Beware of the Biofilm

Jim Curtis

Jim Curtis & Associates, LLC

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

<table>
<thead>
<tr>
<th>Dialysate Type</th>
<th>Allowable CFU (Action Level)</th>
<th>Endotoxin Level (Action level)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Dialysate</td>
<td>&lt; 100 cfu/mL (50 cfu/mL)</td>
<td>&lt; 0.5 EU/mL (0.25 EU/mL)</td>
</tr>
<tr>
<td>Ultrapure Dialysate</td>
<td>&lt; 0.1 cfu/mL</td>
<td>&lt; 0.03 EU/mL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bacteria Level</th>
<th>Exposure during 4 hr tx @ 800 Qd</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 cfu/mL</td>
<td>19,200,000 bacteria</td>
</tr>
<tr>
<td>50 cfu/mL</td>
<td>9,600,000 bacteria</td>
</tr>
<tr>
<td>0.1 cfu/mL</td>
<td>19,200 bacteria</td>
</tr>
</tbody>
</table>
Why do we want high purity water for our patients?
INFLAMMATION

PROTEIN AND LIPID OXIDATION

CYTOKINES (IL-1β, TNFα)

O2

UREMIC TOXINS

NADPH OXIDASE

C5

C5a

NEUTROPHIL

BLOOD

MONOCYTE

MEMBRANE

DIALYSATE

ENDOTOXIN and OTHER MICROBIAL PRODUCTS

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

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

CARPAL TUNNEL SYNDROME IN PATIENTS TREATED WITH ULTRAPURE WATER

EFFECT OF IMPROVED WATER QUALITY ON ANEMIA CORRECTION


EFFECT OF DIALYSATE PURITY ON NUTRITION

EFFECT OF FILTERED DIALYSATE ON MUSCLE MASS AND SERUM ALBUMIN

<table>
<thead>
<tr>
<th>Study</th>
<th># Patients</th>
<th>Inflammation</th>
<th>Anemia Correction</th>
<th>Nutritional Status</th>
<th>β₂-microglobulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arizono (2004)</td>
<td>23</td>
<td>+↑+</td>
<td>+↑+</td>
<td>+↑+</td>
<td>+↑+</td>
</tr>
<tr>
<td>Baz (1991)</td>
<td>226</td>
<td></td>
<td></td>
<td></td>
<td>+↑+</td>
</tr>
<tr>
<td>Furuya (2005)</td>
<td>16</td>
<td>+↑+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Go (2007)</td>
<td>61</td>
<td>+↑+</td>
<td>+↑+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hsu (2004)</td>
<td>34</td>
<td>+↑+</td>
<td>+↑+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Izuhara (2004)</td>
<td>84</td>
<td>●↔●</td>
<td>●↔●</td>
<td>●↔●</td>
<td>●↔●</td>
</tr>
<tr>
<td>Kleophas (1998)</td>
<td>399</td>
<td></td>
<td></td>
<td>+↑+</td>
<td>+↑+</td>
</tr>
<tr>
<td>Lamas (2006)</td>
<td>78</td>
<td>●↔●</td>
<td>●↔●</td>
<td>●↔●</td>
<td>+↑+</td>
</tr>
<tr>
<td>Matsuhashi (2002)</td>
<td>27</td>
<td>+↑+</td>
<td>+↑+</td>
<td>●↔●</td>
<td></td>
</tr>
<tr>
<td>Molina (2007)</td>
<td>107</td>
<td>+↑+</td>
<td>+↑+</td>
<td>●↔●</td>
<td></td>
</tr>
<tr>
<td>Ouseph (2007)</td>
<td>105</td>
<td>●↔●</td>
<td>●↔●</td>
<td>+↑+</td>
<td>+↑+</td>
</tr>
<tr>
<td>Rahmati (2004)</td>
<td>342</td>
<td>+↑+</td>
<td>+↑+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schiffl (2000)</td>
<td>89</td>
<td></td>
<td></td>
<td>+↑+</td>
<td></td>
</tr>
<tr>
<td>Schiffl (2001)</td>
<td>48</td>
<td>+↑+</td>
<td></td>
<td></td>
<td>+↑+</td>
</tr>
<tr>
<td>Sitter (2000)</td>
<td>30</td>
<td>+↑+</td>
<td></td>
<td></td>
<td>+↑+</td>
</tr>
</tbody>
</table>

**Legend:**
- **+↑+** IMPROVED
- **●↔●** NO CHANGE
- **↓↓** WORSENED
## POOLED ESTIMATES OF CHANGE FOLLOWING INTRODUCTION OF ULTRAPURE DIALYSATE

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

* 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 Leeuwenhoek
* 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/
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

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.

Figure 2. Bacteria growing on and near an oil droplet 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”

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

“Dental plaque as seen under a scanning electron microscope”

“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

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

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 layers of the biofilm
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
“...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).”
“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
Inside An RO Membrane
THE GOAL

Where We Are Today  ➔  Where We Want To Be

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

Fig. 2. Tubing segment, showing extensive biofilm formation, from a standard water treatment system.
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 with 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 with 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/cm² minimum dose should be 16 mW-s/cm²
- 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 your 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?