Wound Medicine 5 (2014) 9–15

Contents lists available at ScienceDirect

Wound Medicine

journal homepage: www.elsevier.com/locate/wndm

Electrospun soy protein scaffolds as wound dressings: Enhanced reepithelialization in a porcine model of wound healing

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ARTICLE INFO

Article history:
Received 18 April 2014
Accepted 24 April 2014
Available online 2 May 2014

Keywords:
Soy protein
Electrospinning
Scaffold
Wound healing
Porcine animal model
Reepithelialization

ABSTRACT

In this study we describe the use of an electrospun soy protein-based tissue scaffold (SPS) as a bioactive wound dressing in a pig model of full thickness excisional wound healing. The time course of wound healing and the quality of the healing tissue were evaluated using histology (H&E and Masson's trichrome staining). While the overall rate of wound closure was similar in the SPS-treated *vs.* untreated control wounds covered with Tegaderm®, there were significant qualitative differences between the two groups. Two weeks after a single application of SPS at the time of wounding, the SPS treated wounds showed robust signs of reepithelialization, which was absent in the control wounds. After 4 weeks, the SPS treated wounds contained a stratified epithelial layer in the epidermis that looked essentially normal, while the connective tissue in the dermis was attaining a cellular, organized appearance. By contrast the nascent epidermis of the untreated controls appeared immature, while the dermis was still replete with numerous inflammatory/immune cells. Masson's trichrome staining confirmed the increased presence of collagen in the dermis of the SPS treated wounds at 4 weeks, while the control wounds were largely devoid of collagen. Finally, in addition to enhanced reepithelialization and dermal tissue regeneration, 4 weeks after application of the SPS dressing, we observed the presence of dermal appendages, such as sweat glands and hair follicles. No such appendage formation was observed in the untreated controls during the entire duration of our study. Taken together, the histological data clearly indicate that our soy protein based scaffolds accelerated and enhanced a more natural mode of tissue regeneration in the porcine model of full thickness excisional wound healing. Given the similarities between porcine and human wound healing, we anticipate that SPS will also be advantageous in clinical applications.

1. Introduction

The current gold standard for treating chronic wounds is to use autologous skin grafts, of which there is a limited supply. Skin substitutes are plentiful, ranging from transplantation of cadaveric skin, allogeneic cell-based skin substitutes and acellular tissue scaffolds. Yet each of these alternatives comes with their own limitations. Skin substitutes containing human cells exhibit large variability when used in full thickness wounds and there is a need to delay treatment while these cells are cultured [1]. Acellular cadaveric and allogeneic skin substitutes have the potential of transmitting disease and some patients will also oppose them on religious grounds [2]. Some of the "natural" xenogeneic acellular scaffolds are derived from decellularized animal tissue and carry the risk of disease transfer and may also carry an ethno-cultural stigma. Scaffolds made from synthetic materials have also been widely investigated, but they do not elicit biological cues similar to native extracellular matrix (ECM), they do not have mechanical properties similar to that of skin, and in many cases they are not biodegradable [3].

Acellular tissue scaffolds made from non-animal materials, specifically of plant constituents, do not exhibit any of these limitations. Plant derived scaffolds are being used in an increasing number of tissue engineering applications [4]. Such "green" scaffolds can be manufactured from a variety of alimentary plant

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http://dx.doi.org/10.1016/j.wndm.2014.04.007

products, such as purified plant proteins, and by a number of distinct processes, including electrospinning [5–13]. Electrospinning is a platform technology originally developed to create nonwoven fabrics in the textile industry. Today it is being applied in the biomedical field to build nanofibrous three-dimensional scaffolds to mimic the natural ECM [14].

Recently, a number of laboratories, including ours have successfully electrospun purified soy protein isolate (SPI) under a variety of conditions [11–16]. Our previous studies have shown that SPI can be electrospun without any cross-linkers into a stable tissue scaffold that has mechanical properties similar to those of human skin [11,12]. In addition, SPI resorbs into the body and contains bioactive, "cryptic" peptides [17]. For example, two of these peptides, lunasin and Bowman–Birk inhibitor, inhibit cancer cells cultured *in vitro* and suppress carcinogenesis in animal models *in vivo* [18,19].

We hypothesized that wound dressings made of SPI (operationally termed Soy Protein Scaffolds, SPSs) might contribute to a more natural healing process and be a suitable alternative or adjuvant to currently available treatments for chronic cutaneous wounds. Previously, we optimized conditions for consistently electrospinning bead-free submicron-size fibers from SPI with a minimal addition of high molecular weight synthetic polymer, poly(ethylene oxide) (PEO), to increase chain entanglement [11,12]. *In vitro*, these scaffolds are cytocompatible, promoting adhesion and proliferation of primary human dermal fibroblasts [11,12].

There are a number of animal models of wound healing for testing these scaffolds *in vivo*. Amongst the advantages of rodent models are the ease of handling/housing and the relatively low costs [20,21]. As disadvantages, rodent skin is distinctly different from that of humans in terms of physical properties, such as thin epidermis and dermis and the amount of body hair, and how they heal, which is mostly by wound contraction [22]. The pig is a larger animal; porcine skin lesions heal similarly to man, mainly through reepithelialization [22]. The porcine model also has other features in common with human skin, such as a thick epidermis and a similar dermal–epidermal thickness ratio. Moreover, there is a similar distribution, size and orientation of blood vessels and sparse body hair [22]. For this reason we tested the efficacy of SPSs in full thickness excisional wounds in a porcine model. Our results indicate the SPSs enhance reepithelialization and tissue regeneration including restoration of skin appendages, such as sweat glands and hair follicles.

2. Materials and methods

2.1. Preparation of protein solutions

Soy protein isolate (SPI) was obtained from Cargill Health and Food Technologies (Minneapolis, MN, USA). SPI and poly(ethylene oxide) (PEO; Sigma, St. Louis, MO, USA) were combined by first dissolving 0.5% w/v PEO in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP; Sigma) and adding appropriate volumes from this stock solution to 7% SPI in HFP. Solutions were left to stir at room temperature for at least 8 days before electrospinning to ensure complete and homogeneous dissolution.

2.2. Electrospinning

Soy Protein Scaffolds (SPSs) (5 cm × 5 cm) were electrospun essentially as previously described [11,12,23]. Briefly, a digital precision syringe pump (KD Scientific Single Syringe Infusion Pump, Fisher Scientific) was set up horizontally to eject the SPI/PEO solution from a disposable syringe (BD Biosciences, Franklin Lakes, NJ, USA) through a blunt 18-gauge needle. The electrospinning parameters were as follows: Delivery rate – 0.8 mL/h, air gap distance – 15 cm and accelerating voltage of 12 kV. Under the optimized conditions we consistently produced bead-free fibers that formed scaffolds (mats) with a randomly oriented fibrous structure [12].

2.3. In vivo *pig model*

All animal procedures were carried out according to protocols approved by Drexel University's Institutional Animal Care and Use Committee. For the results reported here 3 Yorkshire pigs (35–40 pounds) were purchased from John Meck LLC (Refton, PA). After acclimatization in the animal facility for at least 1 week, the animals were anesthetized with isoflurane gas. The surgical site was sterilized before proceeding. Sixteen square target areas (3 cm × 3 cm) were marked with a surgical pen, with eight wounds on each side of the spine in two rows of four wounds (see Fig. 1).

At each time point (4 weeks, 2 weeks, and 1 week prior to euthanasia) four or five full thickness wounds were excised at the location of the markings. SPSs were applied as dry scaffolds and hydrated *in situ* with a sterile solution then trimmed to fit the wound (Fig. 1, panels A and B). Some of the wounds were left untreated and served as controls. All wounds were secured

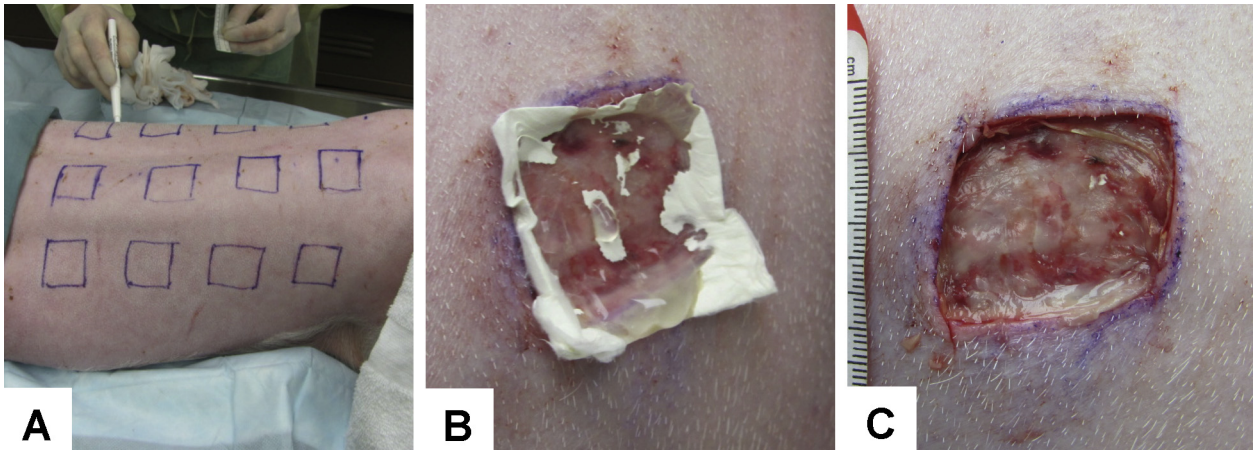

Fig. 1. Placement of wounds onto the back of a pig model and treatment of an excisional wound with soy protein scaffold. Panel A: 16 wounds were mapped out on the pig's back. Panel B: Application of a dry Soy Protein Scaffold (SPS) to a full thickness excisional wound. Panel C: Appearance of the SPS treated wound immediately after full hydration of the scaffold.

individually with a translucent occlusive wound cover (Tegaderm®) to keep them moist and prevent bacterial/fungal contamination. All wounds were then covered with a layer of gauze, cast padding and a self-adherent wrap to prevent pigs from removing the individual bandages by rubbing against the walls of their pens. At the end of the study (4 weeks), the pigs were euthanized using sodium pentobarbital and phentoyin as per IACUC protocol.

2.4. Histology

Upon euthanasia, the wounded skin area and ~2 cm of healthy skin surrounding it were excised down to the muscle and fixed in 10% buffered formalin first for one hour at room temperature and then for at least 24 h at 4 °C. The tissue sections were embedded in paraffin and then cut into 5 μm thick sections and mounted on glass microscope slides. The samples underwent routine histological processing for hematoxylin and eosin (H&E) staining or were stained with Masson's trichrome stain (MTS), following established procedures [24–26]. The slides were dehydrated, mounted in Richard-Allan Cytoseal-60 mounting medium and imaged with a Nikon Microphot-FX with a Leica DFC310 FX camera using Leica Application Suite 4.3.0.

3. Results and discussion

The porcine model of full-thickness excision wounds is widely accepted as one that closely mimics human dermal healing [22]. Due to the animals' large size, up to 16 wounds (3 cm × 3 cm) can be generated on each pig, concomitantly, or, as in this study, sequentially (see below), with each wound spaced far enough apart so that inflammation from any two wounds did not overlap (Fig. 1, panel A).

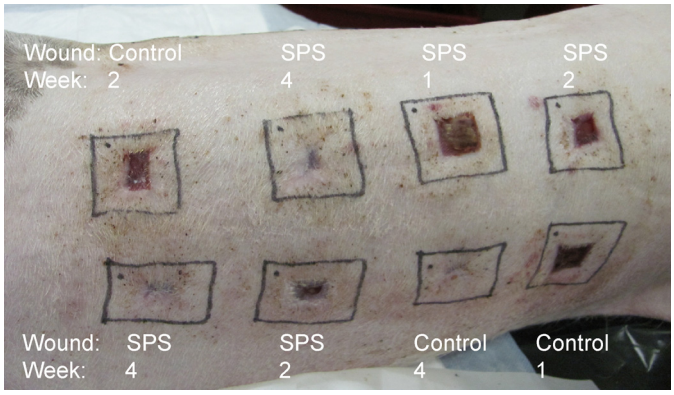

Fig. 2. Appearance of a representative experimental field on the back of a pig following euthanasia. The markings outline the areas of tissue collected for histological examination. Top row: Control-2 weeks; SPS-4 weeks; SPS-1 week; SPS-2 weeks. Bottom row: SPS-4 weeks; SPS-2 weeks; Control-4 weeks; Control-1 week.

In order to compare the time course of healing of Soy Protein Scaffold (SPS)-treated and untreated wounds, each pig received 16 square full thickness excisional wounds (3 cm × 3 cm), which were applied in a staggered fashion on three time points, 4 weeks, 2 weeks, and 1 week prior to euthanasia. At each time point, wounds were either treated by directly applying SPS (Fig. 1, panels B and C) or they were left untreated (sham) as a control. In this way, we were able to establish the time course of wound healing independently and individually. Using this staggered approach,

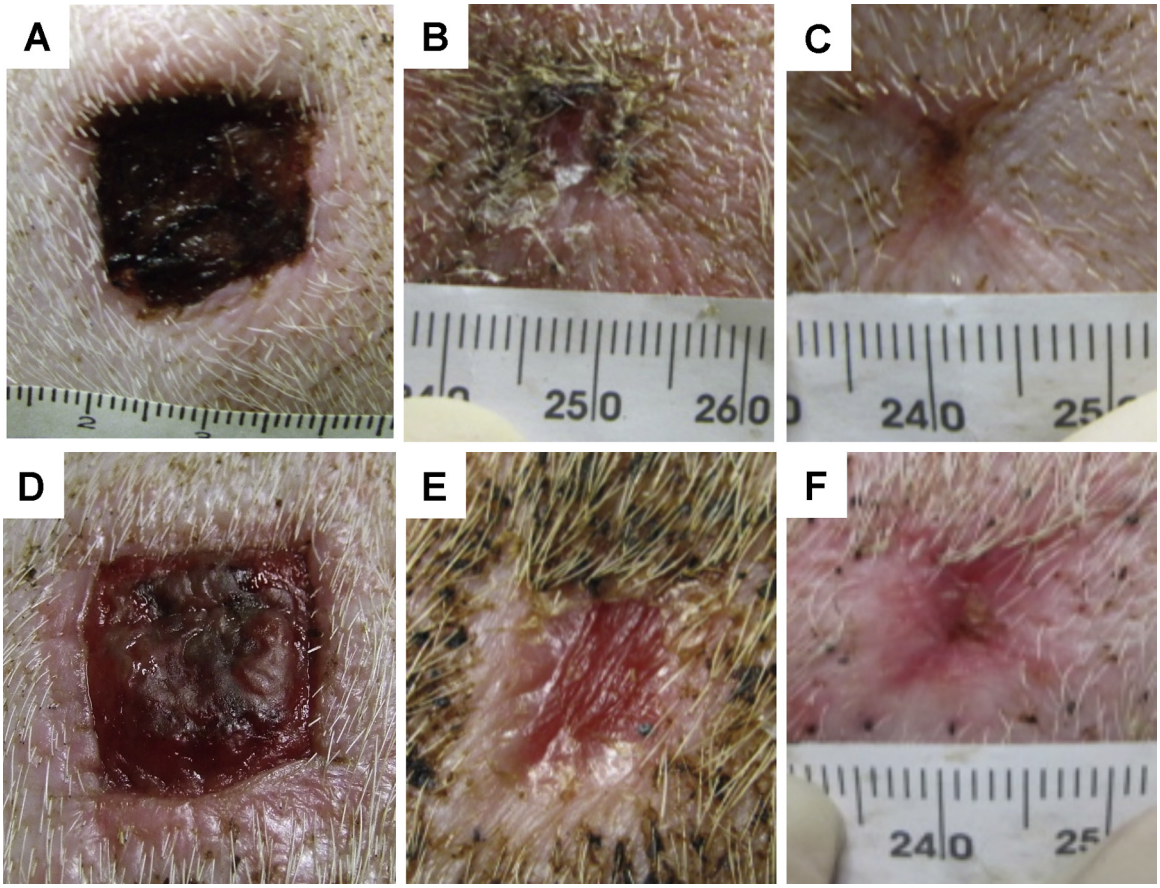

Fig. 3. Close-up photographs of the healing process of full thickness excisional wounds. Panels A–C: Photographs of one representative wound treated with SPS. Images taken on days 5, 9, and 15, respectively. Panels D–F: Following one representative untreated control wound. Images taken on days 5, 9, and 15, respectively.

all tissues could be concomitantly harvested and processed at the termination of the experiments and, importantly, each animal also served as its own control. This is significant, as we observed that each pig had a slightly different overall healing rate.

Shown in Fig. 2 is the overall appearance of a representative experimental field of eight wounds following euthanasia of the animal. Seen in this photograph are control and SPS-treated wounds at different stages of healing (1 week, 2 weeks, and 4 weeks). In terms of overall external appearance the kinetics of wound closure appears to be quite similar (Fig. 2), albeit there are some differences in terms of the quality of the wounds, as more clearly visible in the representative close-up photographs of SPS treated and control wound at days 5, 9 and 15 (Fig. 3).

Taken together, these photographs did not reveal a significant acceleration of wound closure *per se*, as already observed in preliminary studies using a rat model [11]. Rather the quality of the wounds, in terms of the reduced oozing and inflammation (redness), was significantly improved in the SPS treated samples.

The beneficial effects of SPSs on the wound healing process, especially in terms of reepithelialization and regeneration of skin appendages became evident upon histological analysis of the wounds (Fig. 4). Shown in Fig. 4, panels A–D are low and higher magnifications of histological sections (H&E stains) of healthy tissues.

Panels A and B show the appearance of normal skin (taken from an area of the pig's back that had not been wounded), including transverse (cross)-sections of some hair shafts. Shown in panels C and D are micrographs of histological sections of tissue taken at the "normal" edges of untreated control wounds, indicating some infiltration of inflammatory/immune cells beyond the level found far from the wounds, as seen in panels A and B.

Two weeks after wounding, the dermis of the untreated controls (Fig. 4, panels E and F) is composed of disorganized granulation tissue replete with copious amounts of inflammatory/immune cells. Notable is the absence of any epidermal layer, as reepithelialization has not yet occurred. By contrast, in the SPS-treated samples (Fig. 4, panels G and H), the dermis appears more organized, there seem to be far fewer inflammatory/immune cells, and there is clear evidence for the reestablishment of the beginnings of a stratified epithelial layer.

Shown in Fig. 4, panels I–L, are micrographs taken 4 weeks after injury at the center of untreated controls (panels I and J) and wounds treated on day 0 with SPSs (panels K and L). The tissue in the untreated controls begins to show signs of organization and rebuilding of the connective tissue (including fibroblast/smooth muscle cells), the number of inflammatory/immune cells is diminished and there is a an epithelial layer which lacks normal stratification (panels I and J).

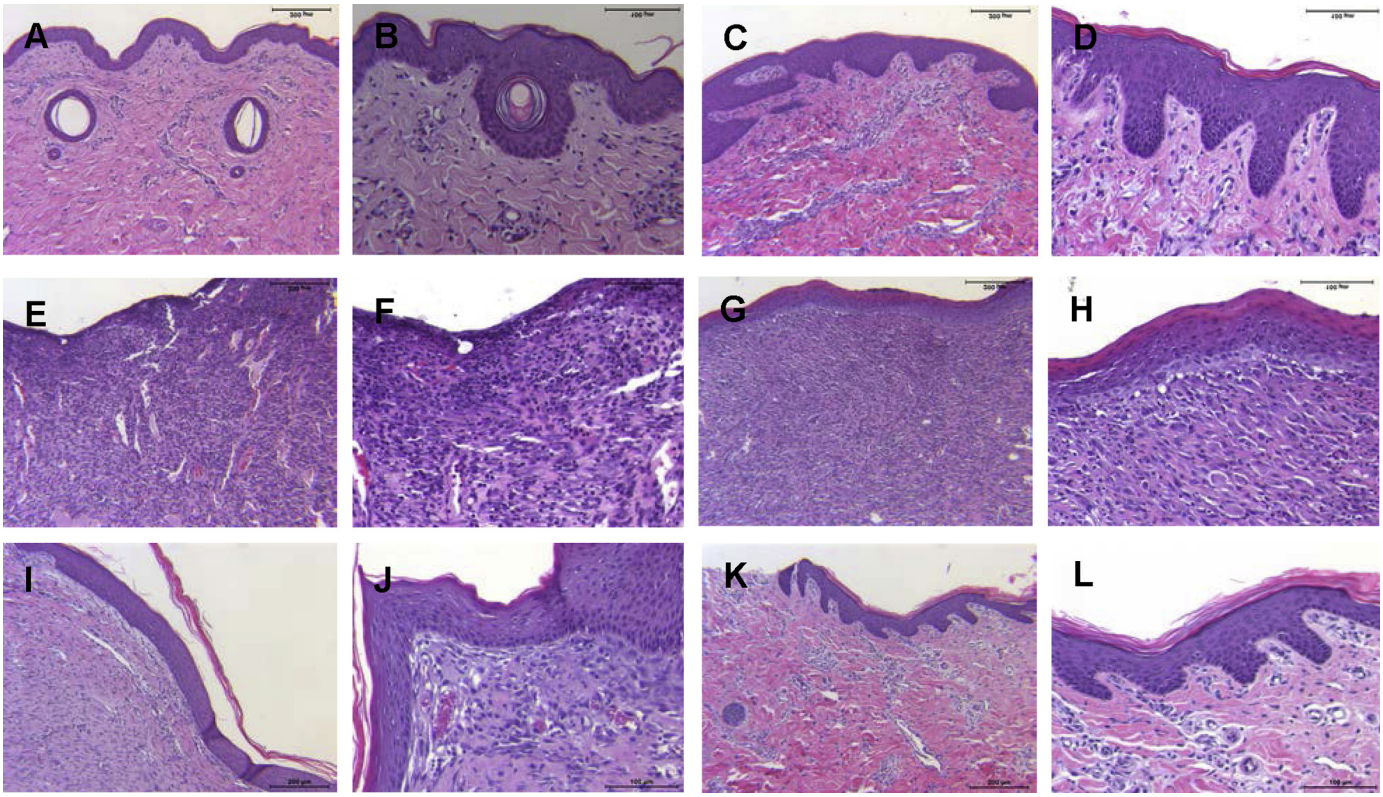

Fig. 4. Micrographs of H&E stained tissues. Panels A–D: Control panels. Micrographs of normal healthy tissue at low and high magnifications. Panels A and B: A control sample of never wounded skin taken from an area far away from any wound (original magnification 63×, 200×, respectively). Panels C and D: The "healthy margin" of untreated controls samples harvested 2 weeks after injury. Note the increased number of inflammatory cells in the "healthy margins" (original magnification 63×, 200×, respectively). Panels E–H: 2 weeks after injury. Panels E and F: Micrographs of the central wound area in untreated controls (original magnification 63×, 200×, respectively). Note the abundance of inflammatory/immune cells and absence of an epithelial layer in the disorganized granulation tissue. Panels G and H: Wounds treated on day 0 with SPS (original magnification 63×, 200×, respectively). Wounds treated with SPS exhibit significantly fewer inflammatory/immune cells, the tissue itself is more organized/structured and there is a well-formed stratified epithelial layer. Panels I–L. 4 weeks after injury. Panels I and J: Micrographs taken 4 weeks after injury at the center of untreated controls (original magnification 63×, 200×, respectively). The tissue in the untreated controls (panels I and L) begins to show signs of re-organization and rebuilding of the connective tissue, the number of inflammatory/immune cells is diminished and there is a less stratified, but clearly discernable epithelial layer. Panels K and L: Wounds treated on day 0 with SPS (original magnification 63×, 200×, respectively). In the wounds treated with SPS, the number of inflammatory/immune cells is greatly diminished, the connective tissue in the dermis is much better organized/structured and has developed dermal papillae and there is a well-formed stratified epithelial layer with clear evidence for stratification. Note also the abundance of small blood vessels in the dermis close to the epidermis.

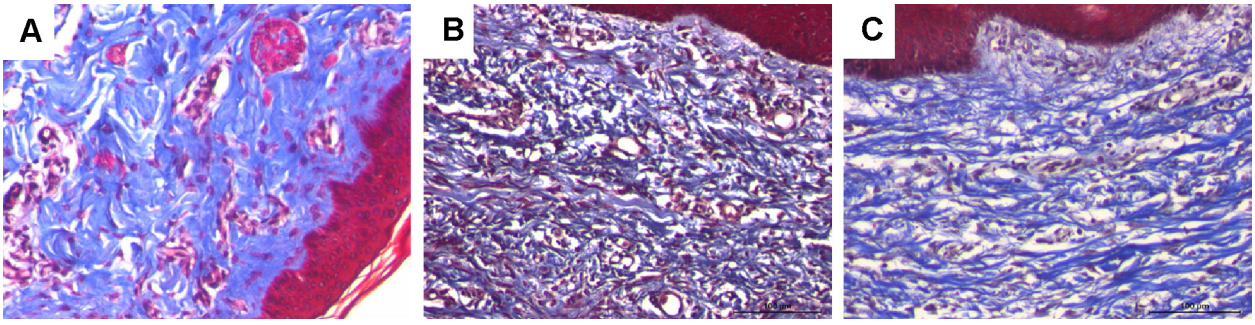

Fig. 5. Micrographs of tissue 4 weeks after injury and stained with Masson's trichrome stain (MTS). Original magnification 200×. Panel A: "Healthy" skin taken not far from untreated control. Panel B: Wound area of an untreated control. Panel C: Wound area of SPS treated wound.

By contrast, in the wounds treated with SPSs (panels K and L), while the tissue is still not quite normal (see panels A and B) the number of inflammatory/immune cells is greatly diminished, while the connective tissue is much better organized/structured and contains ample stromal cells (fibroblast and muscle cells). The dermis has developed dermal papillae and there is a well-formed stratified epithelial layer with clear evidence for stratification, including basal layer and well developed stratum corneum. Note also the abundance of small blood vessel in the dermis close to the epidermis (panels K and L).

One of the hallmarks of tissue regeneration is the formation of collagen, which is critical for the function and structure of healthy skin. Shown in Fig. 5 are histological sections of control skin, untreated and SPS treated wounds (4 weeks) stained with Masson's trichrome, in which collagen is stained in blue. As seen in panel A, collagen is abundantly present in the dermis of normal tissue at the margin of untreated controls. By contrast collagen is largely missing in the untreated wounds, even after 4 weeks, the tissue lots of inflammatory/immune cells (panel B). By contrast, the SPS-treated wounds, albeit still not completely normal, show significant levels of collagen (panel C). Taken together the histological data clearly indicate that our soy protein based scaffolds accelerated and enhanced a more natural mode of tissue regeneration in the porcine model of full thickness excisional wound healing.

In addition to enhanced reepithelialization, 4 weeks after application of the SPS dressing, we observed the presence of dermal appendages, such as sweat glands and hair follicles (Fig. 6). No such appendage formation was observed in the untreated controls during the entire duration of our study. The overview low magnification H&E stained micrographs in Panels A and B show the emergence of sweat glands in the SPS-treated wounded areas close to the epidermis. The higher resolution micrographs in panels C and D show a growing hair shaft and nascent sweat gland, respectively, embedded into a fairly well organized connective tissue environment.

We note that in this pig model, the soy scaffolds seem to exert their beneficial effects not so much in terms of accelerating wound closure (Figs. 2 and 3) but rather in the quality of wound healing (Figs. 4–6). Conventionally, the superiority of a new "device", wound dressing, or skin substitute to enhance wound healing is often assessed in terms of accelerating wound closure. However, we posit that acceleration of wound closure may lead to enhanced contraction and scar tissue formation by enhanced myofibroblast differentiation [27] and hypothesize that the functional tissue restoration will require restoration of "normal" tissue morphology, as seen with SPS treatment, rather than rapid closure/contraction. Of particular interest in this context is the more rapid and more natural/complete reepithelialization of the SPS-treated wounds, including formation of the Malpighian layer (stratum basale and

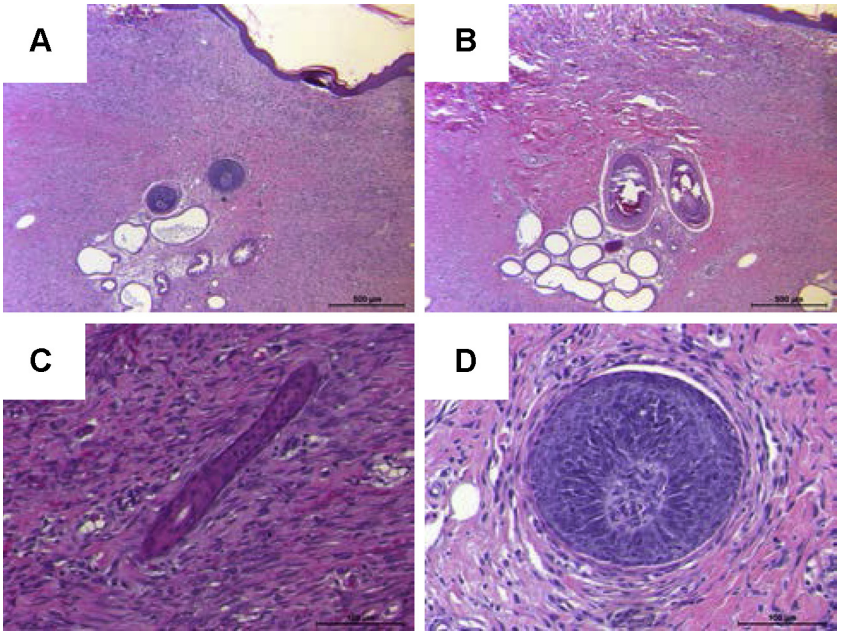

Fig. 6. Formation of appendages in SPS treated wounds after four weeks. Panels A and B: Representative micrographs of histological sections taken at center of two different SPS-treated wounds. Original magnification 25×. Panels C and D: Higher magnifications of appendage formation in wounds treated with SPS. Original magnification 200×.

stratum spinosum) and the regeneration of critical dermal appendages, such as sweat glands and hair shafts/follicles.

The mechanism of action by which SPS enhances a more natural, regenerative wound healing remains to be determined. In preliminary studies we determined that the soy scaffolds are susceptible to proteolytic degradation *in vitro* [11] and that upon s.c. implantation the scaffolds are rapidly degraded and completely absorbed in about 14 days (Y. Har-el and P.I. Lelkes, unpublished data). As seen from the histological micrographs (Fig. 4), soy protein scaffolds are rapidly degraded during the wound healing process and fully adsorbed after 4 weeks. We therefore hypothesize that proteolytic degradation of SPSs might liberate some of the cryptic peptides contained in the soy proteins and that some of these peptides might enhance the wound-healing process by as yet unknown mechanisms. It is well known, that proteolytic soy protein degradation products, such as lunasin or beta-conglycinin are bioactive. For example, lunasin reportedly inhibits inflammation by suppressing the NFkB signaling pathway [28] whereas beta-conglycinins might inhibit cancer cells [29] or prevent alopecia [30]. The fact that we see significantly less inflammatory/immune cell influx in the SPS-treated samples (Fig. 4) than in the granulation tissue in the untreated controls may be related to the anti-inflammatory properties of lunasin or some as yet unidentified soy-degradation products with similar anti-inflammatory properties The reported anti-alopecia properties of beta-conglycinins may be related to our finding of enhanced regeneration of skin appendages, such as hair shafts in SPS treated wounds, but not in the untreated controls (Fig. 4). Of potential significance for the enhanced wound healing capabilities of SPS, some of the soy degradation products reportedly exhibit pro-angiogenic properties promoting neovascularization [31,32], which is an important component of the healing process. Indeed, careful evaluation of our micrographs shows exuberant (neo) vascularization in the vicinity of the regenerating epithelium in SPS treated wounds (see Fig. 4). Current studies focus on the mechanistic understanding of why and how SPS enhances the healing process and reepithelialization in our porcine model.

Currently we are designing several follow-up studies to (a) evaluate the mechanisms by which soy-protein based scaffolds enhance wound healing and (b) to directly compare SPSs with commercially available products such as Oasis™. The latter study will also have to address the question whether a single application of SPSs at the time of wounding, as in this report, will suffice or whether we can enhance the efficacy of soy-based scaffolds by following multiple application protocols developed for other bioactive wound dressings.

4. Conclusions

This works presents the potential of our soy protein scaffolds (SPSs) to be used in the treatment of chronic wounds in humans. In preclinical tests conducting in the porcine model, the SPSs were shown to promote reepithelialization and a more natural, tissue-like healing. Results of wound treatments in this model have shown strong correlations to results obtained in humans [22]. The use of our plant-derived scaffold circumvents current and emerging problems with animal protein-based skin substitutes. It also meets an unmet clinical need, as it provides affordable, bioactive scaffolds and dressings for wound healing.

Disclosures

PIL is the chief scientific consultant for Eqalix Inc., the biotechnology company that sponsored, in part, this research. Eqalix did not affect the design, interpretation, or reporting of any of the experiments described herein.

Ethical approval

All animal procedures were carried out according to protocols approved by Drexel University's Institutional Animal Care and Use Committee.

Acknowledgements

This study was funded, in part by grant C000046982 provided by The Nanotechnology Institute, which is supported by the Commonwealth of Pennsylvania's Ben Franklin Technology Development Authority through the Ben Franklin Technology Partners of Southeastern Pennsylvania, a grant-in aid from the Coulter Foundation and a sponsored research agreement with Eqalix, Inc., Reston VA. We are grateful to Janet Schulenberg for providing excellent assistance in all veterinary procedures and thank Kimberly Wasko for her valuable contributions to the design of the animal study. We gratefully acknowledge the contributions of Daniel Hakakian, Marion Hirshberg, Mario Mata, and Adrianna Urbano, undergraduate researchers in the laboratory who enthusiastically participated in the staining and evaluation of the myriads of histological slides.

References

[1] Mohamed A, Xing M. Nanomaterials and nanotechnology for skin tissue engineering. Int J Burn Trauma 2012;2(1):29–41.
[2] Enoch S, Shaaban H, Dunn KW. Informed consent should be obtained from patients to use products (skin substitutes) and dressings containing biological material. J Med Ethics 2005;31:2–6.
[3] Metcalfe AD, Ferguson MW. Tissue engineering of replacement skin: the crossroads of biomaterials, wound healing, embryonic development, stem cells and regeneration. J R Soc Interface 2007;4:413–37.
[4] Tuzlakoglu K, Bolgen N, Salgado AJ, Gomes ME, Piskin E, Reis RL. Nano- and micro-fiber combined scaffolds: a new architecture for bone tissue engineering. J Mater Sci Mater Med 2005;16:1099–104.
[5] Woerdeman DL, Ye P, Shenoy S, Parnas RS, Wnek GE, Trofimova O. Electrospun fibers from wheat protein: investigation of the interplay between molecular structure and the fluid dynamics of the electrospinning process. Biomacromolecules 2005;6:707–12.
[6] Gao C, Li X, Song T. Electrospinning and crosslinking of zein nanofiber mats. J Appl Polym Sci 2007;103:380–5.
[7] Selling GW, Biswas A, Patel A, Walls DJ, Dunlap C, Wei Y. Impact of solvent on electrospinning of zein and analysis of resulting fibers. Macromol Chem Phys 2007;208:1002–10.
[8] Santos MI, Fuchs S, Gomes ME, Unger RE, Reis RL, Kirkpatrick CJ. Response of micro- and macrovascular endothelial cells to starch-based fiber meshes for bone tissue engineering. Biomaterials 2007;28:240–8.
[9] Santos MI, Tuzlakoglu K, Fuchs S, Gomes ME, Peters K, Unger RE, et al. Endothelial cell colonization and angiogenic potential of combined nano- and microfibrous scaffolds for bone tissue engineering. Biomaterials 2008;29:4306–13.
[10] Ghanaati S, Fuchs S, Webber MJ, Orth C, Barbeck M, Gomes ME, et al. Rapid vascularization of starch-poly(caprolactone) in vivo by outgrowth endothelial cells in co-culture with primary osteoblasts. J Tissue Eng Regen Med 2011;5:136–43.
[11] Lin L. Electrospun soy protein-based scaffolds for skin tissue engineering and wound healing.[dissertation] Philadelphia, PA: Drexel University; 2011.
[12] Lin L, Perets A, Har-el Y, Varma D, Li M, Lazarovici P, et al. Alimentary electrospun scaffolds for skin regenerative engineering. J Tissue Eng Regen Med 2013;7:994–1008.
[13] Xu X, Jiang L, Zhou Z, Wu X, Wang Y. Preparation and properties of electrospun soy protein isolate/polyethylene oxide nanofiber membranes. Appl Mater Interfaces 2012;4:4331–7.
[14] Lelkes PI, Li M, Perets A, Lin L, Han J, Woerdeman DL. Electrospinning of natural proteins for tissue engineering scaffolding. In: Reis RL, editor. Handbook of natural-based polymers for biomedical applications. Cambridge, England: Woodhead Publishing Ltd; 2008. p. 446–82.
[15] Vega-Lugo AC, Lim LT. Electrospinning of soy protein isolate nanofibers. J Biobased Mater Bioenergy 2008;2(3):223–30.
[16] Fung WY, Yuen KH, Liong MT. Characterization of fibrous residues from agrowastes and the production of nanofibers. J Agric Food Chem 2010; 58(13):8077–84.
[17] Maruyama N, Maruyama Y, Tsuruki T, Okuda E, Yoshikawa M, Utsumi S. Creation of soybean beta-conglycinin beta with strong phagocytosis-stimulating activity. Biochim Biophys Acta 2003;1648(1–2):99–104.
[18] Armstrong WB, Wan XS, Kennedy AR, Taylor TH, Meyskens Jr FL. Development of the Bowman–Birk inhibitor for oral cancer chemoprevention and analysis of

new immunohistochemical staining intensity with Bowman–Birk inhibitor concentrate treatment. Laryngoscope 2003;113(10):1687–702.
[19] de Lumen BO. Lunasin: a cancer-preventive soy peptide. Nutr Rev 2005; 63(1):16–21.
[20] Birch M, Tomlinson A, Ferguson MWJ. Animal models for adult dermal wound healing. Methods Mol Med 2005;117:223–35.
[21] Galiano RD, Michaels J, Dobryansky M, Levine JP, Gurtner GC. Quantitative and reproducible murine model of excisional wound healing. Wound Repair Regen 2004;2(4):485–92.
[22] Sullivan TP, Eaglestein WH, Davis SC, Mertz P. The pig as a model for human wound healing. Wound Repair Regen 2001;9(2):66–76.
[23] Li M, Mondrinos MJ, Gandhi MR, Ko FK, Weiss AS, Lelkes PI. Electrospun protein fibers as matrices for tissue engineering. Biomaterials 2005;26(30): 5999–6008.
[24] Mondrinos MJ, Koutzaki S, Jiwanmall E, Li M, Dechadarevian JP, Lelkes PI, et al. Engineering three-dimensional pulmonary tissue constructs. Tissue Eng 2006;12(4):717–28.
[25] Mondrinos MJ, Koutzaki S, Lelkes PI, Finck CM. A tissue-engineered model of fetal distal lung tissue. Am J Physiol Lung Cell Mol Physiol 2007;293(3): L639–50.
[26] Mondrinos MJ, Koutzaki SH, Poblete HM, Crisanti MC, Lelkes PI, Finck CM. In vivo pulmonary tissue engineering: contribution of donor-derived endothelial cells to construct vascularization. Tissue Eng Part A 2008;14(3):361–8.
[27] Eming SA, Werner S, Bugnon P, Wickenhauser C, Siewe L, Utermöhlen O, et al. Accelerated wound closure in mice deficient for interleukin-10. Am J Pathol 2007;170(1):188–202.
[28] de Mejia EG, Dia VP. Lunasn and lunasin-like peptides inhibit inflammation through suppression of NF-kappaB pathway in the macrophage. Peptides 2009;12:2388–98.
[29] Wang W, Bringe NA, Berhow MA, Gonzalez, de Mejia E. beta-Conglycinins among sources of bioactives in hydrolysates of different soybean varieties that inhibit leukemia cells in vitro. J Agric Food Chem 2008;56(11):4012–20.
[30] Tsuruki T, Takahata K, Yoshikawa M. Anti-alopecia mechanisms of soymetide-4, an immunostimulating peptide derived from soy beta-conglycinin. Peptides 2005;26(5):707–11.
[31] Frigolet MI, Torres N, Tovar AR. Soya protein attenuates abnormalities of the renin-angiotensin system in adipose tissue from obese rats. Br J Nutr 2012;107:36–44.
[32] Medina MA, Quesada AR. Dietary proteins and angiogenesis. Nutrients 2014; 6(1):371–81.