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Chitin nanofibrils/chitosan glycolate composites as wound medicaments

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Abstract

Highly crystalline chitin nanofibrils were isolated from crustacean chitin and characterized by X-ray diffraction and FT-infrared spectrometry. A novel formulation including chitin nanofibrils, chitosan glycolate, and chlorhexidine was manufactured in three presentations: spray, gel, and gauze. The latter included non-woven dibutyryl chitin as a biocompatible support. The products were tested in murine wound models, with phytostimuline-medicated wounds as controls, in two series of experiments, one of which included a concomitant laser treatment capable to activate cells. The gauze consistently induced better epithelial differentiation and keratinization, and better reorganization of the basal lamina. The angiogenetic response induced by the gauze was indicative of satisfactory vascular trophism irrespective of the laser treatment. Immunohistochemical observations showed that the actin cytoskeleton of dermal and epidermal cells was well formed. The gauze was used to treat 75 patients hospitalized for a variety of traumatic wounds with good results in all cases. A single dressing could be kept on site for at least four days, and the healing took place within periods of time similar to those reported for traditional dressings under comparable conditions; in no case secondary infections developed. In conclusion, the spray could be used as a first-aid tool on bleeding abrasions; the gel enhanced physiological repair and was recommended for areas with thin epidermal layers, and the gauze was found to be superior to other dressings insofar as no scar remained. A laser treatment could optionally be applied. The biochemical significance of the chitin/chitosan products was found to accompany their commercial viability in terms of time of healing and clinical labor costs.

Keywords: Chitin nanofibrils; Chitosan; Wound dressing; Scarless healing; Physiological tissue regeneration, Whiskers

1. Introduction

Recent progress in wound management is mainly in terms of physiologic support of healing. Because infections delay healing and worsen scar formation, there is interest in achieving closure as soon as possible. Main goals of wound care are prevention of infection, maintenance of a moist environment, protection of the wound, and minimum scar formation (Clark, 1996). A peculiarity of chitosan is the ability to foster adequate granulation tissue formation accompanied by angiogenesis and regular deposition of thin collagen fibres, a property that further enhances correct repair of dermo-epidermal lesions (Muzzarelli, 1993; Muzzarelli, Mattioli-Belmonte, Pugnaloni, & Biagini, 1999; Shi et al., 2006). In fact, the main biochemical activities of the chitin- and chitosan-based materials are:

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polymorphonuclear cell activation, fibroblast activation, cytokine production, giant cell migration and stimulation of type IV collagen synthesis (Kojima et al., 2004; Minami, Okamoto, Hamada, Fukumoto, & Shigemasa, 1999).

Chitin too has quite a relevant biochemical significance, in particular it accelerates macrophage migration and fibroblast proliferation, and promotes granulation and vascularization. While some chitin and chitosan derivatives also have biochemical significance, some other are rather inert, as it is the case for dibutyryl chitin; in general, however, they are biocompatible. The high biocompatibility of dibutyryl chitin in the form of films and non-wovens has been demonstrated for human, chick, and mouse fibroblasts by various methods in a previous article of ours: this water-insoluble modified chitin was also tested in full thickness wounds in rats with good results (Muzzarelli et al., 2005).

Several chitin-based hemostatic patches and gels are commercially available such as Beschitin® (Unitika), Chitipack-S®, and Chitipack-P® (Eisai), Clo-Sur® (Scion), Chitoseal® (Abbott), Syvek Patch® (Marine Polymer Technologies); Chitopoly® (Fuji Spinning); Crabyon® (Ohmikenshi); and the chitosan-based Hemcon® (Hemcon).

The Syvek Patch® is made of chitin microfibrils from the centric diatom *Thalassiosira fluviatilis* grown under aseptic conditions. It is claimed to be seven times faster in achieving hemostasis than fibrin glue, because it agglutinates red blood cells; activates platelets whose pseudopodia make a robust contact with chitin; promotes fibrin gel formation within the patch; platelets generate force through the clot retraction process and vasoconstriction takes place very soon; and a platelet + chitin + red cells + fibrin plug is formed, due to enzyme and platelets adsorbed on the chitin surface.

The mechanism of hemostasis induction by chitin nanofibrils is redundant insofar various biochemical reactions are involved simultaneously (Fischer et al., 2005); thus the *T. fluviatilis* microfibrils have been tested under the most demanding and crucial conditions requiring hemostasis, such as splenic hemorrage, cardiac catheterization, and bleeding esophageal varices, and found superior to all competing products. While the *T. fluviatilis* microfibrils (60×0.1 μm) are obviously longer than crustacean nanofibrils (typically 350×18 nm), both chitins used in these instances have the same molecular weight (2×10^6 Da) and acetylation degree (>0.90) (Chan et al., 2000; Kulling et al., 1999).

It is therefore reasonable to expect that the crustacean nanofibrils will be at least of comparable efficacy, while being less expensive because their production technology is simpler. The crustacean chitin nanofibrils have been described in a recent book chapter (Muzzarelli & Muzzarelli, 2005) and various articles (Raabe et al., 2005; Raabe et al., 2006).

Early demonstrations of the efficacy of chitin/chitosan in wound healing were based on irregularly shaped, grossly milled powders (Malette, Quigley, & Adickes, 1986). Later, freeze-dried layers permitted scarless restoration of vascularized tissue, and complete healing even in aged patients, in occasional experiments.

Chitin nanofibrils are expected to conform to the wound geometry, and to have immediate contact with cells in all the usual presentations. Molecular recognition phenomena take place on the chitin nanofibril surface (reported area 180 m^2/g), as demonstrated with the aid of fluorescent lectins, therefore various proteins can be retained by chitin nanofibrils. Moreover they can be suspended in gels, including chitosan gels, that dry soon after application thus making a flexible hydrated film.

Films were cast from chitosan solutions containing dispersed α-chitin nanofibrils. The addition of α-chitin nanofibrils did not affect much the thermal stability and the apparent degree of crystallinity of the chitosan matrix. The tensile strength of α-chitin nanofibril-reinforced chitosan films increased from that of the pure chitosan film to reach a maximum at the nanofibril content of 3% (Sriupayo, Supaphol, Blackwell, & Rujiravanit, 2005).

Scope of the present work was the industrially viable preparation of various types of wound medicaments endowed with enhanced healing capacities thanks to the synergistic action of chitosan salts and chitin nanofibrils. Due to the fragile nature of the freeze-dried chitosan sponge and to its high tendency to transform into a gel upon contact with wound fluids, the chitin-bearing freeze-dried chitosan needed to be supported by a flexible and biocompatible non-woven.

Because laser treatment increments biological an metabolic cell activities (Lauto, Stoodley, Avolio, & Foster, 2006; Shomina, Bogatov, & Chervinets, 2005) a further scope of this work was to examine the said innovative dressings in conjunction with laser, with a view to promoting valid healing of difficult-to-repair cutaneous wounds both from the dermatological and the aesthetic viewpoints (Stone, Wright, Clarke, Powell, & Devaraj, 2000). This work was planned to include pre-clinical data for a variety of surgical and traumatic wounds.

2. Materials and methods

2.1. Chemistry

The isolation of chitin nanofibrils was performed from crustacean chitin supplied by Katakura Chikkarin, Tokyo, Japan, according to published protocols (Muzzarelli & Muzzarelli, 2005). Ultrapure water was obtained with a Millipore MilliQ Academic apparatus. From the resulting aqueous suspensions the nanofibrils were obtained in dry form with the aid of a spray-dryer Buchi-190, Flawil, Switzerland, at a feed rate of 10 ml/min; the air inlet temperature was 148 °C, outlet 90 °C, and air flow 600 l/h; the temperature of the collecting cup did not exceed 35 °C. Therefore, the final presentations were the spray-dried nanofibril powder, and suspensions with various chitin contents.

The dibutyryl chitin (DBC) was the one previously used by Muzzarelli, Francescangeli, Tosi, and Muzzarelli (2004): the fibres were produced by wet spinning a 16% DBC solution in dimethylformamide, according to Wlochowicz, Szosland, Binias, and Szumilewicz (2004), and were analysed as described by Van de Velde and Kiekens (2004). The non-wovens were prepared from 6-cm fibres by needle-punching by SFM Speciality Fibres and Materials Ltd., Coventry, England. This collaborative work was done in the frame of the Chitomed Project QLK5.CT.2002-01331 supported by the European Commission.

The cosmetic/food grade chitosan was supplied by Giusto Faravelli S.p.A., Milan, Italy, with average molecular weight 150 KDa (as determined by laser light scattering), X-ray diffraction values 8.22° and 19.30° 2θ, and deacetylation degree 0.87 (as determined by differential UV–vis spectrophotometry (Muzzarelli & Rocchetti, 1985)). Other chemicals were supplied by Sigma–Aldrich, Milan, Italy, unless otherwise specified.

2.2. X-ray diffraction and infrared spectrometry

X-ray diffraction measurements on powder samples were performed with the Bruker AXS General Area Detector Diffraction System (GADDS) equipped with a two-dimensional gas-filled sealed multiwire detector (scattering-angle resolution of 0.02°). Monochromatized Cu Kα radiation ($\lambda = 0.154$ nm) was used. The powder samples were placed in 0.8-diameter Lindemann glass capillaries. The sample to detector distance was 10 cm. The intensity *vs* scattering-angle spectra were obtained after radial average of the measured 2D isotropic diffraction patterns.

A Perkin Elmer Spectrum GX FT-IR spectrometer equipped with a Perkin Elmer Multiscope system infrared microscope (MCT-SL detector) was used to record Attenuated Total Reflection, ATR, spectra. The microscope was equipped with a movable 75×50 mm X–Y stage. Small amounts of the sample, cooled in liquid nitrogen, were ground with KBr and the spectra were obtained by using a Spectra Tech. Diffuse Reflectance (DRIFT) accessory. In both cases, the spectral resolution was 4 cm^{-1}. The absorption spectra were the results of 16 scans. Treatments of the data were achieved with a Perkin Elmer Spectrum and with a Grams/32 Galactic Corp. software package.

2.3. Animals

Eight Whistar male rats aged 8–10 weeks and weighing 300 ± 20 g were subjected to comprehensive genetic and health quality controls (specific-pathogen free) by a veterinary surgeon before being housed in individual cages at the animal care certified facilities of INRCA, Ancona, Italy. Twenty-four hours before the operation and 36 h afterwards, each rat received 800 mg amoxicillin powder dissolved in water. Throughout the study rats were kept in a standard environment at a temperature of 20–22 °C, 55% relative humidity, with a 12-h light/dark cycle and *ad libitum* access to water and pellets. After intraperitoneal injection of 242 ng of 2,2,2-tribromoethanol/kg body weight to induce general anaesthesia, four circular dermo-epidermal dorsal incisions 0.5 cm in diameter were made on each rat. Protective dressings were applied to preserve the medicaments. Wounds were medicated every three days using one of the chitosan-based preparations listed above. Eight days after surgery, 4 rats (50%) received one- or two-pass laser treatment of all four wounds using a 810 nm diode Biolaser (Creation S.r.l., Verona, Italy) set as follows: Program, tissue regeneration; Power, 1.5 W; Pulse, 20 s; Mode, continuous; Wand, no contact; Distance, ≈5 cm. Animals were sacrificed 15 days after surgery.

2.3.1. Morphological analysis

Tissue fragments were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer, dehydrated in rising ethanol concentrations and embedded in araldite. Semithin and ultrathin sections were obtained with an LKB ultramicrotome: the former were stained with toluidine blue and examined under a Zeiss light microscope; the latter were treated with uranyl acetate and lead citrate and studied under a Philips CM10 electron microscope.

2.3.2. Immunohistochemistry

From skin fragments stored in liquid nitrogen, 6-μm sections were obtained with a cryotome, left to dry overnight and fixed in acetone for 10 min. Other samples were paraffin-embedded, cut into 6-μm sections and dehydrated through xylene and decreasing ethanol concentrations.

All sections were incubated overnight at 4 °C with anti-VEGF (dil. 1:200, Santa Cruz, CA) and anti-CD34 (dil. 1:20, BD Biosciences, Belgium) monoclonal antibody and processed with the streptavidin-biotin peroxidase technique. They were finally incubated with 3,3-diaminobenzidine, stained with Mayer's haematoxylin and mounted in Paramount. Antibody activity was examined using a Nikon light microscope.

Some frozen sections were processed with immunofluorescence methods using FITC-conjugated phalloidin (to highlight polymerized cytoskeletal actin) after fixing in acetone at 4 °C for 10 min. After rinsing in PBS, sections were permeabilized with 0.1% Triton X-100 in PBS for 15 min and then incubated in 0.1% BSA solution in PBS at 37 °C for 5 min. Tissue samples were then stained with 0.2 μM FITC-conjugated phalloidin at 37 °C for 30 min, washed in PBS and finally mounted in glycerol-base mounting medium (DAKO, Denmark) for observation with a Nikon fluorescence microscope. At least five fields per sample at 250× magnification were studied for the fluorescence and the immunoperoxidase reaction; the number of cells positive for the antibodies tested was expressed as the ratio of positive cells to all counted cells.

2.4. Patients

Seventy-five patients, 45–70 year old, were treated and controlled according to a standard protocol for 2 months.

Following routine methods the necrotic tissue was removed from the wound bed by use of proteases, hydrogels or surgically. In case of infection, antiseptic compounds were used. All patients gave their written informed consent in conformity with the ethics of medical device experimentation and the protocol for the study was reviewed and approved by appropriate Ethics Committee.

3. Results and discussion

3.1. Chemistry

The spray-dried nanofibrils were free-flowing white powders that could be easily re-dispersed in water and simple aqueous media such as chlorhexidine or glycolate solutions. The infrared spectra taken on spray-dried chitin nanofibrils indicated an outstanding definition of the absorption bands, due to the removal of poorly crystallized chitin (Fig. 1). The quality of our spectra was superior to published spectra for commercial chitin, and somewhat better compared to the published spectra of thermally dried nanofibrils. All of the typical bands for chitin were present, among which those at 3445 (NH and OH stretching), 1659 (amide I CO stretching), 1625, 1563 (amide II, NH deformation in the CONH plane), 1418 (CH deformation), 1375 (C–CH_3 amide stretching), 1314 (amide III and >CH_2 wagging), 1155 (COC bridge stretching), 1073 (COC stretching in ring), 1029 (CO stretching), 896 (beta linkage), 692 and 598 cm^{-1}. Some of these bands in Fig. 1 are sharper or more intense than ever seen, in particular the one at 1375 cm^{-1} that is most significant as it refers to the amido group, but also those at 1155, 953, and 896 cm^{-1} (for a comparison see for instance Lu, Weng, & Zhang, 2004).

The main feature of the X-ray diffraction spectrum for spray-dried chitin nanofibrils is the high intensity of the diffraction peak corresponding to $d = 9.624$, indicative of high regularity along the fiber axis, and the presence of clearly detailed minor peaks at other d values (Fig. 2). This spectrum indicates an exceptional degree of crystallinity of the spray-dried chitin nanofibrils. The peak at $d = 7.044$ in the present case is prominent, clearly contoured and 10 times higher than the noise whilst for common chitins it is just above the noise, or absent (for a comparison see for instance Sriupayo et al., 2005, spectrum "g" in Fig. 4; Sun et al., 2006).

DBC-coated test tubes were used to collect freshly drawn citrated or heparinized blood from donors for platelet counting. No significant platelet count variation was reported after 1 h contact. Non-coated test tubes containing a 2.5×0.7 cm DBC film were used as an alternative with the same results as above. Therefore, DBC films were deemed not to promote blood coagulation.

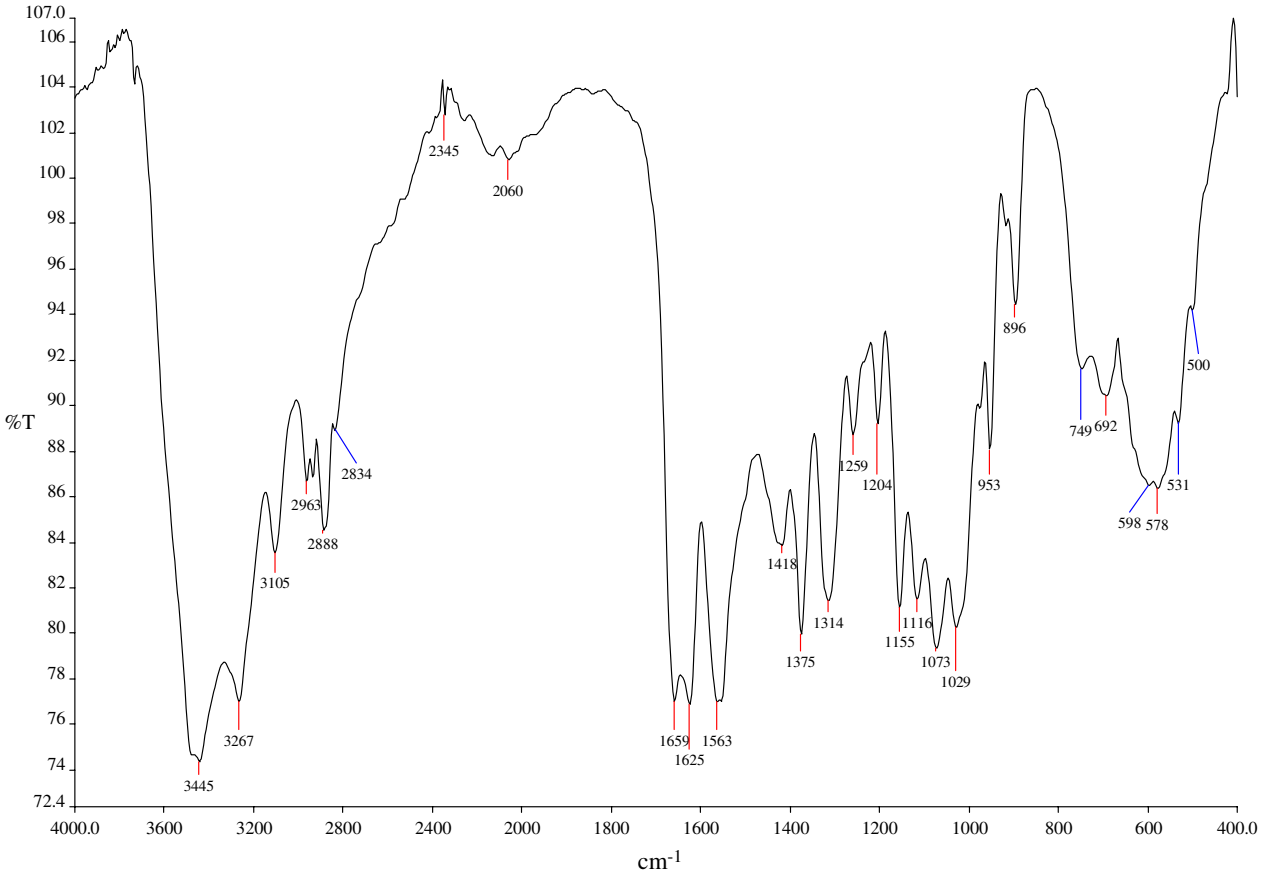

Fig. 1. Fourier transform infrared spectrum of spray-dried chitin nanofibrils.

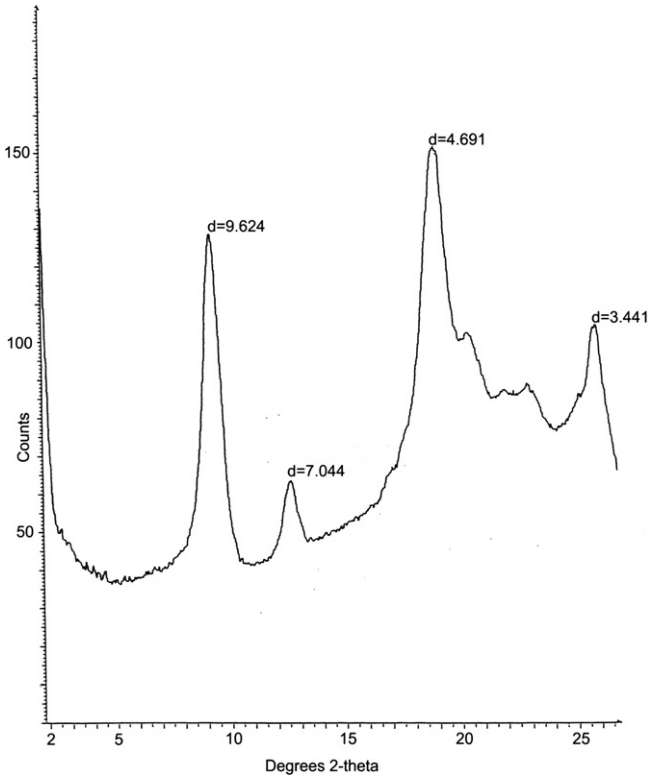

Fig. 2. Wide angle X-ray diffraction spectrum of spray-dried chitin nanofibrils.

3.2. Formulations

For the manufacture of the dressings, the chitin nanofibrils still in suspension after preparation, were incorporated into chitosan glycolate by adding to the suspension the desired amount of chitosan powder and glycolic acid crystals. No aggregation or precipitation of chitin nanofibrils was observed in this medium even after several months from the preparation. The preference given to chlorhexidine as a preservative was based on literature data indicating synergistic antimicrobial action of chitosan and chlorhexidine (Giunchedi, Juliano, Gavini, Cossu, & Sorrenti, 2002). The following cosmetic formulations were prepared:

Spray, a pearly, odourless liquid containing chitin nanofibril suspension (97.5%), 70% glycolic acid (0.56%), chitosan (0.97%), and chlorhexidine (0.40%); pH 5.5, viscosity <100 cps.

Gel, a transparent gel made of a chitin nanofibril suspension (91.1%), 70% glycolic acid (3.13%), chitosan (4.81%), chlorhexidine (0.40%); and sodium hydroxymethylglycinate, pH 4.00, viscosity 16,000–20,000 cps, as described in a previous article (Ricotti et al., 2001).

Gauze, a dressing made of dibutyryl chitin non-woven (0.8 g), chitosan glycolate solution 1.0% (8 g), containing chitin nanofibrils (2 g/l), and chlorhexidine (0.40%); pH 4.5. The preparations were frozen at −20 °C and freeze-dried at −93 °C; they were sterilized with ^{60}Co gamma irradiation at 25 KGy.

In addition, Phytostimuline (Damor S.p.A., Brescia, Italy), a gauze impregnated with aqueous *Triticum vulgare* extract and ethyleneglycol monophenolic ether, was used to medicate control wounds.

3.3. Wounds after medication in the murine model

3.3.1. Medications with no laser treatment

At day 7 the control lesion was already blurred and displayed an irregular greyish surface. The wounds treated with Spray showed a large clot whose amount and persistence indicated that the Spray exerted a significant coagulation activity. The lesions medicated with Gel exhibited limited tissue repair, giving the impression that the Gel had slowed down the proliferative-migratory epidermal reconstruction. In the wound medicated with the Gauze, reconstruction was at an advanced stage, and the lesion area deprived of re-epithelization was however reduced and had regular borders, indicating that correct and effective repair was taking place.

3.3.2. Medications with laser treatment

Two days after laser treatment (and 9 days after surgery) enhanced tissue regeneration was already macroscopically evident in all wounds compared to those being medicated in the absence of laser. In the former animals, acceleration of the healing process could be noticed in the wound medicated with Gauze, which was microscopically more effective than the Spray and the Gel even without the laser treatment. These effects were enhanced in the wounds subjected to double laser exposure compared to a single exposure.

Seven days after laser application (and 15 days after surgery), good tissue repair was observed. The effectiveness of the laser treatment justifies its inclusion into a therapeutic protocol where chitosan is however the principal enhancer of correct and effective tissue repair.

3.4. Morphological study

3.4.1. Gauze alone

A differentiated epithelium displaying the various epidermal layers, including a very thin horny layer in advanced stage of formation was present in the repair tissue dressed with Gauze. Some small vessels and rare inflammatory cells could be detected among collagen bundles (Fig. 3).

These features were partially at variance with those displayed by healed wounds medicated with the other two products. In particular, the Spray induced deposition of repair tissue with fairly irregular histological features such as scattered chitosan globules, some inflammatory cells entrapped in the regenerating epithelium and dermal neo-

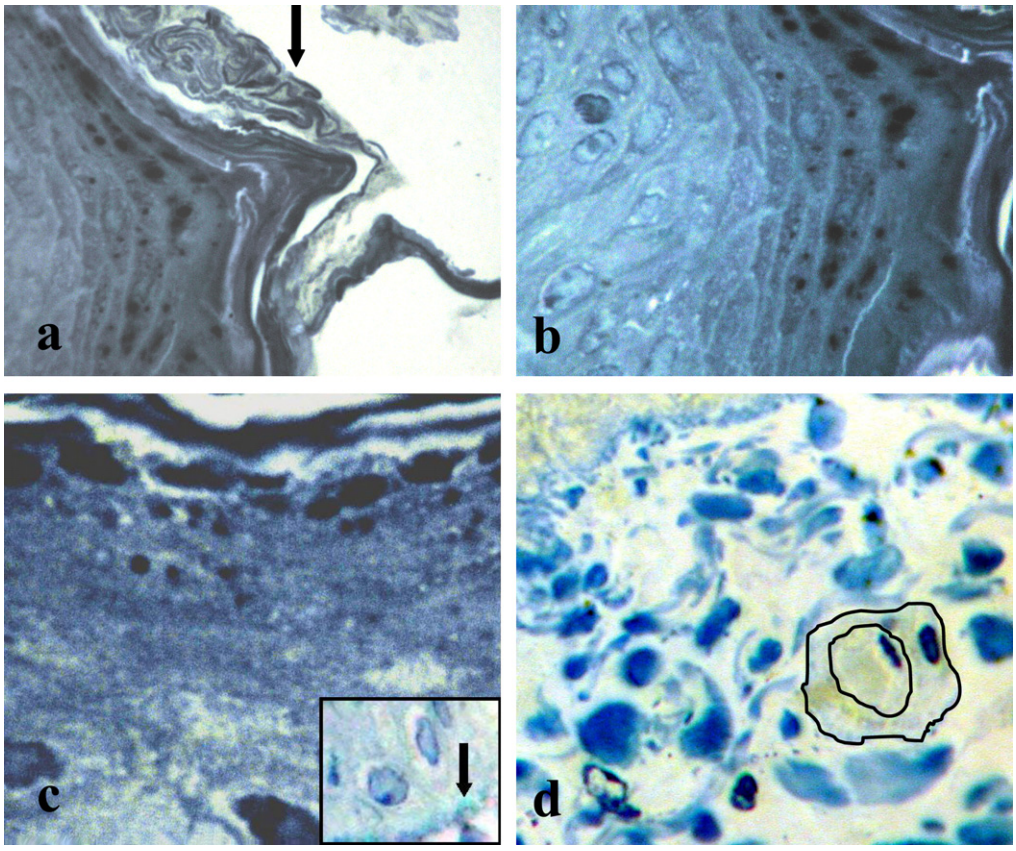

Fig. 3. Wound healing with Gauze. (a) The Gauze enhanced re-epithelization, no laser; incomplete degradation at 14 days (↓). (b) The Gauze stimulated wound repair and multi-layered epidermis formation, even without laser therapy. (c) Correct dermo-epidermal interface with presence of basal lamina after medication with the Gauze and two laser passes (↓). (d) The Gauze (here with laser treatment) induced sub-epithelial neoangiogenesis (*).

vascular structures, consistent with a less mature architecture of the regenerated epidermis compared with Gauze-dressed samples.

The Gel induced fairly good macroscopic tissue repair already at day 7; its relatively slow degradation rate should however be noted. Association with laser therapy appeared to develop effective synergies.

The control wounds exhibited more marked scars and thus failed to restore the physiological structure with an epithelial layer uniformly covering the underlying connective tissue. The control wound medicated with phytostimuline exhibited scarce maturation; the poorly organized cell architecture at the dermo-epidermal interface entailed high vulnerability to mechanical stresses. Even after laser therapy, Phytostimuline did not induce physiological histo-architectural regeneration at the dermo-epidermal interface. A poor reorganization of the basal lamina, with rough and irregularly arranged collagen bundles was observed.

3.4.2. Gauze with laser

Two-pass laser treatment proved to be an effective therapeutic option. The laser beam exerts a dual action, by speeding up extra-cellular matrix synthesis and deposition, as well as resolution of the initial oedematous-inflammatory state typical of skin lesions (Fig. 3).

Wounds medicated with no laser exhibited a less ordered epithelial histo-architecture, with less polarized cells and a larger number of inflammatory cells. The Gauze consistently induced better epithelial differentiation and keratinization.

The association of laser treatment is to be viewed favourably, especially in lesions encompassing both dermis and epidermis and in patients suffering from systemic diseases. The laser procedure appeared to be most effective in combination with the Gauze, although it accelerated healing in the spray-treated wounds. Hair follicles were detected in all treated rats irrespective of the laser treatment.

3.5. Immunohistochemistry

The tissue trophism of the medicated wounds was assessed with immunohistochemical methods using two compounds closely related with angiogenesis: CD34, found in newly formed vessels, and VEGF, a marker of vessel growth. In particular, the angiogenetic response induced

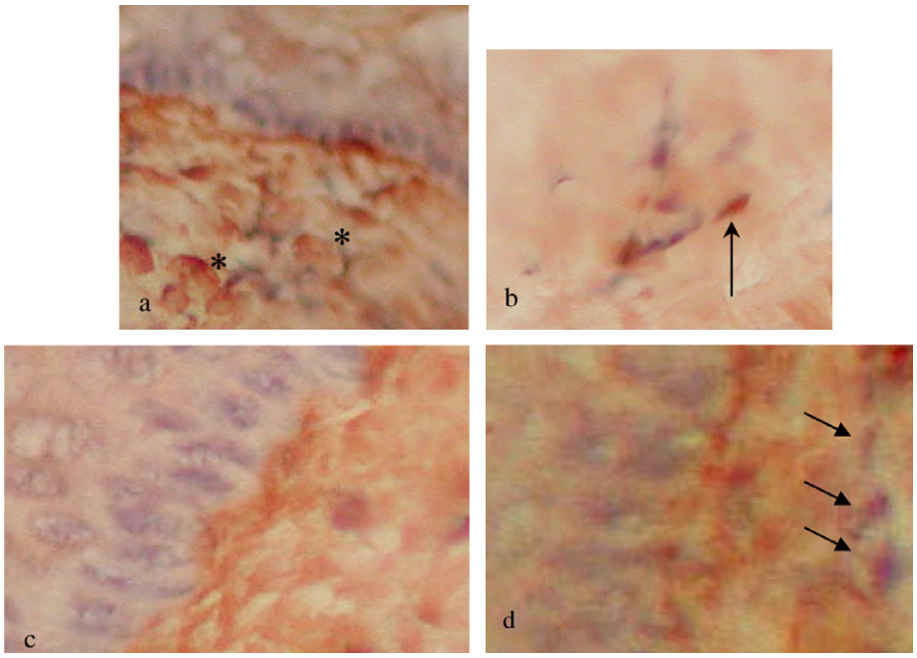

Fig. 4. Immunohistochemical detection of the angiogenic markers CD34 and VEGF in wounds treated with Gauze or Phytostimuline, all with two laser passes. (a) Vascular buds (*) after medication with Gauze (immunohistochemical VEGF reaction). (b) Longitudinal section of a vascular bud displaying CD34 cell staining (→) after treatment with Gauze (immunohistochemical CD34 reaction). (c) Absence of CD34 staining after treatment with Phytostimuline. Collagen bundles can be seen at the level of the dermo-epidermal interface; no vascular structures are evident. (d) Phytostimuline, absence of VEGF staining; intradermal Phytostimuline residues (→).

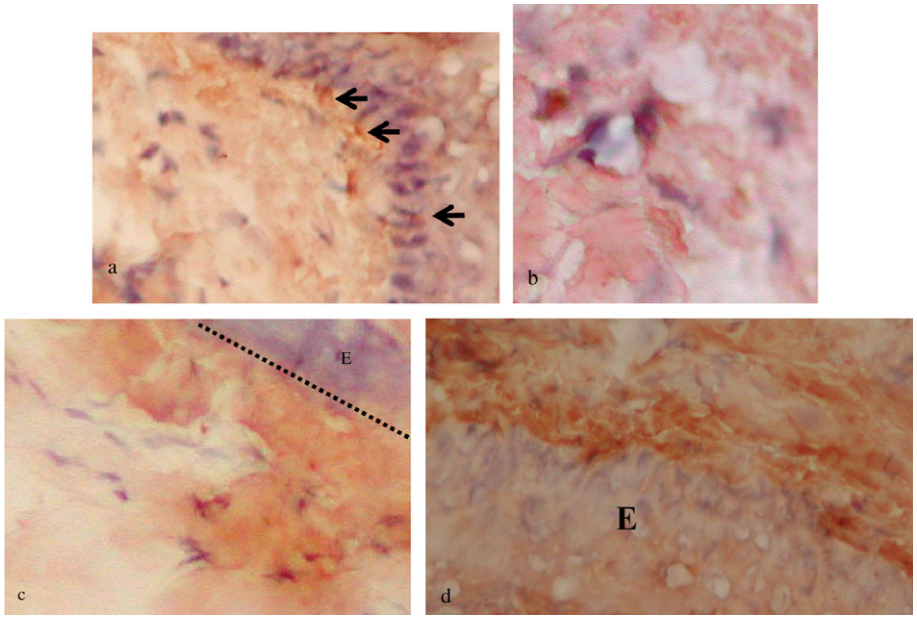

Fig. 5. Immunohistochemical detection of the angiogenic markers CD34 and VEGF in wounds treated with Gauze or Phytostimuline without laser application. (a) The Gauze induced dermo-epidermal regeneration, as shown by focal staining for VEGF at the level of the basal epidermal layer (→) and on the outline of the vascular buds that are forming in dermis (→). (b) The Gauze stimulated angiogenesis, as shown in this small vessel positive for CD34 (a marker of newly formed vessels) in endothelium. (c) Phytostimuline induced rough and irregular dermal repair without clear evidence of angiogenesis. The overlying epidermis (E) appears to lack the demarcation with dermis and even budding papillae (------). (d) Phytostimuline failed to regenerate the dermo-epidermal interface, so that the epidermis (E) ended up lying on a grossly irregular pattern of collagen bundles devoid of vessels and thus of endothelial CD34 staining.

by the Gauze compared with Phytostimuline, albeit not very strong, reflected satisfactory vascular trophism at the level of the dermis irrespective of the laser treatment (Figs. 4 and 5).

The actin cytoskeleton of dermal and epidermal cells was always well formed, but more evident in the wounds treated with the gel and the gauze, irrespective of laser treatment.

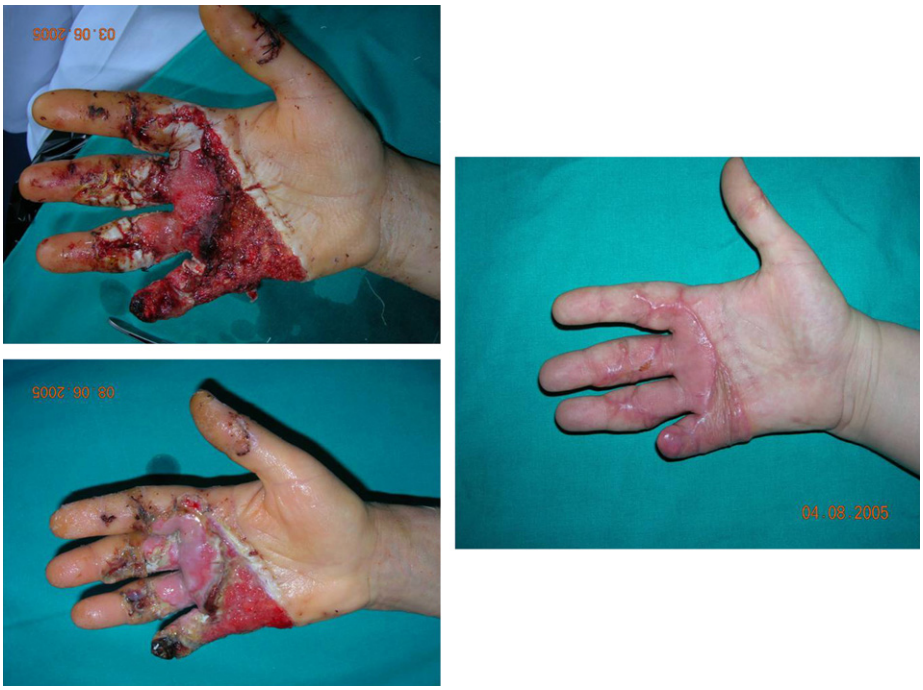

Fig. 6. Traumatic wound of the right hand. Complete healing in 30 days.

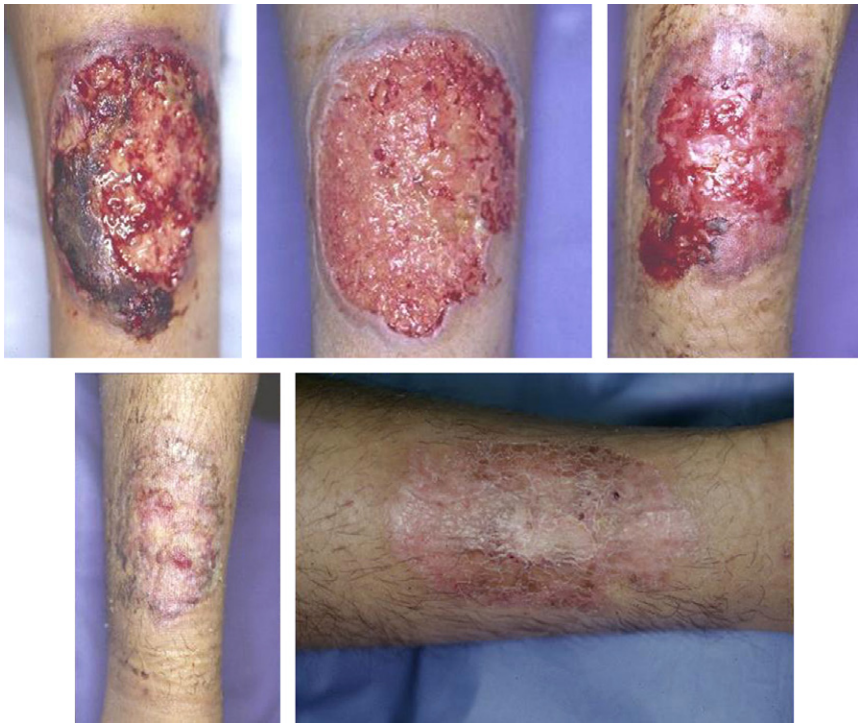

Fig. 7. Gangrenous pyoderma on tibial surface. Treated with Gauze and i.v. therapy of steroids and cyclosporine to ameliorate the wound bed. Complete healing in 40 days.

3.6. Clinical results

The Gauze was used to treat patients hospitalized for a variety of traumatic wounds. It was possible to maintain this new dressing on site for at least four days in all instances. The healing progress for some of the treated wounds is illustrated in Figs. 6–10.

It should be noted that the healing occurred within periods of time comparable to those observed for other dressings under similar conditions: that means that the Gauze

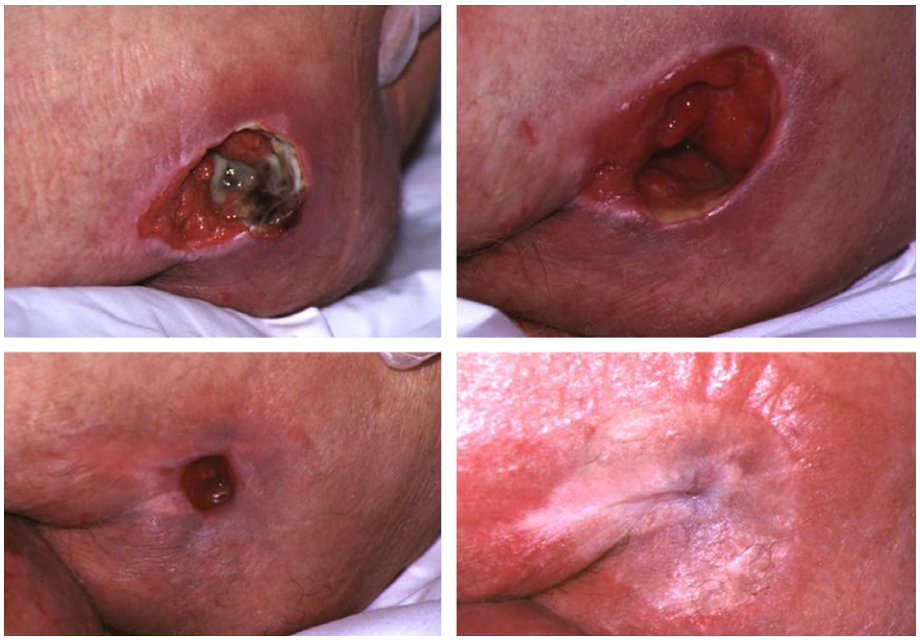

Fig. 8. Ischiatic decubitus ulcer in a paraplegic subject, and complete healing in 60 days.

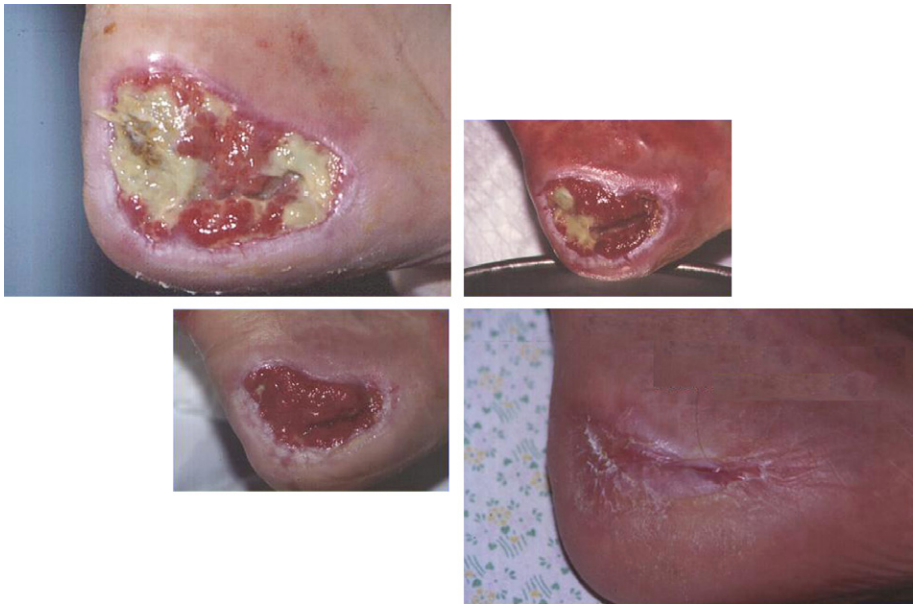

Fig. 9. Decubitus wound treated with protease for 10 days, and then with Gauze for 30 days. Complete healing in 50 days.

has potential commercial viability. In no case secondary infections developed, and there was no need to revert to any other dressings, considering the satisfactory results obtained.

The efficacy and biochemical significance of the Gauze can be appreciated when a comparison with plain DBC non-woven tissues is made: the latter do not prevent secondary infection even in case of daily changes.

4. Conclusions

The novel approach to wound medication involving the use of chitin nanofibrils in association with a chitosan salt has led to amply satisfactory results in all clinical cases examined. The Spray could be used as a first-aid tool for lesions such as abrasions that bleed little but involve a fairly broad skin area (e.g. an abrasion consequent to a fall on gravel). The Gel took longer to induce tissue repair; however, it is certainly capable of enhancing physiological repair, in particular of aesthetically important areas such as those with a thin epidermal layer (e.g. temples, eye area). The Gauze proved to be an effective device to restore a physiological and scarless epidermis.

In the treatment of torpid and slowly healing lesions or ulcers (e.g. in elderly patients with vascular problems and

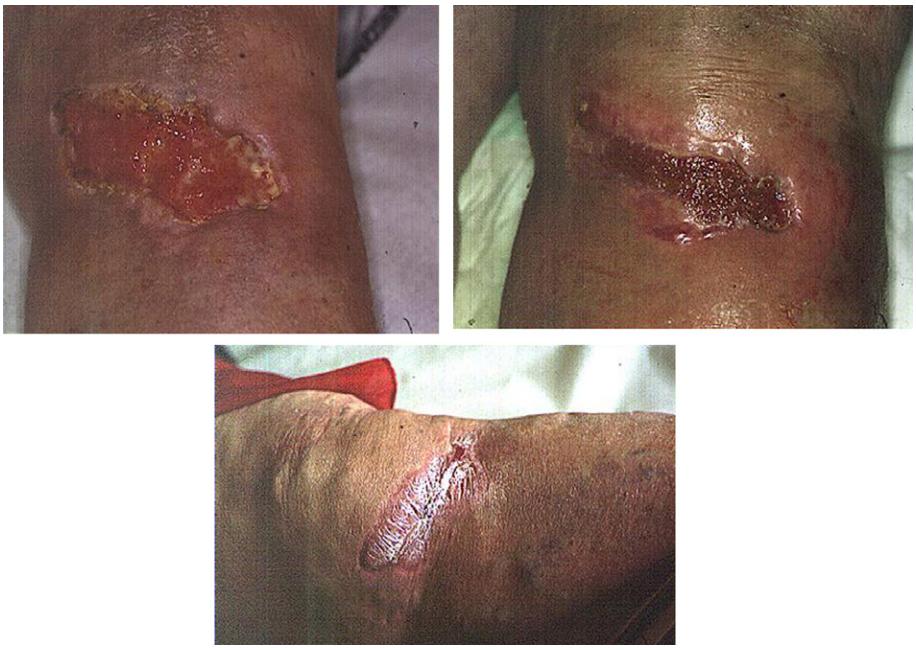

Fig. 10. Traumatic wound of the knee, and complete healing in 45 days.

systemic diseases), the association of the chitin + chitosan gel with a foam-like dressing could effectively induce nearly physiological repair, as opposed to irregular scar tissue observed in controls (that is poorly resistant to mechanical stress).

DBC is apt to support the *per se* fragile freeze-dried chitosan salt/chitin nanofibrils composite. Compared to the commercial wound dressing developed by Hemcon, Portland, OR, the Gauze is much thinner and pliable. One of the complaints with the HemCon, used on the Iraq battlefields, was that the 5-mm thick freeze-dried chitosan acetate pad goes into pieces upon handling when dry, or gelifies to excessive extents when moist. However, it was reported to be a safe and effective hemostatic agent to reduce post-hemodialysis puncture site bleeding (Bachtell, Goodell, Grunkemeier, Jin, & Gregory, 2006; Burkatovskaya et al., 2006; Wedmore, McManus, Pusateri, & Holcomb, 2006).

Of course the DBC non-woven can be replaced by other biocompatible non-wovens manufactured from man-made or natural polymers. Besides the wet-spun mats, today's non-wovens include electro-spun materials made of fibers with diameters typically in the order of a few tenths of a micrometer. Electro-spun fibers can also incorporate nanofibrils.

The nanofibrillar chitin/chitosan glycolate composites appear to be most suitable as medicaments able to exert control over various biochemical and physiological processes involved in wound healing, besides hemostasis: while chitosan provides antimicrobial activity, cell stimulation capacity and filmogenicity, chitin nanofibrils restructure the medium or the gel, slowly release *N*-acetylglucosamine and recognize proteins and growth factors.

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