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Activated Carbon / Dibutyrylchitin (DBC) as Fibrous Antibacterial Noncytotoxic Wound Dressing Material

Abstract
In this study, a noncytotoxic fibrous dressing material was developed using an activated carbon nonwoven, directly overlaid with dibutyrylchitin fibres by electrospinning. This dressing material is designed for wounds that are hard to heal. Its sorption capacity is imparted by highly adsorptive carbon fibres; when measured for distilled water, it is higher than that of commercially available silver-containing carbon dressing, and amounts to 6.63 g/g. As shown by tests, dibutyrylchitin fibres containing a bioactive substance ensure antimicrobial action showing none of the cytotoxic effects that occur in silver-containing dressings. The tests performed for this dressing material confirm that it shows no skin and eye irritating actions, no acute intra-gastric toxicity and no effect on the values of immunoglobulin E and fibrinogen concentration, which indicates that the material produces no allergic reaction.

Key words: *wound dressing material, dibutyrylchitin, activated carbon, biocide.*

Introduction

In recent years, many active dressing materials have been developed for use in healing exudative wounds. A good active dressing material should not only adsorb a secreted substance but also create beneficial conditions for wound healing. Usually, its adsorptive part responsible for the adsorption of exudate is in the form of a gauze, nonwoven or gel layer. It often consists of a common sorbent such as activated carbon, especially fibrous activated carbon of high sorption capacity [1]. An agent facilitating wound healing mostly consists of a properly selected polymer that ensures a moist environment, sometimes additionally enriched with active substances, e.g. noble metal particles showing antibacterial action.

Polymers that beneficially influence wounds include, among others, polyvinyl alcohol, polyvinyl pyrrolidone, polyacrylic acid, polyethylene oxide [2], benzyl hyaluronate [3], carboxymethylated cellulose [4], alginate [4, 5], microbial cellulose [6], chitosan [7, 8], chitin and its derivatives [9, 10]. In a dressing material, they can be used in the form of a membrane, film, spongy product or nonwoven [11 - 14]. The fibrous layer of chitin derivative, i.e. dibutyrylchitin, not only protects the surface of the wound, especially against excessive fluid loss, but also considerably accelerates the process of its healing. Moreover, this layer disappears after fulfilling its function, owing to which the patient's healing comfort is decisively improved [15].

Among the active substances used in dressing materials is silver, commonly found in various medical products as an antibacterial agent [16, 17]. Unfortunately, silver is cytotoxic, and when in contact with a wound under healing, it can adversely affect the restored skin tissue. Only an appropriately low silver content in dressing material is safe for skin, otherwise it may cause undesired effects [18].

The conventional dressings used so far in wound healing meet several essential requirements, such as the protection of the wound against external microbe invasion, effective control of infection if found, pain alleviation and medical personnel protection against the risk of infection. The TIME scheme (tissue-infection–moisture-edge) proposed by EWMA (European Wound Management Association) is aimed at the systematisation of wound assessments to help in selecting proper therapy and dressings that should ensure optimal conditions in a wound bed, including the protection of damaged wound tissue and edges, restoration of optimal humidity, reduction in the number of bacteria and correction of disturbances impeding the progress of healing [19, 20].

Our investigations were aimed at the development of an 'ideal' dressing material, i.e. noncytotoxic, showing a high sorption capacity, ensuring beneficial conditions of wound healing, especially varicose ulceration and infection wounds, having high antibacterial activity as well as being capable of facilitating skin tissue restoration. The dressing material proposed was made of activated carbon fibres and dibutyrylchitin fibres containing a bioactive agent called Microbiocide N750. The material obtained (AC/DBC) was compared with a commercial dressing also designed for wounds that are difficult to heal. The commercial dressing denoted as AC/silver is a woven dressing material based on pure activated carbon impregnated with silver showing adsorption and antibacterial properties. It is used in healing infected wounds with a bad odour and purulent secretion.

The point was to characterise the material obtained as a potential dressing designed for clinical applications and to compare it with the commercial material mentioned above. Considering the different forms and structures of the carbon layer in the materials compared, their sorptive properties were assessed. In view of the differences concerning the antimicrobial agent in the materials under comparison, their effects on the human organism was assessed with respect to the indication of possible side effects resulting from their use.

The tests performed showed the superiority of the material developed over the commercial AC/silver dressing, mainly due to the fact that AC/DBC is not cytotoxic and has a higher sorption capacity measured for distilled water. Moreover, according to literature reports [11, 21], the layer of biodegradable dibutyrylchitin can act as chitin to ensure a moist environment in the wound and to stimulate the regeneration of damaged tissues.

Gliścińska E, Babeł K, Krucińska I, Kowalczyk E. Activated Carbon / Dibutyrylchitin (DBC) as Fibrous Antibacterial Noncytotoxic Wound Dressing Material. *FIBRES & TEXTILES in Eastern Europe* 2012; 20, 2(91): 84-90.

Experimental

The dressing material consisted of an activated carbon nonwoven overlaid with dibutyrylchitin fibres containing a bioactive substance enforcing their antibacterial action. The dibutyrylchitin layer should be on the wound side. Highly adsorptive carbon fibres were used to ensure the sorptive properties of the dressing, a microclimate in the wound environment and an antibacterial barrier at the surrounding border. The layer of biodegradable dibutyrylchitin was to act as chitin to ensure a humid environment and stimulate the regeneration of damaged tissue; and owing to the presence of a bioactive substance, it should show antibacterial and antifungal properties. Moreover, thanks to the fine filaments of 1 μm in size and developed external surface, dibutyrylchitin should contribute to accelerating bioreadsorption.

The dressing material in question was prepared by three processes:

1. Pyrolysis of a precursor nonwoven to obtain a carbon nonwoven
2. Activation of the carbon nonwoven
3. Deposition of dibutyrylchitin fibres containing a bioactive substance on the activated carbon nonwoven.

Materials

A viscose spunlace nonwoven from 'Lentex' S.A. (Lubliniec, Poland) was used to prepare the activated carbon nonwoven. The layer of diburyrylchitin fibres was obtained with the use of krill dibutyrylchitin of the following characteristics: intrinsic viscosity: 1.94 dl/g, measured in dimethylacetamide (DMAC) solution at 25 °C, molar weight: 127100 g/mol, measured by the technique of gel chromatography, and Mw/Mn: 2.79 (distribution width or polydispersion degree). Ethanol was used as a solvent of dibuturylchitin. As a bioactive substance added to dibutyrylchitin solution, Microbiocide – N750 from INTER-IODEX Sp. z o.o. Tarnowo Podgórne, Poland, was used. This preparation in form of a homogeneous liquid contained the following: N,N, n,n,-didecyl-N,N-dimethylammonium chloride (> 25%), Bis-(3-aminopropyl)-dodecylamine (< 5%), and 2-propanol (< 20%).

Pyrolysis process

Pyrolysis was carried out in a chamber reactor, in an emitting vapour and gas atmosphere free of air. The nonwoven was heated up to 500 °C at a rate of 3 °C/min and then stored in a thermostat at the final temperature for 1 h. The process yield was at a level of 20.5%.

Activation process

The process conditions ensuring the preparation of a nonwoven with a large total pore volume were as follows: temperature: 850 °C, duration: 60 min, and quantity of activating gas (CO_2) : 60 l/h. The process yield was 26.1%.

Deposition of dibutyrylchitin fibres on the surface of activated carbon nonwoven

The dibutyrylchitin fibre layer was deposited on activated carbon nonwoven directly using the electrospinning process by the conventional method with a capillary. The electrospinning process parameters were as follows: distance between the capillary tip and collector: 25 cm, applied voltage: 17.5 kV, and capillary diameter: 0.8 mm. The spinning solution consisted of 8% krill dibutyrylchitin solution in ethanol containing 0.5% of Microbiocide-N750 (concentration of active substance in the solution: about 0.15 wt.%). It was assumed to use a minimum quantity of this modifier (for economical reasons), but sufficient to obtain antibacterial and antifungal effects.

Physical properties

Physical properties were determined according to proper standards: thickness according to PN-EN ISO 9073-2:2002, surface weight according to PN-EN 29073-1:1994, and air permeability according to PN-EN ISO 9237:1998.

The transverse dimensions of dibutyrylchitin fibres produced by the electrospinning technique were determined by means of SEM microphotography (scanning microscope JEOL JSM 5200 LV, Jeol LTD., Japan) and the Lucia G image analysis software program (Laboratory Imaging s.r.o., Czech Republic).

Mechanical properties

Tensile strength tests were performed by means of a tensile tester (model 4204 from Instron, USA) according to PN-EN 29073-3:1994. Compressive strength tests were carried out with the use of a KAWABATA KES FB -3- AUTO measurement system (Japan).

Water extract

Tests were performed in an aqueous medium as the most neutral one. Water extract of the dressing material under investigation was tested for the presence of chlorides, sulfates and starch [22].

Antimicrobial activity

The principle of the qualitative assessment of antibacterial activity consists in placing a sample on an agar substrate containing bacterial culture and observing its growth under and around the sample. The antimicrobial activity of the sample tested is shown in the form of the so-called inhibition zone of microbial growth measured in mm. The bactericidal effect in relation to Gram-positive bacteria *Staphylococcus aureus* ATCC 6538 and Gram-negative bacteria *Escherichia coli* ATCC 8739 was determined according to Standard PN-EN ISO 20645:2005. The fungicidal effect in relation to yeast *Candida albicans* ATCC 10231 and fungi *Trichophyton mentagrophytes* Ł 0572 was determined according to the AATCC Test Method 147 – 1998. These tests were performed for the AC/DBC material with and without the bioactive substance in dibutyrylchitin fibres. The samples for testing were radiation-sterilised with a dose of 25 kGy.

Sorption properties/porous structure

Comparative tests of the AC/DBC material and commercial AC/silver were performed by the technique of surface liquid sorption with the use of SORP 3 apparatus (Textile Research Institute, Poland).

The porous structure of the activated carbon fibres was investigated using nitrogen adsorption-desorption isotherms at –196.15 C, determined in a sorptometer - ASAP 2010, manufactured by Micromeritics Instrument Corp. (USA). Prior to the isotherm measurements, the samples were outgassed at 300 °C for 10 h to a constant vacuum (10 - 4 kPa). Their specific surface area was calculated by the BET method (S_{BET}); the total pore volumes (V_{tot}) were determined from the last point of the isotherm at a relative pressure of 0.99 using the BJH model; the pore volume and pore size distributions were derived from the adsorption branches of the isotherms (V_{mes}). The micropore volume (V_{mic}) was calculated by the t-plot method.

Cytotoxicity tests

Tests were performed for both AC/DBC and AC/silver dressing materials previously sterilized with a dose of 25 kGy, in accordance with Standard PN-EN ISO

10993-5:2001 'Biological assessment of medical products – part 5: Cytotoxicity tests in vitro."

These tests were carried out by the method of direct contact with a monolayer culture of L929 cells (a line of fibroblast-like cells obtained from the hypodermic fatty tissue of a mouse (ATCC CCL 1)) at a temperature of 37 °C in an atmosphere of 5% CO_2. Qualitative and morphological changes induced by the materials tested were assessed after 24, 48 and 72 h by means of a phase-contrast microscope. To determine the quantity of dead cells, samples were dyed with trypan blue.

Irritating action, acute intragastric toxicity and effect on selected blood parameters

Considering the future of the material developed, i.e. its registration as a dressing material containing therapeutic agents for sale without prescription, appropriate tests were carried out under consent No. 29/ŁB468/2009-9 by the Local Bioethic Commission for Experiments with Animals acting at the Medical University of Łódź, Poland. The materials for testing were radiation-sterilised with a dose of 25 kGy.

Irritating effects

Irritating action was performed with 9 male rabbits BN according to guidelines 405 – acute irritation/eye injury – procedure OECD TG 405:2001 for the assessment of eye irritating effects (3 rabbits) and in accordance with the requirements of the Polish Pharmacopea, edition VI of 2002 for the assessment skin irritating effects (6 rabbits).

The material under testing was deposited on scarified rabbit skin on the right hand side of the back. The opposite side was used for comparative assessment. The test results were assessed after 30 min, 4, 48 and 72 h from the moment of material deposition. After 72 h the dressing was taken off, and after successive 24 and 48 h the areas of dressing deposition were reassessed. The assessment took into account erythema, desquamation and swelling according to a scale from 0 to 4. The assessment of irritating action was performed according to a scale from 0 to 8.

Acute intragastric toxicity

Acute intragastric toxicity tests were performed on 5 male rats of the Wistar strain according to guidelines 425 – acute toxicity – oral administration – procedure OECD TG 425 of 2001. The dosage level of an aqueous suspension of the dressing tested was 2 g/kg.m.c.

Effect on selected blood parameters and histopathologic image

Laboratory tests were carried out on both AC/DBC and AC/silver materials with the use of 30 male rabbits BN with an average body weight of 2.5 kg. The total level of immunoglobulin E (IgE), fibrinogen concentration, prothrombine time (PT), and partial thromboplastin time after activation (APTT) were determined. Histopathological tests were also performed.

Results and discussion

A carbon nonwoven prepared by pyrolysis was activated to obtain an activated carbon nonwoven with a high sorption capacity, which was then overlaid with a layer of dibutyrylchitin fibres containing a bioactive substance by electrospinning. The dibutyrylchitin fibres were in the form of twisted ribbon, whose smaller side of the cross-section amounted to about 1300 nm (***Figure 1***). The content of these fibres in the dressing material, determined as a quantity of solution covering 1m^2 of the nonwoven surface, was 40 ml/m^2.

Physical properties of the material after successive modification stages are listed in ***Table 1***.

The pyrolysis process causes a considerable decrease in the surface weight of the precursor material and its thickness. A similar effect, but to a lesser extent, is due to the activation of carbon material. The direct spinning of a low amount of dibutyrylchitin fibres onto the activated carbon nonwoven slightly increases the material weight, but there is also an almost unnoticeable decrease in thickness resulting from the partial solidification of fibres on the carbon nonwoven, as well as their partial sinking into the nonwoven structure. The air permeability of the material after pyrolysis and activation increases, but after the deposition of dibutyrylchitin fibres it is at the level of the initial material, i.e. the precursor nonwoven, which seems to be connected with the formation of an external dense fibre layer impeding air flow.

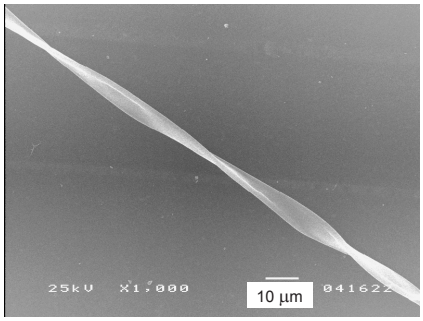

Figure 1. *SEM image of dibutyrylchitin nanofibres obtained from 8 wt.% DBC/ethanol solution with 0.5 wt.% of MICROBIOCIDE-N750.*

Mechanical properties of the material after successive modification stages are presented in ***Tables 2*** & ***3***.

In consequence of the pyrolysis and activation, the tensile strength of the material is drastically reduced; its breaking force decreases from 88.67 N to 2.09 N and then to 0.82 N, respectively. However, after the deposition of dibutyrylchitin fibres, the breaking force slightly increases to 2.12 N. Similar dependences are observed in the case of the elongation at break.

Test results for the compression strength show that the linearity of the nonwoven increases after pyrolysis and activation, while after the deposition of dibutyrylchitin fibres it returns to the initial value, i.e. to that of the precursor nonwoven. The material's compression energy finally drops, but the strain after compression increases, amounting to a low level of 71.80%.

Chemical test results of the nonwoven aqueous extract after successive modification stages are listed in ***Table 4***.

From the test results it follows that the pH of the aqueous extracts for the nonwoven after carbonisation and activation shifts towards basic values. Such a change results from the fact that during the thermal treatment of nonwoven, it is the surface acidic groups that are decomposed first . The dibutyrylchitin overlay changes the pH only to an insignificant extent. Moreover, no chlorides, sulfates or starch are observed in the aqueous extract.

Antimicrobial effects

From the test results it follows that dibutyrylchitin fibres show good antimicrobial properties against Gram-positive bacteria *Staphylococcus aureus*

and Gram-negative *Escherichia coli* as well as against the fungi *Trichophyton mentagrophytes,* while their action effectiveness in relation to yeast *Candida albicans* is worse. Activated carbon nonwoven overlaid with a dibutyrylchitin fibre layer without any bioactive substance shows good antibacterial properties due to the presence of an inhibition zone around the sample and to no growth in the zone of contact with the substrate. No growth of *Trichophyton mentagrophytes* was observed under the nonwoven sample, which indicates that in this case the sample treatment resulted in a sufficient effect in relation to the micro-organism tested. However, it was observed that in the zone of sample-medium contact, the growth of *Candida albicans* yeast was reduced by 50%. Therefore, it was expedient to enrich dibutyrylchitin fibres with a bioactive substance to improve their antifungal action.

The activated carbon nonwoven overlaid with dibutyrylchitin fibres containing the bioactive substance showed both good bactericidal and fungicidal properties, as confirmed by the fact that no growth of the micro-organisms tested in the zone of fibre-medium contact was observed, and around the samples tested an inhibition zone from 1mm to 3.5 mm was formed (***Table 5***).

Sorption properties/porous structure

Sorption parameters values of the activated carbon nonwoven depend on its porous structure. From the comparison of activated carbon nonwoven (AC) with no dibutyrylchitin fibres and commercial AC/silver dressing material it follows that the former has a more developed porous structure (***Table 6***) and, consequently, a higher sorption capacity. Water sorption values of the AC/DBC and AC/silver materials are listed in ***Table 7*** (see page 88).

The analysis of the sorption capacity values, assumed as the amount of liquid in grams sorbed by 1 g of the material tested, has shown that the sorption capacity of AC/DBC dressing is 56% higher than that of commercial AC/silver dressing. Sorption kinetic curves for particular samples are shown in ***Figure 2*** (see page 88).

The prolonged wetting time of AC/DBC containing the bioactive substance is associated with the surface layer nanostruc-

Table 1. *Physical properties of the material; (*) Air permeability determined by means of a TEX Test/Instrument. Air negative pressure: 0010 Pa, pressure foot pattern: 5 (measurement area diameter: 5 cm²).*

Sample	Thickness, mm	Surface weight,g/m²	Air permeability,dm³/sm² (*)
Precursor	0.764	184.44	22.4
Carbon nonwoven	0.455	59.34	33.2
Activated carbon nonwoven	0.390	47.82	29.6
AC/ DBC	0.360	50.77	23.4

Table 2. *Tensile strength of the material.*

Sample	Breaking force, N	Standard deviation of breaking force, N	Elongation at break, %	Standard deviation of elongation at break, %
Precursor	88.67	3.70	19.569	0.91
Carbon nonwoven	2.09	0.11	2.552	0.32
Activated carbon nonwoven	0.82	0.51	1.056	0.43
AC/ DBC	2.12	0.21	1.056	0.35

Table 3. *Compression strength of the material.*

Sample	Linearity, 1	Compression energy, J/m²	Strain after compression, %
Precursor	0.342	0.222	43.34
Carbon nonwoven	0.408	0.168	69.58
Activated carbon nonwoven	0.408	0.211	62.92
AC/ DBC	0.343	0.162	71.80

Table 4. *Chemical compounds in the aqueous extract from the material; The pH value of water is 5.51.*

Sample	pH of the aqueous extract	Chlorides	Sulfates	Starch
Precursor	6.80	absence	absence	absence
Carbon nonwoven	9.02			
Activated carbon nonwoven	9.65			
AC/ DBC	9.52			

Table 5. *Assessment of the bactericidal and fungicidal action of AC/DBC dressing material.*

Sample	Micro-organism	Growth assessment		Assessment of the bactericidal and fungicidal action
		Average inhibition zone, mm	Growth under nonwoven	
AC/DBC without the bioactive substance	1. *Staphylococcus aureus* ATCC 6538	1.0	no growth	good effect
	2. *Escherichia coli* ATCC 8739	1.0		
	3. *Candida albicans* ATCC 10231	0.0	average growth	reduced effectiveness
	4. *Trichophyton mentagrophytes* Ł 0572	0.0	no growth	good effect
AC/DBC with the bioactive substance	1. *Staphylococcus aureus* ATCC 6538	3.5	no growth	good effect
	2. *Escherichia coli* ATCC 8739	1.5		
	3. *Candida albicans* ATCC 10231			
	4. *Trichophyton mentagrophytes* Ł 0572	0		

Table 6. *Porous structure of AC and commercial AC/silver materials.*

Sample	Specific surface area, m²/g	Pore volume, cm³/g			V_{mic}/V_{mes}
		Total V_{total}	Micropore V_{mic}	Mesopore V_{mes}	
AC	1336	0.6517	0.5632	0.0885	6.3
AC/silver	1140	0.5453	0.4777	0.0676	7.1

Table 7. *Parameters of the water sorption of AC/DBC and commercial AC/silver materials; where: S_{max} – maximal sorption, V_{max} – sorption rate, t_0 – initial time, t_{max} – total time of sorption, m – sample weight, d – sorption capacity.*

Sample	S_{max}, µl/cm²	V_{max}, µl/(cm²s)	t_0, s	t_{max}, s	m, g	d, g/g
AC/ DBC	31.1	3.56	0.0	70.5	0.072	6.63
AC/silver	57.7	14.0	0.5	25.5	0.209	4.24

Table 8. *Cytotoxic changes in fibroblast-like cells L929.*

Time, h	Material	Morphological changes	Average of 3 cultures			Toxicity degree in a scale from 0 to 4
			Total	Dead, %	Alive, %	
After 24	AC/DBC	Living cells form a uniform layer. No dead and detached cells.	6.7×10^5	3	97	0 Absence
	AC/silver	Shrunk cells, many detached and dead cells.	3.3×10^5	23	78	3 Average
	Control culture L929	Cells of proper morphology adhere to the substrate in the form of a uniform layer. No dead and detached cells.	7.4×10^5	0	100	0 Absence
After 48	AC/DBC	Living cells form a uniform layer.	5.4×10^5	4	96	0 Absence
	AC/silver	Shrunk cells, many detached and dead cells.	2.9×10^5	53	46	4 Strong
	Control culture L929	Cells of proper morphology adhere to the substrate in the form of a uniform layer. No dead and detached cells.	7.2×10^5	3	97	0 Absence
After 72	AC/DBC	Living cells form a uniform layer.	5.0×10^5	3	97	0 Absence
	AC/silver	Shrunk cells, many detached and dead cells.	2.3×10^5	62	38	4 Strong
	Control culture L929	Cells of proper morphology adhere to the substrate in the form of a uniform layer. No dead and detached cells.	7.6×10^5	2	98	0 Absence

ture. The accompanying relatively high sorption capacity, amounting to 6.63 g/g, together with biomedical properties indicates that this material can be used in the production of dressing materials.

Cytotoxic action

Cytotoxic changes in fibroblast-like cells L929 after appropriate incubation times are presented in ***Table 8*****,** and microscopic images of the nonwoven samples are shown in ***Figures 3-5*****.**

From the toxicity tests it follows that AC/DBC containing Mcrobiocide-N750 is not toxic for the cells of line L929, while commercial AC/silver dressing shows a toxic effect.

Irritating action, acute intragastric toxicity and effect on selected blood parameters

The exposure of animals to the effects of the material tested did not affect their behaviour. No pathologic symptoms were observed in the animals during tests.

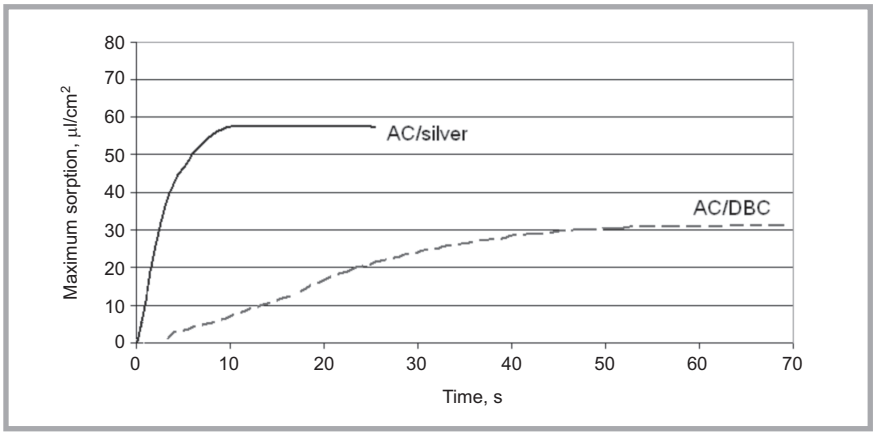

Figure 2. *Sorption kinetic curves of distilled water for AC/DBC material and commercial AC/silver dressing.*

Irritating action

For each time point, i.e. after 30 min, 24, 48 and 72 h of the exposure of scarified skin to the effects of the material tested, the arithmetical average of the point calculations obtained was 0. After 24 and 48 h from the removal of dressing, no skin changes were observed. The arithmetical average of the point calculations was also 0.

As no skin irritating action was observed, the action of the material tested on the anterior eye section was assessed. The conjunctiva, cornea and iris were assessed with the use of a slit lamp after 1, 24, 48 and 72 h from the material deposition. At none of the time points mentioned were corneal opacification or ulceration observed, and the blood vessels of conjunctiva were normal with no conjunctiva swelling. The iris was also regular.

The active carbon nonwoven overlaid with dibutyrylchitin fibres containing Microbiocide – N750 does not irritate skin and eyes. Even the basic reaction of the material aqueous extract does not contribute to irritating action.

Acute intragastric toxicity – Pathological test

None of the animals participating in the test died during observations; they showed toxic effect symptoms and only underwent the sacrifice required. All the animals after 14 days of testing were subjected to macroscopic examination during dissection. No deviations from the initial state were found.

LD_{50} cannot be determined for AC/DBC nonwoven containing Microbiocide – N750. This material causes no acute intragastric toxicity with the boundary dose - 2000 mg/kg. m. c. (Dose 2000 mg/kg m. c. is a boundary dose, and according to guidelines 425 – Acute toxicity – Oral administration - Procedure OECD TG 425 of 2001, it is the maximal permissible dose for experimental animals).

Effect on selected blood parameters and histopathological image

In laboratory tests, results were obtained only for IgE and fibrinogen. The PT and APTT times, despite different determination methods, in successive tests always reached values below the sensitivity threshold both before the application of

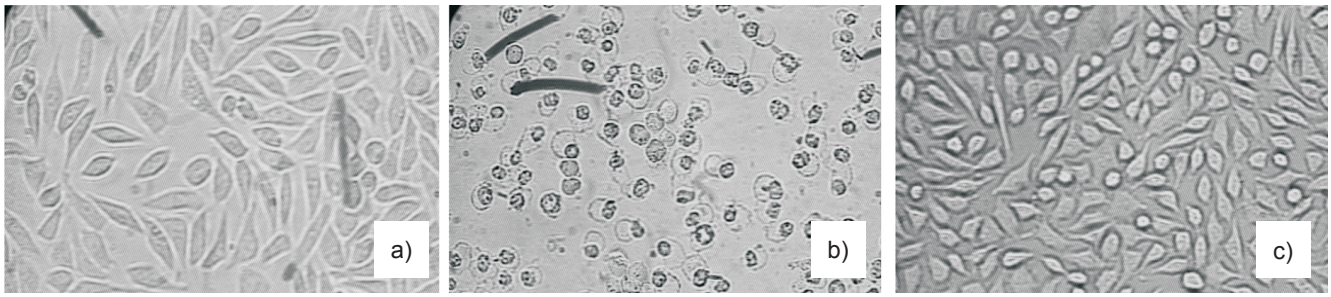

Figure 3. *Images of cell growth after 24 h: a) AC/DBC, b) AC/silver, c) L929-control.*

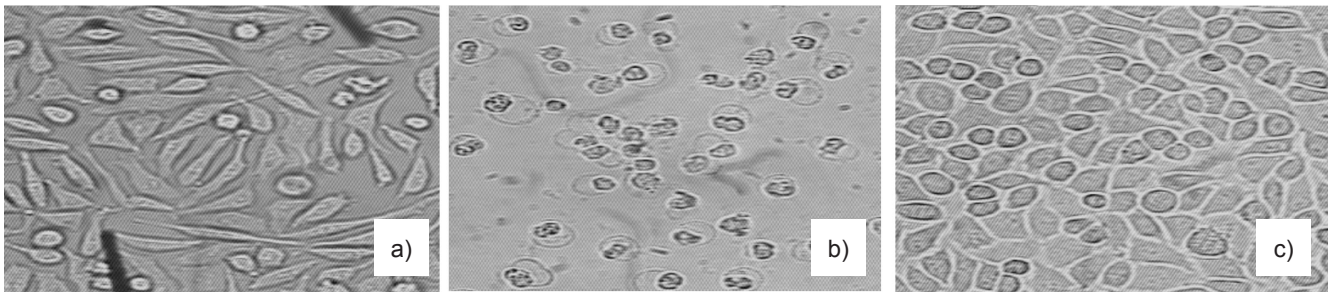

Figure 4. *Images of cell growth after 48 h: a) AC/DBC, b) AC/silver, c) L929-control.*

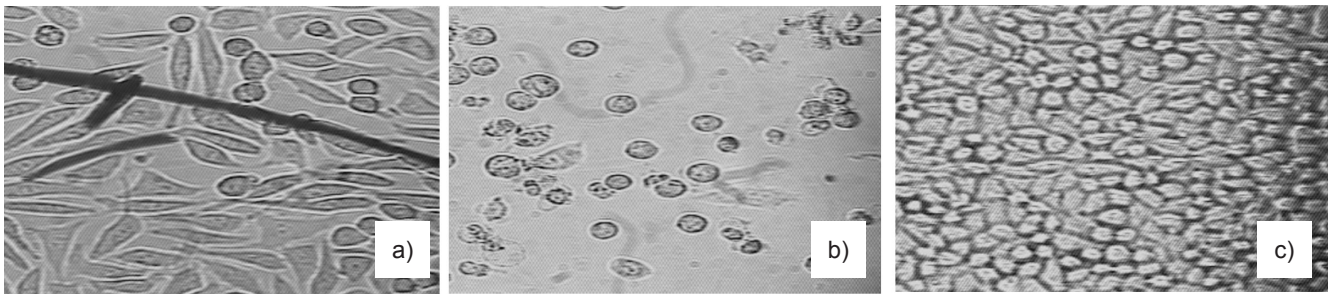

Figure 5. *Images of cell growth after 72 h: a) AC/DBC, b) AC/silver, c) L929-control.*

dressing and 14 days afterwards. Assuming the PT and APTT times are individually variable, tests of this type cannot be performed on rabbits using the methods commonly used for people.

The total IgE in all tests before and after dressing application amounted to a value of < 0.24ng/ml. (IgE participate in the immunological response of the organism, being an antibody committed in allergic reactions. The lack of increases in the IgE value after skin exposure to the dressings tested allows one to assume that these do not cause any allergic reaction).

The dressings tested do not influence the value of fibrinogen in blood (***Table 9***). (The determination of fibrinogen is used in the diagnostics of inbred hypo- and afibrynogenemia and hypofibrynogenemia from wear. The increase in fibrinogen growth is observed in posthemorrhagic states and in some malignant diseases.) The fibrinogen values observed are contained within the laboratory standards for men (200 to 400 mg/dl).

Histopathological tests have confirmed that AC/DBC nonwoven containing Microbiocide – N750 causes smaller histopathological changes than those brought about by commercial AC/silver dressing. In the first case, one could observe focally moderately intensified fibrosis under epidermis, dispersed small clusters of lymphoid infiltration and few granulocytes under epidermis, while in the case of AC/silver dressing, in addition to the changes mentioned above, there appeared places of hydropic degeneration in the basic layer of epidermis.

Table 9. *Average fibrinogen concentration in blood serum before and after dressing application.*

Average fibrinogen concentration, mg/dl			
AC/DBC		AC/silver	
before	after	before	after
272.0 ± 42.4	275.0 ± 61.8	264.8 ± 51.4	267.3 ± 68.1

Conclusions

The AC/DBC dressing material developed in this study, containing a bioactive additive, is characterised by a good bactericidal effect against Gram-positive bacteria *Staphylococcus aureus* ATCC 6538 and Gram-negative bacteria *Escherichia coli* ATCC 8739 as well as against fungi *Trichophyton mentagrophytes* and yeast *Candida albicans*. It shows no skin and eye irritation, no intragastric toxicity and no effect on the values of immunoglobulin E and fibrinogen concentration, which implies that this material induces no allergic reaction. Moreover, it does not induce cytotoxic effects and shows a higher sorption capacity, measured for distilled water, than that of commercial AC/silver dressing of active carbon woven fabric.
From the histopathological tests it follows that in the specimens of skin exposed to the materials tested, morpho-

logical changes of similar character are observed, but in the case of AC/DBC material, the intensification of changes is minimal in comparison to that observed in the animals exposed to AC/silver dressing.

Acknowledgment

- *The study was sponsored by the Polish Ministry of Science and Higher Education, Grant No R08 031 01.*
- *The authors would like to thank the following institutions for their participation in this research: Institute of Fermentation Technology and Microbiology, Technical University of Lodz, for assessment of the bactericidal and fungicidal properties; Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, for assessment of the cytotoxic action; Medical Laboratory Synevo in Lodz for biochemical tests of blood; Medical University of Lodz, for assessment of the irritating action on the eye; Consulting Room Patomorfologia' for the histopathological assessment; and the Animal House of the Medical University of Lodz for assessment of the toxicity and skin irritating action.*

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Received 07.09.2011 Reviewed 06.10.2011