Marc E. Gottlieb, MD, FACS
A Professional Corporation

PLASTIC & RECONSTRUCTIVE SURGERY
Board Certification ••• ••• ••• Plastic Surgery ••• Hand Surgery ••• General Surgery

Specializing in the treatment, reconstruction, and management of
Acute and chronic wounds • Diseases and defects of the soft tissues • Injuries, diseases, and defects of the hand and extremities • Defects of the head and trunk

Office: 1415 N. 7th Avenue • Phoenix, AZ 85007 **Phone** 602-252-3354 **Fax** 602-254-7891 megott@arimedica.com

Marc E. Gottlieb, MD, FACS

1415 N. 7th Avenue
Phoenix, AZ 85007

Phone 602-252-3354
Fax 602-254-7891

megott@arimedica.com

Apligraf – Re-Engineered Living Skin – Biotechnology and Chronic Wounds

Original presentation October 6, 2005, Dallas, TX.
Sponsored by Organogenesis.

This file is partially annotated, containing text notes supplied by Organogenesis to accompany the company's "blue" slides.


APLIGRAF
RE-ENGINEERED LIVING SKIN
Marc E. Gottlieb, MD, FACS
BIOTECHNOLOGY AND CHRONIC WOUNDS
Sponsored by Organogenesis

Chronic and Pathological Wounds

Nullifying the Rules of Ordinary Surgery

The Paradigms of Wound Repair

ONE: The native wound module.

Topical care in support of contraction and epithelialization.

TWO: Conventional wound repair surgery: direct closure, grafts, and flaps.

Simple repairs and grafts succeed when host and target are healthy and wound healing competent.

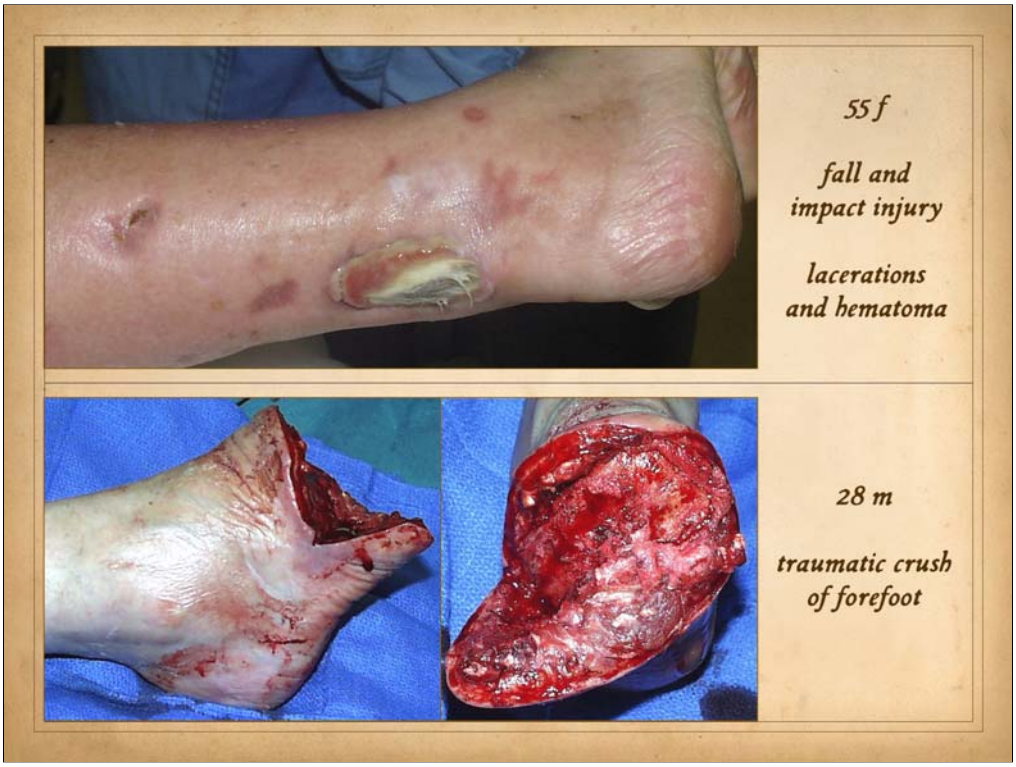
If the wound is pathological and incompetent to heal, but the host is generally healthy, then repair and grafts will fail, but good flaps succeed.

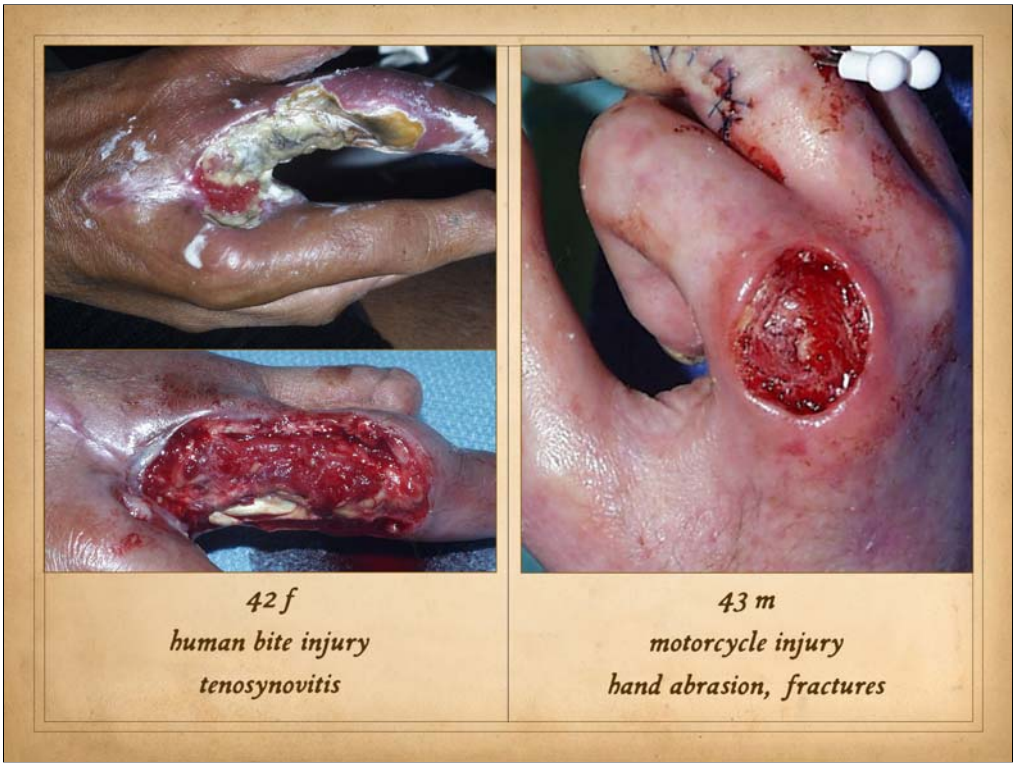
The Caveats of Wound Repair

ONE: The usual paradigms of repair have a common biological basis:

They all depend on the physiological process of reactive wound healing – the post-inflammatory proliferative wound module of fibroplasia, contraction, and epithelialization.

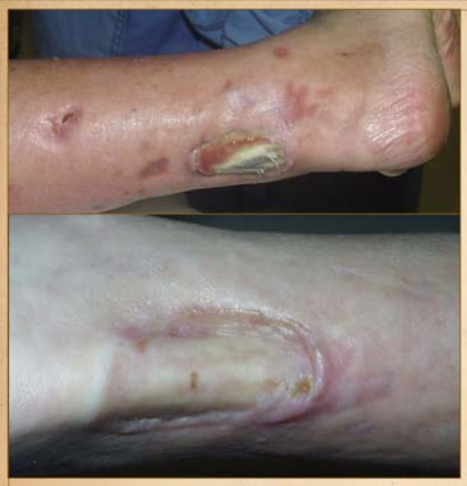
TWO: When systemic illness or wound healing pathologies are the basis of a wound, then the wound becomes chronic, and the classic paradigms of care and surgery do not work.


55 f
fall and impact injury
lacerations and hematoma
28 m
traumatic crush of forefoot



42 f
human bite injury
tenosynovitis

43 m
motorcycle injury
hand abrasion, fractures



55 f, fall, hematoma

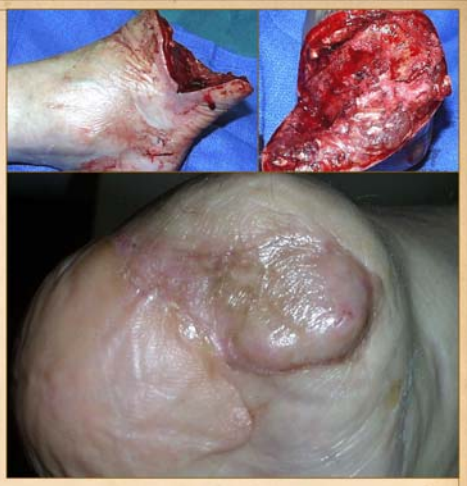
65 f

Wegener's granulomatosis

Caveats

Active immunopathy.

Severe pulmonary disease.



28 m, traumatic crush

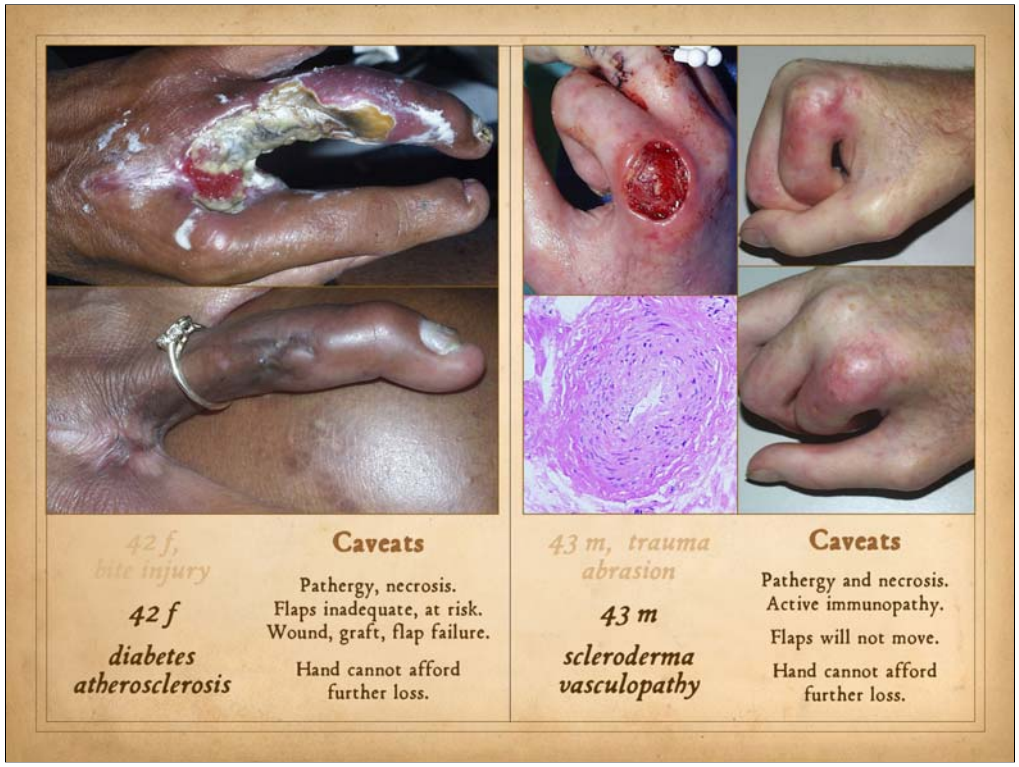
73 m

aso, pvod embolic necrosis

Caveats

No local flaps.
Skin grafts ineligible.
No free flaps.

Cardiovascular risks.



42 f, bite injury

42 f

diabetes atherosclerosis

Caveats

Pathergy, necrosis.
Flaps inadequate, at risk.
Wound, graft, flap failure.

Hand cannot afford further loss.

43 m, trauma abrasion

43 m

scleroderma vasculopathy

Caveats

Pathergy and necrosis.
Active immunopathy.

Flaps will not move.

Hand cannot afford further loss.

Chronic and Pathological Wounds

Issues that interdict or endanger ordinary surgery

Anything that makes the patient too ill
Anything that keeps risk factors active
Anything that renders wound healing incompetent

Disease effects (blood, immune, metabolic)
Pathergy, necrosis, ulceration, failed wound healing
Hematological disorders can kill tissues and flaps
Immunopathic disorders can kill tissues, flaps, and grafts
Immunopathic disorders damage wound repair
Wound failure and dehiscence

Vascular effects
Pathergy, necrosis, failed wound healing
Vascular disease may not support a graft
Vascular disease may kill a flap
Atherosclerosis precludes a free flap

Anatomical factors
Flaps inadequate (not available, won't reach, etc.)
Flaps can sacrifice useful parts and create disabilities
Failed flaps waste anatomy and limit further options

Injury & inflammation effects
Pathergy, necrosis, ulceration, dehiscence
Active inflammation suppresses repair
Effects of infection
Local flaps can be within zone of injury

General risks
Illness and comorbidities make surgery risky
Donor site complications can make the problem larger

Not every "surgical problem" can be cured by surgery.

Chronic and Pathological Wounds

Technology in support of new paradigms of care

High Tech - Low Tech Drugs - Dressings - Devices

Environment regulating therapies

Promogran & Prisma

VAC

Wound stimulatory therapies

PDGF - Becaplermin

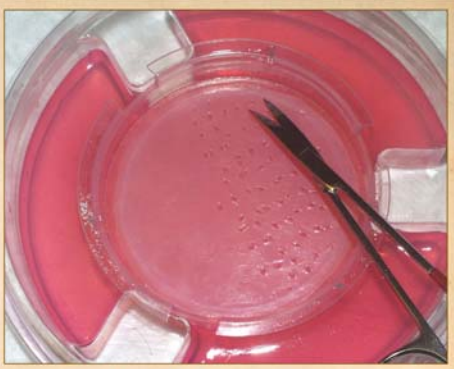
Dermagraft

Apligraf

Regenerative therapies

Integra


APLIGRAF
RE-ENGINEERED LIVING SKIN
A Wound Stimulatory Therapy
ORGANOGENESIS, INC.
LIVING TECHNOLOGY



Apligraf can be used on ulcers of any cause,

e.g. venous, arterial, immunopathic,
diabetic, pressure, mechanical

as long as all other necessary components
of diagnosis-specific care are enforced.

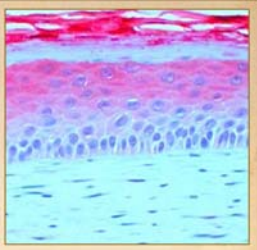
Apligraf is typically applied
during outpatient encounters.

The wound must meet
customary criteria for closure.

Curettage or similar preparation.
Affix with dressings.

Examine and rewrap weekly or half-weekly.
No debridement or regular care for 2 - 4 weeks.

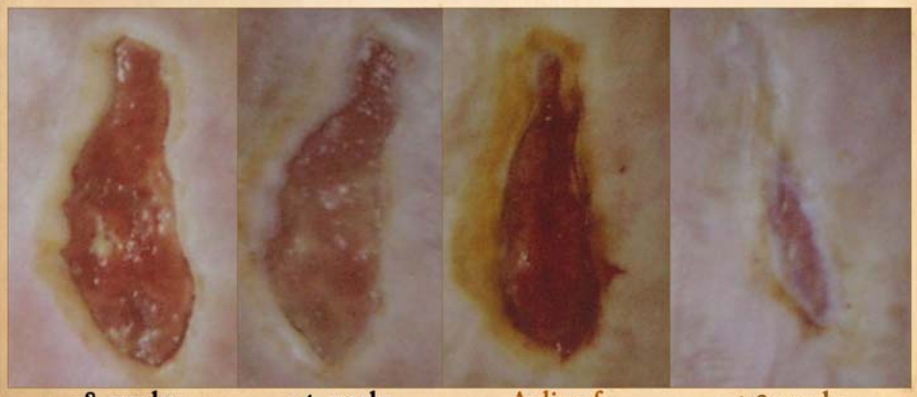
Apligraf



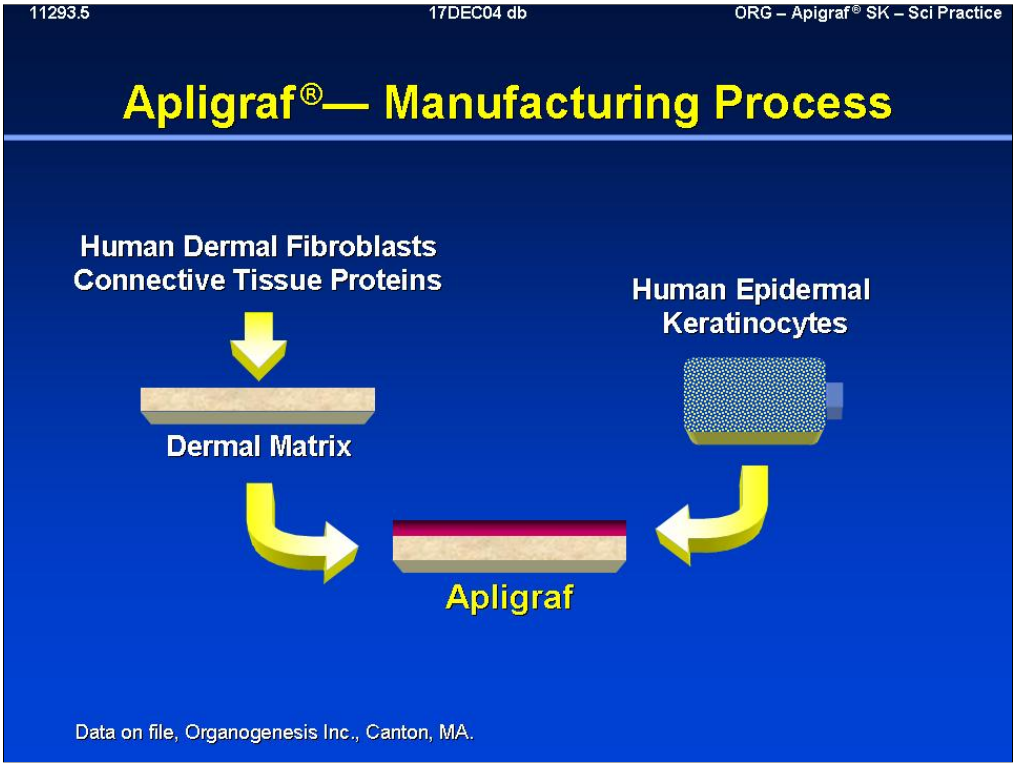
Re-engineered human living skin equivalent.

It functions as a pharmaceutical – a drug packaged in a living vehicle.

It stimulates repair in chaotic or retarded wounds.



- 8 weeks | - 4 weeks | Apligraf | + 3 weeks



Apligraf®— Manufacturing Process

Apligraf is produced using state-of-the-art tissue engineering technology with a unique manufacturing process. Development of the bi-layered product is dependent on the isolation and expansion of 2 basic components: living human keratinocytes and fibroblasts (the principal cells of the epidermal and dermal layers of skin)

Step 1: The Dermal Component

In vitro production of Apligraf begins with the formation of the dermal layer. For dermal layer formation, acid-extracted bovine type-1 collagen plus neutralizing medium is cast on a permeable polycarbonate membrane. This is followed by addition of living human fibroblasts to the collagenous surface. During the 6-day incubation period, the fibroblasts multiply and begin to produce new collagen. The collagen filaments contract, and the dermal matrix forms and condenses

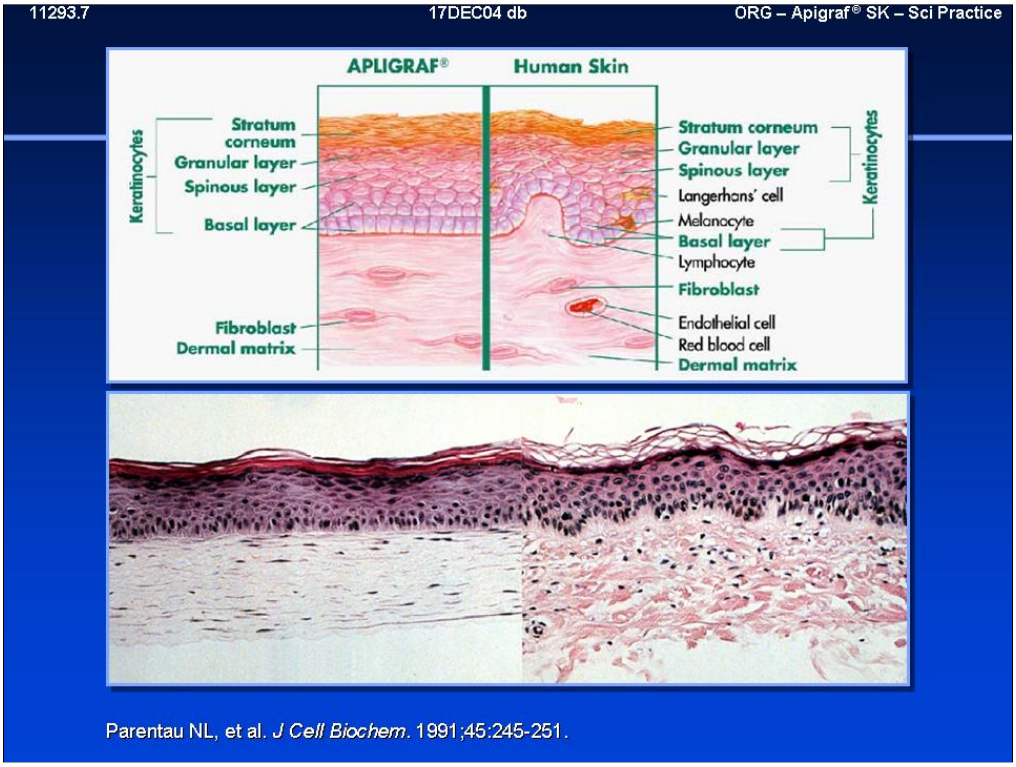
Step 2: The Epidermal Component

Human keratinocytes are seeded onto the contracted dermal matrix in a culture medium. Following attachment onto the matrix, the keratinocytes proliferate and differentiate such that distinct epidermal and dermal layers are present within 4 days of incubation

Step 3: Development of a Stratum Corneum

During the final 10-day incubation, the keratinocytes are exposed to air and further mature (cornify) into a multilayered epidermis with a protective stratum corneum at the surface

Apligraf is now ready for clinical use



Apligraf® has 4 Active Components of Living Skin

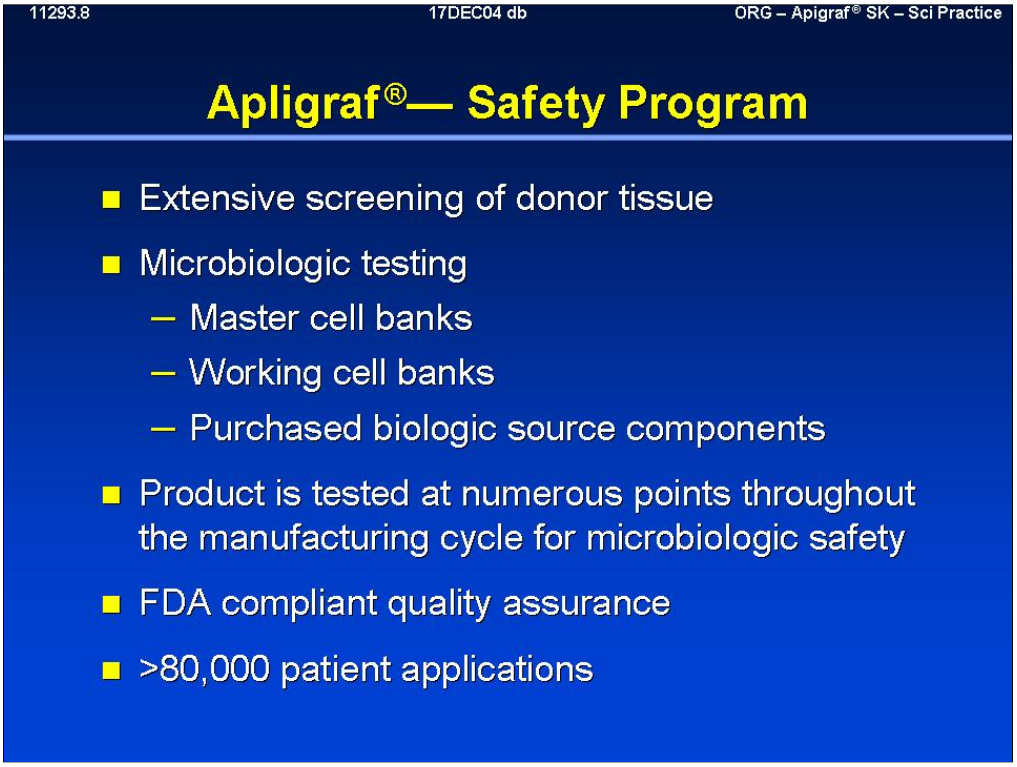
The illustration and the stain above is the final product; note the similarities of Apligraf with normal human skin; we will discuss in the subsequent section the science of Apligraf.

Fully differentiated, 4-layered epidermis: serves as a protective barrier

Epidermal keratinocytes: close wounds and produce growth factors and cytokines

Dermal fibroblasts: produce matrix proteins, growth factors and cytokines

Extracellular collagen matrix: promotes in-growth of patient cells, blood vessels and ECM synthesis



Apligraf®— Safety Program

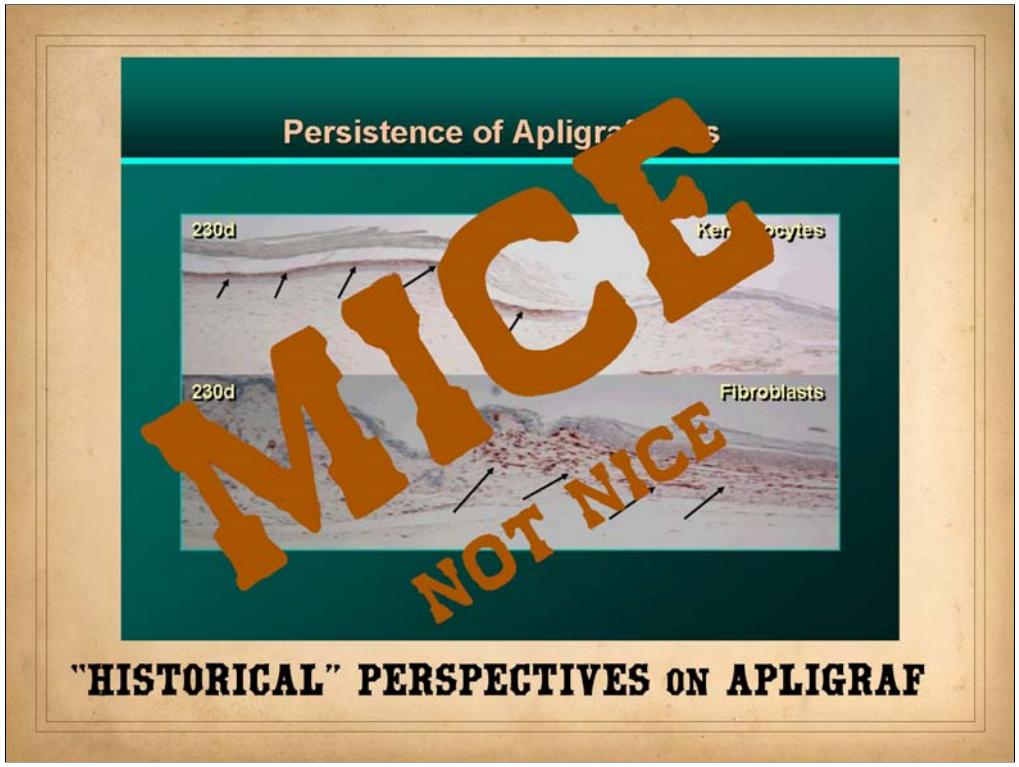
The living nature and complexity of Apligraf® require special safety considerations and testing

This applies to:

microbiological risks

tumorigenic risks

immunological risks



"HISTORICAL" PERSPECTIVES ON APLIGRAF


CARREL
LANDSTEINER
MEDAWAR
MURRAY
STARZL
TRANSPLANTHEON
BARRIERS TO ALLOGENEIC
TRANSPLANTATION CANNOT BE
SO EASILY OVERCOME

THE ART OF GRAFTS

TRANSPLANT BIOLOGY

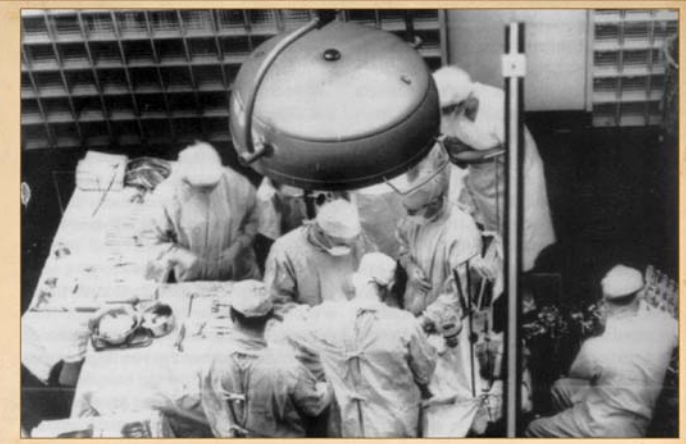
IMMUNOLOGY

WOUND PATHOLOGY

TECHNIQUE

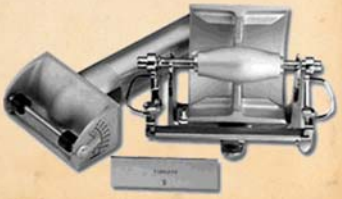
CLINICAL CARE

CF. APLIGRAF ALLOGENEIC SKIN

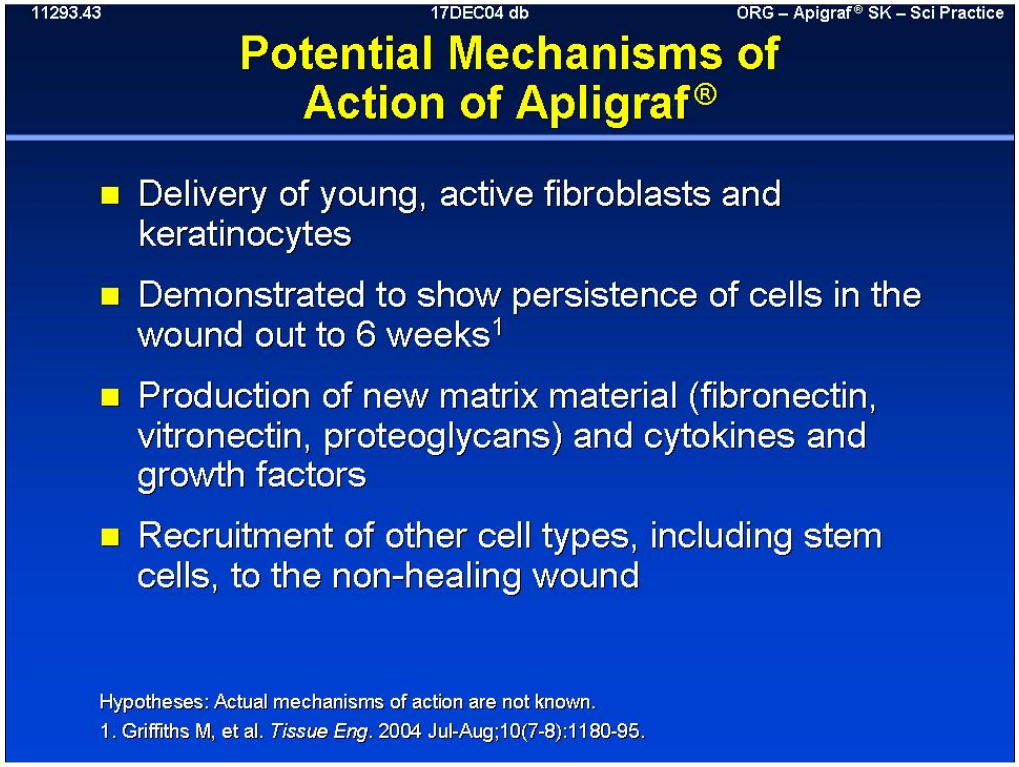


PLASTIC SURGERY RESEARCH COUNCIL

Barcelo, 1804




APLIGRAF
Re-Engineered Living Skin
The Underlying Science
WE KNOW ONLY PIECES OF THE PUZZLE



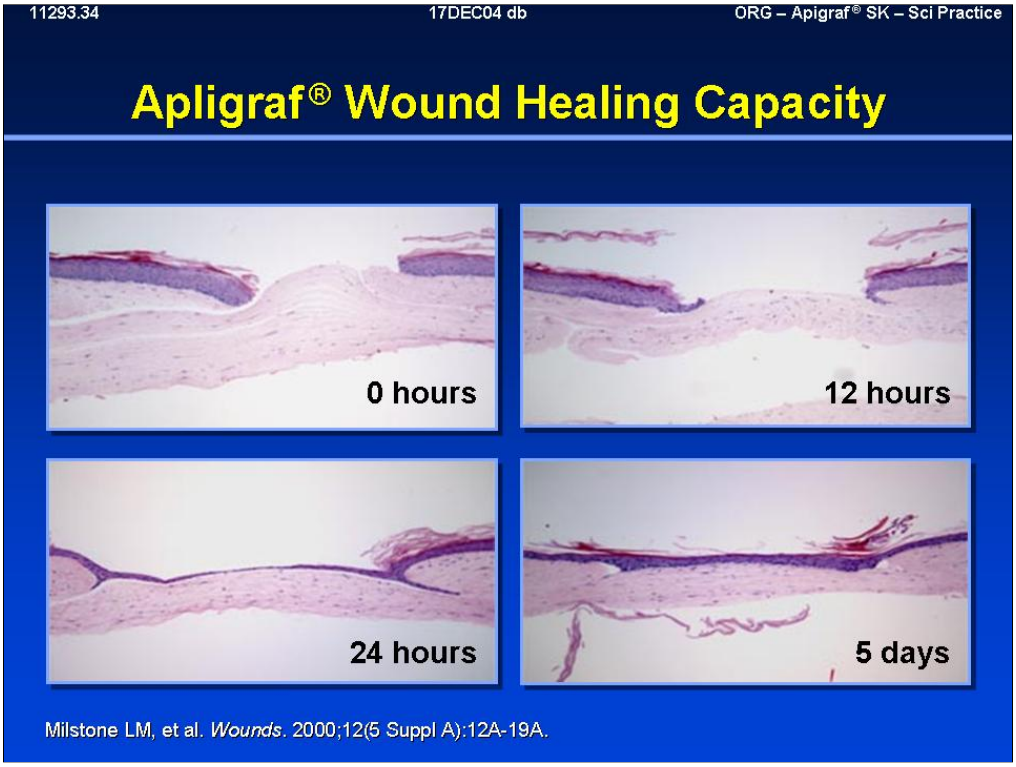
Potential Mechanism of Action of Apligraf®

Delivery of young, active fibroblasts and keratinocytes

Demonstrated to show persistence of cells in the wound out to 6 weeks[1]

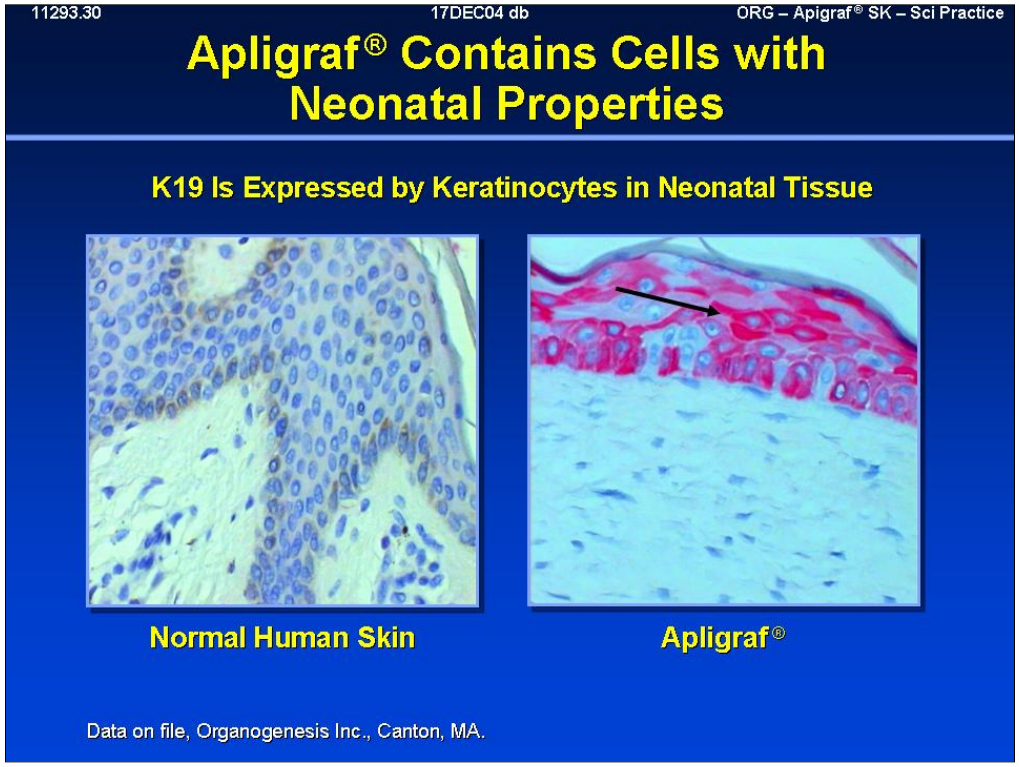
Production of new matrix material (fibronectin, vitronectin, proteoglycans) and cytokines and growth factors

Recruitment of other cell types, including stem cells, to the non-healing wound



Apligraf® Wound Healing Capacity

Wounded Apligraf were placed on a fibroblast-constructed matrix to assess its self-healing capacity. At 12 hours following wounding epiboly was observed at the wound edge. Complete re-epithelialization was observed at 24 hours after wounding and complete closure and maturation with stratum corneum formation occurred by 5 days



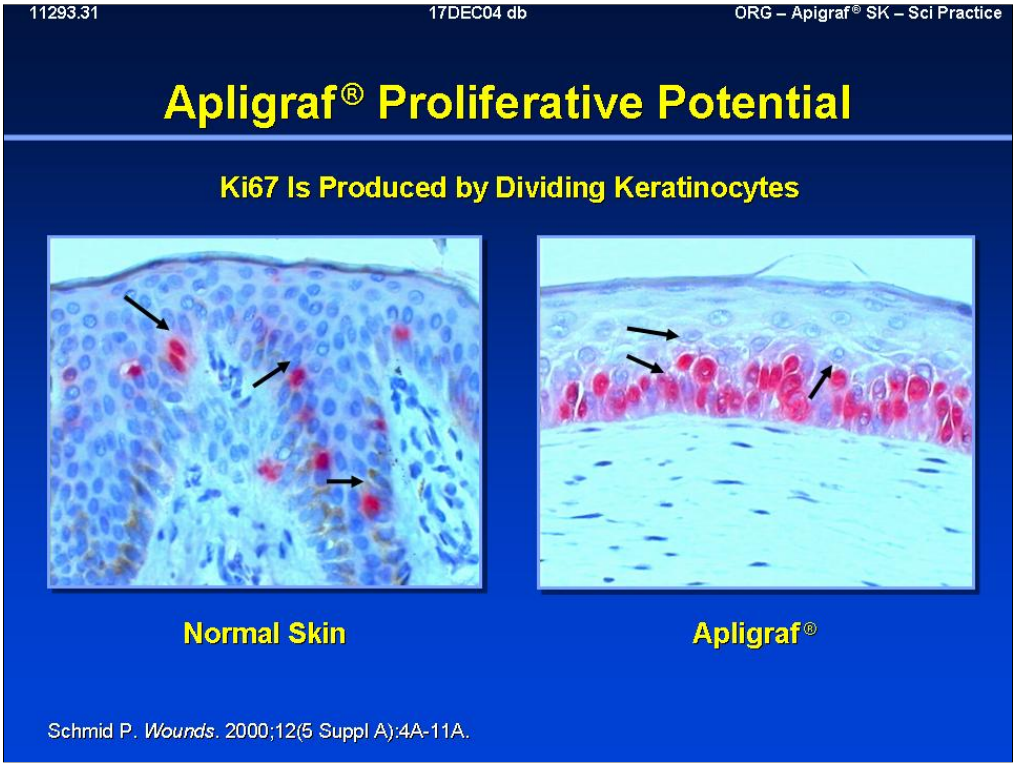
Apligraf® Contains Cells with Neonatal Properties

Proteins expressed by activated keratinocytes: Keratin-19

Localization of keratin 19 in Apligraf epidermis.

Keratin 19 is a marker for putative stem-cell containing epithelial compartments and is highly expressed in fetal cutaneous tissue

This suggest a potential fetal-tissue like regenerative (as opposed to adult somatic wound healing) character for Apligraf



Apligraf® Proliferative Potential

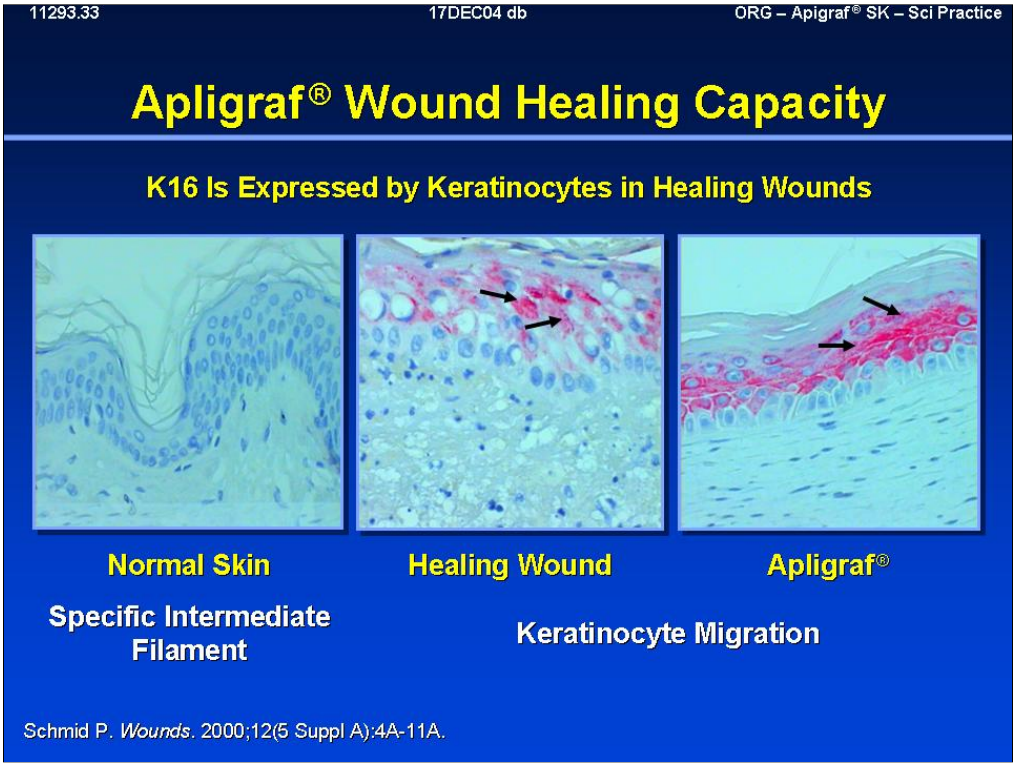
Proteins expressed by activated keratinocytes:

Cell proliferation markers

Ki67 is a nuclear protein involved in cell cycling progression. It is expressed in cycling cells but not in resting cells (marks cells entering G1a from G0 arrest phase of cell cycle)

The proliferative potential of Apligraf keratinocytes where compared with normal adult skin by immunostaining of Ki67 antibody. Similar to normal skin, Apligraf epidermis showed Ki67 positive cells in the basal layer of keratinocytes. However, the intensity and number of positive cells were significantly more pronounced in Apligraf

This finding suggest that although skin constructs can display similar histology than normal skin the metabolic pathways and cellular activities are highly enhanced in Apligraf showing a “highly metabolic active” tissue



Apligraf® Wound Healing Capacity

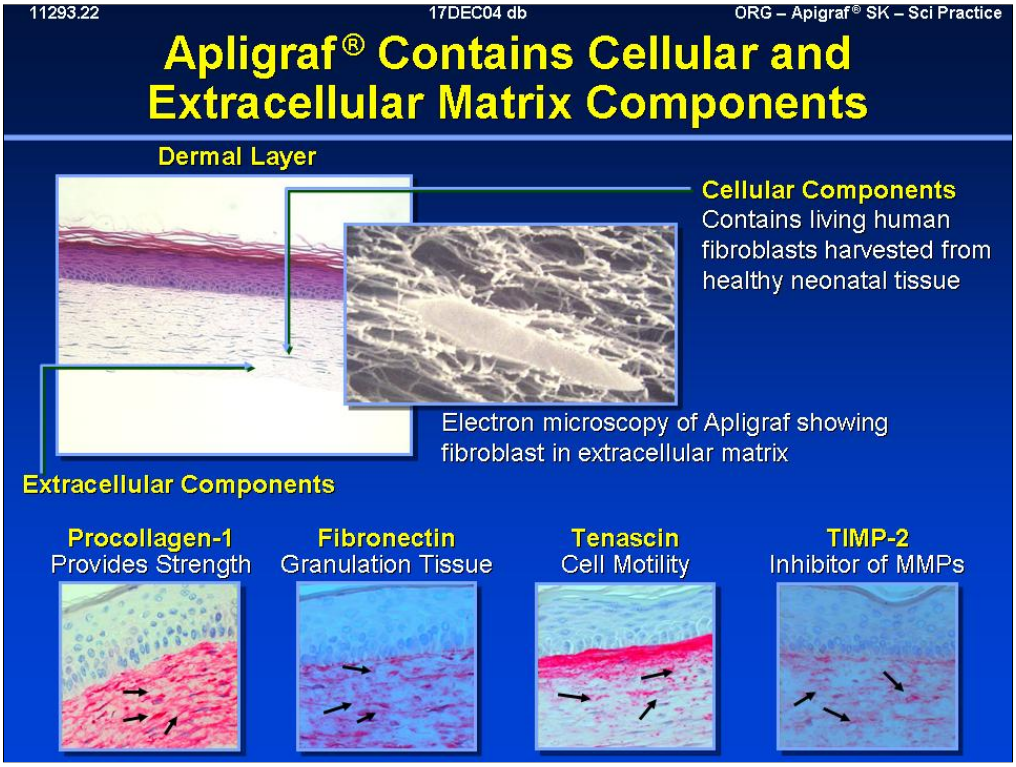
Proteins expressed by activated keratinocytes:

Intermediate filament proteins

Keratin 16 is a specific intermediate filament protein involved in keratinocyte migration

Keratin 16 staining is not detectable in the interfollicular epidermis of normal skin while its staining is present in the epidermis of healing wounds and in high levels in Apligraf

This suggests that the Apligraf cells display an activated phenotype and have a high regenerative capacity



Apligraf® Contains Cellular and Extracellular Matrix Components

Hematoxylin/eosin stained thin section of Apligraf showing the details of the dermal layer

Apligraf contains living human fibroblasts harvested from healthy neonatal tissue

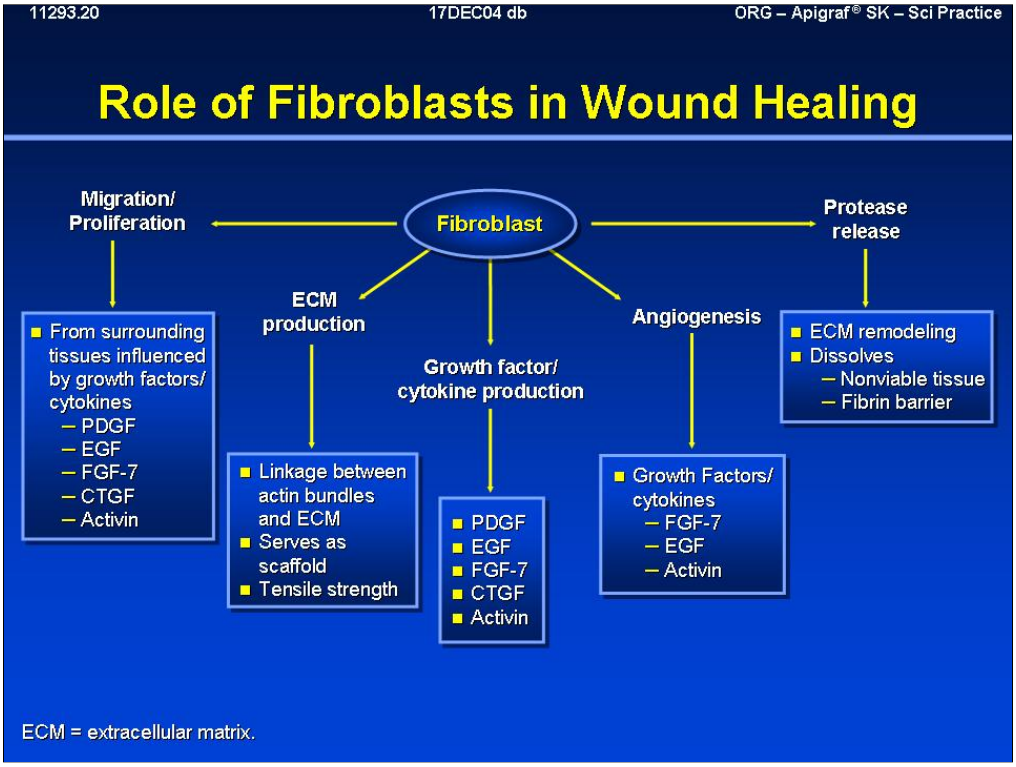
The insert is an electron microscopy of Apligraf, demonstrating fibroblast in the extracellular matrix

> Micrograph shows a single fibroblast cell body embedded in a dense collagen type I lattice

Recent studies indicate that much of the collagen present at the end of the manufacturing process is the product of the human fibroblasts

The following are the functions of the extracellular components in human skin

> Procollagen-1: provides strength
>
> Fibronectin: a key component of granulation tissue
>
> Tenascin: skin tenascin modulates cell motility in granulation tissue
>
> TIMP-2: In injured human tissue inhibitor of metalloproteinase (TIMP), inhibit protein-degrading enzymes called matrix metalloproteinase's (MMPs)



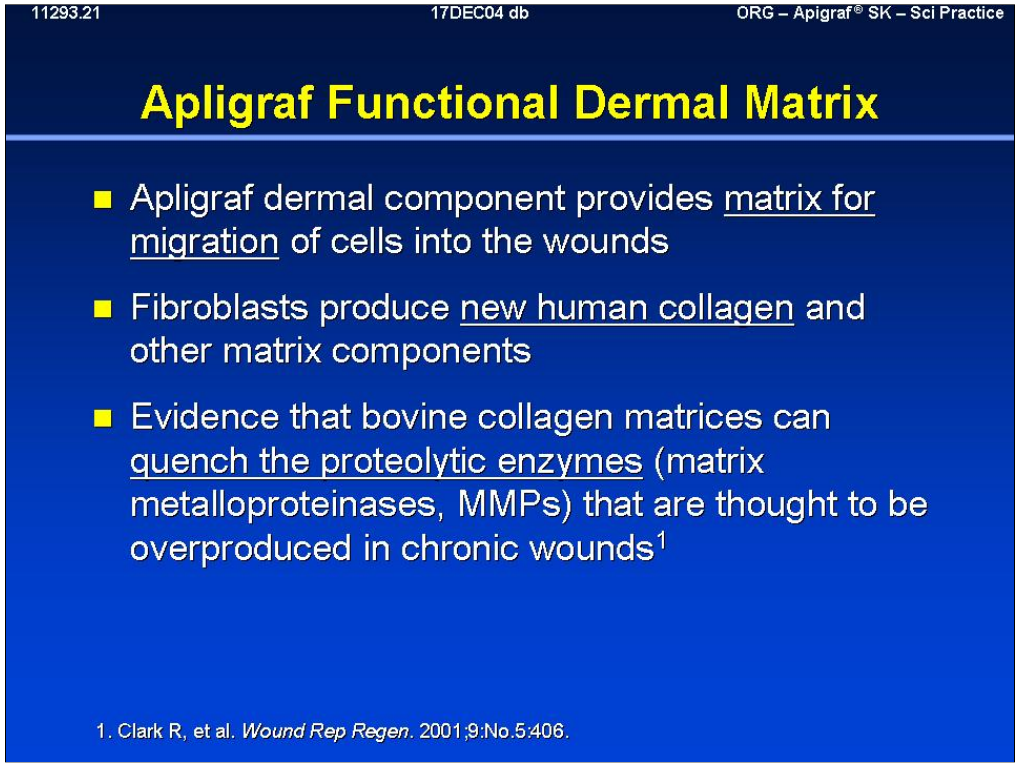
Role of Fibroblasts in Wound Healing

Fibroblasts migrate into the wound site from the surrounding tissue and begin to proliferate within several days of injury

Fibroblasts release a variety of growth factors and cytokines involved in the wound healing process, such as FGF-7 (KGF), CTGF (connective tissue growth factor), PDGF, EGF, and activin[1-3]

Extracellular matrix produced by fibroblasts serves as a scaffold for angiogenesis and also contributes to the tensile strength of the wound[2]

Through protease release, the extracellular matrix undergoes remodeling[1]

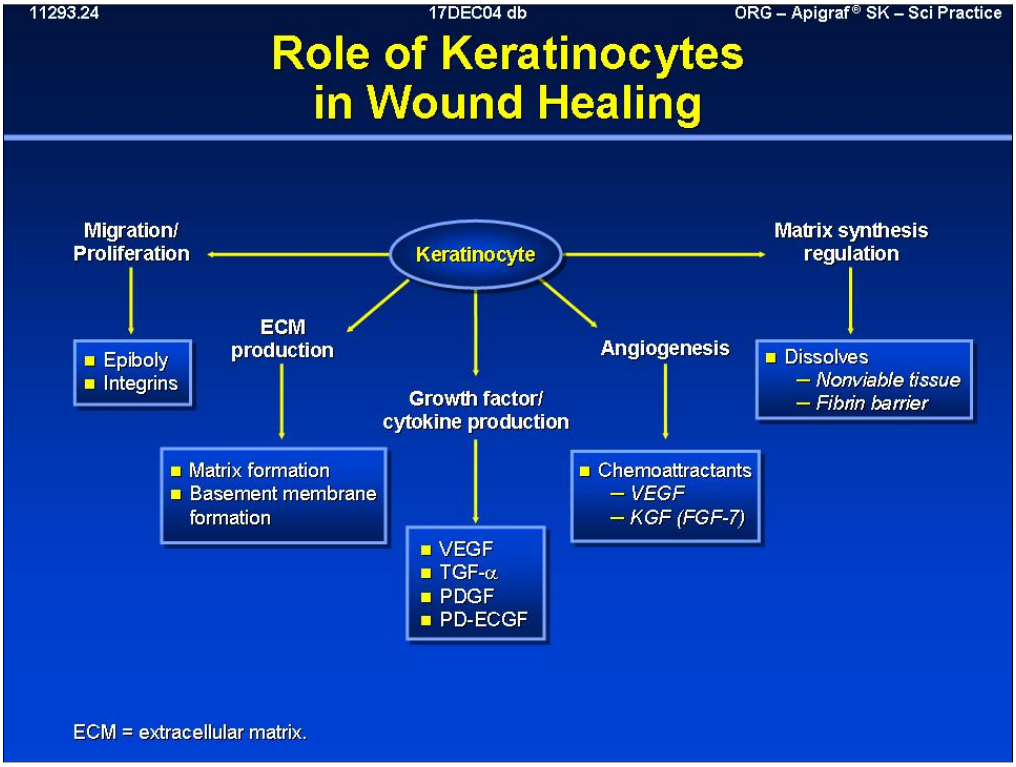


Apligraf Functional Dermal Matrix

Apligraf dermal component provides matrix for migration of cells into the wounds

Fibroblasts produce new human collagen and other matrix components

Evidence that bovine collagen matrices can quench the proteolytic enzymes (matrix metalloproteinases, MMPs) that are thought to be overproduced in chronic wounds[1]



Role of Keratinocytes in Wound Healing

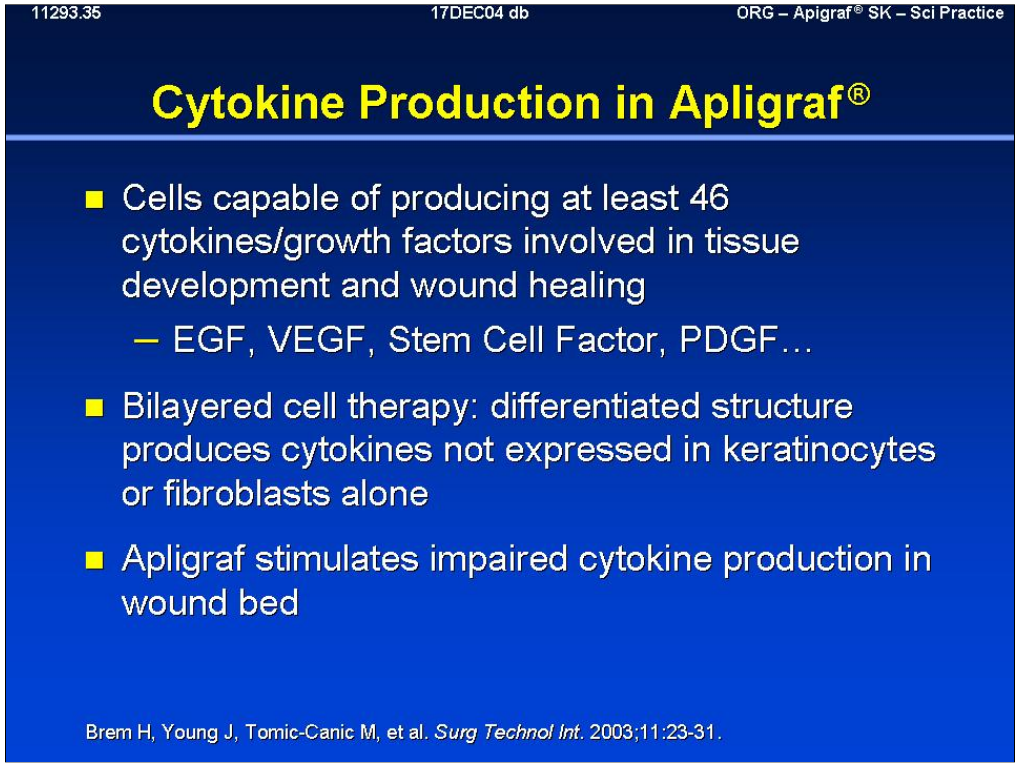
Keratinocytes migrate via lamellipodial crawling (epiboly) mechanism across the wound surface; this is influenced by the expression of integrins.[1] Once keratinocytes cover the surface of the wound, a newly differentiated and stratified epidermis becomes established[1]

Through protease expression, early keratinocytes dissolve nonviable tissue and the fibrin barrier at the wound margin[1,2]

The activity of keratinocytes in the extracellular matrix production results in the formation of a provi-sional matrix and the basement membrane[2]

Keratinocytes release a variety of growth factors and cytokines involved in the wound healing process, such as vascular endothelial growth factor (VEGF),TGF-α, PDGF, and platelet-derived endothelial cell growth factor (PD-ECGF)[1]

Keratinocytes provide a stimulus to angiogenesis by releasing chemoattractants, such as vascular endothelial growth factor, possibly in response to keratinocyte growth factor[1]



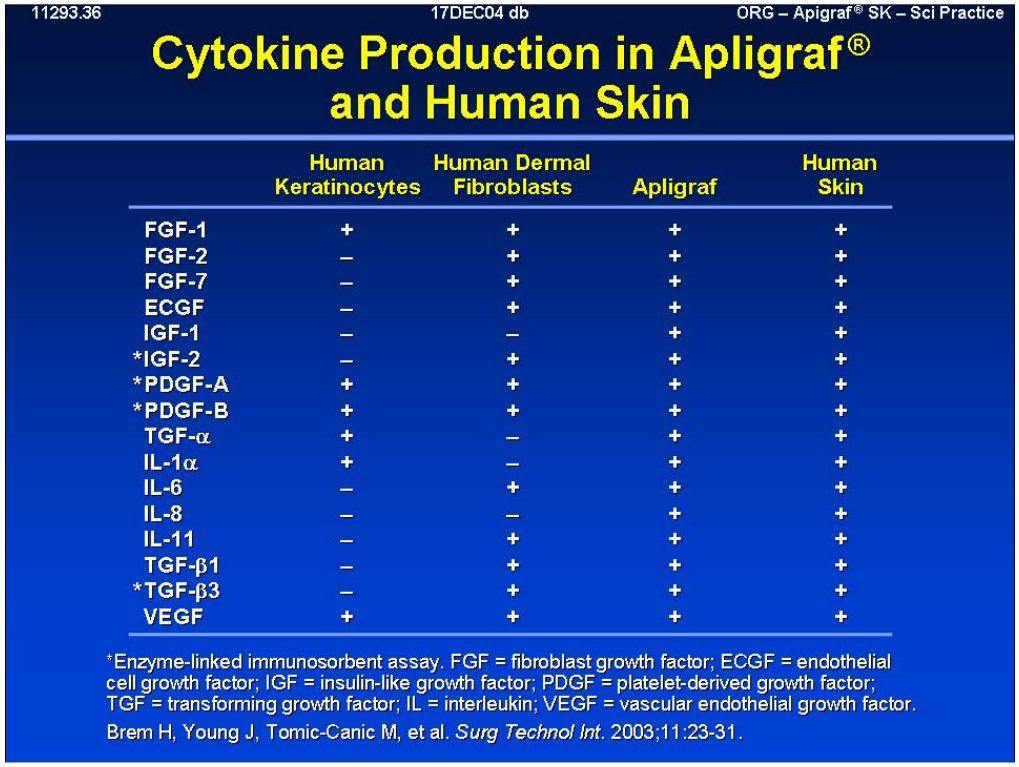
Cytokine Production in Apligraf®

Cells capable of producing at least 46 cytokines/growth factors involved in tissue development and wound healing

EGF, VEGF, Stem Cell Factor, PDGF

Bilayered cell therapy: differentiated structure produces cytokines not expressed in keratinocytes or fibroblasts alone

Apligraf stimulates impaired cytokine production in wound bed

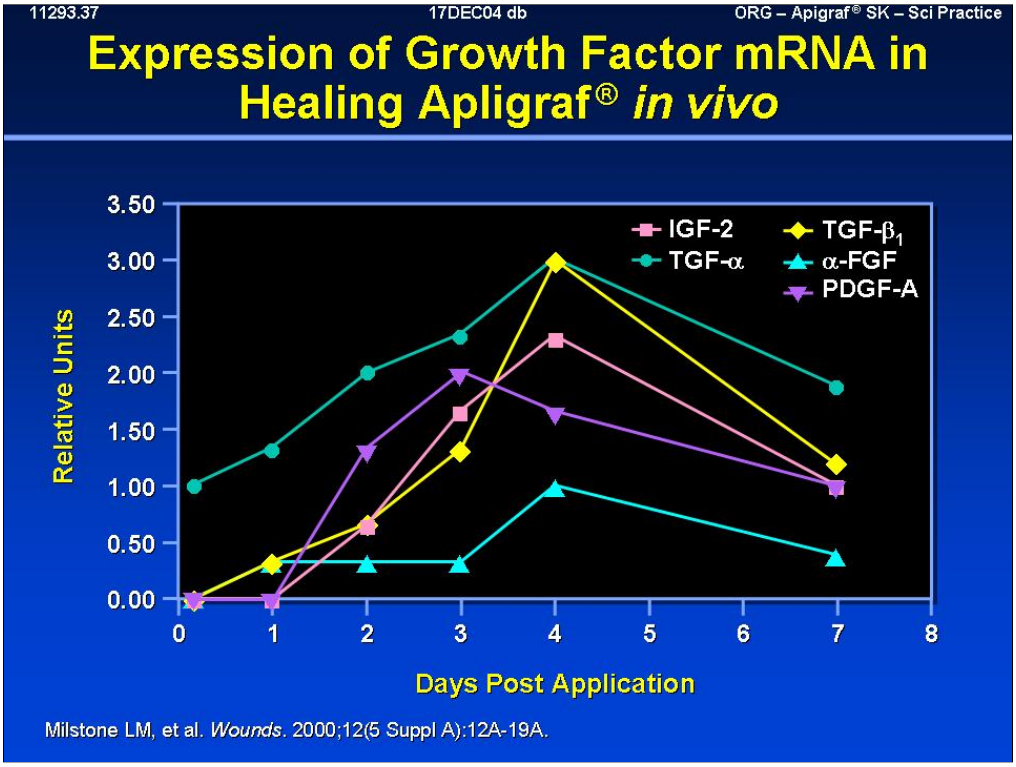


Cytokine Production in Apligraf® and Human Skin

Cytokine and Growth Factor Profile of Apligraf and its Cellular Components Compared to Human Skin

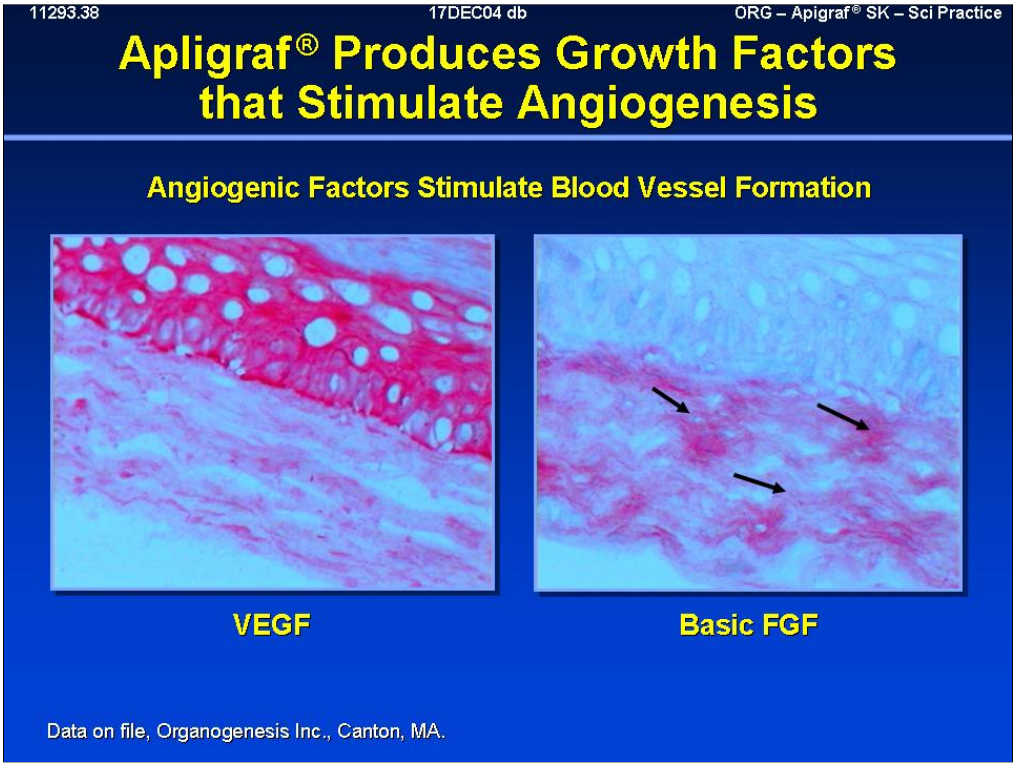
This table depicts a partial list of cytokines and growth factors expressed by intact Apligraf and its purified cellular components in comparison to normal skin

Note that some cytokines such as IL-8 and IGF-1 are made only by intact Apligraf, implying and interaction between the cellular components of Apligraf



Expression of Growth Factor mRNA in Healing Apligraf® *In vivo*

Kinetics of cytokine expression during wound healing of wounded Apligraf. Gene expression of growth factor associated with tissue remodeling such as transforming growth factors, fibroblast growth factor, insulin growth factor and platelet derived growth factor was increased during the healing response of wounded Apligraf



Apligraf ® Produces Growth Factors that Stimulate Angiogenesis[1]

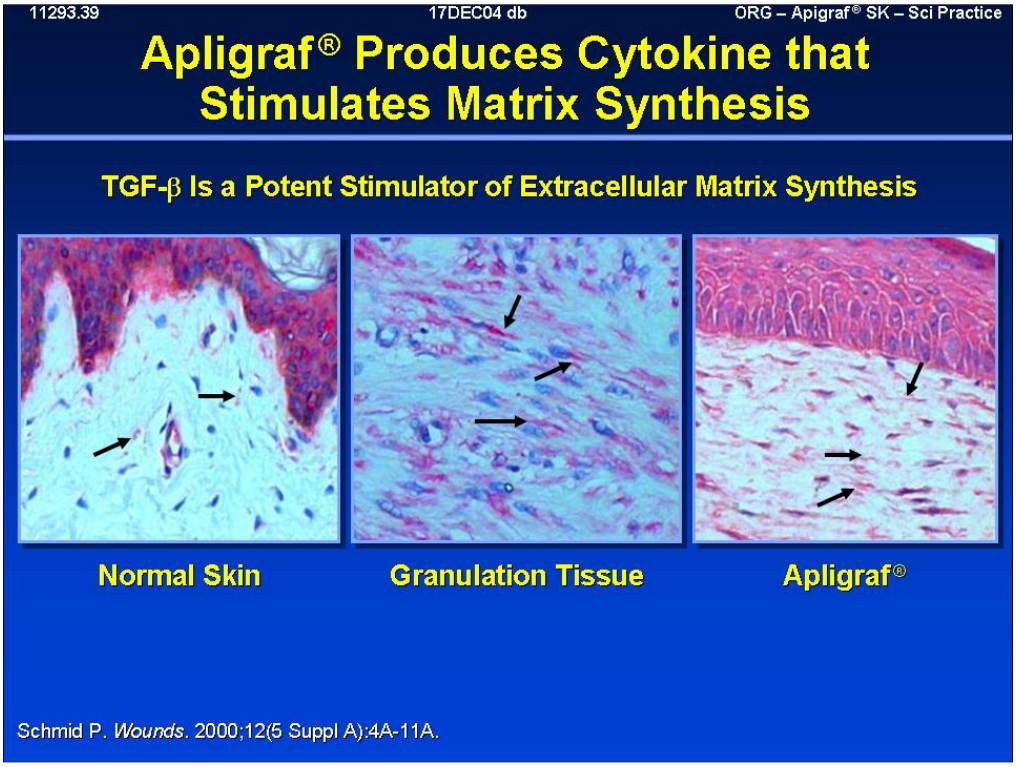
Proteins expressed by activated fibroblasts: Angiogenic growth factors

Vascular endothelial growth factor (VEGF)

- Homodimeric protein secreted by a variety of vascularized tissues induces endothelial proliferation and vascular permeability
- Induced in wound edge keratinocytes and macrophages, possibly in response to KGF and TGF-α

Basic Fibroblast Growth Factor (bFGF)

- bFGF or FGF-2, released by damaged endothelial cells and macrophages at wound site, is an important mediator of angiogenesis[2]
- bFGF induces VEGF in vascular endothelial cells



Apligraf® Produces Cytokine that Stimulates Matrix Synthesis

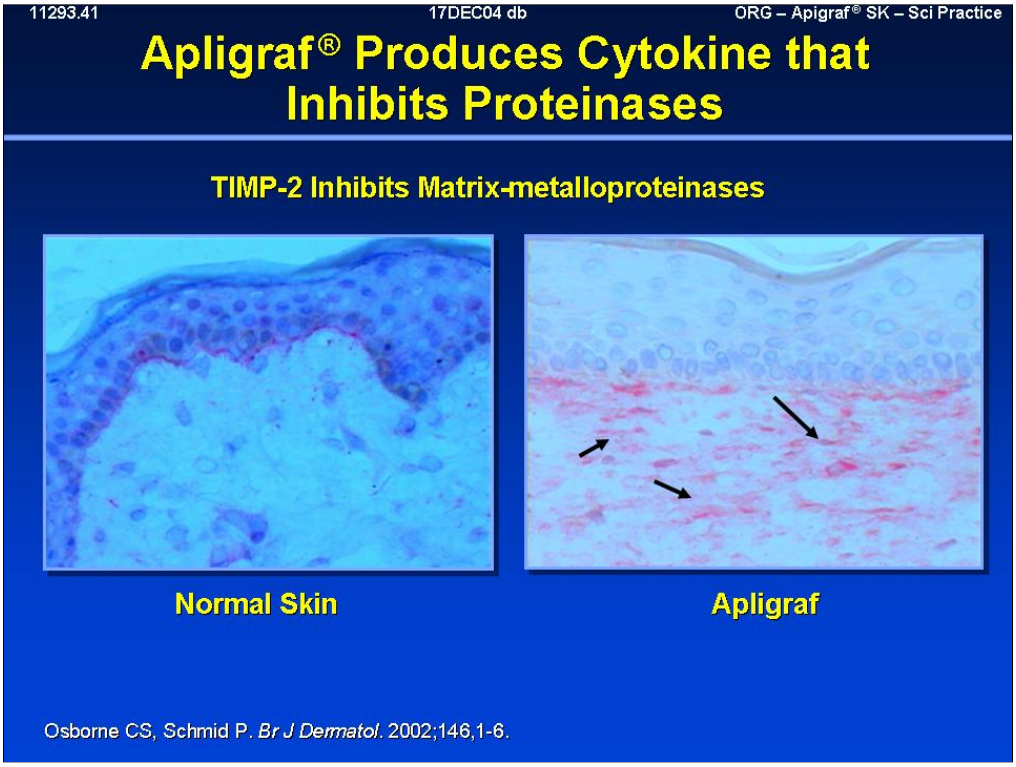
Immunostaining of TGF-β

Proteins expressed by activated fibroblasts: Fibrogenic growth factors

Transforming growth factor-beta (TGF-β)

- Family of homologous dimeric peptides which are released by platelets, activated macrophages and fibroblasts
- Chemotactic effects on monocytes and fibroblasts
- Stimulates angiogenesis
- Stimulates fibroblasts to produce enhanced levels of extracellular matrix proteins

Note high levels of expression in Apligraf and in granulation tissue of normal healing wounds but little or no expression in unwounded normal skin



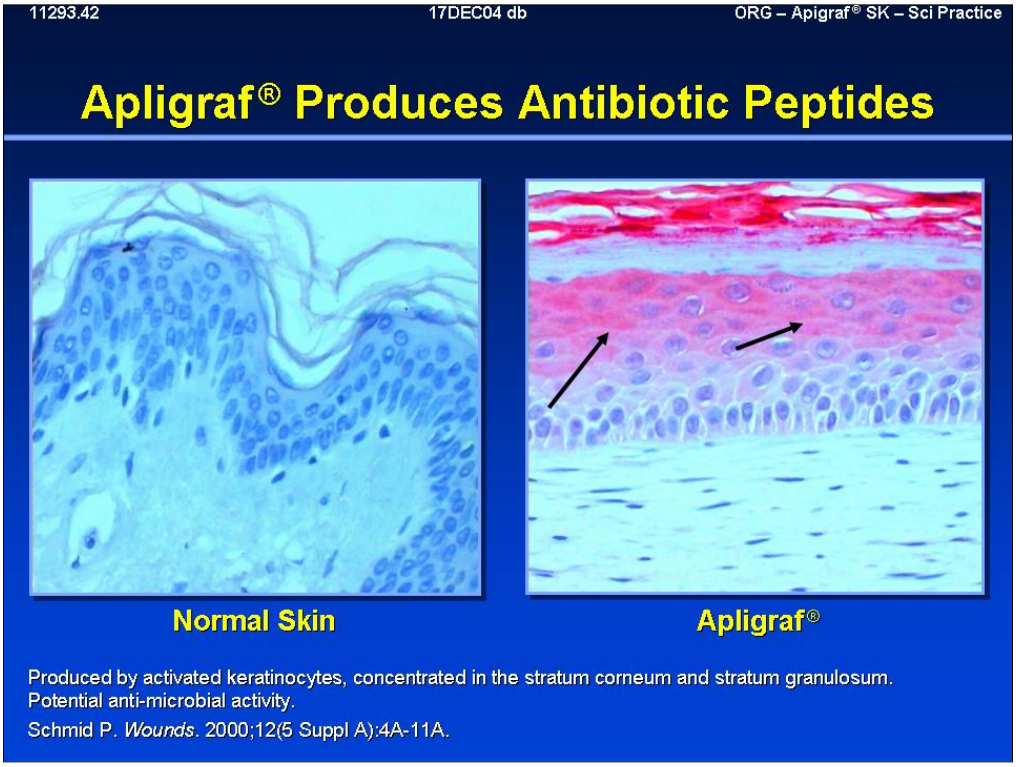
Apligraf® Produces Cytokine that Inhibits Proteinases

Immunohistochemical Staining of Tissue Inhibitor of Matrix Metalloproteinases- 2 (TIMP-2) in Apligraf

Frozen section demonstrating strong TIMP-2 immunostaining in the dermis and basement membrane of Apligraf

Normal skin shows only very faint staining for TIMP-2

Conclusions: These studies provide evidence that epidermal-dermal interactions suppress epidermal MMP activity. In addition, expression of TIMPs (and fibronectin) in Apligraf dermis suggests that Apligraf has the potential to counteract the imbalance between matrix production and degradation in chronic wounds and thus may support wound re-epitheliazation



Apligraf® Produces Antibiotic Peptides

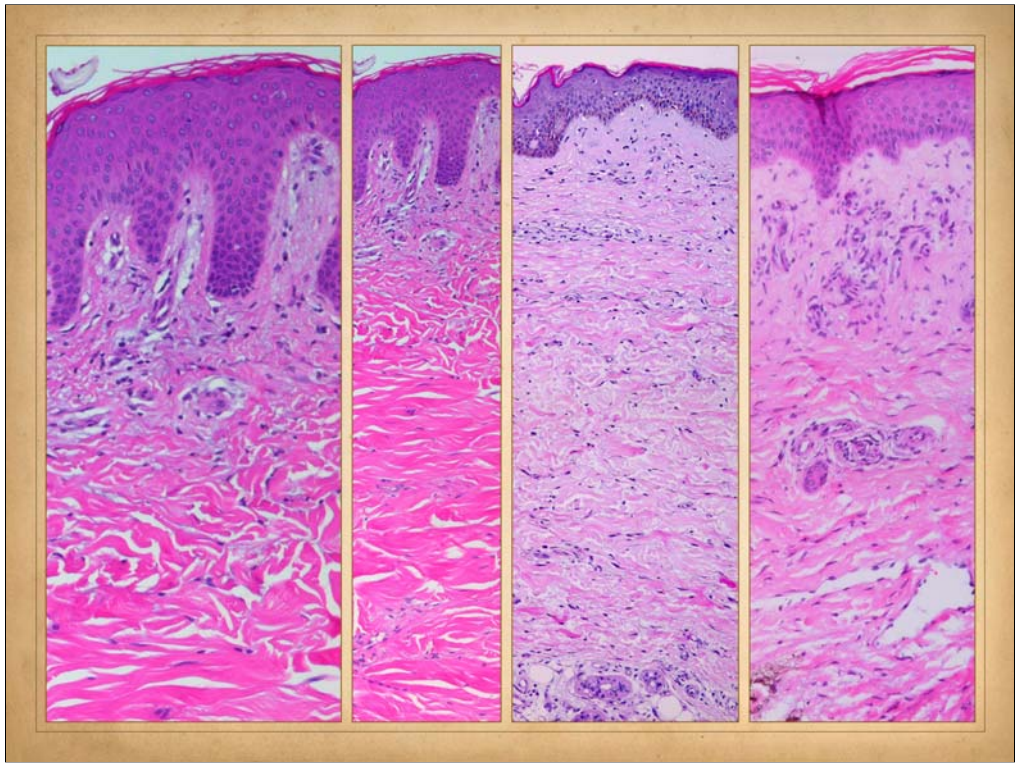
Human β-defensin 2 (HBD-2); defensins represent a family of small cationic peptides

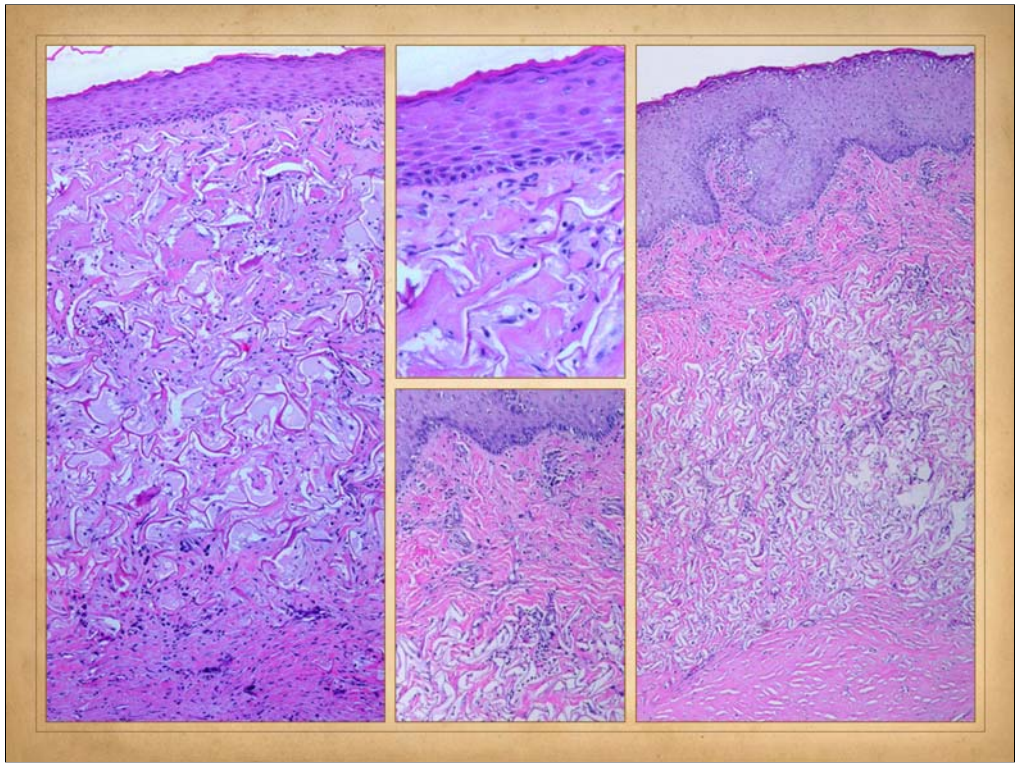
Exhibit natural antibiotic activities:

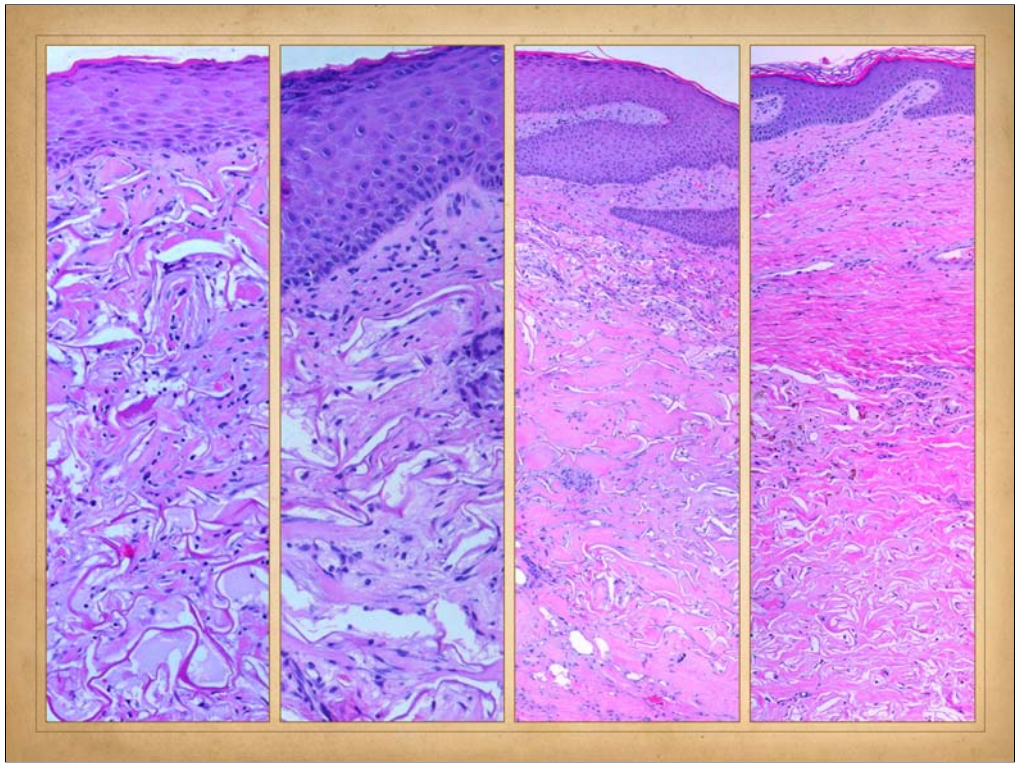
- gram-positive (*Staphylococcus aureus*)
- gram-negative (*Pseudomonas aeruginosa*)
- yeast (*Candida albicans*)

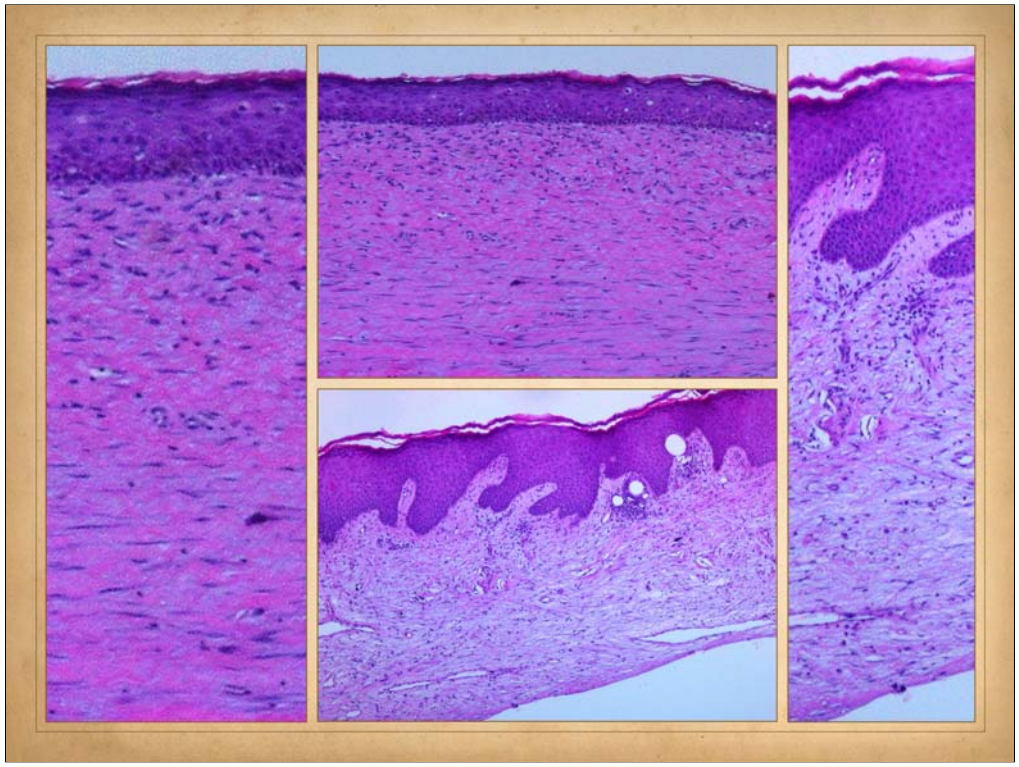
Subdivided into α- and β-defensins based on sequence relationship

Immunostaining of human beta defensin-2 in paraffin sections of human normal skin and Apligraf®. Human beta defensin-2 is not detectable in normal adult skin, but it is expressed in Apligraf. This data suggest that HBD-2 is a marker for activated keratinocytes and may help to protect skin lesions from infection

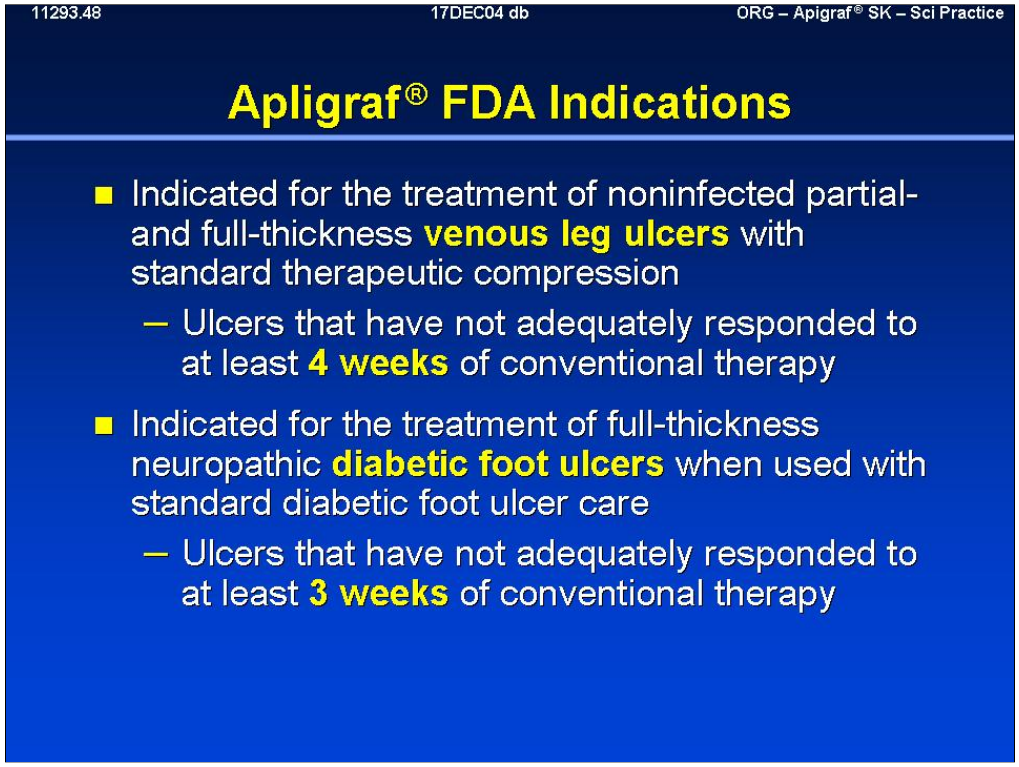








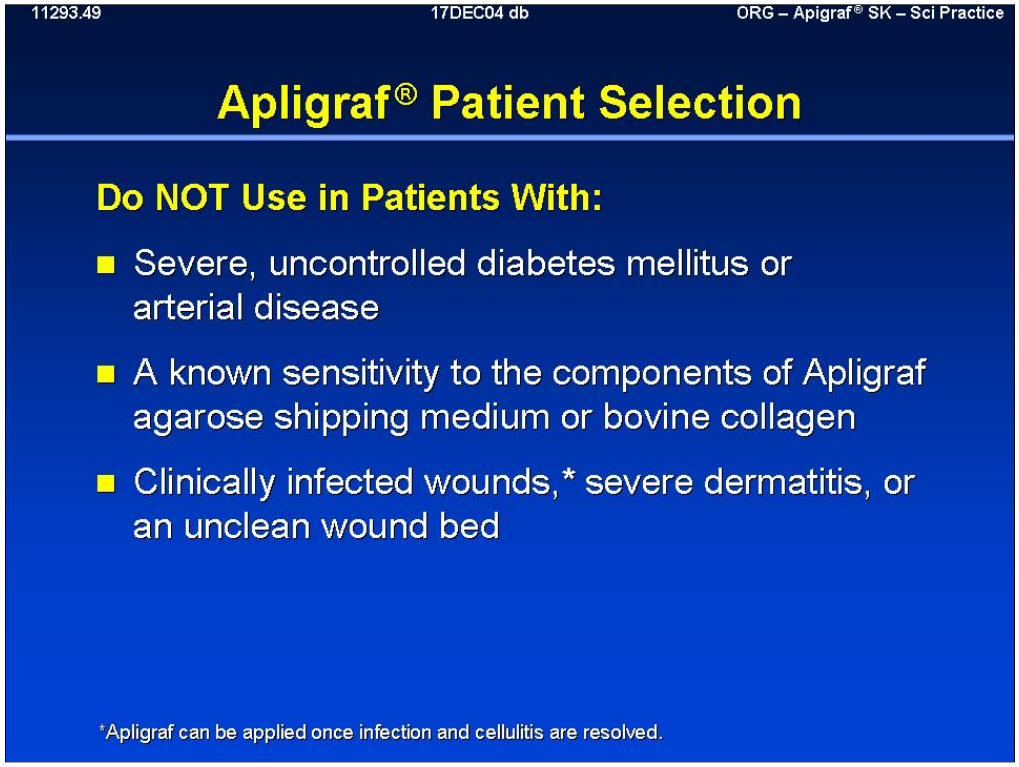

APLIGRAF
Re-Engineered Living Skin
APLIGRAF in USE
METHODS AND MANAGEMENT



Apligraf® FDA Indications

Indicated for the treatment of noninfected partial- and full-thickness venous leg ulcers with standard therapeutic compression (ulcers that have not adequately responded to at least 1 month of conventional therapy)

Also indicated for the treatment of full-thickness neuropathic diabetic foot ulcers when used with standard diabetic foot ulcer care (ulcers extending through the dermis but without tendon, muscle, capsule, or bone exposure, that have not adequately responded to at least 3 weeks of conventional therapy)



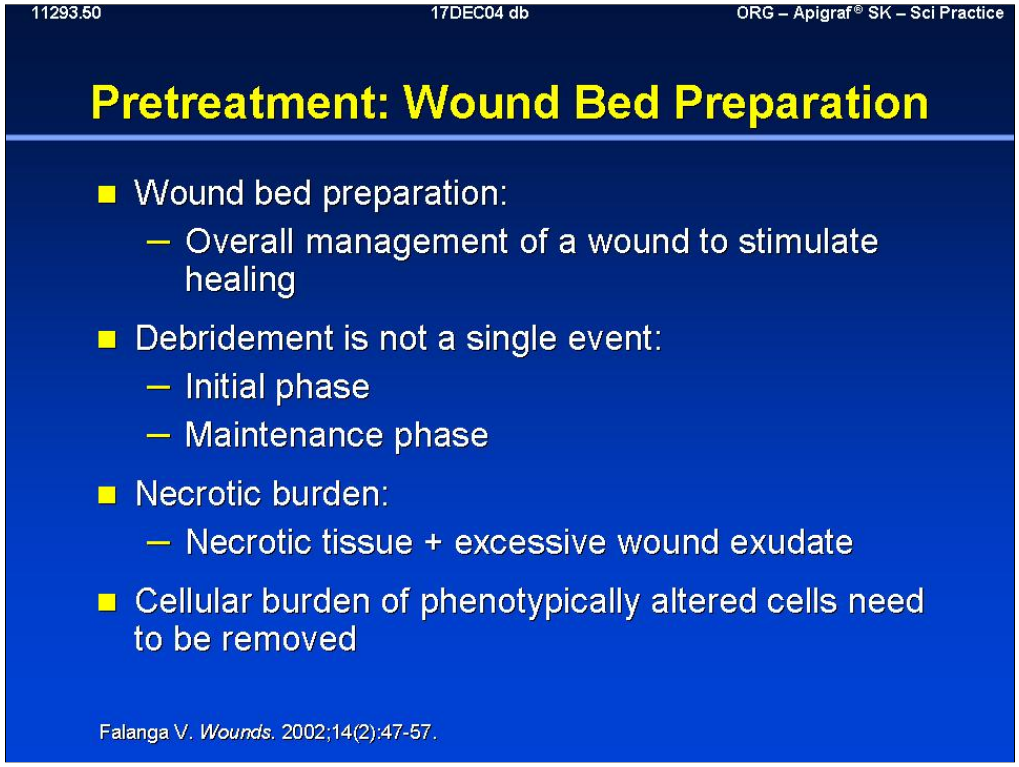
Apligraf® Patient Selection

Do NOT use in Patients With:

Severe, uncontrolled diabetes mellitus or
arterial disease

A known sensitivity to the components of Apligraf agarose shipping medium or bovine collagen

Clinically infected wounds,* severe dermatitis, or an unclean wound bed



Pretreatment: Wound Bed Preparation

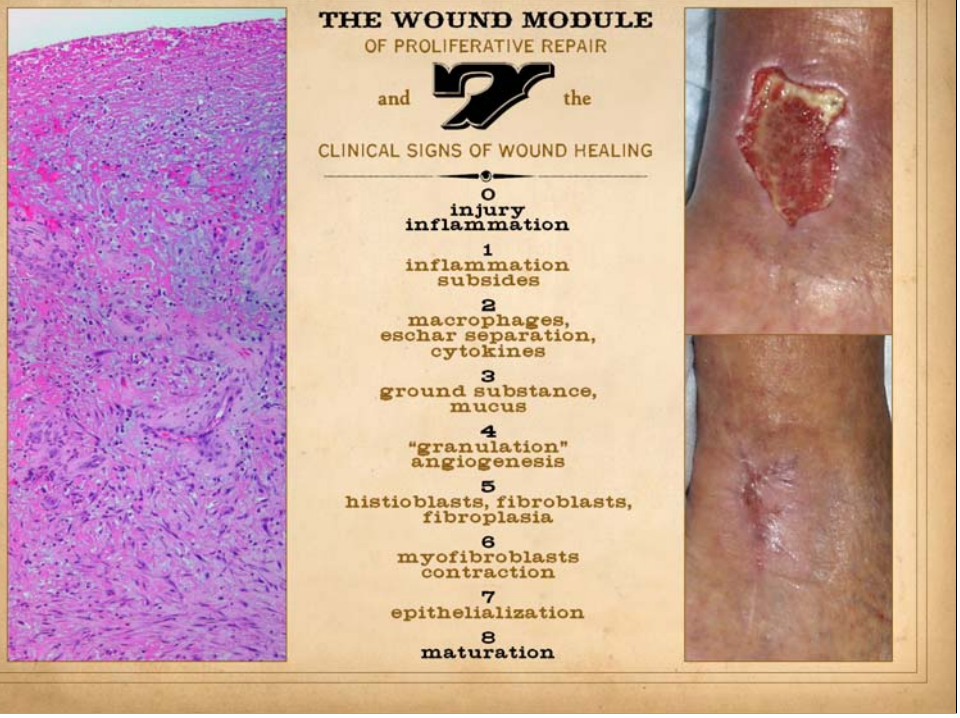
Emerging Concepts:

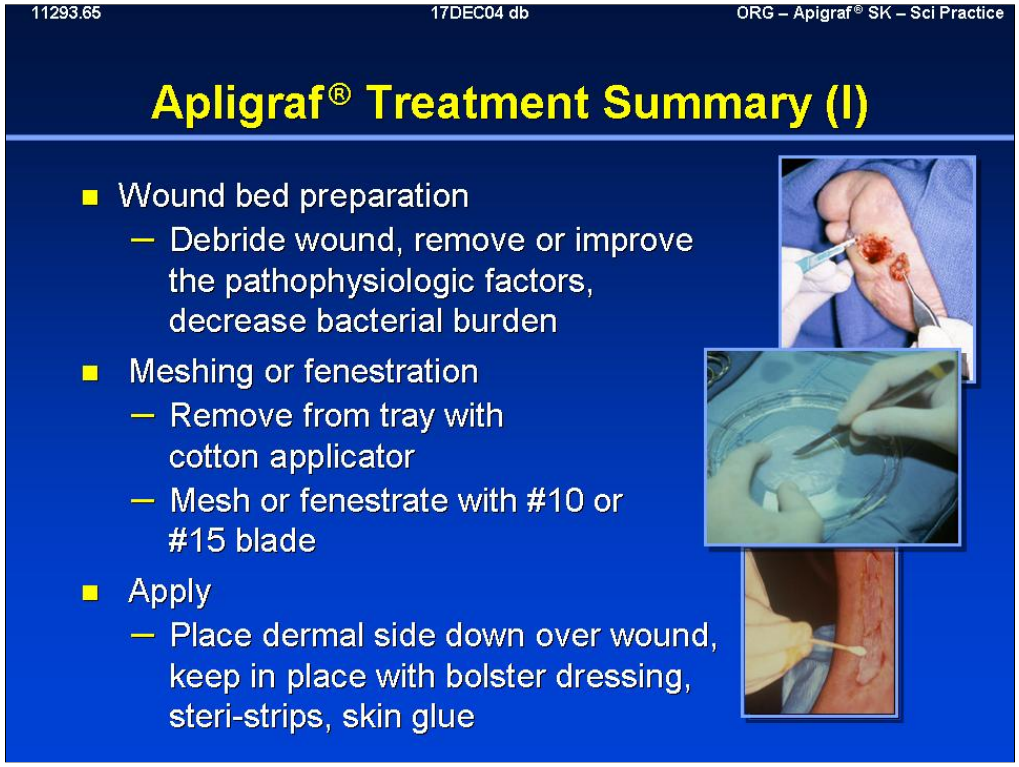
Wound bed preparation may be defined as the overall management of a wound to stimulate healing or to enhance the efficacy of additional therapies

Wound bed preparation must be differentiated from debridement alone. Debridement is not a single event but a continuum of care. Initial phase consisting of a sharp debridement with maintaining that wound bed with either enzymatic or mechanical techniques

The term necrotic burden refers to both necrotic tissue and wound exudate, thus inhibiting the proliferation and function of normal cellular activity. Wound exudate contains proteases that can break down extracellular matrix proteins

An important step in the wound bed preparation is the removal of senescent cells that are phenotypically altered (cellular burden)


THE WOUND MODULE
OF PROLIFERATIVE REPAIR
and
the
CLINICAL SIGNS OF WOUND HEALING
0
injury
inflammation
1
inflammation
subsides
2
macrophages,
eschar separation,
cytokines
3
ground substance,
mucus
4
"granulation"
angiogenesis
5
histioblasts, fibroblasts,
fibroplasia
6
myofibroblasts
contraction
7
epithelialization
8
maturation



Apligraf® Treatment Summary (I)

Prepare

Apligraf should be used with good wound care including off-loading and comprehensive diabetes management

Apligraf should be applied to a clean granulating wound bed. Wound should receive a sterile scrub, saline rinse, and be debrided of fibrotic and necrotic material. Debridement should extend to healthy, viable, bleeding tissue

Apply

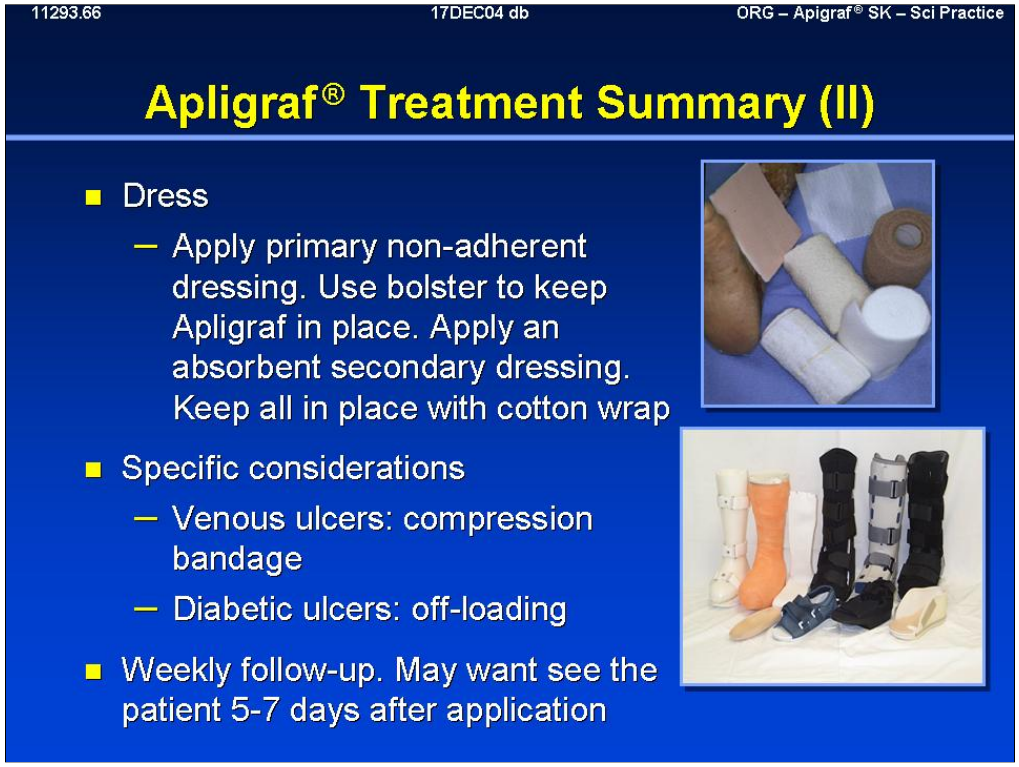
Apply Apligraf immediately after removing from packaging

Apligraf is packaged dermal (glossier) layer down, epidermal (matte) layer up. Keep track of product orientation after removing Apligraf from the storage dish; the dermal layer must be placed in direct contact with the wound bed

Loosen Apligraf from the storage dish with sterile gloves and/or sterile forceps

Be sure not to pierce or remove the polycarbonate membrane supporting Apligraf in the storage dish

Apligraf can be held in place by bolstering. Other anchoring techniques such as suturing, skin glue, or Steri-Strips® may also be used at the physician's discretion



Apligraf® Treatment Summary (II)

Dress

Cover Apligraf with a nonadherent primary dressing (eg, Adaptic® [Johnson & Johnson Medical], Mepitel® [Mölnlycke] Xeroform® [Kendall], Tegapore® [3M Health Care]), then apply and secure a secondary dressing

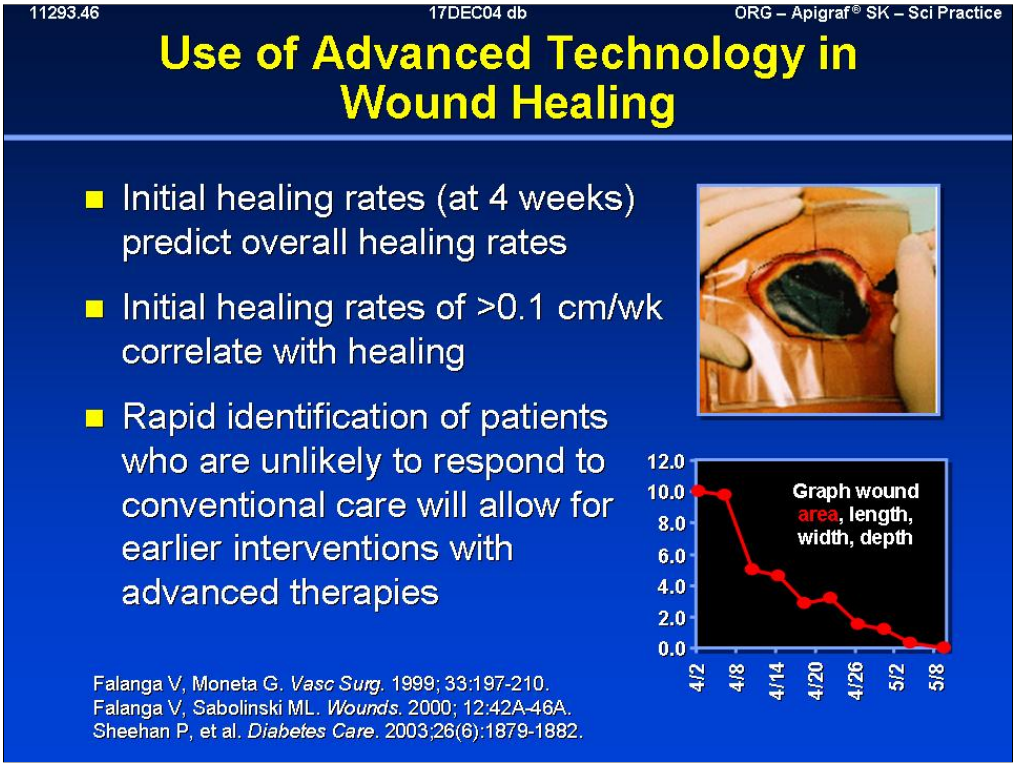
> Secondary dressings (eg, SurePress® [Conva Tec], Kling® [Johnson & Johnson Medical] cotton wrap) should be changed 5 to 7 days after initial application. The primary dressing should be left in place for 7 to 14 days. Removing the primary dressing too early may strip Apligraf away from the wound; subsequently, dressings may be changed once a week or at the discretion of the physician

Considerations

The patient should be placed in an appropriate off-loading device (at the physician's discretion). Failure to off-load causes ongoing mechanical trauma, which may prevent wound healing

Follow-up

The application site should be inspected after the primary dressing is removed. Apligraf appearance may vary from patient to patient

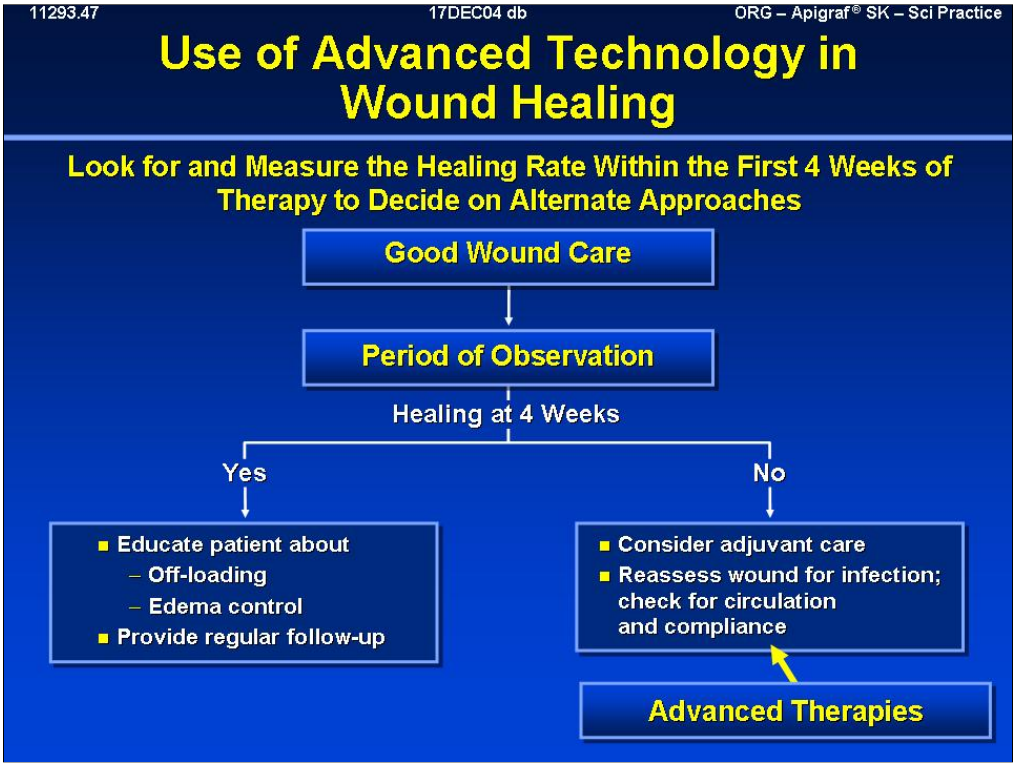


Use of Advanced Technology in Wound Healing

Initial healing rates (at 4 weeks) predict overall healing rates

Initial healing rates of >0.1 cm/wk correlate with healing

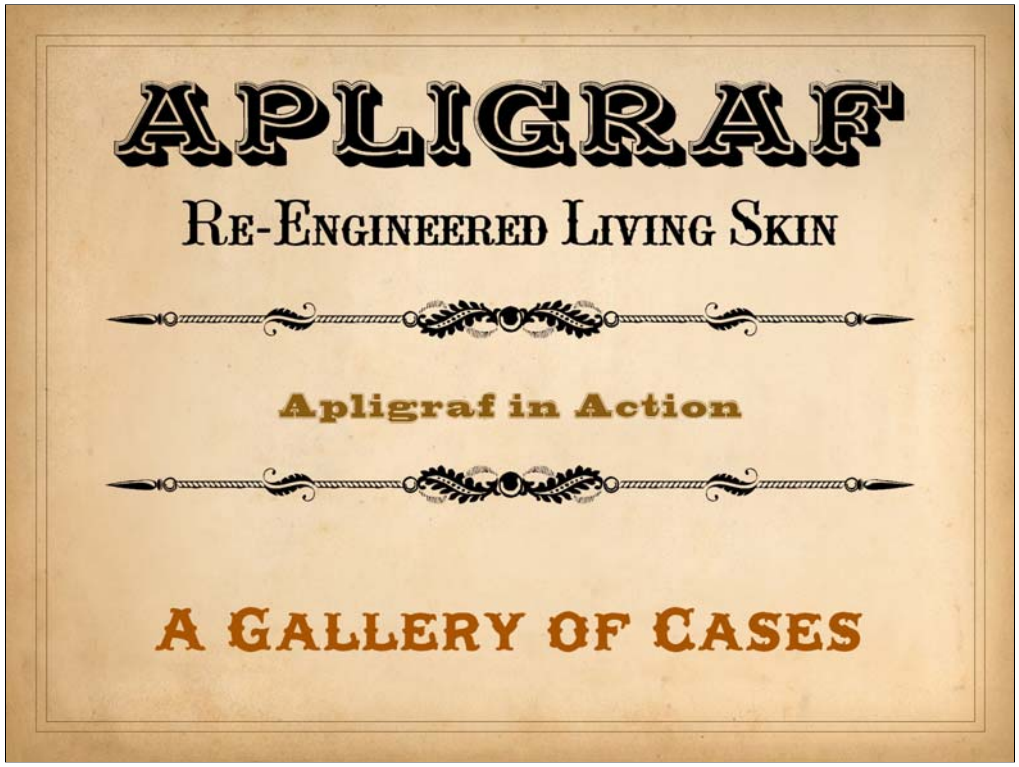
Rapid identification of patients who are unlikely to respond to conventional care will allow for earlier interventions with advanced therapies

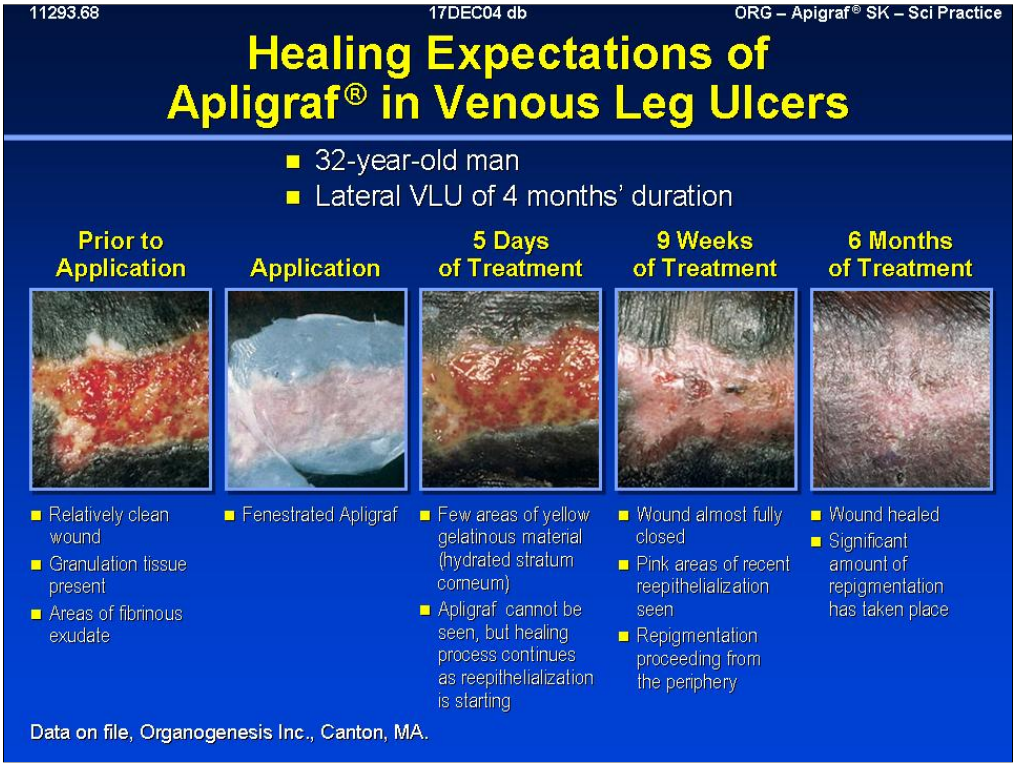


Use of Advanced Technology in Wound Healing

The above flow chart is a simple schematic on when to use advanced therapies. Data supports key time points (4-6 weeks) as an appropriate time to add advanced therapies to good wound care

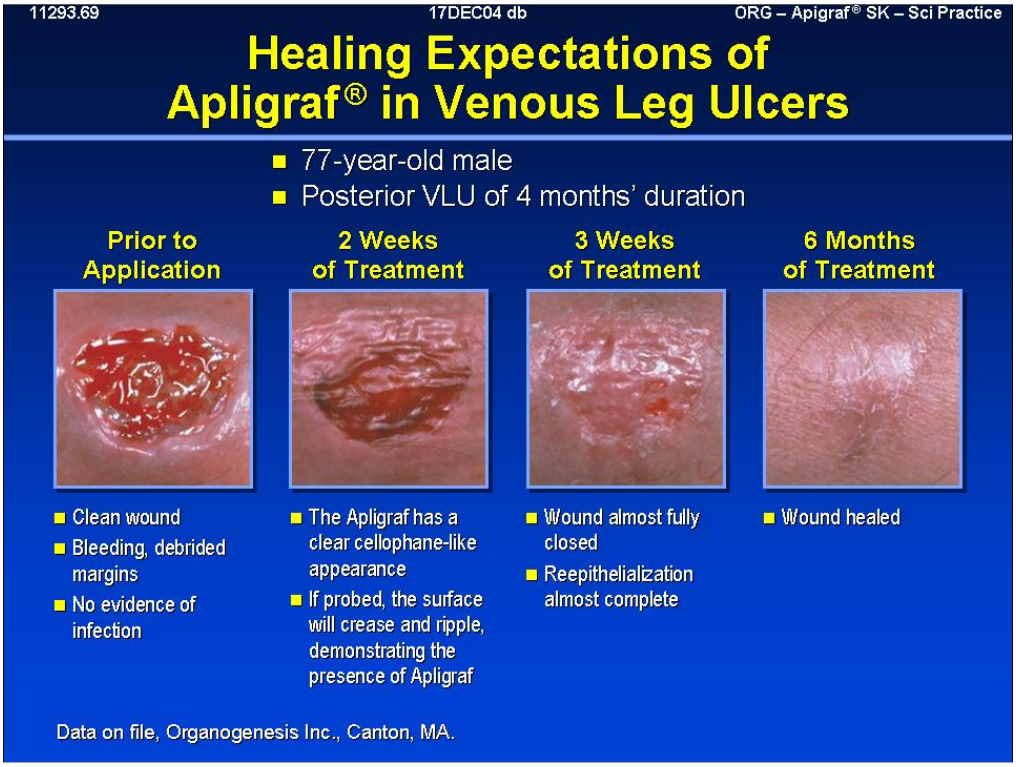
The data continues to evolve on the timing and triggering factors, however the 4 week time point has been evaluated in several studies


APLIGRAF
Re-Engineered Living Skin
Apligraf in Action
A Gallery of Cases



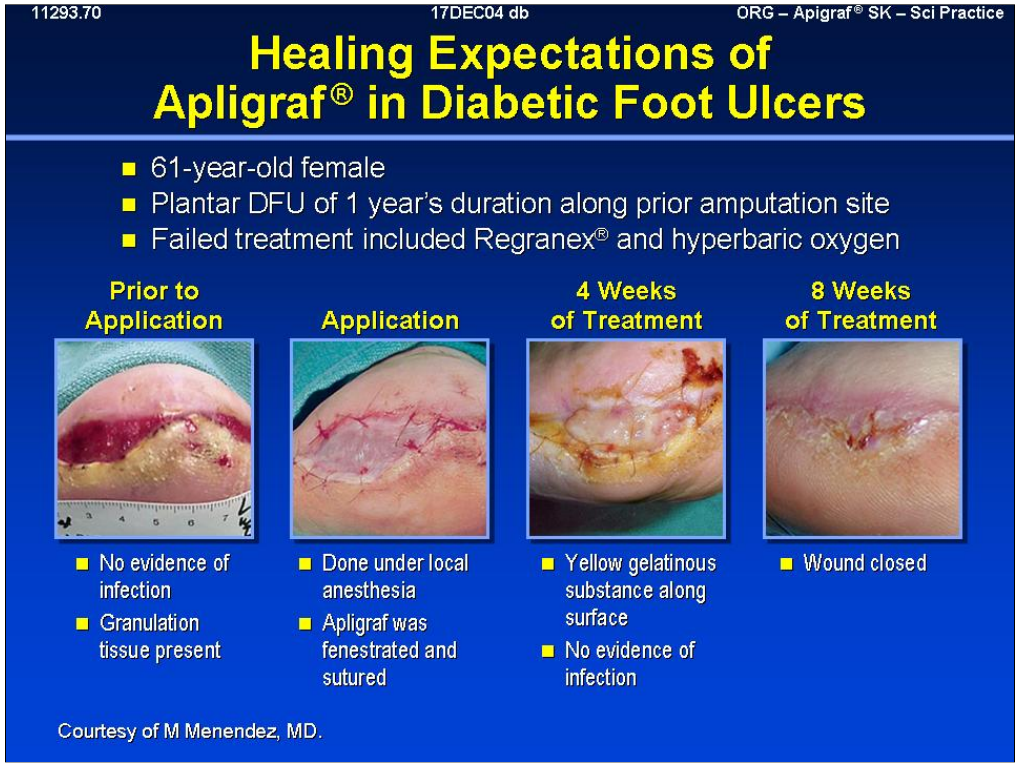
Healing Expectations of Apligraf® in Venous Leg Ulcers

Unlike autografts, which carry the melanocytic makeup and resulting pigment match of their donor site, Apligraf does not contain melanocytes. The repigmentation observed after treatment with Apligraf occurs from ingrowth of the patient's own melanocytes



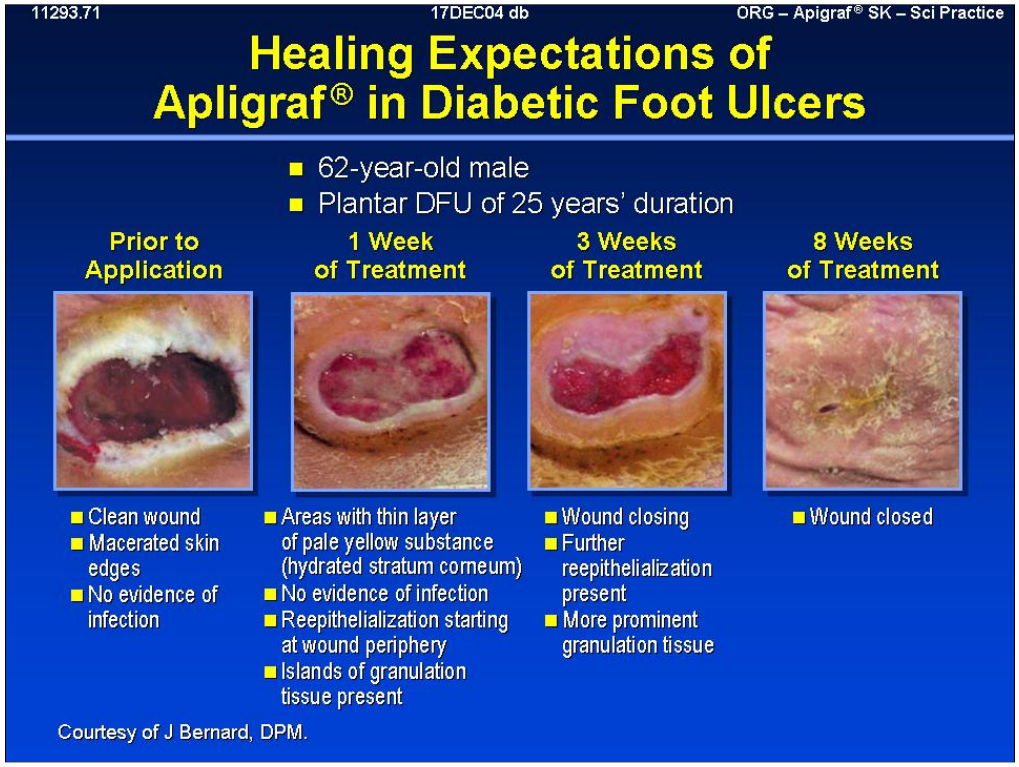
Healing Expectations of Apligraf® in Venous Leg Ulcers

Living fibroblasts and keratinocytes become an integral part of the healing site. Growth factors and cytokines present in normal wound healing have also been detected in Apligraf



Healing Expectations of Apligraf® in Diabetic Foot Ulcers

The yellow substance is attributed to hydrated stratum corneum. Do not mistake this for pus or fibrinous exudate. Wiping or debriding the wound may remove beneficial keratinocytes and fibroblasts in which cytokines and growth factors have been detected. If signs of infection, such as surrounding redness and fever, do occur, the infection should be worked up and treated appropriately



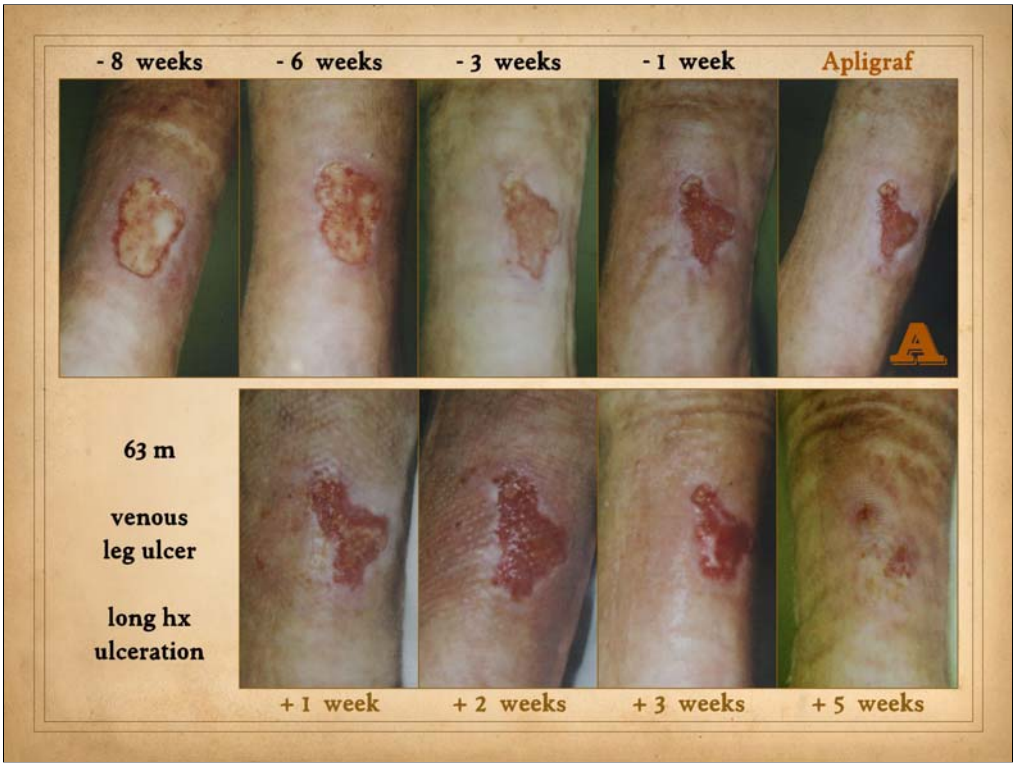
Healing Expectations of Apligraf® in Diabetic Foot Ulcers

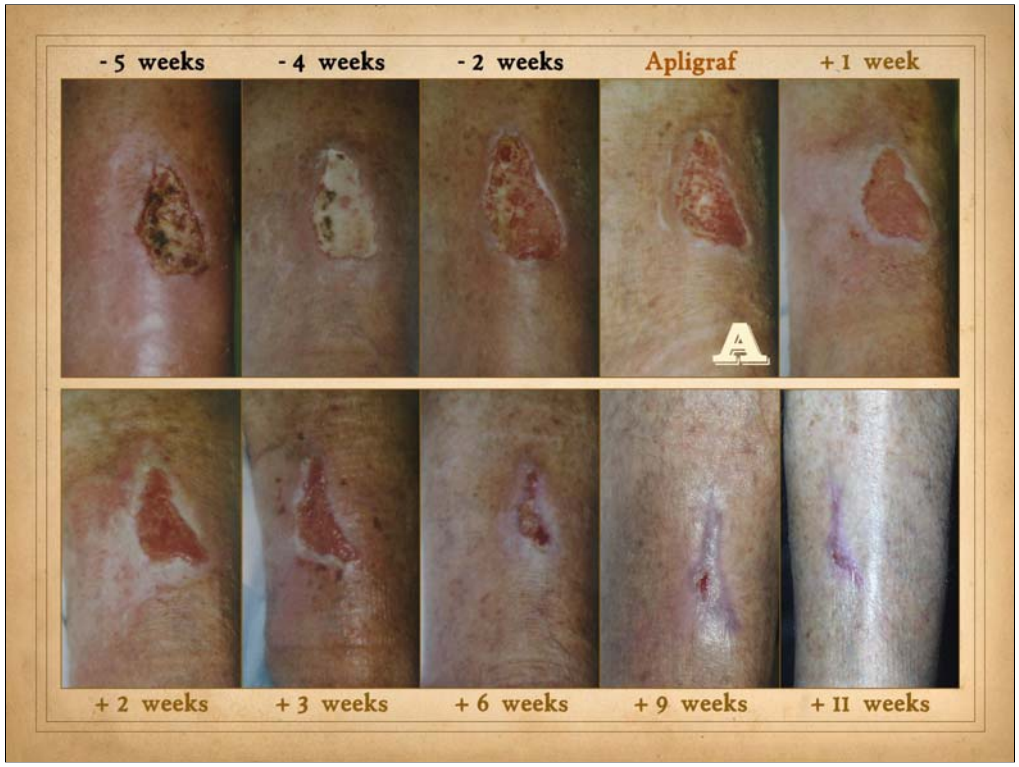
Prior to Apligraf application the wound has a clean appearance, with mild maceration surrounding the wound edges, that could have been further debrided. There were no clinical signs of infection

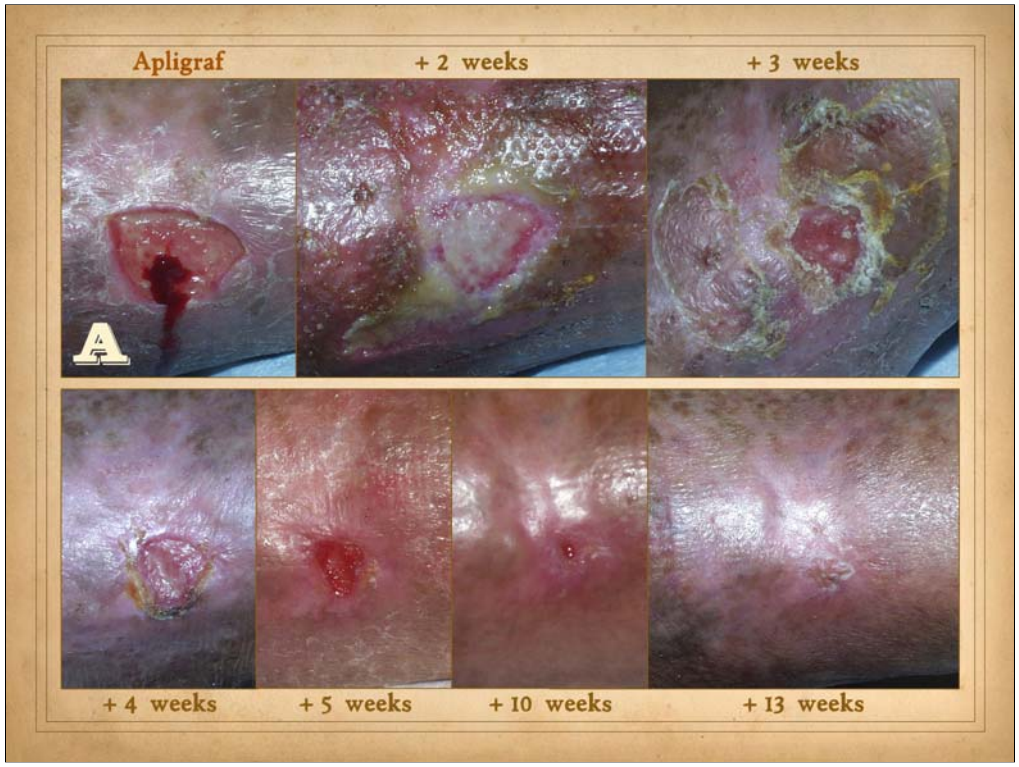
Following Apligraf application post 1 week, there areas with a thin layer of hydrated stratum corneum appears. There are no sign of infection and reepithelialization is starting at the wound margin and islands of granulation tissue are present

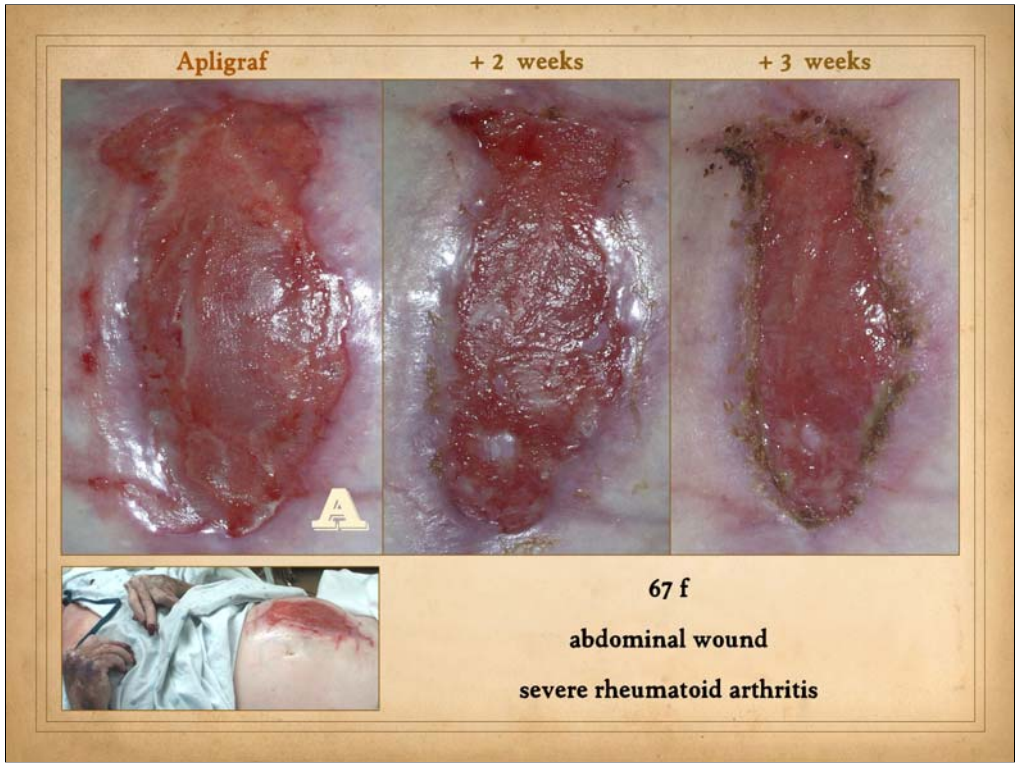
At 3 weeks post Apligraf application the wound has significantly reduced in size with additional granulation tissue present

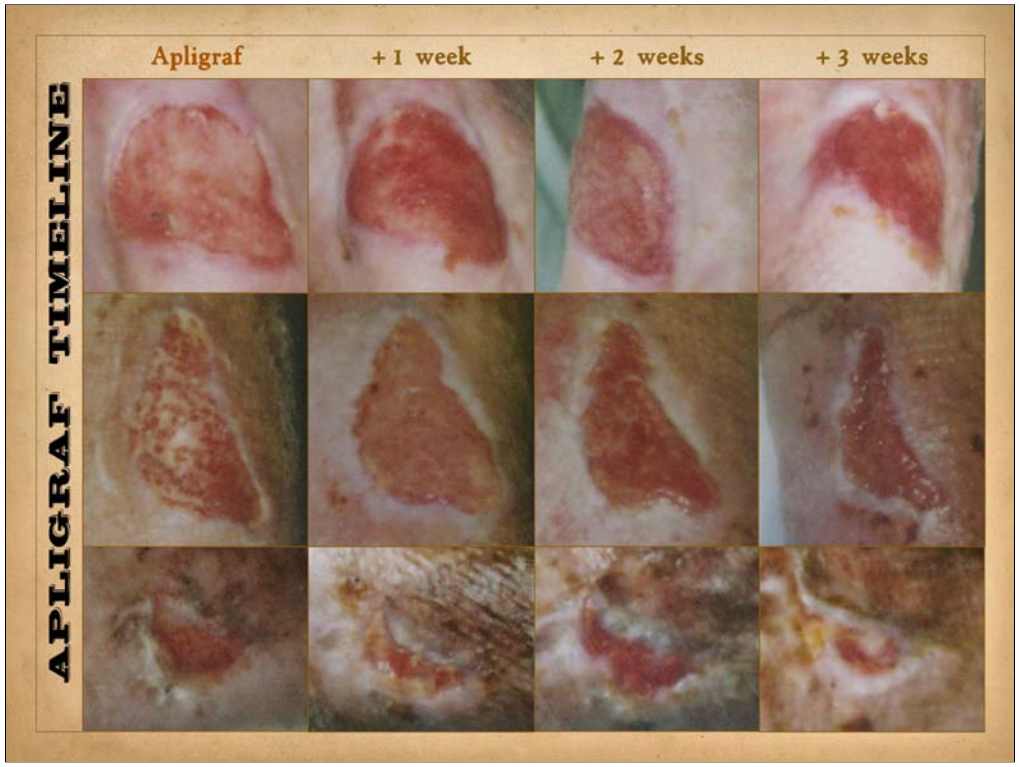
At 8 weeks the wound has completely closed

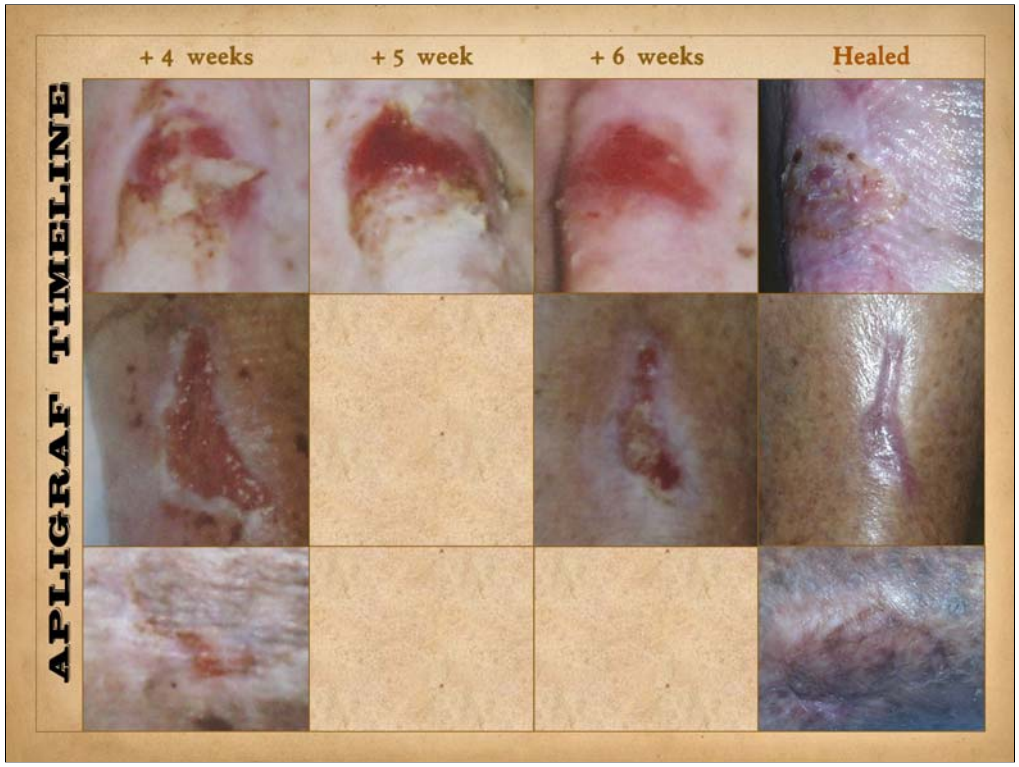

- 8 weeks
- 6 weeks
- 3 weeks
- 1 week
Apligraf
63 m
venous
leg ulcer
long hx
ulceration
+ 1 week
+ 2 weeks
+ 3 weeks
+ 5 weeks

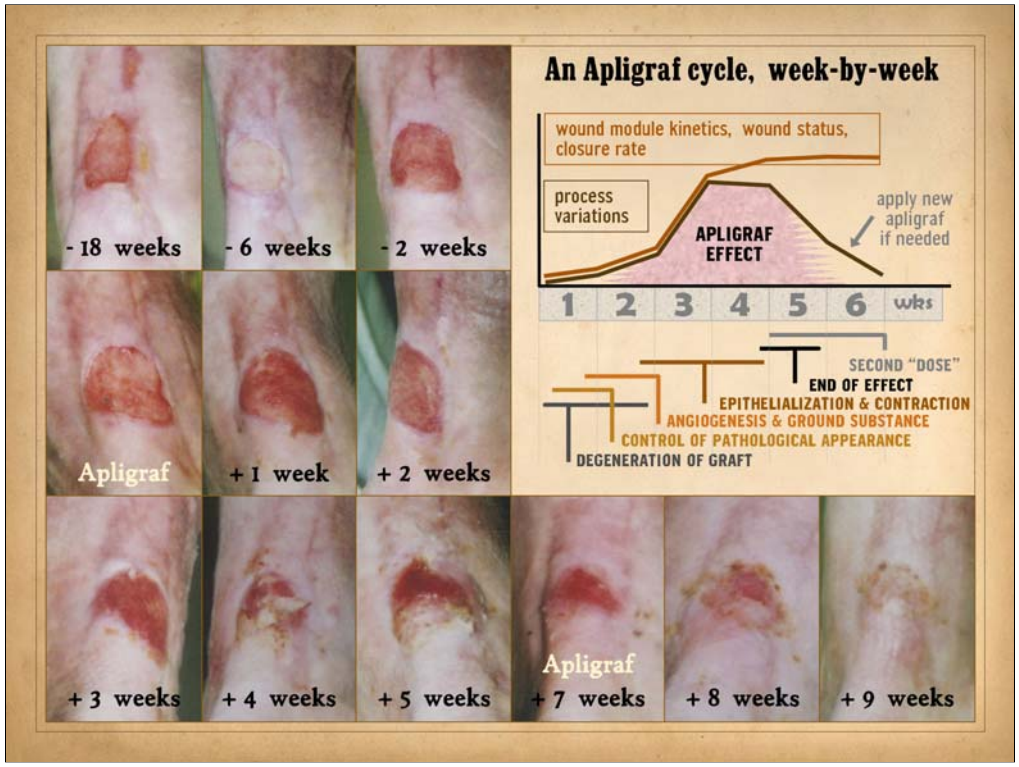

- 5 weeks
- 4 weeks
- 2 weeks
Apligraf
+ 1 week
+ 2 weeks
+ 3 weeks
+ 6 weeks
+ 9 weeks
+ 11 weeks

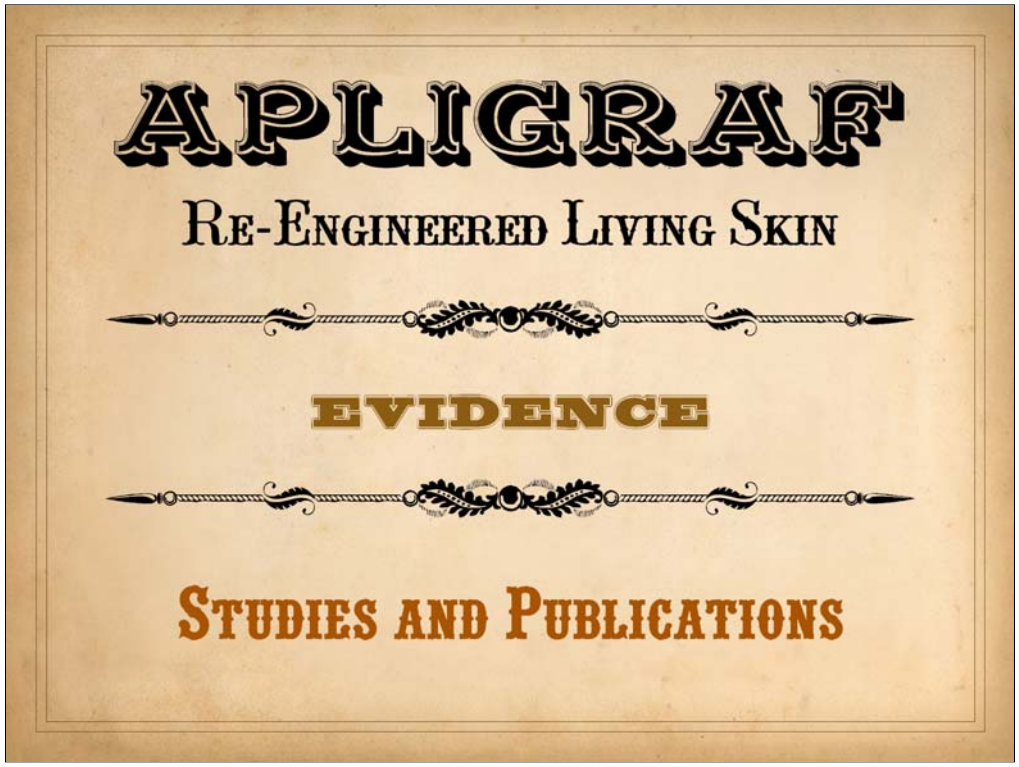

Apligraf
+ 2 weeks
+ 3 weeks
+ 4 weeks
+ 5 weeks
+ 10 weeks
+ 13 weeks

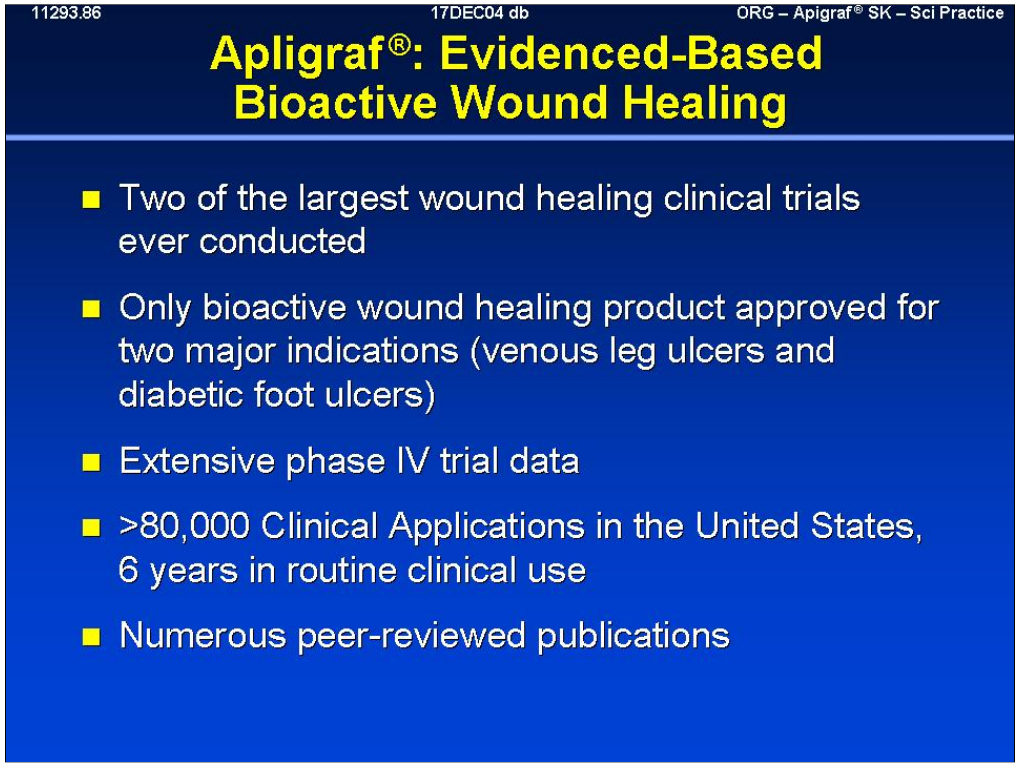

Apligraf
+ 2 weeks
+ 3 weeks
67 f
abdominal wound
severe rheumatoid arthritis


APLIGRAF TIMELINE
Apligraf
+ 1 week
+ 2 weeks
+ 3 weeks


APLIGRAF TIMELINE
+ 4 weeks
+ 5 week
+ 6 weeks
Healed


- 18 weeks
- 6 weeks
- 2 weeks
Apligraf
+ 1 week
+ 2 weeks
+ 3 weeks
+ 4 weeks
+ 5 weeks
Apligraf
+ 7 weeks
+ 8 weeks
+ 9 weeks
An Apligraf cycle, week-by-week
wound module kinetics, wound status, closure rate
process variations
APLIGRAF EFFECT
apply new apligraf if needed
1 2 3 4 5 6 wks
SECOND "DOSE"
END OF EFFECT
EPITHELIALIZATION & CONTRACTION
ANGIOGENESIS & GROUND SUBSTANCE
CONTROL OF PATHOLOGICAL APPEARANCE
DEGENERATION OF GRAFT


APLIGRAF
Re-Engineered Living Skin
EVIDENCE
Studies and Publications



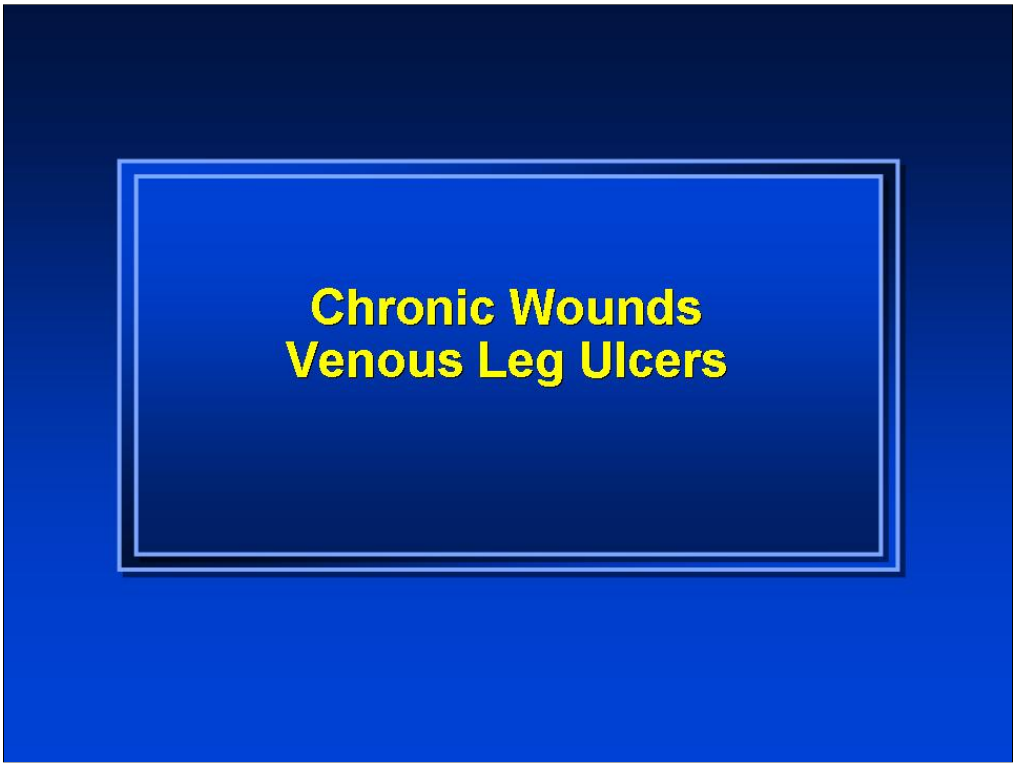
Apligraf ®: Evidence-Based Bioactive Wound Healing

Apligraf is the only bioactive wound healing product FDA approved for both venous leg ulcers and diabetic foot ulcers. The FDA approved Apligraf in 1998 for venous leg ulcers based on the pivotal trial data of 240 patients with venous leg ulcers. In 2000 Apligraf was approved for diabetic foot ulcers based on the clinical trail evaluating 208 patients

There have been numerous late phase trials with Apligraf conducted in the United States and Europe

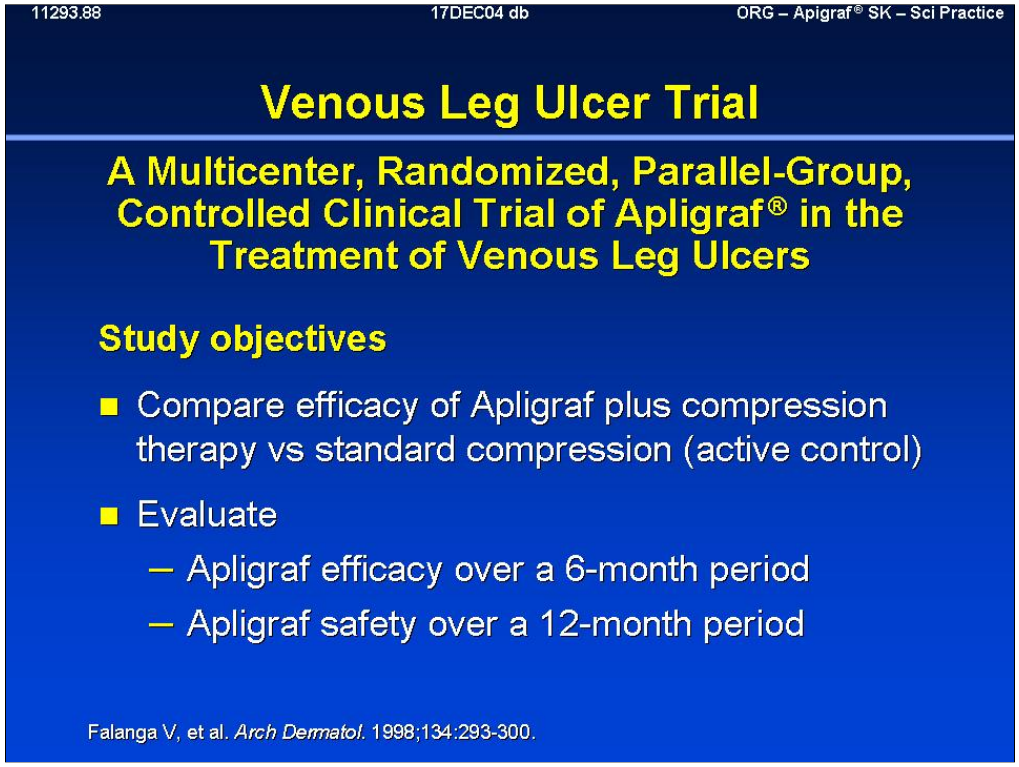
Currently greater the 80,000 clinical applications in the United States and Apligraf has been utilized in 6 years of clinical practice

There has been greater than 150 peer-reviewed publications on Apligraf



Chronic Wounds

Venous Leg Ulcers



Venous Leg Ulcer Trial[1]

A Multicenter, Randomized, Parallel-Group, Controlled Clinical Trial of Apligraf ® in the Treatment of Venous Leg Ulcers

This prospective study compared Apligraf plus compression therapy with active control (standard multilayer compression therapy with Unna's boot). This pivotal trial is the first large-scale study to evaluate Apligraf for wound healing and is the largest prospective, randomized, controlled venous leg ulcer trial performed to date

The study involved 297 patients aged 18 to 85 years with venous leg ulcers secondary to venous insufficiency that had been open for at least one month and that had failed previous treatment

240 patients were evaluated for efficacy over a 6-month period: twice during the first week, weekly for the next 7 weeks, at month 3, and at month 6

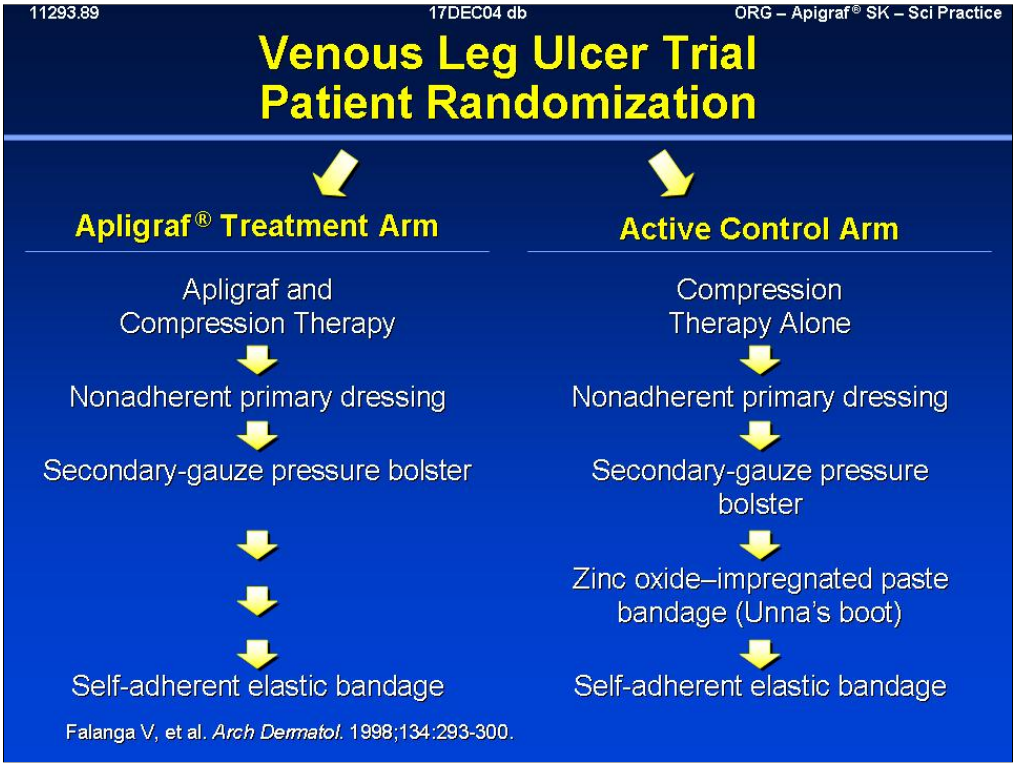
Safety evaluations were conducted for 297 patients over a 6-month period in parallel with efficacy evaluations followed by an additional 6 months for follow-up[1]

Patients with severe arterial insufficiency, diagnosed by an ankle-brachial index of 0.65 or less, or other medical conditions known to impair wound healing, such as uncontrolled diabetes, were excluded from the study

Combination Compression Systems

Images above represent 2 types of compression systems: short stretch + elastic bandage and a multilayer compression bandaging system

Compression therapy, achieved using a variety of techniques, including gradient elastic stockings, paste gauze bandages, multilayer elastic wraps/bandages, or pneumatic pumps, continues to represent a primary treatment option for chronic venous ulcers



Venous Leg Ulcer Trial Patient Randomization

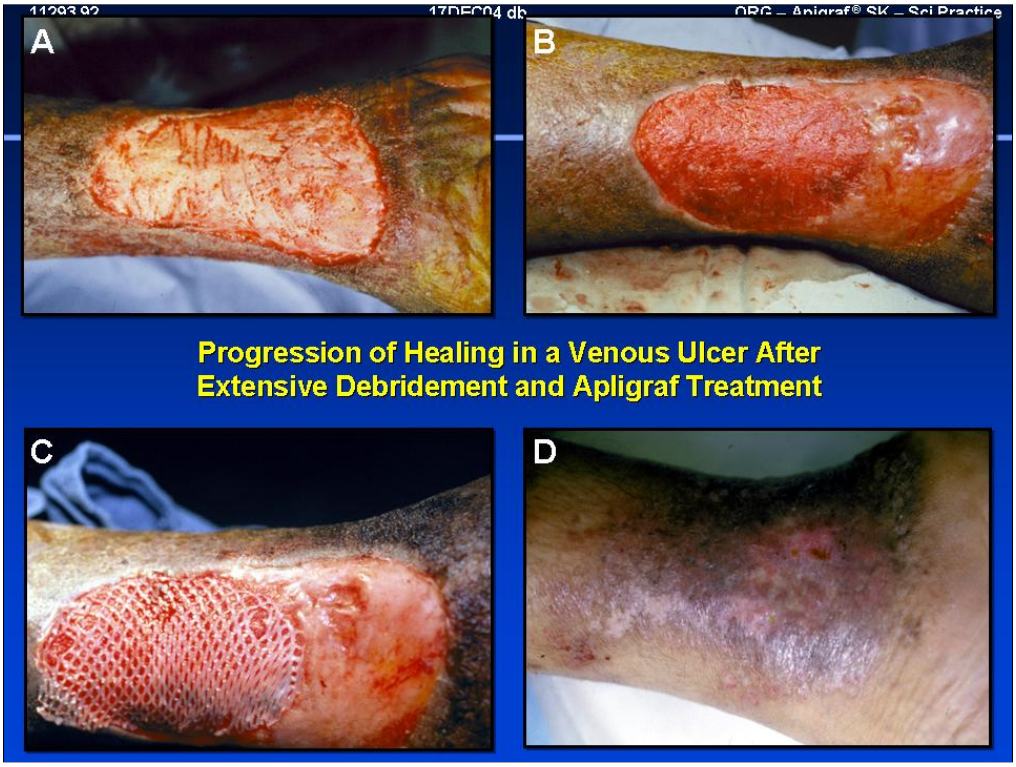
Patients were randomly assigned to receive either Apligraf plus compression therapy or active control therapy consisting of stan-dard compression therapy alone (Unna's boot)

In the Apligraf treatment group, Apligraf was placed directly on the ulcer site, followed by a 3-layer compression wrap consisting of

- A nonadherent primary dressing (Tegapore®, 3M Health Care, or Adaptic®, Johnson and Johnson Medical)
- A secondary gauze cloth pressure bolster
- A self-adherent elastic bandage (A zinc oxide—impregnated paste bandage **was not** applied)

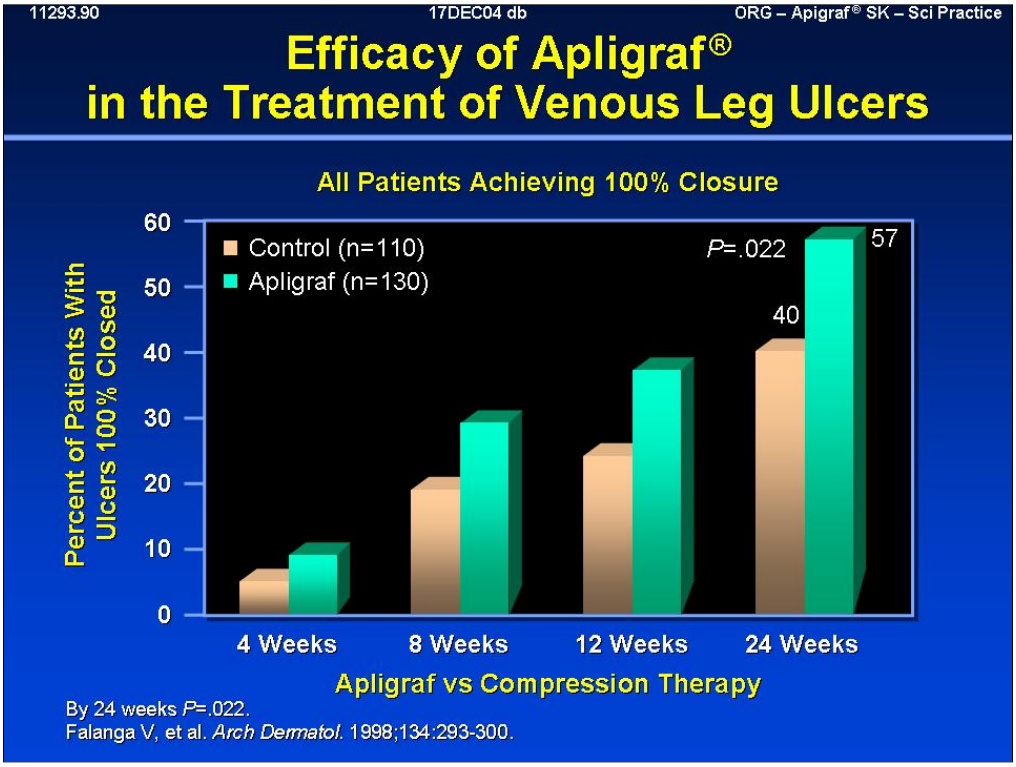
In the active control group, patients received standard multilayer compression therapy con-sisting of

- A nonadherent primary dressing (Tegapore or Adaptic)
- An overlay with a gauze pressure bolster
- A zinc oxide—impregnated paste bandage (Unna's boot)
- A self-adherent elastic bandage



Example of Apligraf® Healing a Venous Leg Ulcer

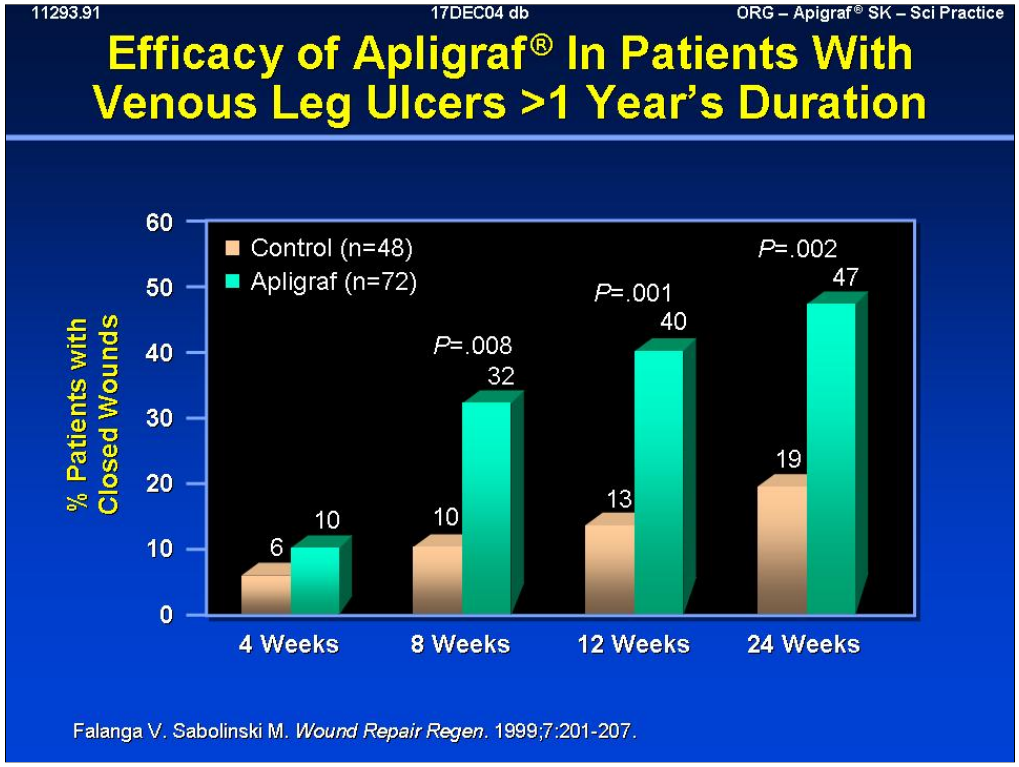
Following the basic tenets of wound care for this venous leg ulcer, Apligraf demonstrated significant healing



Efficacy of Apligraf® in the Treatment of Venous Leg Ulcers

Apligraf plus compression therapy was more effective in achieving complete wound closure by week 24 (57% vs 40%, *P* =.022*) than compression therapy alone[1]

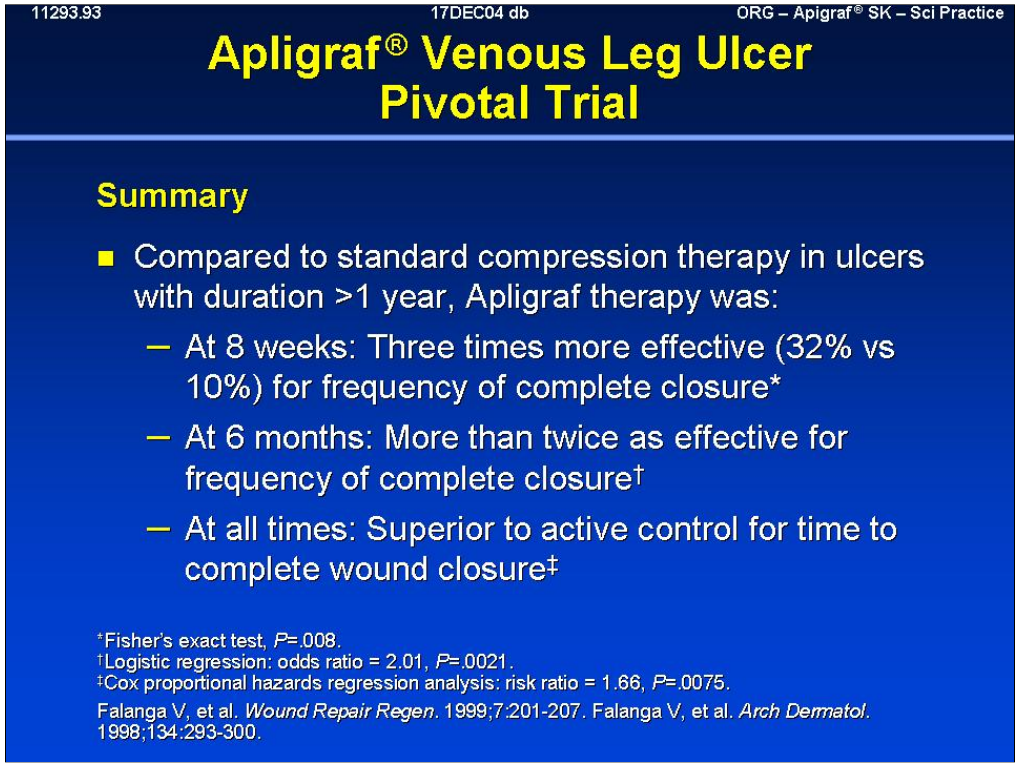
In patients with ulcers >1 year's duration (n=120), Apligraf plus compression therapy was more than twice as effective in achieving complete wound closure by week 24 (47% vs 19%, *P* =.002).[2] These data compare with 66% vs 73% (Apligraf vs control), *P* =.434, for patients with ulcers <1 year's duration (n= 120)



Efficacy of Apligraf® In Patients With Venous Leg Ulcers >1 Year's Duration

In the clinical trial, Apligraf was proven to be beneficial in patients with ulcers of greater than 1 year's duration

A total of 120 patients had wounds that were of greater than 1 years duration (72 in the Apligraf group, 48 in the active control group). Of those, significantly more patients treated with Apligraf achieved complete wound closure at 6 weeks, 8 weeks, 12 weeks, and 6 months than did active control patients (P=.048, $P \sim$ 008, P =.001, and P =.002, respectively)



Apligraf ® Venous Leg Ulcer Pivotal Trial

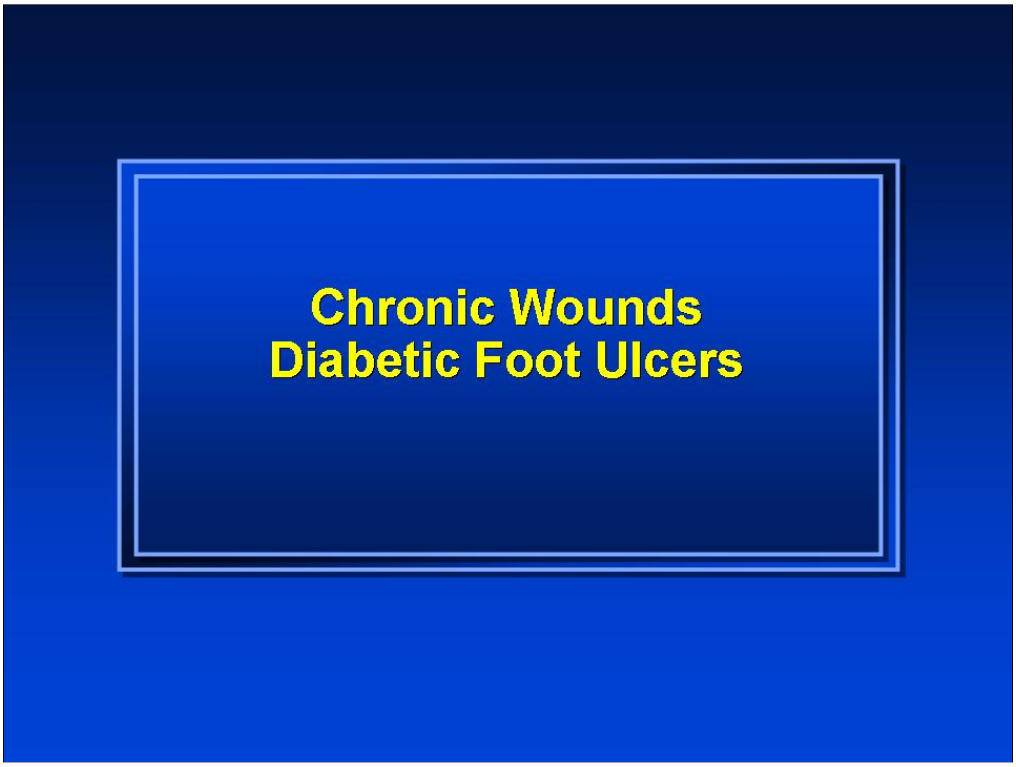
Apligraf was more than twice as effective in achieving wound closure at 6 months than was standard compression therapy alone in patients with hard-to-heal ulcers

Apligraf was 3 times more effective in achieving wound closure at 8 weeks when compared with standard compression therapy alone in patients with hard-to-heal ulcers

For patients with hard-to-heal ulcers of greater than 1 year's duration, Apligraf treatment was superior to active control for time to complete wound closure

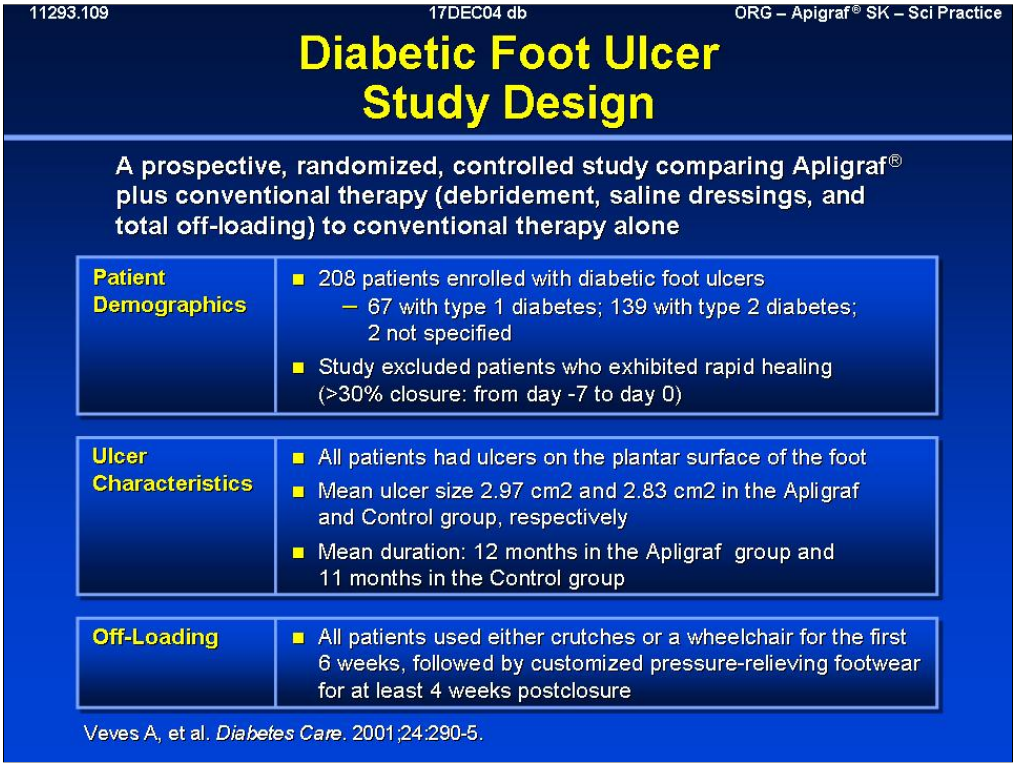
Apligraf ® Evidence-based Healing:
Multiple Clinical Trials in Venous Leg Ulcers

Listed above a peer-reviewed publication in which Apligraf demonstrated effective healing in patients with venous leg ulcers



Chronic Wounds

Diabetic Foot Ulcers



Diabetic Foot Ulcer Study Design

A Multicenter, Prospective, Randomized, Controlled Clinical Trial of Apligraf ® in the Treatment of Diabetic Foot Ulcers

Demographic data were comparable between Apligraf and control groups with no significant differences

All patients were instructed to avoid weight-bearing on the affected foot throughout the duration of the study. During the first 6 weeks, patients were instructed to use crutches or a wheelchair. All patients received customized tridensity sandals at the initiation of the study and wore them throughout the study, or for a minimum of
4 weeks **after the** ulcer achieved complete wound closure

Patient Enrollment

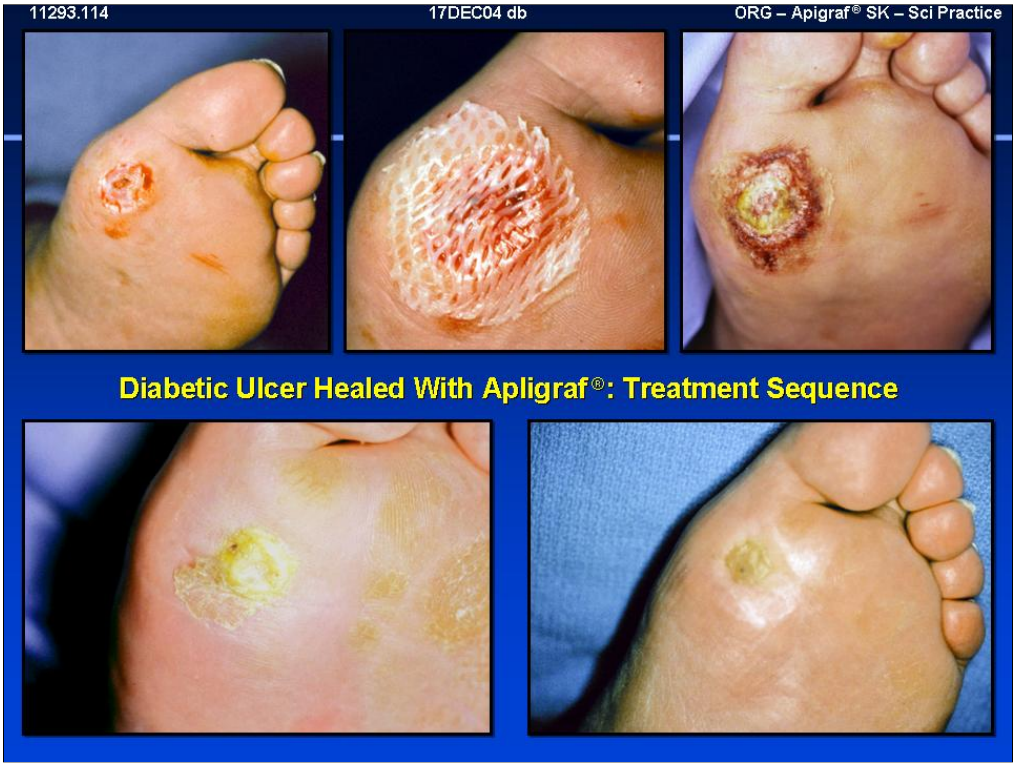
A total of 24 sites participated in the trial. 277 patients were randomized during die screening visit but 69 were disqualified when they were seen a week later because they did not fulfill the inclusion/exclusion criteria

The 208 patients who were enrolled into die treatment phase were randomized to receive Apligraf treatment (112 patients) and control (standard care) treatment (96 patients)

Patient demographics and baseline characteristics were comparable between groups

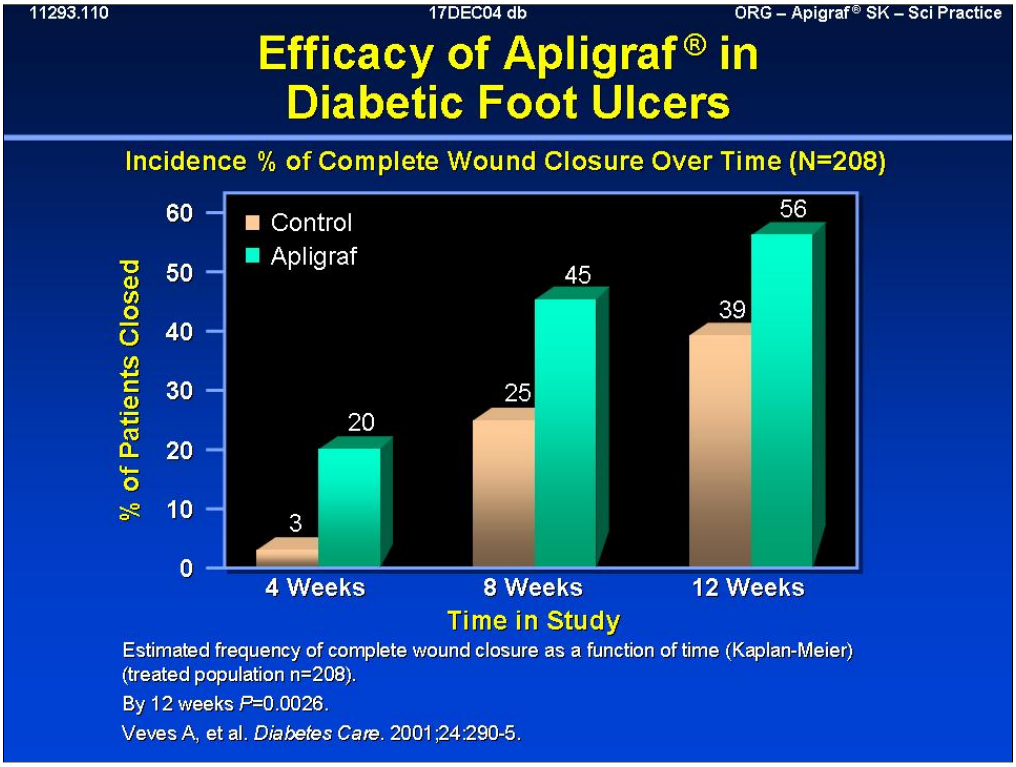
Mean baseline ulcer area was 3.0 cm^2 for the Apligraf group and 2.8 cm^2 for the control group

Mean duration of study ulcer was 12 months for the Apligraf group and 11 months for the control group



Example of Apligraf ® Healing a Diabetic Foot Ulcer

Following the basic tenets of wound care for this diabetic foot ulcer, Apligraf demonstrated significant healing



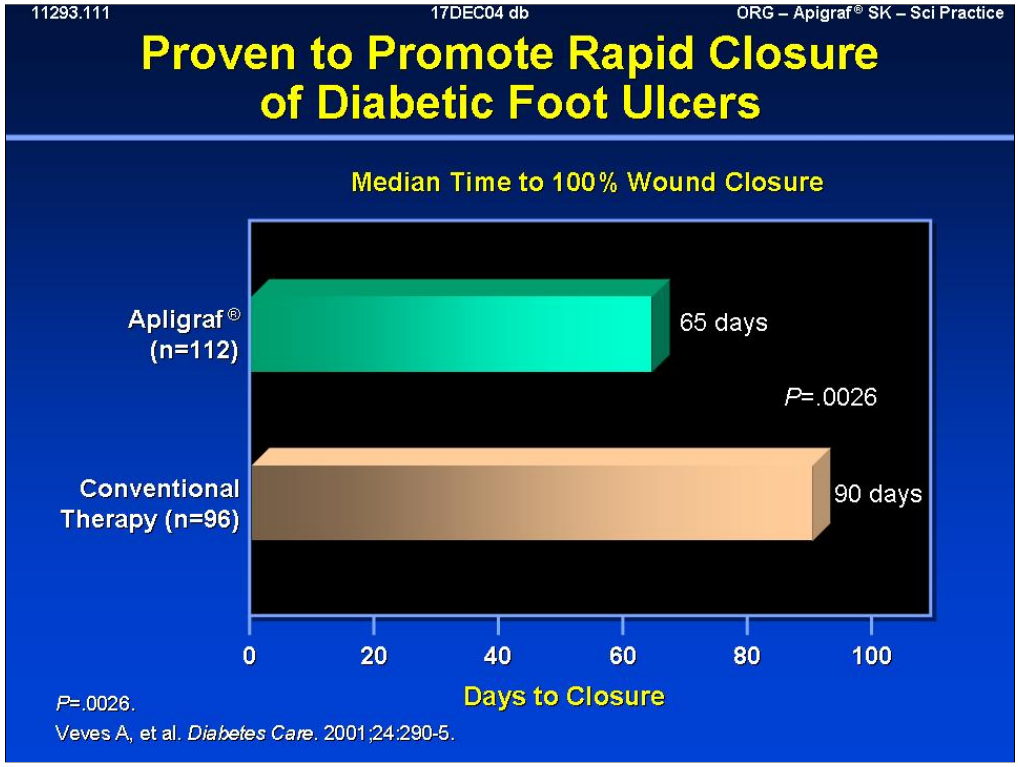
Efficacy of Apligraf ® in Diabetic Foot Ulcers

Complete wound closure of the study ulcer was defined as 100% epithelialization with an absence of drainage. Epithelialization was defined as a thin layer of epithelium visible on the wound surface

Evaluations were performed weekly for the first 12 weeks, with follow-up visits at 4, 5, and 6 months

By 12 weeks of treatment, 56% (63/112) of diabetic foot ulcers (DFUs) treated with Apligraf plus conventional therapy (debridement, saline dressings, total off-Ioading) were 100% closed, compared to 39% (36/96) of ulcers treated with conventional therapy alone (*P* =.0026)

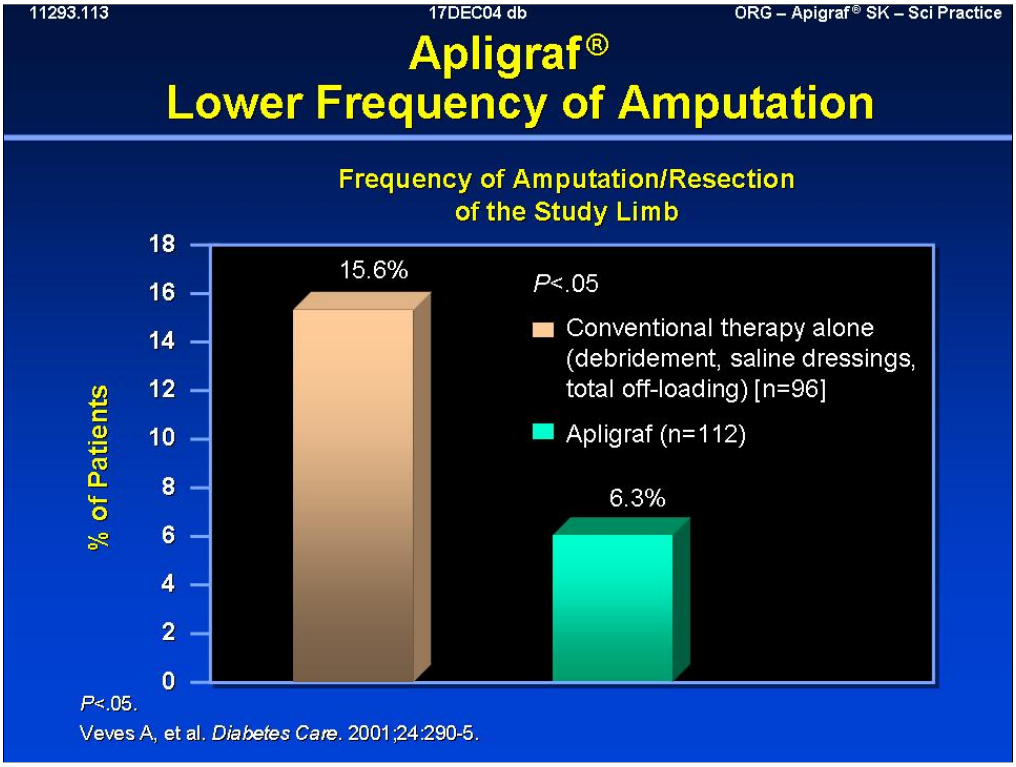
Estimated frequency of complete DFU wound closure as a function of time by Kaplan-Meier analysis



Proven to Promote Rapid Closure of Diabetic Foot Ulcers

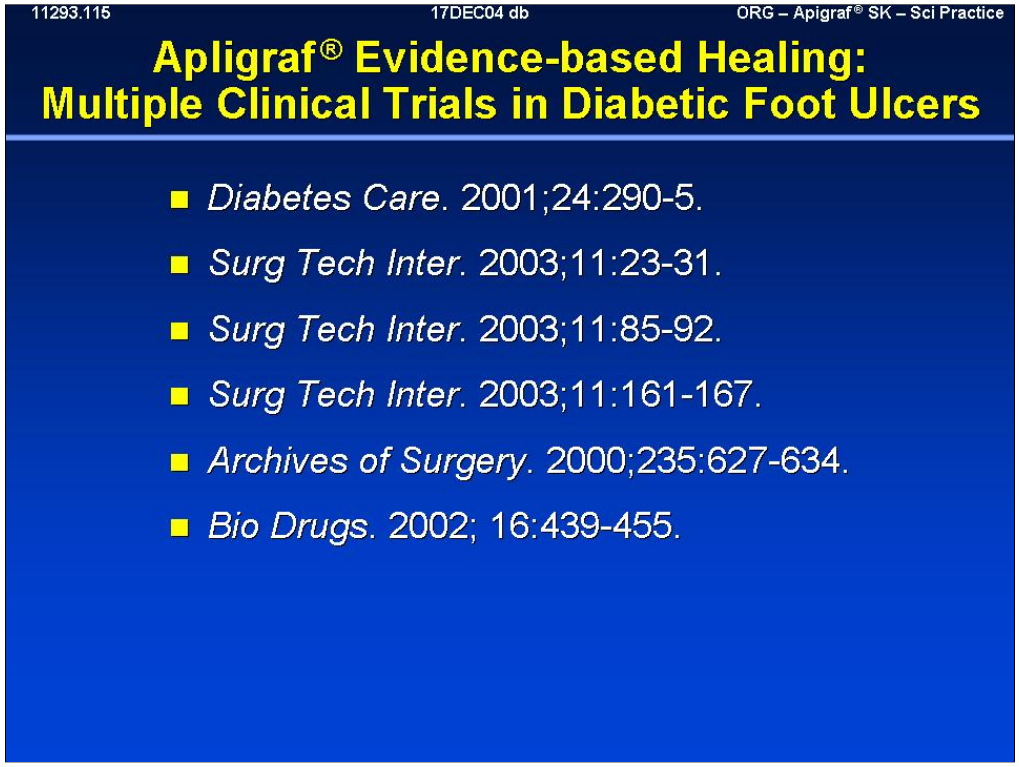
Median time to 100% wound closure

The median time to 100% wound closure was 65 days for diabetic foot ulcers treated with Apligraf plus conventional therapy compared to 90 days for ulcers treated with conventional therapy alone (P =.0026)



Apligraf ® — Lower Frequency of Amputation

Apligraf treated patients required significantly fewer amputations/resections of the study limb. (6.3% vs 15.6%) (P<.05) compared to patients treated with conventional therapy at 6 months



Apligraf ® Evidence-based Healing: Multiple Clinical Trials in Diabetic Foot Ulcers

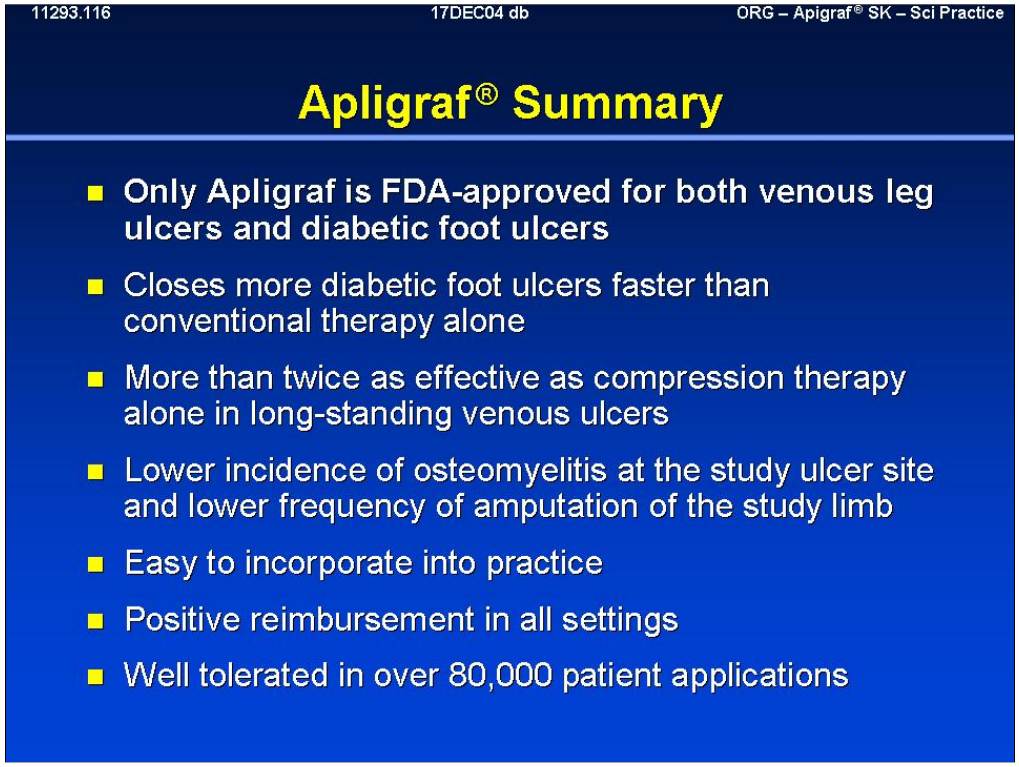
Listed above a peer-reviewed publication in which Apligraf demonstrated effective healing in patients with diabetic foot ulcers

SUMMARY

Apligraf

Re-engineered human living skin equivalent.
It functions as a pharmaceutical – a drug packaged in a living vehicle.
It stimulates repair in chaotic or retarded wounds.
Treated wounds have a predictable response of 4-6 weeks duration.

"Official" indications for venous & diabetic ulcers, supported by large studies.
Medical indications for any chronic and pathological wound.
Its use must be preceded and accompanied by comprehensive good wound care.
Statistical therapeutics, and the economics of any given wound and patient.
Early use appropriate.



Apligraf ® Summary

Only Apligraf is FDA-approved for both venous leg ulcers and diabetic foot ulcers

Closes more diabetic foot ulcers faster than conventional therapy alone

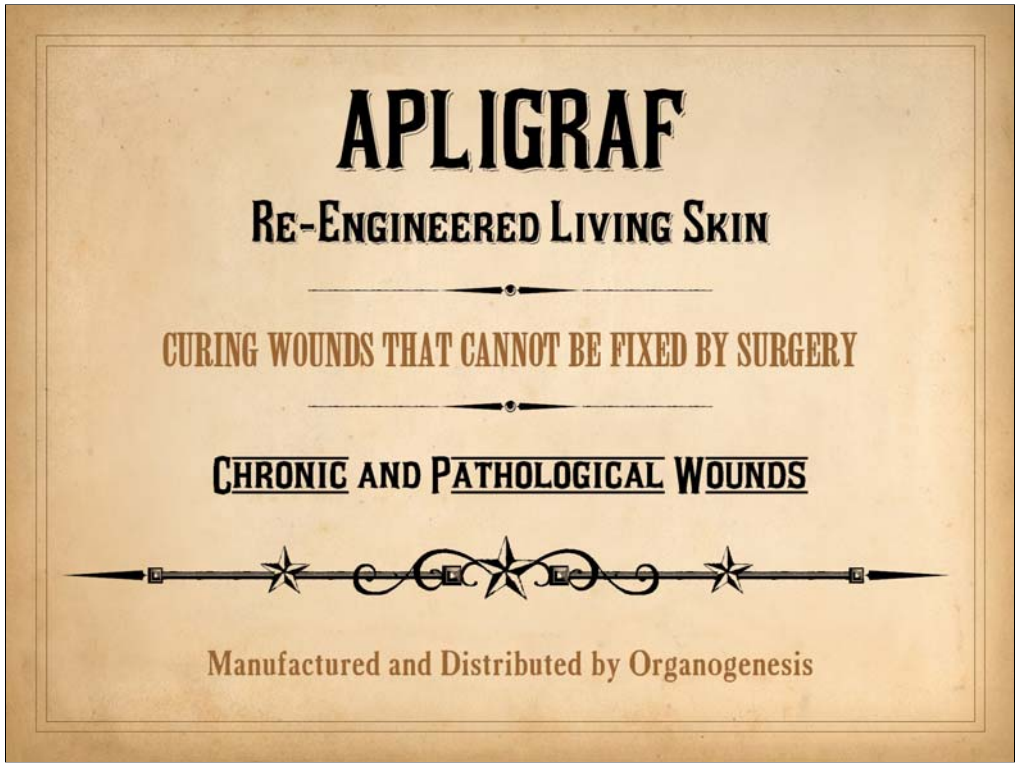
More than twice as effective as compression therapy alone in long-standing venous ulcers

Lower incidence of osteomyelitis at the study ulcer site and lower frequency of amputation of the study limb

Easy to incorporate into practice

Positive reimbursement in all settings

Well tolerated in over 80,000 patient applications in the United States


APLIGRAF
RE-ENGINEERED LIVING SKIN
CURING WOUNDS THAT CANNOT BE FIXED BY SURGERY
CHRONIC AND PATHOLOGICAL WOUNDS
Manufactured and Distributed by Organogenesis

Marc E. Gottlieb, MD, FACS
A Professional Corporation

PLASTIC & RECONSTRUCTIVE SURGERY
Board Certification ••• Plastic Surgery ••• Hand Surgery ••• General Surgery

Specializing in the treatment, reconstruction, and management of
Acute and chronic wounds • Diseases and defects of the soft tissues • Injuries, diseases, and defects of the hand and extremities • Defects of the head and trunk

Office: 1415 N. 7th Avenue • Phoenix, AZ 85007 **Phone** 602-252-3354 **Fax** 602-254-7891 megott@arimedica.com

Marc E. Gottlieb, MD, FACS

1415 N. 7th Avenue
Phoenix, AZ 85007

Phone 602-252-3354
Fax 602-254-7891

megott@arimedica.com

Apligraf – Re-Engineered Living Skin – Biotechnology and Chronic Wounds

Original presentation October 6, 2005, Dallas, TX.
Sponsored by Organogenesis.

This file is partially annotated, containing text notes supplied by Organogenesis to accompany the company's "blue" slides.