An Integrated Approach to Wound Healing Through the Use of Biofilm-Based Wound Management and Proven Living Cellular Therapies

This educational activity is supported by an educational grant from Organogenesis Inc.
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Disclosures

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Off-Label Disclosures

• The faculty have disclosed the following off-label/unapproved uses of drugs and/or devices will be discussed: BIOGUARD™, NIMBUS®, and PuraPly™ Antimicrobial.

• This continuing medical education activity includes device brand names for participant clarity purposes only, due to the presence of different branded versions of the same device. No product promotion or recommendation should be inferred.
Learning Objectives

• Evaluate the role of extracellular matrices (ECM) in facilitating constructive remodeling in wounds

• Differentiate degradation profiles between various ECMs

• Analyze how ECM persistence in various wound environments can generate more precise treatment regimens

• Explore cases on the clinical application of ECM devices in wound management
The Role of PHMB Antimicrobial in Managing Bioburden and the Combination of Collagen with Adjunctive Therapy to Manage Chronic Wounds
Contamination

- Presence of non-replicating microorganisms on the wound surface that evoke no clinical host response
  - All chronic wounds are contaminated
  - Bacterial colony counts low
  - Wound healing occurs despite the presence of bacteria

Colonization

- Bacteria that
  - Have adhered to superficial tissue (sessile)
  - Have begun forming colonies without generating a host immune response
  - Are not typically associated with a delay in healing

Critical Colonization

- The inability of the wound to maintain a balance between altered bioburden and an effective immune system
- Results in unexplained delay in healing
- No overt signs of clinical infection or wound deterioration

Biofilm

- Densely packed communities of microbial cells that grow on surfaces and surround themselves with EPS
- Biofilms develop defenses from topical agents and impair wound healing
  - Inflammatory immune response
  - Impair granulation tissue formation
  - Impair epithelialization

EPS = extracellular polymeric substance.
Prevalence and Impact

- 60% of chronic wounds possess biofilm
- Biofilm may delay and impair the healing process
  - Each year, as many as 17 million new biofilm infections occur in the United States
  - 65% to 80% of all human infectious disease is caused by biofilm

Principles of Biofilm-Based Wound Care

- **Frequent debridement** of wounds to physically remove biofilm communities
- **Use an effective microbicidal dressing** after debridement to prevent reformation of biofilms
- **Alter topical and systemic antimicrobial treatments** to prevent emergence of dominant bacteria from polymicrobial populations
  - Use DNA bacterial identification techniques
- **Biofilm-based wound care** is part of wound bed preparation (TIME)

How Quickly Do Biofilms Form?

- Strongly attached micro-colonies
  - 2-4 hours

- Develop initial EPS
  - 6-12 hours

- Evolve into fully mature biofilm colonies
  - 2-4 days

- Rapidly recover from mechanical disruption (ie, debridement)
  - Within 24 hours

Chronic Wound
Static healing, moderate improvement with repeated rounds of oral antibiotics

Suspected biofilm

Reduce biofilm burden → debridement / vigorous cleansing

Prevent recontamination with microorganisms → barrier dressing AND Suppress biofilm reformation → sequential topical antimicrobials

Reassess healing

Healed

Treatment Strategy (cont)
Treatment Challenges

Systemic antibiotics
• Fail to reach adequate local tissue levels
  – Topical antiseptics in conjunction with systemic therapy may be more effective

Debridement
• Frequent debridement allows for treating agents to be most effective
• Effective in the clinic setting?
  – Biofilm rapidly reconstitutes itself on the surface within 24 hours

Topical antimicrobials
• Tissue compatibility?
• Broad spectrum?
• Resistance?

- Purified type-1 collagen matrix coated with broad-spectrum antimicrobial PHMB
- Acute and chronic wound management across a variety of wound types

Contraindications

- Patients with known sensitivity to porcine material
- Patients with third-degree burns
- Patients with known sensitivity to PHMB
• EDC cross-linking increases collagen bonds and increases resistance to enzymatic degradation in the wound

• Two layers of collagen matrix also provide greater surface area for PHMB coating

EDC = 1-ethyl-3-(3-dimethyl aminopropyl)carbodiimide.

**PuraPly™ Antimicrobial**

Collagen cross-linking between/within two layers

Fenestrations

PHMB on surface and between/within layers
Purification Technology

What it is
- Chemical treatment of the porcine tissue to remove cells and other non-collagen materials (eg, DNA, lipids, glycosaminoglycans)

What it does
- Removes materials that can cause an inflammatory response
- Inactivates viruses and bacteria

Advantages
- Preserves the natural structure of collagen, which is important for strength, function, and biocompatibility
- Product is very homogeneous and consistent

Importance of Collagen

• Collagen is the main structural protein in the extracellular space of connective tissue

• ECM controls many cellular functions, including cell shape and differentiation, migration, and protein synthesis

• 28 types of collagen identified so far

• Type-1 collagen
  – Most common (80%-90%)
  – Found in all tissues
  – Primary collagen in a healed wound

ECM = extracellular matrix.
ECMs that retain native tissue structure were found to inhibit a wider range of MMPs, including collagenases, gelatinase, and neutrophil elastase.

Oxidized regenerated cellulose/collagen shown only to inhibit gelatinases.

Conclusion: Native biomaterials that are capable of inhibiting both upstream (eg, collagenases) and downstream (eg, gelatinases) proteases are more likely to halt collagen proteolysis.
PHMB Provides an Effective Microbial Barrier

Well-studied, extensive clinical experience

- Covers a broad antimicrobial spectrum
  - Gram-positive and gram-negative bacteria (eg, MRSA, *P aeruginosa*)
  - Biofilm-forming bacteria and fungi
- Low cytotoxicity, high tissue compatibility
  - Does not impair wound healing, unlike other antimicrobials
- No bacterial resistance reported in vitro or clinically
- Efficacy not impaired in wound fluid, blood, or tissue

PHMB: Mechanism of Action

- Interacts with negatively charged phospholipids in the bacterial membrane (leading to disruption)
- Inhibits bacterial cell metabolism
- Shown to effectively remove biofilm through blocking microbial attachments to surfaces
- Binds to cellular surfaces for sustained effect over hours

Microbicidal Mechanism of Polycationic Molecules

Normal bacterial membranes (panel A) are stabilized by Ca\(^{+2}\) ions binding anionically charged phospholipids. NIMBUS\(^{®}\) quat-polymer rapidly displaces Ca\(^{+2}\) (panel B) leading to loss of fluidity (panel C) and eventual phase separation of different lipids. Domains in the membrane then undergo a transition to additional smaller micelles.

BEFORE

AFTER

BEFORE

AFTER
PuraPly™ Antimicrobial effectively inhibited microorganisms

*Aspergillus niger*
*Candida albicans*
*S aureus*

*MRSA*
*P aeruginosa*
*Escherichia coli*

*United States Pharmacopeia Antimicrobial Effectiveness Test showed reduced concentrations at days 7, 14, and 28.
†Zone of inhibition test demonstrated efficacy in vitro.
Preclinical Partial-Thickness Wound Model

PuraPly™ Antimicrobial wound matrix demonstrated greater reduction in MRSA vs other products

Bacterial Count at 72 Hours

PuraPly™ Antimicrobial Wound Matrix Is Indicated for a Variety of Wounds

1. Postsurgical Wound Dehiscence (Failed Flap)
2. Postsurgical Wound Dehiscence (Mohs Surgery)
3. Vascular Ulcer
4. Pressure Ulcer
5. Trauma Wound: Skin Tear
6. Trauma Wound: Skin Laceration
7. Diabetic Foot Ulcer
8. VLU

Biofilms Are Highly Tolerant to Antibiotics

Tobramycin vs *P. aeruginosa* Biofilm

Tobramycin rapidly kills planktonic *P. aeruginosa* (■) very effectively, but is not effective against biofilm (●).

Biofilms Provide Clinical Benefit in Three Ways

1. Purified type I collagen matrix is a durable, biocompatible scaffold

2. Effective barrier against a wide range of microorganisms

3. PHMB is known to inhibit the formation of biofilm on the wound surface

Case Presentations
• 52-year-old man with diabetes mellitus, history of heel ulceration, calcaneal osteomyelitis

• Past medical history: diabetes mellitus, hypertension, coronary artery disease

• Previous treatment: partial calcanectomy, multiple debridements, NPWT, BATG
12/28/15

After first application
After second application
• 59-year-old woman with a history of a puncture wound and subsequent ulceration sub-fifth metatarsal wound that extends dorsally to a dorsal wound

• Past medical history: Peripheral artery disease, peripheral neuropathy

Plantar

Dorsal

Prior to first application
After first application

Plantar

Dorsal
After second application

Plantar

Dorsal
After third application
• 67-year-old African American man presented with a pressure ulcer on the left heel at the site of a previously closed wound, likely caused by shearing in a Charcot Restraint Orthotic Walker (CROW boot) while walking on prosthesis on the right

• Wound present for 2 months and was previously treated with NPWT

• Past medical history: Diabetes mellitus, peripheral vascular disease, hypertension, neuropathy, gout, end-stage renal disease, hyperlipidemia, anemia, osteomyelitis (right heel)

• Surgical history: Partial calcanectomy bilaterally

• May 6-13, 2014: Right below-the-knee amputation and surgical resection of left heel
Pressure Ulcer (Heel) Closed after Nine Applications

Before

After
Wound with significant slough, devitalized tissue, and edema

Pre-debridement
Date: 6/29/15
Wound Size: 4.0 x 4.5 x 0.2 cm
Wound Area: 18.0 cm²

Wound Bed Prep: Sharp debridement
Primary Dressings: Restore
Secondary Dressings: Calcium alginate, Kling® rolls, Ace™ bandage
Off-loading: TCC

TCC = total contact cast.
Reduction in wound size with increased granulation tissue

**Pre-debridement**
**Date:** 7/6/15  
**Wound Size:** 3.7 x 4.2 x 0.2 cm  
**Wound Area:** 15.54 cm²

**Wound Bed Prep:** Sharp debridement  
**Primary Dressings:** Restore  
**Secondary Dressings:** Calcium alginate, Kling® rolls, Ace™ bandage  
**Off-loading:** TCC
Continued reduction in wound size—despite complete wound dressing change during the prior week

**Pre-debridement**

**Date:** 7/13/15  
**Wound Size:** 2.4 x 4.0 x 0.2 cm  
**Wound Area:** 9.6 cm²

**Wound Bed Prep:** Sharp debridement  
**Primary Dressings:** Restore  
**Secondary Dressings:** Calcium alginate, Kling® rolls, Ace™ bandage  
**Off-loading:** TCC
Continued reduction in wound size

**Pre-debridement**
**Date:** 7/20/15  
**Wound Size:** 2.3 x 4.0 x 0.2 cm  
**Wound Area:** 9.3 cm²

**Wound Bed Prep:** Sharp debridement  
**Primary Dressings:** Restore  
**Secondary Dressings:** Calcium alginate, Kling® rolls, Ace™ bandage  
**Off-loading:** TCC
Continued reduction in wound size with some peri-wound maceration; additional nursing visits added to manage drainage

**Pre-debridement**
**Date:** 7/27/15
**Wound Size:** 2.1 x 3.3 x 0.1 cm
**Wound Area:** 6.93 cm²

**Wound Bed Prep:** Sharp debridement
**Primary Dressings:** Restore
**Secondary Dressings:** Calcium alginate, Kling® rolls, Ace™ bandage
**Off-loading:** TCC
Sixth Application

Continued reduction in wound size and maceration improved

**Pre-debridement**
- **Date:** 8/3/15
- **Wound Size:** 1.4 x 3.0 x 0.1 cm
- **Wound Area:** 4.2 cm²

**Wound Bed Prep:** Sharp debridement
**Primary Dressings:** Restore
**Secondary Dressings:** Calcium alginate, Kling® rolls, Ace™ bandage
**Off-loading:** TCC
Continued reduction in wound size

Pre-debridement
Date: 8/10/15
Wound Size: 1.2 x 2.5 x 0.1 cm
Wound Area: 3.0 cm²

Wound Bed Prep: Sharp debridement
Primary Dressings: Restore
Secondary Dressings: Calcium alginate, Kling® rolls, Ace™ bandage
Off-loading: TCC
Continued reduction in wound size with no signs of peri-wound maceration

- **Pre-debridement**
  - **Date:** 8/17/15
  - **Wound Size:** 0.7 x 2.1 x 0.1 cm
  - **Wound Area:** 1.47 cm²

- **Wound Bed Prep:** Sharp debridement
- **Primary Dressings:** Restore
- **Secondary Dressings:** Calcium alginate, Kling® rolls, Ace™ bandage
- **Off-loading:** TCC
Continued reduction in wound size

Pre-debridement
Date: 8/24/15
Wound Size: 0.2 x 0.7 x 0.1 cm
Wound Area: 0.14 cm²

Wound Bed Prep: Sharp debridement
Primary Dressings: Restore
Secondary Dressings: Calcium alginate, Kling® rolls, Ace™ bandage
Off-loading: TCC
Patient received 9 applications of PuraPly Antimicrobial

Complete Wound Closure
Data: 8/31/15
Wound Size: Closed

Wound Healed: Off-loading continued
Off-loading: TCC
Summary

- All wounds have some level of bioburden
- 90% of chronic wounds have biofilm
- Excessive bioburden can adversely affect tissue repair and delay healing
- Excess protease activity in chronic wounds degrades the collagen matrix, thereby inhibiting healing

Summary (cont)

- A new paradigm that adds the management of biofilm and suppression of bioburden to pre-existing standard modalities

- Staged wound healing
  - Appropriate off-loading
  - Wound bed preparation
  - Progression to closure
Assessing when to move to cellular therapies and in what stages of wound healing cellular therapies are most effective
Time Driven?

- Increased bacterial load
- Excessive proteases
  - Degraded
    - Growth factors
    - Matrix proteins
    - Cell surface receptors
- Prolonged inflammation
- Cellular senescence
- Inadequate / inappropriate treatment

Patient Driven?

- Diseases or conditions
  - Competing for O₂ or metabolic resources
  - Autoimmune diseases
  - Other medical comorbidities
- Medications
  - Steroids
  - Immunosuppressive agents
  - Chemotherapy
- Patient adherence
  - Diet / Blood glucose
  - Smoking
  - Offloading
Focus on Time to Healing: Use of Prognostic Indicators

- **Venous Leg Ulcers**
  - <40% reduction in wound size by week 4
  - Unlikely to achieve complete wound closure by 24 weeks\(^1,2\)

- **Diabetic Foot Ulcers**
  - <50% reduction in wound size by week 4
  - Unlikely to achieve complete closure at 12 weeks\(^3\)

- **Pressure Ulcers**
  - >47% percent area reduction by two weeks predictive of healing

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When to Move to Advanced Therapies

- Following comprehensive assessment of the patient and the wound to identify and address known comorbidities and risk factors for delayed healing.
- When initial appropriate conservative treatment has failed to demonstrate significant progress in an appropriate period of time.
- When wound bed preparation has been accomplished in an optimal fashion for the wound being treated.
  - Use of advanced products on a poorly or suboptimally prepared wound bed will lead to treatment failure and wasted health care dollars!
Initial Conservative Treatment - DFU

- Arterial supply
- Meaningful offloading
- Effective debridement
- Management of bioburden
- Moist wound care – based on exudate

No Yes
Initial Conservative Treatment - VLU

- Adequate arterial supply
- Venous reflux exam
- Multilayer compression
- Moist wound care
- Bioburden management

Yes  Maybe  No
Initial Conservative Treatment – Pressure Injury

- Pressure redistribution
- Bed support surface
- Chair cushion
- Heels
- Debridement
- Nutrition consultation
- Turning and repositioning
- Moist wound care
- Management of infection
- Early surgical consultation stage 3 & 4.
Clinical Benefits of Advanced Wound Care Products

- Earlier control of symptoms
- Promotion of wound closure
- Addition of cells and growth factors to wound bed
  - Fibroblasts and keratinocytes
    - Human fibroblasts and keratinocytes/stem cells in bioabsorbable matrix
  - Fibroblasts
    - Human fibroblasts in bioabsorbable matrix
  - Stem Cells
    - Keratinocyte stem cells in bilayer construct as above
    - Neonatal MSCs in cryopreserved amnion product
  - PDGF (becaplermin)
Additional Clinical Benefits

- Binding and quenching of proteases
  - Native collagen products
    - Ovine forestomach
    - Porcine small intestinal submucosa
  - Reconstituted collagen and collagen/ORC dressings
- Reduction of bioburden and prevention of reformation of biofilm
  - Native collagen and PHMB construct
- Rate of collagen resorption may be diagnostic of protease activity?
Other Benefits

- Improved/faster outcomes
- Lower overall cost
- Increased patient satisfaction

• **Triple Aim**

- How hard could that be?

![Diagram](image-url)
Patient Selection: Thought Processes

- Type, history and duration of wound
  - Consider wounds in the context of comorbid conditions (ICD-10)
    - Traumatic wound in presence of known venous insufficiency
    - Post-surgical wound in presence of diabetes
- Wound bed preparation: is it ready?
  - Debridement
    - Bioburden management
- Ability to offload/compress
- Insurance coverage
  - Avoid making financial decisions for patients
- Start early; consider prognostic indicators
In the absence of adequate wound bed preparation not only will wounds not progress to healing, advanced therapies will fail.

Wound Bed Preparation: Removal of Barriers to Healing

Wound Bed Preparation

“Healability”

- **Healable**
  - With adequate blood supply that can be healed as long as the underlying problem can be addressed

- **Maintenance**
  - Have healing potential, but also have patient or health system barriers compromising healing, including patient non-adherence to treatment plan or healthcare resource limitations

- **Non-Healable**
  - Including palliative wounds, cannot heal because of irreversible causes or associated illnesses including critical ischemia or non-treatable malignancy
Case Examples
Purified Collagen Matrix with PHMB

- 53 year old female with Stage IV breast cancer presented with 3 month history of non-healing wound following Bevacicumab (anti-VEGF) Chemotherapy
- Underwent 6 weekly applications of product with adhesive bordered foam cover dressing.
- Closed in 6 weeks
Changing the Trajectory…

- 74 year old gentleman, referred for second opinion
- PMH: HTN, RA, type 2 Diabetes, Hx. of DVT
- Medications: clopidogrel, furosemide, Prednisone, Methotrexate, Atenolol, Lisinopril, Pravastatin
- Previous history of diabetic foot ulcers; resolved
- PSH: Hip replacement right, Lumbar spine surgery, CABG/Carotid surgery 2011
- Previous treatment: Various dressings, history of being unable to tolerate compression
Venous Ulcers x 2, Left Leg x 14 Months: Proximal Ulcer Pre & Post Debridement
Venous Ulcers x 2 Left Leg x 14 Months: Distal Ulcer Pre & post debridement
Initial Management

- Collagenase santyl
- Pigmented PVA foam
- Tubular elastic bandage
1st Apligraf Application

BCT = bilayer cellular therapy.
2 Weeks: 2nd Application
Apligraf in Place
Week 4: 3\textsuperscript{rd} Application
6 Weeks: 4th Application
8 Weeks
Closed @ 9 weeks (open for 14 months)
46 y/o, w/m, type II DM with 18 month hx large plantar ulcer on charcot foot. Debridement, offloading with TCC, monolayer human fibroblast CTP x 3, closed in 18 wks.
Infected AICD Site

- 34 year old patient, PMH of diabetes, ischemic cardiomyopathy, ventricular tachycardia
- Meds: coumadin, lisinopril, clopidogrel, aldactone, furosemide, simvastatin, coreg, ASA, spironolactone, novolin
- Site of AICD (Automatic implantable cardiac defibrillator) became infected and was removed.
- Patient was required to wear a “Life Vest” until healed
Initial Visit: Day 0

- 2.9 x 1.2 x 0.2 cm (2.73 cm²)
- Post Debridement

Initial treatment: Becaplermin 0.01% gel, collagen and foam cover dressings
Day 21 Cellular Amnion Product Applied

- **Pre-debridement & Pre-application**

- **Post-debridement and post-application**

3.3 x 2.2 x 0.2 (5.7 cm²)
Day 21, Wound Closed
Pressure Ulcers

- 51 year old female
- Paraplegic for 32 years; secondary to entrapment in falling building from house fire
- No other significant medical problems
- Smokes cigarettes, 1 ppd
- History of multiple pressure ulcers over time.
Right Heel: Pre and post Debridement

- Initial visit
- Patient states ulcer present for 1 week
- Initial debridement done, cellular amnion product applied and covered with a foam cover dressing
- Ulcer measures 1.5 x 2.0 x 0.3 cm (2.36 cm²)
3 Weeks Post Second Application
Q & A