Curriculum Vitae

Howard Myles Kimmel, DPM,MBA
14401 Snow Road, Suite 102
Brook Park, Ohio 44142
(216) 267-0304
Email: buckeyefootcare@sbcglobal.net

Education

2013-2014	Cardiff University, Cardiff, Wales: Took classes towards a Masters of Science in Wound Healing and Tissue Repair
1997-2000	Masters of Business Administration, Health Care Administration Cleveland State University, Cleveland Ohio
1986-1990	Doctor of Podiatric Medicine Ohio College of Podiatric Medicine, Cleveland Ohio
1983-1986	Bachelor of Arts, Major: Chemistry University of South Florida, Tampa Florida

Internship /Residency/Fellowship

2016	Cleveland Clinic Micro-Surgery Course Training in micro surgical procedures, under the direction Of Maria Siemionow MD,Phd
2010	Istituto Clinico Humanitas, Milan, Italy Advanced training in Illizarov Techniques, under the direction of Alexander Kirienko, MD
2009	Illizarov Institute, Kurgan, Russia Advanced training in Complex Foot and Ankle Deformities under the direction Sergey I. Shved MD and V.I. Shevtsov MD
1990-1992	Podiatric Surgical Residency (PSR24) Hawthorne Hospital/Baja Project for Crippled Children Los Angeles, California Chief Resident of the Baja Project for Crippled Children: Was responsible for the training of junior and senior residents from 7 residency programs from Southern California

Altruism

1994-Present	Free Clinic of Cleveland: Volunteer medical services on a monthly basis
1994-2000	Bellfaire Jewish Big Brothers/Big Sisters: Board Member and participated having 2 little brothers over the 6 years; Did weekly events with the little brothers
2010-2011	MedWish International Mission Trip to El Salvador and Nicarauga
2011	University Circle Judson Smart Living Award for Volunteerism

Certifications

1999- Present	Board Certified, American Board of Podiatric Surgery, Recertified 2008
1992- 1999	Board Qualified, American Board of Podiatric Surgery

Licensure

1994- Present	State of Ohio
1992-1994	State of North Carolina
1991-1993	State of California

Hospital Affiliations

Cleveland Clinic Foundation Hospitals: Euclid/Marymount/South Pointe/Fairview/Lutheran

University Hospitals: Cleveland Medical Center/Parma Hospital/St. Johns Westshore Hospital/Southwest General Hospital

Lake Hospital Systems

Academic Positions and Appointments

2008- Present	Senior Clinical Instructor, Department of Surgery Case Western Reserve University School of Medicine Cleveland, Ohio
2006- May 2016	Residency Director, PSR-36 Program at the Louis Stokes Department of Veterans Affairs Hospital, Cleveland, Ohio Responsible for the education and training of 9 surgical residents
2001- Present	Core Clinical Faculty, Ohio College of Osteopathic Medicine, Athens Ohio Third and Fourth year medical students rotate within my practice as an elective in wound care and diabetic lower extremity problems
1999- Present	Faculty Member St. Vincent's Charity Residency Program, Cleveland Ohio Responsible for training first, second, and third year surgical residents in a variety of settings including and outpatient clinic and operating room; give instruction in hospital protocol. Responsible for facilitating journal club for the residents
1994- Present	Chief of Podiatric Medicine and Surgery Free Clinic of Cleveland, Cleveland Ohio Responsible for running the podiatric clinic and training medical students, podiatric medical students from the AAWP organization, and physician assistants
1994-Present	Adjunct Clinical Professor of Surgery Kent State College of Podiatric Medicine, Cleveland Ohio From 1994 to 1997, Students received semi-weekly instruction in operating room protocol including hand ties, suturing, sterile technique and casting/bandaging

Organizations

Member, American Podiatric Medical Association
Fellow, American College of Foot and Ankle Surgeons

Work Experience

9/2007-Present	Case Western Reserve University School of Medicine, IQ Group Facilitator
11/2006- May 2016	Department of Veterans Affairs, Cleveland, Ohio: Residency Director and Senior Surgical Podiatrist; Responsible for training 9 surgical residents
9/2003- 2006	St. Vincent Charity Wound Care Center, Cleveland Ohio Actively see and treat patients with a variety of acute and chronic wounds in a clinical setting. Interact closely with vascular surgery and internal medicine. Perform a variety of limb salvage procedures. Educate the podiatric surgical residents in both conservative and surgical treatment of wounds.
3/2000-8/2000	Next MED Systems/New Health Management, Cleveland Ohio Internship requirement for MBA in Health Care Administration (Part-Time); was a physician liaison between the firm and hospitals to help develop components of e-healthcare including electronic medical records, practice management software and electronic patient consulting; helped develop expertise in creating efficiency in department budgets
1994-Present	Private Practice, Buckeye Foot Care, Cleveland Ohio Practice all aspect of podiatric medicine and surgery; including an emphasis on wound care, diabetic lower extremity disease, and reconstructive foot and ankle surgery
1992-1993	Private Practice, Shelby, North Carolina

CME Presentations

October 2016	Heal Ohio, Akron Ohio: Lecture entitled "Treating complex Wounds in a Veteran Population"
November 2015	Desert Foot Meeting, Phoenix, Arizona; Lecture entitled "The Use of Decellularized Dermis in Lower Extremity Wounds"
April 2014	SAWC Meeting, Orlando, Florida; Lecture entitled "Offloading the Diabetic Foot"
April 2013	Midwest Podiatry Conference, Chicago IL Lecture entitled "The Use of Evidence Based Algorithms for the Treatment of Diabetic Foot Ulcers and Venous Leg Ulcers"
September 2012	SAWC Meeting, Baltimore, MD moderating and lecturing on the topic "Wound Care in the VA Setting"
August 2012	APMA National Meeting, Washington DC Presenting an oral abstract entitled, "The Use of the Amnion/Chorion in Wounds and Surgery"
January 2011	Florida Podiatric Medical Association, SAM Conference, Florida Presented lectures entitled" The Use of Locking Plates in Complex Reconstructive Surgery and The Use of Porcine Dermis in Reconstructive Surgery"
November 2010	Desert Foot Conference 2010, Scottsdale, Arizona Presented a lecture entitled "Diabetic Foot Ulcer Management: Current Concepts"
October 2010	Oral abstract presented at the 3rd World Congress of External Fixation, Barcelona, Spain "Combined Use of the Monobody Dynamic External Fixator and Internal Fixation for Ankle"
June 2010	New Cardiovascular Horizons, New Orleans, Louisiana Presented a lecture entitled " Surgical Correction of Diabetic Foot Ulcers"
January 2010	Florida Podiatric Medical Association, SAM Conference, Florida Presented a lecture entitled " Hallux Rigidus and Limitus Solutions: Resurfacing, Total Joint Arthroplasty and Plating"
November 2009	Michigan Podiatric Medical Society, Troy, MI Presented a lecture entitled "1st MPJ Implants"

October 2009	Suffolk County Podiatric Medical Society Seminar, Islandia, NY Presented a lecture entitled "1st MPJ Implants"
September 2009	Podiatry Institute Seminar, Atlantic City, NJ : presented a lecture entitled "1st MPJ Resurfacing"
September 2009	Chicago Lower Extremity Symposium, Chicago, IL : presented a lecture entitled "Advances in Wound Care"
July 2009	APMA National Meeting Toronto, Canada: presented an abstract Entitled "The Use Mesenchymal Stem Cells in Foot and Ankle Surgery" and poster abstracts entitled "Bone Stimulators- It's not just bone" and "Comparison of Human Fibroblast vs. Extra-Cellular Matrix"
June 2009	OPMSA Seminar, Columbus, Ohio; Lectured on Amputations of The lower extremity
January 2009	Presented a lecture entitled, "The Use and Abuse of External Fixation", at the 17th Annual OPMSA/AAWP Podiatric Clinical Symposium, Cleveland, Ohio
January 2009	Presented Abstracts entitled, "Use of Pulsed Radio Frequency Energy in the Treatment of Dehisced Complex Wounds", and "The effectiveness of the PACT Program on Ulcers and Limb Amputations" at The 1st International Workshops on Wound Technology, Paris, France
November 2008	Faculty Member of the 5th Annual Multi-Disciplinary Management of the High Risk Diabetic Foot Conference; Lectured on Surgical Amputations
October 2008	Presented a lecture entitled, "The Uses of External Fixation in Foot and Ankle Surgery", at the Society of Chiropodists and Podiatrists, Bournemouth, England
September 2008	Faculty Member of the New Cardio Vascular Horizons Seminar, New Orleans, LA; Lectured on Surgical Amputations
September 2008	Presented an Abstract entititled, "Surgical Correction of the Charcot Ankle" at the National Institutes of Health Seminar, Bethesda, Maryland

March 2008	Presented an Abstract entitled "The Use of Pulsed Electromagnetic Fields in Wound Care" at the American Professional Wound Care Association, Ft. Worth, Texas
February 1997	Presented a paper entitled, "Comparative Analysis of Soft Tissue Anchors", at the 55th annual meeting of the American College of Foot and Ankle Surgeons
1994- 1999	Class Lectures to the third year students at the Ohio College of
1999	Podiatric Medicine
September 1995	Lectured at the OCPM Fall Seminar
July, 1994	Lectured at the Carnegie Surgery Center Hallux Valgus Seminar, lectured entitled "The Akin Osteotomy"
October 1993	presented four papers at the First World Congress of Podiatry in Brighton, England. The papers were entitled, "Treatment of In Toe Gait", "Surgical Correction of the Adult Clubfoot", "Case Presentation of Deletion of Chromosome 6p", and "The Use of Bone Stimulators in Fracture Management"
February 1993	presented two papers entitled "Surgical Correction of Congenital Pes Plano Valgus" and "The Use of Bone Stimulators in the initial treatment of Fractures", at the 51st annual meeting of the American College of Foot and Ankle Surgeons in San Diego, CA.
February, 1992	presented a paper entitled, "Takedown of the Rigid Neglected Clubfoot, at the 50th annual meeting of the American College of Foot and Ankle Surgeons in Orlando, FL (As a resident)

Papers and Publications

Kimmel, Howard and Gittleman,Haley , Retrospective observational analysis of the use of an architecturally unique dermal regeneration template (Derma Pure®) for the treatment of hard-to-heal wounds. Int Wound Journal, Early Access Online (2016)

Kimmel, Howard M., Ditata J, and Grant,T " The Presence of Oxygen in Wound Healing" WOUNDS- A COMPENDIUM OF CLINICAL RESEARCH AND PRACTIVE September 2016

Kimmel, Howard M., The Use of a Decellularized Dermal Allograft in the Treatment of Chronic Venous Leg Ulcers, Abstract SAWC Conference, Atlanta, GA 2016

Kimmel, Howard M., The Use of a Decellularized Dermal Allograft in the Treatment of Chronic Venous Leg Ulcers, Abstract SAWC Conference, Atlanta, GA 2016

Kimmel, Howard M., Comparison of Amniotic Membrane* and Umbilical Cord# Tissues for Treating Chronic, Non-healing Wounds, Abstract SAWC Conference, Orlando, FL 2014

Kimmel, Howard M., and Angela L. Robin. "An evidence-based algorithm for treating venous leg ulcers utilizing the Cochrane Database of Systematic Reviews." *WOUNDS-A COMPENDIUM OF CLINICAL RESEARCH AND PRACTICE* 25.9 (2013): 242-250.

Podiatry Today May 2013, Issue 5 "Point-Counterpoint: Are Acellular Dermal Matrices More Effective Than Fibroblast-Derived Dermal Substitutes?" Wrote the point that acellular dermal matrices are more effective

Kimmel, Howard M, DPM, MBA 1 and Jennifer Regler, DPM, MS 2 An Evidence Based Approach to Treating Diabetic Foot Ulcerations in a Veteran Population **;** The Journal of Diabetic Foot Complications, 2011; Volume 3, Issue 3, No. 2, Pages 50-54 © All rights reserved.

Kimmel, Howard, Michael Rahn, and Thomas W. Gilbert. "The clinical effectiveness in wound healing with extracellular matrix derived from porcine urinary bladder matrix: a case series on severe chronic wounds." *The Journal of the American College of Certified Wound Specialists* 2.3 (2010): 55-59.

Kimmel, Howard M. "The Use of Human Derived Fibroblast Dermal Substitute in Diabetic Ulcers with Significant Peripheral Vascular Disease." *DIABETES*. Vol. 59. 1701 N BEAUREGARD ST, ALEXANDRIA, VA 22311-1717 USA: AMER DIABETES ASSOC, 2010.

Kimmel, Howard M., and Sara Borkosky. "The Molecular Effects of Wound Healing Using Pulsed Radio Frequency Energy." *DIABETES*. Vol. 59. 1701 N BEAUREGARD ST, ALEXANDRIA, VA 22311-1717 USA: AMER DIABETES ASSOC, 2010.

KIMMEL, HOWARD MYLES, MELANIE JOHNSON, and C. Valentine. "The use of Cascade platelet rich plasma membrane in healing chronic diabetic ulcers." *J Wound Technol* 10 (2010): 10.

Kimmel, Howard and Johnson,Melanie "Pulsed radio frequency energy and angiogenesis in chronic wounds." *J Wound Technol* 6 (2009): 6-10.

Abstracts at the Diabetic Foot Global Conference, Los Angeles, California, March 2010, "The Surgical Use of Extra Strength Porcine Urinary Bladder Matrix in Treating Full Thickness Diabetic Wounds**",** **"**The Surgical Use of Extra Strength Porcine Urinary Bladder Matrix in Treating Full Thickness Diabetic Wounds**",** and "The Use of Porcine Urinary Bladder Matrix Powder in the Treatment of Diabetic Wound Dehiscence's**"**

Abstract at the 2nd Annual International Workshop of Wound Technology Seminar, Paris France, January 2010, "The Use of Dermagraft for the Treatment of Diabetic Foot Ulcers"

Journal of Wound Technology, October 2009, Number 6, Volume 1, "Angiogenesis and Pulsed Radio Frequency Energy"

Podiatry Management August 2009 roundtable discussion of Wound Care

Podiatry Today July 2009 "Key Insights of Surgical Offloading of Diabetic Foot Ulcers"

Abstract at the NIH Charcot Workshop September 2008, Bethesda, MD entitled "Surgical Correction of the Charcot Ankle"

Podiatry Management August 2008 roundtable discussion on Wound Care

Abstracts at the 3rd Congress of the World Union of Wound Healing Societies, Toronto Canada June 2008; "The Effectiveness of the PACT Program in Reduction in Limb Amputation" and "The Use of Pulsed Electro Magnetic Fields in the Treatment of Lower Extremity Wounds"

Podiatry Today December 2007,
"Current Insights on Custom and Pre-Fabricated Foot Orthoses"

Clinics in Podiatric Medicine and Surgery, April 1996 13:2,
"A Comparison of End to End Versus "V" Arthrodesis Procedures for the Correction Digital Deformities"

Submitted a chapter on Endoscopic Plantar Fasciotomy, for Levy and Hetherington's, Principles and Practice of Podiatric Medicine

Hospital Affiliations

UH Case Medical Center, Cleveland, Ohio

Southwest Hospital, Middleburgh Heights, Ohio

UH Parma Hospital, Parma, Ohio

Cleveland Clinic Foundation: Euclid/Fairview/Lutheran/South Pointe Hospital, Cleveland, Ohio

Lake Hospital, Willoughby, Ohio

International Wound Journal

International Wound Journal ISSN 1742-4801

ORIGINAL ARTICLE

Retrospective observational analysis of the use of an architecturally unique dermal regeneration template (Derma Pure®) for the treatment of hard-to-heal wounds

Kimmel Howard[1] & Gittleman Haley[2]

1 Department of Surgery, Buckeye Foot Care, Brook Park, OH USA
2 Case Comprehensive Cancer Center, Cleveland, OH USA

Key words
CD31; DermaPure; Observational analysis; PROK2

Correspondence to
Howard Kimmel
Department of Surgery
Buckeye Foot Care
14401 Snow Road
Suite 102
Brook Park
OH 44142
USA
E-mail: buckeyefootcare@sbcglobal.net

Howard K, Haley G. Retrospective observational analysis of the use of an architecturally unique dermal regeneration template (Derma Pure®) for the treatment of hard-to-heal wounds. Int Wound J 2016; doi: 10.1111/iwj.12667

Abstract

The purpose of this analysis was to evaluate the use of DermaPure, a decellularised human skin allograft, in the treatment of a variety of challenging wounds. This retrospective observational analysis reviewed a total of 37 patients from 29 different wound clinics across the USA. Each patient received one application of DermaPure which was followed until complete closure. A statistical analysis was performed with the end point being complete healing. All wounds on average, had a duration of 56 weeks and healed in an average time of 10·58 weeks. Individual wound categories included diabetic foot ulcers, which healed in 8·21 weeks; venous leg ulcers, which healed in 11·29 weeks; and surgical/traumatic wounds, which healed in 11·8 weeks.

Introduction

It has been estimated that in the USA, there are approximately 2.5 –4.5 million people living with chronic wounds (1). Richmond *et al*. stated that these ulcers last approximately for 12 months, have a high reoccurrence rate and can cause significant morbidity (1). Ulcers are classified as vascular (either arterial, venous or mixed), diabetic or pressure ulcers, which in the lower limb are most commonly found on the heel. Other wound types that prove challenging in terms of facilitating closure include those caused by trauma or as a result of dehiscence following surgery. Even with an appropriate standard of care, these wounds do not always heal as expected. They may remain open and in a stalled state for extended time periods, putting additional pressure on clinical and financial resources within health care settings. Chronic wounds can be defined as wounds that fail to proceed through the normal phases of wound healing in an orderly and timely fashion. Factors associated with delayed healing include persistent inflammation, infection or the possible presence of a biofilm that could be resistant to many forms of treatment. The presence of senescent fibroblasts that fail to respond to normal wound-healing stimuli could also contribute to delayed healing. From a physiological standpoint, chronic wounds have an excessive level of proinflammatory cytokines, proteases, reactive oxygen species (ROS), senescent cells, persistent infection and a deficiency of stem cells (2). The increase of ROS production causes damage to the extracellular matrix (ECM) proteins and also causes cell damage. This process unfortunately leads to enhanced stimulation of proteases and proinflammatory cytokines (3). Higher levels of proteases, compared to their inhibitors, lead to the destruction of the ECM, preventing the wound to transition to the proliferative phase and attracting more inflammatory cells (4). High levels of senescent cell populations with impaired proliferative capacities lead

Key Messages

- This was a multicentred retrospective observational analysis of DermaPure, a decellularised allograft. The product was used on different wounds (a total of 37 patients) with only one application. DermaPure was compared to similar products with published studies. It was found that DermaPure had a quicker rate of closure. DermaPure was also used on wounds, such as necrotising fasciitis and traumatic wounds, that had no published similar studies. Complete wound closure was achieved.

to unresponsiveness to typical wound-healing signals, directly correlating with failure of the wound to heal (5).

Even with site-specific optimal standards of care, many wounds do not heal and require the use of advanced wound care therapies (6). Currently, there is a large number of such products consisting of wound dressings and a growing segment of biological wound matrices, with the majority of these being acellular in composition (2). Some of these decellularised therapies include dehydrated amniotic/chorionic membrane, porcine intestine, porcine bladder and dermal/epidermal allografts. These decellularised therapies leave in situ many constituents of dermal ECM, which can perform a number of key functions that will direct the healing process. For example, they can function as a substrate into which cells can migrate to promote/initiate angiogenesis and tissue regeneration (7). As an integral component of the residual scaffold, the ECM plays a significant role in regeneration through a dynamic interaction with the body's host cells and growth factors (8). ECM elasticity and porosity play key roles in regulating dynamic interactions between cells and matrix components as well as mediating the binding or release of sequestered growth factors. Consequently, ECM characteristics significantly influence infiltration and cellular positioning within matrices, as well as the proliferation, differentiation and secretion profiles of resident cells (9). The ECM also contains functional components such as glycosaminoglycans, glycoproteins and proteoglycans, which are key to replacing a defective/injured ECM (10).

One type of decellularised therapy is a human dermal allograft, which is harvested from screened donors and prepared using a proprietary process to decellularise the dermis while maintaining the natural structures of the ECM (11). Prospective studies have shown that decelluarised human dermal allografts help heal stalled diabetic foot ulcers and other types of chronic wounds in a timely manner (11 – 16). Most recently, Walters *et al*. completed a 16-week prospective multicentre assessment of an acellular dermal matrix on diabetic foot ulcers (DFUs), in which they attained 67·9% closure of all wounds treated (17).

DermaPure, decelluarised human dermal skin allograft

DermaPure (Tissue Regenix, San Antonio, TX) is a unique and architecturally distinct decellularised human skin allograft harvested from screened tissue donors. Once harvested, it is minimally processed according to current FDA guidelines. The end result is a dermal scaffold, the porosity of which is optimised for guided cell infiltration (Figure 1). Using a proprietary dCell® process, the tissue is preserved and found to be 99% free of any donor DNA. This is an important attribute associated with a product of this kind. The minimal DNA content sets DermaPure apart and minimises any possible risk of disease transmission associated with residual DNA that remains in the tissue (18). Much higher levels exist with other technologies that exist in this category of skin substitutes.

The first study of DermaPure in the treatment of chronic wounds was performed by Greaves in 2013 (19). A total of 22 patients were enrolled who had minimal or absent response to standard of care of their chronic wounds after 3 months. Half of these patients had ulcers for longer than 1 year with an average wound age of 4.76 years. The ulcers treated were venous,

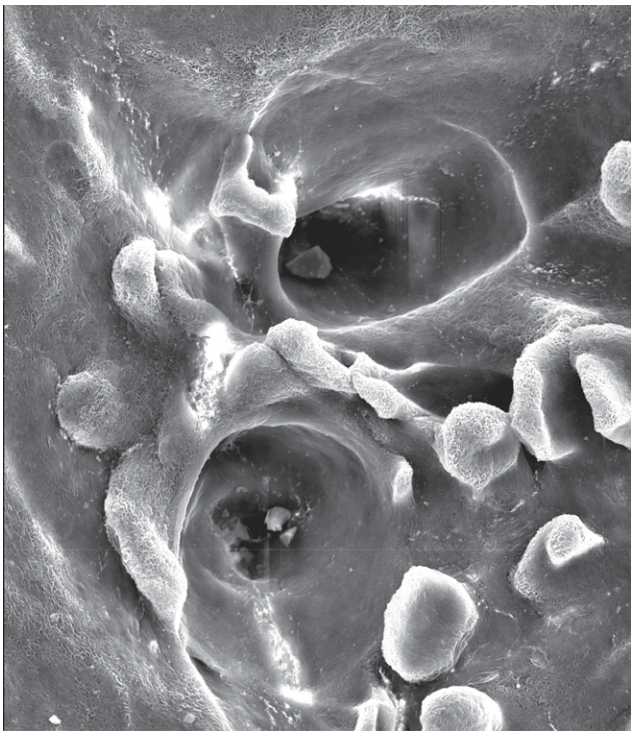

Figure 1 DermaPure imaging on a FEI Quanta 400 (ESEM). Tracts consistent with vascular channels were found, highlighted by organization of collagen around the tract. Vessel sizes reminiscent of capillaries in the papillary dermis and larger venules/arterioles in the reticular dermis.

diabetic or of mixed aetiology and were all on the lower limb. All patients had hydro-surgical debridement of their wound with Versajet◇ followed by a single application of DermaPure. Negative pressure wound therapy was then applied for 1 week. Prior to application, all patients had non-invasive vascular testing and a 1 week course of oral antibiotics. A full-thickness skin biopsy at the wound margin was taken at the time of surgery, and wound biopsies were also obtained at 3 and 6 weeks. Patients were then followed up weekly for 6 weeks, and final observations were made at 4 and 6 months. The primary outcome measure was wound surface area reduction. The authors also evaluated changes in vascularity, collagen levels and fibronectin. Primary outcomes showed wound reduction of 49·51% at 6 weeks, 80% after 4 months and 87% after 6 months. It was also shown that at week 6, there was an increase in haemoglobin flux, which is consistent with an increase in angiogenesis and restoration of vascular channels. Biopsies at week 3 showed that the graft was colonised by host fibroblasts, lymphocytes and neutrophils. These are significant observations because they show how the graft becomes an integral part of the host.

A prospective study on acute wounds using DermaPure was published in 2015 (20). The hypothesis of this study was that there were structural and biochemical variations of biomaterials that may induce differential scar formation after injury. Within this study, 50 healthy subjects had four biopsies of their inner arm, with each site allowed to heal in a different manner: site 1 was allowed to heal by secondary intention; Integra® (Plainsboro, NJ) was applied to site 2; DermaPure was applied to site

3; and site 4 had an autograft which was the biopsy intact tissue which was placed in the defect. Subjects were divided into five groups, with a biopsy performed at day 7, 14, 21 or 28. The histological results showed that the ECM-like DermaPure promotes stable focal adhesions facilitating tissue formation, while softer matrices encouraged transient adhesions and increased cell motility. In turn, cells exert contractile forces on ECM, which modulate matrix components over time. As a result, structural and biomechanical similarities between DermaPure and autografts may contribute to reduced fibrosis noted in the appropriately stained biopsies. The authors also contended that DermaPure resembled the angiogenic properties of an autograft. The authors concluded that DermaPure might stimulate more of a regenerative process than a reparative process.

A similar study was performed evaluating angiogenesis and the acute wound (21). This study mirrored the previous study, with the only minor difference being one less biopsy at day 42. Skin microcirculation was evaluated by analyzing the levels of haemoglobin flux and oxyhaemoglobin concentrations through non-invasive measures. Biopsy samples were evaluated for endothelial marker CD31, and these samples were also evaluated for gene expressions of PROK2, HIF2A, HIF3A and MT6-MMP. The former markers are genes associated with angiogenesis. The results demonstrated that both DermaPure and the autograft had organised vascular channels at the graft/host interface at Day 21, while the test comparator with the softer matrix did not. An increased expression of the pro-angiogenic PROK2 and MT6-MMP and CD31 was also seen in the DermaPure group, with maximum expression of CD31 at week 3. Both haemoglobin flux and oxyhaemoglobin concentrations were also elevated at week 3 in the DermaPure group compared to all the other groups, coinciding with the re-establishment of the vascular channels at week 3.

The hierarchy of laboratory, clinical and histological evidence leads to the conclusion that DermaPure may offer a very promising addition to the armamentarium of products designed to promote wound healing. The uniqueness of structure, biomechanical properties and biologically derived human components has been shown to address deficiencies of repair in both acute and chronic wounds. To further add to the consistency of this growing evidence base, an opportunity arose to conduct a retrospective, observational analysis of the clinical use of this dermal regeneration template in a large number of wound clinics across the USA.

Materials and methods

Design

The current study reports a retrospective observational analysis of 37 patients who received a single application of DermaPure for treatment of their wounds that had resisted attempts to achieve closure. The wound types reviewed included DFUs (n = 14), venous leg ulcers (VLU, n = 7), surgical/traumatic wounds (N = 12) and other (n = 4). The primary endpoint was the complete closure of the wound. Secondary outcome measures evaluated wound healing by level of chronicity and wound size. All patients reviewed had wounds > 1 cm^2 in size and a wound duration of >30 days. Wound size was measured on a weekly basis for 20 weeks or until closure. The graft was applied and secured with a non-adherent dressing over it. Common components of standardised care across all sites included debridement, infection control, off-loading if a plantar DFU was present and compression if the wound was a VLU. Complete healing/closure was defined as 100% epithelisation.

Statistical analyses

Descriptive statistics were prepared using SAS version 9.4 (SAS Institute, Inc., Cary, NC) and R Version 3.12 (R Core Team (2014) R: a language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria). Average time to heal in weeks was determined for each wound type along with wound age, duration at application (<1 year old versus≥1 year old) and wound size at application (<5 sq. cm versus≥5 sq. cm). Further stratification was performed for wound age and size at application for each wound type. The proportion of wounds completely healed at week 12 was also examined by wound size quartile. Multivariate logistic regression was used to evaluate the association between the proportion of healed wounds after 12 weeks with wound size and age duration at the time of initial application. Overall healing rates with 95% confidence intervals were examined using the Kaplan–Meier method. Patients who did not heal by 24 weeks were considered unhealed. The time to heal by each different wound type was also analysed.

Results

There were a total of 29 centres that treated a total of 37 patients. Patient characteristics are presented in Table 1. A high proportion of patients (51·4%, n = 19) had wounds located on their foot, with wounds on the leg being the second most common location (27·0% n = 10). The most common wound type was DFUs (37·8%, n = 14), followed by VLUs (18·9%, n = 7), with the remaining wounds being either traumatic or surgical. The average wound size at application for all wounds was 12.88 cm^2 (SD = 18·68 cm), and the average wound age at application was 55·8 weeks (SD = 27·89 weeks). The average time to heal for all wounds was 10·58 weeks (SD = 6·76 weeks). Complete healing for DFUs was 52% at 4 weeks, 73% at 8 weeks and 85% at 12 weeks. Complete healing for VLUs was 49% at 4 weeks, 70% at 8 weeks and 81% at 12 weeks.

Average time healed

DFUs had the lowest average time to heal (8·21 weeks), while traumatic wounds had the highest (20 weeks). VLUs had an average time to heal of 11·29 weeks, whilst surgical wounds healed within 15·67 weeks (Table 2). The majority of wounds were less than 1 year old, with an average age of approximately 32 weeks. Wounds that were less than 1 year old at application had a lower average time to heal compared to wounds that were 1 year old or older at application (10·08 weeks versus 13·30 weeks, respectively). Wounds that were less than 5 sq. cm at application had a lower average time to heal compared to wounds that were greater or equal to 5 sq. cm at application (8·14 weeks versus 12·77 weeks, respectively). Regardless of

Table 1 Patient characteristics (n = 37).

Wound location (%)	
Foot	19 (51·4%)
Leg	10 (27·0%)
Arm	1 (2·7%)
Breast	1 (2·7%)
Chest	1 (2·7%)
Elbow	1 (2·7%)
Lip	1 (2·7%)
Sacral	1 (2·7%)
Shoulder	1 (2·7%)
Toe	1 (2.7%)
Wound type (%)	
DFU	14 (37·8%)
VLU	7 (18·9%)
Surgical	6 (16·2%)
Trauma	6 (16·2%)
Other	4 (10·8%)
Wound size at application (cm^2) [mean (sd)]	12·88 (18·68)/DFU 13.23/VLU 14/Surg. and Traumatic 12·25
Wound age at application (weeks) [mean (sd)]	55·88 (27·89)/DFU 36·6/VLU 40·23/Surg. and Traumatic 11
Weeks to heel [mean (sd)]	10·58 (6·76)

Table 2 Average time to heal in weeks

Group	Average time healed	Standard deviation
All	10·58 weeks	6·76 weeks
DFUs	8·21 weeks	3·89 weeks
VLUs	11·29 weeks	4·15 weeks
Surgical wounds	15·67 weeks	8·55 weeks
Wounds < 1 year old	10·08 weeks	6·07 weeks
Wounds ≥1 year old	13·30 weeks	8·08 weeks
Wound size <5 cm	8·14 weeks	5·93 weeks
Wound size ≥ 5 cm	12·77 weeks	6·70 weeks

Group	Subgroup	Average time healed	Standard deviation
Stratified by wound age at application			
Wounds < 1 year old	DFUs	8·00 weeks	4·24 weeks
	VLUs	10·25 weeks	2·99 weeks
	Surgical wounds	9·33 weeks	3·79 weeks
Wounds ≥ 1 year old	DFUs	9·00 weeks	2·65 weeks
	VLUs	12·67 weeks	5·77 weeks
	Surgical wounds	22·00 weeks	6·93 weeks
Stratified by wound size at application			
Wound size < 5 sq. cm	DFUs	6·88 weeks	3·44 weeks
	VLUs	6·00 weeks	Only one patient
	Surgical wounds	12·00 weeks	Only one patient
Wound size ≥ 5 sq. cm	DFUs	10·00 weeks	4·00 weeks
	VLUs	12·17 weeks	3·76 weeks
	Surgical wounds	16·40 weeks	9·34 weeks

DFU, diabetic foot ulcers; VLU, venous leg ulcers.

Table 3 Logistic regression results

Outcome: healed by 12 weeks Effect	Odds ratio	95% CI	P -value
Wound size at application	0·949	(0·902, 1·000)	0·0490
Wound age at application	0·976	(0·944, 1·009)	0·1459

wound duration, DFUs healed in the shortest period of time. Of the wounds that were less than five sq. cm at application, VLUs had the lowest average time to heal (6·00 weeks), while surgical wounds had the highest average time to heal (12·00 weeks). In contrast, of the wounds that were at least 5 sq. cm at application, DFUs had the lowest average time to heal (10·00 weeks), while surgical wounds had the highest average time to heal (16·40 weeks).

Proportion healed

The average proportion of wounds healed by 4 weeks was 49·58% (SD = 31·79%). The proportion of wounds healed by 12 weeks was examined by size quartile: 93·67% of the first size quartile (0·02 – 2·4 cm), 100% for the second size quartile (2·55 –6·33 cm), 82·33% for the third size quartile (7.36 – 10 cm) and 82·38% for the fourth size quartile (12·88 – 72 cm).

Logistic regression

The binary response of being healed by week 12 was modelled by wound size and wound age at application (Table 3). Only wound size at application was found to be statistically significant (P = 0·0490). For every centimetre increase in wound size, the odds of being healed by 12 weeks significantly reduced by 5·1% (OR = 0·949). For every month's increase in wound duration at application, the odds of being healed by 12 weeks reduced by 2·4% (OR = 0·976). This finding was not statistically significant at the α = 0·05 level (P = 0·1459).

Kaplan–Meier

The proportion of patients who remained unhealed was plotted over time in weeks (Figure 1). Patients who did not heal by 24 weeks were considered unhealed. Of the 37 patients, 36 were healed by 24 weeks. The median healing time was 10·5 weeks [95% CI: (6 weeks, 13 weeks)]. This proportion was also plotted over time in weeks by wound type (Figure 2). All patients with DFUs and VLUs healed by 24 weeks, whereas one patient with surgical/trauma wounds did not heal by 24 weeks. Patients with necrotising fasciitis had the lowest median heal time (4·5 weeks), followed by DFUs (7·5 weeks), VLUs (11 weeks), surgical wounds (15 weeks) and trauma wounds (17·5 weeks) (Figure 3).

Discussion

This retrospective review of the efficacy associated with the use of a human-derived novel dermal regeneration template targeted the most common yet challenging wound types (Figure 4). The majority of patients had long standing DFUs >24 weeks, which met the universally accepted definition of hard-to-heal wounds. According to Sheehan, DFUs with >50% healing within 4 weeks have a greater chance to heal (22). Our data showed that 52% of patients achieved 100% healing at 4

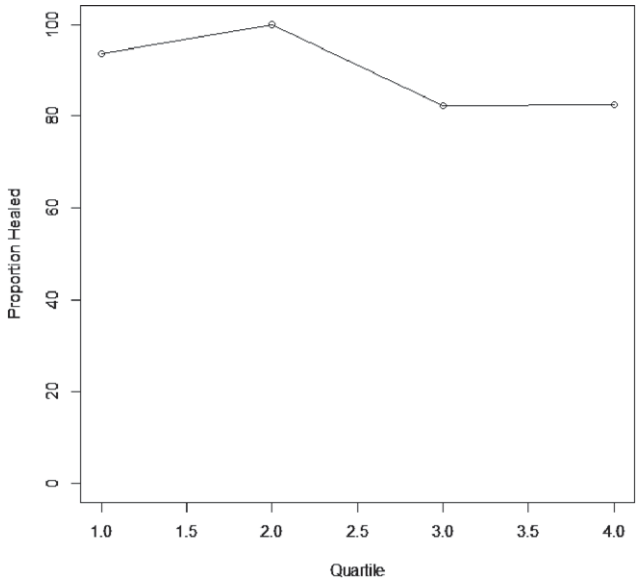

Figure 2 Proportion of wounds completely healed at week 12 by size quartile.

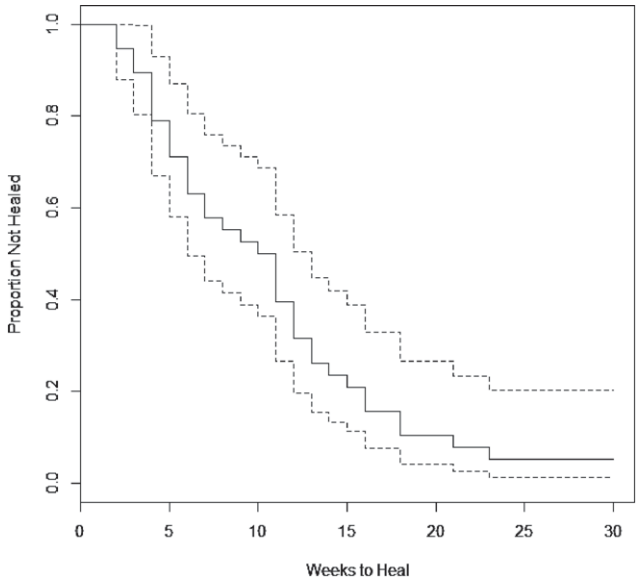

Figure 3 Overall healing rate.

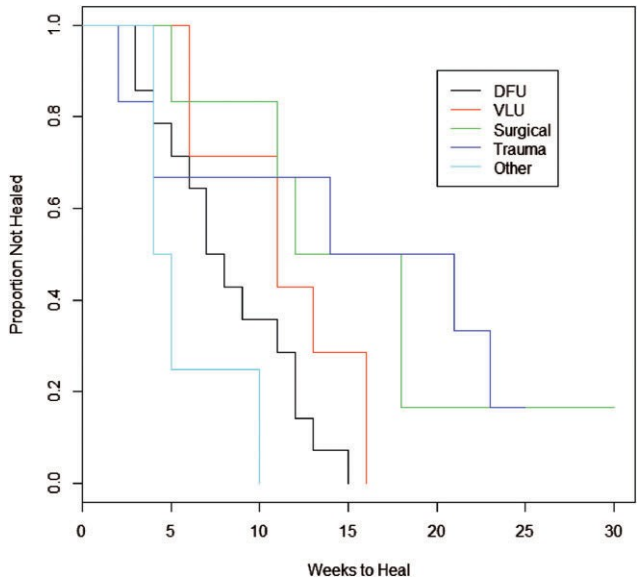

Figure 4 Healing rate by wound type.

weeks. Previous retrospective studies with a similar graft type, for example, Williams and Holewinski, reported their results for 16 patients with DFUs and achieved an average healing time of 10·96 weeks; unfortunately, there was no average ulcer duration listed (18), which makes a direct comparison difficult. Martin *et al*. reviewed 17 consecutive patients with DFUs of a mean wound size of 4·5 cm^2 who received a single application of an acellular human dermis. The average wound duration was 29·8 weeks. The average time to healing was 8·9 weeks (23). In a larger retrospective study, Winters *et al*. reviewed the outcomes of 100 DFUs (13). The average wound age was 20·4 weeks, and the average time to complete healing was 13·8 weeks. There have been two randomised controlled trials (RCTs) and two pilot studies of acellular human dermis for DFUs (12,17,24,25).

There have been only two multicentre RCTs evaluating technologies similar to the one described in this article. In 2009, Reyelman and coworkers published results from a 12 week prospective multicentred study where 47 patients received a single application of an acellular human dermis (12). The average ulcer duration was 23·3 weeks, with an average ulcer size of 3·6cm^2, and 70% of the ulcers were healed at 12 weeks. Winters *et al*. conducted a similar study examining two different products comprised of acellular human dermis compared to conventional standard of care (17). The 12-week endpoints of healing for both acellular human dermis products were 65% and 56·3%, respectively. When comparing all retrospective trials conducted using an acellular dermis, DermaPure healed similar challenging ulcers statistically faster. Reviewing all the data, both retrospective and prospective, ulcers treated with DermaPure were present for a longer duration (33·7 weeks) and were larger in size (13·24cm^2), yet healed 8·21 weeks faster.

In a retrospective study of DFUs and VLUs using a cryopreserved human dermis, a healing rate of 67% was reported with an average of 3·23 applications (26). In this study, the average baseline wound size was 6·2 cm^2 in the DFU group and 11·8 cm^2 in the VLU group, with an average wound duration of 18·7 weeks. Desman published a study looking at DFUs, VLUs and surgical/traumatic wounds treated with a similar acellular human allograft (27). The study had a total of 36 patients with 7 DFUs, 18 VLUs and 11 surgical/traumatic wounds. There were, on average, 3·3 applications of the matrix, with an average time to closure of 11·2 weeks for DFUs, 8·2 weeks for VLUs and 9·6 weeks for traumatic wounds, with an overall closure rate of 9·2 weeks for all wounds. The endpoints are nearly identical to the current analysis with some exceptions. At 20 weeks, the total wounds healed in the Desman study were 58%, while the current analysis had 100% healing. The Desman study also used, on average, more than one application. When compared

to all the previously described studies, DermaPure healed all wounds with one application and had nearly 100% healing by 20 weeks for wounds that were larger and were of a longer duration. When comparing DermaPure to the two other studies that included VLUs, the healing rate at 20 weeks was better, and while time to heal was similar, the other studies required more than one application to heal.

The replacement of areas of skin destruction represents a formidable challenge to the attending health care professional. Solutions were developed in the form of Dermal Regeneration Templates (DRTs), with the clinical goal of providing early wound coverage and neodermis formation, minimising the need for autograft dermis. Other advantages of such an approach include simplicity and reliability of technique and pliability and expected superiority of the cosmetic appearance of the resulting scars. Skin substitutes comprise of a range of heterogeneous biomaterials designed to accelerate wound healing through the process of guided cell attraction to the scaffold element of the template, which culminates in the provision of ECM, which facilitates the process of wound closure. Skin substitute characteristics include biocompatibility, porosity and elasticity that strongly influence cellular behaviour during the healing process and may induce differential scar formation after cutaneous injury (20).

A more practical and physiological approach would be to develop scaffold-based solutions from decellularised human cadaveric skin that has comparable biomechanical properties to the injured tissue. Cells would intuitively be primed to do what they do in situ, hence restoring normality to an abnormal situation. This would result in the restoration of skin architecture with successful scar outcomes. DermaPure is a bioengineered skin substitute that mimics native skin in terms of structure and rapidly integrates with surrounding tissue to actively stimulate cell migration, angiogenesis and epithelialisation (28). Through a patented, gentle decellularisation process, a graft is produced that consists of much less immunogenic ECM, which allows it to serve as an initial permanent implant that can be repopulated with the recipient's cells. During the healing process, fibrosis is an ill-defined term to describe ECM deposition from normal wound healing to pathological scarring (20). The whole wound healing process results in a differential development of fibrotic tissue, which will have a major impact on aesthetic outcomes. Recent findings have shown that the use of DermaPure in human wounds resulted in reduced dermal fibrosis compared to equivalent injuries treated with a bovine-derived matrix and those healed by secondary intention (20). Differences in matrix composition, architecture and cellular content between biomaterials may account for this variability. Therapies to ameliorate the fibrotic response to injury remain elusive. An exciting property associated with the use of DermaPure is that it could be used to create a shift in the processes associated with scarring to a more regenerative form of healing. This raises the exciting thought that the future direction of tissue-based products will not just be focused on dermal regeneration but also on the concept of dermal refinement, in which restoration of normal skin architecture with minimal scarring is the primary goal.

Living cell-based skin substitutes have been studied in RCTs for the treatment of DFUs and VLUs (29 – 32). The healing rates, wound age, wound size and number of applications in all of the living cell-based trials were significantly different than the current retrospective analysis. Clinicians have to determine the most efficacious way of healing an ulcer while being fiscally conscious. Redekop performed a cost-effectiveness study in 2003 (33). Within this study, he compared the 12-month cost of an advanced wound care skin products to the standard of care for DFUs. The conclusion was that the higher cost of the advanced wound care product was offset by the decrease in amputations and serious infections. Although the cost of DermaPure might be higher than the traditional standard of care, DermaPure was the most cost effective of all the advanced wound care products because it usually only requires a single application to heal.

Limitations

There are some limitations within the paper. Although statistical analysis was performed, being a retrospective cohort study, it is still considered level 2 evidence. There were numerous trial sites, but each site allowed the clinician to perform what they considered to be standard of care. There were no inclusion or exclusion criteria in this analysis. Patient's comorbidities along with critical lab values were not included in this analysis.

Conclusion

A single application of DermaPure results in the complete healing of stalled DFUs in approximately 2 months, VLUs in < 3 months, surgical wounds < 4 months and traumatic wounds < 5 months. A comparison of DermaPure to other prospective trials of acellular human dermis used to treat DFUs showed that DermaPure healed more effectively with fewer applications. No prospective trials on the treatment of VLUs with acellular human dermis exist. Comparisons to two retrospective trials reveal that DermaPure is more effective at healing with fewer applications. DermaPure heals chronic wounds in both an efficient and timely manner and also has the added economic benefit of being cost effective.

Acknowledgement

This manuscript was funded by Tissue Regenix US. Dr. Kimmel is on the speakers' bureau of Tissue Regenix.

References

1. Richmond NA, Maderal AD, Vivas AC. Evidence-based management of common chronic lower extremity ulcers. *Dermatol Ther* 2013;**26**:187–96.
2. Frykberg RG, Banks J. Challenges in the treatment of chronic wounds. *Adv Wound Care* 2015;**4**:560 –82.
3. Schreml S, Szeimies RM, Prantl L, Karrer S, Landthaler M, Babilas P. Oxygen in acute and chronic wound healing. *Br J Dermatol* 2010;**163**:257–68.
4. McCarty SM, Percival SL. Proteases and delayed wound healing. *Adv Wound Care (New Rochelle)* 2013;**2**:438–47.
5. Stanley A, Osler T. Senescence and the healing rates of venous ulcers. *J Vasc Surg* 2001;**33**:1206 –11.
6. Margolis DJ, Kantor J, Berlin JA. Healing of diabetic neuropathic foot ulcers receiving standard treatment. A meta-analysis. *Diabetes Care* 1999;**22**:692–5.

7. Mulder GHK, Kirsner R, Lee D, Serena T. International Consensus. Acellular matrices for the treatment of wounds. An expert working review. *Wounds Int* 2010.
8. Schultz GS, Davidson JM, Kirsner RS, Bornstein P, Herman IM. Dynamic reciprocity in the wound microenvironment. *Wound Repair Regen* 2011;**19**:134–48.
9. Greaves NS, Ashcroft KJ, Baguneid M, Bayat A. Current understanding of molecular and cellular mechanisms in fibroplasia and angiogenesis during acute wound healing. *J Dermatol Sci* 2013;**72**:206–17.
10. Badylak SF, Freytes DO, Gilbert TW. Extracellular matrix as a biological scaffold material: Structure and function. *Acta Biomater* 2009;**5**:1–13.
11. Greaves NS, Iqbal SA, Baguneid M, Bayat A. The role of skin substitutes in the management of chronic cutaneous wounds. *Wound Repair Regen* 2013;**21**:194–210.
12. Reyzelman A, Crews RT, Moore JC, et al. Clinical effectiveness of an acellular dermal regenerative tissue matrix compared to standard wound management in healing diabetic foot ulcers: a prospective, randomised, multicentre study. *Int Wound J* 2009;**6**:196–208.
13. Winters CL, Brigido SA, Liden BA, Simmons M, Hartman JF, Wright ML. A multicenter study involving the use of a human acellular dermal regenerative tissue matrix for the treatment of diabetic lower extremity wounds. *Adv Skin Wound Care* 2008;**21**:375–81.
14. Yonehiro L, Burleson G, Sauer V. Use of a new acellular dermal matrix for treatment of nonhealing wounds in the lower extremities of patients with diabetes. *Wounds* 2013;**25**:340–4.
15. Iorio ML, Shuck J, Attinger CE. Wound healing in the upper and lower extremities: a systematic review on the use of acellular dermal matrices. *Plast Reconstr Surg* 2012;**130**:232S–41.
16. Capito AE, Tholpady SS, Agrawal H, Drake DB, Katz AJ. Evaluation of host tissue integration, revascularization, and cellular infiltration within various dermal substrates. *Ann Plast Surg* 2012;**68**:495–500.
17. Walters J, Cazzell S, Pham H, Vayser D, Reyzelman A. Healing rates in a multicenter assessment of a sterile, room temperature, acellular dermal matrix versus conventional care wound management and an active comparator in the treatment of full-thickness diabetic foot ulcers. *Eplasty* 2016;**16**.
18. Williams ML, Holewinski JE. Use of a human acellular dermal wound matrix in patients with complex wounds and comorbidities. *J Wound Care* 2015;**24**:261–2, 4–7.
19. Greaves NS, Benatar B, Baguneid M, Bayat A. Single-stage application of a novel decellularized dermis for treatment-resistant lower limb ulcers: positive outcomes assessed by SIAscopy, laser perfusion, and 3D imaging, with sequential timed histological analysis. *Wound Repair Regen* 2013;**21**:813–22.
20. Greaves NS, Iqbal SA, Hodgkinson T, et al. Skin substitute-assisted repair shows reduced dermal fibrosis in acute human wounds validated simultaneously by histology and optical coherence tomography. *Wound Repair Regen* 2015;**23**:483–94.
21. Greaves NS, Lqbal SA, Morris J, et al. Acute cutaneous wounds treated with human decellularised dermis show enhanced angiogenesis during healing. *PLoS One* 2015;**10**:e0113209.
22. Sheehan P, Jones P, Caselli A, Giurini JM, Veves A. Percent change in wound area of diabetic foot ulcers over a 4-week period is a robust predictor of complete healing in a 12-week prospective trial. *Diabetes Care* 2003;**26**:1879–82.
23. Martin BR, Sangalang M, Wu S, Armstrong DG. Outcomes of allogenic acellular matrix therapy in treatment of diabetic foot wounds: an initial experience. *Int Wound J* 2005;**2**:161–5.
24. Brigido SA, Boc SF, Lopez RC. Effective management of major lower extremity wounds using an acellular regenerative tissue matrix: a pilot study. *Orthopedics* 2004;**27**:s145–9.
25. Brigido SA. The use of an acellular dermal regenerative tissue matrix in the treatment of lower extremity wounds: a prospective 16-week pilot study. *Int Wound J* 2006;**3**:181–7.
26. Landsman AS, Cook J, Cook E, et al. A retrospective clinical study of 188 consecutive patients to examine the effectiveness of a biologically active cryopreserved human skin allograft (TheraSkin(R)) on the treatment of diabetic foot ulcers and venous leg ulcers. *Foot Ankle Spec* 2011;**4**:29–41.
27. Desman E, Bartow W, Anderson LH. Human skin allograft for patients with diabetic foot ulcers, venous leg ulcers, or surgical/traumatic wounds retrospective, descriptive study. *Ostomy Wound Manage* 2015;**61**:16–22.
28. Greaves NS, Morris J, Benatar B, Alonso-Rasgado T, Baguneid M, Bayat A. Acute cutaneous wounds treated with human decellularised dermis show enhanced angiogenesis during healing. *PLoS One* 2015;**10**:e0113209.
29. Edmonds M, European, Australian Apligraf Diabetic Foot Ulcer Study G. Apligraf in the treatment of neuropathic diabetic foot ulcers. *Int J Low Extrem Wounds* 2009;**8**:11 –8.
30. Veves A, Falanga V, Armstrong DG, Sabolinski ML, Apligraf Diabetic Foot Ulcer S. Graftskin, a human skin equivalent, is effective in the management of noninfected neuropathic diabetic foot ulcers: a prospective randomized multicenter clinical trial. *Diabetes Care* 2001;**24**:290–5.
31. Marston WA, Hanft J, Norwood P, Pollak R, Dermagraft Diabetic Foot Ulcer Study G. The efficacy and safety of Dermagraft in improving the healing of chronic diabetic foot ulcers: results of a prospective randomized trial. *Diabetes Care* 2003;**26**:1701–5.
32. Harding K, Sumner M, Cardinal M. A prospective, multicentre, randomised controlled study of human fibroblast-derived dermal substitute (Dermagraft) in patients with venous leg ulcers. *Int Wound J* 2013;**10**:132–7.
33. Redekop WK, McDonnell J, Verboom P, Lovas K, Kalo Z. The cost effectiveness of Apligraf treatment of diabetic foot ulcers. *Pharmacoeconomics* 2003;**21**:1171–83.

REVIEW

The Presence of Oxygen in Wound Healing

Howard M. Kimmel, DPM, MBA[1]; Anthony Grant, DPM[2]; and James Ditata, BSN, RN, CNOR[2]

Wounds 2016;28(8)???-???

From the [1]Case Western Reserve University School of Medicine, Cleveland, OH; and [2]Louis Stokes VA Medical Center, Cleveland, OH

Address correspondence to:
Howard M. Kimmel, DPM,MBA
Case Western Reserve University
School of Medicine
Cleveland, OH
buckeyefootcare@sbcglobal.net

Disclosure: The authors disclose no financial or other conflicts of interest.

Abstract: Oxygen must be tightly governed in all phases of wound healing to produce viable granulation tissue. This idea of tight regulation has yet to be disputed; however, the role of oxygen at the cellular and molecular levels still is not fully understood as it pertains to its place in healing wounds. In an attempt to better understand the dynamics of oxygen on living tissue and its potential role as a therapy in wound healing, a substantial literature review of the role of oxygen in wound healing was performed and the following key points were extrapolated: 1) During energy metabolism, oxygen is needed for mitochondrial cytochrome oxidase as it produces high-energy phosphates that are needed for many cellular functions, 2) oxygen is also involved in the hydroxylation of proline and lysine into procollagen, which leads to collagen maturation, 3) in angiogenesis, hypoxia is required to start the process of wound healing, but it has been shown that if oxygen is administered it can accelerate and sustain vessel growth, 4) the antimicrobial action of oxygen occurs when nicotinamide adenine dinucleotide phosphate (NADPH)-linked oxygenase acts as a catalyst for the production of reactive oxygen species (ROS), a superoxide ion which kills bacteria, and 5) the level of evidence is moderate for the use of hyperbaric oxygen therapy (HBOT) for diabetic foot ulcers, crush injuries, and soft-tissue infections. The authors hypothesized that HBOT would be beneficial to arterial insufficiency wounds and other ailments, but at this time further study is needed before HBOT would be indicated.

Key words: components of wound healing, hyperbaric oxygen therapy, HBOT

Oxygen is a significant factor in wound healing. In general, living tissue needs oxygen and nutrients to thrive, and with wounds, it is needed to regenerate healthy tissue. In normal wound healing, the wound either requires conditions of hypoxia or normal levels of oxygen (ie, normoxia). These different conditions occur in all phases of wound healing. A wound is dependent on both the supply of oxygen to the wound tissue, which is determined by the pulmonary gas exchange, and the blood hemoglobin level. The cardiac output of the patient, the perfusion rate, and the amount of capillaries around the wound along with the consumption rate of parenchymal and stromal cells determine these levels.[1] This paper will discuss the role of oxygen in healthy wound healing.The discussion will examine how oxygen is produced, consumed,

and used in the various stages of wound healing at both a molecular level and a cellular level. Finally, there will a brief discussion on the use of oxygen as therapy.

Oxygen at the Molecular Level

In the aerobic metabolism of glucose, cells use oxygen as the final electron acceptor to generate adenosine triphosphate (ATP), which fuels the majority of cellular processes during wound healing.[2] Healing tissue requires an increased energy demand.[3] This additional energy is generated from the oxidative metabolism which in turn increases the oxygen demand of the healing tissue.[4] Thus, the ATP that is generated from this process helps supply the power for tissue repair. During the inflammatory phase of wound healing, platelets and disintegrating cells can contribute ATP.[5] This extracellular ATP can act as a signalling mechanism for many aspects of wound healing such as the immune response, inflammation, epithelial cells, and angiogenesis.[6] When ATP is released during an injury to the skin, it acts as an early signal in an epidermal-like growth factor which, downstream, signals epidermal growth.[7] Another signalling function of ATP is that it is released from the cells in the injured tissue, thereby activating NADPH oxidasis, which is required to produce the redox signals in wound healing.[8] The first discussion of the killing of bacteria by an oxidase occurred in 1978.[9] When the phagocytosis of bacteria occurs, the immune system increases oxygen consumption through NADPH oxidase (NOX) that in turn generates metabolites.[10] These metabolites catalyse the production of a reactive oxygen species (ROS) by cells that then stimulate a high demand for oxygen or "respiratory burst."[11] The majority of the oxygen consumed by neutrophils occurs during this respiratory burst.[12] Nicotinamide adenine dinucleotide phosphate oxidase is vital in the survival of macrophages, and it also enables phagocytosis of dead cells.[13]

Redox Signalling

Initially, free radicals were thought to be destructive to normal tissue, and it also was thought that these free radicals should be bound to antioxidants to stop their destructive nature.[14] Low level free radicals were then later recognized as possibly serving as signalling messengers.[15] Inflammation after an injury occurs as a site for significant production of ROS due to the amount of phagocytosis occurring. As wound healing progresses, things like cell proliferation and migration are present due to redox signalling of ROS.[16] Production of hydrogen peroxide also occurs during wound healing.[8] When hydrogen peroxide is decomposed, it generates oxy- gen as an end product.[17] Redox signals are generated, and decreased tissue oxygen and tissue hypoxia will limit the signalling of redox; thus disabling the func- tion of several growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth fac- tor (VEGF), and also limit some molecular mechanisms such as leukocyte recruitment.[15]

Oxygen and Wound Healing Phases

Nearly every step in the wound healing process requires oxygen.[18] Even though acute hypoxia stimulates wound healing, oxygen recovery (tissue oxygenation) is required, because chronic hypoxia will impair the healing.[1]

During the inflammatory phase, the most significant cellular processes occur when oxygen is involved in the oxidative phosphorylation in the mitochondria which results in the production of ATP.[19]The ROS have more roles than just the oxidative killing of bacteria; after hemostasis, hypoxia occurs and activates the initial steps of wound healing by boosting ROS activity. Hypoxia also activates platelets and endothelium by inducing cytokines released from platelets, monocytes, and growth factors.[20] This usually occurs at low concentrations. Hypoxia-induced factor (HIF) results in a transcription HIF, which binds to hypoxia response elements in gene promoter regions.

These regions upregulate glucose metabolism, control vessel tone, and angiogenesis.[21] Hypoxia-induced factor regulates oxygen hemostasis in the wound, and ROS stimulates cytokine and chemokine-receptor activation as well as other functions necessary for wound repair.The main effect of these mediators is the recruitment and activation of neutrophils and macrophages to the wound site and the activation of fibroblasts.[22] Once the cytokines and chemokines are secreted, they activate the oxygen-dependent complement cascade. At this time, a set of growth factors are released that stimulate and attract the major components of wound healing such as wound leukocytes and fibroblasts. Hydrogen peroxide has been shown to be a mediator of these interactions. In an experiment by Niethammer and colleagues,[23] a Zebra fish larval tail fin had a mechanically created wound induced. This was done to prove hydrogen peroxide arrives first to a new wound site from the epithelial cells of the tail fin. Eventually

the hydrogen peroxide recruits leukocytes and fibroblasts in this study of inflammatory and regenerative chemical response to wounds.[23]

Once the skin and vasculature is disrupted, there is an increased amount of oxygen consumption which in turn creates a hypoxic event.[19] Reactive oxygen species activity is initiated by hypoxia, which causes platelets and monocytes to release transforming growth factor beta (TGF-β), VEGF, and tumor necrosis factor alpha (TNF-α).[24] Neutrophils and monocytes produce ROS as described in this respiratory burst, consequently inducing neutrophil chemotaxis.[24] Certain antibiotics, such as aminoglycosides, have been shown to work synergistically with oxygen.[25] Oxygen is known to have a preventive effect against anaerobic wound infections.[26] A prospective study of 300 patients with a colorectal resection was randomized into 2 groups.[27] The first group of 148 patients received 80% oxygen supplementation intraoperatively and 80% postoperatively for 6 hours, while the other group of 143 patients received 30% supplementation intraoperatively and 30% oxygenation postsurgically for 6 hours. The latter group (30% oxygen) had a greater rate of infection in contrast to the group receiving 80% oxygen. In conclusion, it was demonstrated that patients receiving higher concentrations of oxygen resulted in lower rates of postcolon or postrectal surgery infections.[27]

In the proliferative phase, hypoxia has been shown to increase keratinocyte motility. This was shown in vitro producing proteins that are involved in cell motility.[28]

Human keratinocytes in patients more than 60 years of age have been shown to have slower motility than people half their age.[29] It has been hypothesized that matrix metalloproteinases (MMPs) 1 and 9 are required in keratinocyte migration on type I and type IV collagen, respectively. These MMPs in young keratinocytes are induced by hypoxia yet not induced in older keratinocytes.[30]

Transforming growth factor beta one (TGF-β1) is the growth factor responsible for the transcription of the procollagen gene, which has been proven to increase the migration of young cultured human fibroblasts.[31] Siddiqui et al[32] have also demonstrated that acute hypoxia increases fibroblast proliferation, collagen synthesis, and expression of TGF-β1 messenger RNA (mRNA). Oxygen is needed in the later steps of collagen synthesis for proline and lysine hydroxylation and cross-linking.[33] For fibroblasts to lay collagen down properly, oxygen tensions are needed to be between 30-40 mm Hg because the production of collagen is proportional to the oxygen tension.[34] Oxygen is needed for lysine and proline hydroxylation, which is the step required for collagen to be released from cells.[35] In order for collagen to form a triple helix, oxygen must be present. Without oxygen, the pro-alpha peptide chains fail to form the triple helix.[36]

Hypoxia stimulates angiogenesis but cannot sustain the process.[37] The most influential growth factor for angiogenesis is VEGF.[38] In vitro studies have proven the expression of VEGF increases in both states of hypoxia and hyperoxia.[39] Angiogenesis will proceed and can only be maintained when there is sufficient oxygen and VEGF will be released at higher oxygen tensions.[40]

Epidermal keratinocytes differentiate, proliferate, and

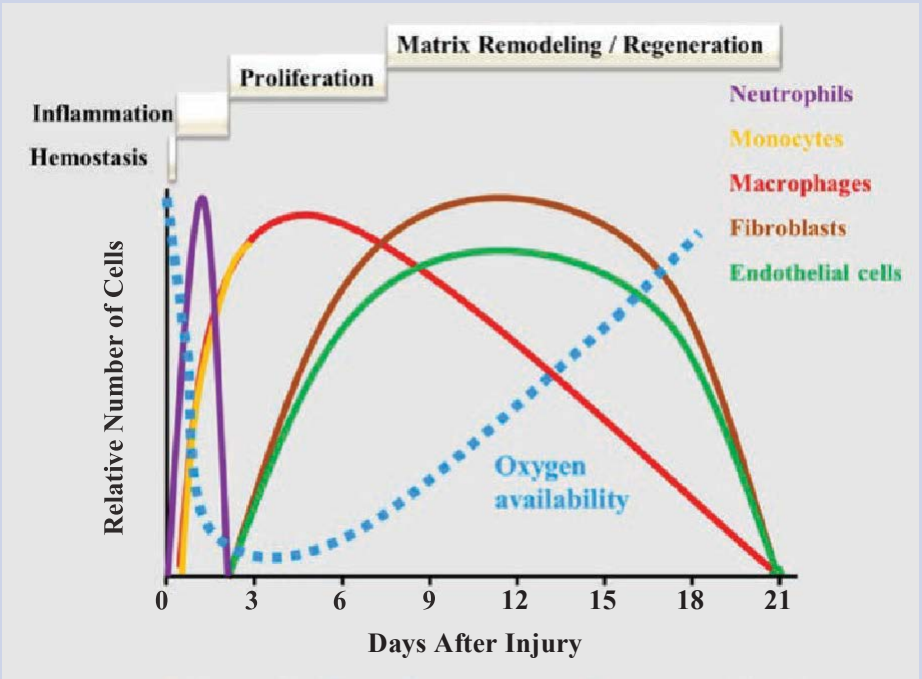

Figure 1. Schematic review of the phases of wound healing over time with oxygen availability. Reproduced with permission.[65]

migrate on the wound surface to start the reepithelization of a wound. Wound injury causes stress pathways to be activated which then cause the oxygen-dependent release of certain cytokines and chemokines, such as keratinocyte growth factor (KGF), epidermal growth factor (EGF), PDGF, insulin-like growth factor (IGF), and tumor necrosis factor (TNF) superfamily.[41] The TNF is the main cytokine that seems to stimulate epidermal cells at the wound edges and hair follicles in an autocrine manner, which is an oxygen-dependent process.[42] In turn, cells develop a process in which structures are developed for adhesion to the extracellular matrix and developing actin filaments for cell migration.[43] There has to be a significant cell migration accompanied with oxygen-dependent cell proliferation for large wounds to close. Cytokines and chemokines that are most likely released from keratinocyte stem cells stimulate the proliferation of keratinocytes in a process called a "proliferative burst."[44] This process has a high amount of metabolic activity since there are different steps that require oxygen and ROS.

The last step or phase of wound healing is remodeling which can last up to 2 years. Gradually, the provisional collagen, which is mostly type III, is replaced with type I collagen produced strictly in oxygen- dependent fibroblasts. The wound then gains tensile strength, and the collagen fibers contract so the wound shrinks.[45] The most prominent mediators of this col- lagen process are MMPs and tissue inhibitors of metal- loproteinases (TIMPs), which are released by macro- phages, keratinocytes, endothelial cells, and fibroblasts, which are all dependent on oxygen.[46]

Oxygen Sensing

Throughout the phases of wound healing there is a control of oxygen maintained in a narrow range. This point of normoxia is important because it is used to prevent abnormal periods of hypoxia or hyperoxia which can create damage to cell membranes.[5] This point of normoxia is the state of oxygenation where the cell or tissue does not report hypoxia nor does it report hyperoxia which would be oxygen toxicity.[47] If there were a change, the cells or tissue would react by switching on either a hypoxic or hyperoxic response. Depending on the organ of the body, the normoxic set point would be different due to the amount of oxygen required.[48] Hypoxia sensing and response is implicated in ischemic disease conditions, but is required for development where there is a changing state of oxygen-

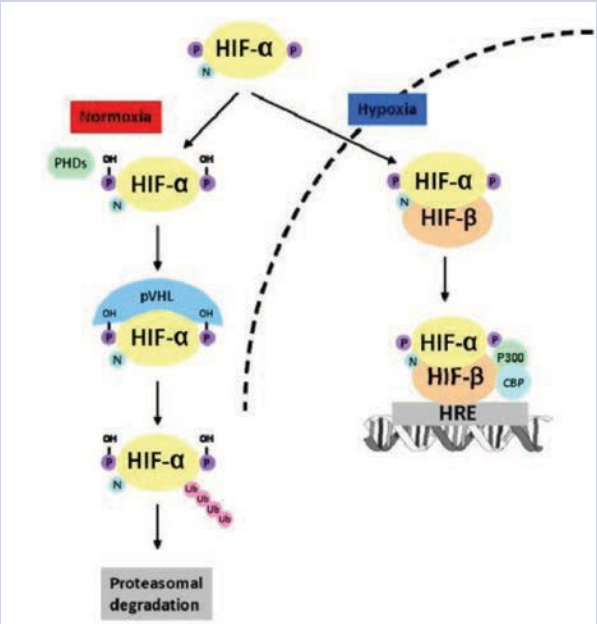

Figure 2. A schematic representation of the oxygen-dependent regulation of hypoxia-inducible factor 1-alpha (HIF-α). Reproduced with permission.[65]

ation sending a signal to continue the wound-healing process. This sensing is either considered HIF-dependent or HIF-independent.[21]

Intermittent hypoxia, a periodic exposure to hypoxia, is interrupted by a return to normoxia where less hypoxic periods occur in many circumstances.[49] This intermittent hypoxia is mostly found in obstructive sleep apnea, but in a study by Khayat et al,[50] the authors have shown patients with this condition commonly have nonhealing wounds. Even though hyperoxia may induce some positive effects, if this occurs for a period of time exceeding the normoxic set point it can be a risk factor.[51] In areas where the wound has pockets of hypoxia, the goal is to reestablish normoxia in the areas of hypoxia without exposing the wound to high levels of oxygen which might cause oxygen toxicity.[52] Wound healing might be delayed in extreme hyperoxia which can cause growth arrest and cell death by mitochondria apoptosis.[53] The normoxic set point can be tuned when the cells are exposed to modest changes of oxygen and there is a physiological change that can possibly be an adaptive process.[54]

Oxygen Therapy

This review would be incomplete without a brief

discussion of the use of oxygen in the treatment of wounds. Hyperbaric oxygen therapy (HBOT) is usually administered in a single patient or multipatient chamber that delivers 100% oxygen at 2 atmospheres of pressure. Hyperbaric oxygen therapy has proven to raise tissue oxygen 10 to 20 fold above room air.[55] One theory as to why HBOT might work is the synergy with PDGF since PDGF requires oxygen-derived hydrogen peroxide for functioning.[56] Another oxygen therapy is topical oxygen. This therapy utilizes either a chamber or a plastic bag to create a closed environment to deliver 100% oxygen converted from room air.[57] It is hypothesized that 100% oxygen applied locally to a wound increases VEGF expression, which may induce angiogenesis.[58] The evidence for clinical use of HBOT is moderate at best. In a review from the Cochrane Library Database, Kranke et al[59] presented 12 randomized trials that included participants with foot ulcers/wounds and diabetes. Short-term (up to 6 weeks) HBOT was found to be effective in improving healing but there were no significant findings that the wounds were completely healed after 1 year. For chronic wounds in patients with decreased blood supply or pressure ulcers, no evidence could confirm or deny any effects of HBOT.[59] Another study by Fedorko[60] consisted of a randomized, placebo-controlled study for patients with both types I and II diabetes, diabetic wounds, or lower extremity injuries. Hyperbaric oxygen therapy did not offer any additional advantages in wound care nor did the therapy support a reduction in lower limb amputations or wound size in patients with diabetic foot ulcers over a 12-week period.

Conclusion

Throughout all phases of wound healing, oxygen plays a substantial role. Its effects vary depending on whether the wound is in a hypoxic, normoxic, or in a hyperoxic state.The following are the key points. First during energy metabolism, oxygen is needed for mitochondrial cytochrome oxidase.[61] This in turn produces high-energy phosphates which then are needed for many cellular functions.[40] Second, in collagen synthesis oxygen is involved in the hydroxylation of proline and lysine into procollagen which leads to collagen maturation.[62] Third, in angiogenesis, hypoxia is required to start the process, but it has been shown that if oxygen is administered it can accelerate and sustain vessel growth.[63] Finally, the antimicrobial action of oxygen occurs when converted by leukocytic NADPH oxidase to a superoxide ion which kills bacteria.[64]

References

1. Schreml S, Szeimies RM, Prantl L, Karrer S, Landthaler M, Babilas. Oxygen in acute and chronic wound healing. *Br J Dermatol*. 2010;163(2):257-268.
2. Ichioka S, Ando T, Shibata M, Sekiya N, Nakatsuka T. Oxygen consumption of keloids and hypertrophic scars. *Ann Plast Surg*. 2008;60(2):194-197.
3. Im MJ, Hoopes JE. Energy metabolism in healing skin wounds. *J Surg Res*. 2010;10(10):459-464.
4. Gupta A, Raghubir R. Energy metabolism in the granulation tissue of diabetic rats during cutaneous wound healing. *Mol Cell Biochem*. 2005;270(1-2):71-77.
5. Sen CK. Wound healing essentials: let there be oxygen. *Wound Repair Regen*. 2009;17(1):1-18.
6. Bours MJ, Swennen EL, Di Virgilio F, Cronstein BN, Dagnelie PC. Adenosine 5′-triphosphate and adenosine as endogenous signaling molecules in immunity and inflammation [published online ahead of print June 19, 2006]. *Pharmacol Ther*. 2006;112(2):358-404.
7. Yin J, Xu K, Zhang J, Kumar A, Yu FS. Wound-induced ATP release and EGF receptor activation in epithelial cells [published online ahead of print Feb 6, 2007]. *J Cell Sci*. 2007;120(Pt5):815-825.
8. Roy S, Khanna S, Nallu K, Hunt TK, Sen CK. Dermal wound healing is subject to redox control [published online ahead of print Aug 26, 2005]. *Mol Ther*. 2006;13(1):211-220.
9. Babior BM. Oxygen-dependent microbial killing by phagocytes (second of two parts). *N Engl J Med*. 1978;298(13):721-725.
10. Lambeth JD, Kawahara T, Diebold B. Regulation of Nox and Duox enzymatic activity and expression [published online ahead of print April 1, 2007]. *Free Radic Biol Med*. 2007;43(3):319-331.
11. Wang X, Wang Y, Kim HP, Choi AM, Ryter SW. FLIP inhibits endothelial cell apoptosis during hyperoxia by suppressing Bax [published online ahead of print Feb 28, 2007]. *Free Radic Biol Med*. 2007;42(10):1599-1609.
12. Allen DB, Maguire JJ, Mahdavian M, et al.Wound hypoxia and acidosis limit neutrophil bacterial killing mechanisms. *Arch Surg*. 1997;132(9):991-996.
13. Brown JR, Goldblatt D, Buddle J, Morton L, Thrasher, et al. Diminished production of anti-inflammatory mediators during neutrophil apoptosis and macrophage phagocytosis in chronic granulomatous disease (CGD). *J Lukoc Biol*. 2003;73(5):591-599.
14. Clark IA, Cowden WB, Hunt NH. Free radical-induced

pathology. Med Res Rev. 1985;5(3):297-332.
15. Sen CK. Redox signaling and the emerging therapeutic potential of thiol antioxidants. *Biochem Pharmacol.* 1998;55(11):1747-1758.
16. Sen CK, Roy S. Redox signals in wound healing [published online ahead of print Jan 18, 2008]. *Biochim Biophys Acta.* 2008;1780(11):1348-1361.
17. Roy S, Khanna S, Sen CK. Redox regulation of the VEGF signaling path and tissue vascularization: hydrogen peroxide, the common link between physical exercise and cutaneous wound healing [published online ahead of print Jan 19, 2007]. *Free Radic Biol Med.* 2008;44(2):180-192.
18. Hopf HW, Rollins MD. Wounds: an overview of the role of oxygen. *Antioxid Redox Signaling.* 2007;9(8):1183-1192.
19. Tandara AA, Mustoe TA. Oxygen in wound healing — more than a nutrient [published online ahead of print Feb 17, 2004]. *World J Surg.* 2004;28(3):294-300.
20. Görlach A, Brandes RP, Bassus S, et al. Oxidative stress and expression of p22phox are involved in the up-regulation of tissue factor in vascular smooth muscle cells in response to activated platelets. *FASEB J.* 2000;14(11):1518-1528.
21. Semenza GL. HIF-1 and human disease: one highly involved factor. *Genes Dev.* 2000;14(16):1983-1991.
22. Gurtner GC,Werner S,BarrandonY,Longaker MT.Wound repair and regeneration. *Nature.* 2008;453(7193):314-321.
23. Niethammer P, Grabher C, Look AT, Mitchison TJ. A tissue-scale gradient of hydrogen peroxide mediates rapid wound detection in zebrafish [published online ahead of print June 3, 2009]. *Nature.* 2009;459(7249):996-999.
24. Freinkel RK, Woodley DT. *The Biology of the Skin.* Boca Raton, FL: CRC Press; 2001.
25. Verklin RM Jr, Mandell GL. Alteration of effectiveness of antibiotics by anaerobiosis. *J Lab Clin Med.* 1977;89(1):65-71.
26. Gottrup F. Oxygen in wound healing and infection [published online ahead of print Feb 17, 2004]. *World J Surg.* 2004;28(3):312-315.
27. Belda FJ,Aguilera L, García de la Asunción J, et al; Spanish Reduccion de la Tasa de Infeccion Quirurgica Group. Supplemental perioperative oxygen and the risk of surgical wound infection: a randomized controlled trial. *JAMA.* 2005;294(16):2035-2042.
28. O'Toole EA, Marinkovich MP, Peavey CL, et al. Hypoxia increases human keratinocyte motility on connective tissue. *J Clin Invest.* 1997;100(11):2881-2891.
29. Xia et al. 2001 – AU: Please include citation info; thank you.
30. Salo et al. 1994 – AU: Please include citation info; thank you.
31. Mogford JE,Tawil N, Chen A, Gies D, Xia Y, Mustoe TA. Effect of age and hypoxia on TGFβ1 receptor expression and signal transduction in human dermal fibroblasts:impact on cell migration. *J Cell Physiol.* 2002;190(2):259-265.
32. Siddiqui A, Galiano RD, Connors D, Gruskin E,Wu L, Mustoe TA. Differential effects of oxygen on human dermal fibroblasts: acute versus chronic hypoxia. *Wound Repair Regen.* 1996;4(2):211-218.
33. Hopf HW, Gibson JJ,Angeles, et al. Hyperoxia and angiogenesis. *Wound Repair Regen.* 2005;13(6):558-564.
34. Hunt, T. and Hussain, Z. Wound microenvironment. *Wound Healing: Biochemical & Clinical Aspect*s. Cohen IK, Diegelmann RF,Lindblad WJ, eds. Philadelphia, PA:WB Saunders;1992,274-281.
35. Hutton JJ Jr,Tappel AL, Udenfriend S. Cofactor and substrate requirements of collagen proline hydroxylase. *Arch Biochem Biophys.* 1966;118(1):231-240.
36. Tuderman, L. et al. 1977. Mechanism of the prolyl hydroxylase reaction. 1. Role of co-substrates. *Eur J Biochem.* 1977;80(2):341-348.
37. Fries RB, Wallace WA, Roy S, et al. Dermal excisional wound healing in pigs following treatment with topically applied pure oxygen [published online ahead of print Aug 18, 2005]. *Mutat Res.* 2005;579(1-2):172-181.
38. Sen CK, Khanna S, Baboir BM, Hunt TK, Ellison EC, Roy S. Oxidant-induced vascular endothelial growth factor expression in human keratinocytes and cutaneous wound healing [published online ahead of print June 14, 2002]. *J Biol Chem.* 2002;277(36):33284-33290.
39. Sheikh AY, Rollins MD, Hopf HW, Hunt TK. Hyperoxia improves microvascular perfusion in a murine wound model. *Wound Repair Regen.* 2005;13(3):303-308.
40. Patel V, Chivulkula IV, Roy S, et al. Oxygen: from the benefits of inducing VEGF expression to managing the risk of hyperbaric stress. *Antioxid Redox Signal.* 2005;7(9-10):1377-1387.
41. Dimitrijevich SD, Paranjape S, Wilson JR, Gracy RW, Mills JG. Effect of hyperbaric oxygen on human skin cells in culture and in human dermal and skin equivalents. *Wound Repair Regen.* 1999;7(1):53-64.
42. Kairuz E, Upton Z, Dawson RA, Malda J. Hyperbaric oxygen stimulates epidermal reconstruction in human skin equivalents. *Wound Repair Regen.* 2007;15(2):266-274.

43. Paladini RD, Takahashi K, Bravo NS, Coulombe PA. Onset of re-epithelialization after skin injury correlates with a reorganization of keratin filaments in wound edge keratinocytes: defining a potential role for keratin 16. *J Cell Biol*. 1996;132(3):381-397.
44. Werner S, Smola H, Liao X, et al.The function of KGF in morphogenesis of epithelium and reepithelialization of wounds. *Science*. 1994;266(5186):819-822.
45. Wrobel LK, Fray TR, Molloy JE, Adams JJ, Armitage MP, Sparrow JC. Contractility of single human dermal myofibroblasts and fibroblasts. *Cell Motil Cytoskeleton*. 2002;52(2):82-90.
46. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat Rev Mol Cell Biol*. 2002;3(5):349-363.
47. Khanna S, Roy S, Maurer M, Ratan RR, Sen CK. Oxygen-sensitive reset of hypoxia-inducible factor transactivation response: prolyl hydroxylases tune the biological normoxic set point. *Free Radic Biol Med*. 2006;40(12):2147-2154.
48. Porwol T, Ehleben W, Brand V, Acker H. Tissue oxygen sensor function of NADPH oxidase isoforms, an unusual cytochrome aa3 and reactive oxygen species. *Respir Physiol*. 2001;128(3):331-348.
49. Fitzgerald RS, Shirahata M, Balbir A, Grossman CE. Oxygen sensing in the carotid body and its relation to heart failure. *Antioxid Redox Signal*. 2007;9(6):745-749.
50. Patt BT, Jarjoura D, Lambert L, et al. Prevalence of obstructive sleep apnea in patients with chronic wounds. *J Clin Sleep Med*. 2010;6(6):541-554.
51. Brahimi-Horn MC, Pouysségur J. Oxygen, a source of life and stress [published online ahead of print June 19, 2007]. *FEBS Lett*. 2007;581(19):3582-3591.
52. Prince LS. Hyperoxia and EGFL7: saving cells from too much of a good thing [published online ahead of print Nov 9, 2007]. *Am J Physiol Lung Cell Mol Physiol*. 2008;294(1):L15-L16.
53. Wang Y, Zeigler MM, Lam GK, et al. The role of the NADPH oxidase complex, p38 MAPK, and Akt in regulating human monocyte/macrophage survival [published online ahead of print Aug 24, 2006]. *Am J Respir Cell Mol Biol*. 2007;36(1):68-77.
54. Minamishima YA, Moslehi J, Bardeesy N, Cullen D, Bronson RT, Kaelin WG Jr. Somatic inactivation of the PHD2 prolyl hydroxylase causes polycythemia and congestive heart failure [published online ahead of print Dec 20, 2007]. *Blood*. 2008;111(6):3236-3244.
55. Mathieu D, ed. Handbook on hyperbaric medicine. Dordrecht,The Netherlands: Springer; 2006.
56. Sundaresan M, Yu ZX, Ferrans VJ, Irani K, Finkel T. Requirement for generation of H202 for platelet-derived growth factor signal transduction. *Science*. 1995;270(5234):296-299.
57. Kalliainen LK, Gordillo GM, Schlanger R, Sen CK. Topical oxygen as an adjunct to wound healing: a clinical case series. *Pathophysiology*. 2003;9(2):81-87.
58. Heng MC, Harker J, Csathy G, et al. Angiogenesis in necrotic ulcers treated with hyperbaric oxygen. *Ostomy Wound Manage*. 2000;46(9): 18-28, 30-32.
59. Kranke P, Bennett MH, Martyn-St. James M, Schnabel A, Debus SE, Weibel S. Hyperbaric oxygen therapy for chronic wounds. *Cochrane Database Syst Rev*. 2015;6:CD004123. doi: 10.1002/14651858.CD004123.pub4.
60. Fedorko L, Bowen JM, Jones W, et al. Hyperbaric oxygen therapy does not reduce indications for amputation in patients with diabetes with nonhealing ulcers of the lower limb: a prospective, double-blind, randomized controlled clinical trial [published online ahead of print Jan 6, 2016] *Diabetes Care*. 2016;39(3):392-399.
61. Sibbald RG, Woo KY, Queen D. Wound bed preparation and oxygen balance — a new component? *Int Wound J*. 2007;4(Suppl 3):9-17.
62. Sheikh AY, Gibson JJ, Rollins MD, Hopf HW, Hussain Z, Hunt TK. Effect of hyperoxia on vascular endothelial growth factor levels in a wound model. *Arch Surg*. 2000;135(11):1293-1297.
63. Domínguez-Rosales JA, Mavi G, Levenson SM, Rojkind M. H(2)O(2) is an important mediator of physiological and pathological healing responses. *Arch Med Res*. 2000;31(1):15-20.
64. Hunt TK, Ellison EC, Sen CK. Oxygen: at the foundation of wound healing—introduction [published online ahead of print Feb 17, 2004]. *World J Surg*. 2004;28(3):291-293.
65. Nauta, TD, van Hinsbergh VWM, Koolwijk P. Hypoxic signaling during tissue repair and regenerative medicine. *Int J Mol Sci*. 2014;15(11):19791-19815. dx.doi.org/10.3390/ijms151119791

An Evidence Based Approach to Treating Diabetic Foot Ulcerations in a Veteran Population

Authors: Howard Kimmel, DPM, MBA [1] and Jennifer Regler, DPM, MS [2]

The Journal of Diabetic Foot Complications, 2011; Volume 3, Issue 3, No. 2, Pages 50-54

Abstract:

With the advent of evidence based medicine, some physicians have decided to change their practice patterns. At our facility including both residents and students, an evidence-based algorithm for treating diabetic foot ulcers has been developed incorporating published data. Patients are initially assessed and are assigned to a low, moderate, or high risk category. Basic wound care principles are followed (off-loading , moist wound environment, debridement, and control of infection). Vascular assessment is made and if ankle-brachial indices are <0.8, an appropriate vascular referral is made. In the low risk patient, wounds are assessed and measured. If there are minimal changes after 2 weeks, therapy is changed. After 4 weeks, if the ulcer has not decreased more than 50%, a living skin equivalent, such as a single layered dermal equivalent is used. For the moderate to high risk patients, a living skin equivalent is used initially. Expeditious and complete wound healing is the definitive goal in treating DFUs. The longer the ulcer is open, the greater the chance for infection and amputation. Using an evidence based approach helps determine which patients are best suited for Advanced Therapies (Living Skin Equivalents), thereby allowing the clinician to facilitate improved outcomes in healing chronic ulcers in patients with diabetes.

Key words: Ulcer, Algorithm, Diabetes

Corresponding author:

Howard Kimmel, DPM, MBA
Louis Stokes VA Hospital
19791 East Blvd Cleveland, Ohio 44106
Email: *howard.kimmel@va.gov*

1. Director of Residency Training, Louis Stokes Department of Veterans Affairs, 10701 East Blvd., Cleveland, Ohio 44106

2. Submitted as a third year resident

Introduction

Data reported as recently as 2007 by the Centers for Disease Control (CDC), has estimated that nearly 7.8% of the US population or about 23.6 million Americans are diabetic, with the population growing at alarming rates leading to severe impacts on American society[1]. According to the CDC National Diabetes Fact Sheet, there were a reported 1.5 million new cases of diabetes in 2006 among individuals 20 years or greater, and diabetes was the 7th leading cause of death listed on 2006 US death certificates[1]. It has been further estimated that by the year 2025 nearly 300 million people worldwide will be diagnosed with diabetes[2], showing that the population of diabetic persons is expected to greatly increase over the next 15 years. Among this population, the lifetime incidence of developing a diabetic foot ulcer (DFU) has been estimated to be as high as 15%[3].

Despite the numerous available treatments, these ulcerations commonly become chronic wounds. This presents a huge burden to patients with diabetes as well as to the healthcare system, with costs estimated at nearly $13,200 per ulcer-related episode[4].

Hospitalization for ulcer care as the reason for hospital admission is $3000 a day and amputations at over $50,000 not considering the collateral risk of revision and mortality.

The most cost effective way to minimize complications is to attain wound closure as expeditiously as possible. Therefore, as the population of diabetic persons continues to rise in the future, finding a method to quickly and adequately close and/or prevent ulcerations will be of the utmost importance.

Based on the 1999 American Diabetes Association[5] consensus statement on DFU care, it is generally believed that foot ulcerations in patients with diabetes become chronic wounds due to the numerous co-morbidities compared to the average patient. Co-morbidities commonly encountered in the diabetic patient include abnormal biomechanics, vascular and/or arterial compromise, diminished protective sensation, renal disease, and altered nutritional status. These factors not only put the diabetic patient at risk for the development of ulcerations, but also impede the effectiveness of treatments.

Typically, conventional care techniques for the treatment of DFUs have focused on four major concepts: debridement of necrotic or devitalized tissue, controlling infection, offloading, and maintaining a moist wound environment. Although there may be variations as to the exact means employed, these concepts have been the basis of several published DFU treatment guidelines. In 2003, Sheehan and colleagues noted that an ulcer that fails to reduce in size by at least 50% at the 4 week mark has less than a 10% chance of closing by 12 weeks with good conventional care.[6]

The authors of this study therefore felt that achieving at least a 50% reduction in wound size in 4 weeks time could strongly predict whether a wound will go on to closure. In 2006, the Wound Healing Society published evidence-based treatment guidelines supporting the re-evaluation of wound treatment for chronic wounds that have shown less than 50% reduction in area after 4 weeks of treatment with standard care methods alone.[7] This was based on data collected in a prospective multicenter study of 203 patients with DFU's, which suggested that the four week mark is a good point to evaluate wound healing. It is at this juncture, the failure of a wound to reduce in size by 50% in 4 weeks, that Boulton et al suggested the use of additional advanced wound care products[8]. Such products include Pre-market approval (PMA) approved products, negative pressure, hyperbaric oxygen (HBO) and pulsed radio frequency therapies. Such advanced therapies could therefore be considered to achieve wound closure in a timely manner, and thus prevent any further morbidity so commonly associated with chronic DFUs.

Aims

With the advent of Evidence-Based Medicine (EBM) and the importance that this evidence has in directing patient care, many physicians have begun to take a step back and revaluate their practice patterns. Therefore, the aim of this work is to provide a reference and guidance (algorithm) to what the data indicate regarding timeliness and treatment options, realizing that no two patients are the same and all care should be individualized to the patient

Methods

Utilizing the evidence that has been published, our algorithm (Figure 1) has been developed and is currently being used for the treatment of numerous DFUs. The systematic approach begins with initial patient assessment in which patients are classified, based on clinical criteria, as being either low risk or moderate to high risk DFU patients. Low risk DFU patients are generally patients who develop new foot ulcerations, without a previous history of ulcerations, show no evidence infection being present, and who have documented palpable pedal pulses. Patients that fall into the moderate to high risk category tend to be patients with wounds probing to bone, ulcerations greater than 30 days duration, or patients with additional co-morbidities including renal disease, a previous history of ulceration or amputation, an elevated HbA1c, and decreased albumin/prealbumin levels.

Anyone with one or a combination of these factors is someone who may be at a higher risk for experiencing a non-healing ulceration and therefore, may have a greater chance of developing a serious complication.

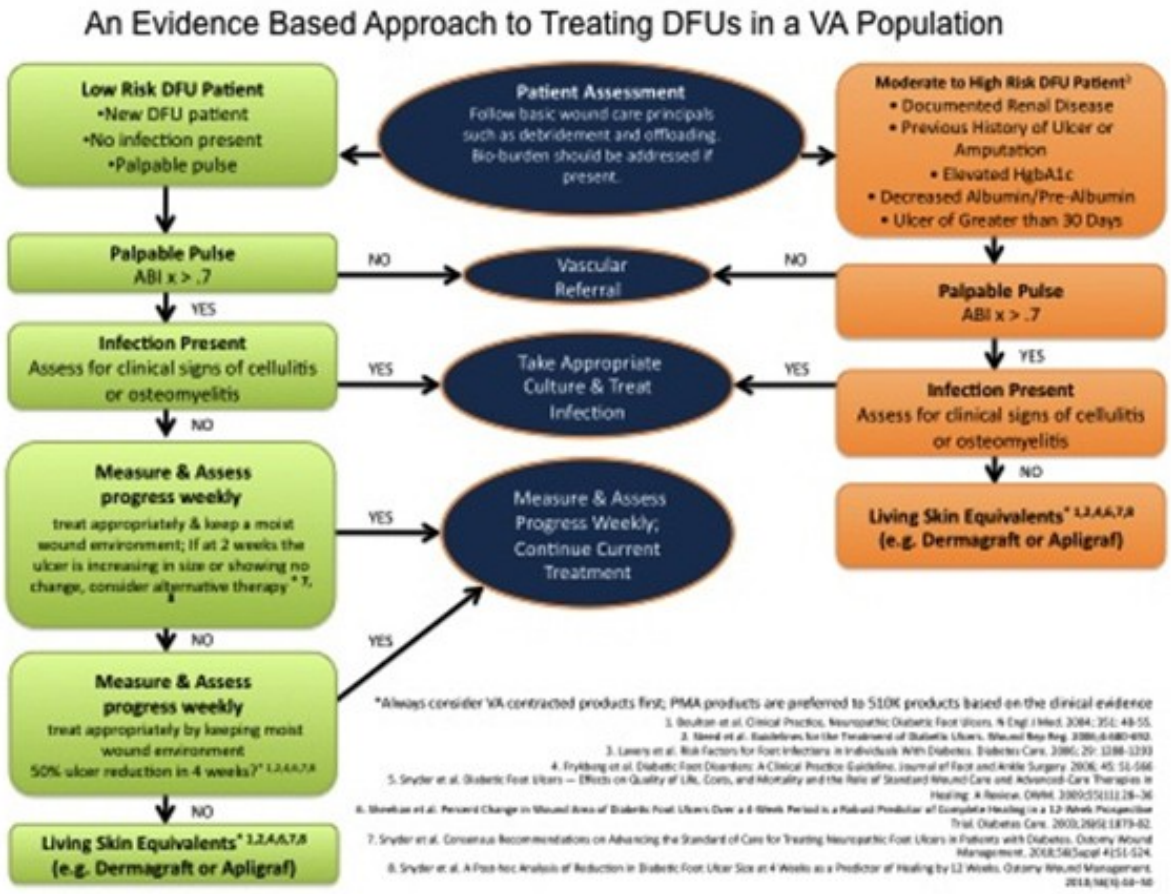

Figure 1: Evidence Based Approach Algorithm to treating DFUs

After the initial patient assessment, a complete medical history and exam along with a comprehensive lower extremity exam is performed. The lower extremity exam includes a visual assessment of the lower extremity, vascular assessment with a Doppler probe, and a neurological exam, including 10-G monofilament assessment, vibratory sensation, proprioception, and reflex testing. , The orthopedic exam includes testing of muscle strength, gait analysis, range of motion of the foot and ankle, as well as visual inspection for any structural deformities, such as bunions or hammertoes. From this history and physical assessment, patients can be assigned a risk category which will direct what treatment path to follow. Basic wound care principles are followed for both groups and include debridement of necrotic and devitalized tissue, infection control, offloading of the ulceration, and maintenance of a moist wound environment. Throughout the treatment, vascular assessment is made and monitored. For any patient with an ankle brachial index (ABI) measurement of less than 0.8, an

appropriate vascular referral is made. Infection is also closely monitored and patients are assessed at each visit for signs of cellulitis or osteomyelitis. Controlling infection is extremely important as several studies have found infection to be strongly correlated with increased risk of amputation[9]. In fact, a large cohort study conducted by Lavery and colleagues (2006) found that an infected DFU increased the risk of hospitalization by nearly 56 times and amputation by nearly 155 times.[10] Interestingly, all independent risk factors for infection identified in the study mirror the at-risk comorbidities or patient history (ulcer probing to bone, ulcer history of greater than 30 days, peripheral vascular disease, recurrent ulcer and traumatic etiology) that place our Veterans in a moderate to high risk category. One thing that also needs to be considered is that many of our patients present with multiple risk factors that multiply their risk for complications. Signs or symptoms of infection that most commonly present with DFUs include: increased redness, increased warmth, swelling, purulent exudate, increased pain or tenderness, and constitutional symptoms (nausea, vomiting, fever, chills). With the development of these symptoms, wound cultures are taken and confirmed infections are treated with appropriate measures.

For low risk patients, the algorithm specifies that wounds are measured and progress is assessed weekly. Wounds are treated appropriately following conventional wound care guidelines, and, if at 2 weeks the ulcer is increasing in size or showing no change, an alternate form of therapy may be considered. As long as the wound continues to show weekly progress, the current form of treatment is continued. At 4 weeks, if the wound does not show at least 50% reduction in ulcer area, an advanced form of therapy, such as a living skin equivalent (LSE; i.e. Dermagraft® or Apligraf®), is recommended due to the stagnant nature of the wound. Again, as long as the wound shows at least 50% reduction in area in 4 weeks, the wound is measured or assessed weekly and the current modality of treatment is continued. For moderate to high risk patients, the algorithm outlines that in these patients an advanced form of therapy, such as an LSE, should be used initially as long as infection is controlled and appropriate vascular status is present. While these moderate to high risk patients are often excluded from Food and Drug Administration (FDA) clinical trials, we have seen good success with a human fibroblast derived dermal substitute (i.e. Dermagraft®) at closing ulcers and reducing complications.

The typical requirement for FDA approval is to demonstrate a 12-week closure mark significantly faster than conventional wound care. The LSEs have demonstrated faster closure when used weekly per FDA approvals to treat DFUs. They have also proven to reduce the complications such as infection and amputation. Negative pressure and pulsed radio frequency are not approved under the PMA process because they are not intended to provide direct closure. There is also reported clinical experience in using the human fibroblast-derived dermal substitute in combination with both therapies to promote closure in patients with exposed bone and deep wounds.[11,12] Collagen-based products and extracellular matrix products are considered alternative dressings because they provide collagen to the wound. While they can be beneficial to some patients they have not demonstrated faster closure than wet-dry dressings in FDA approved trials.

Results

Within our clinic, we have noticed that by employing this evidence based algorithm, we have been able to significantly reduce our closure time of chronic DFUs. By expediting the rate of closure, we have been able to reduce the infection rate, decrease the level of hospitalizations due to complications of chronicity, and reduce the overall number of clinic visits in our diabetic patient population.

Conclusion

Expeditious and complete wound healing is the definitive goal in the treatment of DFUs. The longer a wound remains open, the greater the risk of complications, such as infection and subsequent amputation. Using an evidence-based approach helps determine which patients and when those patients are best suited for advanced therapies such as LSEs. This therefore allows the clinician to facilitate improved outcomes in healing chronic ulcers in patients with diabetes. By following this algorithm it is possible to increase closure rate of DFU's, decrease complications associated with chronic ulcers, as well as prevent future amputations which often are the result of longstanding DFUs.

References

1. Centers for Disease Control and Prevention. National Diabetes Fact Sheet: general information and national estimates on diabetes in the United States. 2007.

2. Snyder R, Cardinal M, Dauphinee D, et al. A post-hoc analysis of reduction in diabetic foot ulcer size at 4 weeks as a predictor of healing by 12 weeks. Ostomy Wound Manage 2010;56(3):44-50.

3. Sanders, LJ. Diabetes mellitus: prevention of amputation. JAPMA 1994; 84:322-328.

4.Stockl K, Vanderplas A, Tafesse E, et al. Costs of lower-extremity ulcers among patients with diabetes. Diabetes Care 2004;27(9):2129-2134.

5. American Diabetes Association. Consensus Development conference on diabetic foot wound care. Diabetes Care. 1999 ; 22 1354-1356

6. Sheehan P, Jones P, Caselli A, et al. Percent change in wound area of diabetic foot ulcers over a 4-week period is a robust predictor of complete healing in a 12-week prospective trial. Diabetes Care 2003;26:1879-1882.

7. Steed DL, Attinger C, Colaizzi T, et al. Guidelines for the treatment of diabetic ulcers. Wound Repair Regeneration 2006;14:680-692.

8. Boulton AJ, Kirsner RS, Vileikyte L. Clinical practice. Neuropathic diabetic foot ulcers. N Engl J Med 2004;351:48–55

9. Snyder RJ and Hanft JR. Diabetic foot ulcers - effects on quality of life, costs, and mortality and the role of standard wound care and advanced care therapies in healing: a review. Ostomy Wound Management 2009;5(11):28-38.

10. Lavery LA, Armstrong DG, Wunderlich RP, et al. Risk factors for foot infections in individuals with diabetes. Diabetes Care 2006;29(6):1288-1293.

11. Frykberg RG, Tierney E, Tallis A, Klotzbach T: Cell Proliferation Induction: Healing Chronic Wounds Through Low-Energy Pulsed Radiofrequency. Int J Low Extrem Wounds. 8:45-51, 2009

12. Frykberg R, Martin E, Tallis A, Tierney E. A case history of multimodal therapy in healing a complicated diabetic foot wound: negative pressure, dermal replacement and pulsed radio frequency energy therapies. Int Wound J 2011; 8:132–139

An Evidence-Based Algorithm for Treating Venous Leg Ulcers Utilizing the Cochrane Database of Systematic Reviews

Howard M. Kimmel, DPM, MBA, FACFAS[1,2]; and Angela L. Robin, DPM[1]

WOUNDS 2013;25(9):242-250

From [1]Louis Stokes Cleveland VA Medical Center, Cleveland, OH; [2]Case Western Reserve University School of Medicine, Cleveland, OH

Address correspondence to:
Howard M. Kimmel, DPM, MBA, FACFAS
Louis Stokes Cleveland VA Medical Center
10701 East Boulevard
Cleveland, OH
buckeyefootcare@sbcglobal.net

Disclosure: The authors disclose no financial or other conflicts of interest.

Abstract: *Background*. This literature review serves to develop an evidence-based algorithm for the treatment of venous ulcerations and the development of a guideline to systemically treat venous leg ulcerations (VLUs) that may improve outcomes, restore function of the affected limb, and reduce health care costs. *Methods*. The Cochrane Database and PubMed search engine were utilized to accumulate literature concerning venous ulcerations and their treatment. The most relevant literature was reviewed to develop an algorithm to guide treatment of VLUs. *Results*. An algorithm was established outlining the use of compression therapy in VLUs present for < 4 weeks. If a wound is present after 4 weeks of therapy and has not reduced in size by ≥ 40%, bilayered living skin equivalents may be indicated. *Conclusion*. An algorithm was established to guide the treatment of venous ulcerations. By utilizing a systematic approach in treating VLUs, clinical outcomes may be improved.

Key words: evidence-based algorithm, venous leg ulcers, chronic venous disease

The treatment of chronic venous disease (CVD) and its complications can be frustrating. Chronic venous disease can be defined as an abnormally functioning venous system caused by venous valvular incompetence with or without associated venous outflow obstruction. Venous leg ulcers (VLUs) are defined as an area of discontinuity of epidermis and dermis on the lower leg, persisting for 4 weeks or more.[1] The occurrence of venous leg ulcer is strongly associated with venous disease (eg, varicose veins and deep vein thrombosis) contributing to sustained venous hypertension; arterial disease is present (alone or in combination with venous disease) in approximately 20% of cases.[2] The etiology of VLUs includes inflammatory processes resulting in leukocyte activation, endothelial damage, platelet aggregation, and intracellular edema. Other factors contributing to VLUs include immobility, obesity, trauma, vasculitis, older age, diabetes, and neoplasia.[3] Outflow obstruction, valvular obstruction, and venous hypertension contribute to venous ulceration risk. Arterial and ischemic ulcerations generally occur on the anterior tibia, lateral leg, and distal toes, all areas which are susceptible to trauma. The

inability to heal these wounds stems from vascular congestion and artherosclerotic changes, particularly in the feet and toes.[4] The accepted statistics indicate that VLUs require an average of 24 weeks to heal; approximately 15% never heal; and recurrence is found once or multiple times in 15%-71% of cases.[5,6] In reported populations of venous ulcers, 15%-71% are found to be recurrent lesions.[7] Healed ulcerations possibly can have a 5-year recurrence rate as high as 40%.[8]

Venous leg ulcers are a common chronic recurring condition and a major cause of morbidity and disability. Epidemiological evidence suggests that approximately 1% of the United States (US) adult population, or about 3 million Americans, have VLUs.[9,10] The prevalence of VLUs increases with age, with rates of about 8% in patients > 80 years.[11] Approximately 1.7% of persons > 60 years develop a new VLU within 2 years.[12]

Venous leg ulcer outcomes are optimized when patients receive multidisciplinary care and evidence-based wound management.[13,14] Dermatology, geriatrics, podiatry, and surgery are just a few specialties that may be utilized to improve outcomes.[15] Adherence to multidisciplinary guidelines was associated with 6.5-fold and 2.5-fold increases in the likelihood of healing among US and British patients with VLUs, respectively.[16] Significant decreases in healing time and costs were also associated with guideline adherence. Among veterans with VLUs, those who receive guideline-concordant wound care are 2.5 times more likely to achieve wound healing than are those who receive non-concordant care.[17]

Several comprehensive clinical guidelines for the diagnosis and management of VLUs have been developed in recent years, but the widespread implementation of evidence-based VLU management has not been achieved. Common barriers to the adoption of VLU consensus guidelines include misdiagnosis, under-recognition of VLUs, inadequate training, absence of structured care delivery plans, and lack of coordination among providers.[18,19] The costs of VLUs include direct costs associated with medical resource utilization, indirect costs related to loss of productivity, and patient impact.[19] In 2006, Khan and Davies[20] stated that the direct treatment costs of VLUs in the US are about $1 billion annually, and that the average lifetime cost of VLUs for 1 patient exceeds $400,000. In 2011, O'Donnell and Balk[21] wrote "the management of VLUs consumes considerable resources in health care systems and accounts for up to 1% of health care budgets in some industrialized countries." The indirect costs of VLUs are primarily due to time lost from work because of illness or disability. Since the treatment of VLUs often involves multiple office visits for debridement, dressing changes, and other procedures, and VLU may be associated with significant loss of productivity and ability to engage in leisure activities, these costs are likely to be substantial.

Gelfand et al[22] conducted a large cohort study examining 56,488 venous ulcerations. A venous ulceration was defined in the study as a chronic wound of the lower extremity in the gaiter area. These wounds were less than 2 cm in depth and did not involve tendon, ligament, or bone and were less than 150 cm^2. The study concluded that change in wound area at 4 weeks was a strong indicator of healing at 12 weeks or 24 weeks. When examining full thickness ulcerations however, van Rijswijk[23] found that > 30% reduction in ulcer area at 2 weeks of treatment was a predictor of both treatment outcome and time required for healing. The depth of the ulceration is an important consideration as full thickness wounds take longer to heal.[24] When comparing partial thickness venous ulcerations and full thickness ulcerations, full thickness wounds take approximately twice as long to heal.[24]

An additional factor in predicting healing time and potential is ulcer duration. Margolis et al[25] evaluated 260 patients over a 2-year period with chronic venous ulcerations. The patients received weekly multilayered compressive dressings. The study found that those wounds that were < 5cm^2 and those ulcerations present for < 6 months were more likely to heal by week 24. The multilayered compressive dressings healed 85% and 88% of these wounds, respectively.[25]

Comorbid illnesses are common in patients with VLUs and may contribute to delayed wound healing and an increased risk of VLU recurrence.[26,27] Performing a comprehensive clinical history and physical examination is critical to the identification of underlying comorbidities and provides important information regarding the etiology of VLUs. Management decisions in patients with chronic VLUs are often influenced by comorbidities. Factors such as obesity, malnutrition, intravenous drug use, and coexisting medical conditions may affect prognosis and suitability for invasive and noninvasive interventions. When VLUs fail to respond to treatment or heal in a timely manner, clinicians should consider further diagnostic investigations and referral to specialists to identify occult etiologies and ensure underlying comorbidities are being adequately addressed through a multidisciplinary approach.

The purpose of this review is to establish an evidence-based algorithm for treating venous ulcerations by utilizing a systematic review of the Cochrane Database.

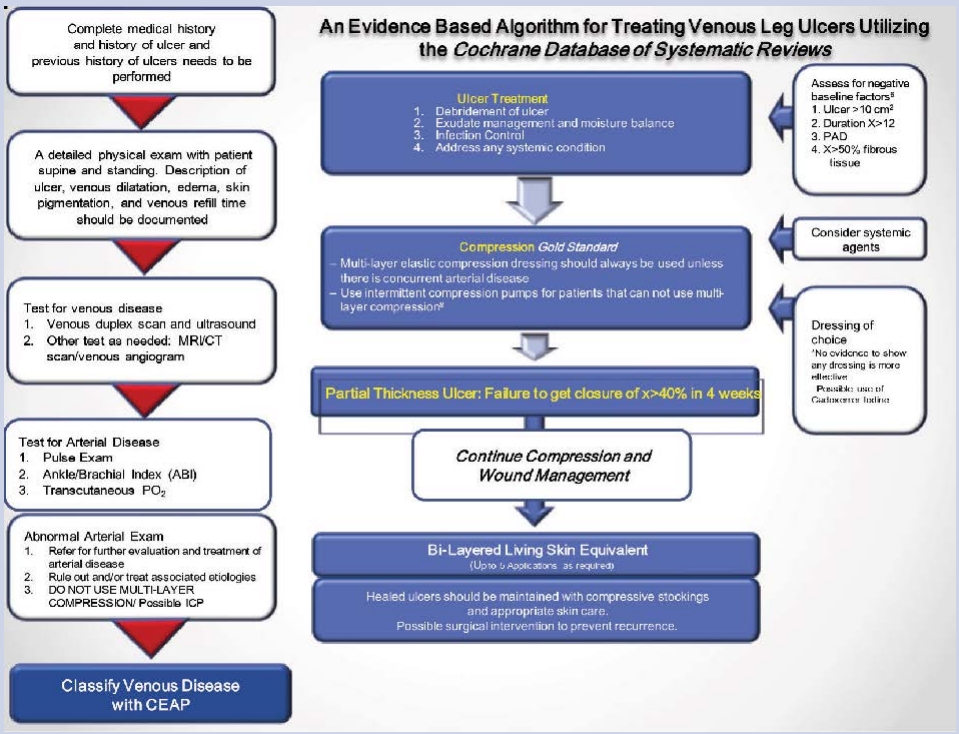

Figure 2. Venous leg ulcer treatment algorithm.

Methods

The algorithm is based on information obtained from multiple sources including the Cochrane Database of Systematic Reviews, the Clinical-Etiology-Anatomy-Pathophysiology (CEAP) classification system, and large VLU cohort studies. In reviewing literature for this algorithm, the Cochrane Database and PubMed were searched. Specific key words included in the search were"VLU algorithm,""history of venous ulcers,""treatment of venous ulcers,""factors affecting VLUs,""CEAP classification," and "VLU dressings." Literature from 1990 to present were included.Cochrane Database revealed 27 publications and the most relevant articles were reviewed.The opinions of clinicians knowledgable in the treatment of VLUs and the most common treatment modalities were used to determine relevance of the modalities chosen. Minimal and low randomized control trials were excluded from the review.

The Cochrane Database of Systematic Reviews is an independent and collaborative source of information about the effects of health care interventions, prepared by > 28,000 independent contributors working in > 100 countries.

Venous Leg Ulcer Treatment Algorithm

Over the years,clinicians have been faced with numerous treatments for VLUs. Some current products and treatments have minimal to no evidence to show that they are effective.The goal of the algorithm is to treat VLUs with the best evidence to close them sooner and more cost effectively. The VLU treatment algorithm is shown in Figure 1.

The CEAP classification system was originally developed in 1994 by an international ad hoc committee of the American Venous Forum and adopted worldwide to facilitate meaningful communication about CVD, and to serve as a basis for scientific analysis of CVD and treatment options.[28] As shown in the algorithm, the management of a patient with a suspected VLU begins with a comprehensive medical history and detailed physical examination.The medical history should include documentation of previous manifestations of CVD and previous and current ulcers.Preceding episodes of malignancy, vasculitis, collagen-vascular diseases, and dermal manifestations of systemic diseases should be identified. Suspected chronic VLUs that increase in size after debridement, or are excessively painful, should be reevaluated for possible underlying etiologies.

An important goal of the physical examination is to document or exclude the presence of CVD. Physical examination should include evaluation of ulcers, venous dilatation, edema, skin pigmentation, and venous refill time.A detailed history should be performed, especially if there is a healed or active ulcer. If there is a current venous ulcer, a physical exam with descriptive terms is important.The exam should be done with the patient both supine and standing.

Venous dilatation should be described and examined by both visualization and palpation. Description of a dilated vein can range from telangiectases to reticular veins to varicose veins.

Edema indicates the disease has progressed and is functionally advanced. The extent of the edema should be described and the limbs should be circumferentially measured.

Skin pigmentation changes, such as venous eczema and lipodermatosclerosis,are important signs of severe chronic disease. Any and all pigmentation changes should be described.

Venous ulceration is the sign of the most advanced disease. The location and the measurement of the ulcer should be well described,along with any healed ulcers that have scarring.

Venous refill time provides an overall measurement of venous reflux.Venous leg ulcers may exist in the presence of mixed arterial/venous pathology, and treatment of only the elevated venous pressure will be unsuccessful when significant arterial disease is present. Gross arterial disease should be ruled out by establishing that pedal pulses are present on physical examination and/or that the ankle brachial index (ABI) is > 0.8. If concurrent arterial disease is present, this should be evaluated and addressed.When present, patients should not have traditional compressive dressings.The complexity of the diagnostic work-up is influenced by the severity of the clinical problem and the degree of disability. An international consensus conference was held in 1994 at the American Venous Forum to develop a new classification system of CVD. The CEAP classification was developed and implemented,[29,30] and is broken down into 4 components:

*Clinical classification.*There are 7 clinical classes from 0 to 6, with 0 indicating no disease; 1 indicating signs of telangiectasia or reticular veins; 2 indicating varicose veins; 3 indicating edema without skin changes; 4 indicating skin changes associated with venous disease; 5 indicating skin changes with healed ulcers; and 6 indicating skin changes with active ulceration.

*Etiologic classification.*The type of dysfunction is classified as either congenital, primary, or secondary. Congenital dysfunctions are noted at birth, but don't manifest until later in life. Primary dysfunction is of an unknown cause, while secondary dysfunction is an acquired condition such as deep vein thrombosis.

Anatomic classification. Anatomic sites of venous disease are either superficial, deep, and/or perforating. One system or all systems simultaneously can be involved in the same time.

*Pathophysiologic classification.*Signs or systems of CVD result from reflux, obstruction, or both. This classification system is very detailed and can be used to direct treatment for surgical vs conservative treatment.The one fault of the system is that there is no classification for other concurrent conditions that might affect the severity or treatment of CVD. Important considerations for other diseases, such as diabetes and lymphedema,need to be taken into account because they might affect the treatment and healing times.

Keypoints

- An important goal of the physical examination is to document or exclude the presence of chronic venous disease.
- The exam should be done with the patient both supine and standing, and include an assessment for venous dilatation, edema, skin pigmentation, venous ulceration, and venous refill time.

The diagnostic tests useful in CVD have been classified into 3 levels: I = office testing (eg, history, physical examination, and continuous-wave ([handheld] Doppler studies); II = vascular laboratory (eg, duplex scanning, plethysmography, and venous pressure); and III = phlebography (eg, ascending and descending phlebography and varicography). All patients should undergo level I diagnostic studies, in which the minimal degree of objective testing is achieved by the continuous-wave Doppler examination. Level II diagnostic investigations are done for patients with the simplest and most straightforward problems, and level III diagnostic studies are reserved for difficult cases and preoperative planning, especially for patients undergoing deep venous reconstruction.

Baseline clinical features of VLUs can help identify patients who are likely to respond to conservative treatment and those who may require more aggressive interventions. Margolis et al[31] analyzed a dataset of more than 20,000 patients with VLUs treated with lower limb compression therapy to determine the accuracy of several prognostic models. Initial measures of wound size and duration accurately identified patients who were likely to heal by the 24th week of care. For example, a wound < 10 cm^2 and < 12 months old at the first visit has a 29% chance of not healing by the 24th week of care, while a wound > 10 cm^2 and > 12 months old has a 78% chance of not healing. These criteria may help wound care providers decide when to consider using conservative treatments only, or in addition to, adjuvant therapies early in the course of VLU treatment.

Treatment

The treatment options can be broken down to 5 categories: compression, local wound care, surgical intervention, medical treatment, and advanced technology. Basic wound care principles also need to be followed, such as proper wound environment, control of clinical signs of infection, and debridement. In a recent review of the impact of debridement on healing of VLUs, ulcer surface area reduction was greater in visits after debridement.[32] Attention should

be paid to removal of all necrotic tissue, densely adherent slough and exudates, and reshaping of the ulcer margins.

Compression Therapy

The cornerstones of the VLU management algorithm are wound debridement; management of exudate; and wound moisture, infection control, and management of concurrent systemic conditions. Lower limb compression is the standard of care for patients with VLUs without concurrent arterial disease and provides the basis for the initial treatment recommendation in the VLU algorithm. In 2009, the Cochrane Database reported an extensive evaluation of the clinical effectiveness of compression bandage or stocking systems in the treatment of VLU.[33]The analysis was designed to determine if the application of compression bandages or stockings aid VLU healing,and if so,which compression bandage or stocking is the most effective.A total of 39 randomized clinical trials that evaluated any type of compression bandage system or compression hosiery were included in the analysis.The evidence strongly suggests that VLUs heal more rapidly with compression than without,and that multicomponent compression achieves better healing outcomes than single-component systems. When competing systems comprising 2 components were compared, there was some evidence suggesting those including an elastic component may be more effective than those composed mainly of inelastic constituents; a similar finding was noted for alternative 3-component systems.[33]

A substantial proportion of patients with VLUs are not helped by compression bandaging, or are unwilling or unable to wear it.Other patients with VLUs may be unsuitable candidates for compression bandaging due to concurrent arterial disease. Intermittent pneumatic compression (IPC) is an alternative method of delivering compression that utilizes an air pump to periodically inflate/deflate bladders incorporated into sleeves applied to affected limbs. Multiple techniques for providing IPC are available using single or multiple chambers/bladders, different types of pumps and compression cycles, and variations in inflation and deflation times.

Clinical evidence of the effectiveness of IPC in increasing healing rates in patients with VLUs was extensively reviewed by Nelson et al[34] and reported in the Cochrane Database.A total of 7 randomized controlled trials including 367 patients were included in the analysis. Compared with no compression,IPC was associated with a 2.27-fold increase in the likelihood of VLU healing.Trials of IPC and compression vs compression alone provided inconsistent results,with no differences in healing rates reported in some trials,and modest benefits of combination treatment reported in others. Rapid IPC was associated with greater VLU healing rates and shorter time to complete VLU healing than slow IPC. No significant differences in pain scores were observed between patients receiving IPC and those treated with Unna's boot.

Dressings

Wound dressings are usually applied beneath the compression to aid healing, enhance comfort, prevent adherence of the bandage to the ulcer, and control exudate. A wide variety of dressing products and types are available including hydrocolloids,foams, alginates,hydrogels, and others.A Cochrane review of 42 randomized controlled studies with a total of more than 1000 patients found no evidence that any one dressing type was better than others in terms of number of ulcers healed.[35] Furthermore, the more expensive hydrocolloid dressings were not shown to provide healing benefits over the lower-cost simple nonadherent dressings.Without clear evidence to support the use of one dressing over another, the choice of dressings for VLUs can be guided by cost,ease of application,and patient and physician preferences.

Studies have shown that modern dressings, particularly if the wound is < 4 weeks old, do not provide a significant improvement in healing rates of chronic venous ulcerations.[36] Chaby et al[36] concluded that only a weak level of evidence existed for clinic efficacy of products such as hyaluronic acid, hydrogels, and silver-impregnated products when compared to saline or paraffin gauze.Statistical significance in wound healing did not occur in venous ulceration healing until week 6 in a literature review by Kerstein et al.[37] Impregnated gauze, hydrocolloid dressings, and human skin constructs were evaluated, and it was concluded that advanced products may not be cost-effective in early treatment. Cambal et al[38] conducted a small study of 20 patients with chronic venous ulcerations with compressive sclerotherapy and maggot debridement therapy. In these patients, 95% showed a significant clinical improvement. A review conducted by Simms and Ennen[39] determined that no dressing was superior to another, and compression is the necessary gold standard of treatment.

Systemic Therapy

The use of systemic agents should be considered in patients with chronic or recurrent VLUs and in those with negative prognostic factors. Systemic agents may be used alone or in combination with compression and other mechanical modalities. Despite a number of studies designed to examine the efficacy and safety of adjunctive systemic therapy

in patients with VLUs, and possibly as monotherapy, the cost-effectiveness of this approach has not been established. Pentoxifylline is an inhibitor of platelet aggregation which reduces blood viscosity and, in turn, improves microcirculation.The Cochrane Database reported an extensive review of randomized trials comparing pentoxifylline with placebo or other therapy in the presence or absence of compression in patients with VLU.[40] The authors found that pentoxifylline is more effective than placebo in terms of complete ulcer healing or significant improvement (RR, 1.70). Pentoxifylline plus compression proved more effective than placebo plus compression (RR, 1.56), and pentoxifylline in the absence of compression was more effective than placebo or no treatment (RR 2.25). More adverse effects were reported in patients receiving pentoxifylline (RR 1.56) and most of the reported adverse effects were gastrointestinal. [40]

Like pentoxifylline therapy, aspirin (300 mg per day) combined with compression therapy has been shown to decrease ulcer healing time and reduce ulcer size compared with compression therapy alone.[41] The therapeutic role of aspirin in VLUs is supported by observed increases in levels of fibrinogen, coagulation factor VIII, von Willebrand factor, and plasminogen activator inhibitor-1 in patients with VLUs compared with healthy controls.[42] The addition of aspirin therapy to compression bandages may be useful in the treatment of VLUs as long as there are no contraindications to its use.

Bacterial colonization and superimposed bacterial infections are common in VLUs and contribute to poor wound healing. However, a recent Cochrane Review of 22 randomized control trials of systemic and topical antibiotics and antiseptics for VLU treatment found no evidence that routine use of oral antibiotics improves healing rates.[43] Oral antibiotics may be indicated in patients with VLUs and suspected cellulitis. Suspected osteomyelitis warrants an evaluation for arterial disease and consideration of intravenous antibiotics to treat the underlying infection.Only topical cadexomer iodine showed promising results. [43]

Oxygen is essential to wound healing.Local tissue hypoxia,caused by disrupted or compromised vasculature,is a key factor that limits wound healing.[44] Clinical use of oxygen to promote wound healing began in the 1960s with the administration of systemic full body hyperbaric oxygen therapy (HBOT) to treat wounds.[45] Today, HBOT is usually administered in single- or multiplace chambers utilizing pressures of 2,500 mb and higher.There has been only 1 study on VLUs that indicated a significant reduction in wound area at 6 weeks following the administration of HBOT.[46] The problem with HBOT is the possible complications such as damage to the ears,sinuses,and lungs from the effects of pressure,temporary worsening of short-sightedness, claustrophobia, and oxygen poisoning.Although serious adverse events are rare, HBOT cannot be regarded as an entirely benign intervention. Furthermore, as an adjunct to standard therapy HBOT may be associated with increased costs,and any cost/benefit advantage should be carefully considered.[47]

Treatment Response

The "4-week" Model. The initial healing rate of VLUs and the percentage change in the ulcer area after treatment initiation have been shown to predict ulcer healing.[48] The use of a valid surrogate marker for complete VLU healing may allow for the identification of patients who are not likely to heal by standard methods early in the course of treatment, thereby allowing for expedited referral to specialty centers or the earlier initiation of advanced wound healing therapies.

The VLU treatment algorithm recommends > 40% wound closure after 4 weeks of conventional therapy as a surrogate marker for the identification of patients who are likely to achieve complete wound closure with continued conservative treatment. Patients with < 40% closure at 4 weeks are unlikely to achieve complete wound healing and may benefit from alternative or advanced interventions.[49]

The algorithm recommendation is based on an analysis of wound characteristics and healing rates in 29,189 patients with 56,488 VLUs.[49] The median wound size was 189 mm^2 and the median wound duration was 3 months. By the 12th week of care, 45.2% of patients had healed.Those that healed had smaller wounds at baseline and wounds of shorter duration as compared with those that did not heal (all P values < 0.001).The continuous surrogates percent change in wound area, log healing rate, and log area ratio at weeks 2, 4, and 6 were shown to discriminate between a wound that healed by 12 weeks of care and one that did not. The 4-week surrogate maximized accuracy and minimized the time to surrogate endpoint. Dichotomization of the surrogate markers at week 4 demonstrated that a wound's healing status at 24 weeks can be correctly classified at a rate of 66%-69% depending on the marker utilized. These surrogates were further validated by demonstrating that established risk factors for not healing, such as wound size and wound duration, are also important risk factors for not achieving the surrogate endpoint.

Skin autografts, allografts, and xenografts. A Cochrane Review was conducted to assess the effect of various skin grafts for treating VLUs.[50] The types of skin grafts examined in this review included autografts (from the patient's own

skin), fresh or frozen allografts (from other human sources), and xenografts (from pigs).

The review found that the randomized controlled trials were of generally poor methodological quality, characterized by flaws including lack of reported inclusion criteria, unclear descriptions of randomization methods,lack of baseline comparability,and lack of blinded outcome assessments. The authors concluded there was not enough evidence to recommend any of these types of grafts for the treatment of VLUs. It was recommended that further research be conducted to improve methods for identifying patients amenable to treatment with skin grafts and to assess whether skin grafts increase healing for VLU patients.

*Bilayered and single-layered bioengineered cellular technologies.*The Cochran Review of skin grafting for VLUs also examined the available evidence for bioengineered cellular technologies.[50] These advanced technologies feature living human cells and differ from traditional skin grafts in that they do not engraft or persist long-term, but instead delivery a cascade of growth factors and cytokines that stimulate healing in the recipient.

The single layer technology (Dermagraft, Shire Regenerative Medicine, Inc, San Diego, CA) contains only the dermal component and is comprised of human fibroblasts seeded onto a vicryl mesh. The Cochrane Review analyzed data from 2 single-layer technology VLU trials which employed different dosage regimes (1 piece, 4 pieces, and 12 pieces) and found there was no evidence of benefit associated with any of these dosage protocols.

The bilayered living cellular construct (Apligraf, Organogenesis, Inc, Canton, MA) contains 2 layers of living human cells—an epidermal layer of differentiated keratinocytes and a dermal layer of fibroblasts in a collagen matrix.The safety and efficacy of this bilayered living cellular product in treating VLUs was evaluated in a large prospective randomized controlled trial where patients were eligible to receive up to 5 applications.[51] The results showed a significantly higher proportion of ulcers healed in the Apligraf treatment group, and also reported a shorter time to complete healing. Based on these findings, the authors of the Cochrane Review concluded that applying a bilayered living cellular construct with compression increases the chance of healing a venous ulcer compared to compression alone. Based on these Cochrane conclusions, the algorithm recommends applying bilayered living cellular constructs to VLUs that failed to reduce in size > 40% following 4 weeks of conventional care.

Surgical therapy. Direct surgical intervention may be helpful in patients with VLUs not responding to conservative management, but is generally performed to decrease the likelihood of VLU recurrence. In patients with first-time VLUs, healing rates with surgery are comparable to those achieved with conservative treatment. The benefits of surgical treatment may outweigh those of conservative treatment in patients with recurrent VLUs. Other factors favoring surgical intervention include medial ulceration, older age, and larger VLU size.Direct surgical intervention on the deep venous system is generally reserved for patients who do not respond to treatment of the superficial system or are not candidates for superficial venous intervention.

Surgical correction of superficial venous reflux does not increase healing rates in patients with VLUs receiving compression therapy. In 500 patients with open or recently healed VLUs and superficial venous reflux, healing rates at 3 years were 89% for the compression group and 93% for the compression plus surgery group ($P = 0.73$).[52] Rates of ulcer recurrence at 4 years were 56% for the compression group and 31% for the compression plus surgery group ($P < 0.01$). Patients receiving compression plus surgery experienced significantly longer absolute (100 weeks vs 85 weeks, $P = 0.013$) and proportional (78% vs 71%, $P = 0.007$) ulcer-free time up to 3 years compared to those receiving compression alone. These findings support the role of surgery and compression therapy in patients with chronic wounds. Surgical correction of superficial venous reflux in addition to compression bandaging reduces the recurrence of VLUs at 4 years and results in a greater proportion of ulcer-free time.

Maintenance therapy. Appropriate maintenance therapy following healing of VLUs may help prevent the occurrence of new VLUs and reduce the incidence of ulcer recurrence. Well-designed randomized controlled trials of maintenance strategies following VLU healing are rare. Maintenance treatment with compressive stockings and appropriate skin care should be considered in all patients with healed VLUs. The identification of patients who are likely to benefit from posthealing VLU surgery is difficult. It is unclear which type of compression stocking may be most suitable for maintenance therapy, and the choice may be based on cost issues and patient and provider preferences.Further studies,including economic evaluations,are needed to help determine the optimal maintenance strategies in patients with VLUs.

Functional restoration. Patients with VLUs experience significant functional impairment including loss of mobility, decreased work capacity,limitations in leisure activities, and challenges with activities of daily living. In addition to ulcer healing and prevention of recurrence, functional restoration, defined as a return to pre-VLU levels of activity, may be an appropriate endpoint in VLU clinical trials and a useful

Keypoints

- The venous leg ulcer treatment algorithm recommends > 40% wound closure after 4 weeks of conventional therapy as a surrogate marker for the identification of patients who are likely to achieve complete wound closure with continued conservative treatment.
- Patients with < 40% closure at 4 weeks are unlikely to achieve complete wound healing and may benefit from alternative or advanced interventions.[49]

marker of VLU treatment success. From the patient's perspective, pain relief and restoration of functional capacity may be the most important outcomes of VLU treatment. Providers should monitor changes in functional capacity during VLU treatment and consider lack of functional restoration as a possible marker of inadequate treatment.

Conclusion

This paper describes an evidence-based algorithm for the treatment of VLUs. The algorithm is based on current and unbiased analysis of randomized clinical trials. Widespread implementation of the VLU treatment algorithm has the potential to improve outcomes, restore function, and reduce costs associated with VLU.

Previous Presentation

Poster presented at: The National 2012 American Podiatric Medical Association Annual Scientific Meeting; August 16-19, 2012; Washington DC.

References

1. Royal College of Nursing Institute. *The Management of Patients with Venous Leg Ulcers: Recommendations for Assessment, Compression Therapy, Cleansing, Debridement, Dressing, Contact Sensitivity, Training/Education and Quality Assurance*. London: Royal College of Nursing, 1998.
2. O'Brien JF, Grace PA, Perry IJ, Burke PE. Prevalence and aetiology of leg ulcers in Ireland. *Ir J Med Sci*. 2000;169(2):110-112.
3. Simon DA, Dix FP, McCollum CN. Management of venous leg ulcers. *BMJ*. 2004;328(7452):1358–1362.
4. McCulloch JM, Kloth LC. *Wound Healing: Evidence Based Management*. Philadelphia PA. F.A Davis: 2010:248-250, 263.
5. Kurz N, Kahn SR, Abenhaim L, et al., eds. VEINES Task Force Report, The management of chronic venous disorders of the leg (CVDL): an evidence based report of an international task force. McGill University. Sir Mortimer B. Davis-Jewish General Hospital. Summary reports in: *Angiology*. 1997;48(1):59–66; and *Int Angiol*. 1999;18(2):83-102.
6. Heit JA. Venous thromboembolism epidemiology: implications for prevention and management. *Semin Thromb Hemost*. 2002;28(suppl 2):3-13.
7. Negus D, Coleridge-Smith PD, Bergan J. *Leg Ulcers. Diagnosis and Management*. 3rd ed. London: Hodder Arnold; 2005.
8. Margolis DJ, Berlin JA, Strom BL. Risk factors associated with the failure of a venous leg ulcer to heal. *Arch Dermatol*. 1999;135(8):920-926.
9. Jankūnas V, Rimdeika R, Jasėnas M, Samsanavičius D. Changes in patient's quality of life comparing conservative and surgical treatment of venous leg ulcers. *Medicina (Kaunas)*. 2004;40(8):731-739.
10. Bergan JJ, Schmid-Schönbein GW, Smith PD, Nicolaides AN, Boisseau MR, Eklof B. Chronic venous disease. *N Engl J Med*. 2006;355(5):488-498.
11. Moffatt CJ, Franks PJ, Doherty DC, Martin R, Blewett R, Ross F. Prevalence of leg ulceration in a London population. *QJM*. 2004;97(7):431-437
12. Takahashi PY, Chandra A, Cha SS, Crane SJ. A predictive model for venous ulceration in older adults: results of a retrospective cohort study. *Ostomy Wound Manage*. 2010;56(4):60-66.
13. Simon DA, Freak L, Kinsella A, et al. Community leg ulcer clinics: a comparative study in two health authorities. *BMJ*. 1996;312(7047):1648-1651.
14. Morrell CJ, Walters SJ, Dixon S, et al. Cost effectiveness of community leg ulcer clinics: randomised controlled trial. *BMJ*. 1998;316(7143):1487-1491
15. Association for the Advancement of Wound Care (AAWC) Venous Ulcer Guideline. National Guideline Clearing House. Rockville, MD: Agency for Healthcare Research and Quality. http://www.guideline.gov/content.aspx?id=36081. Accessed August 23, 2013.
16. McGuckin M, Waterman R, Brooks J, et al. Validation of venous leg ulcer guidelines in the United States and United Kingdom. *Am J Surg*. 2002;183(2):132-137.
17. Olson JM, Raugi GJ, Nguyen VQ, Yu O, Reiber GE. Guideline concordant venous ulcer care predicts healing in a tertiary care Veterans Affairs Medical Center. *Wound Repair Regen*. 2009;17(5):666-670.
18. Walker NK, Vandal AC, Holden JR, et al. Does capture-recapture analysis provide more reliable estimates of the incidence and prevalence of leg ulcers in the community? *Aust N Z J Public Health*. 2002;26(5):451-455.
19. Van Hecke A, Grypdonck M, Defloor T. Guidelines for the management of venous leg ulcers: a gap analysis. *J Eval Clin Pract*. 2008;14(5):812-822.
20. Khan MN, Davies CG. Advances in the management of leg ulcers--the potential role of growth factors. *Int Wound J*.

2006;3(2):113-120.
21. O'Donnell TF Jr, Balk EM. The need for an Intersociety Consensus Guideline for venous ulcer.. *J Vasc Surg.* 2011;54(6 suppl):83S-90S.
22. Gelfand JM, Hoffstad O, Margolis DJ. Surrogate endpoints for the treatment of venous leg ulcers. *J Invest Dermatol.* 2002;119(6):1420-1425.
23. van Rijswijk L. Full-thickness leg ulcers: patient demographics and predictors of healing. *J Family Pract.* 1993;36(6):625–632.
24. Bolton L, McNees P, van Rijswijk L, et al. Wound-healing outcomes using standardized assessment and care in clinical practice. *J Wound Ostomy Continence Nurs.* 2004;31(2):65-71.
25. Margolis DJ, Berlin JA, Strom BL. Which venous leg ulcers will heal with limb compression bandages? *American J Medicine.* 2000;109(1):15-19.
26. Margolis DJ, Knauss J, Bilker W. Medical conditions associated with venous leg ulcers. *Br J Dermatol.* 2004;150(2):267-273.
27. Finlayson K, Edwards H, Courtney M. Factors associated with recurrence of venous leg ulcers: a survey and retrospective chart review. *Int J Nurs Stud.* 2009;46(8):1071-1078.
28. Robson MC, Cooper DM, Aslam R, et al. Guidelines for the treatment of venous ulcers. *Wound Repair Regen.* 2006;14(6):649-662
29. Eklöf B, Rutherford RB, Bergan JJ, et al. Revision of the CEAP classification for chronic venous disorders: Consensus statement. *J Vasc Surg.* 2004;40(6):1248-1252.
30. Kistner RL, Eklof B, Masuda EM. Diagnosis of chronic venous disease of the lower extremities: The "CEAP" classification. *Mayo Clin Proc.* 1996;71(4):338-345.
31. Margolis DJ, Allen-Taylor L, Hoffstad O, Berlin JA. The accuracy of venous leg ulcer prognostic models in a wound care system. *Wound Repair Regen.* 2004;12(2):163-168
32. Cardinal M, Eisenbud DE, Armstrong DG, et al. Serial surgical debridement: A retrospective study on clinical outcomes in chronic lower extremity wounds. *Wound Rep Reg.* 2009;17(3):306–311.
33. O'Meara S, Cullum NA, Nelson EA. Compression for venous leg ulcers. *Cochrane Database Syst Rev.* 2009;(1):CD000265.
34. Nelson EA, Mani R, Thomas K, Vowden K. Intermittent pneumatic compression for treating venous leg ulcers (Review). *Cochrane Database Syst Rev.* 2011;(2): CD001899.
35. Palfreyman SJ, Nelson EA, Lochiel R, Michaels JA. Dressings for healing venous leg ulcers. *Cochrane Database Syst Rev.* 2006;(3):CD001103.
36. Chaby G, Senet P, Vaneau M, et al. Dressings for acute and chronic wounds: a systematic review. *Arch Dermatol.* 2007;143(10):1297-304
37. Kerstein MD, Gemmen E, van Rijswijk L, et al. Cost and cost effectiveness of venous and pressure ulcer protocols of care. *Dis Manage Health Outcomes.* 2001;9(11):651-636.
38. Cambal M, Labas P, Zonca P, Hrbaty B, Janik M, Polakovicova S.. The combined algorithm of venous ulcer therapy. *Bratislava Med J.* 2012;113(5):285-288.
39. Simms KW, Ennen K. Lower extremity ulcer management: best practice algorithm. *J Clin Nurs.* 2010;20(1,2):86-93.
40. Jull AB, Arroll B, Parag V, Waters J. Pentoxifylline for treating venous leg ulcers. *Cochrane Database Syst Rev.* 2012;12:CD001733.
41. Layton AM, Ibbotson SH, Davies JA, Goodfield MJ. Randomised trial of oral aspirin for chronic venous leg ulcers. *Lancet.* 1994;344(8916):164-165.
42. Ibbotson SH, Layton AM, Davies JA, Goodfield MJ. The effect of aspirin on haemostatic activity in the treatment of chronic venous leg ulceration. *Br J Dermatol.* 1995;132(3):422-426.
43. O'Meara S, Al-Kurdi D, Ovington LG. Antibiotics and antiseptics for venous leg ulcers. *Cochrane Database Syst Rev.* 2008;(1):CD003557.
44. Gordillo GM, Sen CK. Revisiting the essential role of oxygen in wound healing. *Am J Surg.* 2003;186(3):259-263.
45. Hunt TK, Ellison EC, Sen CK. Oxygen: at the foundation of wound healing – introduction. *World J Surg.* 2004;28(3):291-293.
46. Hammarlund C, Sundberg T. Hyperbaric oxygen reduced size of chronic leg ulcers: a randomized double-blind study. *Plast Reconstr Surg.* 1994;93(4):829-833.
47. Kranke P, Bennett M, Debus S, Roeckl-Wiedmann I. Hyperbaric oxygen therapy for chronic wounds. *Cochrane Database Syst Rev.* 2004;(2):CD004123.
48. Tallman P, Muscare E, Carson P, Eaglstein WH, Falanga V. Initial rate of healing predicts complete healing of venous ulcers. *Arch Dermatol.* 1997;133(10):1231-1234.
49. Phillips TJ, Machado F, Trout R, Porter J, Olin, Falanga V. Prognostic indicators of venous ulcers. *J Am Acad Dermatol.* 2000;43(4):627-630.
50. Jones JE, Nelson EA. Skin grafting for venous leg ulcers. *Cochrane Database Syst Rev.* 2007;(2):CD001737.
51. Falanga V, Sabolinski M. A bilayered livin skin construct (APLIGRAF) accelerates complete closure of hard-to-heal ulcers. *Wound Repair Regen.* 1999; 7(4):201-207.
52. Gohel MS, Barwell JR, Taylor M, et al. Long term results of compression therapy alone versus compression plus surgery in chronic venous ulceration (ESCHAR): randomised controlled trial. *BMJ.* 2007;335(7610):83.