# Beware of the Biofilm

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Several slides in this presentation are borrowed from Richard Ward and Jo-Ann Maltais.

# Questions for today

* Why do we want high purity water for our patients?
* Where does the microbiological contamination come from?
* How do we minimize our patients' exposure to microbiological contamination?

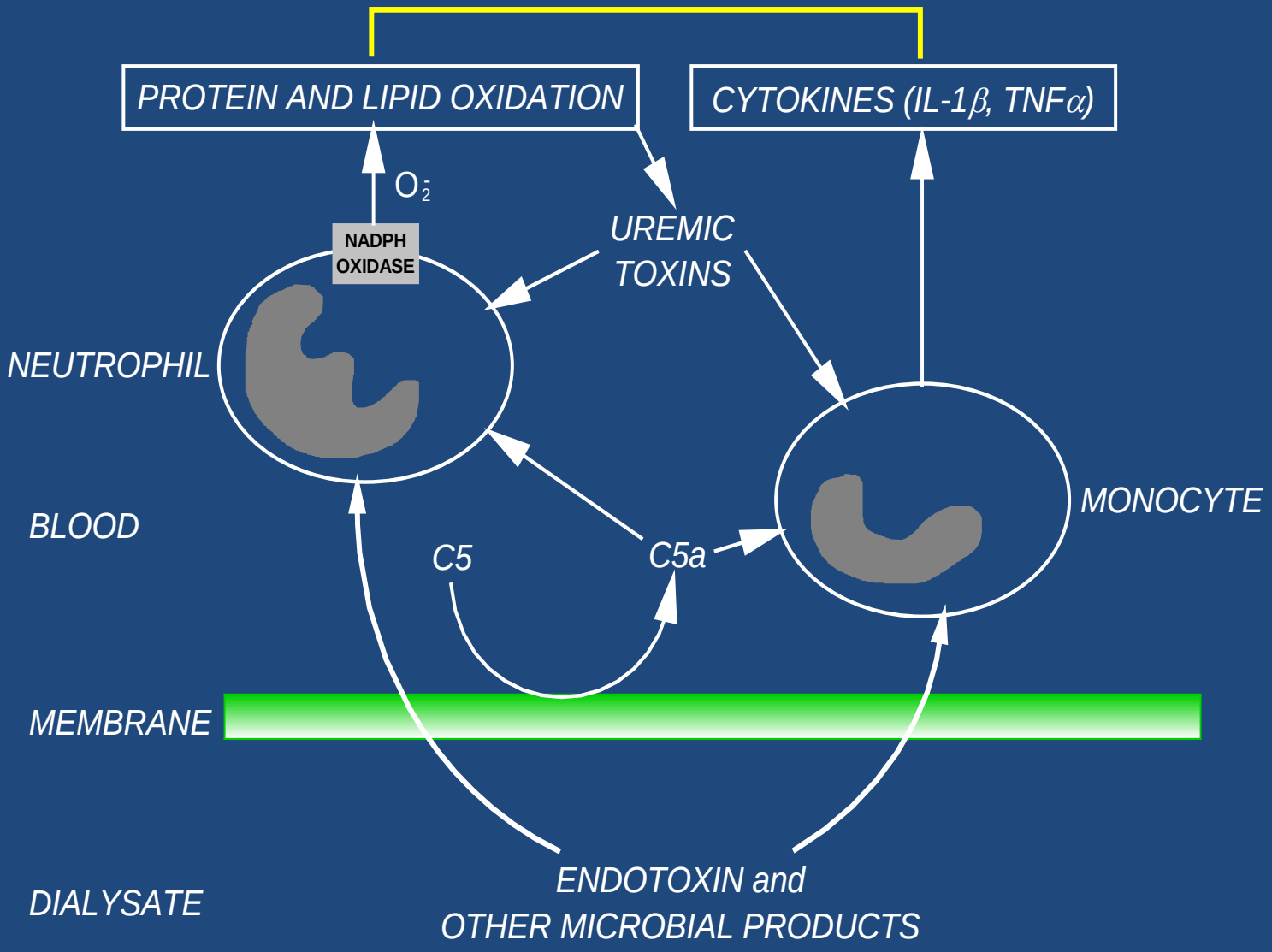
# AAMI Dialysate Standards

| Dialysate Type | Allowable CFU (Action Level) | Endotoxin Level (Action level) |
|---|---|---|
| Standard Dialysate | < 100 cfu/mL (50 cfu/mL) | < 0.5 EU/mL (0.25 EU/mL) |
| Ultrapure Dialysate | < 0.1 cfu/mL | < 0.03 EU/mL |

| Bacteria Level | Exposure during 4 hr tx @ 800 Qd |
|---|---|
| 100 cfu/mL | 19,200,000 bacteria |
| 50 cfu/mL | 9,600,000 bacteria |
| 0.1 cfu/mL | 19,200 bacteria |

# Why do we want high purity water for our patients?

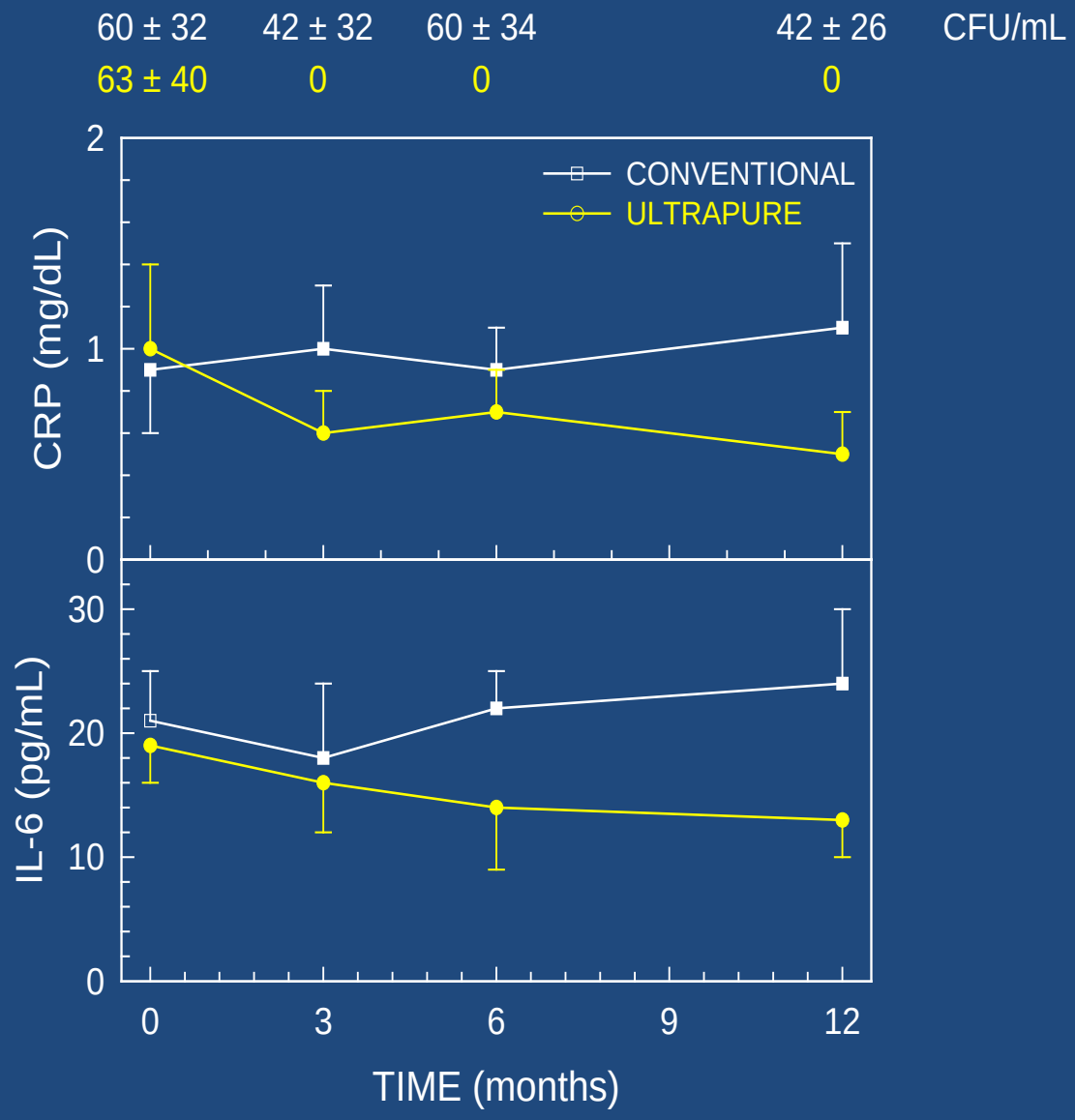
# INFLAMMATION



# POTENTIAL ADVANTAGES OF WATER AND DIALYSATE OF HIGH MICROBIOLOGICAL PURITY

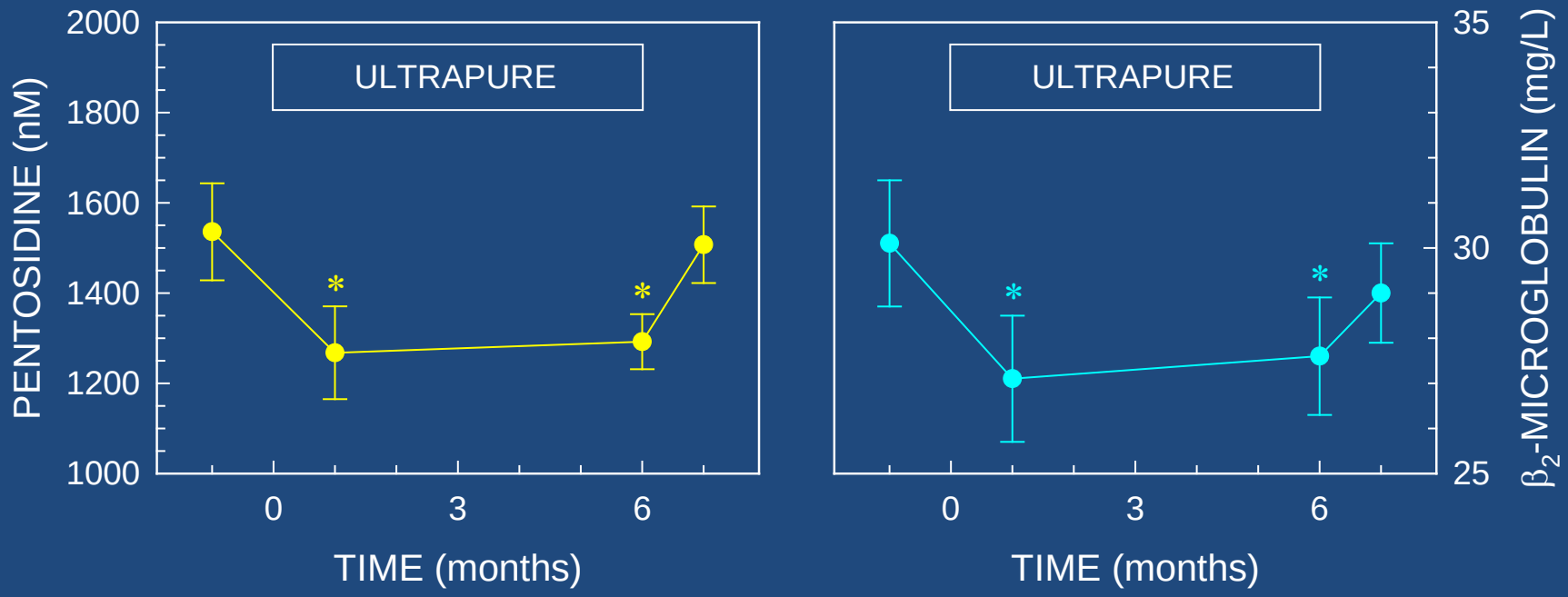
- LESS INFLAMMATORY STIMULUS
- LESS MORBIDITY ASSOCIATED WITH INFLAMMATION
  - Reduced incidence of $\beta_2$-microglobulin amyloid disease.
  - Improved responsiveness to erythropoietin.
  - Improved nutritional status.
  - Improved preservation of residual renal function.

# EFFECT OF DIALYSATE PURITY ON INFLAMMATION



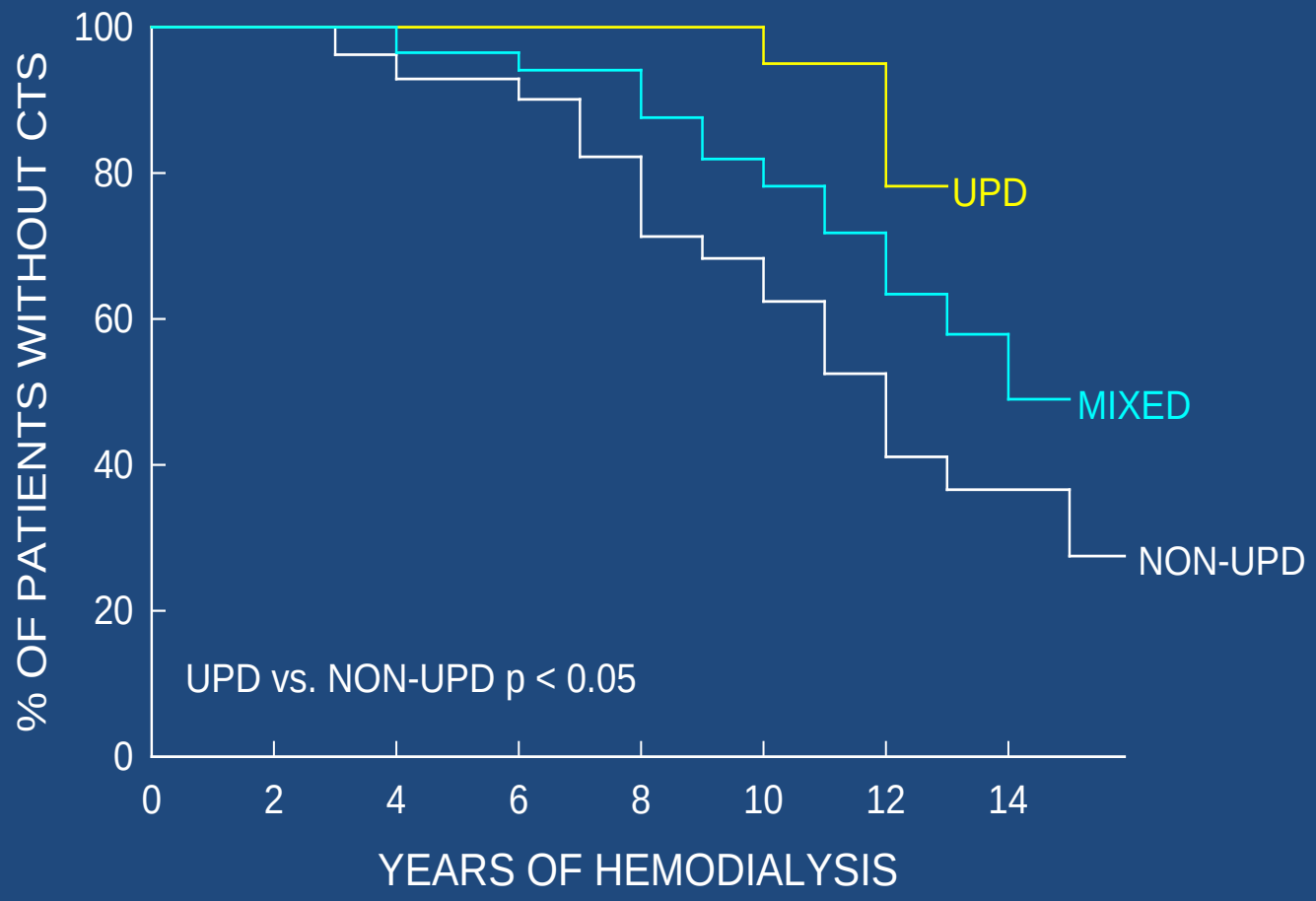

Schiffl H et al. *Nephrol Dial Transplant* 16:1863-1869, 2001

# EFFECT OF WATER QUALITY ON OXIDANT STRESS AND $\beta_2$-MICROGLOBULIN



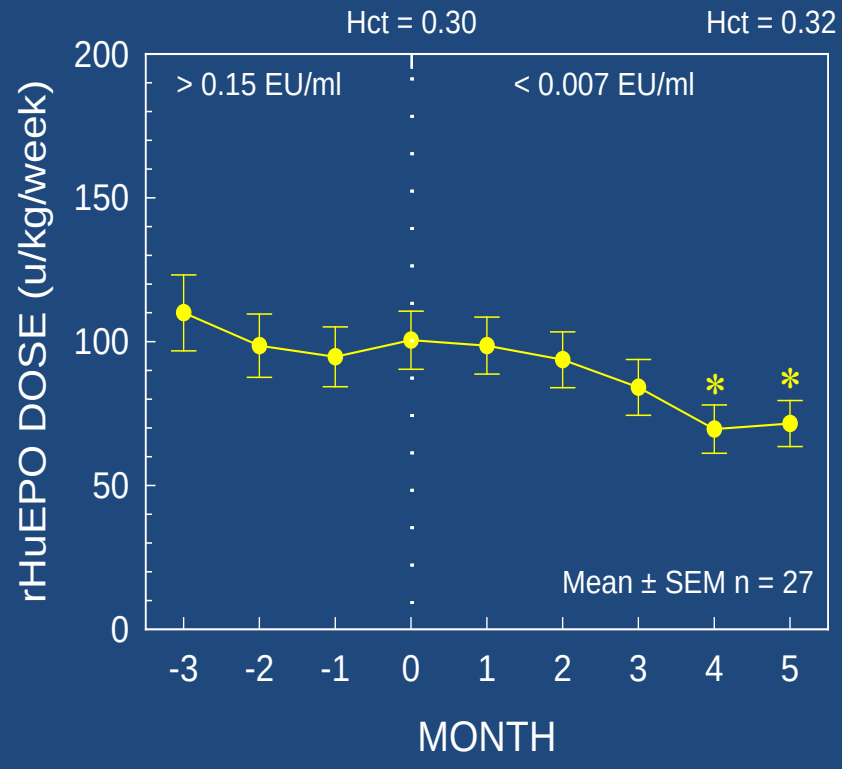

Furuya R, et al. *Blood Purif* 23:311-316, 2005

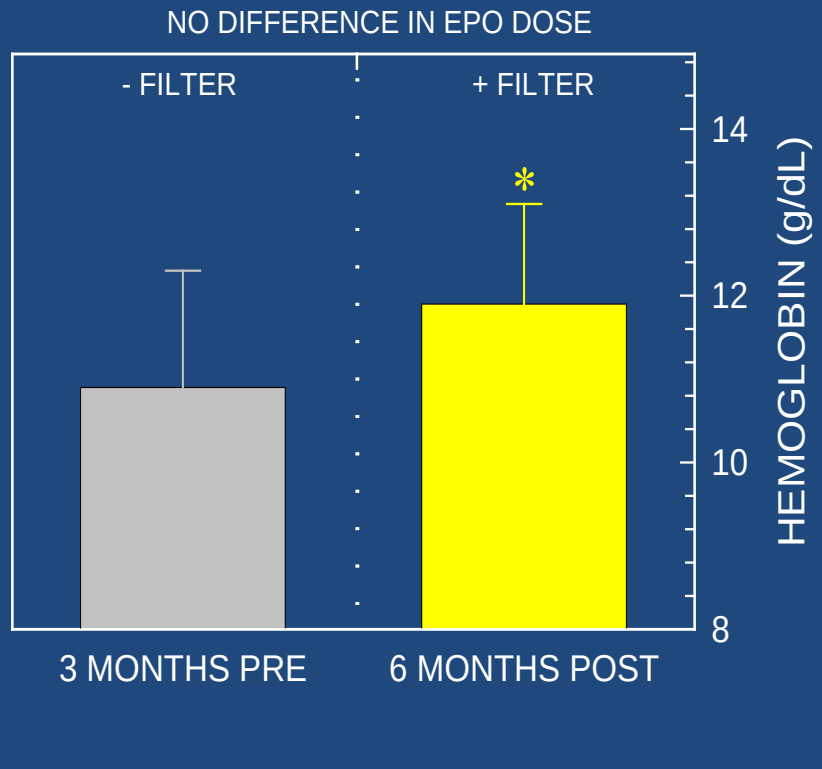
# CARPAL TUNNEL SYNDROME IN PATIENTS TREATED WITH ULTRAPURE WATER




Baz M et al. *Int J Artif Organs* 14:681-685, 1991

# EFFECT OF IMPROVED WATER QUALITY ON ANEMIA CORRECTION

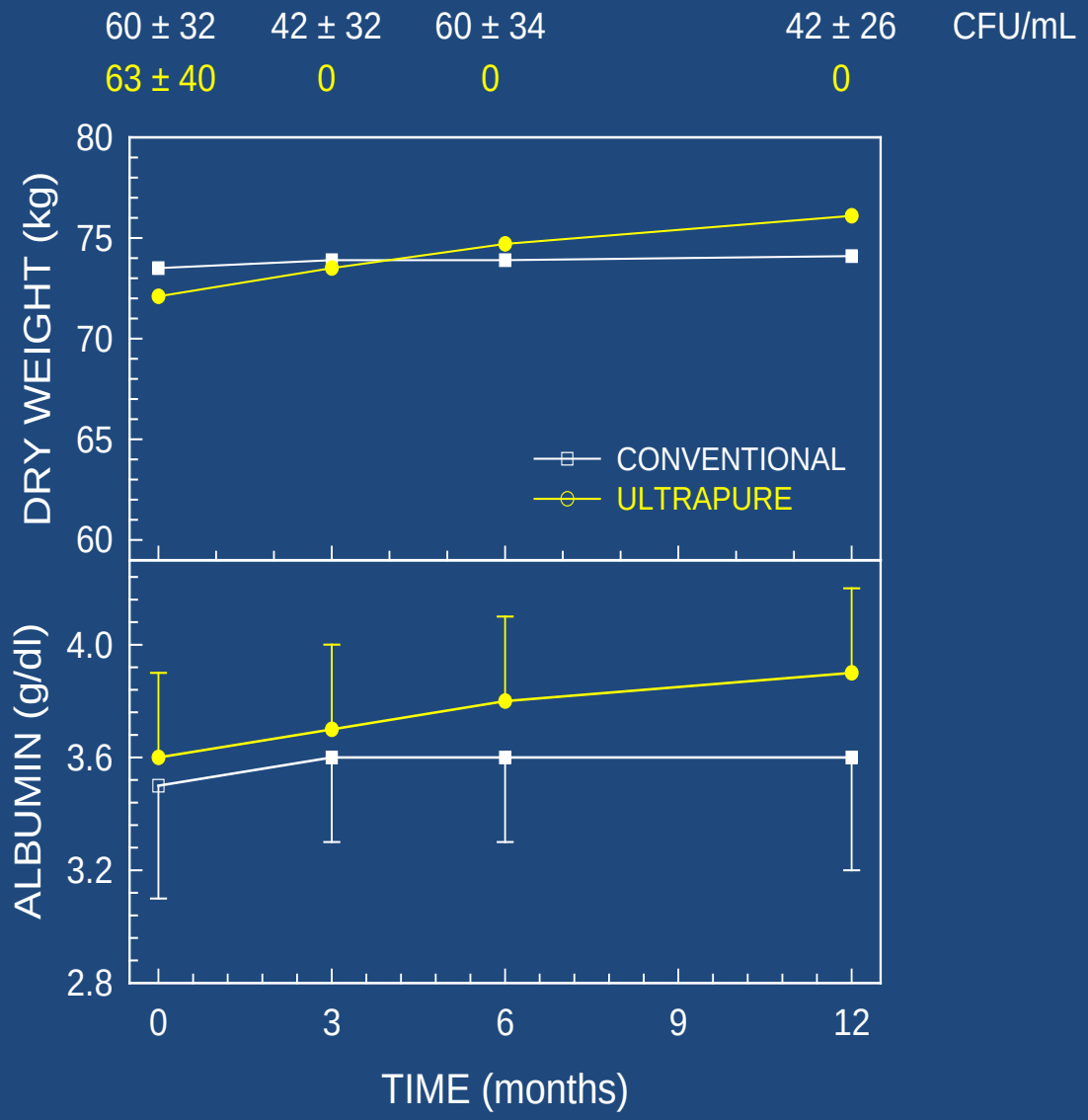



Rahmati MA et al. *Int J Artif Organs* 27:723-727, 2004

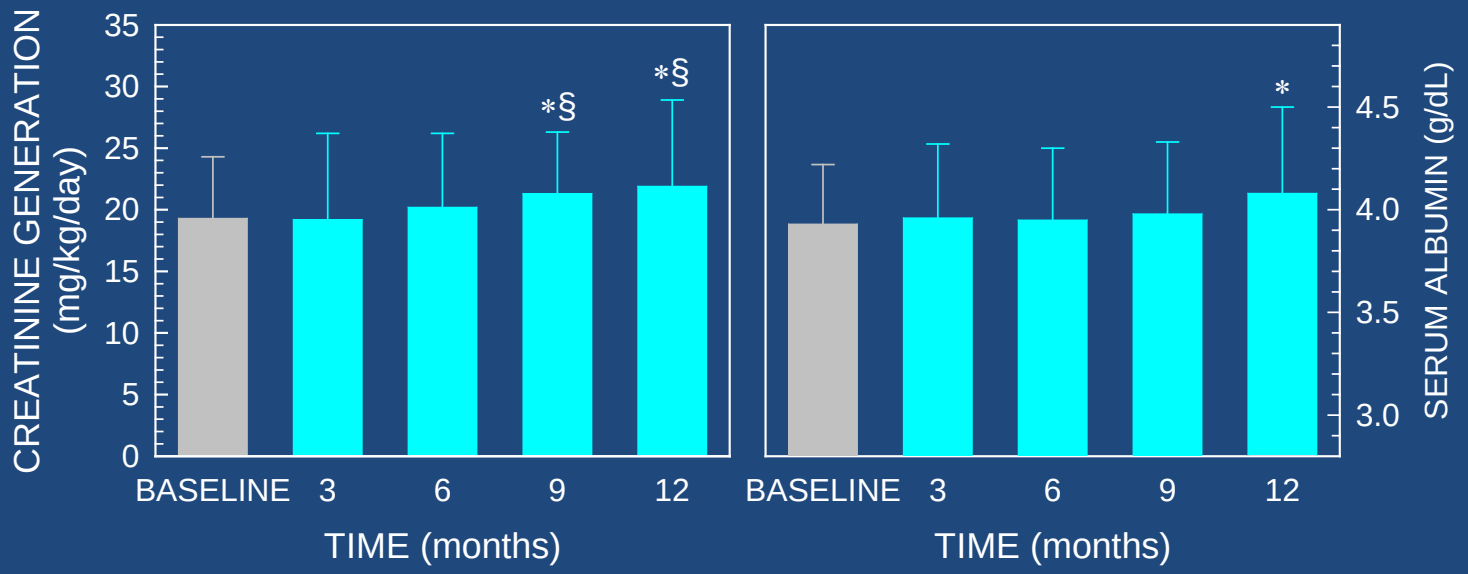
Matsuhashi N and Yoshioka T. *Nephron* 92:601-604, 2002

# EFFECT OF DIALYSATE PURITY ON NUTRITION




Schiffl H et al. *Nephrol Dial Transplant* 16:1863-1869, 2001

# EFFECT OF FILTERED DIALYSATE ON MUSCLE MASS AND SERUM ALBUMIN




Ouseph R, et al. *Nephrol Dial Transplant* 22: 2269–2275, 2007

| | # Patients | Inflammation | Anemia Correction | Nutritional Status | $\beta_2$-microglobulin |
|---|---|---|---|---|---|
| Arizono (2004) | 23 | +↑+ | +↑+ | +↑+ | +↑+ |
| Baz (1991) | 226 | | | | +↑+ |
| Furuya (2005) | 16 | +↑+ | | ●↔● | +↑+ |
| Go (2007) | 61 | +↑+ | +↑+ | | |
| Hsu (2004) | 34 | +↑+ | +↑+ | ●↔● | |
| Izuhara (2004) | 84 | ●↔● | | ●↔● | ●↔● |
| Kleophas (1998) | 399 | | | +↑+ | +↑+ |
| Lamas (2006) | 78 | ●↔● | ●↔● | ●↔● | |
| Matsuhashi (2002) | 27 | +↑+ | +↑+ | ●↔● | |
| Molina (2007) | 107 | +↑+ | +↑+ | ●↔● | |
| Ouseph (2007) | 105 | ●↔● | ●↔● | +↑+ | +↑+ |
| Rahmati (2004) | 342 | +↑+ | +↑+ | +↑+ | |
| Schiffl (2000) | 89 | | | | +↑+ |
| Schiffl (2001) | 48 | +↑+ | | +↑+ | |
| Sitter (2000) | 30 | +↑+ | +↑+ | | |

+↑+ IMPROVED ●↔● NO CHANGE −↓− WORSENED

# POOLED ESTIMATES OF CHANGE FOLLOWING INTRODUCTION OF ULTRAPURE DIALYSATE

| | BEFORE* | AFTER* | DIFFERENCE |
|---|---|---|---|
| CRP (mg/L) | 9.9 (5.7, 14.2) | 7.0 (4.8, 9.1) | -2.9 |
| IL-6 (ng/L) | 15.4 (6.4, 24.5) | 10.7 (5.9, 15.5) | -4.7 |
| Albumin (g/dL) | 3.80 (3.70, 3.89) | 3.89 (3.78, 3.99) | 0.09 |
| EPO (U/week) | 8336 (3526, 13145) | 9218 (4323, 14114) | 882 |
| $\beta_2$-Microglobulin (mg/L) | 32.1 (30.8, 33.4) | 28.5 (26.0, 31.0) | -3.6 |

* Pooled mean (95% confidence interval)

We know that even low levels of contamination affect our patients.
So, where does the microbiological contamination come from?

In Wine there is Wisdom

In Beer there is Happiness

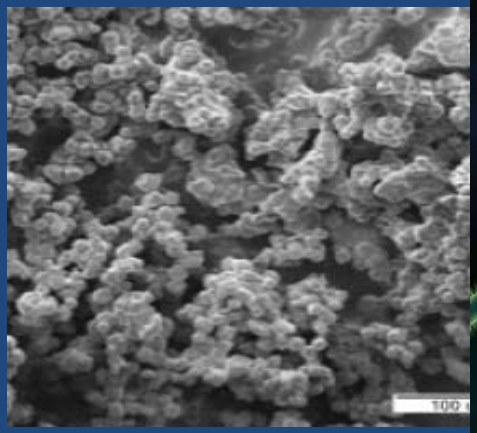
In Water there is Bacteria

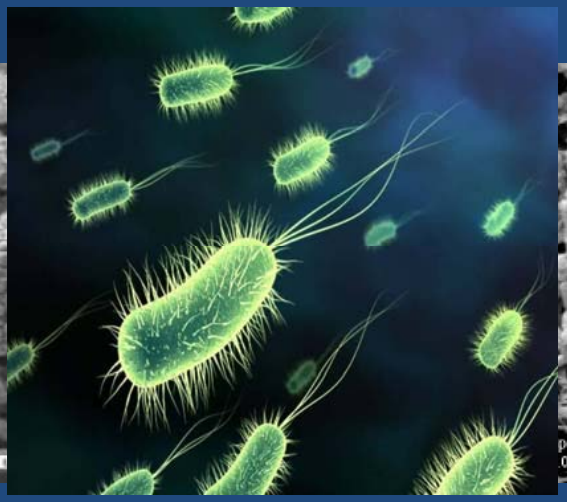
# Bacteria in Water Systems

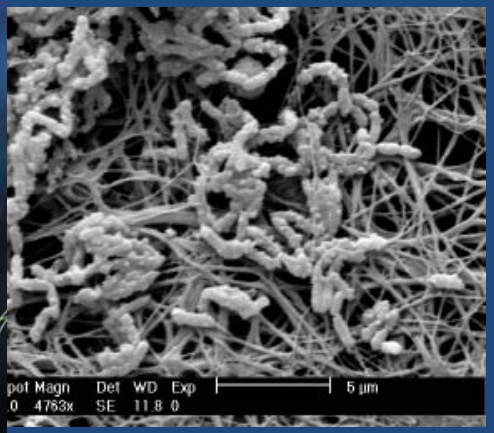
* The municipal water feeding the water system in your dialysis facility contains low levels of bacteria
* The level of bacteria in Tap water does not pose a hazard to dialysis patients. However, bacteria are living organisms and will reproduce in the system to very high levels that are a hazard.
* Bacteria in the water can adhere to the plumbing in the system and create biofilms, making it difficult to eliminate them.

# What on Earth is a Biofilm?

* Survival mechanism
* Community of bacteria
* Symbiotic relationships
* Slimy Matrix








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

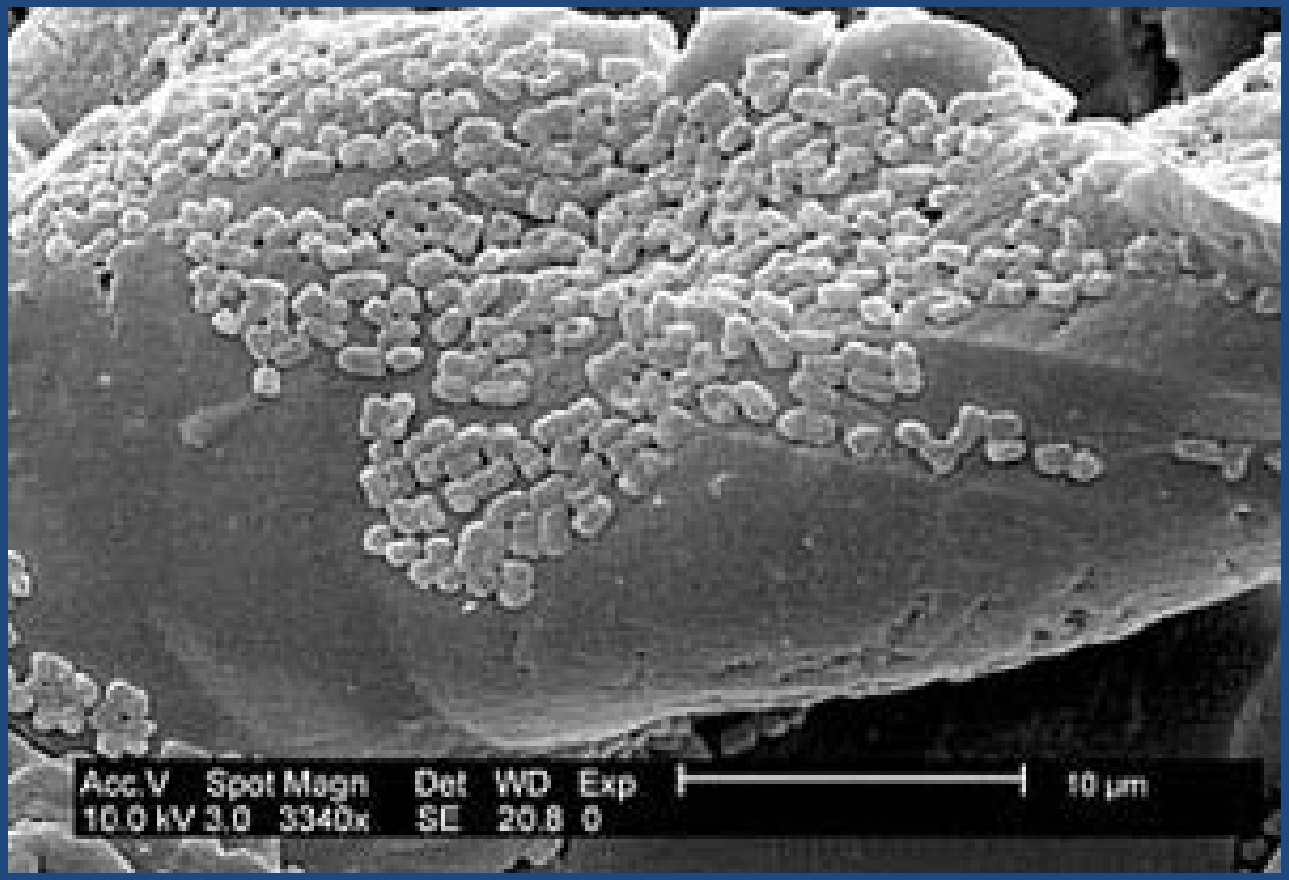
Donlan, RM. Biofilm Laboratory. CDC

# Bacteria Have Been Here a Lot Longer Than We Have

* Bacteria were here 3.6 million years ago
* Man came 100,000 years ago
* Bacteria first discovered in 1670's by Ludwig van Leeuvenhoek
* Biofilm first described by Costerton (1978)
* >60% of human infections estimated to be caused by biofilms

# Benefits of Living in a Biofilm

* Built to suit the specific environment
* Food co-op
* Modern plumbing
* Security System
  * Reduced effects of UV and disinfectants




*A biofilm on a piece of lettuce*

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


Permissions

**Figure 4. Microscopic View of a Shower Curtain**


*Biofilm in acidic pools at Yellowstone National Park*

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

## *Oil Drops Suspended in Water*

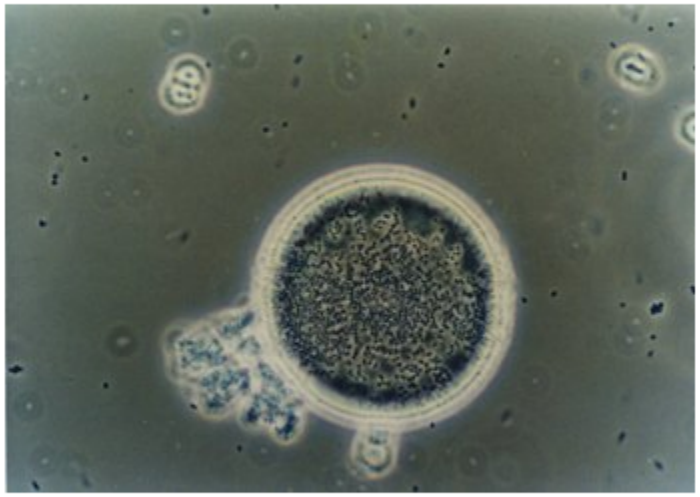


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**Figure 2. Bacteria growing on and near an oil droplet suspended in water**

It is fortunate that many microorganisms are capable of metabolizing hydrocarbons. Due to natural and human caused contamination large amounts of hydrocarbons are annually contaminating soil, fresh water and marine environments. Many genera of bacteria including *Pseudomonas*, *Alcaligenes*, and *Flavobacterium* are capable of mineralizing oil and other HCs to carbon dioxide and water. This image shows a population of bacteria actively degrading a droplet of oil suspended in water.

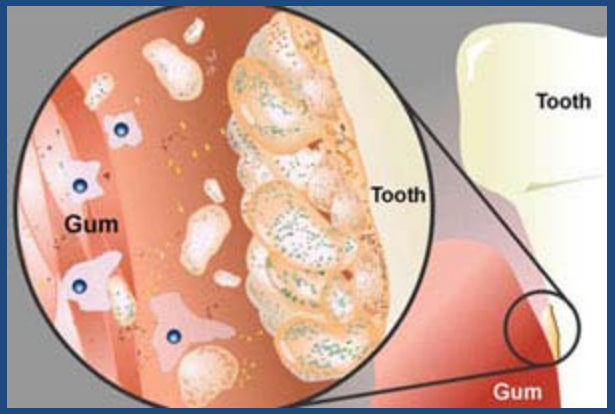
# Did You Know?

* Biofilm can develop & survive on the surfaces of a jet plane
* Biofilm forms faster on plastic pipes than on metal pipes

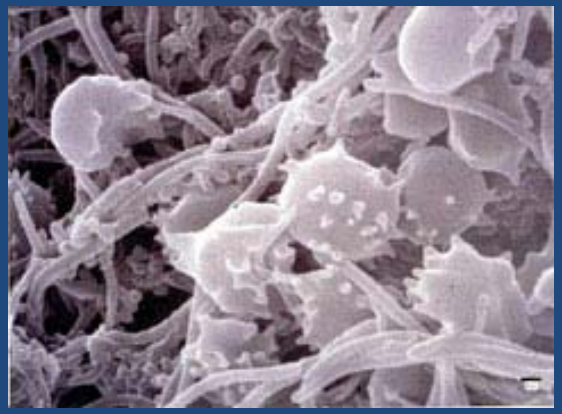
# Biofilms

AND

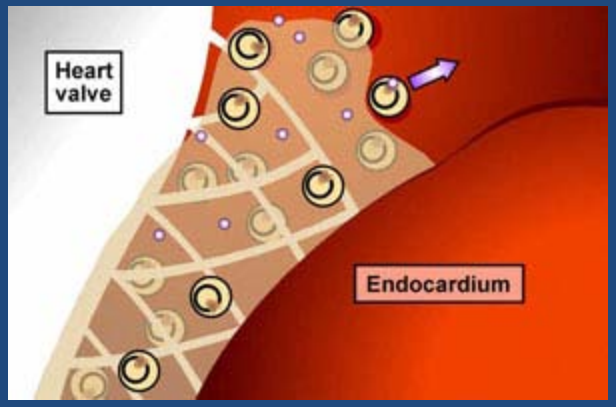
Patient Safety



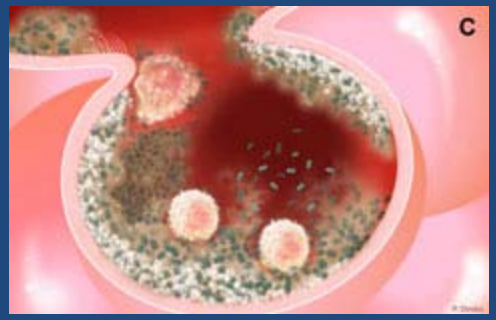

*"Hundreds of microbial biofilm colonize the human mouth, causing tooth decay and gum disease"*



*"Dental plaque as seen under a scanning electron microscope"*

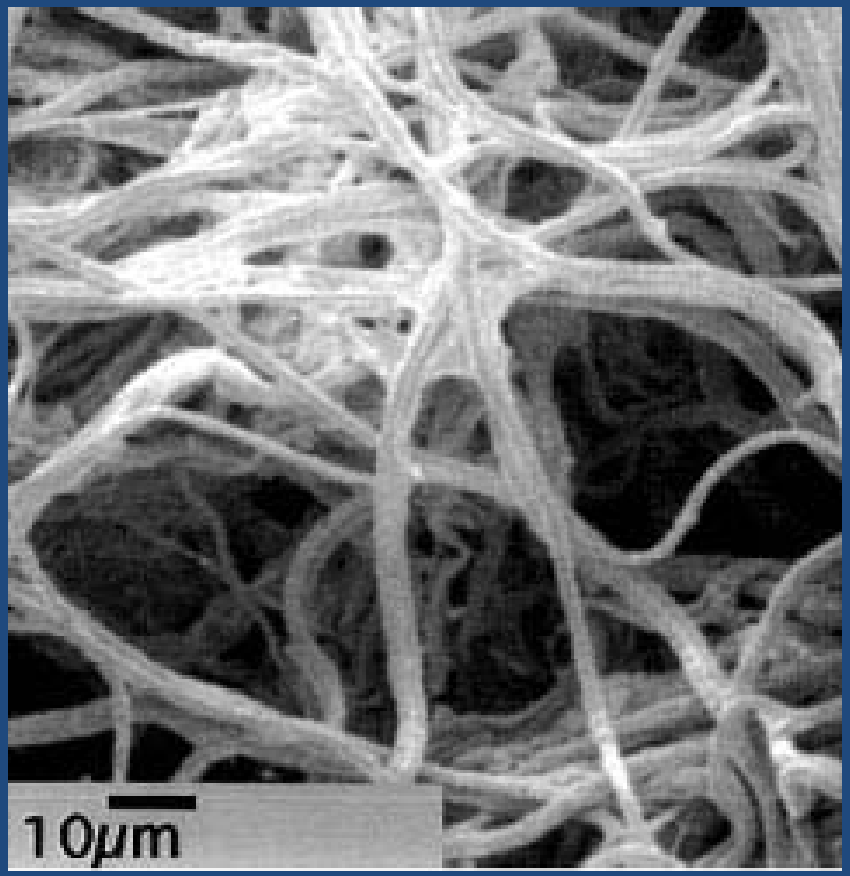



*"Cells of* Staphylococcus epidermidis *causing devastating disease as they grow on the cuff at a mechanical heart valve"*




*"When the immune response is compromised,* Pseudomonas aeruginosa *biofilms are able to colonize the alveoli, and to form biofilms"*

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

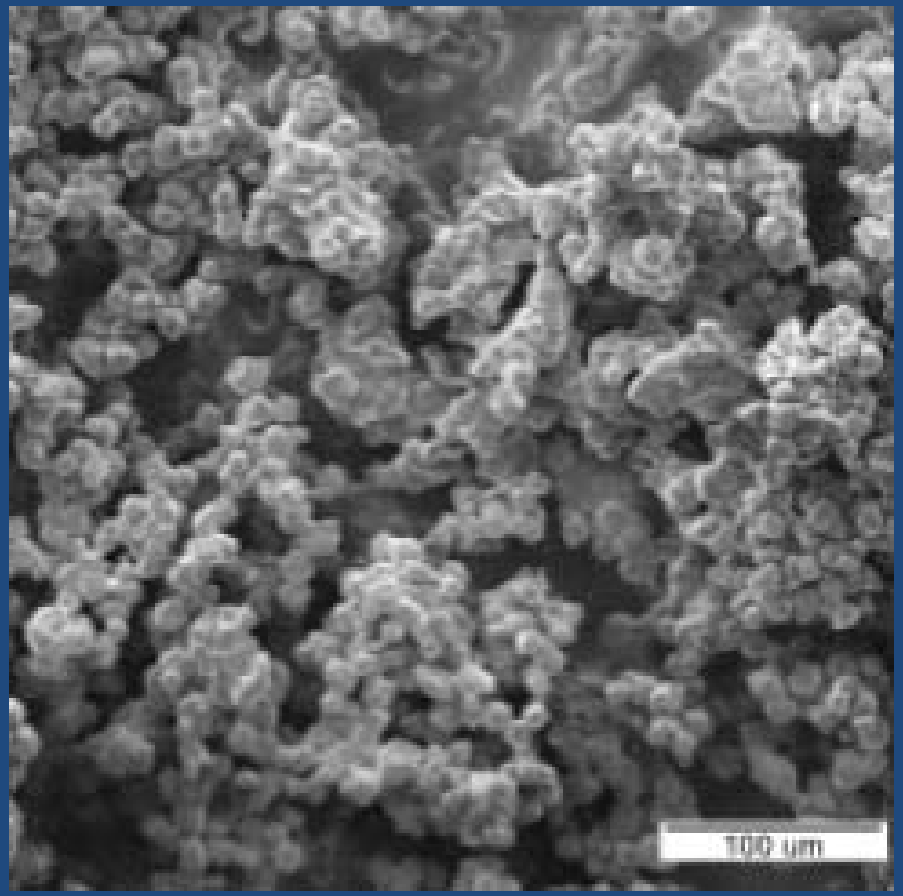



*After antibiotics are applied to a biofilm, a number of cells called "persisters" are left behind*



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

# Biofilms in Water & on Medical Devices



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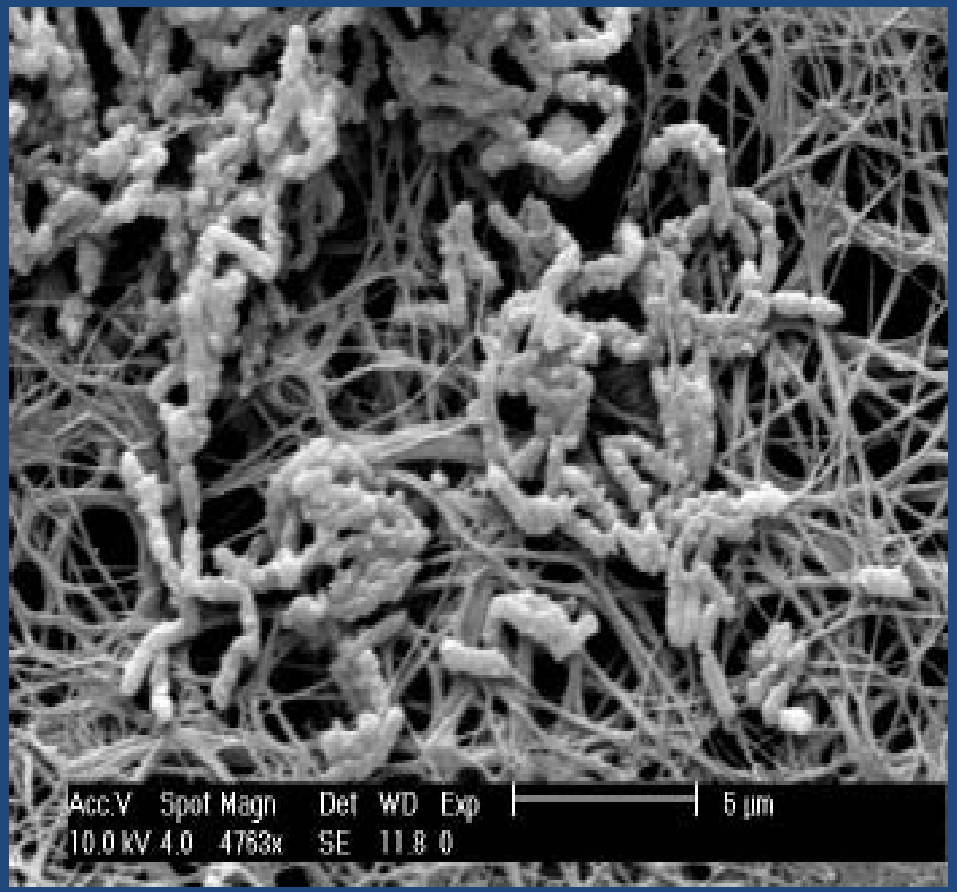
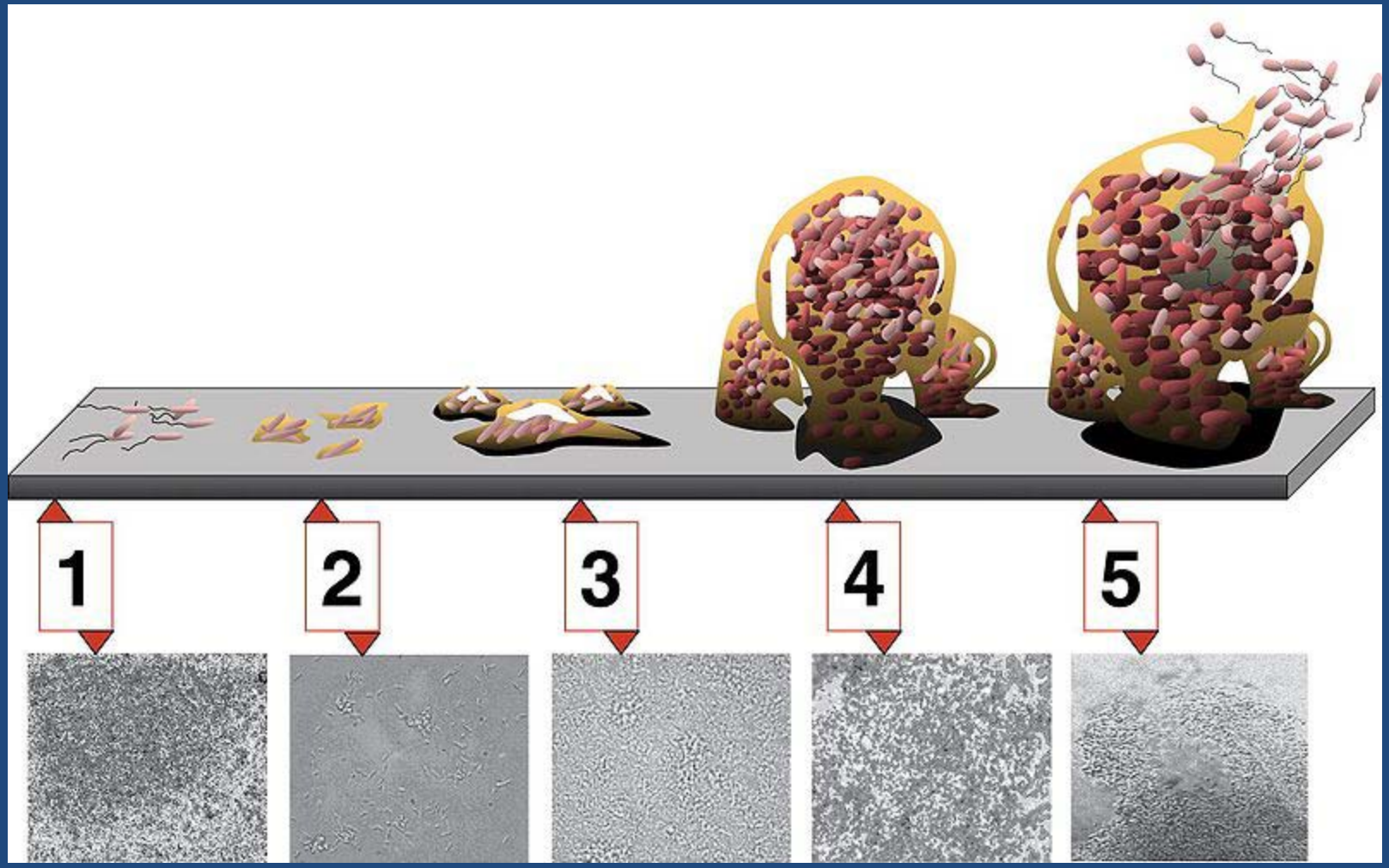



Donlan, RM. Biofilm Laboratory. CDC

# How Does Biofilm Form?

# Bacteria Are the Primary Source of Biofilm Formation

# Stages of Biofilm Development




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

# How Biofilm Happens

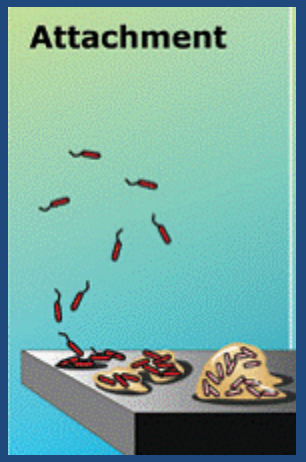
* A solid surface is submerged or exposed to a fluid such as water
* Free-floating, planktonic bacteria adhere to the surface to begin biofilm development
  * Only certain species can attach on their own
  * Weak, reversible adherence
  * More permanent adherence if not immediately flushed off
* A slimy 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

# Factors Affecting Biofilm Formation

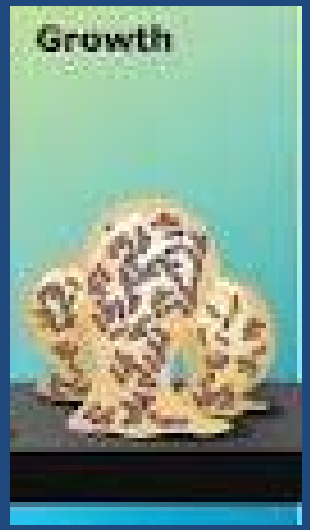
* Environment
  * pH
  * Temperature
  * Presence of nutrients
* Microbial Interactions
* System materials of construction
  * Surface properties
  * Corrosion
* System hydraulics
  * Flow rates
  * Dead legs

# 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



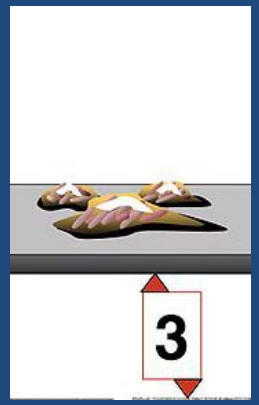
# Stage 2: Irreversible Adherence

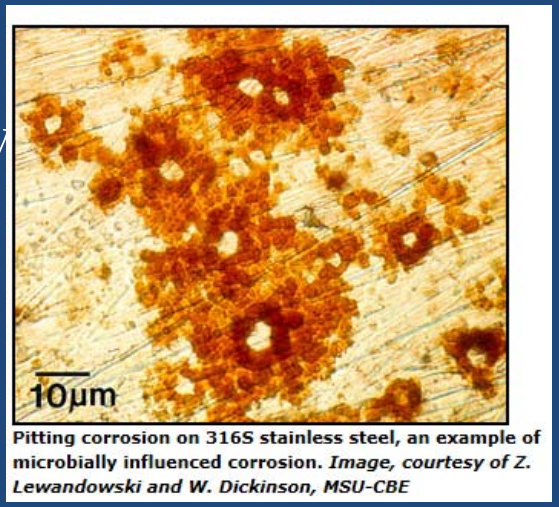



* In 12 minutes, attached bacteria increase
  * Production of proteins
  * Excretion of polysaccharides (slime layer)
  * Rapid cell division—exponential bacterial growth
* Slime layer prevents dislodgement of biofilm
  * Resistant to shear forces of flowing water
  * Keeps bacteria attached to surface

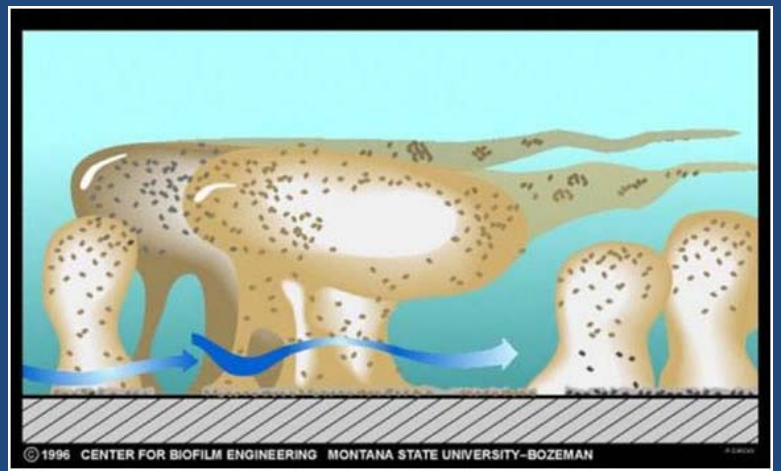
# Stage 3: Aggregation

* 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 lay of the biofilm



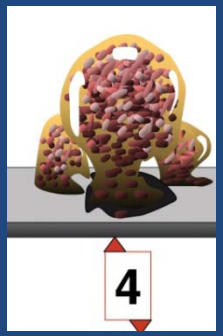
Pitting corrosion on 316S stainless steel, an example of microbially influenced corrosion. *Image, courtesy of Z. Lewandowski and W. Dickinson, MSU-CBE*

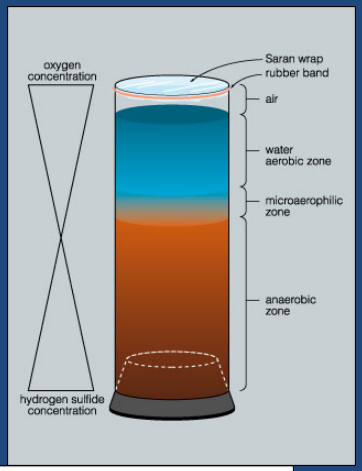


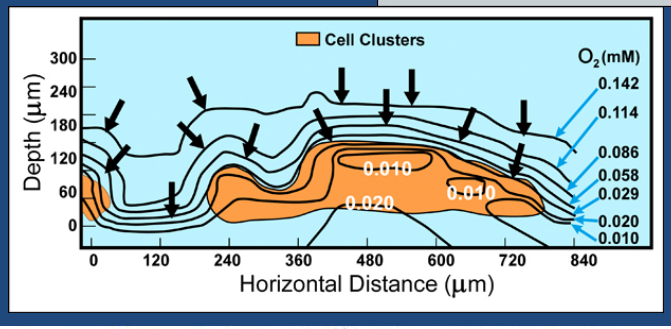
# Stage 4: Maturity--Composition

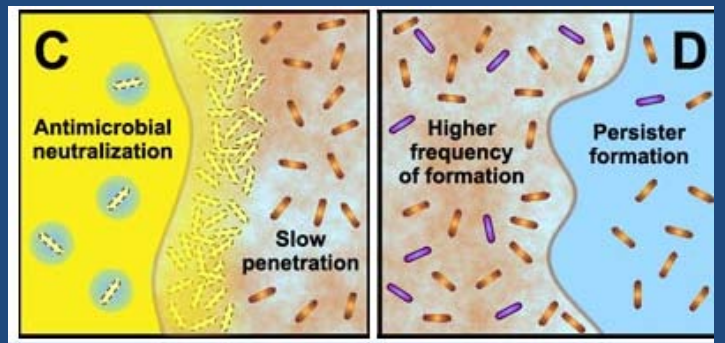
* Biofilm Composition
  * 10-75% Bacteria
  * 90-25% Slime
* Oxygen gradient
* 1000x More Resistant

to Disinfectants



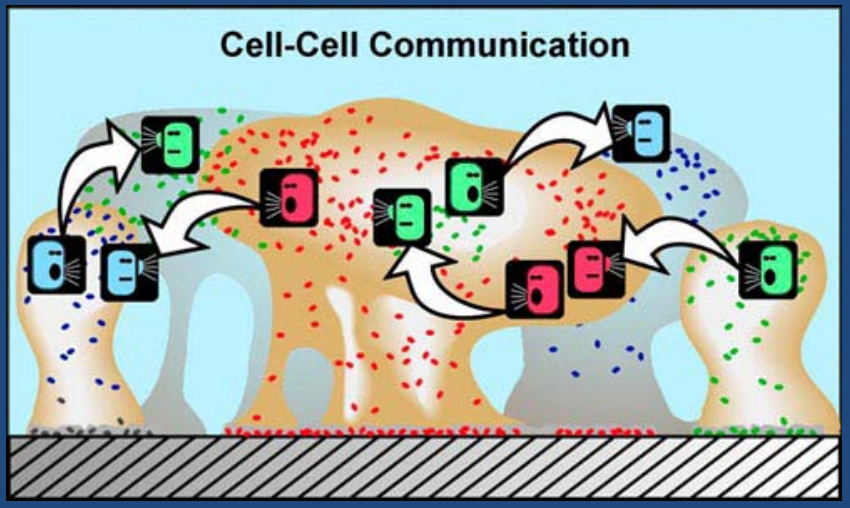



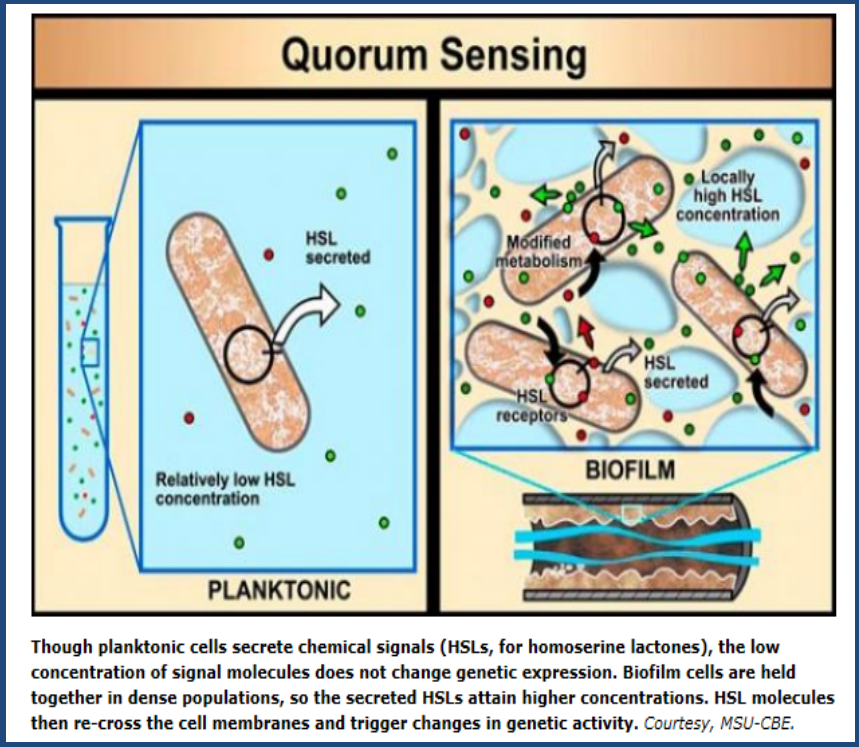



# Stage 4: Maturity — Biofilm Communication

* 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.*

"…Bacteria use at least four of the five senses. In addition to smell, the organisms respond to light (sight), to physical contact with others of their species (touch), and to direct contact with chemicals (taste)."

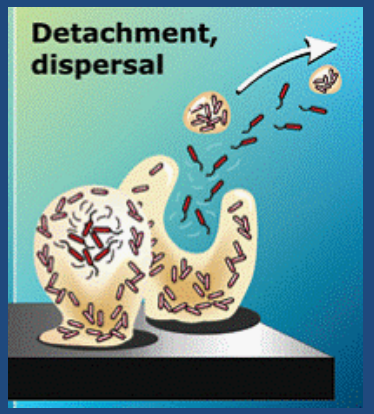
Brian Handwerk National Geographic News

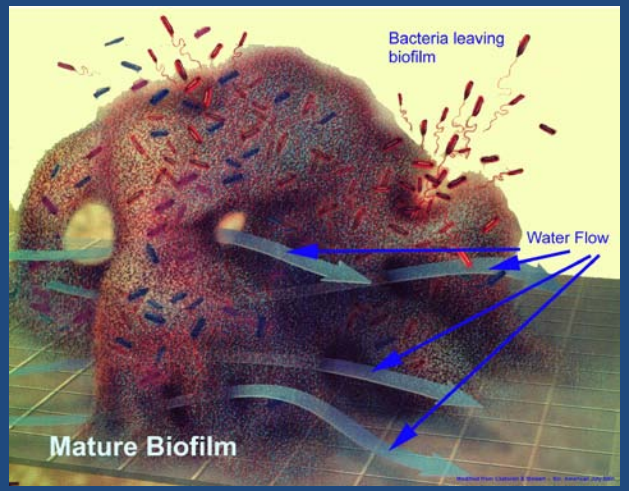
Published August 18, 2010

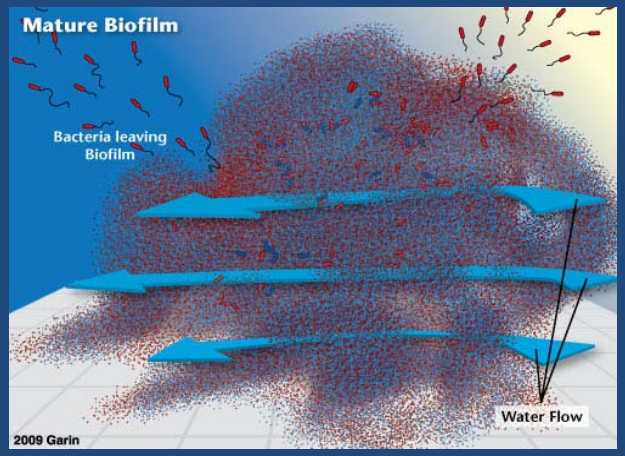

*"Biofilm bacteria can move in numerous ways: Collectively, by rippling or rolling across the surface, or by detaching in clumps. Individually, through a "swarming and seeding" dispersal."*

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

# Stage 5: Dispersal



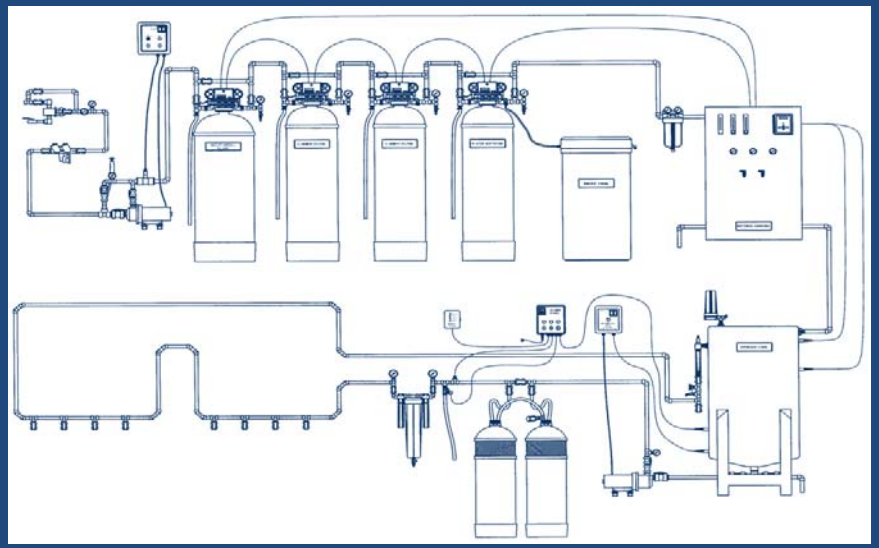




*This is the Biofilm's Most Vulnerable Time!*

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

# 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

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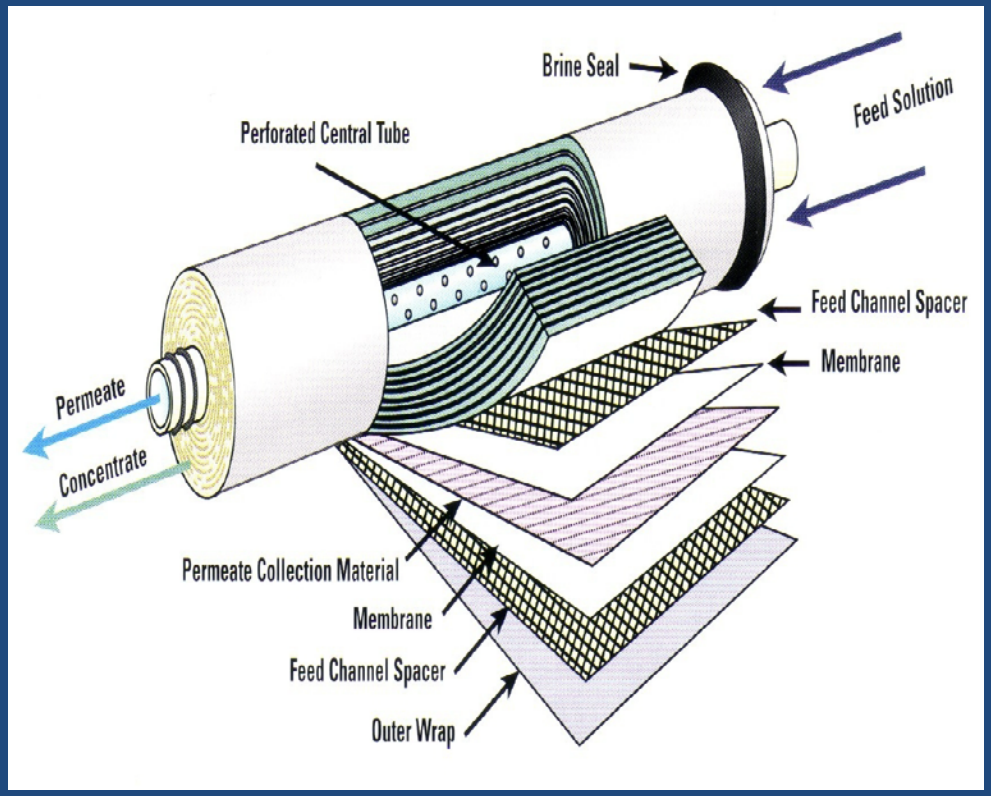
# Inside An RO Membrane



# RO Membrane Biofilm

# THE GOAL

## Where We Are Today

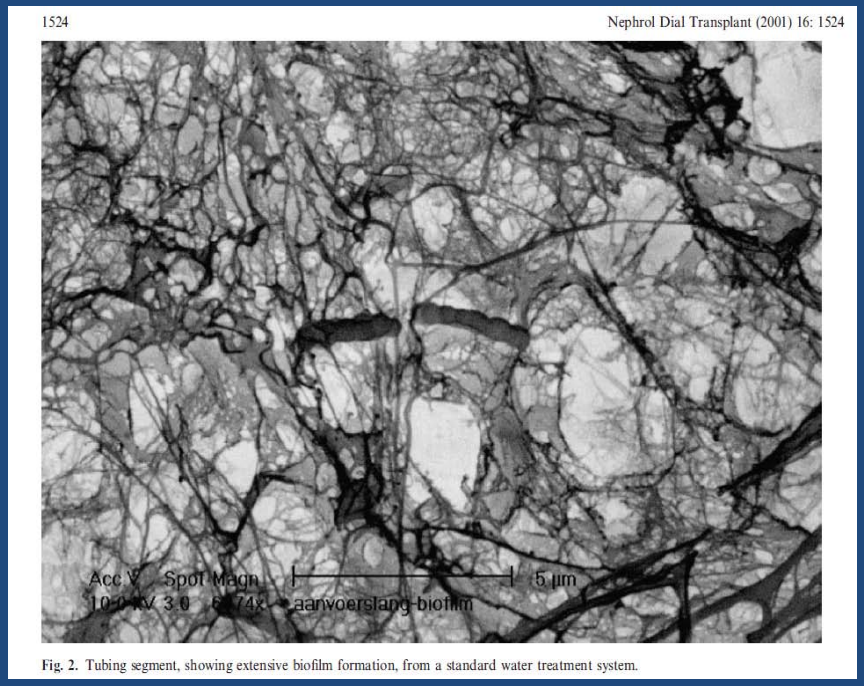



Fig. 2. Tubing segment, showing extensive biofilm formation, from a standard water treatment system.

## Where We Want To Be

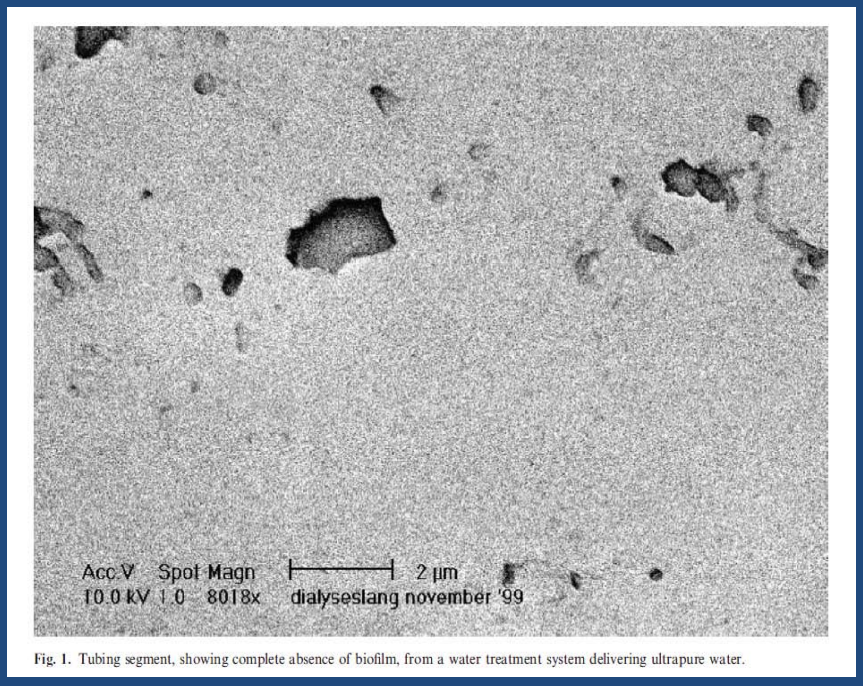



Fig. 1. Tubing segment, showing complete absence of biofilm, from a water treatment system delivering ultrapure water.

How do we minimize our patients' exposure to microbiological contamination?

# 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
* When?
  * Disinfection strategies in systems in dialysis should be designed to be proactive rather than reactive
  * If you are disinfecting based on positive culture results you already have a biofilm problem.

# Disinfectant Choices

* Ozone
* Bleach
* Peracetic Acid/H2O2 Mixtures
* ~~Formaldehyde~~
* ~~Glutaraldehyde~~
* Heat
* UV

# Effectiveness of Disinfection 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

# Ozone

* Concentration levels of 0.3-0.7 ppm for disinfection purposes
* Kills microorganisms by oxidation
* Fairly aggressive towards biofilm – but rarely completely removes it.
* Residual level must be below 0.1 ppm. There has been discussion within the AAMI committee of reducing this to 0.05 as low levels of ozone can affect a person's immune response.
* Not compatible will all distribution piping materials.
* Not compatible with RO membranes

# Bleach

* Concentration level of 1% (~500 ppm) for disinfection purposes
* Kills microorganisms by oxidation
* Fairly aggressive towards biofilm – but rarely completely removes it.
* Residual level must be below 0.1 ppm.
* Not compatible will all distribution piping materials or dialysis machine components.
* Not compatible with RO membranes

# Peracetic Acid/H2O2 Mixtures

* Concentration level of 1% for Water system disinfection purposes (3% for dialyzer reprocessing)
* Kills microorganisms by oxidation
* Not particularly aggressive towards biofilm
* Residual level must be below 0.1 ppm.
* Good compatibility will all distribution piping materials and dialysis machine components.
* Compatible with RO membranes

# Heat Disinfection

* Heat disinfection for 20 minutes at 85°C will kill most microorganisms
* Does not kill bacterial or fungal spores (white "cheese" at the tip of the drain line)
* Will help prevent biofilm formation if done frequently
* Will not remove a biofilm

# Ultraviolet Disinfection

* Emits light at wave length of 254 nm
* Normally provides a dose of radiant energy of 30 mW-s/$cm^2$ minimum dose should be 16 mW-s/$cm^2$
* Should be equipped with a calibrated UV intensity meter and sized for the maximum flow in the system.
* If the UV radiant out put is not adequate and/or the flow too high, the result can be the mutation of the organisms to UV resistant strains.

# Disinfection Strategies

Should be designed to prevent formation of biofilm. If you disinfect your system based on bacteria results hitting the AAMI action level (50 cfu/mL), then you already have biofilm formation in your system.

# Disinfection Frequency

* IDEAL: Since we know that biofilm can form in you water system in about 12 minutes, disinfecting every 11.5 minutes would be ideal.
* REAL WORLD: How often is it practical for you to disinfect your system?
  * CMS requires monthly disinfection of the water system.
  * We normally disinfect our dialysis machines on a daily basis with heat, and 1-2 times weekly with bleach.

# So What Is Practical?

* Many water systems currently in use require a time consuming manual disinfection process, which makes frequent disinfections difficult and expensive.
* Water treatment companies are (or have) developed more sophisticated systems that decrease labor time and automate the process.

# Thank you!

Questions?