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Chitin Nanofibrils: a Natural Multifunctional Polymer *Physicochemical characteristics, effectiveness and safeness.*

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Introduction

Every year about 300 billion tons of industrial and agricultural waste are generated, deriving from processing of plant raw materials into intermediates or final products [1]. Only 3% (13 billion tons/year) of world plant biomass is used for making goods, whilst 20% of 154 billion tons/year of fishery and crustacean's processing are transformed into chitin, chitosan and oligosaccharides, producing waste of 30 million/tons [2, 3]. Hence, a comprehensive overview of the amount of by-products generated in different industrial and agricultural sectors in each country is timely and much needed [4] in order to transform the waste in useful goods by means of environmentally friendly processes (Figure 1.1).

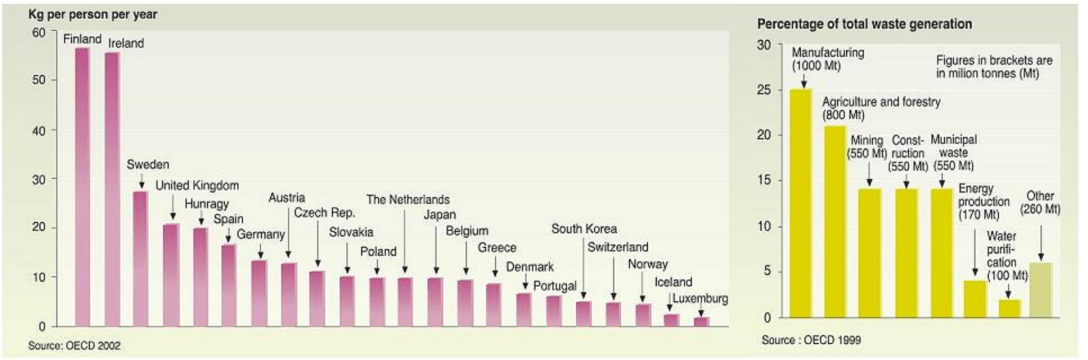


FIGURE 1.1
Waste generated by person per year in different Industrial Sectors and in different Countries.

The recycling and reuse of waste using green technologies will reduce water consumption, greenhouse gas emissions and worldwide pollution without impoverishing of the environment with precious and fundamental materials whilst saving the biodiversity of the Earth [5].
Natural ingredients play an important role in consumer culture, and knowledge and ethic of traditional sourcing of biodiversity have increased strongly, particularly in emerging economies like Brazil and China (Figure 1.2) [5]. In China, 98% of consumers buy cosmetic products based on natural ingredients, and 94% pay close attention to the source of the ingredients for food. A significant increase in the amount of waste resulting from the industrial processing of seafood has became a problem for environmental and processing plants. About 45% of processed seafood consists of 50-70% of shrimps exoskeleton and cephalothorax waste. Naturally, the amount of the discarded raw material depends on the processing conditions, species, body parts, and season's changes [3, 6-8].

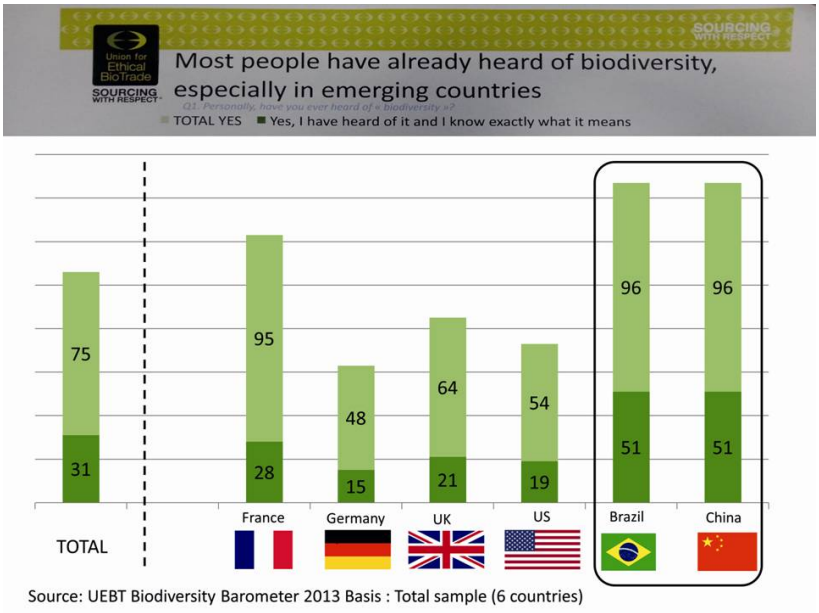


FIGURE 1.2

Chitin is the main structural polysaccharide of arthropods and fungal cell walls containing of about 50-100% of N-acetyl-D-glucosamine and 50-0% of D-glucosamine together with mineral salts and proteins. It is the second most abundant natural biopolymer on Earth after cellulose. Owing to its sugar-like character, this natural polymer is promising in several areas due to its biocompatibility, biodegradability, antimicrobial properties and high tensile strength. The deacetylated form of chitin refers to chitosan (Figure 1.3).

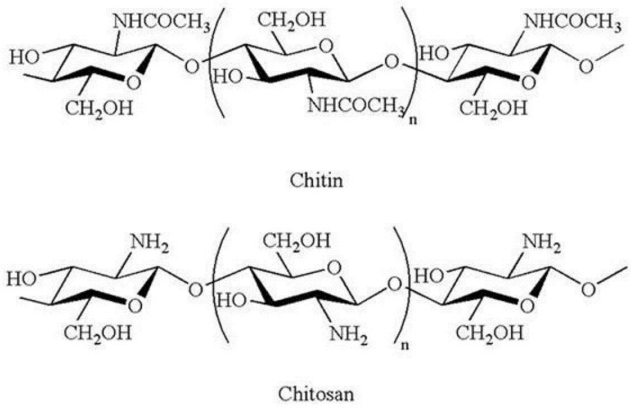


FIGURE 1.3
Chemical structures of Chitin and Chitosan. When the degree of N-acetylation (DA) is greater than 50%, the polysaccharide is considered to be chitin. When the DA is less that 50% , the polysaccharide is considered to be chitosan.

However, contrary to chitosan, only recently, chitin received little industrial attention due to its poor solubility. Chitin Nanofibrils (CN) have been already used in interesting delivery systems, goods and biomaterials [9-13].
CN being positively charged polymeric nanoparticles of crystal nature, easily form complexes with any natural or synthetic electronegative polymer containing entrapped active ingredients of different kind inside or outside their structure [14-16]. In addition to solve the delivery problems associated with poorly soluble substances, CN are promising carriers for controlled delivery and release of active ingredients by offering new nanotechnological solutions safe for both human and environment [17-19].
Nanobiotechnology is a multidisciplinary science acquiring knowledge from physical sciences, molecular engineering, biology, chemistry, and biotechnology that gives a great advantage for advanced pharmaceutical, healthcare and food products [20-22].

Chitin Nanofibrils

Physicochemical characteristics

Chemistry

Chitin Nanofibrils, known also as nanowhiskers or CN, are slender rods with diameter and length of about 30-40 nm and 600-800 nm, respectively (Figure 1.4).

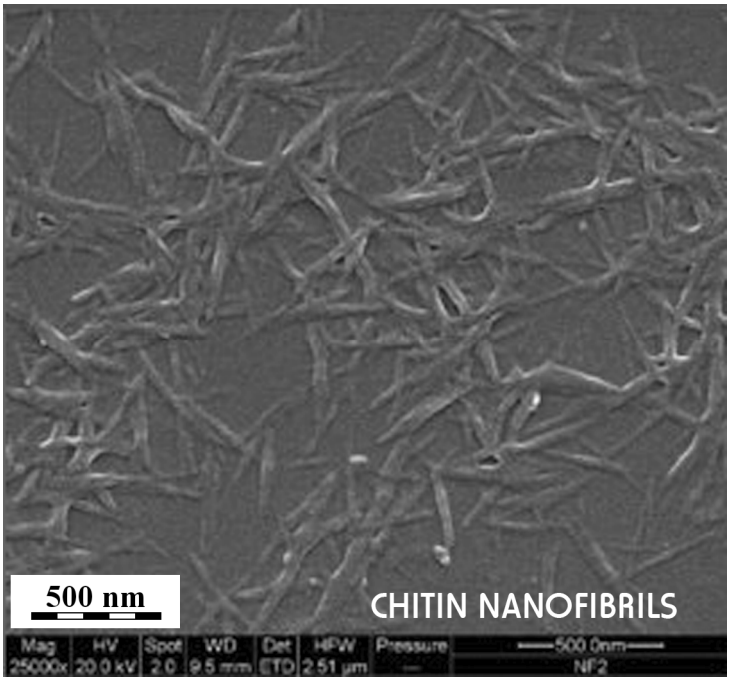


FIGURE 1.4
Chitin Nanofibrils at SEM.

They are the purest crystal form of chitin. This natural, renewable and biodegradable block copolymer consists of N-glucosamine and N-acetyl-D-glucosamine units attached to each other through β-(1-4) glycosidic bonds. Each chitin nanofiber is composed of linear intertwined chitin chains containing about 18-25 units.

Hyaluronic acid, (HA) [23] is an amorphous substance (Figure 1.5) with the same structural unit (N-acetyl-D-glucosamine) similarly to chitin but N-glucosamine units in HA are displaced with glucuronic acid ones.

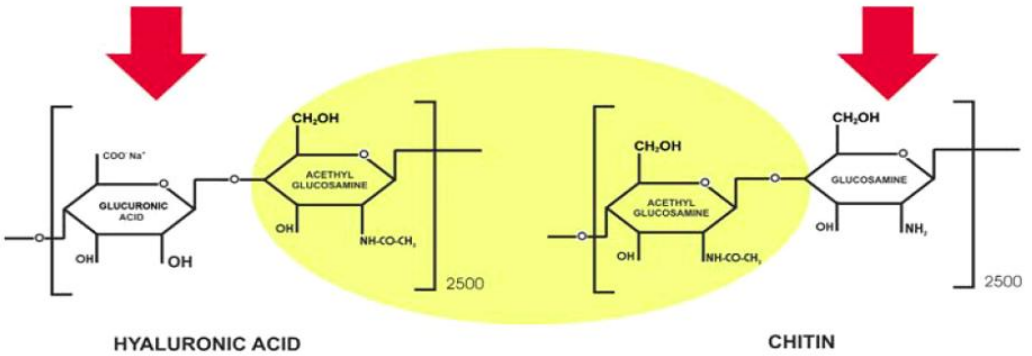


FIGURE 1.5
Chitin has the same backbone of hyaluronic acid.

Despite its accessibility, chitin is still an under utilized natural renewable resource due its insolubility in water and common organic solvents. Recently, the crystalline chitin nanofibrils have been obtained as a stable aqueous suspension from commercial chitin using the green process patented world wide [24]. CN have been used in making innovative cosmetics, drug delivery systems, and advanced medications [25-27]. In the dry form, CN [27] were obtained using a spray-dryer (Buchi-190, Flawil, Switzerland), at the following operation conditions: (the feed rate of 10ml/min; the air inlet and outlet temperature of 148°C and 90° C, respectively; the air flow of 600 l/h). Their structure was analyzed using SEM (SEM/EDY, Philips XL30).

A single chitin nanofibril appears as needle-like crystal with the medium dimensions of 240×7×5 nanometers (nm) (Figure 1.6), Its medium weight is evaluated to be equal to 0.074×10^6 ng and the water uptake achieves of about 400 wt% At pH interval (2-4), one ml of the aqueous colloidal CN dispersion contains of about 2×10^{14} (i.e. 300 trillions!) nanocrystals, which are enveloped with water molecules preventing CN from flocculation. Protonation of free amino groups on the CN surface provides the positive charge. The diluted colloidal CN dispersion is stabilized owing to electrostatic repulsive forces between chitin nanofibrils bearing the same electrostatic charge [27-29]. The surface charge density can be evaluated from the values of the average particle volume (1.1×10^5 nm^3), the crystal surface (2.0×10^4 nm^2), and the chitin density (1.425g $\times cm^3$). If the content of amino groups per 1 nm^3 of a CN nanocrystal is equal to ~15,000 [27-29], then 10 nm^3 is occupied with about 7.6 charges. As a rule, CN suspension also contains fragments of chitin nanocrystals having irregular shape and associated or collapsed micro/nanocrystals. The size distribution, width of distribution, and zeta potential of dried CN dispersion re-suspended in distilled water, were determined using a Zetasizer (Nano ZS model Zen 3600, Malvern Instruments, Worchestershire, UK) (Figure 1.6). Their length and width usually ranged from 100 nm up to 600 nm, and from 4 to 40 nm [28], respectively. More than

75% of the CN crystals obtained have an average length and width of about 240 and 7 -5 nm (Figure 1.7) respectively.

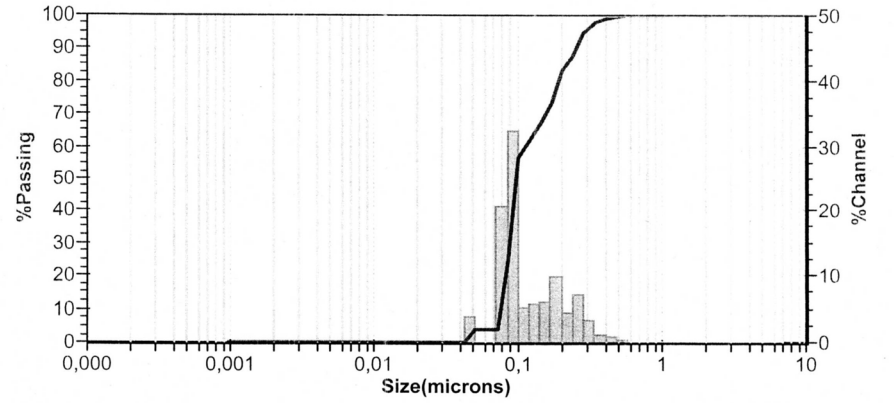


FIGURE 1.6
Size distribution of Chitin Nanofibrils in water suspension.

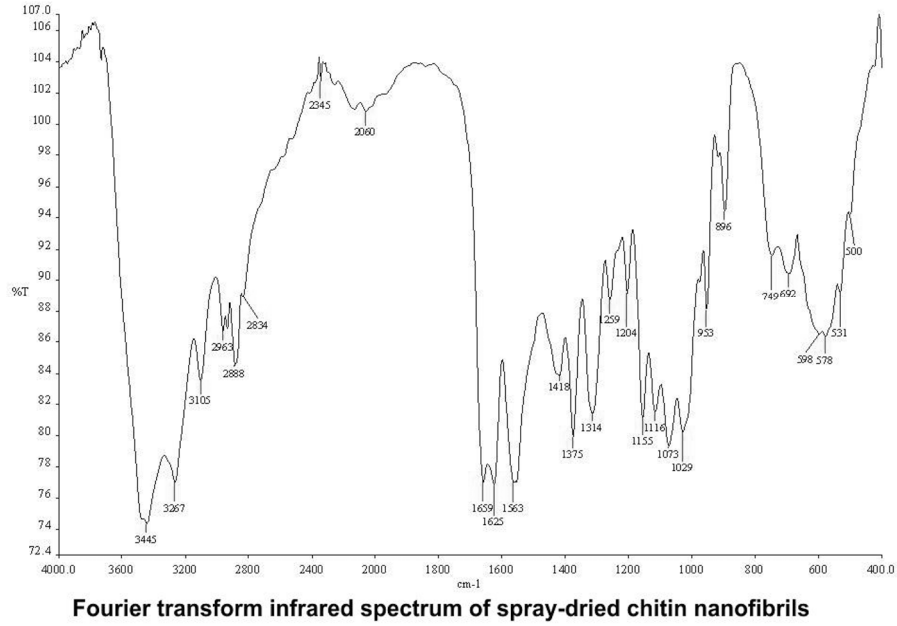


FIGURE 1.7
Chitin Nanofibril crystal form, compared with the commercial chitin, has shown a superior quality, as evident by the obtained Infrared bands 1375,1155.

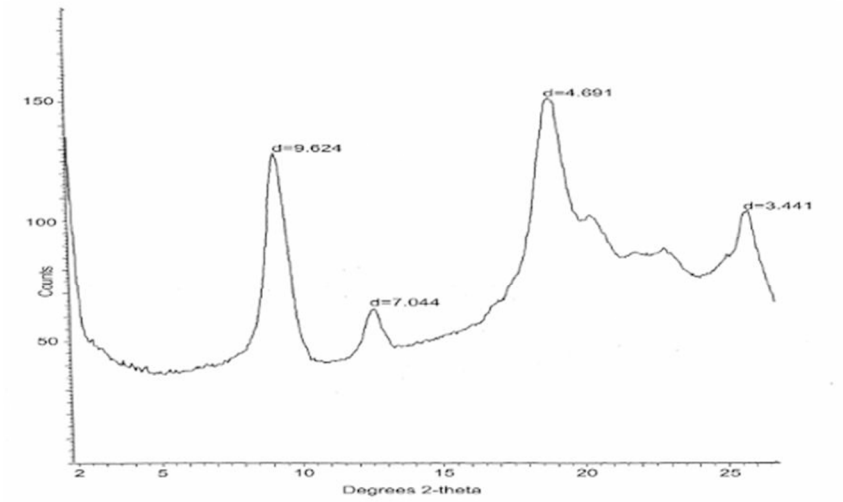
X-ray diffraction and infrared spectroscopy

CN samples dried using spray-drying have been characterized with FT-IR spectroscopy and X-ray diffraction. [27,28]. X-ray measurements were performed using a Bruker AXS General Area Detector

Diffraction System equipped with a two-dimensional gas-filled sealed multi-wire detector (scattering-angle resolution of 0.02°) mono-chromatized by CuK α-radiation (λ= 0.154 nm). The powder samples were placed in 0.8-diameter Lindmann glass capillaries at a distance to detector of 10 cm. The spectra of intensity *vs* scattering-angle were obtained after radial average of the measured 2D isotropic diffraction patterns.

The Attenuated Total Reflection (ATR) spectra were recorded using a Perkin Elmer Spectrometer GX FT-IR equipped with a multiscope system of an infrared microscope with a movable 75×50 mm X-Y stage (MCT-SL detector) [27, 28]. Small amount of the dried CN powder was cooled in liquid nitrogen and ground with KBr to obtain the spectra using a Spectra Tech with a Diffuse Reflectance (DRIFT) accessory.

The spectrum obtained (Figure 1.8) was the results of 16 scans with a resolution of 4 cm^{-1}, which were treated using a Grams/32 Galactic Co. Software package.



Wide angle X-ray diffraction spectrum of spray-dried chitin nanofibrils

FIGURE 1.8

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.

Typical bands of chitin are present in the spectrum of CN (Figure 1.7): bands at 3445 cm^{-1} and 3267 cm-1, which are assigned to vibration of N-H, O-H groups and N-H group of the secondary amide in trans-configuration, respectively; the band at 3110 cm^{-1} confirms the vibration of NH-CO grouping in chitins; the bands at 2963 cm^{-1} and 2888 cm^{-1} reflect the vibration of methylene (-CH_2-) in –CH_2OH and in pyranose ring and methyl (CH_3-) in CH_3CONH-groupins, respectively. The vibrations at 1625 cm^{-1} and 1659 cm^{-1} are attributed to C-N of the later grouping of chitin in crystalline and amorphous states, respectively. The presence of acetylated and deacetylated amine groups is confirmed by the bands at 1375 cm^{-1} and 1563 cm^{-1}, which are assigned to vibration of CH_3 group and N-H in amine, respectively. Finally, the absence of the vibration band at 1540 cm^{-1}, which is assigned to proteins, is an evidence of high purity of CN obtained by the patented process [24]. The absence of any trace of proteins, being possible cause of allergic and sensitizing phenomena, ensures safety of CN for medical applications.

The structure of α-chitin with aniparallel chains packing has been determined by using X-ray diffraction analysis based on the intensity data (8) [27, 28].

The chains form hydrogen-bonded sheets linked by C=O...H–N bonds approximately parallel to the α-axis. Each chain has an O-3'–H...O.5 intra-molecular hydrogen bond, similar to that in cellulose. The results indicate also that a statistical mixture of CH_2OH orientations is present, equivalent to half oxygen on each residue, each forming inter- and intra-molecular hydrogen bonds. As a result, the structure contains two types of amide groups, which differ in their hydrogen bonding, and account for the splitting of the amide I band in the infrared spectrum. The inability of this chitin polymorph to swell on soaking in water is explained by the extensive intermolecular hydrogen bonding [27, 29].
The type of crystallinity with its strong intermolecular hydrogen bonding determines the structure and morphology of chitin nanofibrils. They are perfect crystals with uni-planar orientation [30]. In fact, CN, as α-chitin, contains two anti-parallel chains, which are held tightly by a number of strong C=O...H-N inter-chain hydrogen bonds.

Biodegradability

Biodegradability, non-toxicity, biocompatibility and ability to promote the synthesis of hyaluronan are the main specific characteristics of natural chitin-derived polymers in general and of CN in particular. Metabolism of chitin in nature is controlled by enzymatic systems, which produce and break down its molecule by chitin synthases and chitinases. Thus, chitin and chitosan are easily degraded not only by enzymes such as lysozyme [31], N-acetyl-D-glucosaminidase and lipases [32], but also by chitotriosidase (HCHT) belonging to 18 family of chitinases secreted by humans [33].
It is interesting to underline that the level of HCHT in blood is up-regulated in a series of human diseases such as cardiovascular risk and coronary artery disease [34], prostatic hyperplasia [35], and other medical conditions or antiparasite responses [36] and can be considered as a biomarker. It seems that HCHT represents better defence against chitin-containing pathogens. It is primarily expressed in human macrophages [37, 38] and activated by human microbiota [39].
This specific enzyme degrades chitin and chitosan primarily via the endo-processive mechanism showing an absolute preference for acetylated polymers compared with the deacetylated ones because of a relative weak preference for an acetylated unit in the -2, -1, and +1 subsites, respectively [40, 41]. Thus, CN are easily degraded because of higher content of acetylated glucosamine groups in comparison to chitosan resulting in enhancing the production of hyaluronan and collagen glycosaminoglycans which are the fundamental components of extracellular matrix (ECM) [22]. As a result, a risk of hypertrophic formations of scars and keloid and a slowdown of intra-peritoneal adhesion and intestinal structures are considerably reduced [42, 43]. A probable reason could be the increase of chito-oligosynthase DG42 protein, which has been recovered, for example, during the embryo genetic process and acted as primer in the synthesis of hyaluronan [44]. Another reason may be associated with contemporary activity the chitin oligosaccharides shown in modulating the collagen synthesis [22, 28].
Formation of scars depends on the continued synthesis and catabolism of collagen, which has to be balanced for preventing the formation of keloids and hypertrophic scars during the wound-healing process [43, 45]. Therefore, CN acts as a template for both the synthesis of hyaluronan and glycosaminoglycan and as a carrier capable to modulate the collagen production by disposition of its fibers during the healing process [43-46].
Studies in chitin-treated lesions suggest that N-acetylglucosamine serve as a substratum for reinforcement of wounded tissues, while the histiocytes induced by chitin, are activated to produce fine collagen fibres [45-47]. It is clearly seen that chitin can stimulate the activity of fibroblasts to balance its synthesis. In turn, the production of fine collagen fibres increases in the early wound healing stage. In the subsequent healing stages, an appropriate amount of synthesized collagen is degraded by

collagenase produced from macrophages, epidermal cells, neutrophils and fibroblasts to balance its synthesis [48-50].

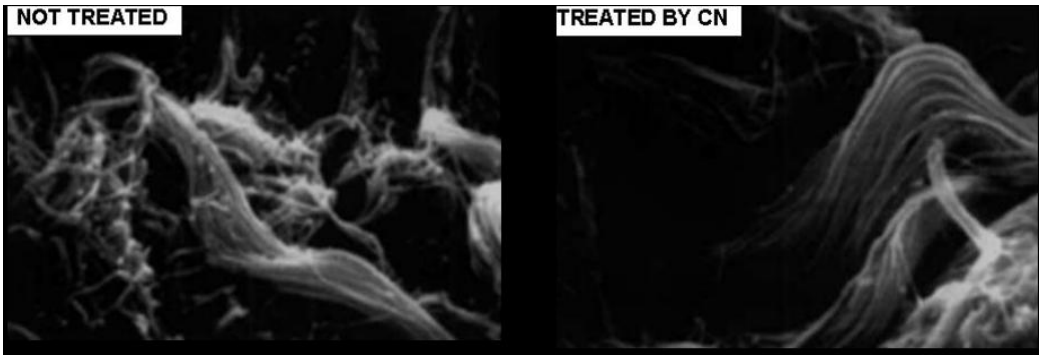


FIGURE 1.9
Chitin Nanofibrils activity to faster the skin granulation phenomena accompanied by angiogenesis and regular deposition of collagen fibers.

Wound healing, consists of a complex series of biochemical processes regulated by hormonal factors and anti-inflammatory mediators, resulting in the rebuilding of tissue and protection against infections [45-50]. Regulating factors include some biochemical substances, growth factors and immunological mediators, whose influence can be decisive, particularly during the early phase of tissue rebuilding [51, 52]. Skin cells interact with the extracellular environment via surface proteins such as integrins, defensins and fibronectin, which trigger various metabolic pathways of important roles in processing their shape, mobility, and proliferation [53, 54]. Owing to purity and polysaccharide nature, CN can constitute a cell micro-environmental stimulus, influencing the correct trophism of the skin and its appendages, and control the molecular relationship of the mesenchymal epithelium and the hair follicle cycle [52, 54]. While, adequate extracellular signalling inputs prompt a local and diffuse cellular response, their extracellular adhesion, cell proliferation and migration leads to the cell dynamic rearrangement [55]. These hyaluronan-mediated signals induced by CN are transmitted partially by activation of protein phosphorylation cascades, cytokine release and stimulation of cell cycle proteins. In this way, CN exhibits an enormously developed surface interacting with the signalling cell enzymes, platelets, and other cell compounds present in living tissue to regulate the cell life continuously. Thus, the recovered peculiarity of wound healing with CN consists in the ability for the faster formation of an adequate granulation tissue accompanied by angiogenesis and regular deposition of collagen fibers, with the consequently enhanced and correct repair of derma-epidermal lesions [52-55] (Figures 1.9 & 1.10).

FIGURE 1.10
Cicatrizing activity of Chitin Nanofibrils on skin wounds.

The main functions characterizing the activity of chitin/chitosan are: (a) chemo-attraction and activation of macrophages and neutrophils to initiate the healing process; (b) promotion of granulation tissue and riepithelization; (c) limitation of scar formation and retraction; (d) analgesic and haemostatic activities; (e) activation of immunocytes; (f) release of glucosamine, N-acetylglucosamine, and oligomers that stimulate cellular activities being used as building blocks in the synthesis of the ECM; (g) own antimicrobial activity [55-58].
Studies from our group [20-22] show the nanocrystalline form of chitin enhances all these functions with relevant biological significance, favouring extremely high cells migration, activating polymorphonuclear cells and fibroblasts, modulating cytokine release and collagenase, metallo proteinases activity and ATP synthesis.
Amongst the cumulative environmental and endogenous skin damages, a decrease of hyaluronan synthase and mitochondrial ATP production takes place together with elevation of level of proteolytic enzymes such as collagenase, elastase and other matrix metallo proteinases [59, 60]. Figure 1.11 shows that CN alone influence the hyaluronan synthase a boosting activity when complexed with vitamin E, melatonin, and B-glucan probably stimulating the HAS-2 gene expression of fibroblasts. CN also inhibit the collagenase activity (Figure 1.12), and increase the ATP production of irradiated keratinocytes (Figure 1.13).

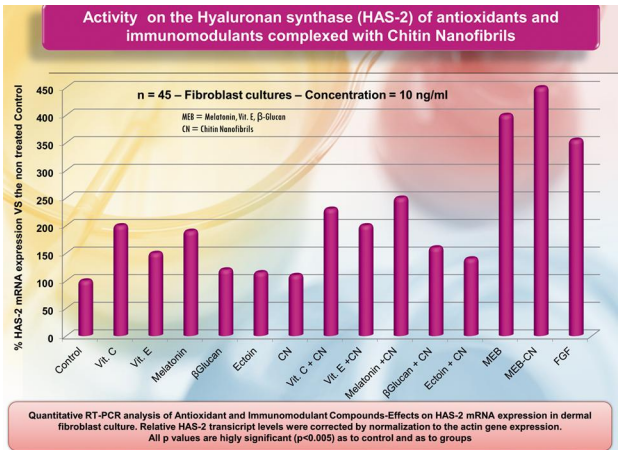


FIGURE 1.11

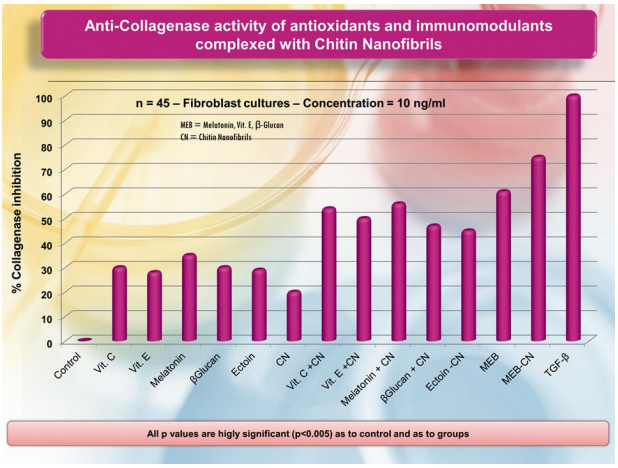


FIGURE 1.12

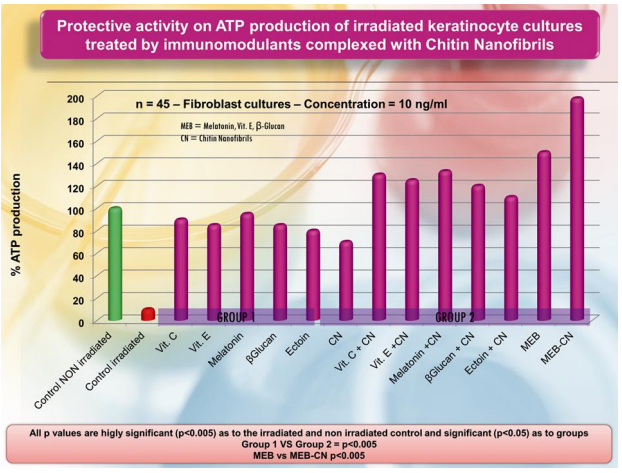


FIGURE 1.13

Biocompatibility

Biocompatibility is the ability of a biomaterial to perform desired function with respect to a medical therapy without eliciting undesirable local or systemic effects in the recipient or patient.
A further characteristic of a biocompatible material is the ability to generate the most appropriate beneficial cellular or tissue response in a specific context [61, 62]. As shown in Figures 1.14 and 15 [16], the biocompatibility of CN was verified for the cultures of keratinocytes and fibroblasts by the MTT method on culture of keratinocytes and fibroblasts.

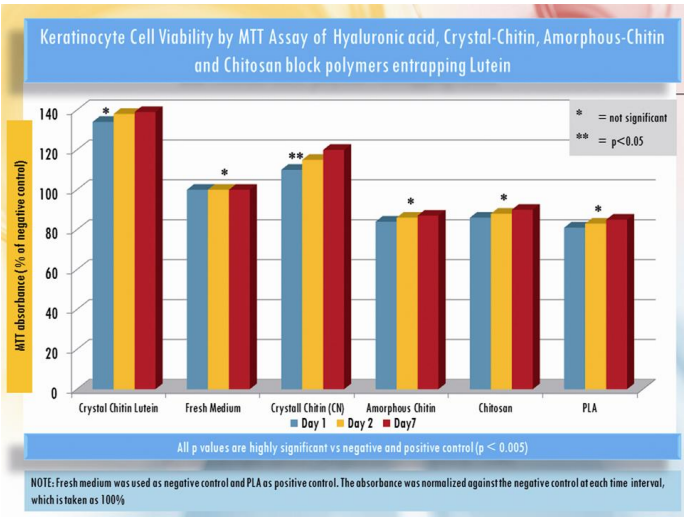


FIGURE 1.14

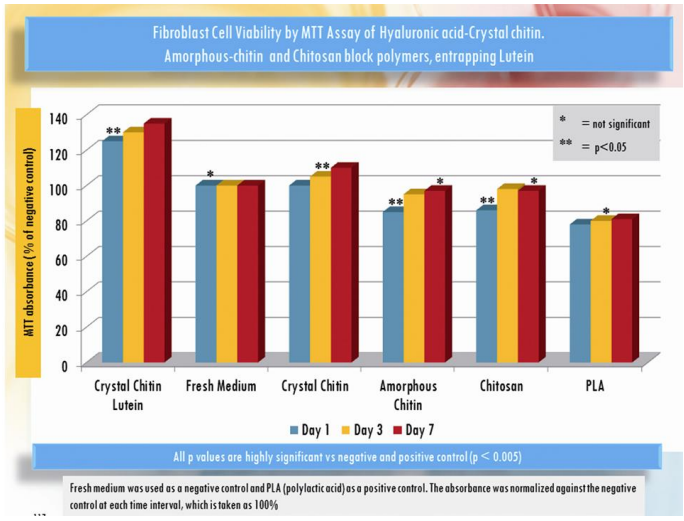


FIGURE 1.15

Block copolymeric nanoparticles

CN have a reactive surface, on which hydroxyl, amine and possibly some acetylated amine groups belonging to polysaccharide chitin chains are exposed. They are able to participate in multiple interactions via van der Waals attractions and hydrogen bonds. The functionality of the surface of CN can be changed by involving the most reactive amine groups in chemical reactions.
These modifications create specific functions, for expanding applications of this natural compound. Owing to protonation of amine groups the CN surface acquires the positive charge and simply interacts with different synthetic or electronegative polymers obtained from animal or vegetal wastes [63-65]. In

this way and without using any chemical ingredients or toxic solvents, complexes can be made from the used block copolymeries (BCC) capable to entrap or encapsulate different kind of physiologically *active* substances [66, 67]. These CN-based complexes can be easily prepared in the form of micro/nano particles.

Combining of CN and other natural lignocellulosic polymers, such as cellulose, hemicellulose and pectin, the compounds obtained are biodegradable, biocompatible, and environmentally friendly.

By forming these complexes through gelation of aqueous CN dispersions with hyaluronan as negatively charged polymer (Figure 1.16) it is possible to obtain micro/nanolamellae or globular nanoparticles with a mean size between 250 and 400 nm (Figure 1.17). They can contain the entrapped different active ingredients able to regularly release them in time, as was demonstrated for carotenoid lutein (Figure 1.18) [20, 66-68].

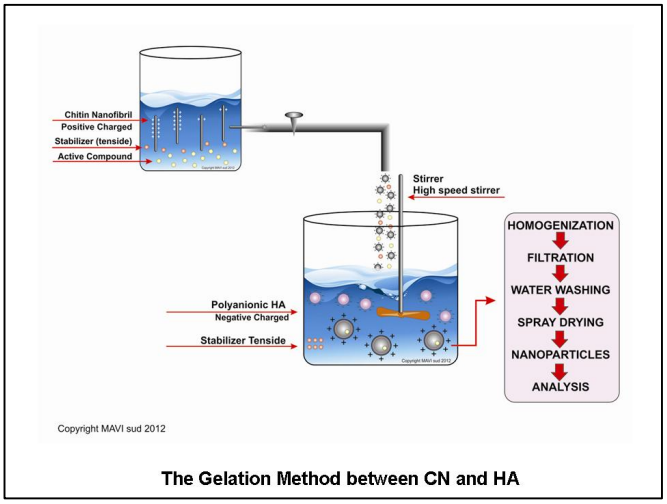


FIGURE 1.16
Chitin Nanofibrils form block-copolymeric nanoparticles with electro-negative polymers, such as Hyaluronic Acid (HA), by the gelation method.

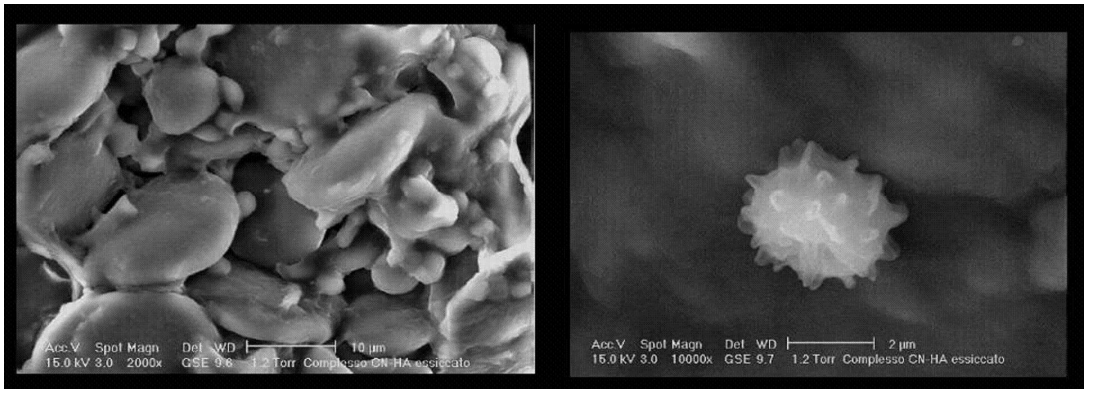


FIGURE 1.17
Nanolamellae and Nanoparticles of Chitin Nanofibrils (CN)–Hyaluronic Acid (HA) at SEM.

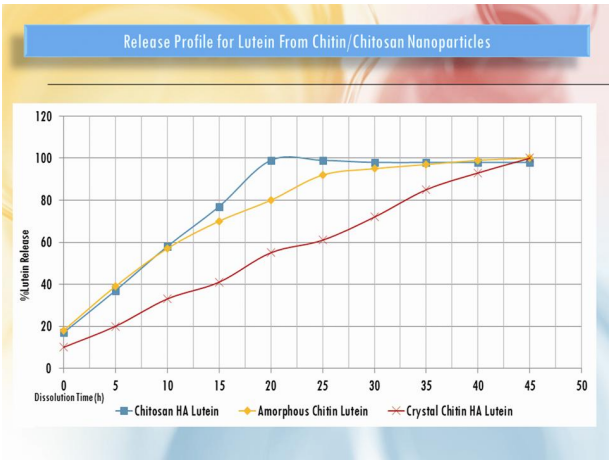


FIGURE 1.18

As the design of complexes based on polyelectrolyte polymers (PEPs), some important aspects should be taken into considerations such as their water solubility, the possibility of controlling anionic and cationic BCC assembley and reversibility of their functionality by changing pH, ionic strength, type of counterions and the solvent effects. Naturally, the quality of the BCC obtained plays an important role in their self-assembly and the ability to disintegrate in living organisms.

The driving force for the spontaneous adsorption of the negatively charged PEPs onto a positively changed surface is primarily the entropically favoured release of small counterions into solution.

The process of dipping a cationic (as CN) polymeric polyelectrolyte substrate into a suspension of anionic (e.g. hyaluronic acid) one, rinsing with water and then dipping the negatively charged complex obtained into a oppositely charged polymer (e.g. cationic) may be referred to as the layer-by-layer deposition shown schematically in Figure 1.19.

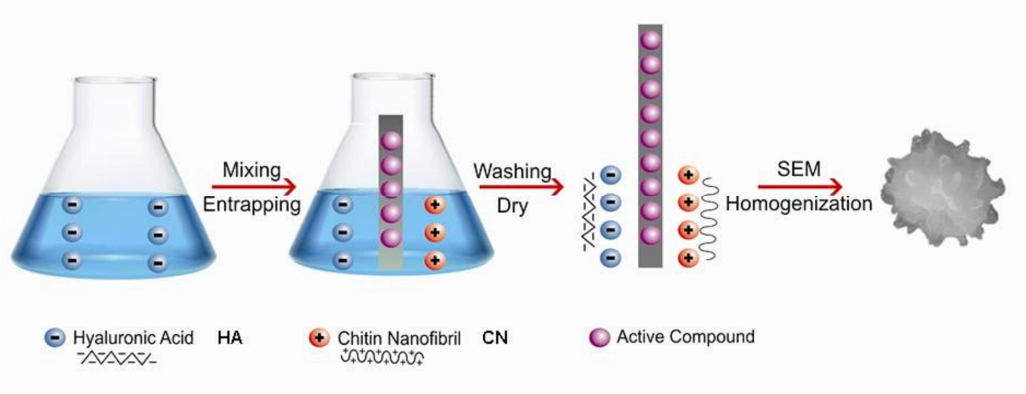


FIGURE 1.19
Method for producing CN-HA block polymeric nanoparticles.

Our studies show that the entrapment efficacy of a component, its loaded content, and the release of active ingredients depend on the crystallinity, size, and the electrical charge covering the CN surface (Table I) [14-16, 66-68]. Positive charges of nanoparticles seem to have the interesting ability to disturb the tight lamellar layers of the *stratum corneum*, enabling a better diffusion of the entrapped active compounds through the skin, (Figure 1.20), by using the stripping method *in vivo* [16].

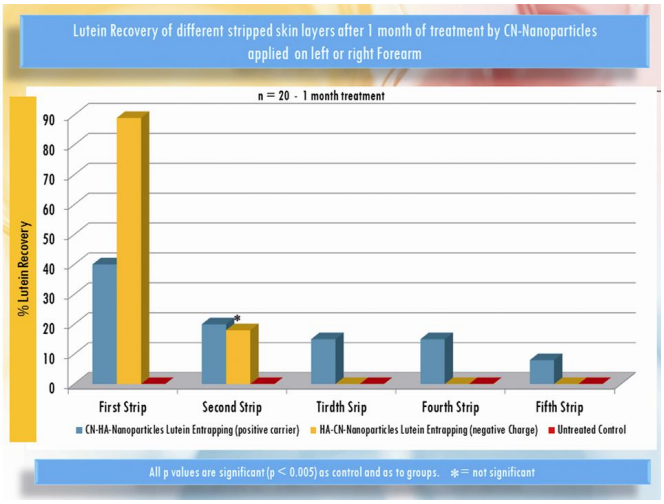


FIGURE 1.20

Naturally, the electrical charge, size, the balance of hydrophilicity/hydrophobicity of CN as *carrier* and the used *active ingredients* determined in advance by the *designed formulation,* will control the release of the *active substances* during the predicted time for obtaining the expected efficacy of the final product. On the one hand, the choice of CN as *active* nanoparticles for preparation of polyelectrolyte complexes has to be based on the reason of administration and the properties of the selected *active ingredients* (their stability, hydrophilicity/hydrophobicity and etc.) [60, 66-68]. On the other hand, the release of the *active ingredients* from these BCC–nanoparticles can occur through either outer absorption (burst release) or a continuous release, depending on the type of the polymer materials and the nature of the entrapped active ingredients [69] including the electrical charge of nanoparticles (Figure 1.21).

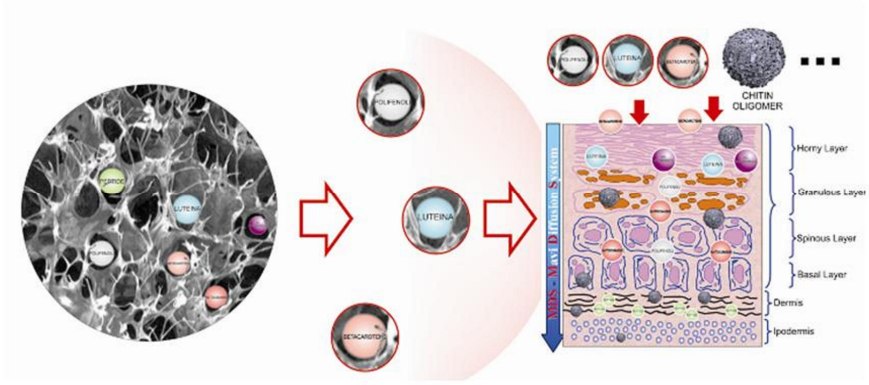


FIGURE 1.21
Skin penetrability of nanoparticles depends on their size, superficial charge and type and polymer used.

Active ingredients can be dissolved, absorbed, entrapped and/or encapsulated into/or onto the selected BCC whilst the rate limiting step of the kinetics of release could be doubled: a) diffusion of active ingredient and carrier and/or b) dissolution of the carrier itself [70].
The appropriate choice of the carrier material together with the methods adopted to encapsulate the active ingredients are the decisive factors for regulating the release of active ingredients and achieving the effective dosage.
Finally, the influence of positively charged CN on cell biology seems to be related also to the osmotic stress induced by the particular hydrophilicity of the BCC obtained e.g. from CN and hyaluronic acid or collagen [13-16]. When skin cells contact with these complexes, they initially swell up with water, and subsequently shrink back to close their previous volume due to an inflow of ions and osmolytes, which induce an outflow of water and skin hydration.
BCC with different entrapped active ingredients and obtained by the interaction of chitin nanofibrils with hyaluronic acid (CN-HA), have shown interesting activities both *in vitro* and *in vivo* when introduced into cosmetic emulsions. These emulsions have been tested *in vitro* on keratinocytes and fibroblast cultures and *in vivo* on 60 healthy women showing signs of photoaging in the multicenter randomized study [22].
The BCC with different *activate ingredients*; have accelerated the collagen formation *in vitro* (Figure 1.22), and the synthesis of chaperon HSP-47 (Figure 1.23) at the level of fibroblasts' culture. An interesting antioxidative activity, re-equilibrating its imbalance, occurs during the oxidative stress (Figure 1.24, 25) [16, 22].

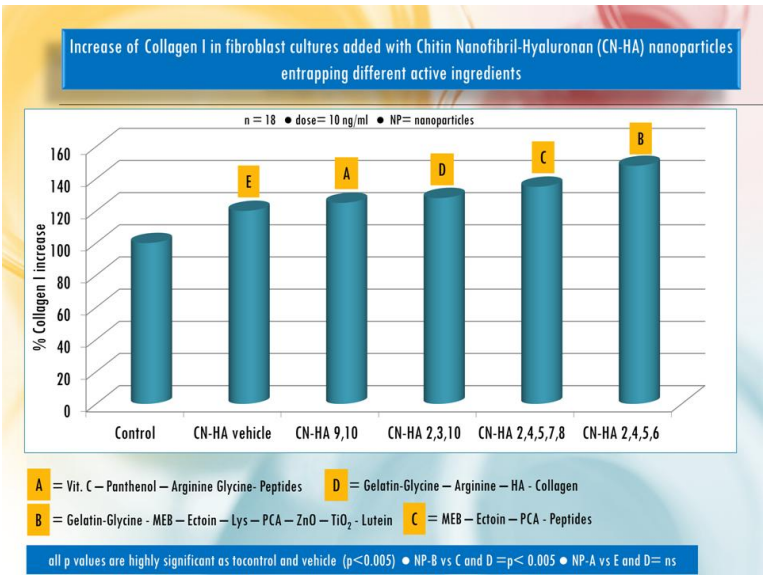


FIGURE 1.22

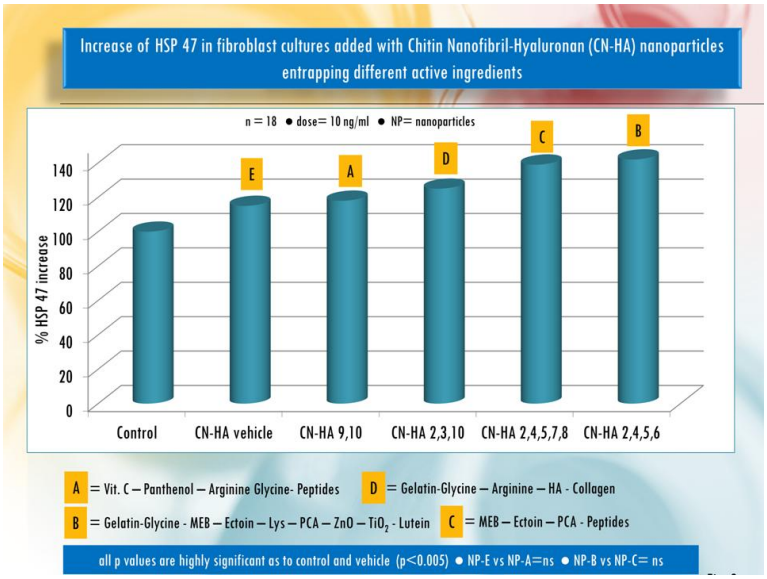


FIGURE 1.23

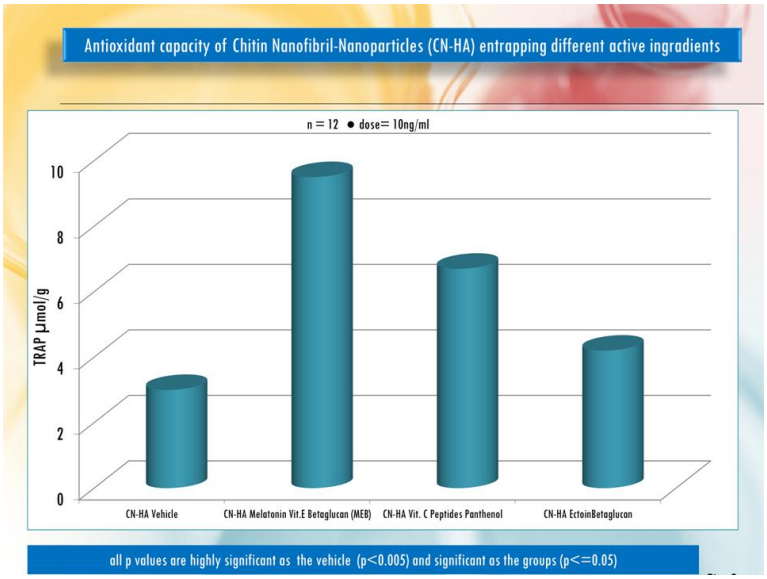


FIGURE 1.24

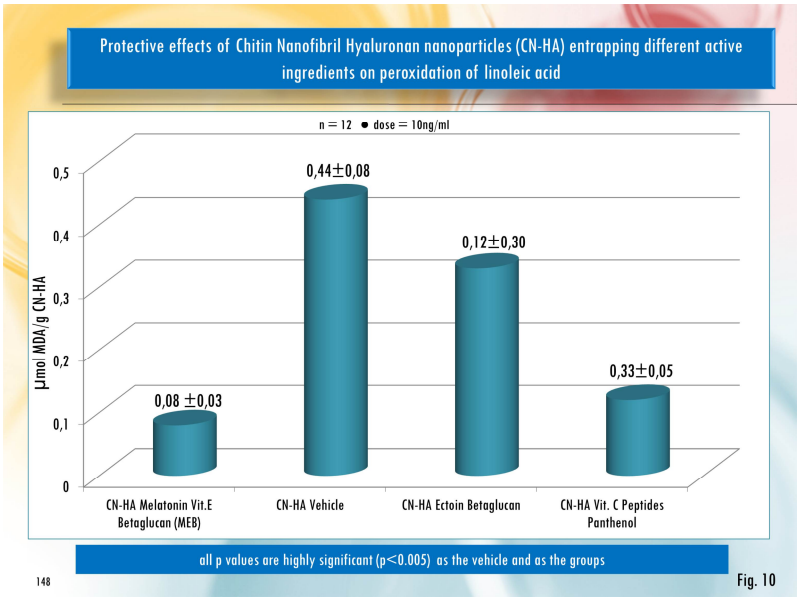


FIGURE 1.25

Furthermore, the topical *in vivo* application of the different daily-used emulsions in the multicenter randomized vehicle controlled preliminary study has shown increase in the skin hydration (Figure 1.26) with a contemporary decrease of both TEWL (Figure 1.27) and black spots (Figure 1.28) for the treated voluntary women. These results supported the previous data reported by our group [10, 20, 28, 67].

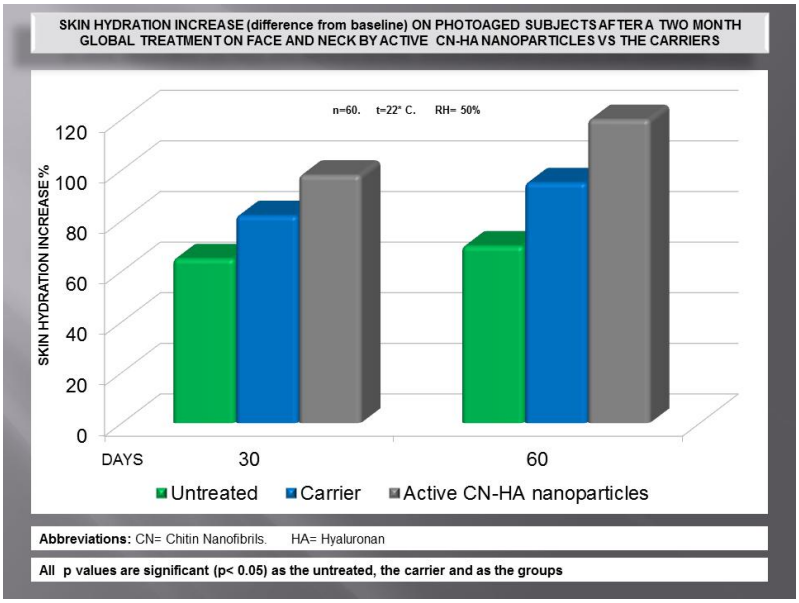


FIGURE 1.26

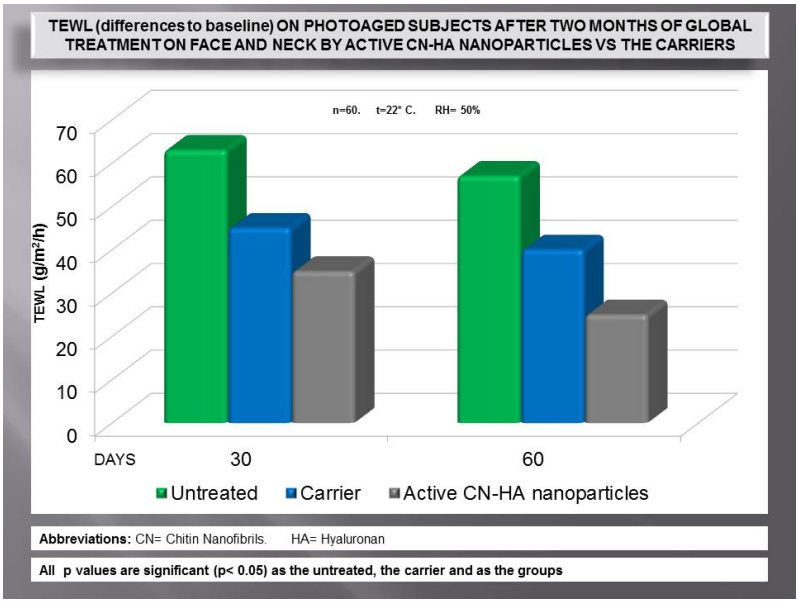


FIGURE 1.27

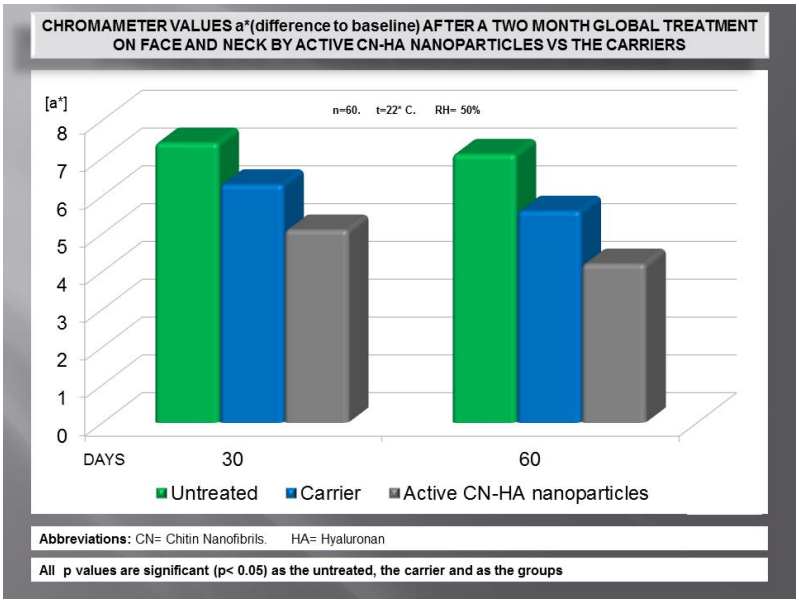


FIGURE 1.28

The exposure to either UV or other aggressive agents generate reactive oxygen or nitrogen species (ROS and RNS) resulting in premature entry of the skin into the senescent state [71]. Thus, in photoaged skin (extrinsic aging), collagen fibres become disorganized, abnormally cross-linked with elastin-containing material [72]. In genetic aging (intrinsic aging), the decline in signalling molecules

(cytokines and chemiokines) and cell receptors induce fibroblast senescence and alteration in the synthesis and maturation of both collagen and scaffold-stress proteins, as HSP-47. Hence, they play a key role in the formation of the adaptive immune system [73] and regulation of the collagen folding [74]. Moreover, BCC have stimulating activity on ATP production (Figure 1.29) and Langerhans cell density (Figure 1.30) due to UVB irradiation and on fibroblasts proliferation (Figure 1.31) [15, 75]. It is interesting to underline also that CN increase the antidandruff activity of zinc pyrithione (Table II) improving also the mechanical properties of UV-damaged hairs (Figure 1.32) [76]. This natural polysaccharide seems to possess a boosting activity in comparison with zinc ions and pyrithione by increasing their antidandruff activity. It is also able to repair the hair's cortex proteins, ameliorating its modulus and surface gloss (Figure 1.33).

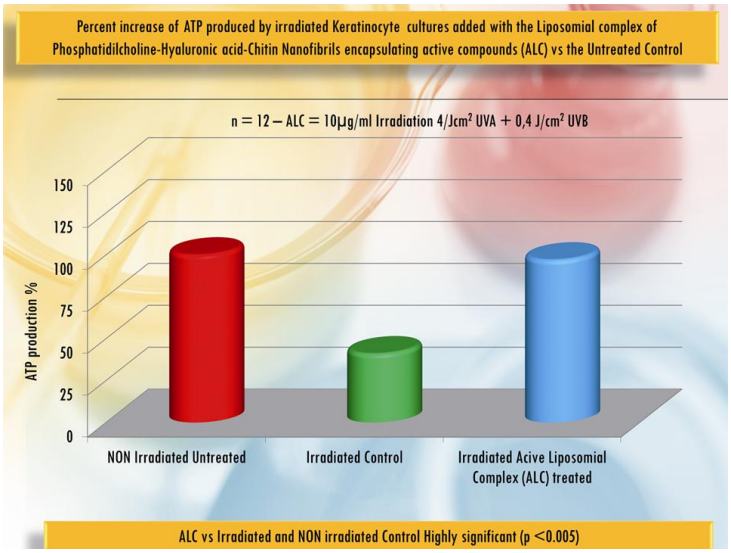


FIGURE 1.29

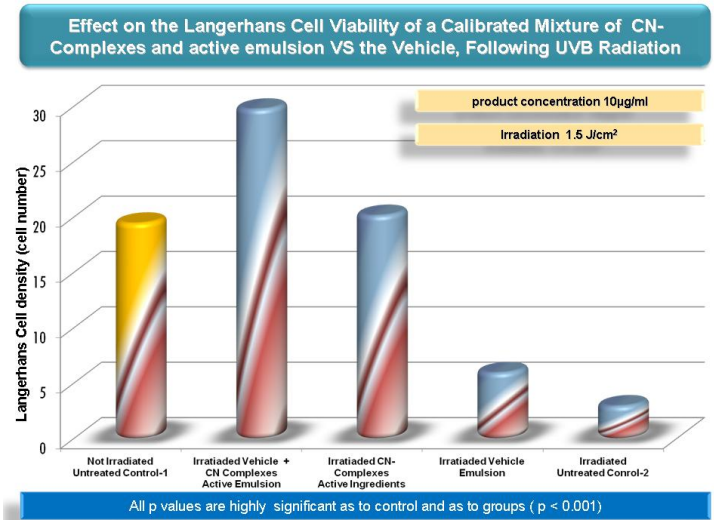


FIGURE 1.30

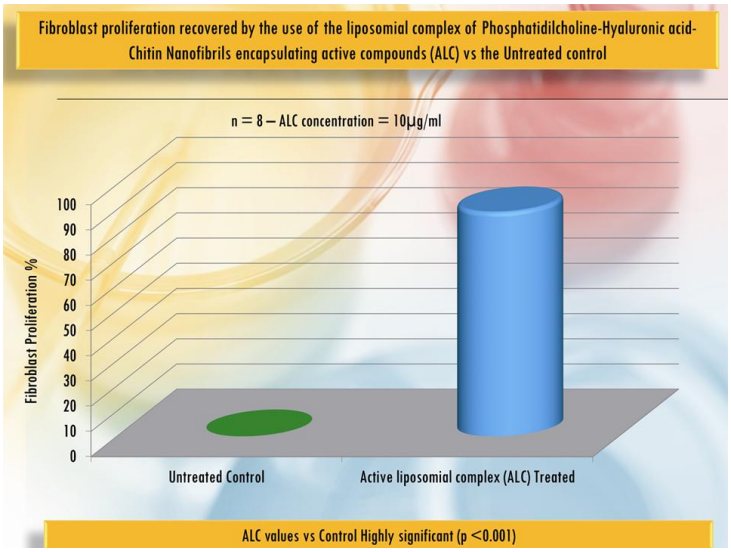


FIGURE 1.31

TABLE I

Nanoparticles yield, Lutein loading content and entrapment efficiency of different kind of chitin and chitosan complexed with hyaluronic acid.

Polymer	Nanoparticle yield (%)	Lutein loading content (%)	Entrapment efficacy (%)	Particle mean size (nm)
Chitosan -HA- Lutein	33 ±9	10 ±3	32 ±5	458 ±14
Amorphous Chitin -HA-Lutein	31 ±10	18 ±3	40 ±5	355 ±13
Crystal-Chitin HA (CN) Lutein	42 ±9	35 ±3	66 ±6	185 ±13

NOTE: all measurements were performed in triplicate
ABBREVIATIONS: CN = Chitin Nanofibrils; HA = Hyaluronic acid

TABLE II

Dandruff scales on the scalp of subjects affected by oily scalp with dandruff treated by Zn-CN Shampoo and conditioner VS traditional treatment and unaffected

RH = 50% - t = 22° C | n = 10 +20 + 20 + 20 | 60 days of treatment

	SUBJECT	0	30	60	TREATMENT
Control unaffected	10	855.384 $\pm 4 \times 10^4$	832.500 $\pm 7 \times 10^4$	861.721 $\pm 6 \times 10^4$	NO treatment
Dandruff affected	20	1,356.821 $\pm 1.56 \times 10^5$	915.743 $\pm 1.88 \times 10^4$	878.903 $\pm 3 \times 10^4$	Product B Zn Shampoo CN-ZPT Conditioner
Dandruff affected	20	1,512.300 $\pm 5 \times 10^4$	1,117.281 $\pm 5 \times 10^4$	972.333 $\pm 5 \times 10^4$	Commercial antidandruff products (shampoo+conditioner)
Oily scalp	20	955.764 $\pm 3 \times 10^4$	871.615 $\pm 5 \times 10^4$	863.487 $\pm 4 \times 10^4$	Zn-Shampoo + CN-&Cationic-LPO Conditioner

all p values are significant as to groups and as untreated ($p<0.05$)

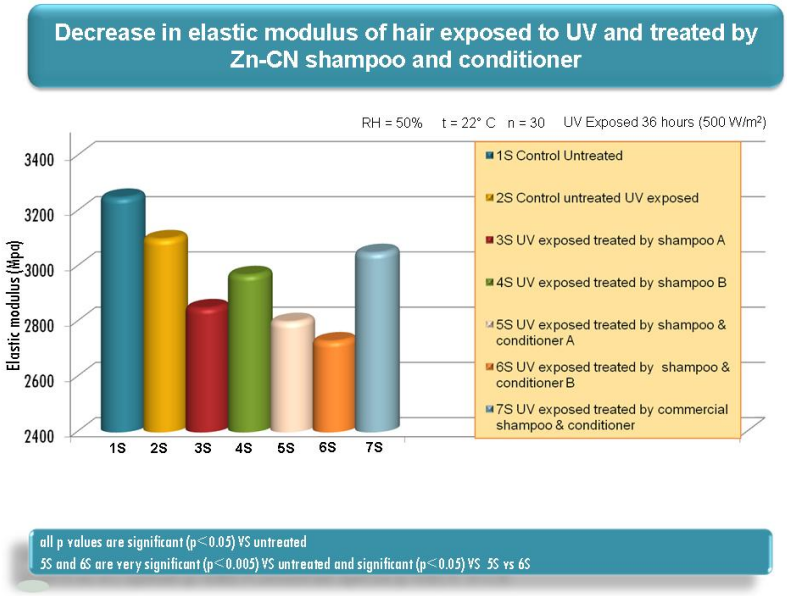


FIGURE 1.32

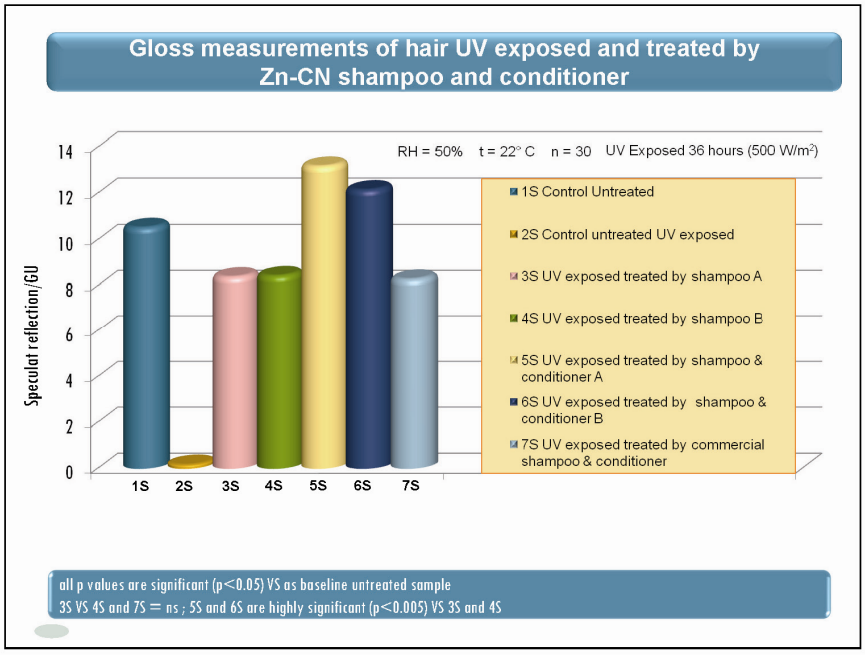


FIGURE 1.33

Non-woven tissues and films

Chitin nanofibrils are obtained as aqueous suspension and may be used for reinforcing of water-soluble polymers in preparation of the environmentally friendly biodegradable nanocomposite materials with high performance.

CN-based biomedical nanocomposites can be used for drug/gene delivery, for tissue engineering as scaffolds and cosmetic orthodontics [25, 26, 77] because they are able to support the growth of cells inducing tissue regeneration. Best results are obtained when a scaffold or non-woven tissue has a proper architecture, which is designed in such a way that the cellular response desirable for biological function of specific organs is triggered [78-80].

One of the most versatile techniques of polymer processing for this purpose is electrospinning. It allows generating micro- and nano- fibers for production of nonwoven tissues (scaffolds) [78]. During the electrospinning process, a jet of a polymer is formed from a viscous solution/suspension in the presence of the high voltage. Electrospinning seems to have high potential efficacy to produce various nonwoven fibers with high surface/volume ratio. If different active ingredients are incorporated into the fiber, the scaffolds prepared have better healing effect. The structural features of scaffolds influence on their therapeutic effect. When the structure of scaffolds made from electrospun fibers are comparable with that of native extra cellular matrix (ECM) of the skin, the cellular adhesion, proliferation, and guide cell differentiation increase (Figure 1.34) (unpublished data). It has been shown that the antimicrobial activity (Figure 1.35) [63] and enhanced effect on healing of human skin is observed if Ag-ions have been entrapped into both a CN-containing gel emulsion [43] and nanocomposite chitosan films made by casting.

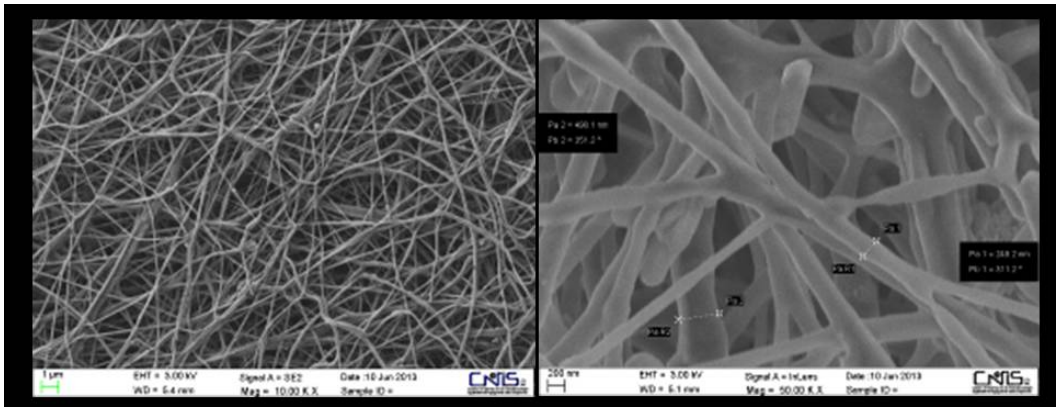


FIGURE 1.34
Non-woven tissue made by Chitin Nanofibrils at SEM.

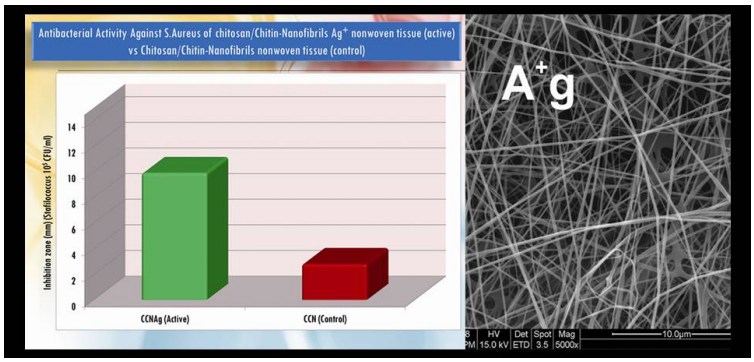


FIGURE 1.35

A polymer composite from ligninocellulosic compounds and CN having the same ECM architecture of skin may also form the multifunctional medical tissues or beauty masks with the entrapped antiseptic mineral ions (e.g. Ag+) or with other kind of *active ingredients* useful for ameliorate skin appearance. As usual, the addition of fillers in a fiber- or film-forming polymer is a standard method for improving the mechanical behaviour of a composite material. The CN-filled chitosan films (Figure 1.36a,b) exhibit enhanced tensile strength, thermal stability and water resistance [81], which increase with increasing CN content. Since CN has capability to bind ions and other polymers by electrostatic interactions they can store and deliver them for long period of time. Their antimicrobial and antifungal effectiveness for different microorganisms was found out. Investigations, which were supported by the projects BIOMIMETIC (www.biomimetic-eu-project.eu) and n-CHITOPACK (www.n-chitopack.eu), have revealed the viability of both keratinocytes and fibroblasts on the non-woven tissues and nanocomposte films (unpublished data). Both non-woven tissues and films made by electrospinning (Figure 1.37) [82] or casting [83], respectively, are in progress to use CN with entrapped different ions and active ingredients as storage matrix in skin care.

For medical applications, various composite fibers, nonwoven tissues, films or gels can be prepared on the basis of homogeneous aqueous CN suspension and cellulosic polymers or chitosan by using environmentally friendly processes. A huge diversity of different nanocarriers able to penetrate in certain human tissues owing to their physicochemical properties can be also developed be means of different technical methodologies.

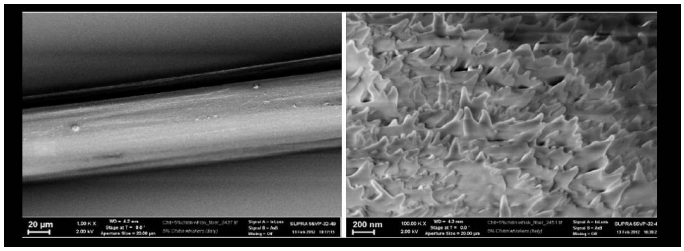


FIGURE 1.36a
The smooth surface of chitosan/CN composite fibers (a) shows a regular disposition of CN into its inner structure (b). The fiber contains 1 wt.% of CN.

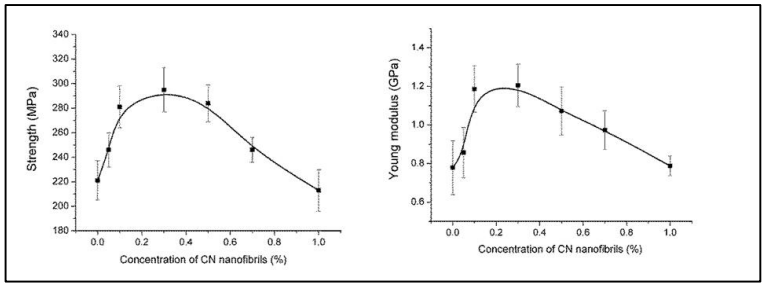


FIGURE 1.36b
Dependences of tensile strenght (a) and Young Modulus (b) of the chitosan/CN composite fibers on the content of the chitin nanofibrils.

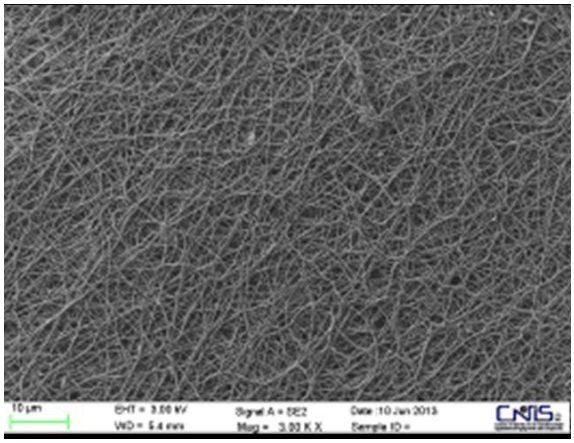


FIGURE 1.37
Non woven Tissue obtained by electrospinning at SEM.

Conclusions

Engineered nanomaterials constitute a large number of classes and subclasses of diverse materials having features in common: one, two or three of their dimensions are within the interval of 1-100 nm [84]. If only one dimension equals or less than 100 nm, everyone deals with nanoflakes. Materials with fibrous as CN or tubular structures or those having cubic shape are characterized with two or three nanodimensions, respectively.

CN providing numerous advantages including their easy availability, non-toxicity, renewability, biodegradability, good biocompatibility, reproducibility, and easy chemical and mechanical modification [16, 23, 26, 81] are much better choice for using as nanofillers than the traditional inorganic ones in fabrication of the various so called *green composites* [1-8, 63-65], i.e. environmentally friendly biopolymers such as e.g. CN and different ligninocellulosic compounds [1-8, 63-65].

According to innovative BioEconomy, green compounds have advantages not only from the ecological but also from the economical point of view [85]. This is the reason why in its 2020 strategy the European Union highlights nanotechnology as one of the fundamental basis of BioEconomy and sustainable technology, capable of providing prosperity and social stability to its citizens [85, 86]. Being multifunctional compounds, CN act, first of all, as reinforcing agents giving to polymeric nanocomposites some additional properties.

If CN are incorporated to biopolymers, the formed innovative biomaterials (templates) ensure the successful tissue development in the process of skin regeneration owing high cyto-compatibility of CN with human cells. Nanocomposite films for food packaging prepared from CN-filled chitosan slurries are safety for humans because of antimicrobial activity and non-toxicity of both CN and chitosan. The same effect can be expected for CN-based biotexiles [87] for production of both sportswear and various hygienic and medical biomaterials. The latter are highly advantageous for patients suffering from dermatitis or psoriasis because the risk of secondary infections is remarkable reduced.

Being produced from low cost raw chitin by using the environmentally friendly process of CN fabrication, the ultrafine nanofibril-based porous membranes prepared by electrospinning may surpass conventional membranes in-water purification owing to the impressive high flux efficiency.

A new class of thin CN-based films and membranes with barrier permeability for gases can be manufactured by using casting [64] owing to excellent film-forming properties of chitosan. The chains are bound with each other and CN through multiple hydrogen bonds and hydrophobic interactions. The porous CN-based polymeric membranes can be prepared by electrospinning.

Drug delivery with CN is highly effective since the positively charged CN surface, due to protonation of glucosamine groups is able to attract and complex many negatively charged polymers. The formed nano-lamellae or nanoparticles entrap water- or lipo-soluble active ingredients, which are used for pharmaceutical and cosmetic purposes. Moreover, due to their biological and safe characteristics, CN may be used for the production of innovative and advanced medications using both the Electrospinning and the Casting technology.

As previously reported, the physicochemical properties of the cosmetic nanoparticles, tissue-nanofibers, and/or casting-films made of CN and other natural polymers, may be predicted and designed at the molecular level, whilst their real shape, size, and electrical charges can be controlled and optimized for each specific application.

There is also a tendency to predict the physicochemical properties of the CN-containing cosmetic nanoparticles, nanofibers for tissue engineering and films from chitosan and other natural polymers with the aim of designing them at the molecular level and comparing the obtained theoretical calculations with experimentally determined characteristics (shape, size, electrical charge and etc.) of the prepared CN complexes.

Interestingly, chitin being a fishery waste, is the second most abundant polysaccharide existing in nature as component of the invertebrates' exoskeleton, proceeded only by cellulose obtained from the vegetable biomass. As a consequence, polymers obtained from these waste materials using green processes, will reduce the worldwide pollution, ameliorating the quality of our life.
Many interesting possibilities exist in different fields of nanotechnology, especially when raw materials used are classified as natural or possibly obtained from the waste and by-products such as CN. It is important to underline the possibility of using the same raw material for producing, for example, innovative cosmetics, advanced medications, dedicated textiles, and also in air and water filtration, or drug delivery, to reduce pollution and transform waste materials into goods.
In conclusion, it is imperative requirement to develop new strategies for designing effective and possibly low cost biomaterial for practical usage [78, 80, 88] in order to enter into a real *green era*.

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