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Nanochitin and Nanolignin: Activity and Effectiveness

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Abstract

Worldwide consumers are considering the need to change their daily habits for having a more positive effect on the environment, boosting both health and economy. Thus they are seeking for natural-based, biodegradable, skin- and environmentally-friendly products, producing zero waste and being able to maintain the declared efficacy. Consumers became aware of waste and specifically of plastics by-products that, when discarded, are no more sustainable for land and the oceans 'equilibrium. Microplastics, in fact, consumed as food from fishes, marine birds and mammals are entering into human food together with their content of toxic compounds. Thus the necessity to produce and use biodegradable goods and tissues made by natural, sugar-like polymers, such as chitin and lignin. These polymers, obtainable from fishery's and agro-forestry biomass respectively, are biodegradable, nontoxic, having a low cost also. Thus on the one the physicochemical characteristics and the relative market of both chitin and lignin have been reported, while on the other hand the method to produce some of their complexes at the nanosize has been described. Chitin nanofibrils, in fact, covered by electropositive charges, are easily complexed with the nano lignin' nano-fibers negatively charged, to obtain micro/nanoparticles which may encapsulate various active ingredients, such as nicotinamide, glycyrretyc acid, nanosilver, etc.

Different obtained nanoparticles, therefore, have been bound to natural biodegradable fibers for characterizing and making non-woven tissues to be used in the medical and cosmetic field, as innovative products.

Keywords: Chitin Nanofibrils, Lignins, Polysaccharides, Non-Woven Tissues, Films, Waste, Plastics,

Introduction

In recent years consumers are increasingly seeking for drugs, food and cosmetic products characterized for their effectiveness and safeness because made by biodegradable and natural Ingredients [1]. Thus, the holistic approach is becoming a kev motivator behavior that, with a cleaner environment and a reduced production and consumption of food, goods and waste converges with convenience, wellbeing and longevity [2]. As a consequence, consumers spending is focused on public health and sanitary services rather than on private ones, searching for a better organization of the public hospitals, necessary to stop the worldwide COVID-19 diffusion. Moreover, on the one hand they are looking for a reduced business traveling with a contemporary increase of video conferencing. On the other hand they are seeking for an innovation that, driving future political, social, scientific and economic ethics, could ameliorate the human interactions.

Consequently to this holistic approach, it is necessary to change our way of producing and consuming, reducing also the great loss of food and the packaging waste, made prevalently by plastic containers. Thus the necessity to find new natural raw materials possibly obtained from the actual food waste, contemporary reducing production and consumption of synthetic polymers and plastic packaging, the majority of which is discarded into landfills and oceans [3].

At this purpose, i Food Agriculture Organization (FAO) estimated that 20-30% of fruits and vegetables have been lost or discarded as waste during the past harvest, handling for an amount of 1.3 billion tonnes per year (i.e.one third of food produced for human consumption) [4].On the other hand, the annual global production of plastics, exploded from 1.5 million metric tons in 1950 to nearly 350 million metric tons in 2017, is expected to tripled in 2050, accounting for a fifth of the global consumption. Unfortunately, 40% of this nonbiodegradable plastics are actually disposed to landfills as waste with a global Greenhouse gas (GHG) emission of 2.8 billion tones CO2 (fig 1) [3]. Moreover, this polluted toxic material as a pervasive and increasing threat of the worldwide marine ecosystems, results dangerous for sea mammals, entering as microplastics in their food chain [5-7].

Thus, development and production of bio-based polymers result essential to slow down the increasing global environmental pollution, directly linked to the production of synthetic non-bio-degradable polymers and plastics. For this reason ,natural polymers such as polysaccharides are attracting considerable interest in biomedicine, also due to their biocompatibility, non-toxicity, biodegradability, renewability, and mild processing conditions. Therefore, these bioactive polymers, obtainable from animals and plant biomass, are regarding as economic and environmental favorable resource of raw materials [8]. Additionally, their particular structure can be modified and improved to reach the designed objective, creating for example, biodegradable micro/nanocomposite scaffolds and biological membranes that can match the bio-mechanical properties of the skin tissue, mimicking the natural extracellular matrix(ECM) [9].

ECM, in fact, is a three-dimensional network of extra-cellular macromolecules, which provides structural and biochemical support to its surrounding cells [9].

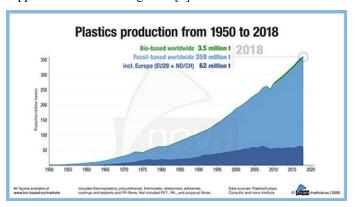


Figure 1 Global plastic production from 1950 to 2017(by courtesy of Eu-ropean Bioplastic-Neova Institute)

Naturally, the obtained scaffolds have to elicit specific cellular skin responses (i.e. cell adhesion, proliferation and differentiation), while the polymers structure should exhibit a controllable breakdown into non- toxic degradable compounds.

It is to remember that these scaffolds, made as smart tissues by synthetic and/or natural polymeric nano-composites, serve as platform to guide the development of a new functional regenerated skin, necessary to repair, for example, wounds, burns or other diseases.

At this purpose, biomaterials, such as the polysaccharides, play a pivotal role to provide three-dimensional templates and extracellular matrices for tissue regeneration, due to their capacity to emulate or interact with natural ECM and the biological membranes, as previously reported [9-11].

Among the many natural polymers, chitin and lignin are considered the more interesting biomolecules for regenerative medicine because nontoxic, biodegradable, bio- and ecocompatible, first of all in their micro/nano dimension. Moreover the decreased size and width of their nanofibers, compared to microfibers, add unique optical, mechanical and electrical characteristics for the development of innovative medical and cosmetic applications.

Finally their more intensive use could contribute to safeguard the conservation of natural raw materials, being easily obtainable from the fishery's and plant biomass respectively, which represent around 300 billion/year of underutilized industrial and agroforestry by-products [12].

Chitin Nanofibrils

Chitin nanofibril (CN),known also as whisker or nanochitin, is the second polymer component recovered in nature after cellulose, characterized for its interesting biocompatibility, biodegradability, and nontoxic properties [12]. While cellulose is a structural component in plant, and in the cell wall of some fungi and algae, chitin is largely found as structural backbone in exoskeleton of insects, mollusks and crustaceans. However, both the polymers are of support for the cell structure, being almost similar polysaccharide compounds: cellulose contains a hydroxyl group, whereas chitin contains an acetamide group (fig 2).

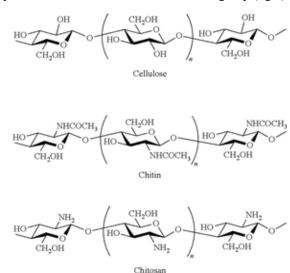


Figure 2 Cellulose Versus Chitin And Chitosan

CN is composed by a hierarchical, twisted and plywood structure of molecules assembled under the form of ordered crystal-line micronano fibrils. This polymer, having a high mechanical strength and biological qualities results a suitable candidate as pharmaceutical, food and cosmetic carrier and as reinforcement agent in polymer nano-composites (fig 3) [13-15].

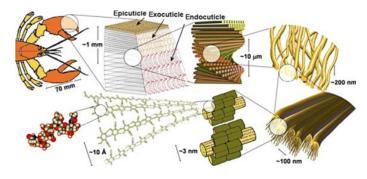


Figure 3 The Twisted Play Wood Structure of Chitin(by Courtesy of Raabe 2005)

As sugar-like polymer linked by glycosidic bonds, chitin is a key and abundant component in several biological structural materials, so that every year around 100 billion tons of chitin is produced on the earth by crustaceans and other related organisms [16]. Chemically CN is composed of N-acetyl-2-amido-2-deoxygenated-D-glucose units linked by beta(1-4)bond, but when produced industrially it possesses D-glucosamine groups also, just as chitosan but in a lower quantity. Thus, CN and its deacetylated compound chitosan have a different degree of N-acetylation and polymerization which, depending to the adopted method of production, result important parameters to dictate their use: CN, produced industrially, has a degree of deacetylation (DD)from 50 to 60% while the DD of Chitosan is generally from 73 to 83%.

Additionally, by the enzymatic or acidic hydrolysis of these polymers, it is possible to obtain further degraded compounds, known as chito oligosaccharides (COS) [17-19].

Differently from chitin and chitosan these compounds, completely water soluble, are prevalently used as elicitor, activating many defense mechanisms of the plant response to pathogens invasion, specifically binding them in the cell wall by chitin and glucan. Moreover, they promote the plant growth improving the photosynthetic efficiency and modulating the antioxidant enzymatic activity [17-19].

However, although CN and chitosan have a similar structure, their chemical reactions are distinct because of the different grade of solubility. Both the polymers possess reactive hydroxyl (-OH) and amino g r o u p s (-N H 2), but chitosan is less crystalline t h a n chitin. Moreover, chitin nanofibers are linked each to other by a number of hydrogen bonds including the strong -CO and -NH bonds that maintain their molecular chains at a distance of around 0.48 nm along the axis of the unit cell [20]. Moreover CN, consisting of molecules oriented in parallel manners, has amorphous and crystallin domains easily separated by physicochemical methods [21]. Each CN crystal, obtained industrially from a food grade chitin by a patented technology, is represented by a polymer composed of about 20 linear molecular chains of N-Acetyl glucosamine and glucosamine with structure needle-like and a mean dimension of 240X7x5nm (fig4).

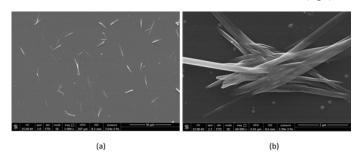


Figure 4 Needle Like Structure of Chitin Nanofibrils By SEM(-by courtesy of Coltelli et al [72]

The CN nanocrystals, obtained as a 2% water acidic suspension containing around 300 trillion/ml of the polymer, are characterized by a surface positively charged. Thus, its protonated state provides the electrostatic repulsion which stabilizes the nano-

crystals' suspension [20, 21], differently to cellulose the crystallites which tend to be aggregated into larger bundles. Therefore the nonbonding pair of electrons of the CN' primary amino group units, performing the role of protons' acceptor (i.e.positively charged), react easily with negatively charged polymers, such as hyaluronic acid and nanolignin. About the permeation enhancement of CN, applied on the skin surface, it seems associated with the opening of the epithelial tight junctions due to the electrical interaction between the positive charges of CN and the negative charges of the cell membranes (unpublished data). However the block polymeric complexes, based on the use of chitin nanofibrils, may be defined as bio-macromolecules which, able to fulfill different structural or biological roles in living organisms, could be also used to produce biological membrane. For this reason the biomimetic approach is gaining a great interest together with a constantly growing of research studies, based on marine-derived or marineinspired materials, such as chitin.

Therefore, for its particular reactivity CN and its block polymeric complexes with nano-lignin may be used to design micro/nano particles (NPs) or polymeric scaffolds encapsulating different active ingredients. These smart structures, for their specific dimensions, cargo-release activity, and the healthy and skin-friendly characteristics, may be embedded into gel/emulsions or linked to natural fibers, used not produce ter innovative biodegradable films or non-woven tissues (fig.5) [22, 23]. Moreover, NPs may be also used to stabilize Pickering emulsions for their ability to establish fibrillar networks in the O/W or W/O continuous phase [24].

However, these smart polyglucoside complexes, may be employed to make specific designed nano-carriers or biological membranes in which sugar-like polymers are the key components as well as may be used for micro encapsulation of active ingredients to be delivered through the skin layers [24]. At this purpose, it is to remember the various biological functions these innovative structures may have.

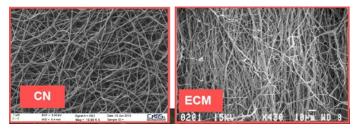


Figure 5. Structure At Sem of A Non-Woven Tissue Made By Cn (Left) Compared As Scaffold To The Native ECM(right)

They, in fact, may play crucial roles in cellular protection as well as may be used for the control and transport of nutrients which, constituting an interface between cells and their surroundings, could favor molecular recognition and cellular adhesion and growth [25]. At this purpose CN alone and/or associated with other biopolymers has been used from our research group to produce innovative beauty masks for aged skin or wound dressings [26]. It has been shown that these innovative biodegradable non-woven tissues and films provide an improved healing effectiveness on burns of first and second grade, slowing down the

inflammatory cascade and stimulating the production of defensine-2 [27-31]. This antimicrobial peptide, in fact, plays an important role in cutaneous immune defense, contributing to repair the impaired skin barrier [29]. Consequently, the skin repairing process has been faster, while the scars appeared smoother in comparison with a normal medication used as control. A more enhanced vascularization and a probably continuous supply of chitooligomers to the wound were probably the key factors in the effective physiological rebuilding of the skin, treated in vivo by the CN-tissue [32, 33]. It seems, in fact, that N-acetylglucosamine and glucosamine could serve as a substratum for reinforcement of wounded tissues by a stimulated production of proteoglycans and fine collagen fibers in absence of excessive inflammatory reaction [32, 33]. Chito-oligomers, in fact, could stimulate a correct deposition, assembly and orientation of the collagen fibrils, incorporated into the native ECM (fig.6) [34].

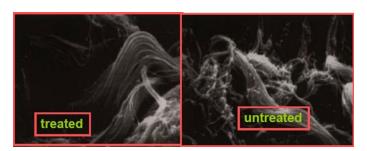


Figure 6 Assembly and Orientation of Collagen Fibrils of a Skin Tissue Treated (left)and Not Treated (right) by Polysaccharide Compounds(by courtesy of Tucci et all [34]

Moreover,by the results obtained from different research studies in vitro and in vivo, it seems that chitosan, CN and their complexes with nanolignin may provide antimicrobial activity and filmogenicity with skin adherence, when used as gel or tissue [27, 28, 30, 31]. On the other hand, both CN and CN-nanolignin complexes(CN-LG), linked to polysaccharidic fibers of a non-woven tissue/films, have shown a restructuring effectiveness, releasing N-acetyl glucosamine and glucosamine which recognize proteins and grow factors. Naturally, the different active ingredients, encapsulated into CN complexes and linked to non-woven tissues, may be used to make innovative beauty masks with an antioxidant, moisturizing and anti-aging effectiveness or advanced medications with an anti-inflammatory and repairing activity [30, 31, 35, 36].

In addition all the ingredients, encapsulated into CN-LG and embedded into or linked to non-woven matrices, are protected from the oxidative processes generally enhanced from the light, thus resulting more active and effective. Moreover, for its filmogenicity, CN and its derived compounds and complexes have the ability to form an elastic film on the surface, for example of hair, imparting softness and physical strength to its fibers [37], as well as may be also used in tooth pastes, mouth washes and chewing gums for their anti-microbial activity (Data not reported). Finally this natural polymer may be used not only as reinforcing filler for chitosan and poly lactic acid (PLA) sheet to produce environmentally friendly and biodegradable nano-composite materials

for drug delivery or nano engineered tissue-scaffolds, but also to make soft or hard biodegradable packaging [28, 31, 38-44].

Chitin Market

In 2015 the global availability of annual chitin and derived compounds was estimated to be over 10 million tons, 1.5 billion of which produced in Asia-pacific [45].

According to FAO, fisheries resources have exceeded 160 million tons in recent years, while around 750,000 tons of crustaceans' shell waste is generated annually in Europe, around 40% of which is represented by shells with comprise 30-40% of protein,30-50% calcium carbonate, and 30% of chitin (fig 7) [45, 46].

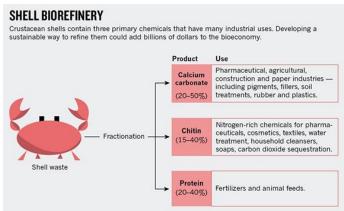


Figure 7 Chemicals and Polymers Content of Crustacean' Shell of Indus-Trial Use(by Courtesy of Ning and Chen, 2015)

On the one hand worldwide demand for chitin in 2015 was above 60,000 tons, while the worldwide production of chitin and derivatives in the same year was 28,000 tons with an estimated market value previsional of 63 billion tons by 2024 [47]. Due to the COVID-19 impact, the research studies on chitin and its derived compounds of the various companies involved, report different provisional market data. According to these data their global market should reach 4.2 billion tons by 2021 from 2 billion tons in 2016, with a compound annual growth rate(CAGR) of 15.4%. The economic value with a prevision- al growth rate of US\$ 35 million in 2014 and US\$43 million in 2019 could have a further expansion to US\$ 56/63 million and more by 2024 with CAGR between 15 to 20% [47-50]. It is to remember, in fact, that chitin & chitosan derivatives, being naturally occurring polymers with a wide range of applications, are used in major industries, such as food &beverages, agrochemicals, personal care, biomedicine, bioprinting, water cleaning, and others. Moreover, more intensive R&D activities are also projected to augment the overall demand of chitin in the foreseeable years, especially focused on the discovery of novel applications, such as biodegradable surgical masks and dressings, along with development of the existing and new discovered technologies [47-50].

However, chitin has a future huge potential market which include food grade and industrial grade. Thus the necessity to increase the industrial production of this polymer at high purity and good performance, further improving the market of its micro/nano size. The high purity chitin, in fact, will be useful for the biomedical and cosmetic field, projected to constitute more than 18.5% of the market size in the next years for manufacturing innovative anti-aging cream-tissues.

Lignin

Lignin inter-wined with hemicellulose and cross-linked with cellulose microfibrils through covalent and non-covalent bonding (fig 8), is the most frequently used natural polymer which provides the mechanical backbone and strong structural protection for the high plant vascular cell wall [51, 52]. It is one of the most abundant and highly renewable natural resource on Earth obtainable from plant and readily available in bulk quantity and at low cost. The polymer, in fact, is produced worldwide in the Annual quantity of 100 million tons as by

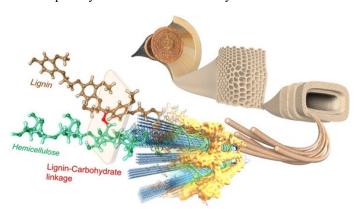


Figure8: Crosslinking between Lignin, Hemicellulose and Cellulose at Cell Plant (by courtesy of Nishimura et al [52].

product of lignocellulosic biomass, obtained from the paper industry for around 50%.

Lignin, constituting from 20% to 30% of the Earth Plant biomass, is a polymer formed by the polymerization of 3 different phenyl propanoid units: conipheryl, pcoumaryl coumaryl- and sinapsyl alcohol linked together by different linkages to form a macromolecule, organized by a fibrillar structure (fig 9) [52, 53]. This structure is composed by a great number of monomers named phenylpropanols, molecules having 6 carbon atoms organized as a ring and rich of side chains bound by hydrogen bonds. These fibrillar molecules, protect plants from collapsing and grow straight, determining the physicochemical and biological feature of the lignocellulosic biomass. The well-ordered fibrils, electro negatively charged and organized in a similar way of chitin nanofibrils, show a strong tendency to self-associate in water also for the dominant role played from their hydroxyl groups, as previously reported.

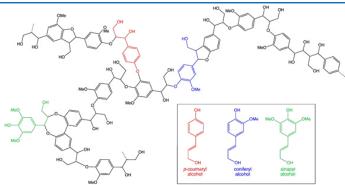


Figure 9 Pholiphenolic Structure of Lignin Made by the Polymerization of 3 Propanoid Units (coumaryl-,coniferyl- and sinapyl alcohol)

Lignin is composed of modules containing about 20 monomers, further polymerized and cross-linked into super modules containing about 500 monomers [53]. Due to the H- and -C-C bonds cross linking and branching the primary monomers, the final composition of the polymer depends on the raw material' source, such as softwood, hardwood or crop-based, which affects its macromolecular structure and organization, as well as its degradability. Thus, the hydroxyls content of lignin affects its ability of cross-linking which varies with the extraction process and the lignocellulosic source, also because branching and bonds are higher in softwood lignin than in hardwood ones. At this purpose, it is important to remember that hydrogen bondings play an important role in the thermal and mechanical properties of polymers. This is probably the reason why the ability of the lignin' carboxylic groups to form intermolecular hydrogen bonds seems to affect its mechanical properties [54].

However, due to its phenol and quinone groups, the polymer Possesses antioxidant and UV barrier properties that may be used as natural sun blocker for broad-spectrum sunscreens in partial substitution of the chemical molecules used [55-57]. Also for this reason the growing demand for anti-aging, moisturizing, anti-oxidant, UV-screening, and whitening products coupled with population aging is driving both cosmetics and lignin market, with a worldwide production by 2015 of 100 million tons per year and a value of US\$ 732,7 million, Unfortunately,98% of lignin is burned and 2% only is used for producing goods [51]. However, for its increasing use in dispersants, adhesives, removal of heavy metals from waste water, cosmetics, food and other bio-products, it is expected over the coming years a higher growth of this natural material ranging a value of US\$ 913.1 to 2025 with a CAGR of ~2.2% from 2016 [51]. Thus, the development and use of other value-added compounds and goods based on lignin, will represent a sustainable alternative to petrol-derived products, contributing to reduce waste and the greenhouse gas(GHG)emissions also [51, 58].

Lignin Market

Actually about 85% of lignin is produced worldwide by the sulphate cooking process (KRAFT) in the high quantity, as reported previously. Recently, plant biomass has been highlighted as an alternative to petrol source for fossil fuels and other useful chemical compounds. Consequently it is expected that lignin production from biomass will increase rapidly, also for its

conversion in high-value aromatic compounds, such as vanillin widely used in the food and cosmetic industry [59].

The global lignin market size has been estimated differently from various research companies. Thus Gran View reported an expected size at US\$ 954.5 million in 2019 ranging US\$ 1.12 billion by 2027 with an expansion at a CAGR of 2.0% in term of revenue from 2020 to 2027 due to the increasing demand in animal feed, paper and natural products [60]. According to this research study the demand for lignosulphonates accounted for the largest market share of 85.5% and is expected to expand at a CAGR of 1.6% over the forecast period. Major pulp and paper manufacturers will support the lignin market growth with an increasing demand of macromolecules and aromatics that positively will influence the future market with a revenue share of more than 5.5%. About the Regions, EU has been the largest market in 2019 for the consumption of this polymer with a revenue share of 39.5% anticipated to ascend at a CAGR of 3.6% over the estimated period. Asia Pacific is expected to register the fastest CAGR of 2.9% until 2027 with a revenue of US\$ 23 million in 2019 for Kraft lignin, while China was the largest market projected to continue leading for the forecast period.

On the other hand for the MarketWatch study, the worldwide market of lignin and its derived compounds is expected to grow at a CAGR roughly 8.1% over the next five years, reaching US\$ 1.090 billion by 2024. These polymers will be used as a green alternative to many petroleum-derived substances, such as fuels, resins, rubber additives thermoplastic blends, nutraceuticals and pharmaceuticals [61]. According to this study, North America holds the major revenue share in the global lignin market and is estimated to continue its dominance owing to the usage in concrete additives in the construction industry, while EU holds the second position.

At this purpose, global construction Industry is spending over US\$ 10 trillion lignin per year, showing a huge opportunity for product demand. Their derivatives play also a significant role in improving the animal diet from an early stage, providing high fiber content together with energy necessary to fight against infections [62]. It is to remember that global animal feed market size is estimated to surpass US\$ 335 billion by 2025.

In addition lignin is also added to insecticides & pesticides and bio-pesticides because of the effectiveness to reduce microbial attack and control weed production. Consequently, the bio-pesticide market alone is expected to grow from US\$ 3.8 billion in 2018 to almost US\$ 10 billion by 2025 at a 17 %CAGR. Moreover, global lignin market de-mand from aromatic applications likely to surpass US\$535 billion by 2025.

Thus global Kraft market size is expected exceed US\$ 15 billion by 2025 according to Global Insight Co [62]. In conclusion, the increasing focus on naturally derived raw materials and increasing concern towards reducing GHGs emissions for both industrialized and emerging countries are likely to stimulate the lignin market growth.

Thus the necessity of a continuous R&D activity for trying to obtain from waste biomass renewable and more characterized pure lignin to be used massively by pharmaceutical and cosmetic companies. The production of a purer polymer from agroforestry by- products will generate substantial opportunity for industrial development, safeguarding also the consumer requests of natural-derived and eco-sustainable ingredients. Therefore, it is necessary to explore at atomic and molecular level the relationship between structure and function of lignin at biological level, as well as the reactivity of its complexes with other biopolymers, such as chitin nanofibrils. So doing it will be possible to better understand the intra- and intermolecular interactions of this polymer which, underlining the fundamental roles of life processes in terms of chemical substances, energy transport and electrical charges, may help to better discover the complicate and al- most unknown mechanisms of natural phenomena.

Nanochitin-Nanolignin Polymer Complexes

Nanoparticles (NPs)have an important impact on the active ingredient activity through their effect on physicochemical properties of the realized formulations, because of their nano dimension and their high surface/weight ratio. They may regulate the active ingredients discharge, increasing their adherence or retention at level of the skin, acting also as penetration agents and active reservoirs in the Stratum corneum. In particular, the activity of CN-derived compounds seems to be particularly effective when bound on the surface or into the fiber' structure of a natural non-woven tissue or film (22, 23, 35, 36) As previously reported, our research group has made polymeric nanoparticles utilizing the spontaneous electrostatic assembly between chitin nano-fibrils (CN) and nano-lignin(LG)in water solution, at room temperature and under normal atmospheric conditions [63]. The production of this innovative NP system is based, in fact, on the inter- actions between the opposite electric charges of two natural polymers, chitin of cationic nature and lignin of anionic ones. The micro- capsule-like system obtained, able to entrap both lipophilic and hydrophilic active ingredients, may be embedded or bound to a fibrous tissue by different methodologies, such as spray dried impregnation, electrospinnig or melt blowing [63,64]. However, to obtain the right release of the active ingredients at the designed time, it is important to verify in advance the main characteristics of the micro capsule-system, such as the physicochemical properties of the polymers selected, their biodegradation kinetics and the thermodynamic compatibility between the polymer and the active ingredients used.- Naturally the final films or non-woven tissues realized, will be characterized in function of the different polymers used and the nano-capsules embedded or bound to their fibers. At this purpose, due to the variability of the commercial lignin and chitin our research group selected not only the polymers to be electrospun, but also and first of all the specific purity of these two polymers. Thus as Lignin, the so called Biolignin extracted from wheat straw by the patented CIMV' technology, was selected and used as phenolic Polymer characterized for is linear and low molecular weight [65]. CN was produced directly in our lab by the use of a food grade chitin and a patented methodology without solvents and at zero waste. Moreover, for the research study's necessities, CN,LG and all their complexes were produced as water suspensions and/or as powder. To powdering the CN-LG solution

by the spray drying process, it was necessary to pre-cover the nanoparticles by a thin soft corona of PEO (fig 10), to avoid the agglomeration and maintain their micro/nano dimension [66].

NANOPARTICLE UNCOVERED AND COVERED BY A SOFT OR HARD CORONA Soft corona Hard corona NANOPARTICLE Nanoparticle

Figure 10 The Nanoparticle' Corona(soft or hard) for Covering Its Structure to Avoid the Agglomeration Phenomena.

Naturally for going on by this process, chitin has been turned into its nano-fibril form to overcome the drawbacks related to its poor water solubility. Successively, it was complexed with the bio-lignin turned into its nano size and embedded into biodegradable films or tissues made by polysaccharide bio-nano-composites fibers as a viable alternative to synthetic ones. The obtained tissues may be considered appropriate organic

matrices(membranes) for the incorporation of CN-LG particles able to form inter and intramolecular hydrogen bonds with the functional groups of the polymeric surface. These innovative matrices could be used to remove environmental pollutants from the skin as well as to realize smart carriers for a controlled delivery of selected active ingredients. In this contest, the structure of the tissues, used to produce beauty facial masks, surgical masks ,and advanced medications, were made by well-defined networks of natural fibers interwined to form biocompatible systems(film/tissues) able to bound bioactive molecules (fig 11) [67].

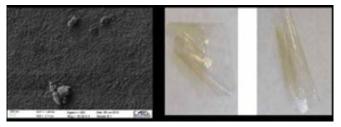


Figure 11 Film-Matrix Made by CN and the Casting Technology :Film at- SEM(left) and Film Produced (right)

To Make These Innovative Tissues Our Group Selected Two Techniques

,electrospinning and melt-blowing, also if the majority of them were realized by electrospinnig. This method, in fact, characterized for its simplicity, versatility and flexibility, is the easiest technology for making non-woven tissue at high porosity and capacity to entrap various kind of molecules into its matrix (fig 12) [68, 69]. It, in fact, requires three major parts only: a syringe needle, a high voltage power and a grounded collecting rotating plate(collector)(68). Additionally these tissues, made by natural sugar-like composites, have shown to be particularly promising in the biomedical and cosmetic fields for their high area to volume ratio, high porosity and high permeability and, naturally the particular skin bio-compatibility and eco-compatibility versus the environment [41-43, 69].

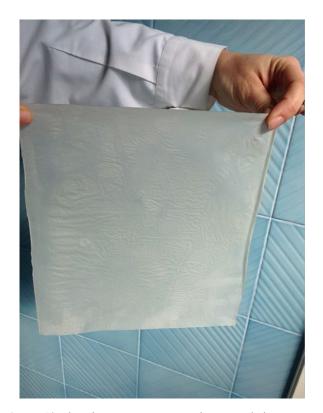


Figure 12 Electrispun Non-Woven Tissue Made by CN-LG

Moreover the surface modification of fibers and tissues used, enabling attachment of active compounds on their polymeric material, increased active ingredients effectiveness of the obtained products. Thus this methodology may be used to functionalize the tissues by the active ingredients selected for producing an antioxidant, antimicrobial, antiinflammatory or skin repairing effectiveness, according to the designed project. Surface modification, for example, enables attachment of active compounds on the polymeric material giving them a better

availability. At this purpose different study have been realized to underline the thermal stability of CN-LG Complexes together with the capacity they have to entrap various active ingredients [70]. For The active ingredients selected for the first study were:

Niacinammide, glycirrhetic acid ,vitamins C and E nano-structured and encapsulated into different CN-LG complexes, in the range from 0.2% to 5% calculated on chitin weight. It was possible, therefore, to design and make Advanced Medications and Face Beauty Masks, the first one with a supposed antiaging activity, the second with a skin repairing activity.

To control the thermal stability of the obtained complexes, the thermo- gravimetric analysis(TGA)was used. This control was considered necessary for verifying the stability of the complexes CN-LG, made by the spray dried method, before applying them on the surface or bound to the fibers of the film/tissues realized by the casting or the electrospinning techniques respectively. All

these methodologies require high temperature for short times and therefore the necessity to control stability of both encapsulated ingredients and carriers.

Moreover, to verify the concentration of the encapsulated ingredients and their chemical and morphological structures the Fourier transform Infrared Spectroscopy (FT-IR) (fig 13)and the Scanning electron Microscopy (SEM)(fig.14) have been used, while their cell bio compatibility and effectiveness were controlled on human Keratinocyte 'cell cultures(HaCAT cells) [30, 31, 71, 72].

As reported in figure 15,the thermal stability decreased from CN-LG- Ag to CN,CN-LG-Lutein, CN-LG-Glycyrrhetinic, CN-LG-Vit E,CN-LG- Niacinamide, CN-LG Vit C respectively, while the better cell compatibility decreased from CN to CN-LG,CN-LG-Vit C,CN-LG- Niacinamide, CN-LG-Vit E,CN-LG-Lutein, and CN-LG-Ag nano [70-72]. On the other hand, the best options supposed for obtain Beauty Face Masks or the Advanced medications, characterized for effectiveness and safeness, are reported in figure 16 [71, 72]. Recently, further studies have been realized by the NIR Hyperspectral imaging technology to determine the quality and quantity distribution of Chitin nanofibrils-nanolignin encapsulating glycyrrhetic acid on the non-woven tissue made by pullulan(GA) [73].

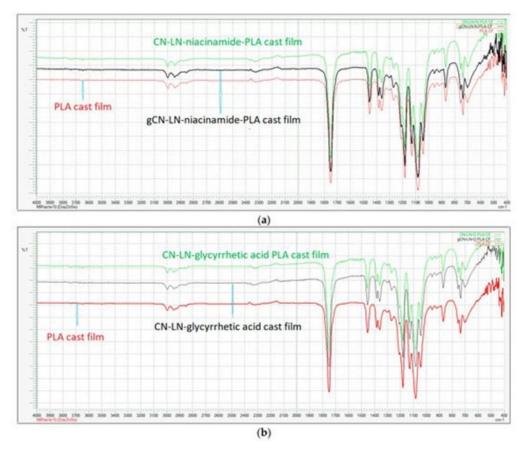


Figure 13 FTIR Spectra of Polylactic Acid (PLA) Cast Film With CN-Nano- Lignin(LN) Complex Encapsulating Niacinamide and Glycyrrhetic Acid

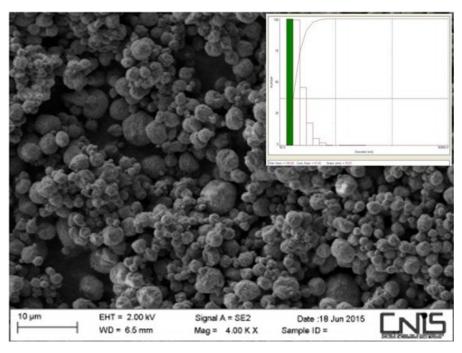


Figure 14 Chitin nanofibril-Lignin Complex Encapsulating vit C by SEM

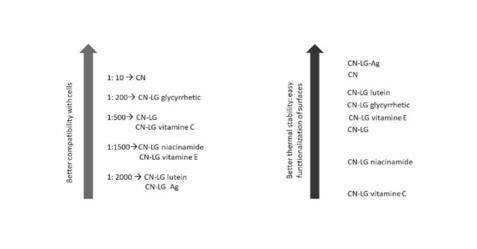


Figure 15 Biocompatibility and Thermal Stability of Different CN-LG com- plexes at Different Concentrations

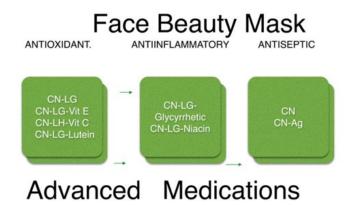


Figure 16 Different Effectiveness of Beauty Masks and Advanced Medications According to the Different Content in Active Ingredients.

By this technique, in fact, it is possible to combine conventional imaging and infrared spectroscopy collecting spatial and spectra information. Naturally, other studies have been previously conducted *in vitro* to verify on Keratinocyte culture the anti- bacterial, anti- inflammatory, immunomodulating and skin repairing activity of both nanocapsules and tissues as well as their effectiveness in vivo on aged and diseased skin affected by burns of 1st and 2nd grade [27, 28, 30, 31].

Conclusions

In conclusion these innovative tissues made by resorbable biomaterials could offer the most valuable opportunities as smart nano-carrier/dressings used to transport and deliver the bioactive ingredients to the target tissue improving their permeation throughout the skin layers. They, in fact, not only could enable in vivo cell migration, but also may act as scaffolds which, replicating the tissue morphology, have the healing capacity to repair lesioned tissues or rejuvenate aged and photo aged skin [40,41]. It is to Remember, in fact, that electrospun CN is probably involved directly in allowing the functional regeneration of tissues and organs being a Biomaterial able to mimic the function of the body's extracellular Matrix (ECM) [40, 71]. However it is to underline that chitin represents a fundamental part of the reported carrier-scaffolds able to promote and regulate the release of certain antiinflammatory cytokines. The polymer, in fact, because of its nano-size seems able to modulate the inflammatory cascade as immunomodulant agent, contributing to the skin repairing activity [41]. Thus, as previously reported in figure 16, the beauty masks showing an higher effectiveness, seem to

be those made by tissues containing no ingredients or vit E (CN-LG-vit E) as antioxidant compound and/or the antiinflammatory glycirrhetic acid. However, selection of active ingredients and the processing conditions have to be into great attention. For the Advanced medications, where antiinflammatory and antiseptic properties are the most required, CN-LG-Ag and CN-LG-Glycyrrhetic seem to represent the best options, confirmed by our previous studies also [22, 23, 26-28, 30, 31].

Concerning the ability to regulate the expression of defensine-2 (HBD-2), thus possessing an indirect antibacterial activity, the most efficient compound seems to be represented by CN, also if the incorporation of stable nano-silver, which increases its antiinflammatory and antimicrobial properties resulted to be an interesting strategy.

Apart the skin and eco-friendly activities the health function of these innovative tissues has to be taken in consideration, remembering that wellness economy during the years 2015-2017 grew 6.4%, nearly twice as fast as economic growth output (3.6%), with a personal care market of around US\$ 1000 (fig.17) [74]. In this market beauty standards are continually evolving and consumers need to feel safe more than ever, embracing who they are and why they are looking for cosmetics which may maintain all the body young and healthy [75]. Therefore consumers are requesting products able to really ameliorate the health and beauty skin condition, capturing the intersection between beauty health and wellness



Figure 17: Global Wellness Economy: a USD 4.5 Trillion Market (by courtesy of USA Global Wellness Institute. [74])

Thus people are looking for innovations which may help to provide the dreams of more natural beauty cosmetics and diet supplements, having the capacity to positively influence their life-style [75]. As the saying goes, they know that: we are what we eat! Therefore the necessity to find cosmeceuticals and nu-

traceuticals(fig 18)able to increase beauty and wellness based on novel and validated scientific research studies, transparency, natural raw materials and sustainable production.

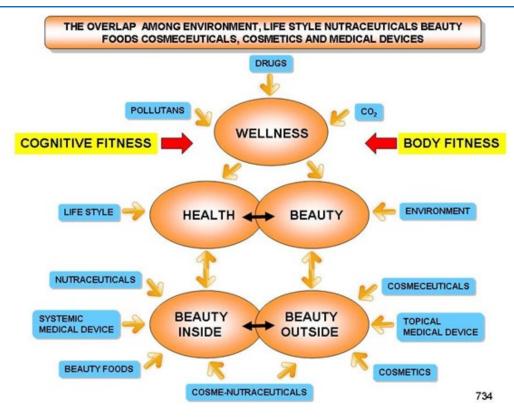


Figure 18. Cosmeceuticals and Nutraceuticals to Maintain a Healthy lifestyle.

Millennials (consumers between age 18-34)" feel the need for their re-empowerment more strongly than other generations" wishing products uniquely for them and their lifestyle [76, 77]. For these consumers, who are the largest potential buying group accounting for about 32% of the world's population compared to 17% for those over 55 years old (baby boomers), are more important to purchase healthy and clean products [78]. They are

Source: AlixPartners Global Health and Wellness Study, 2018

looking for quality and products allergen-free, reflecting their aspiration for a healthy lifestyle and ethical environmental standards (fig.19). Moreover they want to know where ingredients originate and how products are made and act freely and don't want to feel old(fig 20). However, all consumers wish to take back control over what to eat, to drink and put on the skin,

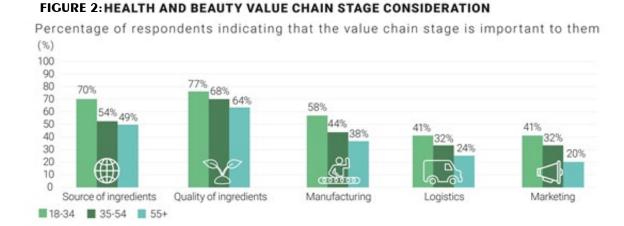


Figure 19 Consumers are Looking for the Quality of the Cosmetic Prod- Ucts(by courtesy of AlixPartners study [77])

FIGURE 3: IMPORTANCE OF ATTRIBUTES IN BEAUTY AND PERSONAL CARE PRODUCTS

Attributes ranked by importance for each age group across all regions



1. Attributes were tied

Source: AlixPartners Global Health and Wellness Study, 2018

Figure 20 Consumers are looking to know the sources of ingredients and the Manufactoring methodologies (by courtesy of Alix-Partners study [77])

prioritizing the mental and physical balance, shifting from aging to longevity (76-78). Therefore they are moving to companies making investments in high-quality products and are enjoying for their way of living "exploring the push-pull between nature and science", while don't be wary of the future. Moreover, they are looking for products plastic free, including their reusing and up cycling wherever possible, also if circularity appear at moment very difficult [75-77]. However, a sustainable development is considered extremely important due to the limited natural sources of the Planet, also because the actual daily consumption and production of goods is growing day by day, causing waste and climate changing [79]. Thus the necessity to transform the linear economy in a circular economy, paying much attention to making profit without forgetting to maintain the environment and social aspects. Consequently a better management of recycling, reusing and reducing could be of great help as well as the use of industry 4.0, which enables automation, digitation and integration with the correct sustainable development goals [79].

In conclusion, production and use of the proposed innovative tissues to make Surgical Masks, Advanced medications, Face Beauty Masks and biodegradable dressings, seem in line with the circular economy goals. The raw materials used, such as chitin, lignin and other biodegradable polymers, in fact, are obtained from recycled food waste and therefore protective of the environment, while the tissues realized are skin and environmentally friendly. Thus, the proposed facial mask represents a very and promising innovative cosmetic product. It is effective, easy and quick to apply, and accessible with instant effects and totally biodegradable as well as the surgical masks made by the proposed tissues Moreover. It is interesting to underline that consumerism and waste produced from both Beauty- and Surgical Masks are notably increasing. The spreading of COVID-19 pandemic, in fact, is causing further waste due to the use of surgical masks and gloves by a population of ~ 7 billion people who wears one to two masks per day [80]. As a consequence the global sales of beauty and personal care products are valued today at over US\$

500 billion and expected to reach US\$800 billion by 2023 with a compound annual rate (CAGR) of just over 7%(77), Increasing with a CAGR over 12% for natural and organic beauty industry which is expected to reach around US\$ 22 billion by 2024, from the actual US\$11 billion. Into this niche-market the sheet face masks, which dominated the skincare scene in the last years, had a global selling estimated in US\$ 282.8 million in 2018 by products not fully compostable, recyclable or biodegradable. Thus, cosmetic products together with the shampoo bottles, are causing, for example, in USA an annually waste nearly to 1,200 football stadium! On the other hand, the surgical masks made prevalently by the Petrol-derived polypropylene exceeded a value of US\$ 74.90 billion in Q1 of 2020 and is expected to grow at an annual CAGR of 53.0% from 2020 to 2027 [81].

In conclusion the use of biodegradable natural biopolymers, such as chitin and lignin, if used in the right way in substitution of the petrol-de- rived ones, will certainly reduce the actual waste safeguarding environment biodiversity and the human health, contemporary maintaining the natural raw materials for the future generations.

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