Biofilm

Where Does It Come From & Why Is It Such a Problem?

NANT 33rd Annual National Symposium

Jo-Ann Maltais, Ph.D.

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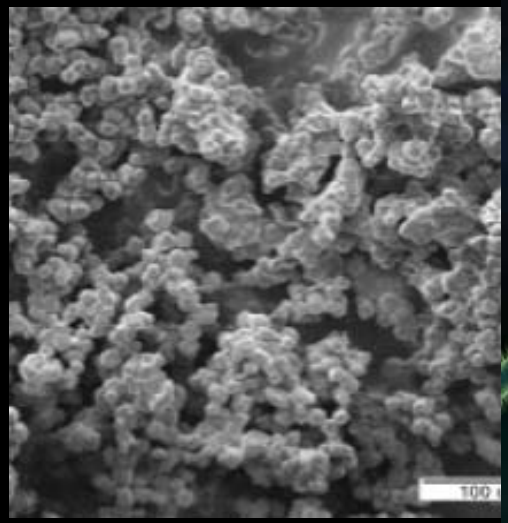
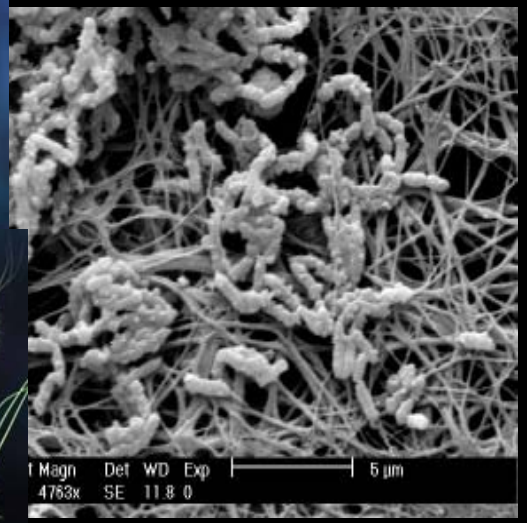
BIOFILM & ENDOTOXINS—METHODS, CULTURES, SAMPLING TECHNIQUES

WHY DO BACTERIA FORM BIOFILMS?

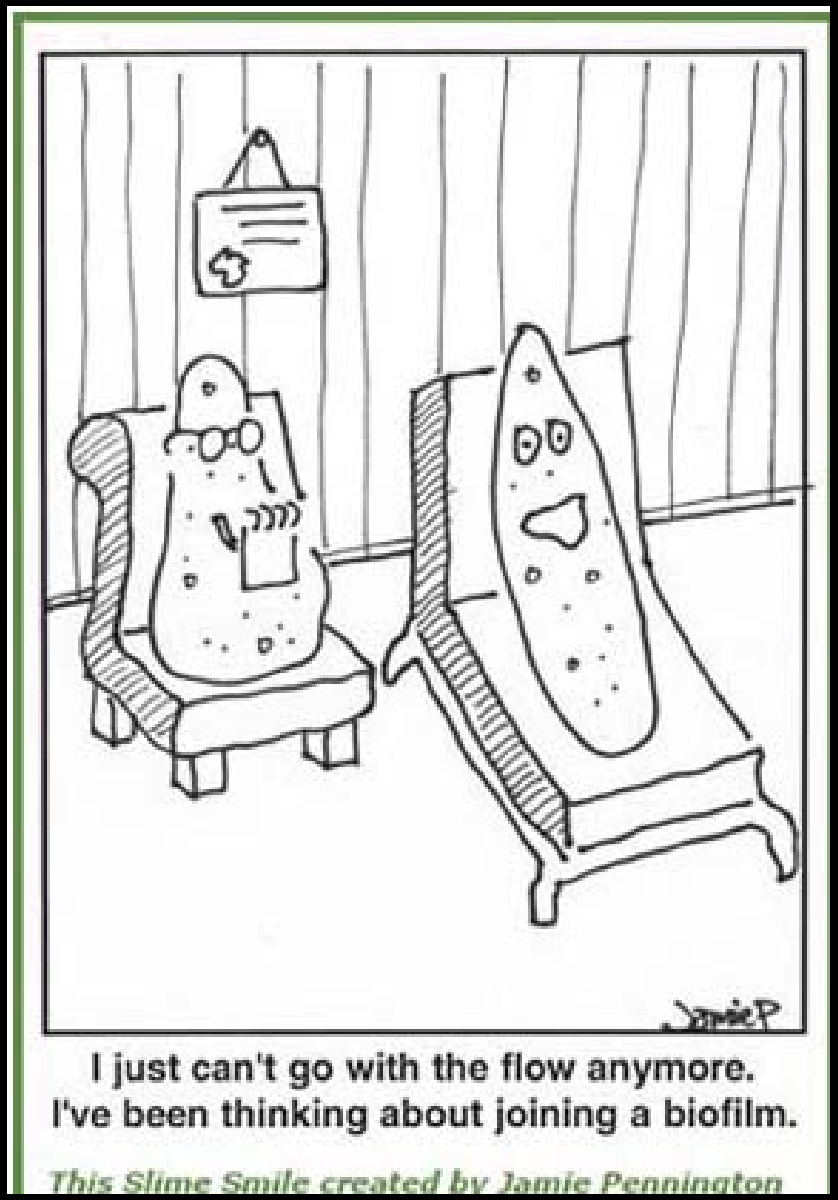
- Survival mechanism
- Community
- Symbiotic relationships
- Slimy Matrix for protection



Ryder, M. Medical Biofilm Research
TargetBSI.com Webinar 7/28/09



Donlan, RM. Biofilm Laboratory. CDC


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

WHAT IS A BIOFILM?

A collection of microorganisms, (single or multiple species) which stick to a surface, form a protective, extracellular matrix or slime layer, attract other organisms and nutrients to enhance survival.

INGREDIENTS NEEDED FOR BIOFILM FORMATION

Surface

- Hydrophobic e.g. plastics, Teflon, PVC
- Hydrophilic
- Non-polar
- Rough texture
- Conditioned surface

Bacteria

- Biofilm forming properties
  - Surface charge
  - Fimbriae
  - Flagella
  - Alginate

Fluid dynamics

- Water
- Dialysis Fluid
- Low Flow/Stagnant Areas
- Nutrients
- Low shear forces

COMPOSITION OF A BIOFILM

- Bacteria
  - Filamentous
  - Fimbriae
  - Flagella
- Extracellular Matrix (EPS)
- Feeding & Water Channels
- Polysaccharide sugars
  - Glucose
  - Mannose
  - Galactose
  - Xylose
  - N-acetylglucosamine
- Divalent cations—Ca^{++} & Mg^{++}
- Lipid A

BACTERIA IN BIOFILMS

- Single bacteria biofilm—thinner
- Multiple bacteria biofilm—thicker
- Quorum sensing—cell to cell communication
  - Attachment & detachment role
- Exchange genetic material (plasmids)
- Migration between microcolonies
- Up to 1.0×10^9 cells per biofilm clump
- Up to 12 EU/cm^2 endotoxin reported

BACTERIA OF BIOFILMS IN DIALYSIS

- Mycobacteria can also form biofilms
- Autotropic bacteria may be a source of organic matter for biofilm
- Hydrogen oxidizing and nitrogen fixing bacteria are likely secondary colonizers of biofilm
- Staphylococcus, Streptococcus can be indicators of mature biofilms

Gomila, M et.al., 2005. FEMS Microbiology Ecology (2005)52:101-114

FLUID PROPERTIES THAT AFFECT BIOFILM FORMATION

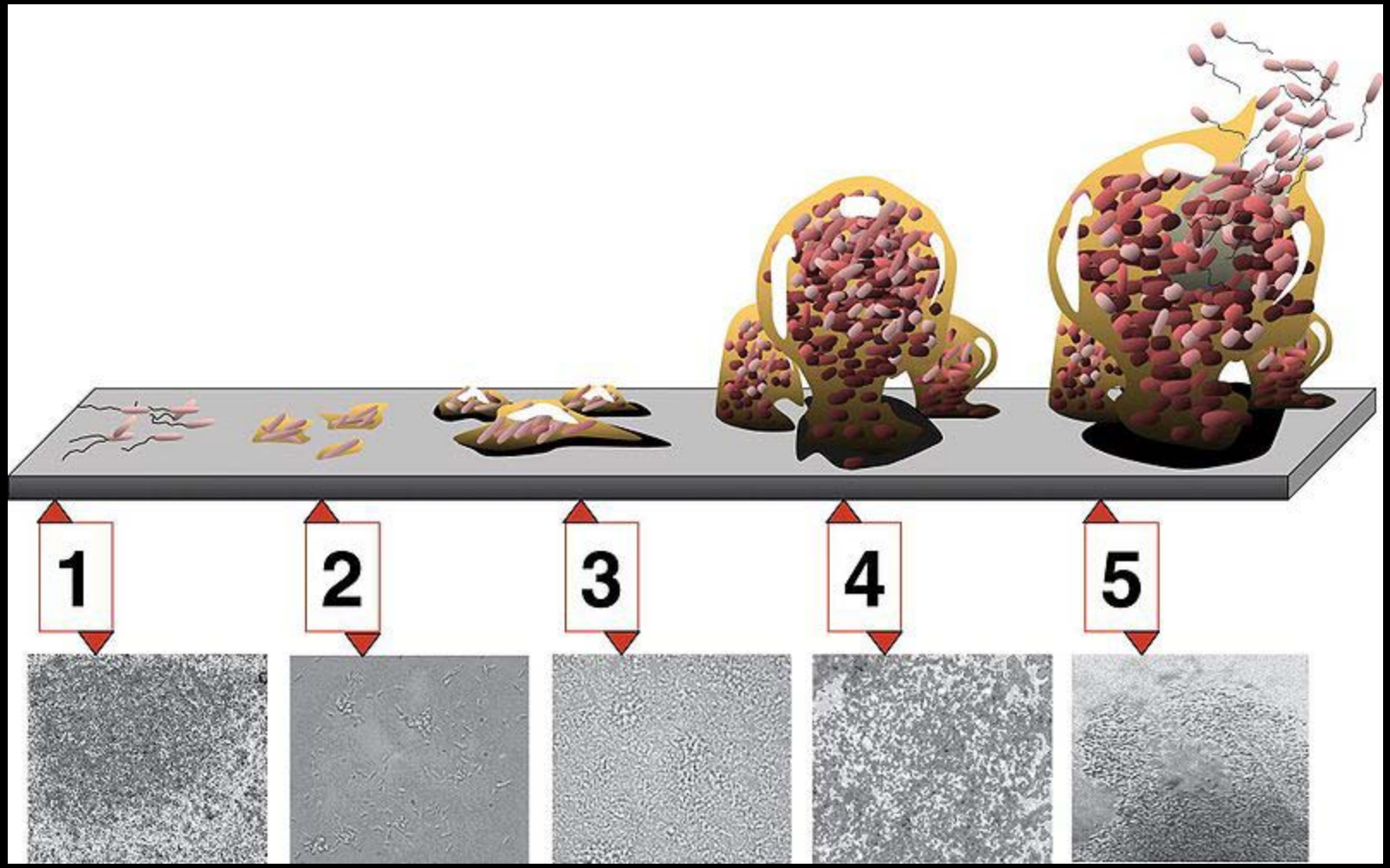
- pH
- Nutrient Levels
- Ionic strength
- Temperature
- Seasonal effects
- Flow velocity
- Surface properties
- Hydrophobic vs hydrophilic surface attachment
  - Hydrophobic attachment—fimbriae, protein, mycolic acid (Gram + bacteria)
  - Hydrophilic attachment—EPS & LPS

HOW DOES BIOFILM FORM?

HOW BIOFILM HAPPENS

- A solid surface is submerged or exposed to a fluid such as water
- Free-floating, planktonic bacteria adhere to the surface to begin biofilm development
  - Only certain species can attach on their own
  - Weak, reversible adherence
  - More permanent adherence if not immediately flushed off
- A slimy matrix is excreted to protect residents
- Other bacteria adhere to initial colonists or to the matrix
- Growth of bacteria in the biofilm & recruitment of more residents occurs

STAGES OF BIOFILM DEVELOPMENT



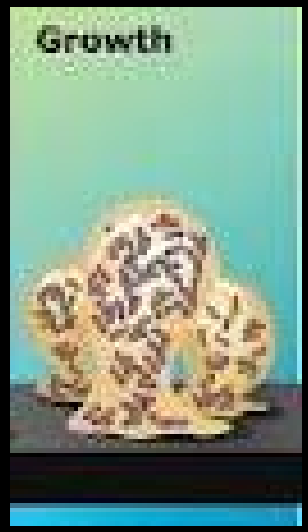
http://bacteriality.com/2008/05/26/biofilm/

STAGE 1: ATTACHMENT TO SURFACES

- Low flow, laminar areas of surfaces
- Surface conditioning
  - Dead cells
  - Protein
- Bacteria touching hard surface
  - Fimbriae, pili, flagella, adhesion proteins
- Biofilm residents sends out signal molecules to attract other bacteria to join them
- Reversible process at this stage

STAGE 2: IRREVERSIBLE ADHERENCE

- In 12 minutes, attached bacteria increase
  - Production of proteins
  - Excretion of polysaccharides (slime layer)
  - Rapid cell division—exponential bacterial growth
- Slime layer prevents dislodgement of biofilm
  - Resistant to shear forces of flowing water
  - Keeps bacteria attached to surface



ADHERENCE

Micro-organism	Number of samples isolated			Adhesion to inert surface		
	Water	Dialysate	Total	Strong	Moderate	Weak
Burkholderia cepacia	14	6	20	3	10	7
Pseudomonas aeruginosa	3	12	15	4	6	5
Acinetobacter haemolyticus	2	10	12	9	2	1
Pseudomonas stutzeri	1	6	7	2	4	1

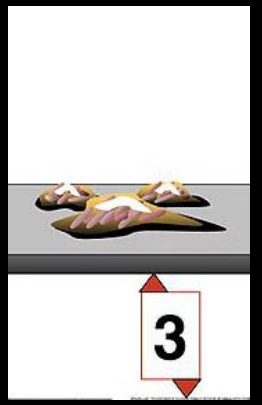
Table 2 Prevalence of the principal micro-organisms isolated from samples of water and dialysate and the profile of adhesion to inert surface

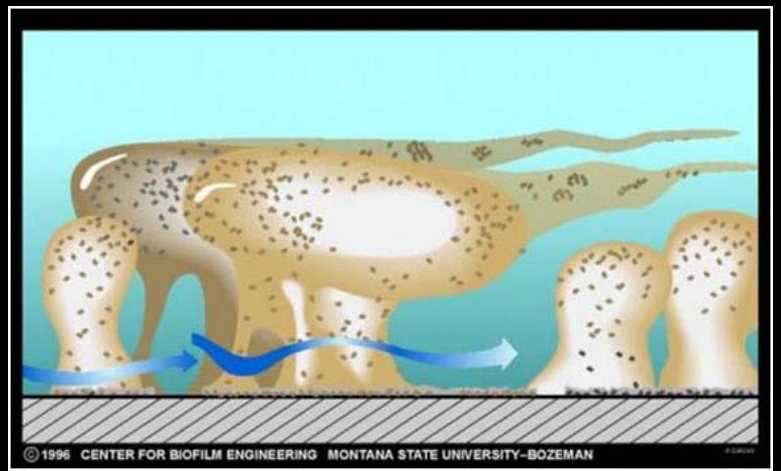
Journal compilation © 2007 The Society for Applied Microbiology, Journal of Applied Microbiology **103** (2007) 1791–1797

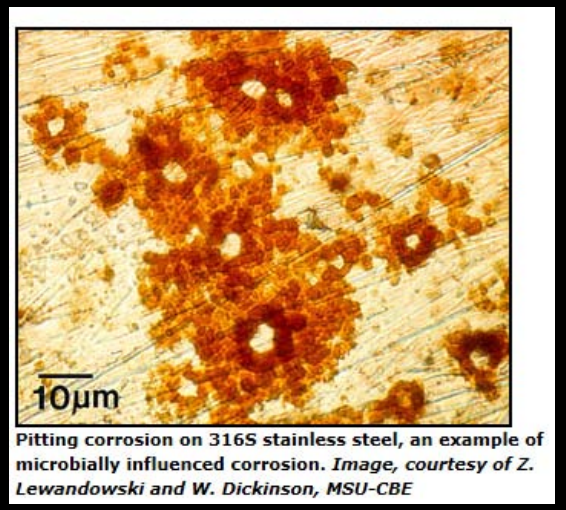
Vincent, et. al. 2007

STAGE 3: AGGREGATION

- Location in Biofilm = Specific Responsibilities
  - Outermost Layer = Defensive, aerobic bacteria
  - Higher Layers = Food Gathering
  - Lower Layers = Waste Removers (Sewage Tx), anaerobic bacteria
  - Bottom Layer = Adherence of Biofilm to Surface
- More slime production
  - Creates water channels
  - Allows diffusion of nutrients to inner layers of the biofilm



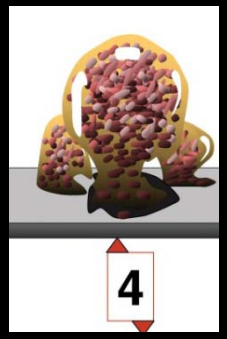


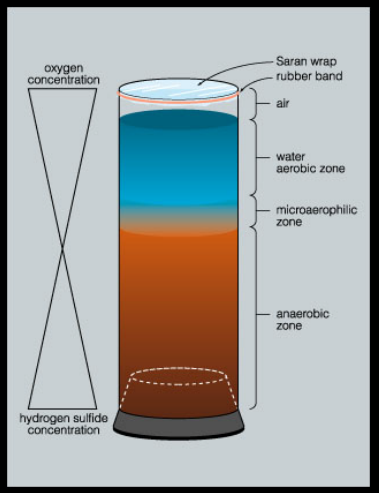


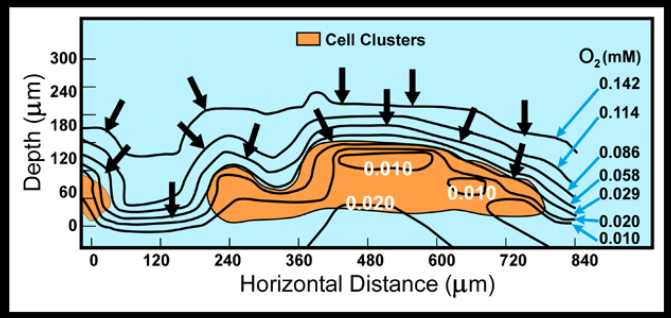
Pitting corrosion on 316S stainless steel, an example of microbially influenced corrosion. *Image, courtesy of Z. Lewandowski and W. Dickinson, MSU-CBE*

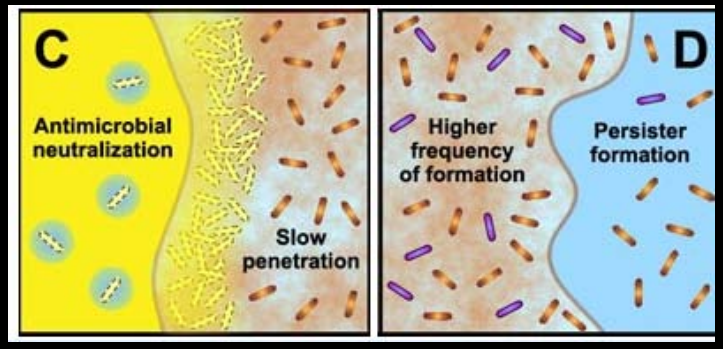
STAGE 4: MATURITY--COMPOSITION

- Biofilm Composition
  - 10-75% Bacteria
  - 90-25% Slime
- Oxygen gradient
- 1000x More Resistant to Disinfectants



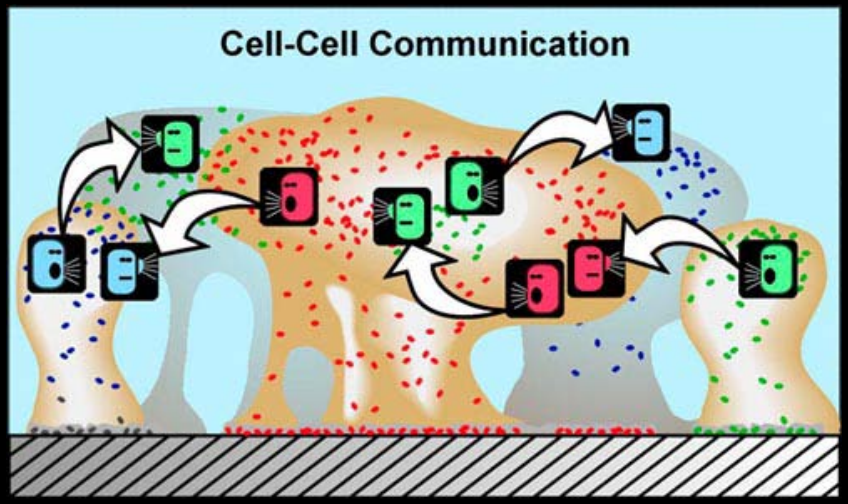


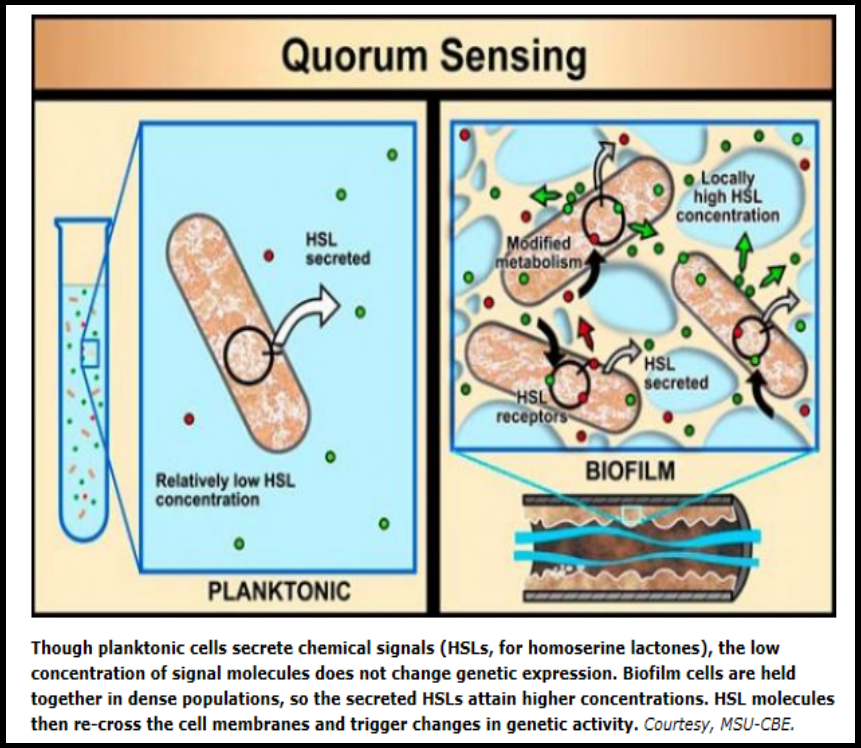




STAGE 4: MATURITY—BIOFILM COMMUNICATION

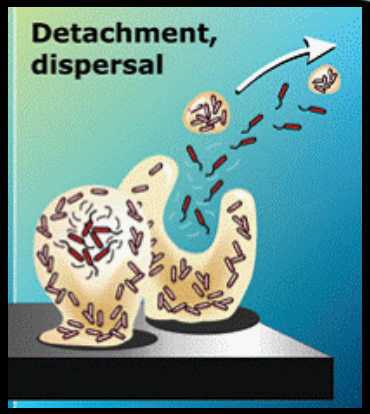
- Quorum sensing
- Communicate changes in environment
- Alter behavior

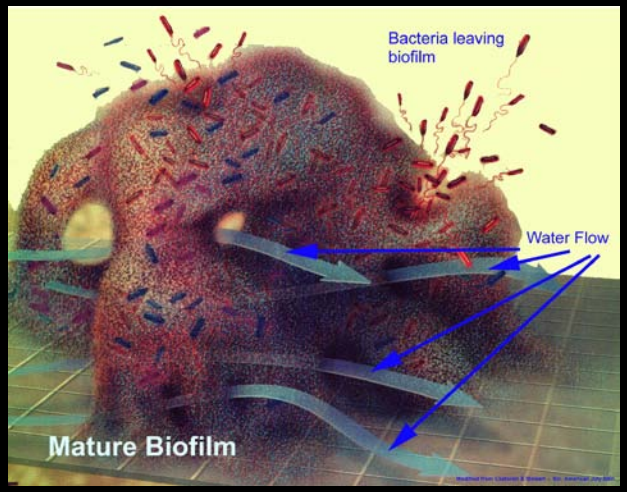


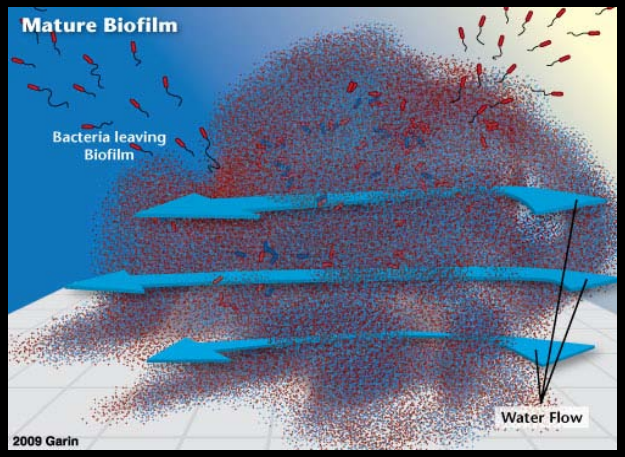


Though planktonic cells secrete chemical signals (HSLs, for homoserine lactones), the low concentration of signal molecules does not change genetic expression. Biofilm cells are held together in dense populations, so the secreted HSLs attain higher concentrations. HSL molecules then re-cross the cell membranes and trigger changes in genetic activity. *Courtesy, MSU-CBE.*

STAGE 5: DISPERSAL







This is the Biofilm's Most Vulnerable Time!

- Releases Single Cell Bacteria or Cell Plaques
  - Start new biofilm colonies
- Releases cytokine inducing substances
  - Endotoxin, peptidoglycans, DNA fragments

FACTORS AFFECTING DETACHMENT OF BIOFILM

- Shedding daughter cells
- Quorum sensing effects
- Shearing of aggregates (continuous low level removal)
- Sloughing (rapid & massive)
- Maturity of biofilm
- Flow effects—increase in fluid flow velocity

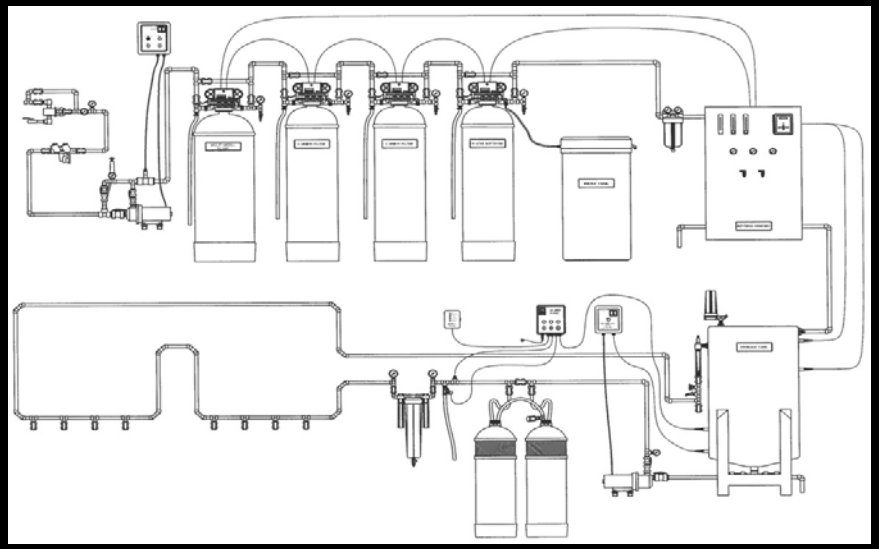
"Biofilm bacteria can move in numerous ways: Collectively, by rippling or rolling across the surface, or by detaching in clumps. Individually, through a "swarming and seeding" dispersal."

http://bacteriality.com/2008/05/26/biofilm/

WHERE IS BIOFILM FOUND IN DIALYSIS?

WHERE BIOFILM CAN DEVELOP IN DIALYSIS H2O TREATMENT SYSTEMS

- Feed water
  - Well Water vs Surface Water
- Water Softener Brine Solution
- Softener exchange resin
  - Provides large surface area for bacteria to attach
  - Captures nutrients for bacterial growth
- Carbon Bed
- Ion Exchange Resin Beds
- Membranes
  - RO
  - Filters
- Break Tank



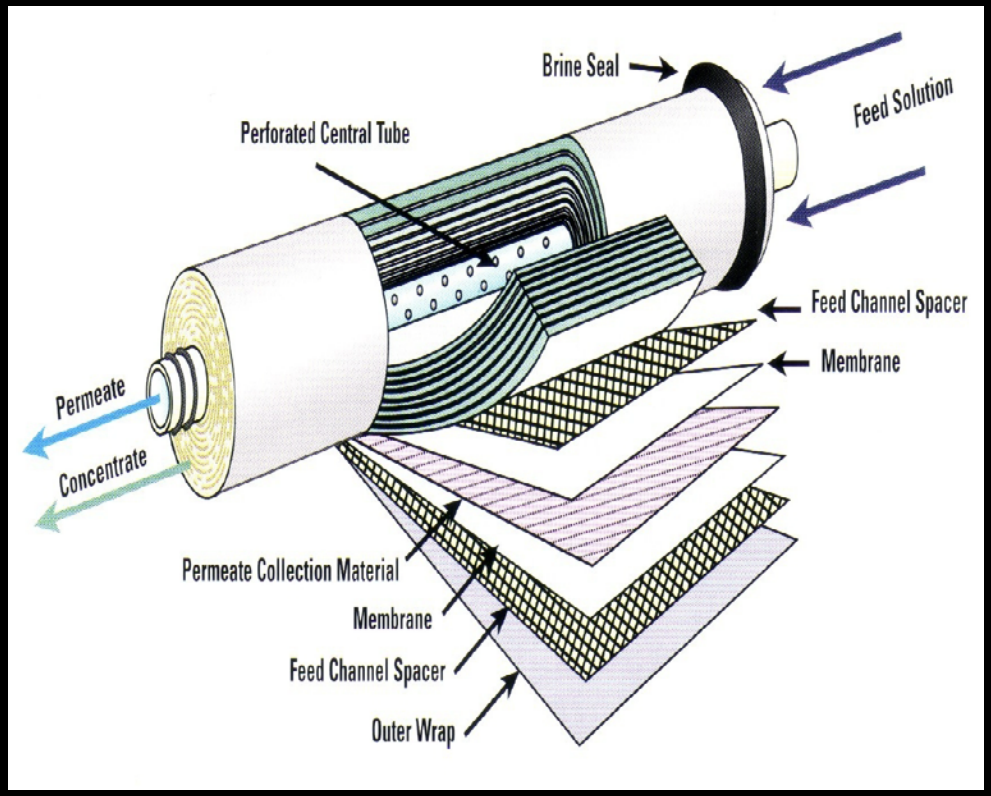
POST H2O TX SYSTEM BIOFILM SITES IN DIALYSIS SETTINGS

- Permeate loop
  - Piping
  - Joints
  - Taps
  - Storage Tank
- Dialysis Machine Water Inlet Line
- Dialysis Machine Hydraulic Path
- Bicarbonate Concentrate Mixing System
- Bicarbonate Concentrate Jugs

BIOFILM IN PIPES & TUBING

Ryder, M. Medical Biofilm Research
TargetBSI.com Webinar
7/28/09

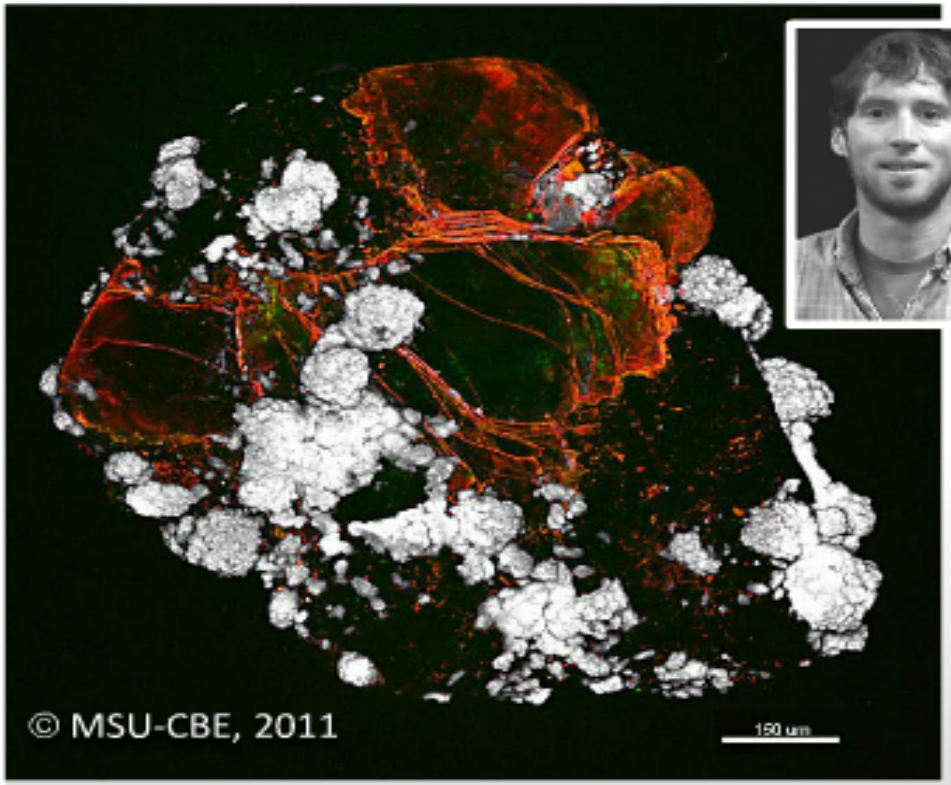
INSIDE AN RO MEMBRANE



RO MEMBRANE BIOFILM

BIOFILM AND CARBONATE

Confocal Scanning Laser Microscopy — **MSU Center for Biofilm Engineering**



James Connolly, CBE PhD student in environmental engineering, NSF-IGERT awardee

"This image is a CSLM reconstruction of a sand grain colonized by *Sporosarcina pasteurii*, where calcium carbonate (white) has been precipitated. Healthy cells can be seen as green dots. Regions with cells that have compromised membranes or contain extracellular nucleic acids appear as red."

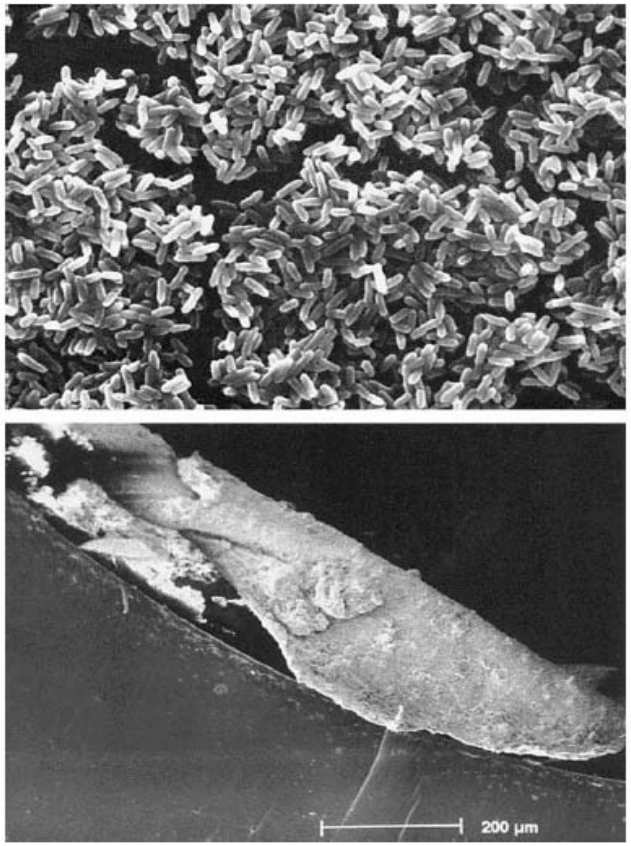


Fig. 3. Typical biofilms in a nephrological setting are found in water lines of dialysis monitors and in catheters either from venous, peritoneal or bladder origin. Upper part is a SEM vision of a silicon dialysate line heavily contaminated with *Pseudomonas aeruginosa*; lower part is a SEM analysis of a silicon central venous catheter showing a protein layer with bacteria.

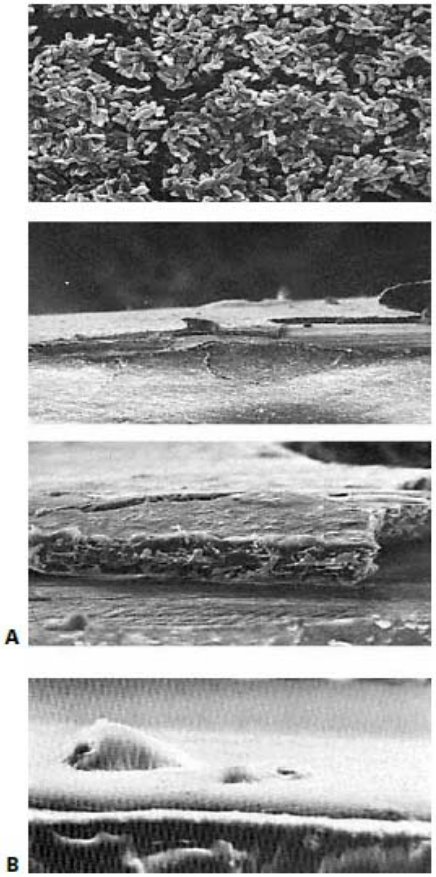


Fig. 4. Effect of chemicals on biofilm from dialysate lines (SEM analysis). **A** Acetic acid in the presence of heavy organic contamination (upper figure) results in a fixative effect (centre and lower figure. **B** Hypochlorite results in slime formation.

Capelli, et al 2000 Blood Purif 18:224-230

EFFECTS OF BIOFILM

- Reduce in fluid flow
- Masks actual bioburden levels
- Increase in endotoxins
- Release of toxic products
- Reduction in heat transfer
- Damage to equipment
- Increased resistance to disinfectants

CURRENT CHECKLIST

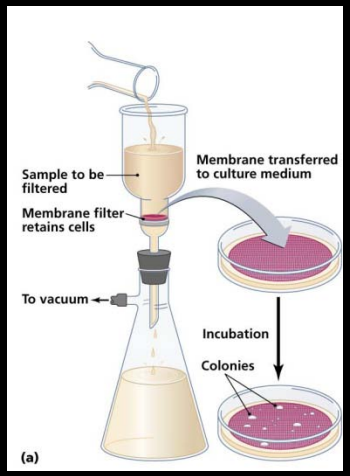
TOTAL VIABLE COUNT ACCEPTABLE LEVELS DIALYSIS WATER & DIALYSATE

Contaminant	ISO Max Level*	ISO Action Level*	RD 52 Max Level**	RD 52 Action Level**
Total Viable Bacteria Count (TVC)	<100 CFU/ml	50 CFU/ml	<200 CFU/ml	50 CFU/ml

**ISO 13959:2014; ANSI-AAMI 13959:2014*
***ANSI-AAMI RD52:2004*

SAMPLING & CULTURE METHODS

- ISO 23500
  - Membrane Filtration
  - Spread Plate Technique
  - Pour Plate Technique
  - TGEA or R2A 7-23C, 7 days or equivalent
- RD52
  - Membrane Filtration
  - Spread Plate Technique
  - Dip Samplers with QA program
  - TSA 48h, 35-37C



CURRENT FREQUENCY OF SAMPLING & ANALYSIS

- Dialysis water & dialysate
  - At least once a month
- Dialysis machines
  - At least 2 per month or a sufficient number to test every machine at least once per year

CULTURE METHOD DEFICIENCIES FOR BIOFILM DETECTION

- Only about 30% of total bioburden recovered
- Planktonic and not sessile organisms accounted for
- Detects only viable & replicating bacteria not dead or injured organisms
- Biofilm bioburden not accounted for

WHY IS BIOFILM SUCH A PROBLEM?

- Risk to patient
- Adverse effects on equipment & processes
- Can’t be prevented only controlled
- Difficult to remove
  - Disinfectant resistance
  - Complex construction
  - Multiple component chemistry
- Economic impact

EFFECT OF BIOFILM PRESENCE ON DIALYSIS PATIENTS

- Biofilm contains contaminants that can be transferred to patients thru dialysate
- Most undetectable with current testing methods
- Pyrogenic reactions
- Results in chronic micro-inflammation

- Contaminants
  - Bacteria
  - Debris
    - Endotoxin
    - Exotoxin
    - Peptidoglycan
    - LPS, Lipid A
    - DNA & RNA fragments
    - Low molecular weight by products of bacterial metabolism
    - Carbohydrate slime layer
    - Matrix Proteins
    - Cytokine inducing substances

LONG-TERM EFFECTS ATTRIBUTED TO CHRONIC MICRO-INFLAMMATION

- Malnutrition
- Low albumin
- Muscle protein wasting
- Protein catabolism
- Increased CRP
- Atherosclerosis
- Low cholesterol synthesis
- Increased ferritin levels
- Resistance to EPO therapy
- Bone disease, cysts, fractures
- Sleep disorders
- Anti-endotoxin antibodies

ECONOMIC IMPACT

- Servicing of Equipment and Systems
- Downtime for equipment
- Replacement of components or systems
- Additional cleaning & disinfection required
- Clinical operations may be impacted
- Patients adversely affected
  - Interruption of treatment
  - Hospitalization

WATER SYSTEM COMPONENTS AT RISK FOR BIOFILM

- Water Softener—High Risk
  - Cannot be disinfected
  - Provides nutrients to captured bacteria
  - Back-flushing does not remove all adhering bacteria
  - Brine solution selects for salt-loving bacteria

WATER SYSTEM COMPONENTS AT RISK FOR BIOFILM

- RO—Medium Risk
  - Short interval flushing & frequent disinfection reduces bacteria
  - Endotoxin not inactivated
  - Bacteria, endotoxin, cytokine inducing substances can pass through to permeate side

WATER SYSTEM COMPONENTS AT RISK FOR BIOFILM

- Break Tank & Permeate Loop—Low Risk
  - Frequent disinfection can help control biofilm

BICARBONATE CONCENTRTATE RISK FOR BIOFILM

- High levels of bacteria in bicarbonate concentrate
- Inadequate disinfection of bicarbonate storage tank
- Not rinsing bicarbonate jugs & pick-up tubes daily
- Not disinfecting bicarbonate jugs and pick-up tubes at least weekly
- Allowing bicarbonate concentrate or post rinse/disinfection fluid to sit jugs/pick-up tubes
- Poor quality of water used to prepare concentrate

DIALYSIS MACHINE RISK FOR BIOFILM

- Dialysis water not meeting microbial &/or endotoxin quality levels
- Not rinsing out dialysate post treatment
- Not disinfecting after each day of use
- Not disinfecting inlet water line
- Not following manufacturer recommendations for use, cleaning, disinfection and maintenance

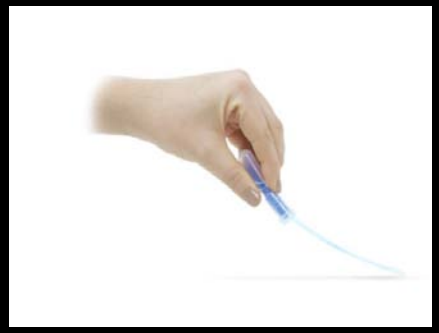
HOW DO WE KNOW WE HAVE BIOFILM?

CRITERIA FOR INITIATING BIOFILM REMEDIATION

- Routine disinfection is inadequate to keep bacteria and endotoxin levels below the required action levels
- Culture results erratic
- Culture results negative or below action level, but endotoxin levels increasing
- Bacteria culture numbers, endotoxin levels, TOC all increasing

SAMPLING FOR BIOFILM DETECTION

SURFACE SWAB & CULTURE



http://www.testkitcentral.com/prodImages/ultrasnapatpswabs.jpg

http://www.luminultra.com/luminultra-biofilm-dsa/

SENSORS & IN-LINE DISKS

http://www.processinstruments.net/products/water-analyzers/biofilm-monitor/?gclid=CIHng_uow8sCFYIBaQodJpgA9A

http://www.slideshare.net/mfornalik/early-biofilm-detection-presentation?qid=93f56975-b73d-4c16-8efd-e34c7e8f747e&v=&b=&from_search=1

METHODS OF BIOFILM ANALYSIS

- Microscopy
  - Phase contrast or Epifluorescence
    - Early cell attachment & migration
  - EM—1D
  - SEM-2D
  - Confocal Scanning Laser—3D
    - Ultrastructure
- ATP
- PCR
- Staining & fluorescence absorbance
- Tissue culture plate
- Congo Red Agar method
- Tube method
- Piezoelectric sensors
- Disruption and plate count

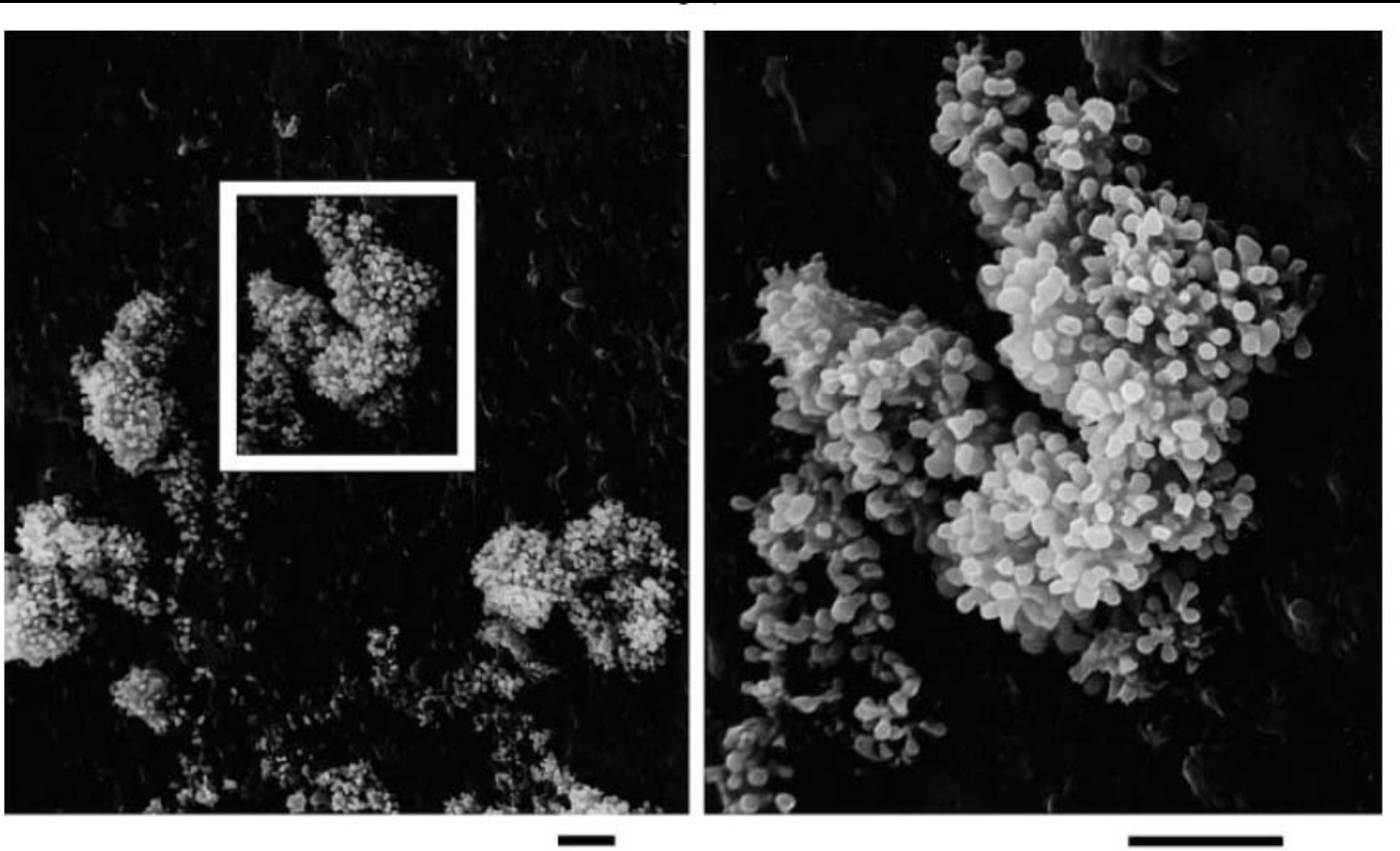
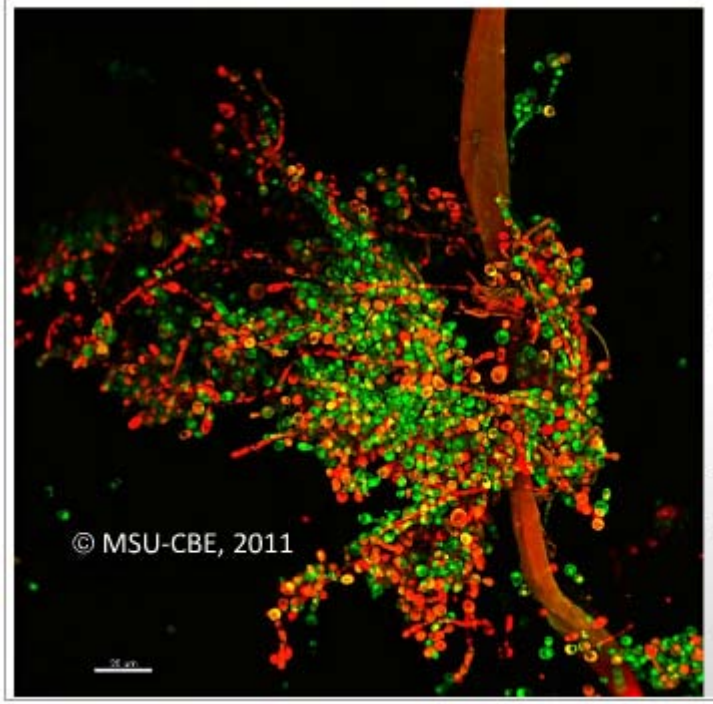


FIG. 2. Representative scanning electron micrograph of staphylococcal biofilm microcolonies on the outer surface of a peritoneal catheter: magnified view of the inset showing details of the glycocalyx-covered microcolonies.

Dasgupta, MK. 2002. Seminars in Dialysis 15(5): 338-346

CONFOCAL SCANNING MICROSCOPY

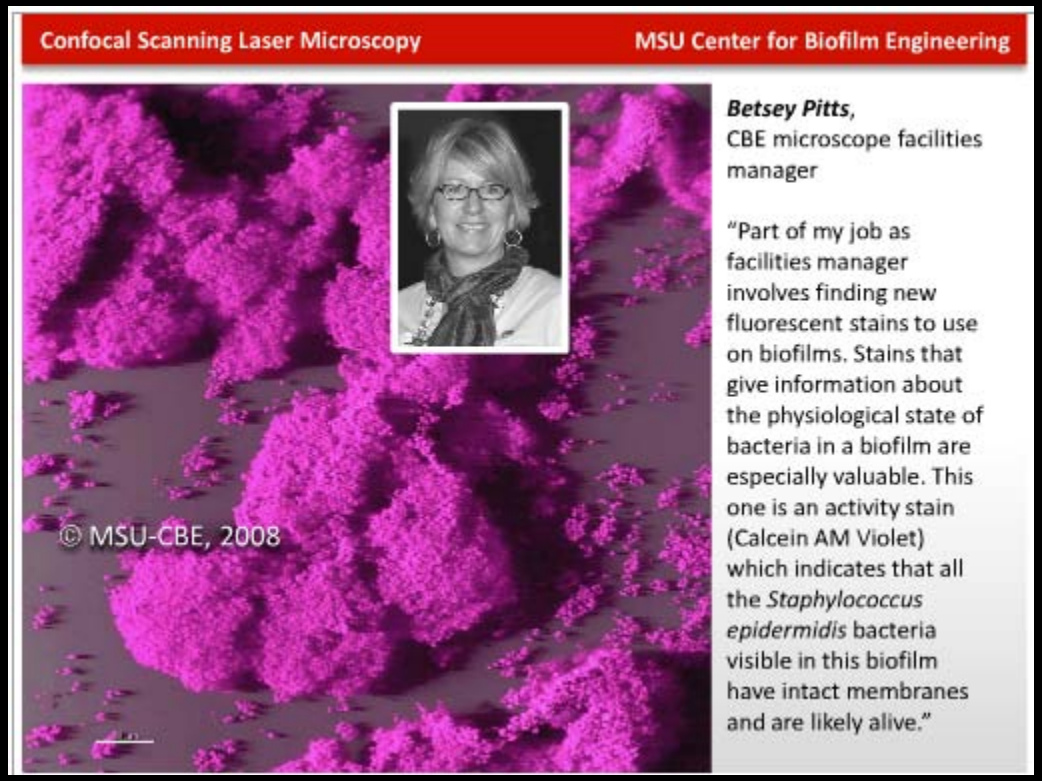
Confocal Scanning Laser Microscopy — **MSU Center for Biofilm Engineering**



Alessandra Agostinho, DDS, CBE research scientist

"This *Candida albicans* biofilm grown on a PMMA coupon is stained to show live cells as green and non-viable cells as red. PMMA—poly (methyl methacrylate)—is used in dental fillings, denture material, and bone cement."

FLUORESCENT PROBES



How Do We Deal With Biofilm?

THE GOAL

Where We Are Today → Where We Want To Be

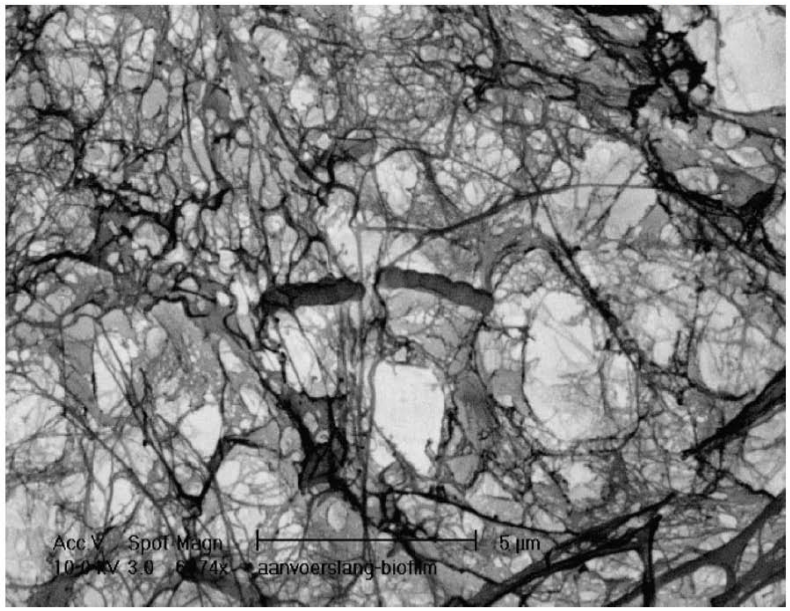


Fig. 2. Tubing segment, showing extensive biofilm formation, from a standard water treatment system.

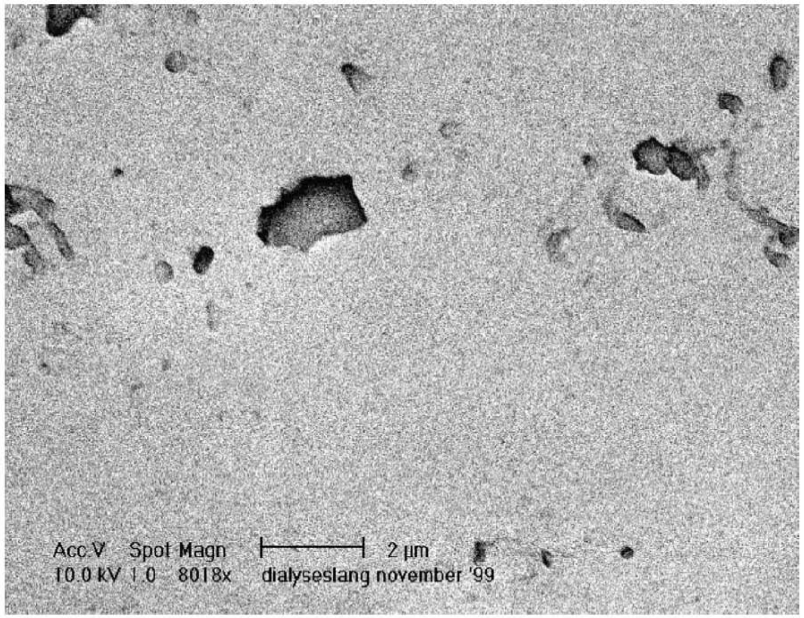


Fig. 1. Tubing segment, showing complete absence of biofilm, from a water treatment system delivering ultrapure water.

STRATEGIES FOR BIOFILM CONTROL

- Proper Water Treatment System Design
- Proper Operation & Maintenance of Systems & Equipment
- No oversized piping or dead legs
- Routine Monitoring and Trending
- Regular & Frequent Flushing, Cleaning & Disinfection
  - Water treatment & distribution systems
  - Storage tank
  - Hemodialysis machines
  - Valves
  - Line between distribution system & dialysis machine
- Use of in-line concentrate generators
- Use of ultrapure water &/or dialysate

WAYS TO CONTROL BIOFILM

- Control flow rates and piping sizes
- Avoid long periods of no flow
- Avoid dead legs and rough surfaces
- Use of UV pre RO
- Use of ultrafilters
- Proper disinfectant choice, concentration, dwell time, frequency
- Respond early to action levels or increasing trends

METHODS TO REMOVE BIOFILM

- Mechanical cleaning
- Agents to kill bacteria
- Agents to remove biofilm matrix components
  - Hypochlorous acid (acidified bleach)
  - Basic agents such as NaOH
  - Surfactants
- Remove essential nutrients
- Inhibit/reduce attachment surfaces
- Promote biofilm detachment
  - Chelating agents e.g. citric acid
- Depolymerize polysaccharides and/or extracellular DNA

CLEANING & DISINFECTION

- Acetic acid
  - Acts on calcium carbonate salts to dissolve
  - Converts organics in dialysate into deposits strongly affixed to surfaces
- Bleach kills bacteria but can oxidizes organics in dialysis lines, furthering biofilm matrix production
- Acidified bleach—hypochlorous acid—affects biofilm matrix
  - Reacts with
    - Protein sulfhydryl groups
    - Protein amino groups
    - DNA & nucleotides
    - Lipids
- Oxidizing agents (e.g. bleach, peracetic acid) kill bacteria but may oxidize proteins in biofilm
- Proteolytic enzymes can release attached bacteria from biofilm

CAUTIONS

- Descaling & disinfection procedures may induce biofilm matrix formation (Morin et al, 2000; Cappelli et al 1998; Dasgupta, 2002)
- Ultrafilters capture bacteria, biofilm formation during low or no flow periods
  - Disinfect with bleach &/or peracetic acid as compatible
  - Follow manufacturer’s recommendations
- Activated carbon (GAC) filters, biofilm of Gram negative bacteria forms on surface; large surface area

KNOW WHAT SOLUTION TO APPLY

- Cleaning
- Disinfection
  - Chemical Disinfection
    - Chemical types, concentrations, temperature & dwell time
    - Certain chemicals may be incompatible with system materials
    - Bacterial tolerance to disinfectants increases within biofilm
    - Biofilm matrix may inactivate or reduce the effectiveness
  - Heat disinfection
    - Can help reduce biofilm formation if done daily
    - Once biofilm exists, heat disinfection will not remove it
    - Endotoxin and CIS remain active
- Combination treatments

DISINFECTION

- What should be disinfected?
  - Water Treatment & Distribution Systems
  - Hemodialysis machines
  - Line between water distribution system & dialysis machines
  - Water storage tank
  - Bicarb jugs
  - Bicarbonate Concentrate Mixing Systems
  - Ultrafilters
- When?
  - At Least Monthly for Water System & Hemodialysis machines
  - After maintenance or replacement of components
  - Indications of biofilm
  - Bacterial & endotoxin levels rising (>action level)

BICARBONATE CONCENTRATE DISINFECTION

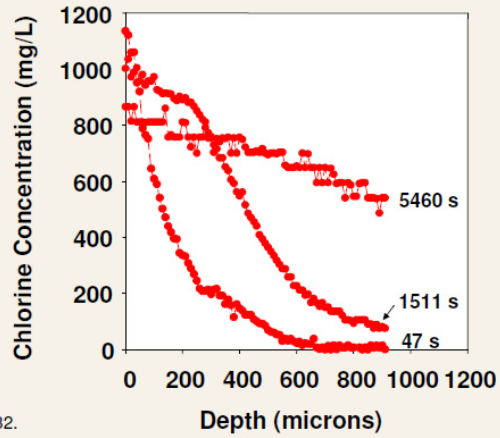
- When to disinfect
  - Bicarb jugs—
    - Rinse daily, disinfect **Weekly**
  - Concentrate mixing & distribution systems
    - **Weekly** or as per manufacturers instructions
    - Facility designed system-cleaned & disinfected by validated procedure
    - Routinely meets standards requirements
    - Record data per disinfection cycle in dedicated log

DISINFECTANT CHOICES

- Ozone
- Acidified Bleach
- Bleach 1:10
- Peracetic Acid
- Formaldehyde
- Glutaraldehyde
- Bleach 1:100
- Heat
- UV

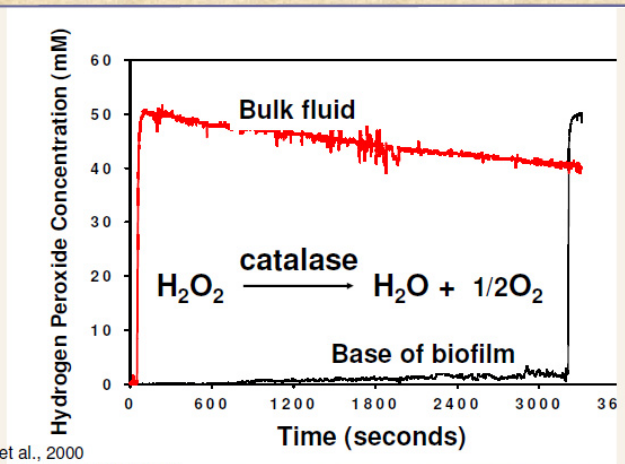
FACTORS TO KEEP IN MIND

Hypochlorite Penetrates Biofilm Slowly



2. Stewart et al., 2001
Appl Microbiol 91:525-532.

H_2O_2 fails to Penetrate Biofilm



3. Stewart et al., 2000
Appl Environ Microbiol 66:836-838.

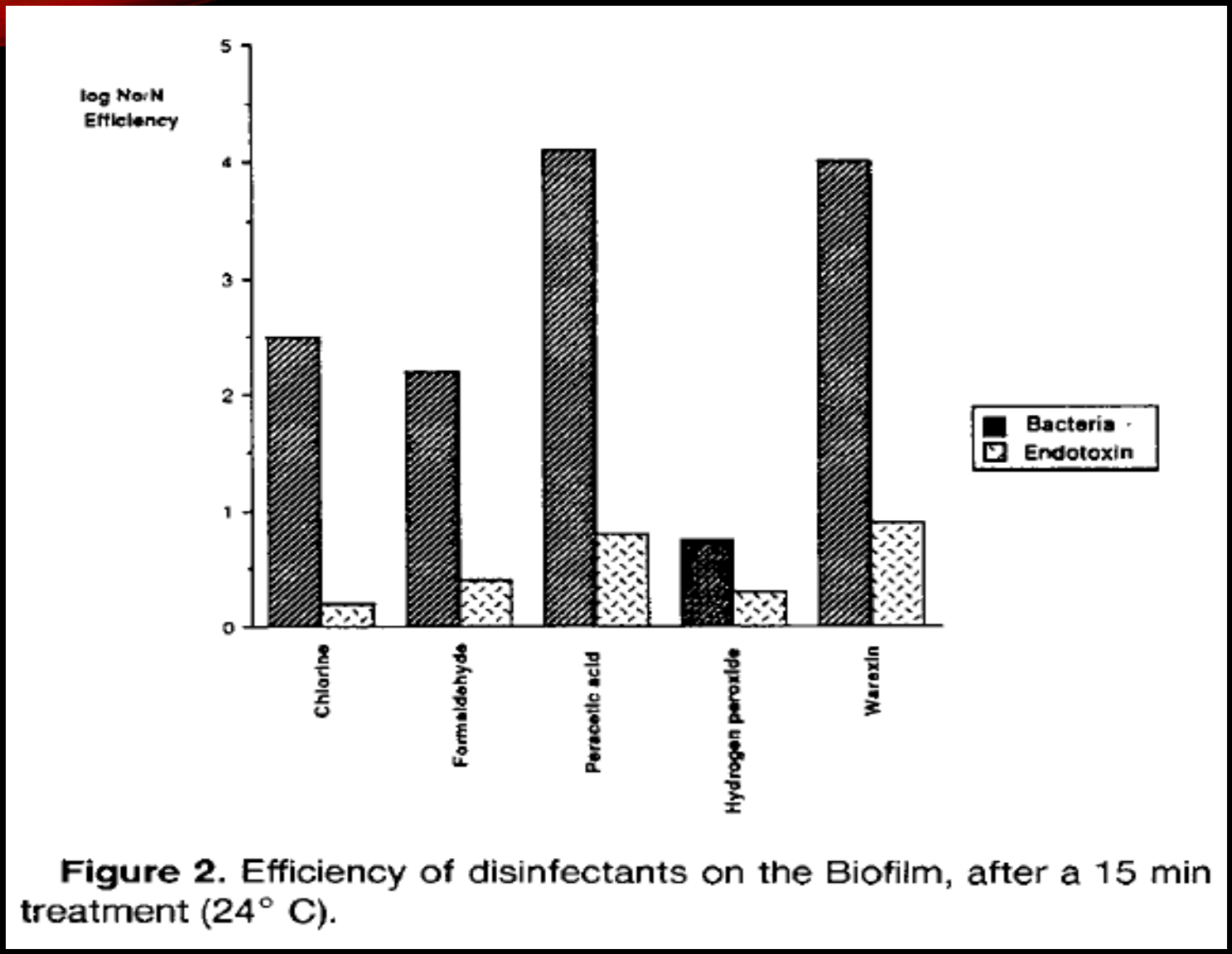


Figure 2. Efficiency of disinfectants on the Biofilm, after a 15 min treatment (24° C).

Vincent, et.al. 1989. Trans Am Soc Artif Intern Organs 35:310-313.

OTHER THINGS TO REMEMBER ABOUT DISINFECTION

- Effectiveness depends on
  - Adequate concentration
    - **Test for Potency**
  - Adequate Dwell Time
  - Correct choice of disinfectant for the problem
  - Biofilm presence or not
  - Design of System
  - Getting disinfectant to all surfaces
- Summer months may require more frequent disinfection

DISINFECTANT RESISTANCE

- Resistance to sodium hypochlorite 500 ppm, 10min, 10% sodium hypochlorite
  - 53.3% Pseudomonas aeruginosa
  - 25% Acinetobacter haemolyticus
- Mycobacterium and Methylobacterium more resistant to chlorine disinfection
- Sensitive to peracetic acid 2000ppm, 8-24h

IF ALL ELSE FAILS REPLACE ALL OR PART OF THE WATER SYSTEM

MYTHS

WATER TREATMENT SYSTEM MYTHS

- ∅Once monthly disinfection is adequate for all water treatment systems
- ∅No detectable bacteria and/or acceptable levels of endotoxin = No Biofilm
- ∅Turbulent flow in the loop prevents any biofilm formation
- ∅Filters only need to be changed at manufacturer recommended intervals

DIALYSIS MACHINE MYTHS

- ∅The dialyzer prevents transmission of bacteria and endotoxin to patient so water and dialysate levels are irrelevant
- ∅Post disinfection sampling tells you the machine meets AAMI acceptable levels
- ∅As long as monthly monitoring results are OK on a % of the dialysis machines, all of them are within limits and I don’t need to be concerned or have to trend my data

CONCENTRATE & DIALYSATE MYTHS

- It is OK to mix old and new batches of bicarbonate concentrate
- Biofilm doesn't form in bicarbonate concentrate
- Containers don't need to be disinfected once emptied of bicarbonate concentrate
- Water quality doesn't matter for Acid Concentrate
- Dialysate contaminants don't cross dialyzer membranes

SUMMARY

- Biofilms are organized communities
- Once established they are difficult to eliminate completely
- Currently, prevention and understanding of how, why & where biofilms develop is the best approach to removal & control
- Lint, debris, pieces of biofilm, organic material & particulate matter act as “crystals” for additional biofilm development
- Bacteria can survive a long time even on dry surfaces, but best in biofilms
- Lack of cleaning, proper disinfectant concentration,dwell time, pH, water quality and temperature can affect biofilm formation, growth and control
- Start with a good system design, keep system cleaned and disinfected, monitor and control feed water quality and respond to data--It is the best we have for NOW!

ENDOTOXIN

WHAT IS ENDOTOXIN

- Major component of the cell wall of Gram negative bacteria
- Pyrogenic (fever causing) component—LPS lipid A
- Structure & level of potency of endotoxin varies by species and strain of bacteria
- Two parts linked
  - Hydrophilic polysaccharide (sugar) chain
  - Hydrophobic lipid—affinity for surfaces
- Heat stable
- Some endotoxins more soluble than others
- 3,000 to 25,000 daltons in size
  - Influenced by pH, [salt], surfactants
- Lack of correlation with bacterial counts
- Aggregates in aqueous environments
- Potency greatest in disaggregated form
- Pseudomonas aeruginosa—43,000 cells = 1EU/mL endotoxin activity
- Can transfer from dialysis fluid to blood via backfiltration/backdiffusion
- Activity quantifiable by LAL test
  - Endotoxin can be protected from reaction by biofilm matrix (EPS)

EPS
EXTRACELLULAR POLYMERIC SUBSTANCES

- Comprises 50-90% of TOC in biofilms
- Primary matrix material
- Gram positive biofilm—cationic
- Gram negative biofilm—anionic—attracts Ca & Mg-tighter binding biofilm
- Most biofilm have hydrophobic & hydrophilic areas
- Some EPS soluble and others not
- Inhibits transport of antibiotics and diffusion of disinfectants into biofilm
- Attracts metal ions, cations, proteins, DNA, lipids

ENDOTOXIN ACCEPTABLE LEVELS DIALYSIS WATER & DIALYSATE

Contaminant	AAMI & ISO Max Level*	AAMI & ISO Action Level*	RD 52 Max Level**	RD 52 Action Level**
Endotoxin Water	<0.25 EU/mL	0.125 EU/mL	<2 EU/mL	1 EU/mL
Endotoxin Dialysate	<0.5 EU/mL	0.25 EU/mL	<2 EU/mL	1 EU/mL

**ISO 13959:2014 & ANSI-AAMI 13959:2014*

***ANSI-AAMI RD52:2004*

ENDOTOXIN MEASUREMENT METHODS

- LAL (Limulus Amebocyte Lysate) Test
  - Limulus Polyphemus (horseshoe crab)
  - Amebocyte (blood cell of crab) lysate
  - Reaction with Lipid A
  - Sensitivity 0.01-0.03 EU/mL
- Rabbit pyrogen test (USP)

TYPES OF LAL TESTS

- Gel clot*
  - Sensitivity—0.03-0.06 EU/mL
  - Types
    - Limit
    - Semi-quantitative
- Kinetic
  - Chromogenic
    - Sensitivity—0.005-0.03 EU/mL
  - Turbidimetric
    - Sensitivity—0.001 EU/mL
- End-point
  - Chromogenic
  - Turbidimetric
- Fluorescent
  - Sensitivity—0.01 EU/mL

*Reference method

SOMETHINGS TO REMEMBER ABOUT ENDOTOXIN

- Endotoxin adheres to glass
- Some plastics may either enhance, inhibit or be contaminated with endotoxin
  - Check with lab for acceptable sample collection containers/tubes

MOVE BEYOND THE CHECKLIST

Control Biofilm & Endotoxin for Patient Safety