Battling Biofilms

Winning the War in Wounds

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Learning Objectives

- Review the four sequential phases of normal wound healing and recognize the BENEFICIAL effects of CONTROLLED INFLAMMATION and PROTEASE ACTIVITIES

- Understand the link between CHRONIC INFLAMMATION caused by PLANKTONIC and BIOFILM BACTERIA and ELEVATED PROTEASE ACTIVITIES that DESTROY proteins that are essential to healing (extracellular matrix, growth factors, receptors)

- Recognize the high TOLERANCE of BIOFILM bacteria to most antibiotics, antiseptics and disinfectants

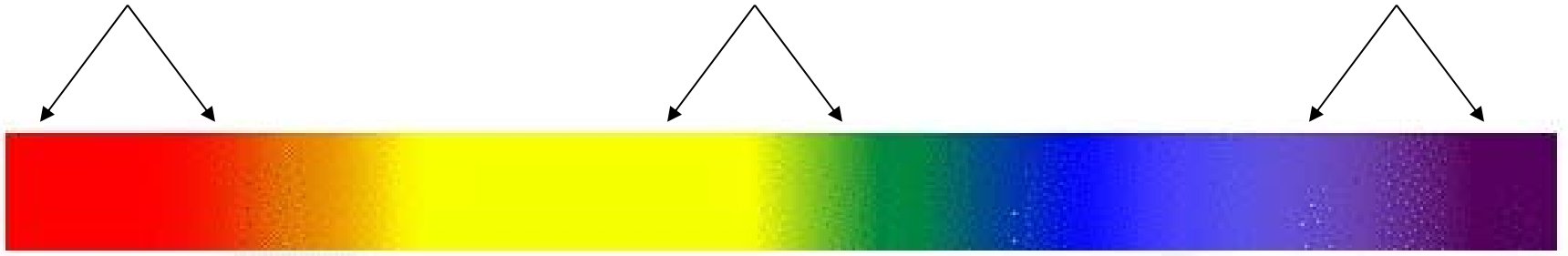
- Describe key principles of BIOFILM-BASED WOUND CARE that emphasize DEBRIDING BIOFILMS and PREVENTING REFORMATION OF BIOFILMS as part of the STEP-DOWN-STEP-UP approach for effective therapies

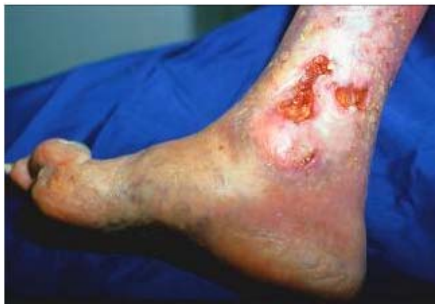
Think of Wound Healing as a Spectrum of Clinical Outcomes

Inadequate Healing (Chronic)

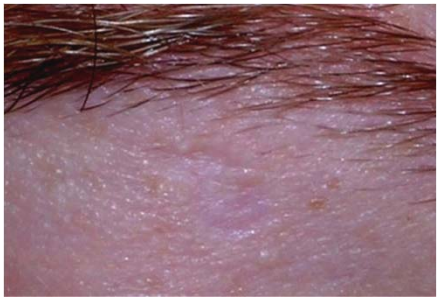
Normal Healing (Repair)

Excessive Healing (Fibrosis)

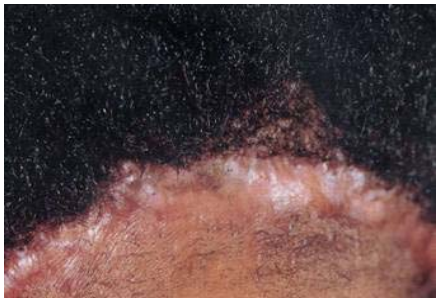




Venous Leg Ulcer

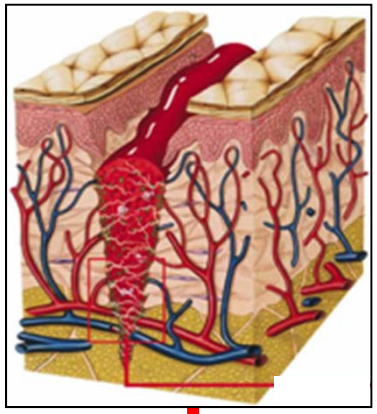


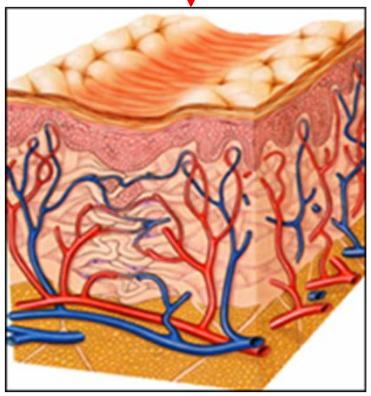
Good Skin Scar

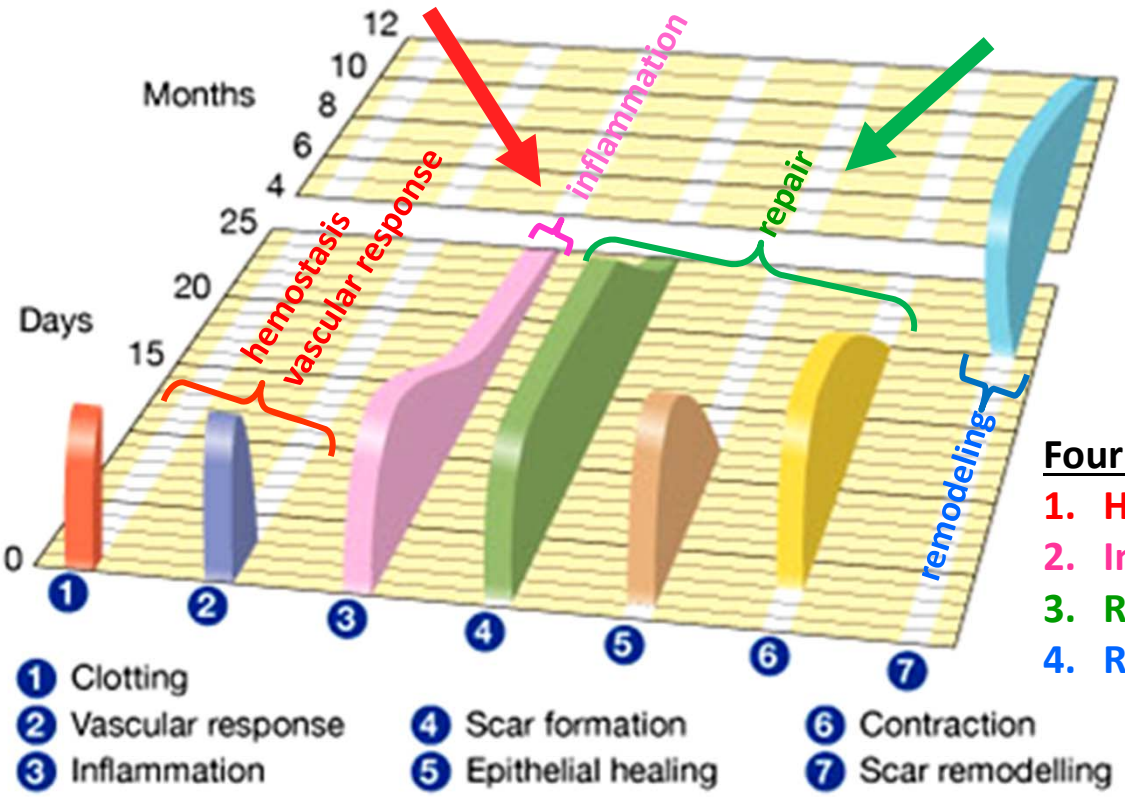


Hypertrophic Scar

Sequence of Molecular and Cellular Events in Skin Wound Healing



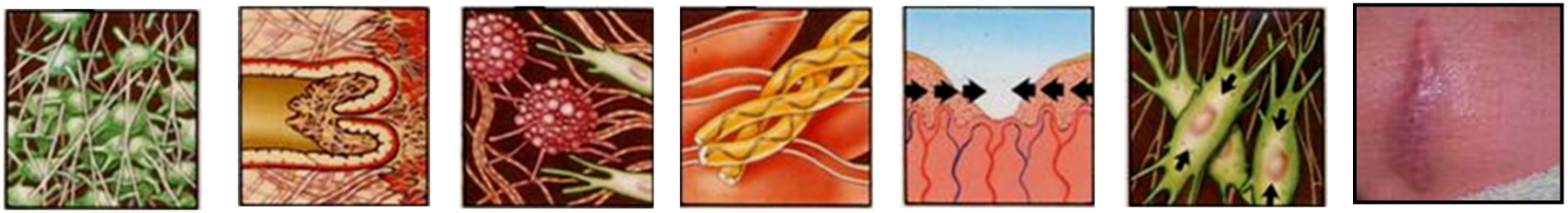




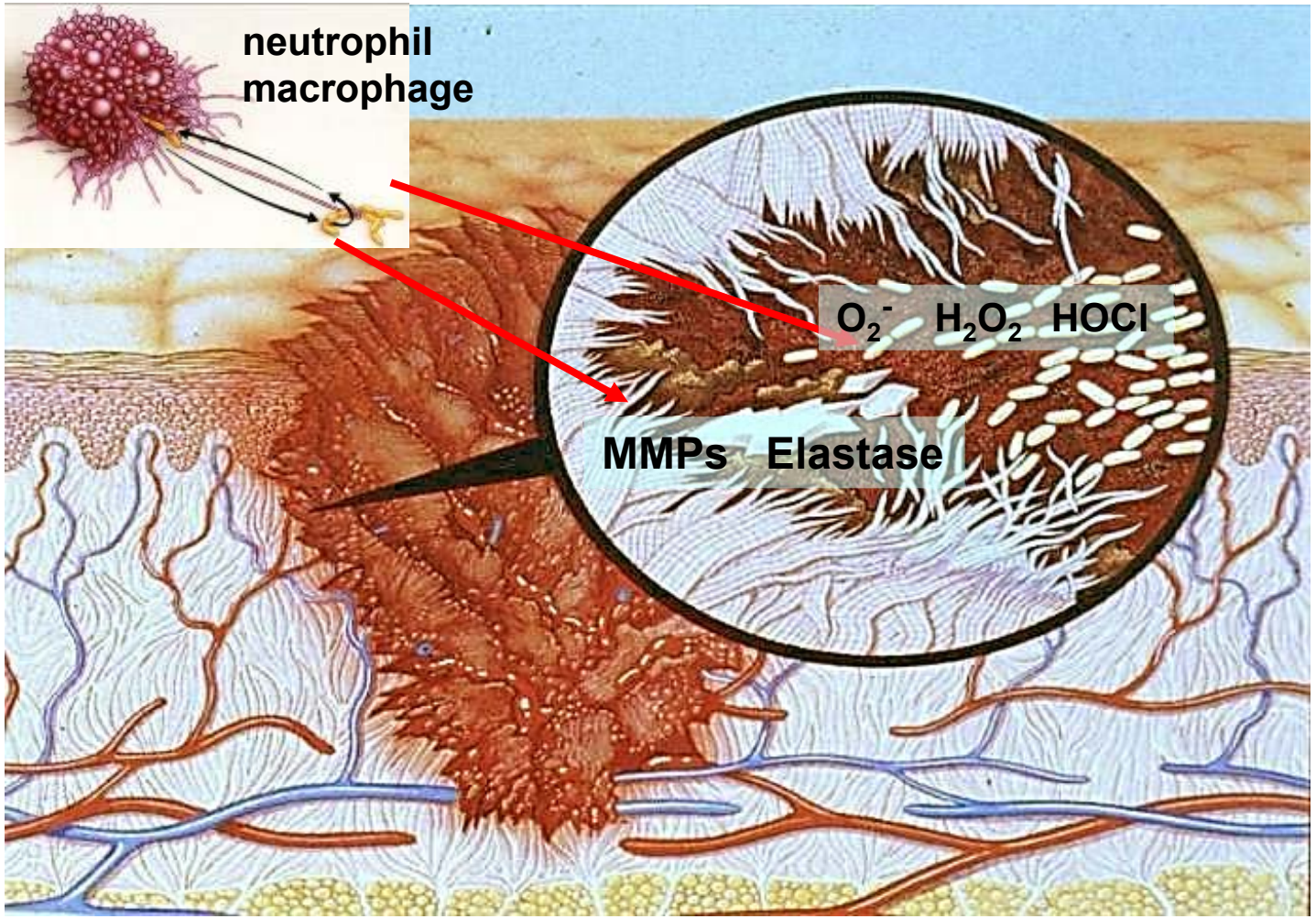
Four Phases of Healing

1. **Hemostasis**
2. **Inflammation**
3. **Repair**
4. **Remodeling**

1	2	3	4	5	6	7
Clotting	Vascular Response	Inflammation	Scar Formation	Epithelial Healing	Contraction	Scar Remodeling

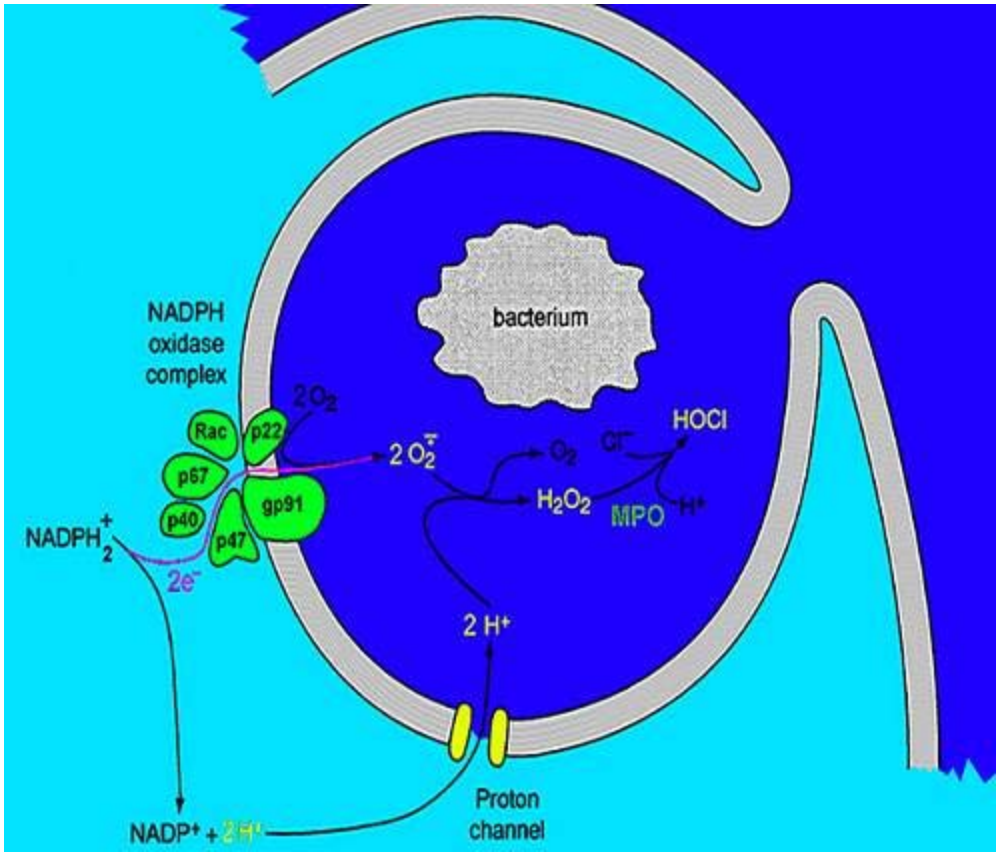


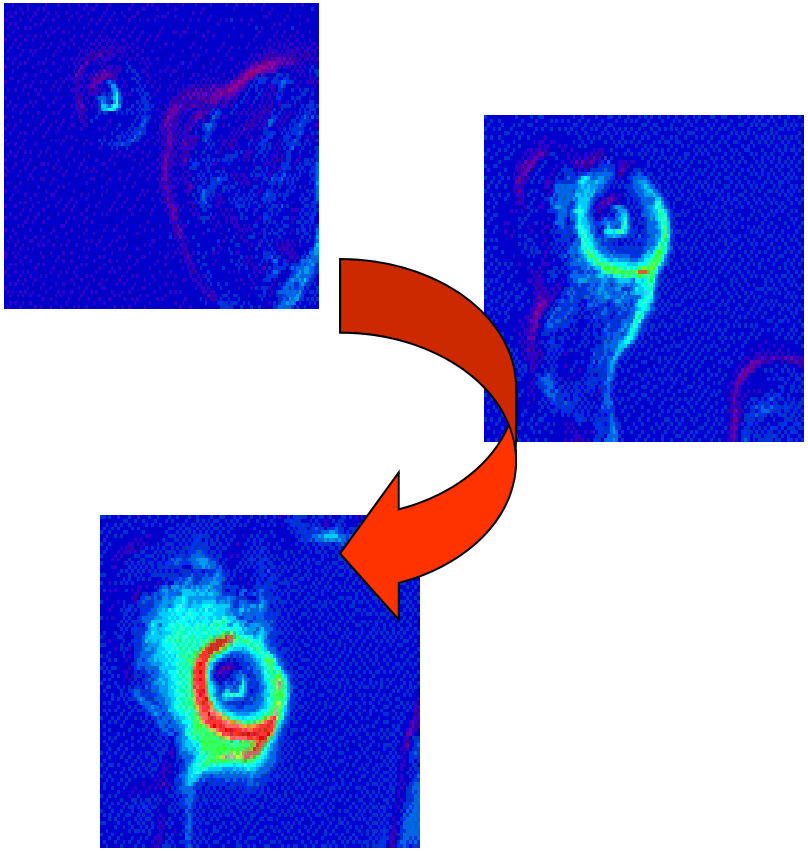
Controlled Wound Inflammation Is Beneficial



Inflammatory cells kill microorganisms and release proteases (MMPs, elastase) that remove denatured ECM components and permit wound healing to proceed. Wounds that are contaminated by bacteria and fungus must not be closed.

Respiratory Burst In Neutrophils & Macrophages Produces Reactive Oxygen Species (ROS) That Kill Bacterial & Fungi





In the membranes of neutrophils, NADPH oxidase generates superoxide (O_2^-), which spontaneously dismutates to H_2O_2, and is converted to hypochlorous acid (HOCl) by myeloperoxidase (MPO). These reactive oxygen species (ROS), especially HOCl, participate in the killing of bacteria. The right panels show a bacteria being phagocytized and production of ROS (red color) surrounding the yeast cell.

Question: **What happens when the respiratory burst is impaired?**

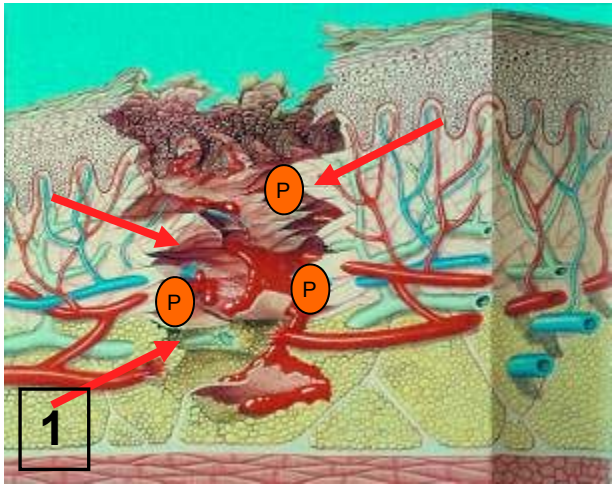
Answer: **Severe impairment of host resistance to infection occurs. Clinical condition - Chronic Granulomatous Disease is due to mutated NAPDH oxidase. Characterized by predisposition to bacterial and fungal infections**

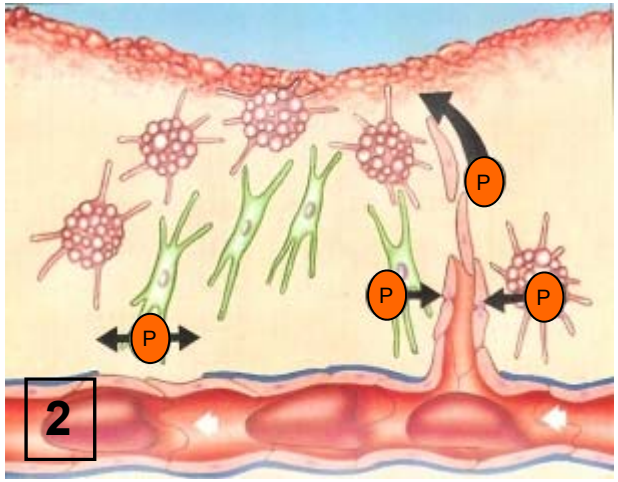
decreased levels of:

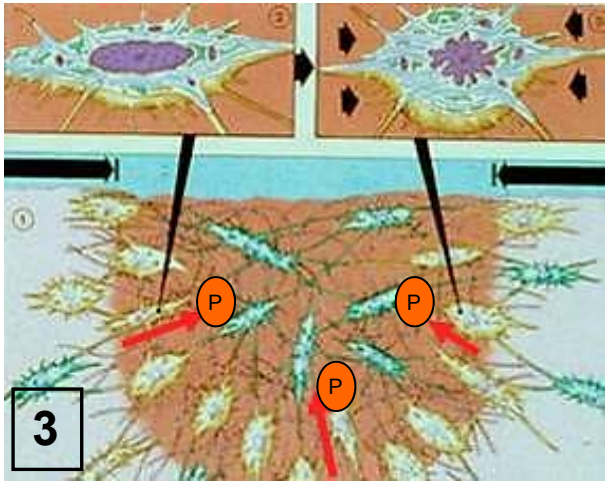
- **hydrogen peroxide (H_2O_2)**
- **peroxynitrite anion ($ONOO^-$)**
- **oxyhalides (HOCl hypochlorous acid)**

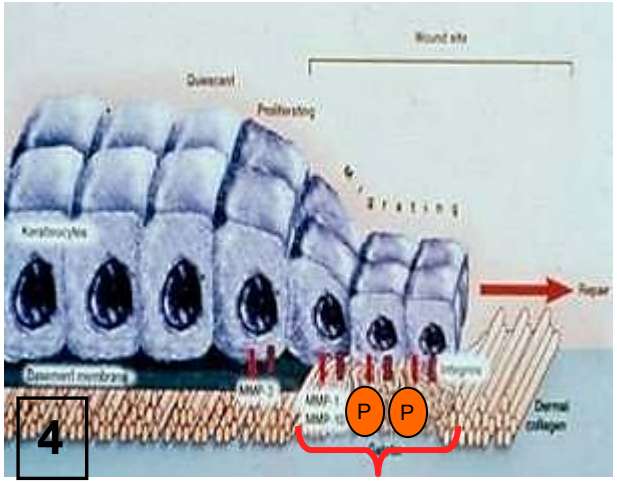
Controlled MMPs Are Necessary for Wound Healing

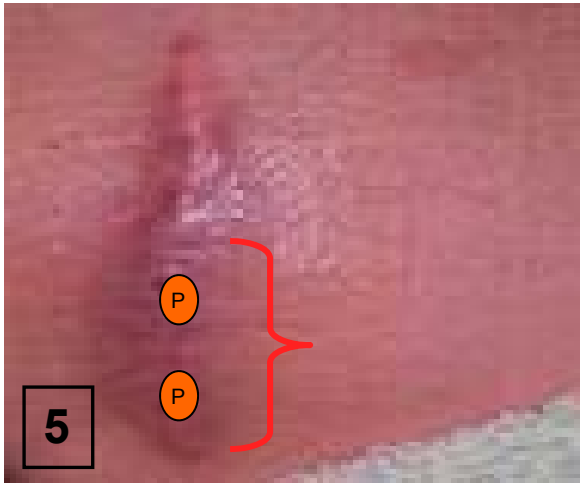
Debridement, Angiogenesis, Contraction, Epithelial Migration, Remodeling







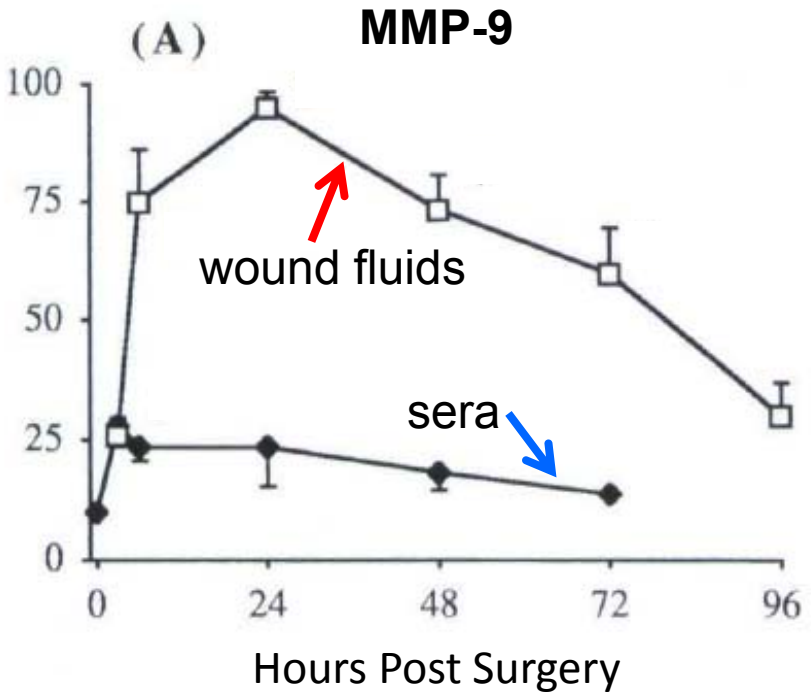




MMPs ARE NECESSARY FOR SEVERAL KEY PROCESS IN WOUND HEALING

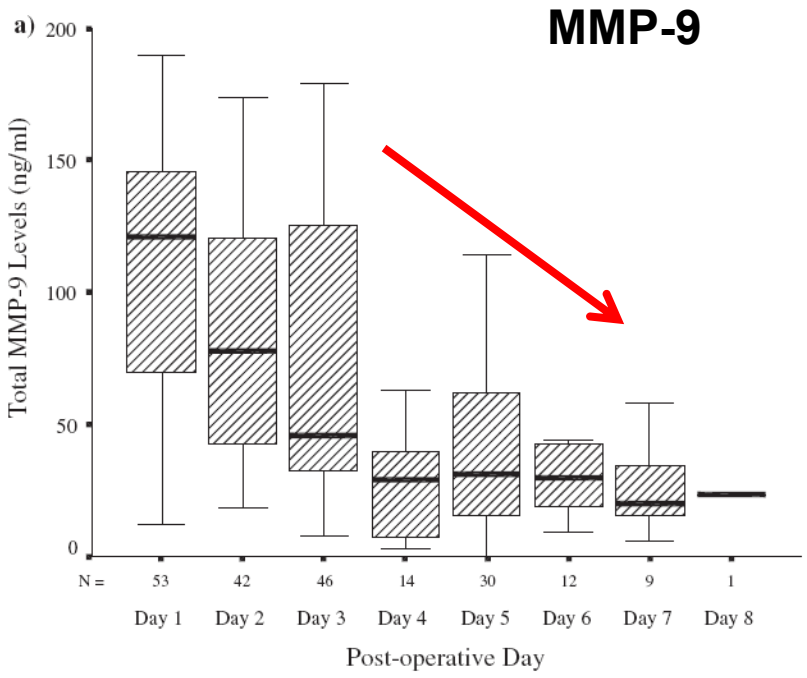
1. removing denatured matrix
2. degrading capillary basemen membrane for angiogenesis
3. contraction of ECM by myofibroblasts
4. migration of epidermal cells
5. remodeling of scar

Profiles of MMP-9 in Acute Healing Wound Fluids



Profiles of MMP-9 in mastectomy wound fluids (□) and matched sera (◆) during early wound repair in nine patients.

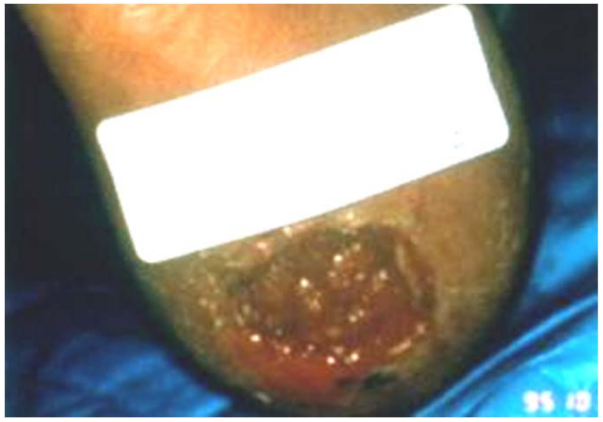
Tarlton, J.F., Vickery, C.J., Leaper, D.J., Bailey,A.J. Postsurgical wound progression monitored by temporal changes in the expression of matrix metalloproteinase-9. **Br J Dermatol** 137:506, 1997



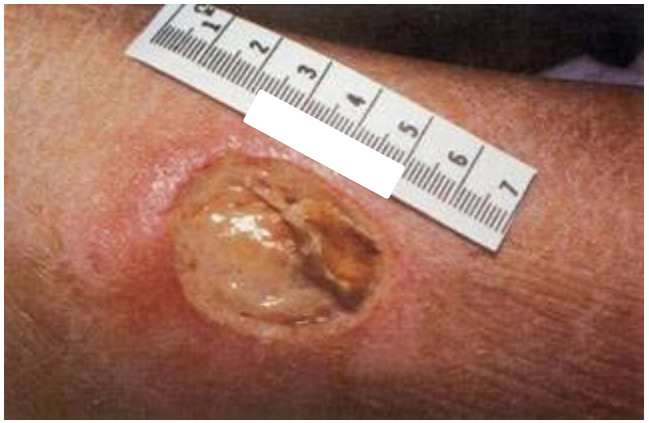
Levels of total MMP-9 protein in intraperitoneal drainage fluid from 58 patients undergoing elective colorectal surgery.

Baker, E.A.and Leaper, D.J. Profiles of matrix metalloproteinases and their tissue inhibitors in intraperitoneal drainage fluid: relationship to wound healing. **Wound Rep Reg** 11:268-274, 2003

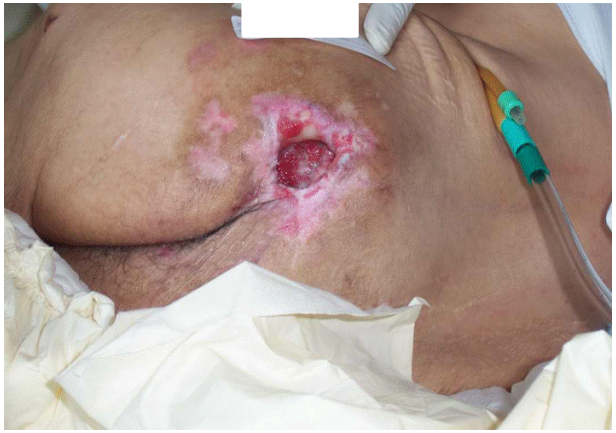
Is There a Common Molecular Pathology Of Chronic Wounds??



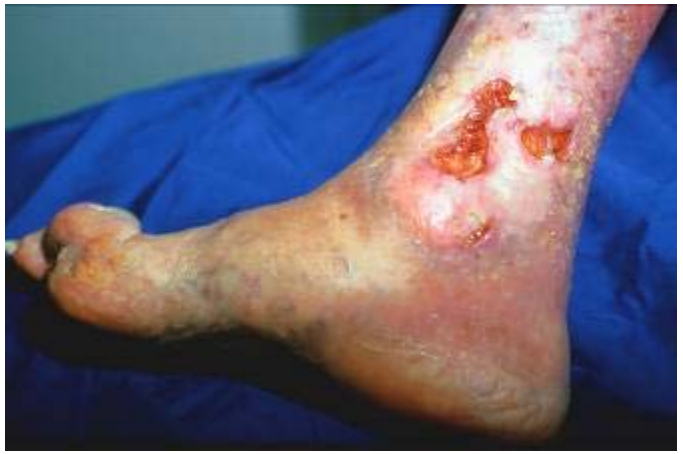
Diabetic foot ulcer



Arterial ulcer

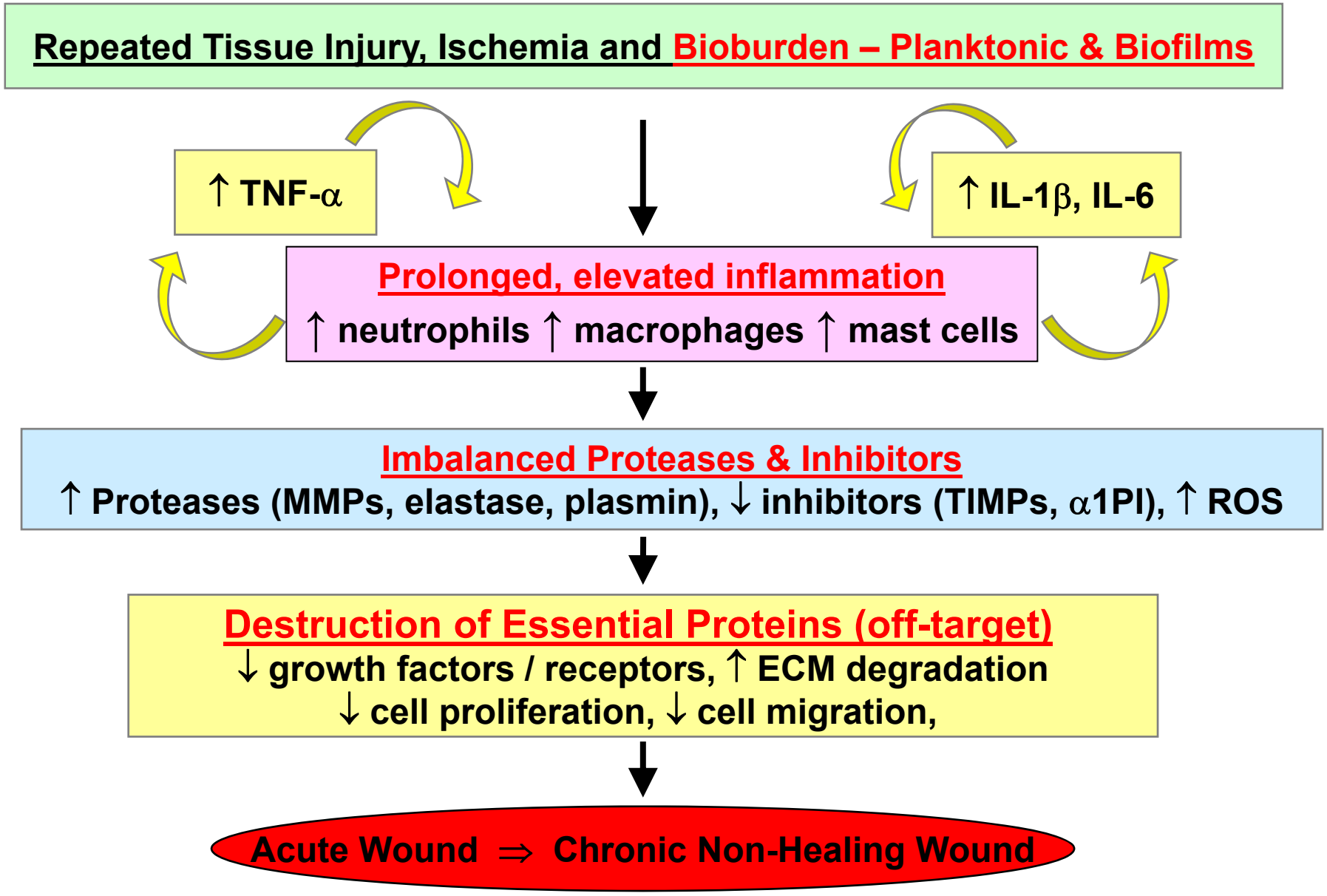


Pressure ulcer



Venous ulcer

Hypothesis Of Chronic Wound Pathophysiology

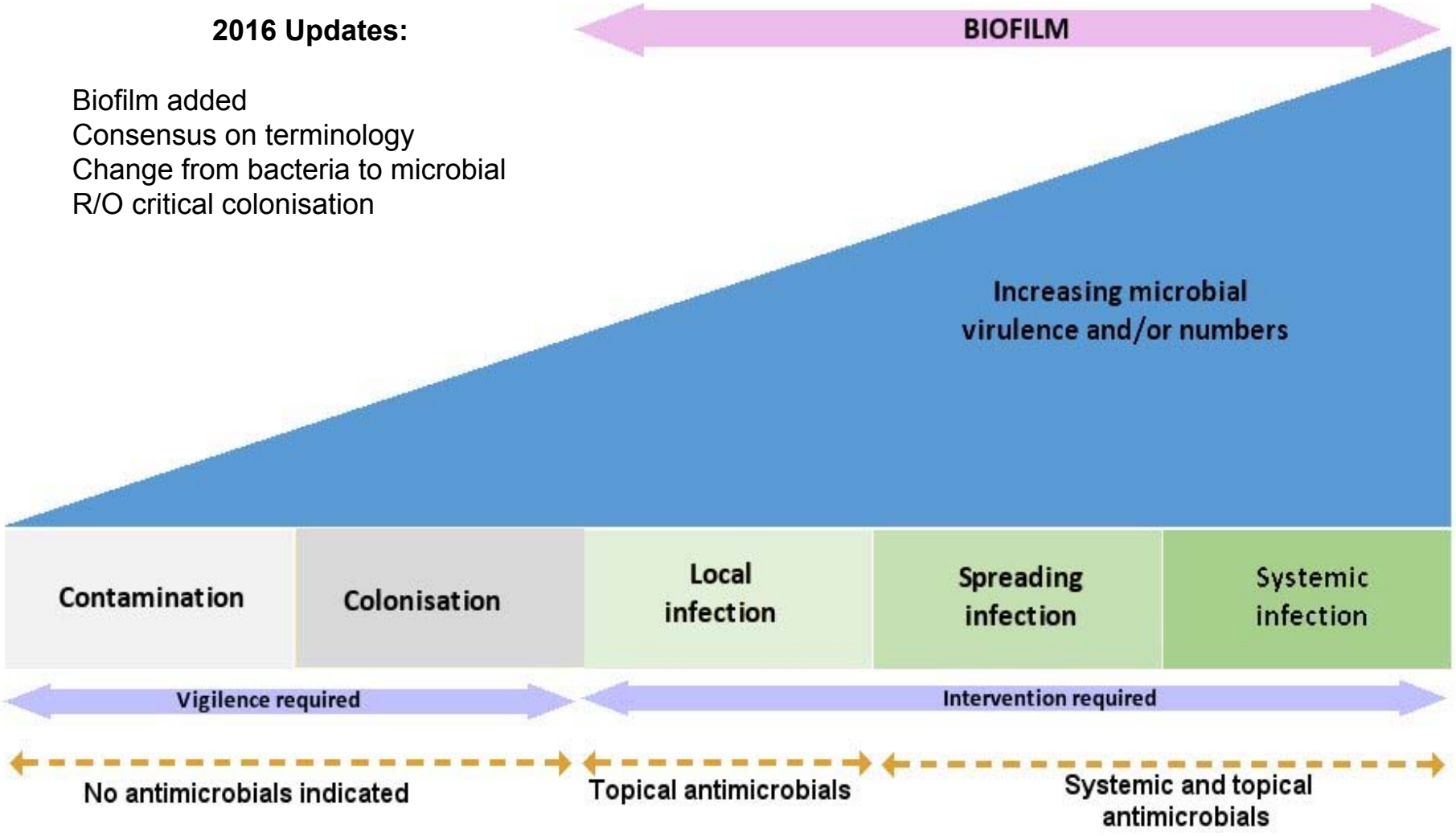


B.A. Mast and G.S. Schultz. Wound Rep Reg 4:411-420, 1996.

Wound Infection Continuum

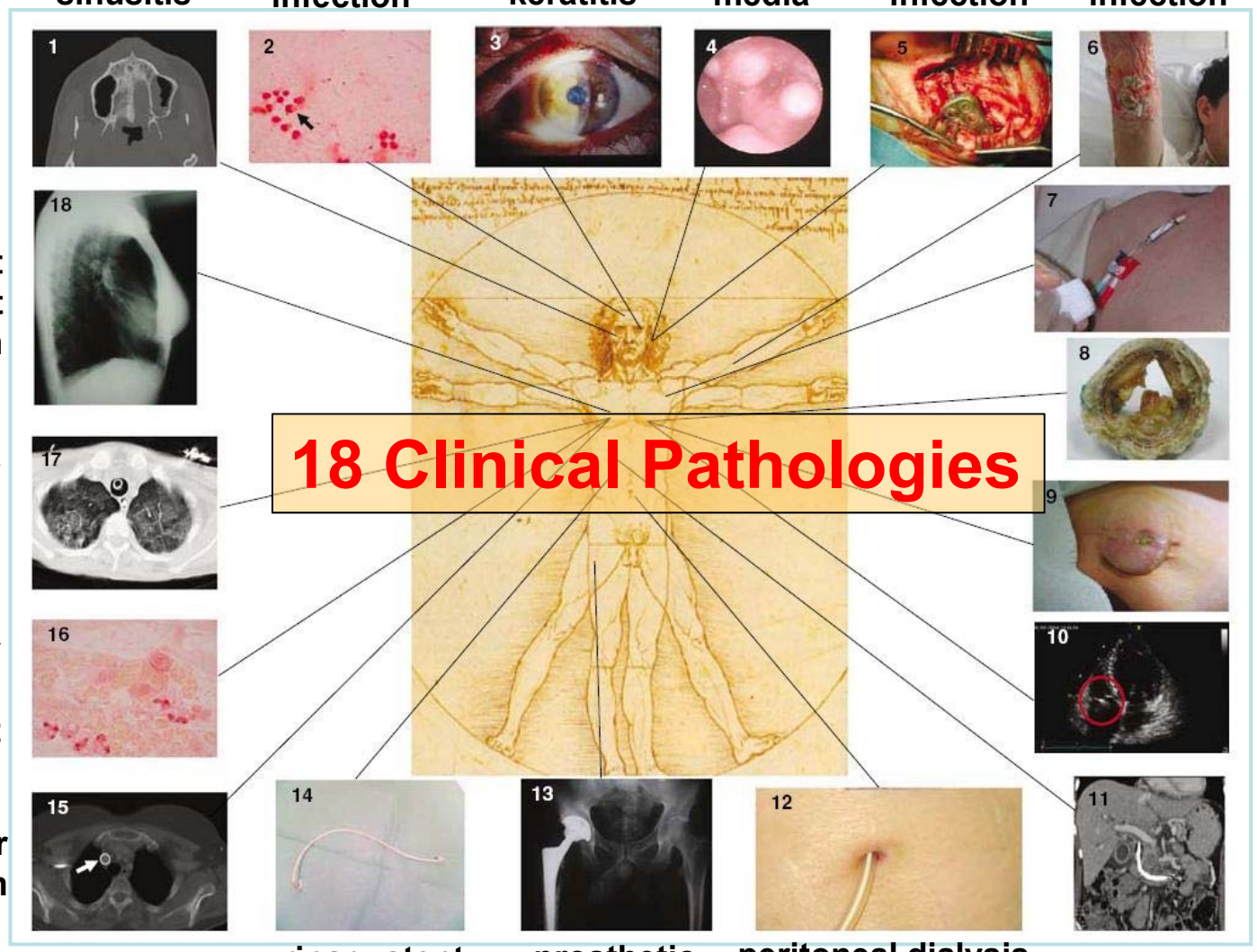
2016 Updates:

Biofilm added
Consensus on terminology
Change from bacteria to microbial
R/O critical colonisation



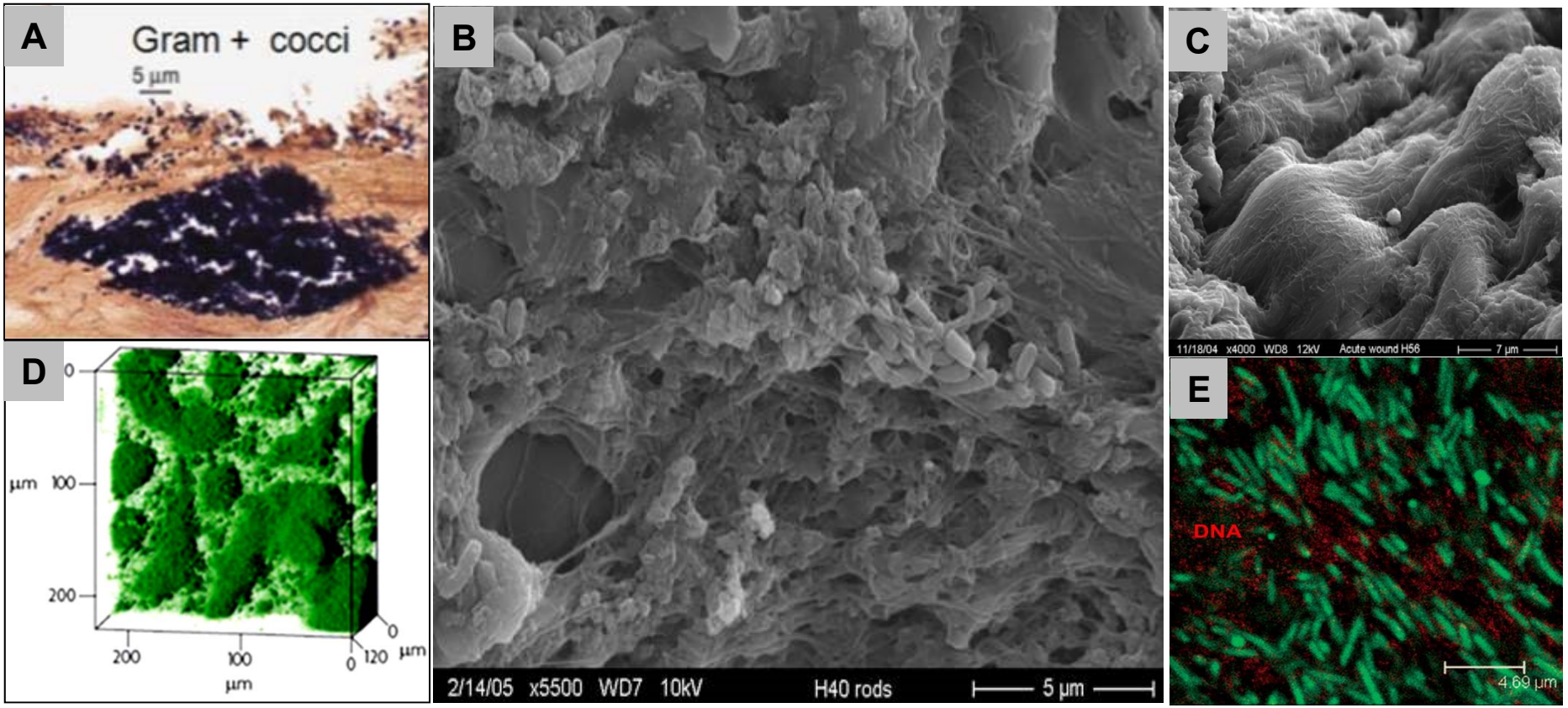
http://www.woundinfection-institute.com/2016/11/httpwww-woundinfection-institute-comwp-contentuploads201707iwii-consensus_final-2017-pdf/

Chronic Infections Causes by Medical Biofilms



del Pozo and Patel. The Challenge of Treating Biofilm-Associated Bacterial Infections. C*lin Pharm Ther* 82:204-20, 2007

Biofilms Identified in >80% of Biopsies of Chronic Wounds but in Only 6% of Acute Wounds



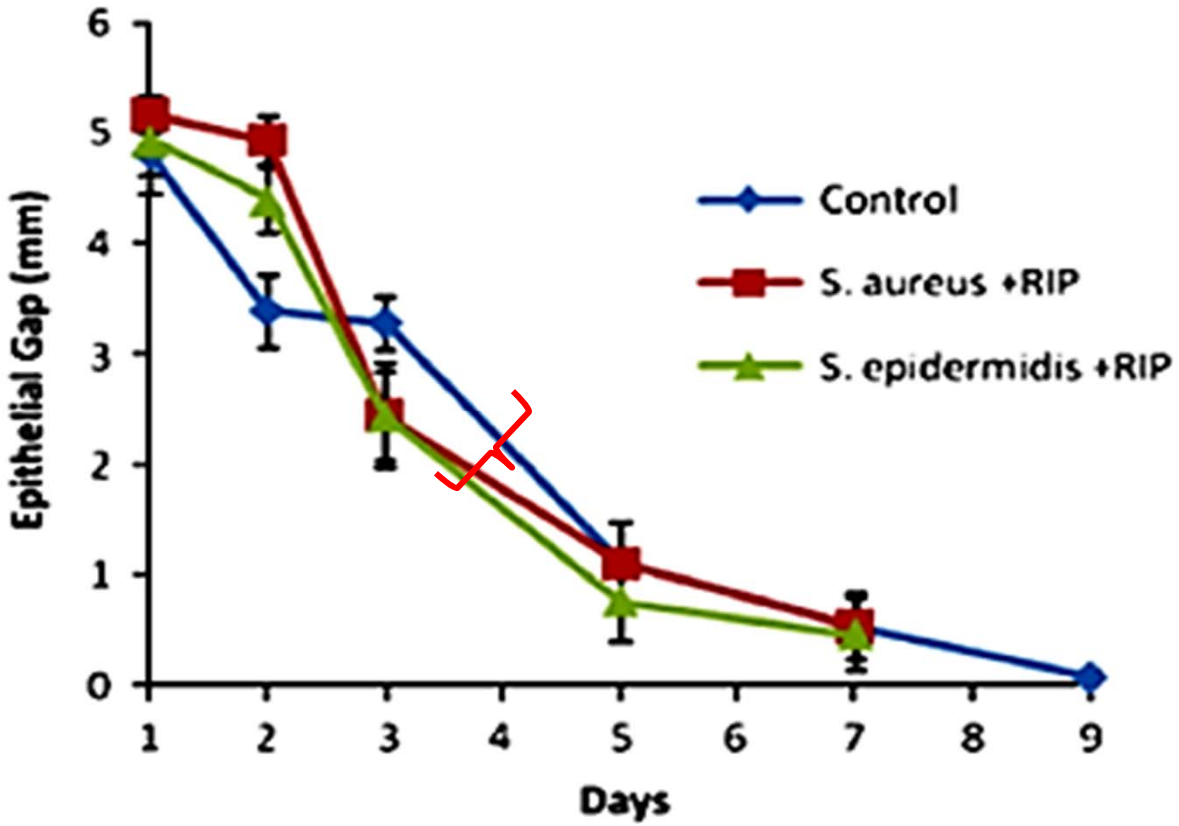
Panels A, B & C: G. James, E. Swogger, R. Wolcott, E. Pulcini, P. Secor, J. Sestrich, J. Costerton, P. Stewart. Wound Rep Regen, 16:37-44, 2008
Panel D: HC Flemming, J Wingender The Biofilm Matrix, Nature Rev Microbiol, 8:623-633, 2010
Panel E: SR Schooling , A Hubley, TJ Beveridge. J Bacteriol 191:4097-4012, 2009

M. Malone, T. Barjnsholt, A. McBain, G. James, P. Stoodley, D. Leaper, M. Tachi, G. Schultz, T. Swanson, R. Wolcott. Prevalence of biofilms in chronic wounds: a systematic review and meta-analysis of published data, J wound Care, in press

Wound Biofilms Are Linked To Delayed Healing

Mouse model showed presence of *S. aureus* and *S. epidermidis* biofilms significantly delayed re-epithelialisation.[1]

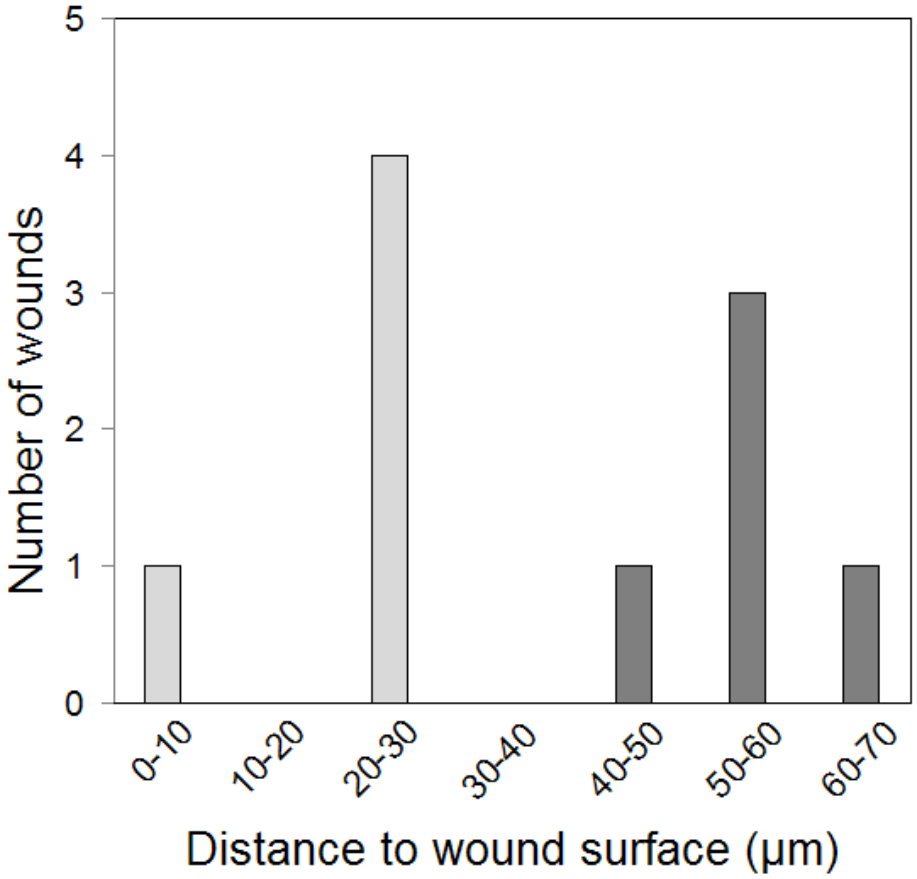
Negative impact of biofilm on healing verified by other studies [2,3]

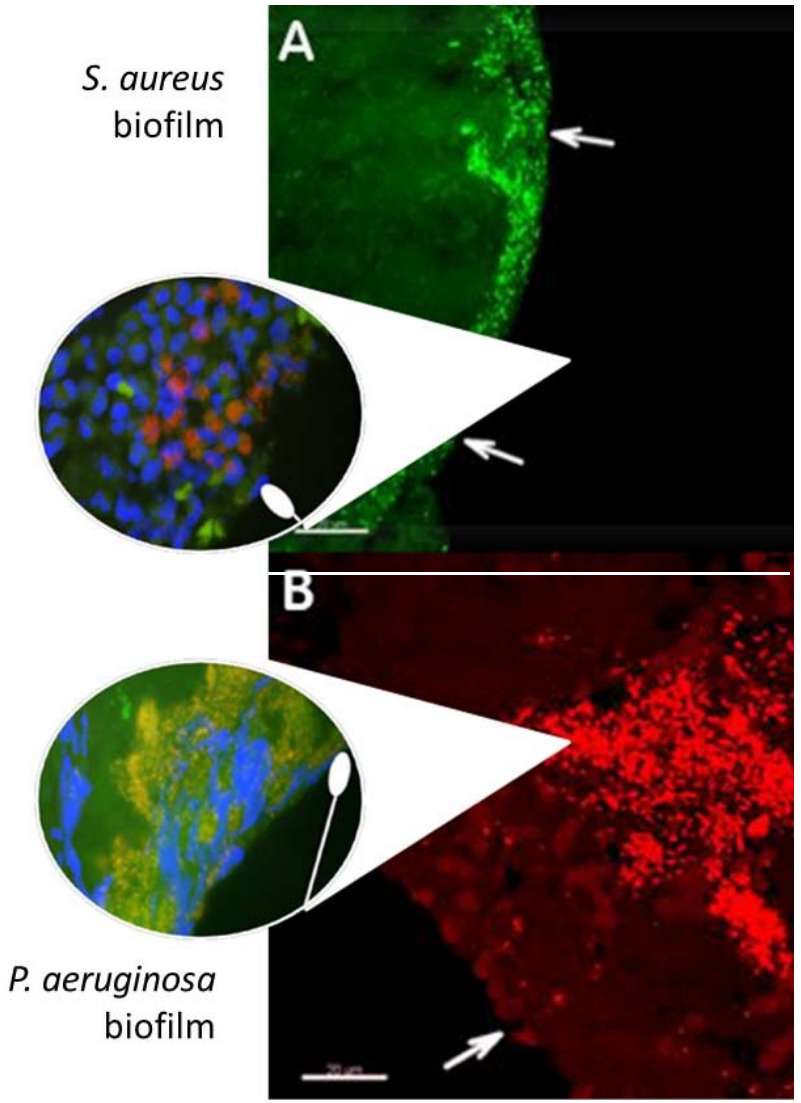


Effect of *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms on wound re-epithelialization.[1]

1. Schierle, C. F., *et al.*. *Wound Repair Regen.* 17, 354–9 (2009).
2. Zhao, G. *et al. Wound Repair Regen.* 20, 342–352 (2012).
3. Roche, E. D. *et al.*. *Wound Repair Regen.* 20, 537–43 (2012).

Distribution of Bacterial Species in Wound Beds

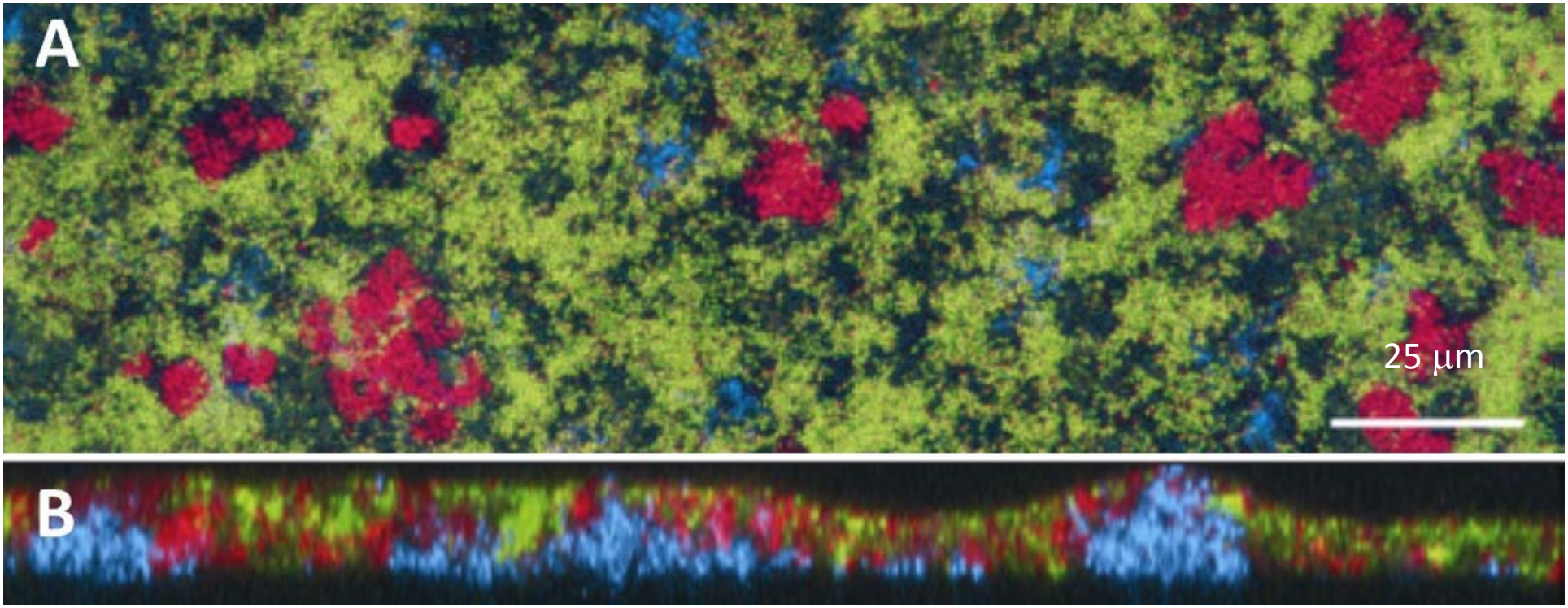




Fazli and Bjarnsholt et al: J Clin Microbiol. 2009 Dec;47(12):4084-9

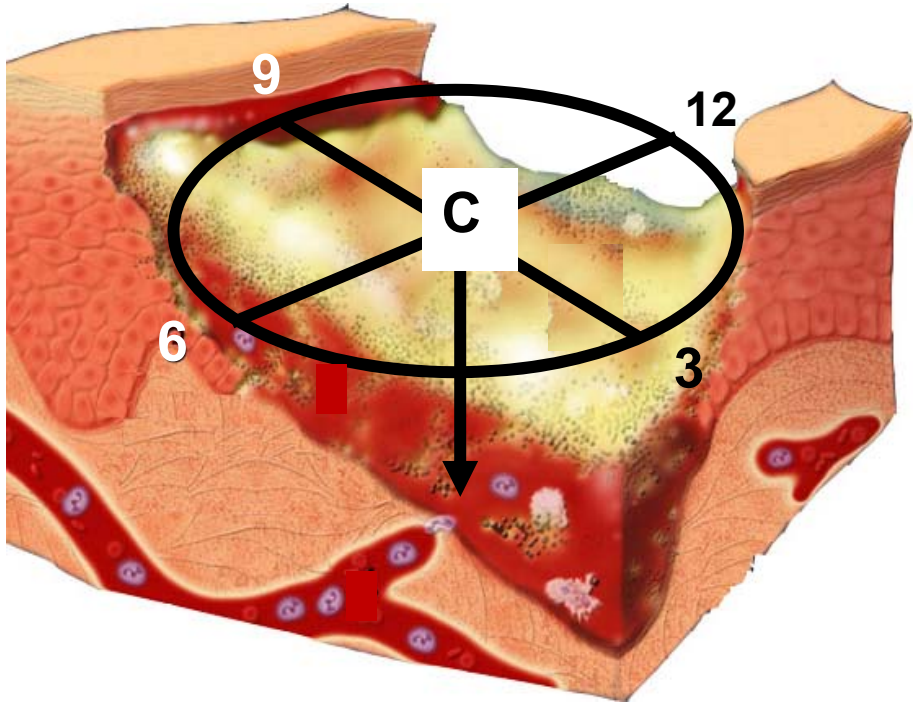
Images from Prof Bjarnsholt with permission

Polymicrobial Dental Biofilms Form Multilayered Mosaic Structures With Clusters of Bacteria



Confocal Laser Scanning Microscopic (CLSM) images of 48-h in situ dental biofilms stained simultaneously with all-bacterium-specific EUB338 probe (red), a Streptococcus-specific STR405 probe (yellow-green), and Actinomyces-specific ACT476 probe (blue) and red represent streptococci. (A) Maximum projection image of relative thin 48-h biofilm showing complete surface coverage with the dominance of streptococci. Well-defined microcolonies of large coccoid non-streptococci are observed as well as microcolonies of A. naeslundii. Scale bar = 25 mm. (B) Sagittal (x-z, y-z) section of a multilayered dental biofilm. Note that A. naeslundii (blue) is predominantly located in the inner part of the biofilms next to the surface (bottom of the images). Some microcolonies of A. naeslundii extended almost throughout the entire thickness of the biofilm. Burmølle, M. et al. Biofilms in chronic infections – a matter of opportunity – monospecies biofilms in multispecies infections. FEMS Immunol. Med. Microbiol. 59, 324–336 (2010).

Heterogeneous Distribution Of Bacteria In Chronic Wounds



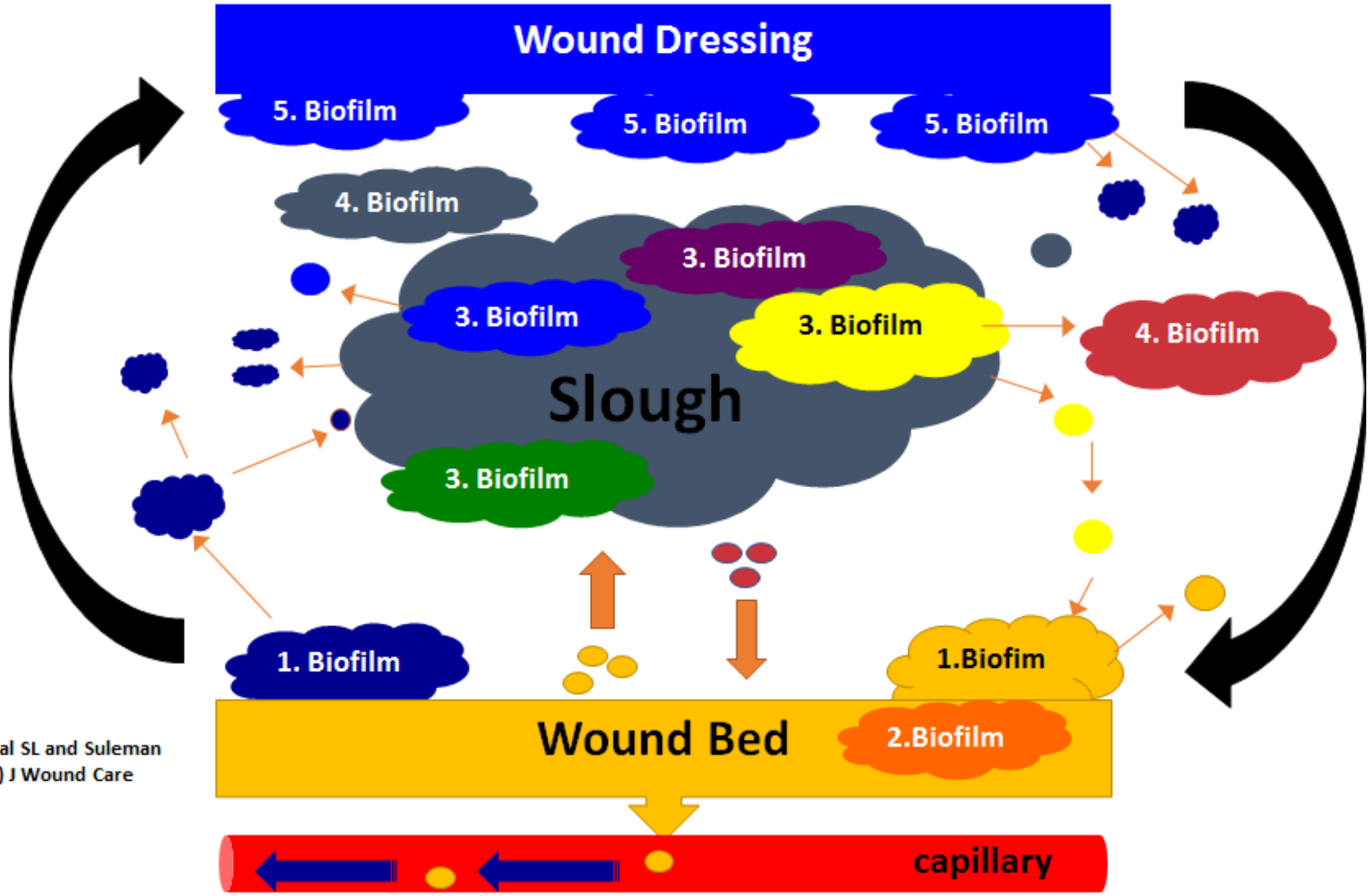
qPCR *Pseudomonas aeruginosa*

Position	Wound 1	Wound 2
C	510±18%	920±9%
3	No sample	300±13%
6	760±7%	8200±8%
9	47±9%	800±10%
12	280±3%	15±5%

Picture from homepage of Montana State University with permission

Thomsen TR, Aasholm MS, Bjarnsholt T, Givskov M, Kirketerp-Møller K, and Nielsen PH. The bacteriology of chronic venous leg ulcer examined by culture-independent molecular methods. *Wound Repair Regen,* 18(1):38-49, 2010

Biofilm Bacteria Are Present In Multiple Locations

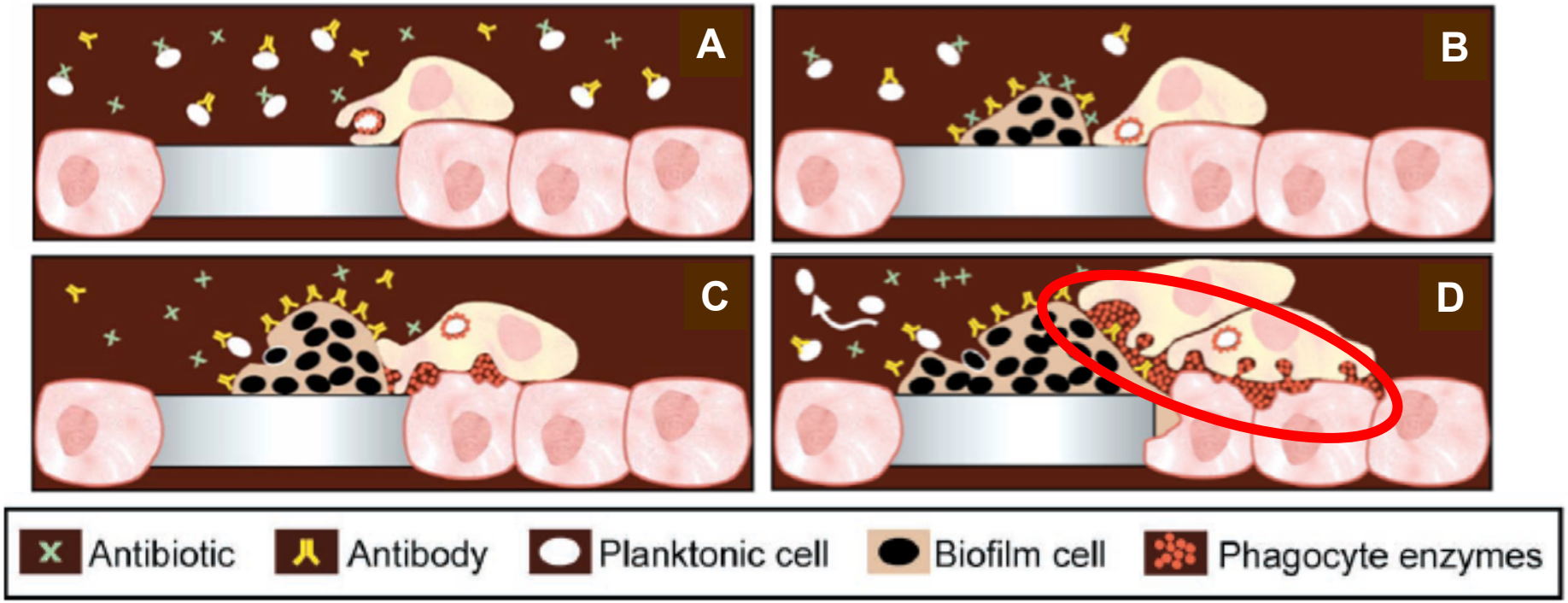


1-Surface of wound bed; 2-Deep in wound bed; 3-Slough; 4-Wound fluid; 5-Wound dressing

Question: How do biofilms impair healing of skin wounds?

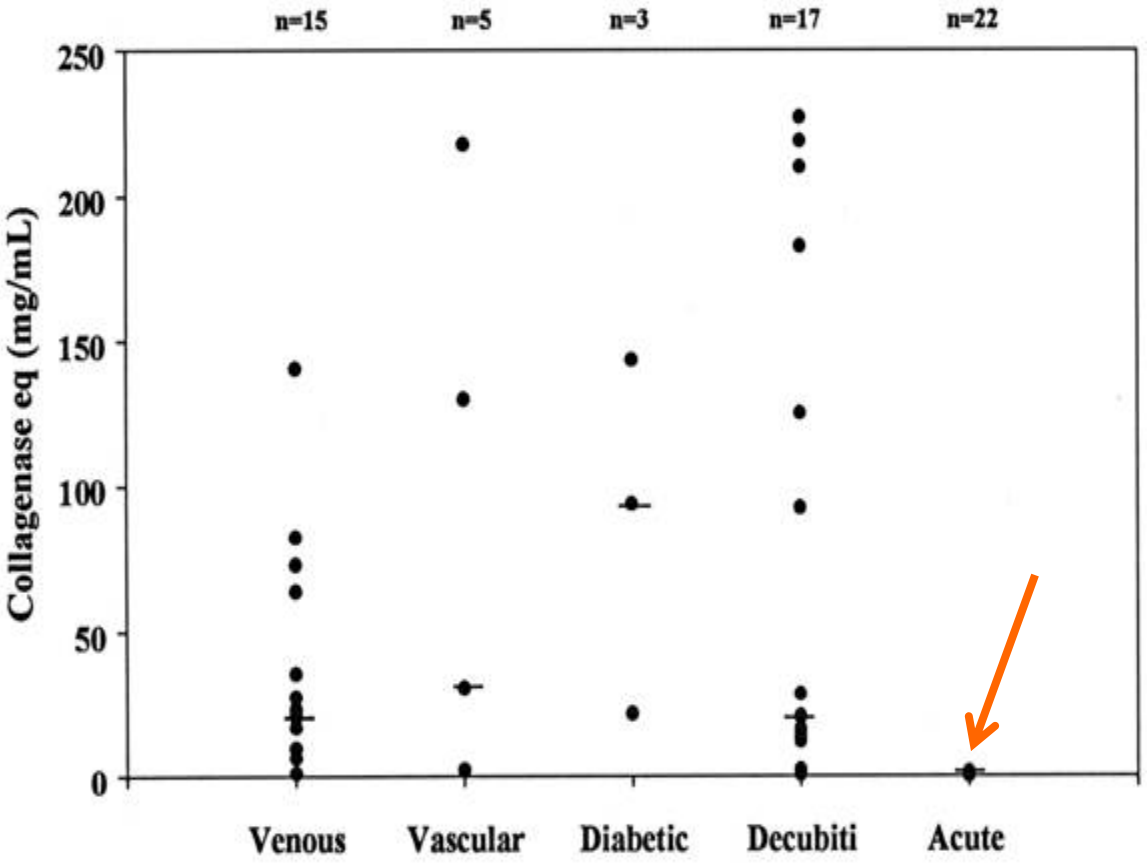
Answer: Biofilms stimulate *chronic inflammation* by increasing release of proinflammatory cytokines that leads to highly *increased levels of proteases and reactive oxygen species* that *degrade proteins* that are *essential for healing*.

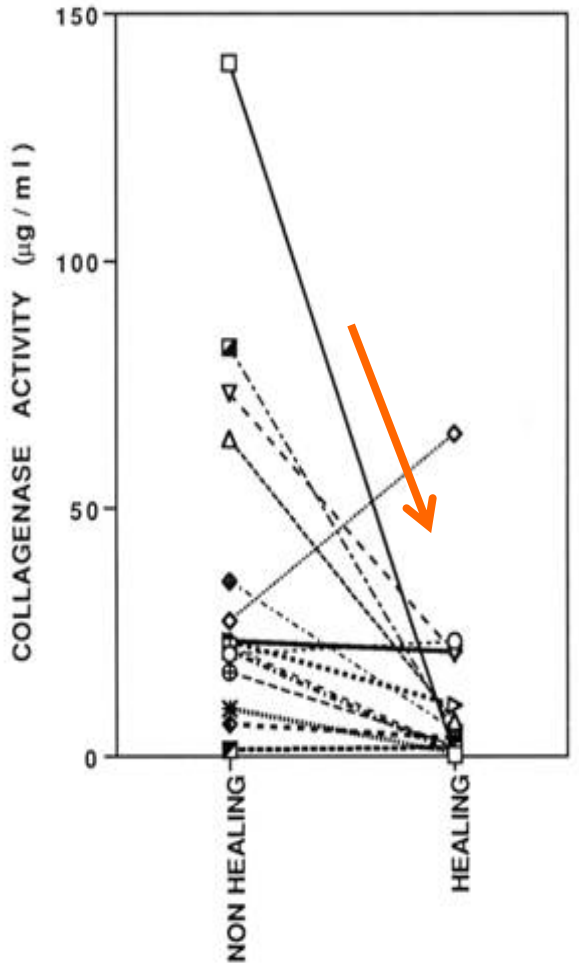
How Does The Immunological Response to Biofilms Cause Tissue Damage and Impair Healing?



In Panel A, planktonic bacteria can be cleared by antibodies, phagocytosis, and are susceptible to antibiotics. Adherent bacterial cells (Panel B) form biofilms preferentially on inert surfaces or devitalized tissue, and these sessile communities are tolerant to antibodies, phagocytosis and antibiotics. Neutrophils (Panel C) are attracted to the biofilms, but cannot engulf biofilm. Neutrophils still release proteases and reactive oxygen species. Phagocytic enzymes (Panel D) damage tissue around the biofilm, and planktonic bacteria are released from the biofilm, causing dissemination and acute infection in neighboring tissue. **Costerton, Stewart, Greenberg, Science 284, 1999**

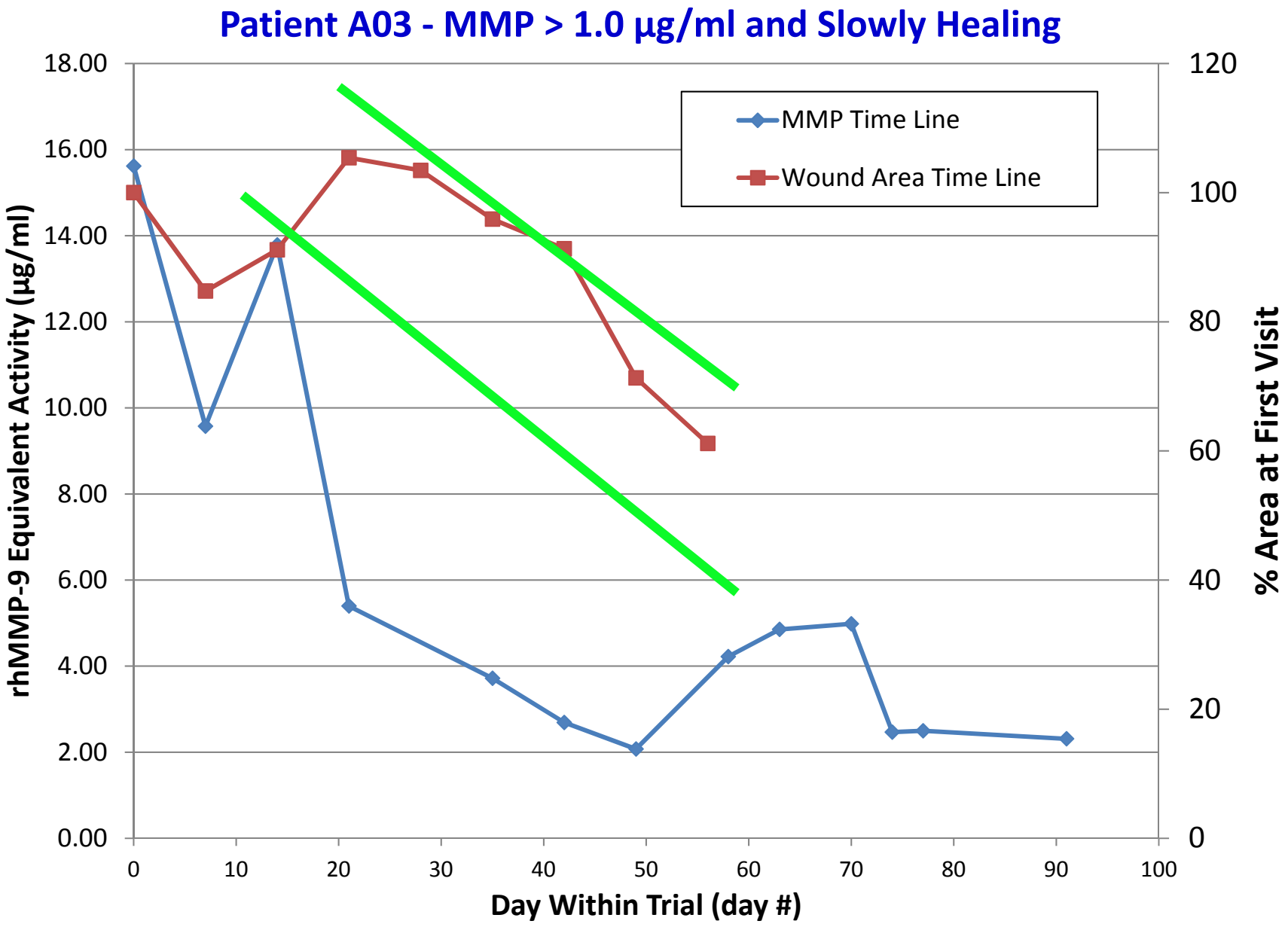
High Levels of MMP Activity in Chronic Wounds Decrease as Wounds Heal



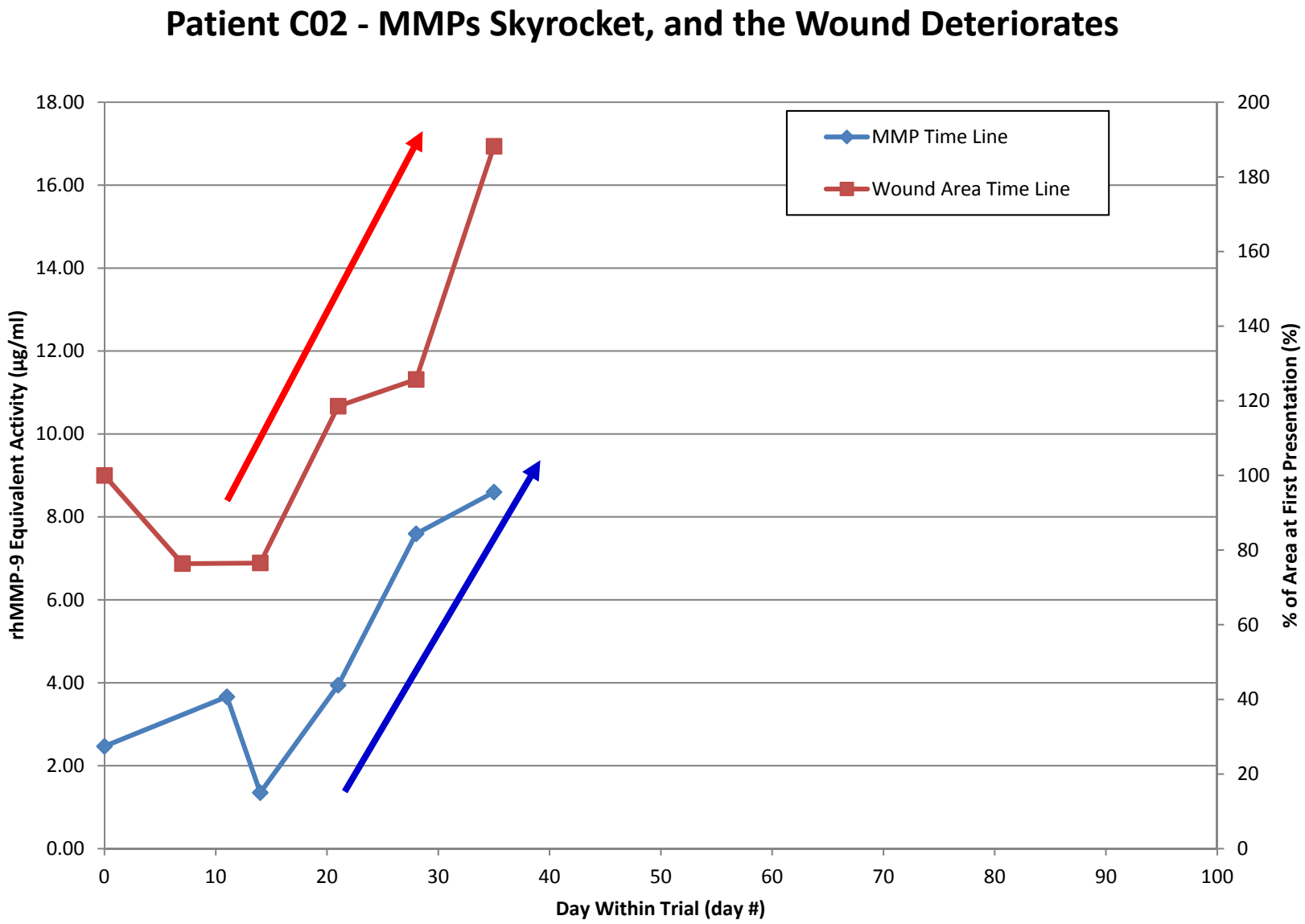


Trengove, Stacey, Macauley, Bennett, Gibson, Burslem, Murphy, Schultz. Wound Rep Reg 7:442-452, 1999

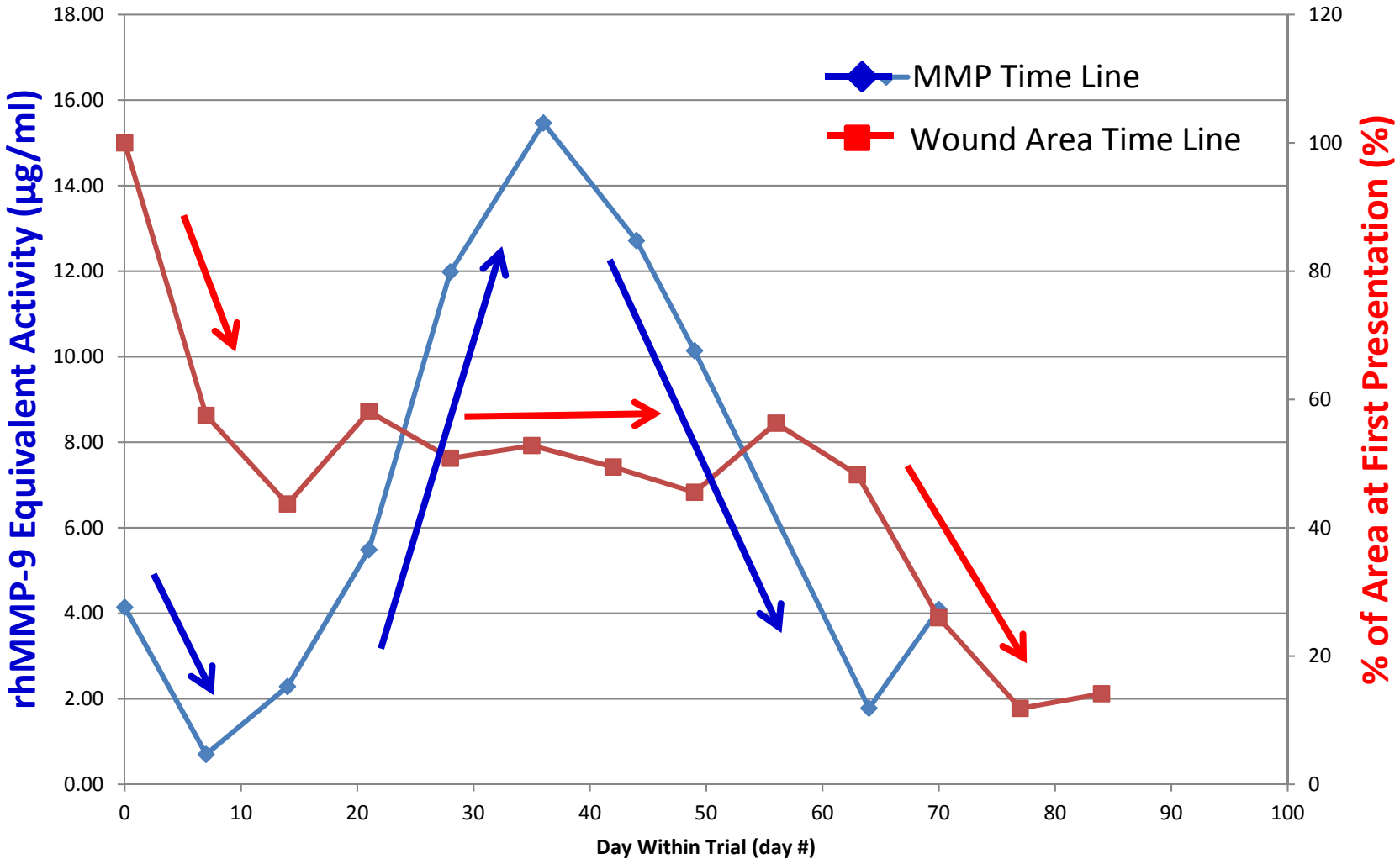
Clinical Study Patient #3



Clinical Study Patient #2

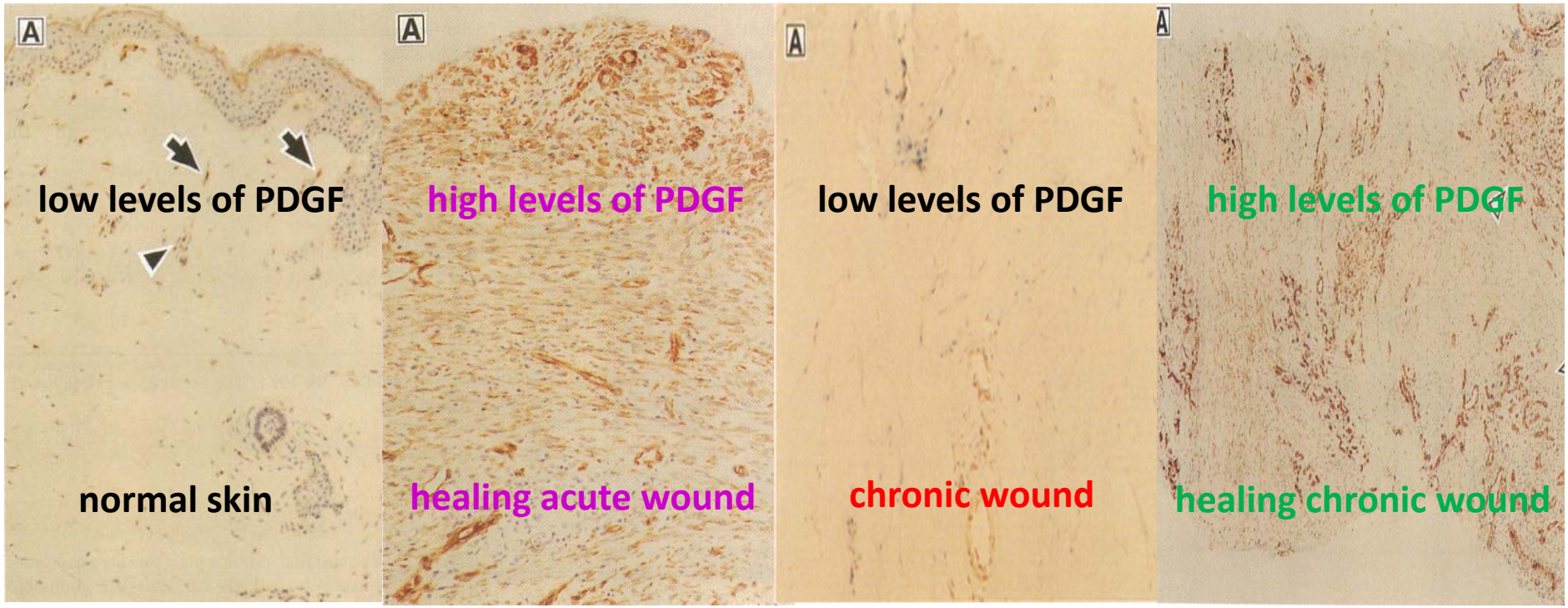


MMP-9 Activity Correlates With Wound Healing Time Course



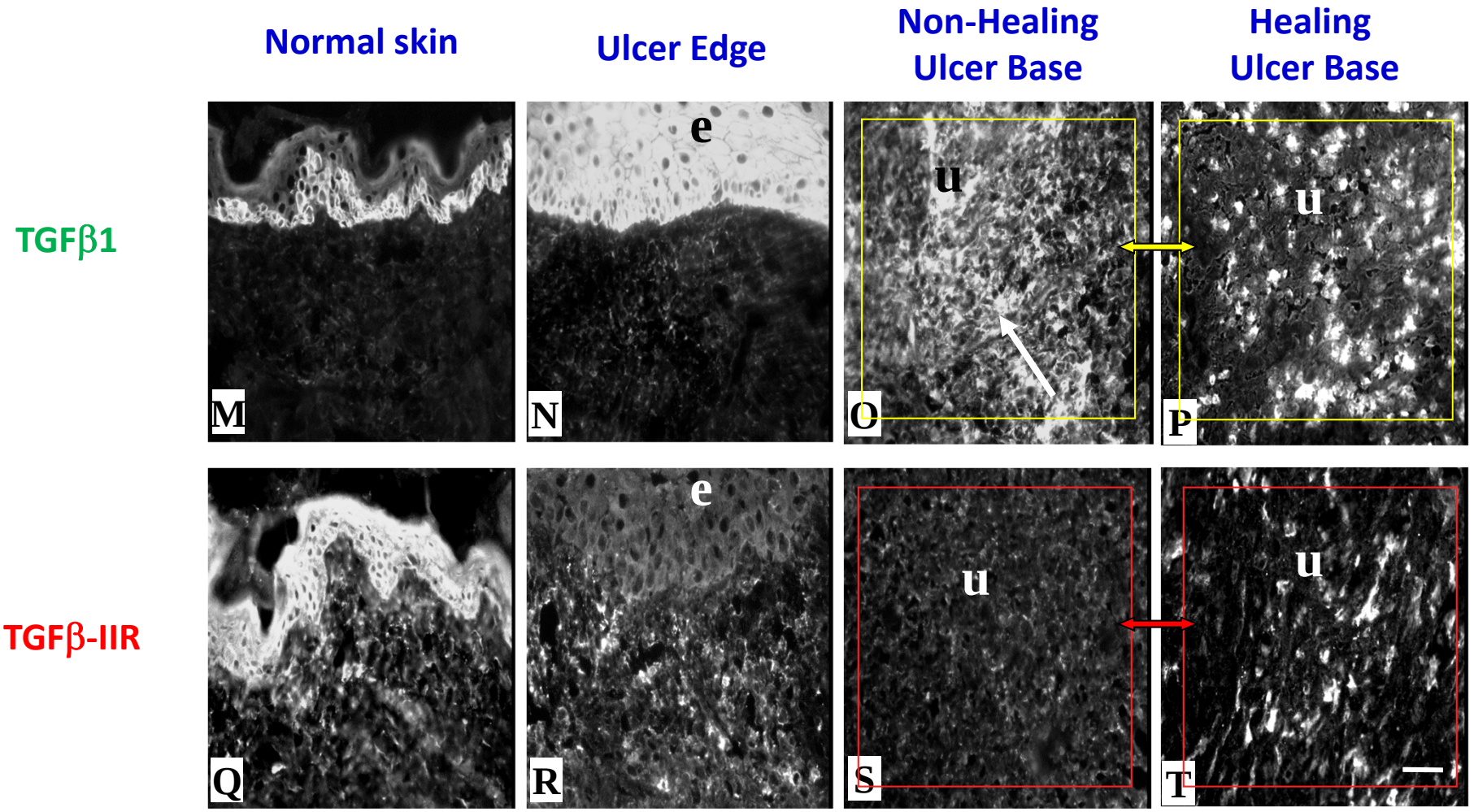
G. Bohn, B. Liden, G. Schultz, Q. Yang, D.J. Gibson. Ovine-Based Collagen Matrix Dressing: Next-Generation Collagen Dressing for Wound Care. Advances Wound Care 6(1):1-6, 2016.

PDGF-AA Immunostaining in Normal Skin, Acute Healing Wound, Chronic Wound, and Healing Chronic Wound



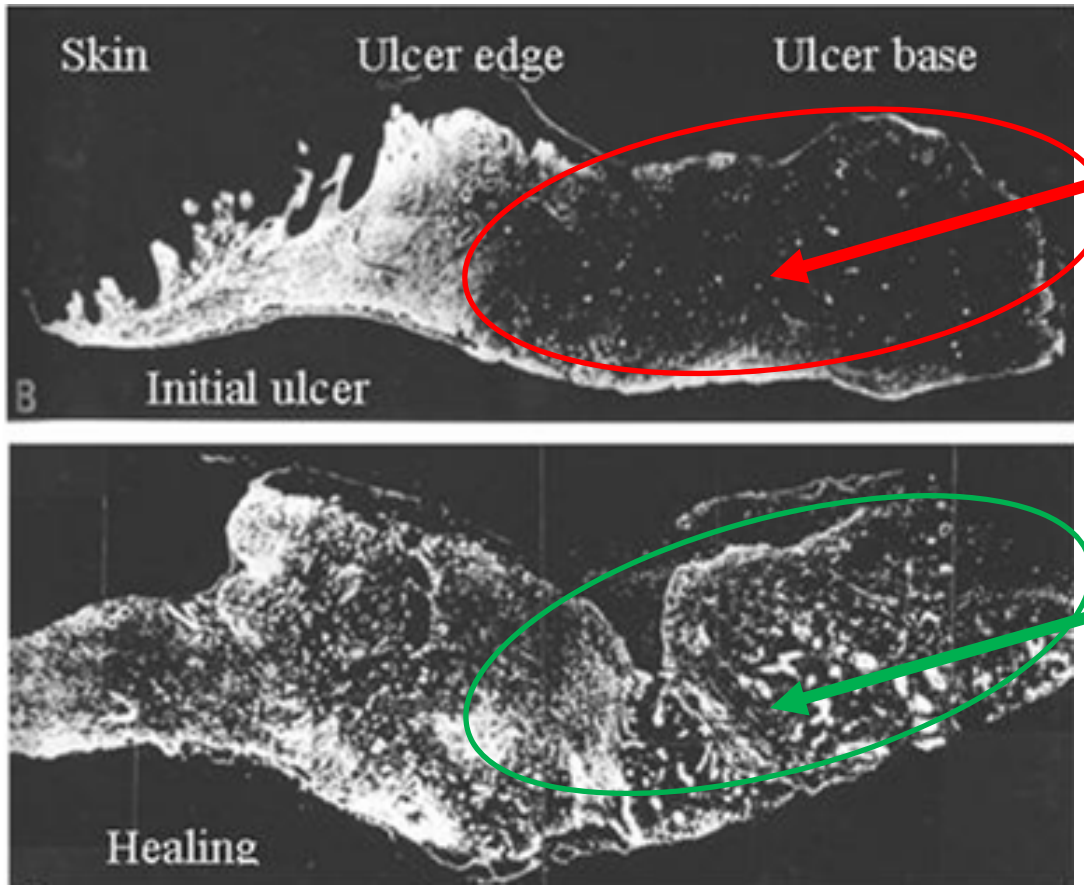
Pierce et al, J Clin Invest 96, 1336-50, 1995

TGFb-Type II Receptor Is Decreased In Chronic Venous Ulcers And Increases With Healing



A.J. Cowin, N. Hatzirodos, C.A. Holding, V. Dunaiski, R.H. Harries, T.E. Rayner, R. Fitridge, R.D. Cooter, G.S. Schultz and D.A. Belford. Effect of Healing on the Expression of Transforming Growth Factor-βs and Their Receptors in Chronic Venous Leg Ulcers. **J Invest Dermatol** 117:1282-1289, 2001.

Degradation of Fibronectin in Base of Chronic Venous Ulcers Reverses With Initiation of Healing



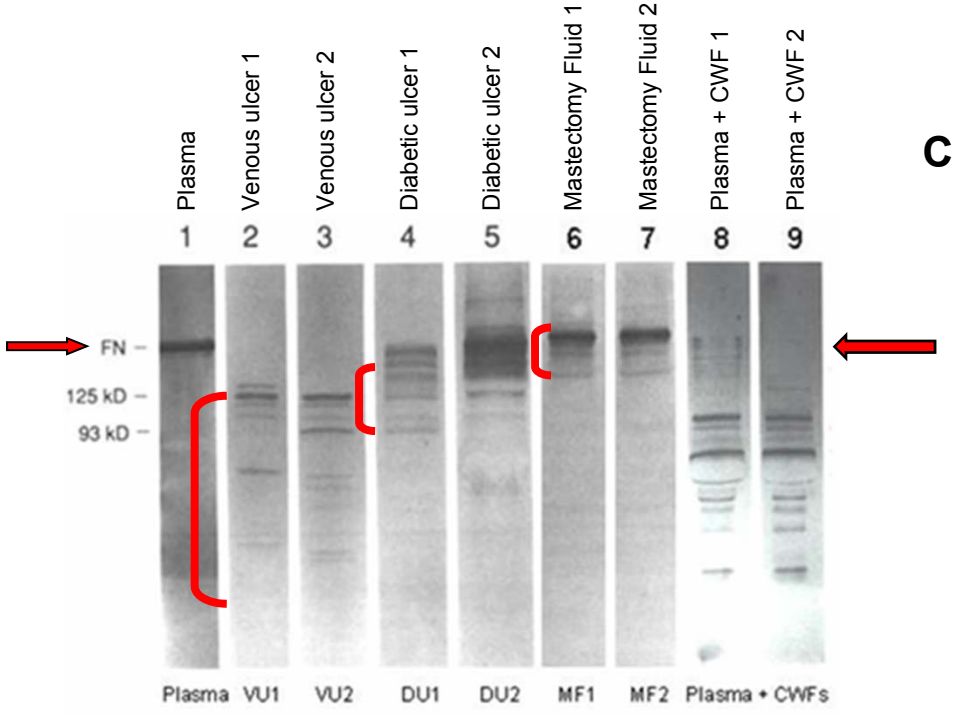
Fibronectin is degraded in non-healing ulcer

Fibronectin is present in healing ulcer

Summary: fibronectin is absent (degraded) in base of chronic venous ulcer, but fibronectin reappears (stable) as ulcer heals

Herrick, Sloan, McGurk, Freak, McCollum and Ferguson. Am J Pathol 141, 1992.

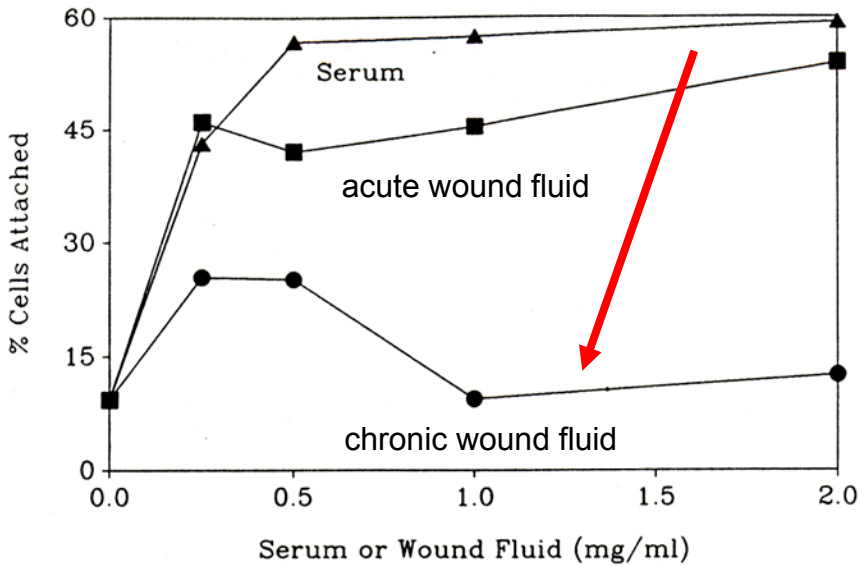
Fibronectin is Degraded by Chronic Wound Fluids & Chronic Wound Fluid Reduces Cell Attachment



Fibronectin profile in plasma shows a single intact band at 250 kDa. In contrast, fibronectin is degraded to lower molecular weight fragments in venous stasis ulcers and in diabetic ulcers.

Wysocki and Grinnell. Lab Invest 63:825, 1990

Chronic Wound Fluid Reduces Cell Attachment



Incubation of fibroblasts with increasing concentrations of serum or acute wound fluid enhances cell attachment to culture dishes. In contrast, incubation of fibroblasts with chronic wound fluids reduces cell attachment.

Wysocki and Grinnell. Lab Invest 63:825, 1990

Conclusion: **Inflammation in chronic wounds must be reduced to levels that lead to low protease activities that allow wounds to heal.**

Action: **Bacterial levels (both planktonic and biofilm) must be reduced for healing - how to do that?**

Principles of Biofilm Based Wound Care

1. Frequent sharp debridement of wounds to physically remove biofilm communities
2. Use an effective, fast acting microbicidal dressing after debridement to manage residual biofilm bacteria and to prevent reformation of biofilms e.g. Cadexomer Iodine
3. Alter topical & systemic antimicrobial treatments to prevent emergence of dominant bacteria from polymicrobial populations; utilize DNA bacterial identification techniques
4. Step-Down-Step-Forward treatment should be used to rapidly decrease biofilms and proteases that impair healing

Wolcott,R.D.; Rhoads,D.D. A study of biofilm-based wound management in subjects with critical limb ischaemia. J.Wound.Care 17:145-154, 2008

Healing of Diabetic Foot Ulcers Increases with Frequency of Debridement

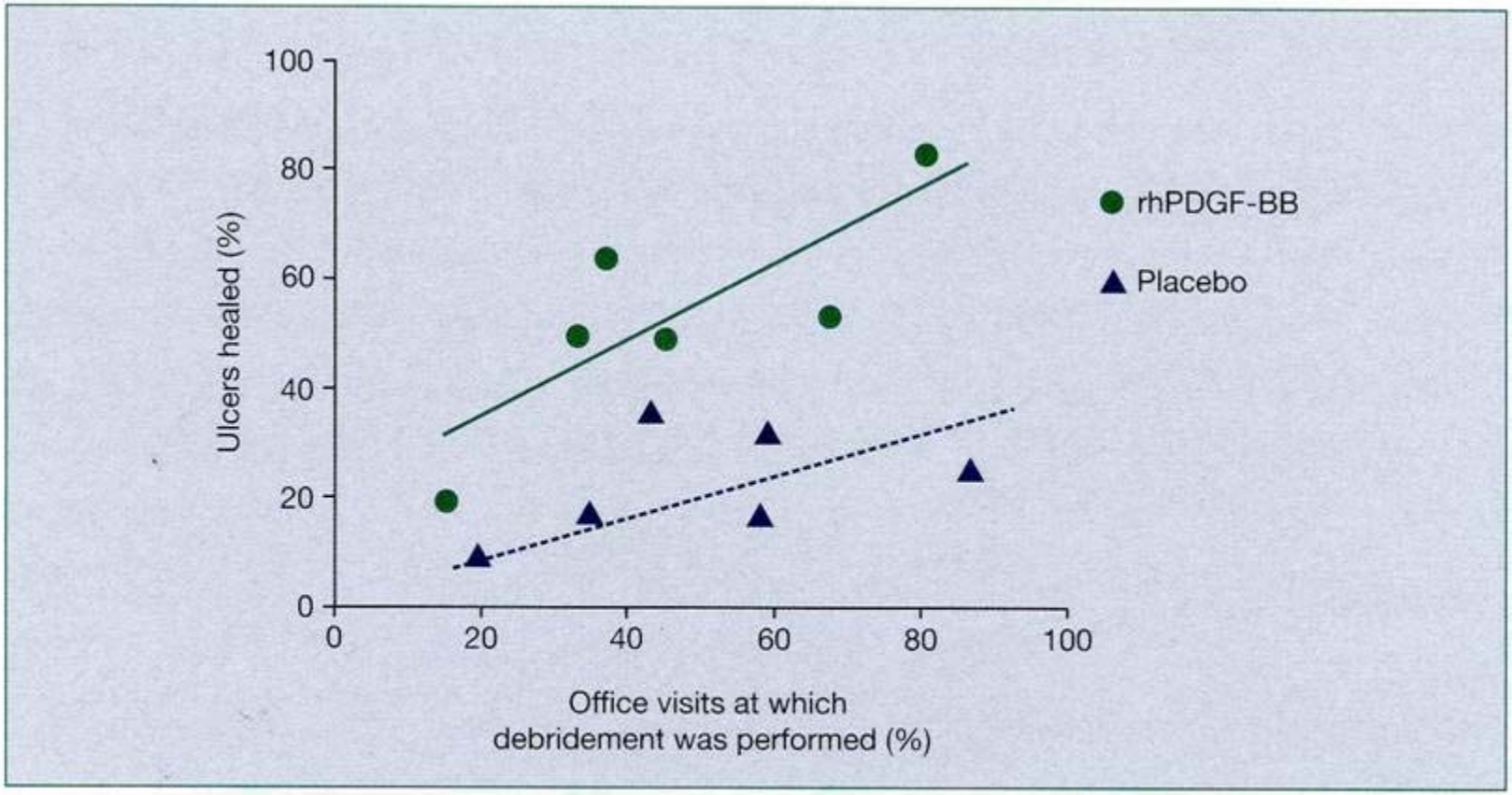
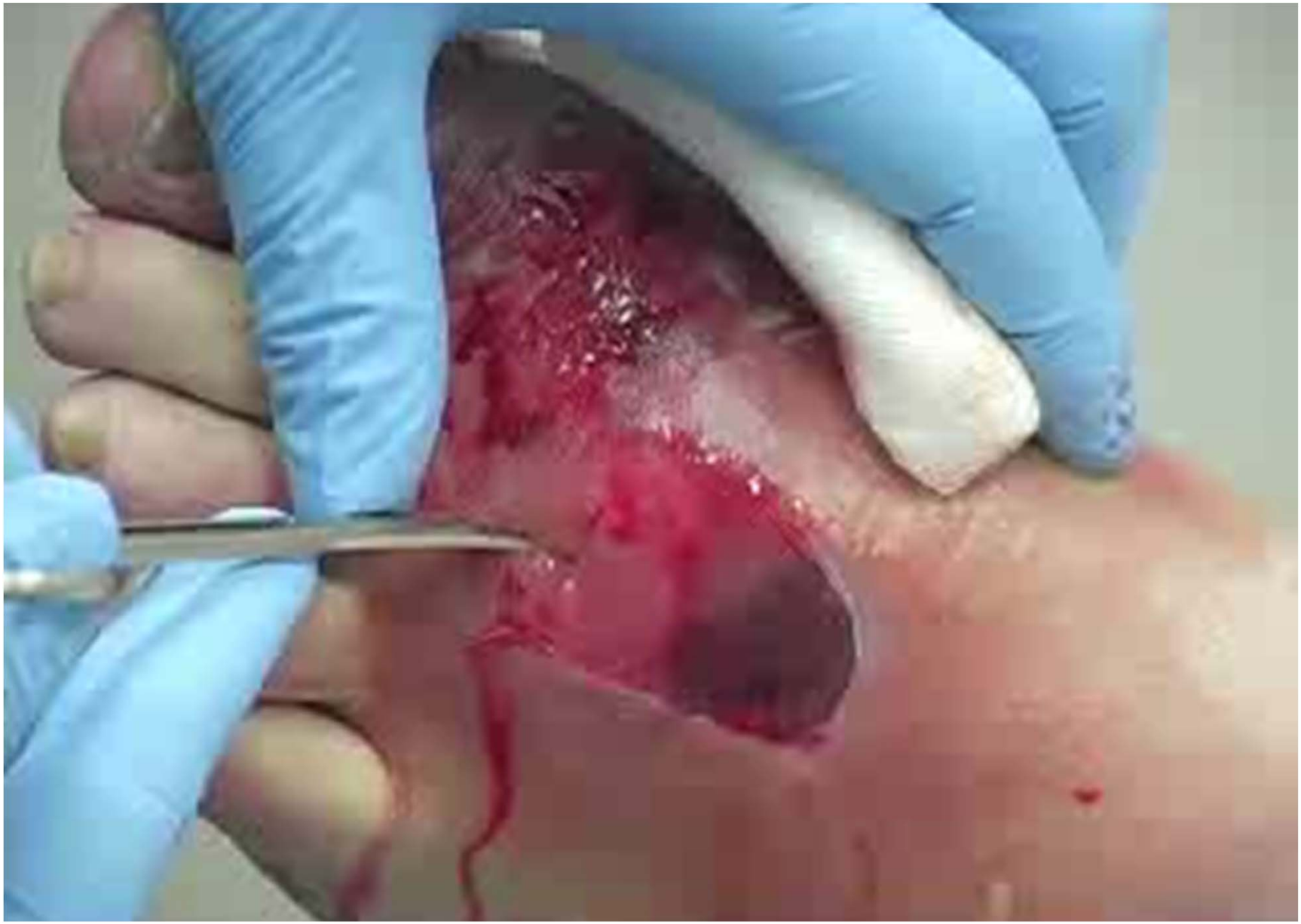


Figure 4. The incidence of complete healing increases with frequency of debridement in patients receiving rhPDGF-BB or placebo gel. When the frequencies of debridement are equal, the incidence of complete healing is approximately 2 to 3 times as high in patients receiving REGRANEX Gel compared with that of patients receiving placebo gel. **Steed et al., J Am Col Surg, 183: 61, 1996c**

Surgical Debridement of Infected Wounds

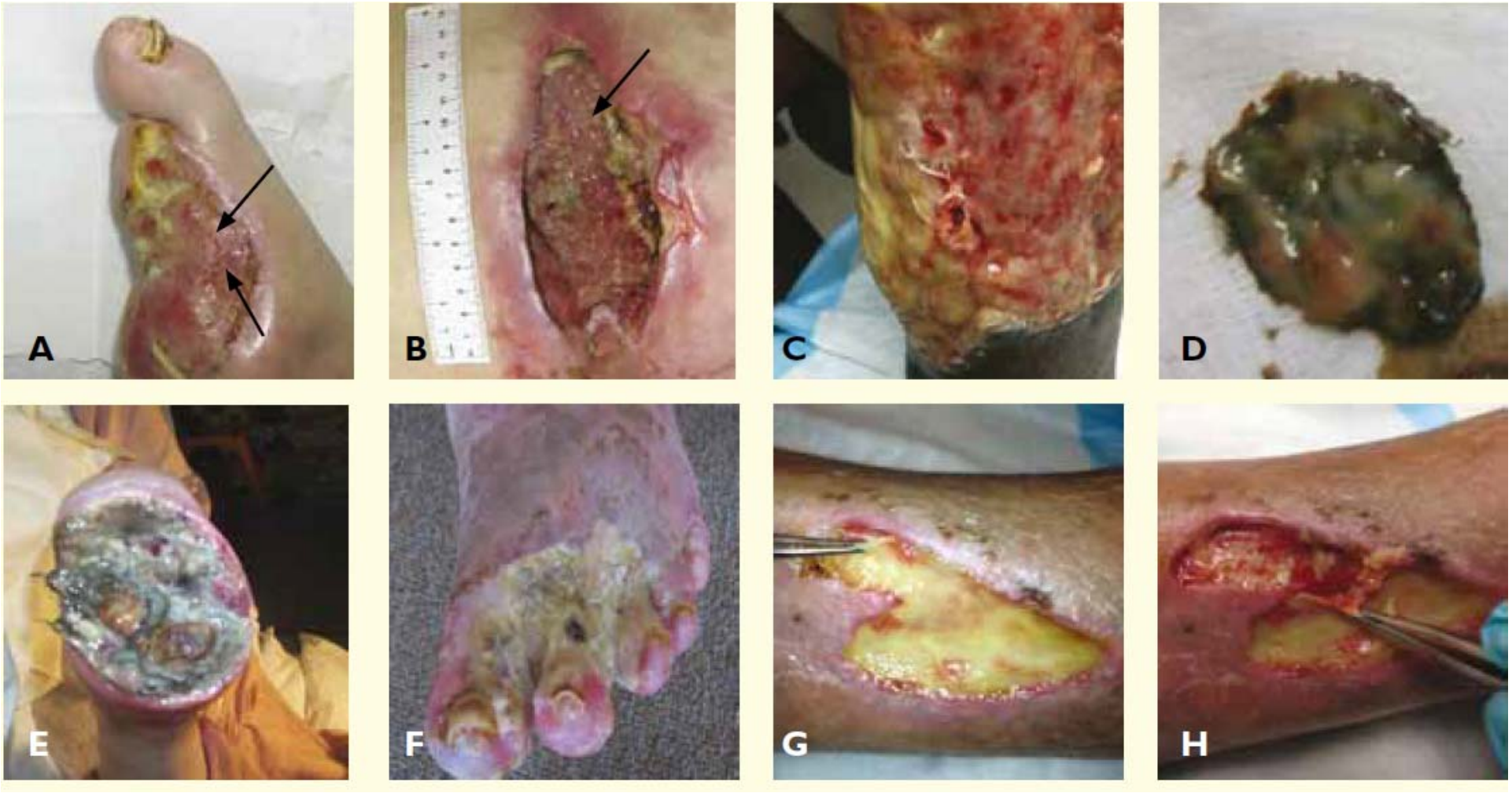


Question: Can you see biofilms on the surface of wound beds?

Answer: YES or NO

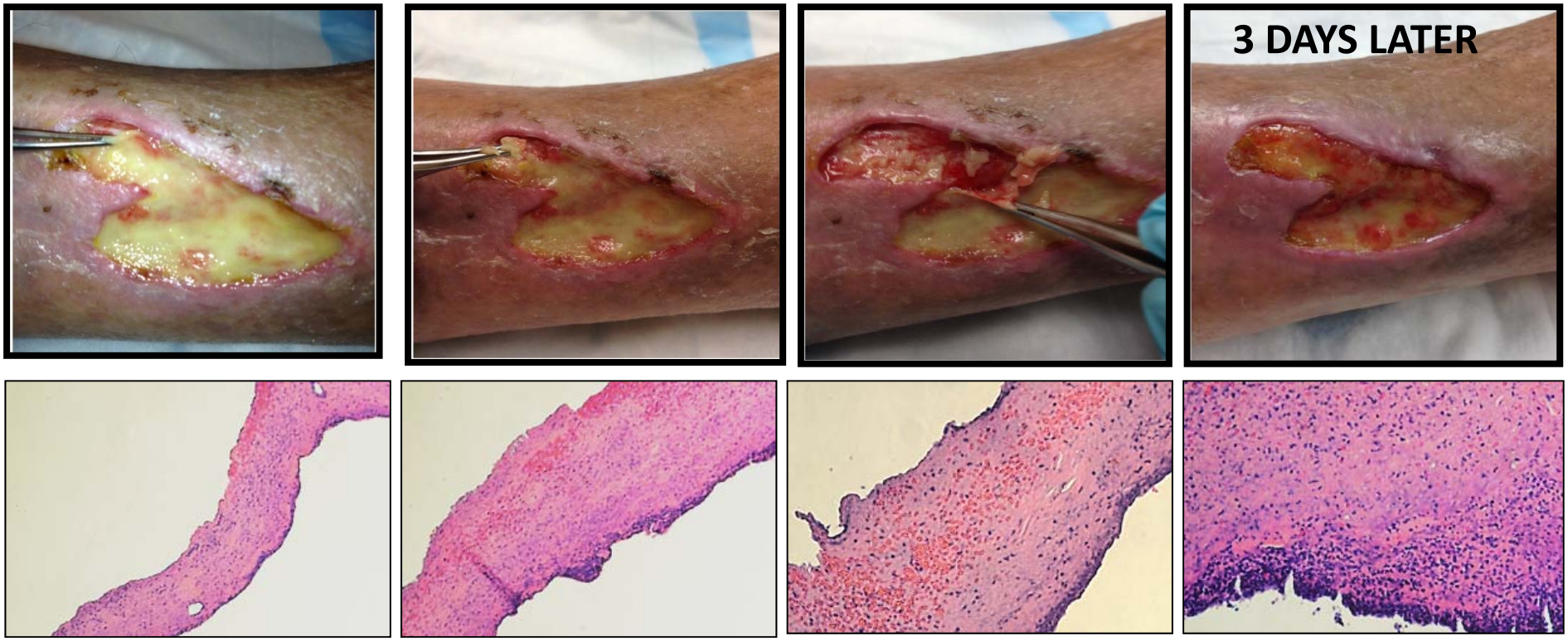
Most biofilms are **NOT VISIBLE** on the surface of a wound bed, and much of the biofilm is **BENEATH** the surface of the wound bed where it is very inflammatory!

What Are These Shiny Substances on Wound Beds?



D.G. Metcalf, P.G. Bowler, J. Hurlow. A clinical Algorithm for Wound Biofilm Identification. J Wound Care 2014.

What is This Thick Wound Slough?



G. Schultz, unpublished data

Can you see a biofilm in this wound?

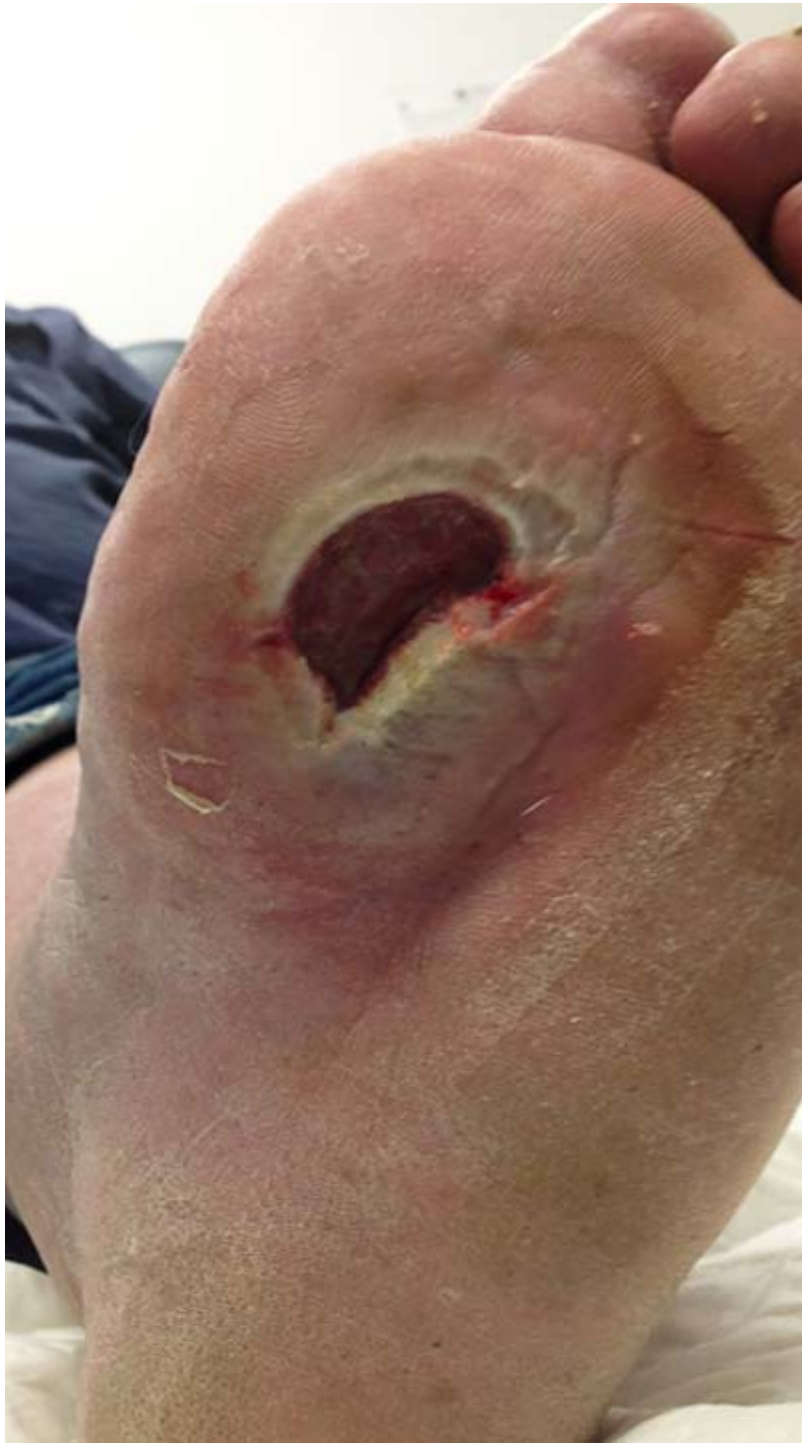


Photo provided by Dr Matthew Malone

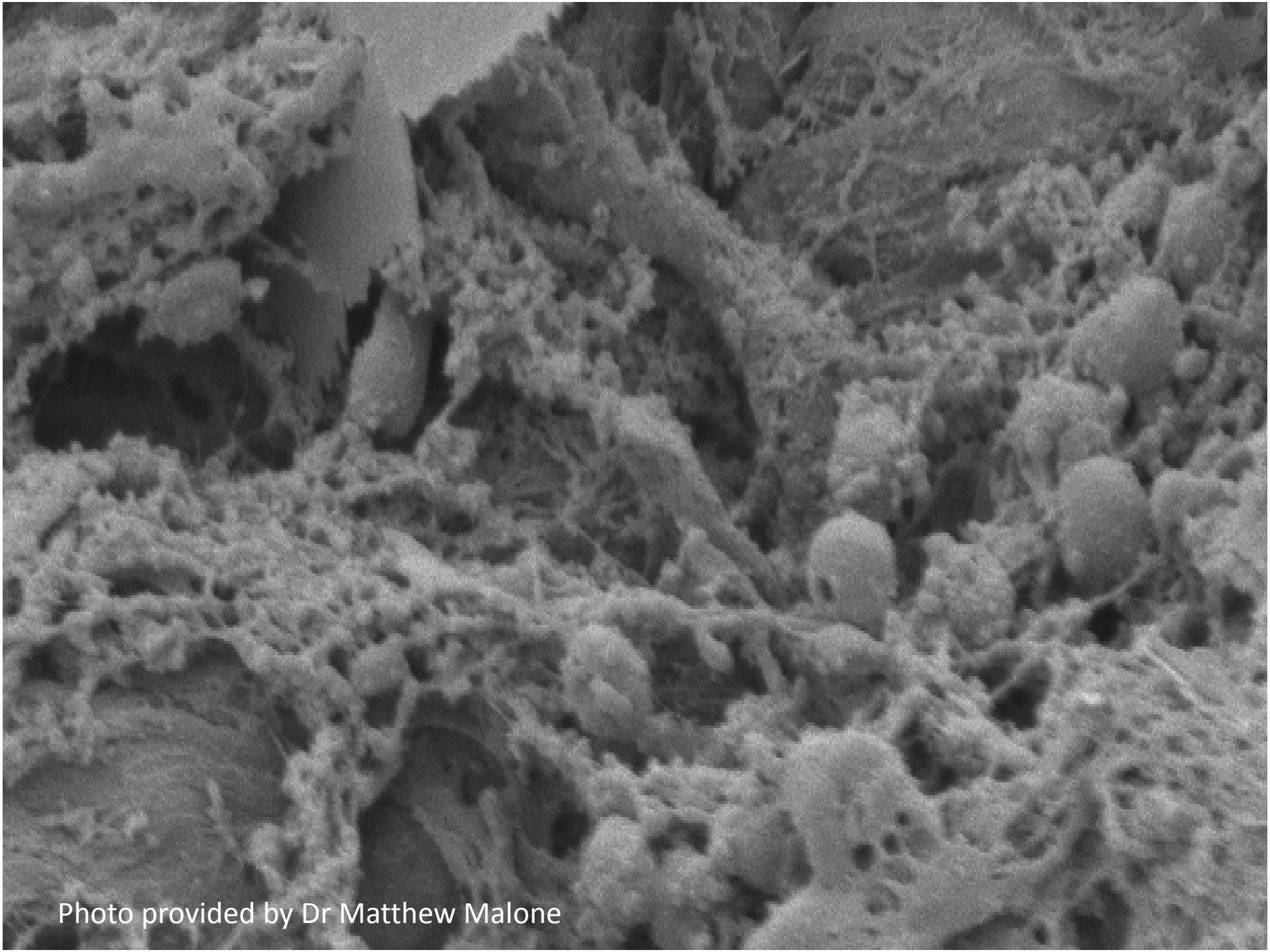


Photo provided by Dr Matthew Malone

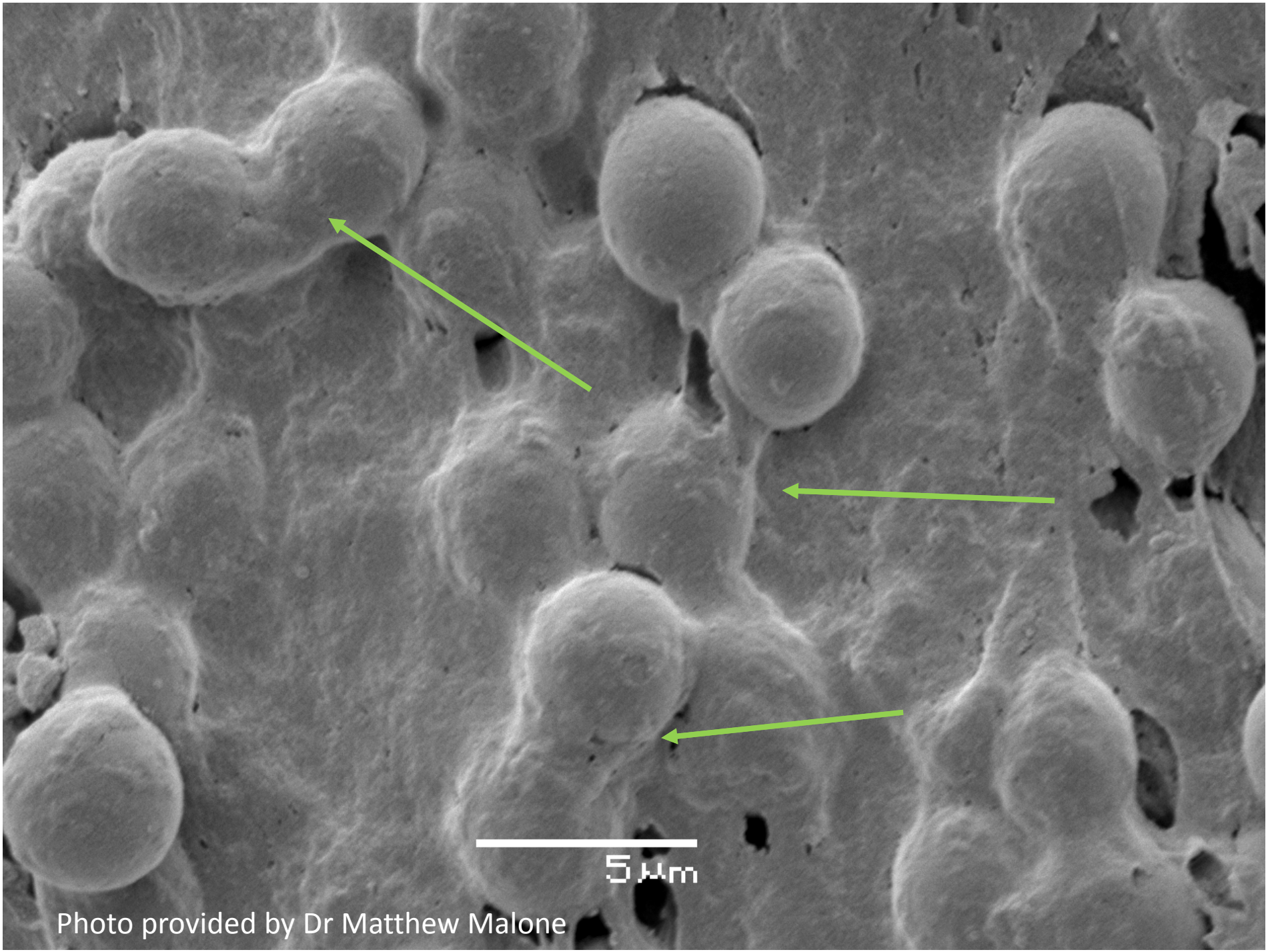
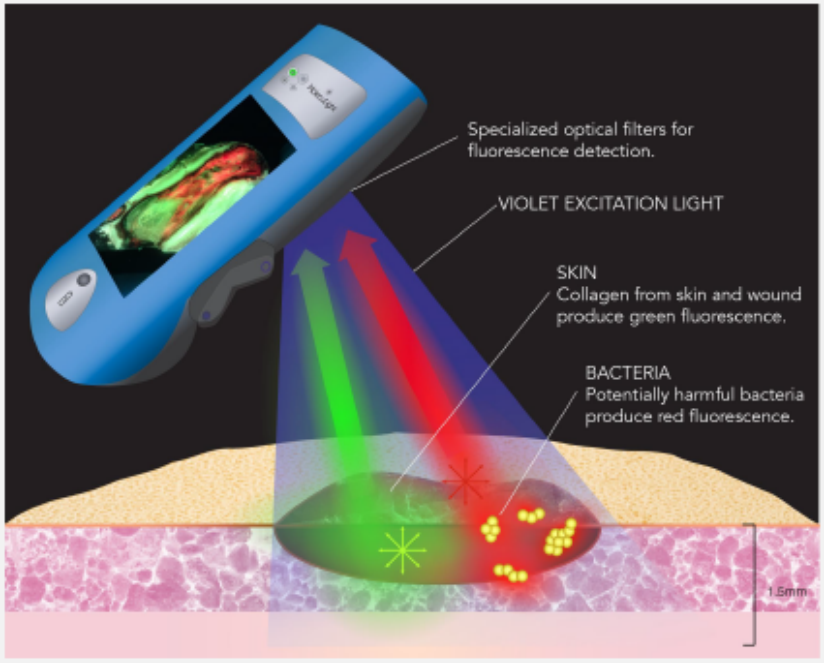


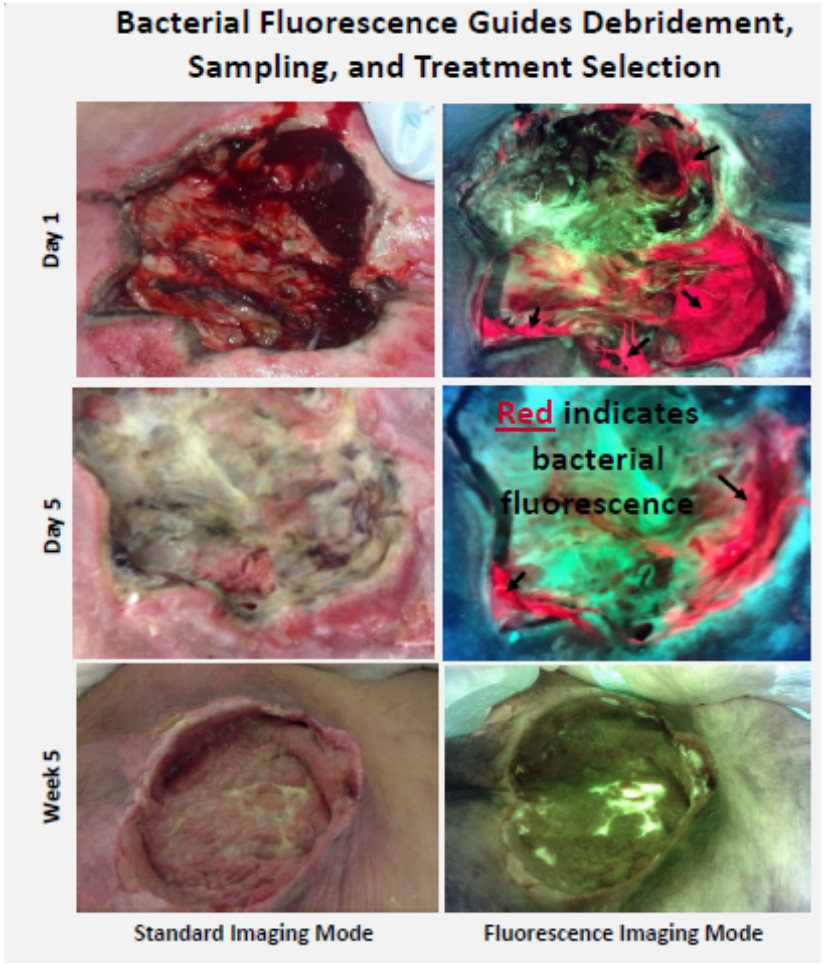
Photo provided by Dr Matthew Malone

Bacterial Fluorescence Imaging

Bacterial Fluorescence Imaging (MolecuLight *i:X*)

- When excited by 405 nm violet light, tissues fluoresce green while bacteria fluoresce red (porphyrin-producers, e.g. *Staphylococcus aureus*) or cyan (pyoverdine-producing *Pseudomonas aeruginosa*).
- This enables real-time, point-of-care detection and localization of bioburden within and around wounds[2-4].



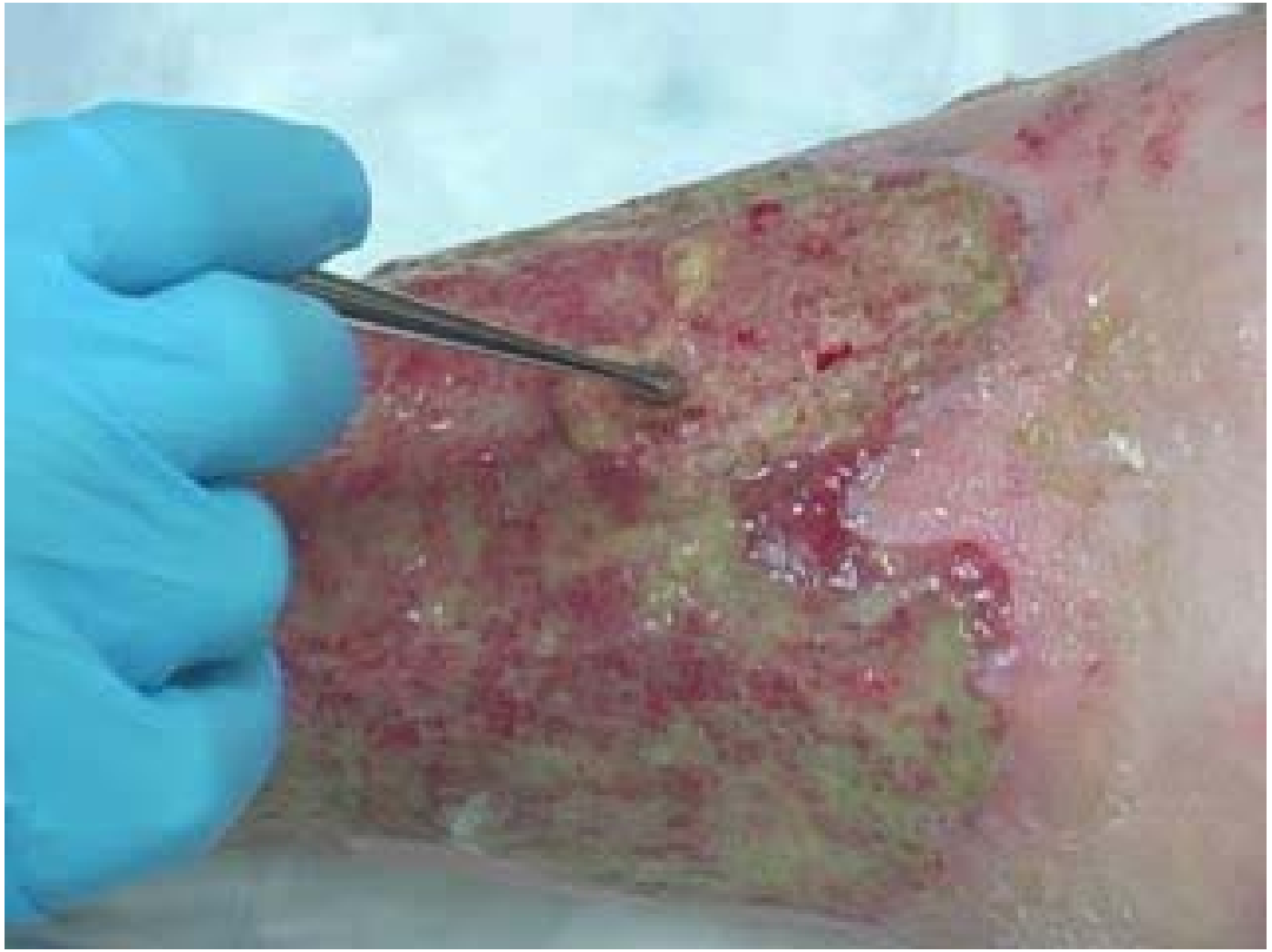


Pilot Study

- 40 wounds with diverse etiologies were imaged with the fluorescence imaging device at various stages of the wound healing process. 6 cases (3 bacterial fluorescence positive, 3 bacterial fluorescence negative.
- Wounds that were positive for red or cyan fluorescence signal were considered to have clinically significant bacterial loads. This real-time information guided immediate treatment decisions.
- All instances of bacterial fluorescence were confirmed via swab cultures. **All cultured regions of bacterial fluorescence exhibited moderate to heavy pathogenic bacterial growth.**

Rosemary Hill, Joshua Douglas , Lions Gate Hospital, Vancouver, Canada

What is This Filmy Wound Slough?
Mainly Fibrin - Surrogate Biomarker for Inflammation



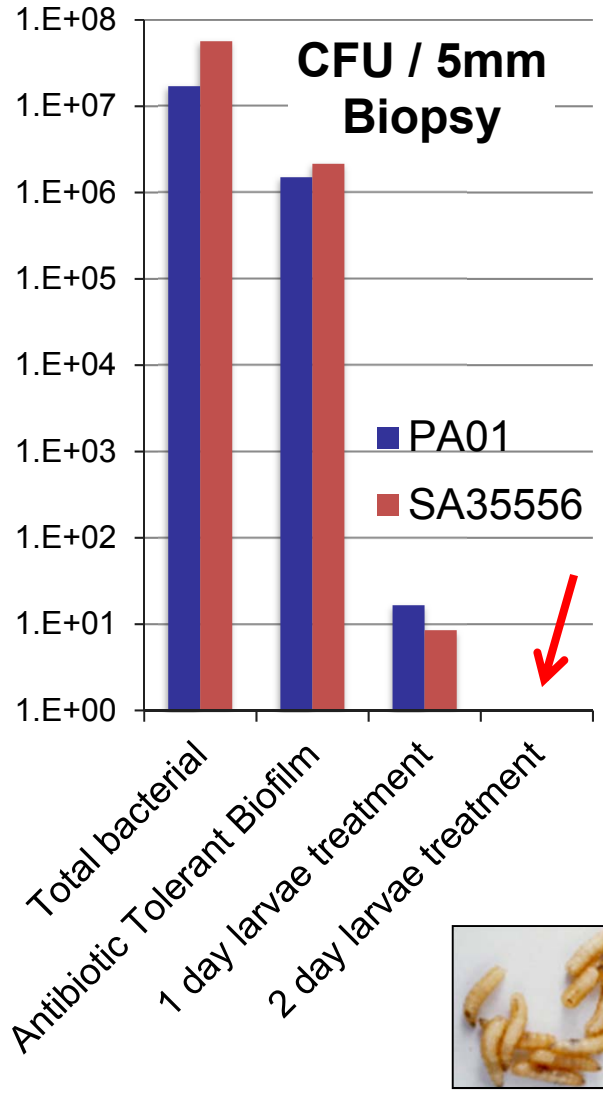
Dr Randy Wolcott

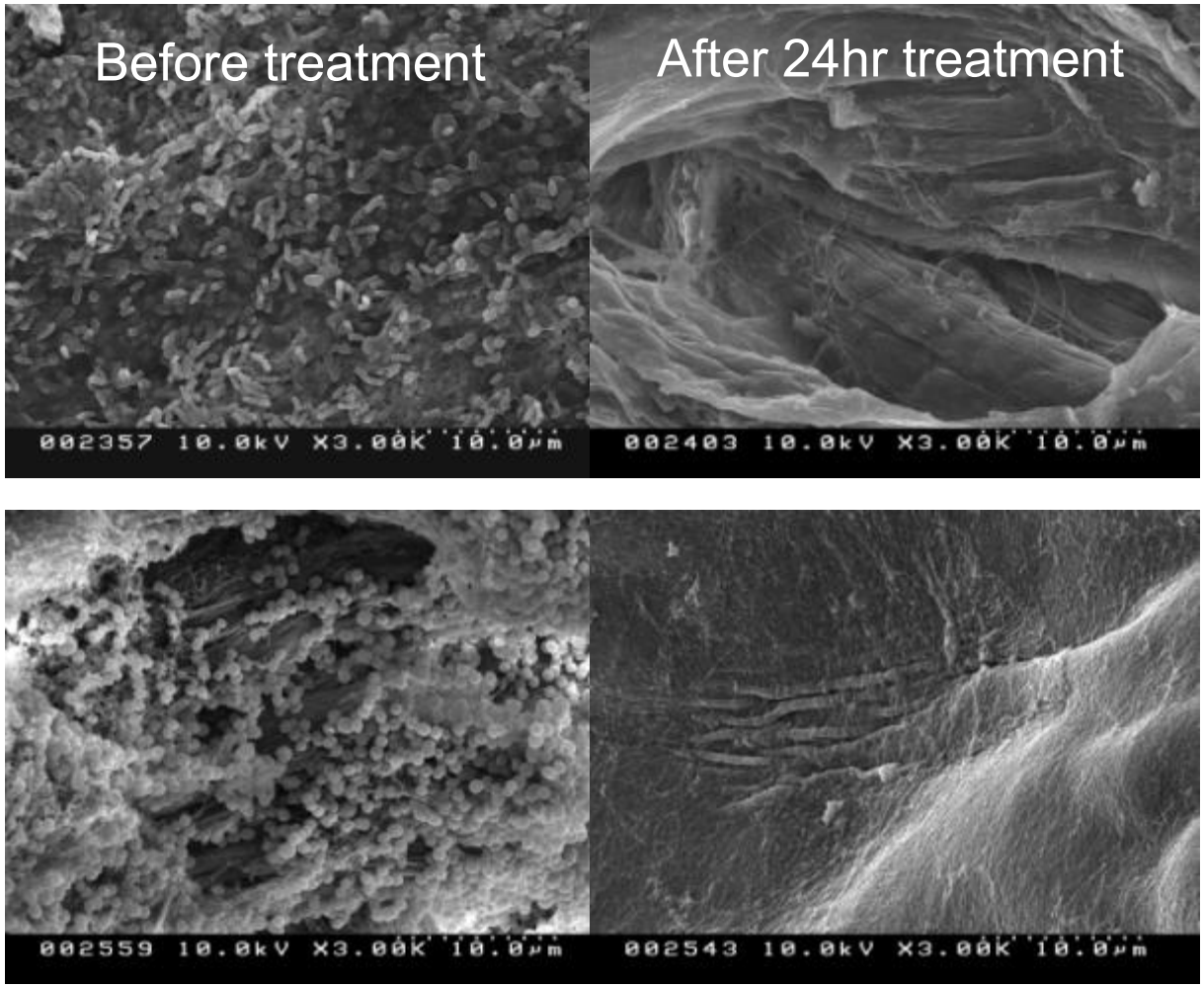
Question:

What effects do different debridement techniques have on removing and killing biofilms on dermal explants?

- Larval debridement
- Non-contact ultrasonic debridement
- Negative pressure wound therapy with instillation
- Concentrated surfactant gel

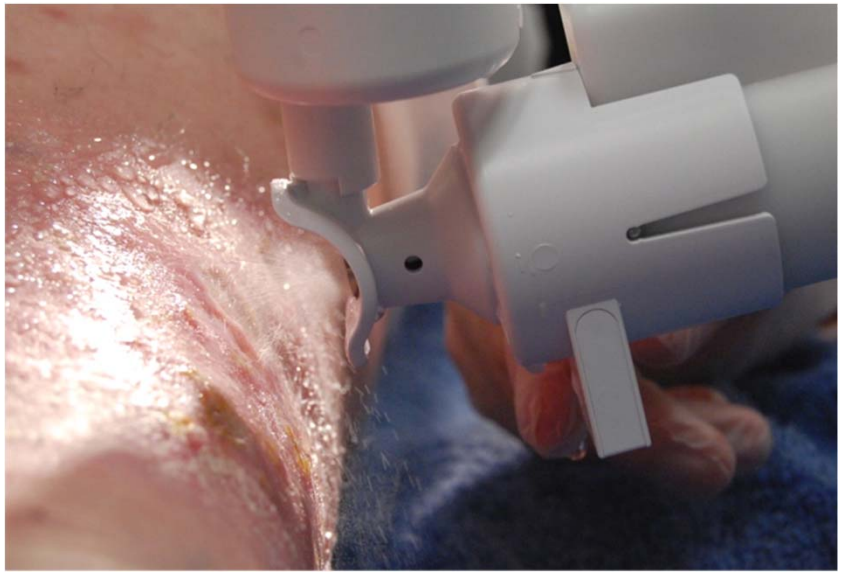
Larval Debridement Therapy



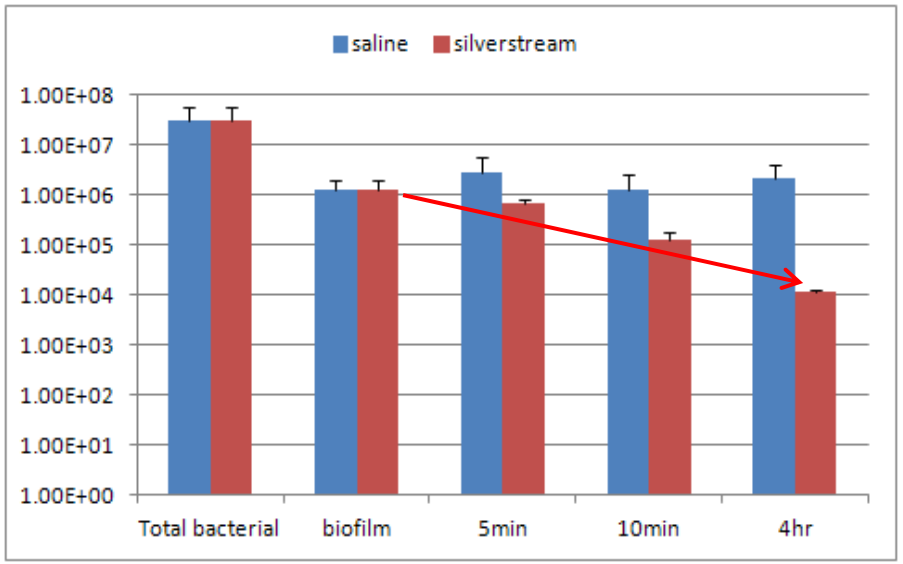


L. Cowan, J. Stechmiller, P. Phillips, Q.P. Yang and G. Schultz. Chronic Wounds, Biofilms and Use of Medicinal Larvae, **Ulcers**, Article ID 487024, 7 pages; http://dx.doi.org/10.1155/2013/487024, 2013.

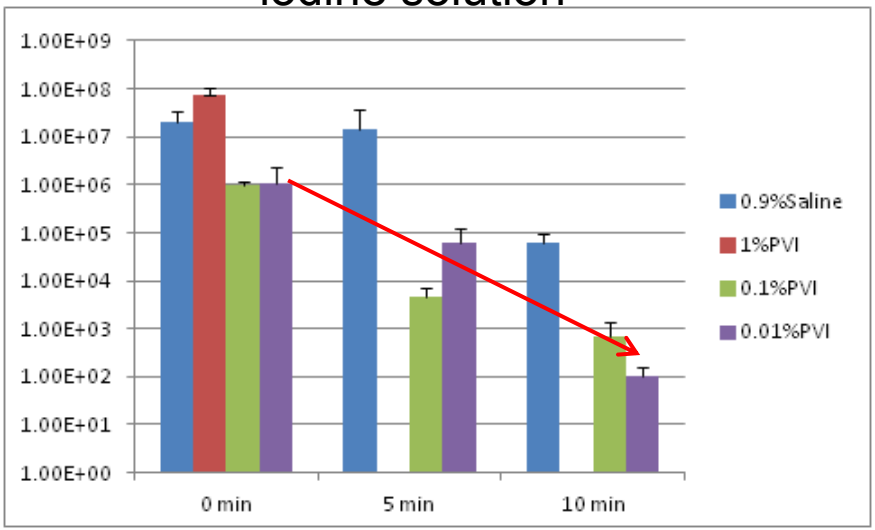
Effects of Non-Contact Ultrasonic Wound Cleansing on Biofilms



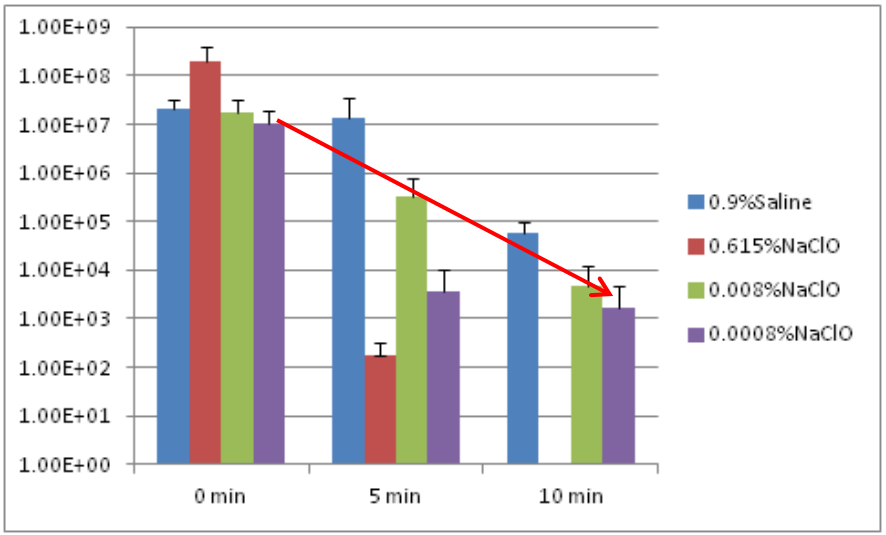
silver solution



iodine solution



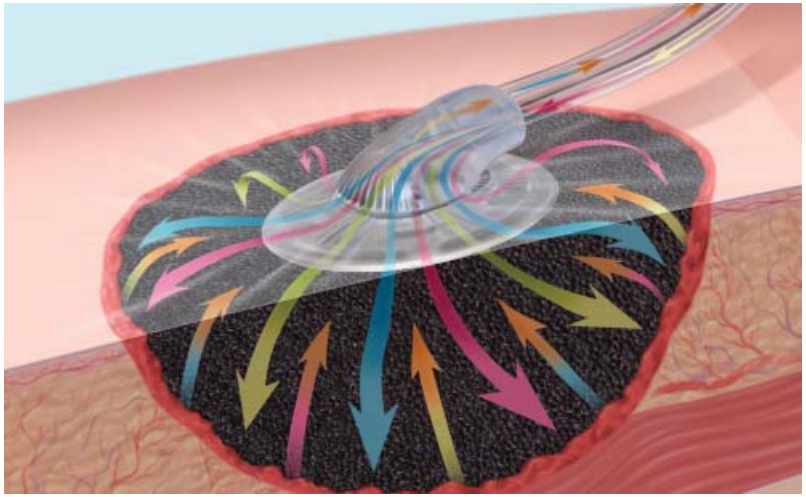
bleach solution

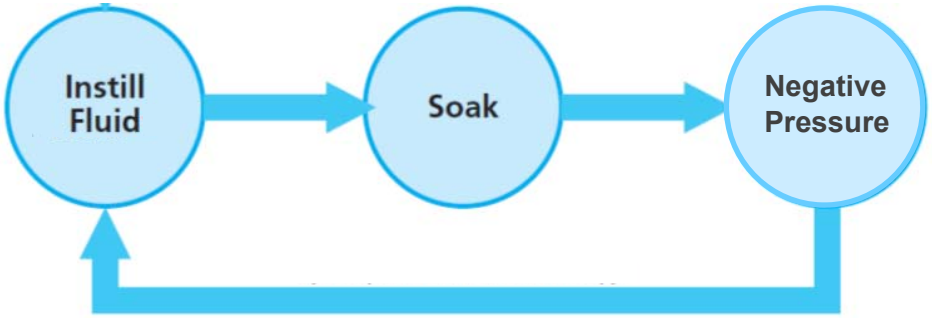


NPWT with Instillation Therapy

NPWT with instillation therapy combines the benefits of vacuum therapy with automated solution instillation and removal which can help:

- **Cleanse** the wound with instillation of topical wound cleansers in a consistent, controlled manner
- **Treat** the wound with the instillation of appropriate topical antimicrobial and antiseptic solutions and the removal o infectious material
- **Heal** the wound and prepare for primary or secondary closure

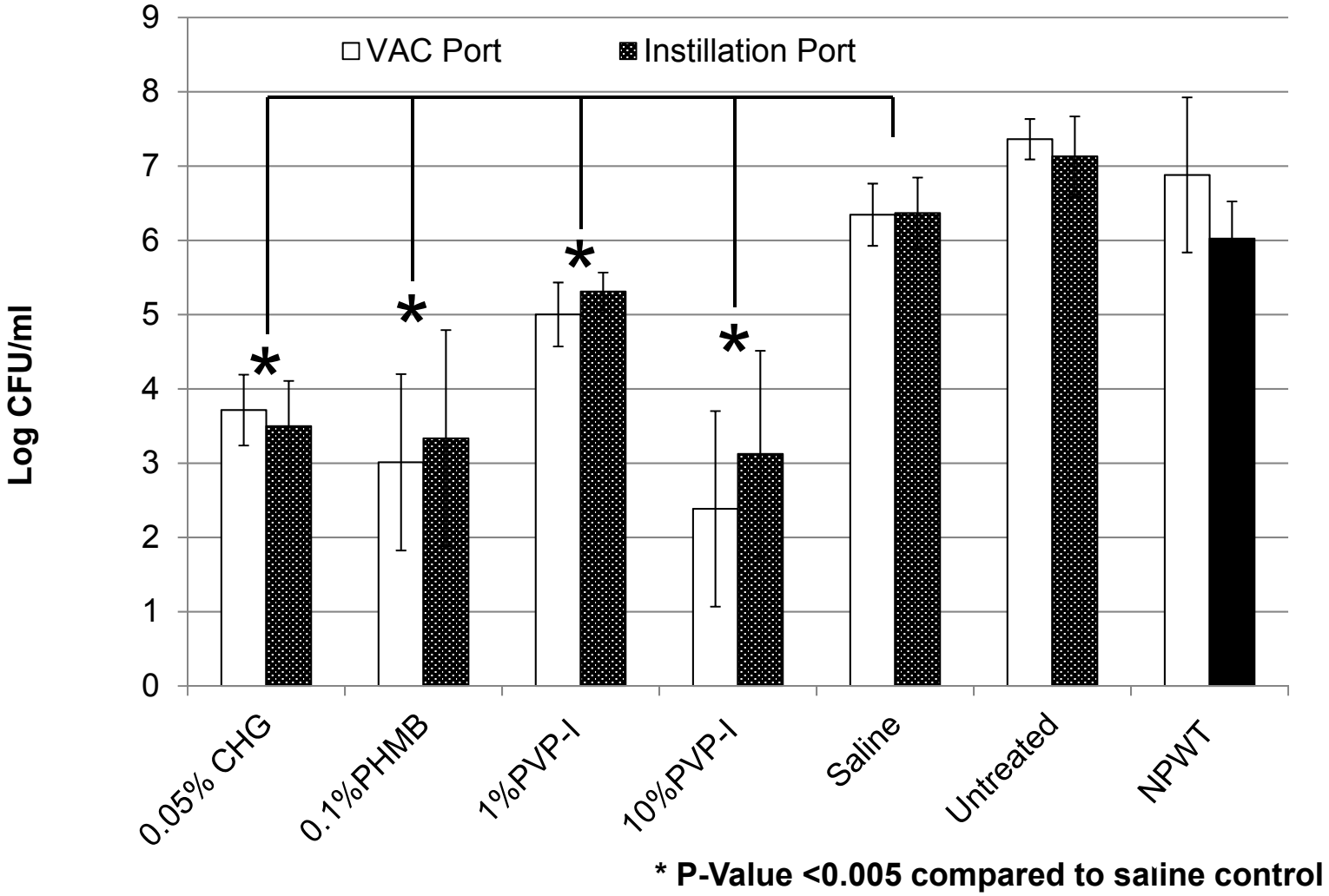




cycle repeats for duration of therapy

Effects of 6-Cycles of NPWT-Instill Treatments Over 24 Hours on *P. aeruginosa* Biofilm Grown on Pig Skin Explants

k2



P.L. Phillips, Q. Yang, G.S. Schultz. Effect of Negative Pressure Wound Therapy with Periodic Instillation Using Antimicrobial Solutions on Pseudomonas aeruginosa Biofilm on Porcine Skin Explants. **International Wound J**, 10 (suppl. 1) 48-55, 2013.

Slide 46

k2 On far right bars, change "VeraFlow" to " NPWT"

kci, 12/4/2012

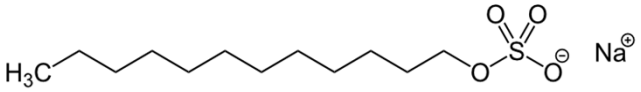
Types of Surfactants

SURF- **-ACT-** **-ANT**

Surface **Acting** **Agent**

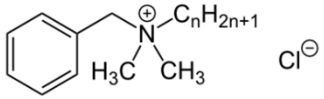
A compound that lowers the surface tension between two liquids or between a liquid and a solid.

- Anionic



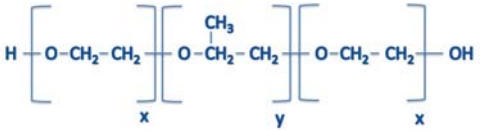
  – soap, detergent, sodium dodecyl sulfate (SDS)

- Cationic



  – Quaternary ammonium compound (BAK)

- Non-ionic

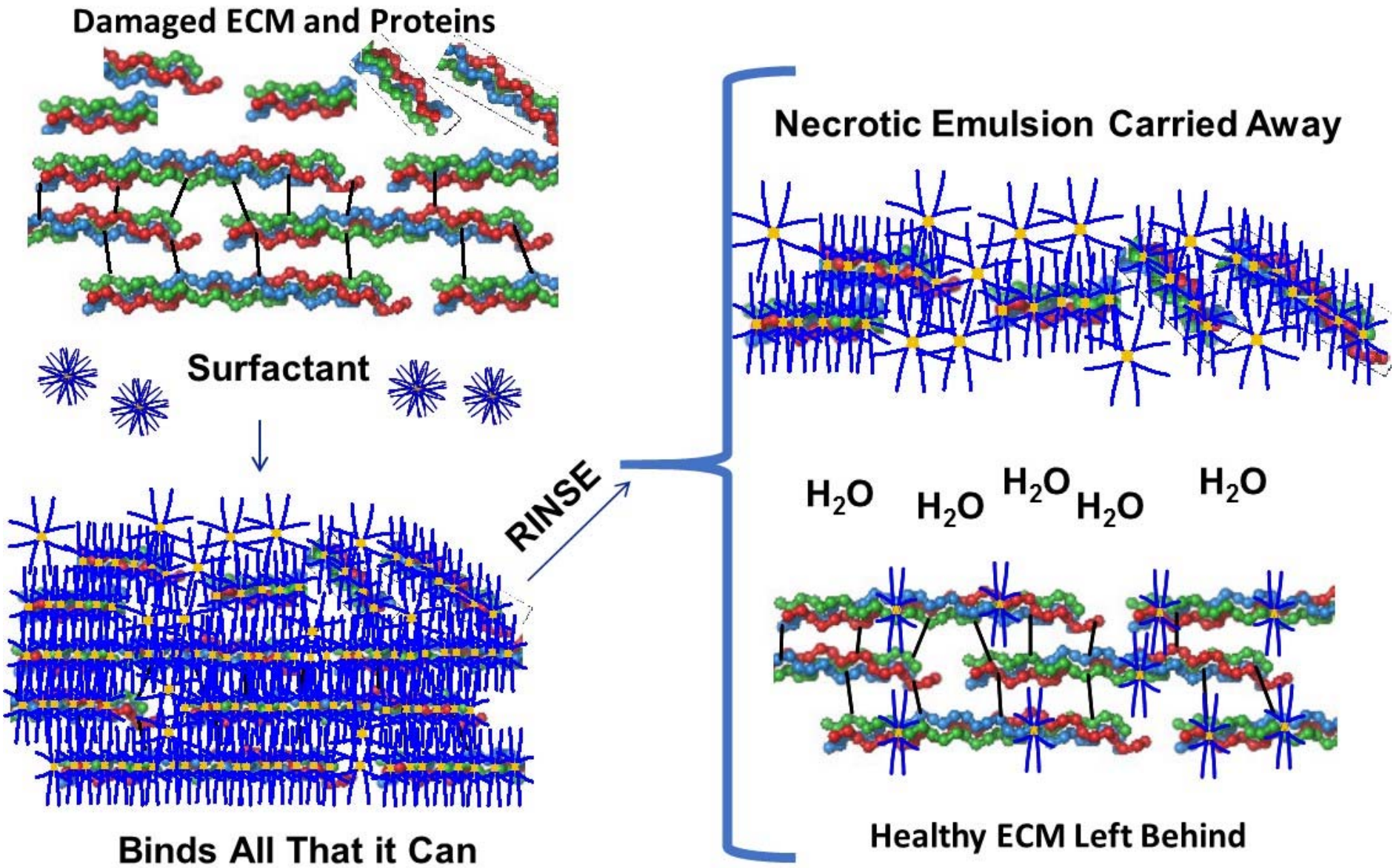


  – Triton-X, Tween 80, P-188

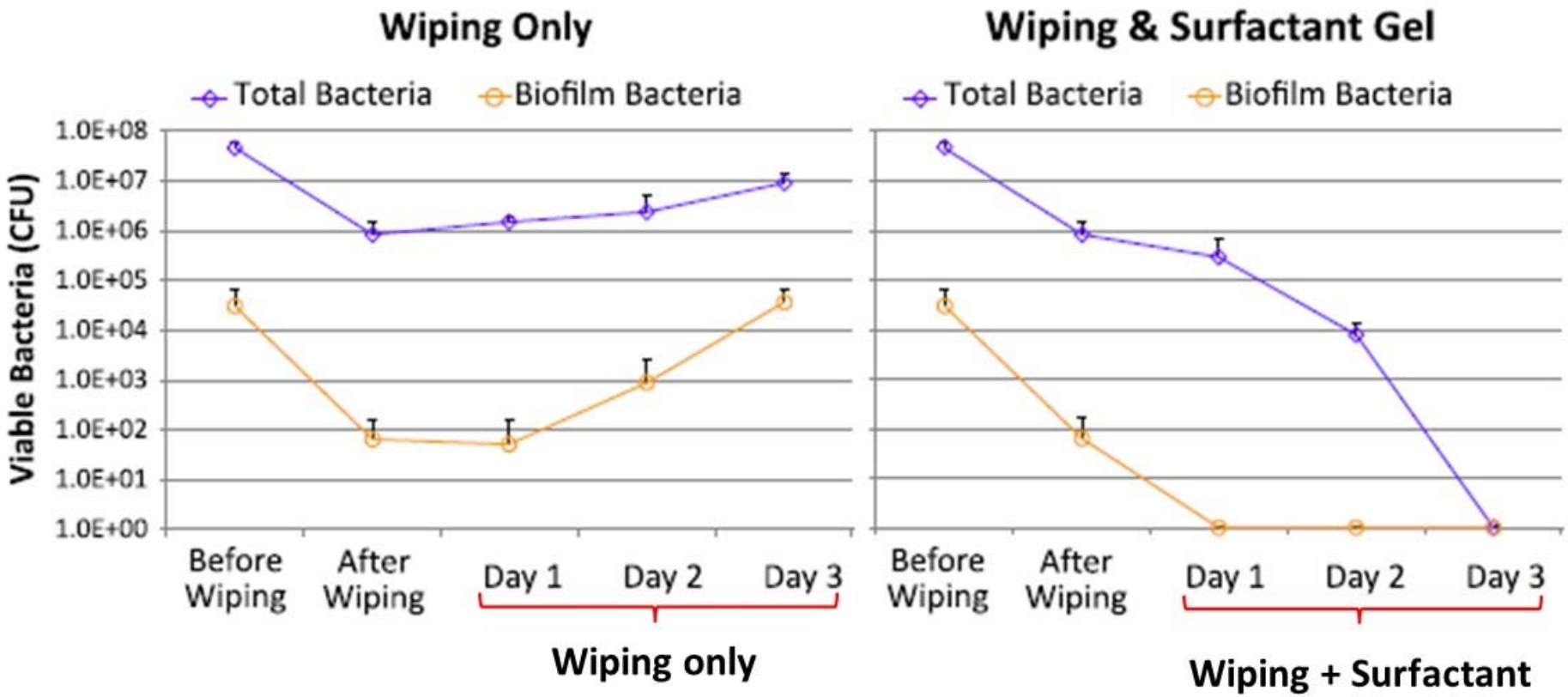
Cytotoxic

Concentrated Surfactant Gel
CSG

Non-Ionic Concentrated Surfactant Gel Removes Degraded ECM

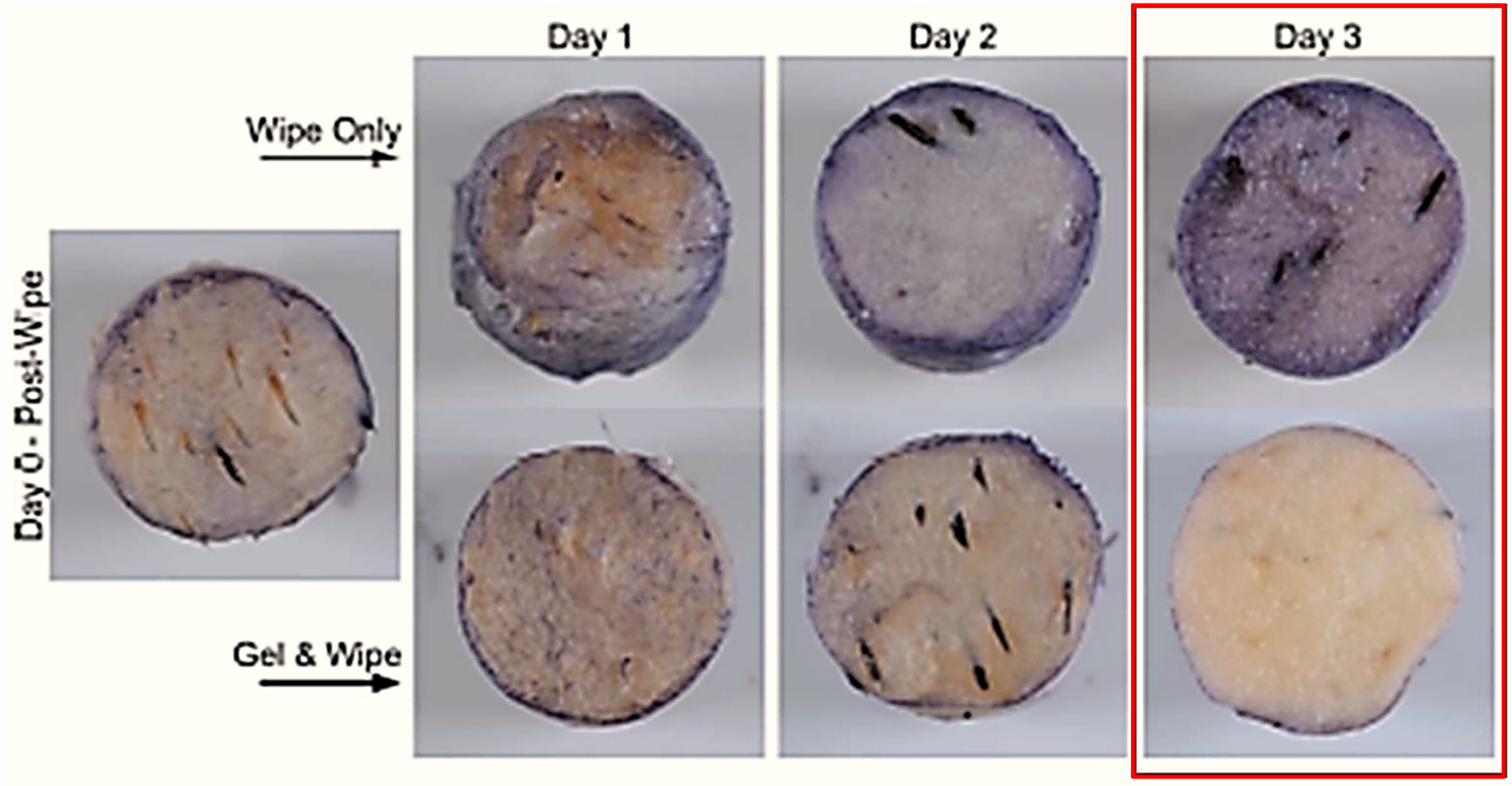


Concentrated Surfactant Gel Eliminated Bacterial Biofilms Grown on Porcine Skin Explants After Daily Treatments for 3 Days



Yang Q, Larose C, Porta AD, Della Porta AC, Schultz GS, Gibson DJ. A surfactant-based wound dressing can reduce bacterial biofilms in a porcine skin explant model. Int Wound J, 2016

Effect of Daily Wiping + Concentrated Surfactant Gel on PA Bacteria Biofilms



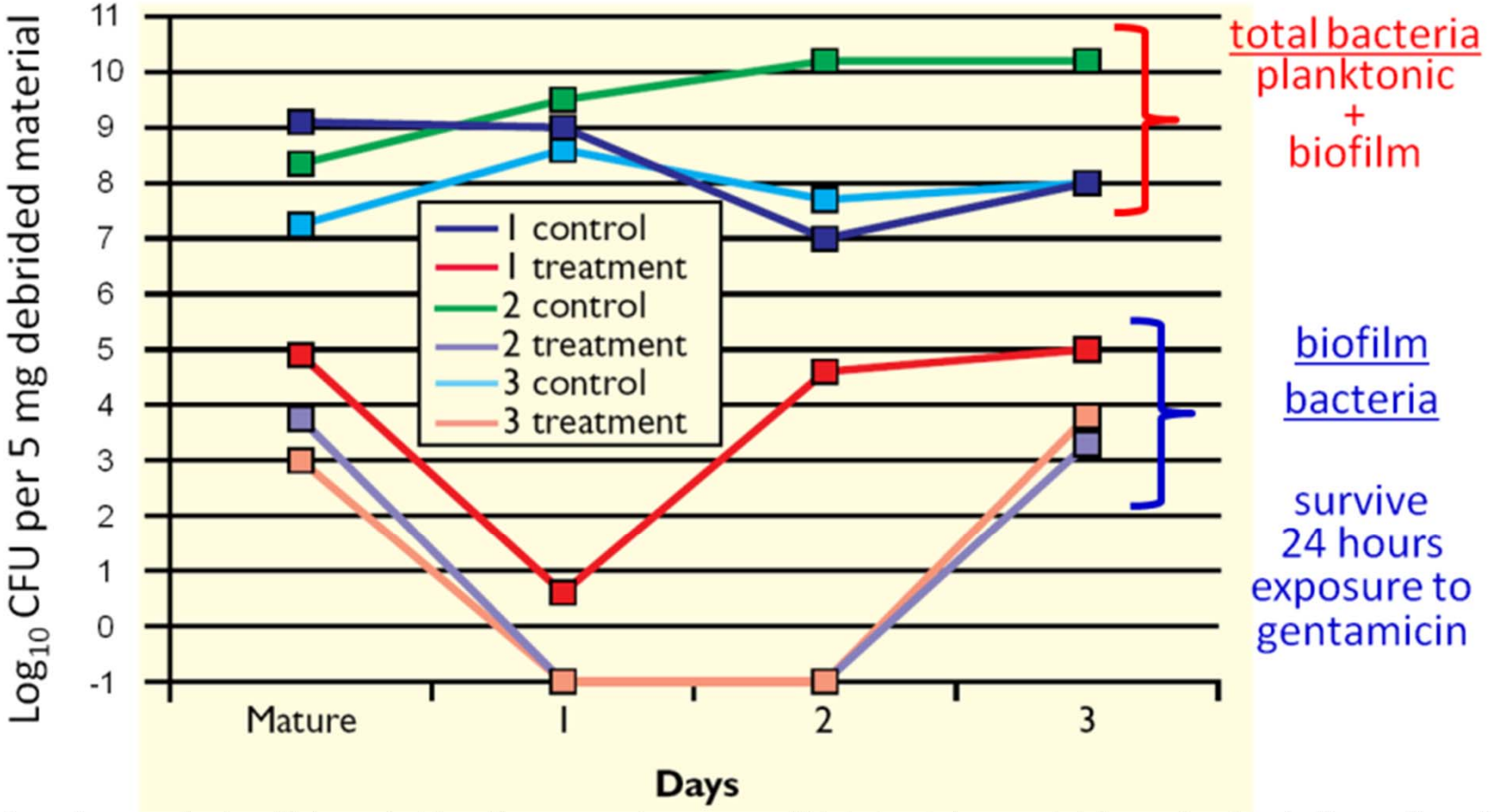
Q. Yang, D. Porta, G. Schultz, D. Gibson. The Mechanism of Action of an Anti-Biofilm Surfactant-Based Dressing, submitted

Question: How quickly can planktonic bacteria form protective biofilms in wounds after sharp debridement?

Which answer is true?

1. 7 days
2. 5 days
3. 3 days
4. 1 day

Biofilm Maturity Studies Indicate Sharp Debridement Opens a Time-Dependent Therapeutic Window

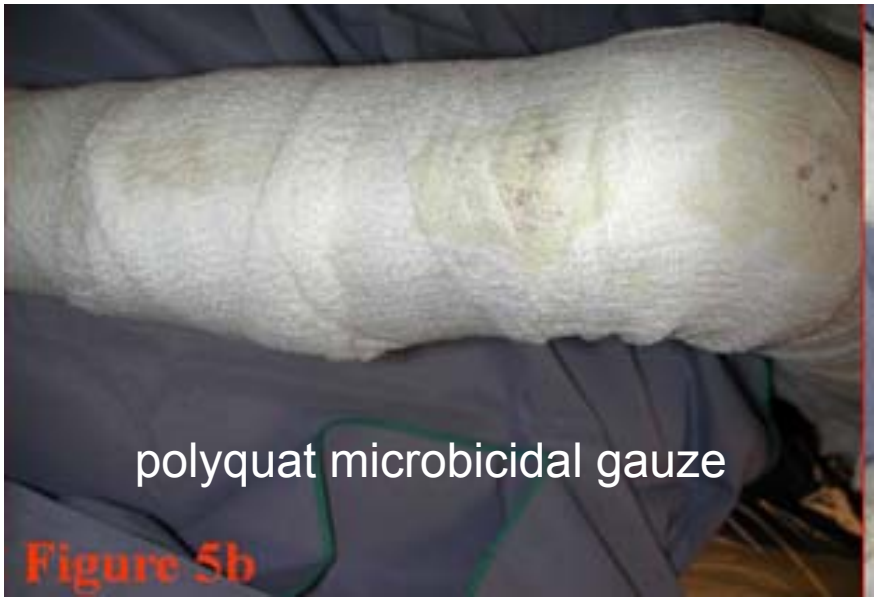


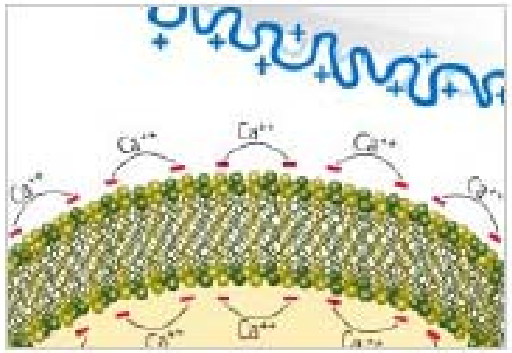
Biopsies from three patients with large (>10 cm^2) venous ulcer were split into two tubes containing saline (control) or saline with 200 ug/ml gentamicin (treatment), and after 24 hours of incubation, samples were disperse biofilm into microcolonies and CFU/5 gm were measured. Total levels of bacteria at 0, 1, 2, and 3 days after initial debridement remained consistently high. However, in two of the three wounds, all bacterial were "planktonic" at 1 and 2 days after debridement (full kill by exposure to gentamicin), but by 3 days post-debridement, all three wounds had re-established substantial levels of biofilm bacteria (10^3 – 10^5 CFU/5 gm).

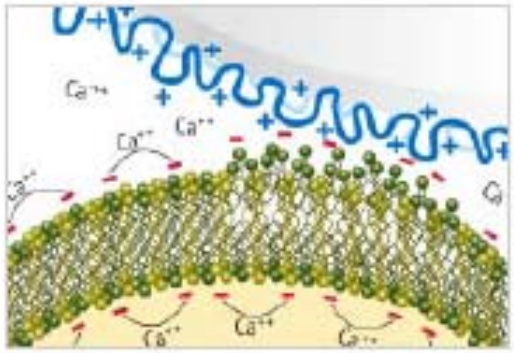
R.D. Wolcott, K.P. Rumbaugh, G. James, G. Schultz, P. Phillips, Q. Yang, C Watters, P.S. Stewart, S.E. Dowd, J Wound Care 19: 320-328, 2010.

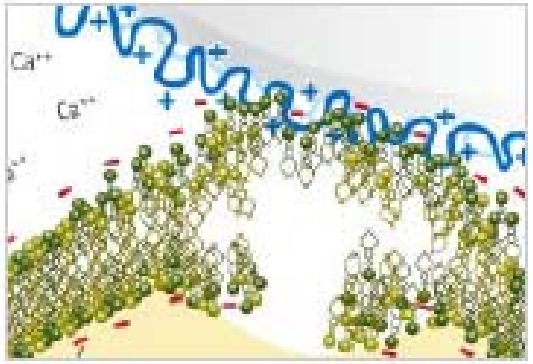
Reformation of Biofilms – Or Bad Terminators

Anti-Microbial Non-Leaching Gauze



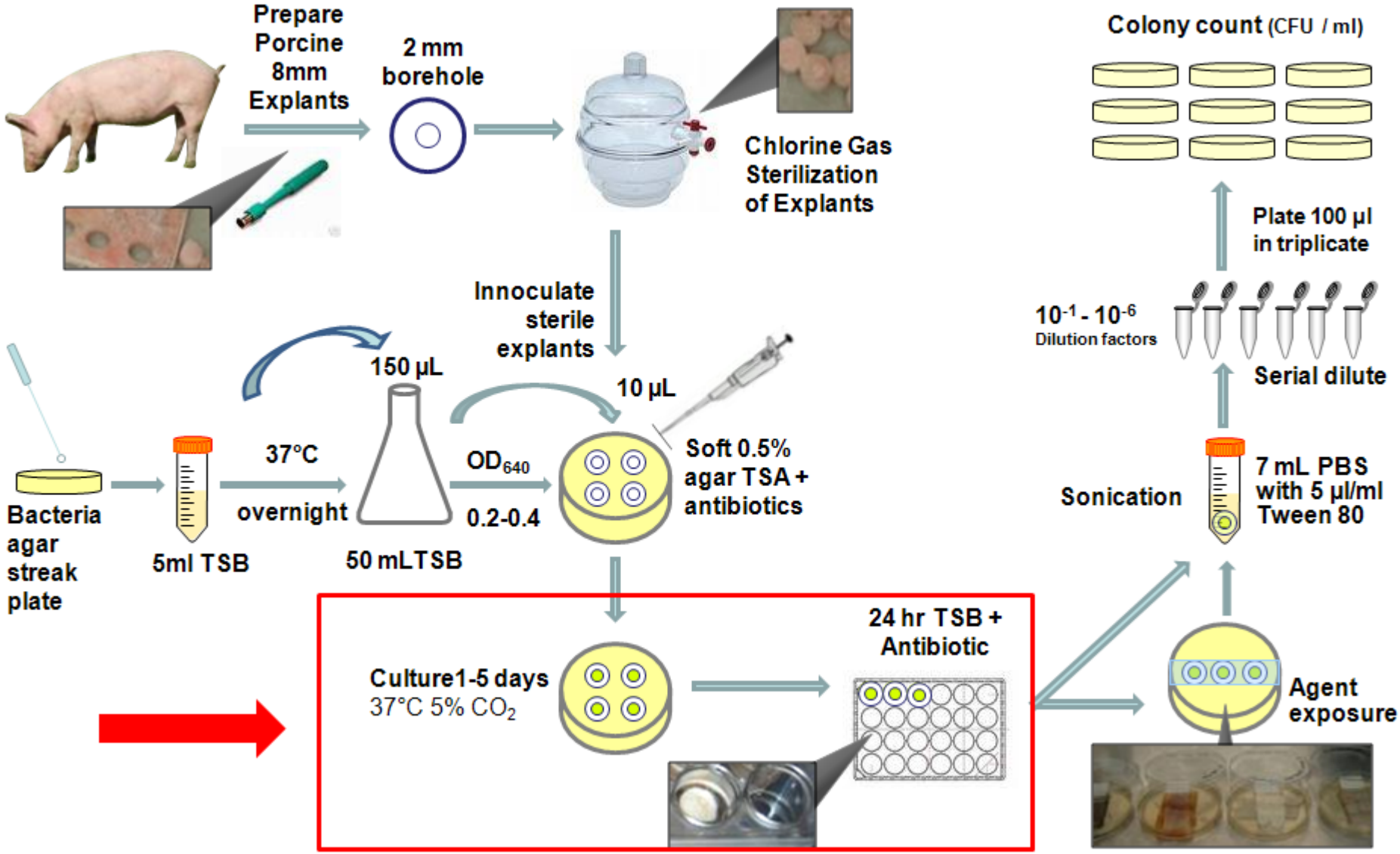






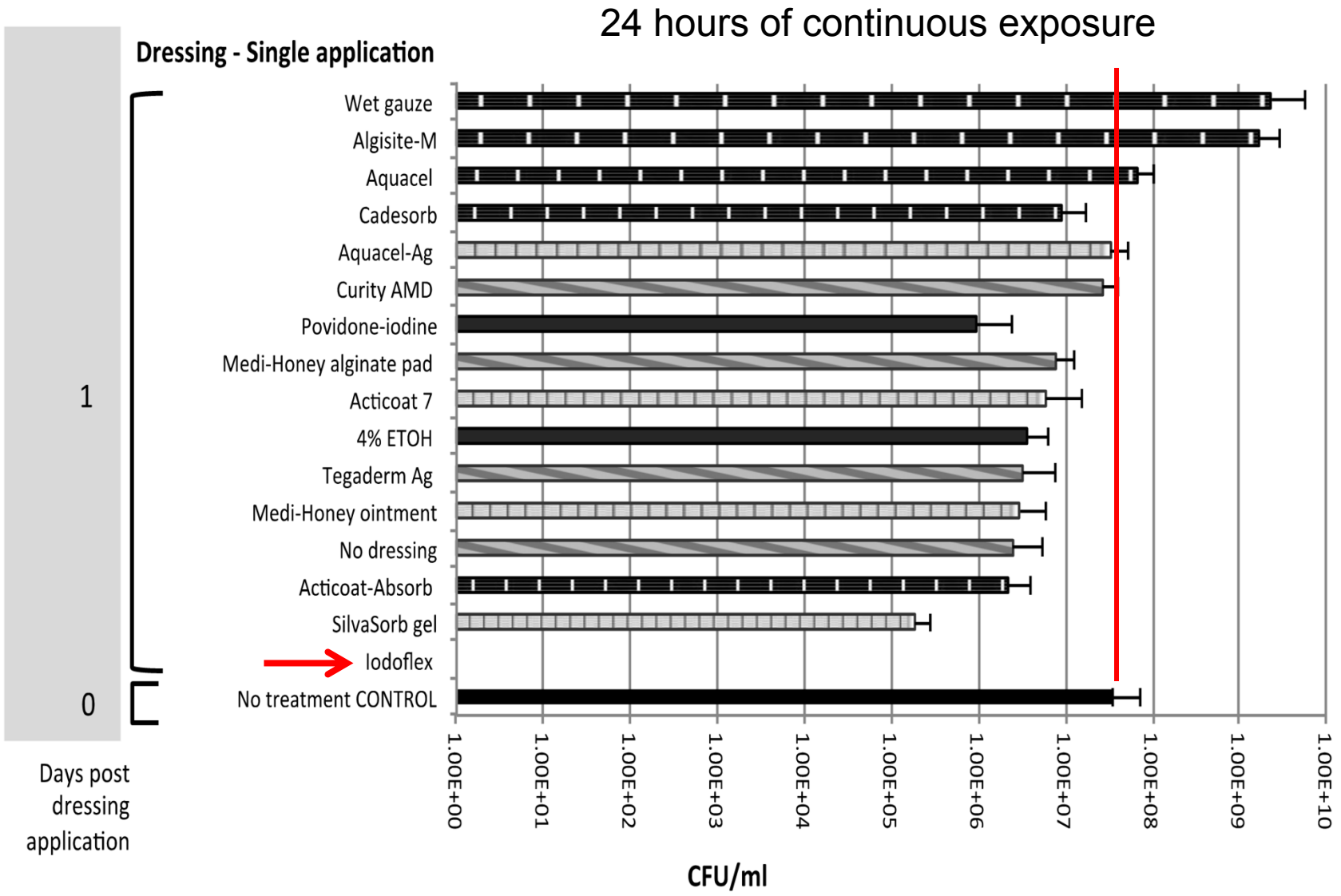
Rob Nappo and Lisa Young, UF Burn Unit

Can Most **Dressings** Disrupt & Kill Mature Biofilms?



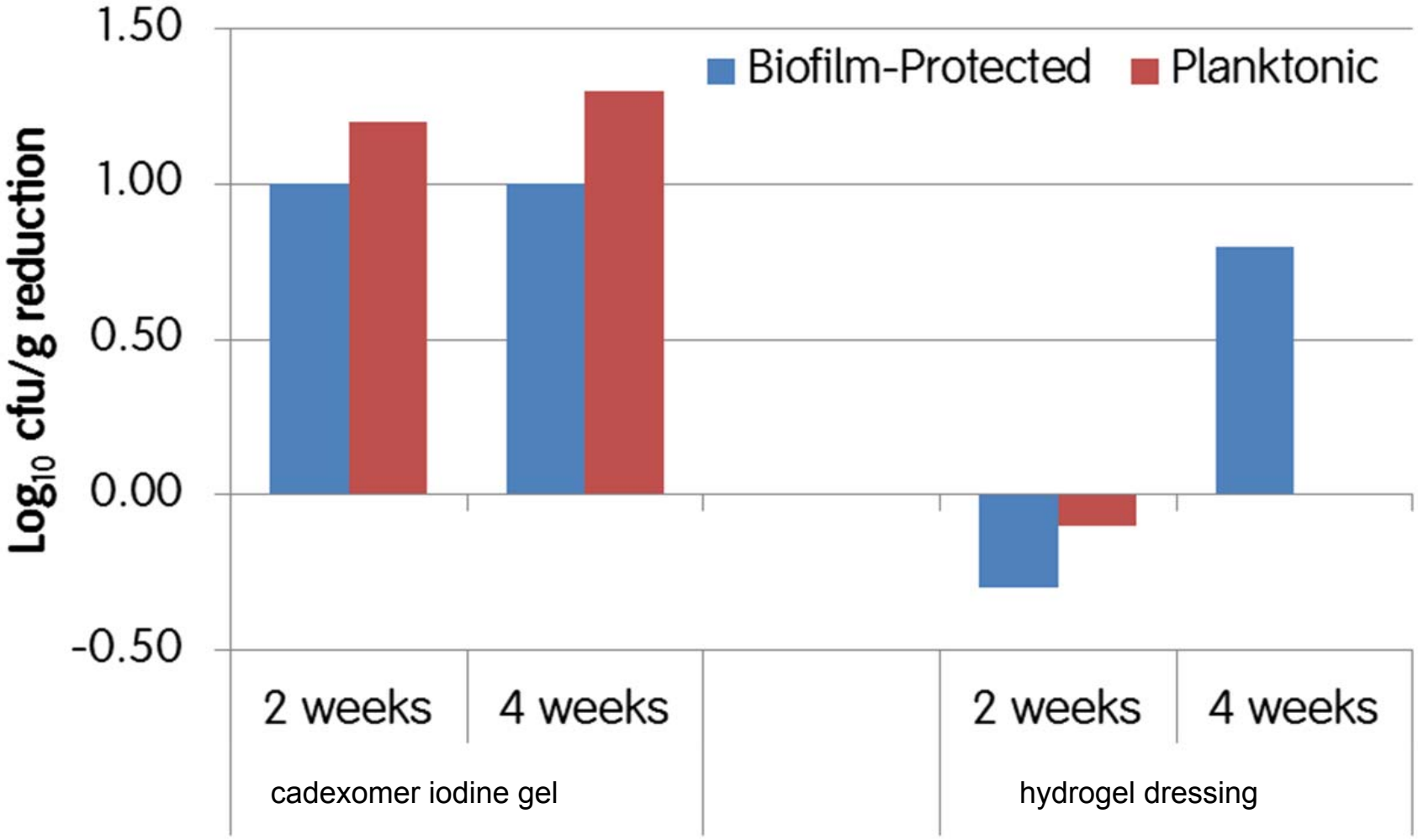
PL Phillips, Q Yang, E Sampson, GS Schultz. Effects of antimicrobial agents on an in vitro biofilm model of skin wounds. Adv Wound Care 2010; 1: 299-304.

Effects of Antimicrobial Agents on Mature Biofilms on Pig Skin Explants



P.L. Phillips, Q. Yang, E. Sampson, G. Schultz. Effects of Antimicrobial Agents on an In Vitro Biofilm Model of Skin Wounds, **Advances Wound Care**, 1: 299-304, 2010.

Cadexomer Iodine Gel Reduced Planktonic and Biofilm Bacteria in DFUs Compared to Hydrogel Dressing



Lantis J, Schultz G, et al. World Union Wound Healing Societies, Florence, Italy 2016

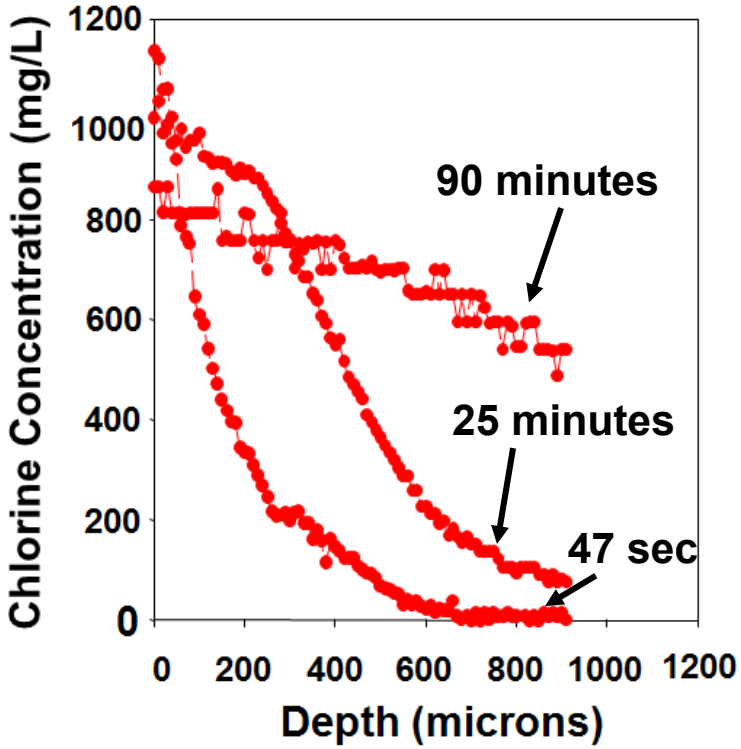
Question: Why are bacteria in biofilms hard to kill?

Answer:

- **Exopolymeric material (EPM) of the biofilm**
  - Hydrophobic proteins of some EPM (*B. subtilis*) reduces penetration
  - EPM materials chemically react (neutralize) microbicides
  - Negative charges of polysaccharides and DNA bind cationic molecules like Ag^+, antibiotics, $PHMB^+$
- **Persister bacteria have low metabolic activity**
  - Antibiotics only kill metabolically active bacteria
- **Oxygen diffusion to center of biofilm is limited**
  - Promotes growth of anaerobic bacteria
- **Synergism between different bacteria**
  - MRSA secrete resistance proteins
  - Pseudomonas secrete catalase that destroys H_2O_2

Hypochlorous Acid Very Slowly Penetrates Biofilm Matrix – Reaction-Diffusion Problem

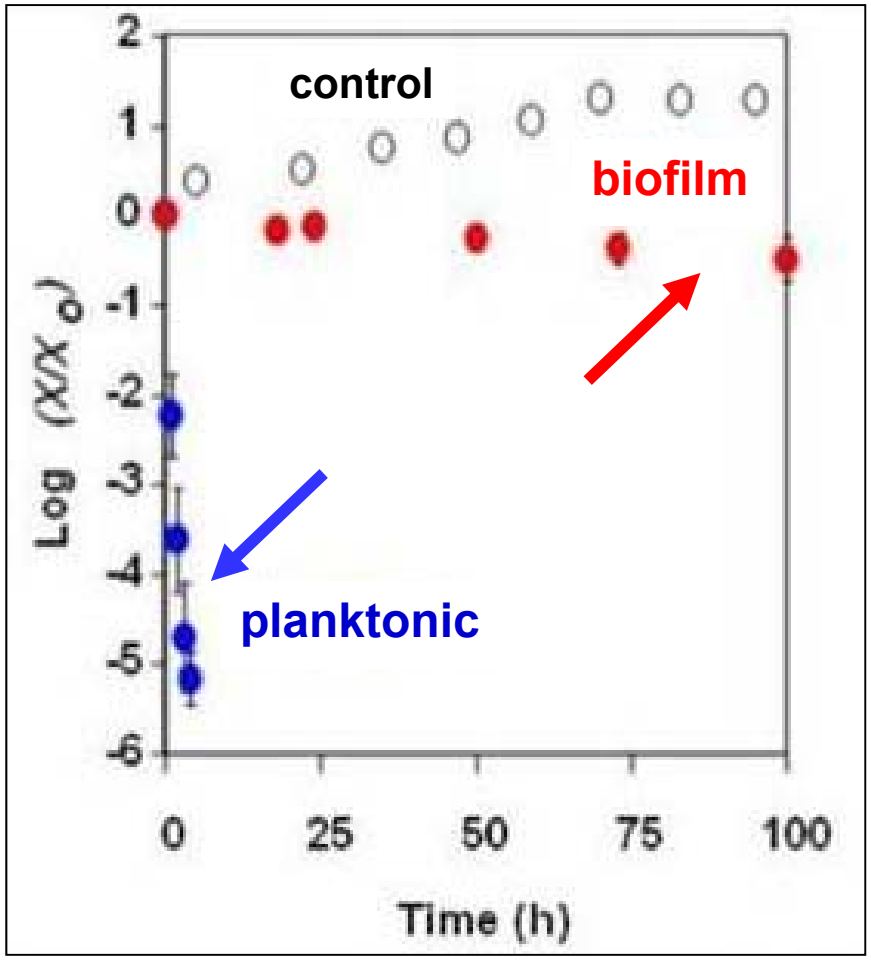
After 60 minutes of exposure to dilute bleach (Dakin's solution), many bacteria in this biofilm were dying (**green cells**), but many cells in the interior of the biofilm were still alive (**orange cells**) Costerton, Sci Am, 2001

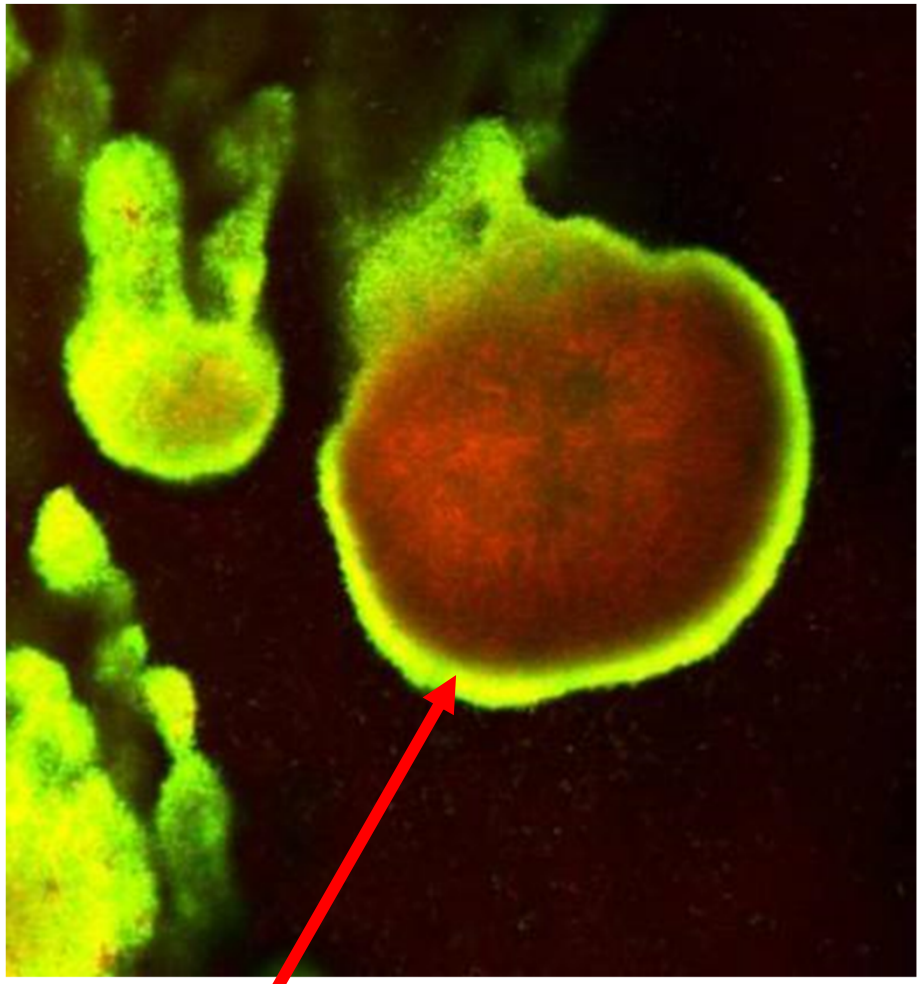


Reaction-Diffusion Problem

Hypochlorous acid rapidly reacts with molecules that form the biofilm exopolymeric matrix, which limits its diffusion into the center of the biofilm colony. Stewart, P.S. et al. Biofilm penetration and disinfection efficacy of alkaline hypochlorite and chlorosulfamates. J Applied Microbiol 91:525-532, 2001.

Tobramycin Kills Metabolically Active Planktonic Pseudomonas Bacteria But Is Ineffective Against Metabolically Dormant Bacteria in Biofilm





P. Stewart, Controlling Biofilms, Chapter 7, in The Biofilms Hypertextook, published by Montana State University, A.B. Cuningham, J.E. Lennox & R.J. Ross, eds, 2010.

-- Only fluorescent bacteria are metabolically active
-- Only located in outer layers of the biofilm matrix
-- Antibiotics only kill metabolically active bacteria

International Consensus Guidelines on Identifying and Treating Biofilms

10 global experts in biofilms

- 5 scientists
- 5 wound care clinicians

One goal…. Bridging the gap between scientific understanding and clinical practice addressing core issues in wound biofilm understanding, diagnosis and treatment variables

woundbiofilm.expert
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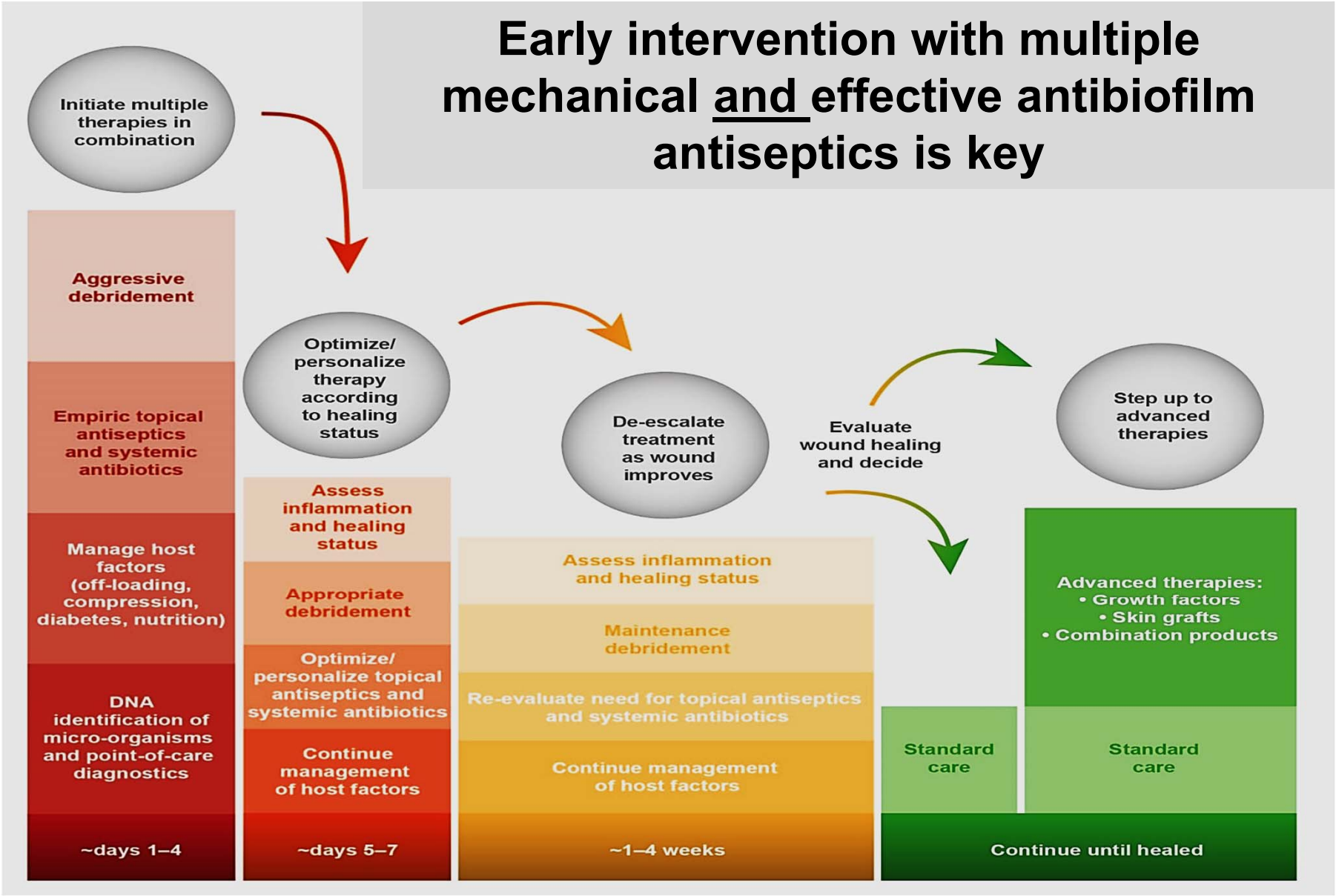
Dr Garth James
Dr Andrew McBain
Prof Masahiro Tachi

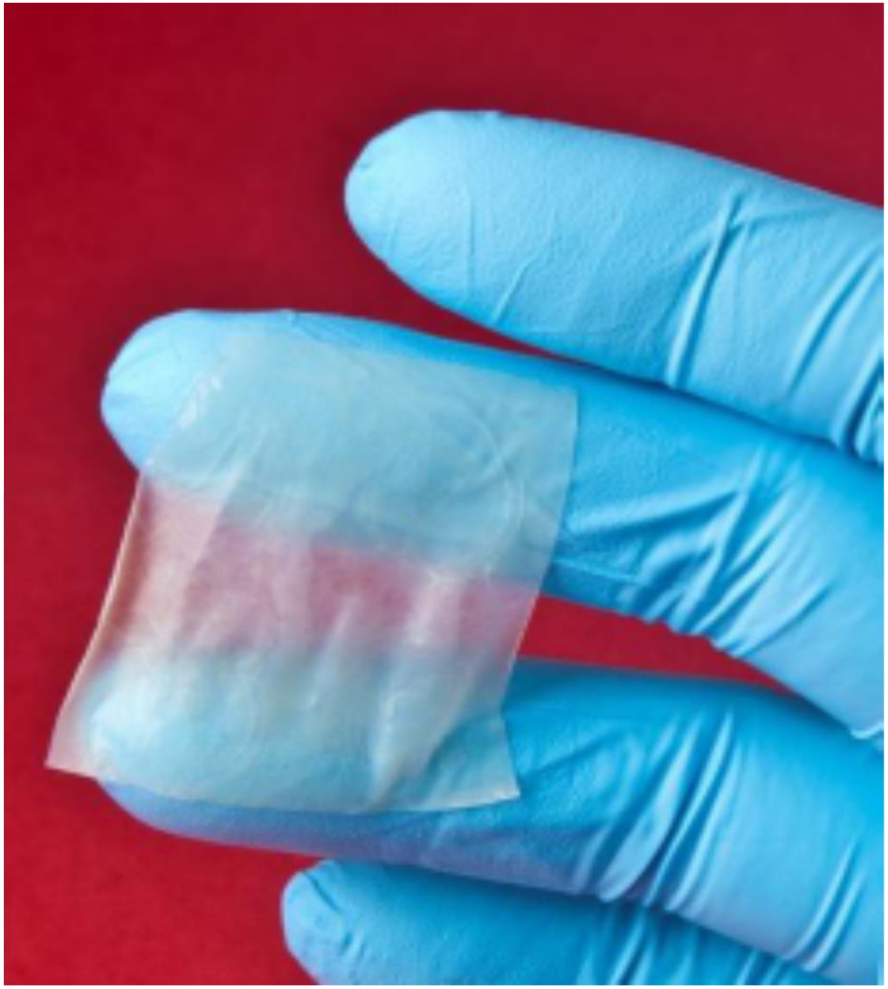
Step-Down Then Step-Up Treatment Strategy

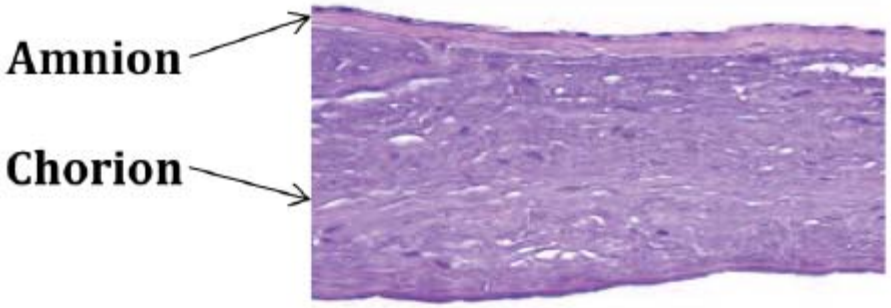


Schultz, G., Bjarnsholt, T., James, G.A., Leaper, D.L., McBain, A. J., Malone, M., Stoodley, P., Swanson, T., Tachi, M., Wolcott, R.D.; for the Global Wound Biofilm Expert Panel. *Wound Repair and Regeneration*: 25(5): 744-757, 2017

Advanced Wound Treatments

Human Amniotic Membrane

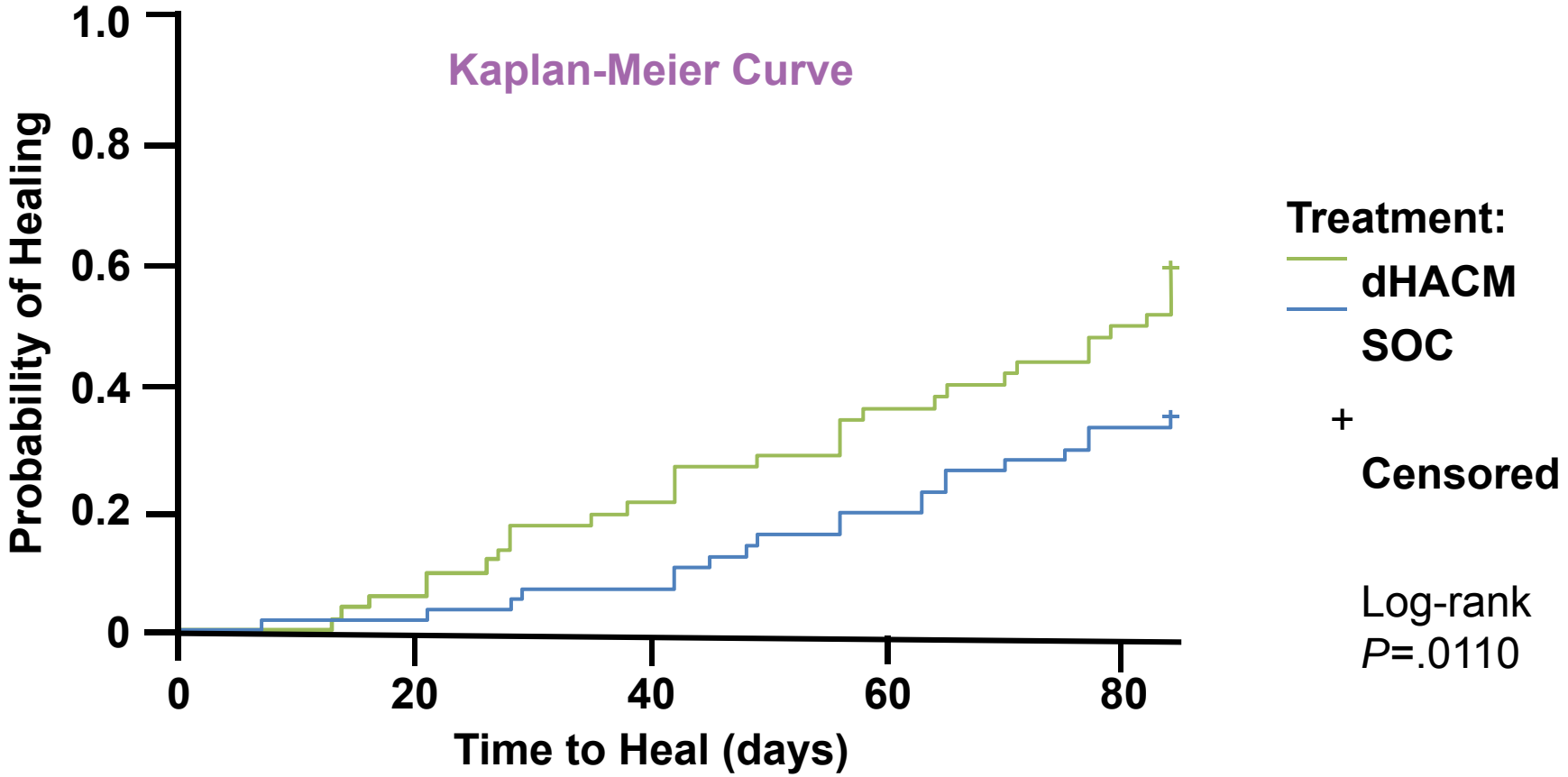




Contains

Type IV basement membrane collagen
Type I collagen, laminin
Tissue inhibitors of proteases (TIMPs)
Biologically active growth factors, cytokines (TGFb1, VEGF, FGF, PDGF)
Total of 285 regulatory proteins

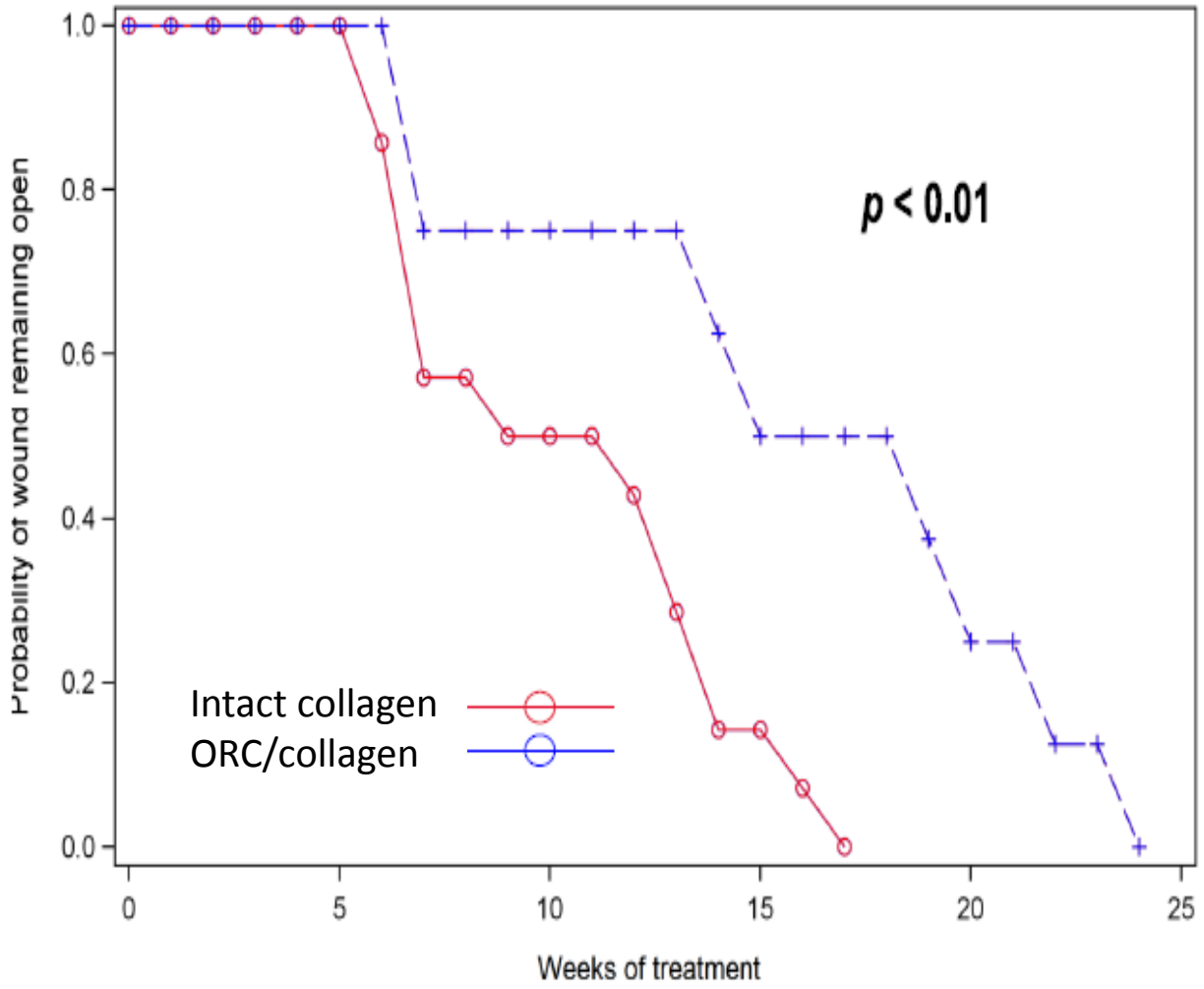
Dehydrated Human Amnion/Chorion Allograft Improved Healing of Venous Leg Ulcers Compared to Standard of Care



Kaplan-Meier analysis of 109 subjects enrolled in a randomized, controlled, multicenter, clinical trial showed a significantly improved time to healing using a dehydrated human amnion/chorion membrane (dHACM) dressing (log-rank P=.0110). Cox regression analysis showed that subjects treated with the allograft had a significantly higher probability of complete healing within 12 weeks (HR: 2·26, 95% confidence interval 1.25-4.10, P=.01) versus without dHACM.

Bianchi C, et al. *Int Wound J*. 2018;15(1):114-122.

VLU Wounds Close Faster With Non-Denatured Ovine Collagen Dressing than Denatured ORC-Gelatin Dressing



Bohn G. A New Ovine Collagen Dressing Demonstrates Cost Effectiveness in the Treatment of Venous Leg Ulcers SAWC Spring 2013 Denver CO

biofilms made easy

Wounds

Introduction

This article describes what biofilms are and the important roles they appear to play in disrupting wound healing. In addition, it discusses potential interventions aimed at removing/reducing biofilms and preventing their reformation in wounds.

Authors: Phillips PL, Wolcott RD, Fletcher J, Schultz GS
Full author details can be found on page 5.

What are biofilms?

Biofilms are complex microbial communities containing bacteria and fungi. The microorganisms synthesise and secrete a protective matrix that attaches the biofilm firmly to a living or non-living surface[1].

Biofilms are dynamic heterogeneous communities that are continuously changing[2]. They may consist of a single bacterial or fungal species, or more commonly, may be polymicrobial, ie contain multiple diverse species[3,4]. **At the most basic level a biofilm can be described as bacteria embedded in a thick, slimy barrier of sugars and proteins.** The biofilm barrier protects the microorganisms from external threats.

How are biofilms relevant to wounds?

Biofilms have long been known to form on surfaces of medical devices, such as urinary catheters, endotracheal and tympanostomy tubes, orthopaedic and breast implants, contact lenses, intrauterine devices (IUDs) and sutures[5,6]. They are a major contributor to diseases that are characterised by an underlying bacterial infection and chronic inflammation, eg periodontal disease, cystic fibrosis, chronic acne and osteomyelitis[7,8].

Biofilms are also found in wounds and are suspected to delay healing in some. Electron microscopy of biopsies from chronic wounds found that 60% of the specimens contained biofilm structures in comparison with only 6% of biopsies from acute wounds[9]. Since biofilms are reported to be a major factor contributing to multiple chronic inflammatory diseases, it is likely that almost all chronic wounds have biofilm communities on at least part of the wound bed.

How do biofilms form?

Stage one: reversible surface attachment

Microorganisms are commonly perceived to be free-floating and solitary (ie planktonic). However, under natural conditions most microorganisms tend to attach to surfaces and eventually form biofilms[1,9] (Figure 1). The initial attachment is reversible.

Stage two: permanent surface attachment

As the bacteria multiply, they become more firmly attached (sessile) and differentiate, changing gene expression patterns in ways that promote survival[8,9]. This is usually the result of a type of bacterial communication known as quorum sensing[10].

Stage three: slimy protective matrix/biofilm

Once firmly attached, the bacteria begin to secrete a surrounding matrix known as extracellular polymeric substance (EPS)[11]. This is a protective matrix or 'slime'. Small bacterial colonies then form an initial biofilm[12].

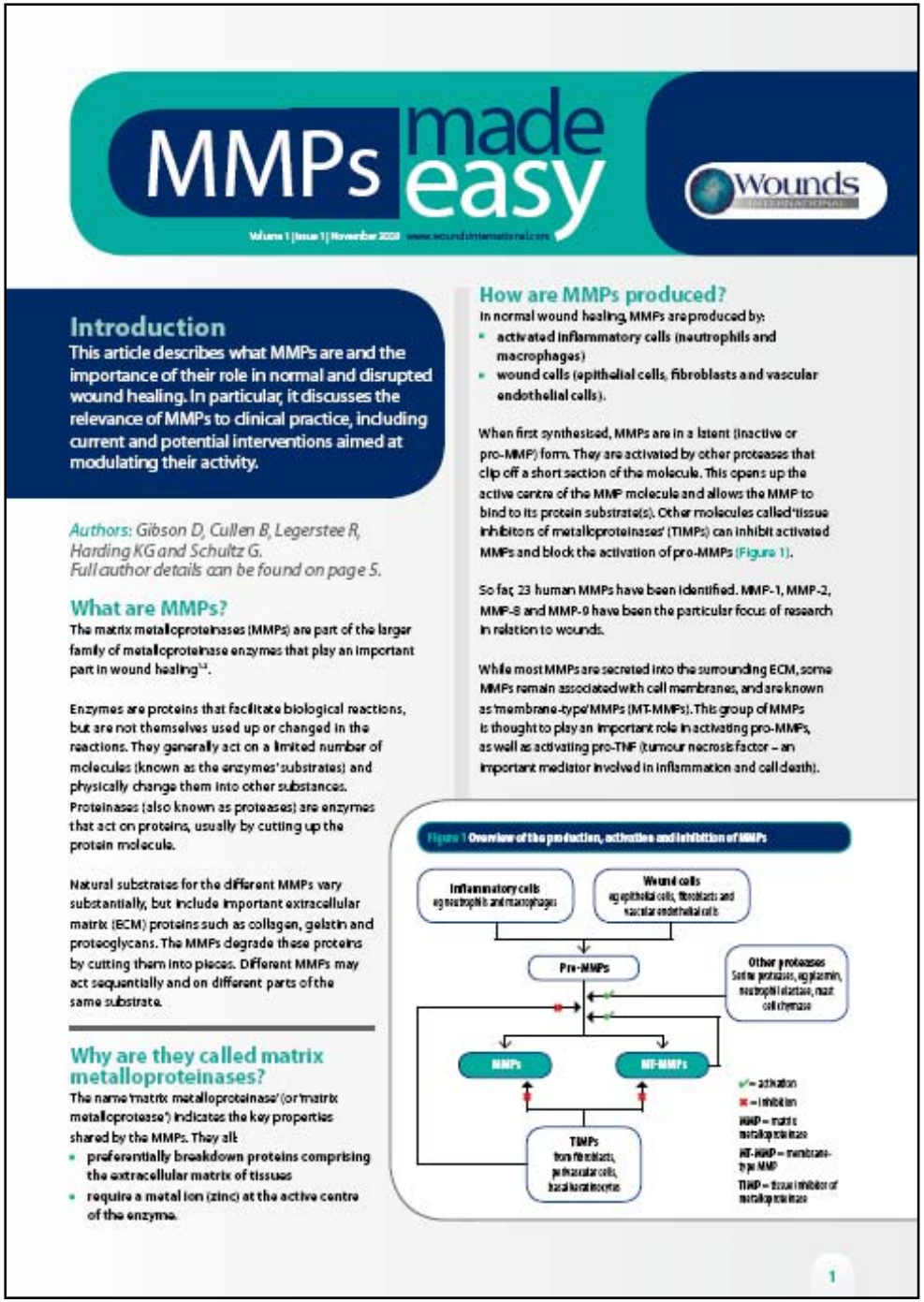
The exact composition of EPS varies according to the micro-organisms present, but generally consists of polysaccharides, proteins, glycolipids and bacterial DNA[10,11]. Bacterial DNA released by living or dead bacteria is thought to provide an important structural component for biofilm EPS matrix[12]. Various secreted proteins and enzymes help the biofilm to become firmly attached the wound bed[9].

Fully mature biofilms continuously shed planktonic bacteria, microcolonies and fragments of biofilm, which can disperse and attach to other parts of the wound bed or to other wounds, forming new biofilm colonies[2,4].

Figure 1 Schematic representation of polymicrobial biofilm formation (adapted from [9])

Free download from Wounds International

P. Phillips, R. Wolcott, J. Fletcher, G. Schultz. Biofilms Made Easy. **Wounds International**, 1(3): 1-6, 2010.



MMPs made easy

Wounds International

Volume 1 | Issue 1 | November 2010 www.woundsinternational.com

Introduction

This article describes what MMPs are and the importance of their role in normal and disrupted wound healing. In particular, it discusses the relevance of MMPs to clinical practice, including current and potential interventions aimed at modulating their activity.

Authors: Gibson D, Cullen B, Legerstee R, Harding KG and Schultz G.
Full author details can be found on page 5.

What are MMPs?

The matrix metalloproteinases (MMPs) are part of the larger family of metalloproteinase enzymes that play an important part in wound healing[1,2].

Enzymes are proteins that facilitate biological reactions, but are not themselves used up or changed in the reactions. They generally act on a limited number of molecules (known as the enzymes' substrates) and physically change them into other substances. Proteinases (also known as proteases) are enzymes that act on proteins, usually by cutting up the protein molecule.

Natural substrates for the different MMPs vary substantially, but include important extracellular matrix (ECM) proteins such as collagen, gelatin and proteoglycans. The MMPs degrade these proteins by cutting them into pieces. Different MMPs may act sequentially and on different parts of the same substrate.

Why are they called matrix metalloproteinases?

The name 'matrix metalloproteinase' (or 'matrix metalloprotease') indicates the key properties shared by the MMPs. They all:

- preferentially breakdown proteins comprising the extracellular matrix of tissues
- require a metal ion (zinc) at the active centre of the enzyme.

How are MMPs produced?

In normal wound healing, MMPs are produced by:

- activated inflammatory cells (neutrophils and macrophages)
- wound cells (epithelial cells, fibroblasts and vascular endothelial cells).

When first synthesised, MMPs are in a latent (inactive or pro-MMP) form. They are activated by other proteases that clip off a short section of the molecule. This opens up the active centre of the MMP molecule and allows the MMP to bind to its protein substrate(s). Other molecules called 'tissue inhibitors of metalloproteinases' (TIMPs) can inhibit activated MMPs and block the activation of pro-MMPs (Figure 1).

So far, 23 human MMPs have been identified. MMP-1, MMP-2, MMP-8 and MMP-9 have been the particular focus of research in relation to wounds.

While most MMPs are secreted into the surrounding ECM, some MMPs remain associated with cell membranes, and are known as 'membrane-type' MMPs (MT-MMPs). This group of MMPs is thought to play an important role in activating pro-MMPs, as well as activating pro-TNF (tumour necrosis factor – an important mediator involved in inflammation and cell death).

Figure 1 Overview of the production, activation and inhibition of MMPs

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D. Gibson, B. Cullen, R. Legerstee, K.G. Harding, G. Schultz. MMPs Made Easy. **Wounds International**, 1(1): 1-6, 2010.

KEY POINTS

1. **Biofilms are communities of bacteria encased in a matrix of polysaccharides, protein and DNA that provides high levels of tolerance to antibodies, antibiotics and antiseptics.**

2. **Biofilms are present in a high percentage of chronic wounds and they impair healing by stimulating chronic inflammation, leading to elevated levels of proteases and ROS that degrade proteins that are essential for healing.**

3. **Debridement is a critical first step in Biofilm-Based Wound Care; several techniques reduced levels of biofilm bacterial, but biofilms can reform quickly (~3 days) so combine it with antimicrobial treatment**

4. **Step-Down-Step-Up (SD-SU) therapy is based on starting with the therapies that most effectively reduce biofilms, inflammation, and proteases then (Step-Down) to general antimicrobials that control planktonic bacteria then (Step-UP) to advanced therapies that enhance repair of the wound bed (growth factors, collagen dressings, biological membranes, and NPWT).**