Secreted HoxA3 Promotes Epidermal Proliferation and Angiogenesis in Genetically Modified Three-Dimensional Composite Skin Constructs

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Objective: Homeobox (HOX) transcription factors coordinate gene expression in wound repair and angiogenesis. Previous studies have shown that gene transfer of HoxA3 to wounds of diabetic mice accelerates wound healing, increasing angiogenesis and keratinocyte migration. In this study, we examined whether HoxA3 can also improve angiogenesis, epidermal integrity, and viability of composite skin grafts.

Approach: To determine the effects of HoxA3 on composite skin grafts, we constructed bilayered composite grafts incorporating fibroblasts engineered to constitutively secrete HoxA3. We then transplanted these composite grafts *in vivo*.

Results: The composite grafts produced a stratified epidermal layer after seventeen days in culture and following transplantation *in vivo*, these grafts exhibit normal epidermal differentiation and reduced contraction compared to controls. In addition, HoxA3 grafts showed increased angiogenesis. Quantitative polymerase chain reaction (PCR) analyses of HoxA3 graft tissue reveal an increase in the downstream HoxA3 target genes MMP-14 and uPAR expression, as well as a reduction in CCL-2 and CxCL-12.

Innovation: Expression of secreted HoxA3 in composite grafts represents a comprehensive approach that targets both keratinocytes and endothelial cells to promote epidermal proliferation and angiogenesis.

Conclusion: Secreted HoxA3 improves angiogenesis, reduces expression of inflammatory mediators, and prolongs composite skin graft integrity.

INTRODUCTION

CHRONIC WOUNDS REPRESENT a significant burden to patients, health-care professionals, and the U.S. health-care system, affecting 5.7 million patients and costing an estimated 20 billion dollars annually.¹ Two common chronic, nonhealing wounds are diabetic foot ulcers, with up to 25% lifetime incidence risk among diabetics,² and venous stasis ulcers, affecting 2.5 million U.S. patients annually.³ Due to recent advances in technology, clinicians are

now presented with a wide array of adjunct treatment modalities to standard care, including but not limited to, growth factor treatments, enzymatic debridement, and bioengineered skin constructs.

Introduced in 1988, bioengineered bilayered skin constructs, such as Apligraf, comprised of a dermal layer of neonatal fibroblasts embedded in collagen, overlaid with an epidermal layer of neonatal keratinocytes, have been shown to accelerate wound healing,^{4–6} and provide an enticing

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Abbreviations and Acronyms

CCL2 = chemokine (C-C motif) ligand-2

CxCL12 = chemokine (C-X-C motif) ligand-12, stromal cell-derived factor-1

DMEM = Dulbecco's modified Eagle's medium

Hox = homeobox

IHC = immunohistochemistry

K5 = keratin-5

K10 = keratin-10

MMP-14 = matrix metalloproteinase-14

PCR = polymerase chain reaction

qPCR = quantitative polymerase chain reaction

sHoxA3 = secreted Hox A3

sHoxA5 = secreted Hox A5

TSP-2 = thrombospondin-2

uPAR = urokinase receptor

option for the treatment of chronic wounds. Currently, Apligraf is Food and Drug Administration approved for the treatment of full-thickness neuropathic diabetic foot ulcers that have not responded to at least three weeks of conventional therapy,⁷ and noninfected venous leg ulcers with standard therapeutic compression that have not adequately responded to at least four weeks of conventional therapy.⁸ Although the exact mechanism of the observed accelerated wound healing is unknown, bilayered skin constructs like Apligraf are thought to deliver growth factors and extracellular matrix proteins,^{9,10} and attract differentiated cells, including fibroblasts and endothelial cells to the wound bed.

While bilayered skin constructs are often thought of as grafts, previous studies have shown that true engraftment or prolonged persistence of cells from these composite constructs does not occur.^{11,12} Subsequently, multiple applications of the composite constructs are required for complete healing of a wound. In addition, compared to autologous grafts, there is scant evidence of neovascularization with bilayered skin constructs,¹³ which may contribute to the limited viability of these constructs. Thus, methods to improve the viability of bilayered constructs, including improved neovascularization, would also improve their clinical utility.

Our laboratory has previously identified roles for members of the Homeobox (Hox) family of transcription factors that when topically applied to diabetic wounds lead to a marked increase in angiogenesis and accelerated wound healing.^{14,15} In addition to their well-established roles in development and morphogenesis, Hox genes also influence adult tissue remodeling by regulating expression of downstream target genes, including extracellular matrix proteins, matrix-degrading proteinases, and integrin adhesion receptors.^{16–18} Specifically, we have previously shown that *HoxA3* stimulates angiogenesis as well as keratinocyte migration via upregulation of uPAR and MMP-14 and topical application of *HoxA3* accelerates wound healing in diabetic mice.¹⁵

CLINICAL PROBLEMS ADDRESSED

To determine whether the stimulatory effects of *HoxA3* on wound healing could be exploited to also improve the viability of composite skin constructs, we constructed composite grafts, which constitutively express a secreted form of *HoxA3* that could act on both endothelial cells and keratinocytes, and evaluated the viability of these grafts following transplantation into nude mice.

MATERIALS AND METHODS

Cells, culture conditions, transfection of cell lines, and composite grafts

Both HaCAT and NIH-3T3 murine cell lines were obtained from the University of California, San Francisco Cell Culture Facility. Cells were maintained in the Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with 10% fetal bovine serum, 2 mM glutamine, and 0.05 mg/mL gentamicin. Secreted *HoxA3* and *HoxA5* vectors were constructed as previously described^{15,19} by fusing the *HoxA3* or *HoxA5* coding region in frame to the IgG leader sequence using the pSecTag2 cassette. The resulting vectors *pSecTag2B-HOX-A3* and *pSecTag2A-HOX-A5* produced secreted forms of *HoxA3* or *HoxA5* proteins, respectively.

Transfection of NIH-3T3 fibroblasts were performed using the Amaxa Nucleofector System (Lonza) according to the manufacturer's instructions, and pools of stably transfected cells were selected using 50 μ g/mL of Zeocin (Invitrogen) incubated at 37°C for seven days. Polymerase chain reaction (PCR) and quantitative polymerase chain reaction (qPCR) of the transfected 3T3 cells confirmed stable expression of *HoxA3* and upregulation of known downstream targets of *HoxA3*, uPAR, and MMP-14. Transfected fibroblasts were cocultured with unmodified HaCAT cells using modified Boyden chambers. Chambers were coated with 20 μ g/mL type I collagen (Invitrogen) for 2 h at 37°C, and cells were plated and incubated in fibroblast basal media for 24 and 48 h to allow the secreted HOX protein to migrate to the lower chambers.

Composite grafts were fabricated using a modification of an established protocol by Garlick and Taichman.²⁰ Untransfected and transfected 3T3 fibroblasts were embedded into a type I collagen matrix (1 mg/mL; Invitrogen) and allowed to grow for seven days in Boyden chambers. The immortalized human keratinocyte HaCAT cell line was then seeded on top of the fibroblast–collagen matrix and allowed to proliferate in epithelialization media for seven days, and subsequently allowed to stratify for an additional ten days at an air–liquid interface using stratification media. Composite grafts were then harvested for analysis or transplanted *in vivo*. Epithelialization and stratification media were prepared according to the protocol using DMEM as the core media.

Quantitative PCR of HOXA3 and HOXA5 in cells and grafts

Total RNA was isolated from the transfected NIH-3T3 cells and cocultured with nontransfected HaCaT cells after 24 and 48 h of exposure to serum containing the secreted HOXA3 myc/his fusion

protein using the RNeasy Isolation Kit (Qiagen). Quantitative PCR was performed using primers for murine and human (respectively) *MMP-14*, *uPAR*, and *thrombospondin 2* (Applied Biosystems) with undisclosed sequences. *MMP-14*, *uPAR*, *thrombospondin 2*, and control (*GUS*) amplification plots from independent samples of control, *pSecTag2A/2B*, and *pSecTag2B-HOX-A3* and *pSecTag2A-HOX-A5* exposed cells were generated using an ABI Prism 7000 Sequence Detection System and analyzed using ABI 7000 software. *MMP-14*, *uPAR*, and *thrombospondin 2* expression were normalized to *GUS* expression for each case; analysis was performed at least three times for each sample, and the results were averaged. Statistical significance was determined using a two-tailed *t*-test.

Murine skin/graft areas were harvested at the specified time points indicated and flash-frozen on dry ice and stored at -80°C or stored in RNA later (Ambion) at 4°C . Tissue was then briefly rinsed in RNAase-free phosphate buffered saline (Ambion). RNA was isolated by tissue homogenization in Trizol (Invitrogen) with a rotor-starter homogenizer (Omni International). Murine primers of *MMP-14*, *uPAR*, *thrombospondin 2*, *Ccl-2*, and *CxCl-12* and control (*GUS*) of undisclosed sequences (Applied BioSystems) were used to analyze the expression of each gene as described above.

Western blot analysis

Western blots of HaCAT cells cocultured with unmodified 3T3 fibroblasts, 3T3 fibroblasts transfected with a secreted form of the control vector (*pSecTag 2B*) and 3T3 fibroblasts transfected with secreted HoxA3, were probed for the His₆ epitope tag. Total protein was isolated from cell lysates of exposed HaCAT cells and 40 μg were loaded onto a 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis gel, transferred onto an Immobilon-P membrane and blocked in the manufacturer's blocking solution. Blots were incubated with a 1:100 dilution of a monoclonal antibody against a His₆ epitope tag (Novus Biologicals), followed by goat anti-mouse IgG, and detected with an enhanced chemiluminescence Wallac machine.

Immunohistochemistry of composite skin constructs and *in vivo* graft areas

All antibody staining was performed on 10- μm deparaffinized sections of the composite skin constructs and *in vivo* graft areas. Keratin-5 and Keratin-10 (Covance) staining was performed as previously described.^{15,21} CD31 staining was performed using a 1:50 dilution of rat anti-murine CD31 antibody (PharMingen) followed by a 1:200 dilution of biotinylated anti-rat antibody (Pierce).

Animal skin grafting model

All animals used in this study were housed at the University of California, San Francisco animal care facility. The Committee on Animal Research approved all procedures. Male nude Swiss mice were used, and they were between six and eight weeks of age at the time of wounding. All mice were anesthetized with 3% isoflurane in oxygen at 2 L/min. The dorsum of the mouse was sterilized with betadine and a 1.5-cm-diameter open wound was excised, including the panniculus carnosus layer. Composite skin constructs incorporating (1) unmodified 3T3 fibroblasts, (2) 3T3 fibroblasts secreting a control vector, (3) 3T3 fibroblasts secreting HoxA5, and (4) 3T3 fibroblasts secreting HoxA3 were transplanted directly onto the open wounds. Animals received the analgesic/bactrim mixed solution for pain. Graft areas were measured every seven days by planimetry. At least three animals were used in each group. For molecular and immunohistological analyses, skin/graft areas were harvested at the described time points by sacrificing the animal and removing the entire graft area, including a 2-mm region outside the graft edge and the graft itself. Tissue samples were snap-frozen in dry ice and stored at 80°C or RNA later solution until processing for RNA isolation. Tissues were fixed in formalin and embedded in paraffin before sectioning and immunohistochemical analyses.

Statistical analyses

All statistical analyses were performed with the Student's *t*-test. Statistical significance was determined by a *p*-value of <0.05 .

RESULTS

Generation of 3T3 fibroblasts constitutively secreting HoxA3 protein

To allow access of HoxA3 to both endothelial cells and keratinocytes, we fabricated composite grafts using fibroblasts capable of expressing a secreted form of HoxA3. The secreted HoxA3 (sHoxA3) was created by fusing the *HoxA3* coding region to an IgG leader sequence using the *pSecTag2* cassette. In addition, most type I Hox genes, including *HoxA3*, contain an endogenous 16-amino acid sequence within the third homeodomain that directs translocation of the protein across biological membranes and subsequent targeting to the nucleus to carryout transcriptional regulation.²² Fibroblasts were stably transfected with control or sHoxA3 expression plasmids. To confirm that the sHoxA3 produced by transfected fibroblasts was incorporated and transcriptionally active in adjacent cells, we cocultured control

Figure 1. Analysis of nontransfected keratinocytes cocultured with transfected fibroblasts after 48 h. **(A)** Western blot analysis of proteins isolated from nontransfected keratinocytes exposed to the serum of untransfected fibroblasts (3T3-UT), fibroblasts transfected with secreted HOXA3 (3T3-Hox A3), and fibroblasts transfected with an empty vector (3T3-Cntrl) after 48 h. Western blot done with probe against the His₆ epitope tag of secreted HoxA3 protein. **(B)** Quantitative polymerase chain reaction analysis of mRNA isolated from the same keratinocytes using primers against proangiogenic downstream HOXA3 targets, MMP-14 and uPAR. * $p < 0.05$, 3T3-UT used as the reference group. Hox, homeobox; MMP-14, matrix metalloproteinase-14; uPAR, urokinase receptor.

or sHoxA3 transfected fibroblasts with an immortalized human keratinocyte line (HaCAT). Western blot analysis of HaCAT cell lysates harvested after 48 h, cocultured with fibroblasts, confirms that the secreted His₆ epitope tagged HoxA3 protein was taken up by and is transcriptionally active in the HaCAT cells (Fig. 1A). To evaluate the functional response of HaCAT cells exposed to secreted HoxA3, we performed qPCR for HoxA3 target genes by harvesting RNA from HaCAT cells cocultured with control or sHoxA3 expressing fibroblasts. We observed significant increases in expression of the previously established down-

stream targets of *HoxA3*, uPAR, and MMP-14 in keratinocytes cocultured with fibroblasts secreting HoxA3 compared to control fibroblasts (Fig. 1B).

Composite grafts using modified fibroblasts permit development of stratified keratinocyte layers in culture

Composite grafts were subsequently created using a modification of an established protocol²⁰ whereby the immortalized human keratinocyte HaCAT cell line was seeded on top of control or sHoxA3 expressing 3T3 fibroblasts embedded into a type I collagen matrix (Fig. 2A). As an additional

Figure 2. Composite grafts in culture. **(A)** Schematic showing the fabrication of composite grafts. Transfected fibroblasts are embedded in a layer of collagen I to form a representative dermal layer. Next, untransfected keratinocytes are plated and allowed to grow at an air–liquid interface to form a stratified epidermal layer. The Hox protein secreted by the transfected fibroblasts can affect both the overlying keratinocytes, as well as host endothelial cells adjacent to transplanted grafts. **(B)** hematoxylin & eosin staining of composite grafts fabricated with HaCAT cells and fibroblasts transfected with an empty plasmid (3T3-Cntrl), sHoxA3 expression plasmid (3T3-Hox A3), or sHoxA5 (3T3-Hox A5) seventeen days after plating of keratinocytes. sHoxA3, secreted HoxA3. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

control, we also used 3T3 fibroblasts transfected with a secreted form of another *Hox* gene, the anti-angiogenic *HoxA5*.¹⁹ Within ten to fourteen days, stratified keratinocyte layers began to form in all groups and mature, stratified epidermal layers were evident by seventeen days in cultures of each group (Fig. 2B).

Stratified, differentiated epidermal layers are maintained in transplanted composite grafts *in vivo*, but graft viability is prolonged with sHoxA3

To evaluate the viability of the composite grafts *in vivo*, we subsequently transplanted mature composite grafts (seventeen days postkeratinocyte seeding) onto the backs of nude mice and assessed graft parameters for an additional seven to fourteen days (Fig. 3A).

Graft tissue was subsequently harvested and hematoxylin & eosin staining revealed that keratinocytes from all grafts maintained the stratified differentiated morphology up to fourteen days postimplantation (Fig. 3B). Immunohistochemistry (IHC) staining for basal and superficial keratinocyte markers keratin 5 (K5) and keratin 10 (K10) revealed that keratinocytes maintained both basally expressed K5- as well as K10-positive superficial layers in each group (Fig. 3B). Keratinocytes in each group also expressed similar levels and localization of involucrin, another marker of mature, differentiated keratinocytes (not shown).

To evaluate the viability of the transplanted composite grafts, we measured the mean graft areas of transplanted composite grafts at zero, seven, and fourteen days post-transplantation. Graft areas with control or sHoxA5 producing grafts were

Figure 3. Immunohistochemistry of composite grafts transplanted *in vivo* at fourteen days. **(A)** Photomicrograph showing the appearance of sHoxA3 composite grafts at day zero (a) and day fourteen (b) post-transplantation. (c) Hematoxylin and eosin staining of a complete harvested graft on day fourteen, arrow indicates the border between native skin (*left*) and graft (*right*). **(B)** Upper panel shows hematoxylin and eosin staining of graft areas fourteen days post-transplantation. Middle panel shows immunohistochemistry for expression of keratin-5, and lower panel shows staining for keratin-10 in epidermal layers of grafts from control sHoxA3 or sHoxA5 producing grafts. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

reduced by over 70% within seven days and continued to decline through fourteen days (Fig. 4). In contrast, the areas covered by sHoxA3 producing grafts were reduced by <15% after seven days and significantly larger grafts were maintained through fourteen days compared to control or sHoxA5 producing grafts (Fig. 4). Thus, the inclusion of sHoxA3 in composite grafts not only enhances graft viability, but also maintains normal keratinocyte differentiation and epidermal stratification to enhance graft integrity.

Composite grafts with secreted HoxA3 maintain characteristic changes in target gene expression *in vivo*

To confirm that the improved viability of sHoxA3 grafts was linked to changes in target gene expression *in vivo*, one-half of the graft area was harvested and RNA was isolated for quantitative PCR analysis. We observed a significant increase in both uPAR and MMP-14 mRNA levels at seven days post-transplantation in composite grafts secreting HoxA3, compared to control groups (Fig. 5A, B) and increased uPAR and MMP-14 expression persisted through at least fourteen days post-transplantation. In contrast, whereas grafts secreting the anti-angiogenic HoxA5 showed a corresponding upregulation of its known target gene thrombospondin-2 (TSP-2), no change in TSP-2 expression was noted in sHoxA3 or control grafts (Fig. 5C). Together these results confirm that alterations in gene expression in composite grafts *in vivo* were specific for the *Hox* gene used and were maintained in grafts for up to fourteen days following transplant.

Figure 4. sHoxA3 maintains graft integrity. Graft integrity as measured by graft areas in control, sHoxA3 or sHoxA5 expressing grafts at seven and fourteen days after transplantation. * $p < 0.05$, 3T3-UT used as reference group. $N = 3$ for each group.

sHoxA3 composite grafts reduce expression of inflammatory chemokines CCL-2 and CxCL-12

Our previous data also showed that *HoxA3* can attenuate the excessive inflammatory responses linked to poor wound healing.²³ To evaluate whether composite grafts with sHoxA3 may similarly modulate the inflammatory response when transplanted *in vivo*, we performed qPCR on the harvested graft areas seven days and fourteen days post-transplantation to evaluate changes in the mRNA expression of inflammatory markers CCL-2 and CxCL-12. We found that the graft areas exposed to secreted HoxA3 exhibit decreased expression of CCL-2 and CxCL-12 at seven days and fourteen days post-transplantation (Fig. 5D), suggesting that sHoxA3 may modulate inflammatory responses following transplantation of the grafts *in vivo*.

sHoxA3 composite grafts exhibit increased angiogenesis *in vivo*

To evaluate whether the improved viability of grafts producing sHoxA3 was linked to increased angiogenesis following graft transplantation, we assessed the capillary density on grafts harvested at seven days and fourteen days post-transplantation. Vascular density was assessed by IHC for CD31, a marker for endothelial cells, and revealed that sHoxA3 composite grafts displayed increased expression of CD31 and a concomitant increase in the number of small capillaries (Fig. 6A, B) compared to grafts lacking sHoxA3.

DISCUSSION

We have previously shown that topical application of the transcription factor *HOXA3*, which is lacking in poorly healing diabetic wounds, promotes both epithelial cell migration and angiogenesis and accelerates wound repair.¹⁵ In the current study, we now show that the ability of *HoxA3* to act positively on both keratinocytes and endothelial cells can be exploited to improve the viability and functionality of composite skin grafts.

Bioengineered composite tissue grafts have proved to be useful tools in the treatment of chronic wounds. Their use, however, has been limited by incomplete engraftment and lack of direct stimulation of angiogenesis.¹³ Previous attempts to enhance the therapeutic potential of composite skin constructs have focused on increasing the expression of the various growth factors to act on either keratinocytes or to directly enhance vascularization.^{24–26} Whereas grafts delivering platelet derived growth factor and keratinocyte growth factor

Figure 5. Hox-induced changes in target gene mRNA are maintained following transplantation of composite grafts *in vivo*. Real-time polymerase chain reaction (PCR) analysis of MMP-14 (A) and uPAR (B) and TSP-2 (C) mRNA levels in sHoxA3 or sHoxA5 grafts seven and fourteen days after transplantation (relative to control grafts). (D) Real-time PCR analysis of CCL-2 and CXCL-12 mRNA levels in sHoxA3 or sHoxA5 grafts harvested fourteen days after transplantation (relative to control grafts). For each panel, relative mRNA expression levels are expressed as percentages of the mRNA expression in the control groups, with the control group expression = 1. * $p < 0.05$ using control as the reference group. $N = 3$ for each group.

are moderately successful at promoting keratinocyte proliferation and migration, they do not directly stimulate angiogenesis.^{24,26} Recent studies that have genetically modified bilayered constructs to express increased levels of vascular endothelial

growth factor have been shown to increase angiogenesis, but do not stimulate epidermal proliferation or migration.²⁵

Others have noted that direct coseeding of fibroblasts with keratinocytes also improves the

Figure 6. Increased vascular density in transplanted grafts with sHoxA3. (A) Immunohistochemical staining for the endothelial marker CD31 in day fourteen composite grafts. (B) Quantitative analysis of CD31-positive cells per field of view (FOV). * $p < 0.05$ using control as the reference group. $N = 3$ for each group. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

viability and performance of composite skin substitutes compared to keratinocytes alone.²⁷ The addition of fibroblasts not only increases the epidermal thickness, but also resulted in increased angiogenesis. Importantly, the improved performance of these keratinocyte/fibroblast grafts was accompanied by reduced contraction and greater maintenance of their original size. Others have noted that inclusion of mesenchymal stem cells also reduces contraction of split-thickness autografts, although the precise mechanism by which this improved graft integrity was not defined.²⁸

In the current study, we have shown that inclusion of fibroblasts engineered to secrete HoxA3 to act on both the adjacent keratinocytes and underlying endothelial cells, significantly improves graft viability and reduces contraction for up to twenty-one days *in vivo*. We observed a robust increase in angiogenesis directly linked to improved graft viability and reduced contraction. Others have noted that keratinocyte-derived fibronectin or alterations in collagen crosslinking also factor in graft contractions²⁹; however, we did not investigate whether exposure to HoxA3 also impacted these variables. Similarly, HoxA3 may also act on fibroblasts and our unpublished observations indicate that fibroblasts secreting HoxA3 exhibit higher levels of uPAR, which may also contribute to the improvement in angiogenesis and graft viability. The full impact of HoxA3 on fibroblast function, however, was not explored in the current study.

The improved viability of grafts is also selective for HoxA3. As a control, we also constructed grafts that secreted HoxA5, which we previously showed to be a potent anti-angiogenic factor via increased expression of TSP-2.^{19,30} As expected, these grafts showed increased TSP-2 expression, reduced angiogenic responses, and no change in graft contraction or integrity was observed compared to controls.

In addition to increasing angiogenesis, we have also shown that composite skin constructs with secreted HoxA3 or HoxA5 exhibit decreased expression of inflammatory mediators CCL-2 and CxCL-12, which play key roles in attracting monocytes, macrophages, and other immune cells to wounds. Although the current studies were performed in nude mice with reduced immune responses, our previous studies in immune competent db/db mice showed that topical application of HoxA3 to poorly healing wounds reduced recruitment of CD45⁺ leukocytes to levels close to those observed during normal healing in wild-type animals.²³ Thus, the reduced recruitment of leukocytes by HoxA3, would be expected to further

KEY FINDINGS

- sHoxA3 composite grafts maintain characteristic target gene expression *in vivo*.
- sHoxA3 composite grafts reduce expression of inflammatory chemokines CCL-2 and CxCL-12.
- sHoxA3 composite grafts increased angiogenesis *in vivo*.
- sHoxA3 composite grafts exhibit improved graft viability.

contribute to prolonged integrity and viability of composite skin constructs secreting HoxA3. Together, the combined actions of HoxA3 on endothelial cells and keratinocytes lead to an increase in angiogenesis, normal epidermal differentiation, reduced expression of inflammatory mediators, and reduced graft contraction and suggest that HoxA3 may have therapeutic benefits and may improve the integrity of composite skin grafts.

INNOVATION

Although engineered bilayered composite grafts have become a useful therapeutic adjunct in the treatment of chronic wounds, their use is limited by lack of angiogenesis and true engraftment.

This study shows that the inclusion of fibroblasts engineered to secrete HoxA3 in composite tissue grafts, which act on both adjacent keratinocytes and underlying endothelial cells, promotes normal epithelial differentiation, increased angiogenesis, and reduces inflammatory mediators and results in less graft contraction. Thus, the inclusion of secreted HoxA3 in the construction of these composite grafts may further improve their therapeutic potential.

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