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.

Clinical Activity of Innovative Non-Woven Tissues

Tommaso Anniboletti, Marco Palombo, Simone Moroni, Agostino Bruno, Paolo Palombo and Pierfrancesco Morganti

Abstract: Tissue engineering and the study of engineered materials are ever-expanding disciplines for the development of technological applications, focused on the reconstruction of mammalian tissue. In this study, we explained the surgical problems connected with the wound healing of burned skin, reporting the solutions recovered by the use on non-woven tissues (that we called MAVI dressing) made prevalently by chitin nanofibril (CN)-binding Ag^+ ions. Chitin-based materials and their derivatives, in fact, are receiving increased attention in tissue engineering because of their unique and appealing biological properties, useful to support the skin anatomical structure and its physiological functions. To this purpose, the in vivo results obtained on 30 patients will be shown by photographic images. The biocompatibility and the histological immune responses are shown by these advanced medications connected with the modulating effectiveness of the cytokine cascade. The obtained results recovered in vitro on a culture of keratinocytes and fibroblasts, were confirmed by the quick regenerative activity shown on people affected by burns of the first and second grade. In conclusion, these medications have shown that, while CN seems to be a natural polymer of choice for rapidly regenerating the burned skin, Ag^+ ions, bonded to its fiber structure in very low concentration, possess a sufficient antibacterial effectiveness to control the skin microbiological growth without showing side effects. Therefore, this hybrid biomaterial (chitin nanofibril/Ag nanoparticle composite) can be used for manufacturing advanced medications, solving both the problem of increasing its effectiveness for wound healing, and to slow down the bacterial growth connected with the wounded and burned skin. Moreover, the use of these advanced medications reduces the cost/h of plastic surgeons and the ancillary costs, shortening the time of the wound healing process.

Regenerative medicine has developed extremely rapidly during the last few years, so that the ideas, aspirations, and expectations of cell biologists, material scientists, engineering chemists, biochemists, and of course dermatologists and plastic surgeons have flourished [1–3]. Thus, naturally occurring nanostructures and biomaterials useful for the life science sectors have been a source of inspiration for new nanotechnological designs and to make innovative building blocks

and produce products. The biological use of raw biomaterials for wound dressings, such as the natural chitin/chitosan polymers and nanocomposites from fishery waste and the lignocellulosic compounds such as lignin from plant biomass [4–6], has shown that these natural ingredients possess interesting cicatrizing and reparative activity on wounded skin and organs [7–9]. In addition these compounds, totally biodegraded from environmental chitinases and human chitotriosidases [10], have been shown to be non-toxic, skin-friendly and environmentally-friendly, being catabolized to molecules normally used from all the living cells. Among the chitin derivatives, chitin nanofibril (CN) represents the patented purest crystalline form of chitin [11]. This crystalline polymer has been used to make block copolymeric nanoparticles which, embedded into nanoemulsions and non-woven tissues, may release the entrapped ingredients at different skin layers and different times [12–14]. These nanoparticles may be regarded as interesting carrier for gels and emulsions, and important potential filler materials for the enhancement of the physical and mechanical properties of the polymer matrices used to produce the non-woven-tissue that we called Mavi dressing as the name of the factory that furnished the free sample-test for this study [15].

On the other hand, they are useful to entrap active ingredients and Ag-ions that, when embedded into CN electrospun fibers, assume structural similarities compared to the native extracellular matrix (ECM). The obtained Ag-non-woven tissue made of chitin nanofibers (CN) and chitosan (CS) has shown the capacity to reduce the burden bacteria of infected skin (Table 1), and to enhance the reparative capacity of the skin (Figure 1), showing interesting antibacterial activity together with wound healing effectiveness [16,17]. The consensus within wound therapy recommends, in fact, that modern wound dressings should preserve the skin humid environment, while creating a barrier against mechanical stress and secondary infections [18]. Moreover, on the one hand, advanced dressings have to absorb wound exudate and reduce potential microorganism growth, while on the other hand they have to be safe and a non-irritant. Other important properties of these non-woven tissues are their acceptability to the patient and cost per unit [19]. It must be remembered that wound infections are the most serious complications related to burn injuries that affect up 1% of the worldwide population each year. This is the reason that wound therapy represents one of the challenging areas in drug product development; in the USA more than 6.5 million patients are involved annually, with an estimated cost of treatment per year of US$25 billion [20].

While the use of biomaterials for the treatment of wounded and burned skin has revealed to a huge potential for skin repair [21–24], natural fibers, such as CN, form an interesting option for most widely-applied polymers in medical technology, because of their capacity to form scaffolds, mimicking the structure and organization of the extra-cellular matrix (ECM) [25,26].

Table 1. Antibacterial activity of CN-Ag non-woven tissue [27].

Sample	Bacterial Growth (CFU/g)
Agar + culture of bacteria from bioburden skin tissue (CB)	10^7
Agar + CB+ CS − CN nanocomposite film	10^5
Agar + CB + CS – CN − AG nanocomposite film	10^3

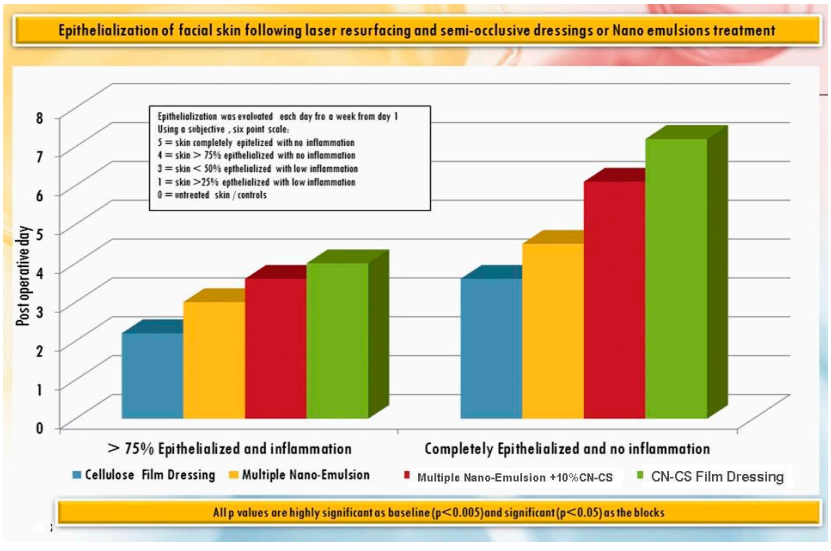


Figure 1. Effect of CN-CS dressing type on the epithelialization of facial skin following laser resurfacing. The epithelialization was evaluated daily during a week beginning from the 1st post-operative day. The appearance of the facial skin was as follows: >25% of the epithelialized skin with low inflammation (1st post-operative day), <50% of the epithelialized skin with low inflammation (3rd), 75% of the epithelialized skin with no inflammation (4th), completely epithelialized skin with no inflammation (5th) [27].

Assesment of antibacterial activity of Naonocomposite CN-CS and Cn-Cs-Ag films after 18 h incubation at 36 °C on agar Containing the Culture of Bacteria taken from Bioburden Skin Tissue. However, only recently the nature of the cellular environment, required for optimal tissue repair and regeneration, has been understood. A major consideration in tissue engineering is, in fact, the first pursuit of scaffolds that provide an architecture on which seeded cells are directed to proliferate and differentiate to form new tissues [27]. This tissue-engineering approach allows the production of new extra-cellular matrices which, resembling the native ECM, replace or regenerate the injured tissues. The creation of this engineered matrix that mimics the ECM requires a scaffold that, serving as a cell carrier, provides structural

support until native tissue is again formed in vivo [28–30]. The pore size of a scaffolding system is, in fact, one of the more important structural design parameters, as it influences cellular infiltration, spreading, intercellular communication, and the transport of nutrients and metabolites [28–32]. On the other hand, the stiffness and mechanical properties of the used substrate, acting as a physical support for the transport of cell signals, influence cellular behavior such as adhesion, spreading, motility, survival, and differentiation.

Together with the physicochemical characteristics of these matrices, the concept of the slow-release of antiseptic agents has been accepted, which, delivering antimicrobial ingredients without being detrimental to the healing process, impedes the micro-organisms proliferation. Wound infections, in fact, continue to be an important complication of chronic wounds in terms of patient morbidity and medical resources. Based on these findings, bioactive, antiseptic ingredients and materials for in situ tissue regeneration play an important role in the control of the skin microenvironment. Over time, many products have been manufactured, particularly in the burns field. In superficial partial-thickness burns, some authors suggest the use of paraffin gauze because there is a low risk of infection. This medication tends to dry out with burn exudate, causing pain in the dressing change and impairment in movement. In deep partial-thickness burns, the incidence of infection is higher than in superficial partial-thickness burns, thus the use of medications that can prevent infection is advocated. The use of silver-containing dressings is therefore recommended. Chitin is a natural, high-molecular-weight linear polymer of β-(1,4) linked *N*-acetylglucosamine (*N*-acetyl-2-amino-2-deoxy-D-glucopyranose) units. Chitosan, a copolymer of glucosamine and *N*-acetyl glucosamine units linked by 1–4 glucosidic bonds, is a cationic polysaccharide obtained by a partial (~60%) alkaline deacetylation of chitin, Industrial chitin nanofibrils (CNs) [4–9] have been shown to be a 1:1 copolymer of N-acetyl glucosamine and glucosamine. However, the role of chitin and chitosan are amazing, attracting increasingly more attention due to their biological and physicochemical characteristics, so that different scientific papers and patents have been published [33,34].

These natural polymers are biocompatible, biodegradable, and nontoxic, showing anti-inflammatory, anti-microbial and hydrating actives, and, therefore, have good biocompatibility and positive effects on wound healing. Previous studies have shown that chitin-based dressings can accelerate the repair of different tissues, facilitating wound contraction and regulating the secretion of inflammatory mediators and innate immunity, depending on their dimensions [35]. Thus, mean-sized chitin has shown pro-inflammatory activity, while small-sized chitin has shown an anti-inflammatory function activating both TNF and IL-10 in macrophages. For this purpose, chitin nanofibrils have a mean dimension of 240 $\times$ 7 $\times$ 5 nm [36].

Chitosan and chitin provide a non-protein matrix for 3D tissue growth and activate macrophages stimulating cell proliferation and a specific hierarchical tissue organization [33,34]. Moreover, they also have a hemostatic activity, which helps in natural blood clotting and blocks nerve endings, hence reducing pain. Both chitosan and chitin gradually depolymerize to release *N*-acetyl-β-D-glucosamine, which, initiating fibroblast proliferation, helps ordered collagen deposition. Finally, the stimulating synthesis and increased production of natural hyaluronic acid at the wound site helps with faster wound healing and scar prevention. This the probably reason why the different CN-Ag-Lignin/PEO non-woven tissues made by electrospinning or casting technologies, have been shown to have interesting anti-inflammatory, anti-microbial [27,36] and cicatrizing [37] activities, without revealing toxic side-effects, being skin-friendly and environmentally-friendly [35–38].

Chapter 1

Burns are a complex skin lesion that are recognized in different depth degrees involving the skin extensive body surface with underlying tissue destruction. In consideration of the histological depth of the loss of tissue, three burn degrees are reported [39].

1st degree burn: only the epidermis is involved with raising of the stratum corneum, dermal edema and vascular dilatation. It heals spontaneously in seven days (Figure 2).

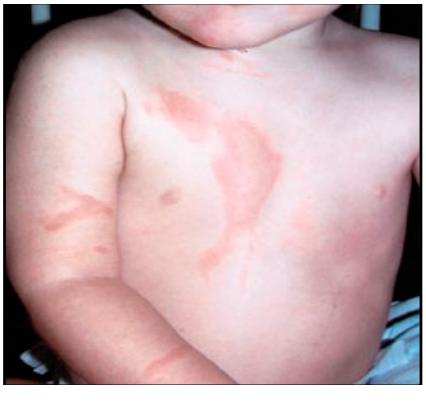


Figure 2. Clinical case of a child with first degree burn on the chest and the right arm.

Superficial 2nd degree burn: in this case the destruction of the epidermis and the superficial layer of the dermis is observed with the development of blisters and a detachment of the epidermis due to the pressure of the transudate fluids. This type of burn is characterized by erythema, edema, blisters and intense pain. Skin is pink,

warm, and painful to touch. Bleeds easily and clears the acupressure. The hairs are present. It heals spontaneously in 15 days (Figure 3).

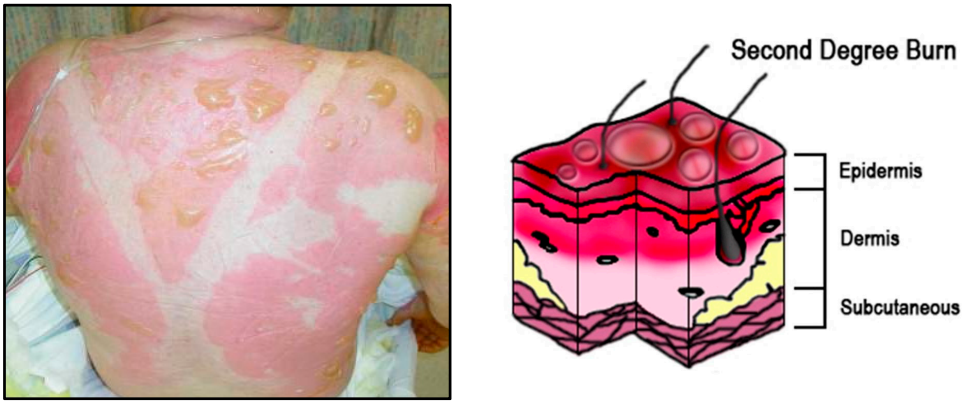


Figure 3. (**Left**): schematic skin section of superficial second-degree burn; (**Right**): clinical case of a patient with superficial second-degree burn on the back due to scald.

Deep 2nd degree burn: Damage that involves the epidermis, dermis, and many superficial neural structures, causes pain and burning. The de-epithelialized areas appear whitish, with eschar expression of more serious damage not uncommon. Under the blisters the skin is pale or bright red (dermal edema and vasodilatation) and it is not painful to touch. Heals in 3–4 weeks with frequent scars and often requires surgical treatment (Figure 4).

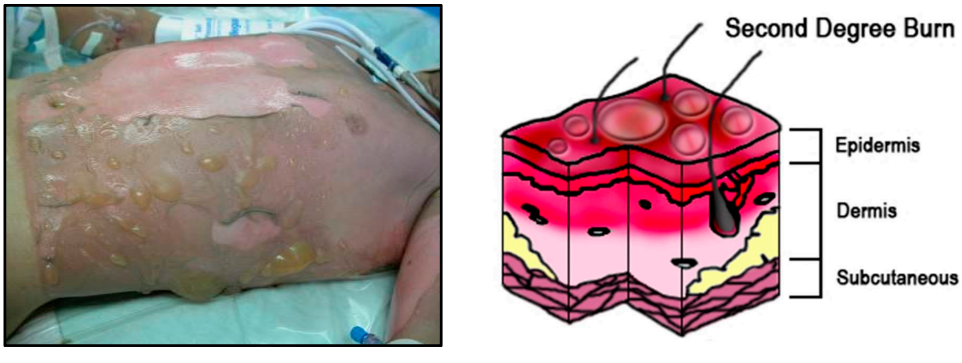


Figure 4. (**Left**): Schematic skin section of a deep second-degree burn; (**Right**): clinical case of a patient with deep second-degreeburn on the trunk.

Third 3rd degree burn: complete destruction of epidermis and dermis is observed. In more severe cases, the exposure of muscles, tendons and bones is possible. If the burning agent is fire or a hot body, the area of necrotic eschar looks blackish or brownish and is dry, cold, hard, painless and non-bleeding. If the agent is a scald, the necrotic area appears whitish or gray in color and of soft consistency. The presence of thrombosed vessels in waxy skin is a pathognomonic sign of this injury. Pain is absent because of the destruction of nerve endings, while only sensitivity to deep pressure is preserved. The hair, even when present, can easily be removed. The treatment is surgical and it heals with scarring (Figures 5 and 6). The goal of the surgical treatment in burns is to prevent invasive local infection and sepsis, to avoid that the burn damage becomes deeper, to get the best skin coverage as quickly as possible, to avoid hypertrophic scars and scar contractures, and to obtain complete healing in the shortest time and with the least number of operations. The operations consist in the removal of all necrotic tissue, which is extended up to the achievement of a vital plane.

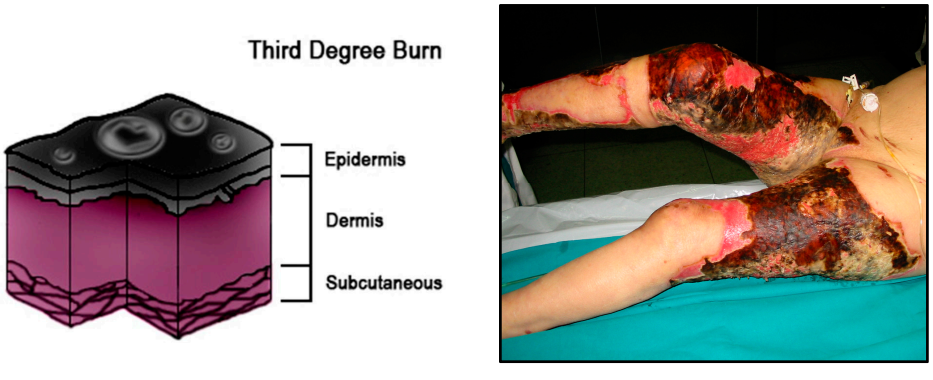


Figure 5. (**Left**): schematic skin section of third-degree burn; (**Right**): clinical case of a patient with third degree burn on the legs due to fire.

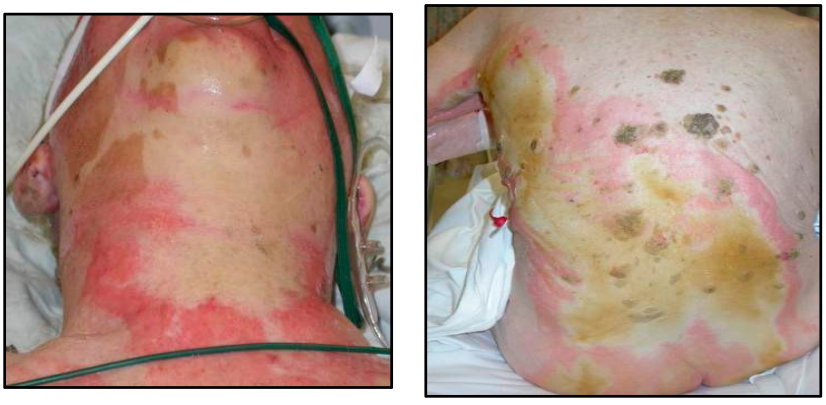


Figure 6. (**Left**): third-degree burn of the back due to scald; (**Right**): third-degree burn of the neck due to scald.

Based on the burn depth, a superficial tangential, a deep tangential and a fascial escharotomy—depending on the depth of the of necrotic tissue removal—can be distinguished. The superficial tangential escharotomy consists of the removal of the superficial dermis, until the dermal capillary plexus is reached; dermal plexus bleeding indicates the achievement of the vital plane on which the skin graft can be performed. The deep tangential escharoctomy is carried out at different levels of depth, sacrificing the entire dermis and hypodermis; the unviable dermis (hard and gray) also does not provide a substrate for skin grafting, but healthy fat looks shiny and yellowish and contains blood vessels that allow a high possibility that the graft will take. In the fascial escharotomy, the excision of necrotic tissue is done to the muscolaris fascia; the subcutaneous tissue is brown, necrotic and hemorrhagic, while the fascial smooth surface is a well-vascularized recipient site (perforating arteries) and allows skin grafts to take easily. Obviously, the main disadvantages of this procedure are the permanent cosmetic deformity (because the fat does not regenerate), risk of nerve and superficial tendon damage (skin denervation and permanent loss of sensation), and bone and tendon exposure. The autologous skin graft is the only definitive method to cover a loss of substance. The donor sites are, in order of preference: the upper and anterior-lateral thigh, the anterolateral area of the legs, upper arms, abdomen, back, chest and buttocks (Figure 7).

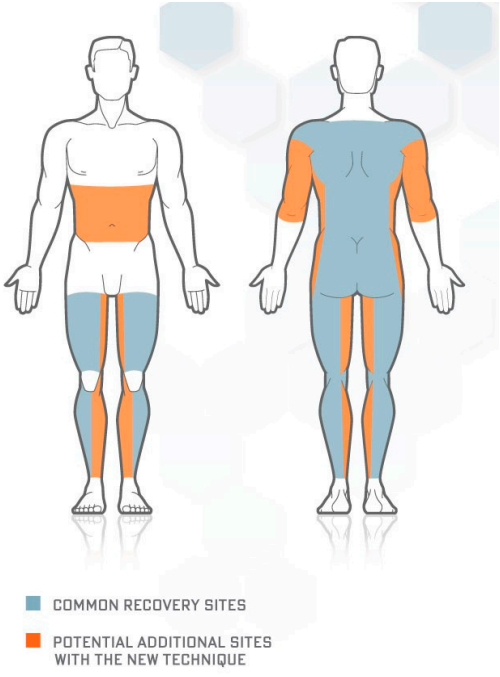


Figure 7. Schematic representation of full and split-thickness skin graft donor sites (Source: by the courtesy of World Press Media).

To take the dermal-epidermal area, skin grafts are used: Brown dermatome (electric or air) electric Padgett or Zimmer dermatome, and a range of freehand knives. Autografts are taken with a thickness of 0.010 to 0.012 cm and applied in a laminar or meshed structure. The mesh graft in the skin produces a series of perforations in a geometric pattern, to allow the expansion of the skin by increasing the initial size. In this way a limited donor area can cover a wider receiver area. The dermal-epidermal skin graft is prepared with mesh graft in ratio of 1 to $1\frac{1}{2}$ or 1 to 3, until even, in some cases, 1 to 6 or 1 to 9.

The skin grafts, both laminar and meshed, lie on the vital area (after adequate and careful hemostasis), are regularized and adapted to the loss of substance, and anchored using mechanical staplers (small grafts and those made in the face are fixed with thin sutures and moulage) (Figures 8 and 9). The meshed skin grafts are used in patients with large burns and limited donor sites. The presence of the "breaks" in the structure of the graft allows excellent drainage of the serous and blood collections that can be formed under laminar skin grafts.

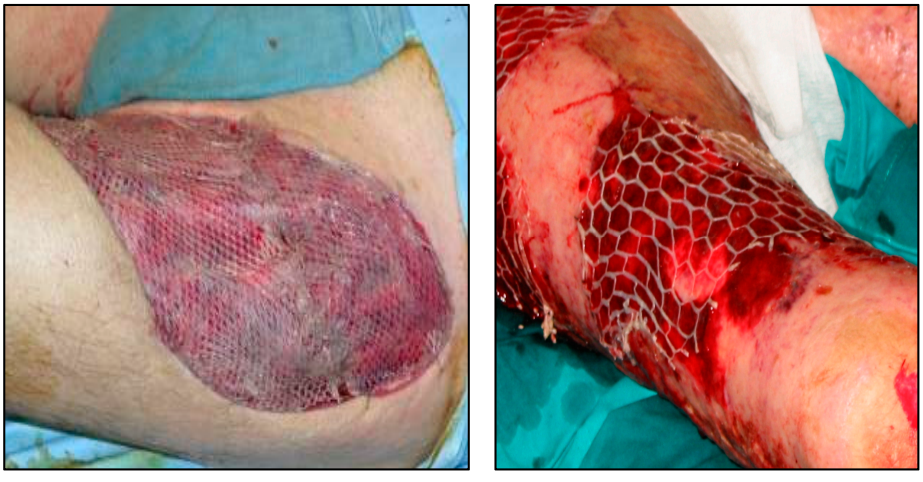


Figure 8. Skin grafts prepared with mesh graft in ratio of 1 to 1:1.5 (**Left**) or 1:6 (**Right**).

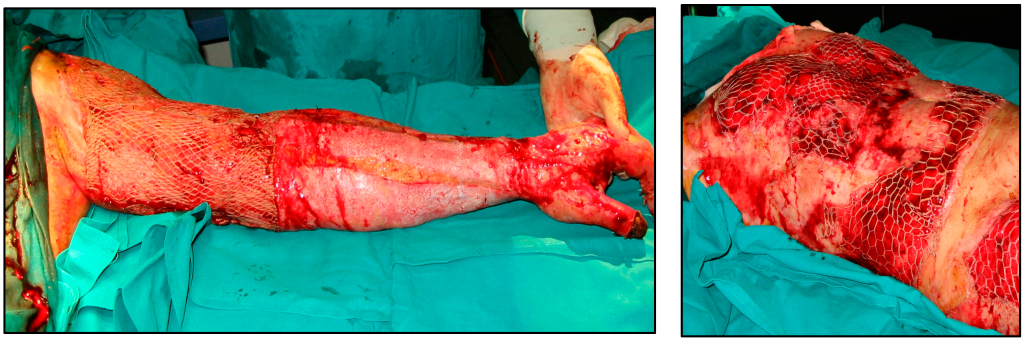


Figure 9. Clinical cases of patients with large burns and limited donor sites treated with meshed split-thickness skin grafts.

Ideal cutaneous wound repair should involve skin regeneration, bringing all the anatomical and physiological capabilities back to normal, without scarring. Unfortunately, wound healing in adult mammals too often results in a fibrotic normal or abnormal scar formation. Abnormal scarring that leads to hypertrophic scar or keloid formation that also invades the healthy tissue seems to be due to a persistent skin-barrier perturbation [29,30]. Thus, the prevention of unsatisfactory scarring begins before the treatment by the selection of both the dressings to be used and the post-operative care. A key part of the preoperative discussion is informed consent, which requires adequate information for the patient so that she/he can to make a decision regarding the procedure, indicating the procedure selected with the relative

risks and benefits. Naturally, with regard to scarring, the patient should be aware of potential scarring that may occur considering which areas of the body can be prone to this unusual wound contraction, as well as she/he has to know that smoking, alcohol and certain medications such as retinoids, may have negative impact on the final scar [31]. However, scarring, which cannot be prevented but controlled, is the inevitable final stage of wound healing. To what degree the resultant scar affects functional and cosmetic outcomes is dependent on early and consistent treatment. This is the reason why our group dedicates great attention to the control of the location and depth of injury, together with the pretreatment and cleansing of the burned/wounded skin, and the relative selection of the non-woven tissues to be used.

Chapter 2

Since natural polymers are an interesting option for most widely-applied fibers in composite non-woven technology, this study aimed to use an innovative non-woven tissue made prevalently by CN, functionalized by the use of marine collagen peptides, lignin and Ag^+ ions.

This innovative engineered composite, proposed as the most promising polymer matrix in wound-dressing development, has been shown in vitro to possess interesting antibacterial properties (Figure 10) connected with good cell adhesion and proliferation capabilities to guide cell differentiation [32].

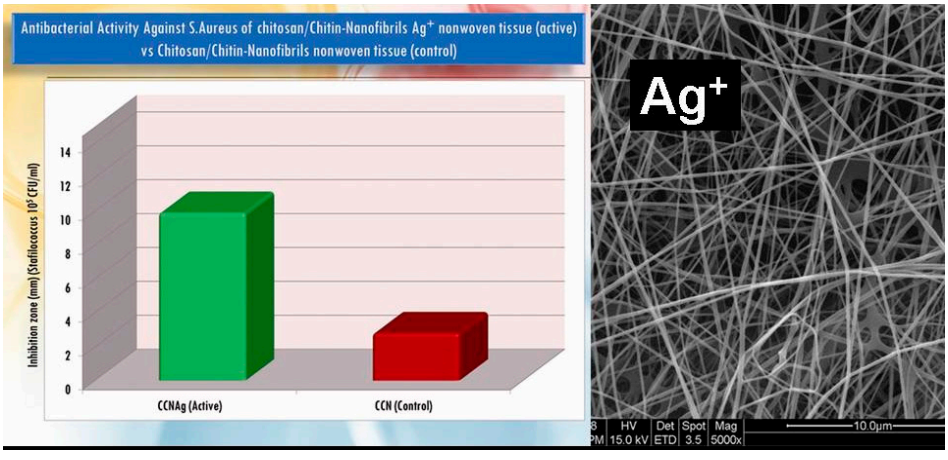


Figure 10. Antibacterial activity of chitosan/chitin fibers Ag^+ treated.

It is interesting to underline that this medical dressing is composed of two different layers. The outside layer (blue in color and composed of polypropylene of pharmaceutical grade) slows down the transcutaneous perspiration, increasing the cicatrizing activity of CN and the bactericidal power of the Ag^+ ions cross-linked to

the chains of the CN-polymer. The inside very thin layer, in direct contact with the wound and composed of natural dispersible materials, mimics the ECM architecture, influencing the cell life and reproduction. The methodology to crosslink Ag^+ to crystal Chitin, before obtaining the non-woven tissue by electrospinning, gave the possibility not only to reduce the toxic effects of the metallic ions (nano-concentrated) at the level of the skin cells, but also to enhance the fibers' anti-inflammatory and bactericidal activity [36–38].

The aim of the study was to use these innovative, biodegradable, and skin-friendly non-woven tissues to facilitate skin repair, serving as either temporary or permanent replacements for burned tissue.

In the treatment of superficial second-degree burns and split-thickness skin graft donor sites, the goal is to achieve complete re-epithelialization. Thus, the effectiveness of MAVI dressing in the healing of split-thickness skin graft donor sites was evaluated on superficial second-degreeburns.

After local institutional ethical committee approval, we collected data from ambulatory patients of our burn center from June 2014 to August 2014.

Exclusion criteria were admitted patients, full-thickness burns, and operated patients that came to the ambulatory facility for postoperative follow up.

Out-patient treatment was selected according to the classification of the American Burn Association: a second-degreeburn with TBSA (total burn surface area) less that 15% in adults (10% in children), burns not involving eyes, ears, face, hands, feet or perineum; burns not derived from electrical injuries, not associated with inhalation, not in poor-risk patients.

The burn depth was clinically assessed. No systemic antibiotics were used. Patient's burns were diagnosed as completely healed up when re-epithelisation was complete in all affected areas. For each patient, we collected the age, sex, cause of burn (scald or flame), type of dressing used and the days required for complete healing (healing time). We used three different preparations of MAVI dressings.

MAVI n.1 was utilized in 10 patients, treating the coverage of split-thickness skin graft donor sites in four patients and superficial second-degreeburns in six patients.

(MAVI n.1 is made of CN-Ag-lignin-PEO and collagen peptides made by electrospinning).

MAVI n.2 was utilized in 10 patients, treating the coverage of split-thickness skin graft donor sites in seven patients and superficial second-degreeburns in three patients.

(MAVI n.2 is made of CN-Ag-lignin-PEO made by electrospinning).

MAVI n.3 was utilized in 10 patients, treating the coverage of split-thickness skin graft donor sites in two patients and superficial second-degreeburns in eight patients.

(MAVI n.3 is made of CN-Ag-lignin-chitosan made by casting technology).

A prospective randomized clinical study was performed on 30 burn patients to investigate the effectiveness, safety and tolerability of MAVI dressings.

The MAVI dressing was applied on superficial second-degree burns and split-thickness skin graft donor sites in patients aged between seven and 71 years old, the mean average age was 43.6. The cause of the burn was scalding in 83% of cases and fire in the remaining 17%. The donor sites and the superficial second-degreeburn wounds were assessed on postoperative days one, five, 14, 21, and long-term for infection, hyperemia, pruritus, pain, exudate level, and adherence to the wound bed (Table 2).

Table 2. Different preparations and applications of MAVI dressings.

	Characteristics	Patients	Donor Sites/Second-Degree Burns
MAVI I	CN-Ag-lignin-PEO and collagen peptides	10	4/6
MAVI II	CN-Ag-lignin-PEO	10	2/8
MAVI III	CN-Ag-lignin-chitosan	10	7/3

At the follow-up visits, the donor sites and the superficial second-degree burn wounds were assessed again for pruritus and pain, patient comfort and convenience for the doctor. Touch-pressure, thermal and pain sensibility tests were performed preoperatively and on postoperative follow-up together with the assessment of color and texture of the re-epithelialized areas.

In all patients, re-epithelialization was completed between five and 13 days (mean eight days) after the application of the MAVI dressing.

Six patients out of 30 required pain killers over the first three days after burns with no significant differences between the type of dressing used.

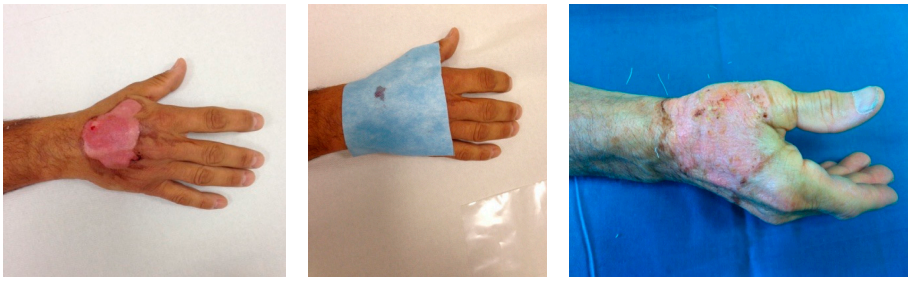
There were neither significant differences between donor sites and the superficial second-degree burn wounds regarding pain, hyperemia, pruritus, exudate, and final appearance (color and texture).

The areas dressed with MAVI completely healed within 5–13 days in a significantly higher proportion than the traditional dressings, showing during the whole study less incidence of exudates and peri-lesional erythema.

The aesthetic outcome of the treated lesions after healing was significantly better for the MAVI dressing and it required very few renewals of the medication during the first week of treatment. The high interval time between dressing changes reduced the amount of medication, patient suffering, overall costs and human resources, according to the reported following cases.

After the application of MAVI n.1 we observed the complete re-epithelialization of the split-thickness skin graft donor sites in three patients and a healing delay due

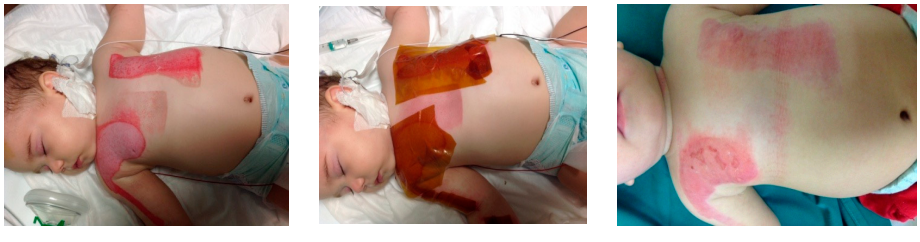
to exudate in one patient; and complete superficial second-degree burn healing in four patients (Figure 11).



(**a**) (**b**) (**c**)

Figure 11. Clinical case n°1: (**a**) superficial second-degree burn on the dorsum of the right hand; (**b**) Application of MAVI dressing number 1; (**c**) Complete re-epithelialization after five days post-burn.

After the application of MAVI n.2, we observed the complete re-epithelialization of the split-thickness skin graft donor sites in five patients and a healing delay due to exudate in two patients; complete superficial second-degree burns healing in two patients and the failure of the procedure in one patient due to infection. After the application of MAVI n.3 (Figure 12), we observed the complete re-epithelialization of the split-thickness skin graft donor sites in all patients, and complete superficial second-degree burns healing in seven patients.



(**a**) (**b**) (**c**)

Figure 12. Clinical case n°2: (**a**) Second-degree burn of the chest and right shoulder; (**b**) Application of Mavi dressing n°3; (**c**) Complete skin re-epithelialization after six days post burn.

Chapter 3

The self-renewing property of the epidermis plays a major role during wound healing. Within moments of wounding, keratinocytes not only inform each other

that the barrier has been broken, but also communicate with the dermis and the local immune system about the urgent need to repair the gap, maintaining a constant flow of information.

The purpose of such complex signaling cascades is to regulate gene targets during wound healing, thus controlling the keratinocyte activation cycle.

The most common initiator of keratinocyte activation is Interleukin-8 (IL-8), while defensine-2 (Figures 13 and 14 [36,37]) was found to be closely associated with the progression of wound healing.

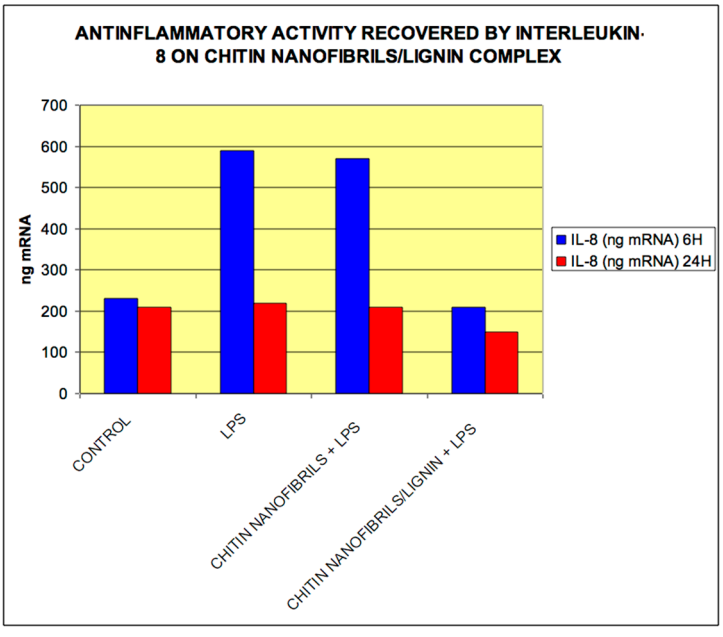


Figure 13. The figure illustrates gene expression modulation in human cell lines of pro-inflammatory cytokines in HaCaT keratinocytes. The cell lines were treated with: Control = untreated cells; LPS (Lipopolysaccharide of *P. Aeruginosa*) = pro-inflammatory substance; LPS + the Complex Chitin Nanofibrils/Lignin; LPS + Chitin Nanofibrils [37].

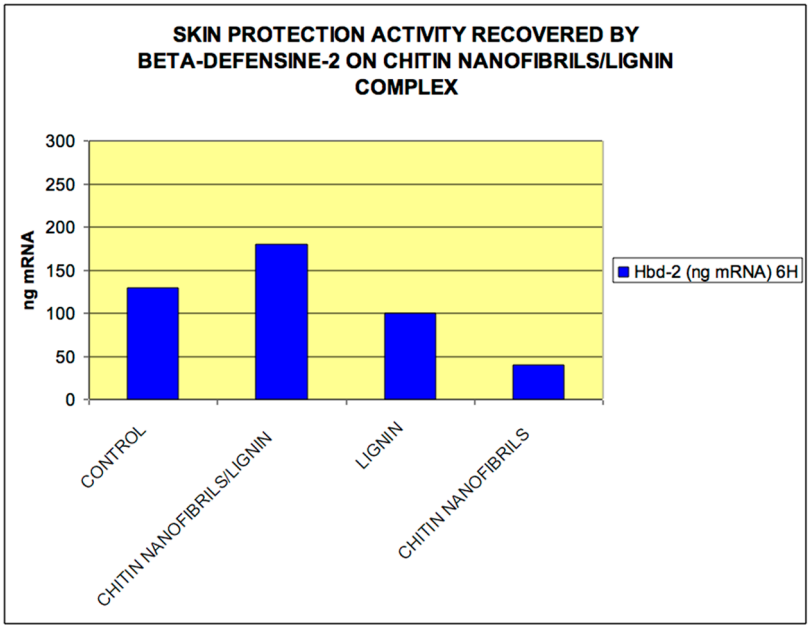


Figure 14. The figure illustrates the modulation of the gene expression of beta-defensin in human keratinocyte HaCaT cell lines. The cell lines were treated with: Control = untreated cells; Lignin; The Complex Chitin Nanofibrils/Lignin; Chitin; An over-expression in the values of these markers is an indication of tissue regeneration activity [37].

Therefore, IL-8 and defensine 2 appear to be accurate prognostic markers of wound repair.

The in vitro results of our studies on keratinocytes cultures were in agreement with the in vivo studies. An interesting modulating activity was observed on both IL-8 and defensine-2 on the non-woven tissue made by the natural fibers embedded in keratinocyte cultures.

On the other hand, the in vivo double-blind study made on the burned and wounded skin of a patient-group recovered in hospital notably accelerated the skin repair and cicatrizing activities of the same non-woven tissues. Moreover, due to the bactericidal and anti-fungal activity of the Ag-metal-ions, capable of regulating microorganisms invasion, it was possible to keep the dressing in place for one week, sensibly reducing the general cost of the in-hospital treatment.

It is interesting to underline how the obtained nano-fibers, made prevalent by the polymer chitin nanofibril bound to lignin and peptides, establishing each with other a stable ionic bonds, show an interesting skin-modulating activity on the synthesis of defensines and interleukines released from keratinocytes, also modulating the

metalloproteinase activity [37]. Moreover, Ag^+ ions, strictly bound to the Chitin chains, gave an interesting antibacterial activity to the medical dressing.

By this approach it seems desirable to use these innovative, chitin-based, non-woven tissues for medical purposes, due to their particular effectiveness in mimicking the size and arrangement of native collagen, accelerating the normal repairing activity of the skin, altered for burn or wound healing. Additionally, the electrospun scaffolds, obtained by a layer-by-layer method for their high surface-to-volume ratio and interconnecting pores, seem capable of facilitating cell adhesion and the formation of cell–cell junctions, accelerating the cicatrizing process [35–39]. These nano-fibrous scaffolds, in fact, seem to induce favorable cell—ECM interactions, increasing the cell proliferation rate, maintaining the cell phenotype, supporting cell differentiation, and promoting in vivo-like three-dimensional matrix adhesion [36–38]. Moreover, they probably activate cell-signaling pathways, by providing the chemical and physical stimuli to cells necessary for faster skin-repairing activity. The sequential, multi-layering electrospinning of the different natural polymers employed in combination with natural peptides, surely enhanced the mechanical integrity and dimensional stability of the electrospun meshes, while the nanostructured Ag^+ silver probably maintained the skin microbiota in equilibrium, impeding the excessive growth of the opportunistic microorganisms [36,37].

These innovative medical dressings seem to represent new, cost effective, non-woven tissues and procedures to be used for skin regeneration and tissue transplantation. Their activity, compared to the traditional non-woven tissues normally used in our hospital, was shown to be more effective in a shorter time to regenerate the skin affected by burns of the first and second degree. It is interesting to underline the capacity that these non-woven tissues have to prevent microbiota growth for the right period of time, extending the medication period. In conclusion, the MAVI dressing seems to be a safe and effective dressing for the re-epithelialization of skin graft donor sites and superficial second-degree burn wounds, showing higher activity than traditional dressings.

The goal of such a treatment strategy based on the use of innovative, bioengineered, non-woven tissues, which are totally biodegradable and environmentally-friendly, is, in fact, to act as smart band-aids, to replace altered or senescent resident cells and reestablish the anatomy and physiology of burned/wounded skin [40,41]. Well-designed randomized clinical trials will involve our group in the next phase in order to scrutinize the true potential of these natural polymers for regenerative medicine.

Acknowledgments: We thank MAVI SUD Srl, Aprilia, Italy, for the sample-test given for this study.

Author Contributions: T.A. and P.M. verified the analytical methods. T.A., A.B., S.M., M.P., P.P. investigated and carried out the clinical part experiments. T.A. and P.M. supervised the findings of this work. P.M. had the idea and realized the tissue engineering dressing. All authors discussed the results and contributed to the final manuscript.

Conflicts of Interest: We declare hat Paolo Palombo, Marco Palombo, Simone Moroni, Agostino Bruno and Tommaso Anniboletti have no conflicts of interest. Pierfrancesco Morganti works as the Head of the R&D Centre of Nanoscience, MAVI SUD, Srl, Italy.

Abbreviations

CS	Chitosan
CN	Chitin nanofibrils
S. aureus	Staphylococcus aureus
CFU	colony forming unit
CB	bio-burden skin bacteria
PEO	polyethylene oxide
Ag	Silver

References

1. Chapekar, M.S. Tissue engineering: Challenges and opportunities. *J. Biomed. Mater. Res.* **2000**, *53*, 617–620. [CrossRef]
2. Chan, G.; Money, D.J. New Materials for tissue engineering: Towards greater control over the biological response. *Trends Biotechnol.* **2008**, *26*, 382–392. [CrossRef] [PubMed]
3. Nieponice, A. Clinical Translation of Tissue Engineering and Regenerative Medicine Technologies. In *Biomaterials and Stem Cells in Regenerative Medicine*; Ramalingam, M., Ramakrishna, S., Best, S., Eds.; CRC Press: New York, NY, USA, 2012; pp. 521–532.
4. Morganti, P.; Del Ciotto, P.; Carezzi, F.; Morganti, G.; Chen, H.-D. From Waste Material a New and Safe Anti Aging Compound: A Chitin Nanofiber Complex. *SOFW J.* **2012**, *138*, 128–138.
5. Morganti, P.; Li, Y.-H. Healthy products from waste materials. *Eurocosmet. China Spec. Issue* **2012**, *20*, 60–63.
6. Morganti, P.; Morganti, G.; Morganti, A. Transforming nanostructured chitin from crustacean waste into beneficial health products: A must for our society. *Nanotechnol. Sci. Appl.* **2011**, *4*, 123–129. [CrossRef] [PubMed]
7. Morganti, P.; Tiscenko, G.; Palombo, M.; Kelnar, L.; Brozova, L.; Spirkova, M.; Pavlova, E.; Kobera, L.; Carezzi, F. Chitin nanofibrils for biomimetic products: Nanoparticles and nanocomposite chitosan films in health-care. In *Marine Biomaterials: Isolation, Characterization and Application*; Kim, S.-K., Ed.; CRC Press: New York, NY, USA, 2013; pp. 681–715.

8. Morganti, P.; Chen, H.D.; Gao, X.H.; Del Ciotto, P.; Carezzi, F.; Morganti, G. Nanoparticles of Chitin Nanofibril-Hyaluronan block polymer entrapping lutein as UVA protective compound. In *Carotenoids: Food Source, Production and Health Benefits*; Nova Science Publishers, Inc.: New York, NY, USA, 2013; pp. 237–259.
9. Morganti, P.; Carezzi, F.; Del Ciotto, P.; Tishchenko, G.; Chianese, A. A Green Multifunctional Polymer from Discarded Material: Chitin Nanofibril. *Br. J. Appl. Sci. Technol.* **2014**, *4*, 4175–4190. [CrossRef]
10. Eide, K.B.; Norberg, A.L.; Heggset, E.B.; Lindbom, A.R.; Varum, K.M.; Eijsink, V.G.H.; Soile, M. Human Chitotriosdase-Catalyzed Hydrolisis of Chitosan. *Biochemistry* **2012**, *51*, 487–495. [CrossRef] [PubMed]
11. Morganti, P.; Muzzarelli, C. Spray-Dried Chitin Nanofibrils, Method for Production and Uses Thereof. U.S. Patent 12/094912, 23 November 2006.
12. Morganti, P.; Fabrizi, G.; Guarneri, F.; Palombo, M.; Palombo, P.; Cardillo, A.; Ruocco, E.; Del Ciotto, P.; Morganti, G. Repair Activity of Skin Barrier by Chitin-Nanofibrils Complexes. *SOFW J.* **2011**, *137*, 10–26.
13. Morganti, P.; Del Ciotto, P.; Morganti, G.; Fabien-Soulé, V. Application of Chitin Nanofibrils and Collagen of Marine Origin as Bioactive Ingredients. In *Cosmeceuticals: Trends and Prospects*; Kim, S.-K., Ed.; CRC Press: New York, NY, USA, 2012; pp. 267–289.
14. Morganti, P.; Del Ciotto, P.; Fabrizi, G.; Guarneri, F.; Cardillo, A.; Palombo, M.; Morganti, G. Safety And Tolerability of Chitin Nanofibrils-Hyaluronic Acid Nanoparticles Entrapping Lutein. Note 1: Nanoparticles Characterization and Bioavailability. *SOFW J.* **2013**, *139*, 12–23.
15. Dobrovol'skaya, I.P.; Katatkin, I.A.; Yudin, V.E.; Ivan'kova, E.M.; Elokhovskii, V.Y. Supramolecular structure of chitin Nanofibrils. *Polym. Sci. Ser. A* **2015**, *57*, 52–57. [CrossRef]
16. Morganti, P. Chitin Nanofibrils and Their Derivatives as Cosmeceuticals. In *Chitin, Chitosan, Oligosaccharides and Their Derivatives. Biological Activities and Application*; Kim, S.-K., Ed.; CRC Press: New York, NY, USA, 2010; pp. 531–542.
17. Morganti, P.; Del Ciotto, P.; Gao, X.-H. Skin Delivery and Controlled Release of Active Ingredients Nanoencapsulated by Chitin Nanofibrils: A New Approach. *Cosmet. Sci. Technol.* **2010**, *20*, 136–142.
18. Hurler, J.; Nataša, Š.-B. Potentials of Chitosan-Based Delivery Systems in Wound Therapy: Bioadhesion Study. *J. Funct. Biomater.* **2012**, *3*, 37–48. [CrossRef] [PubMed]
19. Glossens, A.; Cleenewerck, M.B. New Wound dressings: Classification and Tolerance. *Eur. J. Dermatol.* **2010**, *20*, 24–26.
20. Sen, C.K.; Gordillo, G.M.; Roy, S.; Kirsner, R.; Lambert, L.; Huntb, T.K.; Gottrup, F.; Gusrantir, G.C.; Longsker, M.T. Human skin wound S: A major snowballing threat to Public Health and Economy. *Wound Repair Regen.* **2009**, *17*, 763–771. [CrossRef] [PubMed]
21. Murphy, K.D.; Lee, J.O.; Herndon, D.N. Current Pharmacotherapy for the treatment of severe burns. *Expert Opin. Pharmacother.* **2003**, *4*, 369–384. [CrossRef] [PubMed]

22. Eisner, J.J.; Euozi, D.; Ullman, Y.; Berdicevsky, I.; Shefy-Pleg, A.; Ziberman, M. Novel biodegradable composite wound dressings with controlled release of antibiotics: Results in a guinea pig burn model. *Burns* **2011**, *37*, 896–904. [CrossRef] [PubMed]
23. Kabelitz, D.; Shroder, J.M. *Mechanisms of Epithelial Defence*; Karger Medical and Scientific Publishers: Basel, Switzerland, 2005.
24. Falanga, V. *Cutaneous Wound Healing*; Martin ad Unix Ed.: London, UK, 2001.
25. Rover, D.T.; Maibach, H.I. *The Epidermis in Wound Healing*; CRC Press: New York, NY, USA, 2004.
26. Yudin, V.E.; Dobrovolskaya, I.P.; Neelov, I.M.; Dresvyanina, E.N.; Popryadukhin, P.V.; Ivan'kova, E.M.; Elokhovskii, V.Y.; Kasatkin, I.A.; Okrugin, B.M.; Morganti, P. Wet spinning of fibers made of chitosan and chitin nanofibrils. *Carbohydr. Polym.* **2014**, *108*, 176–182. [CrossRef] [PubMed]
27. Morganti, P.; Chen, H-D.; Gao, X-H. Chitin Nanofibril: A Natural Eco-Friendly and Immunoadjuvant Actuve Carrier for Medical Use. *J. Appl. Cosmetol.* **2016**, *34*, 141–154.
28. Kim, J.; Joo, S.; Ko, I.; Atala, A.; Yoo, J.; Lee, S. Smart Biomaterial Scaffold for In Situ Tissue Regeneration. In *Biomaterials and Stem Cells in Regenerative Medicine*; CRC Press: New York, NY, USA, 2012. [CrossRef]
29. Seidi, A.; Ostrovidov, S.; Ramalingam, M. Fabrication and Application of Gradient Hydrogels in Cell and Tissue Engineering. In *Biomaterials and Stem Cells in Regenerative Medicine*; CRC Press: New York, NY, USA, 2012. [CrossRef]
30. Khan, A.J.; Cook, B. Wound healing and scar formation. In *Surgical Technique for Cutaneous Scar Revision*; Harahap, M., Ed.; Marcel Dekker Inc.: New York, NY, USA, 2000; pp. 1–14.
31. Elias, P.M.; Wood, L.C.; Feingold, K.R. The skin and its barrier function: Implications for wound healing. In *Cutaneous Wound Healing*; Falanga, V., Ed.; Martin Dunitz: London, UK, 2001; pp. 5–14.
32. McDonald, M.; Stasko, T. Prevention of unsatisfactory scarring. In *Surgical Technique for Cutaneous Scar Revision*; Harahap, M., Ed.; Marcel Dekker Inc.: New York, NY, USA, 2000; pp. 53–80.
33. Morganti, P.; Carezzi, F.; Del Ciotto, P.; Morganti, G.; Nunziata, M.L.; Gao, X.-H.; Chen, H.-D.; Tischenko, G.; Yudin, E.V. Chitin Nanofibrils: A Natural Multifunctional Polymer. Physicochemical characteristics, effectiveness and safeness. *Nanobiotechnology* **2014**, 1–31.
34. Jayakumar, R.; Prabaharan, M.; Kumar, P.S.; Nair, S.V.; Furuike, T.; Tamura, H. Novel Chitin and Chitosan Materials in Wound Dressing. *Biomed. Eng. Trends Mater. Sci.* **2011**. [CrossRef]
35. Morganti, P.; Febo, P.; Cardillo, M.; Donnarumma, G.; Baroni, A. Chitin Nanofibril and Nanolignin: Natural Polymers of Biomedical Interest. *J. Clin. Cosmet. Dermatol.* **2017**, *1*. [CrossRef]
36. Morganti, P.; Del Ciotto, P.; Stoller, M.; Chianese, A. Antibacterial and antiinflammatory green nanocomposites. *Chem. Eng. Trans.* **2016**, *47*, 61–66.

37. Morganti, P.; Fusco, A.; Paoletti, I.; Perfetto, B.; Del Ciotto, P.; Palombo, M.; Chianese, A.; Baroni, A.; Donnarumma, G. Anti-inflammatory, Immunomodulatory, and Tissue Repair Activity on Human Kerstinocytes by Green Innovative Nanocomposites. *Materials* **2017**, *10*, 843. [CrossRef] [PubMed]
38. Morganti, P.; Stoller, M. Chitin and Lignin: Natural Ingreduents from Waste Materials for making Innovative and Healthy Products for Humans and Plants. *Chem. Eng. Trans.* **2017**, *60*, 319–324.
39. Gibson, A.L.F.; Bennett, D.D.; Taylor, L.J. Improving the histologic characterization of burn depth. *J. Cut. Pathol.* **2017**. [CrossRef] [PubMed]
40. Bottino, M.; Vohra, Y.; Thomas, V. Spatially Designed Nanofibrous Membranes for Periodontal Tissue Regeneration. In *Biomaterials and Stem Cells in Regenerative Medicine*; CRC Press: New York, NY, USA, 2012.
41. Phipps, M.C. Mesenchymal Stem Cell Responses to Bone-Mimetic Electrospun Matrices Composed of Polycaprolactone, Collagen I and Nanoparticulate Hydroxyapatite. *PLoS ONE* **2011**, *6*, e16813. [CrossRef] [PubMed]