Biofilms in Otolaryngology

Joseph E. Kerschner, MD, FACS, FAAP
jkerschner@mcw.edu
Dean and Executive Vice President
Professor, Pediatric Otolaryngology
Medical College of Wisconsin

This is not what I learned in medical school or residency

- What has happened over the past 2 decades:
  - OM as a Biofilm disease:
    - 180 publications since that first paper in 1998
  - Molecular studies regarding OM were in their infancy
  - The relationship between the cytokine inflammatory pathways and middle ear mucosal disease was first being reported
    - Almost 400 papers since

Background

- Otis Media Pathogenesis
  - Eustachian Tube Dysfunction
    - Pressure regulation of the middle ear
    - Pressure changes in the middle ear
    - Unusually substantial eustachian tube function
  - Environment
    - Exposure patterns
  - Risk factors
  - Age of onset
  - Immunology
    - Immune competence
    - Immune dysfunction in otitis-prone children (Coussen & Veenhoven)
  - Microbiology
    - Cytoskeletal components of Neonatal Staphylococcus aureus (Veenhoven & Coussen)
  - Vaccines
  - Microbiology – intratubal factors
  - Genetics and molecular factors
  - Candida genes and specific gene expression
  - Biofilm gene expression
- What is between 2 genomes (Brouwer, J Pediatr 2010;157:571)
- Biofilms

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Biofilms

- Bacterial biofilms
  - Complex organization of bacteria
  - Anchored to a surface
  - Surrounded by exopolysaccharide – Matrix – secreted by bacteria
  - Low metabolic rate
  - Escape host immune surveillance
  - Opposed to planktonic concept of bacteria
Biofilms

- Bacteria growing as biofilms display a different phenotype than free-living planktonic bacteria
  - Reduced metabolic rates that render them resistant to antimicrobial treatment
  - Exopolysaccharide matrix that provides protection from phagocytosis and other host defense mechanisms due to a lack of accessibility by immunoglobulin and complement
  - Reliance on complex intracellular communication system that provides for organized growth characteristics, “quorum sensing”
  - Resistant to standard culture techniques because of altered metabolism
  - Abnormal genetic expression and ability to rapidly share genetic information

Background

- Many chronic infectious processes in humans have been demonstrated to be dependent upon the development of biofilm formation
  - Dental
  - Chronic bacterial prostatitis
  - Cystic fibrosis
  - Medical Implants
    - Orthopedic implants
    - Heart valves
    - Catheters
  - Native valve endocarditis (NVE)
  - Bacteria from oral anaerobic sources
    - Streptococcus (including pneumococci)
    - Neisseria
    - gram-negative bacteria
    - fungi (Candida and Aspergillus spp.)

OM as a Biofilm Disease?

- Chronic infectious process
- Difficulties with culturing effusions
- Recalcitrant to antibiotic therapy

Indirect Evidence of Bacterial Biofilm in Cases of Chronic OM

- Evidence suggesting that otitis media with effusion is not a sterile inflammatory effusion, but rather a vibrant, active bacterial process
  - Bacterial DNA is present in pediatric culturally “sterile” effusion
  - Purified bacterial DNA is cleared within hours whilst DNA from live infectious bacterial DNA persist in sterile effusion for up to 4 weeks
  - Bacterial mRNA is present in culturally sterile, DNA-positive middle ear effusions in children indicating that the bacteria are intact and metabolically active.
  - Bacteria-synthesized proteins are present in sterile effusions

Direct Evidence of Bacterial Biofilms in Otitis Media

- Experimental chinchilla model of OM
  - *H. influenzae* injected via transbullar approach bilaterally
  - Confocal and electron microscopic evidence of biofilm formation


H. influenza 3 Hours Post-Inoculation

H. influenza 24 Hours Post-Inoculation

SEM image of *H. influenzae* Biofilm on Chinchilla Middle-ear Mucosa

Hypotheses

- Otitis media in humans is biofilm mediated
  - Otitis media with effusion (OME)
  - Recurrent otitis media (ROM)
- Direct evidence of *Streptococcus pneumoniae* (SP) and *Haemophilus influenzae* (HI) biofilms is available in children undergoing tympanostomy tube (TT) placement for OM
**Biofilms**

JAMA 2006;296(2):202-211.

**Methods**

- IRB approved evaluation of 40 children undergoing TT placement for OM
- 20 with diagnosis of OME
- 20 with diagnosis of ROM
- 8 control patients with no history of OM
- Broader study of OM pathogens

- Middle ear effusions (MEE) collected (if present)
- Aerobic cultures
- PCR-based assessments for Haemophilus influenzae (HI), Streptococcus pneumoniae (SP) and Moraxella catarrhalis (MC)
- ME tissue biopsies (<1 mm) assessed for bacterial biofilms using confocal laser scanning microscopy (CLSM), vital dyes and specific bacterial assays.

**Direct Assessment for Biofilms**

- Staining with CLSM assessment for biofilms
  - BacLight LD (LiveDead stain for bacteria)
  - Sytox (nucleic acid stain)
  - WGA (wheat germ agglutinin lectin stain)
  - SP specific antibody
  - HF FISH

**Direct Assessment for Biofilms**

- Biofilm Presence
  - Direct evidence by morphology/staining on CLSM of adherent bacteria on ME mucosal membrane with clear biofilm ultrastructure
  - Bacteria
  - Matrix
  - At least one other confirmatory test demonstrating presence of viable bacteria
  - PCR
  - SP Ab
  - FISH

**Culture Results**

- MEE < 25% culture positive
  - SP, HI, MC

- Biofilms are difficult to culture by conventional means

**PCR Results**

- 100% of patients with MEE had positive PCR for SP, HI or MC
  - 53% with 2
  - 10% with all 3
  - Polymicrobial
  - 88% HI
  - 45% SP

- Supports concept of viable bacteria in culture negative MEE (Rupner MS, et al. JAMA. 1988;260:296-6.)
Biofilm Results

- Biofilm positive
  - OM patients
    - 92% positive by 2 or more methods
  - Control patients
    - 0% positive

Discussion

- Compelling evidence that biofilms play a primary role in both OME and ROM
  - Clinical Specimens
  - Multiple steps in processing and washing
  - Human specimens satisfy previous criteria for biofilm disease
  - Chronic disease states
  - Difficult to culture using standard means
  - Evidence of bacterial mRNA by PCR
  - It is the nature of biofilms that make chronic infectious diseases, including otitis, so difficult to treat with current antimicrobial strategies

OM as a Biofilm Disease

- Shift in thinking about this disease
- Not an abandonment of first principles but new frame of debate

  - Anatomy
    - Which aspects of ET dysfunction make biofilm formation likely?
  - Other host factors
    - Which host factors promote biofilm formation?
      - Cytokine polymorphisms
      - Mucin gene expression and mucin gene polymorphisms
Questions About OM Pathophysiology

What is the impact of these findings on current concepts of ReCom?

- New organisms
- Biofilm organisms
- Early onset and increased likelihood of severe disease
- Viral interaction/activation
- Antibiotic usage
- Nasal/systemic
- Tympanostomy tube placement
- Vaccine strategies
- Adenoidectomy

Questions About OM Pathophysiology

What is the impact of these findings on current concepts of ReCom?

- New organisms and biofilm organisms
- We know that the microbiology of the nasopharynx and the middle ear is much more diverse than the big 3 (SIP, NTHi, and Rha)
- We know that within biofilms that microorganisms are continually sharing genetic material and resistance genes
- Does the microbiologic diversity in the ME contribute to these processes?
- Should we be targeting research on other organisms?

Questions About OM Pathophysiology

What is the impact of these findings on current concepts of ReCom?

- Early onset and increased likelihood of severe disease
- We know that children with low cord blood concentrations of pneumococcal antibodies have a higher incidence of being driers prone (Blasen ST, et al. Arch Otolaryngol 2001)
- This is a form of immune incompetence which likely allows for the early set up of biofilms in these children’s ME
- Suggestion that in older prone children that AOM may be more about recrudescence of biofilm bacteria than “new infections”
- Viral interaction/activation

Questions About OM Pathophysiology

What is the impact of these findings on current concepts of ReCom?

- Antibiotic usage
  - Watchful waiting is effective – why?
  - The patient’s immune system
  - Many patients with OM do not have bacterial disease
- However, we know that when strict criteria of OM dx is used that antibiotics significantly reduce the duration of the OM episode and the incidence of OME (Funderson A, et al. JAMA 2011)
- What does this mean for the formation of biofilms and ReCom?
- Tympanostomy tube (TT) placement
  - In patients with ReCom we have substantial evidence that TT:
    - Reduces the incidence of future episodes of OM
    - Improves patient QOL
    - We also know that TT change the ME microenvironment to negatively impact that ability of biofilms to survive
    - Does this provide a pathophysiologic explanation for the benefit of TT in this patient population?

Questions About OM Pathophysiology

What is the impact of these findings on current concepts of ReCom?

- Vaccine strategies
  - Need to consider how vaccines participate as a strategy to limit biofilm formation and pathogenicity
- Adenoidectomy
  - Adenoidectomy – helpful in children with OM – what is the pathophysiology?
- We know that biofilms are also present in the adenoids of children with ReCom and OME
- We know that adenoidectomy is effective in reducing the incidence of ReCom and OME in patients receiving TT (Blasen S, Blasen MT, JAMA Otolaryngol 2014)
- Is the reduction in NP biofilm formation following adenoidectomy the common pathway of improvement?

Questions About OM Pathophysiology

What impact do these findings have on clinical care of patients with chronic OME?

- Adenoidectomy
  - Adenoidectomy is efficacious in patients with OME
  - Adenoid size irrelevance - therefore not a ‘ET obstruction event’
  - Relationship between adenoid biofilms and middle ear biofilms
- Tympanostomy tube placement
  - More than just anatomy
  - Changes in microenvironment
Therapeutic Questions

- Biofilms are involved – now what?
  - OM as a self-limiting disease
  - Impact and feasibility of biofilm eradication
    - Accessibility to the middle ear
    - Impact on normal flora
    - Re-population time

Future Directions

- Middle ear preparations
- Linkage to the nasopharynx
  - Pathophysiologic origin
  - Accessible
- NETs

Neutrophil extracellular traps (NETs)

- Neutrophil strategies:
  - Engagement of microbes (phagocytosis)
  - Secretions of antimicrobials
  - NETs
- NETs are networks of extracellular fibers, primarily composed of DNA from neutrophils, which bind and kill extracellular pathogens while minimizing damage to the host cells.
  - NETs provide for a high local concentration of antimicrobial components
  - In addition to their antimicrobial properties, NETs may serve as a physical barrier that prevents further spread of the pathogens.

NETs

- Middle ear biofilms contain extensive DNA stranding from NETs.
- The NETs contribute to the viscosity of the effusion, potentially contributing to its failure to clear as well as biofilm development.
- Dornase alfa (deoxyribonuclease 1) can fragment these strands and may play a role in future chronic OM treatment.
**Noninvasive *in vivo* optical detection of biofilm in the human middle ear**

- Scanned 13 clinically infected adult patients and 7 normal controls using clinical findings as the gold standard.
- All middle ears with chronic OM showed evidence of biofilms, and all normal ears did not.

**Biofilms Associated with Infected Adenoids**

**Sample Preparation**

CONFOCAL MICROSCOPY

- Live/Dead/EPS
- Bacteria and nuclei

**Fixed Tissue:**

- FISH: (ID Target)
- Syto9: Bacteria and nuclei
- Histology – cytoskeleton / cell type

**PCR (ID of Target)**

- RT (viability and ID of Target)
- IBIS (ID, relative abundance)
- MDA (ID of Target)

**Quantification of The Usual Suspects**

Pathogens in OSA and OM Groups Detected by FISH and PCR
Therapeutic Questions
- Are these biofilms associated with lymphoid hypertrophy and UAO?
- Difficult questions to answer – there is not a ready control population
- Requires novel tests for biofilms in non-surgical patients – Kerschner Lab NP swab study
- Biofilms do exist in children with UAO as well

Chronic Draining Tympanostomy Tubes
- Biofilms have been implicated both within the ME and on the tubes themselves
- Tube removal is effective if the tube is a primary source

SEM Image of Sterile Tympanostomy Tube

SEM image of bacterial biofilm on tympanostomy tube

Biofilms in Other Areas of the H&N
- Chronic Tonsillitis
  - Biofilm forming bacteria have been identified in children with chronic and recurrent tonsillitis
  - Potential for new diagnostic techniques
    - Nitric Oxide increased in patients (Torretta S et al J Laryngol Otol 2015)
- Chronic Sinusitis
  - Systematic literature review (Rameshkrishnan Y et al J Laryngol Otol 2015)
  - The existing evidence supports the role of biofilms (particularly the Staphylococcus aureus phenotype) in more severe, recalcitrant disease and poorer surgical outcomes
  - Multimodality treatment, with a shift in paradigm to incorporate anti-biofilm strategies, is likely to form the mainstay of future recalcitrant chronic rhinosinusitis management
Biofilms in Other Areas of the H&N

- Device Infections
  - Bone-anchored hearing devices
  - Cochlear implants
  - Central lines

Summary

- Biofilms are present throughout the head and neck and contribute significantly to head and neck diseases.
- The understanding of biofilms has substantively changed our way of thinking about these diseases and is leading to new treatment paradigms of new avenues of investigation which hold promise for better outcomes.

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