Wound Infection Day

Consensus and Controversies in Contamination and Bioburden

The Battle against Slough and Microbial Burden

Supported by an educational grant from

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Faculty

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Faculty

- **Sujay Dutta, MD,** has disclosed no relevant financial relationship with any ineligible company (commercial interest).
- **Lisa Gould, MD, PhD, FACS**
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- **Michael Mansour, MD, PhD**
  Advisory Board: Roche, Vericel; Consultant: C-POLAR; Research: Genentech, ThermoFisher, CytoVale
- **Dot Weir, RN, CWON, CWS**
  Consultant, Speakers Bureau: Convatec; LifeNet Health; Lynch Regenerative Medicine; Mölnlycke Health Care; Organogenesis Inc; Smith+Nephew; Solventum, Medical Surgical Business; Urgo Medical North America

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  - Applicable CME staff have no relationships to disclose relating to the subject matter of this activity
  - This activity has been independently reviewed for balance

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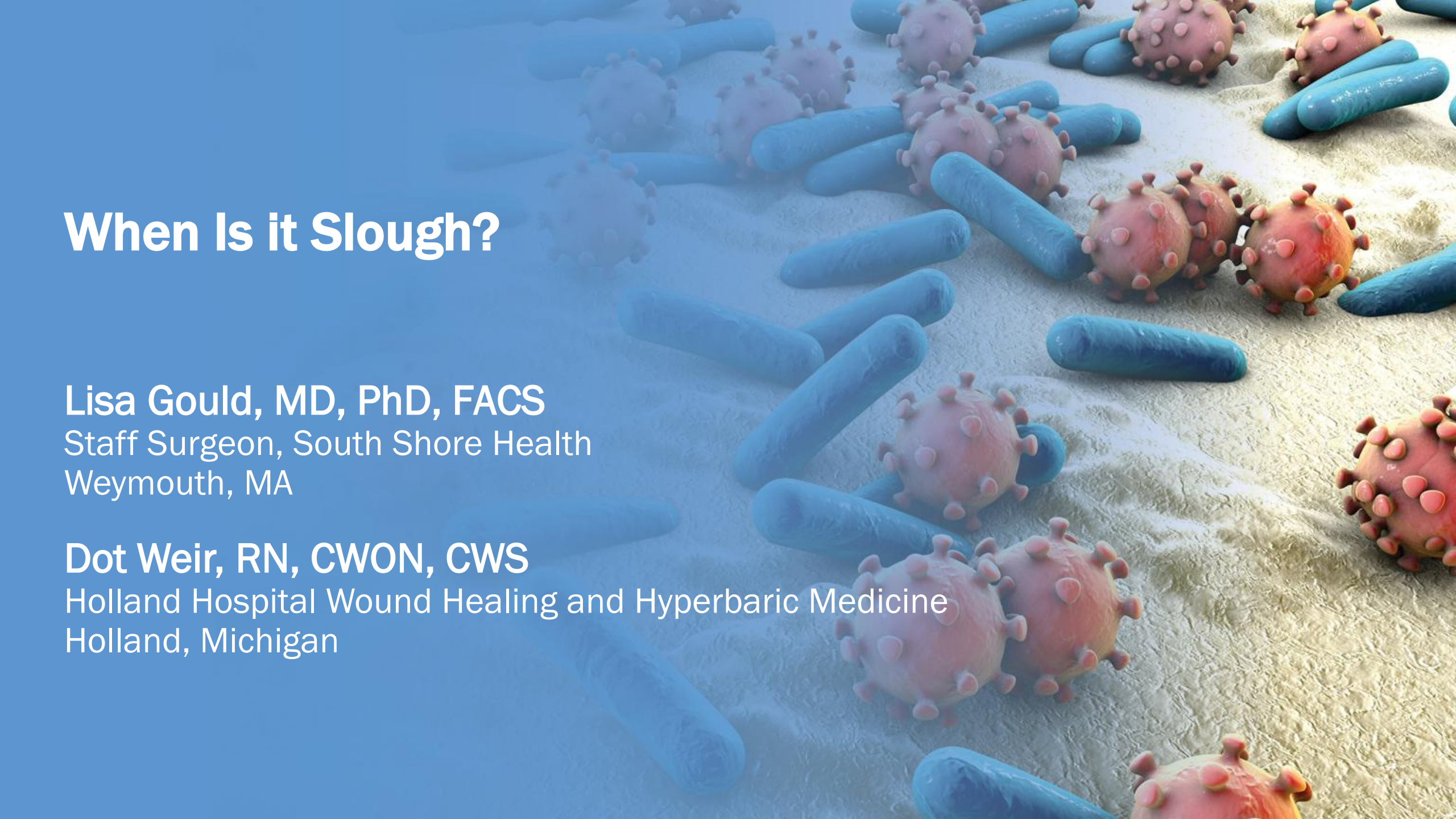
Learning Objectives

- Explain the patient-centric drivers of wound infection today
- Determine if there are gradations of contamination that can be agreed upon, categorize the levels of microbes in the wound, and differentiate modes of treatment for wounds contaminated at various levels
- Assess current techniques, in both inpatient and outpatient settings to determine levels of contamination and identify which method to use and when
- Determine the relationship between slough removal and infection control
- Explain the role of antimicrobial agents (antibiotics, antimicrobial barriers, antimicrobial preserved cleansers) and how to critically assess their efficacy in the management of bioburden
- Examine new and emerging technologies for slough/debris/microbe removal, including hypochlorous acid-based cleansers and charged fiber technology for physical removal of slough and debridement support/amplification
- Explore illustrative case studies on wound infection/slough management and apply current strategies

When Is it Slough?



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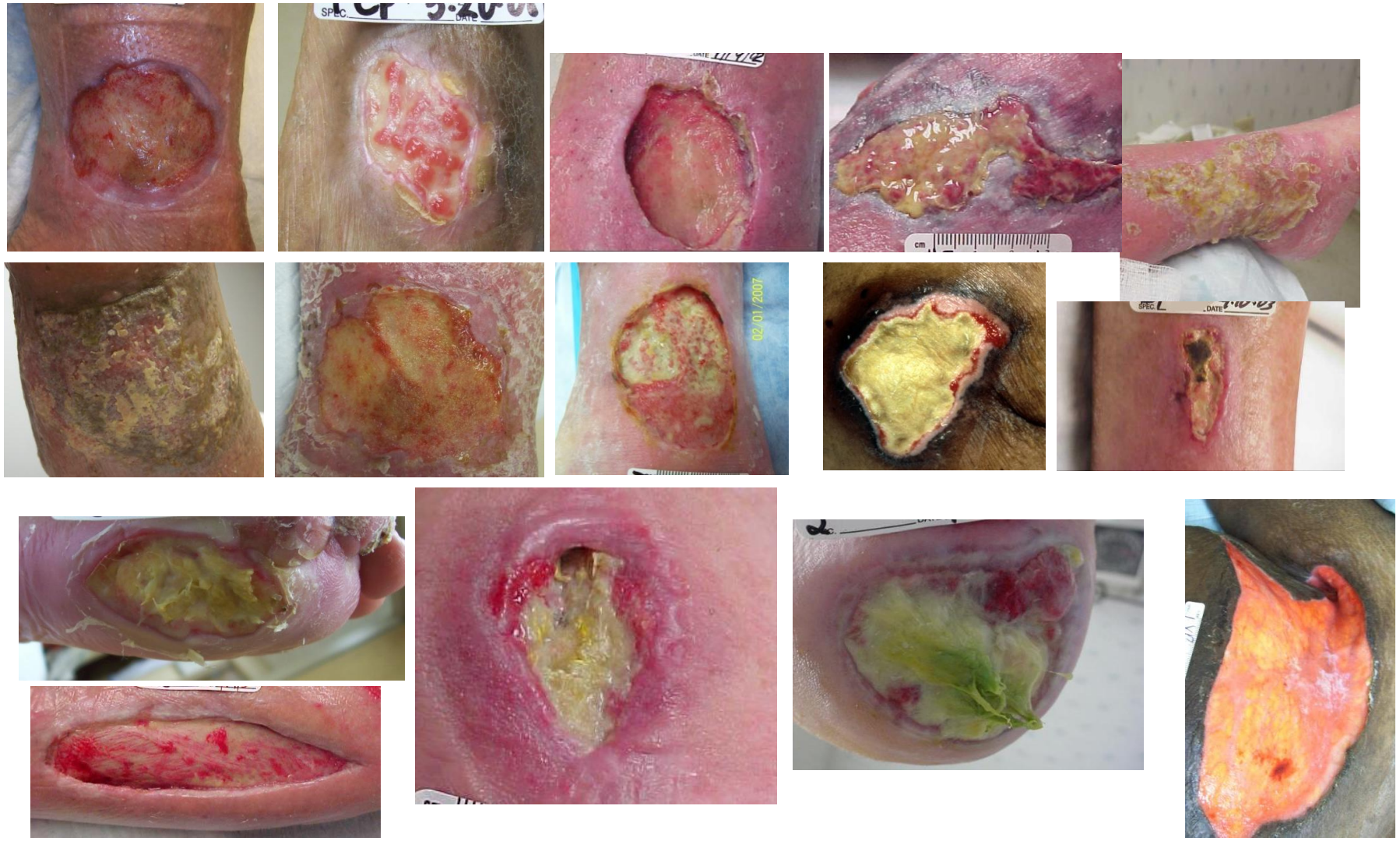
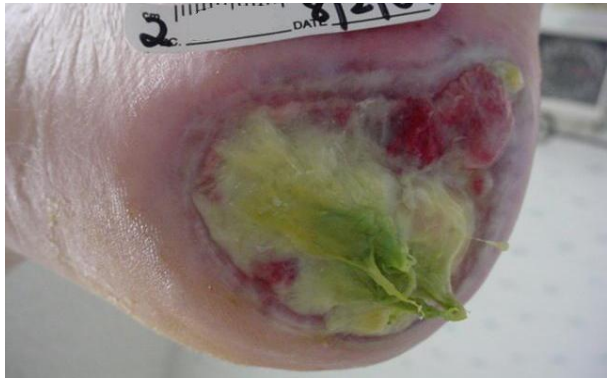
What's That Yellow Stuff?



We *thought* we knew....



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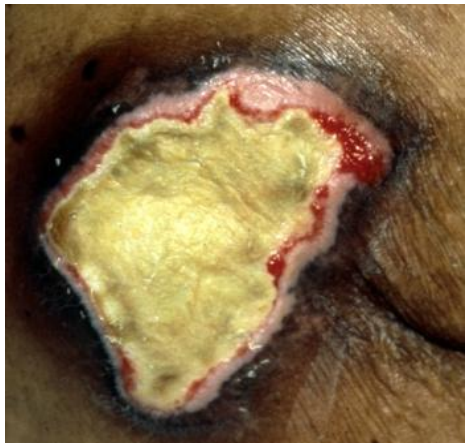
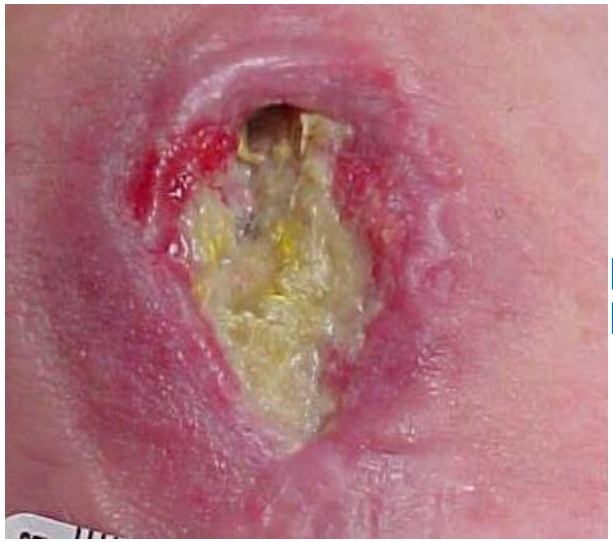
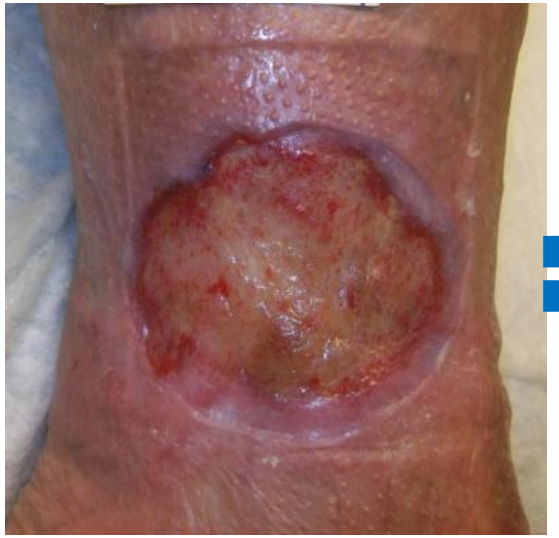


= ***Slough***

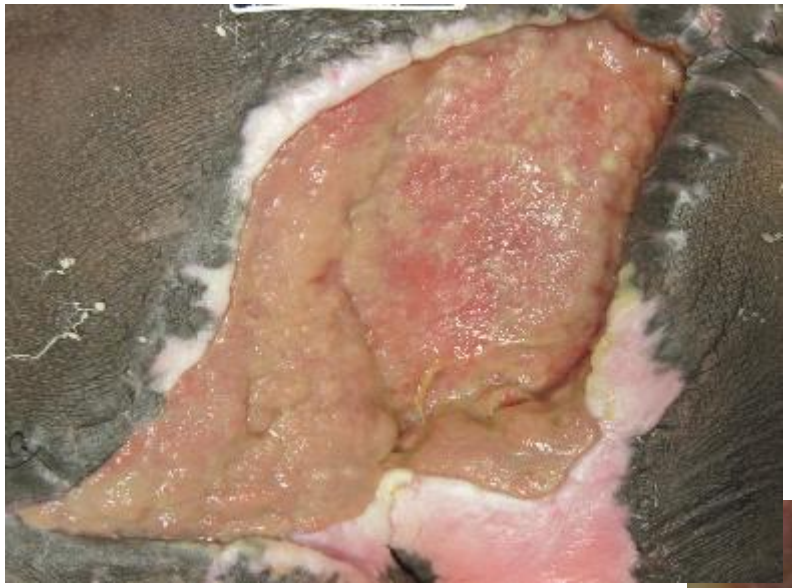


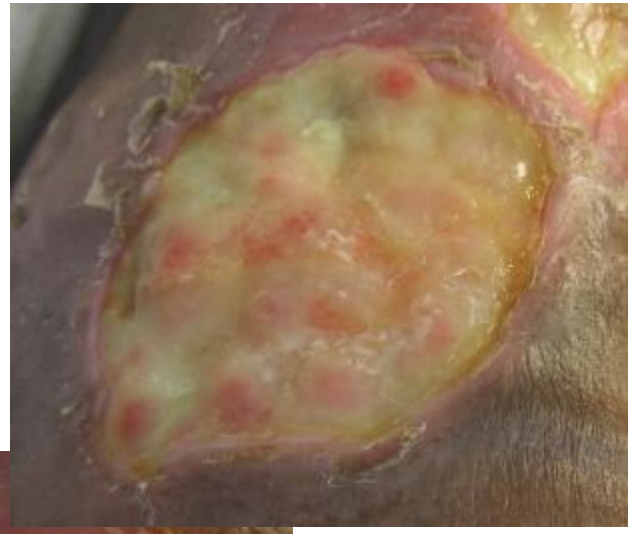
= ***Fat***

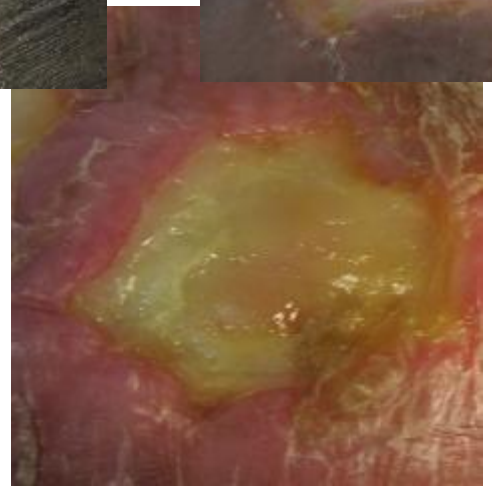


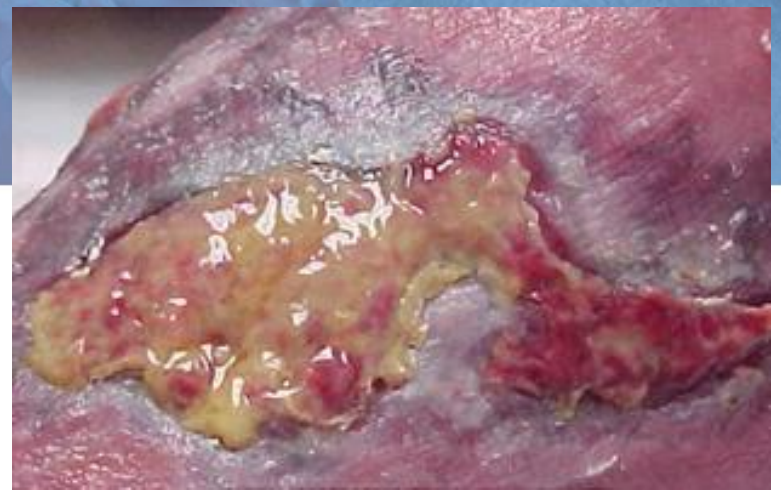
= ***Fibrin***

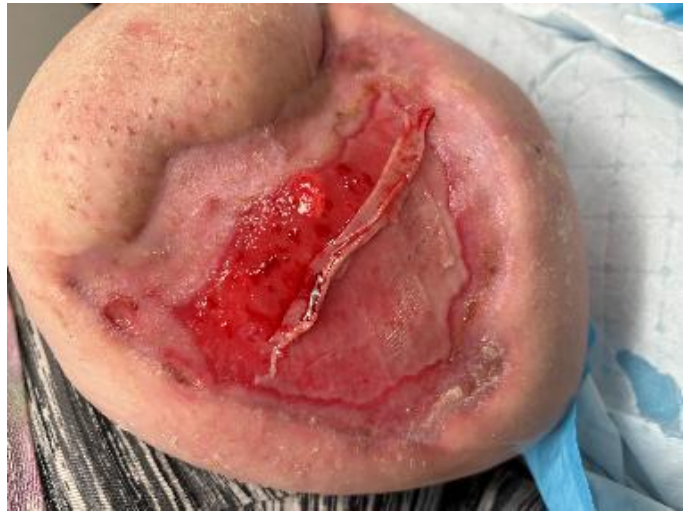
What About Other “Yellow Stuff”







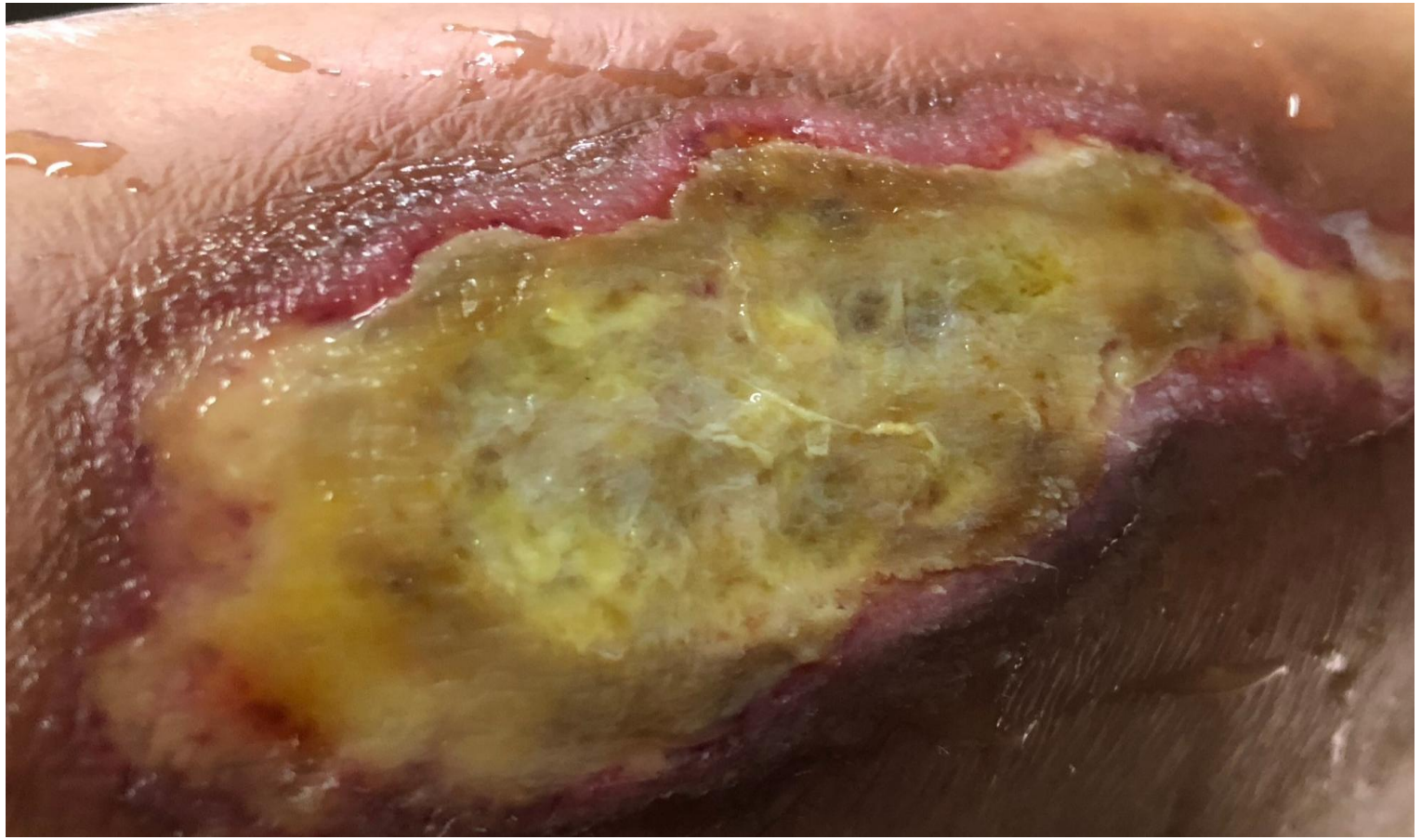




Let's back up.....what do you call this "yellow stuff"?

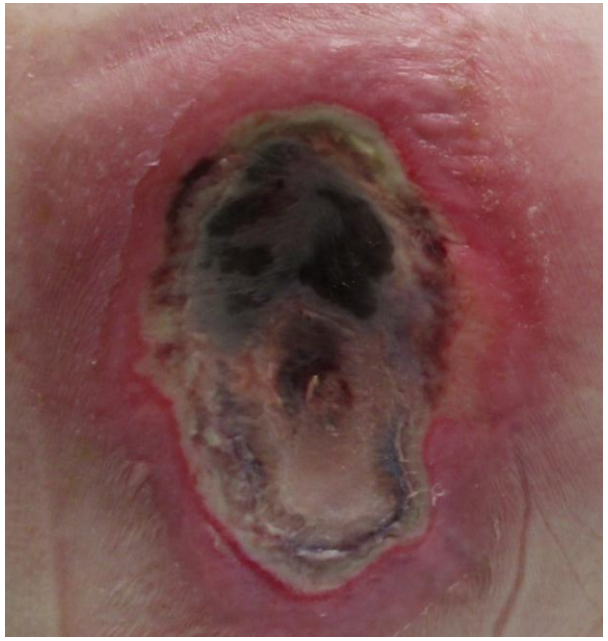
Slough?

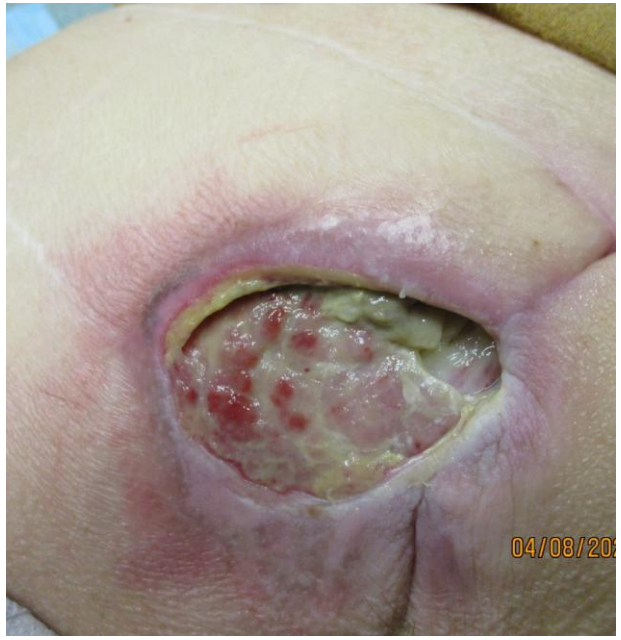
Is that a noun or a verb?

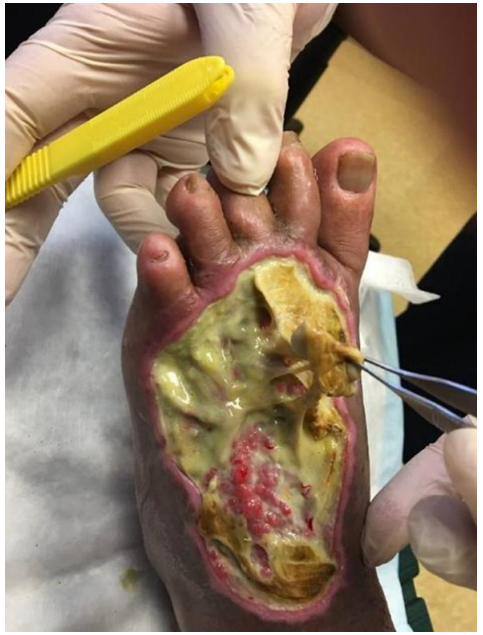


Definitions (Merriam-Webster)

- Noun: a place of deep mud or mire, swamp
- Adjective: a state of moral degradation or spiritual dejection
- Verb: to become shed or cast off; to cast off one's skin; to separate in the form of dead tissue from living tissue







Polling Question

- What is the major component of slough?
  - Dead tissue
  - Neutrophils
  - Bacteria
  - Proteins
  - All of the above

Polling Question

- What is the major component of slough?
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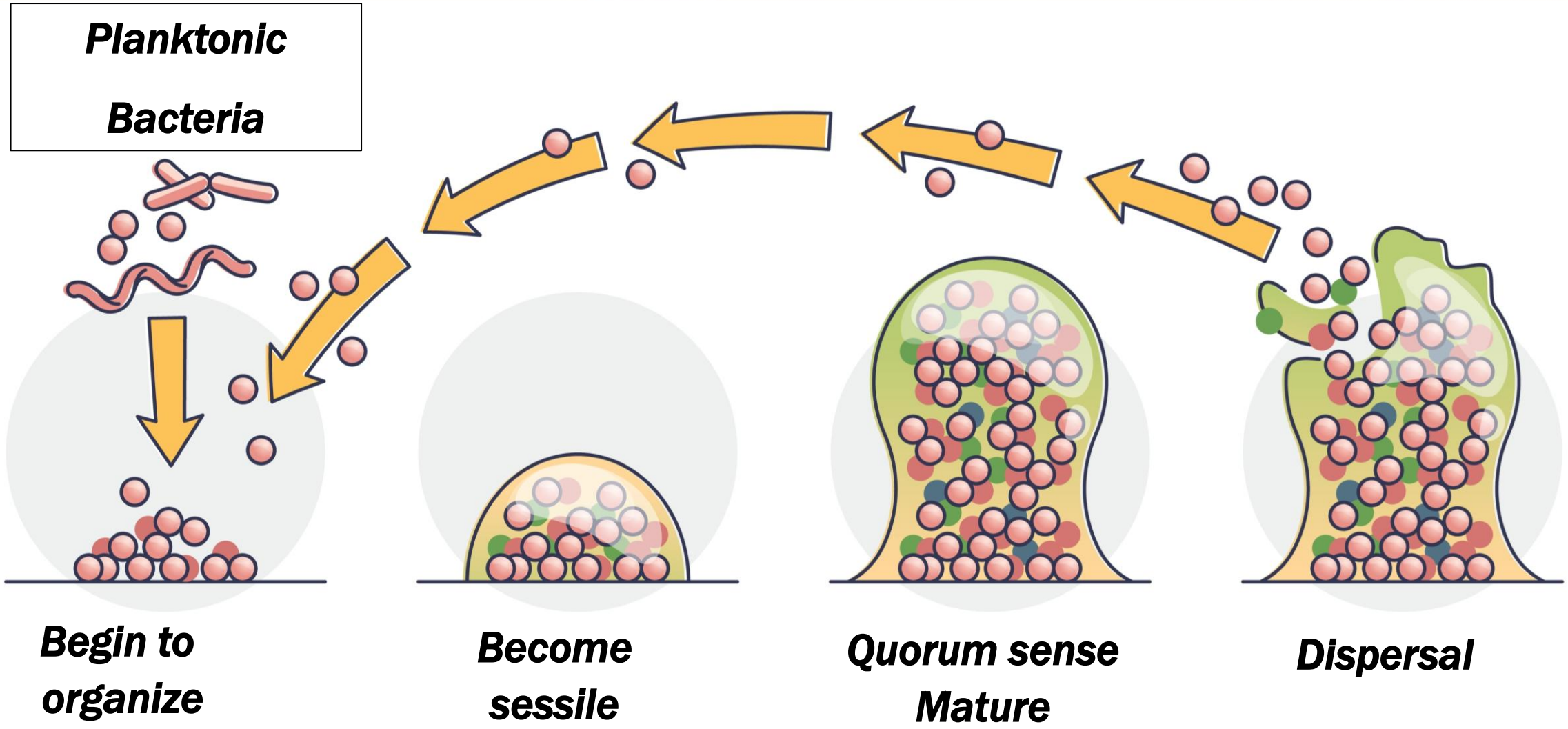
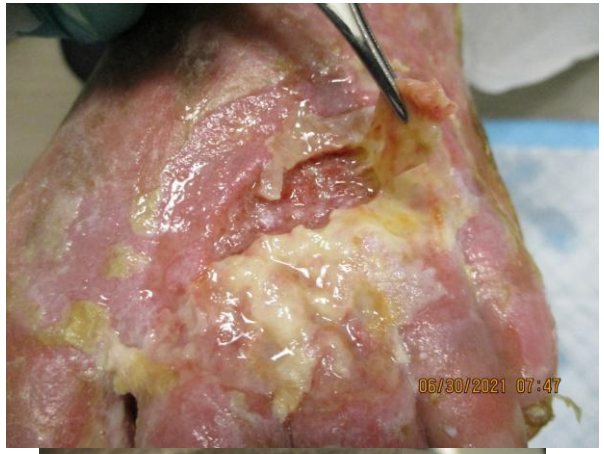
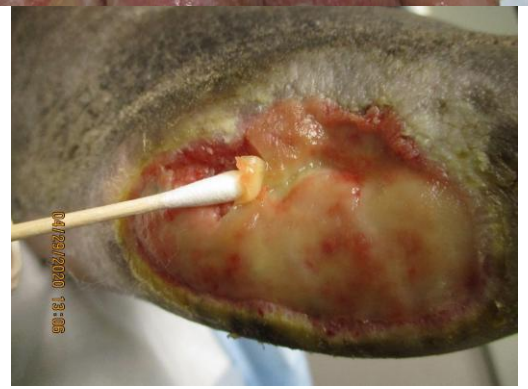
What about Biofilm?



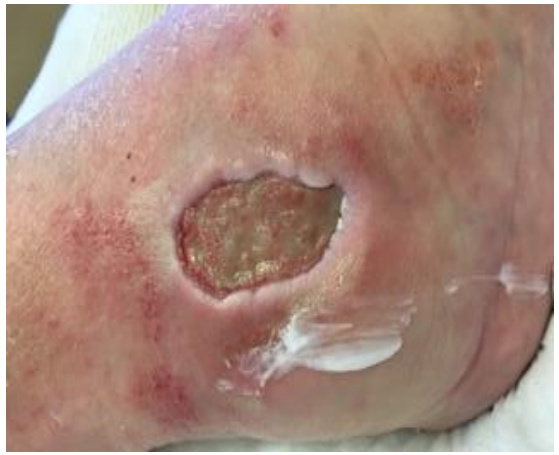
Bjarnsholt T, et al. The impact of mental models on the treatment and research of chronic infections due to biofilms. APMIS. 2021 Oct;129(10):598-606.

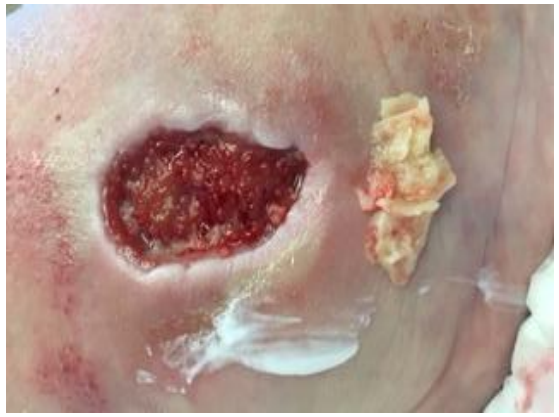
Is it Slough, Biofilm or Infected with Planktonic Bacteria?



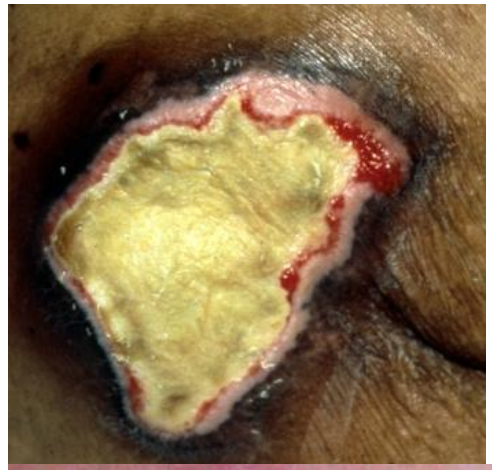


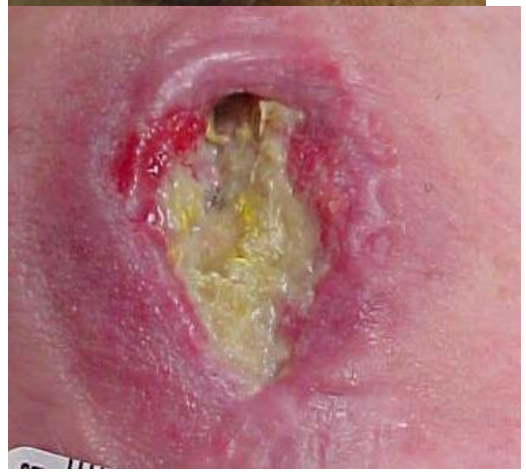
Peels off Easily





Requires a curette



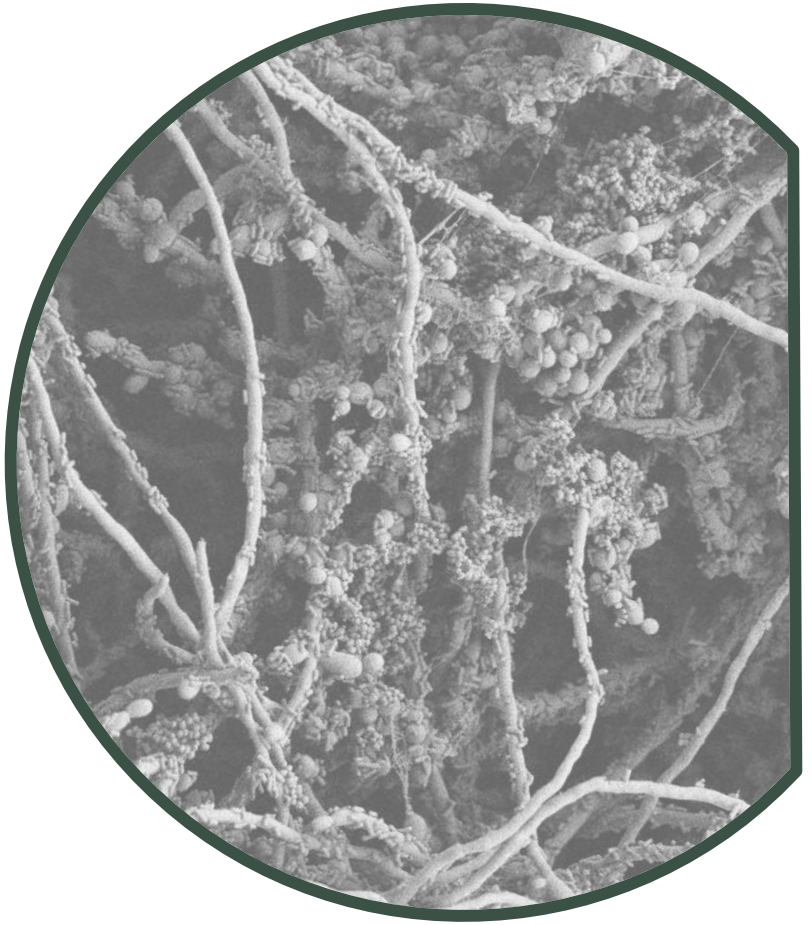


Needs a scalpel

Defining Slough

International Wound Infection Institute

Dr. Lindsay Kalan, University of Wisconsin, USA
Dr. Greg Schultz, University of Florida, USA
Dr. Thomas Bjarnsholt, University of Copenhagen, Demark
Dr. Matt Malone, Western Sydney University, Australia
NP Terry Swanson, South West Healthcare, Australia
Dr. Karen Ousey, University of Huddersfield, UK
Dr. Steven Percival, 5D Healthcare, UK
IWII Committee



The Slough Project

An international collaborative team collected slough samples with high-resolution photography and wound data to characterize the nature of slough:

- Samples were tested for:
  - **Specific host components** (e.g., collagen, fibrin, proteoglycans, serum proteins etc.).
  - The **microbial composition** of each sample
  - Determination of whether the microbes are able to grow as a **biofilm**.
- For each wound, the photograph, level of planktonic and biofilm bacteria, and histology of the wound sample were assessed.
- ***The* most comprehensive assessment of the composition of the 'slough' and its relation to inflammation and biofilm bacteria**.

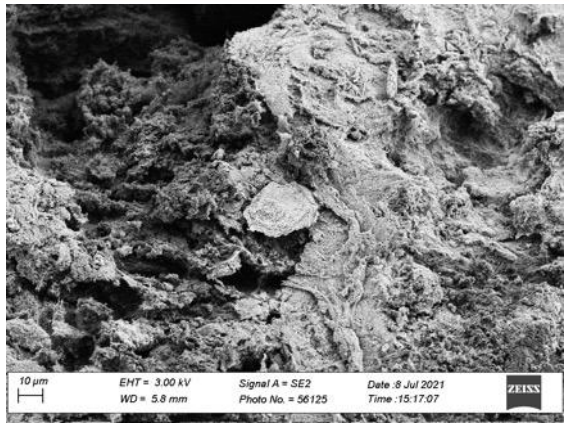
The Slough Project

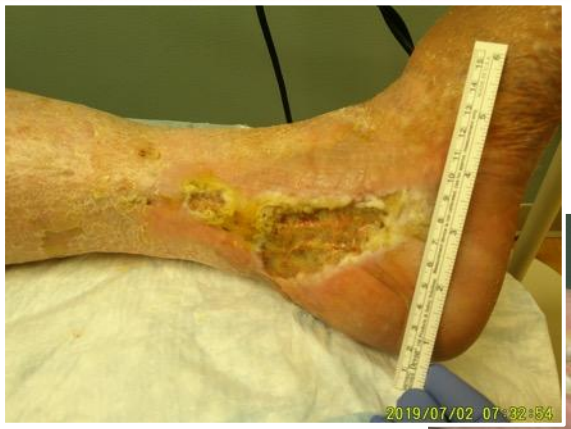
- 23 total subjects, 10 included in the comprehensive analysis including clinical outcomes at 3 months
- Microscopy: Confocal and SEM
  - 30% positive for Biofilm
- Quantitative Culture
  - Wound slough is polymicrobial, diverse bacteria related to wound site
  - 80% of subjects met definition for clinical infection based on $>10^5$ cfu/gm *despite absence of clinical signs of infection*
- Quantitative PCR16s rRNA
  - Correlated with quantitative culture
  - Most common species: Corynebacterium, Staphylococcus, Pseudomonas
- Proteomics: STRONGEST ASSOCIATION WITH WOUND OUTCOME

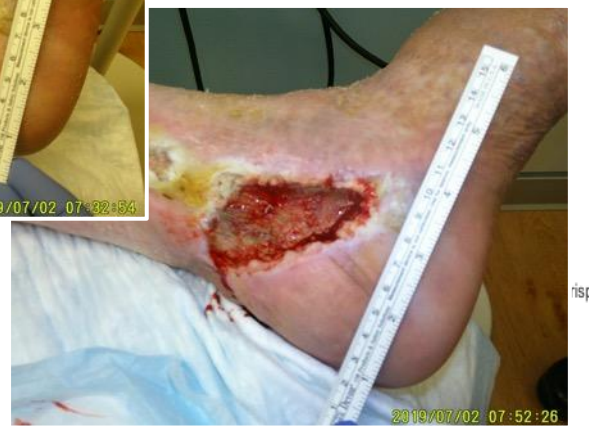
Townsend EC, Cheong JZA, Radzietza M, Fritz B, Malone M, Bjarnsholt T, Ousey K, Swanson T, Schultz G, Gibson ALF, Kalan LR. What is slough? Defining the proteomic and microbial composition of slough and its implications for wound healing. Wound Repair Regen. 2024 Nov-Dec;32(6):783-798

Patient 1

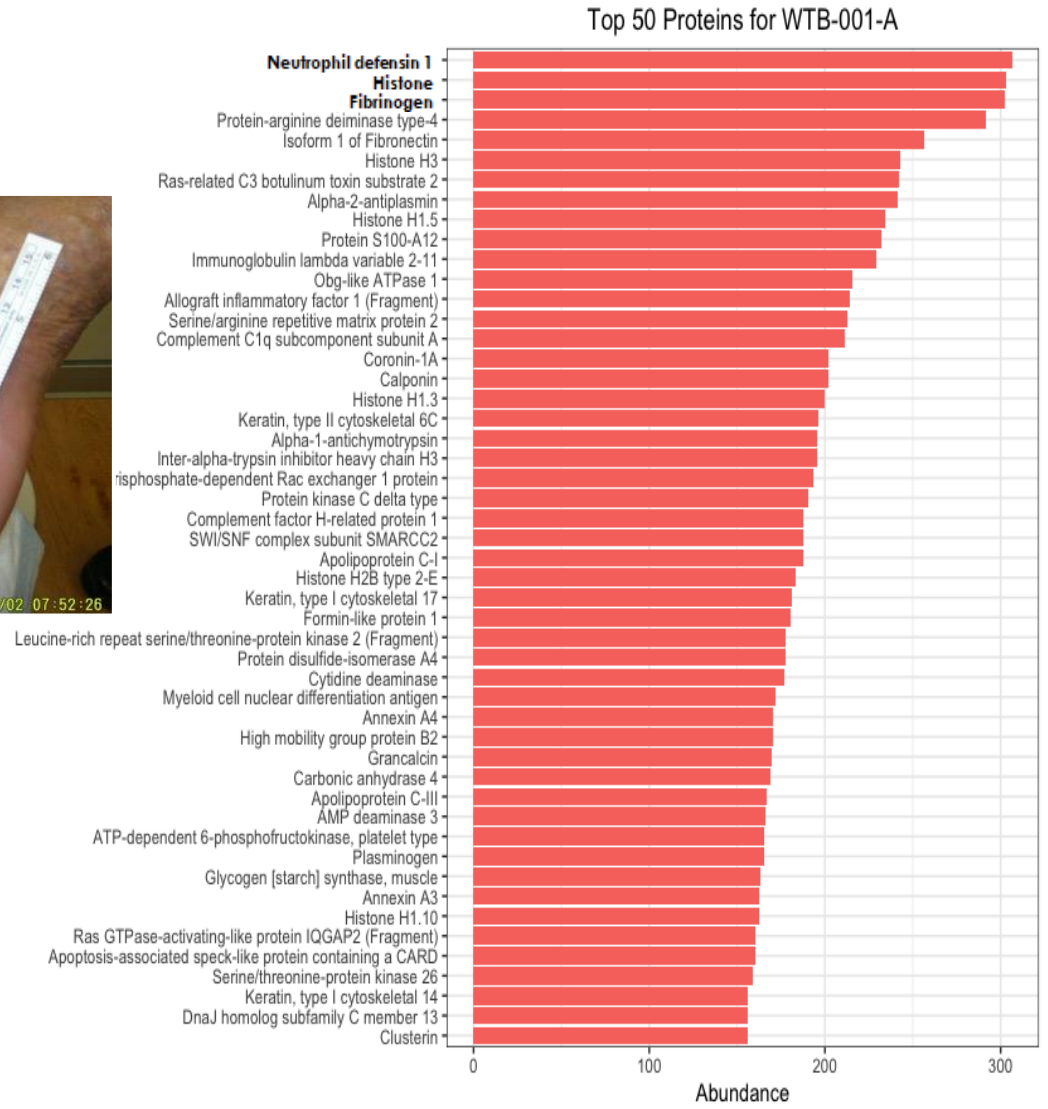
Side	Location	Etiology	Wound Age	Length(cm)	Width(cm)	cm^2	Shape	**CFUs**
Left	Medial Ankle	Lymphedema	15	8.5	4	34	Irregular; Round Oval	1.3×10^7





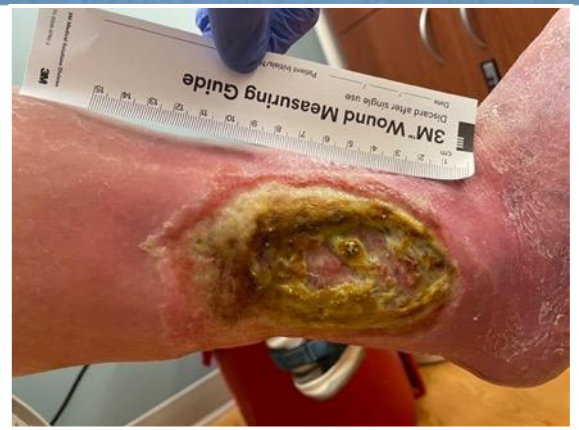


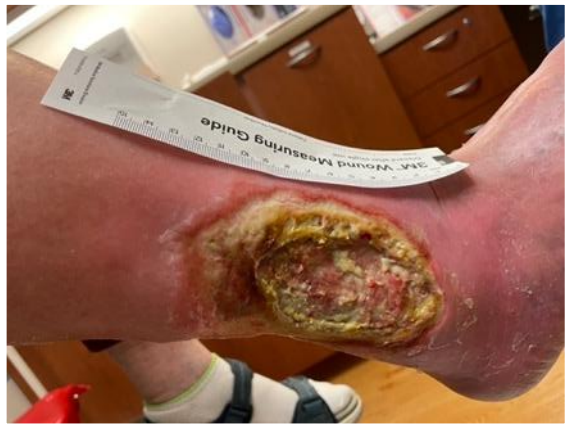
- Loosely adherent yellow slough
- High bioburden 1.3×10^7 CFU
- Polymicrobial (4+ by culture)
- No obvious biofilm by SEM
- Amorphous material



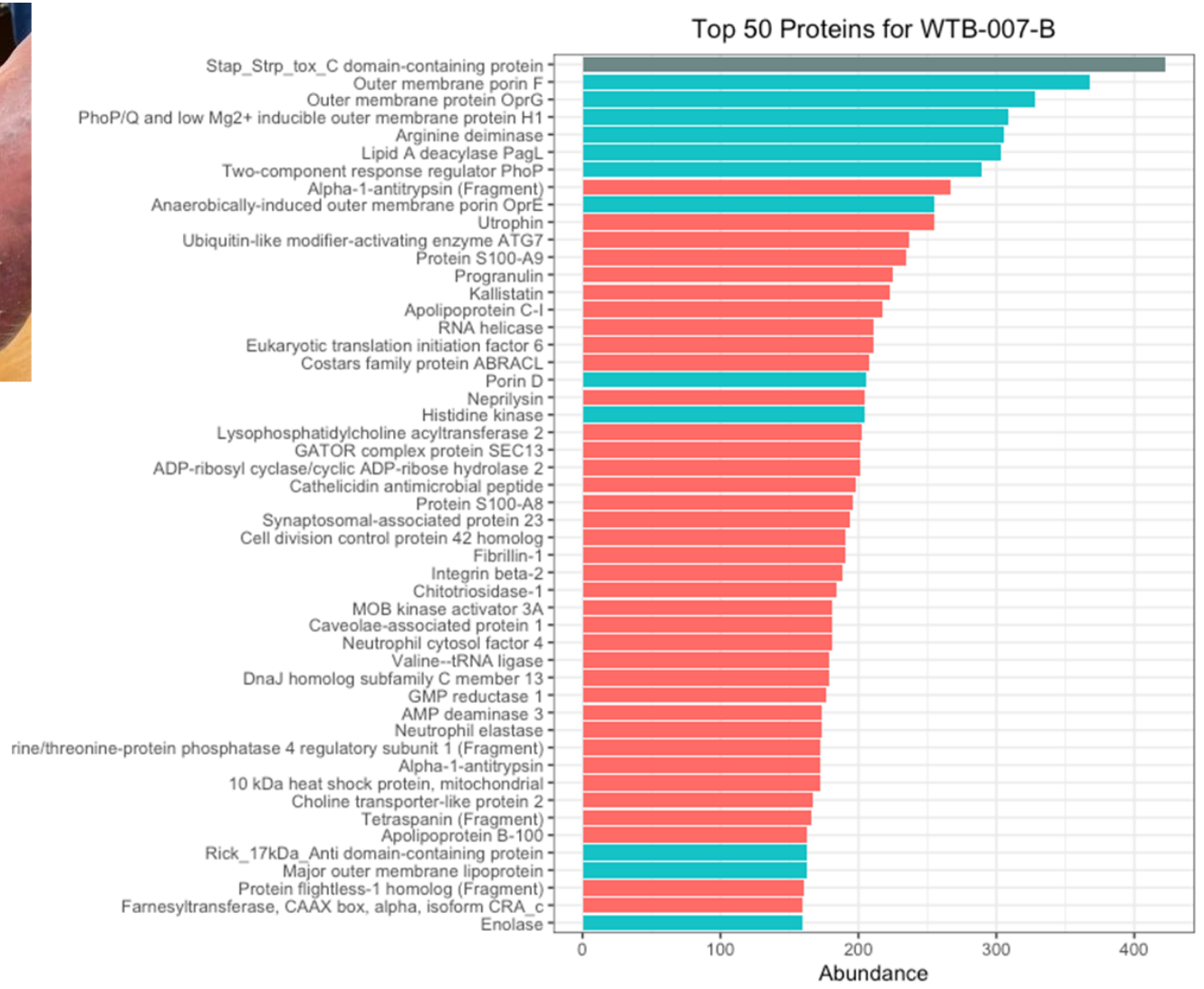
Patient 7

Side	Location	Etiology	Wound Age	Length(cm)	Width(cm)	cm^2	Shape	CFUs
Right	Lateral Ankle	Surgical wound	1.25	8	10.4	93.6	Irregular	8×10^7





- *Adherent*
- *High bioburden*
- *Pseudomonas aeruginosa cultured*
- *No obvious biofilm by SEM*
- *Proteomics identifies P. aeruginosa & S. aureus*
- *16S sequencing is pending*



New insights into the role of biofilm

Wound Healing is Impeded by Metabolically Active Bacteria

Parameter	Result	*p*-value		
		Healing slope	Healing versus non-healing	PAR
Biofilm microscopy score	No association in any test	0.25	0.15	0.19
Total viable bacteria	Less healing in 2 of 3 tests when more bacteria were present	0.94	0.012	0.012
Bleach-tolerant bacteria	No association in any test	0.053	0.97	0.69
Bleach-susceptible bacteria	Less healing in all tests when more bleach-susceptible bacteria were present	0.003	0.0012	0.004

First longitudinal study characterizing presence of biofilm and rate of healing in chronic venous leg ulcers

- 117 patients
- No correlation of healing with biofilm score or bleach tolerant bacteria
- Reduced healing with bleach-susceptible (metabolically active) bactera

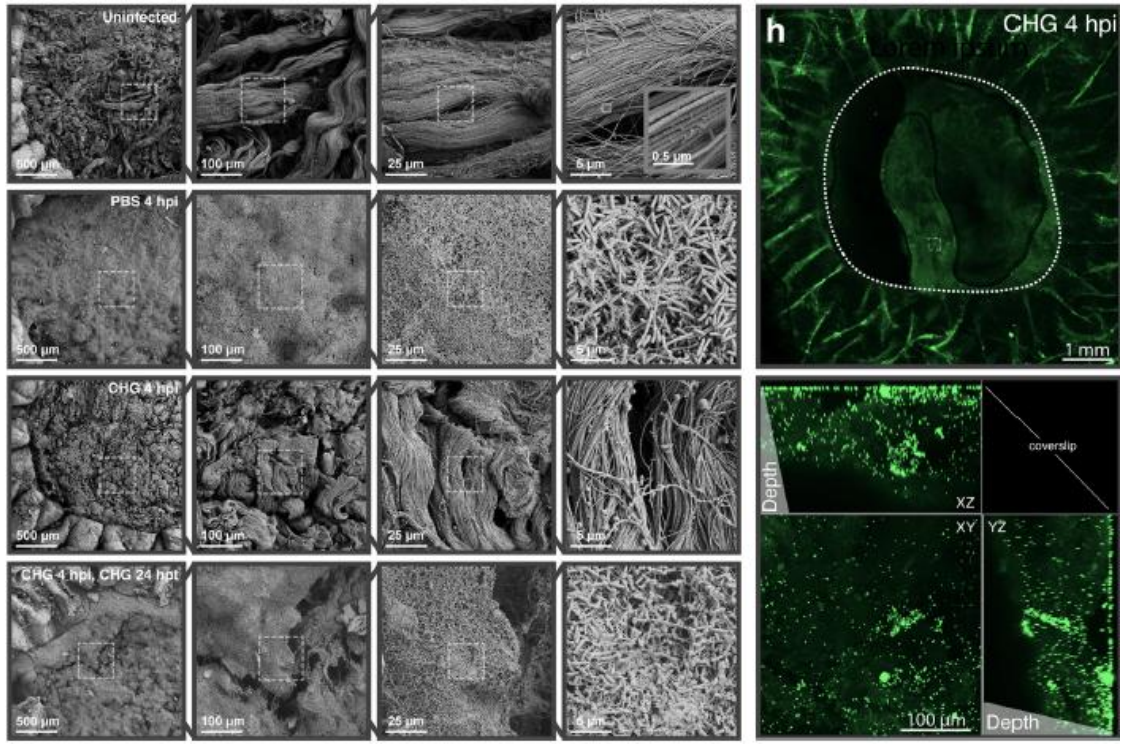
Stewart PS, Kim J, James G, Yi F, Stechmiller J, Weaver M, Kelly DL, Fisher S, Schultz G, Lyon D. Association of biofilm and microbial metrics with healing rate in older adults with chronic venous leg ulcers. Wound Repair Regen. 2024 Nov-Dec;32(6):858-871

Why Debridement is Critical

WHS Fireside Chats

Scanning Electron Microscopy vs Confocal laser scanning microscopy

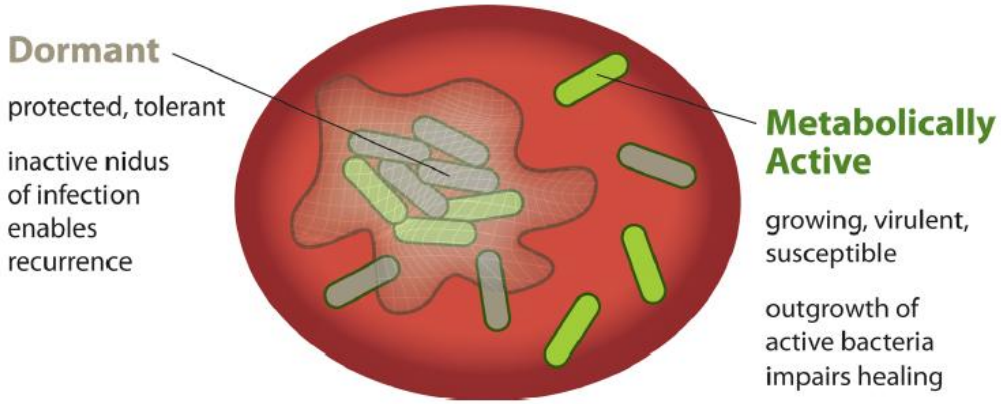
Images compare different depths of infection



Identified reservoirs of P. aeruginosa in deep tissue not seen with SEM

Cheong JZA, Liu A, Rust CJ, Tran CL, Hassan SE, Kalan LR, Gibson ALF. Robbing Peter to Pay Paul: Chlorhexidine gluconate demonstrates short-term efficacy and long-term cytotoxicity. Wound Repair Regen. 2022 Sep;30(5):573-584

A Paradigm Shift and New Model: The Infectious Microenvironment



Bacterial aggregation and Biofilm is a phenotype, not the cause of bacterial persistence

Aggregate size can interfere with phagocytosis

The trajectory of infections is controlled by the metabolism of the bacteria

To understand Wound Infection we need to understand the Infectious Microenvironment

1) Bacterial Metabolism
2) Inflammatory response
3) The metabolic signature of bacteria and host

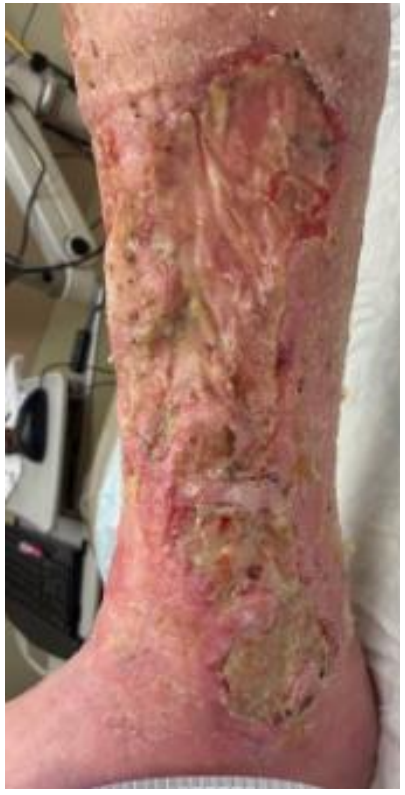
The new research goal is not to grow biofilms but to mimic the microenvironment

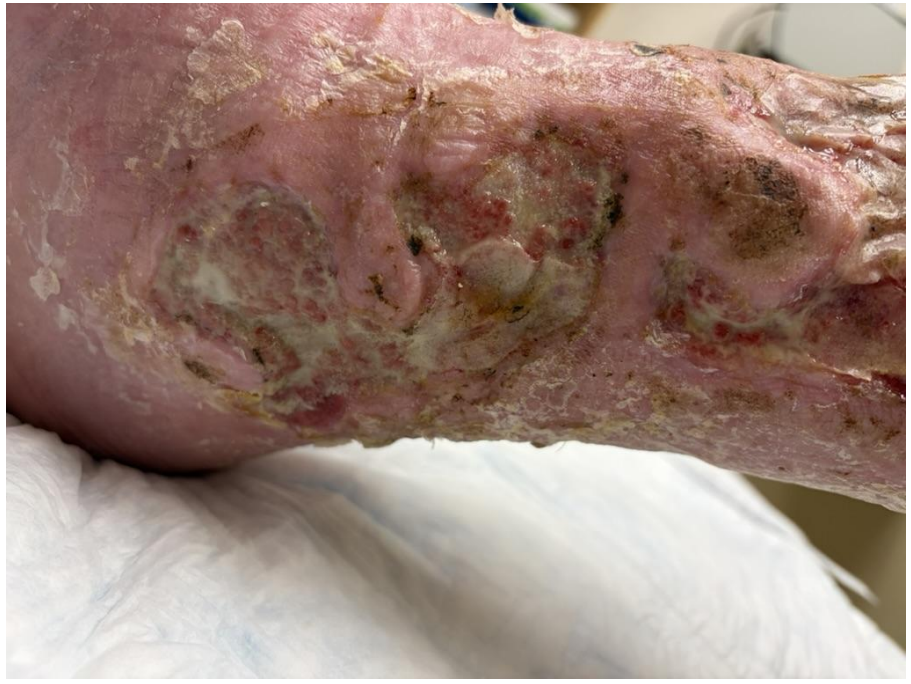
https://woundinfection-institute.com/agm/

Stewart PS, Kim J, James G, Yi F, Stechmiller J, Weaver M, Kelly DL, Fisher S, Schultz G, Lyon D. Association of biofilm and microbial metrics with healing rate in older adults with chronic venous leg ulcers. Wound Repair Regen. 2024 Nov-Dec;32(6):858-871

Slough vs Biofilm vs Planktonic Bacteria?

- 60 y/o male, admitted via the ED with overtly necrotic wounds, pain and cold right foot. Prior femoral to distal popliteal bypass 1 year ago, underwent revision of the bypass and wound treated with operative debridement and topical wound care.
- At his first clinic visit he presented with increased pain, erythema of the right leg, with malodorous drainage





Close up of lateral ankle

Polling question

- The next most important step in this patient's management is:
  - Referral for operative debridement
  - Treat with analgesics and topical wound care
  - Culture
  - Start empiric antibiotics

Polling question

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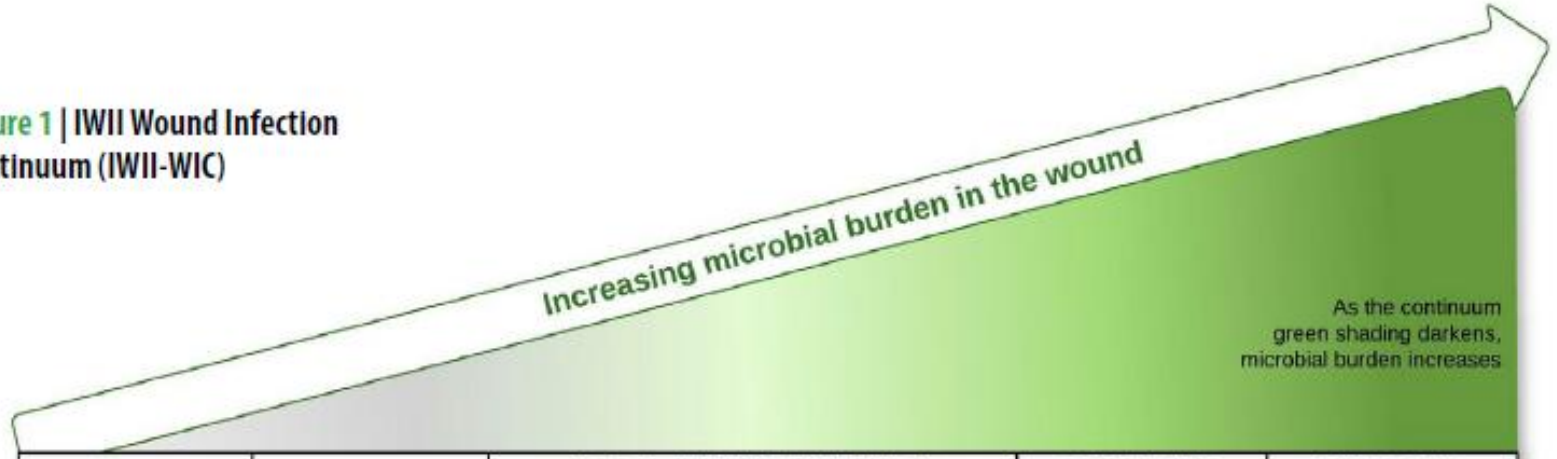
When or WHAT is an Infection?

Figure 1 | IWII Wound Infection Continuum (IWII-WIC)

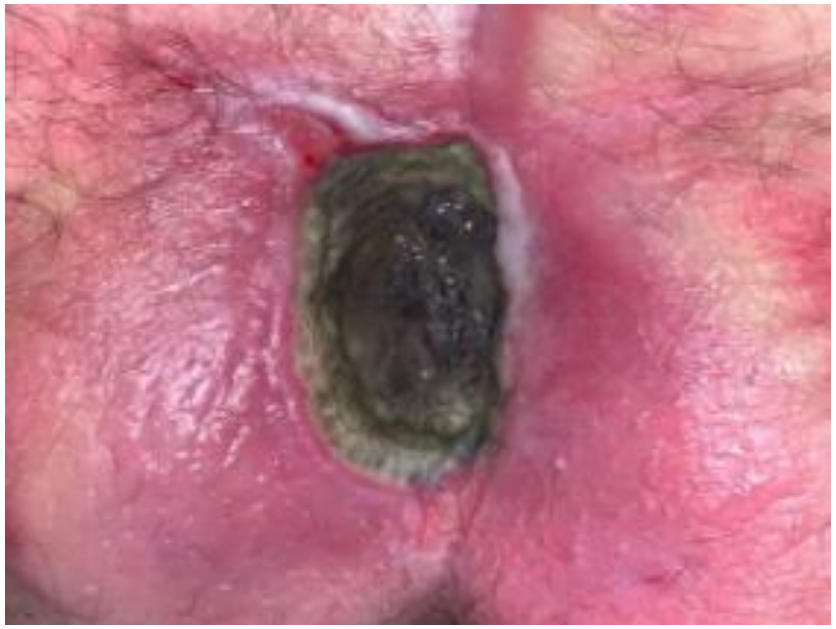


CONTAMINATION	COLONISATION	LOCAL WOUND INFECTION COVERT (subtle)	LOCAL WOUND INFECTION OVERT (classic)	SPREADING INFECTION	SYSTEMIC INFECTION
• Microorganisms are present within the wound but are not proliferating • No significant host reaction is evoked • No delay in healing is clinically observed	• Microorganisms are present and undergoing limited proliferiation • No significant host reaction is evoked • No delay in wound healing is clinically observed	• Hypergranulation • Bleeding, friable granulation • Epithelial bridging and pocketing in granulation tissue • Increasing exudate • Delayed wound healing beyond expectations	• Erythema • Local warmth • Swelling • Purulent discharge • Wound breakdown and enlargement • New or increasing pain • Increasing malodour	• Extending induration • Spreading erythema • Inflammation or erythema >2cm from wound edge • Crepitus • Wound breakdown/ dehiscence with or without satellite lesions • Lymphangitis (swelling of lymph glands)	• Malaise • Lethargy or nonspecific general deterioration • Loss of appetite • Fever/pyrexia • Severe sepsis • Septic shock • Organ failure • Death

https://woundinfection-institute.com/resources/the-iwii-wound-infection-continuum-iwii-wic

Slough vs Biofilm vs Planktonic Bacteria?

- 35 yo male with spina bifida, baseline fecal and urinary incontinence, decreased sensation below T12, presented to the hospital with back pain and noted to have a malodorous, painful sacral pressure ulcer. He is afebrile, WBC wnl, CRP 120, ESR 36. lactate 0.7



Polling question

- Based on the IWII wound infection continuum this patient has a:
  - Colonization
  - Overt infection
  - Spreading infection
  - Systemic infection
  - None of the above: the erythema is from moisture associated skin damage

Polling question

- Based on the IWII wound infection continuum this patient has a:
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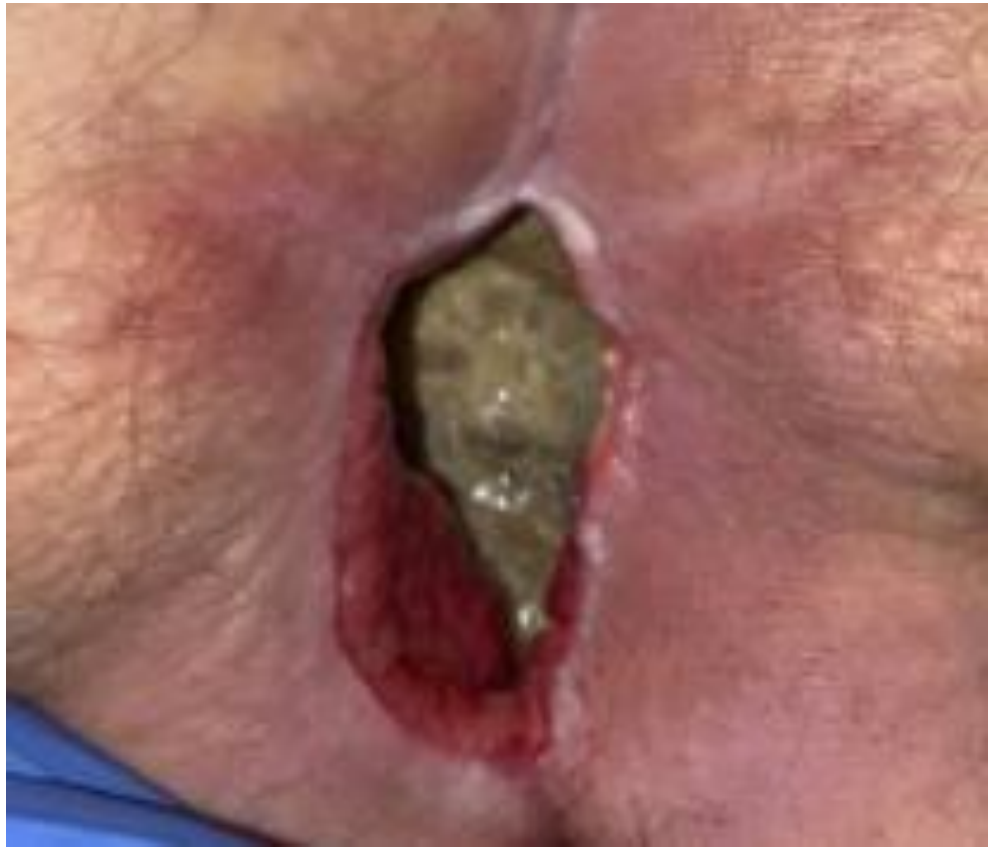
  - None of the above: the erythema is from moisture associated skin damage

When is it not ‘just slough’?



After bedside debridement, 2 weeks of topical moist wound care with hypochlorous acid

Polling question

- The next most important step in this patient's management is:
  - Referral for operative debridement
  - Treat with analgesics and topical wound care
  - Culture
  - Start empiric antibiotics

Polling question

- The next most important step in this patient's management is:
  - Referral for operative debridement
  - Treat with analgesics and topical wound care
  - Culture
  - Start empiric antibiotics

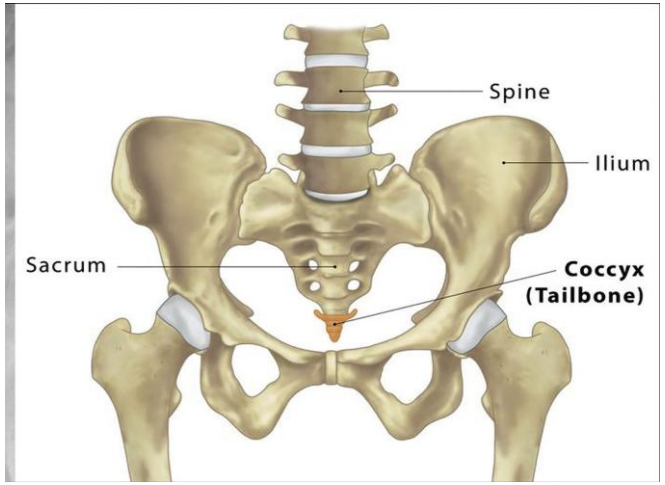
Surgical strategy

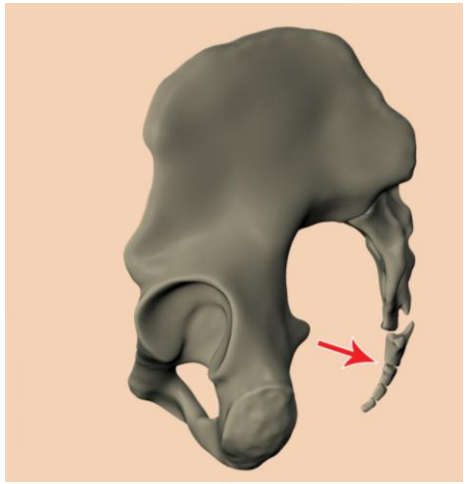
- Operative Debridement and Irrigation
  - Debride to viable tissue
    - Scalpel, Curette, Hydrosurgery
  - Pulse lavage
  - Tissue Biopsy
    - Culture
      - Aerobes, Anaerobes
      - Fungal, Atypical mycobacteria
    - Pathology
      - Wound edge with intact skin
      - Debrided tissue
- Dressing
  - Antimicrobial, antibiofilm
  - Edema reducing
  - Extracellular matrix

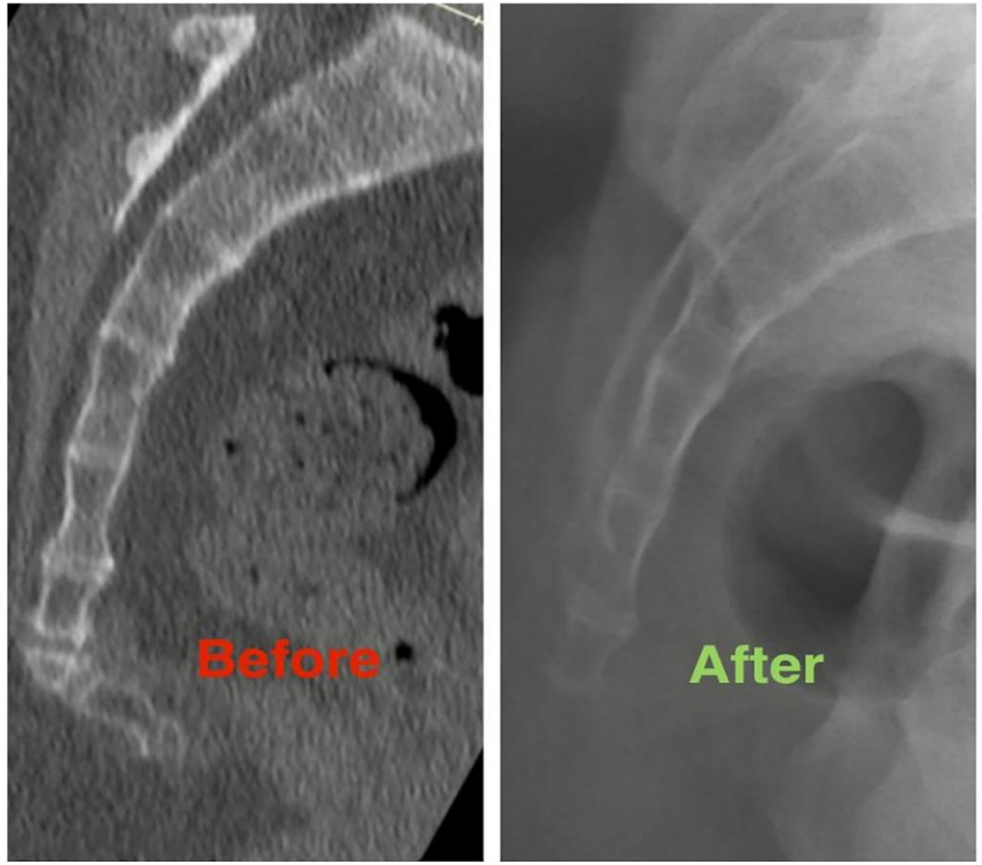
Gould LJ, Alderden J, Aslam R, Barbul A, Bogie K, El Masry M, Graves L, White-Chu E Foy, Ahmed A, Boanca K, Brash J, Brooks, K, Cockron W, Kennerly S, Livingston A, Page J, Stephens C, West V, Yap T. WHS guidelines for the treatment of pressure ulcers—2023 update. Wound Rep Regen. 2024; 32(1): 6-33. doi:10.1111/wrr.13130

No, that was not just slough…

- Decision for operative debridement.
- Coccyx separated from Sacrum
- Sacral bone culture positive for Strep anginosus







Skalski MR. Published Online: July 01, 2020
https://doi.org/10.1148/rg.2020190132
Carayannopoulos N, Montaquila N, Lewis A …**Disorders of the coccyx and sacrococcygeal joint: Etiology, diagnosis, and management strategies**
The American Journal of Medicine, 2025; 139, 405-412

Clinical Pearls

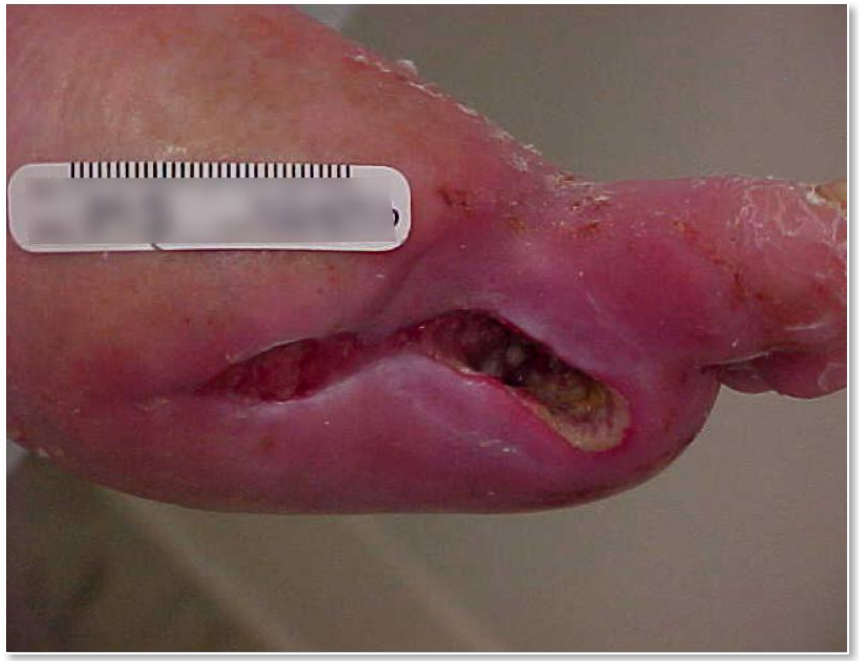
- Debride the wound with appropriate tool to remove slough
- Differentiate between slough and dead tissue
- Culture wounds when there are clinical signs of infection based on IWII continuum
- Refer for surgical debridement when wound does not improve or there is obvious necrotic tissue that cannot be removed at bedside

Culture: Technique, Timing & Interpretation

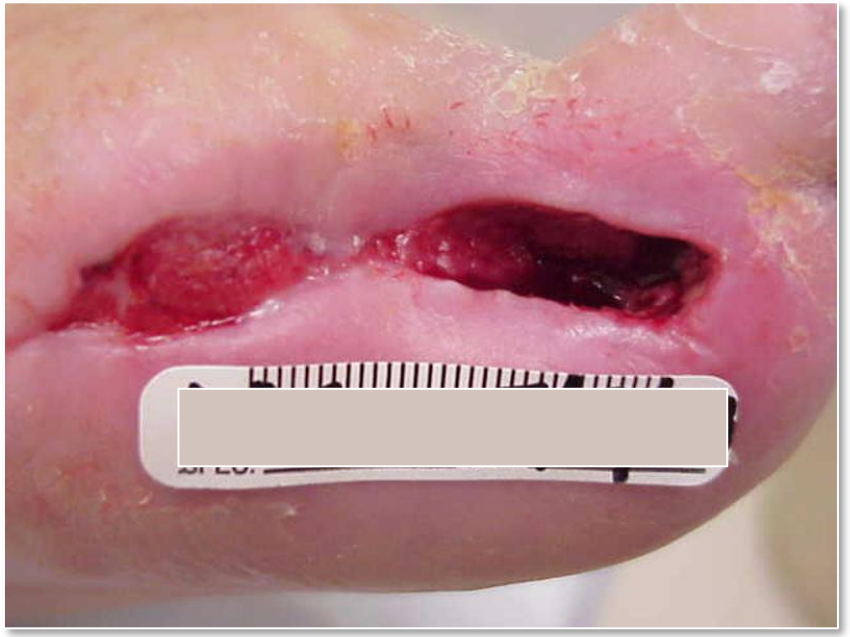
Dot Weir, RN, CWON, CWS
Holland Hospital Wound Healing and Hyperbaric Medicine

Infection: Clinical Picture

- Swelling
- Induration
- Erythema
- Warmth
- Pain
- Odor
- Increased drainage
- Listen to the patient…



5 days prior



Culture with the *intent to treat*

- The diagnosis of infection is based on the clinical findings
- Avoid routine culturing of non-infected wounds!
- Erythema, heat, new or increased pain, swelling/induration, increased exudate, delayed healing, and/or wound deterioration
- May include systemic signs, such as fever, lymphadenitis, streaking, and/or elevated white blood cells and inflammatory markers
- Caution with immunocompromised patients – s/s of infection may be subtle or missing; only sign may be non-healing or deterioration
- Consider starting patient on empiric antibiotics while awaiting culture and sensitivity report

Polling Question

- The most accurate method for obtaining a microbial culture is
  A. Do not cleanse the wound, then swab side-to-side across the wound bed
  B. Cleanse the wound with hydrogen peroxide, then rinse and swab the center of the wound
  C. After cleansing, remove tissue from a non-necrotic area with instrument (scalpel or punch biopsy tool)
  D. Perform a swab culture using the Levine Technique.

Polling Question

- The most accurate method for obtaining a microbial culture is
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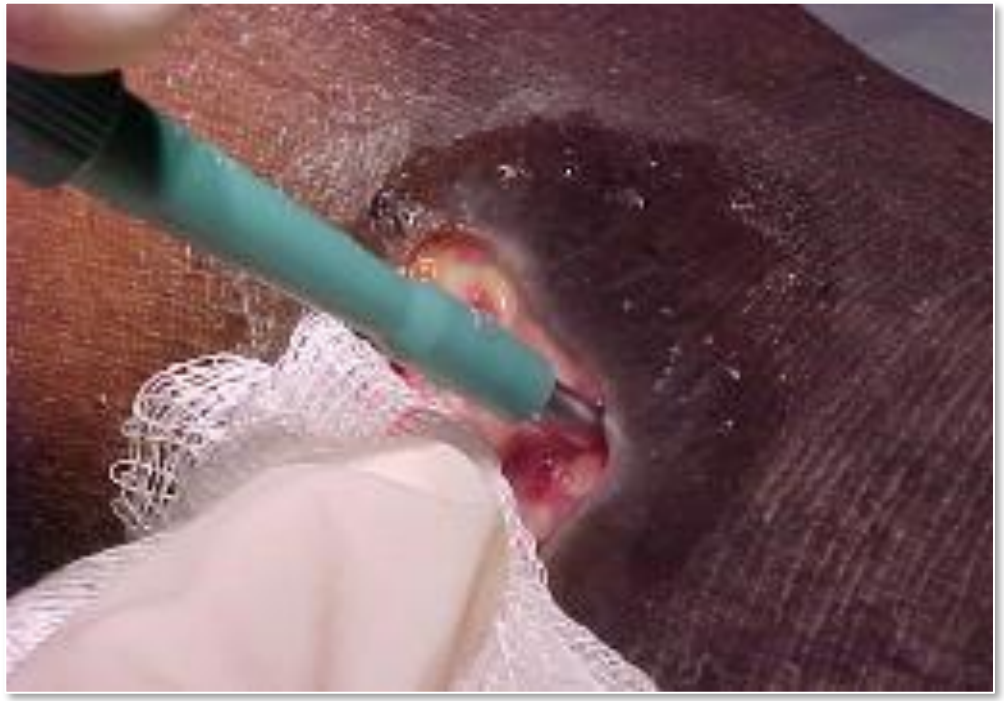
  D. Perform a swab culture using the Levine Technique.

Tissue Biopsy

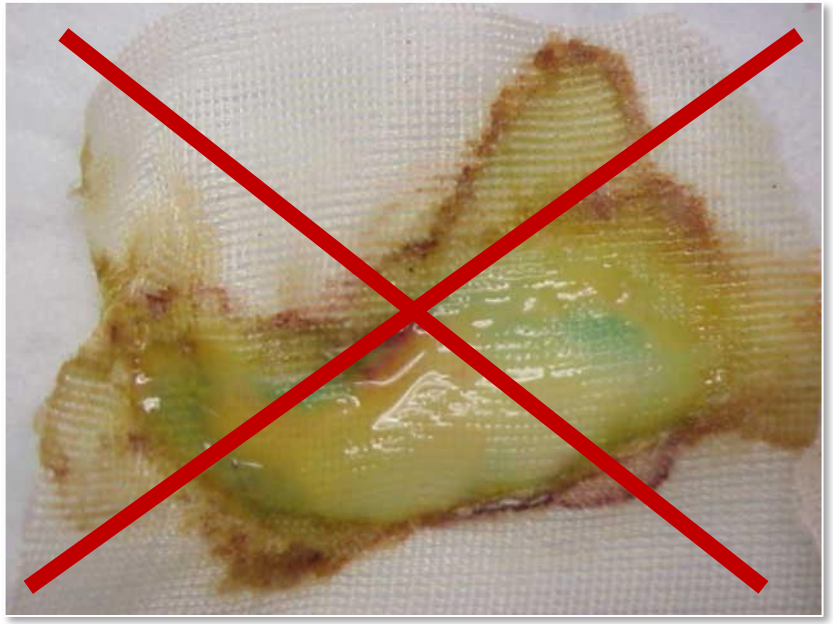
- Historically, the “gold standard” or, at least, best practice
- Painful (needs anesthetic in most cases)
- Skill intensive
  - Unavailable in many settings
- Traditionally, was used in research settings; now used more often in clinical settings

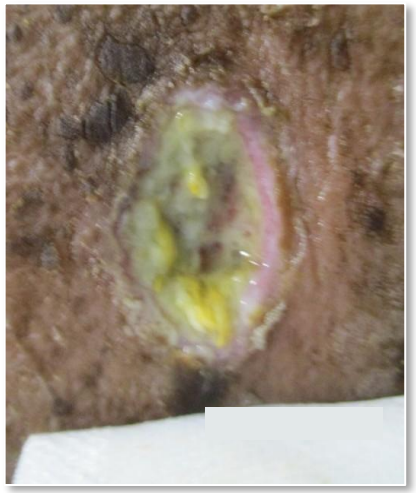


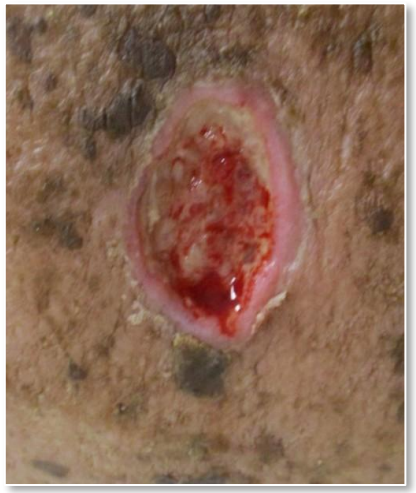
International Wound Infection Institute. *Wound Infection in Clinical Practice.* 2022. Lipsky BA, et al. *J Antimicrob Chemother*. 2016;71:3026-3035.

Do the best swab possible – Levine Technique

- Thoroughly rinse wound surface with non-preserved saline
- Don’t swab through dressing residue, old exudate, necrotic tissue, blood
- Choose area that is free of non-viable tissue, if possible
- Culture post-debridement
- ***Clean again after debridement!***





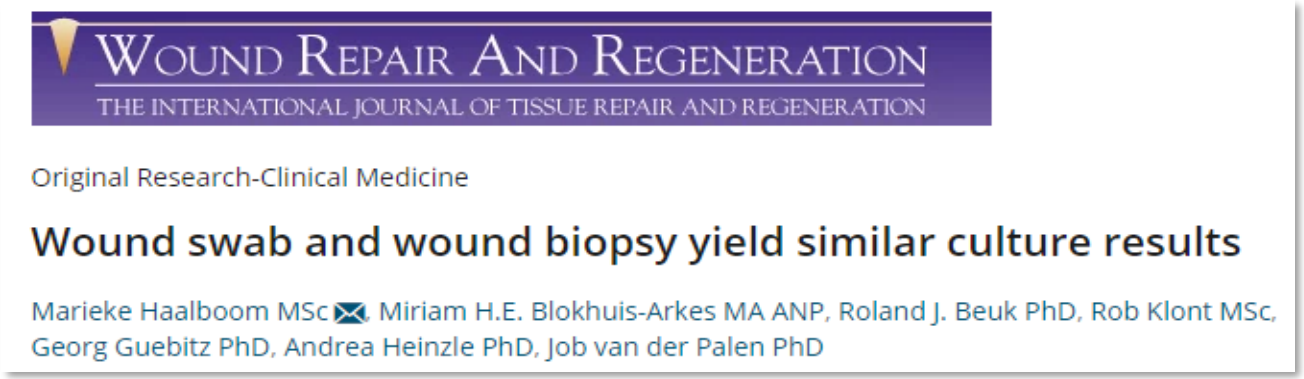


When and How to Perform Cultures on Chronic Wounds

- What is the best method or technique to perform a culture on a chronic wound?
  - 7 studies
  - Results: Quantitative culture of wound tissue is the gold standard to obtain a wound culture (4 studies), but the swab method is an acceptable alternative
  - Two articles demonstrate the Levine technique is more reliable than the Z-technique to determine microbial load in the wound bed

Stallard Y. J Wound Ostomy Continence Nurs. 2018;45(2):179-186.

Levine Technique for Swab Culturing

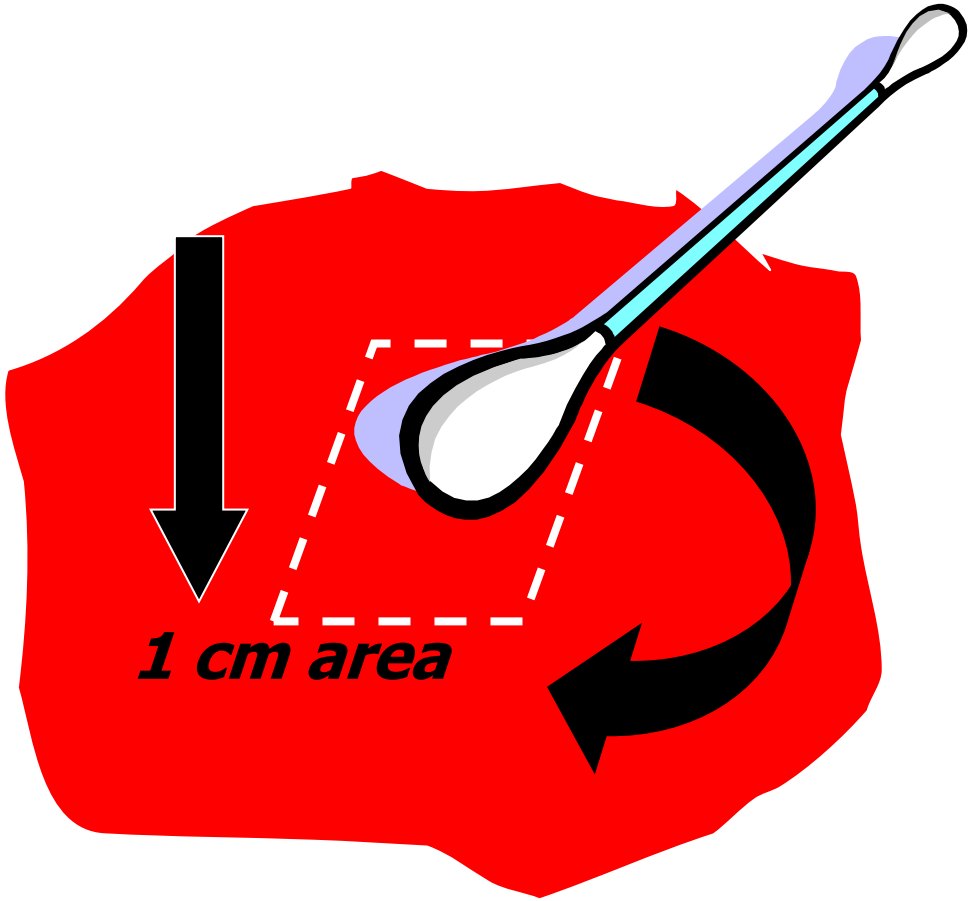


- 180 wounds of different types; swab and biopsy from same site
- Skin flora was more frequently cultured from swabs
- Swabs were able to identify all microorganisms cultured from biopsies in 131 wounds (72.8%) wounds
- Staphylococcus aureus, Pseudomonas aeruginosa, and beta-hemolytic streptococci species most common
  - Correlation was even better when these organisms were present

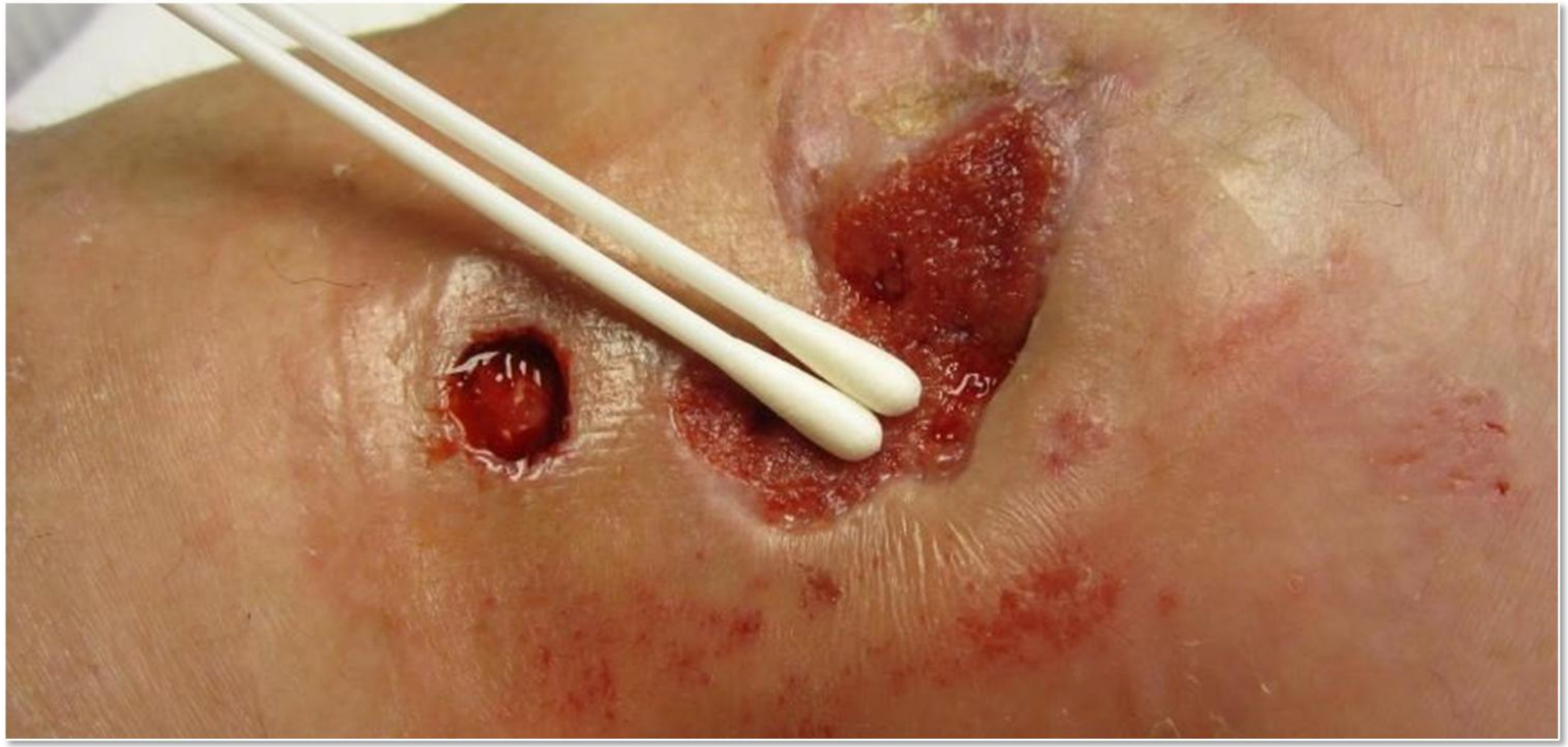
Haalboom M, et al. *Wound Repair Regen*. 2018;26(2):192-199.

Levine Technique

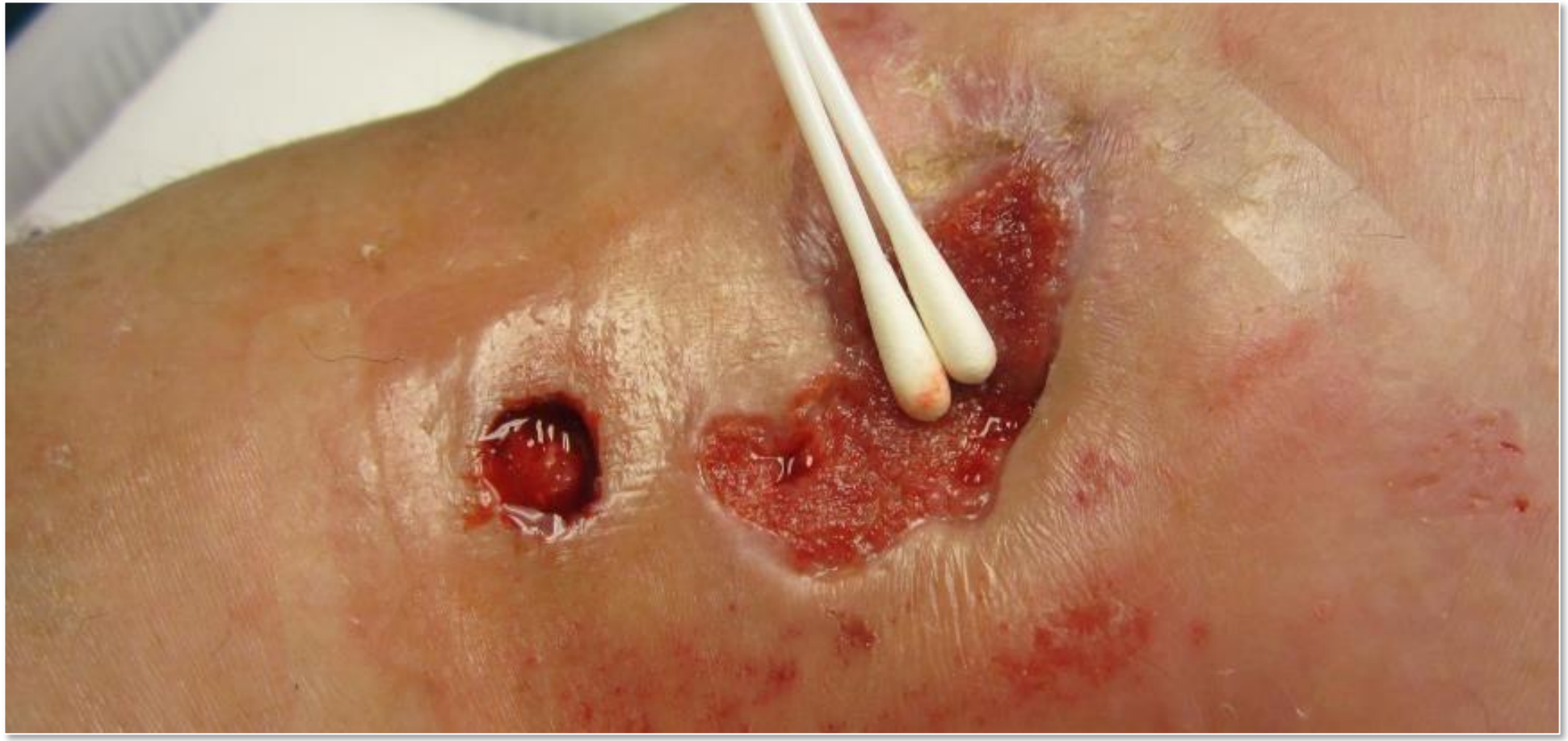
- Surface swab of a 1cm2 area of healthy tissue in the wound
- Press into wound to obtain fluid



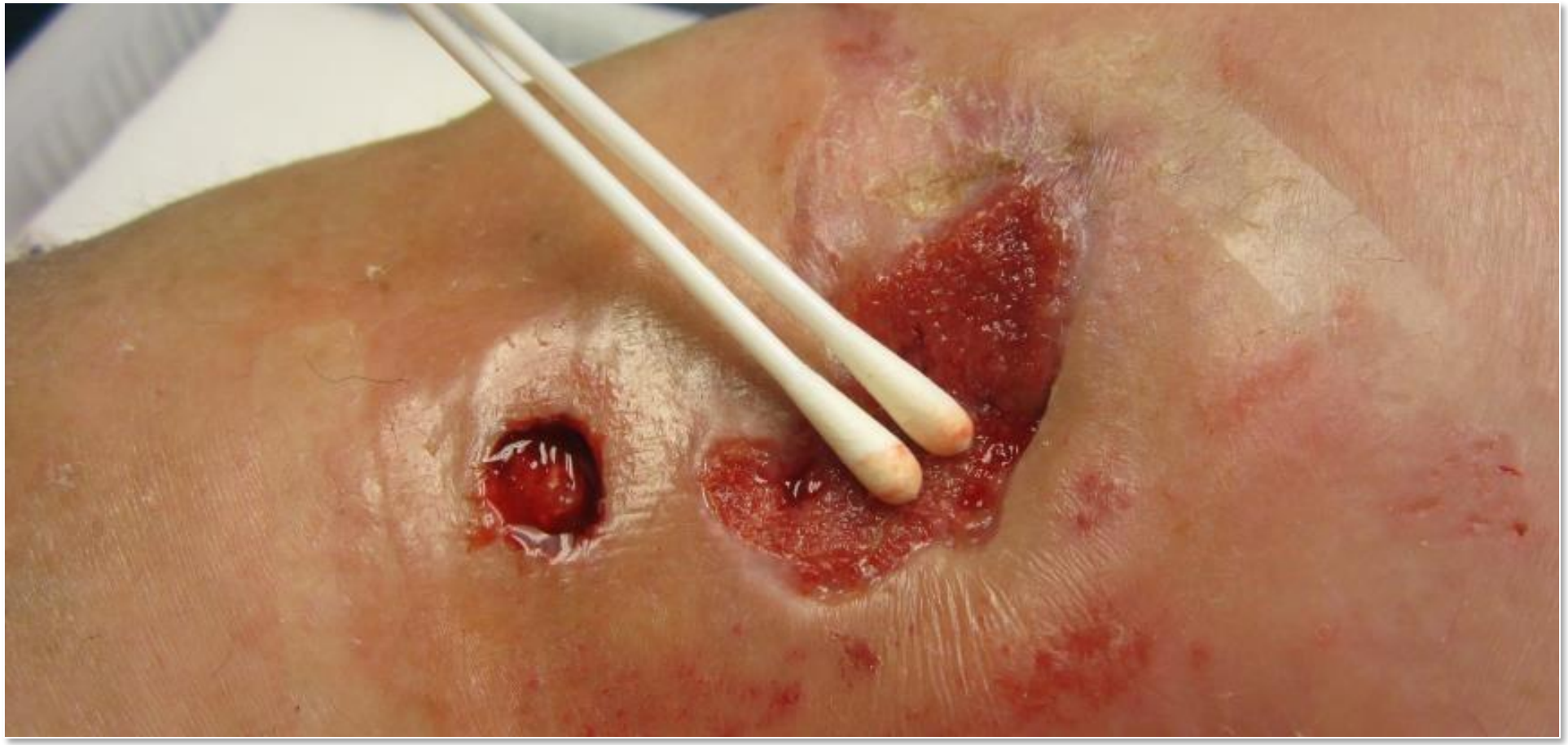
Levine Technique



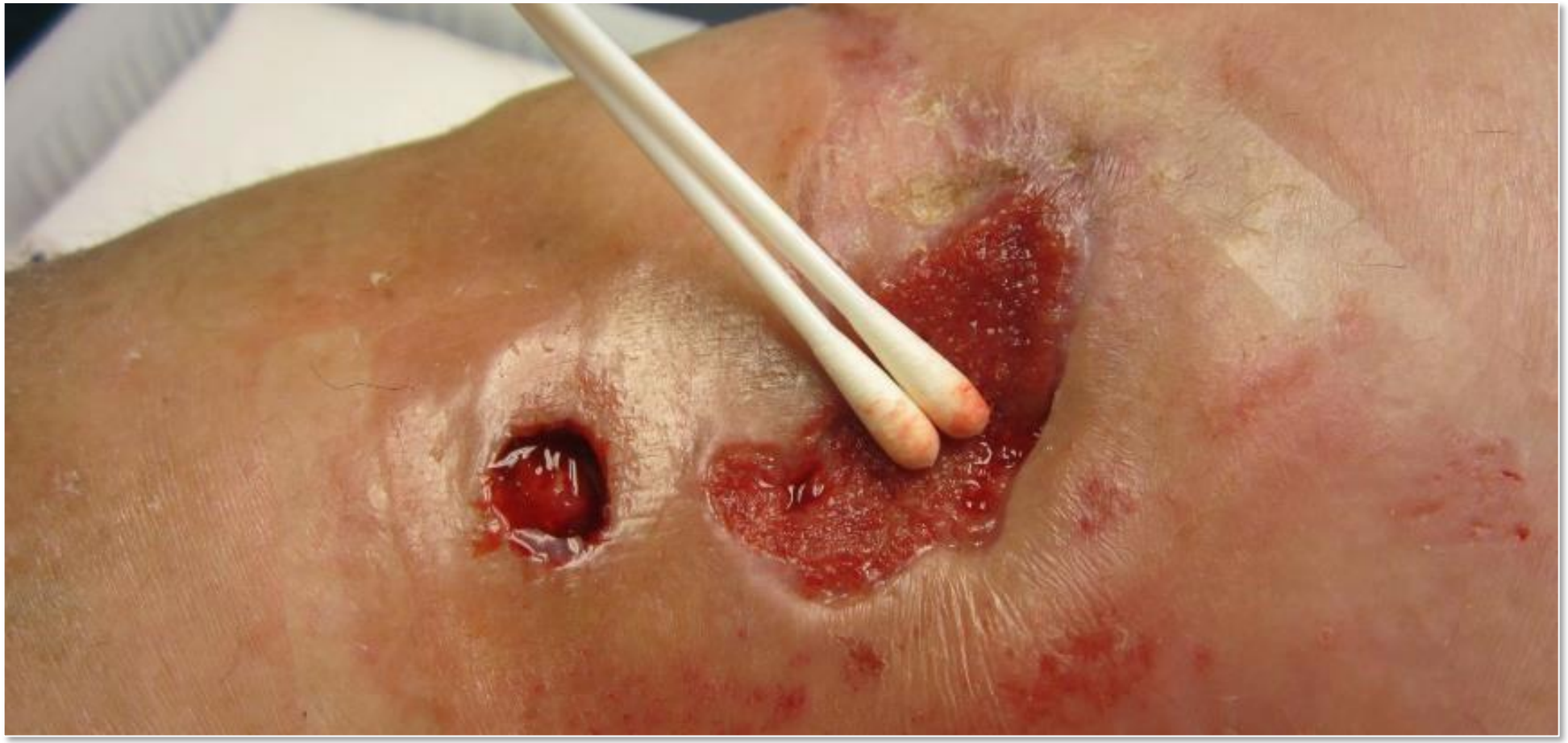
Levine Technique



Levine Technique



Levine Technique

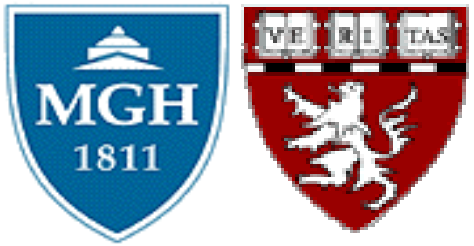


Fungal involvement in chronic wounds

MICHAEL K. MANSOUR, MD, PHD
DIVISION OF INFECTIOUS DISEASES

MASSACHUSETTS GENERAL HOSPITAL

SAWC CONFERENCE, APRIL 10 2026



What are fungi?

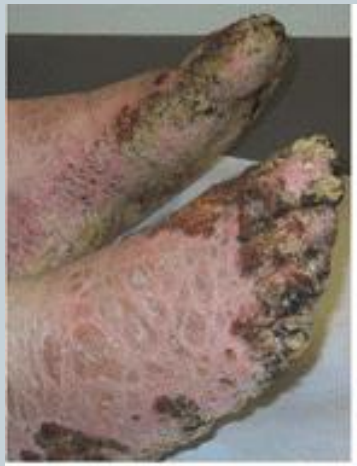
What are fungi?

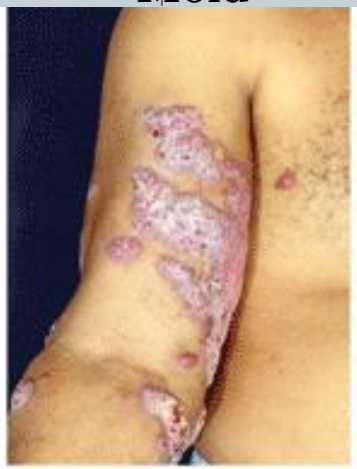
Wide spectrum of disease

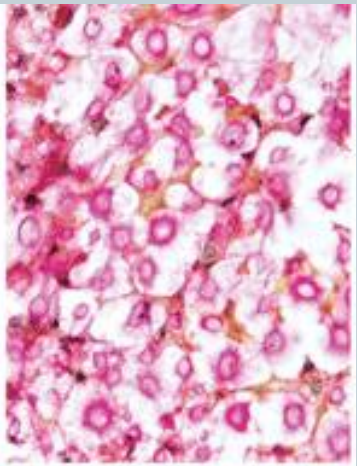
Yeast

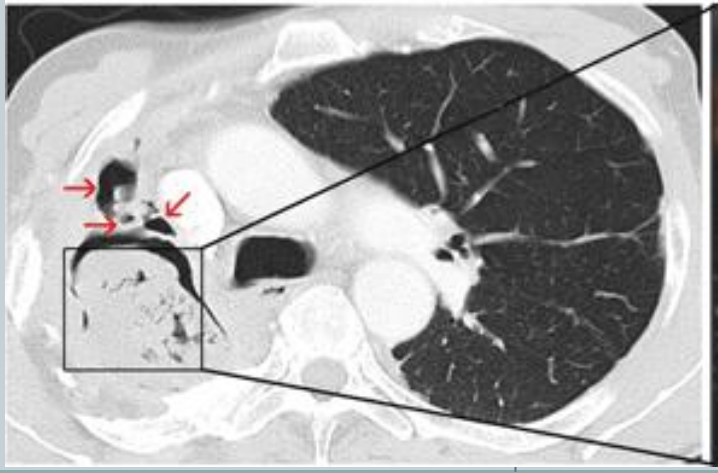
Mold

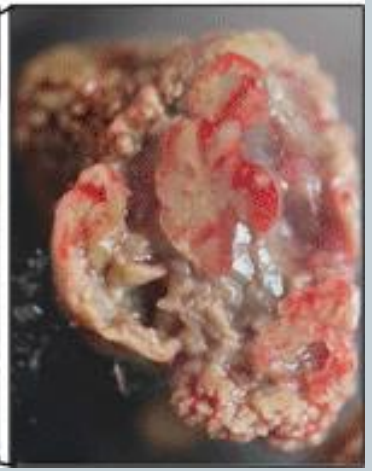
Yeast





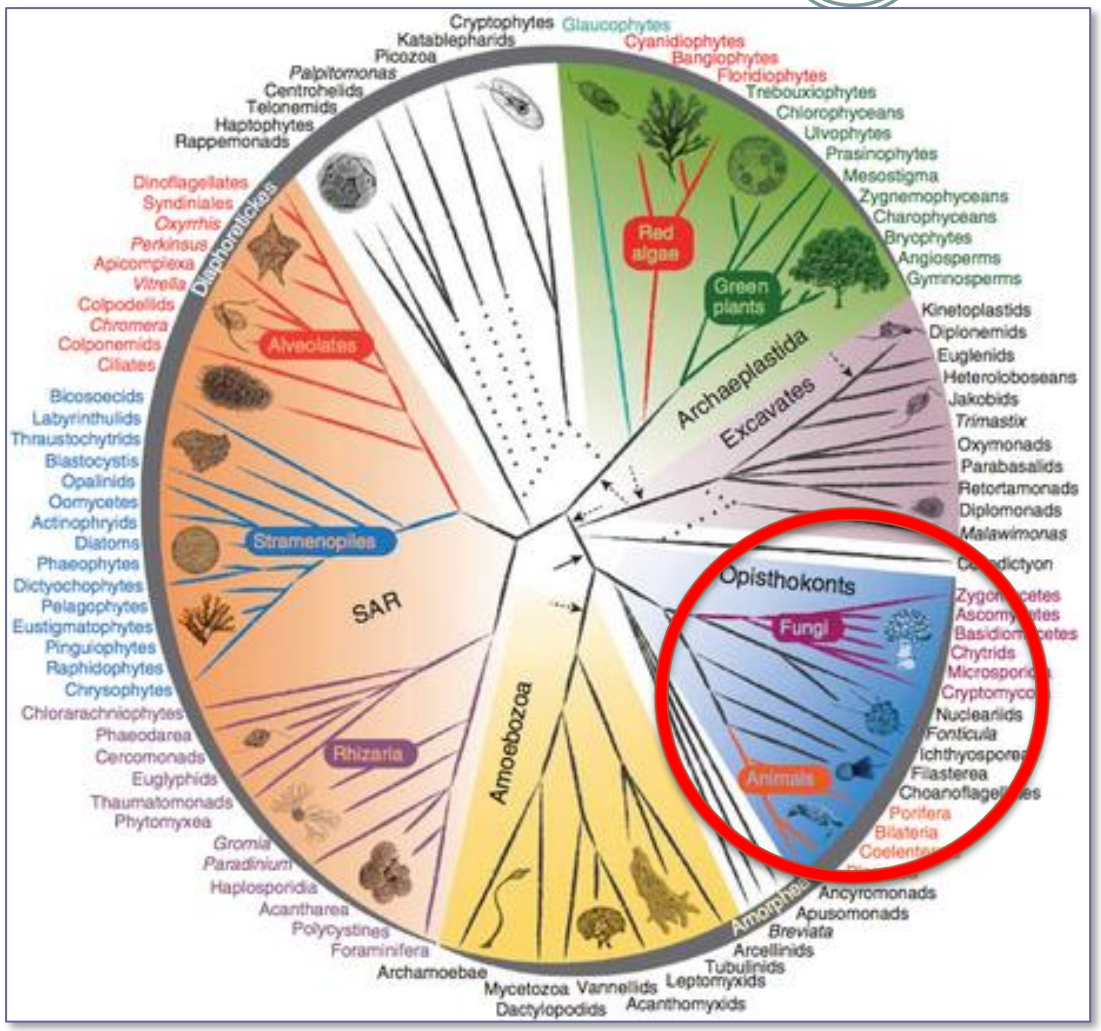






Mold

Fungi are closely related to humans



Animal and fungal kingdoms are closely related

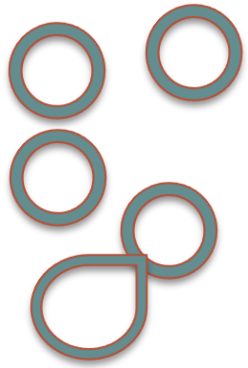
Poses a challenge for antifungal and vaccine development

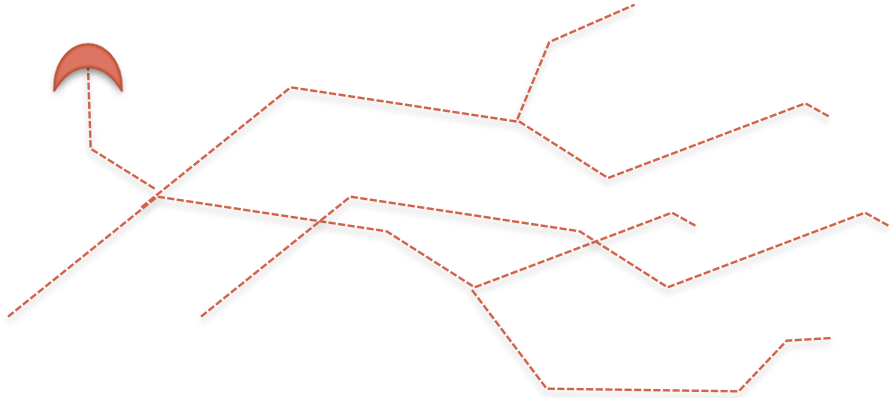
**major exceptions:

- ergosterol
- cell wall (glucan)

Cold Spring Harb Perspect Biol. 2014. 6:a016147 *(slide courtesy of Dr. Stu Levitz)*

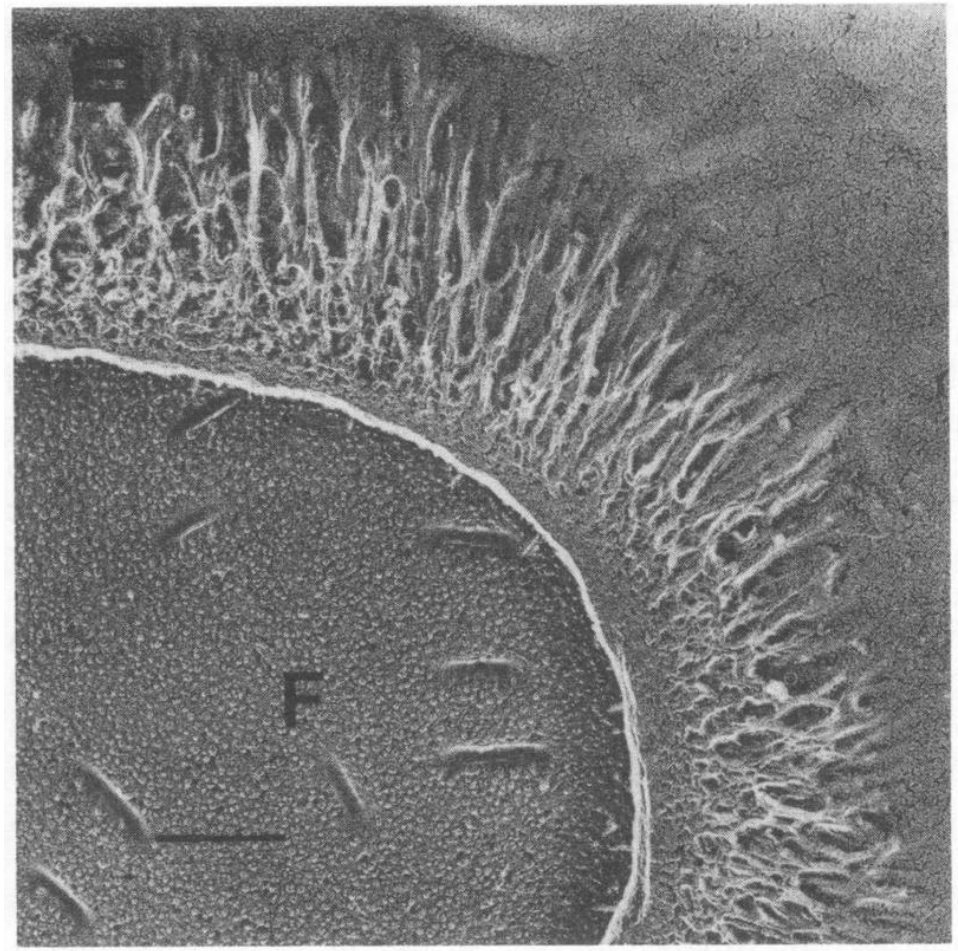
The Fungal Kingdom: *simplified*





Yeasts	<---------->	**Dimorphic**	<---------->	**Molds**
Cryptococcus		Blastomyces		Aspergillus
Saccharomyces		Histoplasmosis		Zygomycetes
Candida glabrata		Coccidiomycoces		Exserohilum
		Candida albicans		

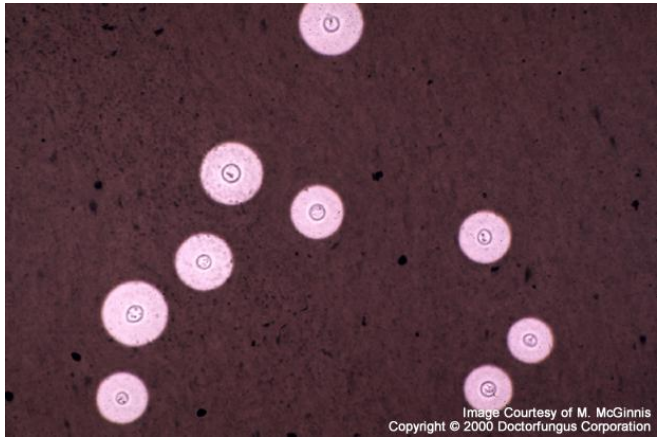
Cell wall is composed of complex carbohydrates

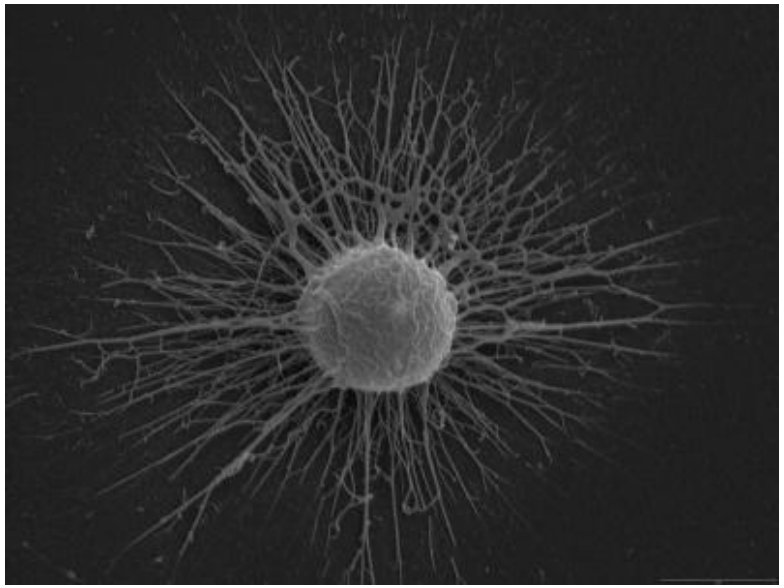


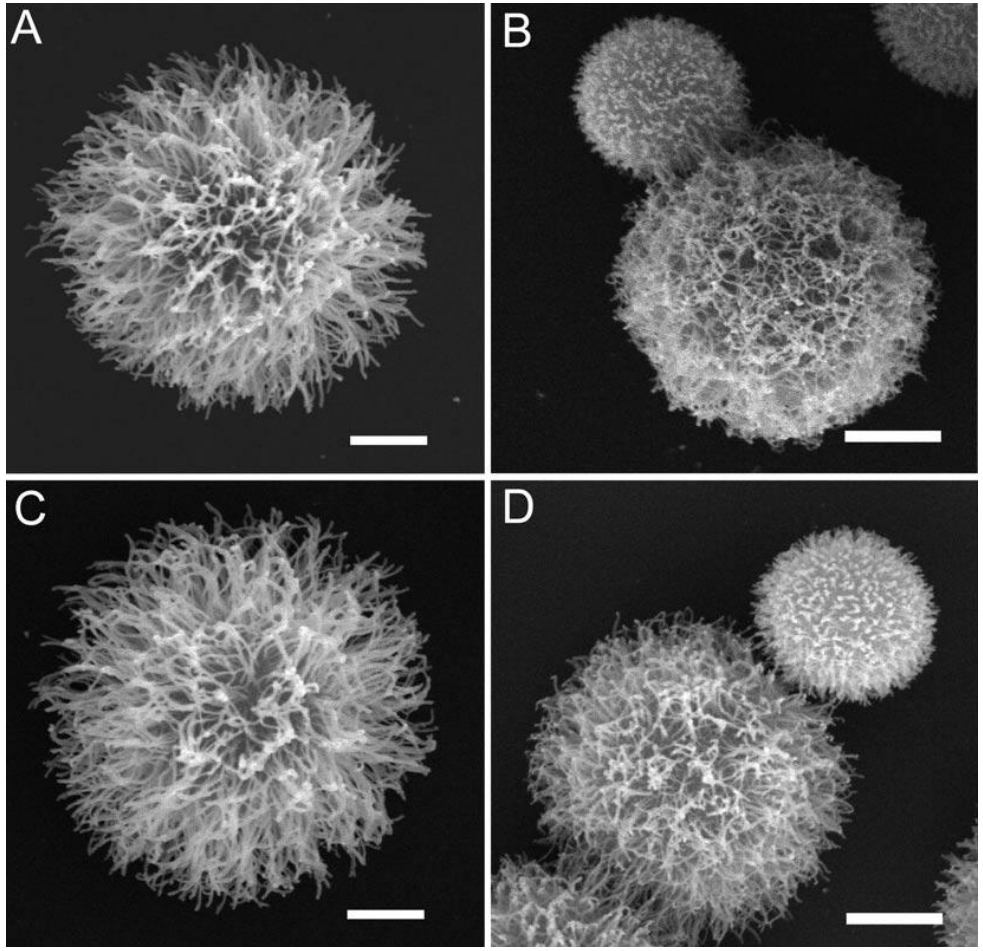
Reid, Curr Opin Immunol, 2009

Hazen, Infect Immun, 1992

Complex cell wall structures

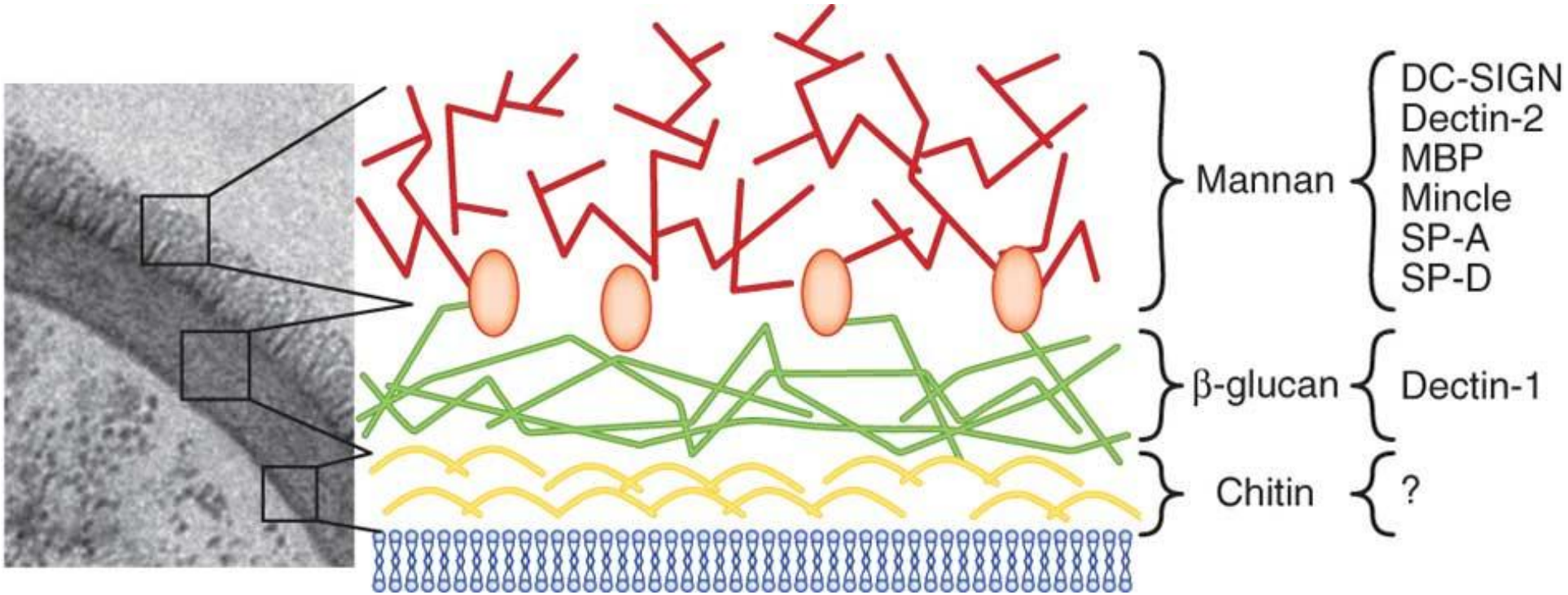






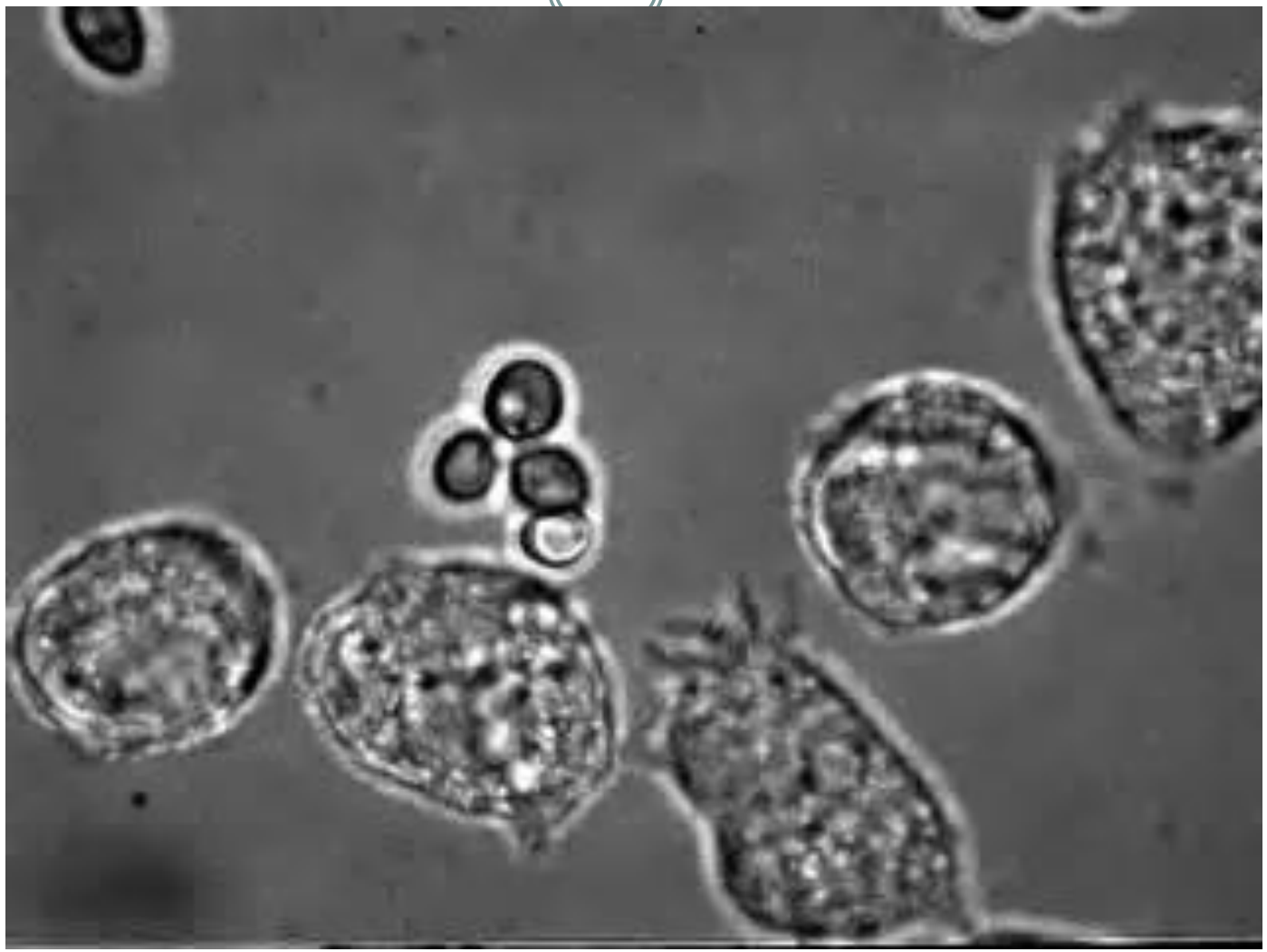
Antimicrob Agents Chemother. 48(6):2014-20.

Fungal recognition by the immune system



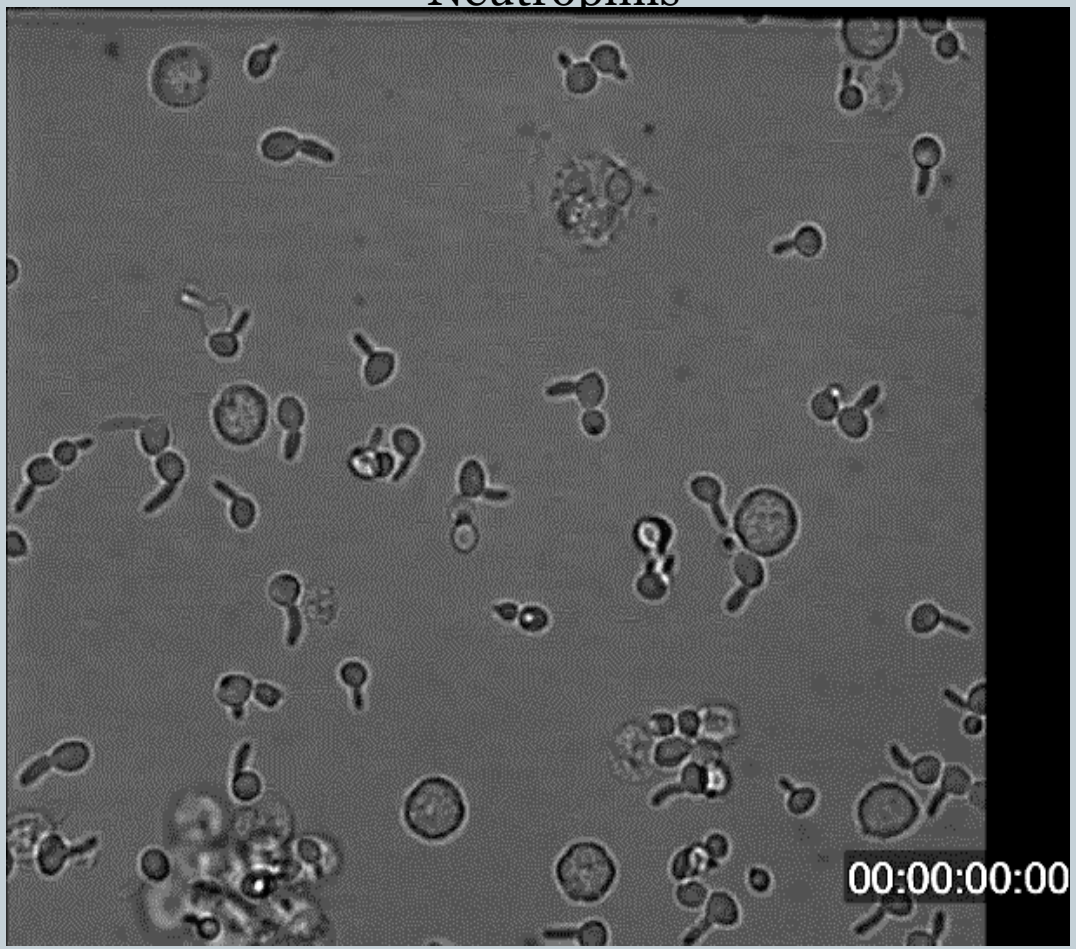
Hardison and Brown, Nature Immunol. 2012

Candida albicans recognized by macrophage



Neutrophils favor *Candida* hyphae

Neutrophils

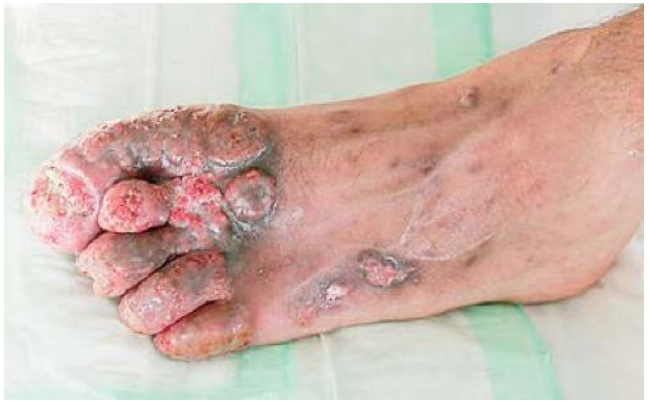


When you can't recognize the fungal cell wall

BRIEF REPORT

Human Dectin-1 Deficiency and Mucocutaneous Fungal Infections

N ENGL J MED 361;18 NEJM.ORG OCTOBER 29, 2009



Science
AAAS

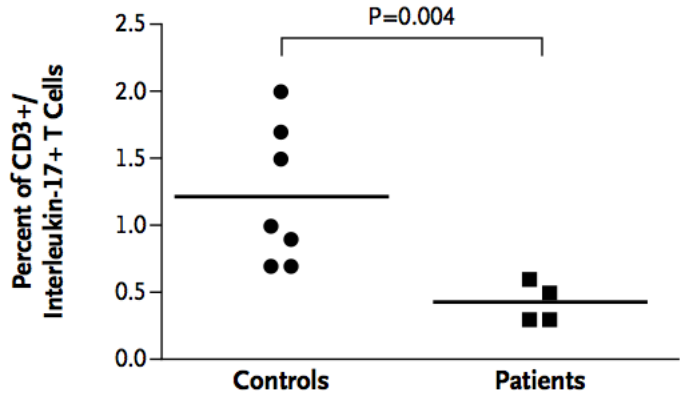
Chronic Mucocutaneous Candidiasis in Humans with Inborn Errors of Interleukin-17 Immunity

Anne Puel *et al.*
Science **332**, 65 (2011);
DOI: 10.1126/science.1200439

ORIGINAL ARTICLE

Deep Dermatophytosis and Inherited CARD9 Deficiency

N ENGL J MED 369;18 NEJM.ORG OCTOBER 31, 2013



Invasive fungi: epidemiology

Invasive Fungal Infections

- >1 Billion individuals have skin/nail infection
- 140 million mucosal candidiasis
- 1 million with invasive *Candida*

- Candida blood stream infections account for 10% of ICU admissions
- Mortality often >30-90%
- Fungal global disease burden (*Aspergillus, Candida, Cryptococcus, Pneumocystis*) is greater than TB or malaria

Bongomin, J Fungi, 2017
Brown, Science Trans, 2012
Tsiodras, Mayo Clin Proc, 2008
Thavarajah, Resp Medicine, 2009
Miceli, The Lancet ID, 2011

HIV burden

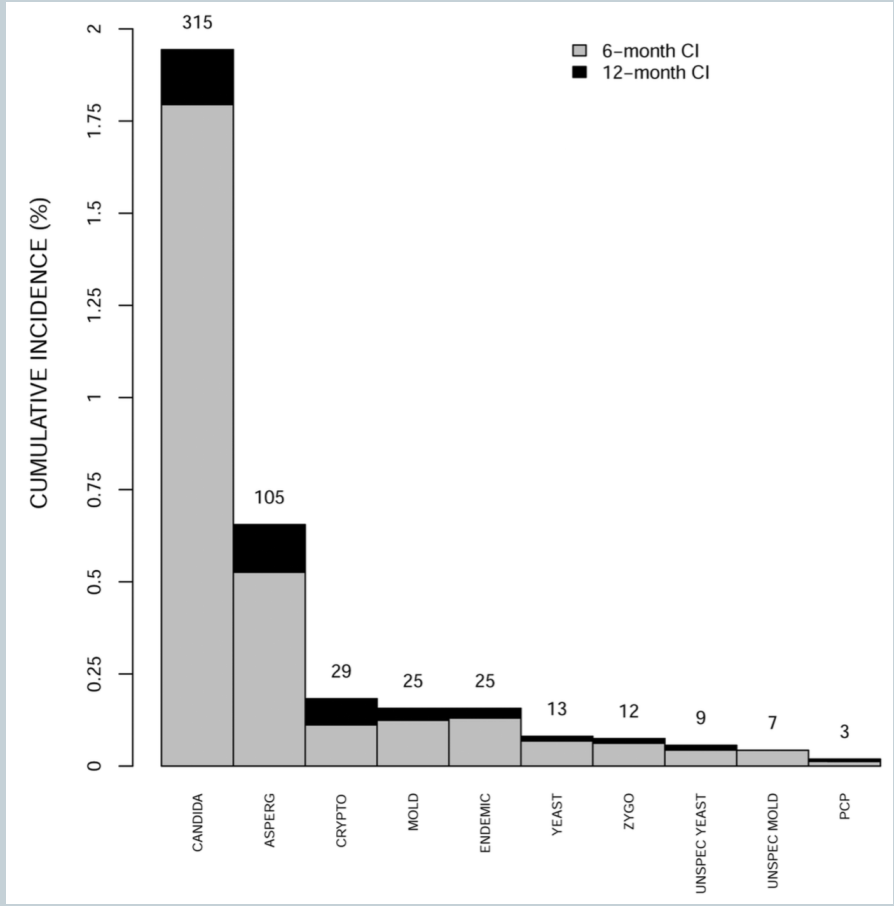
- In 2015, 1.1 million deaths from HIV/AIDS
- Approximately 50% related to fungal disease or about 1,300 deaths per day
- Breakdown:
  - Pneumocystis - 260K
  - Cryptococcus - 181K
  - Histoplasma - 80K
  - Aspergillus - 56K

Bongomin, J Fungi, 2017

Candida most common in Solid Organ Transplant

- TRANSNET consortium
  - 23 centers in the US
  - 2001-2006
  - solid organ transplants
  - 1208 invasive fungal infections

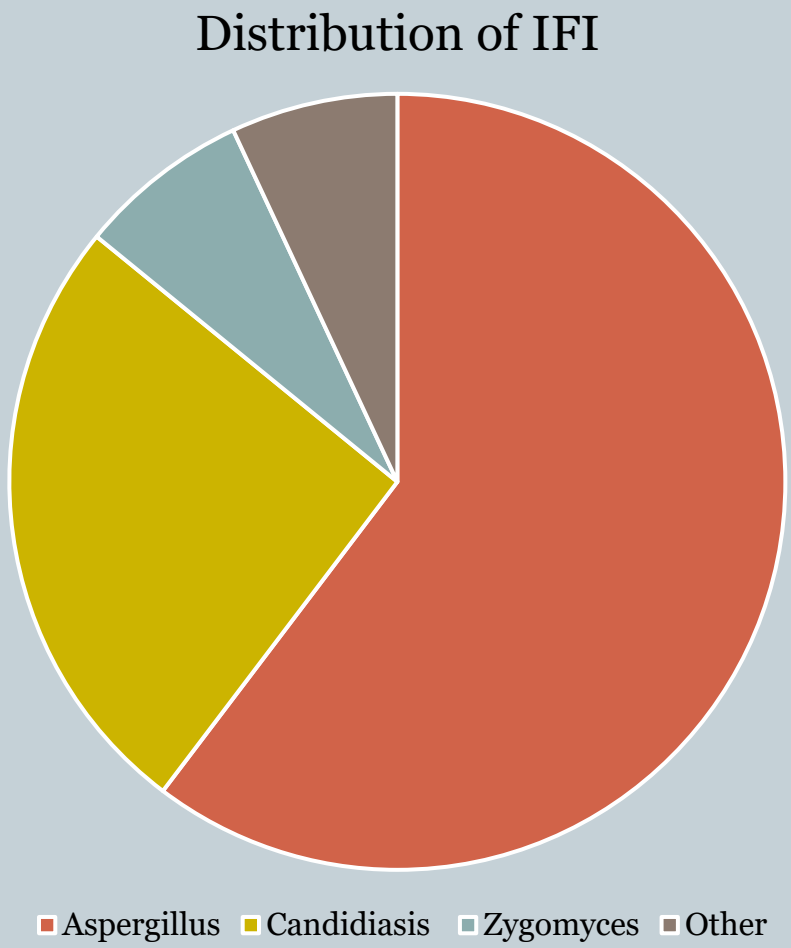
Characteristic	No. (%) of patients
Transplant type (first transplant only)	
Kidney (unrelated donor)	5506 (32.8)
Kidney (living related donor)	3166 (18.8)
Liver[a]	4468 (26.6)
Pancreas[b]	1213 (7.2)
Lung[c]	1195 (7.1)
Heart[d]	1165 (6.9)
Small bowel[e]	71 (0.4)
Other	24 (0.1)



Pappas, P. G., 2010. *Clinical Infectious Diseases*, *50*(8), 1101–1111.

IFI in Hematopoietic Stem Cell Transplantation

- Prospective Antifungal Therapy (PATH) alliance
  - 16 centers in the US and Canada
  - 2004-2007
  - 234 patients, 250 IFI



Neofytos, D., 2009. *Clinical Infectious Diseases*, *48*(3), 265–273.

Distribution of Aspergillus species

Table 3 Infecting *Aspergillus* spp.

Species	Number of isolates[a]	% of identified isolates
A. fumigatus[b]	543	72.6
A. flavus	74	9.9
A. niger	65	8.7
A. terreus	32	4.3
A. versicolor	23	3.1
A. ustus	6	0.8
A. nidulans	3	0.4
A. glaucus	2	0.3

Prospective Antifungal Therapy (PATH) Alliance

Steinbach, W. J., 2012. *The Journal of Infection*, *65*(5), 453–464.

Distribution of *Candida* species is changing

- 1218 candida isolates collected across 52 centers in the US
- 1998-2006
- Blood stream infections

- Results:
  - 50% *C. albicans*
  - 17% *C. parapsilosis*
  - 17% *C. glabrata*
  - 10% *C. tropicalis*
- Overall mortality
  - 38%

Wisplinghoff, 2014. *International Journal of Antimicrobial Agents*, *43*(1), 78–81.

Fungi in Chronic Ulcers

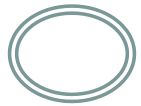
Fungal involvement in chronic ulcers

- Polymicrobial biofilms are thought to inhibit coordinated healing events
- The dominant flora involved in chronic ulceration is bacterial origin
- A smaller fraction of the ulcer microbial composition are fungal pathogens

Fungal pathogens in ulcer

- More invasive owing to both toxin/enzyme and morphologic phase changing structures
- Hyphae are long projections when the fungal cell wall remodels and projects highly invasive protrusions
- Hyphae can form as an obligate structure
- Hyphae can be stimulated after sensing protein, heat, moisture in a dimorphic agent

Fungal diversity in ulcers



AMERICAN SOCIETY FOR MICROBIOLOGY | mBio®

RESEARCH ARTICLE

Redefining the Chronic-Wound Microbiome: Fungal Communities Are Prevalent, Dynamic, and Associated with Delayed Healing

Lindsay Kalan,[a] Michael Loesche,[a] Brendan P. Hodkinson,[a] Kristopher Heilmann,[b] Gordon Ruthel,[d] Sue E. Gardner,[c] Elizabeth A. Grice[a,e]

Clinical characteristics

- Diabetic foot ulcer cohort
- Metrics:
  - White counts
  - Ankle-brachial index
  - Toe-brachial index
  - A1c
  - CRP
  - oxygen levels at the wounds edge
- 100% of participants had neuropathy by monofilament testing
- 30% had some form of complication including osteomyelitis, amputation or wound breakdown

Fungal diversity in ulcers

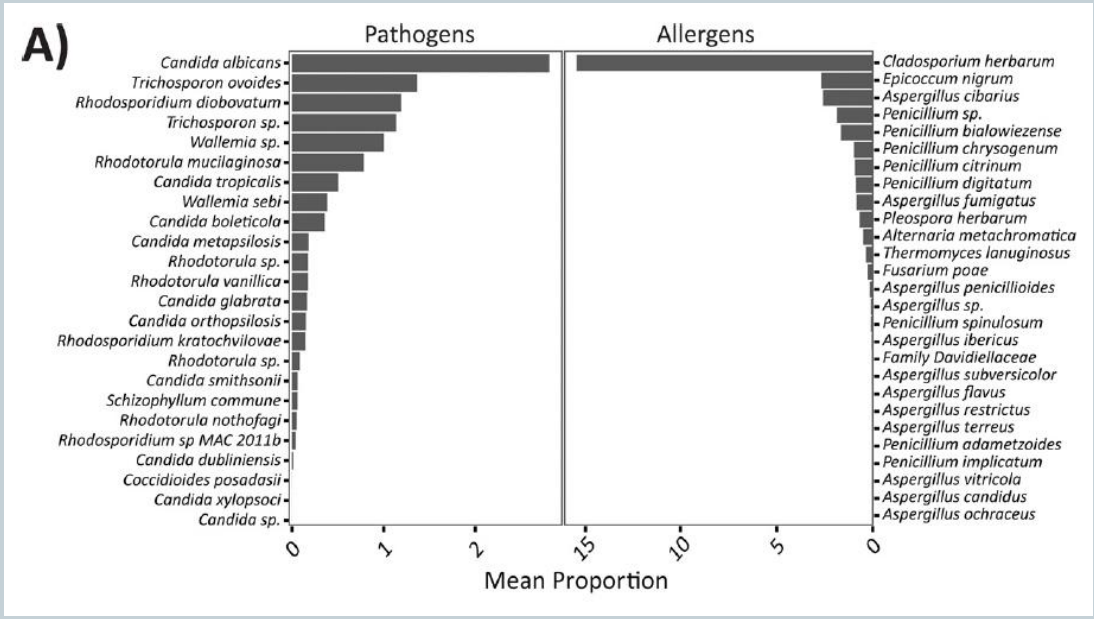
- Examined the association of fungal (mycobiome) with:
  - Clinical outcomes
  - Healing times
  - Establish bacterial and fungal mixed biofilms
- Samples 100 non-healing diabetic lower extremity foot ulcers over time
- Used ribosomal internal transcribed spacer 1(ITS1) sequencing to establish fungal phylogeny

TABLE 1 Subject demographics and wound characteristics

Characteristic	No. of subjects with characteristic or parameter value
Subjects	100
Specimens	384
Sex (male/female)	78/22
Type 2 diabetes	87
Ulcer duration, wk [mean (SD)]	33.1 (41.6)
Ulcer location	
Forefoot	73
Midfoot	20
Heel	7
End of study outcome	
Healed	75
Unhealed	5
Amputation	7
Other infection	3
Dropped study	10
Subjects with detected ITS1	79
Specimens with detected ITS1	275

Results

- Fungal identification in ulcers varied in each individual and over time
- Presence of fungi had a positive correlation with antimicrobial use
- With wounds experiencing >8week delay in healing, specific fungal pathogens were identified



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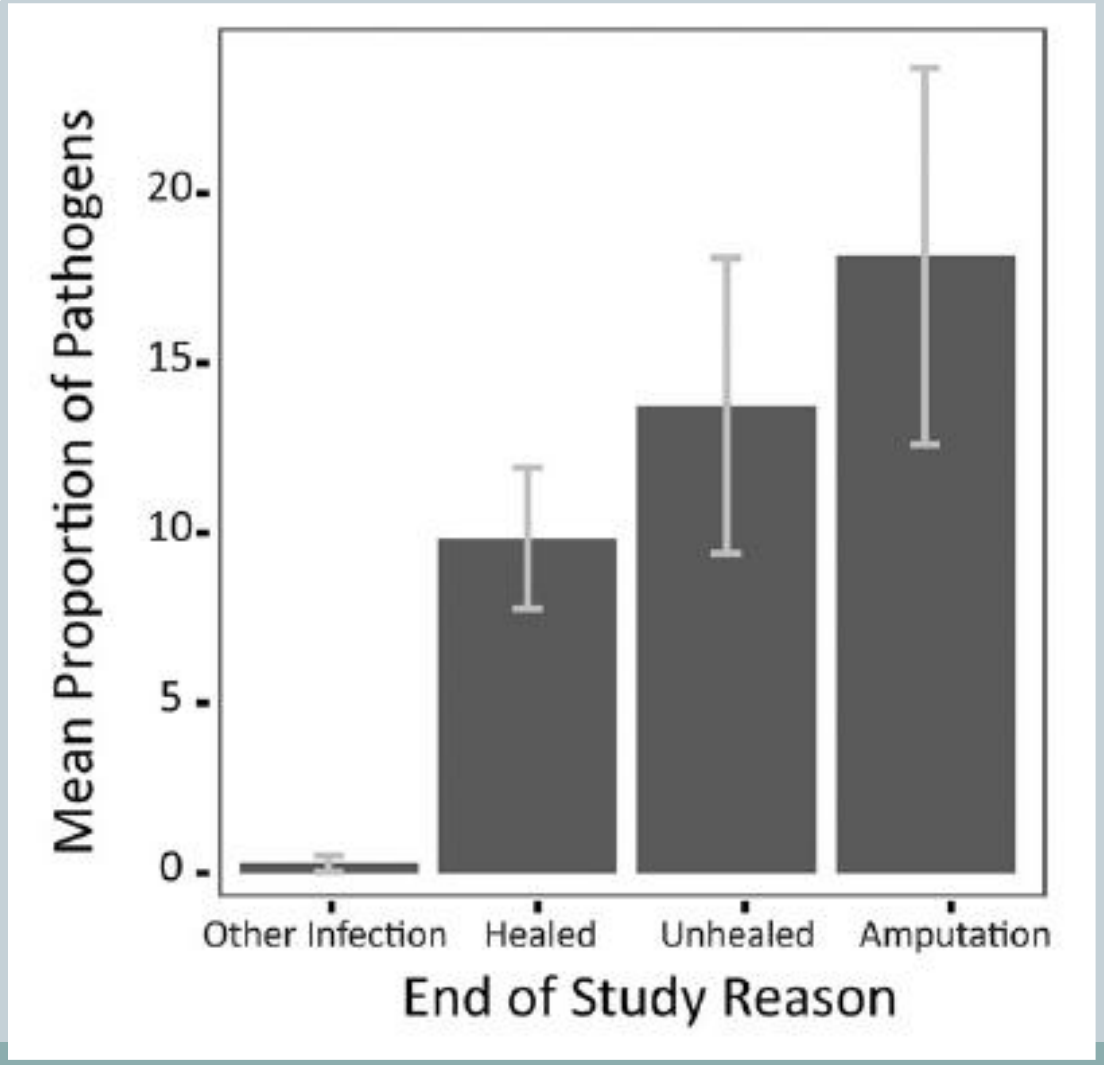

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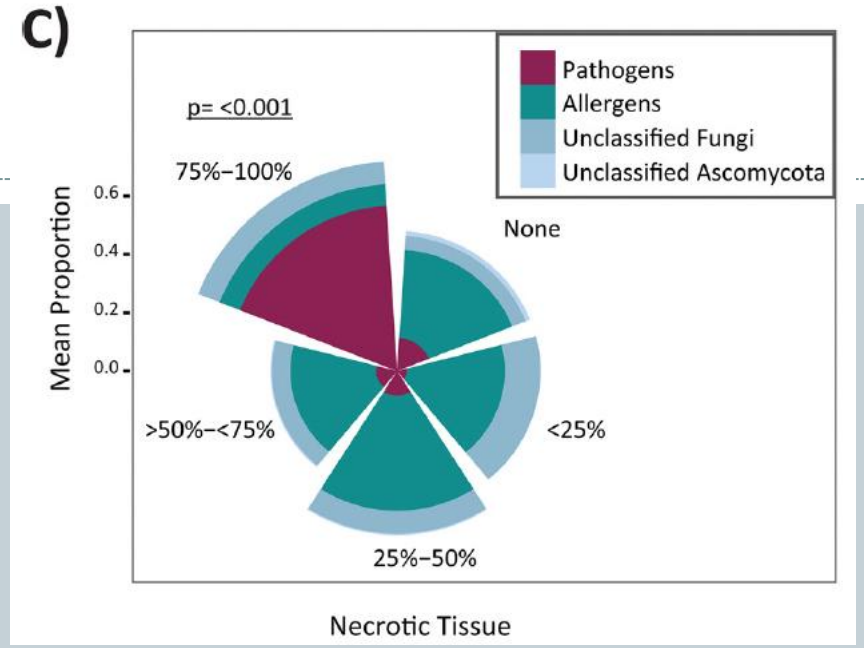
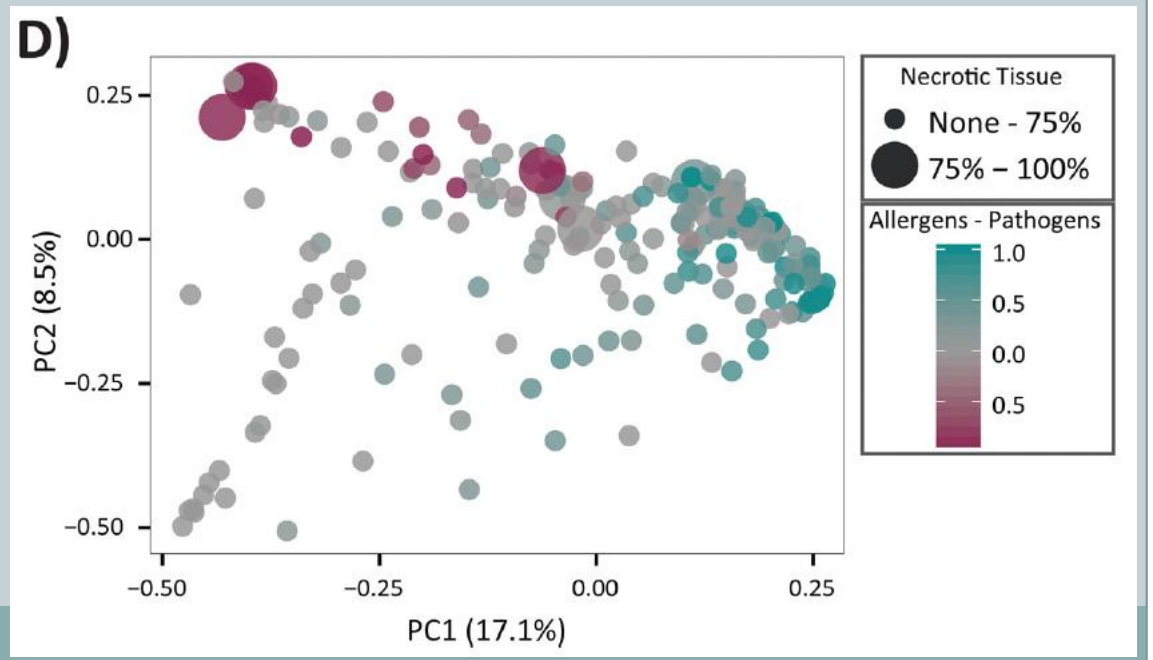
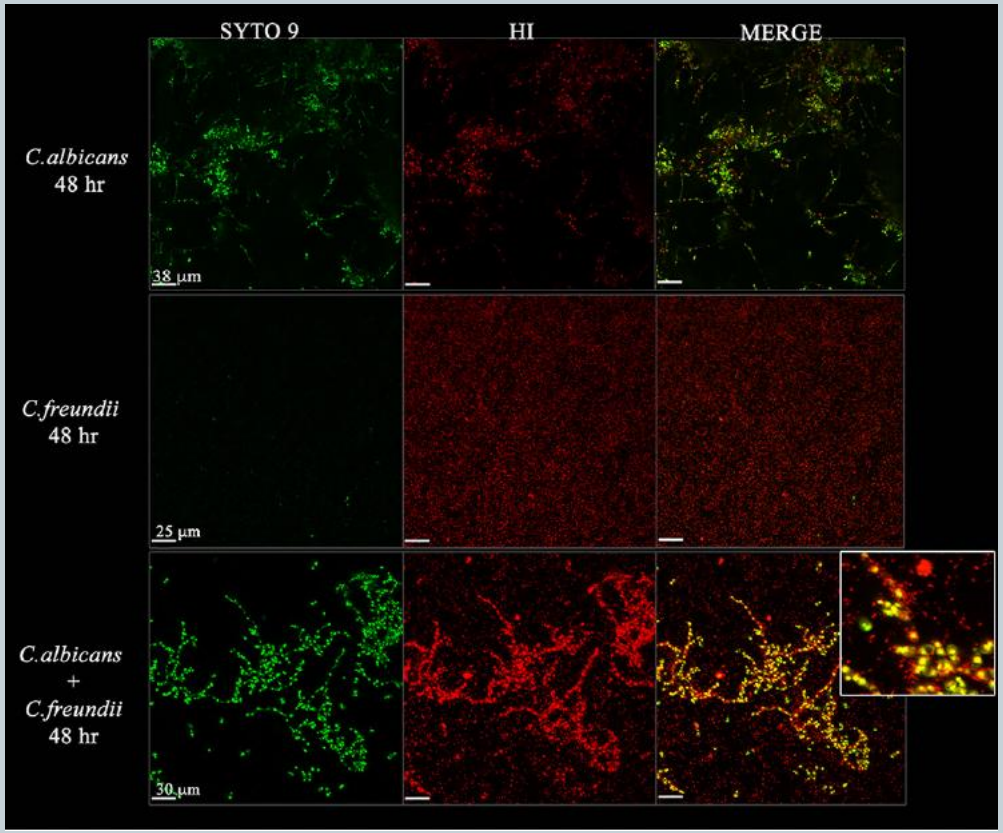




Conclusions

- Necrotic tissue was associated with highly invasive fungal pathogens
- Lower level inflammatory responses, and non-necrotic wounds had taxa consistent with allergy-inducing molds
- Wound with stable colonization were founds to have a large percentage of multicolonization with bacterial and fungal symbionts



Therapeutics

Polymers with antifungal properties

Antifungal polymer	Membrane	Cell wall	Intracellular targets	Toxicity	Reference
Synthetic antimicrobial peptides	Does not appear to permeabilise fungal cell membranes	—	Binds to nucleic acids	Dependent on hydrophobic region length	Ramamourthy et al. (2020)
Synthetic antimicrobial peptides	Membrane permeabilisation	—	Likely	Low haemolytic activity	Dodou Lima et al. (2020)
(Nylon-3 copolymers)	Membrane permeabilisation	—	—	Low toxicity, dependent on hydrophobic region length	Liu et al., 2015 Rank et al. (2018)
PHMB	Membrane permeabilisation	Cell wall target	Nucleus, binds to DNA/RNA	Low	Elsztein et al., 2011
POGH	Membrane permeabilisation likely	—	Likely	Slightly	Luo et al. (2017)
PQ-1	Membrane permeabilisation	Prevents conidia germination	Likely	—	Codling., 2003 Kilvington et al. (2013)
PEI	Membrane permeabilisation	—	Likely Cell membrane depolarisation, binds to nucleic acids	Slightly, dependent on hydrophobic region length	Azevedo et al. (2014)
Chitosan	Membrane permeabilisation	—	Nucleus, binds to DNA/RNA	Low toxicity	Palma-Guerrero et al., 2008
PHMB derivatives (PHMG-P PHMGH)	Membrane permeabilisation	Cell wall target	Likely	Low toxicity but severe toxicity when inhaled	Choi, Kim and Lee, (2017) Olmedo et al. (2018)
(N-(2-hydroxypropyl)-3-trimethylammonium chitosan chlorides) HTCC	Membrane permeabilisation	—	Likely	Low toxicity	Hoque et al. (2016)
Quaternary ammonium chloride derivatives of chitosan	Membrane permeabilisation	—	Likely	Low toxicity	Jung et al. (2020)

Ntow-Boahene W, Cook D, Good L. Antifungal Polymeric Materials and Nanocomposites. Front Bioeng Biotechnol. 2021 Dec 24;9:780328 PMID: 35004642; PMCID: PMC8740302.

Summary of mechanisms of action for polymers with antifungal properties

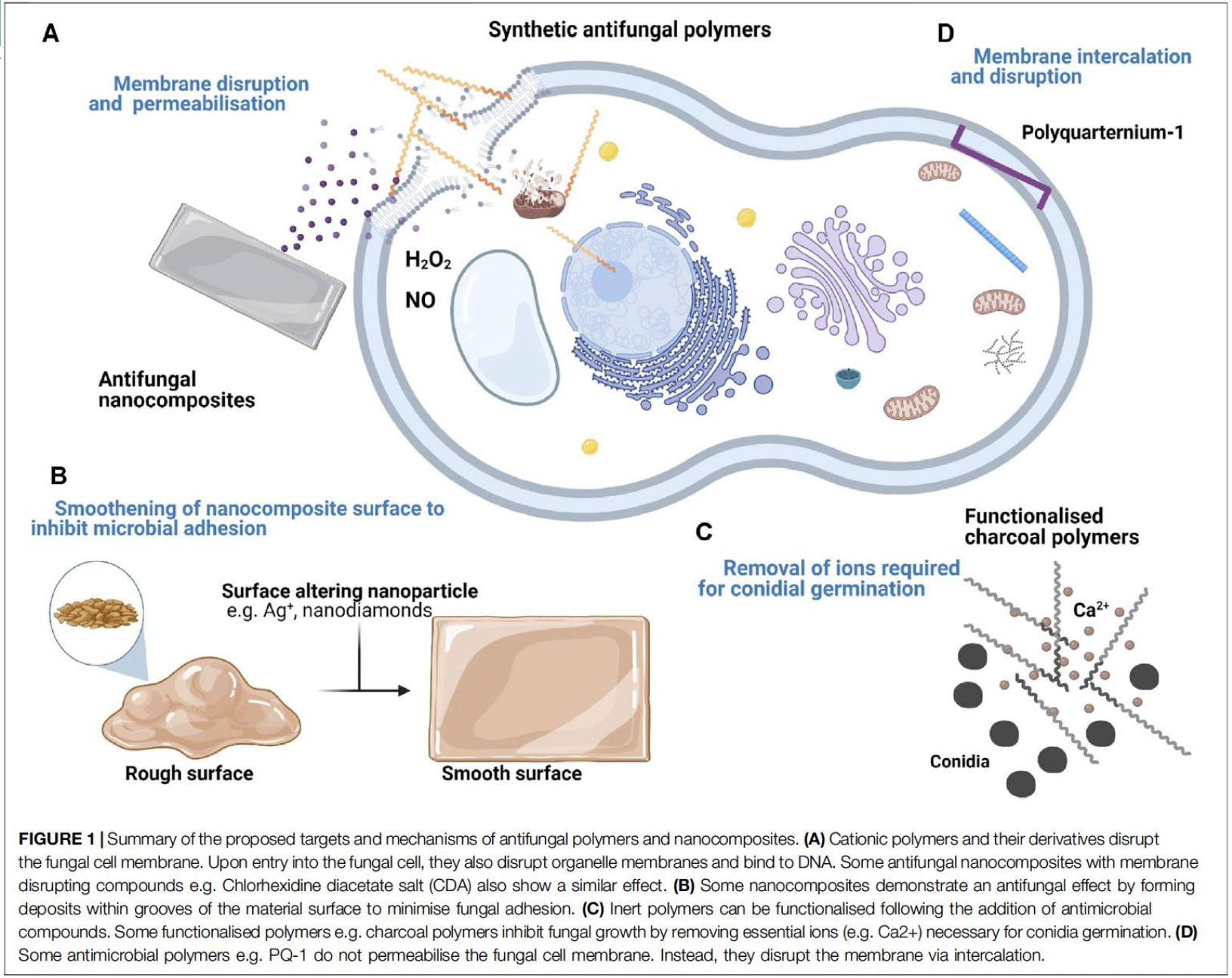
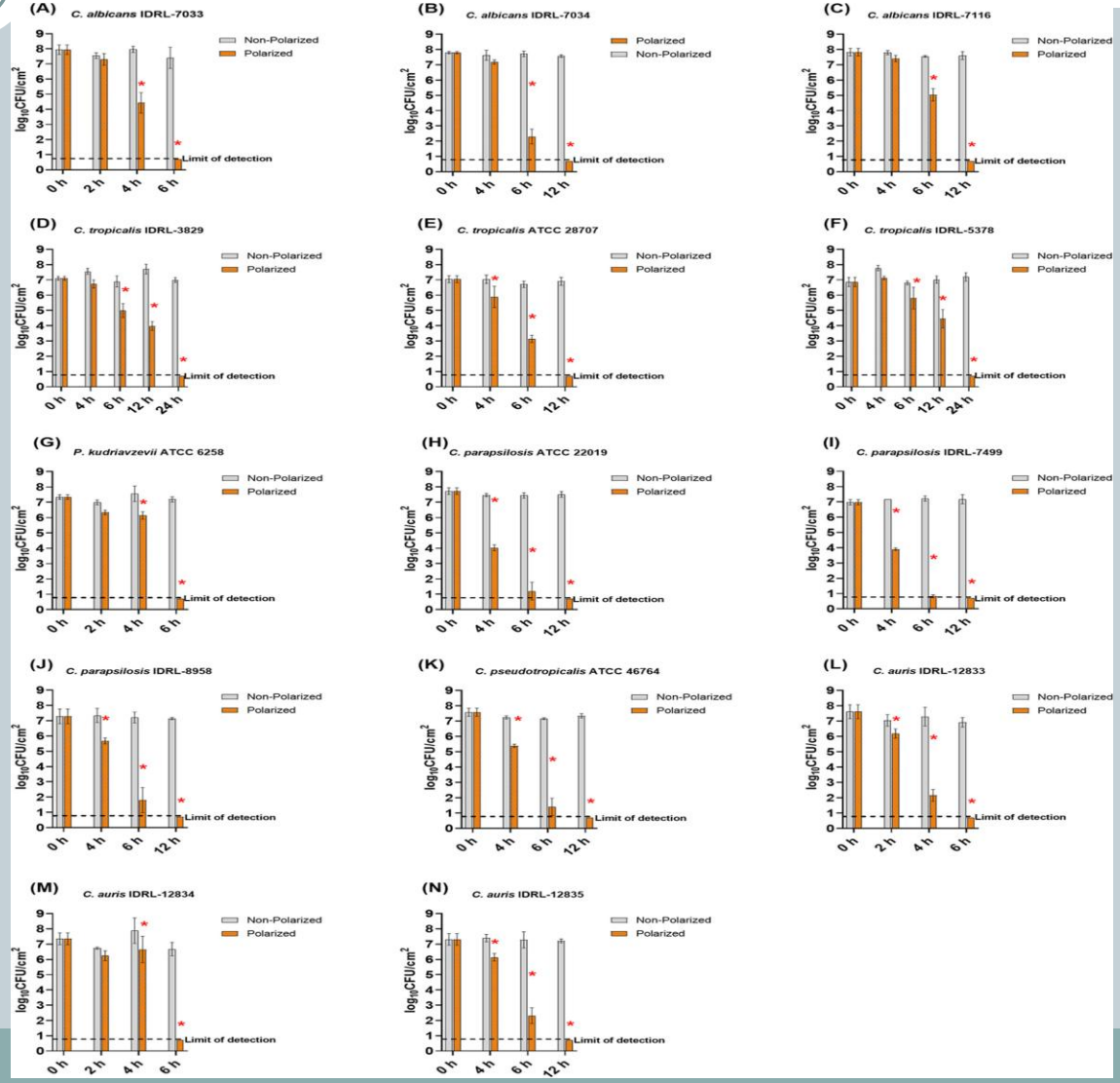


FIGURE 1 | Summary of the proposed targets and mechanisms of antifungal polymers and nanocomposites. **(A)** Cationic polymers and their derivatives disrupt the fungal cell membrane. Upon entry into the fungal cell, they also disrupt organelle membranes and bind to DNA. Some antifungal nanocomposites with membrane disrupting compounds e.g. Chlorhexidine diacetate salt (CDA) also show a similar effect. **(B)** Some nanocomposites demonstrate an antifungal effect by forming deposits within grooves of the material surface to minimise fungal adhesion. **(C)** Inert polymers can be functionalised following the addition of antimicrobial compounds. Some functionalised polymers e.g. charcoal polymers inhibit fungal growth by removing essential ions (e.g. Ca2+) necessary for conidia germination. **(D)** Some antimicrobial polymers e.g. PQ-1 do not permeabilise the fungal cell membrane. Instead, they disrupt the membrane via intercalation.

Ntow-Boahene W, Cook D, Good L. Antifungal Polymeric Materials and Nanocomposites. Front Bioeng Biotechnol. 2021 Dec 24;9:780328 PMID: 35004642; PMCID: PMC8740302.

Hypochlorous agents

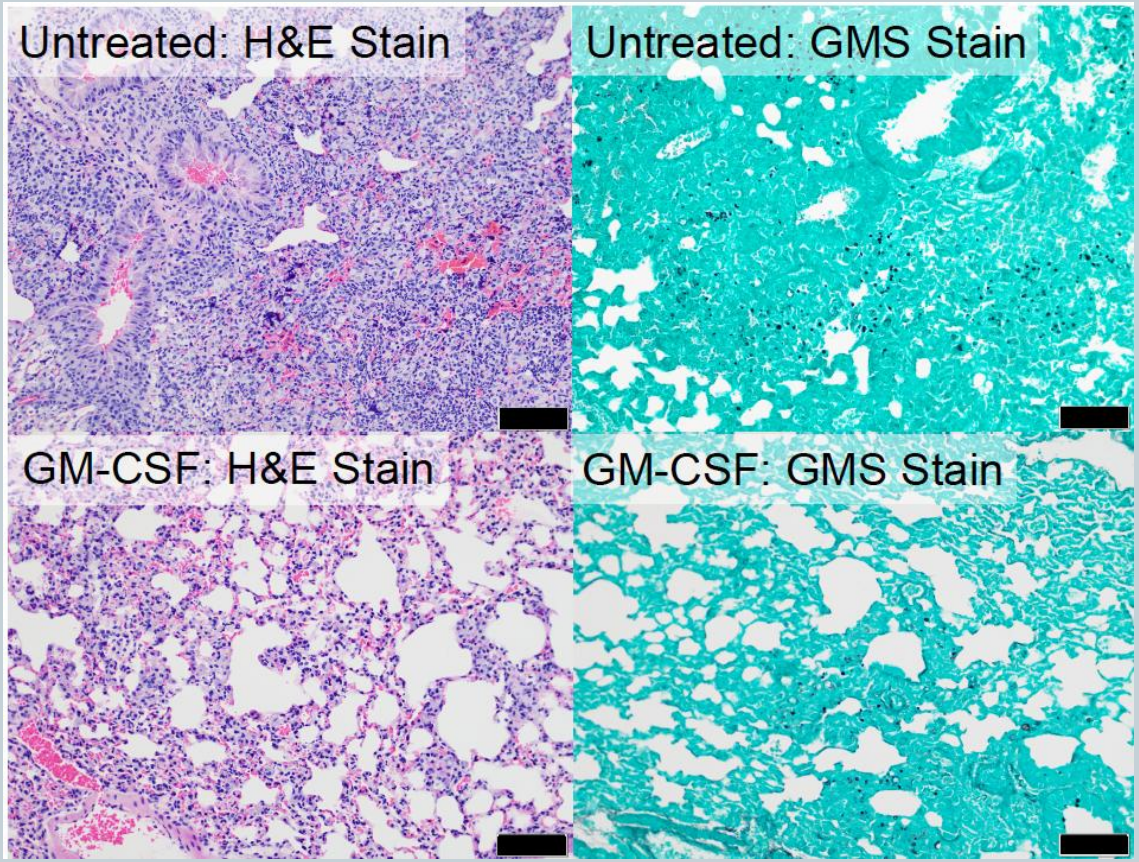
- Stabilized hypochlorous acid
- Active agent with broad anti-fungal activity
- Neutrophils, our first responders generate similar molecules for the elimination of fungal species



Kletzer J. In Vitro Activity of a Hypochlorous Acid-Generating Electrochemical Bandage against Yeast Biofilms. Antimicrob Agents Chemother. 2023.

Other agents: *future concepts*

- Can we augment the immune system?
- Controversial, but supporting evidence is building
- Certain cytokines (FDA approved) can increase immune activity resulting in clearance of fungi (and likely bacteria)



Mansour MK, augmentation of neutrophils against fungal pathogens, unpublished data

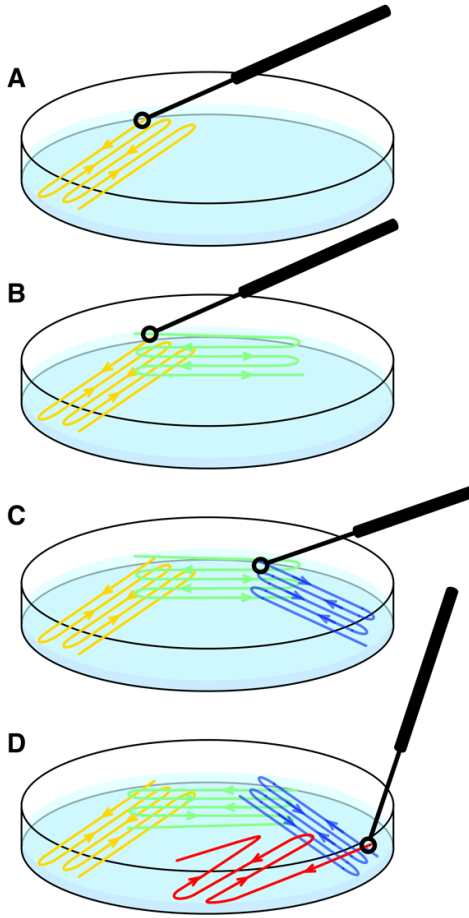
Conclusions

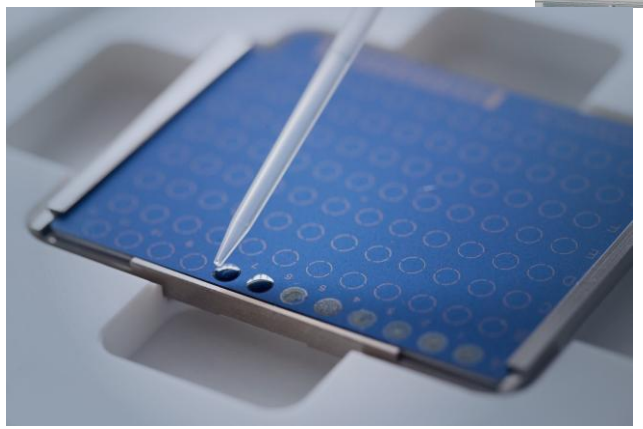
- Host determination is most critical
  - Is your patient immune compromised?
    - Diabetes
    - Transplant
    - Steroid use or other biologics
    - Cancer
- Chronic wounds can be polymicrobial including fungi
  - Species matters; some are quite invasive
- If there no evidence of deep infection (osteo), fungi can be treated with topical agents
- Infectious Diseases is always happy to help with decision making in complex patients where wounds are not responding as expected

Cultures: *real or commensal?*

- Surface cultures are notorious for isolating non-pathogenic species
- Deep biopsy (tissue or bone) always provide an improved clinical outcomes with true pathogen directed therapy
- Approximately 20% of any identified agent will not be an invasive pathogen
- Higher correlation with invasiveness:
  - Bacteria: MRSA, Pseudomonas
  - Fungi: Candida species, Mucorales

What happens when you send down a swab?





Consensus and Controversies in Contamination and Bioburden

Practical Infectious Disease Approach and Antimicrobial Strategy

Sujay Dutta, MD, MS

Does Our Patient Have An Infected Ulcer?

Take a focused history

- How long has the ulcer been open?
- Is the ulcer and surrounding tissue painful, and has pain increased?
- Have the quality and quantity of the discharge changed? If so, how has it changed?
- What treatment(s) – both topical and systemic – have been rendered thus far? What has helped?
- Look for conditions or medications that impair host immunity: DM, PVD, venous insufficiency, HIV infection, use of biologic agents for chronic conditions, chemotherapy, immunotherapy

Physical Examination Clues

General Appearance:
Uncomfortable, change in walking gait, limping, change in mobility. toxic, febrile, diaphoretic

Ulcer:
Change in size, exudate odor, necrotic tissue, condition of periwound – erythema, tenderness, calor, crepitus

Is There an Alternative Reason for the Change:
Poor glycemic control, increased peripheral edema, signs of worsening arterial circulation, tissue ischemia, change in pressure over the ulcer area, medication

Laboratory Clues to Infection

WBC COUNT, LEFT SHIFT, BANDEMIA — Infection may be present even with normal WBC, especially in elderly and immunosuppressed patients

C-REACTIVE PROTEIN — Can be very useful; rises rapidly and falls rapidly. Very helpful in infected diabetic foot ulcers. Look for changes over weeks. More accurate than ESR or WBC in DFU infections

ESR (Westergren most commonly used) – Longer-term marker of infection and inflammation. May be helpful in monitoring efficacy of antibiotics, esp for chronic osteomyelitis.

PROCALCITONIN – Useful in conjunction with other markers

Radiographic Clues for Infection

PLAIN X-RAY – Quick and inexpensive. Moderate specificty for osteomyelitis, but low sensitivity.

MRI (without contrast) – Excellent sensitivity and specificity for osteomyelitis. Adding contrast may help with confirming abscess, but not for osteomyelitis.

TRIPLE PHASE BONE SCAN – Useful older modality, can be very helpful if MRI cannot be done.

SOFT TISSUE ULTRASOUND – Helpful in detection of fluid collections, possible abscess

Culture of Ulcer vs Molecular Methods

- Already discussed in previous presentation
- Interpret results in the context of your local antibiogram
- Be aware of organisms which may be newer, renamed from previous nomenclature, or changes in resistance pattern
- When in doubt or unsure, consult your local infectious disease specialist

When Should We Use Systemic Antibiotics

- Local covert infections: Systemic antibiotic use is not encouraged. IDSA, IWGDF, and other societies support the use of systemic antibiotics for overt infections with signs of cellulitis, osteomyelitis, abscess, or sepsis
- Worsening malodorous drainage
- High bacterial burden (>10^6 cfu/gram of tissue), esp for presence of invasive bacteria: *S. aureus*, beta-hemolytic *Streptococci*
- Lower microbial burden in immunosuppressed hosts

Personal Point of Query

- The role of short courses of systemic antibiotics, appropriately dosed, in conjunction with modern topical antiseptics and appropriate slough removal: Can this shorten the time to healing in chronic wounds that are in the covert local infection state?
- Clinical trials – which were used to support guideline recommendations against using systemic antibiotics – were small, often with unknown or high bias, and more than 10 yrs old
- In the case of both infected venous leg ulcers and diabetic foot ulcers, new well-controlled and robust trials are needed to answer this question

You've Decided to Use Systemic Antibiotics

- Signs of active infection, evolving sepsis: Empiric treatment while waiting for culture results
- Use proper doses, defined durations (eg, 1 wk for cellulitis)
- If no improvement or deterioration, IV antibiotics or hospitalization may be needed
- Consider antibiotics with good bioavailablity: FQ,TMP/SMX, doxycycline>pencillins , cephalosporins
- Addition of rifampin, if possible; has activity vs biofilm
- Modify treatment once culture and sensitivity are back

Should We Use Topical Antibiotics?

Which statement is most appropriate?

A) If the pathogen is sensitive, topical antibiotics can be effective and avoid the use of systemic agents

B) The risk of developing resistant organisms and skin sensitivities is high, and we should not use them

C) Both A and B; benefits outweigh risks, so use it

D) Antibiotic creams and ointments are both useful, depending on the moisture status of the wound

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Resistance Rates with Common Topical Antibiotics

1. Mupirocin: (2022): 10.8% overall
    a) 22.5% for MRSA
    b) 3% for MSSA
    c) Pediatric Population / NYC: 31.3% overall
2. Gentamicin: 17% (USA, Canada) to 38% (India) for *P. aeruginosa*
3. Neosporin (Neomycin, Polymixin B, Bacitracin)
    a)<5% for S. *aureus* and <1% for *P. aeruginosa*
    b) Higher resistance rates for MRSA strains (USA 300 CLONE)
    c) Allergic dermatitis rates for Neosporin (Neomycin, Bacitracin being most allergenic):1%-8%

Silver Sulfadiazine Cream

- Broad spectrum activity
- Resistance rates vs *S. aureus* remain low (<1%), including MRSA strains (however data is older 1997-2010)
- Silver has multimodal action against bacteria, including *S. aureus*
- Binds multiple proteins and causes rapid membrane damage
- Recent study in acute burn patients showed it has excellent antimicrobial effect, but newer dressings (silver-based) had faster healing times
- Effect is diminished in biofilm; silver cannot eradicate biofilms *in vitro*

What Do the Guidelines Favor?

- IWGDF/IDSA Guidelines do not recommend routine use of topical antibiotics in chronic wounds/ diabetic foot infections
- Rising rates of resistant organisms and skin sensitivities are a significant concern
- Clinical studies do not demonstrate significant benefit or are not robust enough to make a firm conclusion
- Topical antiseptics (such as pHA) are recommended
- Anti-fungal agents: Topical vs systemic; insufficient data to support topical anti-fungal agents, more studies are needed
  - Systemic treatment (eg, fluconazole) may be of benefit in faster healing times for diabetic foot ulcers

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Thank You