Silicone-coated non-woven polyester dressing enhances reepithelialisation in a sheep model of dermal wounds

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Abstract Negative-pressure wound therapy (NPWT) also known as V.A.C. (Vacuum-assisted closure), is widely used to manage various type of wounds and accelerate healing. NPWT has so far been delivered mainly via open-cell polyurethane (PU) foam or medical gauze. In this study an experimental setup of sheep wound model was used to evaluate, under NPWT conditions, the performance of a silicone-coated non-woven polyester (N-WPE) compared with PU foam and cotton hydrophilic gauze, used as reference materials. Animals were anesthetized with spontaneous breathing to create three 3×3 cm skin defects bilaterally; each animal received three different samples on each side ($n = 6$ in each experimental group) and was subjected to negative and continuous 125 mmHg pressure up to 16 days. Wound conditions after 1, 8 and 16 days of treatment with the wound dressings were evaluated based on gross and histological appearances. Skin defects treated with the silicone-coated N-WPE showed a significant decrease in wound size, an increase of re-epithelialization, collagen deposition and wound neo-vascularisation, and a minimal stickiness to the wound tissue, in comparison with gauze and PU foam. Taken all together these findings indicate that the silicone-coated N-WPE dressing enhances wound healing since stimulates higher granulation tissue formation and causes minor tissue trauma during dressing changes.

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1 Introduction

Negative-pressure wound therapy (NPWT) is description given to an extremely efficacious therapy for treating a wide range of chronic or difficult cutaneous and soft tissue wounds [1, 2]. Widespread adoption of NPWT over the last 10 years has been driven largely through favourable clinical experience rather than randomized clinical studies or through scientific understanding [3]. Described in one form by Chariker [4] but largely popularized through the work of Argenta and colleagues [5] it was originally developed as an alternative treatment for chronic wounds in debilitated patients. It has rapidly evolved into a widely accepted treatment of diabetic foot, complex leg ulcers, pressure ulcers (stage III and IV), skin grafts, traumatic injuries, open abdominal wounds and dehisced sternal wounds [6, 7]. The ease of technique, a high rate of successes and the cost effectiveness have encouraged its adaptation by thoracic, general, trauma, burn, orthopaedic, urologic, as well as plastic surgeons [5, 8, 9].

NPWT typically uses a vacuum that is applied to tissue beneath an adhesive transparent film dressing almost invariably covering a wound-filler material of some description. The interactions of tissue and dressing result in a cascade of interrelated biological effects including the promotion of peri-wound blood flow, and a stimulation of granulation tissue formation, as originally defined by Argenta and colleagues [5].

The main functions of wound dressings are to facilitate wound healing and minimize scarring. They provide a physical barrier to protect the wound from further physical damages and any contaminations of exogenous organisms. They should also be permeable to moisture and air and to allow the extraction of extra body fluid from the wound area to maintain a partially immobilized moist environment. In addition, the dressing materials should be tidy and non

adherent to the wound so that they can be easily removed after the healing treatment [10].

Although many commercial wound dressing products, such as porous synthetic mesh, treated fine-mesh gauze, and absorbent gauze sponges, have been available, they are still less than ideal in areas ranging from decontamination of bacteria to regulation of microenvironment for skin regeneration [11]. Silicone coating was used to protect the blood from the effects of glass and today serves as a defoaming agent in extracorporeal circulation [12]. Recent studies showed that silicone dressings do not stick to a moist wound but only to the surrounding dry skin, helps to maintain a moist wound healing environment and makes it an ideal technology for non-adherent wound contact dressings [13–16]. For these reasons silicone dressings do not damage newly formed granulating tissue or epithelial cells during changes.

Based on these findings, the present study was aimed at evaluating the performance of a silicone-coated non-woven polyester (N-WPE) dressing in preliminary experiments on a sheep model of dermal wounds treated with NPWT. The sheep model was chosen since it allows the creation of a relatively shallow wound with large surface area that is typical of those wounds that might result from traumatic injury or decubitus ulcers, and which are frequently treated with NPWT. The delivery of negative pressure to the wound through the silicone-coated N-WPE dressing was compared with either open-cell PU foam or medical gauze.

2 Materials and methods

2.1 Silicone coating of N-WPE dressing

The N-WPE dressing (white, thickness 15 mm, fiber diameter $\sim$20 μm) and the silicone [a Simethicone water emulsion, in conformity with the requirements of the UPS (United States Pharmacopea) and FDA (Food and Drug Administration), Regulation 21 CFR 332.10] was kindly supplied by Eurosets S.r.l. (Modena, Italy). The N-WPE dressing was placed between two 12 mm spaced round Teflon® plates (Fig. 1) and bathed in a glass backer filled with a warmed (50 °C) simethicone emulsion for 10 min (dipping technique). After that the N-WPE dressing, inserted between the Teflon® plates, was placed in slow rotation (1 RPM) and let to dry at 80 °C for 100 min in a temperature controlled oven. Finally the silicone-coated N-WPE dressing was removed from the Teflon® plates. The coated dressing was sterilized by low-temperature gas plasma before animal wound healing testing.

2.2 Evaluation of air and liquid permeability

The air permeability of silicone-coated N-WPE dressing was measured through an in vitro circuit. Briefly, samples
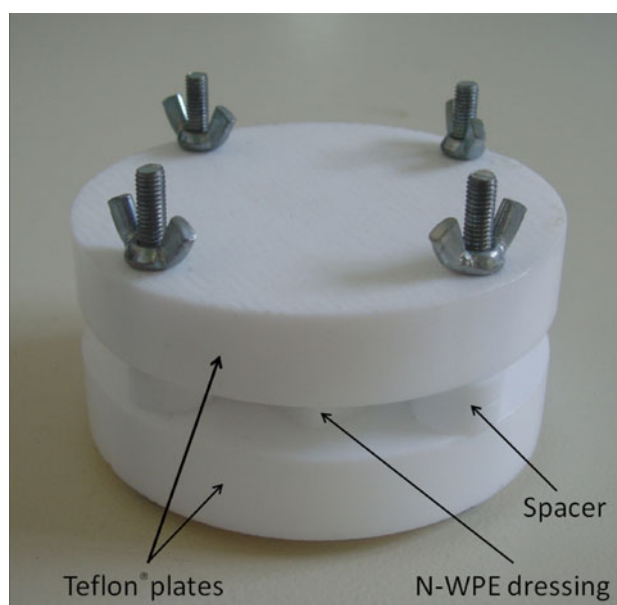
Fig. 1 Set-up for coating the N-WPE dressing. The N-WPE dressing is mounted and hold in position between two Teflon plates. Four cardinal spacers determine the dressing thickness. Once assembled the set-up is slowly dipped in a warmed Simethicone emulsion (further N-WPE processing details are reported in the Sect. 2)

were placed in an airtight Teflon® chamber connected to a flow-meter and a vacuum source (air compressor combined with a Venturi valve). The chamber was provided by a manometer. The vacuum (125 mmHg) was applied in the chamber clamping the tube between samples and flow-meter, then the clamp was removed and the sample strength opposed to air flow was measured by the flow-meter (l/min).

The liquid permeability of silicone-coated N-WPE dressing to saline solution (0.90 % w/v of NaCl) and Haemaccel® (Pierrel Medical Care S.p.A. Potenza, Italy) was evaluated through an in vitro circuit similar to that employed in air permeability measurement. Haemaccel® is a colloidal intravenous infusion solution 3.5 % w/v used as a plasma volume expander in the treatment of hemorrhagic shock. In this study Haemaccel® was employed because of its viscosity is more similar to wound exudates respect to saline solution. Briefly, sample weight was recorded before starting the experiment. A *reservoir* was connected to a suction flask through a filtering funnel in which samples were placed. The Teflon® chamber was connected to the flask and the vacuum (125 mmHg) was applied by the Venturi valve. Then the clamp was removed and the liquid was allowed to pass through the sample for 15 min. At the end of the experiments samples were weighted to determine the amount of absorbed liquid and the volume of liquid in the flask was measured.

The permeability values of silicone-coated N-WPE dressing was compared to that related to uncoated PE dressing used as reference material.

2.3 Animal Study

Three adult sheep weighing 50–60 kg were procured and allowed to acclimate to the research facilities for 2 weeks. All animals received humane care according to guidelines from ''*Dipartimento Alimenti, Nutrizione e Sanità Pubblica Veterinaria—Ministero della Salute*'' which approved this study, based on the Italian Legislative Decree 116/92 regarding animal experimentation. During animals treatment Authors also considered the US guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care International (http://www.aaalac.org/index.cfm).

On the day of surgery, animals were anesthetized with spontaneous breathing with 50 % O_2, 50 % N_2O and isofluorane 3 %. Then auricular venous access was accomplished by catheterization (Angiocath 20 Fr) to administer Propofol (Diprivan®). The anaesthesia was deepened with Propofol (Diprivan®) 0,5-1 mg/kg and maintained with 30 % O_2, 70 % N_2O, isofluorane and endovenous infusion of Propofol 3-6 mg/kg/h.

Sheep was placed in ventral decubitus, back was accurately shaved and prepped with betadine. Under sterile conditions, three 3×3 cm skin defects were created bilaterally down to the deep fascia of the muscles (Fig. 2a).

10 % Enrofloxacin (Baytril®) was given for 10 days as antibiotic therapy and Fynadine® 2 ml/45 kg as analgesic therapy for 3 days. Animals general health was checked daily.

In this study each animal received three different samples (3×3 cm) on each side ($n = 6$ in each experimental group): a silicone-coated N-WPE dressing, a PU foam (V.A.C.® Granufoam® KCI, TX, USA) and a cotton hydrophilic gauze (Kerlix™ AMD™ Antimicrobial Super Sponges, Tyco Healthcare/Kendall, MA, U.S.A.) as reference materials (Fig. 2b). All samples were covered with a cotton gauze (Luigi Salvadori S.p.A., Firenze, Italy) (Fig. 2c) and then sealed with transparent adhesive Incise Drape (Medical Device S.r.l., Arezzo, Italy) (Fig. 2d). Finally, the drainage tubes were connected to the negative pressure wound therapy device (WaterLily™ Suction Head, and WaterLily ™ Reservoir Eurosets S.r.l. Modena, Italy) and subjected to negative 125 mmHg of continuous pressure for 16 days.

At time-points of 1, 4, 8, 11 and 14 days, animals were sedated by spontaneous breathing with 50 % O_2, 50 % N_2O and isofluorane 3 % as previously described and dressings were appropriately changed. Wound macroscopic photographs were taken on the day of surgery and at all
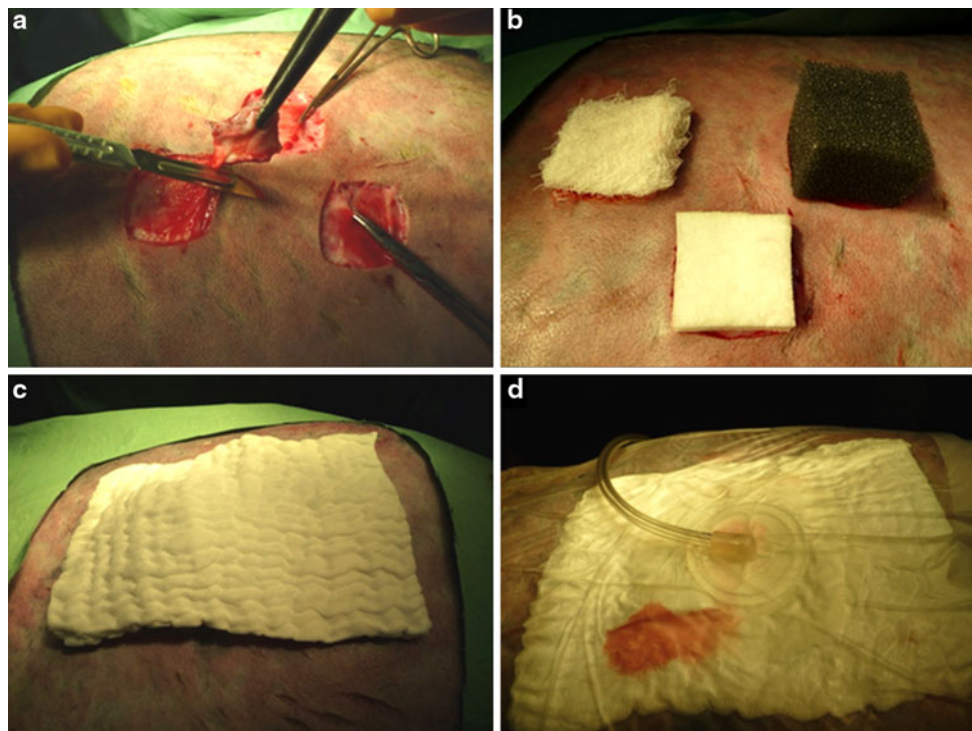
Fig. 2 Identical wounds (3×3 cm) were created on the shaved back of sheep (**a**). Wounds were treated with cotton hydrophilic gauze (on the *left*), polyurethane foam (on the *right*) and silicone-coated N-WPE dressing (on the *bottom*) (**b**). All samples were covered with a cotton gauze (**c**), sealed with transparent adhesive film and connected to the VAC device trough a drainage tube (**d**)

postoperative time-points and wound closure was calculated as a percentage of the final to initial areas by Axio-Vision Rel. 4.6 Software (Carl Zeiss, Gottingen, Germany). In addition, at time-points of 1, 8 and 16 days, a small part of the sub-dressing tissue (3 × 3 mm) of each sample was harvested for histological analysis.

2.4 Histology and wound analysis

The excised sub-dressing tissues were fixed in 4 % buffered formalin for 20 h. All samples were dehydrated through a graded alcohol series and xylene, embedded in paraffin and cut in 7-μm-thick cross-sections. Haematoxylin and eosin (H&E) staining was carried out to evaluate the inflammatory reaction and epithelialization. Collagen fibres were detected by Masson's trichrome staining.

The deparaffinized histological slides for immunohistochemical staining were pre-treated with pH 6 citrate buffer and microwave to facilitate antibody penetration.

For detection of blood vessels, sections were incubated at r. t. for 2 h with a 1:200 dilution of rabbit polyclonal anti-human PECAM-1 primary antibody (Abbiotec, San Diego, CA, USA) in 1 % bovine serum albumin (BSA, Sigma-Aldrich) in PBS. Staining was performed to assess the expression of α-smooth muscle actin (α-SMA) using a 1:25 dilution of a mouse monoclonal to actin primary antibody (Abcam, Cambridge, UK) applied for 30 min at r. t.. The primary antibodies were then detected by Ultra-Vision LP system (Bio-Optica) employing diaminobenzidine substrate and the slides were counterstained with Mayer's hematoxylin.

Representative images of histological sections were taken at 50× and 100× original magnification using an AxioPlan 2 microscope (Carl Zeiss, Jena, Germany) equipped with a color camera (KY-F32, JVC, Milan, Italy) using the image analysis software AxioVision.

3 Results

3.1 Macroscopic evaluation of coating

The silicone coating of the N-WPE original material (Fig. 3a) reduce the overall thickness of the dressing from 15 up to 12 mm and provided a smoother and more homogenous appearance to the N-WPE dressing (Fig. 3b). After silicone coating no free threads have been observed on N-WPE surfaces (Fig. 3d) respect to the uncoated one (Fig. 3c). The single N-WPE threads appeared to be coated by silicone at microscopic observation.
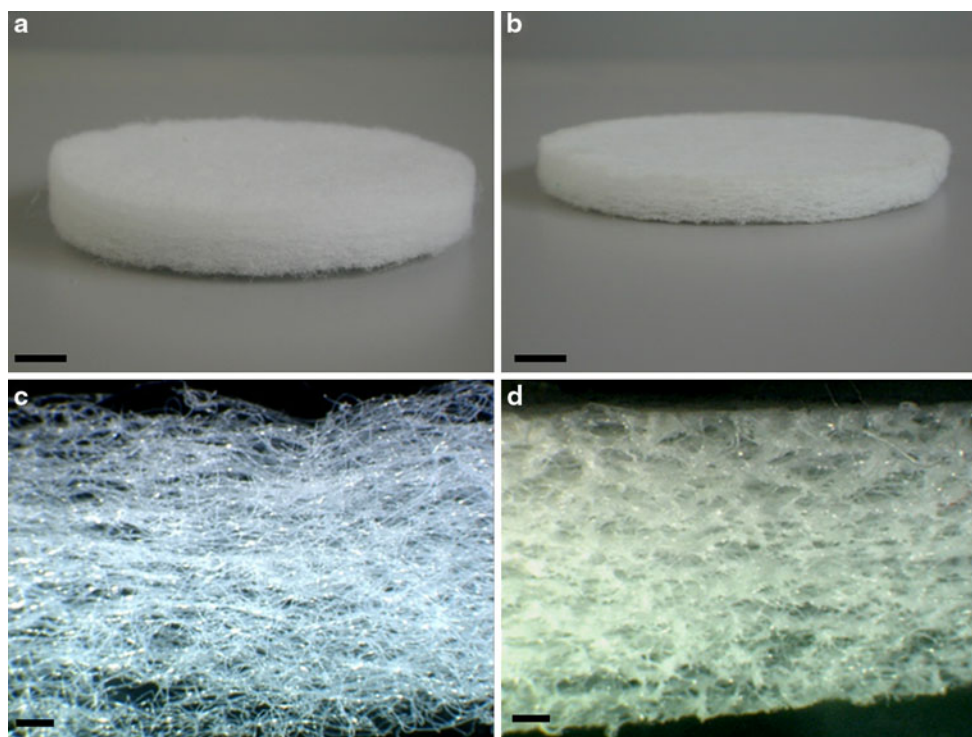
Fig. 3 N-WPE dressing (*white*, thickness 15 mm, fiber diameter ∼20 μm) (**a**); silicone-coated N-WPE dressing (thickness 12 mm) (**b**); transversal section of uncoated N-WPE dressing (O.M. ×7) (**c**); transversal section of silicone-coated N-WPE dressing (O.M. ×7) (**d**). Scale bar = 10 mm in **a** and **b**; scale bar = 1 mm in **c** and **d**

3.2 Evaluation of air and liquid permeability

The air permeability of silicone-coated N-WPE dressing was about 10 l/min, while the permeability value of the uncoated dressing was about 10.5 l/min.

The saline solution permeability, determined by the sum of filtered and absorbed saline, of both coated and uncoated N-WPE dressing was about 200 ml. Finally, the Haemaccel® permeability of silicone-coated N-WPE dressing was about 200 ml, while the permeability value of the uncoated one was about 195 ml.

3.3 Macroscopic analysis of wound healing

During dressing changes the silicone-coated N-WPE showed minor adhesion to the wound tissue in comparison with reference materials allowing minor bleeding and tissue trauma.

The animals and their respective wound gross morphology were followed at the postoperative time-points and the percentage of healing area was calculated respect to original area. Higher percentage indicates a higher healing efficiency. Among all the trial groups, silicone-coated N-WPE dressing showed the highest healing percentage, about 56 %, at 16 days of healing (Fig. 4a). The PU foam, instead, was lower in healing percentage (about 51 %) than the silicone-coated N-WPE dressing, but slightly higher than the cotton hydrophilic gauze (about 46 %) (Fig. 4b, c).

3.4 Histological examination

After 1, 8, and 16 days post-implantation, the harvested biopsy specimens were processed for histology. At all time-points no material fragments or debris were observed in the granulation tissues of silicone-coated N-WPE and reference dressings. At day 1, only muscle and adipose tissues were observed both in sample tissues and in reference materials. After 8 days, the silicone-coated N-WPE dressing induced a slight inflammatory reaction constituted by a few cell number, most of which were judged to be neutrophils and macrophages. 6 out of 6 samples showed neovascularization with numerous capillaries directed to wound sites and pro-collagen deposition (Masson's trichrome staining) (Fig. 5a–c). CD31 and α-SMA positive staining confirmed the presence of capillaries in the tissue (Fig. 5d). At days 8, the cotton hydrophilic gauze and PU foam showed the presence of both inflammatory cells (macrophages and neutrophils) and spindle-shaped cells, considered to be ingrowing fibroblasts within the biopsy specimens and there was no substantial difference among the two dressings in the relative proportion of these infiltrating cell populations (H&E staining) (Fig. 6a–d).

At day 16, the silicone-coated N-WPE dressing promoted an increase in the granulation tissue formation compared to reference dressings. After 16 days there were fewer inflammatory cells than at 8 days. As the healing progressed, new extracellular matrix with collagen fibers was observed. In addition, a mature epithelial layer had formed on the upper surface of the wound site (5 out of 6 samples) (H&E and Masson's trichrome staining) (Fig. 7a, b). Compared with the reference dressings (Fig. 8b), a significantly greater number of positive stained cells for both CD31 and α-SMA antigens in the wound skin treated with silicone-coated N-WPE dressing was observed (Fig. 7c, d). In Fig. 7d a developing epidermis is visible. At day 16, a severe inflammatory reaction with foreign body giant cells was still present in the reference materials (H&E staining) (Fig. 8a–c). The epithelium formation was mostly absent, although in some specimens of both cotton hydrophilic gauze (2 out of 6) and PU foam (3 out of 6) an immature epithelium formation was observed (Masson's trichrome staining) (Fig. 8d).
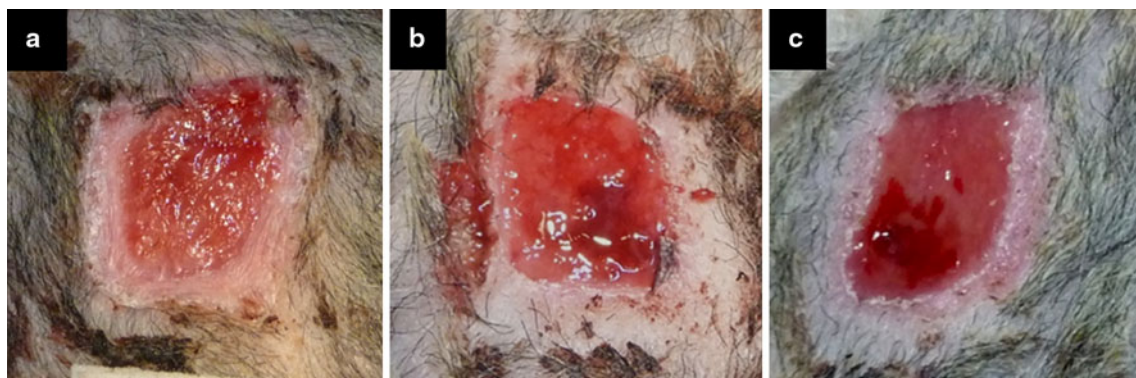

Fig. 4 Gross morphology of skin wounds treated with silicone-coated N-WPE dressing (**a**), cotton hydrophilic gauze (**b**) and PU foam (**c**) on day 16 post-wounding. A representative wound is shown for each group
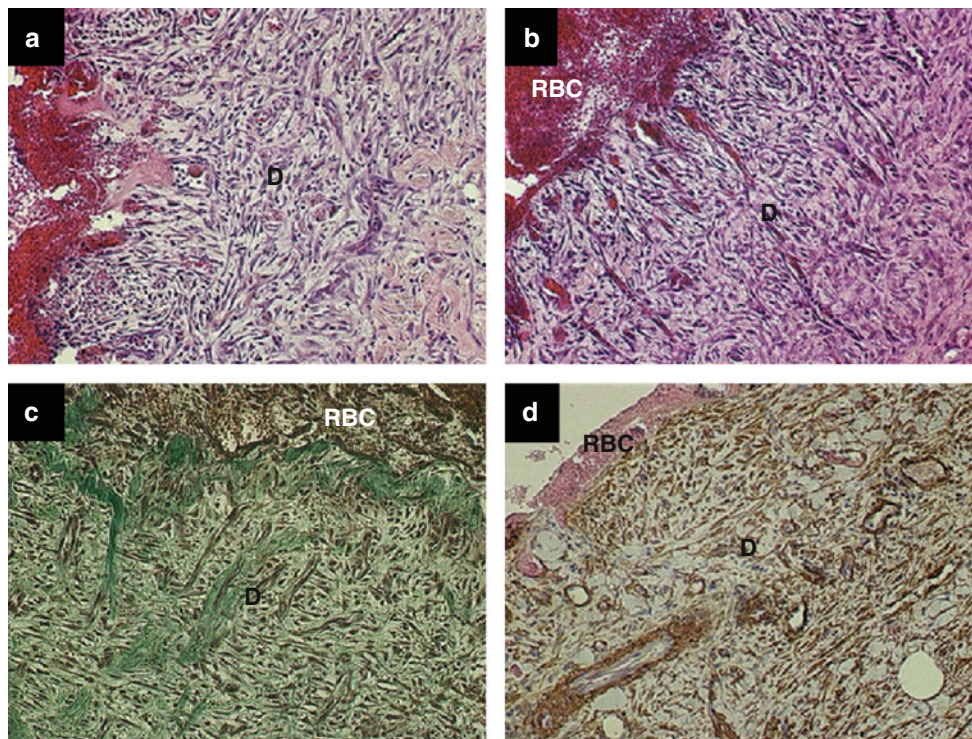
Fig. 5 - Wound cross-section treated with silicone-coated N-WPE dressing harvested on day 8 post-wounding and stained with H&E (*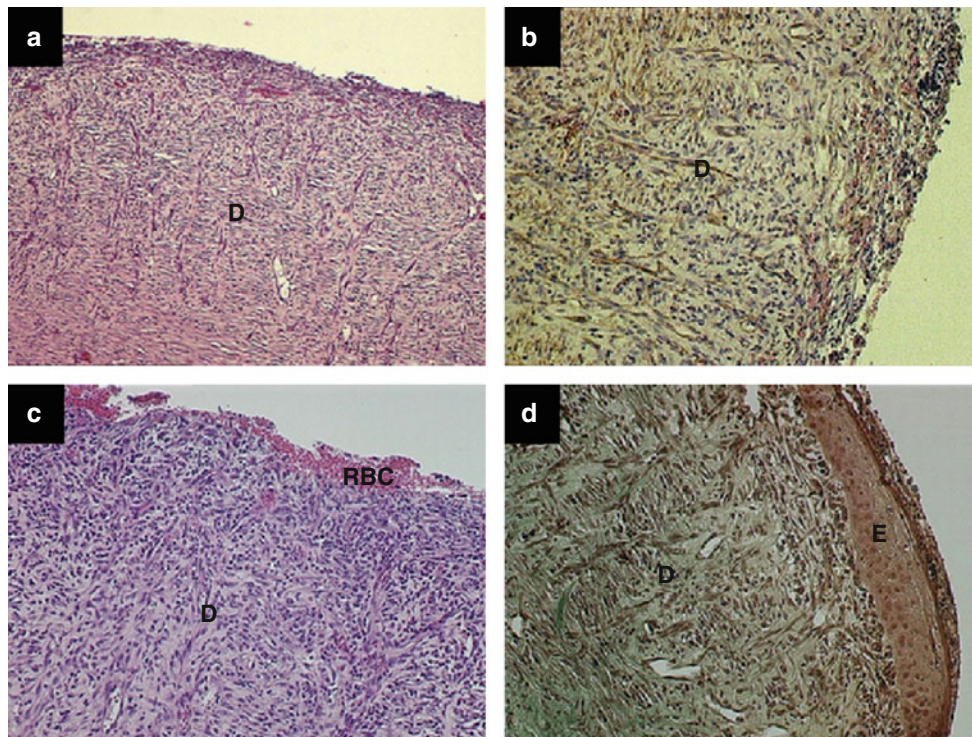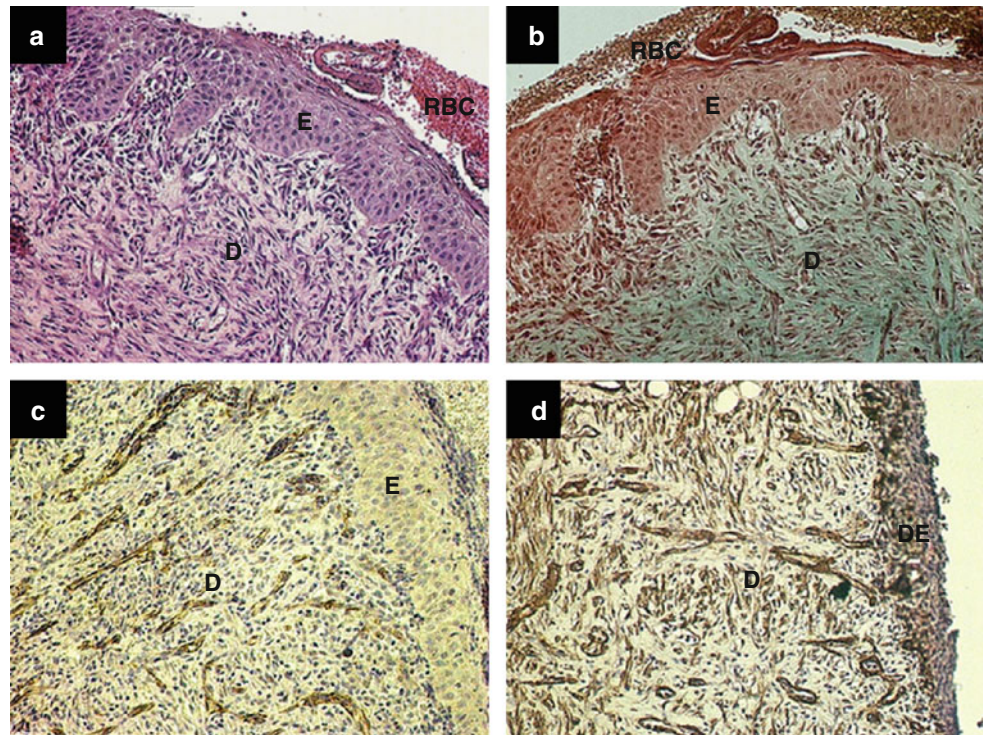*a**, **b**), Goldner's Masson trichrome (**c**) and anti-α-SMA antibody (**d**) (original magnification ×100). Collagen fibers stained with light green SF yellowish appear green; α-SMA positive cells appear brown (DAB substrate employed). *D* dermis. *RBC* red blood cells
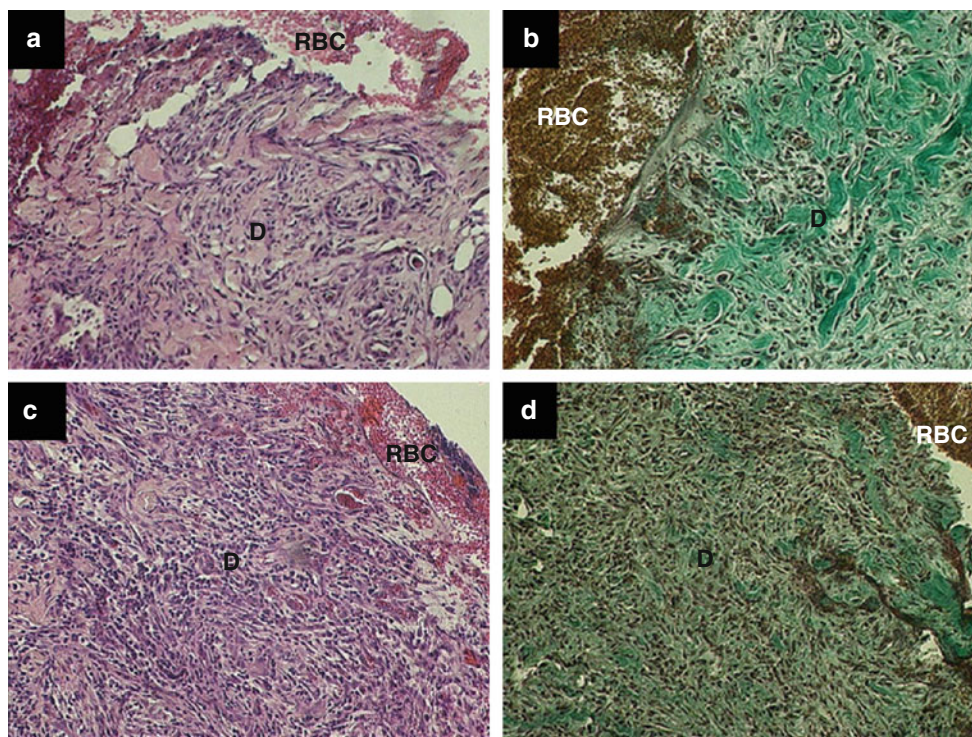
Fig. 6 Wound cross-section treated with cotton hydrophilic gauze harvested on day 8 post-wounding and stained with H&E (**a**), Goldner's Masson trichrome (**b**). Wound cross-section treated with PU foam harvested on day 8 and stained with H&E (**c**), Goldner's Masson trichrome (**d**) (original magnification ×100). Collagen fibers stained with light green SF yellowish appear green. *D* dermis, *RBC* red blood cells

Fig. 7 Wound cross-section treated with silicone-coated N-WPE dressing harvested on day 16 post-wounding and stained with H&E (**a**), Goldner's Masson trichrome (**b**), anti-CD31 (**c**) and anti-α-SMA antibodies (**d**) (original magnification ×100). Collagen fibers stained with light green SF yellowish appear green; CD31 and α-SMA positive cells appear brown (DAB substrate employed). *D* dermis, *E* epidermis, *RBC* red blood cells, *DE* developing epidermis

Fig. 8 Wound cross-section treated with cotton hydrophilic gauze harvested on day 16 post-wounding and stained with H&E (**a**) and anti-α-SMA antibodies (**b**). Wound cross-section treated with PU foam harvested on day 16 and stained with H&E (**c**) and Goldner's Masson trichrome (**d**) (original magnification ×100). Collagen fibers stained with light green SF yellowish appear green and α-SMA positive cells appear brown (DAB substrate employed). *D* dermis, *E* epidermis, *RBC* red blood cells

4 Discussion

The wound healing process is a complex cascade of mechanisms including hemostasis, inflammation, proliferation, contraction, and remodelling, each of these phases involves distinct cell types [17–19]. In the tissue formation phase, angiogenesis, granulation, and reepithelialisation occur, and endothelial cells, fibroblasts, and keratinocytes are mainly involved [17, 18, 20].

Negative pressure wound therapy (NPWT) has remarkable effects on wound healing [5]. A number of studies have examined the mechanisms involved in NPWT and have shown evidence for increased wound edge microvascular blood flow, granulation tissue formation, and exudates removal [21, 22], while its role in reduced bacterial counts is still under discussion [23–26].

Reorganization of damaged tissue requires restoration of appropriate architecture and biomechanical properties. Supplementing the tissue defect with biocompatible scaffolds that may be derived from natural or synthetic sources can augment this aspect [27].

In this study a novel silicone-coated N-WPE dressing was developed to avoid the material debris loss previously observed in scar tissue treated with N-WPE scaffold and the dressing-tissue adhesion that impairs the healing process during dressing changes determining bleeding, lack of granulation tissue and continuous inflammatory stimulus.

The coating of N-WPE dressing was obtained by dipping technique employing a biocompatible Simethicone water emulsion, previously used in biomedical application [12]. The air, saline and Haemaccel® permeability of coated dressing was evaluated in vitro to verify that the silicone coating does not affect the N-WPE permeability, in fact it has been shown that structure dressings influence the delivery of topical negative pressure therapy to the tissue [28]. The results of permeability evaluation suggested that the silicone coating does not hinder the transduction of negative pressure to the wound bed.

The in vivo performance of silicone-coated N-WPE dressing was evaluated in preliminary experiments in full-thickness defects in sheep animal model subjected to NPWT up to 16 days with dressing changes every third days. Two commercial wound filling materials, open-cell PU foam and medical gauze, were subjected to the same experimental conditions of the silicone-coated N-WPE dressing and used as reference dressing. The PU foam is a well documented wound filler frequently used in clinic because it offers a high degree of conformability to the wound surface [5]. The medical gauze is an alternative wound filler used earlier in the development of NPWT but which has only recently become commercially available for the application of NPWT in clinical practice [4].

The new silicone-coated N-WPE dressing material tested in the current study showed adequate pressure transduction to the bottom of the wound, which is crucial in order to assure drainage of excess wound fluid and debris. Moreover, the new wound-dressing showed minor stickiness to the wound tissue in comparison with open-cell PU foam and medical gauze. These findings are in according to what was reported by recent studies, which showed that silicone-based dressings allow atraumatic and pain-free changes [13–16].

The histological findings showed that the silicone-coated N-WPE dressing increased granulation tissue formation and accelerated skin wound healing from day 8 to day 16 after injury, suggesting the speeding up of granulation and reepithelialisation respect to open-cell PU foam and medical gauze. Likewise, no N-WPE filaments or fragments were observed in the scar tissue showing that the material does not shed into the wound, contrarily to what has been experienced with cotton and uncoated non-woven fibre substrates. Furthermore, angiogenesis, which is required to sustain the newly formed granulation tissue, was induced by silicone-coated N-WPE dressing on day 8 after injury.

The results concerning open-cell PU foam and medical gauze showed comparable effects on wound healing in terms of collagen deposition, amount of granulation tissue and immature reepithelialisation on day 16 after injury.

5 Conclusion

In this study we demonstrated that the use of a newly developed silicone-coated N-WPE dressing enhances the proliferation of endothelial cells, fibroblasts, and keratinocytes in wound tissue, leading to an accelerated development of granulation tissue and reepithelialisation that are required for the healing of chronic wounds. Another important aspect to consider is that the silicone-coated N-WPE dressing showed minor stickiness to the wound tissue in comparison with open-cell PU foam and medical gauze, therefore allowing less traumatic dressing changes. Moreover, it can be speculated that the biocompatible silicone coating here described may be a suitable tools to incorporate a broad range of therapeutic agents with the aim to develop bioactive dressing able to sustain a local drug release. These bioactive dressings, jointly with the NPWT, can be useful to potentiate the treatment of chronic wounds, such as venous leg ulceration, a growing challenge in the aging population. At the present, the scale-up and the evaluation in a pilot clinical trial of silicone-coated N-WPE dressing is under planning.

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