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Producing Wound Dressing Materials from Chitin Derivatives by Forming Nonwovens Directly from Polymer Solution

Abstract
Dibutrylchitin is an ester derivative of chitin, characterised by good biomedical properties. Therefore, it can be used as a raw material for wound dressings. The high solubility of dibutyrylchitin in common organic solvents allows to transform it into fibres, and next final fabrics. Technologies of the forming of nonwovens directly from polymer allow to decrease the cost and rate of waste. Two methods of nonwoven production from dibutyrylchitin are described in this paper: the forming of nonwovens by the spraying of polymer solution and the electrospinning of polymer solution. The cytotoxicity and irritation effect of nonwovens made by electrospinning technology was investigated.

Key words: *dibutyrylchitin, electrospinning, spraying of polymer solution.*

Introduction

Chitin is a polysaccharide and because of its macromolecular structure it is barely soluble. Because of this, many chitin derivatives have been created, which are biocompatible and bioresorbable, just like chitin, but at the same time they are easy to dissolve. Thanks to this they can be processed by means of textile techniques.

Dibutyrylchitin [1] is such a derivative, and its biomedical properties have been confirmed by research carried out both in Poland and abroad [2 - 4]. Introducing large ester groups received from butyric acid makes the polymer easily soluble in many solvents, like DMSO, ethyl alcohol, DMF or N-MP. The technology of forming nonwovens directly from polymer solutions is based on that process [4, 5].

Dibutyrylchitin products are proposed as medical products, like wound dressing materials or implants [2 - 5]. Therefore, the solvents used for the production of fabrics must fulfill all the requirements for medical products. On the other hand, it must be easy to remove from the fabric after production. Because of this we chose ethyl alcohol as the main solvent used in our elaborated methods.

Dibutyrylchitin solutions in ethyl alcohol can be transformed into classical fibres, and next into flat fabric or directly into nonwovens. Two main techniques can be used for the production of nonwovens directly from polymer solution: electrospinning and spraying of polymer solution.

Electrospinning is a method where polymer (melted or solved) is forced through spinning nozzles with a diameter of around 1mm [6]. The voltage applied causes the charging of the polymer [7], and solution obtains constant electric potential, which is stabile in its total volume. The semispherical surface of the created drop is deformed into a cone due to the electrostatic field and Culomb forces, and next it is stretched to a stream. The uninominal charges that gather on the polymer stream surface, in combination with the stream's surface tension, cause the stream to bend, as an effect of which the distance covered by the polymer becomes extended. As a consequence of the acting electrostatic field, fibres so manufactured have diameter that vary from ten to several hundred nanometres. Such significant fibre thinning causes the product to be substantially extended and its properties to change. The fibres spun are deported on a bend to form webs of particular thickness The first studies related to the effects of an electrostatic field were carried out by J. Zeleny [8], and then by G. I. Taylor [9].

We stated that as an alternative to electrospinning, dibutyrylchitin can be transformed into nonwovens by the spraying of polymer solution. The polymer solution is forced through spinning nozzles, and the polymer stream is stretched by the technological air, similar to a melt-blown technique. Partial evaporation of solvents from the stream of filaments and solidification of the fibres occur in the air canal. The final solidification of the web takes place on a conveyor band, as well as by the evaporation of solvent or by coagulation in a coagulation bath [10]. This method allows to obtain fleece formed of fibres with a diameter of 20 μm.

Both these methods are differentiated by their way of stretching the polymer solution stream. Both of them use a spinning head, where the polymer stream is formed. For our experiments we designed a spinning head which can be used in both techniques. In the technique of forming nonwovens by the spraying of polymer solution, a head is used which enables to lead the air around each jet individually, and as a result the air stream affects the stream of the polymer solution with greater intensity. In the electrospinning technique fibres are also formed with the use of the element of the same head which forms the polymer stream by the applied voltage. The method of forming

nonwovens directly from dibutyrylchitin solution by the spraying of polymer was firstly elaborated at the Department of Fibre Physics and Textile Metrology, Technical University of Łódź. A laboratory stand was designed and built [10].

The proposed application of the nonwovens manufactured by us as medical fabrics caused that biological evaluation was necessary. As initial parameters the cytotoxicity (according EN ISO 10993, part 5, 2001) and irritation effect (according EN ISO 10993 part 10) were examined. Also, the structure and uniformity of the web and thickness of the fibres were analysed. Results of biological assessment of dibutyrylchitin nonwovens obtained by the spraying of polymer are also presented in [5].

The aim of this work was to compare two methods of obtaining nonwoven directly from a polymer solution; the method of electrospinning and that of blowing-off the polymer stream by air at a particular assumption related to the character of nonwoven as its thickness, and surface mass as thickness of the fibres spun.

Laboratory stands

Two stands were designed and built at the Department of Fibre Physics and Textile Metrology [10, 11]. The first stand used for the spraying of polymer solution, consists of a spinning head, mounted in an air canal. The spinning head is supplied by polymer solution from a solution reservoir by a polymer pump. Technological air is delivered to air canals in the spinning head. The formed and partially solidified polymer streams are collected on a conveyor band, and finally solidified by evaporation of solvent or by coagulation in a coagulating bath. The laboratory stand allows obtain nonwovens by the spraying of polymer under controlled technological parameters, such as:

- the distance between the spinning head and conveyor (from 0.5 to 1.5 m),
- the rate of polymer solution (from 9.5 to 293 cm^3/min),
- the rate of airflow (from 0 to 45 dm^3/s),
- the temperature of the polymer and the temperature of the spinning head (from 20 to 100 °C), and
- the temperature of air (from 20 °C to 100 °C).

Also, the way of solidifying the polymer (evaporation or coagulation) can be selected [10].

The second stand, which enables the electrospinning of the polymer solution, consists of a high voltage generator, which delivers voltage to the polymer solution. The polymer solution is forced through spinning nozzles. An electrostatic field exists between the spinning nozzles and a collector electrode, and allows to stretch the polymer stream. Solidification of the polymer occurs by evaporation of the solvent. Solidified fibres are collected on the conveyor band above the collector electrode.

The spinning head

The spinning head used in both methods was designed and build at the Department of Fibre Physics and Textile Metrology. The spinning head was divided into two parts - an element that forms the polymer jets, and an element that enables to lead the air around each jet individually. During electrospinning only the head element, which forms the polymer jets, was used.

Twelve spinning nozzles, with a diameter of 0.7 mm, were situated in three rows, four spinning nozzles in each. The length of the spinning nozzles was according to the construction and was equal 70 mm. The design of the spinning head can not be described in detail because it is under patent pending.

Obtaining of nonwovens

Raw material

Dibutyrylchitin with a molecular weight of 113 550 g/mol and intrinsic viscosity in DMAc at 25 °C of [η]=1.6 dl/g was dissolved in ethyl alcohol. Solution with a concentration of 10% was used in the technique of spraying polymer solution, and 8% concentration in the electrospinning technique.

Technological parameters

Technological parameters of the production of nonwovens as well as the solution concentration were optimised during numerous experiments using the techniques of spraying polymer solution and electrospinning. The uniformity of the web obtained and the thickness of the fibres were particularly favourable. The main technological parameters of the forming of nonwovens by the spraying of polymer solution were as follows:

- temperature of polymer reservoir: 20 °C,
- temperature of spinning head: 21 °C,
- the rate of technological airflow: 15 cm^3/s,
- the rate of polymer solution: 9.5 cm^3/min,
- the distance between spinning head and conveyor: 0.7 m.

Polymer was solidified by the evaporation of solvent.

The technological parameters of the nonwoven production by electrospinning were as follows:

- applied voltage: 15 kV
- distance between spinning head and conveyor: 15 cm
- rate of polymer: 10 ml/h.

Assessment of nonwovens

The structure of the web obtained was analysed by attaining microscopic views of the web. The structure was analysed using a JEOL SM 522 LV scanning microscope. The photos obtained were next analysed using LUCIA G image analysis software. The diameter of fibres was calculated. The thickness of fibres was measured for 100 fibres from each sample.

The nonwovens obtained were sterilised by γ radiation with a 25 kGy dose. Investigation into the cytotoxic effect was carried out in accordance with Standard PN EN ISO 10993, sheet 5:2001, on the reference line of 3T3/Balb mouse fibroblasts, with the use of the direct contact method. The culture, of 0.5×10^6

Table 1. *Cytotoxic changes in the 3T3/Balb mouse fibroblast culture after 24, 48, and 72 hours of contact with dibutyrylchitin nonwoven samples made by electrospinnng method.*

incubation time	material	average number of cells×10^6			degree of toxicity
		living	dead	total	
24 h	culture with DBC nonwoven	0.68	0	0.68	0
	control culture	0.72	0	0.72	0
48 h	culture with DBC nonwoven	0.98	0	0.98	0
	control culture	1.20	0	1.2	0
72 h	culture with DBC nonwoven	1.86	0.018	1.878	0
	control culture	1.98	0.019	1.999	0

initial number of cells, was conducted under standard incubation conditions at a temperature of 37 °C, in the presence of 5% CO_2. Samples of the test material were put on the culture. A culture of cells incubated under the same conditions, but without contact with the sample tested, was accepted as a control test. The state of the culture was estimated after 24, 48, and 72 hours of incubation. The quantity and morphological changes of the cell cultures were estimated. Aiming at determining the number of living cells, neutral red dye was used; whereas the number of dead cells was determined with the use of triphen blue.

Investigations into irritation effect were carried out in accordance with Standard PN EN ISO 10993, sheet 10:2002(U), by the method of intradermal reactivity, with the use of extracts. Polar extracts with the use of physiological salt and non-polar extracts based on sesame oil were prepared from the samples tested; 120 cm^2 of the tested material coincided with 10 ml of extracted solvent. Physiological salt solution and sesame oil without contact with the material tested were used for control tests, and were incubated under the same conditions as those of the extracts tested. The polar extracts of the nonwoven samples were characterised by a pH of 4.93, whereas that of the polar control test by a pH of 6.42.

The investigations were carried out with the use of albino rabbits of the white New-Zealand breed. Every animal was treated with five 0.2 ml intradermal injections of both test extracts and the liquids prepared for control tests. Estimation of the possible skin alterations was carried out after 24, 48, and 72 hours as well as after injections of the test extracts and control liquids.

Results

Morphological characteristic

Two different webs were obtained independently of the method. The surface mass of the web obtained by the spraying of polymer was 22 g/m^2, and the surface mass of web formed by electrospinning was 17 g/m^2. Better uniformity of web was observed for nonwovens obtained by electrospinning. The thickness of fibres obtained by the spraying of polymer was equal to 17 μm, with a coefficient of variation of 38%. Whereas the mean diameter of fibres formed by electrospinning

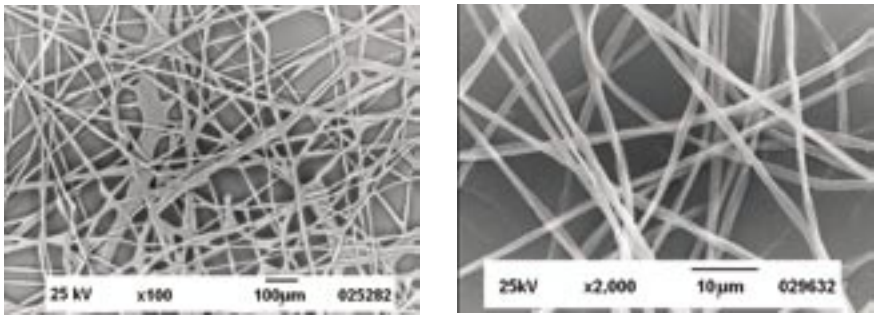

Figure 1. *Structure of web a – obtained by the spraying of polymer, b – obtained by electrospinning; the airflow illustrates that the magnification of the electrospun fibres is 20 times greater than those obtained by spraying the polymer.*

was 1.53 μm, the coefficient of variation was equal to 11%. The structure of the webs obtained is presented in Figures 1.a and 1.b.

While comparing the two photos, the reader should take into account the fact that the magnification of the electrospun fibres is 20 times greater than those obtained by spraying the polymer.

Cytotoxic effect

Results of the investigation of the proliferation of cells in culture after contact with dibutyrylchitin nonwovens obtained by electrospinning and in control are presented in Table 1. Photos showing the morphology of cultures are presented in Figure 2.

The growth of cells in the cultures is presented in Table 1.

An essential increase in the number of living cells from an initial number of 0.5×10^6 up to 0.68×10^6 after 24 hours, to 0.98×10^6 after 48 hours, and 1.86×10^6 after 72 hours was stated as the result of the cell culture being in contact with the dibutyrylchitin nonwoven obtained by electrospinning. Already after 24 hours, the growth of living cells resulted in an essential difference between the number of living cells in the sample tested and their initial number. Dead cells appeared in the cultures only after 72 hours, and their presence was observed on the culture at the same time, which was in contact with the sample tested and the control culture. The difference in the quantity of dead cells between both cultures is unimportant. The proliferation of cells was appropriate during the whole time of incubation, and the cells also grew in direct contact with the sample.

Estimation of the cell culture morphology was carried out with the use of a contrast-phase optical microscope. The photos in Figure 2 present the morphology of the cultures.

No morphological changes were noted after 24, 48, and 72 hours of contact by the cultures and the tested samples of dibutyrylchitin nonwovens made by the electrospinning method, as well as in control cultures. The samples adhered to the base in all cultures and had normal morphological features. The agglutination, vacuolisation, and lysis of cell

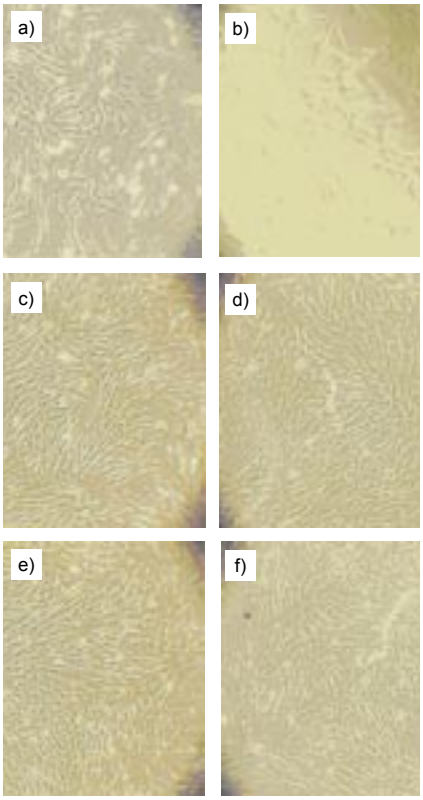

Figure 2. *Culture of 3T3/Balb mouse fibroblast cells; a, c and e after contact with dibutyrylchitin nonwoven made by electrospinning method, b, d and f control cultures; a, b - after 24 hours, c, d – after 48 hours, e and f – after 72 hours*

membranes were not observed. Separate fibres from the sample were observed in the photos.

On the basis of the results obtained, we can state that no cytotoxic action of dibutyrylchitin nonwovens made by electrospinning living organisms exists.

Assessment of the cytotoxic effect of nonwovens obtained by the spraying of polymer solutions shows the same results. No morphological changes were noted during the whole time of examination, and the proliferation of cells in culture was similar (from initial number of 0.5×10^6 up to 0.88×10^6 after 24 hours, to 1.62×10^6 after 48 hours, and 3.14×10^6 after 72 hours [5]).

Irritation effect

No skin alterations in the shape of skin erythema and swellings were observed surrounding the injections of polar extracts and polar control liquids immediately after the injection, as well as after 24, 48, and 72 hours.

Immediately after the injection, as well as after 24 and 48 hours after the injection of the non-polar extracts and non-polar control tests, barely visible skin reddening without swelling was noted. These alterations were observed on every animal. No skin alterations were observed during 72 hours following the injections.

On the basis of the observations carried out, the primary irritation factors for polar and non-polar extracts from dibutyrylchitin nonwoven samples were determined. The factor value amounted to 0.00 for polar extracts and 0.22 for non-polar extracts and was unimportant. A similar reaction of rabbit skin after an injection was observed for extracts prepared from nonwovens obtained by the spraying of polymer solution [5].

Conclusions

Both methods of forming nonwovens directly from dibutyrylchitin solutions in ethyl alcohol allow to obtain a correct web. Biological experiments of cytotoxicity and irritation effects show that nonwovens obtained from polymer solutions do not cause a negative response in living organisms. The results of biological assessment presented are just the basis for future in vivo experiments on animals, including the reaction after implantation, influence on blood coagulation or resorbability. It is expected that changing of the diameter of fibre web forming will differentiate some biological answers. We expect that due to increasing the active surface of fabric, nonwovens made by electrospinning will be better accepted by the human organism and that the resorption process will be accelerated.

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