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MDPI
St. Alban-Anlage 66
4052 Basel, Switzerland

For citation purposes, cite each article independently as indicated below:

LastName, A.A.; LastName, B.B.; LastName, C.C. Chapter Title. In *Bionanotechnology to Save the Environment. Plant and Fishery's Biomass as Alternative to Petrol*; Pierfrancesco Morganti, Ed.; MDPI: Basel, Switzerland, 2018; Page Range.

ISBN 978-3-03842-692-9 (Hbk)
ISBN 978-3-03842-693-6 (PDF)

doi:10.3390/books978-3-03842-693-6

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Production of Electrospun Nonwoven Materials as a Blending of Chitin Nanofibrils and Other Natural Polymers

Angelo Chianese and Paola Del Ciotto

Abstract: Electrospinning is an electrostatic fiber fabrication technique that has evinced much interest and attention in recent years due to its versatility and potential for applications in diverse fields. The sub-micron range spun fibers produced by this process offer various advantages, like high surface area to volume ratio, tunable porosity, and the ability to manipulate nanofiber composition in order to get a set of desired properties and function. This chapter deals with a preliminary investigation on the production of nonwoven materials obtained by the electrospinning of a blending of nanochitin fibrils and lignin, by using polyethylene oxide as the solvent. The adopted blend was carefully prepared as sol-gel material at a suitable temperature, mixing conditions, and time of ageing. The blends were characterized by the measurements of viscosity and electroconductivity. Many factors may influence the quality of the electrospun product, among them the main ones include the following: the applied voltage between the two electrodes, the distance between the tip and the collector, and the rotation of the collector. In this work, these operating parameters have been investigated using a preliminary factorial analysis experimental campaign. For this purpose, a pilot scale electrospinning machine (model Nanospider NS LAB 500 supplied by Elmarco) was used. The characterization of the produced electrospun nanofibers was performed by using the Field Emission Scanning Electron Microscope Auriga Zeiss. This instrument provides images of the nanofibers with an accuracy of less than 10 nm and allows the determination of the chemical composition by using the microanalysis device EDS 123 Mn-KeV supplied by Bruker.

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1. Introduction

In recent years, a large number of research groups have carried out works focused on the development and production of new and improved wound dressings by synthesizing and modifying biocompatible materials [1,2].

In particular, efforts are devoted to the use of biologically derived materials such as chitin and its derivatives, which are capable of accelerating healing processes at the molecular, cellular, and systemic levels. Chitin is a readily available and inexpensive biological material obtained from invertebrate skeletons as well as the cell wall of fungi. It is a linear 1, 4-linked polymer composed of N-acetyl-D-glucosamine residues.

For its chain rigidity, chitin's dissolution in many solvents is a hard task, and for this reason, it is usually used as nanofibril, which are highly crystalline and rigid, thus allowing for the improvement of mechanical performance of composites. Based on infrared spectroscopy and x-ray diffraction data, chitin can be found in one of three crystalline forms: α-chitin, β-chitin, and γ-chitin. The molecules in orthorhombic α-chitin are arranged very tightly in an anti-parallel fashion. In this work, α-chitin, mainly present in shells of crabs, lobsters, and shrimps, was considered. High crystalline chitin is often called chitin nanocrystals, or chitin nanofibrils (CTN).

Chitin and its derivative, chitosan, are biocompatible, biodegradable, nontoxic, antimicrobial, and hydrating agents, and in general act as nanofillers in the reinforcement of both natural and synthetic composites [3]. Due to these properties, they show good biocompatibility and positive effects on wound healing. Previous studies have shown that chitin-based dressings can accelerate the repair of different tissues, thus facilitating the contraction of wounds and regulating the secretion of inflammatory mediators such as interleukin 8, prostaglandin E, interleukin 1 β, and others [4]. The effectiveness of three chitin nanofibril-based preparations, a spray (Chit-A), a gel (Chit-B), and a gauze (Chit-C), in healing cutaneous lesions was assessed macroscopically and by light microscopy immunohistochemistry [5]. These evaluations were compared to the results obtained using a laser co-treatment.

Ja.li Ji et al. [6] have underlined that chitin nanofibrils are an emerging novel filler. They have reinforcing effects on synthetic and natural fibers, and thus they can give rise to efficient scaffolds for tissue engineering. A number of techniques have been developed to fabricate nanofibrous tissue with unique properties. Among these techniques, electrospinning technology has become the most popular for the fabrication of tissue engineering scaffolds in recent years because it is a simple, rapid, efficient, and inexpensive method for producing nanofibers by applying a high voltage to electrically charged liquid [7,8]. Recently, Naseri et al. [9] successfully produced electrospun chitosan-based nanocomposite mats reinforced with chitin nanocrystals for wound dressing. For spinning solutions, chitosan and PEO were blended in a 1:1 ratio and used in a matrix, while CTN was used as reinforcement in order to produce final solutions of 3 wt % polymer in 50% aqueous acetic acid solvent.

The electrospinning process involves the application of a high voltage between a syringe filled with a polymer solution and a collector mounted at a fixed distance from the needle/syringe setup.

An electrical charge builds up on the surface of the solution that is attracted to the collector. The large potential difference overcomes the surface tension of the fluid droplet at the tip of the needle. Under specific conditions of voltage, flow rate, and distance, a jet of fluid is ejected from the needle and subjected to whipping and splaying instabilities due to stresses of electrostatic origin [10]. The solvent evaporates over the jet path, and polymer nanofibers are formed on the collector. Various factors affect the electrospinning process such as solution properties, process parameters (flow rate, voltage, electrode distance), and ambient conditions. Hence, different requirements should be met in order to have an efficient process [11,12].

In this work, chitin-based nanofibers are prepared by a sol-gel material produced by mixing an aqueous solution of chitin with polyethylene oxide (PEOX). The effect of the operating variables of the electrospinning machine was investigated in order to optimize the quality of the electrospun textile.

2. Experimental

2.1. Materials

The materials used in this study include: Chitin, purchased from Primex (Siglufjordur, Iceland), PEOX (polyethylene oxide), purchased from Amerchol (Dow Italia, Italy), Chitin nanofibrils (CN) and CN-Lignin complex purchased from MAVI sud S.r.l. (Aprilia, Italy).

The sol-gel mixture prepared for the electrospinning tests was obtained mixing the CN-Lignin complex (30.1% *w*/*w*) with deionized water (up to 100% *w*/*w*) at temperature of 15 °C for few minutes. Then PEOX (7% *w*/*w*) was added to the solution, under stirring until completely dissolved. This last step took 24 h to obtain a homogeneous gel without agglomerations. The properties of the sol-gel materials are as follows:

- pH: 10.52
- Viscosity: 8.4 P
- Conductivity: 7.8 mS

2.2. Electrospinning

The electrospinning process was performed by using the pilot scale machine Elmarco Nanospider NS LAB 500 based on the nozzle-less technology (S. Petrik, M. Maly, "Production Nozzle-Less Electrospinning Nanofiber Technology", Elmarco s.r.o.). The proof of concept of this technique is that a rotating drum is dipped into a bath of the liquid solution. The thin layer of solution is carried out on the drum surface and exposed to a high voltage electric field. If the voltage exceeds the critical value a number of electrospinning jets are generated. The jets are distributed over the electrode surface with periodicity. This is one of the main advantages of nozzle-less electrospinning, i.e., the number and location of the jets is set up naturally in their optimal positions.

The setting parameters of the machine were:

Voltage: 45–75 kV
Collecting electrode (CE): cylinder
Spinning electrode (SE): cylinder
Distance SE/CE: 10–16 cm
CE rotation: 2–8 rpm
Substrate material: Spunbond, 30 gsm, polypropylene 100% with antistatic treatment

The photo of the electrospinning set-up is reported in Figure 1.

Figure 1. The used electrospinning setup.

2.3. Rheological Measurements

Dynamic rheological properties of the sol-gel material were determined at 25 °C using the rotational rheometer supplied by Brookfield model HA DV-E 230. The applied operating conditions were: spindle code 05, speed of rotation 12 rpm, time 1 min

2.4. Fiber Diameter Characterization

The surface morphology of electrospun nanofibers was characterized by a field emission electron microscope–FESEM Auriga Zeiss, including microanalysis EDS 123 Mn-Kα eV(Bruker) and EBL –7 nm resolution (Raith). Samples cut from the electrospun material mounted on aluminum stubs were coated by an ultrathin layer of platinum for better conductivity during imaging. The samples were observed at magnifications between 100 and 40,000 times their original sizes to visually evaluate the electrospinnability and existence of beads.

Fiber diameters were also determined using Image-J image processing software. For each electrospun material, at least 100 fibers were considered from three different images to calculate the average diameter.

2.5. Factorial Experimental Plan

The main aim of the experimental work has been to determine the best set of the operating parameters of the electrospinning machine in order to optimize the quality of the produced fibers. The process parameters which could be chosen were: the distance between the electrodes, the voltage range applied along each run, and the rotational velocity of the cylindrical electrode. On the basis of a preliminary explorative work it was ascertained that the better operating range of these parameters are those reported in Table 1.

Table 1. The operating conditions of the electrospinning machine.

Electrodes Distance (cm)	Voltage (kV)	Rotational Velocity (rpm)
10	45–60	2
16	45–70/75 (MAX)	8

In order to minimize the experimental runs it was decided to carry out an experimental campaign based on the 2^3 factorial design. In fact, this technique can reduce the number of experiments to be performed by studying multiple factors simultaneously. Additionally, it can be used to find both main effects (from each independent factor) and interaction effects (when both factors must be used to explain the outcome).

Additionally, it can be used to find both main effects (from each independent factor) and interaction effects (when both factors must be used to explain the outcome). The operating conditions of the 8 runs of the experimental campaign are represented in Table 2.

Table 2. The operating conditions of the 8 experimental runs.

Run	Variables Level	Electrodes Distance (cm)	Voltage (kV)	Rotational Velocity (rpm)
(1)	- - -	10	45–60	2
a	+ - -	16	45–60	2
b	- + -	10	45-MAX	2
ab	+ + -	16	45-MAX	2
c	- - +	10	45–60	8
ac	+ - +	16	45–60	8
bc	- + +	10	45-MAX	8
abc	+ + +	16	45-MAX	8

All the operating variables but those ones indicated in Table 1 were maintained constant in all the runs. The investigation was focused on only one effect, i.e., the average diameter of the fibers. In Table 3 the obtained values of this variable, together with the relevant statistical parameters, are reported for all the experimental runs.

Table 3. Average diameters of the electrospun fibers obtained in the factorial campaign.

Run	(1)	a	b	ab	c	ac	bc	abc
Diameter, nm	193	146	184	148	163	144	167	138
Variance	5484	1898	2338	2822	2655	2509	3523	1171
E%	13	10	9	12	10	12	12	8

The average diameter was determined on the basis of more than 100 measurements. The obtained results show significant changes of the fibers diameter for the different operating parameters set, within the overall measured range 138–198 nm. The smaller diameter values are those obtained by adopting the upper value of the electrode distance. In order to evaluate in quantitative way the effect of each operating variable the results of the factorial experiment design were analyzed by means of the ANOVA method. This method is based on the comparison of variance of results corresponding to a single effect or a combination of effects with the variance of the experimental error. In this work, it was assumed that factor interactions are negligible. Under this assumption, the average estimate of high order interactions is considered an estimation of experimental error.

In Table 4 the variance in correspondence of each single effect, in background color, and the zero-variance corresponding to the interactions effect is reported.

Table 4. The variance of single effects and zero variance.

Effect	Variance	Zero Variance	Fisher Factor
A	2145		40.5
B	10		0.19
C	435		8.2
AB	0.71		
AC	151	53	
BC	4		
ABC	55		

Finally, the F-test was made by comparing the ratio between the variance of each single effect and the zero variance with the value of the F distribution at 95% of significance.

In the examined case this value of F distribution is equal to 7.71. By comparing the F-test with the F-distribution it is possible to state that the fiber diameter is strongly affected by the electrode distance, is not affected at all by the voltage and is only slightly affected by the rotational speed of the spinning electrode. The fibers obtained at the best conditions, that is that one of the run abc (upper value of all the three operating variables) is reported in Figure 2.

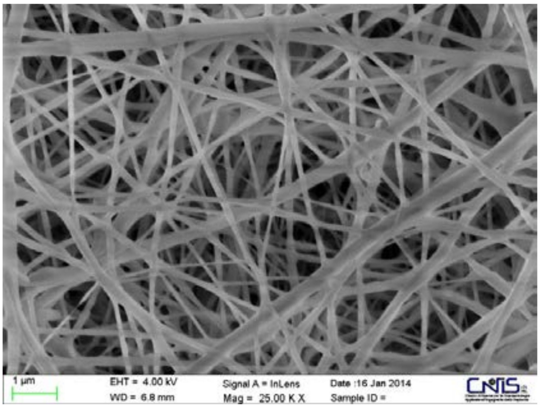

Figure 2. Image of the electrospun nonwoven tissue produced in run abc.

The fibers are elongated and quite regular without evidence of beads. It was not the case for some other runs performed at different operating conditions. For instance, for run b, carried out at lower values of the distance between pin and collector and of the voltage a quite bad tissue was obtained, as shown in Figure 3.

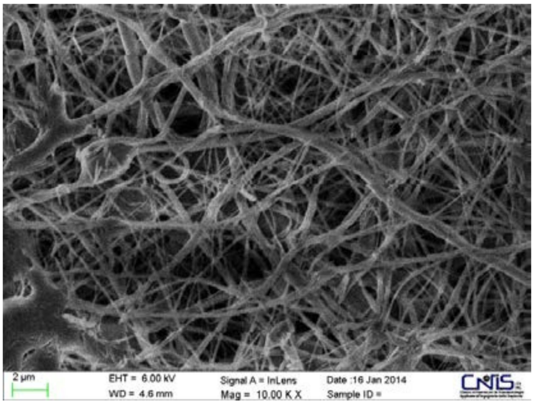

Figure 3. Image of the electron spun nonwoven tissue in run b.

The importance of the distance between tip and collector in order to minimize the beads and to obtain more regular fibers was noticed by other authors [13], in particular it was observed that beads are generated with too small and too large distance and a minimum distance is required to obtain uniform fibers. The applied voltage had no effect on the performances of the electrospinning. This is in agreement with the research work of Reneker and Chun [14], who has showed that there is not much effect of electric field on the fiber diameter with electrospinning of polyethylene oxide. As far as the effect of rotational speed is concerned it is well known that its increase resulted in more uniform and thinner fibers, potentially due to the higher stretching level imposed on them. In our particular case, the increase of the rotational speed from 2 to 8 rpm induces an improving of the electrospinnability of the tissue, even if it is not remarkable.

Obviously, the characteristics of the sol-gel material used for the electrospinning plays an important role. The used material was produced at a relatively low temperature, around 15 °C. If the sol-gel material is produced at a higher temperature, around 20 °C, its viscosity is lowered down to 7 P and the electrospun fibers, produced at the best operating conditions above reported, exhibit a larger size, i.e., of 191 nm. size. It has been found that with very low viscosity there is no continuous fiber formation and with very high viscosity there is difficulty in the ejection of jets from the polymer solution, thus there is a requirement of optimal viscosity for electrospinning. Fong et al. [15] have studied polyethylene oxide (PEOX) to study nanofiber formation at different viscosities and found that a range of viscosity between 1 and 20 poise is suitable for production of uniform nanofibers by electrospinning. The values of viscosity of the sol-gel material produced in this work in presence of PEO were well inside of the suitable range viscosity outlined by Fong et al. [15] and thus it's a confirmation of their results.

3. Conclusions

In this work, the influences of the operating conditions of a rotating electrospinning machine on the characteristics of a non-woven chitin-based tissue has been investigated. In order to minimize the experimental efforts, a factorial campaign of experiments have been designed and performed. The effects of the electrode distance, the voltage range and the rotational speed of the spinning electrode were considered. By means of preliminary experiments, the variables' operating ranges to be adopted were identified. The experimentation showed that the most important operating variable is, in our particular case, the distance between the electrodes, as its increase gave rise to a reduction of the fiber diameter down to less than 150 nm. A second significant effect was exhibited by the collected electrode rotational speed. By operating at the best set of operating variables, a very good electrospinnability of the tissue was obtained with parallel elongated fibers, but the size ratio of the produced fibers was quite high. Future work should be done to find out the best operating conditions and to improve the non-woven tissue, by an investigation within the domain of variables identified by this study.

Conflicts of Interest: The authors declare no conflict of interest.

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