM photo of sponge cross-section (Sponge chit. fibrids/PP bioactive fibres)

**Table 3.** Results of sponge chit. fibrids/PP bioactive fibres bioactivity test

Composition of the sponge FCh/Irga1: bioactive fibres PP – 20 %, chitosan fibrids – 80 %		
Bacteria strain	Bacteriostatic activity	Bactericidal activity
<i>E. coli</i> – gram (-)	3.7	0.4
<i>S. aureus</i> - gram (+)	2.3	0.2
Composition of the sponge FCh/Irga2: standard fibres PP – 10 %, bioactive fibres PP – 10 %, chitosan fibrids – 80 %		
Bacteria strain	Bacteriostatic activity	Bactericidal activity
<i>E. coli</i> – gram (-)	2.8	none
<i>S. aureus</i> - gram (+)	1.2	none

For products obtained, the physico-mechanical properties were determined. The results are illustrated in Table 4.

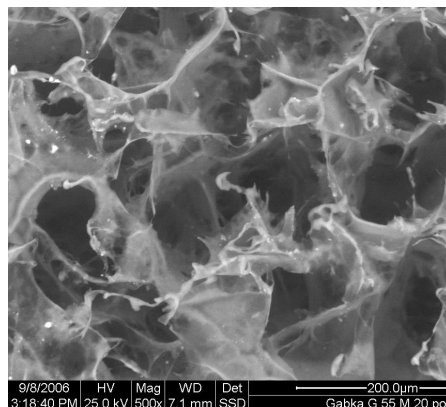
**Table 4.** Some physico-mechanical properties of composite sponges.

Parametr	Sponge FCh/Irga 1	Sponge FCh/Irga 2
Basis weight, g/m <sup>2</sup>	82.7	83.4
Tensile strength, MPa	0.014	0.020
Elongation, %	2.22	7.22
WRV, %	240.0	350.0

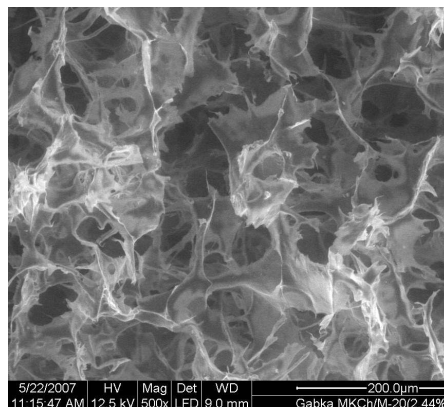
It has been found that larger amount of bioactive fibres in sponge FCh/Irga 1 caused insignificant decrease of tenacity in comparison to sponge FCh/Irga 2. However considerable decreasing of elasticity was noted for sponge FCh/Irga 1. It may be a result of the fact that bioactive fibres characterise by larger stiffness and roughness, so their larger amount in FCh/Irga 1 sponge caused fall of elasticity.

**2. 2. 3. Chitosan sponges containing bioactive agent M20**

Chitosan sponges were manufactured using lyophilization method of aqueous suspension of microcrystalline chitosan (polymer content 2.43 wt% ) containing bioactive agent M-20 in amount of: 1.0; 2.44; 4.75; 9.1 % and plasticizer ( glycerol). Surface and cross-section of sponges is presented on Figures 5 and 6, results of bioactivity tests in Tables 5 & 6.



**Figure 5.** SEM photo of sponge surface with M-20 (2.44 %).



**Figure 6.** SEM photo of sponge cross-section with M-20 (2.44 %).

**Table 5.** Antimicrobial activity of sponge MKCh/M20 against *E. coli* strain.

Symbol of sample	M-20 content, %	Bacteriostatic activity	Bactericidal activity
MKCh/M20/0	0.00	2.8	none
MKCh /M20/1	1.00	7.0	3.9
MKCh /M20/2.5	2.44	7.0	3.9
MKCh /M20/5	4.75	7.0	3.9
MKCh /M20/10	9.10	7.0	3.9

**Table 6.** Antimicrobial activity of sponge MKCh/M20 against *S. aureus* strain.

Symbol of sample	M-20 content, %	Bacteriostatic activity	Bactericidal activity
MKCh /M20/0	0.00	2.8	none
MKCh /M20/1	1.00	3.3	1.3
MKCh /M20/2.5	2.44	5.1	3.1
MKCh /M20/5	4.75	5.5	3.5
MKCh /M20/10	9.10	5.5	3.5

From the results presented in Tables 5 and 6, it may be noted that addition of bioactive preparation M-20 influenced the bacteriostatic and bactericidal effect on Gram-positive and gram-negative bacteria. Sponge containing, even, smallest amount of M-20 (1 wt%) is characterized by quite strong antibacterial effect.



In relation to *S. aureus*, bioactivity is diverse depending on M-20 content. Sponges containing 4.75 wt% and 9.1 wt % of M-20 have shown the highest values of activity, both bacteriostatic and bactericidal.

The physico-mechanical properties of chitosan composite sponges (thickness app. 25 mm) containing M-20 were also tested. The results, given in Table 7 showed that the presence of substance M-20 in amount of 1.0 - 9.1 wt% did not influence the mechanical properties of sponges. However the increasing the M-20 quantity caused decreasing of WRV values even up to 48% for sponge containing the maximal content of this substance.

**Table 7.** Some physico-mechanical properties of composite sponges.

Symbol of sample	M-20 content, %	Basis weight, g/m <sup>2</sup>	Tensile strength, MPa	Elongation at break, %	WRV, %
MKCh/M20/0	0.00	140	0.05	12.3	225
MKCh/M20/1	1.00	140	0.05	12.7	225
MKCh/M20/2,5	2.44	146	0.04	11.0	200
MKCh/M20/5	4.75	147	0.04	10.8	190
MKCh/M20/10	9.10	136	0.04	9.34	178

### 3. Conclusions

1. The method of manufacture of composite, bioactive dressing materials in a form of non-woven or sponge has been elaborated.
2. Bioactive PP fibres containing silver (Irguard B 7000), chitosan microfibrils and commercial antibacterial agent M-20 were used for studies. Techniques of spinning of bioactive PP fibres as well as chitosan microfibrils formation were elaborated at IBWCh.
3. All produced forms of dressings indicated bacteriostatic and bactericidal properties in relation to gram (+) and gram (-) bacteria strains.
4. Sponges made of microcrystalline chitosan with the addition of M-20 containing Ag<sup>+</sup> and Zn<sup>2+</sup> ions characterized by highest bioactivity effect.

### 4. References

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### 4. ACKNOWLEDGMENTS

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