Treatment of Burn and Surgical Wounds With Recombinant Human Tropoelastin Produces New Elastin Fibers in Scars

Hua Xie, MD, PhD,*† Lisa Lucchesi, MS,*‡§ Bo Zheng, MD,*‖ Elena Ladich, MD,¶ Teresa Pineda, BS,‡§ Rose Merten, BS,*§ Cynthia Gregory, PhD,* Michael Rutten, PhD,* and Kenton Gregory, MD*‡

Tropoelastin (TE), the soluble precursor of insoluble elastin fibers, is produced in minimal amounts in adults. Burn injuries result in inflexible collagen-rich scars because of lack of elastin fiber formation. We studied the feasibility of using recombinant human tropoelastin to enable elastin fiber production in burn and surgical scars to improve skin flexibility. In a swine hypertrophic burn scar model, normal skin and 3 × 3-cm^2 partial thickness thermal burns underwent dermatome resection at 1 week post burn and randomized to four subcutaneous injections of saline or TE (either 0.5, 5, or 10 mg/ml) spaced 3 days apart. Two burn sites received TE injections after wound closure (0.5 or 10 mg/ml). At 90 days, skin hardness, flexibility, and histology were evaluated. All injury sites developed hypertrophic scars. New elastin fibers were found in burn scars in all injuries injected after skin closure with low (5/5) and high (6/6) TE doses ($P < .05$). No elastin fibers were observed without TE treatment. No significant differences in skin hardness, flexibility, or inflammation were observed. This is the first report demonstrating that subcutaneous injections of TE into surgical and burn injuries can safely produce new elastin fibers in scars. Despite the development of new elastin fibers, skin flexibility was not improved, possibly because of insufficient elastin fiber maturation or the hypertrophic model used. The ability to restore elastin fiber formation in adult skin after burns, trauma, and surgery may improve skin regeneration and reduce disabling complications of scar formation. (J Burn Care Res 2017;XXX:00–00)

*From the *Center for Regenerative Medicine, Oregon Health & Science University, Portland; †Department of Surgery, Oregon Health & Science University, Portland; ‡Oregon Biomedical Engineering Institute, Wilsonville; §Nike, Inc., Beaverton, Oregon; ‖Department of Hematology, General Hospital of Ningxia Medical University, Yinchuan, China; and ¶CVPath Institute, Gaithersburg, Maryland.*

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Address correspondence to Hua Xie, MD, PhD, Center for Regenerative Medicine, Oregon Health & Science University, 3181 SW Sam Jackson Park Road, Mail Code HRC6RM, Portland, Oregon 97239. Email: xieh@ohsu.edu.

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Burns are one of the most common injuries worldwide, especially among children. Between 2011 and 2013, an annual average of 450,000 burn injury cases presented to emergency departments in the United States with more than 20% suffering injuries severe enough to require hospital admission and intensive burn care.[1] The standard of care for severe burn injury includes analgesia, careful fluid balance, local skin dressings, and antibiotics, followed by surgical interventions for eschar and skin closure and replacement.[2] With improved intensive care and advances in surgical techniques, patients can now survive severe burns affecting more than 90% of their TBSA.[2] Almost all survivors of severe burn injury face lifelong disability and disfigurement because of lack of normal skin regeneration. Scars formed as a consequence of burn injuries are commonly inelastic and permanently restrict mobility of underlying tissue and joints. Hypertrophic skin scarring occurs in 30–75% of severe burn injuries. Hypertrophic

scarring can also complicate up to 35% of surgical skin wounds that do not have burns after 1 year.[3,4] There are extensive physiological and psychological impacts for burn survivors. Prevention and management of the negative effects of wound scarring remain a major challenge.[2,5] Current scar treatment options include pressure garments, corticosteroid injections, laser therapy, 5-fluorouracil treatment, surgical interventions, and others. Unfortunately, there is a high rate of recurrent scarring.[6]

Hypertrophic scars are generally raised, erythematous, hard, inflexible, and contracted. A large proportion of these scars are composed of extracellular matrix, predominantly collagen. Mature elastin fibers are not present in the extracellular scar matrix.[7,8] Elastin proteins are essential for normal skin flexibility and elasticity, and are found in association with fibrillin within the dermis. In addition to its structural and mechanical roles, elastin has inherent cell signaling properties that promote a diverse range of cellular responses including chemotaxis, cell attachment, proliferation, and differentiation.[9] In humans, mature elastin fibers are synthesized primarily in late fetal and early neonatal periods.[10] Tropoelastin (TE) is the soluble precursor or monomer of elastin, the major protein component of polymeric elastic fibers. After 1 year of age, human cells rarely express TE and thus, the insoluble, cross-linked elastin fibers are not normally repaired or replaced after tissue injury and wound healing.[11–13] Treatment of surgical skin wounds in animal models with bovine collagen and elastin digests have been reported to promote dermal elastin fiber formation.[14] Human-pigmented epithelial cells in cell culture do not synthesize TE or elastin fibers but are capable of assembling exogenous TE into an extracellular fibrillar matrix in cell culture.[15] Our group has demonstrated that adult human and swine fibroblasts in cell culture will not normally produce elastin fibers but can synthesize them if given TE (unpublished data). TE, when added to tissue-engineered skin grafts, has been shown to increase elastin fiber formation and neovascularization within the graft in animal models.[16,17]

Here, we hypothesize that subcutaneous injection of recombinant human tropoelastin (rhTE) into the wound bed of a severe burn injury may enhance production of elastin fibers in the tissue repair process, which may improve skin elasticity and mechanic properties. In this study, we investigated timing and dose of rhTE deposition in two types of skin injury: a deep partial-thickness thermal burn after dermatomal surgical excision and a dermatomal surgical excision of normal skin.

METHODS

Preparation of Recombinant Human Tropoelastin Solution

A human fetal heart complementary DNA library was screened for TE genes and a full length transcript representing a splice variant missing exons 22 and 26a (~65 kDa) was isolated, optimized, and expressed from a synthetic gene codon, transfected into *Escherichia coli* BL21-CodonPlus cells.[18] In brief, human TE was expressed in gram quantities in a 10-L *E. coli* fermentation system. Gel electrophoresis determined that the purification procedure resulted in a greater than 99%-pure rhTE protein product. Lyophilized, purified rhTE was suspended in endotoxin-free water and passed through an Acrodisc® Unit with Mustang® Q Membrane (Pall Corp., Port Washington, NY) syringe filter to remove endotoxins. Endotoxin levels were determined by the Kinetic-QCL™ LAL Assay (Lonza, Basel, Switzerland) and host-cell protein contamination was determined by an Immunoenzymometric Assay (Cygnus Tech., Southport, NC) and found to be <5 EU/mg and ≤ 1.54 ppm, respectively. The purified rhTE protein includes all of the functional exons with the exception of exons 1, 22, and 26a. Exon 1 contains the signal sequence, while hydrophobic exon 22 and hydrophilic exon 26a are rarely expressed in mature elastin in humans. The resultant TE exon structure used is the same as a natural isoform produced by normal human fetal heart cells.

An rhTE stock solution was prepared by dissolving rhTE in 1× PBS at pH 7.4 in endotoxin-free water and quantified using a high-performance liquid chromatography (HPLC) trypsin enzymatic digest method. Trypsin was added to stock solution of rhTE and the digested peptides were separated by reverse phase HPLC and compared with digests of pure rhTE standards. From the concentration of peptide, the amount of rhTE in the stock solution was calculated. The stock solutions were diluted in 1× PBS at pH 7.4 to make three rhTE concentrations 10 mg/ml (high dose), 5 mg/ml (medium dose), and 0.5 mg/ml (low dose), and stored at –4°C until the day of treatment.

Swine Model of a Deep-Dermal Partial-Thickness Burn

All procedures were conducted in a surgical facility of the Department of Comparative Medicine under a protocol approved by the Institutional Animal Care and Use Committee (IACUC) and the Department of Defense Animal Care and Use Review Office (ACURO).

This study was a prospective, randomized, blinded, sham-controlled, dose ranging study comparing subcutaneous wound site injections of rhTE with saline. All surgeons, technicians, and research staff were blinded as to wound site treatments and controls. Operators were given numbered vials for injections that were visibly identical. All measurements and statistical comparisons were performed in a blinded fashion as to treatment or control administration. Histologic assessments and comparisons were performed by an independent pathologist, blinded to the treatment administered.

Thermal burn skin injuries in adult female Red Duroc swine have been reported to be similar to human hypertrophic scar that occur owing to burns.[19] A total of seven female Red Duroc swine, average body weight of 36 ± 4 kg, were included in this study.

The swine were socialized to human contact 1 week before surgery to ease stress of post-op handling. The night before surgery, food was withheld and water given ad libitum. Sedation was introduced with intramuscularly injection of Tiletamine-Zolazepam (Telazol®) (4–9 mg/kg) followed by atropine (0.06 mg/kg), and then intubated with 1–3% isoflurane aspiration and ventilation. Intravenous fluid (lactated Ringer's) was given via auricle vein at a rate of 5 ml/kg/hr. After the induction of general anesthesia, swine were placed in a ventral recumbent position with sterile surgical preparation. The study area was scrubbed with chlorohexadine. Ten study sites were created on the dorsal skin of each swine, 5-cm apart, parallel to the spine, randomly assigned to six sites for partial thickness burn injuries plus dermatome excision and four unburned sites for surgical excisions (dermatome incision alone). A square brass rod ($3 \times 3\,cm^2$ skin contact surface and 360 g weight) was heated to 100°C in a water bath and applied perpendicular to, and in direct contact with, the skin for 18 seconds to create a severe partial-thickness injury in the sites assigned to burn injury. At 1 week post burn, all sites underwent dermatome resection and received the assigned treatments (Figure 1; Table 1); of a total of 60 study sites, 36 were burn-plus-excision wounds and 24 were surgical-excision wounds.

Burn Treatments

On the day of burn injuries (day 0), the swine were recovered from anesthesia and returned to their housing facility after surgical procedures. The burn injury sites were securely covered using Mepilex® dressing (Mölnlycke Health Care, Gothenburg, Sweden) and wrapped with layers of sterile gauze.

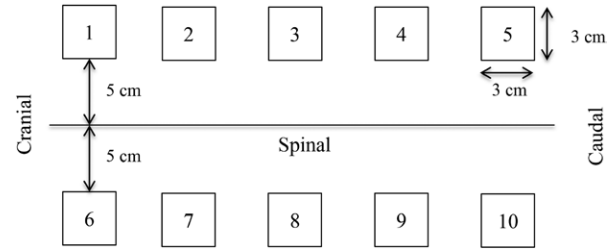

Figure 1. Diagram of study sites.

Seven days later (day 7), the swine were sedated as previously described and the study treatment sites were randomized. Burn-injured and uninjured sites were excised using a Zimmer® electric dermatome (Zimmer, Warsaw, IN) until capillary bleeding and fat cones were visualized. The dermatome settings and number of passes were documented. The wound sites were randomized to receive intradermal injections of saline (control) or one of three concentrations of rhTE (low dosage, 0.5 mg/ml; medium dosage, 5 mg/ml; high dosage, 10 mg/ml) on days 7, 10, 13, and 16 during dressing changes so that each site had four identical treatments during the course of the study. Two groups were randomized to receive rhTE injections of either (0.5 mg/ml) or (10 mg/ml) every 3 days for a total of four injections per site after wound closure, at approximately days 24–32 (Table 1). All injections were 0.5-ml volume of solution and used a 1-ml syringe with a 27-gauge needle (Terumo Medical Corp., Somerset, NJ). All wound sites were again securely wrapped with dressings and the dressings were changed every 3 days and the animals treated with analgesia until wound closure occurred. At day 90, the animals were killed and skin samples were collected for additional investigation.

Measurement of Skin Induration

A Rex® 1700-0 durometer (Rex Gauge Company, Inc., Buffalo, IL) was applied perpendicular to the skin to measure hardness of burn scar area. Measurements were taken for injury sites and the uninjured skin 3-cm adjacent to the injury site. Five readings were taken at each measured site at the following times: before injury and postinjury on day 0; postinjury days 3, 7, 10, 16, 21, 28, 35, 60, and 90.

Assessment of Skin Mechanical Properties

At 90 days following euthanasia, $9 \times 1.5\text{-}cm^2$ skin samples from study sites as well as uninjured areas adjacent to the study sites were harvested and cut into "dog bone" test items using a standard die. Test specimen thickness and cross-sectional gage

Table 1. Comparison among treatment groups

Group (N)	Wound Conditions	Treatments*	Depth of Excision (mm)	Change of Wound Area (cm^2)	Max Stress of Scar (MPa)	Score of Scar Depth	Inflammatory Score	Elastin Fibers in Scar (Ratio, Positive N/ Total N)†
1 (6)	Burn + surgical excision	Saline	1.48 ± 0.05	2.29 ± 2.41	6.4 ± 1.5	2.5 ± 0.8	1.5 ± 0.8	0/6
2 (6)	Surgical excision only		1.54 ± 0.12	5.87 ± 5.06	6.8 ± 4.3	2.5 ± 0.8	1.5 ± 1.0	0/6
3 (6)	Burn + surgical	rHTE, 0.5 mg/ml	1.50 ± 0.14	2.67 ± 3.68	8.9 ± 2.9	2.3 ± 0.8	1.8 ± 1.3	1/6
4 (6)	excision	rHTE, 5 mg/ml	1.76 ± 0.70	2.93 ± 5.10	8.4 ± 3.0	1.8 ± 1.6	1.0 ± 0.6	0/5
5 (6)		rHTE, 10 mg/ml	1.97 ± 0.62	1.46 ± 4.31	8.1 ± 2.8	3.0 ± 0.6	1.0 ± 0.6	2/6
6 (6)	Surgical excision only	rHTE, 0.5 mg/ml	2.05 ± 0.28	4.9 ± 8.33	10.4 ± 4.5	2.2 ± 0.8	1.3 ± 1.4	1/6
7 (6)		rHTE, 5 mg/ml	1.56 ± 0.70	3.61 ± 4.66	9.2 ± 3.8	1.7 ± 0.8	1.7 ± 0.8	3/6
8 (6)		rHTE, 10 mg/ml	1.56 ± 0.13	2.68 ± 6.50	10.6 ± 2.4	1.5 ± 1.2	1.5 ± 0.8	0/4
9 (6)	Burn + surgical	rHTE, 0.5 mg/ml	2.15 ± 1.02	1.11 ± 4.18	6.9 ± 3.7	2.0 ± 1.3	0.7 ± 0.8	5/5
10 (6)	excision	rHTE, 10 mg/ml	1.72 ± 0.05	4.08 ± 3.82	6.8 ± 3.2	2.2 ± 1.0	1.2 ± 1.0	6/6
	Postskin closure							

rhTE, recombinant human tropoelastin.

*All treatments were given every 3–4 days during dressing changes for four treatments. Groups 1–8 were treated at day 7 post burn after surgical excision. Groups 9 and 10 were each given 4 rhTE between days 24 and 32 post burn after wound closure.

†χ^2 test, $P < .05$: group 9 and 10 vs group 1, 2, 3, 4, 5, 6, and 8.

length were recorded. All specimens were tested on a MTS 858 Mini Bionix II Testing System machine (MTS Systems, Eden Prairie, MN) with a 5-lb load cell using pneumatic grips at room temperature for the mechanical test under tension with a crosshead speed of 10 mm/min. Force and displacement measurements were acquired and recorded at 0.1-second intervals. Mechanical properties of the tissue including engineering stress, strain, elastic modulus, ultimate tensile strength, and stiffness were calculated from the data collected on the MTS Testing System.

Histopathological Analysis

Full-thickness skin biopsy was performed using a 4-mm diameter biopsy punch to confirm the depth of burn injury and to evaluate wound healing and hypertrophic scar formation at post burn days 7, 21, 35, and 90. Samples of the uninjured healthy tissue adjacent to study area were also taken. The biopsy samples were fixed in 10% neutral-buffered formalin, dehydrated, and sectioned to embed in paraffin blocks. Each paraffin block was cut with three consecutive sections placed on glass slides and stained with Hematoxylin and Eosin (HE), Masson's Trichrome (MT), or Van Geison's Elastin (VVG) stains. Each slide was evaluated for presence of scarring, the degree of healing (inflammation level scored 0–4, hemorrhage, or necrosis), scar composition (presence of elastin fibers), and the depth of the scar (Table 2). The slides were examined by a study-blinded, board-certified pathologist using an Olympus BH-2 microscope with an Olympus DP71 digital camera (Olympus, Center Valley, PA) at 12.5×, 125×, 250×, and 500× magnifications. Representative pictures were taken at 500×. Burn depth was determined by depth of collagen and depth of vascular injury from biopsies collected on day 7. Wound healing and hypertrophic scar formation were evaluated from biopsies collected on day 21, 35, and 90.

Statistics

All parameter data, such as body weight and length, depth of excision, area of wound scar, skin hardness and stress, were expressed as mean ± SD or SEM with 95% confidence intervals (CI) and analyzed by one-way analysis of variance and two-tailed Student's *t*-test. Pathological score data were assessed using Wilcoxon's/Kruskal–Wallis rank test. Difference in proportion of new elastin fibers in individual scars used a χ^2 test. A P value of less than or equal .05 was considered statistically significant.

Table 2. Scoring table for inflammation and depth of scar

Scores	0	1	2	3	4
Inflammation	No significant	Minimal	Mild	Moderate	Severe
Depth of scar	No scar	<1/2 dermis	1/2–2/3 dermis	2/3–3/4 dermis	>3/4 dermis

RESULTS

Seven female Red Duroc swine were enrolled. One premature death occurred during the study because of a complication from anesthesia and the data were excluded; the other six swine survived to the end of this study without notable complications. A total of 36 deep-dermal partial-thickness burns in the six swine were created and confirmed under microscopy examination. An average depth of dermatome excision was 1.77 + 0.66 mm on all study sites. There were no differences in the depth of excision between burn and surgical excision sites, and among study sites and treatment groups. All study sites, regardless of burned, unburned, or treatment type developed visible hypertrophic scars after surgical excision. The area of wound or scar significantly decreased during wound healing from day 7 to day 38 in all sites. The scar area was observed to have increased at day 90 in all sites, which was probably due to the overall growth of the swine as noted by body weight and length.

Measurement of Skin Induration

Skin durometer tests showed that skin hardness measurements of the excised sites was significantly reduced at day 7 (post excision) compared with pre-excision and with the normal skin adjacent to the study site (39.2 ± 6.4 vs 44.5 ± 4.0 and 44.7 ± 3.5, respectively; $P < .001$) in all sites. Skin hardness increased with time over the duration of the study ($P < .001$, ANOVA) in all sites. The hardness of wound scar sites increased significantly in comparison with its adjacent healthy skin areas postexcision. The hardness of adjacent healthy skin slightly increased during the 90-day study period, but no significant differences were noted (Figure 2). In comparison among treatment groups, the wounded scar area was much harder than adjacent normal skin (Figure 3), but there were no significantly differences in scar hardness between control or treatment sites or between the burned or unburned injury types.

Assessment of Skin Mechanical Properties

Skin mechanical test measured the strain of injured, treated, and normal skin (Figure 4). The max stress of the wound scar area was significantly lower than the adjacent normal skin areas (8.2 ± 3.3 MPa vs 16.9 ± 4.8 MPa, $P < .001$). However, there was no significant difference in max stress between burn or surgical injuries and no evidence of improvement by any of the treatment groups at the time points in this study (Figure 3).

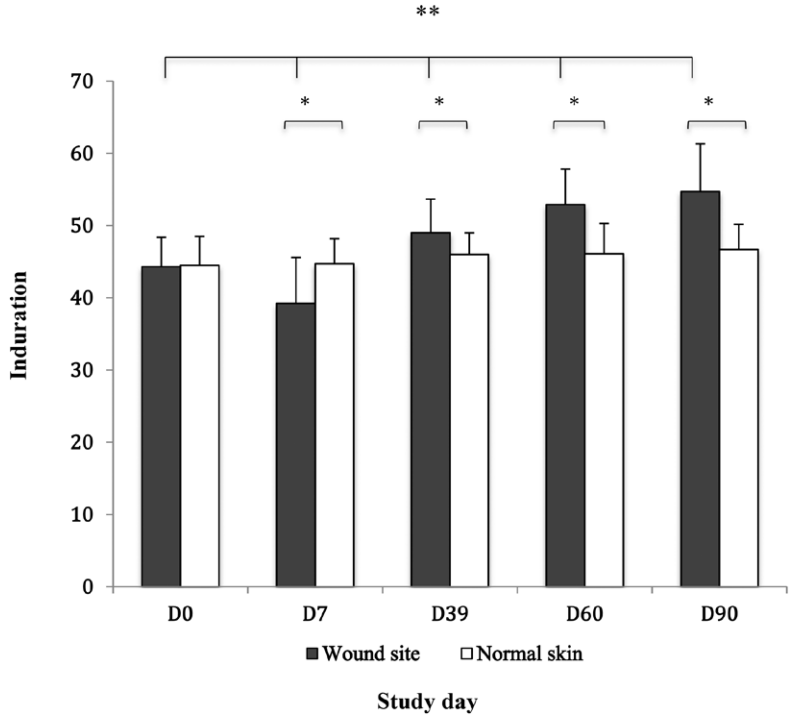

Figure 2. Skin induration reading during healing. Burn injury was created at day 0 (D0), Surgical excision was performed on burned and unburned study sites at day 7 (D7). The significant differences were found at D7, D39, D60, and D90 between excised wound site and normal skin adjacent to wound (*$P < .001$, *t* test), among time points at wound sites (**$P < .001$, analysis of variance).

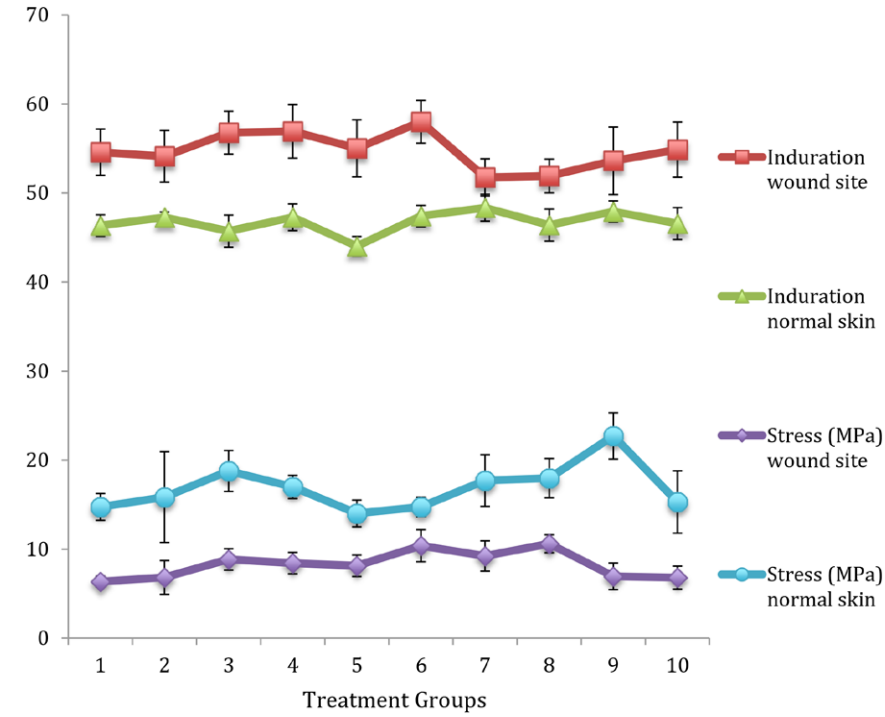

Figure 3. Comparison of skin hardness and stress of wound scar and normal skin at post burn injury day 90 among treatment groups (mean ± SEM). Durometer readings present skin hardness (induration).

Histopathological Analysis

The purpose of this study was to evaluate skin samples from Red Duroc swine 90 days postsurgical excision injury or thermal injury with treatments of either low, medium, and high dose intradermal injections of rhTE or saline control injections. At 90 days, all treatment sites with the exception of the normal skin adjacent to the treatment sites showed mature granulation tissue and dermal scarring composed predominantly of smooth muscle cells and

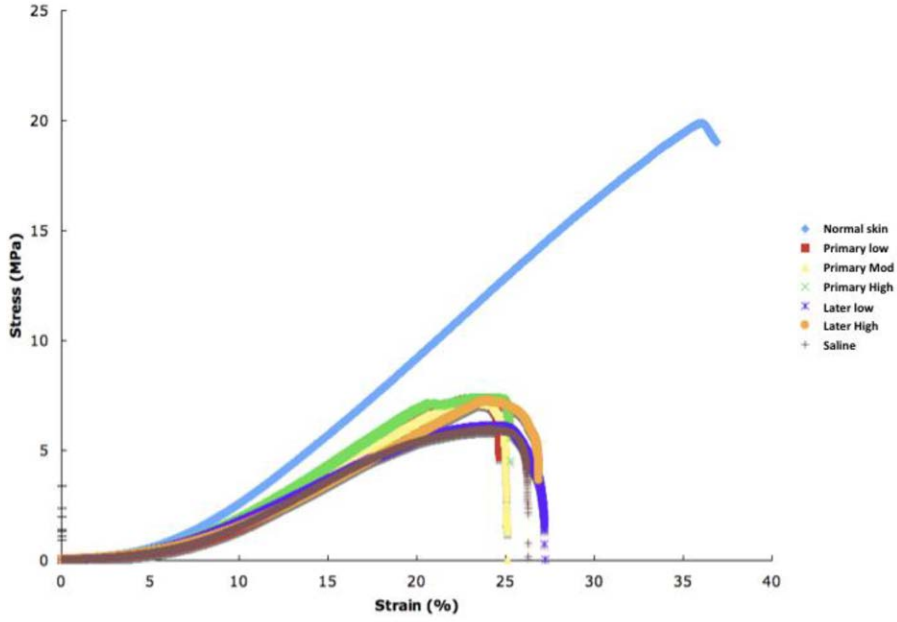

Figure 4. Graph of elastic modulus of skin samples. All skin samples came from burn sites treated with recombinant human tropoelastin or saline except normal skin.

fibroblasts in a collagen matrix with minimal residual inflammation and angiogenesis. Only focal areas demonstrated less-advanced healing as evidenced by greater neo-angiogenesis and chronic inflammation. The epidermis is thickened overlying the scar with a thick keratin layer. The scar tissue ranges from 1/3 to 3/4 of the dermal thickness (Figure 5A). Wound sites treated with rhTE showed elastin fibers within the extracellular matrix in surgical excision scars (low dose, 1/6 and medium dose, 3/6) and burn scars (low dose, 1/6 and high dose, 2/6) in swine treated with rhTE treatment. No elastin fibers were observed in scars without rhTE treatment. In the two treatment groups that received rhTE injections later in wound healing after skin closure, significantly greater numbers of elastin fibers were observed within the dermal scar tissue (5/5 in low dosage; 6/6 in high dosage; $P < .05$). The newly-formed elastin fibers in the tissue samples of both groups, however, were significantly fewer in number and showed a less discrete morphology compared with elastic fibers found in normal dermis adjacent to nonwounded skin (Figure 5). There was no definitive evidence of elastin fiber deposition associated with wound healing in the saline treatment arms, although the rare presence of gray wispy fibers on Von Geison's staining in some sections may represent early elastic fiber formation. There was no evidence of difference in measurements of scar depth or inflammation score among treatments and controls, or among individual swine (Table 1).

DISCUSSION

Despite recent advancements in surgical and medical treatments of severe burns, a large number of patients have insufficient regeneration of normal-functioning skin and this skin is replaced by dense, collagen-rich scars that are inflexible and create significant disability owing to restriction of normal skin movement. The extracellular matrix of normal skin is composed of collagen fibers for structural integrity and elastin fibers to confer elasticity and flexibility. The extracellular matrix created within burn scars do not contain elastin fibers.[7,8] To restore some degree of normal skin flexibility and function, we investigated the possibility that administration of rhTE into the wound bed at various time points during healing of a severe burn injury to determine whether dermal fibroblasts

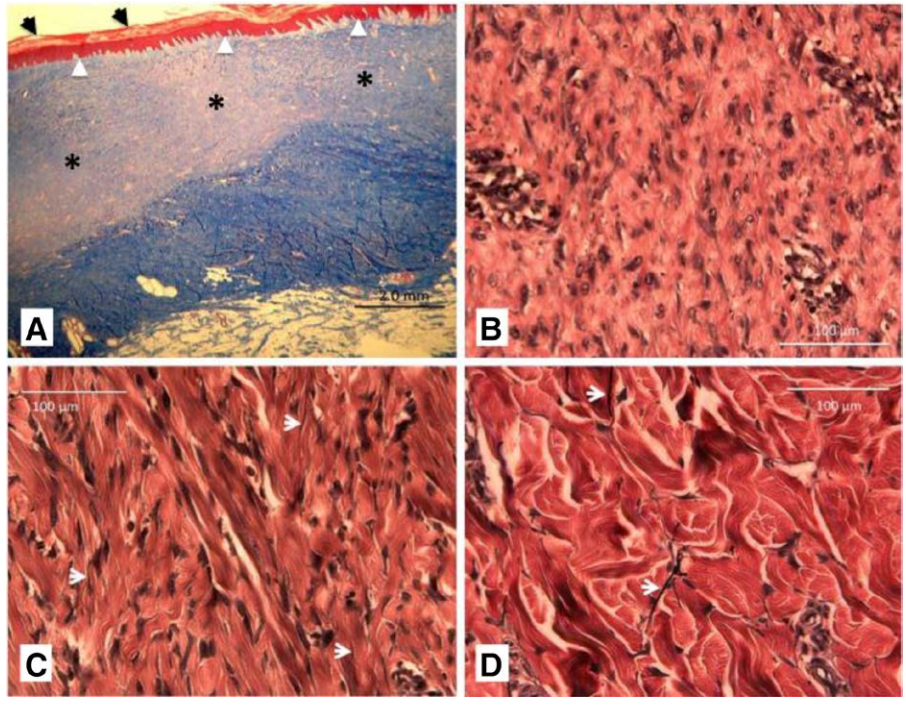

Figure 5. Well-healed burn scar tissue approximately 1/2 dermal wall thickness epidermis (A, lighter blue layer marked by black stars, trichrome stain, 1.25×) with overlying thick keratin (white arrowheads) and necrotic debris layers (black arrowheads). The burn scar region of a saline treatment site shows that mature granulation tissue composed of fibroblasts and small blood vessels (arrows) without definitive elastic fibers (B, Van Geison stain, 20×). Newly formed elastin fibers were found in a rhTE-treated burn scar region (C, arrows, Van Geison stain, 20×) that compare to matured elastin fiber in unburned normal dermis region (D, arrows, Van Geison stain, 20×).

could produce normal elastin fibers and restore the balance of collagen/elastin fibers and improve scar flexibility. Because severe burns have a significant local and systemic inflammatory response that may have an adverse effect on neo-elastogenesis, we also evaluated the possibility of improved elastin fiber production in a wound bed without an inflammatory burn injury utilizing a surgical dermatome injury.

We believe that this study demonstrated, for the first time, that injection of rhTE into a burn or a surgical wound can safely induce the formation of new elastin fibers that are not present in the normal process of scarring in an in vivo model. Importantly, these elastin fibers were formed despite the fact that rhTE was a xenographic protein in the female Red Duroc swine model, which is a model of abnormally severe hypertrophic scarring. One hundred percent of the wounds in this swine model resulted in hypertrophic scarring regardless of burn or surgical wounding or treatment with rhTE. During the 90-day period of observation, the number, length, and distribution of the new elastin fibers were substantially different than that seen in normal skin. Furthermore, these new fibers did not confer any significant measurable increase in scar flexibility compared with wounds treated with saline injections. It is unclear whether the elastin fiber deposition in the scars was simply insufficient to confer improved skin flexibility as desired. It is possible that the density, orientation, or amount of the collagen scar prohibited elastin fiber benefit. The process of wound healing and scar modulation is not complete at 90 days and it is possible that further elastin fiber deposition and maturation along with collagen and cellular maturation of the wound bed would result in a more flexible, skin healing-effect. It is also possible that further treatments later in the wound healing process would improve elastin fiber generation and improve the result.

We investigated the potential effect of three dose levels of rhTE during the process of wound healing beginning 1 week post burn injury, following the standard practice of dermatomal removal of eschar and nonviable skin. Despite the fact that our in vitro cell culture studies have shown rhTE dosage-dependency using either human fibroblasts or pig fibroblasts to assemble new elastin network in the extracellular matrix, we found no dose-related difference in elastin fiber development in a wide range of rhTE doses. We also evaluated the effect of additional rhTE treatments in some animals later in the wound healing period, after skin closure where there was less of an inflammatory milieu and at a time where there was a robust deposition of extracellular matrix. Interestingly, although there was some evidence of elastin fiber deposition after rhTE treatments in the early phases of wound healing, a much more robust deposition of elastin fibers was observed in animals treated in the later phase of wound healing. One explanation of this finding is that early burn wound exudates have high concentrations of neutrophil and leukocyte elastase and other proteolytic enzymes that may result in degradation of the soluble TE proteins.[20–23] Alternatively, there may be inhibitors present in the burn wound that prevent dermal fibroblasts from crosslinking rhTE.[24]

Limitations of the Study

A major potential limitation to this study was the relevance of our female Red Duroc hypertrophic scar model. Although this model has been well described and has been used to evaluate burn and surgical wound treatment approaches, this is a particularly potent genetic variant that predisposes the animal to abnormal skin healing and may not reflect normal human dermal healing after burn or surgical injuries. Our Red Duroc swine model uniformly developed a raised, thickened, and inflexible hypertrophic scar regardless of whether it was a burn or surgical wound, and a treatment effect may have been obscured. Another limitation was the choice of 3 months as the final time endpoint to assess the treatment effect. Histology in this study showed that the newly formed elastin fibers appeared as thin, fragmented, and disorientated structures in scars, which implied that these newly formed elastin fibers may be immature and in development. A 6-month study would have been a more relevant endpoint. Normally it can take 6 to 24 months for complete scar maturation and stabilization.[12,13,25,26]

Our initial therapeutic strategy to deliver rhTE proteins directly to the acute burn wound bed immediately after dermatome excision of devitalized dermis, 1 week after the acute burn, was intended to provide rhTE to the dermal fibroblasts to fabricate elastin fibrils within the healing tissues. Interestingly, our results showed that the best elastin fiber formation occurred in the animals where rhTE was given after wound closure. Newly elastin fiber formation occurred in all rhTE treatment sites, which implied that our choice of rhTE dose ranging was incorrectly projected.

In conclusion, this is the first report that shows the feasibility of using subcutaneous injections of rhTE to enable formation of new elastin fibers in a healing scar after burn and surgical injuries, demonstrated in a swine hypertrophic scar model. Timing the delivery of rhTE until after wound closure was significantly more effective in elastin fiber formation than treatments delivered earlier after wounding. No adverse consequences of multiple doses of this xenogenic protein were observed in this study. Although the generation of new elastin fibers in the hypertrophic scars did not result in desired improvements in skin flexibility compared with scars without elastin fibers at 90 days, it is possible that improved skin flexibility will be seen at longer timepoints when elastin fiber numbers and maturation may be increased. The ability to restore elastin fiber formation in adult skin after burns, trauma, surgery, and aging may ultimately improve skin regeneration, function, and appearance and reduce disabling complications of scar formation that occur, in part, due to lack of expression and production of TE in children and adults.

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