

## Antibacterial and Anti-inflammatory Green Nanocomposites

Pierfrancesco Morganti<sup>\*a</sup>, Paola Del Ciotto<sup>b</sup>, Marco Stoller<sup>c</sup>, Angelo Chianese<sup>c</sup>

<sup>a</sup>Dermatology Depart., 2nd University of Naples, Italy; R&D Director, Nanoscience Centre MAVI, Aprilia (LT), Italy;

<sup>b</sup>R&D, Nanoscience Centre MAVI, Aprilia (LT), Italy.

<sup>c</sup>Chemical Materials Environmental Engineering Department, Sapienza University of Rome, Rome, Italy.

[info@mavicosmetics.it](mailto:info@mavicosmetics.it)

This work deals with the production of a green technology membrane to produce drinkable water from polluted fresh water resources disseminated in rural area, that is by using a small and compact water purification plant. The material adopted for this purpose is non woven-tissue based on green nanocomposites of Chitin Nanofibrils (CN) bonded with nano silver and electrospun with chitosan and polypeptides. Nonwoven materials obtained as electrospun of a blending of nanochitin fibrils and lignin, by using polyethylene oxide as solvent. The adopted blend was carefully prepared as sol-gel material at suitable temperature, mixing conditions and time of ageing. The non-woven tissue was produced by means of a pilot scale electrospinning machine model Nanospider NS LAB 500 supplied by Elmarco. Very uniform membrane with a diameter less than 150 nm were produced. Stress tests showed a good resistance of multiple layer samples.

### 1. Introduction

Nanotechnology could represent the most serious challenge to world economies and societies over the next decades. According to National Science Foundation this new technology could create a trillion dollar industry in the short period by a sustainable development based on the use of alternative sources of raw materials processed by innovative industrial methodologies with a low consumption of water and energy (Lux Reseach, 2009).

In this sense forestry and sea by-products, including wastes from shellfish and finishing processing and algae, as well as food surplus and wastage provide a plentiful resource of potential to transform these wastes in added-value materials and goods (Bowen and Kuralbayeva, 2015). For this purpose, it is to underline that fishery and plant biomass account for about 300 billion tons/year as well as the consumption stage-related food waste accounts for around 35% of all food processed with an estimated cost of US\$ 1 trillion/year (Nellemann et al, 2009; Lipinski et al, 2013). Thus, according to OECD, the role of a *green* biotechnology supported by bioeconomy and applied to agriculture, health, and industry, will be able to provide "food, water, energy, healthcare and other resources and services to a world that will see its population increased by a third in the face of mounting environmental stresses over the next 20 years"(OECD, 2009).

In this contest bioeconomy has to be "global, with heavy involvement from both OECD and non-OECD countries, especially in agricultural and industrial applications". Nevertheless, it should be noted that "while the literature dealing with the social and economic impacts, nanotechnology is characterized by a diversity of opinions and there is a tendency to focus particularly on perceived and potential risks to the exclusion of the potential benefits". However, products enabled with these innovative technologies generated US\$ 254 billion in 2009 in the sectors of medicine, chemistry and electronics so that nanotechnology is evolving towards becoming a general-purpose technology by 2020. It is, therefore, estimated that bionanotechnology "could contribute up to approximately 2.7% of GDP in the OECD by 2030 and an even higher share of GDP in non OECD countries" (OECD, 2009).

For this purpose, MAVI Sud, a SME, located southern of Rome, has developed an advanced non woven-tissue based on green nanocomposites of Chitin Nanofibrils (CN) electrospun with bio-Lignin and poly(ethylene) oxide (PEOX). The purpose has been to produce advanced medications (classified as medical devices) and filters for air and water purification. Chitin is, in fact, one of the most available natural polymer, safe and non toxic (Younes et al, 2015), while chitin nanocrystal (Morganti et al, 2014) or nanofiber (Morganti,

a, 2015) represents its purest form. Additionally, the market of medical devices had a selling turnover of US\$ 30 billion in 2012 (UNEP, 2012), and is expected to grow at a significant increase of 11-12% during the forecast period 2014-2019 (Morganti b, 2015). The main aim of this work has been the optimization of the CN based nonwoven tissues by electrospinning and their characterization. The functionality of the CN based medical masks was also checked.

## **2. Experimental**

### **2.1 Materials**

For the optimization experiments finalized to the determine the best operating condition to be applied in the electrospinning operation the following materials were used: Chitin Nanofibrils in the form of 2% water suspension supplied by MAVI Sud Srl (Italy), bio-lignin by CIMV (France), PEOX was purchased from Amerchol (Dow Italia, Italy). As reagent was used only distilled water.

### **2.2 Preparation of non-woven tissues**

The non-woven tissue was electrospun by the NS Lab 500 (Elmarco, Czech Republic). Before going on with the tissue production by the electrospinning, CN-bio lignin aggregated polymeric nanoparticles were produced by using the spray-dryer DF 500 B9 (JCF, Italy). These nanoparticles were dissolved into water to obtain a gel-mixture, which was then used as starting material for an electrospun material. The sol-gel mixture prepared for the electrospinning tests was obtained mixing the CN-bio-lignin complex with deionized water at temperature of 15 °C for few minutes. Then PEOX was added to the solution, under stirring until completely dissolved. This last step took 24 hours 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.

### **2.3 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.l.). 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: 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

Voltage: 45-60 or 45-70 kV

Substrate material: Spunbond, 30 gsm, polypropylene 100% with antistatic treatment

### **2.4 Characterization of nanoparticles and non-woven tissues**

The particle size distribution of the CN-lignin nanoparticles was measured by means of the dynamic light scattering instrument 90 Plus supplied by Brookhavn. The surface morphology of electrospun nanofibers was characterized by a field emission electron microscope – FESEM Auriga Zeiss, including microanalysis EDS 123 Mn-Ka 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.

Microtensile tests of the produced fiber layers were carried out near the laboratory of advanced material of the Department of Chemical Material Environmental Engineering Sapienza University of Rome by using the machine Zwick/Roell Z010 with 1 kN load cell, according to the directive ASTM 1708-13.

## 2.5 Experimental work and results

First of all, the size distribution of CN-bio lignin aggregated polymeric nanoparticles, produced as a component of the gel to be used for the electrospinning, was measured. A narrow size distribution with an average size equal to 61,9 nm resulted, as shown in Fig. 1.

The SEM analysis of the block co-polymeric nanoparticles of CN-bio-Lignin displayed a nanometer scale textured surface consisting of a tender and regular granular morphology where bio-Lignin is intimately incorporated and linked to the CN nanocrystal to form prevalently nano balls (see Fig. 2). We notice that the bionanocomposite non-woven tissue shows a morphology consisting of randomly assembled nanofibers characterized by a disposition resembling the human Extra Cellular Matrix (ECM) .

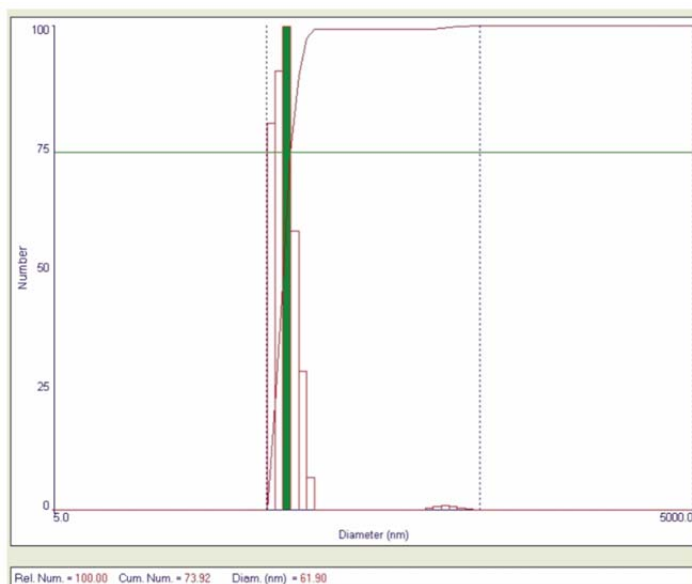


Figure 1: Particles size distribution of the block co-polymeric nanoparticles of CN-bio-Lignin

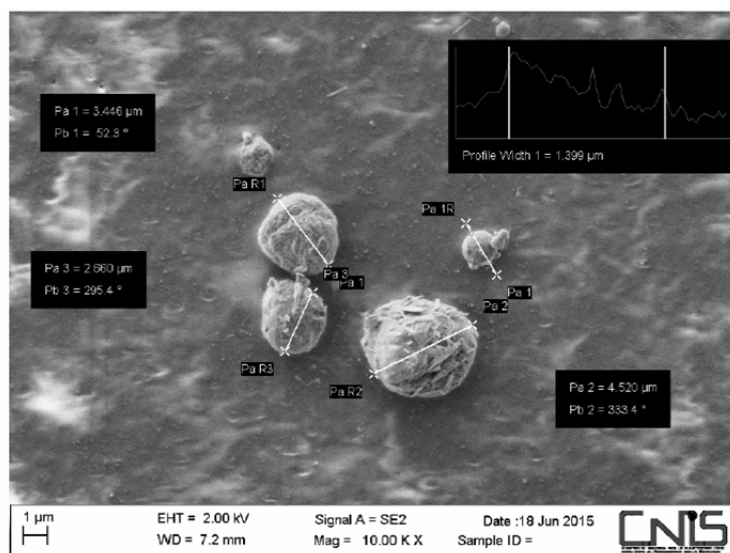


Figure 2: Chitin Nanofibril-Lignin at SEM

After the characterization of the NPs present in the gel, this latter was used for the production of non-woven tissues by electrospinning. The experimental work was first devoted to determine the best set of the operating parameters of the electrospinning machine in order to optimize the quality of the produced tissues. A  $2^3$  factorial campaign was performed, in order to investigate the best value of each operating parameters leading to the minimization of the fiber size of the non-woven tissue. The details of the experimental runs and the

relevant results are reported elsewhere (Chianese and Del Ciotto, 2016). The factorial analyses allowed 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 average thickness of the fibers was in the size range 138 – 193 nm. By operating at the upper value of each operating parameters, i.e. distance SE/CE 16 cm, CE rotation 8 rpm, voltage 45-70 kV, a minimum average size of the fibers equal to 138 nm was obtained. The tissue produced at the best operating conditions, reported in Fig. 3, appears elongated and quite regular without evidence of beads.

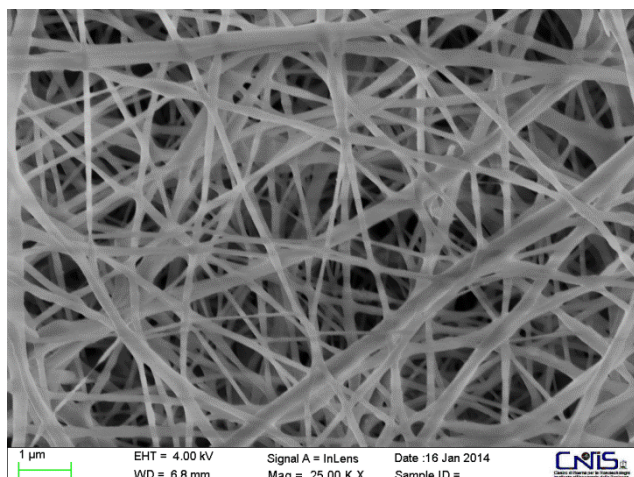


Fig. 3: Image of the non-woven CN non woven tissue produced at best conditions

The produced non-woven samples were submitted to Standard Test Method for estimating the tensile properties by use of microtensile specimens. This standard is issued under the fixed designation D 1708. This test method covers the determination of the comparative tensile strength and elongation properties of plastics in the form of standard microtensile test specimens when tested under defined conditions of pretreatment, temperature, humidity, and testing machine speed. It can be used for specimens of any thickness up to 3.2 mm (1/8 in.), including thin films. The main purpose of these measurements was to evaluate the effect of the layers of the tissue with respect to its resistance. Three examined tissue samples had a different thickness, derived by an increasing number of wounded non-woven tissue layers, respectively equal to 1, 2 and 3. The obtained results are reported in Table 1.

Table 1: Results of microtensile tests.

Sample	Thickness (mm)	Tensile Force (N)	Area (mm <sup>2</sup> )	Tensile strength $\sigma$ (MPa)
A	300	0,301	0,6	0,502
B	450	0,329	0,9	0,366
C	600	0,301	1,2	0,251

Unfortunately, the tensile strength of a single layer tissue resulted quite low with respect to that of electrospun fibers either of PEO (Nandana et al., 2010) or of Chitosan/PVA composite material (Bin Duan et al., 2006). Future work should be focused to improve the characteristics of the PEO/chitin nanofibriles material by changing the composition of the starting sol-gel material, for instance by reducing the ratio CN/PEO or by applying a procedure to improve the cross linking PEO-lignin-CN, for instance by using flexibilizers, ultraviolet light during the hydro gel preparation, etc..

### 3. Functionalization tests

Two kinds of the test were made. The first one was finalized to check the antibacterial property of membranes containing CN. The antibacterial property should be very useful to produce membrane for small and compact water potabilization plant. It is in fact well known that chitin or chitin derivatives (chitosan) nanofibriles have bactericidal properties. As a matter of fact, the chitin, in the shells of crabs and crustaceans, is the natural

defence of these animals to bacteria and algae that may stick on the shell. Therefore, CN incorporated on the membrane, will kill the bacteria present in the feed stream water that comes in contact with the surface. For this purpose, 1% to 5% of CN was added to a homemade polysulphonic ultrafiltration membrane. Membrane surface cytotoxicity was analyzed in a laser scanning confocal microscope (LSCM) according to the following procedure: 50 mL suspension of 107 cells per mL of *Pseudomonas fluorescens* F113 were filtered in a dead-end filtration unit (1 Bar operating pressure) through a modified and non-modified membrane (Ben Guiron University of the Negev, 2010). Preliminary results obtained during dead-end filtration tests using the nanochitin modified membranes show that the modified membrane surface is toxic to bacteria, with very high kill rates and kinetics, thus nanochitin has bactericidal effects. The results were very promising for the use of a membrane with CN for water sanitation.

A second test concerned the effectiveness *in vitro* and *in vivo* of the advanced medications made by CN-biolignin. The particular morphology and composition of the tissue, made prevalently of nanochitin and biolignin fibers randomly assembled, have shown a great cell-affinity by *in vitro* studies and an interesting reparative effectiveness achieved *in vivo* on the burned skin. It is to remember, in fact, that nanochitin, as polymer made of glucosamine and acetyl glucosamine with the same backbone of hyaluronic acid, has the capability to bond a great quantity of water, necessary for all the cell activities (Morganti et al, 2012;2015), as well as to be easily metabolized from the many families of chitotriosidases present into the human body (Eide et al, 2012). Moreover, the bio-lignin, as interesting macromolecule made of many polyphenol units, has shown to have a useful antioxidant activity (Ugartondo et al, 2008 and 2009).

Thus, as above reported, the *in vitro* study on human keratinocytes (Hacat cells) have evidenced that the non-woven tissue has capable to stimulate the protective and antibacterial activity of Betadefensin 2 (see Fig. 4), moreover it decreases the cytokine expression, such as IL-1alpha, IL-8 and TNF alpha and increases the expression of metalloproteinase 2 and 9. (Morganti et al,2016)

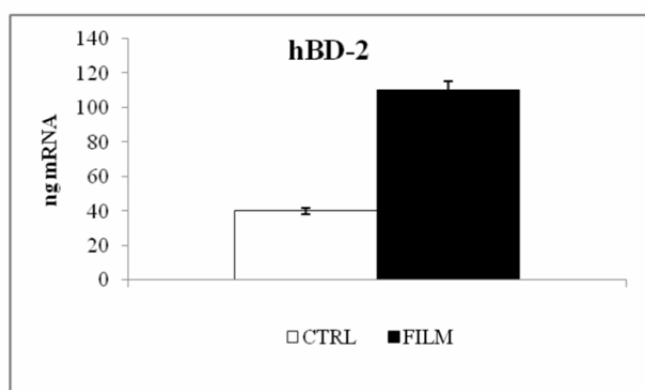


Figure 4: mRNA expression of hBD-2 in Hacat cells after 24 hour of treatment.

#### 4. Conclusions

Natural nanofibers made of CN and bio-lignin may find applications as non-woven tissues for water sanitation and biomedical use. This work has, firstly, shown the feasibility of producing non-woven CN based electrospun, having small thickness, smaller than 140 nm, and uniform mesh, then has reported some interesting applications of the CN as antibacterial and advanced medications. The obtained results are very encouraging for the use of this material both for water sanitation and biomedical use. Finally, the realized CN-bio-lignin non-woven tissue, made by natural raw materials obtained from biomass, has shown to be skin-friendly and 100% biodegradable and therefore useful to decrease the dependence from fossil fuel resources, in agreement to the EOCD and EU programmes on bioeconomy (OECD, 2009; SOER, 2015; EC, 2015).

#### References

- Bin Duan, Xiaoyan Yuan, Yi Zhu , Yuanyuan Zhang , Xiulan Li, Yang Zhang , Kangde Yao, 2006, A nanofibrous composite membrane of PLGA–chitosan/PVA prepared by electrospinning, *European Polymer Journal* 42, 2013–2022 .
- Bowen A and Kuralbayeva K., 2015, Looking for green jobs: the Impact of green growth on employment. Global Green Growth Institute.
- Chianese A. and Del Ciotto P., 2015, Production of Electrospun Nonwoven Materials as a Blending of Chitin Nanofibrils and other Natural Polymers, *Bionanotechnology to Save the Environment*, Ed. P. Morganti IMECHE. Global Food Waste not, Want not, 2013, <<[www.imeche.org/environment](http://www.imeche.org/environment)>>.

- Lipinski B, Hanson C, Lomax J, Kitinoja L, Waite R, Searchinger T., 2013, Reducing Food Loss and Waste. Working Paper, May, 1-39.
- Morganti P, Carezzi F, Del Ciotto P, Tischenko G and Chianese A., 2014, A green multifunctional polymer from discarded material: Chitin Nanofibrils. *Br.J.Appl.Sci Technol.*, 4(20) 4175-4190.
- Morganti P., 2015b, Green economy and Bionanotechnology to transform waste materials in useful goods: Results of EU research projects, *Eurocosmetics.*, 22 (1/2) 12-16.
- Morganti P., 2015, Bionanotechnology & Bioeconomy for a Greener Development., *J Appl Cosmetol*, 33, 51-65.
- Morganti P, Donnarumma G., Palombo M., Fusco A., Del Ciotto P., Baroni A, and Chianese A., 2016, Green Nanotechnology Bioeconomy Serving .Part 2. Advanced Medications made by Green Nanocomposites. In print on *J Appl Cosmetol*.
- Morganti P., Fabrizi G., Tufano M.A., Carezzi F., Cardillo M., Morganti G. and Nunziata M.L., 2015, Green Nanotechnology Bioeconomy Serving. Part 1. Innovative Beauty Masks Made by green nanocomposites. Presented at Nanotech ITALY2015, Bologna, 25-27 novembre.
- Morganti P, Del Ciotto P, Morganti G, and Fabien-Soule' Veronique, 2012, Application of Chitin Nanofibrils and Collagen of Marine Origin as Bioactive Ingredients. In: Se-Kwon Kim Ed *Marine Cosmeceuticals. Trends and Prospects*, New York, CRC Press, 267-289
- Nandana Bhardwaj, Subhas C. Kundu, *Electrospinning: A fascinating fiber fabrication technique*, 2010, *Biotechnology Advances* 28, 325–347
- Nellemann C, MacDevette M, Manders T, Eickhout B, Svilhus B, Prinsa AG, Kalten BP, 2009, The Environmental Food Crisis-The environment's role in averting food crisis. UNEP, GRUD-Arendal, <<[www.grida.no](http://www.grida.no)>>.
- OECD, 2009, The Bioeconomy to 2030: Designing a Policy Agenda. OECD International Futures Programme, <<[www.oecd.org/futures](http://www.oecd.org/futures)>>.
- Ugartondo V., Mitjans M., Vinardell M.P., 2009, Applicability of lignins from different sources as antioxidant based on the protective effects on lipid peroxidation induced by oxygen radicals. *Industrial Crops and Products*, 30 (2) 184-187.
- Ugartondo V., Mitjans M., Vinardell M.P., 2008, Comparative antioxidant and cytotoxic effects of lignins from different sources, *Bioresource Technology*, 99, 6683-6687.
- UNEP., 2012, Building an Inclusive Green Economy for all. Chatelaine, Genève, Switzerland
- Younes I. and Rinaudo M., 2015, Chitin and Chitosan preparation from marine sources. Structure, properties and applications. *Mar. Drugs*, 12, 1133-1174.
- Ben Guiron University of the Negev, Beer Sheva, Israel, 2010, private communication