Risk Reduction of Thrombosis

- Anti-bacterial & Anti-fungal action

Hydromer®

- Duality™ T8B Coating
  - Non-thrombogenic / Anti-microbial /
  - Non-cytotoxic

- Risk Reduction of Thrombosis
- Anti-bacterial & Anti-fungal action
1. Risk Reduction of Thrombus Formation

Study of Blood Component(s) Adhesion

Microscopic examination (stained with Coomassie Blue) of the surface of PU tubing (coated vs. uncoated) after 12 days exposure to citrated human whole blood.

Observation: Under the same experimental conditions, the Hydromer® Duality T8B coating significantly reduced the adhesion of blood components to the surface of PU tubing.
1. Risk Reduction of Thrombus Formation

Study of Protein(s) Adsorption

Duality T8B Coating on Hemolysis of Red Blood Cells

Each cycle involves the blood sample being pumped (2ml/min) for 8 hours at R.T. and then stored @ 4°C for 16 hours, with the exception of the 3rd & 8th Cycle, in which the evaluation covered an 64 hour period following the pumping at R.T. for 8 hours.

\[
\% \text{ Difference} = \left[ \frac{(A_{540\text{nm}} \text{ of Duality T8B} - A_{540\text{nm}} \text{ of uncoated})}{A_{540\text{nm}} \text{ of uncoated}} \right] \times 100
\]

Protein Adsorption Analysis

After 12 days exposed to citrated blood, PU tubes (uncoated & coated, 18 cm length) were rinsed with H₂O and filled with 500 μl buffer (10 mM Tris & 500 mM NaCl, pH 7.4). Any protein eluted into the buffer was analyzed by Bradford protein method.

Observation:
Exposure of Hydromer® Duality T8B coating with whole blood did not result in significant hemolysis of red blood cells compared to uncoated controls and significantly reduced blood protein(s) absorption on to the surface when compared to uncoated controls.
1. Risk Reduction of Thrombus Formation

Study of protein(s) Adsorption

Microscopic examination (800X, stained with Coomassie Blue) of the surface of stainless steel shims (coated vs. uncoated) after 18 hour exposure to plasma proteins or lysozyme

Observation: Hydromer® Duality T8B coating significantly reduced adhesion of plasma proteins and lysozyme to stainless steel shims.
1. Risk Reduction of Thrombus Formation

Study of Platelet Adhesion

Microscopic examination (800X, stained with Coomassie Blue) of the surface of stainless steel shims (coated vs. uncoated) after 1 hour exposure to washed platelets.

Immunohistochemical analysis

Immunohistochemical analysis to determine platelet adhesion to stainless steel using antibodies to CD41 and peroxidase-coupled anti-IgG.
2. Study of Cell Proliferation

Microscopic examination (300X) of the surface of stainless steel shims after seeding with murine L929 fibroblasts for 4 days

Cell Proliferation Assay
Stainless steel pieces were rinsed in PBS and incubated in 1 ml of fresh media with 0.05 ml of CP reagent. The absorbance of a 100 ul aliquot was measured @ 492 nm after 2.5 hours @ 37°C.

Observation: Hydromer® Duality T8B coating significantly reduced adhesion of fibroblast cells to stainless steel shims.
2. Study of Cell Proliferation

Microscopic examination (200X) of the surface of stainless steel shims after seeding with human umbilical vein endothelial cells (HUVEC) for 4 days

Cell Proliferation Assay
Stainless steel pieces were rinsed in PBS and incubated in 1 ml of fresh media with 0.05 ml of CP reagent. The absorbance of a 100 ul aliquot was measured @ 492 nm after 3 hours @ 37°C.

Observation: Hydromer® Duality T8B coating significantly reduced adhesion of endothelial cells to stainless steel shims.
3. Study of Anti-bacterial & Anti-fungal Activity

Polyurethane Tubing

(see methodology on second following page)

Percentage of Inhibition After 4 Weeks of Microbial Exposure

UNCOATED

Duality T8B

**E. coli**

CFU/ml

uncoated

coated

>75 %

**P. aeruginosa**

CFU/ml

uncoated

coated

>68 %

Surface analysis: 1000 X
S. epidermidis

CFU/ml

>55%

S. aureus

CFU/ml

>58%

C. albicans

CFU/ml

>67%

Duality T8B

UNCOATED

coated

uncoated

Hydromer
Reduction of Viability of Bacterial Growth on Uncoated Polyurethane Tubing over Time

As shown above, bacterial overgrowth and loss of viability made further evaluation uncertain.

<table>
<thead>
<tr>
<th>Bacterial Strain</th>
<th>Inhibition Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. Coli</td>
<td>72%</td>
</tr>
<tr>
<td>S. Aureus</td>
<td>81%</td>
</tr>
<tr>
<td>S. Epidermidis</td>
<td>10%</td>
</tr>
<tr>
<td>P. Aeruginosa</td>
<td>69%</td>
</tr>
<tr>
<td>C. Albicans</td>
<td>43%</td>
</tr>
</tbody>
</table>

The data shown on the right is the % inhibition we observed after 6 weeks of study. Because of the decrease in the growth rate of the bacteria in control tubes, these values represent a minimum inhibition level.

**Methodology:** Polyurethane tubing (4 cm length) was incubated in a bacteria culture for four weeks. Tubing was removed, rinsed with PBS, and placed in 4 ml of LB broth. The tubes were sonicated and vortexed to dislodge bacteria colonies. An aliquot was spread on an LB agar plate and incubated overnight. Colonies were counted the following day. A parallel set of tubes were stained for microscopic visualization of surface growth.
3. Study of Antibacterial & Antifungal Activity

Silicone Tubing
(identical methodology applied as with the Polyurethane Tubing)

Percentage of Inhibition After 1 Week of Microbial Exposure*

<table>
<thead>
<tr>
<th></th>
<th>UNCOATED</th>
<th>Coated</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>&gt;74 %</td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>&gt;80 %</td>
<td></td>
</tr>
</tbody>
</table>

* Efficacy studies for additional weeks are ongoing

Surface analysis: 1000 X

Silicone Tubing (identical methodology applied as with the Polyurethane Tubing)
Other HYDROMER® Products & Services:

- Medical Coatings
  - Drug Delivery
  - Anti-microbial
  - Anti-thrombogenic
  - Anti-cell adhesion/proliferation
  - Radio-opaque
- Contract manufacturing/coating
- Aquadapt® Medical Hydrogels
- Cosmetic Intermediaries
  - Anti-fog / Anti-frost condensation control Coatings
- T-HEXX® Animal Health
- OEM Medical Device Manufacturing
- Coating formulation
- Process development
- Device design feedback
- Machine design & build
- Prototype production - GMP/ISO
- Contract manufacturing/coating
- Technology / process transfer

- Analysis
- Testing
- Polymer Synthesis
- Microbiology
- Cell Biology
- Blood Chemistry
- Bio-Polymer Production
- Web Coating/Film Coating
- Tube Coating: External and Internal

A few of Hydromer’s brands:

- AQUAMERE®
  - Hydrogel cosmetic ingredients
- AQUATRIX® II
  - Hydrogels
- Dermasea®
  - Allergen-blocker
- Aquadapt®

We can help make your ideas become reality