Evaluating the Evidence of Biofilm Associated Wound Research: Unmasking the Present Limitations (Looking Back), Highlighting the Future, and Need for Innovative Strategies (Looking Forward)

John G.Thomas, Ph.D., Janice Shreve, APRN, FACCWS, Brian Steffke, FNP-C, WCC, Heidi LaRue, FNP-C, and Linda Dunlap, MBA SC,MT(ASCP)
WVU, School of Dentistry and Nursing Administration and Department of Plastic Surgery, WVUH, Morgantown, WV, USA 26506
International Center for Biofilm Research Linking Disruptive/Restorative Microbial Engineering

SAWC 1

Introduction:

The impact of biofilms on wound pathology has been limited by a lack of global standards to assess anti-biofilm chronic wound management/ intervention.

This has been amplified by the unique bi-phasic nature of wound pathology involving a transition from planktonic to biofilm phenotype via Critical Colonization and the difficulty to demonstrate the presence of a biofilm in the clinical arena in a timely and cost conscience environment. (Figs. 1, 2, 3)

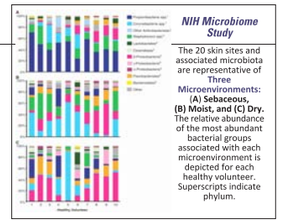

Fig. 1

Biofilm SEM in Chronic Wound

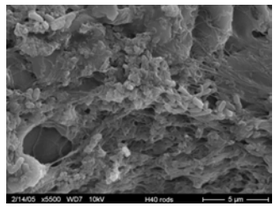

Fig. 2

Objective:

Here, in the Metagenomic era, we wanted to 1) review/ evaluate previous published clinical research (*Looking Back*) to 2) integrate new quantitative anti-biofilm metrics (*Looking Forward*) incorporating the new definitions of a biofilm "super-genome" acting as a "molecular platform" for anti-biotic resistance via "plasticity", and "plurality" of this wound microbiota, invigorating Horizontal Gene Transfer (HGT) and Multi Drug Resistance (MDR).

Methods:

We reviewed published medical/nursing and microbologic articles for the last 15 years keyed to chronic wound biofilms, interventions and outcomes. Manuscripts were placed into a Drop Box.

We used the SORT guidelines (2009) to evaluate evidence based studies of greater than 50 patients and the Parsek-Singh criteria (2003) updated by Hall-Stoodley and Paul Stoodley (2009) and most recently by Hall-Stoodley in 2012 for biofilm presence and detection .

We asked three basic, fundamental questions :

1. What level of evidence was presented for biofilm presence?
2. What were the metrics to measure anti-biofilm management and outcomes?
3. What evidence was presented for significance and pathogenicity of the biofilm associated with the chronic wound?

Results:

210 studies were reviewed. 22 were level 1 by SORT guidelines (good quality), while the majority were level 3 (case series for diagnosis and treatment) with considerable variation among study findings. (Tables 1, 2) 14 studies met 6 point Stoodley/Stoodley criteria for biofilm associated infection with limited distinction between "colonization".

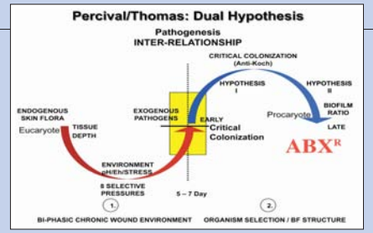

Fig. 3

Fewer met the more detailed criteria for Hall-Stoodley presented in 2012.

Culture criteria were variable, usually mono-species, and inconsistent with limited focus on yeast, emphasizing known 4–6 planktonic isolates. Molecular, non-culture techniques did not generally incorporate phylagenetics or shifts in population dynamics, focusing on 16S RNA, only. (Bacteria)

Looking Back

Table III
- 5 Selection Strategy
- 5 Medical/Nursing/Microbiology since 2000
- Chronic Wounds with biofilm wording
- Clinical Trials w more than 50 Pts

Table IV
- Manuscript Evaluation using SORT Guidelines (2009)
- Level 1 Good Quality , Patient–oriented
- Level 2 Limited Quality, Patient–oriented
- Level 3 Other Evidence

Table V Biofilm Diagnostic Criteria
- Hall-Stoodley and Stoodley (2009)
- Pathogenic Bacteria on Surface
- Aggregates of Bacteria /cells or clusters encased in matrix from direct examination
- Culture negative from BX
- Recalcitrant to antibiotic RX
- Limited host response and clearance in discrete areas

Table VI
- Updated Diagnostic Criteria for Biofilm detection Hall-Stoodley (2012)
- Microbial Evidence of chronic localized Infection
- Molecular ID of microbial pathogen
- Microscopic evidence of cell aggregates
- Medical history of Biofilm predisposing condition
- Documented evidence of antibiotic failure of persistent infection
- Immunologic evidence of recurrence of infection after RX withdrawn

Looking Forward

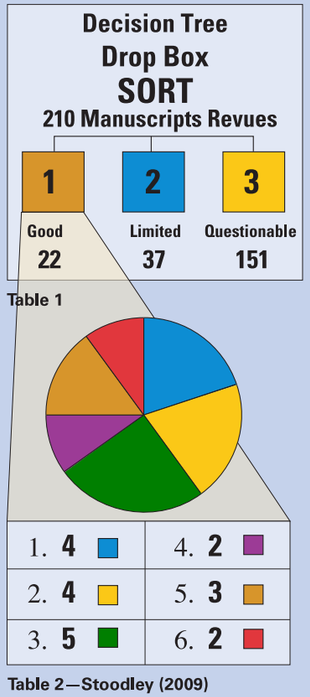

Table 1

1. 4	4. 2
2. 4	5. 3
3. 5	6. 2

Table 2—Stoodley (2009)

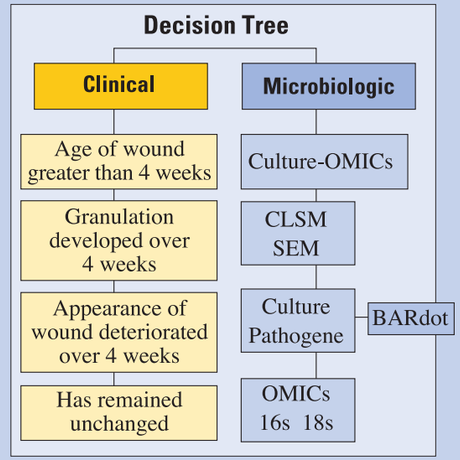

Figure X

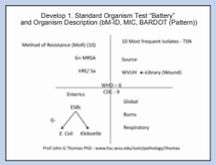

Figure 4

Figure 5

Conclusion:

Looking Back highlighted inconsistent studies with no uniform test method strategy or metric to validate claims, while Looking Forward showed a lack of bridging between new biofilm diagnostic methods, Metagenomics, and wound care assessment. (Fig. X)

There was a particular lack of science in biofilm and collateral antibiotic resistance. Based on these results, we constructed a 10 point standard based upon "culture-OMICs", a "suite" of combined traditional cultures and molecular assays.

This also incorporated a 5 organism Test Battery in biofilm phenotype and FISH, emphasizing the need for a new laboratory Report Form, highlighting biofilm detection with a redefinition of "pathogen/virulence". (Figs. 4, 5)

Discussion:

Future Lab Report Culture-omics
- Traditional Cultures: Acute
- Sensitivities: Planktonic and Biofilm (Poloxamer)
- OMIC's: Molecular (non culture) including 16S and 18S. Phylagenetics (Cluster analysis)
- Biofilm Stage I–IV
- Enterotype: 1, 2, or 3

References:

1. CDC (2008)
2. SORT, (2009)
3. Stoodley, (2009)
4. Stoodley-HAll, (2012)