review-article

MILITARY WOUND CARE

Plastic Surgery Challenges in War Wounded

Anand R. Kumar,[1,2,*] Raymond Harshbarger,[1,3] and Barry Martin[1,3]

[1]Department of Surgery (Plastic Surgery), Uniformed Services University of the Health Sciences (USUHS), Bethesda, Maryland.
[2]Department of Plastic and Reconstructive Surgery, National Naval Medical Center (NNMC), Bethesda, Maryland.
[3]Department of Plastic and Reconstructive Surgery, Walter Reed Army Medical Center (WRAMC), Washington, District of Columbia.

Submitted for publication May 30, 2009.
*Correspondence: Department of Plastic and Reconstructive Surgery, National Naval Medical Center, 8901 Wisconsin Ave., Bethesda, Maryland 20889-5000 (telephone: 301-295-0217; FAX: 301-295-0226; e-mail: arkumar@mac.com).

Background: Military treatment facilities continue to treat injured personnel supporting the Global War on Terrorism. Optimal reconstruction of massive soft tissue and bone defects of the extremities and craniofacial skeleton secondary to high-energy wounding mechanisms remain poorly characterized.
The Problem: The ideal method to care for these injuries has continued to evolve and has yet to be completely defined. Effects of high-energy blast trauma on tissues, and the unusual bacteria (*Acinetobacter baumanii*) existing within the wounds, characterize these unique military wounds. Despite the relatively quick triage time, definitive care is delayed, and multiple concomitant injuries exist per patient.
Basic/Clinical Science Advances: In addition to practicing established concepts from prior military conflicts, new technology for the advancement of trauma care has been applied. Treatment with novel flap reconstructions, distraction osteogenesis, bone grafting, and bone-engineering techniques including use of bone morphogenetic protein-2 has led to improved outcomes.
Clinical Care Relevance: Modern battlefield care in conjunction with treatment protocols at continental United States Military Treatment Facilities has resulted in improved limb and craniofacial reconstruction.
Conclusion: Successful limb salvage and craniofacial reconstruction can be accomplished in the subacute period using treatment protocols, which incorporate the use of novel flaps, fixation devices, and bone-engineering techniques.

AU1 ▶

BACKGROUND

MILITARY TREATMENT FACILITIES continue to treat injured personnel supporting the Global War on Terrorism. Optimal reconstruction of massive soft tissue and bone defects of the extremities and craniofacial skeleton secondary to high-energy wounding mechanisms remain poorly described. The optimal care of these injuries has yet to be defined. Wounds from modern high-energy blast trauma mechanisms are unique. Blast trauma wounds are characterized by severe composite tissue damage, large zones of injury, extensive devitalization, significant foreign body loading, and nearly ubiquitous bacterial colonization with *Acinetobacter Baumanii* (a significant pathogen from Iraq). Despite advances in triage, and access to critical care far forward in the battlefield, definitive care can be delayed. Once stateside, delivery of reconstructive care is often complicated by the multiple concomitant injuries that exist per patient, and prolonged ICU stays. Major civilian principles of early radical debridement, fixation of fractures with bone grafting, and wound coverage with vascularized tissue are followed

Printed in U.S.A.
DOI: 10.1089/awc.2009.0087

when possible. Given the complexity and multiplicity of these blast wounds, along with extensive concomitant injuries, early closure of wounds and fracture fixation can be difficult to achieve. In many cases the subacute (3 weeks after injury) period has been the most appropriate time for definitive wound treatment. The application of traditional wound and fracture treatment techniques along with introduction of novel approaches has met with early success in these difficult military wounds.

CLINICAL PROBLEM ADDRESSED

Evidence in the civilian trauma literature indicates that early wound coverage provides better outcomes and that subacute reconstruction is plagued with high complication rates.[6–12] Conversely, war extremity wounds have been reconstructed in the subacute time period.[13] This variation in the treatment algorithm is necessary due to temporal evacuation factors, large zones of injury, complex desert microflora in battlefield wounds, and multiple concurrent injuries.[1–5]

BASIC SCIENCE CONTEXT

Injured soldiers are treated using evolving protocols developed over the course of the Iraq/Afghanistan conflicts. Extremity-injured patients are initially treated with serial debridement until the wounds appear clinically clean with viable tissue. Between operative wound evaluations, negative pressure dressing therapy was applied to the wounded extremity. Uni-planar external fixation for extremity fractures is placed far forward in the theatre of operation and modified as necessary at the Level V (definitive stateside) military treatment facility.[25] Wounds are then accessed for primary closure, skin grafting, and/or flap reconstruction as indicated. At the time of flap inset, bone defects are filled with OSTEOSET® (Wright Medical Technology, Arlington, TN), antibiotic beads, and INFUSE® Bone Graft (Medtronic, Minneapolis, MN) as indicated in extremity reconstruction. After extremity flap reconstruction, patients are converted within 2 weeks to multiplanar weight-bearing external frames (Taylor Spatial Frame).

Cranial defects are reconstructed in a delayed fashion when necessary, allowing for cerebral edema to resolve. Facial fractures are treated with internal fixation and supplemental bone grafting as indicated and as early as possible. Subsequently, all patients underwent early rehabilitation regaining ambulatory status with functional ability expected for their injuries and preexisting conditions. Intensive traumatic brain injury rehabilitation remains a critical adjunct to the patients' overall physical and mental restoration.

EXPERIMENTAL MODEL OR MATERIAL

Craniofacial reconstruction

Blast trauma with high-energy transfer to the cranial vault has necessitated decompressive craniectomy, a life-saving maneuver performed far forward in the theater of war. Patients once considered expectant have now been aggressively treated and have survived devastating neurologic trauma. Patients surviving a decompressive craniectomy after military blast trauma represent a diverse and unique population that has been poorly studied[16] (Fig. 1). This group of patients ultimately requires reconstruction of the cranial defect not only for brain protection and cosmetic restoration of the calvarium but also for correction of the syndrome of the trephined (dizziness, fatigability, vague discomfort at the site of the defect, mental depression, apprehension and insecurity, and intolerance to vibration). Currently, there are gaps in the literature detailing the incidence, natural history, and appropriate treatment of this unique population of patients.[16–19]

Operation Iraqi/Enduring Freedom has resulted in the largest concentration of penetrating head trauma since the Vietnam War. Due to severe impairment of tissue perfusion and heavy wound contamination at the time of initial injury, reconstruction is often delayed for 6 to 12 months. In the presence of soft-tissue deficiencies, either acute or chronic, calvarial reconstruction is delayed. Soft-tissue reconstruction using tissue expansion or free

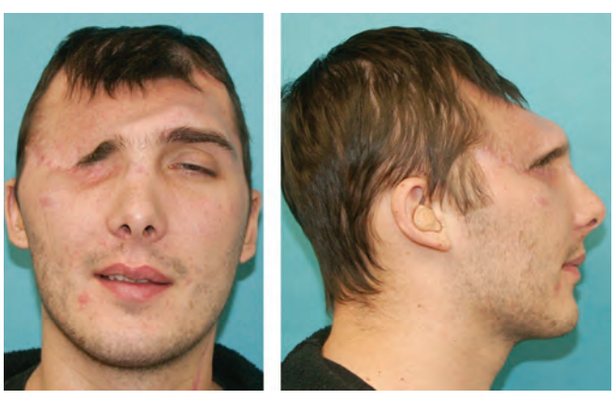

Figure 1. Soldier 5 months post-IED blast injury with loss of right frontal, temporal, and supra orbital bone.

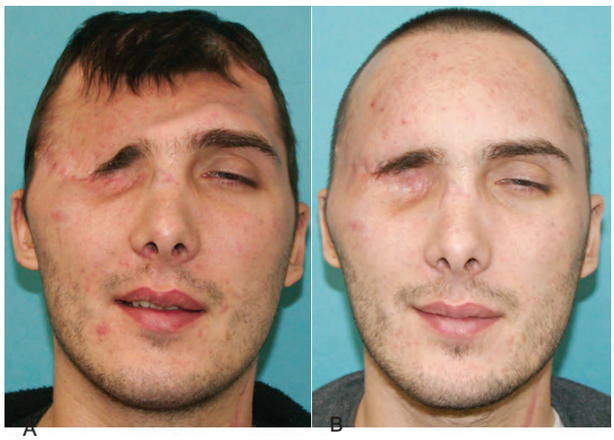

Figure 2. Postoperatively, brain protection and more natural contours have been restored. (**A**) Preoperative anterior view. (**B**) Postoperative anterior view.

tissue transfer has been the main treatment used to augment deficient tissue in the region of the planned cranioplasty. The cranial defect can be safely addressed once adequate soft tissue is available. Hemi-craniectomy defects represent the majority of defects. Due to their large size, the desire to avoid further intracranial surgery on the contralateral hemisphere and concerns with banked cranial bone, implant-based reconstruction has been the first choice of replacement.[18–21] Banked craniectomy bone autograft was utilized for reconstruction during the early phase of the Iraq War. This practice was largely abandoned due to evidence of bone resorbtion and concerns with bacterial colonization/infection. With the advent of rapid prototyping technologies, patient-specific, computed tomography–guided, three-dimensional prefabricated poly methyl methacrylate and Ti Mesh (woven titanium mesh) reconstructive implants are the most common choices (Fig. 2). Although implants have been used to reconstruct the upper orbits (bandeau region), our preference has been cranial or iliac crest bone grafts and cranialization of the frontal sinus as indicated.[18] This strategy separates the alloplastic cranial implants from the facial skeleton and adjacent frontal/ethmoid sinus, lessening potential infectious complications.

Facial fractures in isolation or more commonly in conjunction with major cranial injury are repaired when clinically appropriate. Classic timeframes to reduce and fixate facial fractures are within 7 days of injury. This allows for reconstitution of the skeletal framework before soft-tissue contracture. Military blast trauma craniofacial injury patients typically have severe prolonged swelling (due to mechanism and transport times 3–7 days to definitive care) and multiple concurrent injuries including severe closed head injury with vasospasm. Fixation within 1 week of injury is rarely accomplished. Most of these severely injured soldiers have undergone facial fracture fixation and grafting between 2 and 4 weeks after injury. Combined craniofacial approaches used in civilian practice are often not possible due to severe brain edema.[19,20] Internal fixation with bone grafts is used as indicated. In the critically injured patient, bone grafting may be delayed, and distraction osteogenesis or free tissue transfer is used when indicated.

Torso/extremity reconstruction

For these unique extremity and torso injuries no standard protocol exists. The major principle of early radical debridement and wound coverage with vascularized tissue is followed (Figs. 3 and 4). Modern civilian wound reconstruction principles may be used; however, the timing and type of flap reconstruction for these lower extremity battlefield injuries are generally based on evolving experience and surgeon judgment. Evidence in the civilian literature indicates that early wound coverage provides better outcomes and that subacute

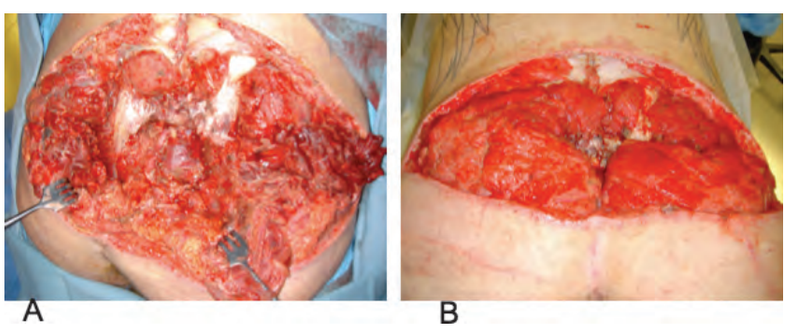

Figure 3. Marine after high-energy torso wound. Massive sacral wound with exposed bone. (**A**) Initial injury with exposed sacral bone. (**B**) Sacral wound after bilateral gluteus maximus advancement flaps.

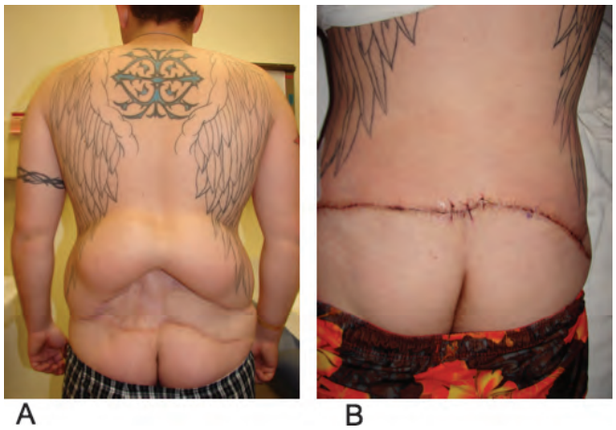

Figure 4. (**A**) Secondary reconstruction with lumbar tissue expanders in place. (**B**) Expanders removed and flaps advanced for complete coverage.

reconstruction is plagued with high complication rates.[6–12] Due to multiple factors including transport times, war extremity wounds are routinely reconstructed in the subacute time period.[13]

TAKE-HOME MESSAGE

Basic sciences

- Bone morphogenetic protein-2 is a potent osteoinductive agent when placed in bone defects at the time of wound reconstruction.
- Hydroxyapatite bone cement with antibiotic impregnation is a useful osteoconductive agent when placed in bone defects at the time of wound reconstruction.

Clinical sciences

- Multiplanar weight-bearing frames used in conjunction with lower extremity reconstruction allow early weight bearing without endosteal blood supply associated with intramedullary nails.
- Transport distraction osteogenesis is a useful technique for bone regeneration even in the setting of adjacent soft-tissue injury.

Clinical care

- Multidisciplinary care incorporating novel bone regeneration techniques along with wound reconstruction with vascularized tissue (flaps) has increased limb and craniofacial salvage.
- Delay in reconstruction has not been associated with increased complications.
- Microsurgical free flap reconstruction has been uniformly successful in craniofacial and extremity wounds using longer pedicled (*e.g.,* anterior lateral thigh flap vs. lateral arm flap) flaps with anastomosis in the relative lesser zone of injury.
- Operating out of the zone of injury is nearly impossible due to the wide blast trauma zone of injury.
- Soft tissue and bone reconstruction can be safely performed in the subacute period when the comorbid patient conditions are controlled.

DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

Subtle variations in care are necessary with the uniquely large zones of injury, complex desert microflora in battlefield extremity wounds, and multiple concurrent injuries.[1–5,22] Unique in military medicine are highly motivated patients who reap benefits from surgeon's constant exposure to complex battle injuries (Fig. 1). In addition to practicing established concepts from prior military conflicts, new technology for the advancement of trauma care has been applied. Treatment with novel flap reconstructions, bone grafting, and bone-engineering techniques including use of bone morphogenetic protein-2 has lead to improved outcomes.[23] Also, early involvement of a wide array of professionals and intense rehabilitation facilities is a critical component of comprehensive care.

We have demonstrated that this delay in reconstruction does not unfavorably affect outcomes.[23] If surgeons adhere to the established principles of creating a clean wound bed and using healthy vascularized coverage, infection and flap failure can be minimized. Compared to the body of orthopedic and plastic surgical literature, our experience demonstrated low flap failure rates and reasonably low infection rates without the need for amputation.[3–5,8] This represents a significant improvement in the management of devastating open lower extremity fractures in the subacute period, also corroborated by the experience at Balboa Naval Hospital.[24]

CAUTION, CRITICAL REMARKS, AND RECOMMENDATIONS

- Soft tissue and bone reconstruction can be safely performed in the subacute period only when the comorbid patient conditions are controlled.
- Wound management must include prompt and serial debridements along with negative pressure dressing therapy during the interval to definitive war wound reconstruction.

FUTURE DEVELOPMENTS

Use of soft tissue and bone regenerate templates in the future may obviate the

need for donor site flap and associated morbidity. Further integration of sophisticated biocompatible synthetic constructs with tissue engineering will improve both form and function in the reconstruction of composite tissue defects. More sophisticated distraction devices will allow for greater soft tissue and bone regeneration.

ACKNOWLEDGMENTS

Wound healing research in the author's institution is not financed by external sources.

AUTHOR DISCLOSURE STATEMENT

No conflict of interest disclosed.

REFERENCES

1. Bermudez LE: Abstract: Microsurgery in war wounds. Presented at the 69th Scientific Meeting of the American Society of Plastic Surgeons (ASPS). Los Angeles, CA: October 14–18, 2000.
2. Stalekar H, Fuckar Z, Ekl D, *et al.*: Primary vs. secondary wound reconstruction in Gustilo type III open tibial shaft fractures: follow up study of 35 cases. Croat Med J 2003; **44:** 746.
3. Byrd HS, Cierny G, III, and Tebbetts JB: The management of open tibial fractures with associated soft tissue loss: external pin fixation with early flap coverage. Plast Reconstr Surg 1981; **68:** 73.
4. Godina M: Early microsurgical reconstruction of complex trauma of the extremities. Plast Reconstr Surg 1986; **78:** 285.
5. Heller L and Levin LS: Lower extremity microsurgical reconstruction. Plast Reconstr Surg 2001; **108:** 1029.
6. Francel TJ, Vander Kolk CA, Hoopes JE, *et al.*: Microvascular soft-tissue transplantation for reconstruction of acute open tibial fractures: timing of coverage and long-term functional results. Plast Reconstr Surg 1992; **89:** 478.
7. Hertel R, Lambert SM, Muller S, *et al.*: On the timing of soft-tissue reconstruction for open fractures of the lower leg. Arch Orthop Trauma Surg 1999; **119:** 7.
8. Khouri RK and Shaw WW: Reconstruction of the lower extremity with microvascular free flaps: a 10-year experience with 304 consecutive cases. J Trauma 1989; **29:** 1086.
9. Hallock GG: Utility of both muscle and fascia flaps in severe lower extremity trauma. J Trauma 2000; **48:** 913.
10. Hallock GG: Lower extremity muscle perforator flaps for lower extremity reconstruction. Plast Reconstr Surg 2004; **114:** 1123.
11. Yildirim S, Gideroglu K, and Akoz T: Anterolateral thigh flap: ideal free flap choice for lower extremity soft-tissue reconstruction. J Reconstr Microsurg 2003; **19:** 225.
12. Yazar S, Lin CH, Lin YT, *et al.*: Outcome comparison between free muscle and free fasciocutaneous flaps for reconstruction of distal third and ankle traumatic open tibial fractures. Plast Reconstr Surg 2006; **117:** 2468.
13. Kumar A: Standard wound coverage techniques for extremity war injury. J Am Acad Orthop Surg 2006; **14(10 Suppl):** S62.
14. Gustilo RB, Mendoza RM, and Williams DN: Problems in the management of type III open fractures. A new classification of type III open fractures. J Trauma 1984; **24:** 742.
15. Caudle RJ and Stern PJ: Severe open fractures of the tibia. J Bone Joint Surg 1987; **69A:** 801.
16. Lee C, Antonyshyn OM, and Forrest CR: Cranioplasty: indications, technique, and early results of autogenous split skull cranial vault reconstruction. J Craniomaxillofac Surg 1995; **23:** 133.
17. Rish BL, Dillon JD, Meirowsky AM, *et al.*: Cranioplasty: a review of 1030 cases of penetrating head injury. Neurosurgery 1979; **4:** 381.
18. Manson PN, Crawley WA, and Hoopes JE: Frontal cranioplasty: risk factors and choice of cranial vault reconstructive material. Plast Reconstr Surg 1986; **77:** 888.
19. Isago T, Nozaki M, Kikuchi Y, Honda T, and Nakazawa H: Sinking skin flap syndrome: a case of improved cerebral blood flow after cranioplasty. Ann Plast Surg 2004; **53:** 288.
20. Schiffer J, Gur R, Nisim U, and Pollak L: Symptomatic patients after craniectomy. Surg Neurol 1997; **47:** 231.
21. Segal DH, Oppenheim JS, and Murovic JA: Neurological recovery after cranioplasty. Neurosurgery 1994; **34:** 729.
22. Pollack AN and Ficke JR: Exremity war Injuries III, session moderators. J Am Acad Orthop Surg 2008; **16:** 628.
23. Kumar AR, Navanjun S, Grewal M, Chung TL, and Bradley JP: Lessons from Operation Iraqi Freedom: successful subacute reconstruction of complex lower extremity battle injuries. Plast Reconstr Surg 2009; **23:**. ◀AU2
24. Geiger S, McCormick F, Chou R, and Wandel AG: War wounds: lessons learned from Operation Iraqi Freedom. Plast Reconstr Surg 2008; **122:** 146.
25. Bagg MR, Covey DC, and Powell ET: Levels of medical care in the global war on terrorism. J Am Acad Orthop Surg 2006; **14(10 Spec No.):** S7.

AUTHOR QUERY FOR 539-41633-2009-0087_1P

AU1: Please note that references 14 and 15 are not cited in the text, and references are not cited in numerical order. Please cite these references and renumber the citations for numerical order.
AU2: In Ref. 23, please mention the inclusive page range.
AU3: Please define IED.
AU4: Figure citations in the text have been edited to match the revised figure legends. Please check.

review-article

GENE AND PROTEIN BASED THERAPIES

AU1 ▶

Smart Growth Factor Gene Delivery for Impaired Wound Healing

María José Escámez,[1] Marta Carretero,[2] Marta García,[1] Lucía Martínez-Santamaría,[1] Fernando Larcher,[2] and Marcela Del Río[1,*]

[1]Regenerative Medicine Unit and [2]Cutaneous Diseases Modeling Unit, Epithelial Biomedicine Division, Basic Research Department, CIEMAT-CIBERER U714, Madrid, Spain.

Background: Acute and chronic wounds of diverse origins remain a chief clinical problem. Tissue engineering and gene therapy come into sight as novel therapeutic tools.
The Problem: Testing novel therapeutic strategies requires reliable wound-healing models and gene delivery systems.
Basic/Clinical Science Advances: Our recent studies address such troublesome matters. Our contribution comprises the development of a bioengineered human skin substitute suitable for clinical applications including acute and chronic wound management, a preclinical skin-humanized mouse model based on permanent human skin regeneration on immunodeficient animals upon grafting of the bioengineered skin equivalent. The humanized model appears to be a useful tool to perform acute healing studies and to design novel gene and protein delivery methods and the characterization of healing-promoting activities of some antimicrobial peptides.
Clinical Care Relevance: Benefits of clinical relevance could be achieved using smart bioengineered products combining cell and gene therapy aiming to enhance tissue repair and control infection concomitantly.
Conclusion: These avenues merge into the development of effective wound-healing management approaches.

Abbreviations and Acronyms

AMP = antimicrobial peptide
KGF = keratinocyte growth factor
VEGF = Vascular endothelial growth factor

Submitted for publication June 9, 2009.
*Correspondence: Regenerative Medicine Unit, Epithelial Biomedicine Division, Basic Research Department, CIEMAT-CIBERER U714, Avda. Complutense, 22, 28040 Madrid, Spain (telephone: +34-91-3466051; FAX: +34-91-3466484; e-mail: marcela.delrio@ciemat.es)

BACKGROUND

NONHEALING ACUTE AND CHRONIC SKIN WOUNDS are a diverse group of lesions of different etiology and manifestation. The most common cause of acute wounds is thermal injury. Chronic wounds include arterial, diabetic, pressure, and venous ulcers. Within this category, we may also include persistent wounds associated to inherited mechano-bullous skin disorders such as Epidermolysis Bullosa. Management of these wounds remains a major clinical challenge involving novel therapeutic strategies such as cell and gene therapy.[1,2] Several more or less complex tissue-engineered skin products aimed at wound-healing enhancement[2] are currently on the market. However, most of these products have limited applications. Our group has developed a tissue-engineered human skin equivalent[3] suitable for a broad spectrum of clinical applications, including permanent skin engraftment and regeneration and chronic wound-healing aid.[4–7] The development of smart bioengineered products acting concomitantly as tissue substitute and a source of soluble factors to improve the healing of wounds and prevent infection has been put forward by various laboratories, ncluding ours.[8–11] Testing these therapeutic strategies requires reliable wound-healing models. Recently, our group has devoted a major effort to

Printed in U.S.A.
DOI: 10.1089/awc.2009.0044

establishing both a preclinical humanized wound-healing system and optimized methods for transient delivery of genes encoding wound healing–promoting factors.[12,13] Robust validation of these approaches has been achieved with keratinocyte growth factor (KGF) and the antimicrobial peptide (AMP) LL-37.

CLINICAL PROBLEM ADDRESSED

Burn injuries and chronic wounds are associated with diminished quality of life, frequent hospitalizations, and increased morbidity and mortality and still represent a major public health problem. The appropriate management of wounds is a standing clinical challenge.[2,14] Although significant progress has been made with state-of-the-art treatments, additional improvements could prove relevant in terms of efficacy. The lack of an appropriately vascularized wound bed and infection are clearly detrimental to the tissue repair process. Several strategies have been proposed by different groups, including ours, to solve these problems.[15–17] A comprehensive understanding of the mechanisms involved in normal and impaired wound healing could lead to smart combinations of tissue engineering with genetic modification of the cells to overproduce key therapeutic factors for satisfactory wound handle and care. Thus, novel approaches combining cell and gene therapy, coupled to the availability of clinically reliable wound-healing models, could be pivotal to the advancement of this field.[2,14,15,17,18]

RELEVANT BASIC SCIENCE CONTEXT

In vivo wound-healing studies in human skin are limited by ethical and practical issues. Current knowledge mainly stems from the use of murine models, including knockout and transgenic strategies. These studies have provided evidence of the relevance of targeted genes and the modulatory effects of many growth factors *in vivo*.[16,19,20] However, based on the significant differences existing between human and murine skin architecture, the question remains as to how far the results can be extrapolated to the human scenario. Studies in large animals such as pigs, whose skin architecture and dynamics resemble that of humans, are an alternative, but troublesome and expensive.[21] Human skin organotypic cultures represent a valid alternative to native skin *in vivo* studies, particularly those endowed with long-term culture life span.[22,23] However, they are restricted, among other constraints, by faulty pivotal mesenchymal responses such as angiogenesis. To circumvent these problems, researchers have often used xenogenic transplantation of donor skin biopsies to immunodeficient mice to perform relevant *in vivo* experimentation in a human context.[23–25] However, in addition to difficulties in sourcing, a major concern for this type of experiments is the big heterogeneity of the grafting material. As a result, regenerating human skin on immunodeficient mice by grafting a bioengineered skin substitute appears to be a balanced compromise between practicality and model fidelity. This methodology enables the generation of numerous mice engrafted with a significant area of single donor–derived human skin, known as skin-humanized mouse model.[7,12,25] This model is suitable as a platform to perform wound-healing studies and to test gene therapy approaches in a humanized context.

EXPERIMENTAL MODEL OR MATERIAL

The contribution of our group to the tissue regeneration field focused on three pivotal points.

Development of a skin equivalent for clinical purposes

The skin equivalent (Fig. 1) is based on a tridimensional fibrin-rich dermal scaffold containing live fibroblasts that supports, mechanically and metabolically, the establishment and maintenance of the epidermal component.[3] The dermal composition of this skin equivalent presents unique characteristics compared to other dermal equivalents, allowing for high proliferation rates of keratinocytes. This skin equivalent (World Patent WO/2002/072800) is being successfully used in clinics in its autologous version for permanent skin regeneration[3,5,6] and, lately, in its allogenic version for treating chronic wounds.[4] In addition, a new chimerical version of this substitute (a skin equivalent composed of autologous patient-derived keratinocytes and allogenic healthy donor–derived fibroblasts) has been awarded the Orphan Drug designation by the EMEA for the treatment of chronic wounds in patients affected with the rare inherited skin disorder Epidermolysis Bullosa (orphan designation number EU/306/369), and it is currently being tested in a phase I–II clinical trial in Spain (http://es.cellerix.com/index.php/Productos/Cx501).

Improvement of graft performance by promoting vascularization and fighting infections

We have proposed a combination of tissue engineering and gene therapy approaches for the treatment of chronic and acute wounds. Vascular endothelial growth factor (VEGF) promotes

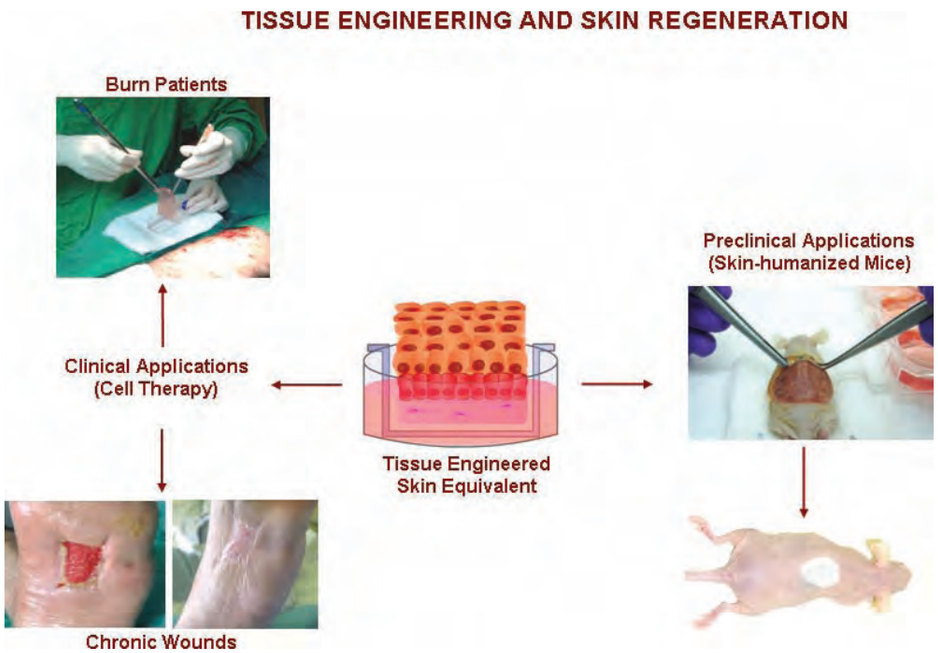

Figure 1. The tissue bioengineered skin equivalent is based on a fibrin matrix containing live fibroblasts as a dermal component and keratinocytes as the epidermal component. This bioengineered human skin has been successfully applied as a cell therapy approach aiming either permanent or temporary cover to treat burn patients and chronic nonhealing wounds, respectively. A skin-humanized mouse model based on the stable engraftment of this setting has been used as a platform for evaluating pharmacological, cell, and gene therapy strategies for wound healing.

cutaneous angiogenesis by inducing potent proliferation and migration of endothelial cells.[16,26] Using *in vivo* models, different laboratories have explored the effect of VEGF as a part of an angiogenic bioengineered material that might improve wound bed conditioning and engraftment.[8,11,27]

Infection is the other major setback for regeneration after skin grafting (either split-thickness or tissue-engineered autografts). The increasing appearance of multidrug-resistant microbes points to the need of using alternative therapies to conventional antibiotics. AMPs appear as good candidates as they present a broad spectrum of activity against microorganisms and, usually, a low induced-resistance due to their mechanism of action.[28] In addition, some AMPs have been suggested to elicit not only bacteria-killing activity but also healing-promoting effects.[29,30]

We have demonstrated the efficacy of adenoviral-mediated gene transfer by overexpressing human β-defensins HBD-2 and −3 and cathelicidin LL-37 in a skin equivalent setting.[9] Moreover, using an *in vivo* approach based on LL-37 adenoviral-mediated delivery to diabetic wounds in ob/ob mice, we have provided early evidence for the potential benefits of using AMP gene therapy in a healing impaired situation.[10] In this model, *in vivo* LL-37 gene transfer increased the re-epithelialization rate and granulation tissue formation. These results evidence a realistic therapeutic option that might contribute to an efficient tissue repair as well as infection control at the wound site[10,31] (Fig. 2). ◀F2

Development of a skin-humanized model to test gene-mediated factor delivery approaches

A reliable preclinical model to validate the efficacy of these and others factors for the treatment of wounds is mandatory. We have generated a skin-humanized mouse model based on the straightforward grafting of a bioengineered human skin equivalent onto the back of immunodeficient mice.[6,25] Once permanent human skin regeneration has been achieved (epidermal maturation and dermal matrix remodeling is accomplished within 9–10 weeks after grafting), the humanized mice are ready to be used in healing experiments. This model also offers the possibility of using genetically modified human keratinocytes and/or fibroblasts. After transplantation, a hybrid system is generated with a skin of human origin that is vascularized and innervated by mouse vessels and nerves. Probably, the main advantage of our system is that full-thickness wounds are performed in a context of a mature, quiescent, homogeneous human skin avoiding the need for volunteers and major differences in tissue architecture and kinetics found in mouse skin. Under these experimental conditions, we have provided *in vivo* evidence that either the native or a green fluorescent protein geneti-

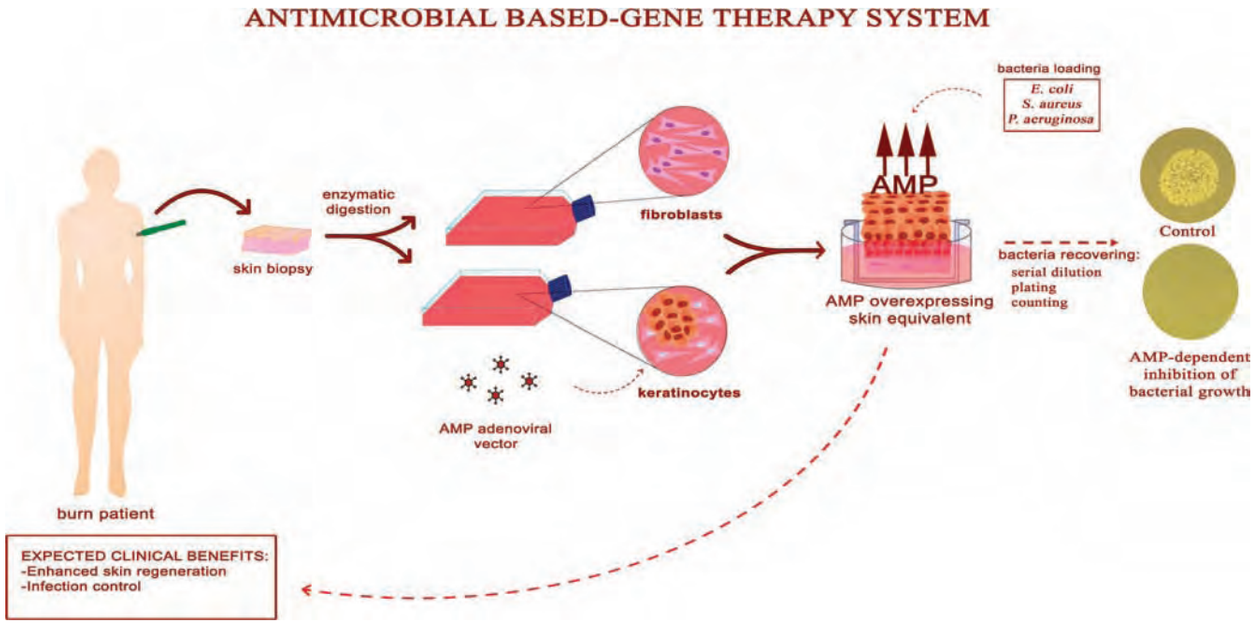

Figure 2. *In vitro* studies demonstrated efficient antimicrobial activity of adenoviral antimicrobial peptide (AMP) gene-transduced keratinocytes as a part of a skin equivalent by inhibiting the growth of different bacterial strains, usually associated to burn wounds. Combined cutaneous tissue engineering in conjunction with antimicrobial gene therapy emerges as a potential therapeutic approach for wound coverage and concomitantly combating infection in burn patients.

cally modified skin-humanized mouse model recapitulates the main features of human wound healing.[12] Further, our wound-healing model allowed us to compare the efficacy of different transient gene transfer strategies aimed at delivering growth factors such as KGF to promote skin repair[13,32] (Fig. 3).

DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

The skin equivalent developed by our team allows for generation of large bioengineered skin surfaces, restoration of both the epidermal and dermal skin compartments, and functional epidermal stem-cell preservation. Further, it has been successfully used, in its autologous version, for permanent skin regeneration in different situations: extensive burns, necrotizing fascitis, removal of giant nevi, and graft-versus-host disease.[3,5,6] In its allogenic version it has also been used as a healing aid to treat chronic wounds.[4]

Growth factors that play a critical role in normal wound healing and in addition are often deficient or inactive in chronic wounds have been highlighted as attractive therapeutic agents.[16–18] Among these, factors with a well-known angiogenic potential are main candidates to improve wound bed preparation.[2,14,33] We and others have thus explored the effect of VEGF overexpression by *ex vivo* gene transfer to keratinocytes.[8,27] VEGF delivered by gene-targeted endothelial cells embedded in a fibrin matrix was also successfully used in the ischemic distal pedicle flap rabbit model.[11]

A critical condition for an efficient engraftment is also the preservation of a sterile wound bed environment. To avoid infection, AMPs have been suggested as an alternative to the use of conventional antibiotics. Besides their role in innate host defense, other activities have been described for some of these AMPs that might positively influence the wound-healing response. In fact, the human cathelicidin LL-37 has been shown to induce an angiogenic response, to enhance adaptive immune responses by recruiting different immune cells, to induce the expression of syndecan in fibroblasts, to induce the production of cytokines and chemokines by keratinocytes, and to enhance the re-epithelialization process.[28] We have provided further evidence for the LL-37–mediated signaling pathways in keratinocytes that are involved in the migratory activity of these cells.[10] We demonstrated that overexpression of this peptide induced

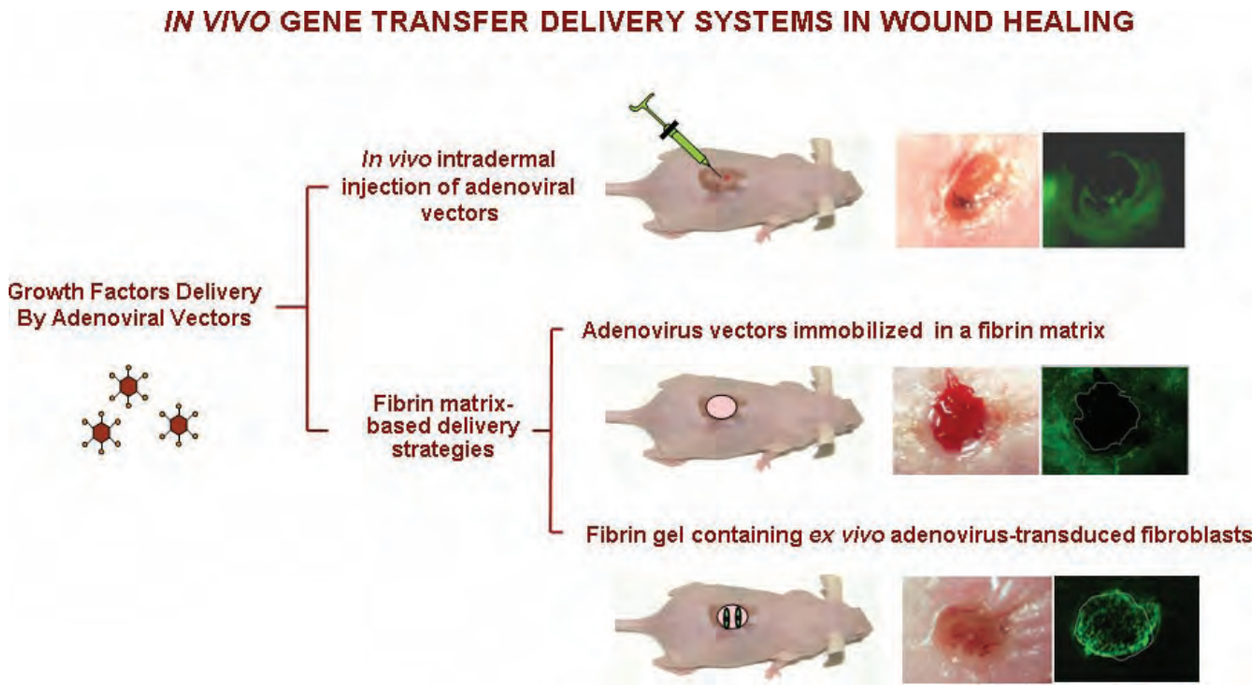

Figure 3. Different *in vivo* growth factor delivery approaches mediated by adenoviral vectors aiming to improve wound healing have been explored using the skin-humanized mouse model. Transient delivery of keratinocyte growth factor to wounds was achieved through direct intradermal adenoviral injection or through fibrin matrix strategies, containing either immobilized adenoviral vector or embedded adenoviral gene-transferred human fibroblasts.

phenotypic changes related to actin dynamics and was associated to augmented tyrosine phosphorylation of proteins involved in focal adhesion complexes. We have also demonstrated that events involved in the epithelial–mesenchymal transition are observed in the presence of the LL-37, such as induction of the Snail transcription factor, presence of discontinuous cell–cell contacts (adherens junctions and desmosomes), activation of matrix metalloproteinases, and activation of the mitogen-activated protein kinase and phosphoinositide 3-kinase/Akt signaling pathways. These signaling events could be mediated not only through the transactivation of epidermal growth factor receptor but also through the induction of G-protein-coupled receptor formyl peptide receptor like-1 expression in these cells. Using an *in vivo* approach based on LL-37 adenoviral-mediated delivery to diabetic wounds in ob/ob mice, we have provided evidence for the potential benefits of using AMP gene delivery for a healing-impaired situation. LL-37 increased the re-epithelialization rate and granulation tissue formation when administered around wound margins. In summary, we showed that gene delivery of factors such as VEGF and AMPs may have a positive impact on wound repair by promoting vascularization and/or controlling infection.

Another important contribution of our group is the development of a reliable *in vivo* tool that allows for preclinical studies to evaluate the benefits of delivering potential therapeutic factors to the wound site.[12,13] The validation of this wound-healing skin-humanized model has been performed by careful examination of a wide spectrum of parameters at different times post-wounding, including migration, proliferation, stratification, and differentiation, dermal remodeling, and dermo–epidermal junction reconstitution. In conclusion, the model mimics the different phases of the human wound-healing process. The biological validity of the model was further confirmed by probing its ability to respond to a known wound healing–promoting factor, such as KGF added exogenously as a recombinant protein.

Using the skin-humanized mouse system, we have compared the efficacy of different *in vivo* approaches involving transient delivery of KGF to wounds by using adenoviral vectors. In the first approach, hKGF was delivered to wounds by intradermal injection of an adenoviral suspension. Although wound acceleration was achieved, the

effect of hKGF was unreliable both in terms of the number of successfully targeted animals (versus the total number of treated animals) and re-epithelialization efficiency. In the second approach, KGF-encoding adenoviral vectors were immobilized in a fibrin gel carrier and applied immediately after wounding. In this case, the proportion of successfully targeted animals was higher than that achieved with the adenoviral injection method, and wound closure rose significantly. A third strategy was explored consisting in delivering hKGF protein from *ex vivo* adenoviral-transduced fibroblasts that were, in turn, embedded in a fibrin matrix and used to treat the wound. In contrast to the two previous methods based on direct adenovirus delivery, this cell-mediated system did not depend on *in vivo* cell transduction. This method depends on the direct transfer of exogenous KGF therapeutic protein from gene-targeted fibroblasts that was, in fact, achieved in all treated wounds, leading to a significant improvement in wound closure. Although all delivery systems achieved KGF protein overproduction at the wound site, with a concomitant re-epithelialization enhancement, only the use of genetically modified fibroblast-containing matrix as an *in situ* protein bioreactor was highly reproducible. This method appears the most reliable means to deliver growth factors to wounds avoiding the potential danger of scoring cases of faulty administration as therapeutic failures and direct exposure to viral vectors. The tissue engineering–based skin-humanized mouse model system emerges as a unique platform for evaluating pharmacological, cell, and gene therapy strategies for wound healing.

CAUTION, CRITICAL REMARKS, AND RECOMMENDATIONS

While cell-based therapy approaches have been successfully used for skin repair, particularly in severe skin losses and most recently for chronic wounds, the combination with genetic modification of cellular components needs to be carefully addressed in appropriate models. In spite of its complexity, normal skin wound healing is an efficient process that is difficult to accelerate or enhance through the activity of exogenous growth factors. Thus, healing-impaired models may better reveal the actual repair-promoting activities of healing-acting molecules. To better mimic a chronic skin repair–deficient condition, it seems worthy to develop new *in vivo* models of impaired wound healing. In fact, we are currently conducting experiments to establish models of impaired wound healing such as diabetic pigs and skin-humanized mice.[34]

FUTURE DEVELOPMENT

A large body of evidence now demonstrates that many currently available cell-based wound therapies are effective in achieving or accelerating cutaneous wound repair and/or regeneration. However, potential technologic improvements may further enhance the applications of this approach. For example, more effective cell preservation techniques could enhance shelf life and minimize problems related to storage and shipping. Issues related to the dermal matrix, important in providing a scaffold for mesenchymal cell homing and allowing better tissue regeneration, have not been properly addressed. Thus, poor durability due to degradation or, conversely, difficulties in matrix remodeling may limit effectiveness of certain cell-based wound therapies and cellular skin substitutes. As mentioned, fibroblast-populated fibrin matrices support keratinocyte growth and are easily remodeled into collagenous (human) dermal

TAKE-HOME MESSAGE

Basic science advances

- Testing cell–gene therapy approaches require valid surrogate markers in the context of appropriate wound-healing preclinical models. The skin-humanized mouse model represents one of such experimental platforms, and its ability to recapitulate physiologic acute human excisional wounds has been extremely useful to design new growth factor delivery strategies.

Clinical science advances

- With relatively few variations, our bioengineered skin equivalent has been shown to be capable of engraftment in cases of large skin acute losses (using autologous epidermal stem cells) or greatly enhancing the patient's own healing in chronic wounds. However, some defiant features such as infection or impaired vascularization compromise the therapeutic efficacy of skin substitutes. A further turn into the enhanced performance of skin substitutes may be the use of genetically modified keratinocytes and/or fibroblasts to provide a favorable niche for regeneration. Pleiotropic factors such as cathelicidin LL-37 appear in sight as potential candidates to perform such a role.

Relevance to clinical care

- Clinical management of skin wounds, from life-threatening acute (e.g., severe burns) to highly prevalent ones such as chronic ulcers, represents a major challenge. Smart bioengineered products should provide both the cellular and soluble factors enabling wound closure for each situation.

tissue *in vivo*, allowing their use for both allogenic and autologous grafting purposes. Due to costs, commercially available live allogenic bilayered products that are a few square centimeters in area are only suitable for treating small wound areas.

Among those working in the field of skin replacement there is a feeling that current use of bioengineered skin products is underdeveloped. Challenges remain regarding the abilities of skin bioengineering to improve cosmetic/esthetic situations such as burn sequels.

A comprehensive understanding of the mechanisms involved in normal and impaired healing could lead to smart combinations with genetic modification of the cells to overproduce various growth factors and cytokines. As many other young therapeutic modalities, skin bioengineering and cutaneous gene therapy will certainly meet further improvements as basic and applied science continue to nourish the field.

ACKNOWLEDGMENTS

This work was supported by Grants SAF2007-61019 from Ministerio de Ciencia e Innovación (Spain), PSE-090100-2006-3 from Ministerio de Educación y Ciencia (Spain), and LSHB-CT-512102 from European Framework Program (UE).

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

REFERENCES

1. Eming SA, Smola H, and Krieg T: Treatment of chronic wounds: state of the art and future concepts. Cells Tissues Organs 2002; **172:** 105.
2. Auger FA, Lacroix D, and Germain L: Skin substitutes and wound healing. Skin Pharmacol Physiol 2009; **22:** 94.
3. Meana A, Iglesias J, Del Rio M, *et al.*: Large surface of cultured human epithelium obtained on a dermal matrix based on live fibroblast-containing fibrin gels. Burns 1998; **24:** 621.
4. Camblor-Santervás L, Meana-Infiesta A, Llaneza-Coto JM, *et al.*: Treatment of chronic vascular ulcers with skin equivalent obtained by tissue engineering. Angiologia 2003; **55:** 21.
5. Llames S, Garcia E, Garcia V, *et al.*: Clinical results of an autologous engineered skin. Cell Tissue Bank 2006; **7:** 47.
6. Llames SG, Del Rio M, Larcher F, *et al.*: Human plasma as a dermal scaffold for the generation of a completely autologous bioengineered skin. Transplantation 2004; **77:** 350.
7. Garcia M, Escamez MJ, Carretero M, *et al.*: Modeling normal and pathological processes through skin tissue engineering. Mol Carcinog 2007; **46:** 741.
8. DelRio MD, Larcher F, Meana A, Segovia J, Alvarez A, and Jorcano J: Nonviral transfer of genes to pig primary keratinocytes. Induction of angiogenesis by composite grafts of modified keratinocytes overexpressing VEGF driven by a keratin promoter. Gene Ther 1999; **6:** 1734.
9. Carretero M, Del Rio M, Garcia M, *et al.*: A cutaneous gene therapy approach to treat infection through keratinocyte-targeted overexpression of antimicrobial peptides. FASEB J 2004; **18:** 1931.
10. Carretero M, Escamez MJ, Garcia M, *et al.*: *In vitro* and *in vivo* wound healing-promoting activities of human cathelicidin LL-37. J Invest Dermatol 2008; **128:** 223.
11. Lasso JM, Del Rio M, Garcia M, *et al.*: Improving flap survival by transplantation of a VEGF-secreting endothelised scaffold during distal pedicle flap creation. J Plast Reconstr Aesthet Surg 2007; **60:** 279.
12. Escamez MJ, Garcia M, Larcher F, *et al.*: An *in vivo* model of wound healing in genetically modified skin-humanized mice. J Invest Dermatol 2004; **123:** 1182.
13. Escamez MJ, Carretero M, Garcia M, *et al.*: Assessment of optimal virus-mediated growth factor gene delivery for human cutaneous wound healing enhancement. J Invest Dermatol 2008; **128:** 1565.
14. Macri L and Clark RA: Tissue engineering for cutaneous wounds: selecting the proper time and space for growth factors, cells and the extracellular matrix. Skin Pharmacol Physiol 2009; **22:** 83.
15. Carretero M, Escamez MJ, Prada F, *et al.*: Skin gene therapy for acquired and inherited disorders. Histol Histopathol 2006; **21:** 1233.
16. Werner S and Grose R: Regulation of wound healing by growth factors and cytokines. Physiol Rev 2003; **83:** 835.
17. Davidson JM, Whitsitt JS, Pennington B, Ballas CB, Eming S, and Benn SI: Gene therapy of wounds with growth factors. Curr Top Pathol 1999; **93:** 111.
18. Eming SA, Krieg T, and Davidson JM: Gene therapy and wound healing. Clin Dermatol 2007; **25:** 79.
19. Scheid A, Meuli M, Gassmann M, and Wenger RH: Genetically modified mouse models in studies on cutaneous wound healing. Exp Physiol 2000; **85:** 687.
20. Davidson J: Experimental Animal Wounds Models. Wounds 2001; **13:** 9.
21. Sullivan TP, Eaglstein WH, Davis SC, and Mertz P: The pig as a model for human wound healing. Wound Repair Regen 2001; **9:** 66.
22. Garlick JA and Taichman LB: Fate of human keratinocytes during reepithelialization in an organotypic culture model. Lab Invest 1994; **70:** 916.
23. Demarchez M, Sengel P, and Prunieras M: Wound healing of human skin transplanted onto the nude mouse. I. An immunohistological study of the reepithelialization process. Dev Biol 1986; **113:** 90.
24. Demarchez M, Hartmann DJ, Herbage D, Ville G, and Prunieras M: Wound healing of human skin transplanted onto the nude mouse. II. An immunohistological and ultrastructural study of the epidermal basement membrane zone reconstruction and connective tissue reorganization. Dev Biol 1987; **121:** 119.
25. Del Rio M, Larcher F, Serrano F, *et al.*: A preclinical model for the analysis of genetically modified human skin *in vivo*. Hum Gene Ther 2002; **13:** 959.
26. Larcher F, Murillas R, Bolontrade M, Conti CJ, and Jorcano JL: VEGF/VPF overexpression in skin of transgenic mice induces angiogenesis, vascular hyperpermeability and accelerated tumor development. Oncogene 1998; **17:** 303.
27. Supp DM, Supp AP, Bell SM, and Boyce ST: Enhanced vascularization of cultured skin substitutes genetically modified to overexpress vascular endothelial growth factor. J Invest Dermatol 2000; **114:** 5.
28. Braff MH and Gallo RL: Antimicrobial peptides: an essential component of the skin defensive barrier. Curr Top Microbiol Immunol 2006; **306:** 91.

29. Niyonsaba F, Ushio H, Nakano N, *et al.*: Antimicrobial peptides human beta-defensins stimulate epidermal keratinocyte migration, proliferation and production of proinflammatory cytokines and chemokines. J Invest Dermatol 2007; **127:** 594.

30. Otte JM, Werner I, Brand S, *et al.*: Human beta defensin 2 promotes intestinal wound healing *in vitro.* J Cell Biochem 2008; **104:** 2286.

31. Thomas-Virnig CL, Centanni JM, Johnston CE, *et al.*: Inhibition of multidrug-resistant *Acinetobacter baumannii* by nonviral expression of hCAP-18 in a bioengineered human skin tissue. Mol Ther 2009; **17:** 562.

32. Davidson JM: First-class delivery: getting growth factors to their destination. J Invest Dermatol 2008; **128:** 1360.

33. Eming SA, Brachvogel B, Odorisio T, and Koch M: Regulation of angiogenesis: wound healing as a model. Prog Histochem Cytochem 2007; **42:** 115.

34. Martinez-Santamaria L, Escamez MJ, Carretero M, *et al.*: Analysis of NGF effects in a humanized model of impaired wound healing. 21st World Congress of Dermatology, Buenos Aires, 2007.

AUTHOR QUERY FOR 539-41633-2009-0044_1P

AU1: Please italicize all gene symbols used in the article.
AU2: Please define LL-37 and hKGF.
AU3: Please expand EMEA and HBD.