EDITOR
Pierfrancesco Morganti
Dermatology Unit, University of Campania "Luigi Vanvitelli", Naples, Italy;
China Medical University, Shenyang, China;
Director of the R&D Nanoscience Centre MAVI, MAVI Sud Srl, Aprilia (Lt), Italy.

Editorial Office
MDPI
St. Alban-Anlage 66
4052 Basel, Switzerland

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

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

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

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

Cover image courtesy of Pierfrancesco Morganti.

Chitin-Hyaluronan Block Copolymeric Nanoparticles for Innovative Cosmeceuticals

Hong-Duo Chen, Li Yuan Hong, and Pierfrancesco Morganti

Abstract: Chitin (C8H13O5N)n is a low-cost available unbranched polysaccharide widely distributed in nature as supporting structure of the cell wall of fungi and exoskeleton of arthropod and insects. Chitin nanofibrils are easily metabolized by the body's endogenous enzymes and thus used in cosmetic dermatology and biotextiles. Hyaluronan is anionic, nonsulfated glycosaminoglycan which, as major component of the Extracellular Matrix (ECM) plays several biological roles, showing expensive age-related changes. A successful model of an innovative block-polymer nanoparticle (BPN) based on phosphatidylcholine, hyaluronan, and chitin nanofibrils entrapping amino acids, vitamins, and melatonin has been formulated. Both the in vitro and in vivo results obtained demonstrate the efficacy of the injected block-polymer nanoparticles in reducing skin wrinkling and ameliorating the signs of aging. Chitin nanofibrils, protecting both corneocytes and intracorneal lamellae, help to maintain cutaneous homeostasis, neutralize the activity of radicals and trap them in their structure, regularizing the correct cell turnover. The BPN seems to be useful for improving the activity of permanent fillers, rendering it useful as an anti-aging remedy for the plastic surgery armamentarium.

Raymond Reed and Albert Kligman brought about the concept of "cosmeceutical" more than 50 years ago [1,2]. According to these authors, *cosmeceuticals* are topical Cosmetic-Pharmaceutical hybrids intended to enhance the health and beauty of skin, being based on the use of biologically active ingredients with medicinal or drug-like benefits.

At this purpose, many substances, either chemically synthesized or extracted from plants or animals, are used as functional ingredients. Nowadays, many cosmetic products with biologically active ingredients have been developed and marketed, though there are discrepancies in relation to their regulations and approvals by the government [3–5]. However, a number of topical cosmeceutical treatments for conditions such as photoaging, hyperpigmentation, wrinkles, and hair damage have come into widespread use.

In the cosmeceutical arena, nanotechnology has played an important role. Using new techniques to manipulate matter at an atomic or molecular level, they have been at the root of numerous innovations, opening up new perspectives for the future of the cosmeceutical industry.

Nanotechnology-based cosmeceuticals, in fact, offer the advantage of diversity in products, increase the bioavailability of active ingredients, ameliorating the aesthetic appeal of cosmeceutical products with prolonged effects [6–8].

There is a great potential in the marine bioprocess industry to convert and utilize most marine resources and marine food by-products as valuable cosmeceutical ingredients. Current available potential cosmeceuticals from marine resources include: seaweed extract, phlorotannins, polysaccharides, carotenoid pigments, fucosterol, microalgae extract, collagen, bioactive peptides, chitooligosaccharide derivatives, enzymes, sea mud, sea water, and minerals. Potential health benefits of marine-derived cosmetic active ingredients on human skin include anti-aging, antioxidant, anti-wrinkling, anti-whitening, cytoprotective, anti-tyrosinase, anti-acne, anti-inflammatory, and UV photo protective effects [9].

Chitin (C8H13O5N)n is a long-chain glucose-derived of a N-acetylglucosamine widely distributed in nature. It is a modified polysaccharide which, containing nitrogen, is distributed in the cell wall of fungi and exoskeketon of arthropods and insects. This polymer available at low cost, has been shown to be bio- and eco-compatible, with a very low level of toxicity. At present, the world offshore disposal of this natural waste material is estimated to be around 250 billion tons per year.

Chitin is an underutilized resource and has the potential to supply a wide range of useful products if suitably recycled, thus contributing to sustainable growth and a greener economy [10,11]. In addition, it is a good inducer of defense mechanisms in plants, being a fertilizer that can improve overall crop yields. Chemically-modified chitin forms edible films as an additive to thicken and stabilize foods and pharmaceuticals. It also acts as a binder in dyes, fabrics, adhesives, a reproducible form of biodegradable plastic, and as a promising substrate for engineering human tissues. Chitin's flexibility and strength make it favorable as surgical thread. Its biodegradibility means it wears away with time as the wound heals and it might have some unusual properties that accelerate the healing of wounds in humans [12].

Recently, it has become possible to industrially produce pure chitin crystals, named "chitin nanofibrils" (CN) for their needle-like shape and nanostructured average size (240 × 5 × 7 nm) [11] (Figure 1).

2).

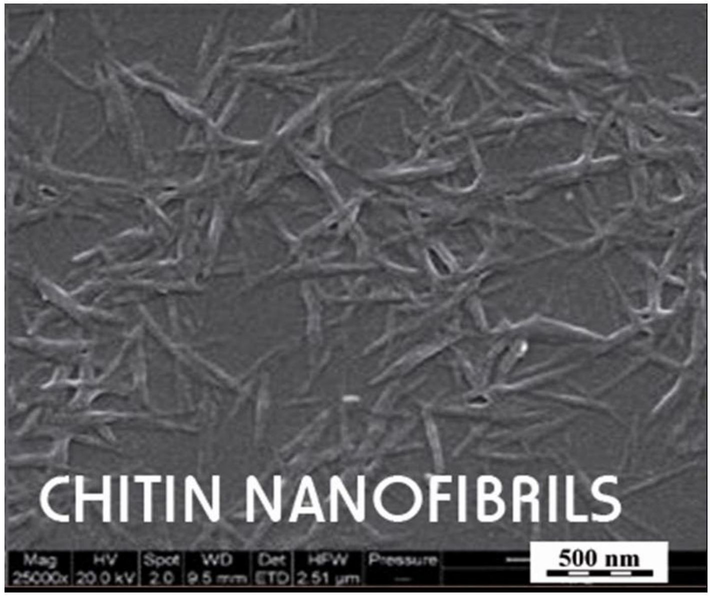

Figure 1. Chitin Nanofibrils at SEM.

Having a backbone like hyaluronic acid, chitin nanofibrils are easily metabolized by the body's endogenous enzymes and thus used in cosmetic dermatology and biotextiles. Moreover, because it occurs naturally and is considered as a safe raw material, it is safe to use. Chitin nanofibrils were recognized as a strong "gelling agent" [13].

As the nanofibril has an average size one-quarter that of a bacterium, 1 g of the product covers a surface area of 400 m^2. Many studies have shown that chitin nanofibrils can activate the proliferation of keratinocytes as well as fibroblasts, regulating not only collagen synthesis but also cytokine secretion and macrophage activity [11,13–15].

Chitosan is a natural biopolymer derived from the deacetylation of chitin and known to have various biological activities such as antifungal, antitumor, and antibacterial activity. It has been applied in various fields including wastewater treatment, agriculture, fabric and textiles, cosmetics, nutritional enhancement, and food processing.

Nanocomposite biomaterials based on chitosan and chitin are widely investigated for their antimicrobial activity, biocompatibility, and biodegradability [11,13]. P. Morganti's group has obtained interesting results showing how chitin nanofibrils can not only ameliorate the appearance of photoaged skin but also promote wound healing by reducing hypertrophic scar formation [6,15–20] (Figure 2).

Figure 2. Cicatrizing activity of a gel based on chitin nanofibrils and chitosan (courtesy of P. Mezzana, MD [20]).

In vitro studies have shown how chitin nanofibrils can increase the reproduction of fibroblasts with a subsequent increase in collagen synthesis and in adenosine triphosphate production. In an in vivo double-blind study, skin hydration and superficial skin lipids were improved, with a simultaneous reduction in lipid peroxides and transepidermal water loss (TEWL) [21–23].

Hyaluronan is an anionic, non sulfated glycosaminoglycan distributed widely throughout connective, epithelial, and neural tissues. Hyaluronan is a polymer of disaccharides, composed of D-glucuronic acid and D-N-acetylglucosamine, linked via alternating β-1,4 and β-1,3 glycosidic bonds. Polymers of hyaluronan can range in size from 5000 to 20,000,000 Da in vivo [24,25].

Hyaluronan is found in many tissues of the body, such as skin, cartilage, and the vitreous humour. Therefore, it is well-suited to biomedical applications targeting these tissues. Native hyaluronan has a relatively short half-life, so various manufacturing techniques have been deployed to extend the length of the chain and stabilize the molecule for its use in medical applications [6,11,22,26].

The first hyaluronan biomedical product was developed in the 1970s and is approved for use in eye surgery. Hyaluronan has been used in attempts to treat osteoarthritis of the knee by injecting it into the joint [27–29].

Dry skin can be treated with a prescription skin lotion containing sodium hyaluronate as its active ingredient. In some cancers, hyaluronan levels correlate well with malignancy and poor prognosis. Hyaluronan is often used as a tumor marker for prostate and breast cancer and may also be used to monitor the progression of the disease [29]. Moreover, it may also be used postoperatively to induce tissue healing, notably after cataract surgery [30], as well as in the synthesis of biological scaffolds for wound-healing applications [31].

Hyaluronan is a common ingredient in skin-care products, so that hyaluronic acid fillers have been injected using a classic sharp hypodermic needle, cutting through nerves and vessels, with increasing risks of causing pain and bruising.

Nanotechnology is not only essential for marketing-oriented chemical companies, but is also a tool for developing science-based solutions for innovative therapeutics and cosmetics, enhancing well-being and addressing anti-aging issues. Nanomaterials and nanobiotechnology have the potential to radically change the way cosmetics and drugs deliver their benefits.

Specifically, nanoparticles are being developed to encapsulate a wide range of ingredients beneficial to the skin. To obtain nanoparticles, two principles approaches are used: (a) the bottom-up method in which nanoparticles are assembled from the molecular dimension; (b) the top-down approach that reduces larger particles through the use of physicochemical methods. In cosmetics, the top-down approach is more commonly used to produce different kinds of structures. Examples of such structures include nanosomes, cubosomes, niosomes, and liposomes [6,32].

A successful model has been formulated by P. Morganti's group: an innovative block-polymer nanoparticles (BPN) based on phosphatidylcholine, hyaluronan, and chitin nanofibrils entrapping amino acids, vitamins, and melatonin.

The specific formulation for each milliliter contained: hyaluronan salt 1 mg; phosphatidylcholine 3 mg; creatine 0.1 mg; caffeine 0.1 mg; ascorbyl tetraisopalmitate 0.5 mg; vitamin E 10 mg; chitin nanofibrils 1 mg; melatonin 0.1 mg; glucosamine 0.1 mg; glycine 0.1 mg; arginine 0.1 mg; sodium phosphate dibasic 2 mg; potassium di hydrogen phosphate 0.2 mg; sodium chloride 9 mg; sterile water for injection to 1 mL.

The injection was based on the mesotherapy technique, using 1 mL solution and a 30 g needle positioned at 45 to the skin surface. The injection rate was at all times less than 0.3 mL/min. A firm massage, with the index finger inside the mouth and the thumb outside, was then used to remove any unevenness. The 1mL quantity is sufficient to treat the entire face [5,6].

Both the in vitro and the in vivo results obtained demonstrate the efficacy of the injected block-polymer nanoparticles in reducing skin wrinkling and ameliorating the signs of aging. Subjects were satisfied with the general aspect of their skin, which appeared softer and more hydrated during the first month of treatment.

In line with their self-evaluation, the appearance of fine wrinkling was notably reduced and the consequent skin softness and firmness enhanced during the entire treatment period. The general amelioration remained during the regression period and 30 days after the interruption of the treatment (Figure 3).

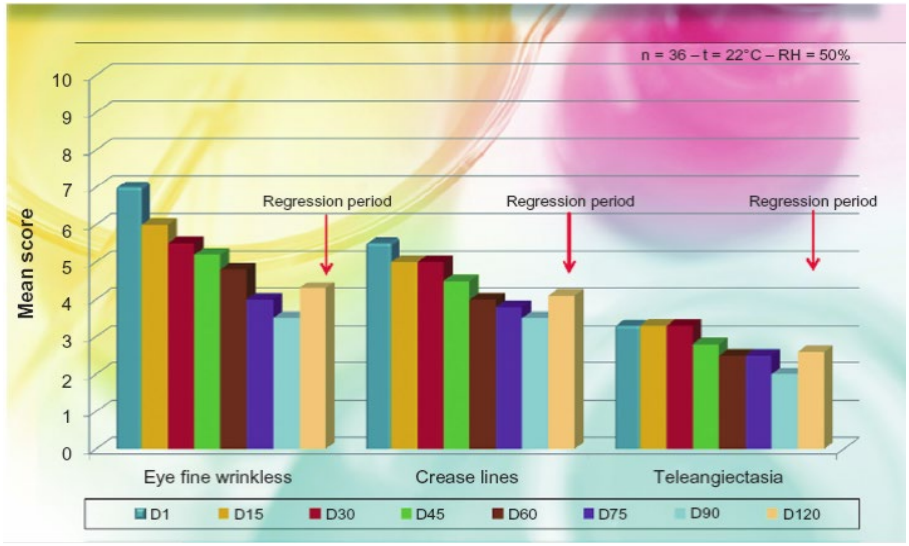

Figure 3. Dermatological mean evaluation on signs of photoaging after injective treatment with phosphatidylcholine-hyaluronic acid-chitin nanofibrils encapsulating active compounds (BPN). Note: All P values are highly significant as to baseline ($p < 0.005$). Abbreviations: BPN, block-polymer nanoparticles; RH, relative humidity.

In this formulation, the high content in linoleic acid of the phosphatidylcholine used allowed the active BPN to quickly reestablish the skin-barrier function. Thus, while the phosphatidylcholine fatty acids of the BPN composition contribute to balancing the disturbed composition and organization of lipids at the level of epidermal keratinocytes and consequently of corneocyte lamellae, the high level of linoleic acid should contribute to reintegrating the reduced level of ceramide 1, structural and stabilizing component of the stratum corneum [5,6,33].

Chitin nanofibrils, protecting both corneocytes and intracorneal lamellae, help to maintain cutaneous homeostasis, neutralize the activity of free radicals and trap them in their structure, and regularize the correct cell turnover [24,34–37]. All these activities are modulated and increased by the chitin nanofibrils/hyaluronan (CN-HA) encapsulation methodology made using the gelation method [38,39] (Figure 4).

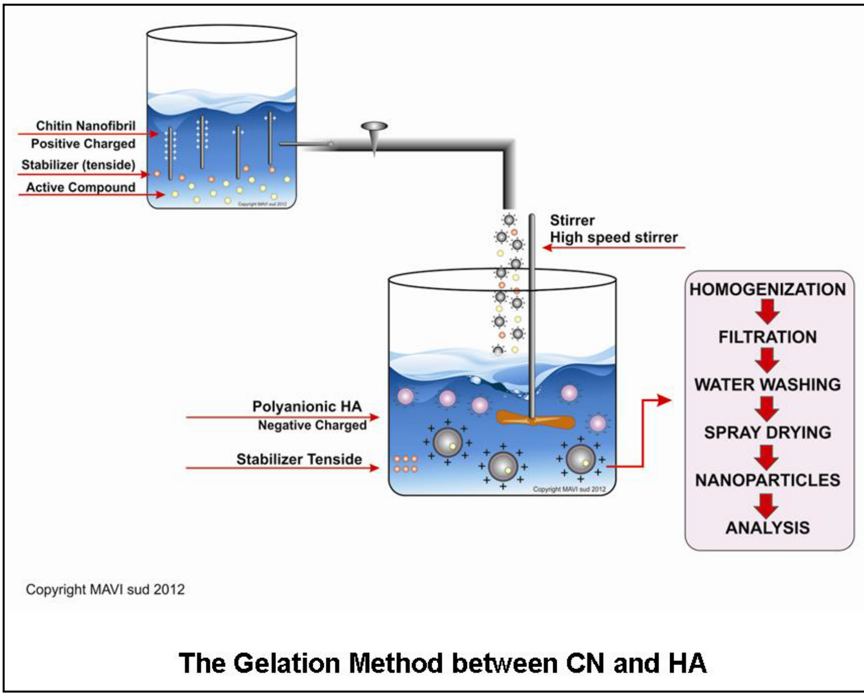

Figure 4. Gelation method for producing Chitin Nanofibril-Yaluronic acid (CN-HA) block polymeric nanoparticles.

In summary, the BPN encapsulating the active ingredient used seems to be useful in improving the activity of permanent fillers, rendering it useful as an antiaging remedy for the plastic surgery armamentarium.

In conclusion, this innovative biostimulating medical device should be used for wrinkle treatment and rejuvenating looks, as well as an adjuvant in soft-tissue augmentation and stretch-mark corrections [6,40].

Author Contributions: all the author contributed to write the paper in the same way.

Acknowledgments: We would like to thank The China Medical University for the help offered in setting up this paper.

Conflicts of Interest: We declare that Hong-Duo Chen and Li Yuan Hong have no conflict of interests. Pierfrancesco Morganti works as Head of the R&D Centre of Nanoscience, Mavi Sud, s.r.l, Italy.

References

1. Reed, R. E. The definition of "cosmeceuticals". *J Soc Cosm Chem* **1962**, *13*, 103–116.
2. Kligman, A.M. Why cosmeceuticals? *Cosm & Toil* **1993**, *108*, 37–40.
3. Mincea, M.; Negrulescu, A.; Ostafe, V. Preparation, modification, and applications of chitin nanowiskers: A Review. *Rev. Adv. Mater. Sci* **2012**, *30*, 225–242.

4. Gao, X.H.; Zhang, L.; Wie, H.; et al. Efficacy and safety of innovative cosmeceuticals. *Clin Dermatol.* **2008**, *26*(4), 367–374.
5. Morganti, P.; Guevara, L.; Palombo, P.; Palombo, M.; Cardillo, A.; Mezzana, P.; Fabrizi, G.; Svolacchia, F. A phosphatidylcholine hyaluronic acid chitin–nanofibrils complex for a fast skin remodeling and a rejuvenating look. *Clinical Cosmetic and Investigational Dermatology* **2012**, *5*, Pages 213–220.
6. Morganti, P. Use and potential of nanotechnology in cosmetic dermatology. *Clinical Cosmetic and Investigational Dermatology* **2010**, *3*, 5–13.
7. Dureja, H.; Kaushik, D.; Gupta, M.; et al. Cosmeceutical: an emerging concept. *Indian J Pharmacol* **2005**, *37*, 155–9.
8. Lohani, A.; Verma, A.; Joshi, H.; et al. Nanotechnology-Based Cosmeceuticals. *ISRN Dermatol.* **2014**, *2014*, 1–14.
9. Li, D.H.; Wu, Z.M.; Marini, N.; et al. Advanced carrier systems in cosmetics and cosmeceuticals: A review. *J Cosmet Sci*. **2011**, *62*, 549–563.
10. Kim, S.K. Marine cosmeceuticals. *J Cosmet Dermatol.* **2014**, *13*(1), 56
11. Morganti, P.; Carezzi F.; Del Ciotto, P.; Morganti, G.; Nunziata, M.L.; Xingua, G.; Hong-Duo, C.; Tishchenko, G; Yudin, V.E. Chitin NAnofibrils: A Natural Multifunctional Polymer: Physicochemical characteristics and safeness. In: *Nanobiotechnology*; Phenix, D.A., Waqar, A., Eds.; One Central Press: Manchester, UK, 2014, pp. 1–31.
12. Morganti, P.; Morganti, G.; Morganti, A. Transforming nanostructured chitin from crustacean waste into beneficial health products: a must for our society. *Nalnotechnol Sci Appl.* **2011**, *6*(4), 123–129.
13. Lee, J.-Y.; Choi, B.; Wu, B.; et al. Customized biomimetic scaffolds created by indirect three-dimensional printing for tissue engineering. *Biofabrication* **2013**, *5*(4), 10.
14. Mikesova, J.; Hasek, J.; Tishchenko, G.; et al. Rheological study of chitosan acetate solution containing chitin nanofibrils. *Carbohydr Polym.* **2014**, *112*, 753–757.
15. Nguyen, T.H.; Kwak, H.S.; Kim, S.M. Physicochemical and biofunctional properties of crab chitosan nanoparticles. *J Nanosci Nanotechnol*. **2013**, *13*(8), 5296–5304.
16. Morganti, P. Use and potential of nanotechnology in cosmetic dermatology. *Clin Cosmet Investig Dermatol.* **2010**, *24*, 5–13.
17. Mikesova, J.; Hasek, J.; Tishchenko, G.; et al. Rheological study of chitosan acetate solutions containing chitin nanofibrils. *Carbohydr Polym.* **2014**, *112*, 753–757.
18. Morganti, P.; Fabrizi, G.; Bruno, C. Protective effects of oral antioxadants on skin and eye function. *Skinmed.* **2004**, *3*(6), 310–316.
19. Biagini, G.; Zizzi, A.; Tucci, G.; et al. Chitin nanofibrils linked to chitosan glycolate as spray, gel and gauze preparations for wound repair. *J Bioact Compat Polym.* **2007**, *22*, 525–538.
20. Mezzana, P. Clinical efficacy of a new chitin-nanofibrils based gel in wound healing. *Acta Chirurgiae Plasticae.* **2008**, *50*(3), 81–84.
21. Biagini, G.; Zizzi, A.; Giantomassi, F.; et al. Cutaneous absorption of nanostructured chitin associated with natural synergistic molecules (lutein). *J Appl Cosmetol.* **2008**, *26*, 69–80.

22. Morganti, P.; Fabrizi, G.; Palombo, P.; et al. Chitin-nanofibrils: a new active cosmetic carrier. *J Appl Cosmetol.* **2008**, *26*, 105–120.
23. Morganti, P.; Morganti, G.; Fabrizi, G.; et al. A new sun to rejuvenate the skin. *J Appl Cosmetol.* **2008**, *26*, 159–166.
24. Morganti, P. Chitin nanofibrils for advanced cosmeceuticals. Clinics in Dermatology **2008**, *26*, 334–340.
25. Morganti, P.; Fabrizi, G.; Ruocco, E.; et al. Chitin nanofibrils improved photoprotection. *Cosmetics and Toiletries* **2009**, *124*(9), 66–73.
26. Fraser, J.R.; Laurent, T.C.; Laurent, U.B. Hyaluronan: its nature, distribution, functions and turnover. *J Intern Med.* **1997**, *242*(1), 27–33.
27. Jin Huang. Organic Chemicals from Bioprocesses in China. Advances in Biochemical Engineering/Biotechnology **2010**, *122*, 43–71.
28. Frampton, J.E.; Hylan G.-F. 20 single-injection formulation. *Drugs Aging.* **2010**, *27*(1), 77–85.
29. Rutjes, A.W.; Juni, P.; da Costa. B.R.; et al. Viscosupplementation for osteoarthritis of the knee: a systematic review and meta-analysis. *Ann Intern Med.* **2012**, *157*(3), 180–191.
30. Josefsson, A.; Adamo, H.; Hammarsten, P. Prostate cancer increases hyaluronan in surrounding nonmalignant stroma, and this response is associated with tumor growth and an unfavorable outcome. *Am J Pathol.* **2011**, *179*(4), 1961–1968.
31. Gritsenko, P.G.; Ilina, O.; Friedi, P. Interstitial guidance of cancer invasion. *J Pathol.* **2012**, *226*(2), 185–199.
32. Shu, X.Z.; Ghosh. K.; Liu, Y. Attachment and spreading of fibroblasts on an RGD peptide-modified injectable hyaluronan hydrogel. *J Biomed Mater Res A.* **2004**, *68*(2), 365–375.
33. Morganti, P.; Palombo, P.; Fabrizi, G.; et al. Biweekly in-office injectable treatment of striae distensae versus a long-term daily use of topical vitamin C. *J Appl Cosmetol.* **2001**, *19*, 107–112.
34. Marenkov, L.N.; Steinert, P.M. Ceramides are bound to structural proteins of the human foreskin epidermal cornified envelope. *J Biol Chem.* **1998**, *273*, 17763–17770.
35. Morganti, P.; Randazzo, S.D.; Giardina, A.; et al. Effects of phosphatidylcholine linoleic acid-rich and glicoli acid in acne vulgaris. *J Appl Cosmetol.* **1997**, *15*, 21–41.
36. Morganti, P.; Agostini, A.; Bruno, C. ; et al. Role of topical glicolic acid and phosphatidylcholine linoleic acid in the pathogenesis of acne. Linoleic acid versus squalene. *J Appl Cosmetol.* **1997**, *15*, 33–41.
37. Morganti, P.; Morganti, G.; Fabrizi, G. ; et al. A new sun to rejuvenate the skin. *J Appl Cosmetol.* **2008**, *26*, 159–168.
38. Morganti, P. Chitin nanofibrils for cosmetic delivery. *Cosmet Toil.* **2010**, *125*, 36–39.
39. Morganti, P.; Fabrizi, G.; Palombo, M.; et al. New chitin complexes and their anti-aging activity from inside out. *J Nutr Health Aging.* **2012**, *16*(3), 242–245.
40. Morganti, P.; Palombo, M.; Palombo, P.; et al. Cosmetic science in skin aging: achieving the efficacy by the chitin nano-strucured chrystallites. *SOFW J.* **2010**, *136*, 14–24.