BIOFILMS IN CHRONIC RHINOSINUSITIS

PRESENTING: ANGELIA NATILI, MD

ATTENDING: BRUCE LEIPZIG, MD

THE UNIVERSITY OF TEXAS MEDICAL BRANCH

DEPARTMENT OF OTOLARYNGOLOGY

GRAND ROUNDS PRESENTATION

FEBRUARY 19, 2014

Series Editor: Francis B. Quinn, Jr., MD, FACS
Archivist: Melinda Stoner Quinn, MSICS

CONFLICTS OF INTEREST

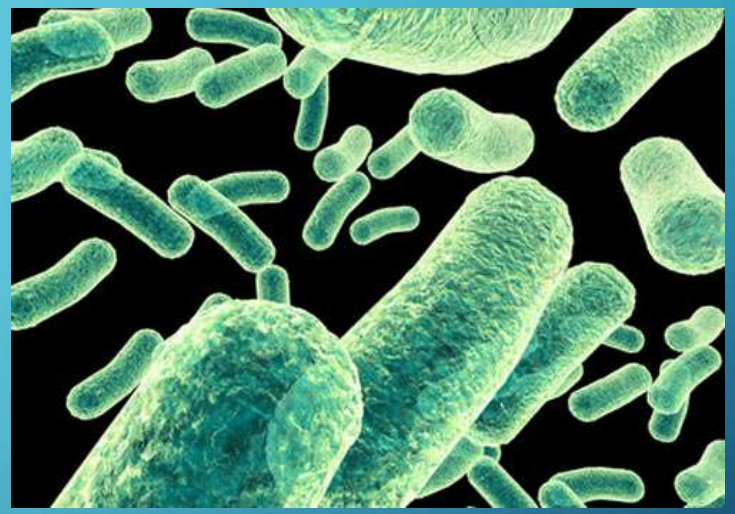
- Neither author has conflicts of interest

Biofilms in Chronic RhinoSinusitis (CRS)

GOALS OF THIS TALK

- At the completion of this talk the listener should be able to:
  - identify common characteristics in biofilm growth and resistance to antibiotics
  - understand current difficulties in clinically identifying biofilms
  - identify several areas of research towards disrupting biofilms

WHAT IS A BIOFILM, ANYWAY?



- Bacteria can exist in two forms
  - Planktonic
  - Biofilm communities
- The planktonic form is a free floating individual organism
  - This form is most commonly conceptualized
  - Treatment modalities such as antibiotics are aimed at the assumption that this is the primary form

Angelia Smith, MD, Farrel Joel Buchinsky, MD, and J. Christopher Post, MD, PhD, MSS,
"Eradicating Chronic Ear, Nose, and Throat Infections: A Systematically Conducted Literature Review of Advances in Biofilm Treatment" Otolaryngology -- Head and Neck Surgery 2011 144: 338

http://www.genengnews.com/insight-and-intelligenceand153/literature-review-bacteria-reprogram-host-cells-in-order-to-survive-and-spread/77899799/

BIOFILMS ARE A LITTLE MORE COMPLEX

The organization of a mature biofilm

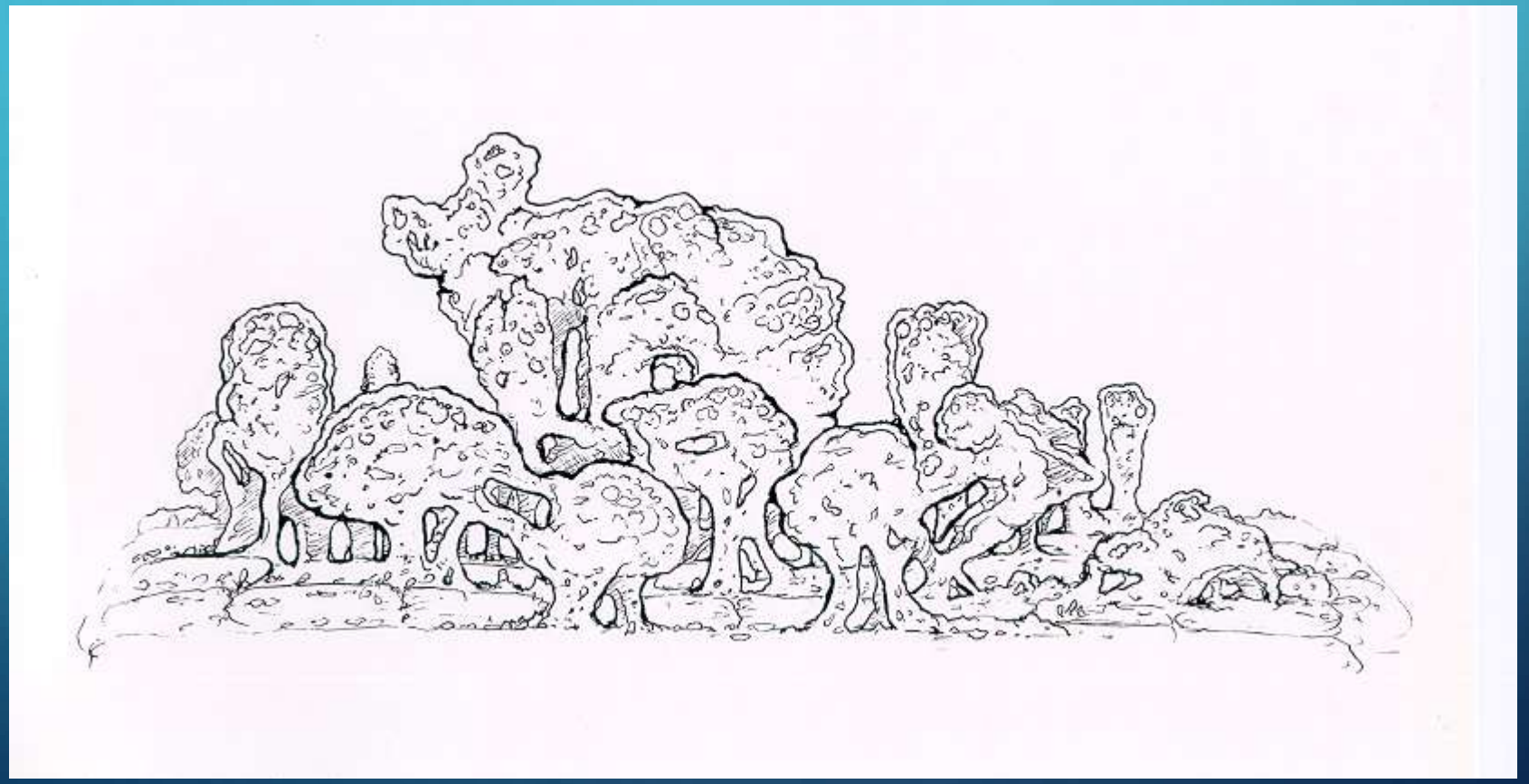
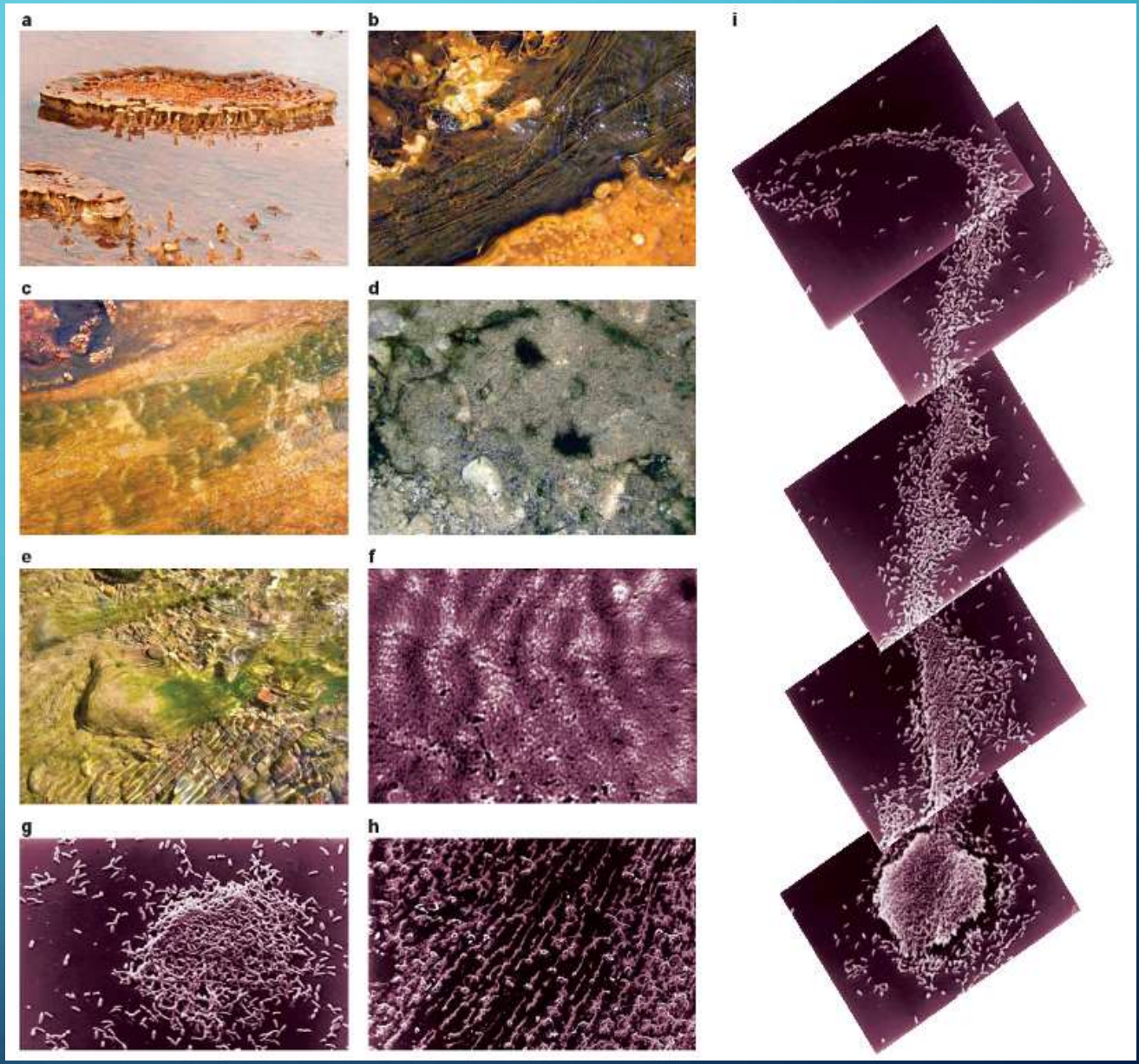


Image used with permission from the Center for Genomic Sciences, Allegheny General Hospital, Pittsburgh PA

BIOFILMS ARE UBIQUITOUS

Biofilms in hot springs, rivers and laboratory flow cells



Hall-Stoodley et al. (2004) Nature Rev Microbiol 2: 95.

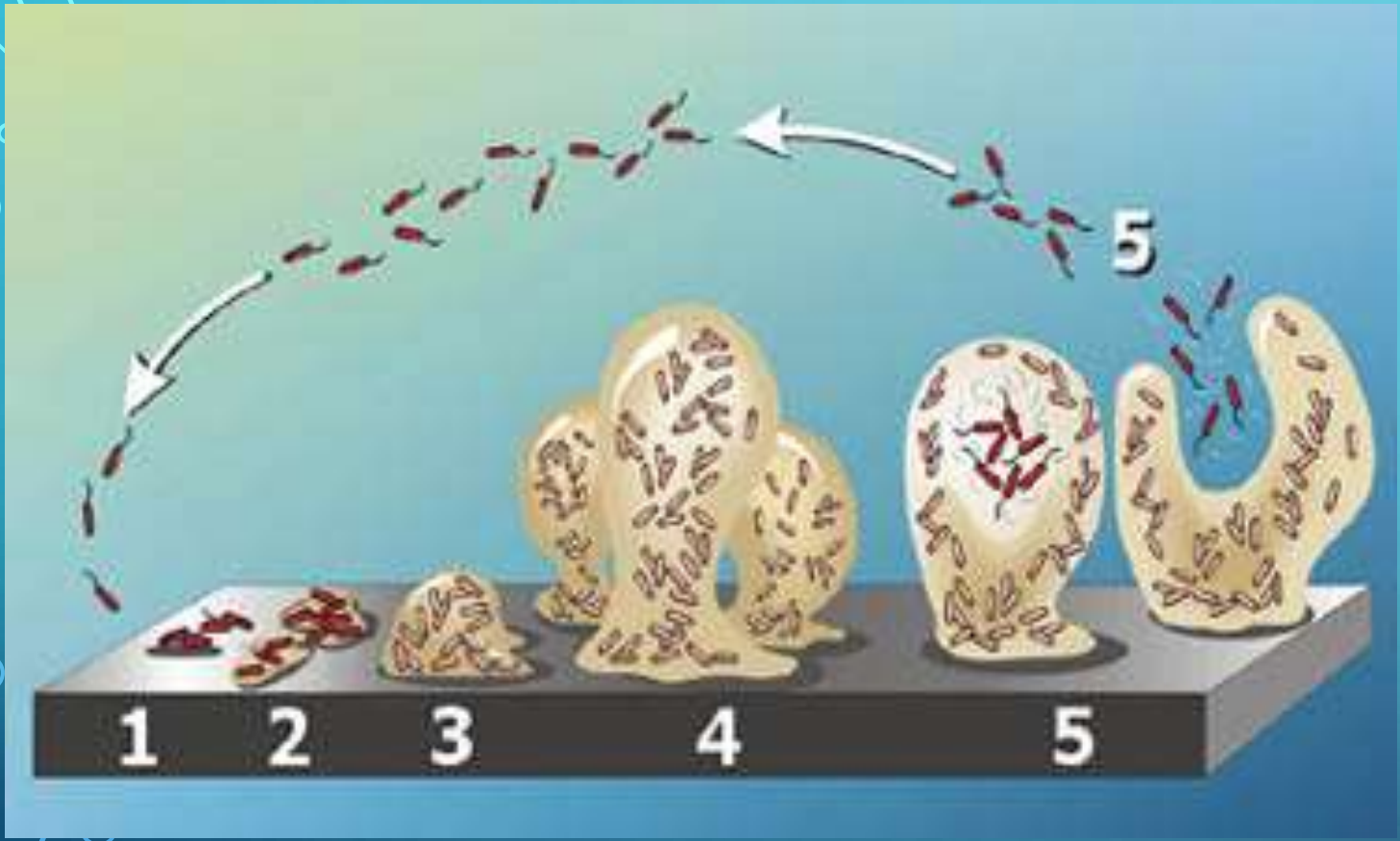
Biofilms are everywhere…

http://www.ladybugsteamvapor.com/wp-content/uploads/2011/03/s03-300x223.jpg

PERHAPS YOU HAVE SOME PLAQUE ON YOUR TEETH…BIOFILM!

http://meadfamilydental.com/2011/11/plaque-vs-biofilm-and-the-research-that-could-change-dentistry-as-we-know-it/

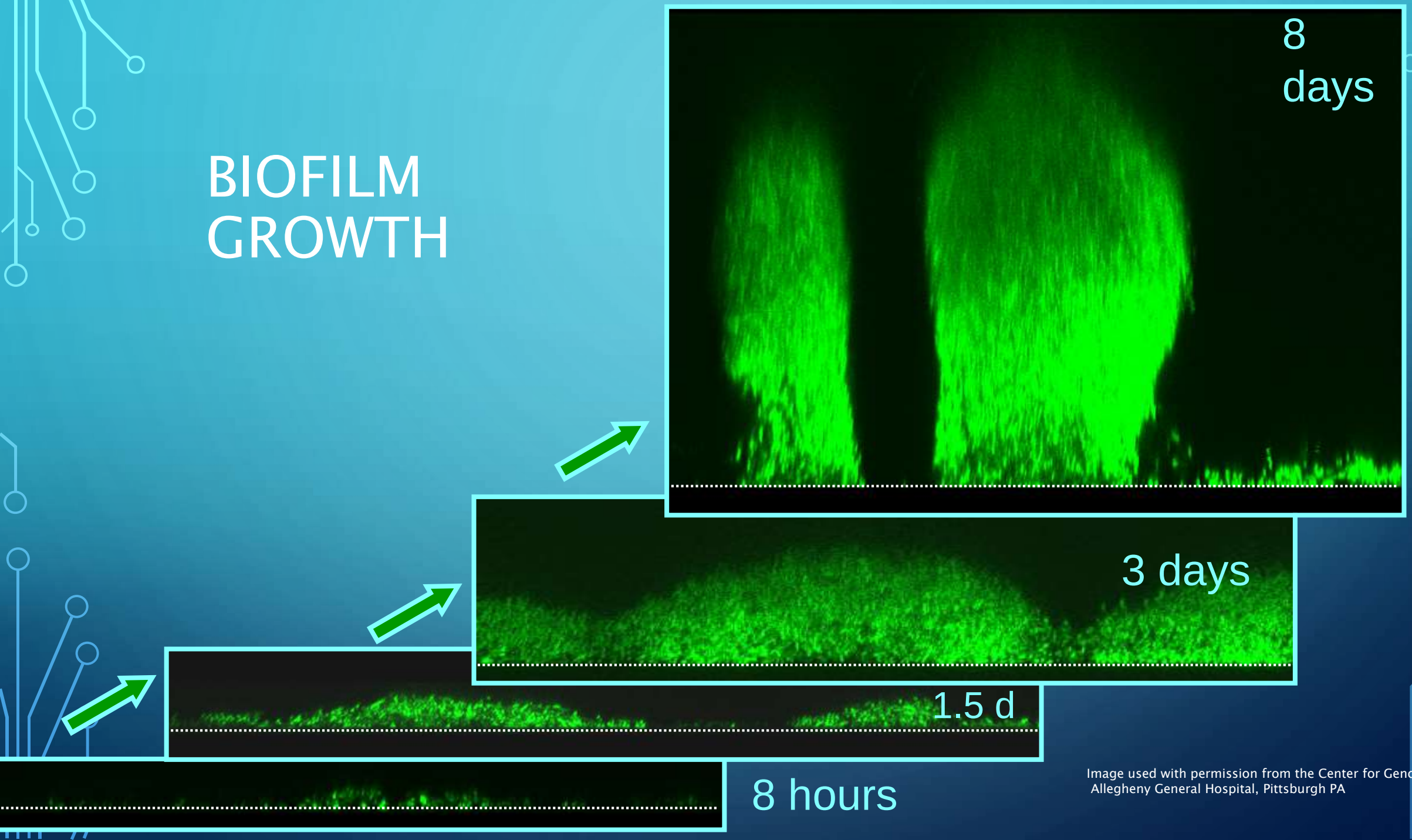
BIOFILM GROWTH



1. Permanent chemical attachment, single layer, bugs begin making slime
2. Early vertical development
3. Multiple towers with channels between, maturing biofilm
4. Mature biofilm with seeding / dispersal of more free swimming micro-organisms
5. Initial reversible attachment of free swimming micro-organisms to surface

http://entkent.com/biofilms.html

BIOFILM GROWTH



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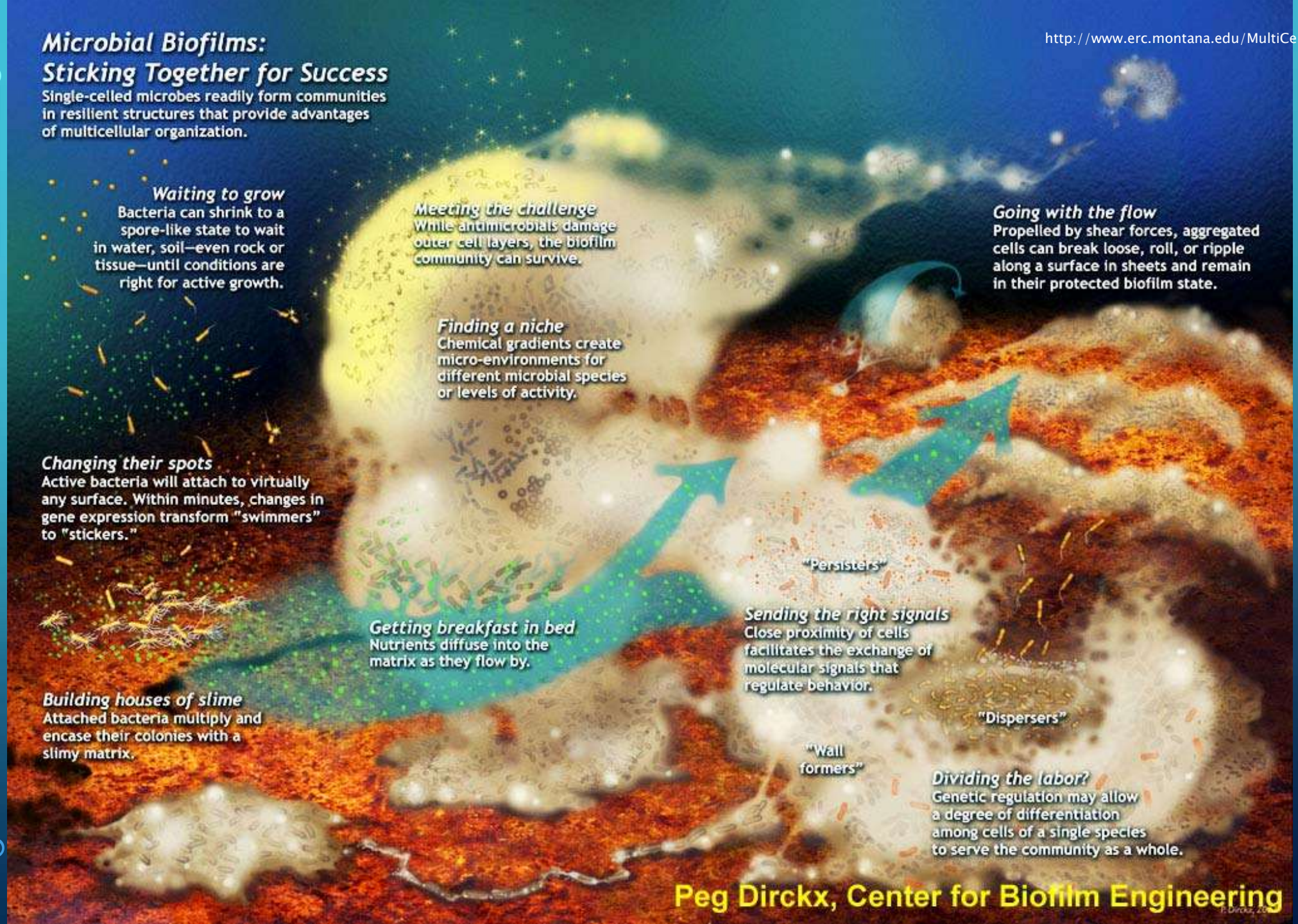
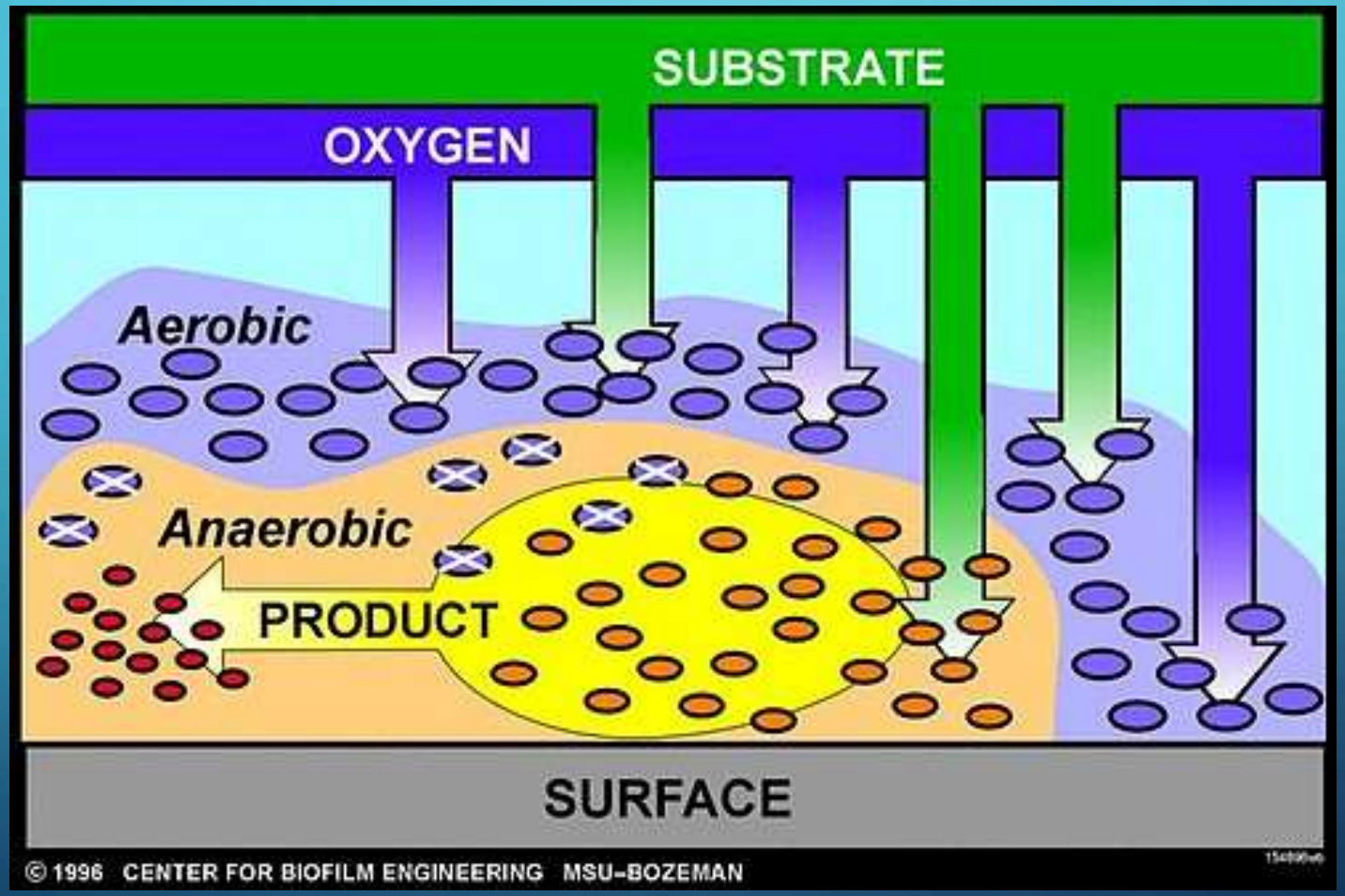
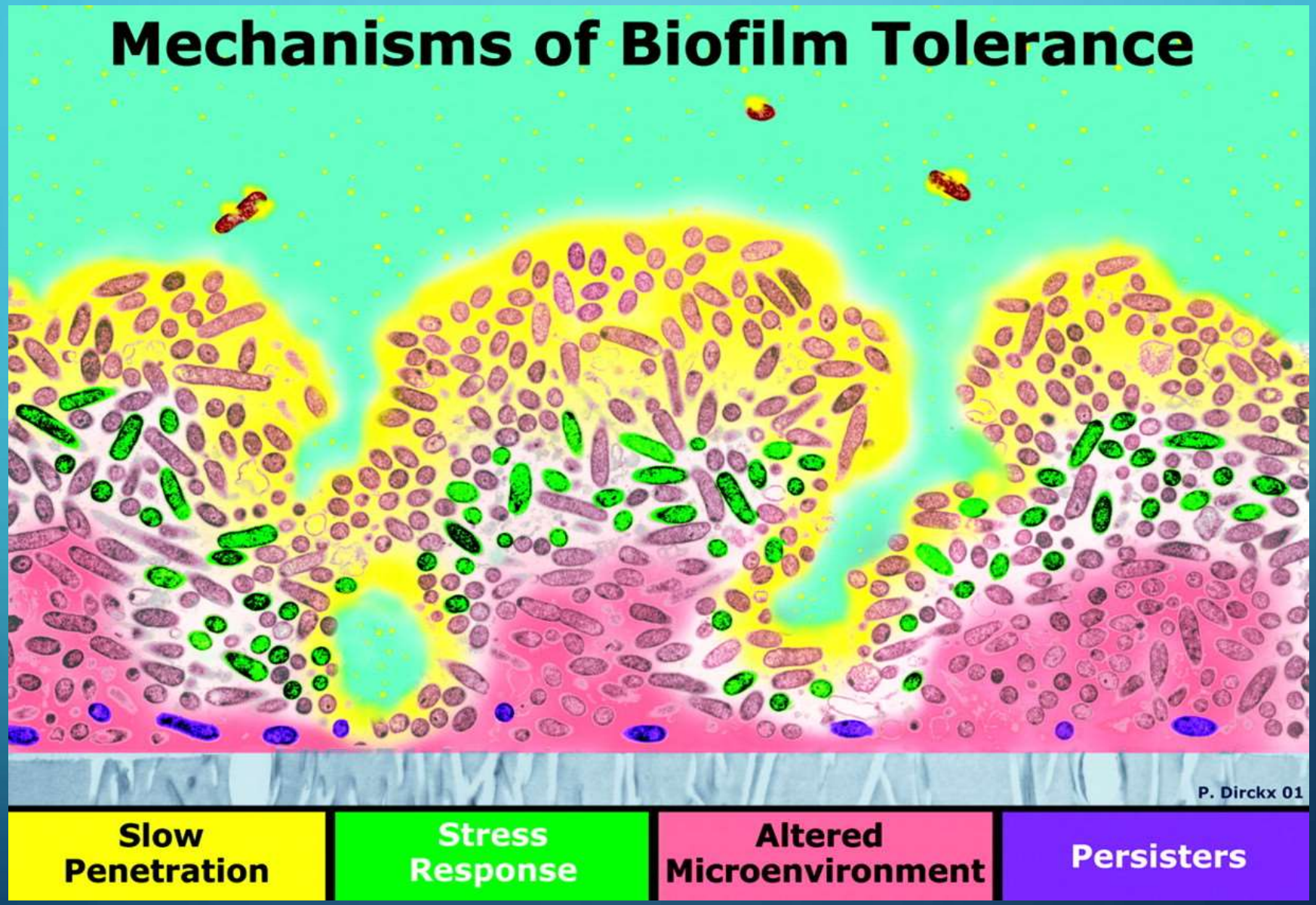

Microbial Biofilms:
Sticking Together for Success
Single-celled microbes readily form communities in resilient structures that provide advantages of multicellular organization.
http://www.erc.montana.edu/MultiCellStrat/default.html.
Waiting to grow
Bacteria can shrink to a spore-like state to wait in water, soil—even rock or tissue—until conditions are right for active growth.
Meeting the challenge
While antimicrobials damage outer cell layers, the biofilm community can survive.
Going with the flow
Propelled by shear forces, aggregated cells can break loose, roll, or ripple along a surface in sheets and remain in their protected biofilm state.
Finding a niche
Chemical gradients create micro-environments for different microbial species or levels of activity.
Changing their spots
Active bacteria will attach to virtually any surface. Within minutes, changes in gene expression transform "swimmers" to "stickers."
"Persisters"
Sending the right signals
Close proximity of cells facilitates the exchange of molecular signals that regulate behavior.
Getting breakfast in bed
Nutrients diffuse into the matrix as they flow by.
"Dispersers"
Building houses of slime
Attached bacteria multiply and encase their colonies with a slimy matrix.
"Wall formers"
Dividing the labor?
Genetic regulation may allow a degree of differentiation among cells of a single species to serve the community as a whole.
Peg Dirckx, Center for Biofilm Engineering

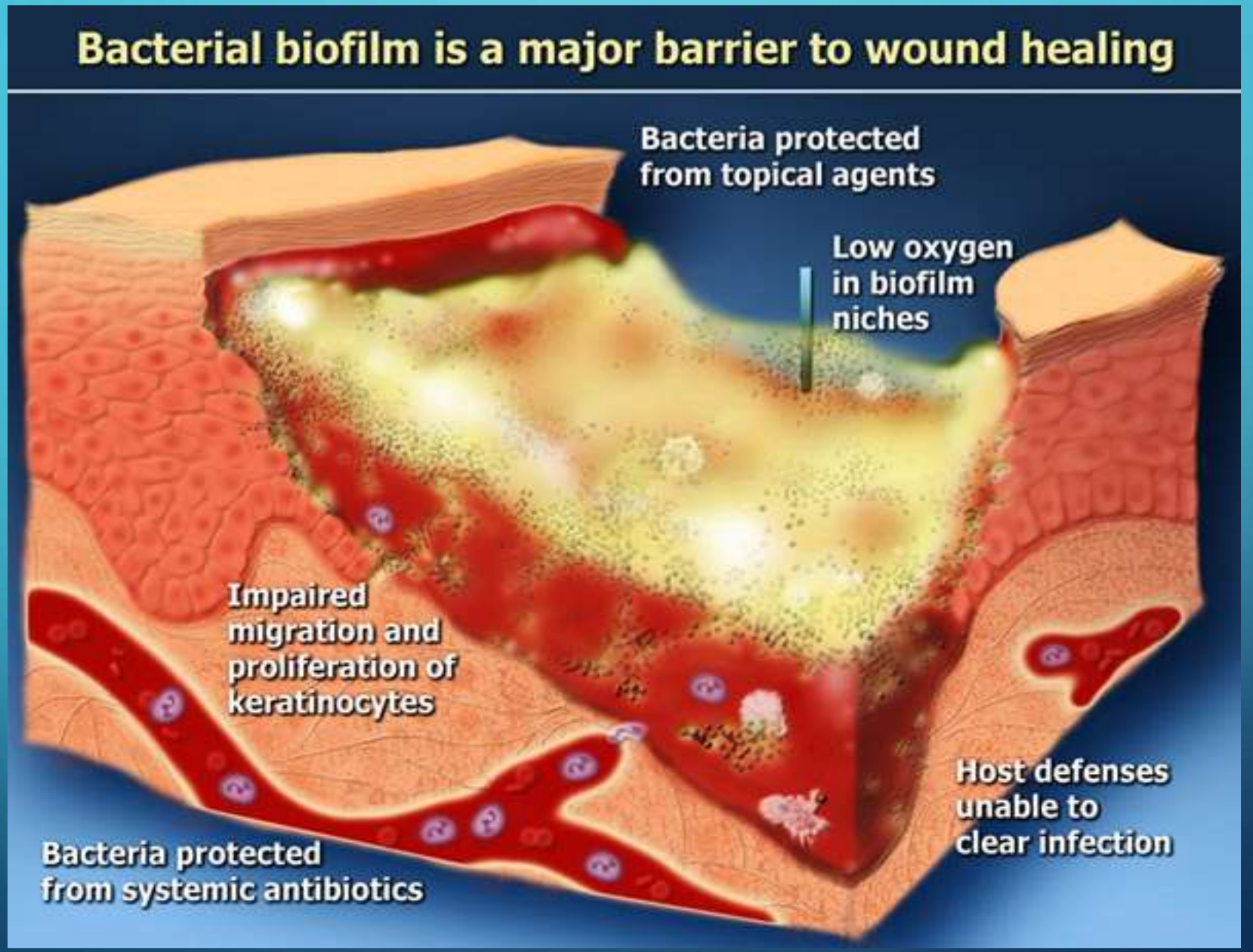
GRADIENTS AT WORK



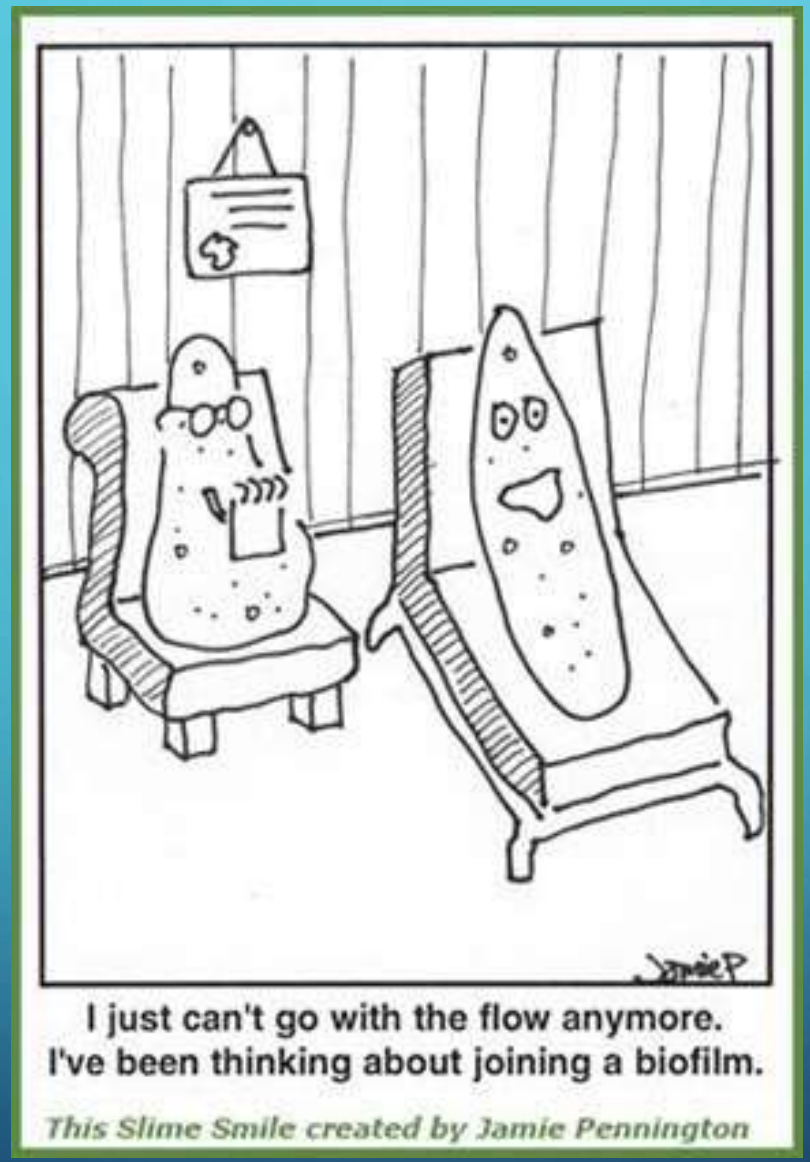
https://www.biofilm.montana.edu/node/2435



Jason D. Chambless, Stephen M. Hunt and Philip S. Stewart A Three-Dimensional Computer Model of Four Hypothetical Mechanisms Protecting Biofilms from Antimicrobials Appl. Environ. Microbiol. March 2006 vol. 72 no. 3 2005-2013



Clark F. Schierle MD, PhD, Mauricio De la Garza MD, Thomas A. Mustoe MD, Robert D. Galiano MD Staphylococcal biofilms impair wound healing by delaying reepithelialization in a murine cutaneous wound model Wound Repair and Regeneration Volume 17, Issue 3, pages 354–359, May/June 2009


I just can't go with the flow anymore.
I've been thinking about joining a biofilm.
This Slime Smile created by Jamie Pennington

HOW CAN WE DETECT BIOFILM?

- Laboratory based research currently uses a few methods
- Scanning electron microscope imaging was used first
  - Older technology
  - Performed under a high vacuum
  - Requires fixation with drying agents such as ethanol or guteraldehyde

Russell, S. D.; Daghlian, C. P. (1985). "Scanning electron microscopic observations on deembedded biological tissue sections: Comparison of different fixatives and embedding materials". *Journal of Electron Microscopy Technique* **2** (5): 489–495.

SEM of Control Chinchilla Middle ear Mucosa

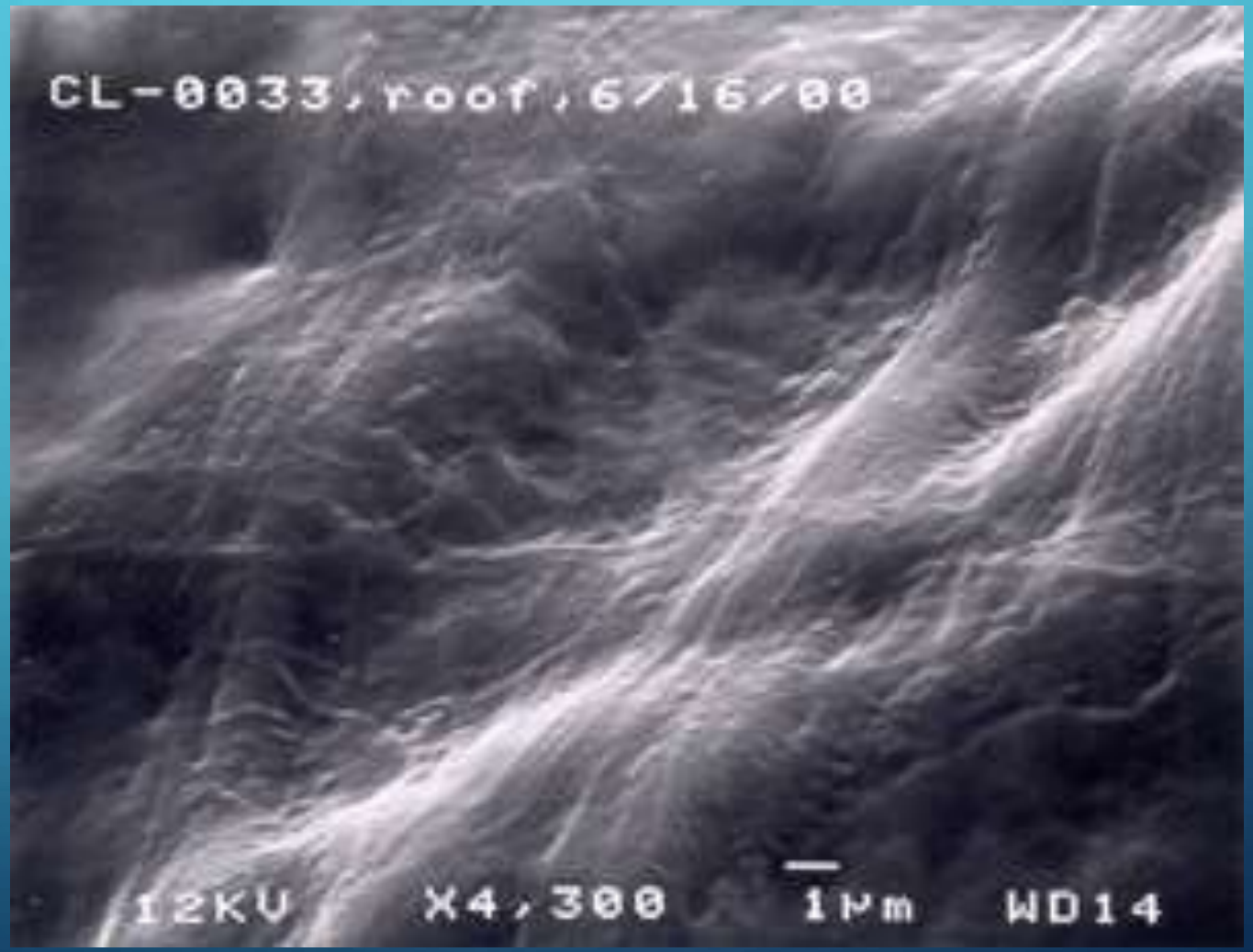
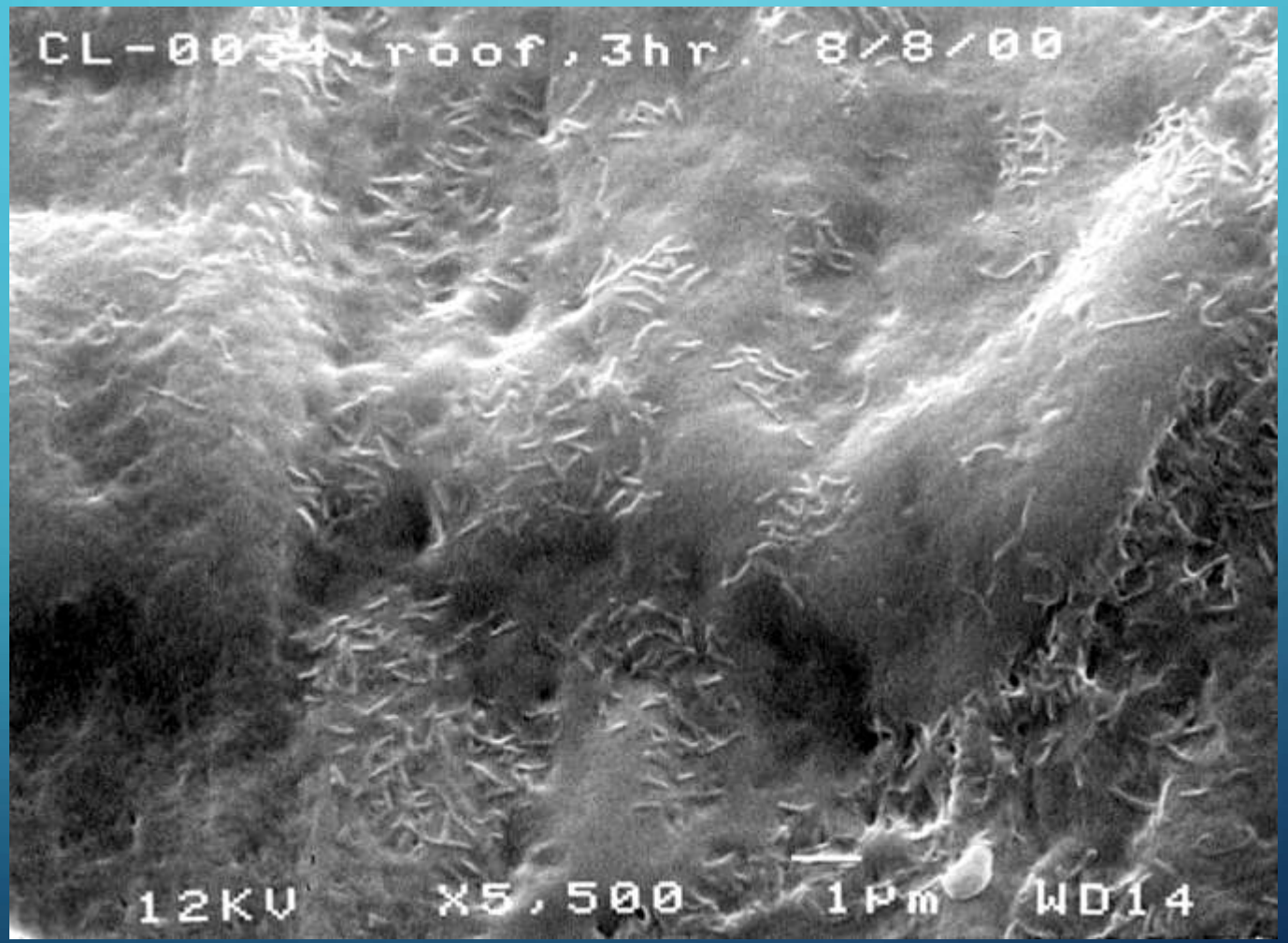


Image used with permission from the Center for Genomic Sciences, Allegheny General Hospital, Pittsburgh PA

H. influenza 3 hours post- inoculation



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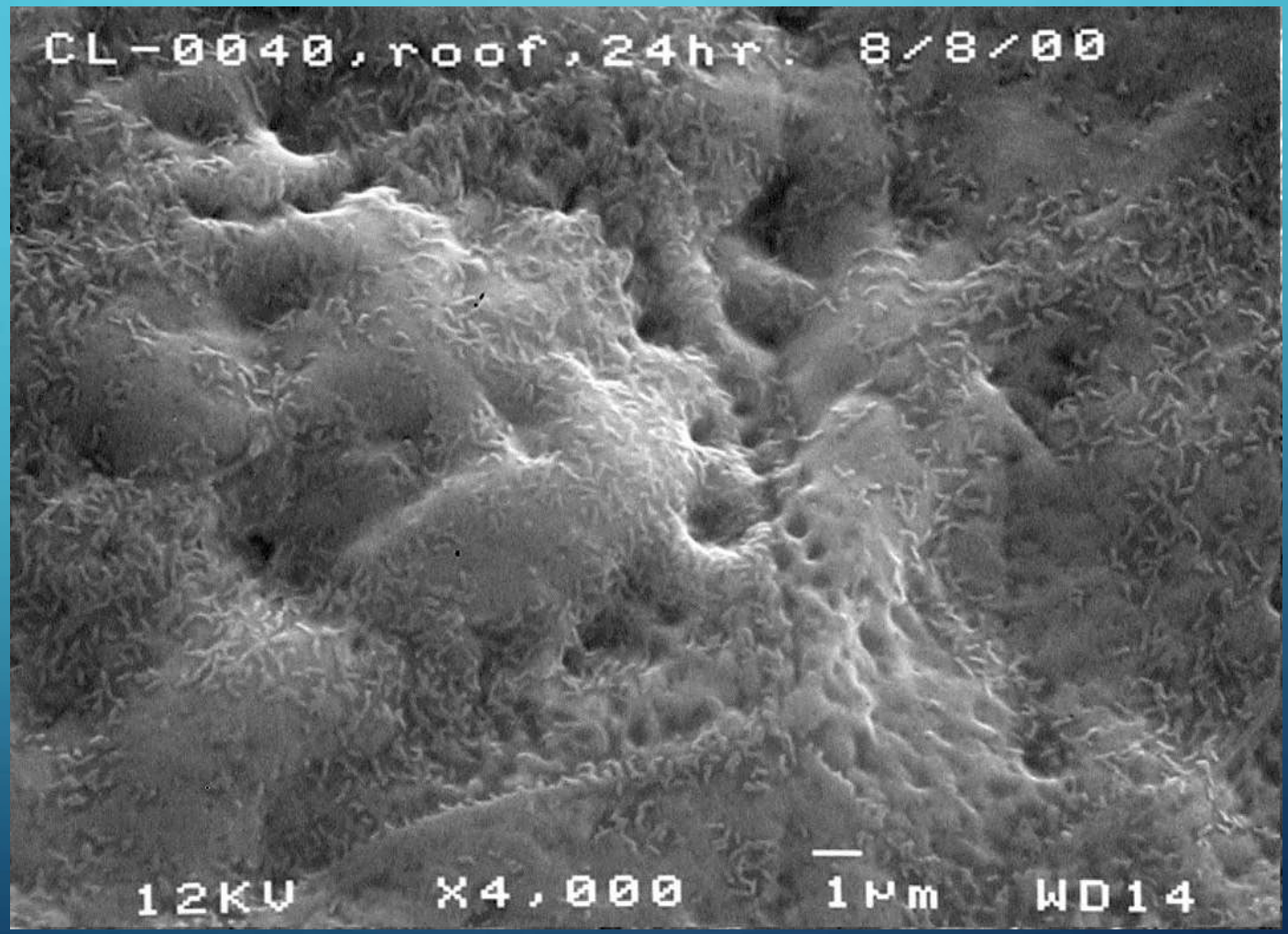
H. influenza 24 hours post- inoculation



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SEM IMAGE OF *H. INFLUENZAE* BIOFILM ON CHINCHILLA MIDDLE-EAR MUCOSA

Does this look like the cartoon?

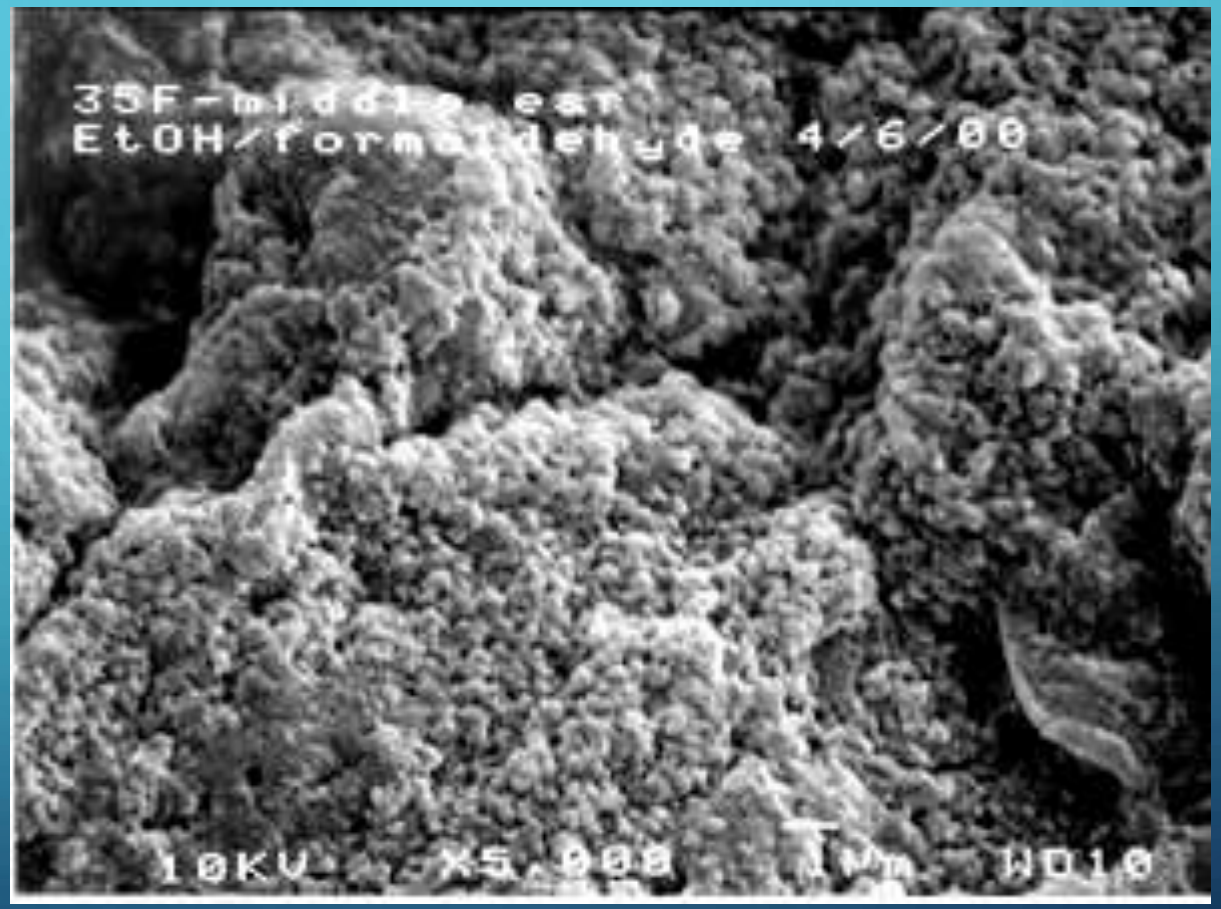


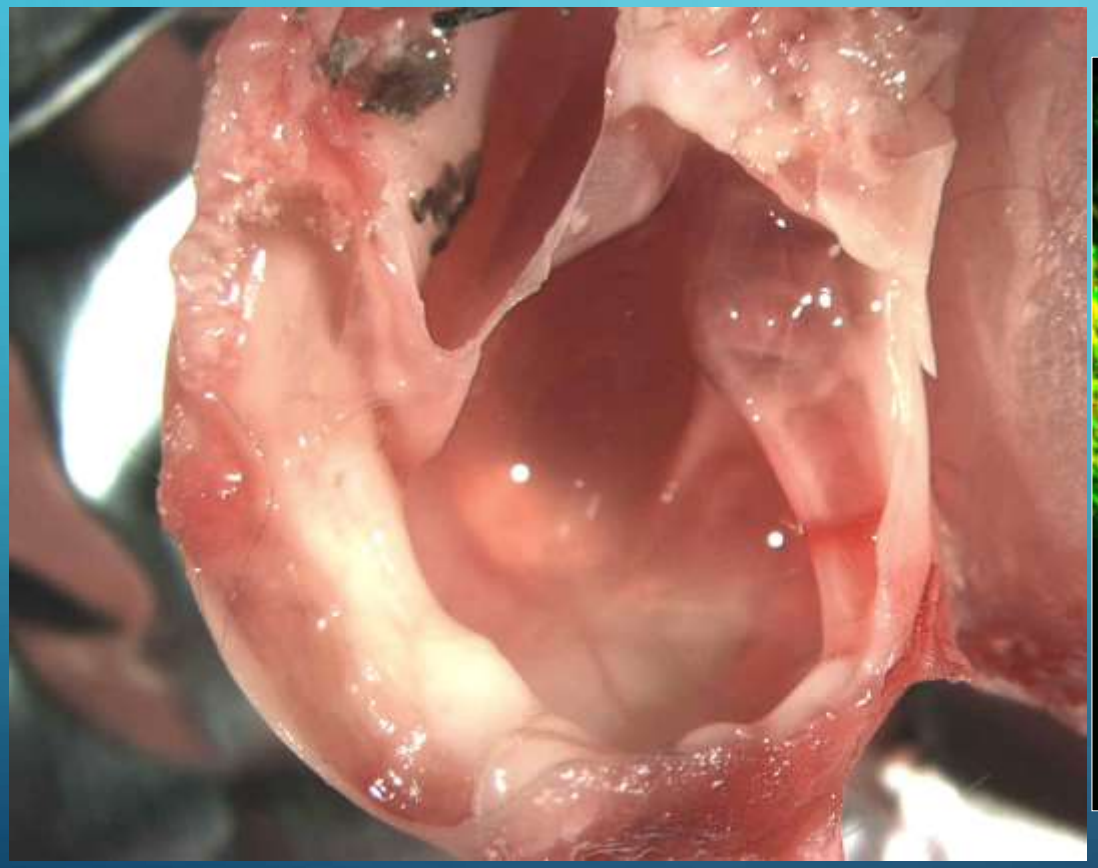
Image used with permission from the Center for Genomic Sciences, Allegheny General Hospital, Pittsburgh PA

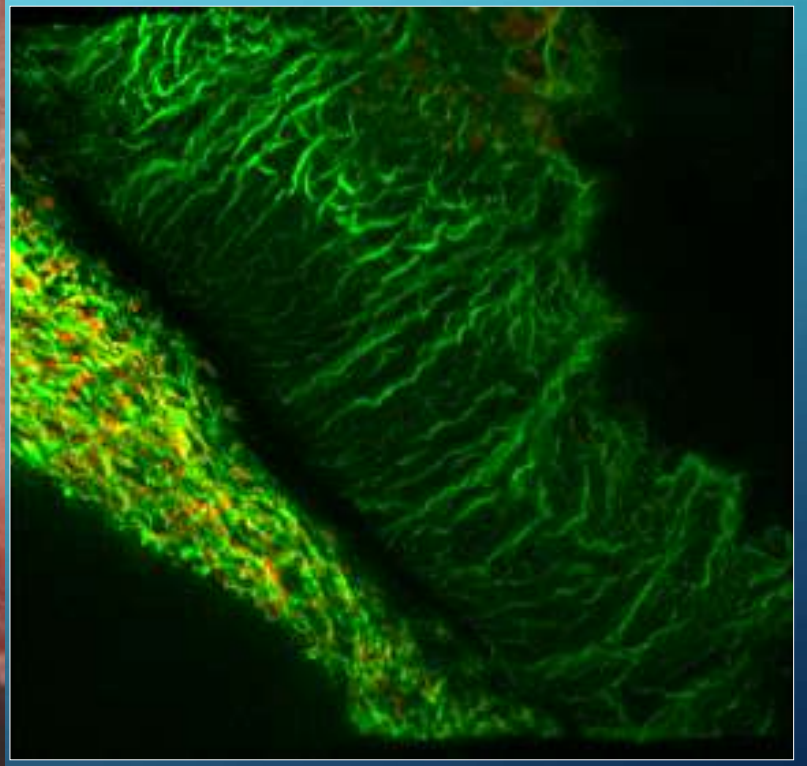
CONFOCAL LASER SCANNING MICROSCOPY

- Confocal microscopy provides the capacity for direct, noninvasive, serial optical sectioning of intact, thick, living specimens with a minimum of sample preparation.
- Fluorescent dyes are often used to enhance the images

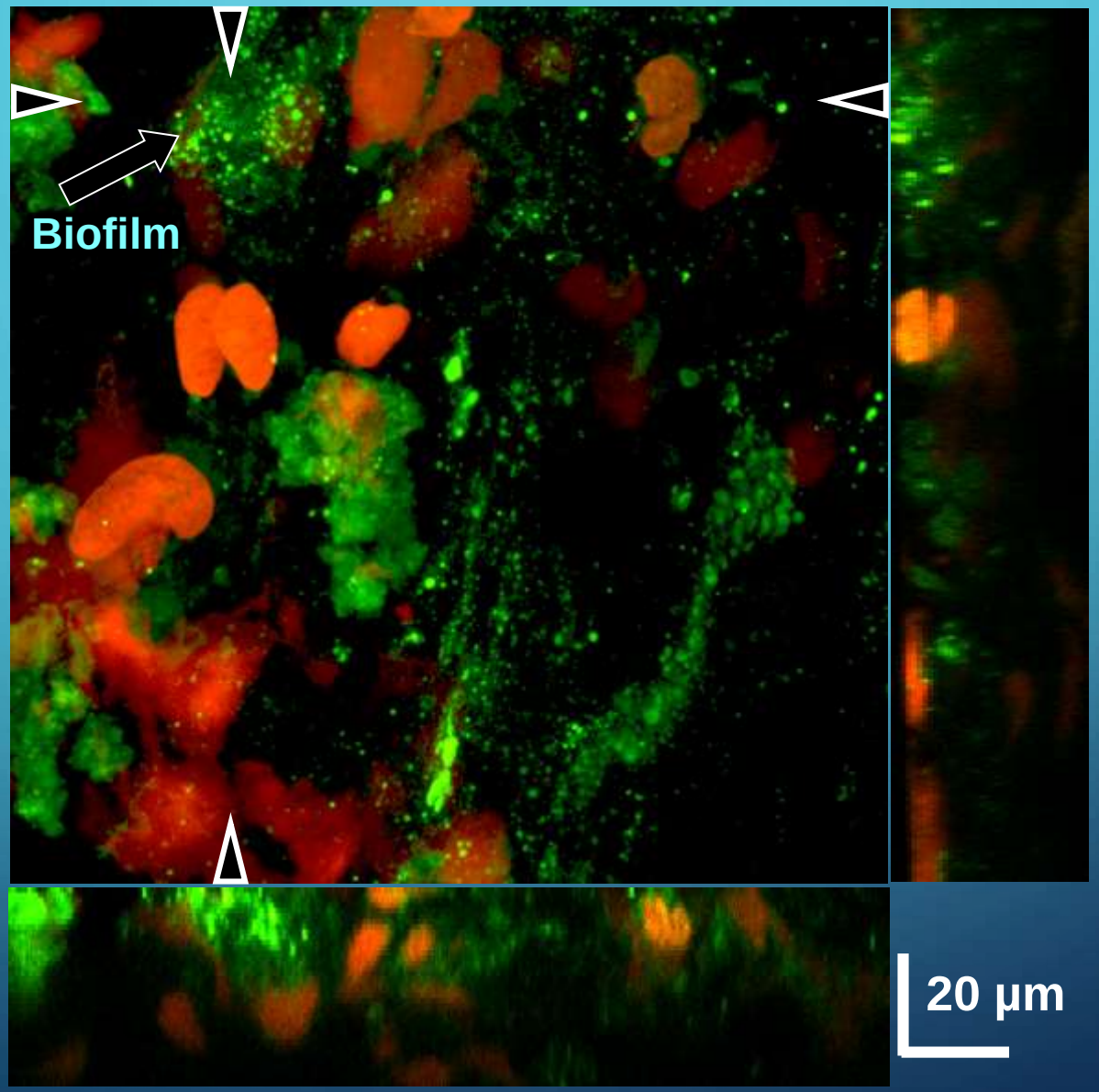
TJ, Davidson MW (2007). "Introduction to Confocal Microscopy". Olympus Fluoview Resource Center. National High Magnetic Field Laboratory. Retrieved 2007-07-25

CONFOCAL IMAGING OF BIOFILM IN THE CHINCHILLA MIDDLE EAR





PEDIATRIC OME MIDDLE EAR MUCOSA



BUT I DON'T HAVE A CLSM IN MY CLINIC…

- (Neither does anyone else)
- Some CRS research has been using H&E combined with gram staining to successfully observe the glycocalyx formation and changes to respiratory epithelium

To´th L, Csomor P, Sziklai I, Karosi T Biofilm detection in chronic rhinosinusitis by combined application of hematoxylin–eosin and gram staining. Eur Arch Otorhinolaryngol (2011) 268:1455–1462

Huihua You, BA, et al, "Factors affecting bacterial biofilm expression in chronic rhinosinusitis and the influences on prognosis" American Journal of Otolaryngology –Head and Neck Medicine and Surgery 32 (2011) 583–590

Polyp without Biofilm

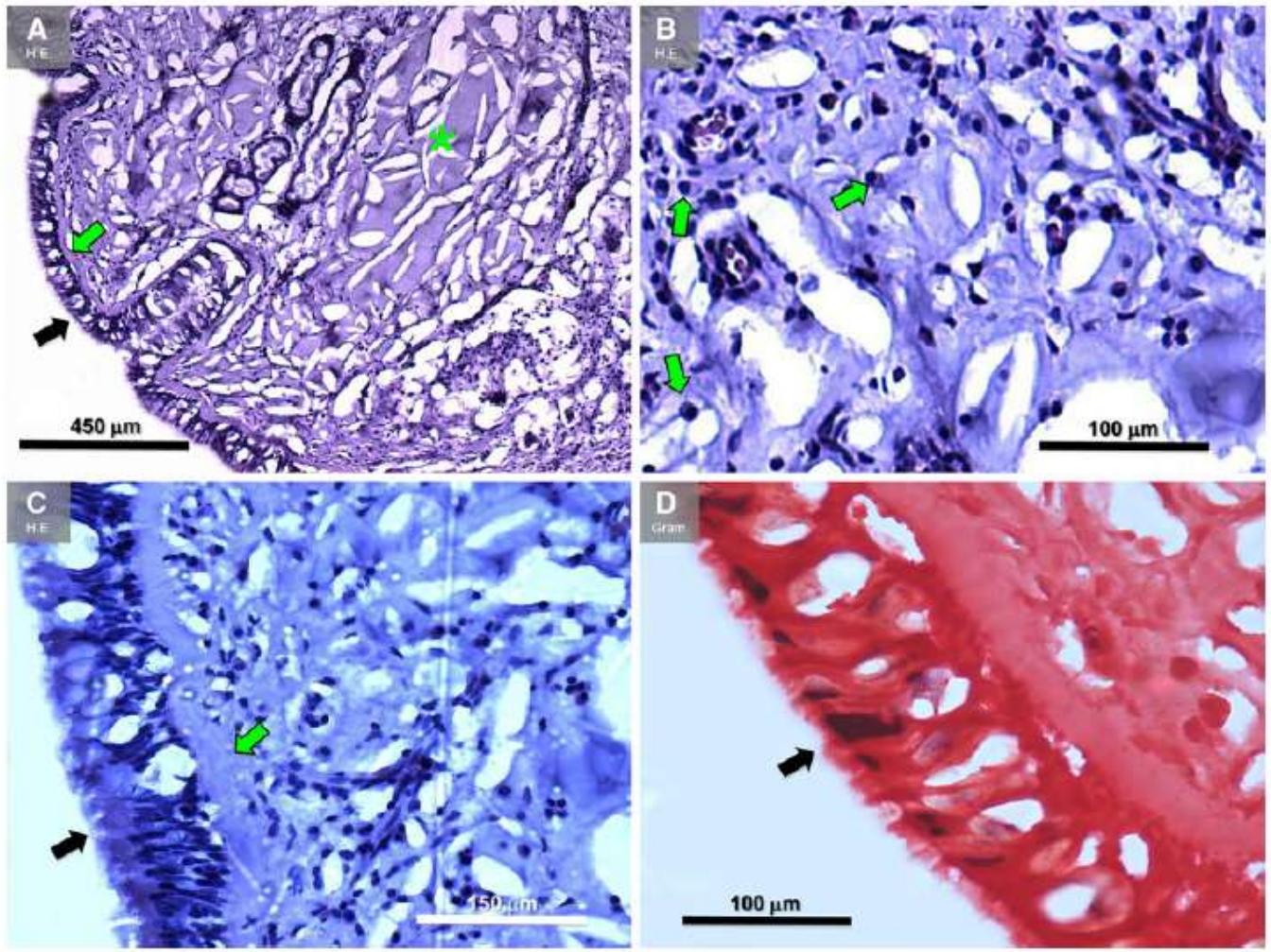


Fig. 1 Histopathological representation of a nasal polyp. **a** At low magnification, regular columnar epithelium and foamy cells can be detected (*black arrow*). Basal lamina is indicated by *green arrow*. The *green star* shows the subepithelial layer. **b** The subepithelial layer is infiltrated by plasmocytes and small lymphocytes (*green arrows*). **c** Biofilm structures cannot be detected on the surface of nasal epithelium (*black arrow*). Basal lamina is a well-identified structure (*green arrow*). **d** Gram staining is negative for bacterial or fungal elements; the polysaccharide matrix is absent (*black arrow*)

To´th L, Csomor P, Sziklai I, Karosi T Biofilm detection in chronic rhinosinusitis by combined application of hematoxylin–eosin and gram staining. Eur Arch Otorhinolaryngol (2011) 268:1455–1462

Polyp with Biofilm

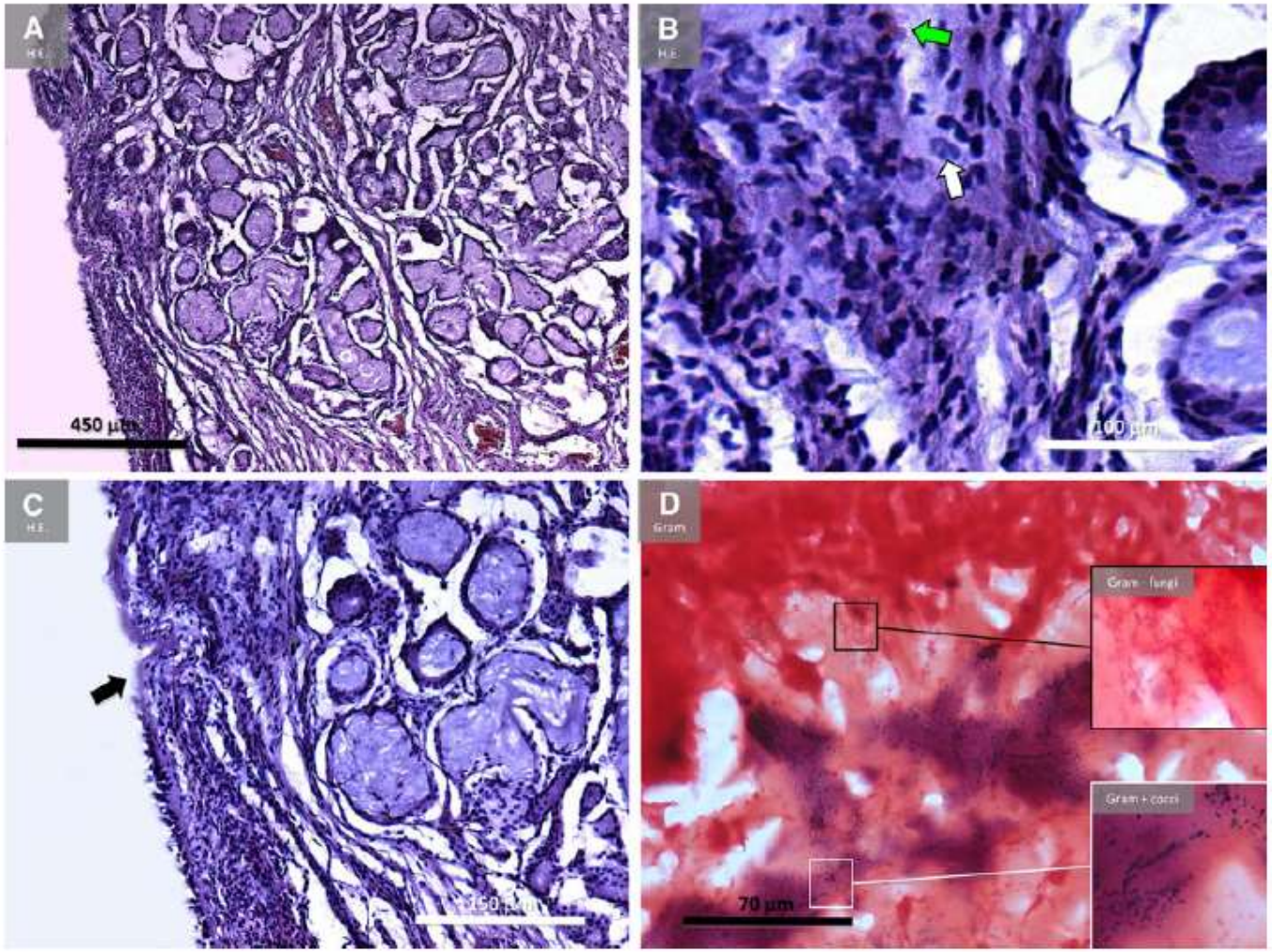


Fig. 2 Histopathological examination of a CRS/NP specimen. **a** The nasal epithelium is destructed and massively infiltrated by inflammatory cells. The stromal layer shows a gland-like structure. **b** The subepithelial layer is infiltrated by eosinophils (*green arrow*) and polymorphonuclear cells (*white arrow*). **c** The epithelial layer is absolutely disintegrated and covered by a complete biofilm showing a homogeneous basophilic staining (*black arrow*). **d** Gram staining reveals a combined biofilm consisting of individual colonies of Gram-negative fungi and Gram-positive cocci (small inserts)

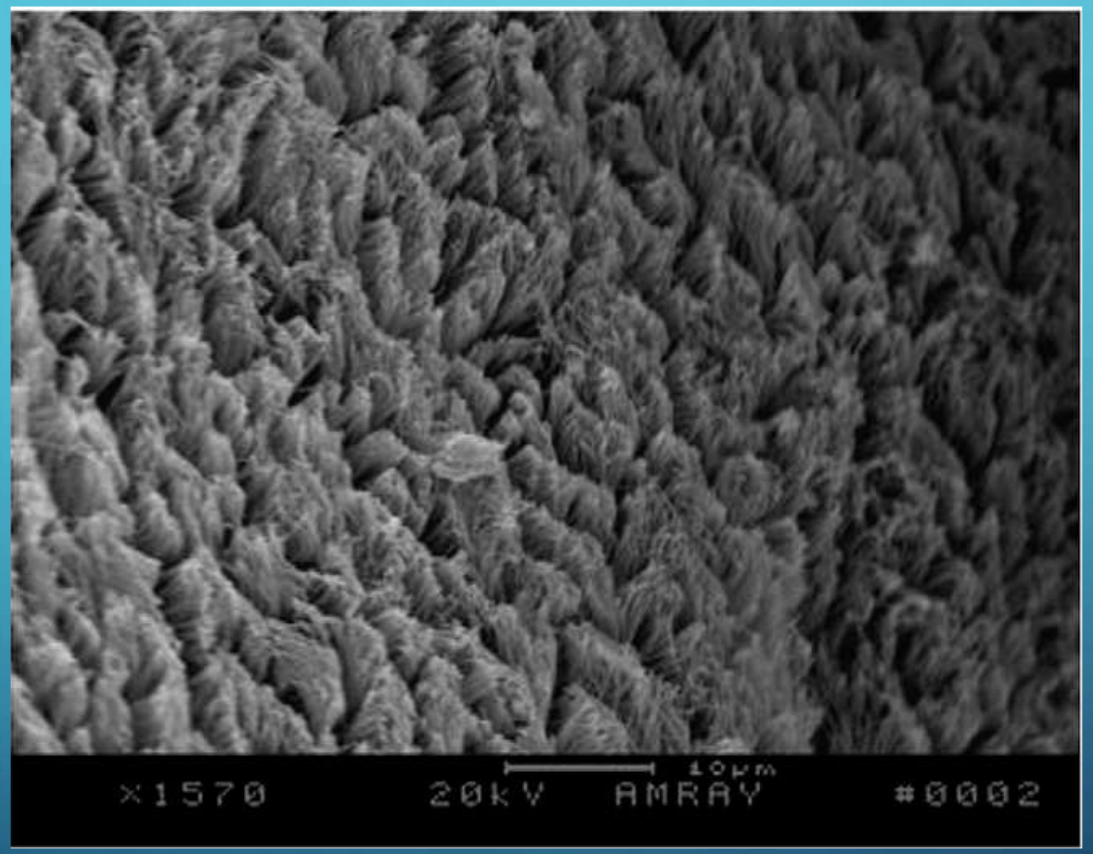
To´th L, Csomor P, Sziklai I, Karosi T Biofilm detection in chronic rhinosinusitis by combined application of hematoxylin–eosin and gram staining. Eur Arch Otorhinolaryngol (2011) 268:1455–1462

CHANGES TO NASAL EPITHELIUM

	Nasal polyps—CRS specimens (HE, $n = 50$)	
	Biofilm present ($n = 44$)	Biofilm absent ($n = 6$)
Epithel	Fragmented—destroyed ($n = 33$) Squamous cell metaplasia ($n = 8$) LPC infiltration ($n = 3$)	Ciliated columnar epithelium—regular respiratory mucosa with foamy cells ($n = 6$)
Stroma	Eosinophil dominancy ($n = 5$) Neutrophil (PMN) dominancy ($n = 36$) Mononuclear and plasmocytic infiltration ($n = 3$)	Eosinophil dominancy ($n = 4$) Neutrophil (PMN) dominancy ($n = 1$) Mononuclear and plasmocytic infiltration ($n = 1$)

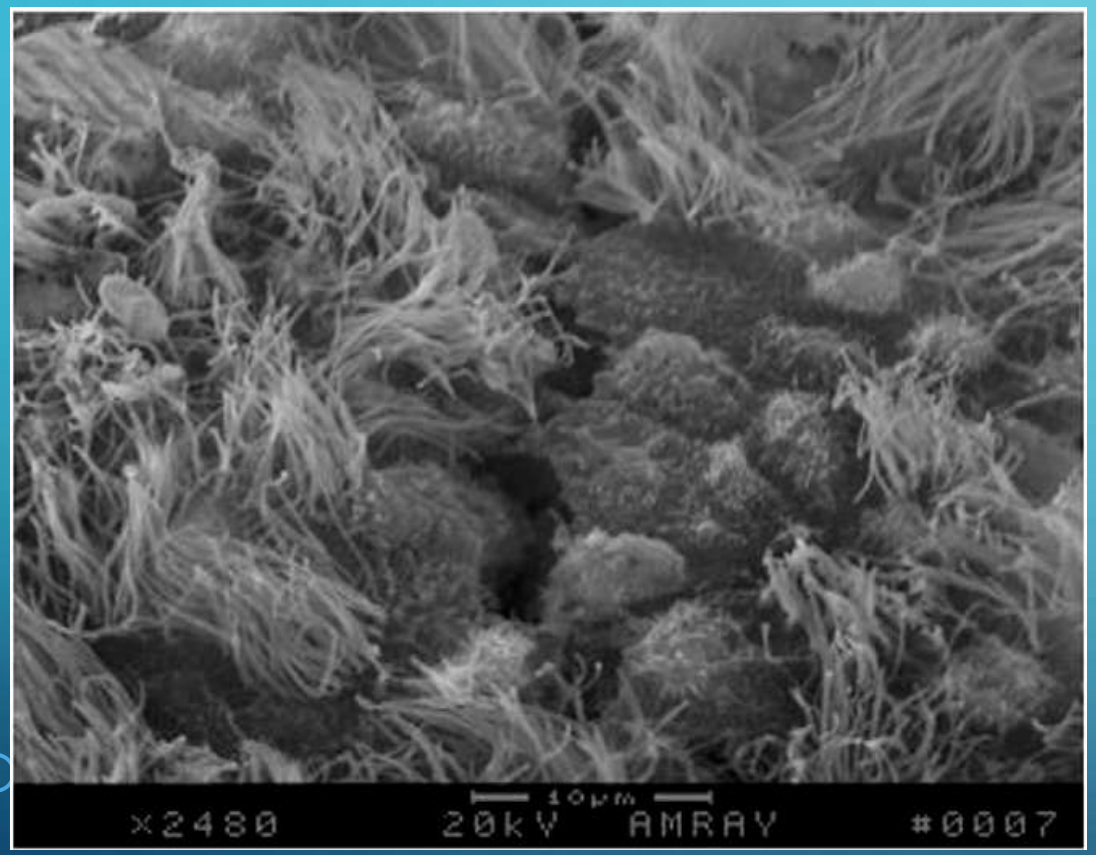
To´th L, Csomor P, Sziklai I, Karosi T Biofilm detection in chronic rhinosinusitis by combined application of hematoxylin–eosin and gram staining. Eur Arch Otorhinolaryngol (2011) 268:1455–1462

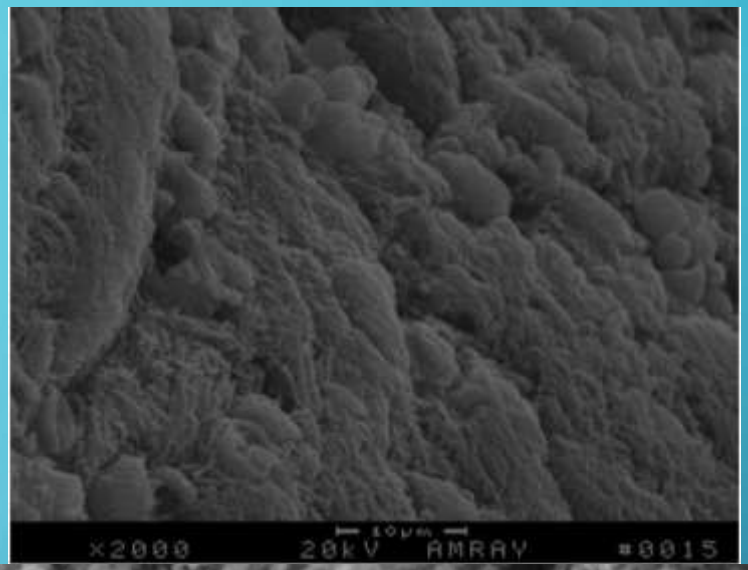
SEM OF HEALTHY MUCOSA

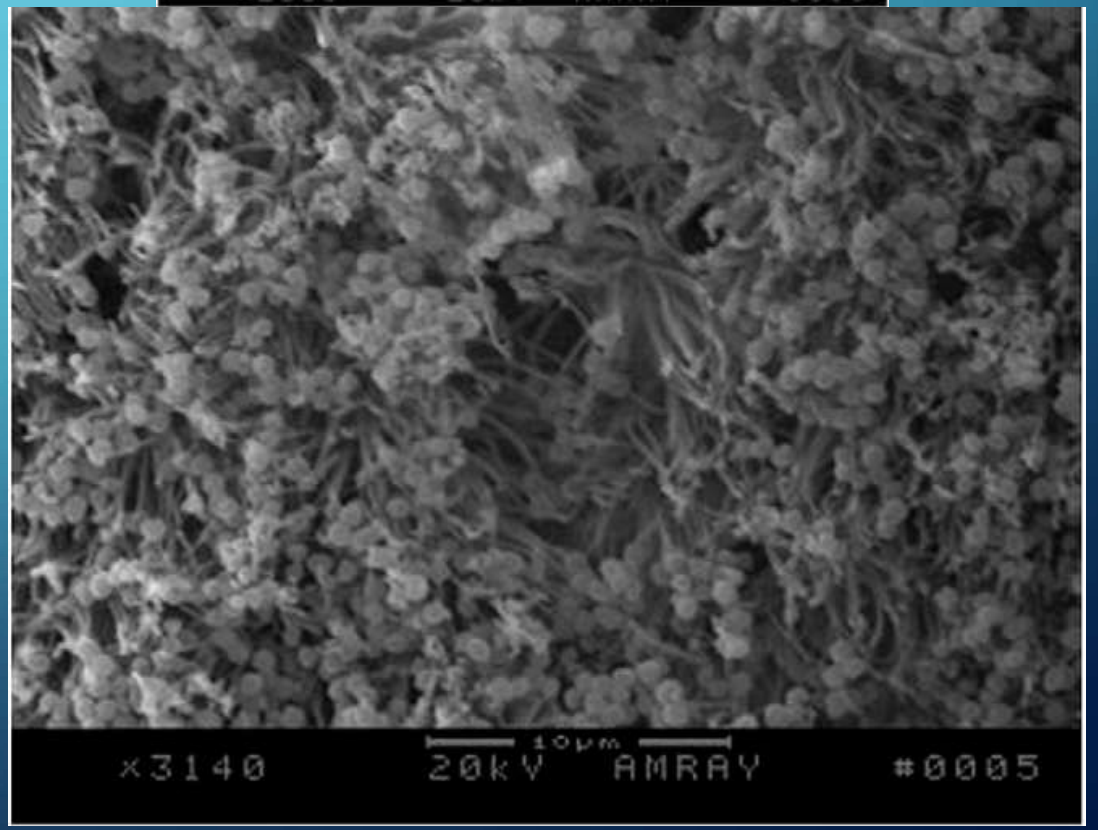


Huihua You, BA, et al, "Factors affecting bacterial biofilm expression in chronic rhinosinusitis and the influences on prognosis" American Journal of Otolaryngology–Head and Neck Medicine and Surgery 32 (2011) 583–590

MUCOSA WITH BIOFILM



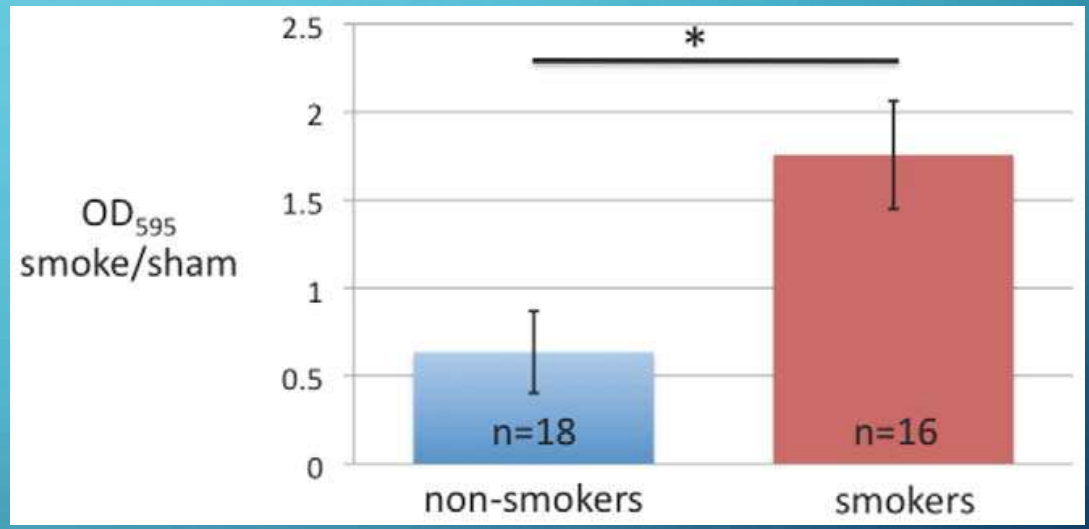




Huihua You, BA, et al, "Factors affecting bacterial biofilm expression in chronic rhinosinusitis and the influences on prognosis" American Journal of Otolaryngology–Head and Neck Medicine and Surgery 32 (2011) 583–590

TOBACCO SMOKE CAN INDUCE BIOFILM

- biofilms grown from endoscopically guided nasal swabs of smokers and nonsmokers
- bacteria from smokers exposed to cigarette smoke grew into biofilms where nonsmoker's bacteria were inhibited



DIAGNOSIS CRS

- Chronic and recurrent rhinosinusitis definitions
- Chronic rhinosinusitis (CRS) Twelve (12) weeks or longer of two or more of the following signs and symptoms:
    - mucopurulent drainage (anterior, posterior, or both)
    - nasal obstruction (congestion),
    - facial pain-pressure-fullness, or
    - decreased sense of smell
  - AND inflammation is documented by one or more of the following findings:
    - purulent (not clear) mucus or edema in the middle meatus or ethmoid region,
    - polyps in nasal cavity or the middle meatus, and/or
    - radiographic imaging showing inflammation of the paranasal sinuses
- Recurrent acute rhinosinusitis Four (4) or more episodes per year of ABRS without signs or symptoms of rhinosinusitis between episodes

HOW DO I KNOW IF MY PATIENT HAS A BIOFILM?

- Failure of maximal medical therapy and ESS
  - In biofilm studies, these CRS patients have a biofilm positive rate of 20-100%
    - wide variety of criteria
    - most studies quote about 60% biofilm positivity
  - One study found biofilm positivity correlated with SNOT-20 items of need to blow nose, cough, and PND.
  - It found no strong correlation with endoscopic or CT findings

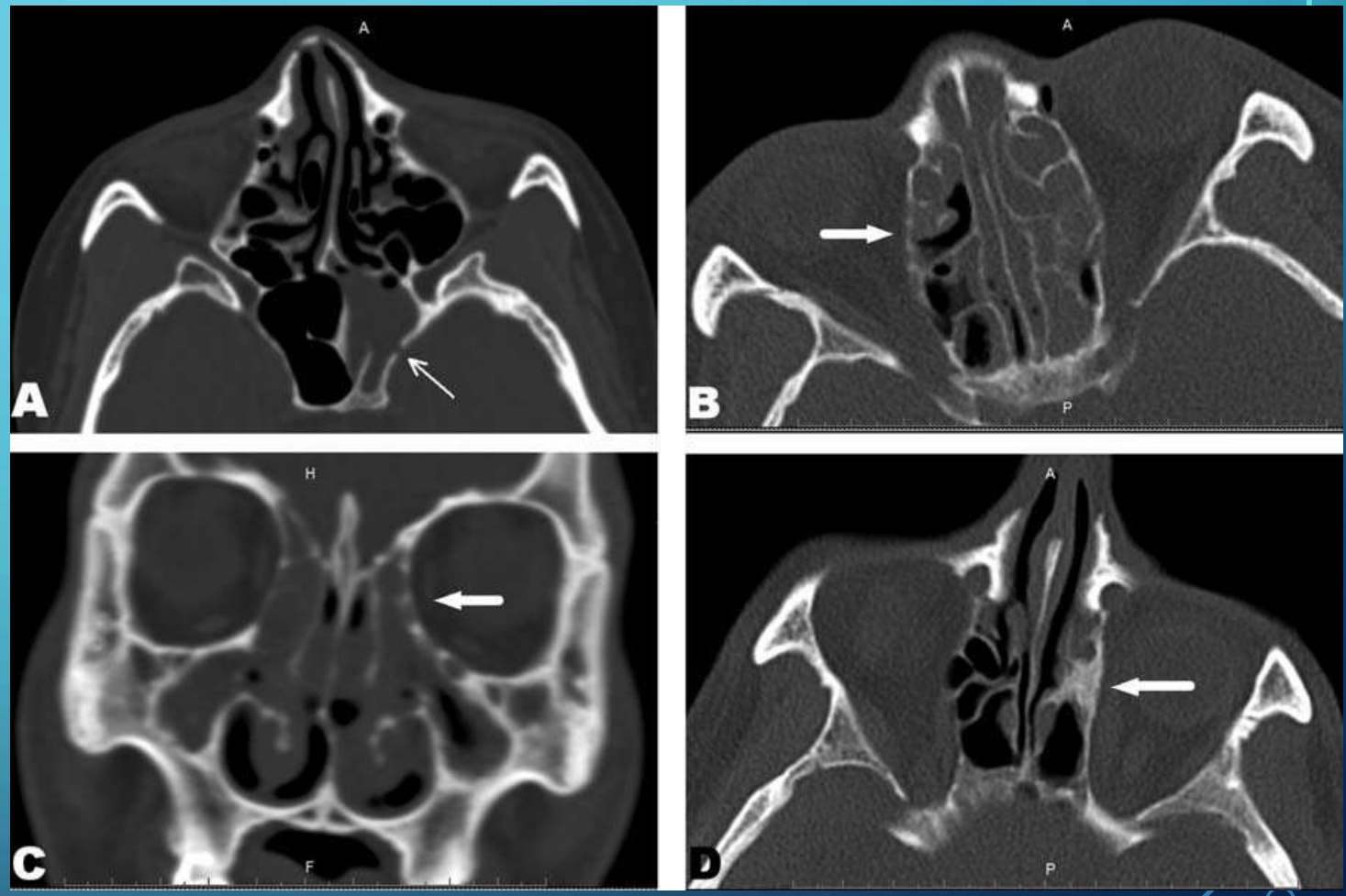
Han Li, et al "Relationship between bacterial biofilm and clinical features of patients with chronic rhinosinusitis" Eur Arch Otorhinolaryngol (2012) 269:155-163

OSTEITIS AND BIOFILM

- One study found strong correlation between grade of osteitis by CT and pathologic evaluation of CRS patients
  - They found pathological and CT evidence of osteitis for 84.8% of the bones underlying mucosa with biofilm in ethmoid sinus
  - confirmed that approximately 46.4% CRS patients were from a subgroup with both biofilm and osteitis

Dong Dong, MM, Zhao Yulin, MD et al "Correlation Between Bacterial Biofilms and Osteitis in Patients With Chronic Rhinosinusitis" Laryngoscope, 00:000–000, 2013

- Examples of radiographic osteitis (arrow) and Global Osteitis Scoring scale (GOSS) score of anterior ethmoid sinus.
  - (A) GOSS score 0, axial computed tomography (CT) slice of a patient having cerebrospinal fluid leakage located at the wall of the sphenoid sinus (open arrow).
  - (B) GOSS score 1, axial CT slice of a chronic rhinosinusitis (CRS) patient, <50% of the sinus walls involved and thickness of osteitic bone<3 mm.
  - (C) GOSS score 2, coronal CT slice of a CRS patient with previous endoscopic sinus surgery (ESS), <50% of the sinus walls involved and thickness of 3 to 5 mm.
  - (D) GOSS score 3, axial CT slice of a CRS patient with previous ESS, <50% of the sinus walls involved and thickness>5 mm.

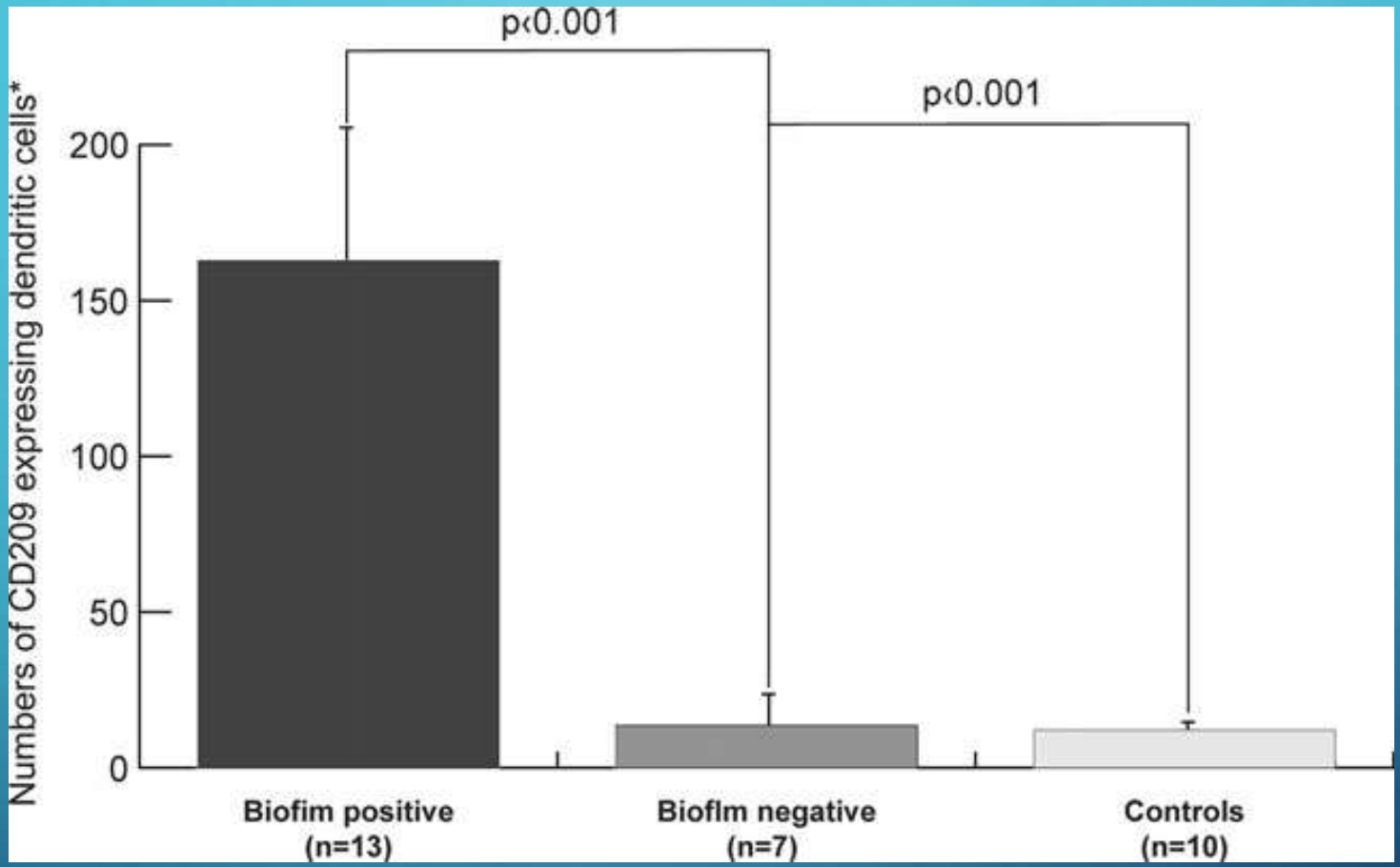


Dong Dong, MM, Zhao Yulin, MD et al "Correlation Between Bacterial Biofilms and Osteitis in Patients With Chronic Rhinosinusitis" Laryngoscope, 00:000–000, 2013

A WIDE VARIETY OF MARKERS ARE BEING INVESTIGATED-CD209

- Increased numbers of dendritic cells expressing CD209 correlates with biofilm positivity
- Dendritic cell immune response releases pro inflammatory cytokines (IL-1 and TNF-a) and growth factors (TGF-b, PDGF, EGF and FGF)
- This response may induce epithelial apoptosis and metaplasia

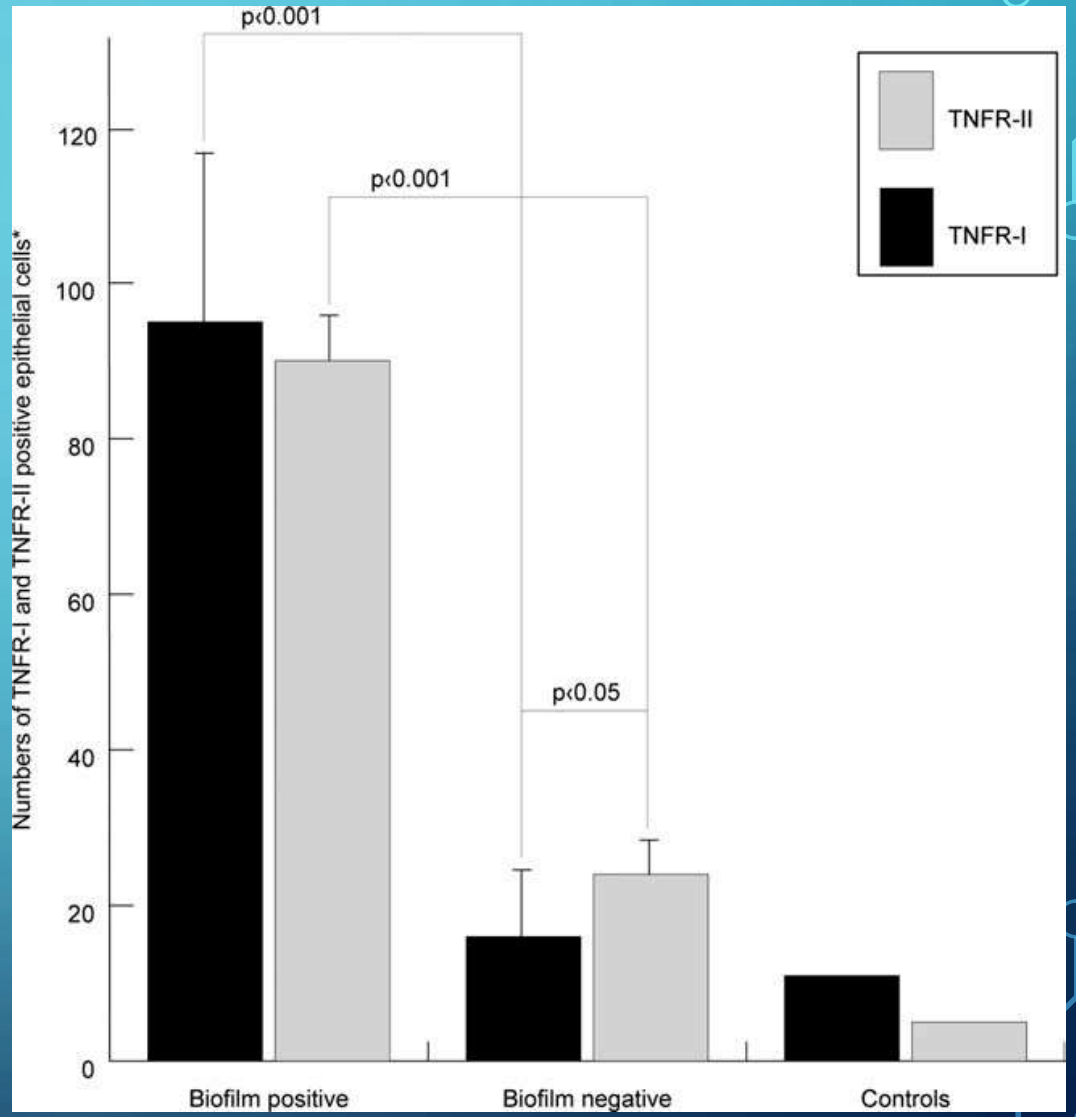
Tama´s Karosi, Pe´ter Csomor, Zolta´n Hegyi, Istva´n Sziklai "The presence of CD209 expressing dendritic cells correlates with biofilm positivity in chronic rhinosinusitis with nasal polyposis" Eur Arch Otorhinolaryngol (2013) 270:2455–2463



Tama´s Karosi, Pe´ter Csomor, Zolta´n Hegyi, Istva´n Sziklai “The presence of CD209 expressing dendritic cells correlates with biofilm positivity in chronic rhinosinusitis with nasal polyposis” Eur Arch Otorhinolaryngol (2013) 270:2455–2463

MARKERS-TNF-A

- Similarly, TNF receptor positivity was found to be upregulated in epithelial cells associated with biofilms in CRS patients



Tama´s Karosi, Pe´ter Csomor, Zolta´n Hegyi, Istva´n Sziklai "Tumor Necrosis Factor-a Receptor Expression Correlates With Mucosal Changes and Biofilm Presence in Chronic Rhinosinusitis With Nasal Polyposis" Laryngoscope, 122:504-510, 2012

EOSINOPHILS AND PLASMA CELLS

- Eosinophil presence as detected by marker for Eosinophil Major Basic Protein (EMBP) and plasma cell presence as detected by CD27 marker found significantly greater counts of these cells in biofilm positive tissue than in controls
- This study also notes higher counts of these cells in patients with polyps

Arjomandi H, Gilde J, Zhu S, Delaney S, Hochstim C, Mazhar K, Wrobel B, Markarian A, Masood R, Rice D, "Relationship of eosinophils and plasma cells to biofilm in chronic rhinosinusitis." Am J Rhinol Allergy. 2013 Jul-Aug;27(4):e85-90.

I FOUND A BIOFILM, WHAT DO I DO NOW?

http://protomag.com/assets/slime-and-the-city?page=2

ANTIBIOTICS DON'T WORK

- Biofilms show resistance to antimicrobial treatment both systemically and topically
  - Cell cycle
  - Multiple species
  - Protection of glycocalyx
  - Diffusion into matrix
  - Resistance is shared

Angelia Smith, MD, Farrel Joel Buchinsky, MD, and J. Christopher Post, MD, PhD, MSS, "Eradicating Chronic Ear, Nose, and Throat Infections: A Systematically Conducted Literature Review of Advances in Biofilm Treatment" Otolaryngology -- Head and Neck Surgery 2011 144: 338

NASAL STEROIDS

- Found to have no effect on presence of biofilm in patients with CRS with polyposis
- However, the pattern of inflammatory cells in the subepithelial layer was altered
  - Steroid users had more monocytes
  - Nonusers had more PMNs
- Steroids have been shown to downregulate release of TNF-a

Pe´ter Csomor, Istva´n Sziklai, Tama´s Karosi “Effects of intranasal steroid treatment on the presence of biofilms in non-allergic patients with chronic rhinosinusitis with nasal polyposis” Eur Arch Otorhinolaryngol, 25 Aug 2013

PHYSICAL DISRUPTION

- Surgery may help to reduce biofilms
  - Reduction of biomass
  - Improving mucociliary function
  - Improved penetrance of topical treatments
- However, ESS has also been implicated in biofilm dispersion
- ESS is also treatment for worst cases of CRS, which may select for higher proportion of biofilm presence

Daniel A. Larson and Joseph K. Han "Microbiology of sinusitis: does allergy or endoscopic sinus surgery affect the microbiologic flora?" Current Opinion in Otolaryngology & Head and Neck Surgery 2011, 19:199–203

IRRIGATION

- 1% baby shampoo and other surfactant irrigations have been found to give some improvement of CRS symptom scores
  - Possibly through mucolytic effects
- They are also purported to have improved penetration of the sinuses and synergy with antibiotic solutions
- However, surfactants potential for nasal irritation and possible ciliotoxicity may limit their use

Rosen PL, Palmer JN, O'Malley BW Jr, Cohen NA. "Surfactants in the management of rhinopathologies." Am J Rhinol Allergy. 2013 May-Jun;27(3):177-80.

MANUKA HONEY

- Active component is methylglyoxal (MGO)
- Irrigations of biofilms in a sheep model at concentrations less than 1.8 mg/ml significantly reduced *S. aureus* biofilm biomass without harming native tissue
- Honey is also known for anti pseudomonal activity

Paramasivan S, Drilling AJ, Jardeleza C, Jervis-Bardy J, Vreugde S, Wormald PJ. "Methylglyoxal-augmented manuka honey as a topical anti-Staphylococcus aureus biofilm agent: safety and efficacy in an in vivo model." Int Forum Allergy Rhinol. 2014

Angelia Smith, MD, Farrel Joel Buchinsky, MD, and J. Christopher Post, MD, PhD, MSS, "Eradicating Chronic Ear, Nose, and Throat Infections: A Systematically Conducted Literature Review of Advances in Biofilm Treatment" Otolaryngology -- Head and Neck Surgery 2011 144: 338

PHOTODYNAMIC THERAPY

- Sinuwave uses a 670 nm red light to illuminate a maxillary sinus model with biofilm growth (antimicrobial photodynamic therapy–aPDT)
  - Area is pretreated with low concentration of methylene blue and EDTA
  - A large number of free radicals are released with photosenstization
- All of the aPDT treatment groups demonstrated statistically significant reductions ($p < 0.005$) in both MRSA and antibiotic resistant *Pseudomonas aeruginosa* biofilms.
- A separate study by the same group shows safe application of MB and aPDT without harm to respiratory epithelium

Biel MA, Pedigo L, Gibbs A, Loebel N. "Photodynamic therapy of antibiotic-resistant biofilms in a maxillary sinus model". Int Forum Allergy Rhinol, 2013; 3:468–473.

Biel MA, et al "The Effect of Antimicrobial Photodynamic Therapy on Human Ciliated Respiratory Mucosa" Laryngoscope, 122:2628–2631, 2012

ULTRASOUND

- In vitro studies of biofilms harvested from patients with biofilm positive nasal polyps were subjected to low-frequency ultrasound with reduction in biofilm load
- Small clinical trial of low frequency ultrasound, 6 sessions over the course of 2 weeks, found the SNOT-20 scores to improve 34% over baseline.

Tamas Karosi, MD, PhD; Istvan Sziklai, MD, DSc; Peter Csomor, MSc, "Low-Frequency Ultrasound for Biofilm Disruption in Chronic Rhinosinusitis With Nasal Polyposis: In Vitro Pilot Study" Laryngoscope, 123:17–23, 2013

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CONCLUSION

- Biofilms are pervasive
- They are implicated in some CRS cases
- Promising improvements in diagnosis and treatment are being developed

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