Research into Developing Antibacterial Dressing Materials

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Abstract
For many years, an increasing demand has been observed for biological dressings which could protect wounds at particular stages of healing. Composite materials with a content of antibacterial fibres and/or different functional forms of chitosan were developed at the Institute of Biopolymers and Chemical Fibres (IBWCh). These materials may be used for dressings applied over the initial healing period, often on wounds accompanied by inflammations. We assumed that the antibacterial content would suppress the infection, whereas chitosan would facilitate the wounds' healing and shorten the recovery period. Tests were carried out in order to manufacture bioactive composite materials in the form of needle-punched nonwovens and sponges. We used modified polypropylene staple fibres with confirmed antibacterial properties and selected chitosan forms in the shape of fibrids. Materials with bacteriostatic properties against Escherichia coli and Staphylococcus aureus were obtained.

Key words: *dressing materials, bioactive fibres, antibacterial activity, chitosan.*

Introduction

Healing chronic wounds is a complex process, and sometimes requires applying several kinds of dressings, depending on the healing phase. The choice of a dressing depends on the kind and dimension of the wound, its positioning on the body, and in particular on the effusion intensity, the depth of tissue damage and the stage of healing. Appropriately-used dressings may prevent complications such as infection, tissue maceration, excessive effusion, swelling, pain, or lasting smell.

Modern dressings prevent wounds from drying, do not stick, are easy to remove, absorb effusions, have suitable gas permeability, prevent infection, do not cause allergies, do not irritate, and do not require frequent changing. Several modern dressings include substances which support healing, such as hydrocolloids, alginians, collagens, chitosan, and antibacterial agents.

An essential problem is the selection of the form of the dressing material. Nonwovens are one of the basic forms used for dressing applications. In order to give antibacterial properties to nonwovens, it is necessary to apply finish techniques, or manufacture nonwovens from blends containing bioactive fibres. The authors have chosen this second option. The cost of manufacturing bioactive fibres on the basis of the technology developed at the Institute of Biopolymers and Chemical Fibres (IBWCh) is only higher than that of non-modified fibres by the cost of the biocide introduced. On the other hand, in the case of using finish techniques, the nonwoven's cost is higher by the costs of the biomodifier, of preparing the finish bath, the energy used to dry the modified nonwoven, and the labour costs. What is more, it should be mentioned that antibacterial dressings belong to the group of special dressings, and their price will always be higher than that of classical dressings. An indisputable advantage of antibacterial dressings is their ability to restrict the growth of bacteria flora within the wound's surrounding, prevent the occurrence of irritations, and shorten the healing period. In this way, a bioactive dressing is handier on training and field grounds.

Wound infections are mainly caused by the gram (+) bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosae*, and gram (-) rod-shaped bacteria, such as *Escherichia coli* [1]. An essential problem connected with infections caused by Staphylococcus is their resistance to metyciline, which practically means resistance to all β-lactam antibiotics [2].

The increase in the resistance of bacteria to antibiotics and other antibacterial agents is an essential problem in medicine. Of significant importance is the fact that bacteria do not develop any resistance to silver ions, in contrast to

some antibiotics currently used. Silver ions kill microorganisms almost instantly after reaching the microbe, by blocking the enzyme of the bacteria respiratory system [3], while at the same time not being toxic to human cells. The efficiency of microbe killing is based not only on the amount of silver ions, but also on the presence of silver radicals released from silver-containing products. The anti-inflammatory effect of silver ions was recognised centuries ago, but most of the reports are only descriptive, identifying the fading of erythema and increased healing.

Several companies use silver compounds in new-generation dressings, such as example Smith & Nephew in the Acticoat dressing, Argentum Medica in the Silverlon dressing, and Coloplast in the Contreet Foam and Contreet Hydrocolloid dressings [4 – 7]. These dressings have different forms, such as nonwovens, knitted fabrics, sponges & gels, and contain various amounts of silver compounds re-calculated per area unit (from 2.7 to 546 mg/100 cm^2).

Chitosan, a deacetylated derivative of chitin, is often used in dressing materials thanks to its biological activity. Chitosan dressings are known in the form of sponges, which release a therapeutic [8] or anti-inflammatory [9] agent. A chitosan dressing with anti-inflammatory and painkilling properties, which accelerates wound healing, has been described in patent [10]. Haemostatic dressings based on chitin and chitosan are known in the USA, for example the Syvek patch and RDH from Marine Polymer Technologies, Clo-Sur PAD from Medtronic/Scion, Chito-Seal from Abbot, and M-Patch & Trauma DEX from Medafor [11, 12]. Research carried out at IBWCh generally falls within the field of application. Thus, PP/PET nonwovens containing antibacterial fibres were manufactured by a classical textile method, which will allow practical implementation. We used PP bioactive staple fibres developed in IBWCh and manufactured with the use of an industrial plant. PP and PET fibres are often used in dressing materials, and are intended for the layer directly contacting the wound. Their task is only to transport the effusion to the dressing's absorption layer and not to absorb anything by themselves. On the other hand, the layer contacting the wound may include antibacterial agents, but its main task is not to stick to the wound and thus hinder changing the dressing. The authors considered this opportunity while developing the antibacterial composite materials with the content of antibacterial fibres, biocides, and/or different chitosan forms, which was the main aim of the presented research work.

Experimental

The following materials were developed in IBWCh within the scope of a research project aimed at obtaining dressing materials with antibacterial properties:

1. needle-punched nonwovens with a bioactive fibre content;
2. dressing sponges from chitosan fibrids obtained by lyophilisation with the addition of
   - antibacterial agents and
   - bioactive fibres.

Materials

Antibacterial additions:

- Irgaguard B 7000 from Ciba, a silicon dioxide with Ag+ ions.
- Triclosan (Irgasan DP 300) from Ciba, a 2,4,4'-trichloro-2'hydroxydiphenylo ether.
- M-20 from Esel Techtra Inc., an aluminosilicate with Ag^+ and Zn^{++} ions.

Bioactive polypropylene fibres

Bioactive modified polypropylene (PP) fibres, manufactured according to a method developed at IBWCh [13, 14] and characterised in Table 1, were used.

Chitosan

Initial chitosan of an average molecular weight of M_v ~ 400 kD, and a deacetylation degree of 80% manufactured by Vanson HaloSource, USA.

Methods

Estimating the antibacterial activity

The antibacterial activity against the gram (-) bacterium *Escherichia coli* (*E. coli*) and the gram (+) bacterium *Staphylococcus aureus* (*S. aureus*) was estimated by the accredited Microbiological Laboratory at IBWCh by the quantitative method, in accordance with standard JIS L 1900:2002.

Assessing the physico-mechanical parameters of the dressings manufactured

The physico-mechanical parameters of the nonwovens were assessed in the Metrological Laboratory of IBWCh, which has an AB 388 accreditation certificate. The tests were carried out in accordance with the Polish standards listed in Table 2.

Table 1. *Selected properties of bioactive PP fibres manufactured in IBWCh.*

Parameter	Staple fibres	Filament cut
Active substance, % wt	Triclosan , 0.5	Irgaguard B 7000, 1.0
Linear density, dtex	5.54	2.76
Tenacity, cN/tex	29.4	12.7
Elongation at break, %	184	66
length of cut, mm	60	3

Table 2. *Standards for tests of the particular parameters.*

Parameter	Nonwovens	Sponges
Thickness	PN-EN ISO 9073-2:2002	PN- ISO 4593:1999
Area mass	PN-EN 29073-1:1994	PN-EN 29073-1:1994
Breaking force, elongation at break	PN-EN 29073-3:1994	PN-EN ISO 527-3:1998

Table 3. *Selected parameters of the needlepunched nonwovens.*

Percentage content of the particular fibres, %	Nonwoven thickness, mm	Area mass, g/m^2	Content of active agent, mg/100 cm^2
100% PET1.60	77.1	0	0.00
100% PP	1.61	69.0	3.45
75 % PP	1.66	78.0	2.93
50 % PP	1.73	83.3	2.08
25 % PP	1.74	76.0	0.95
20 % PP	1.56	99.8	0.99
15 % PP	1.73	80.7	0.60
10 % PP	1.82	111.0	0.55
5 % PP	1.88	124.0	0.31

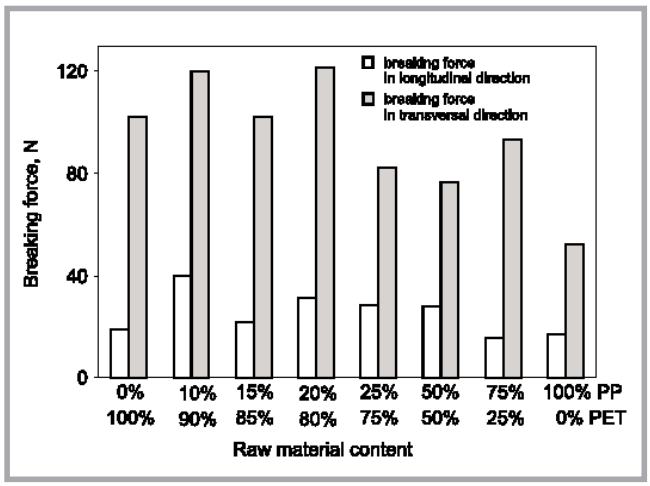

Figure 1. *Breaking force in dependence on the raw material content of the PP/PET nonwovens.*

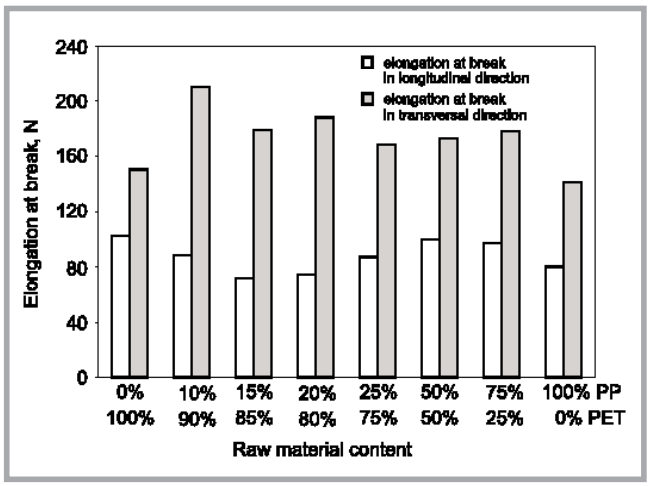

Figure 2. *Elongation at break in dependence on the raw material content of the PP/PET nonwoven.*

Test results and discussion

Needle-punched nonwovens with content of bioactive fibres

The nonwovens were manufactured in the Institute of Teratechnology, Łódź Branch (ITeE). Bioactive PP staple fibres containing 0.5% wt of Triclosan, and PET staple fibres with a linear density of 3.3 dtex and a staple length of 57 mm manufactured in Elana SA, Poland, were used for manufacturing the nonwovens. The webs were manufactured with the use of a modernised laboratory carding machine with a working width of 0.6 m from the Joseph Co. The webs were twice stiched by a Vulkan stitching machine. In order to optimise the nonwovens' content, the percentage content of the modified fibres was changed within a range from 5% wt to 100% wt. Table 3 presents the basic parameters of the nonwovens manufactured.

Figures 1 and 2 present the dependence of the breaking force and elongation at break on the nonwovens' content. The breaking force in the longitudinal direction changed within the range of 52 – 122 N in dependence on the content of the nonwoven's raw material, whereas in the transversal direction it fell within the range of 15.6 to 40 N. The nonwovens manufactured with a bioactive fibre content of 10 – 20% were characterised by the highest strength properties; a decrease in the breaking strength was attested for nonwovens with higher bioactive fibre content.

The nonwovens' elongation at break in the transversal direction was significantly higher than that in the longitudinal direction. The elongations at break were within the ranges of 72 –102% in the longitudinal and 141 – 210% in the transversal direction, and no explicit dependencies on the raw material content of this parameter could be indicated.

Nonwovens of various bioactive fibre contents were tested in the Microbiological Laboratory of IBWCh to prove their activity against *E. coli* and *S. aureus*. The results obtained are presented in Tables 4 and 5.

A distinctive decrease in the antibacterial activity against both the bacteria tested takes place with the decrease in the content of the PP antibacterial fibres. Antibacterial activity against Gram (-) bacterium *Escherichia coli* was characterised by nonwovens manufactured with the use of 75% and 100% modified PP fibres. The nonwovens' activity against gram (+) bacterium *S. aureus* was higher, and a bactericidal effect could be indicated even at a 25% content of modified PP fibres. All the nonwovens prepared containing 5 – 100% of modified fibres were characterised by a bacteriostatic effect against the test bacteria. In order to secure efficient bactericidal activity, nonwovens devoted to dressing materials should contain no less than 75% of modified PP fibres. It should be mentioned that the nonwovens obtained were characterised by low moisture sorption, of a water retention value (WRV) of 5 – 7 %.

Chitosan dressing sponges

The composite chitosan sponges which we prepared as part of this research work

Table 4. *Influence of the nonwovens' raw material content on the nonwovens' activity against the gram (-) bacterium E. coli ATCC 11229 (in accordance with JIS L 1900:2002).*

Nonwoven's content	Bacteriostatic effect	Bactericidal effect
Standard	-	-
100 PP	6.8	3.7
75/25 PP / PET	5.3	3.1
50/50 PP / PET	0.9	-2.2
25/75 PP / PET	0.4	-2.7
15/85 PP / PET	0.6	-3.0
5/95 PP / PET	0.5	-3.1

Table 5. *Influence of the nonwovens' raw material content on the nonwovens' activity against the gram (+) bacterium S aureus ATCC 6538 (in accordance with JIS L 1900:2002).*

Nonwoven's content	Bacteriostatic effect	Bactericidal effect
Standard	-	-
100 PP	5.0	3.5
75/25 PP / PET	5.0	3.5
50/50 PP / PET	5.0	3.5
25/75 PP / PET	5.0	3.5
15/85 PP / PET	2.6	0.2
5/95 PP / PET	0.6	-1.8

represent a quite different bioactive material. Our investigations concerned the two following assortments of sponges manufactured by the lyophilisation technique:

- sponges from chitosan fibrids containing antibacterial fibres, and
- sponges from chitosan fibrids with antibacterial agent content.

The use of chitosan for dressing applications is justified by its following biological properties: its biodegradability, bioconformity, antibacterial and antimycotic activity, the ability to stimulate immunological mechanisms, and its ability to accelerate blood coagulation. The therapeutic usability of dressings made of modified chitosan has been verified during our earlier research works. We indicated that they isolate the wound well from the surroundings, limit the growth of bacterial flora (the bacteriostatic effect against *E. coli*), and increase the growth of epidermis & speed up the healing of infected wounds [15].

Chitosan fibrids manufactured by a method developed in the IBWCh were used in order to obtain the sponges. A Dispax Reactor Labor-Pilot flow-reactor was applied. The fibrids were formed under dynamic conditions by simultaneously feeding streams of a chitosan solution and an alkali bath into the reactor. In order to produce the dressing sponges, chitosan fibrids with a high WRV factor, PP fibres with an 1% wt content of the Irgaguard B 7000 agent or the M-20 antibacterial preparation and a plastificator were used. The lyophilisation was carried out with the use of a sublimation drier of the ALFA-1-4 type from Christ Co. over about 20 hours.

Sponges from chitosan fibrids with addition of bioactive fibres

An aqueous suspension of chitosan fibrids with the addition of glycerine and cut & modified PP fibres of 50% wt related to chitosan was used in order to obtain the sponges. The use of a 50% content of PP fibres with the addition of the active substance Irgaguard B 7000, while manufacturing the chitosan sponges resulted from the recalculation of the amount of this addition in the fibres in relation to the total mass of the sponge. As was indicated by the activity tests of the modified fibres (including microbiological tests) carried out earlier, the resultant modifier concentration in the sponge with the content of these fibres should assure bacteriostatic activity.

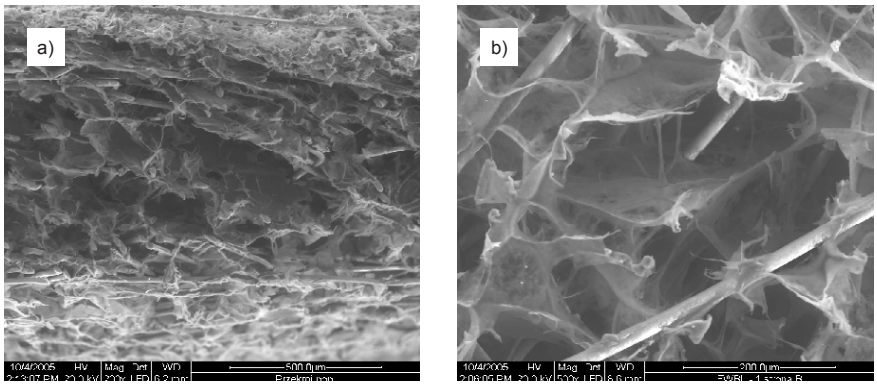

Figure 3. *Chitosan sponge with an addition of PP fibres including Irgaguard B 7000; a) cross-section, magnitude 200×, b) sponge surface, magnitude 500×.*

Figure 3 presents SEM photos of the sponge surface and cross-section of sponges, which include PP fibres with Irgaguard B 7000 incorporated in the sponge matter.

Table 6 lists selected properties of the sponges manufactured, including the activity against gram (-) bacterium *E. coli* and gram (+) bacterium *S. aureus*.

Introducing bioactive fibres worsened the sorption properties (WRV) and decreased the sponges' strength by about 25%, but at the same time secured the bacteriostatic properties against both test bacteria.

Sponges from chitosan fibrids with antibacterial agent content

An aqueous suspension of chitosan fibrids, in which the M-20 antibacterial agent was dispersed in the presence of glycerine, was used to manufacture the sponges. Four concentrations of biocide within the range from 0.25% to

Table 6. *Selected property parameters of the chitosan sponges.*

Parameter	Sponge, non-modified	Sponge with PP fibres with Irgaguard B 7000 agent
Area mass, g/m^2	83.4	82.7
Thickness, mm	1.53	1.50
Breaking force, cN,	46.5	34.6
WRV, %	320	160
Bacteriostatic effect against: *E. coli* *S. aureus*	 0 0	 2.8 1.2

Table 7. *Physico-mechanical properties of the sponges manufactured from chitosan fibrids including a M-20 addition.*

Sample designation	Amount of M-20, %	Area mass, g/m^2	Thickness, mm	Breaking force, N	WRV, %
M20-0.25	0.25	130	2.5	25.4	370
M20-1.0	1.00	132	2.5	22.6	380
M20-2.5	2.50	140	2.5	24.0	350
M20-5.0	5.00	145	2.6	25.5	310
M20-10.0	10.00	148	2.6	24.6	290

Table 8. *Antibacterial activity of chitosan sponges with a M-20 addition against E. coli and S. aureus, in accordance with JIS L 1900:2002.*

Sample designation	Activity against *Escherichia coli*		Activity against *Staphylococcus aureus*	
	bacteriostatic	bactericidal	bacteriostatic	bactericidal
M20-0.25	0	0	0	0
M20-1.0	7.0	3.9	3.3	1.3
M20-2.5	7.0	3.9	5.1	3.1
M20-5.0	7.0	3.9	5.5	3.5
M20-10.0	7.0	3.9	5.5	3.5

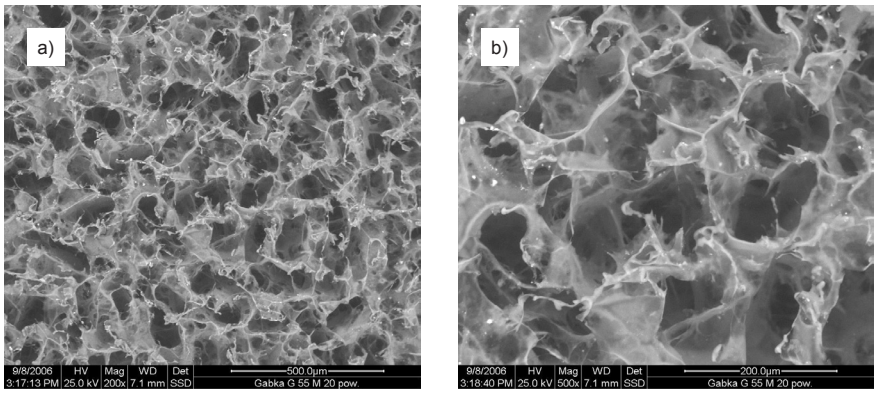

Figure 4. *Surface of M20-5.0 chitosan sponges; a) magnification 200×, b) magnification 500×.*

10.0% were used in the dispersion. In order to improve the strength properties, the sponges were joined during the lyophilisation with a polyester-viscose nonwoven of the hydronin type with an area mass of 60 g/m^2 from Lentex Co. The properties of the two-layer sponges obtained are presented in Table 7.

The two-layer composite sponges manufactured as described above were not only characterised by high mechanical strength, which depends mainly on the nonwoven, but also by a relatively high WRV value, higher than that of the sponges modified by bioactive PP fibres. The modifier particles dispersed in the sponge's structure are clearly visible in photo b) of Figure 4.

Table 8 presents the results of estimating the antibacterial activity of chitosan sponges including M-20 against gram (-) bacterium *E. coli* and gram (-) bacterium *S aureus*.

All the sponges tested, which were manufactured with an addition of the M-20 agent in an amount no smaller than 1.0% wt, were characterised by bacteriostatic and bactericidal activity against *E. coli* and *S aureus*. The slightly higher activity of the sponges against E. coli was indicated. We can assume that chitosan sponges which include only 1.0% wt of M-20 used in dressing materials will be characterised by effective antibacterial action.

Summary

We developed various forms of composite materials to limit bacterial growth which were prepared for dressings. The antibacterial activity depended on the form and amount of the biocide in the composite. The area mass of the materials developed was within the range of 80 to 150 g/m^2 (the latter in the case of sponges joined with a nonwoven). The water retention value (WRV) factor depended on the structure and material composition. Sponges from chitosan fibrids were characterised by a WRV within the range of 310 – 350%, which ensured good sorption and absorbency properties. The high WRV results of the properties of the half-finished product and the manufacturing technique used (in this case, chitosan fibrids and lyophilisation respectively) provide appropriate porosity, which is visible in the microscopic photos presented. On the other hand, PP/PET composite nonwovens were characterised by low sorption, which predestines such material for the dressing layer, positioned directly on the wound (after decreasing their area mass). Some of the materials developed will be further tested by biological and medical investigations within the scope of bioconformity, cytotoxicity, and irritation effects.

We intend for the materials we proposed to be used for developing dressings useful to heal wounds which are infected and require much time and care to heal, and which would need additional application of antibiotics when applying standard dressings.

We also intend to carry out comparative investigations to estimate the antibacterial properties of commercial dressings and those developed by us, but only after optimum dressing material forms will be selected from among those which we are preparing. A comparative analysis will be very difficult, as the antibacterial dressings cited in our work differ not only in their form but also by the amount of the biocide introduced; what is more, the type of bactericide agent used by the manufacturer is not commonly known.

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