A CHITIN NANOFIBRIL-BASED NON-WOVEN TISSUE AS A MEDICAL DRESSING: THE ROLE OF BIONANOTECHNOLOGY

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5.1. INTRODUCTION

The *Stratum Corneum* (SC), the outermost layer of keratinized cells, called corneocytes, is a specific skin protective barrier consisting of an intercellular lipid-like substance with a lamellar crystalline gel structure, which acts as a mortar between the corneocytes. It is highly active in lipid enzymatic synthesis and has the ability to adapt to the environment (Figure 1) [1].

Figure 1. Lamellar structure of *Stratum Corneum*

Barrier recovery and skin homeostasis, in fact, are the result of restoration of these lipids, that also involves control and normalization of keratinocyte turnover (Figure 2) [2]. When barrier perturbation becomes persistent, the processes of lipid and keratin synthesis can escalate, and if it becomes chronic, a pathogenic sequence of hypertrophic scars and keloids may appear in wound healing [3].

Figure 2. Skin homeostasis with keratinocyte turnover

Defects created by severely damaged skin caused by large burns or chronic wounds, modify the synthesis of the extra cellular matrix (ECM) and therefore impede the skin's ability to breathe, retain or expel water, and defend itself against harmful bacteria, oxidants and toxins. Thus, the main function of the SC is to provide protection for preserving the body from external insult. In fact, the skin represents the first line of defence and is the guardian of the body's organs; as a consequence, when the skin is burned beyond a certain percentage of total body area, death may occur. This is the reason why injured skin needs to be immediately covered with a dressing capable of restoring tissue integrity, maintaining homeostasis, and preventing invasion of toxic substances and pathogens. The four main goals of burn wound care are, in fact: (a) prevention of infection, (b) maintenance of a moist environment, (c) protection of the wound from external aggressions, and (d) reduction of scar formation [4].

Therefore, a medical dressing has to establish a barrier to environmental irritants, impede microbial growth, maintain a moist environment, and allow exchange of gaseous and nutritive ingredients. Moreover, it should not adhere to the wound, in order to allow new tissue growth, and it must be easily removed. For this purpose, specialized non-woven tissues made from engineered biomaterials are used that are biocompatible, non-allergenic, and non-toxic. They also promote wound healing because they can modulate ECM synthesis and regulate microbial growth.

In addition, the application of appropriate fibres capable of making the non-woven tissues free of binder and chemicals, and of carrying active ingredients for tissue repair and tissue regeneration, are becoming important concerns in the field of medical textiles. For better skin tissue regeneration, in fact, natural

polysaccharides like chitin or chitosan are required (Figure 3), since they seem to promote production and deposition of ECM in a tissue-specific way [5].

Figure 3. The polysaccharide chemical structures of cellulose, chitin and chitosan

Chitin, in fact, is the second most abundant biopolymer after cellulose and is found mainly in the exoskeletons of shellfish and insects, and in the cell walls of mushrooms. When nanostructured, it is considered to have great potential for application in tissue engineering scaffolds, drug delivery and dressings, as well. For this purpose, Morganti *et al.* developed and patented a single closed method for the preparation of chitin nanofibrils (CN) as shown in Scheme 1.

Scheme 1. Chitin nanofibril production cycle

It has been shown that this natural polymer, when nanostructured and electrospun, organized as a porous ECM-like structure (Figure 4), allowing cells to be seeded; thus having favourable biological properties for tissue regeneration [6-8].

Figure 4. ECM-like porous structure of a non-woven tissues based on the use of CN at scanning electron microscope (SEM)

For these reasons, our group has produced non-woven tissues based on CN cross-linked with hyaluronic acid (HA) by use of the gelation method and electrospinning technology (Figure 5) [9].

Figure 5. Non-woven tissues have been obtained by the gelation method and electrospinning technology, using HA and CN block-copolymeric nanoparticles.

The advantage of these natural CN-based non-woven tissues over the others is the capacity to control the thickness and composition of nanofibres, as well as the porosity of the obtained meshes. It is possible, in fact, to synthesize fibres of the desired diameters by the simplest and most economical method. The pore size and density, in reality, play an important role in many biomedical applications, resembling the architecture of the ECM [10]. The main parameters in controlling the diameter and uniformity of the electrospun nanofibres are: polymer molecular weight and concentration, viscosity, surface tension and conductivity of the solution / suspension, and the voltage selected. Thus, electrospinning, which also provides the possibility of combining different nanomaterials (NM) to produce polymeric nanocomposites, offers enormous advantages over traditional non-woven tissues made by macro-or-micro sized polymers.

5.2. THE MARKET

As recently reported [11], the available medical non-woven market is projected to reach US \$20 billion by 2017, and the market size for nanotechnology is expected to grow to over US \$3 trillion [12]. In these growing markets, "non-woven tissues remain the component of choice for providing appropriate protection to the skin, due to their ability to create barriers either from the structure of the non-woven itself or from an additional active coating for personal protective apparel" [13].

According to this report, advanced medications are used to a large extent in healthcare for making products designed to provide appropriate "*protective barriers*" between the patient and himself, patient and physician, and between different patients. The reason for such barriers is to reduce the spread of hospital infections, which are still rising worldwide along with antibiotic-resistance.

What about the production locations? Even though China is the current leading global exporter of non-woven roll goods, for the future of manufacturing, converting and using these medical dressings will represent potential opportunities of growth for the home markets, primarily because of geopolitical instabilities, unpredictable fuel costs, and increasing regulations [14,15]. In any case, to be competitive in the global market, product innovation is necessary, especially to enhance the quality of life of elderly or disabled people [16]. In the wound care industry, the goal is to create thinner dressings that are made by sustainable bionanotechnology with the use of active and green nanoparticles that have similar absorption properties as foam dressings.

Incoming wound care dressings must provide the ability to assess the speed and progress of wound healing, and permit the use of antimicrobial agents to minimize the potential for infections. In this field, nanotechnology may have a positive and strong impact. To this end, the possibility of directly binding

antimicrobial components, such as Ag^+ ions or other active ingredients, into the CN fibre before producing the non-woven tissue has been shown by electrospinning technology [17]. With this new technique it has been possible to reduce the concentration of Ag^+ ions in the final product, and strongly reduce its potential toxicity, and its environmental impact while maintaining its antibacterial effectiveness. However, the industrial sustainability of the entire life cycle of any medical dressing has to involve the use of safe, biodegradable, and recyclable active ingredients and fibres, causing less burden overall on human beings and the environment.

5.3. CHITIN NANOFIBRIL-HYALURONIC ACID NANOPARTICLES

The use of CN, HA and their derivatives are gaining popularity because they are natural polymers produced from the by-products of fisheries, according to the European Union (EU) and Organisation for Economic Co-operation and Development (OECD) programs for a forthcoming greener economy [18,19]. Both of these polymers are totally biodegradable, biocompatible, ecologically-friendly, and compostable. It is interesting to underline that, while HA is a polymer distributed along our body, as a fundamental component of the ECM, chitin has the same backbone (Figure 6) and is an important component of human cartilage that is necessary for bone articulation. For instance, chitin's reduced synthesis during the aging process is one of the causes of osteoporosis (Figure 7).

Figure 6. Chitin has the same backbone of HA

Figure 7. The reduced synthesis of glucosamine is one of the cause of osteoporosis: right normal tissue, left osteoblasts tissue of a patient affected by osteoporosis

Nonetheless, it is important to remember that while the environment is rich in chitinase enzymes, capable of metabolizing chitin-derived compounds, the human body possesses 18 families of the same enzyme, named chitotridase [20], which are necessary to catatabolize chitin / chitosan into its components: glucosamine and acetyl glucosamine. These important molecules, useful for the synthesis of glycosaminoglycans (Figure 8), are also involved in the process of glycosylation, which is necessary to bond amino acids and glycoside compounds (Figure 9).

This is the reason that CNs are non-toxic and completely safe for both humans and for the environment, when recovered as composites of the micro dimension or as single components of nanodimensions.

Figure 8. Glucuronic acid, a component of HA, and glucosamine, a component of chitin are molecules necessary to produce glycosaminoglycans

Figure 9. Physiological process of glycosylation involving glucose and *N*-acetyl glucosamine

The block copolymeric micro / nanoparticles CN-HA have been obtained by the gelation method in distilled water followed by refinement of the micro lamellae, as the result of the combination of electropositive CN and electronegative HA (Figure 10). Control of their size and dimensions has been verified by a Zetasizer and by SEM [21,22]. It is interesting to underline that during the gelation method, before mixing together the two water suspensions of CN/HA, different active ingredients, both water and oil-soluble, may be solubilised in advance by the use of surface active agents. According to the designed method, the nanoparticles can entrap the ingredients into their structure before the refinement process. Moreover, it is possible to regulate the size of the particles and the surface electrical charges, thereby modulating their penetrability towards targeted delivery. The obtained nanoparticles, in fact, have shown an ability to disturb the SC *lamellae* and increase their diffusion through the skin layers, because of the cationic character of CN.

Figure 10. Copolymeric nano *lamellae* and nanoparticles obtained by complexing CN with HA, by SEM

During the patented chitin nanocrystals (nanofibrils) production process, the final fibril composition is about 30 units of glucosamine / acetyl glucosamine in about a 1 : 1 ratio, in contrast to natural chitin in crustaceans where acetyl glucosamine is predominant (Figure 11).

Figure 11. Chemical structures of CN and Chitosan. When the degree of *N*-acetylation (DA) is greater than 50 %, the polysaccharide is considered to be CN. When the DA is less than 50 %, the polysaccharide is considered to be chitosan.

For this reason, both CN and chitosan, which are positively charged, can form a complex with many types of negatively-charged polymers and large molecules, such as HA or lignin.

This property of CN-based composites has been shown to be significant in the modulation of cell behaviour during tissue regeneration [23-25]. It seems possible that positively-charged CN, interacting with negatively charged glycosaminoglycans and proteoglycans, fundamental components of the ECM, may have an important role in tissue regeneration. This could be the reason for

the regenerative activity in repairing burned skin that was recently shown by the use of non-woven tissues made by CN as the main component (Figure 12) [26].

Figure 12. Repairing activity on a burned skin obtained by the use of a non-woven tissue made by CN bonded with Ag^+ ions

Of course the final composition of the obtained nanoparticles is fundamental to their activity, for example, at the skin level. Following exposure, in fact, some NM have been shown to penetrate beyond the skin, with the extent of penetration being dependent on the ability of the NM to cross biological barriers. However, skin penetration is also a function of NM size and other properties, such as surface charge, as well as formation of the protein or lipid corona on the NM surface [27]. Moreover, the excellent mechanical properties of CN combined with its good gas barrier properties also may find many applications in the food packaging industry. Thus, as a producer of CN, our research group participated in the EU project n-CHITOPACK (www.n-Chitopack.eu).

5.4. CONCLUSIVE REMARKS

Nanoscience constitutes a challenging scientific frontier capable of engineering materials on the scale of a nanometer, *i.e.* a billionth of a meter (Figure 13.), revolutionizing modern life. Thus, nanotechnology, as one of the key enabling technologies (KETs) identified by the EU commission [28] for contributing to sustainable development of high-tech applications, is expected to stimulate industrial growth innovation and development, not only in electronics but also in plant growth, in food packaging, and in cosmetic and pharmaceutical fields (Scheme 2). It has been predicted, therefore, that by 2020 nearly every industry will be affected by this new scientific frontier.

Figure 13. The nanometer scale

On the nanoscale, in fact, common materials can take on new physicochemical and biological properties, opening up new possibilities of exploitation for commercial small, medium enterprises (SMEs). For these reasons, the use of bionanomaterials is steadily increasing day by day due to the new properties addressed by the reduced dimensions of the ingredients used.

Scheme 2. Display of expected nanotechnology's stimulation of industrial growth innovation and development in electronics, in plant growth, in food packaging, and in cosmetic and pharmaceutical fields

This new technology particularly impacts industrial sectors, such as cosmetics, drugs and advanced medications, where safety and social health are important elements.

To this end, the last published data show a significant increase in the production of the most representative nanomaterials with an expected growth of 2 billion jobs by the end of 2015, and more than 1000 nano-enabled products currently available on the market in more than 20 different countries.

In this context, the EU is responsible for 30 % of nanomaterial manufacturing and use, including polymers containing nano-reinforcements. It is important to underline, in fact, that one of the main applications of nanotechnology in material science is the development of polymer nanocomposites, which are polymers reinforced with a low quantity of nanosized organic or inorganic ingredients dispersed into the polymer matrix.

5.5. ROLE OF CN AND CHITIN-DERIVATIVES IN COMPOSITE DEVELOPMENT

Thus, in composite manufacturing for the development of biocomponent fibres by meltdown (casting) and spunbound (electrospinning) technologies, the use of CN or CN-HA nanoparticles offers enormous advantages over traditional macro- or micro-sized fillers and applications across a wide range of industrial sectors, such as food packaging and advanced medications, as revealed by the recent results of the EU research projects n-CHITOPACK (www.n-Chitopack.eu), Bio-Mimetic (www.biomimetic.eu), and Chitofarma (www.mavicosmetics.it).

Through these projects, in which the MAVI Nanoscience Centre has been involved as an owner of CN technology and processes, thin transparent and flexible food-packaging based on the casting technology (Figure 14), hard food packaging for meat and / or coffee-caps (Figure 15), advanced medications and beauty masks (Figure 16) have been obtained.

Step by step preparation procedure of n-CHITOPACK films

Note: Poly(ethylene terephthalate) and Dura Lar films or stainless steel sheets were used as supports for casting the (CS/CN)/glycerol slurries

Figure 14. The casting production at lab level (By courtesy of G. Tischenko)

Figure 15. Coffee-caps and hard food containers made by PLA and CN

Figure 16. Advanced medications (right) and beauty masks (left) made by the use of CN

All of these containers, based on the use of CN, are totally biodegradable and compostable, and are safe for both humans and the environment. Moreover, it is interesting to underline how the mechanical properties of the films made of CN, chitosan and glycerol, generally used to temporary pack sandwiches, have shown more than three times higher elasticity and mechanical strength compared with those of cellulosic paper. On the other hand, hard containers made of poly(lactic acid) (PLA) reinforced by CN, have shown better resistance to the aging process, but still possess the same interesting compostability, and easy biodegradability recovered from the advanced medications also.

Thus the one-off use of this film and daily use of these hard food containers could decrease environmental pollution by the major utilization of chitin waste and reduced consumption of petrol-derived polymers. In addition, deforestation and the presence of greenhouse gas emissions also will be reduced because of the major use of the plant biomass.

In conclusion, the intricate composite network obtained by the meltdown and electrospun natural fibers, such as CN from crustacean waste and lignocellulosic compounds and manmade PLA from plant biomass, open new perspectives to produce promising biomimetic candidates for practical applications in biomedicine and tissue engineering [29], as well as in the food packaging field.

As previously reported, chitin-based biomaterials have favourable biological properties for tissue regeneration, since they are capable of restoring function and architecture of both aged and damaged skin [30]. These polysaccharide polymers play major roles in maintaining body contours by providing mechanical cushion and protection in recovering epithelium and soft tissue for their regeneration [31,32], making them safe for the human body and the environment.

In addition, it is important to underline the capacity that CN have shown for delivering active ingredients in the optimum dosage, increasing their bioavailability at skin level, and enhancing their efficacy compared to normal chitin, probably because of CN's nanodimensions of $240 \times 7 \times 5$ and needle-like crystal form (Figure 17).

Figure 17. CN by SEM

According to our first obtained results on the use of CN as a skin regenerative ingredient [33], new efforts of our group are focused on better understanding the activity of CN when used to make block copolymeric nanoparticles for pharmaceutical and cosmetic use, or to produce polymers for making

nanocomposites with other natural fibres for making biomedical non-woven tissues and/or innovative food packaging.

Our future goal is to use CN, lignin, and other lignocellulosic compounds to produce innovative anti-aging emulsions / beauty masks and regenerative advanced medications, as well as to use PLA for making innovative food packaging that is completely biodegradable and compostable.

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REFERENCES

1. P.M. Elias, G.K. Menon. *Adv. Lipid. Res.* **24** (1991) 1–26.
2. G.K. Menon, K.R. Feingold, M.P. Elias. *J. Invest. Dermatol.* **98** (1992) 279–289.
3. P.M. Elias, J.C. Ansel, L.C. Woods, K.R. Feingold. *Arch. Dermatol.* **132** (1996) 1505–1506.
4. M. Cho, T.K. Hunt, in *Cutaneous Wound Healing*, V. Falanga (Ed.) London, UK, Martin Dunitz, 2001, pp. 141–154.
5. T.L. Yang, T.H. Young. *Biomaterials* **29** (2008) 2501–2508.
6. M.V. Risbud, M. Sittering. *Trends Biotech.* **20** (2002) 351–356.
7. A. Di Martino, M. Sittering, M.V. Risbud. *Biomaterials* **26** (2005) 5983–5990.
8. C.E. Ayers, B.S. Jha, S.A. Sell, G.L. Bowlin, D.G. Simpson. *Wiley Interdiscip. Rev. Nanomed. Nanobiotech.* **2** (2010) 20–34.
9. D.H. Remember, I. Chun. *Nanotechnology* **7**(3) (1996) 216–223.
10. S.D. McCullen, S. Ramaswamy, L.I. Clarke, R.E. Gorga. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **1** (2006) 369–390.
11. O. Gia. Medical Nonwoven Disposables: A Global Strategic Business Report. Global Industry Analysts, Inc., San Jose, CA, March 2012.
12. Lux Research Inc., Nanomaterials of the Market. www.luxresearchinc.com (2009).
13. V.A. Barbur. The Future of Nonwovens in Medical Markets, http://www.nonwovens-industry.com/issues/2012-08/view_features/the-future-of-nonwovens-in-medical-markets (2012)
14. J. Prigneaux. Nonwoven Trade Reaches Record in 2011. *EDANA*. Nonwoven Industry, May 2012.
15. E. Krenzer, M. Evans. Stay Relevant in a High Volume, Commoditized, Medical Device Product Line in Manufacturing (Insourced and Outsourced). *Medical Device Manufacturing Summit*, Las Vegas, 2012.
16. H. Meinander, M. Varheenma. Clothing and Textiles for Disabled and Elderly People. *VTT Tiedotteita Research Notes*, 2002.
17. P. Morganti, G. Tishchenko, M. Palombo, I. Kelnar, L. Brozova, M. Spirkova, E. Pavlova, L. Kobera, F. Carezzi, in *Marine Cosmeceuticals. Trends and Prospects*, S.K. Kim (Ed.), New York, CRC-Press, 2013, pp. 681–715.

18. European Commission (EC), *Innovation for Sustainable Growth: A Bioeconomy for [europe.COM](#) final*, EC, Brussels, Belgium, 2012.
19. Organization for Economic Cooperation and Development (OECD), *The Bioeconomy to 2030: Designing a Policy Agenda, Main Findings*, OECD, Paris, France, 2009.
20. K.B. Eide, A.L. Norberg, E.B. Heggeset, A.R. Lindbom, K.M. Varum, V.G.H. Eijsink, M. Sorlie. *Biochemistry* **51** (2012) 487–495.
21. P. Morganti, P. Del Ciotto, G. Fabrizi, F. Guarneri, A. Cardillo, M. Palombo, G. Morganti. *SOFW-Journal* **139**(1/2) (2013) 12–23.
22. P. Morganti, G. Di Massimo, C. Cimini, P. Del Ciotto. *Pers. Care Eur.* **6**(9) (2013) 61–66.
23. P. Mezzana. *Acta Chirurgiae Investig. Dermatol.* **3** (2008) 5–13.
24. M. Mattioli-Belmonte, A. Zizzi, G. Lucarini, F. Giantomassi, G. Biagini, M.G. Tucci, F. Orlando, M. Provinciali, F. Carezzi, P. Morganti. *J. Bioact. Compat. Polym.* **22** (2007) 525–538.
25. P. Morganti, M. Mattioli-Belmonte, M.G. Tucci, G. Ricotti, G. Biagini. *J. Appl. Cosmetol.* **18** (2000) 125–130.
26. M. Palombo, Innovative Solutions for Advanced Medications: Biochemical and Clinical Data, *Proceeding of the 11th ISCD International Congress of Cosmetic Dermatology*, Beijing, China, 2014, p. 38.
27. F. Got Schalk, B. Nowack. *J. Environ. Monitoring* **13** (2011) 1145–1155.
28. E. Van de Velde, C- Rammer, P. Padilla, P. Scliessler, O. Slivke, B. Gehrge, V. Bilsen, R. Lukach, *Key Enabling Technologies*, European Union, Brussels, Belgium, 2012.
29. M. Wysokowski, A.L. Stelling, D. Stawski, T. Jesionowski, H. Ehrlich. *Polymers* **7** (2015) 235–265.
30. K. Hemmrich, D. Von Heimburg, R. Rendchen, C. Di Bartolommeo, E. Milella, N. Pallua. *Biomaterials* **26** (2005) 7025–7037.
31. K. Kohima, Y. Okamoto, K. Zkojima, K. Miyatake, H. Fujise, Y. Shigemasa, S. Minami. *J. Vet. Med. Sci.* **66** (2004) 1595–1598.
32. J. Sharma, M. Lizu, M. Stewart, K. Zygula, Y. Lu, R. Chaiman, X. Yan, Z. Guo, E.K. Wujcik, S. Wei. *Polymers* **12** (2015) 1936–1963.
33. P. Morganti, F. Carezzi, P. Del Ciotto, G. Morganti, M.L. Nunziata, Y.H. Gao, H.D. Chen, G. Tischenko, W.E. Yudin, in *Nanobiotechnology*, D.A. Phoenix, W. Ahmed (Eds.), Manchester UK, One Central Press, 2014, pp. 1–31.