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A Green Multifunctional Polymer from Discarded Material: Chitin Nanofibrils

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Authors' contributions

This work was carried out in collaboration between all authors. Author PM designed the study, and together with the authors FC and PDC performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors GT, AC and VEY managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

Commentary

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ABSTRACT

Chitin Nanofibril (CN) is the purest crystal form of chitin obtained industrially from fishery and crustacean waste. Characterized by positive charges covering its surface, this natural polymer has the ability to form block copolymeric nanoparticles with electronegative natural or man-made compounds, entrapping active ingredients also.
Due to its own biological properties, CN complexed with hyaluronic acid and entrapped with different active ingredients has been included into cosmetic emulsions, electrospun fibers and transparent films obtained by the casting technology.
The antiaging activity of the obtained emulsions has been reported as well as the first technical characteristics of the non-woven tissues and the transparent films made by two EU research projects:
BIOMIMETIC (www.biomimetic-eu-project.eu) and n-CHITOPACK (www.n-chitopack.eu).

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Keywords: Chitin nanofibrils; non-woven tissues; food packaging; natural polymers; gelation method; electrospinning; casting technology.

1. INTRODUCTION

Chitin Nanofibril (CN) is a natural polymer with a medium dimension of 240x7x5 nm. This polysaccharide, copolymer of N-acetyl-D-glucosamine and D-glucosamine units linked with Beta-[1-4] glycosidic bond, is obtained from fishery and crustacean waste in a pure crystalline form by a new green patented process [1] it has the ability to link many different ingredients for making block copolymeric skin-friendly nanoparticles for many applications as: innovative cosmetics, textiles fibers, and diet supplements [2-5]. Characterized by infrared and X ray spectra, CN is obtained industrially as a suspension containing about 3 trillions molecules/ml of a medium weight of 0. 074 10^6 ng with about 15,000 amino-group per nanocrystal [5,6]. Nanoparticles, made of CN-Hyaluronan have shown interesting biological peculiarity favouring, for example, fibroblasts proliferation, cytokines modulation and giant cells migration, as well as capturing enzymes, growth factors, proteins, and active ingredients [6-8]. Cosmetic emulsions, based on the use of this natural polymer, show to have an anti-inflammatory efficacy, to increase skin elasticity and hydration, decrease skin hyperpigmentation, possessing in conclusion an antiaging efficacy [9-13]. Moreover, CN may be linked to other polymers to give rice to edible particulates for food packaging or to produce micro/nano fibers by the electrospinning technology useful for advanced medical non-woven tissues. [14-17].

2. PHYSICOCHEMICAL PECULIARITY

Chitin Nanofibril has the same backbone of hyaluronic acid (Fig. 1) and its purest crystal form, compared with the commercial chitin, has shown a superior quality as evident by the obtained Infrared bands 1375,1155, and 896 (Perkin Elmer Spectrometer GX FT-R with MCT-SL Detector) and the high intensity of the 9. 624 X-Ray diffraction spectrum (Bruker AXS Detector Diffraction System) (Figs. 2 and 3) [17]. Being its structure covered by electropositive charges, it easily forms block-copolymeric nanoparticles with electro-negative polymers, such as Hyaluronan (HA), by the gelation method as shown on Fig. 4 [18,19].

The block copolymers CH-HA appear at SEM as nano-lamellae or nano particles, depending on the method of preparation and the homogenizer process used (Fig. 5) [15,19].

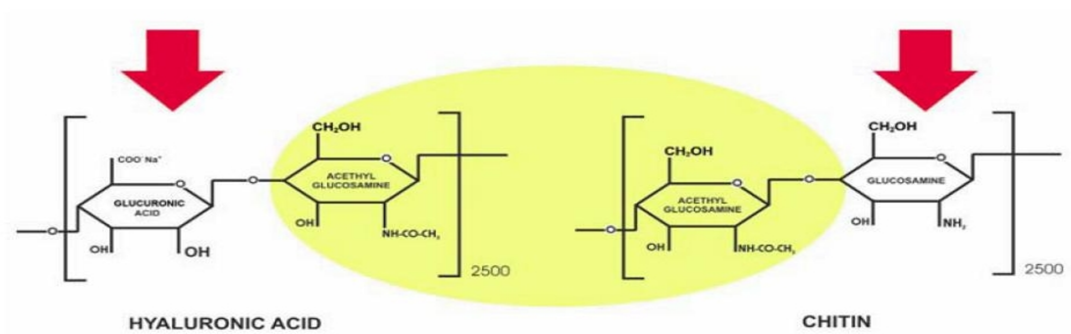


Fig. 1. Chitin nanofibril has the same backbone of hyaluronic acid

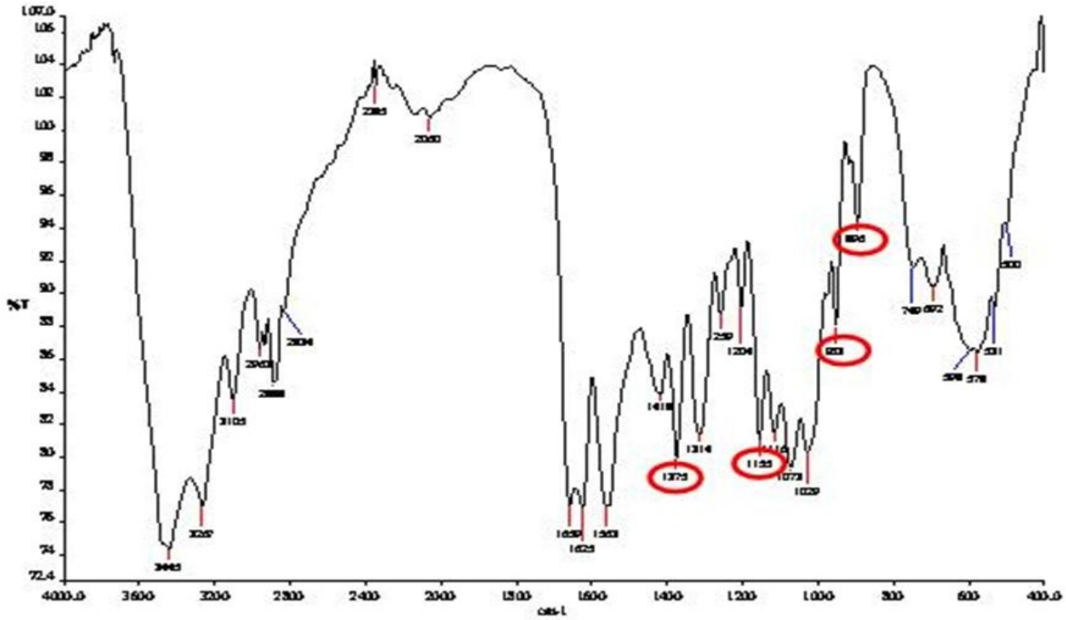


Fig. 2. Chitin nanofibril crystal form, compared with the commercial chitin, has shown a superior quality as evident by the obtained infrared bands 1375,1155

Fig. 3. Chitin nanofibril crystal form, compared with the commercial chitin, has shown a superior quality as evident by the high intensity of the 9. 624 X-ray diffraction spectrum

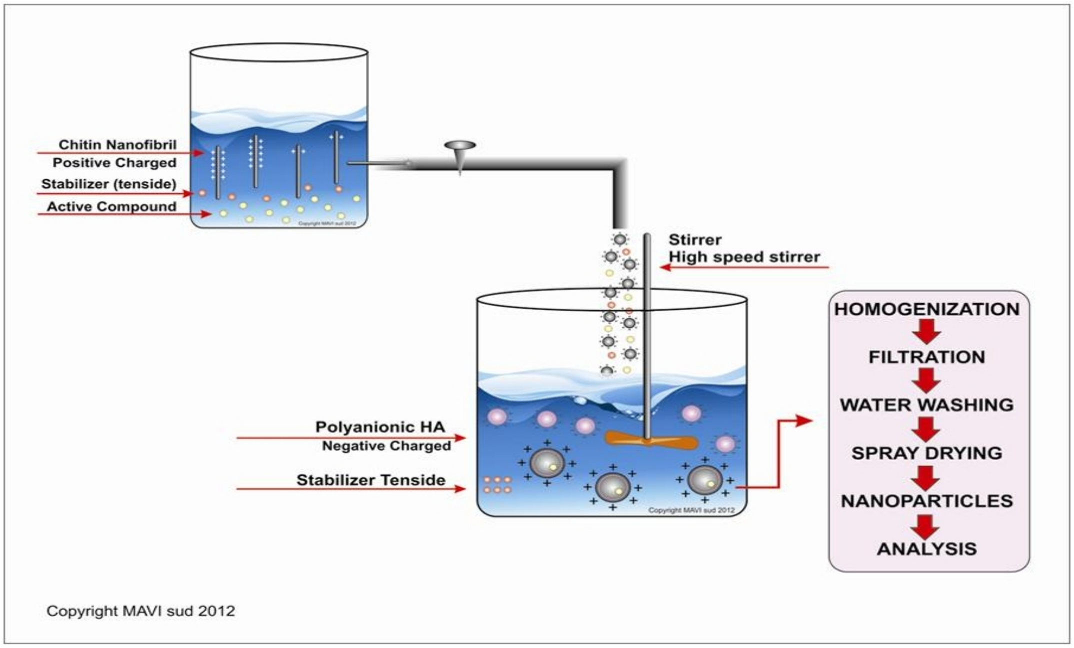


Fig. 4. Chitin Nanofibrils form block-copolymeric nanoparticles with electro-negative polymers, such as hyaluronan (HA), by the gelation method

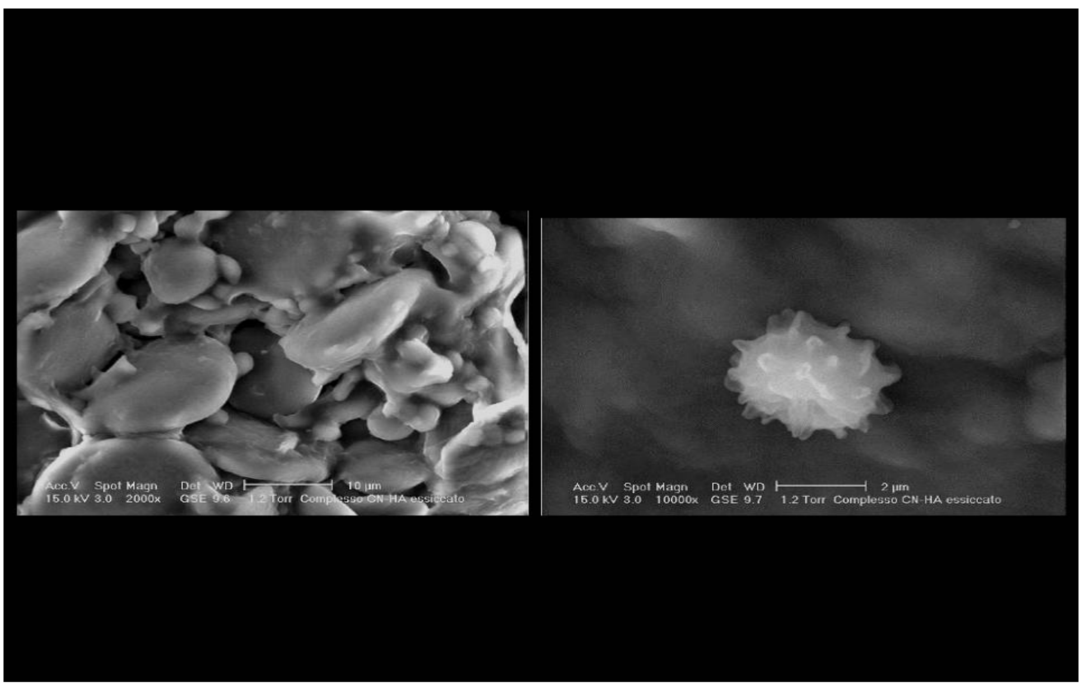


Fig. 5. The block copolymers CH-HA appear at SEM as nano-lamellae or nano particles, depending on the method of preparation and the homogenizer process used

3. BIOLOGICAL PECULIARITY

As previously reported, CN and its block polymers exhibit an enormous surface development, which allows them to interact with enzymes, platelets and other cell compounds present in living tissues [6-9]. Thus the recovered peculiarity and the ability to faster the skin granulation phenomena is accompanied by angiogenesis and regular deposition of collagen fibers, with the consequent enhanced and correct repair of dermo epidermal lesions (Fig. 6) [5-8].

In fact, they possess relevant biological significance capturing enzymes, grow factors, proteins, drugs and, being biocompatible with human cells, induce and support haemostasis.

Moreover, they are immune stimulating, safe to ingest, deprived of allergenicity, fully biodegraded by soil and marine bacteria, and by the18 families of human chitotriosidases (chitinases) capable to digest chitin to glucosamine or $H2O$ and $CO2$ [4,20,21,34].

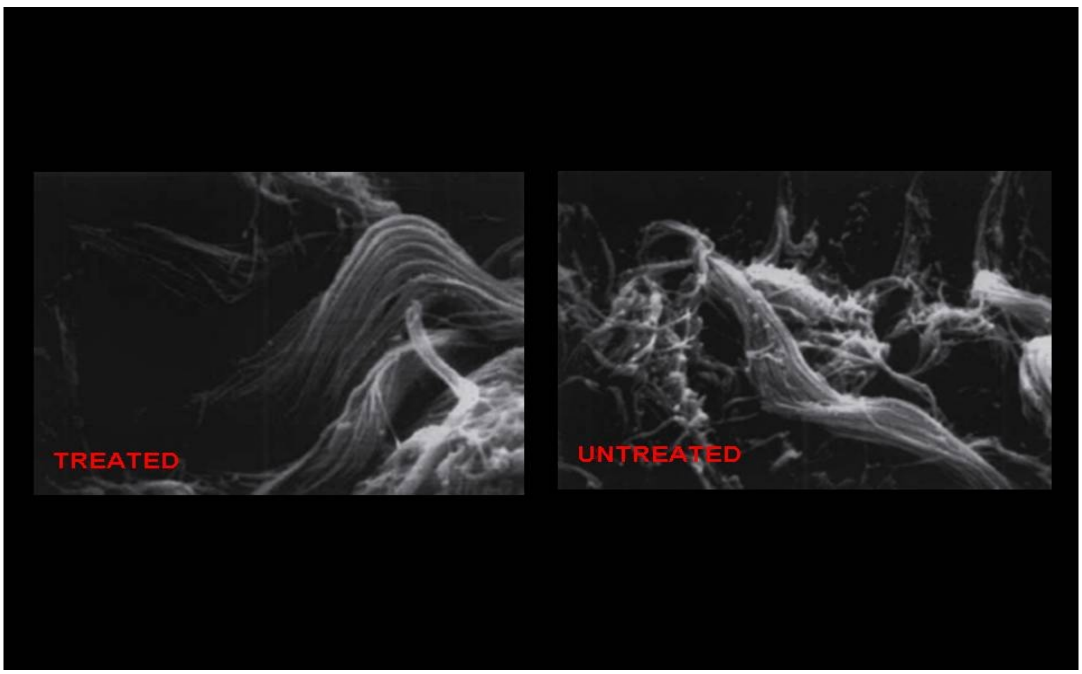


Fig. 6. Chitin Nanofibrils ability to faster the skin granulation phenomena accompanied by angiogenesis and regular deposition of collagen fibers

4. CHARACTERIZATION AND CONTROL

Anyway CN nanoparticles, entrapping or encapsulating different active ingredients, may be covered outside their surface by positive or negative electrical charges, evidencing different skin' effectiveness not only for the size but for the charge also. The positive charges, in fact, generating electrostatic interactions with the stratum corneum, seem have the ability to disturb the tight lamellar layers, enabling a better diffusion of the entrapped active ingredients through the skin [18-20,22-27].

The block copolymeric nanoparticles, obtained by the gelation method, purified by centrifugation and atomized in a stream of hot air, were characterized by the use of zeta potential (NanoZS model Zen 3600, Malvern Instrument) and SEM (SEM/EDY, Philips XL30), while non-woven tissues, made by the use of electrospinning (NS LAB 500S - Elmarco, Chz) and films produced by casting technology (SMK AFA L800, USA), were controlled by SEM,TEM, AFM (Multi Mode Digital Instrument NanoScope Dimension IIIa) and Instron 5800 to verify their images and mechanical properties (tensile strength and Young's module).

Depending on the method of production, it is possible to produce nanoparticles, particulate-films, and non-woven tissues that may act as carriers, to deliver entrapped molecules into or across the skin, as skin penetration enhancers, as depot for the sustained release of active ingredients, or as site-limiting membrane barrier to preserve food [28-35].

5. RESULTS FROM OUR STUDIES

By the use of CN nanoparticles, entrapping different active ingredients, our group obtained interesting biological and clinical results, such as skin moisturizing activity [10,12,13] (Fig. 7), black spots reduction [13] (Fig. 8), anti-inflammatory efficacy [11] (Fig. 9), increase of skin elasticity [11] (Fig. 10), anti-wrinkles efficacy [10,12,13,37,38] (Fig. 11), anti-acne activity (Fig. 12), and hair protective activity [4,10-12,36-39] (Fig. 13).

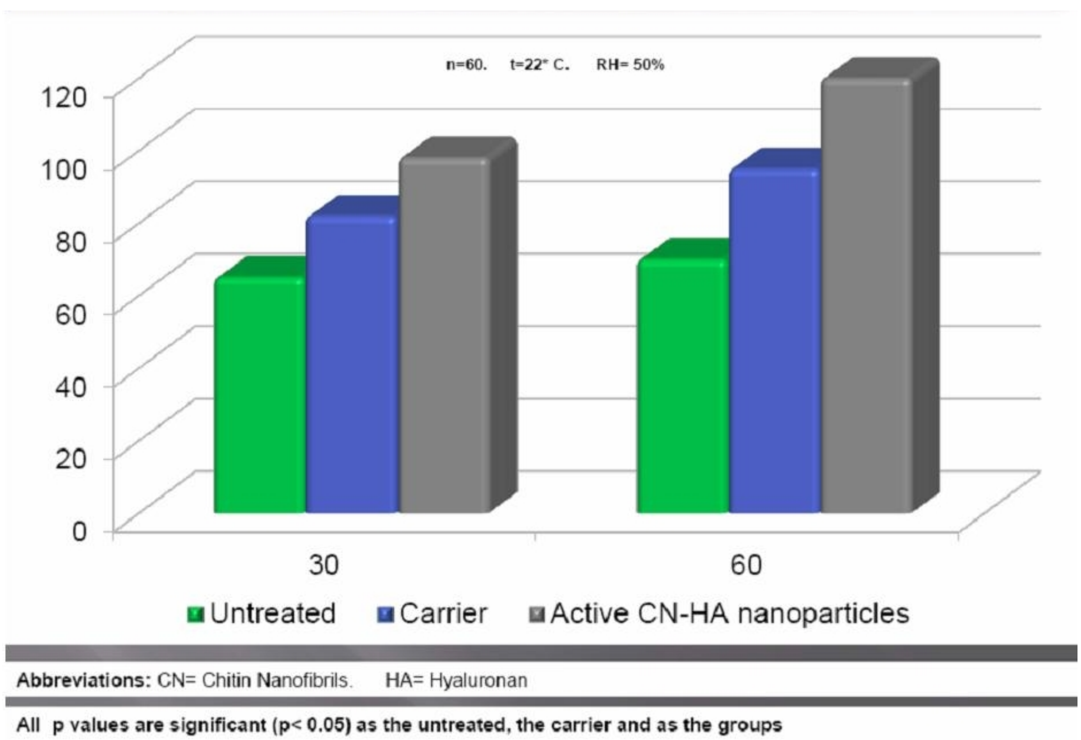


Fig. 7. Skin hydration increase (difference from baseline) on photoaged subjects after a two month global treatment on face and neck by active CN-HA nanoparticles vs the carriers [13]

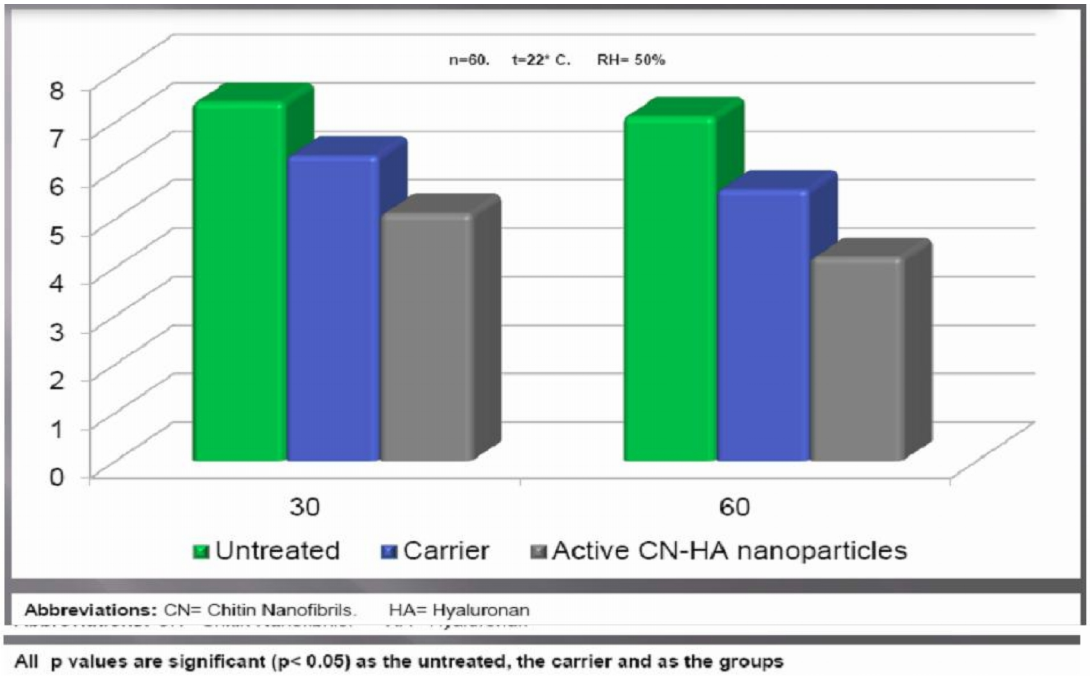


Fig. 8. Chromameter values a* (difference from baseline) after a two months global treatment on face and neck by active CN-HA nanoparticles vs the carriers [13]

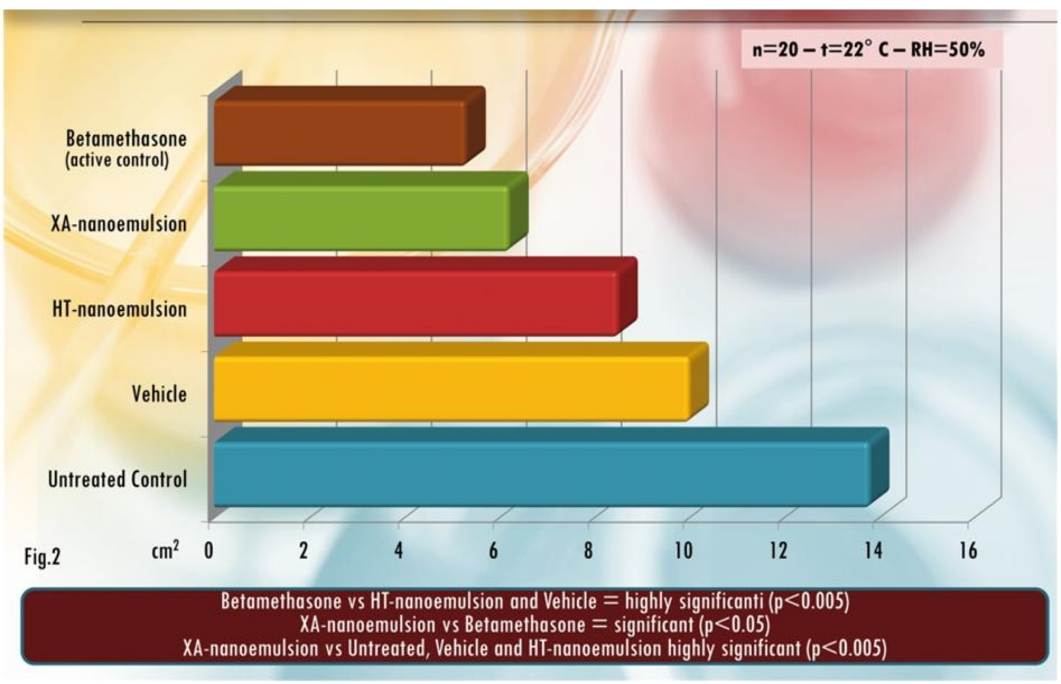


Fig. 9. Erythema area in cm^2 after a 12 hour treatment with XA-nanoemulsion chitin nanofibrils vs hydrocortisone nanoemulsion HT and the vehicle [11]

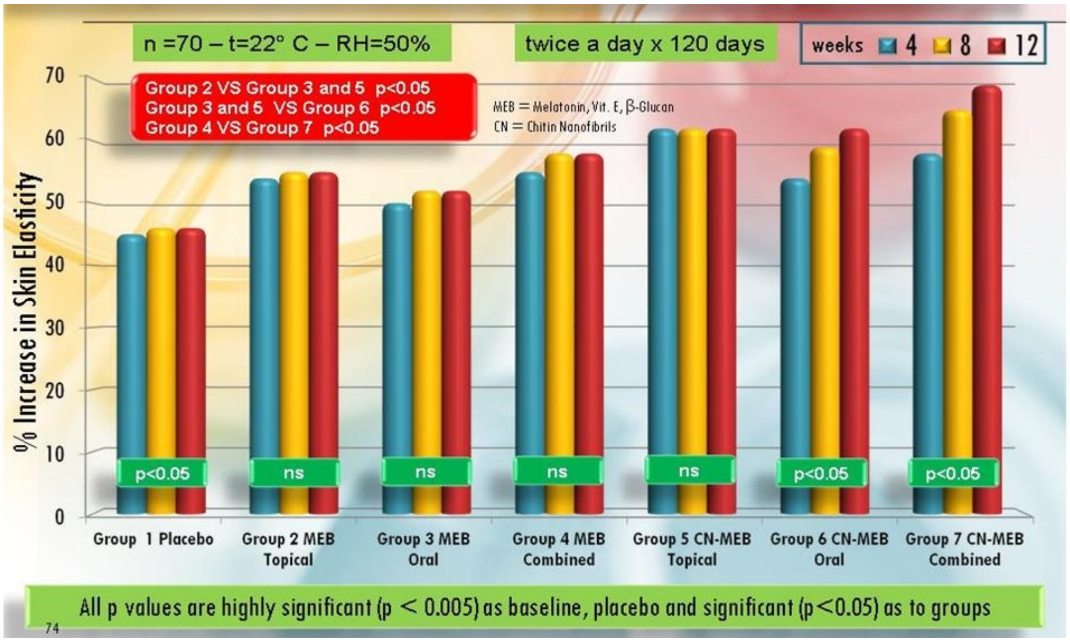


Fig. 10. Skin elasticity of photoaged healthy subjects treated topically and/or orally by antioxidant compounds complexed with chitin nanofibrils (% increase vs baseline values). MEB = melatonin, vitamin E, betaglucan entrapped into chitin nanofibrils applied topically by an emulsion or orally taken [4]

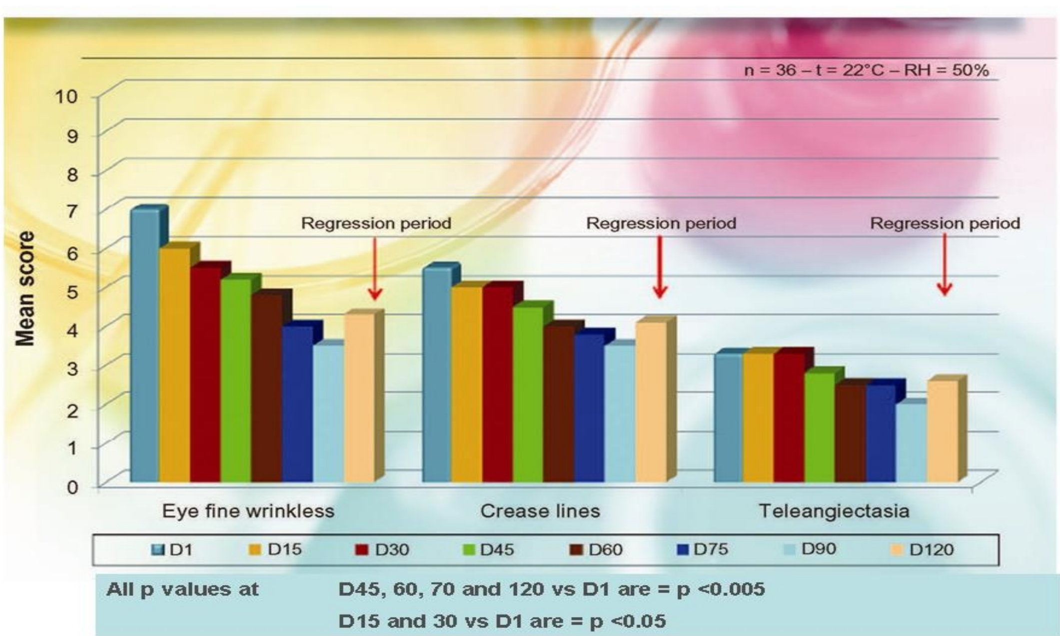


Fig. 11. Antiwrinkles efficacy on photoaged healthy subjects treated topically and/or orally by antioxidant compounds complexed with chitin nanofibrils [12]. D stays for days

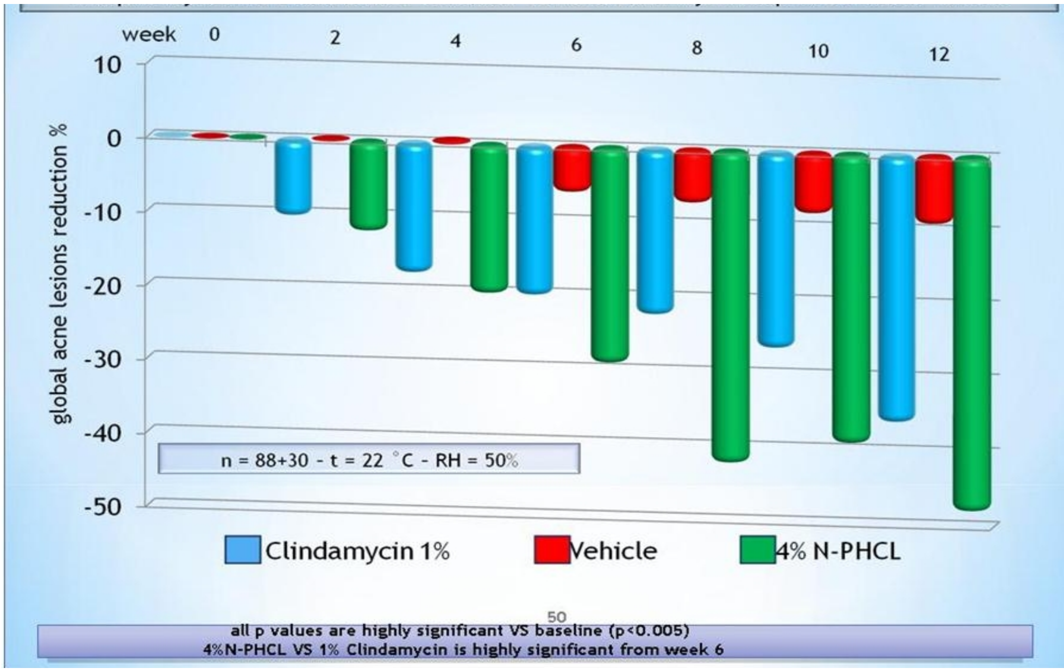


Fig. 12. Mean reduction of global acne lesions after a 12 week treatment by 4%-nicotinamide phosphatidylcoline linoleic-rich chitin nanofibrils vs 1%-clindamicyn phosphate and the vehicle [52]

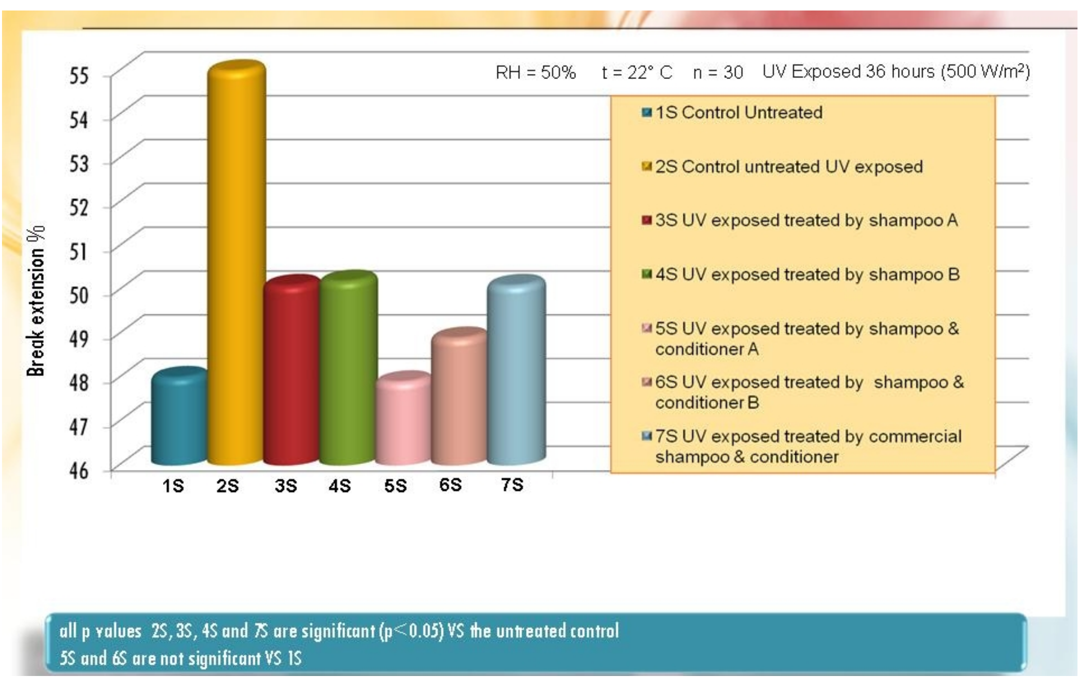
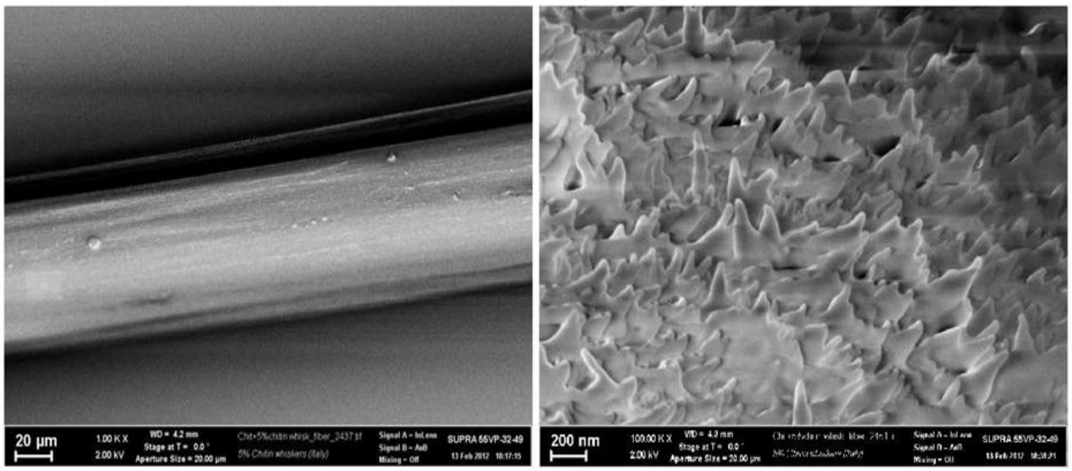


Fig. 13. Increase in break extension of hair exposed to UV and treated by Zn-CN shampoo and conditioner [39]

About toxicity, both CN and CN-block polymeric nanoparticles have shown to be safe, on the viability of keratinocytes and fibroblasts' cultures, while resulting also totally degraded by the enzymatic activity [18-20,34,37,38].

Moreover, from the results of our national and recent international collaborations, it was shown the possibility CN and its nanoparticles have to orient the fiber deposition of nanocomposite films made, for example, with chitosan (Fig. 14) increasing the strength and Young modulus (Fig. 15) of composite fibers, increasing the swelling of the final film (Fig. 16), and their hydrophobicity (Fig. 17) [40-43].



a b

Fig. 14. CN and its nanoparticles are able to orient the fiber deposition of nanocomposite films made with chitosan

Mechanical properties of chitosan and composite fibers

Composite Fibers	Tensile Strength MPa	Tensile Modulus MPa	Elongation at break, %
Chitosan 100%	199	7950	7.2
+0.5% Chrysotile	292	13740	7.6
+1.0% Chrysotile	220	8510	8.5
+1.5% Chrysotile	241	11370	8.5
+2.0% Chrysotile	187	7310	4.8
+3.0% Chrysotile	214	9200	4.7
+ 5% CN	**391**	**18240**	**6.2**
+ 20% CN	**411**	**20700**	**3.9**

Fig. 15. CN and its nanoparticles are able to orient the fiber deposition of nanocomposite films made with chitosan increasing the strength and young modulus

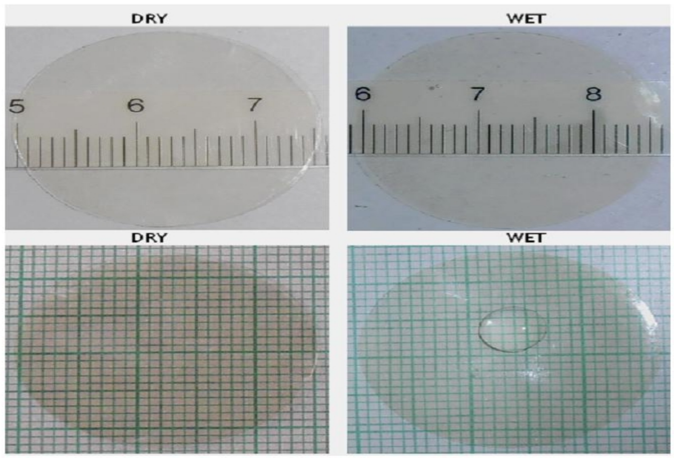


Fig. 16. CN and its nanoparticles are able to orient the fiber deposition of nanocomposite films made with chitosan increasing the swelling of the final film

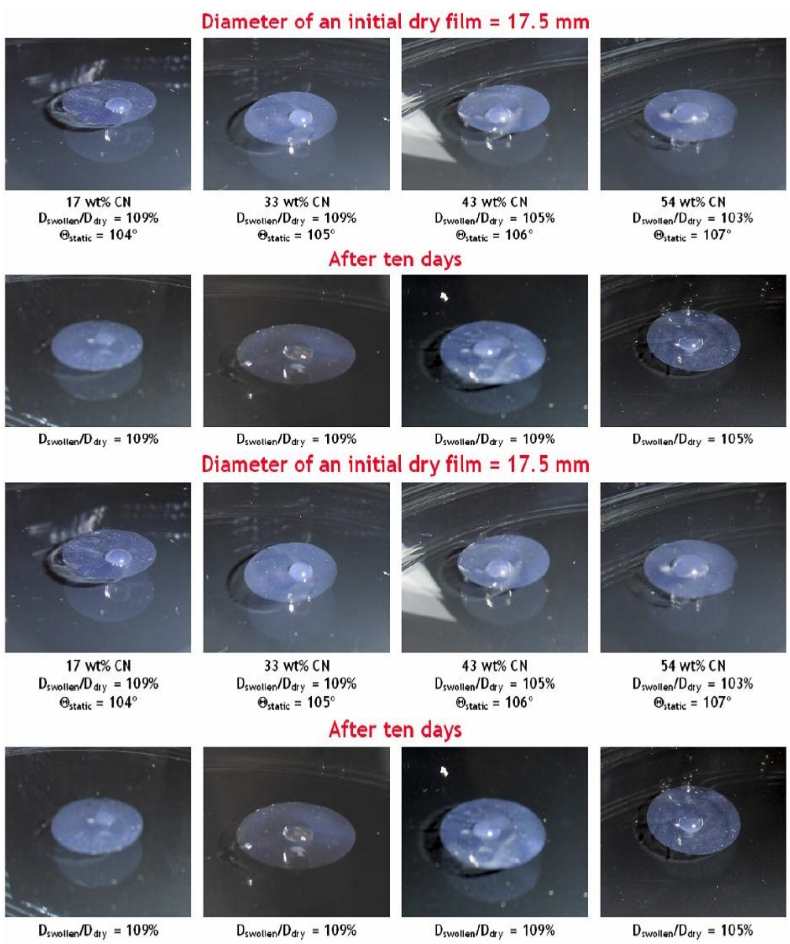


Fig. 17. CN and its nanoparticles are able to orient the fiber deposition of nanocomposite films made with chitosan increasing the final film hydrophobicity

6. DISCUSSION

Technological advancements in nanoscience have led to the expansion of novel nanoscale biomimetic materials, such as biocompatible and eco-compatible nanolamellae, nanospheres, biofilms, and advanced medications made by non-woven tissues. The efficacy both *in vitro* and *in vivo* of the studied antiaging nanoemulsions, based on the use of CN-nanoparticles and entrapping different active ingredients, have shown to increase from 15 to about 40% the production of collagen type I, III and IV at different skin levels, safeguarding the activity of chaperon HSP47, to decrease of about 50% the release of IL-8 and MMP1 (*in vitro* on fibroblast and keratinocytes culture), as well as to increase of about 40% the skin hydration, decreasing notably TEWL and the skin wrinkling appearance by a double blind study *in vivo* on 60 photoaged women as shown elsewhere) [12,13]. This activity is probably due to the composition of chitin made by about 15/20 molecules of glucosamine and easily hydrolized by the human chitotriosidases [21]. Probably these molecules favour the collagen synthesis and deposition, while glucose may give energy to the cell to anabolise and catabolise CN and the different collagen molecules at different skin levels. Moreover, the polymeric films, realized by the use of CN -reinforced chitosan by the casting technology (n-CHITOPACK (www.n-chitopack.eu) have shown to possess significant and a better tensile strain (elongation at break), good hydrophobicity, and swelling capacity and maximum tensile stress at break in comparison with chitosan alone [40-52], while the non-woven tissues, realized with the use of CN and other natural polymers from plant biomass by electrospinning, have shown to be safe, totally biodegradable and useful to support the skin granulation process regulating the normal and regular deposition of collagen fibers, thus favouring the relative wound healing (unpublished data).

7. CONCLUSION

The high effectiveness, safety, non toxicity and biodegradability of CN and its derivatives, give rise to produce innovative biomimetic goods in a wide variety of industrial fields, reducing also the environment pollution because of its production from waste materials (Fig. 18).

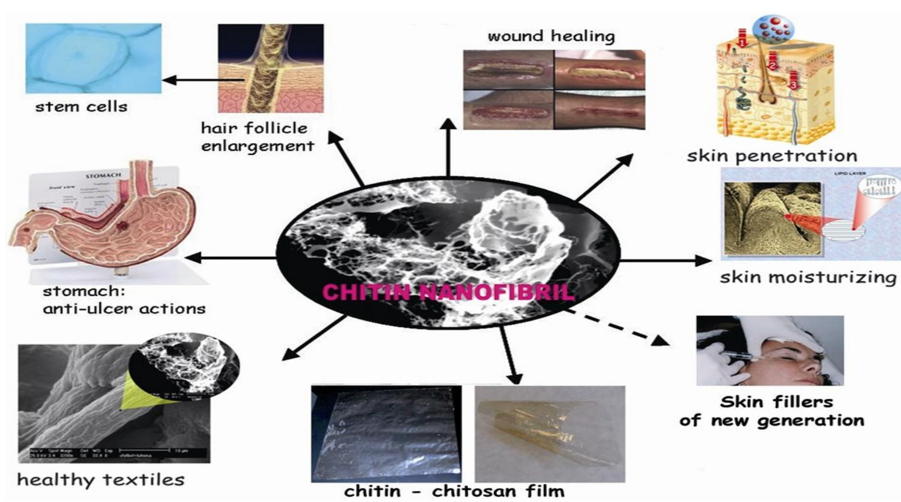


Fig. 18. CN and its derivatives are useful to produce innovative biomimetic goods in a wide variety of industrial fields

Further development of bio-nanotechnology by the use of raw material, obtained from fishery's and plant biomass, will represent a great intellectual challenge for a global sustainability.

Mimicking the molecular structures and processes adopted in nature by the use of crystal chitin, is the goal of our in progress projects, to produce innovative nanoemulsions for cosmetic products: nanofibers for non-woven medical dressing, smart textiles, and nanostructured films for advanced food packaging.

COMPETING INTERESTS

Drs. Morganti, Carezzi and Del Ciotto served as consultants in MAVI SUD; Prof. Tischenko, Chianese and Yudin have no conflict of interests to declare.

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