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RESEARCH ARTICLE

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Combination product of dermal matrix, preconditioned human mesenchymal stem cells and timolol promotes wound healing in the porcine wound model

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Abstract

A combination product of human mesenchymal stem/stromal cells (MSCs) embedded in an extracellular matrix scaffold and preconditioned with hypoxia and the beta-adrenergic receptor antagonist, timolol, combined with sustained timolol application post implantation, has shown promising results for improving wound healing in a diabetic mouse model. In the present study, we extend those findings to the more translatable large animal porcine wound model and show that the combined treatment promotes wound reepithelialization in these excisional wounds by 40.2% and increases the CD31 immunostaining marker of angiogenesis compared with the matrix control, while maintaining an accumulated timolol plasma concentration below the clinically safe level of 0.3 ng/mL after the 15-day course of topical application. Human GAPDH was not elevated in the day 15 wounds treated with MSC-containing device relative to wounds treated with matrix alone, indicating that the xenografted human MSCs in the treatment do not persist in these immune-competent animals after 15 days. The work demonstrates the efficacy and safety of the

Hsin-ya Yang and Fernando Fierro contributed equally to this study.

combined treatment for improving healing in the clinically relevant porcine wound model.

KEYWORDS
mesenchymal stem/stromal cells, swine, timolol, wound healing

1 | INTRODUCTION

A combination of preconditioned human mesenchymal stem/stromal cells (MSCs) embedded in an extracellular matrix scaffold, followed by a regimen of daily topical administration of timolol, a nonselective antagonist for the beta-adrenergic receptor, has shown promising results for improving healing of wounds in a diabetic mouse model.[1] However, the porcine wound model, with its similarity to human skin architecture and the human skin wound healing process can better predict the potential for translation and address safety concerns. Rodent wounds heal primarily by contraction due to the panniculus carnosus structure in the loose skinned animals,[2,3] whereas porcine skin heals by reepithelialization with limited contraction, as does human skin.[3] The porcine wound model is recommended in the FDA guidelines for investigators developing therapies for chronic wounds.[4] In a literature survey of 25 wound therapies in human, porcine and rodent models, 78% of the results from the porcine model are in concordance with the human studies, compared with only 53% of those from the rodent model;[3] thus, there is an advantage for potential translation from a porcine model to human therapeutics.

The components of the combined wound therapeutic described here were chosen with the following rationale: Human bone-marrow-derived, allogeneic MSCs are well characterized, have a strong safety profile in the clinic,[5] can be expanded and cryo-preserved, prescreened for their cytokine profiles, and prepared for clinical applications in a short period of time.[1] Allogeneic MSCs can also suppress local immune responses and evade host rejection, and hypoxic preconditioning increases their persistence at the wound site,[6] both of which potentially allow for their extended therapeutic potential.[7] The beta-adrenergic receptor antagonist, timolol, has been safely used to treat cardiovascular diseases and glaucoma for over 30 years.[8] Recent clinical trials have suggested that topical application of timolol can promote wound closure in chronic ulcers.[9,10] To determine if the combination treatment, MSCs preconditioned in timolol and hypoxia on wound scaffolds (MSC/T/H/S) that improves healing in mice, has the potential for successful translation to humans, we examined its ability to improve healing in a porcine, large animal wound model.

2 | MATERIALS AND METHODS

2.1 | Preparation of MSC/T/H/S device

Human bone marrow MSCs were purchased from Stem Express (Placerville, CA, USA), isolated, cultured, characterized, and cryopreserved as previously described.[1] The combined treatment of MSC/T/H/S with timolol solution is summarized in Figure 1(A–C). Five days prior to the wounding surgery, MSCs were seeded on 16 mm diameter circular scaffolds (Integra® Wound Matrix Thin, catalog #54051T, Integra LifeSciences, Plainsboro, NJ, USA) at a density of 2.5×10^5 cells/cm^{2,} and incubated at 37°C for 4–12 h, and then preconditioned by continued cultivation for 48 h in 1% oxygen (hypoxia), in the presence of 1 μM timolol (timolol maleate, Sigma-Aldrich, St. Luis, MO, USA).

2.2 | Fluorescent imaging of MSC/T/H/S device

The seeded MSC/T/H/S scaffolds (1×10^5 cells/cm^2 for imaging) were paraformaldehyde fixed for 30 min and blocked in 2% bovine serum albumin (BSA blocking solution) at 4°C overnight. For staining, scaffolds were incubated for 1 h in the BSA blocking solution containing 2 U/mL Texas Red-X Phalloidin (Life Technologies, Grand Island, NY, USA) and 3.5 μM To-Pro®-3 (Life Technologies) and mounted in Vectashield® Mounting Medium (Vector Labs, Burlingame, CA, USA). Scaffolds were cross sectioned relative to their top-side seeding orientation and the cross-sectional interior of the scaffold was imaged on an Olympus Fluoview FV10i confocal microscope with Olympus FV10-ASW software.

2.3 | Animal training, surgery, and wound care

The porcine wounding protocol is in accordance with the AAALAC guidelines and approved by the Institutional Animal Care and Use Committee at UC Davis. The University of California, Davis has an PHS Animal Welfare Assurance (A3433-01, valid until March 31, 2023 with the Office of Research). Yorkshire/Landrace-cross female pigs (*Sus scrofa domesticus*, 27–40 kg, from the Swine Teaching and Research Center at UC Davis) were singly housed in a temperature and daylight-controlled vivarium (70°F, 12-h light/dark cycles) with a minimal of 15 ft^2 space/animal and environment enrichment. Pig feed (Nutrena, Country feed grower/finisher pig feed) was supplied at 1.5 lbs/animal twice a day with ad libitum water. The animals were acclimated and trained for 7–10 days using positive reinforcement (food treats) to voluntarily enter a portable Panepinto-like sling (Lomir, cat# SF H1PU) for handling up to 30 min/day.[11–14] This modified protocol for sling-training allowed for daily examination and treatment of wounds on awake, low-stress animals without anesthesia, a potential confounder.[15,16]

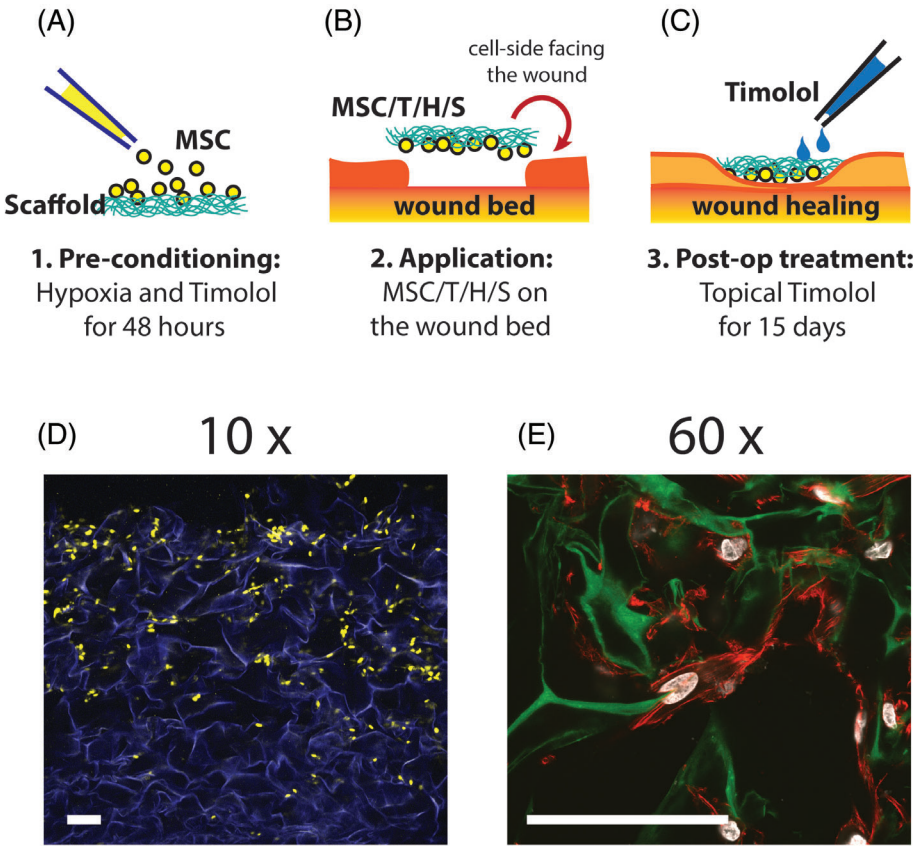

FIGURE 1 The combined MSC/T/H/S treatment with timolol for promoting wound healing. (A–C) A schematic to demonstrate the steps of treatment, including the preconditioning of the MSC/T/H/S (MSC cells are not to scale), the application of MSC/T/H/S on a wound, and the topical treatment with timolol postoperationally. Layers of the wound dressings are omitted in the schematic. (D) Fluorescent images of the MSCs on the cross sections of a wound scaffold by confocal microscopy at 10×. The MSC/T/H/S is in the same configuration as in (A). The MSC nuclei are pseudo-colored in yellow and the collagen in the scaffold is pseudo-colored in blue. (E) A high magnification image of the MSC/T/H/S at 60×. The nuclei were stained with To-Pro®-3 (shown in white), actin was stained with Texas Red-X Phalloidin (shown in red), and the autofluorescence from collagen fibers in the scaffold is shown in green. Scale bar =100 μm

Animals were fasted >12 h prior to the surgery and induced under telazol (4–8 mg/kg, intramuscular). After endotracheal intubation, animals were maintained under isoflurane (0.5–4% in 100% oxygen) for the wounding procedure. The heart rate, respiratory rate and body temperature were monitored, and Ringer's lactate solution was given (5–10 mL/kg/h, intravenous) during the surgery. Ten milliliters of blood were collected via ear vein in EDTA tubes prior to the surgery, one hour post timolol treatment, and at the endpoint of each experiment.

After depilation the back, skin was prepared with 2% chlorhexidine and isopropyl alcohol rinses. Sixteen or eighteen 16 mm circular, full-thickness excisional wounds (2 cm^2) were created in two paraspinal columns between the crest of the shoulders and the ilium on each animal (Figure 2A,B). The Integra wound matrix alone or the MSC/T/H/S device was applied to the wound bed, and each wound was treated with subsequent daily application of either saline (control, 300 μL) or timolol solution (0.047 mg timolol in 300 μL saline) (Figure 2C). Wounds were dressed with Conformant 2 Wound Veil Sheet (Smith & Nephew, Watford, England, UK), bolster foam/Optifoam Basic Non-Adhesive Dressing (Northfield, IL, USA), and sealed with Tegaderm Transparent Film Dressings (3M, Maplewood, MN, USA) (Figure 2B). The entire back of the animal was then covered with Reston Self-Adhering Foam Dressing Pads (3M) and a tear-resistant coat to protect the wounded area. A fentanyl patch (5 μg/kg/h for 72 h) was applied for post-op pain management and buprenorphine (0.01 mg/kg, intramuscular) was administrated at extubation. Post-op wound care was performed while the animal rested in the sling as described above. Daily treatments of saline or timolol were injected under the Tegaderm dressing into the matrix or the MSC/T/H/S layer, and dressing changes were performed as needed. At day 15, animals were humanely euthanized (>100 mg/kg pentobarbital injection intravenously) and wound tissue collected along with 1 cm of normal skin adjacent to the wound at a depth below the initial wound bed and bisected through the widest diameter of the wound to be fixed either in 4% paraformaldehyde, or preserved in RNALater (Invitrogen, MA).

2.4 | Wound reepithelialization

Histological analysis was performed as previously described.[1] The wound tissue was processed in a Tissue-Tek VIP Processor 6 (Sakura Finetek, Torrance, CA, USA) with modifications for the porcine tissue: 4% paraformaldehyde fixation for >72 h, 70% Ethanol for 90 min (2 cycles), 80% Ethanol for 90 min, 95% Ethanol for 110 min (2 cycles), 100% Ethanol for 110 min (2 cycles), 100% Xylene for 110 min (2 cycles), and Paraffin for 75 min (4 cycles) for embedding. Five micron sections were made for H&E staining to quantify the reepithelialization of the wounds.[1] The histological sections were imaged and analyzed on a BioRevo BZ-9000

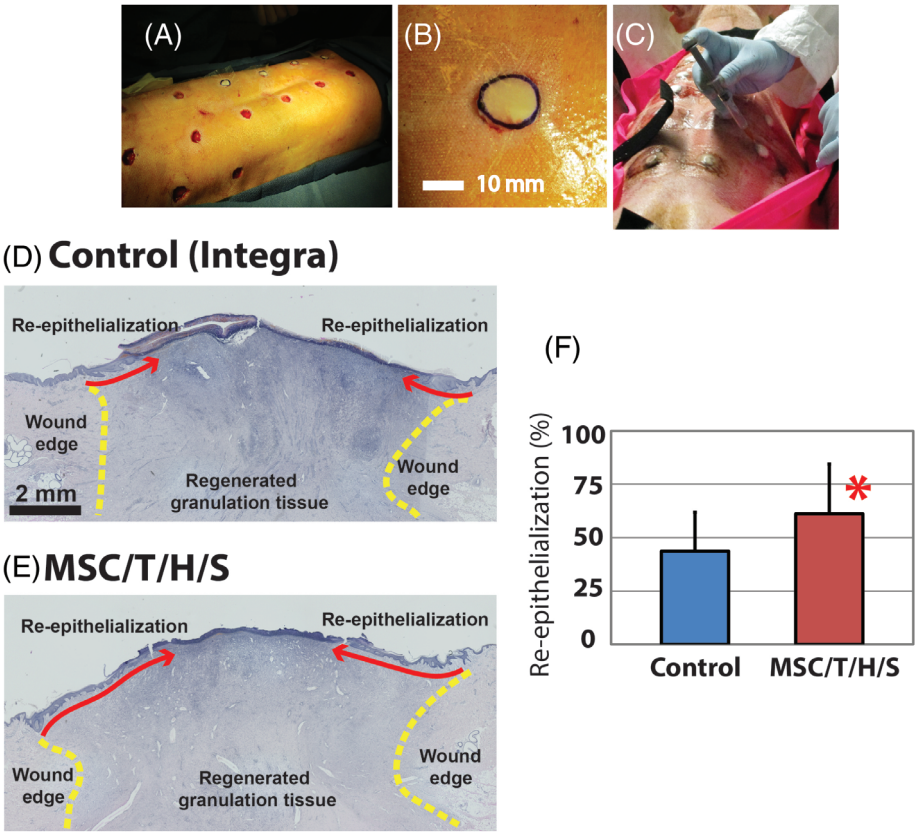

FIGURE 2 Wound healing in domestic pigs. (A) Multiple wounds were created on the back skin while the pig was under anesthesia. (B) A wound scaffold (Integra control) or the MSC/T/H/S treatment was applied to the wound bed, and layers of dressings including wound veil, a foam bolster, and Tegaderm were used to seal the wound (scale bar = 10 mm). (C) daily postoperational wound care. The dressings were replaced and the wound treatment (saline or timolol solution) was injected to the Integra matrix layer while the pig rested in the sling. (D) A representative histological image of pig wound treated with Integra® wound scaffold (control). The wound edges (yellow dotted lines) are defined by absence of hair follicles and the newly generated granulation tissue (dark purple staining) and the reepithelialization was manually tracked (red arrows). (E) A representative histological image of pig wound treated with MSC/T/H/S device. (F) Quantitation of the wound reepithelialization. The 15-day reepithelialization rate is 43.5% ± 18.1% in the control (Integra® Wound Matrix Thin Skin + saline) and increases to 61.0% ± 23.4% with the MSC/T/H/S treatment. (Mean ± STDEV, n = 15 wounds from four pigs, statistical analysis performed in Excel by Student's *t*-test, $^*p < .05$, scale bars for D and E = 2 mm)

inverted microscope with the BZ-II viewer and analyzer (Keyence, Osaka, Japan). The wound edges were defined by absence of all dermal appendages including hair follicles and the newly generated granulation tissue (Figure 2D,E). The outgrowth of the newly formed epidermis was tracked and the percentage of the combined length of the reepithelialization to the total length of the wounds was calculated (Figure 2F).

Four to six replicate wounds were created in each animal, and the wound treatments were not randomized, but were rotated at different locations (near shoulder or ilium) in each animal. Four repeat experiments were performed. The reepithelialization scoring was measured by an investigator blinded to treatment groups and the results from 15 wounds were combined. Wounds were excluded from analysis if the Integra® matrix or the MSC/T/H/S device was damaged or detached from the wound before the end of the experiment, or if the wound edge could not be clearly identified by the H&E staining.

2.5 | Immunohistochemistry staining for CD31

Wound sections (5 μm) were deparaffinized and rehydrated, steamed in sodium citrate solution for 15 min to retrieve antigen, blocked in TBS/0.025% Triton with 10% goat serum and 1% BSA at room temperature for 1 h, incubated with anti-CD31 primary antibody (Rabbit anti-pig CD31, 1:100, catalog # nb100-2284, Novus Biologicals, Centennial, CO, USA) overnight at 4°C, and then with secondary antibody (Goat anti-rabbit IgG conjugated with alkaline phosphatase, 1:300, catalog# ab98505, Abcam, Cambridge, UK) for 1 h at room temperature. To visualize the results, the slides were stained with ImmPACT Vector Red substrate (magenta color, Vectors Labs, catalog# SK-5105) and nuclei counter-stained with methyl green solution (green color, Vectors Labs, catalog# H-3402-500) for 5 min at 60°C. Then the slides were dehydrated again and mounted in xylene-based Cytoseal mounting medium (VWR, catalog# 48212-187, Radnor, PA, USA). The histological sections were imaged on a BioRevo BZ-9000

inverted microscope and the BZ-II viewer with 2× and 20× objectives (Keyence, Osaka, Japan).

2.6 | Timolol quantitation

Timolol was measured in the collected plasma samples from each animal as described.[1] Timolol was extracted by cation-exchange solid phase extraction and analyzed by reverse-phase HPLC with UV/visible detection at 284 nm (uHPLC and neurotransmitter analyzer, Antec Scientific, Zoeterwoude, the Netherlands).

2.7 | Cytokine expression and MSC persistence in wound tissue

Wound tissue was homogenized using a Bullet Blender Storm 24 (Next Advance, NY). Total messenger RNA was isolated with the RNeasy Mini Kit (Qiagen, Venlo, Netherlands). One microgram of RNA was reverse transcribed to cDNA using the Quantitect Reverse Transcription Kit (Qiagen). The Qiagen pig primers were used: RT^2 qPCR primer assay for *IL1B* (cat # PPS00015A-200), for *IL-6* (cat # PPS00991A-200), for *TNFa* (cat # PPS00426A-200), for *IGF1* (cat # PPS00801A-200), for *VEGFA* (cat # PPS00495A-200), for *TGFB1* (cat # PPS00418A-200), for *GAPDH* (cat # PPS00192A-200), and for *RPL19* (cat. # PPS00333A-200). For qPCR analysis, the Quantitect Sybr Green master mix was used (Qiagen). Each sample was run in duplicate. Relative normalized expression of the target gene was calculated via ΔΔCt against the average ΔCt of the control samples within each sample and normalized to GAPDH and RPL19. To examine the persistence of human MSCs in the porcine wounds at the end of the treatment, quantitative RT-PCR was performed with human and pig specific primers to GAPDH by TaqMan® Gene Expression Assays (Applied Biosystems, Waltham, MA, USA, pig GAPDH Ss03375629_u1 and human GAPDH: Hs02758991_g1) according to the manufacturer's protocol. The ratios of the relative expression levels (ΔΔCt) of human and pig GAPDH mRNA were compared in the day 15 control and the MSC/T/H/S-treated samples.

2.8 | Statistical analysis

Wound samples were excluded from analysis if the Integra® matrix (control or the MSC/T/H/S) was detached from the wound before the end of the experiment, or if the wound edge could not be identified in the histological images. The percentage of wound reepithelialization was not transformed or normalized. In the qPCR assays, each fold change dataset was analyzed by the Grubbs Test to remove outliers. The bar graphs are presented as mean ± STDEV. Fifteen wounds from four pigs were combined and a two-sided Student's *t*-test was performed to determine statistical differences (α or threshold of significance = 0.05) between the treatment groups in Excel (Microsoft, Redmond, WA, USA).

3 | RESULTS

Most of the seeded MSCs were found concentrated in the upper half (the seeding side) of the scaffold as previously reported[17] (Figure 1D, the cells are in the same configuration as in Figure 1A). The MSCs appear to be aligned directly upon the collagen fibers within the Integra ® scaffold (Figure 1E). In order to ensure the direct contact of MSCs and the wounded tissue, the cell seeded side of the scaffold was flipped to face wound bed during the device application (Figure 1B). The wound and the MSC/T/H/S device was then protected by wound dressings during the course of the experiments. Histological analysis revealed that wounds that were treated with the MSC/T/H/S device and daily timolol application demonstrated reepithelialization that was 40.2% higher than wounds that received the control treatment of the matrix/saline (Figure 2A–C), demonstrating improved healing with the MSC/T/H/S device.

MSCs are known to improve angiogenesis by facilitating the formation of polarized, tubular structures with endothelial cells.[18,19] The paracrine effectors of angiogenesis, including platelet derived growth factor, epidermal growth factor, fibroblast growth factor, and nuclear factor-kappa B signaling pathway proteins, have also been identified in MSC-derived exosomes.[20] To examine if the MSC/T/H/S treatment improves angiogenesis in the pig wounds, we performed CD31 immunohistochemistry (IHC) staining on the day 15 tissue sections (Figure 3). In the representative images from the MSC/T/H/S treated wounds, the CD31 staining (Figure 3B, location #3, positive staining shown in magenta color and by the block arrows) is increased in the newly regenerated dermal tissue adjacent to the MSC/T/H/S device, indicating that in addition to improving reepithelialization in the wound, the MSC/T/H/S treatment promoted angiogenesis.

To address a potential safety concern for the absorption of topically applied timolol that could result in elevated systemic levels and adverse effects (such as bradycardia and bronchoconstriction), we monitored heart rates and pulmonary status in the pigs prior to (Table 1, $T = 0$ min) and at the onset of timolol action at 20 min post application (Table 1, $T = 20$ min). The heart rates did not decrease more than two beats per minute at the post timolol administration examination time, nor was there any pulmonary wheezing on auscultation (Table 1).

Timolol levels were also measured in plasma obtained before timolol treatment ($T = 0$ h), and 1 h post timolol application ($T = 1$ h for the peak concentration[21]) on the wound surgery day. To determine cumulated values, blood was collected after the 15-day course of topical application ($T = 24$ h, post timolol treatment). In each animal, the daily accumulated applied dosage of timolol was 0.564 mg (0.047 mg/wound × 12 treated wounds/animal). In 1 out of 12 samples, timolol concentration detected in plasma was between 0.3–0.5 ng/mL, but in the remaining 11 samples timolol concentrations were below the limit of detection, 0.3 ng/mL (Table 1, $T = 1$ and 24 h).

To assess whether the increased reepithelialization observed in the MSC/T/H/S device-treated wounds was due to dampened inflammatory responses, we examined levels of cytokines associated with

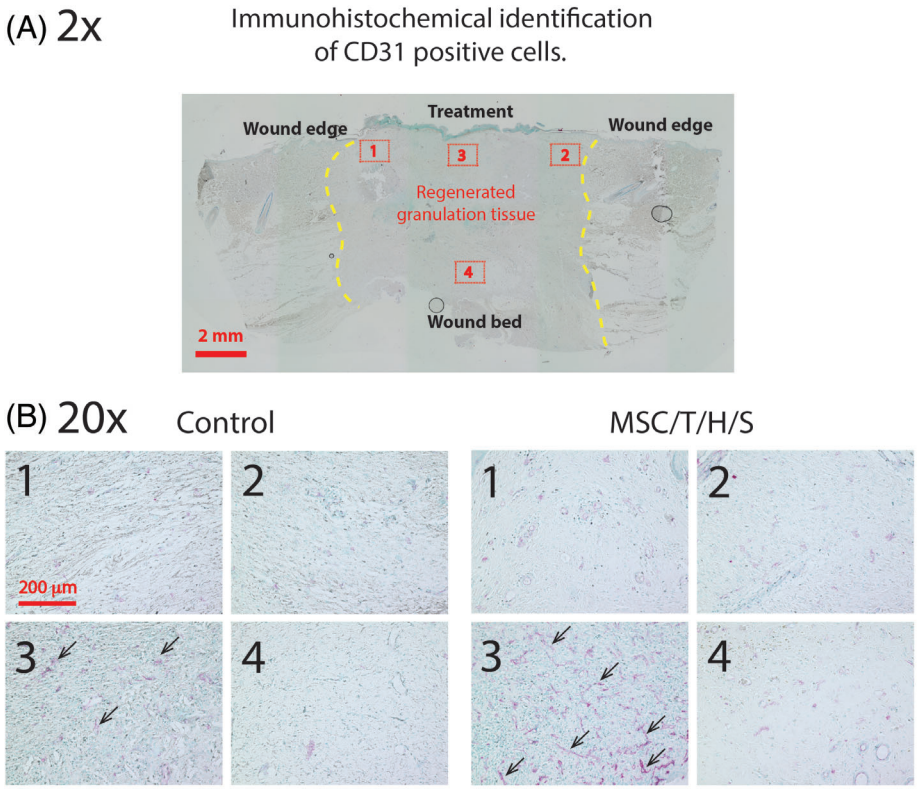

FIGURE 3 MSC/T/H/S treatment increased angiogenesis in wounds. (A) A representative stitched image of CD31 IHC in the wound at 2×. Four locations of each section were further examined at 20× (marked by the red boxes). Location #1, left wound edge; location #2, right wound edge; location #3, next to the Integra matrix or the MSC/T/H/S treatment; location #4, next to the wound bed. Several air bubbles (the black circles near the wound bed and one on each side of the wound edges) were trapped in this slide. (B) Representative images of each location from the control and the treated groups. Positive CD31 staining is shown in magenta color and nuclear staining is shown in green. Scale bar in (A) = 2 mm and in (B) = 200 μm. Black arrows indicate the positive CD31 staining

TABLE 1 Heart rate monitoring and plasma concentrations after timolol treatment

	Timolol	Heart rates (beat/min)			Timolol in plasma (ng/mL)		
Animals/weight on surgery day	Daily accumulative dosage (mg/pig)	$T = 0$ min	$T = 20$ min	beat change/min	$T = 0$ h (surgery day)	$T = 1$ h (surgery day)	$T = 24$ h (Day 15)
Pig #1 (45.0 kg)	0.564 mg	100	99	−1	<0.3	<0.3	<0.3
Pig #2 (42.2 kg)	0.564 mg	110	108	−2	<0.3	<0.3	<0.3
Pig #3 (37.7 kg)	0.564 mg	88	92	+4	<0.3	<0.3	<0.3
Pig #4 (40.5 kg)	0.564 mg	103	105	+2	<0.3	$0.3 < x < 0.5$[a]	<0.3

[a]The value is trace positive. The result is above the limit of detection but below the limit of quantitation and cannot be quantified accurately.

wound healing. TNFa and IL-6 are both cytokines that are involved in the inflammatory phase of wound healing by recruiting neutrophils and macrophages to the site of the injury and inhibiting action by myofibroblasts.[22–24] Previous studies showed that IL-6 indirectly promotes keratinocyte proliferation via fibroblast activation.[25] We also examined the expression levels of growth factors associated with the proliferative phase of wound healing. TGFb, VEGF, and IGF1 are classically known for their pro-healing functions in wound healing such as inducing cellular proliferation, endothelial cell migration and angiogenesis, and hemostasis.[22,23,26] There are no significant differences in the relative amounts of mRNA of the inflammatory cytokines *Il1b* (undetected; data not shown), *Tnfa*, *Il6*, growth factors *Tgfb*, *Vegf*, and *Igf1* between the matrix control and the MSC/T/H/S treatment from the day 15 wound tissue (Figure 4A–F). Interestingly, the mRNA transcripts of IL-6, a cytokine exerting both pro-inflammatory as well as pro-reparative functions in wound healing, and of TGFB1 show an upward trend in the treated wounds, but the change does not reach statistical significance when compared with the control group (Figure 4B,C). These results suggest that changes in pro-inflammatory cytokines on day 15 were not associated with increased wound reepithelialization. However, since they are more highly expressed during the early stage of wound healing,[27–30] it is possible that analysis of wound tissue isolated at earlier or later time points may reveal differences in these genes between the treatment groups.

The persistence of human MSCs in the pig wounds after the 15 days of treatment was also examined. Total mRNA extracted from the day 15 wound tissue was probed for human and porcine GAPDH. The ratios of the human/pig GAPDH relative expression levels remained the same in both the control (Integra only, without MSCs) and the MSC/T/H/S treated wounds (no statistically significant difference, Figure 4F) at the end of the experiments, suggesting that after 15 days the xenografted human MSCs do not persist in these immune-competent animals.

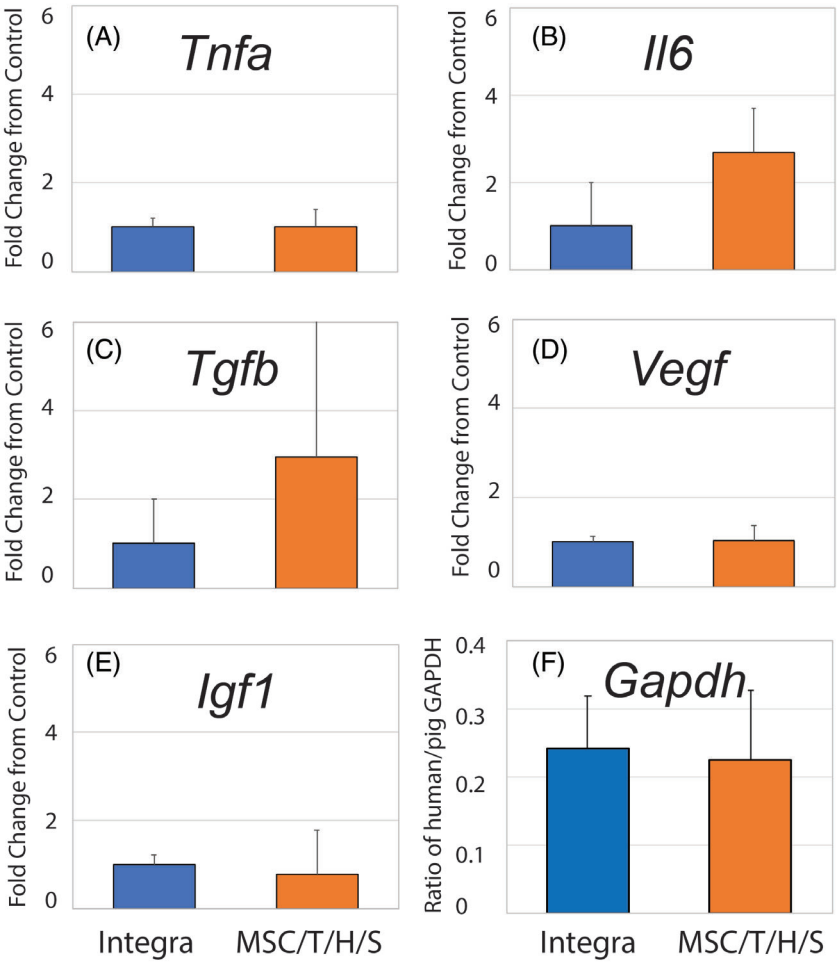

FIGURE 4 Cytokine and growth factor expression and MSC persistence in day 15 wound tissue. (A–E) There are no statistically significant differences in the expression levels of *Tnfa*, *Il6*, *Tgfb*, *Vegf*, and *Igf1* between the Integra® only and the MSC/T/H/S treated wounds at day 15. (F) The ratios of human/pig GAPDH expression level are similar in the Integra control and the MSC/T/H/S treated wounds (n = 15 wounds/group from 4 pigs, mean ± STDEV, statistical analysis performed in Excel by Student's *t*-test)

4 | DISCUSSION

A previous study applying a combination MSC/T/H/S product to wounds in diabetic mice showed improved wound healing by 65.6% relative to control.[1] By demonstrating efficacy in a large animal model, we now demonstrate that the beneficial effects of the treatment to wound closure and its safety can move to the next step for translation to human clinical care, a randomized clinical trial.

Full FDA approval of the MSC/T/H/S as an Investigational New Drug will require us to address additional safety concerns for this device. Systemic absorption of timolol is a concern since ocular administration of a 0.25%–0.5% aqueous timolol solution results in plasma concentration of 0.46–1.72 ng/mL, which can decrease resting heart rate up to 11 beats per min and decrease pulmonary flow, as well as induce wheezing and orthostatic hypotension.[31] Here, we show that the plasma timolol level (<0.3 ng/mL) in the pigs both 1 h after application (peak absorption) to a wound similar in size to human chronic wounds, as well as after 15 days of treatment with topical timolol, is lower than the reported absorption from clinical ophthalmic use, suggesting that the MSC/T/H/S treatment with the regimen of daily topical timolol application could provide local beneficial effects on wound healing without inducing systemic adverse effects.

Other cell types have been incorporated into dermal matrices to improve healing. Of note, a noncultured autologous skin cell suspension seeded onto Integra® dermal matrix is reported to improve healing in porcine excision wounds and human burn wounds.[32] However, these reports lack histological quantitation of wound reepithelialization, the gold standard for determining wound closure. Allogeneic MSC-containing devices provide for easier translation since a separate skin harvesting procedure is not needed, and these MSC-containing devices can be off-the-shelf for point-of-care applications in the clinic. Stem cell-based therapies are considered to be cell transplantation in which tumorigenicity of the product is a potential adverse event. The human MSCs used here have been extensively tested in other systems using rule-out tumorigenicity studies in immune deficient mice.[6,33] Since the same cell batches[1,6] have already been tested in sensitive assays in immune-deficient mice which cannot reject transplanted MSCs, we believe that the MSCs are safe for the future development of the MSC/T/H/S device.

The porcine wound model used here, with modified sling training, allows for daily interventions without the need for repeated anesthesia, and provides a facilitated pathway for preclinical testing of the device to better emulate the long-term human patient treatment regimens (8–12 weeks). In addition, there are reports of impaired healing in wounds in pigs that have been rendered diabetic.[34] Using the

modified sling technique can facilitate further testing of the device in compromised healing scenarios, such as preclinical models for human diabetic foot ulcers. These advances portend well for a facilitated pathway for translation to clinical use.

5 | CONCLUSION

Applying the combination product MSC/T/H/S with subsequent topical application of timolol solution to wounds results in improved wound healing in the swine excisional wound model. The treatment promotes wound reepithelialization and increases angiogenesis compared with the control, while the accumulated timolol concentration in plasma remains below the clinically safe level of 0.3 ng/mL after the 15-day course of treatment.

ACKNOWLEDGMENTS

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DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are provided in the article.

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