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Biological Activity of Innovative Polymeric Nanoparticles and Non–Woven Tissue

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Abstract: In the last few years many studies have focused their attention on different potential biomedical applications for biocompatibile polymers, especially nanopolymers, in shape of nanotubes, nanofibers, and nanoparticles. Polymer nanocomposites arising from different chemistries and constructions include aliphatic polyester such as polylactide (PLA) and poly (DL-lactic-cd-glycolic acid) (PLGA), poly (ε-caprolactone) (PCL), poly(*p*-dioxanone) (PPDO), poly(butylenes succinate) (PBS), poly (hydrdroxyalkanoate), and natural biopolymers, such as starch, cellulose, chitin, chitosan, lignin, and protein. In medicine, they contribute to applications in surgery, dentistry, and pharmachology for scaffolds for tissue regeneration, tissue engineering, drug delivery devices, and gene transfection. Bionanopolymers can be used in many other fields including food, cosmetics and agriculture. The research efforts are focused on the study of new polymeric nano-constructs that exploit the body's natural biological response and are environment-friendly.

1. Introduction

For thousands of years humans made tools and devices from naturally available compounds, and later artificial compounds were created to make them.

The interdisciplinary field of biotechnology and particularly nanobiotechnology, which combines biology, chemistry, engineering, nanopharmacology, and nanomedicine, is revolutionizing the development of drug delivery systems and tissue engineering, such as tissue engineered for vascular grafts and wound healing. Research in this area has provided unlimited potential to improve human health [1–3]. A large variety of materials can be used and are classified as synthetic, natural, or hybrid. The synthetic materials can be further classified in degradable and non-degradable materials. The synthetics include polymers, such as (poly(ethyleneglycol), *N*-(2-hydroxypropyl) and methacrylamide co-polymers, Natural polymers can be classified as those obtained from natural sources such as animal, microbial, and vegetable sources. They are usually natural proteins or polysaccharides, such as chitin, chitosan, (dextran (α-1,6 polyglucose), dextrin (α-1,4 polyglucose), lignin, collagen, gelatin, and hyaluronic acid. Interestingly, in the interaction of nanopolymers with the human immune system, is that chitin and hyaluronic acid show the same backbone (Figures 1 and 2).

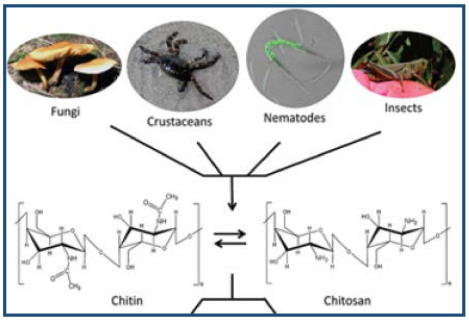

Figure 1. Chemical structures of Chitin and Chitosan from natural sources such as crustacean exoskeleton and fungi wall. Adapted from Morganti, P. et al. [4].

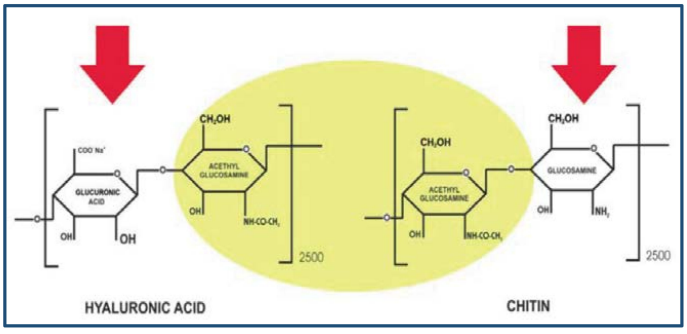

Figure 2. Chitin and hydrauronic acid structures. Adapted from Morganti, P. et al. [4].

Others include linear polyamidoamines and pseudosynthetic polymers the man-made poly (amino acids) poly(L-lysine), poly(L-glutei acid), poly(malic acid), and poly(aspartamides).

Natural polymers have the disadvantage of high biodegradability, while synthetic polymers can be synthesized and modified in a controlled manner to produce constant and homogenous physical and chemical properties and stability. However, the latter are biologically inert and do not offer the therapeutic advantages of natural polymers. Ongoing research is focused on the development of hybrid biodegradable materials for specific applications using new resorbable biomaterials and applying computational and combinatorial approaches to develop biomimetic polymer structures with unique chemistries, thus increasing diversity [4–8]. Tsao and coworkers [9] used a polyelectrolyte complex consisting of chitosan and (-poly (glutamic acid) (-PGA) as a wound dressing material. This complex combination offered good mechanical properties, suitable moisture content, and favorable removal without the damage of regenerated tissue. To form a suitable wound dressing Kim and coworkers [10]

used chitosan with poloxamer. A semi-interpenetrating polymer network provided enhanced compatibility and mechanical strength. Novel wound dressing applications have been devised by novel bioprocesses and advances in organic chemistry, thus enabling the development of enhanced smart polymers as candidates for specialized wound dressings that elicit favorable biological, physical, and chemical responses. Hydrocolloid dressings, the most widely used dressings, are obtained from colloidal materials that are gel-forming agents combined with other materials such as gelatin, elastomers, carboxymethylcellulose, pectin and adhesives. These agents can be bonded together to produce a thin film, sheet, or foam with the properties of hydrocolloids, thus forming a gel on the wound surface to promote moist wound healing Recent advances have been made in wound healing and dermal substitution. The main component of every wound is the connective tissue matrix, thus there is an overall consensus that in order to heal wounds effectively, it is necessary to ensure the effective substitution of the main component. Wound dressings are an essential part of wound management and care in order to enhance the natural wound healing process. The development of new intelligent dressings is under way, and these promise to play an active role in promoting healing of both acute and chronic wounds [11].

Other important nano-biopolymer applications are drug delivery devices and tissue engineering. Innovative delivery strategies based on nano and micro-particulate systems are currently being investigated for pulmonary delivery to improve drug transport to its target [12], and to develop a compatible small-diameter engineered tissue as a scaffold for vascular graft. This requires a compliant polymer scaffold to which endothelial cells can adhere, form an anti-thrombogenic luminal surface, exhibit vasoactive properties, and improve patency, and within which smooth muscle cells can migrate, deposit functional vascular ECM, and become contractile [13]. When choosing a material for surgical or pharmacological devices, it must meet specific requirements, and of utmost importance is the biocompatibility and biodegradability. The aim of this chapter is to provide an overview of the biological activity of innovative nanoparticles and non–woven tissue.

2. Bionanotechnology

Recent improvements have been made in the technique to produce biopolymers in the form of nanosized fibers [13], biodegradable polymeric nanospheres, nanorods, and nanotubes. Several important characteristics can be seen by reducing the diameters of polymer fiber materials from micrometers to submicron or nanometers, such as a very large surface area to volume ratio; the ratio of nanofiber in comparison with that of a microfiber may be as much as 10^3 greater. This also provides greater mechanical support in the functionalities, such as tensile strength and stiffness, and improved flexibility when compared to any other known form of the same material [14,15].

Electrospinning, a spinning technique, is a widely used technology for electrostatic fiber formation, which utilizes electrical forces to produce polymer fibers with diameters ranging from 2 nm to several micrometers using polymer solutions of both natural and synthetic polymers. This is a unique approach to produce fine fibers from polymer solutions or melts, which have a larger surface area and a thinner diameter (from nanometer to micrometer) than those obtained from conventional spinning processes. There are two standard electrospinning setups, vertical and horizontal [14,16]. With the expansion of this technology, more sophisticated systems that can fabricate more complex nanofibrous structures in a more controlled and efficient manner have been developed. Electrospinning is conducted at room temperature under atmospheric conditions. An electrospinning system has three components: a high voltage power supply, a spinneret (e.g., a pipette tip), and a collecting plate (usually a metal screen, plate, or rotating mandrel). It utilizes a high voltage source to inject a charge of a certain polarity into a polymer solution or melt, which is then accelerated towards a collector of opposite polarity. Most polymers are dissolved in solvents before electrospinning, and when they completely dissolve, they form a polymer solution. The polymer fluid is then inserted into the capillary tube for electrospinning. In the electrospinning process, a polymer solution held by its surface tension at the end of a capillary tube is subjected to an electric field, thus inducing an electric charge on the liquid surface. When the electric field applied reaches a critical value, the repulsive electrical forces overcome the surface tension forces. A charged jet of the solution is then ejected from the tip of the Taylor cone and an unstable and rapid whipping of the jet occurs in the space between the capillary tip and collector, which leads to the evaporation of the solvent, leaving a polymer behind. The jet is only stable at the tip of the spinneret and after that instability starts [17–19] (Figures 3 and 4). A wide range of polymers that are able to form fine nanofibers within the submicron range suitable for varied applications are used in electrospinning. Electrospun nanofibers have been produced from natural polymers, synthetic polymers, or a blend of both, including nucleic acids, proteins, and even polysaccharides. More than 200 polymers have been electrospun successfully from natural polymers and characterized according to their applications. When used in biomedical applications, naturally occurring polymers normally exhibit good biocompatibility and low immunogenicity when compared to synthetic polymers. An advantage of natural polymers for electrospinning is their capacity to bind to cells since they carry specific protein sequences, such as RGD, arginine/glycine/aspartic acid sequences [17]. Nanoparticles can be defined as particulate matter having at least one dimension that is less than 100nm and an exceptionally high surface to volume ratio, which contributes to their unusual properties and behavior. Furthermore, because of their high surface area, the surface structure also differs from that of the core. Specific and suitable functional groups

can be attached to the surface of a nanoparticle to allow it to reach its target and interact with the biological system [11].

Polymeric nanoparticles (NP) are a promising resource for drug delivery. These carriers must be designed for a specific purpose since the material used affects the distribution inside of the body and the uptake into certain cells. The NP size affects the ability to penetrate barriers in the body. It is important to select an appropriate approach for the specific drug (hydrophobic vs. hydrophilic) and delivery route. Drug incorporation by adsorption or entrapment can alter the size and physicochemical properties that determine the NP interactions [20,21].

Different methods are used for gelatin NP preparations: the desolvation method and the water-in-oil emulsification method. The protocols for chitosan NPs include the ionotropic gelation method and another method that includes complex coacervation, emulsion evaporation, nanoprecipitation, and radical polymerization [22]. For synthetic polymer preparations, such as PLGA nanoparticles, which are made from a copolymer of polylactic acid and polyglycolic acid, the salting out method or nanoprecipitation, also known as solvent diffusion or solvent displacement, is used. The emulsification methods include emulsion diffusion, a top-down technique that starts by dissolving the pre-formed polymer in an organic solvent that is partially miscible with water (e.g., ethylacetate, dichloromethane, or acetone/methanol) and emulsion evaporation, another top-down method used for the formation of PLGA NPs. The emulsion dispersion polymerization method is used to prepare polyalkyl(cyano)acrylate (PCA) nanoparticles [23].

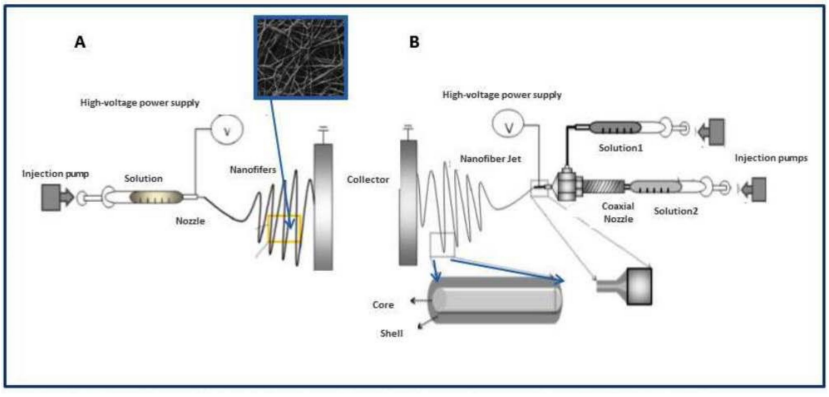

Figure 3. Schematic diagram of electrospinning setup consisting of injection pump/s, power supply, nozzle and conducting collector. A: One type of solution. B: Two type of solution and a core-shell nozzle design used to encapsulate drugs within the nanofiber. Adapted from Shin, S.H. et al. [24].

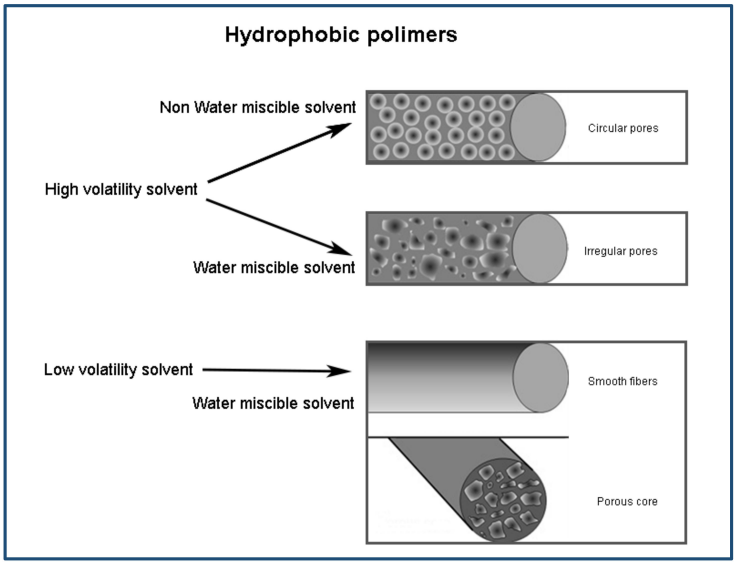

Figure 4. Fibers for hydrophobic polymers, electrospun at high percent RH, obtained with different solvent, show different morphology. Adapted from Nezarati, R.M. et al. [19].

3. Biological Activity

Like any biomaterial, a functional polymeric system aimed at serving for a limited period of time before degradation and elimination from the body must first fulfill strict criteria related to biocompatibility and biofunctionality. Therapeutic devices made from biomaterials, especially polymers, must permit in vivo: no or acceptable toxicity, no immune reaction, no carcinogenic mutation and no thrombus formation to meet the strict criteria of biocompatibility. Regarding biofunctionality, these devices must offer adequate mechanical, chemical, thermal, physical, and biological properties, and must be sterile and easy to handle [23]. Increasing attention to the environment has brought about an in-depth study of the biological activity of natural polymers or artificial polymers that respect the characteristics of biodegradability and biocompatibility, such as PLA or PLGA. Besides the application of chitin as a starting material for the synthesis of chitosan and chito-oligosaccharides, chitin itself has been a center of many therapeutic applications, and is thought to be a promising biomaterial for tissue engineering and stem-cell technologies. In 2013, Bae and coworkers [25] demonstrated that the oral administration of chitin (α and β forms) is beneficial in preventing food allergies; the oral administration of chitin was accomplished by milling it to particle size less than 20 m and mixing it with the feed. Their results showed that the α-form reduced the serum levels of peanut-specific IgE and both the forms decreased the levels of

interleukin IL-5 and IL-10, and increased the levels of IL-12. Dietary supplementation of chitin was shown to exert positive immunomodulatory effects; the antibacterial activity of chitin, prepared from shrimp-shell waste, was reported by Benhabiles et al. [26]. Chitosan, a natural nontoxic biopolymer that is produced by the deacetylation of chitin, has been noted for its application as a film-forming agent in cosmetics, a dye binder for textiles, a strengthening additive in paper, and a hypolipidic material in diets. It has been used extensively as a biomaterial owing to its immune-stimulatory activities, anticoagulant properties, antibacterial and antifungal action, and its action as a promoter of wound healing in surgery. The antimicrobial activity of chitosan has been demonstrated against many bacteria, filamentous fungi, and yeasts. Chitosan has a wide spectrum of activity and high killing rate against Gram-positive and Gram-negative bacteria but a lower toxicity toward mammalian cells. Due to the presence of hydroxyl, amine, and acetylated amine groups, chitosan, low molecular weight chitosan, and chito-oligosaccharides (COS) interact with various cell receptors that trigger a cascade of interconnected reactions in organisms, which results in anti-inflammatory, anticancerogenic, antidiabetic, antimicrobial, anti-HIV-1, antioxidant, antiangiogenic, neuroprotective, and immunostimulative effects [27]. Both in the form of nanoparticles or nanofibers these biopolymers play an important role in helping the human body in its outstanding ability to self-repair.

3.1. Nanoparticles

Nanoparticles can be defined as ultradispersed solid supramolecular structures with a submicrometer size ranging from 10 to 1000 μm, used especially to produce drug delivery devices (Figure 5). The nanoparticle matrix with the drugs (dissolved, entrapped, encapsulated, or attached) acts as a reservoir for particulate systems and plays an important role as a drug delivery device, e.g., in oncology [28]. Nanoparticles fabricated from polysaccharides, proteins and biocompatible/biodegradable polymers, such as polyethylene glycol (PEG), poly(γ-benzyl l-glutamate) (PBLG), poly(D,L-lactide), poly(lactic acid), poly(D,L-glycolide), poly(lactide-co-glycolide), polycyanoacrylate, chitosan, gelatin, and sodium alginate are called PNPs [27]. The various materials used in NP preparation show different drug release kinetics, which can be achieved through desorption of surface-bound drugs, diffusion through the NP polymer wall, or erosion of the NP matrix. Drugs can be released with a burst mechanism or in a sustained manner. Synthetic polymers can be more easily engineered to produce a sustained release than natural polymers. In addition to the choice of polymer, the loading method has a strong impact on the release rate. However, the choice of the loading method depends on the drug, as certain drugs are more effective when released in a large burst, but controlled release is better for long-term effects; the size of the particle is equally important. The drug encapsulated will have an impact on the nanoparticle size, but it is difficult to predict which

method of encapsulation will impact the size. This parameter is crucial to determine which organs the particle may reach and whether or not it will be opsonized by macrophages. Once NPs are in the circulation they can pass, according to their size, through fenestrations in the endothelial barrier. Table 1 shows the materials commonly used for NP preparation.

Chitosan has been the impetus for the development of safe and effective drug delivery devices. Its primary hydroxyl and amine groups that are located on the backbone permit chemical modifications to control its physical properties. The interaction of the hydrophobic moiety with a chitosan molecule determines an amphiphile that can form self-assembled nanoparticles that are able to encapsulate a quantity of drugs and deliver them to a specific site of action. Chemical attachment of the drug to the chitosan throughout the functional linker may produce useful pro-drugs, exhibiting the appropriate biological activity at the target site [29].

Synthetic polymers, when compared to natural polymers, are generally more homogenous in composition and have a higher purity, thus making the preparation of NPs more reproducible. However, it should be noted that not all of the synthetic polymers are suitable for drug delivery as they need to be biodegradable and exert low cytotoxicity. NPs made from synthetic polymers are polyesters, which include poly(lactic acid), poly(glycolic acid), their co-polymer: poly(lactide-co-glycolic acid) (PLGA), as well as polyalkyl(cyano)acrylates. These are not new and have been extensively used in clinical settings, e.g., PLA has been used for surgical sutures and implants, while PCAs have been studied for their use in sealing wounds. Knowledge of the degradation kinetics of such polymers allows for the preparation of better formulations for a more controlled release. Because of the large amount of materials used for NP preparations (Table 1) and the extensive field of application as drug delivery devices, it is not possible to provide a comprehensive overview. We have limited our intervention to some recent studies that investigated the use and biological activity of different NPs.

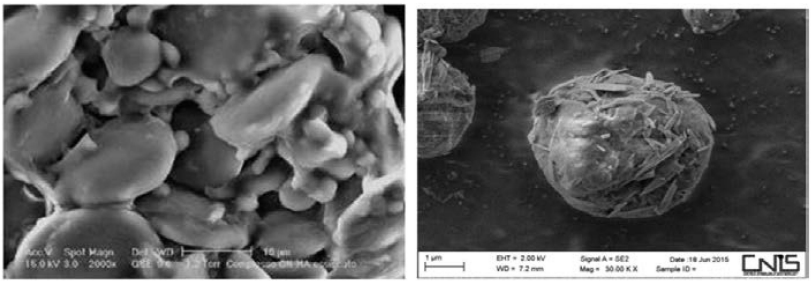

Figure 5. Micro/nanoparticles of the complex chitin nanoparticle-nanolignin. From Morganti, P. et al. [4].

Table 1. List of organic materials used to formulate nanoparticle drug carriers.

Polymeric Based Nanoparticles	Non-Polymeric Based Nanoparticles
Natural sources	
Gelatin Chitosan Alginate Nano-crystalline cellulose	Carbon-based carriers Liposomes Solid lipid nanoparticles
Synthetic sources	
Poly(lactic-co-glycolic)acid Poly-n-(cyanoacrylate) Polycaprolactone	

Lai [22], Dinavard [30], Nitta [31], and coworkers offer a good overview of the preparation and ability of polymeric nanoparticles as a drug-delivery system for gene deliver, anticancer drug delivery, and antibiotics delivery. Recently, in the treatment of pulmonary infections, the use of antibiotics for inhalation has gained increasing attention, particularly for cystic fibrosis (CF) patients. Aerosolized antibiotics offer an interesting way to deliver high drug concentrations directly to the site of infection, which reduces the toxicity and enhances the therapeutic potential of the antimicrobial agents against resistant microorganisms.

In 2009, Tahara et al. [32] demonstrated in vitro that chitosan-modified PLGA NSs (CS-PLGA NSs) are preferentially taken up by human lung adenocarcinoma cells (A549). Cellular uptake of PLGA NS was confirmed using fluorescence spectrophotometry and was visualized in A549 cells with confocal laser scanning microscopy (CLSM). The cytotoxicity of non-and CS-PLGA NS systems were compared in vitro using a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2Htetrazolium (MTS) assay; CS-PLGA NSs did not show cytotoxicity to A549 cells. The cellular uptake of non- and CS-PLGA NSs is a time-, temperature-, and concentration-dependent saturable event mediated by clathrin-coated pit endocytic pathways, and that of PLGA NSs is related to a particle diameter. CS modification through electrostatic interactions between the CS adsorbed to the NS surface and the negatively charged cell membrane improved the cellular uptake of PLGA NSs. PLGA NS and CS-PLGA NS (200-nm) were internalized by A549 cells through adsorptive endocytosis started by nonspecific interactions between NS and cell membranes, triggered partially by a clathrin-mediated process. NSs have been evaluated for the delivery of different types of therapeutic agents, such as DNA, peptides, and proteins. Moreover, NSs protect the encapsulated drugs from enzymatic degradation and therefore provide a sustained release. In conclusion,

CS-PLGA NSs are better drug carriers because of a high cellular uptake due to their strong interaction with the cells and a low cytotoxicity.

Ungaro and coworkers [33] designed and developed a pulmonary delivery system for antibiotics based on spray-dried lactose/PLGA nano-embedded microparticles (NEM), engineered at the nanosize and microsize levels. To test the use of NEM, Tobramycin (Tb), the first choice antibiotic in lung infections for CF treatment, has been chosen. The results showed that to improve the size and modulate the surface properties of Tb-loaded PLGA NPs, poly(vinyl alcohol) (PVA), and chitosan are essential, and the use of alginate (Alg) allows an efficient Tb entrapment within NPs and its release up to one month. The optimized formulations of NP spray-drying with lactose offered NEM with particular flow and aerosolization properties without altering the NP features. The Tb-loaded PLGA NPs showed good in vitro antimicrobial activity against *P. aeruginosa* planktonic cells. In addition, the bio-distribution studies showed that PVA-modified Alg/PLGA NPs reached the deep lung, while CS-modified NPs remained in the upper airways lining lung epithelial surfaces. In conclusion, the composition of PLGA NP plays a crucial role in determining the technological features of NPs, and when NPs are processed in the form of NEMs, their in vitro/in-vivo deposition pattern is also modified

Recently Piras et al. [34] evaluated as an antimicrobial protein drug model, chitosan in a new formulation of nanoparticles loaded with lysozyme (LZ). LZ-loaded nanoparticles (LZ-NPs) of 150 nm diameter were prepared by inotropic gelation. They demonstrated that, these nanoparticles preserved the antibacterial activity of the loaded enzyme, which was slowly released over three weeks in vitro and remained active against *Staphylococcus epidermidis*, up to five days of incubation. So, beyond the intrinsic antibacterial activity of CS and LZ, the LZ-NPs showed a sustained antibacterial activity that resulted in about a 2-log decrease in the number of viable *S. epidermidis* compared to plain CS nanoparticles, and showed a full in vitro cytocompatibility towards murine fibroblasts.

It is well known that the development of small interfering RNA (siRNA)-controlled-release NPs may improve the therapeutic efficacy of RNA interference (RNAi) by prolonging their release to allow long-term gene silencing. In 2014 Shi et al. [35], proposed an NP platform with sustained siRNA-release properties, which can be self-assembled using biodegradable and biocompatible polymers and lipids, with an excellent silencing efficacy. The temporal release of siRNA from the NPs continued for over one month. When tested in vitro on luciferase-expressed HeLa cells and A549 cells after short-term transfection, the siRNA NPs showed greater sustained silencing activity than lipofectamine 2000-siRNA complexes. More importantly, the NP-mediated sustained silencing of prohibitin 1 (PHB1) generates more effective tumor cell growth inhibition in-vitro and in vivo than the lipofectamine complexes.

Morganti and coworkers [36] are working on the formulation of complex chitin–hyaluronan nanoparticles as a multifunctional carrier to deliver anti-aging active ingredients through the skin. They are evaluating in vitro its antioxidant capacity, anti-collagenase activity, and metalloproteinase, and anti-inflammatory mediator release. These copolymeric nanoparticles are able to entrap different kinds of active ingredients and release them at different times, depending on the productive process adopted and fine size of the micro/macro particles designed [37,38]. It is interesting to underscore how these nanoparticles, based on the use of chitin nanofibrils and obtained from crustacean waste, support the industrial sustainability [39] and respect the indications of the in-progress green economy [40].

3.2. Non-Woven Tissue

The current direction in the research area of non-woven tissue is to create a scaffold that mimics the structure and function of the native extracellular matrix (ECM) (Figure 6). The best scaffold for clinical use is one that has both structural integrity and allows for normal cellular function and interaction [41]. There is growing evidence that nanofibers amplify certain biological responses, such as cellular contact guidance and differentiation [42]. According to the medical application, i.e., wound dressing, small-diameter vascular graft, several parameters must be analyzed, such as: material selection, scaffold design, porosity, mechanical properties, fiber morphology, and cytocompatibility. Chitosan (CS) is a natural chitin-derived polysaccharide that is extensively used as a biomaterial in different engineering applications due to its low cost, large-scale availability, antimicrobial activity, biodegradability, and biocompatibility [43–46]. Besides nanoparticles, many authors have focused their studies to evaluate in vitro the biocompatibility of new co-polymer based on chitosan.

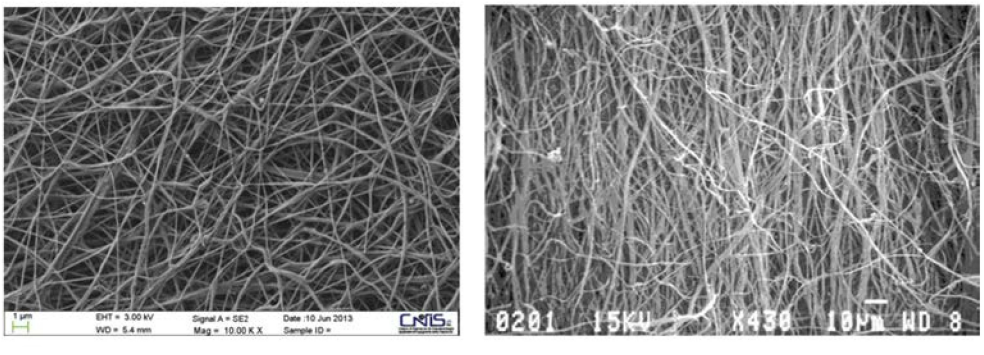

Figure 6. Similarity between the structure of Chitin Nanofibril scaffold (left) and skin ECM (right). From Morganti, P. et al. [4].

In 2013 Wang et al. [13], evaluated the biocompatibility of an electrospun chitosan/collagen complex as a scaffold in vascular tissue engineering to support the cell adhesion, proliferation, and phenotypic expression of endothelial cell markers by PIECs in-vitro. The chitosan/collagen scaffold showed endothelial cell adhesion, and did not adversely affect cellular function. In addition, the chitosan/collagen scaffold (w/w; 50/50, 20/80), in particular, showed the highest potential for vascular tissue engineering.

Naseri and coworkers [47], developed electrospun chitosan/polyethylene oxide-based randomly oriented fiber mats, reinforced with 50 wt % chitin nanocrystals (ChNC) for wound dressing. The results showed that the electrospun porous random mats comprising ChNC were free from any defects because of a homogeneous dispersion of ChNC in the chitosan matrix, indicating good chemical compatibility between the matrix and the chitin. The addition of chitin nano-crystals improved the moisture stability of these mats and facilitated water-mediated cross-linking processes. The cross-linked nanocomposite fiber mats with 50 wt % chitin nano-crystals had a high surface area (35 m^2 g^{-1}), a high tensile strength of 64.9 MPa and modulus of 10.2 GPa, and were at the same time flexible, and, therefore, were considered as beneficial for wound healing. The water vapor transmission rate of these mats was between 1290 and 1548 g m^{-2} day^{-1}, and was in the range for injured skin or wounds. The compatibility towards adipose-derived stem cells of the electrospun fiber mats confirmed their potential use as wound dressing materials [32]. Many other associations have been tested in the last few years. Enrione et al. [48] designed a gelatin/chitosan/hyaluronic acid biopolymer using a thermophysical approach for use in tissue engineering. The gelatin/chitosan/hyaluronic acid biopolymeric scaffold was made by applying a modification of the method described by Liu et al. [49]. Its thermal characterization was performed using differential scanning calorimetry (DSC), and its physical characterization by gas pycnometry and scanning electron microscopy. The effects of the gelatin (Ge) content and cross-linking on the thermophysical properties were evaluated by means of a factorial experiment design (central composite face centered). The Ge content was the main factor that affected the thermophysical properties (microstructure and thermal transitions) of the scaffold for tissue engineering, which were studied by seeding skin cells on the biopolymers. Different amounts of Ge did not affect cell attachment, while the cell growth rate increased linearly with the decrease in the Ge content. This relationship, together with the thermophysical characterization, can be used to design scaffolds for tissue engineering. Finally, the authors concluded that a Ge stock solution of 0.8% was adequate to formulate Ge/Ch/Ha-scaffolds to seed fibroblasts.

Different types of chitin- and chitosan-based wound dressing materials are commercially available. Chitin and chitosan in the form of composites, gels,

nanofibers, films, non-wovens, and scaffolds have been used to regenerate wounded tissues. Nowadays, commercial products are available for topical application in wound repair based on chitin nanofiber polymers. Our research group [50] tested in vitro a novel combination of chitin nanofiber/lignin in different ratios for their anti-inflammatory and wound repair activity in experimental models of human keratinocytes. For the evaluation of the anti-inflammatory effect of chitin /lignin nanofibers, the IL-8, IL-1α and TNF-α expressions were analyzed on human keratinocytes treated with lipopolysaccharide of *P. aeruginosa* (LPS). Also evaluated were the expression of the beta-defensin 2 (hBD-2) and metalloproteinases 2 and 9 (MMP-2 and -9), which are well known to be involved in the mechanisms of tissue regeneration. The polymer tested significantly reduced the pro-inflammatory cytokines that were induced by LPS in human keratinocytes and modulated the expression of MMPs and hBD-2. This suggests that the association may improve the ability of chitosan/chitin polymers in their scaffold function.

Bacterial cellulose also seems to be a promising material for the construction of polymers in a nano, micro, and macro scale to use in wound dressing. It is composed of a pure cellulose nanofiber mesh that is spun by bacteria. Its high water content contributes to its biocompatibility [51]. Recently, Harkins et al. [52], tested a novel composite containing chitosan and cellulose for its antimicrobial activity, absorption of anticoagulated whole blood, and anti-inflammatory activity through the reduction of tumor necrosis TNF-α and IL-6, and the biocompatibility with human fibroblasts. The composites tested inhibited the growth of both Gram-positive and -negative microorganisms, such as *Escherichia coli* (ATCC 8739), methicillin-resistant *Staphylococcus* (ATCC 33591), and vancomycin-resistant *Enterococcus faecalis* (ATCC 51299) by 78, 36, and 64%, respectively. In addition, they showed no toxicity *vs*. fibroblasts responsible for the formation of the connective tissue matrix. The composites proved to be a good absorbent for anticoagulated whole blood and were able to maintain a moisture balance for wound healing. For successful tissue repair, several factors, including blood clotting and cellular survival are necessary. Therefore, the dressing material should possess anti-inflammatory activity, since proinflammatory cytokines (TNF-α and IL-6) contribute to the inflammation in chronic wounds, which stalls and prevents them from proceeding to the proliferative phase of tissue regeneration. The significant reduction in TNF-α and IL-6 by stimulated macrophages obtained with the Cel + CS composites clearly indicates their biodegradability, biocompatibility, and non-toxicity.

Advances in medicine have led to a significant increase in life expectancy but significant advances have also been made in cosmetics. The increasing proportion of women and men interested in skin rejuvenation has created a rapidly growing demand for anti-aging remedies to rejuvenate photo-damaged skin. Wrinkling, slackening, and irregular pigmentation, and symptoms of age-associated skin

damage, are in fact influenced by environmental factors, particularly lifetime sun exposure. In the last few years, different techniques for rejuvenation, such as injections with fillers and bio stimulating agents for wrinkle treatment, correction of scars, and soft-tissue augmentation, have been proposed. To obtain beauty and wellness inside and outside, there has been an increased demand from plastic surgeons for new effective medical devices and procedures, and an increased use of cosmeceuticals and nutricosmetics. Morganti and coworkers [53] developed and studied a new medical device to treat facial lines and body contours by balancing the skin-cell turnover and metabolism. To this purpose, block-polymer nanoparticles (BPN) of linoleic acid–rich phosphatidylcholine nanocomplexed with hyaluronan and chitin nanofibrils (PHHYCN) were formulated by encapsulating into them cholesterol, creatine, caffeine, melatonin, vitamins E and C, and the amino acids glycine and arginine. The BPN quickly re-establish the skin-barrier function thanks to their high content in linoleic acid and phosphatidylcholine. The phosphatidylcholine-fatty acids contained inside the BPN contribute to balancing the disturbed composition and organization of the lipids at the level of the epidermal keratinocytes, and consequently of corneocyte lamellae, while the high content of linoleic acid contributes to reintegrating the reduced level of ceramide 1, a structural and stabilizing component of the stratum corneum. These BPN seem to be useful in improving the activity of permanent fillers, making them useful as an anti-aging remedy in plastic surgery. This innovative biostimulating medical device may be used for wrinkle treatment and skin rejuvenation, as well as an adjuvant in soft-tissue augmentation and stretch-mark corrections.

4. Conclusions

Developments in the field of nanotechnology and the increasing interest for the environment have promoted the formulation of new nano-polymeric materials based on natural raw materials, and/or biodegradable and biocompatible synthetic polymers to use as innovative strategies in the fields of medicine, pharmacology, agriculture, and cosmetology [54–56]. Thus, the increased use of natural polysaccharides, such as chitin derivatives and lignocellulosic polymers, can reduce greenhouse emissions and improve our way of living.

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