Control of Surface Chemistry in a Biomimetic Therapeutic Device for Treating Bacteremia and Sepsis

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An Emergence Company
Whole Blood Affinity Apheresis

The process of removing specific components from *whole* blood by passing it through an *adsorption bed* and returning it to the patient.

- Lowering the concentration of inherently harmful elements (e.g., pathogens and toxins) and/or those that are harmful due to an acute increase in their concentration (pro-inflammatory cytokines).
A Simple Extracorporeal Circuit for Whole Blood Apheresis

“Seraph™ Microbind™ Affinity Blood Filter (Seraph)”
Requirements for an Effective and Safe Whole-Blood Affinity Apheresis Therapy

- Non-thrombogenic adsorption media
- Rapid, selective adsorption
- Good *hemodynamics* and high blood flow rates in extracorporeal circuits
- High binding capacity with minimum blood hold-up
- Scale-able for different clinical applications
- Robust, low-cost manufacturing processes
Heparin

Heparin’s AT Binding Sequence

- Discovered by Jay McLean (1926)
- First clinical use by Jorpes & Crafoord at Karolinska Inst. (1940’s)
- Currently the most commonly-used blood anticoagulant
  - Binds antithrombin III (AT) increasing its activity >1000X
  - The bound AT inactivates thrombin and factor Xa
- End-point attachment to surfaces by Larm, et al. (1983)*
  - Carmeda CBAS®
- Use of end-point attached heparin in DLT by ExThera AB (2005)
- Broad-spectrum mixed bed adsorbents by Exthera Medical (2010)

Seraph’s Biomimetic Mechanism

- Heparan sulfate proteoglycans, known as syndecans, are naturally found on cell membranes
  - Syndecans bind and regulate many inflammatory factors through heparan sulfate segments
- Many pathogens bind to heparan sulfate, a key receptor site, to subvert the host immune response
- Heparin is very similar in chemical structure to heparan sulfate and is able to bind most of the same molecules and pathogens. Examples include but are not limited to:
  - Staph. aureus and MRSA, Dengue, CMV, Group A Strep., Plasmodium falciparum, Candida, HIV, Hep-C, TNF-α
Methicillin Resistant *Staphlococcus aureus* (MRSA)

- **MRSA Bacteremia Incidence**
  - Leading cause of bacteremia in hospital patients, 18.8%
  - 2nd leading cause in outpatients, 14.4%
  - An emerging global pandemic

- **Metastatic Complications**
  - Infective endocarditis
  - Medical device infection
  - Organ involvement (lung, vertebra, spleen, CNS, soft tissue)

- **Mortality**
  - Over 36% from MRSA
## S. aureus and MRSA Strains Tested

<table>
<thead>
<tr>
<th>Strain</th>
<th>% Removed in One Pass</th>
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<tbody>
<tr>
<td>SA 1800T</td>
<td>62%</td>
</tr>
<tr>
<td>MRSA 485</td>
<td>85%</td>
</tr>
<tr>
<td>MRSA 251</td>
<td>59%</td>
</tr>
<tr>
<td>MRSA 860</td>
<td>70%</td>
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<tr>
<td>USA 300</td>
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</tbody>
</table>

### Results:

59 to 85% of MRSA (\( \bar{X} = 71\% \)) is removed by one pass through Seraph adsorption media. (Therapeutic use will involve \( \approx 10 \) passes.)
Removal of *S. aureus* α-Toxin*
Texas A&M University

\[ \approx 73\% \text{ of the } S. \text{ aureus } \alpha \text{-toxin was removed in a single pass of USA300 MRSA through Seraph media and by a (thrombogenic) control media.} \]

*Kills by creating pores in cell membranes*
1 ml of human blood containing $10^{11}$/mL of radiolabelled HSV-1 or HSV-2 virus particles was passed through a miniature column packed with 1 ml of Seraph media.

<table>
<thead>
<tr>
<th>Virus</th>
<th>HSV-1</th>
<th>HSV-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Removed</td>
<td>99.1</td>
<td>99.8</td>
</tr>
</tbody>
</table>

[HSV] Control = 100%
Removal of TNF-α from Porcine Plasma by Human-Size Seraph Cartridge: Univ. of MN

- 5 liters of platelet-poor plasma dosed with high concentration of TNF-α
- Recirculated at 150 mL/min through a prototype Seraph cartridge at 37°C using a dialysis pump

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S. aureus targets heparan sulfate during its pathogenesis. S. aureus and MRSA bind to heparinized surface under \textit{in vitro} flow conditions.

S. epidermis does not target heparan sulfate. S. epidermis does not bind appreciably to heparinized surface under \textit{in vitro} flow conditions.
Developing a Safe, **Very** Broad-Spectrum “95%” Adsorption Media

- End-point-attached heparin is an inherently anti-thrombogenic adsorbent surface
- Heparin binds many, but not all pathogens, toxins, and cytokines
- Other potentially useful adsorbents may be inherently thrombogenic or inflammatory
- **What to do??**
Utilize heparin’s catalytic effect to counteract thrombogenicity of nearby thrombogenic sites

• Use heparin in very close proximity to thrombogenic sites to achieve broad-spectrum adsorption capability
  – Don’t overcoat the thrombogenic sites
  – Maintain molecular-scale distances on the same media particle  
    OR More Simply
  – Maintain nano-scale or micro-scale distances on adjacent particles
• Endotoxemia is a common during gram-negative sepsis.

• Endotoxins are also found during gram-positive sepsis: could be translocation from the intestinal track.

• Endotoxins bind to cationic surfaces, but cationic surfaces are generally highly thrombogenic.

• Can the presence of heparinized media in close proximity to thrombogenic surfaces prevent thrombosis??

- Human blood inoculated with LPS at 10 ng/mL
- Reference: Incubated, not treated
- Samples circulated for 2 hr. at 150 mL/min through human-size mixed bed column

LPS Reduction at 2 hours: \( \overline{X} = 88\% \) (n=3)

* Lipopolysaccharide endotoxin
Electron Micrographs of ‘Mixed Media’ Column after Exposure to Blood for 2 hr.

Heparin

Mixed

Heparin
Mini-Column for CLP Rat Study

Miniature Column
Rat CLP Study with a Single Heparin-only Column

- **48-hour Survival** ($n = 10$)
  - Treated: **100%**
  - Control:* 70%
  - Untreated:** ≈ 50%

- **Organ Injury:** TBD

- Circuit with empty tubing with same free volume as adsorption column. Lovenox needed to prevent clotting in control circuit only.

Large Animal Safety Study of Seraph™ Microbind™ Affinity Blood Filter

- Catheter Placement
- Filling While Displacing Saline
- Experiment In Progress
- Rinsed Column at 3.5 hrs.
Seraph’s Broad Spectrum Capability and Inherent Safety in Contact with Blood:

Creates an organ preservation strategy that reduces morbidity and mortality by:

1. **Removing Blood-borne Pathogens**, such as MRSA at first sign of bacteremia (optionally before pathogen is identified)

2. **Removing Endotoxin and Exotoxins**: bacterial byproducts activating inflammation

3. **Shortening the Duration of Bacteremia via 1. and 2. to reduce metastatic complication and the progression to sepsis and septic shock**: goal of initial clinical trials

4. **Restoring Balance to the Inflammatory System** by down-regulating cytokine imbalance
1. End-point-attached heparin surfaces are known to be antithrombogenic, & inherently safe for blood contact

2. Heparin-based affinity apheresis is capable of rapidly removing pathogens, cytokines and toxins from whole blood

3. Very broad spectrum removal may be safely achieved with mixed media that includes heparin

4. Pathogen/toxin removal by heparin-based affinity therapy may become a useful treatment for bacteremia and sepsis: Clinical trials start Q1 2013
Acknowledgements

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• **Murine CLP Study:** Profs. Peter Ward and Jean Nemzek, University of Michigan

• **LPS Removal:** Profs. Soheyl Bahrami and Heinz Redl, Ludwig Boltzmann Institute, Vienna

• **Anthrax Cytokine Study:** Ricardo Carrion, Ph.D., Texas Biomedical Research Institute, San Antonio
# Some Pathogens that Bind to Heparin/Heparan Sulfate

<table>
<thead>
<tr>
<th>Bacteria and (Toxins)</th>
<th>Viruses</th>
<th>Parasites</th>
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</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em> and MRSA (α-toxin, β-toxin)</td>
<td>HIV</td>
<td><em>Plasmodium</em> spp.</td>
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<tr>
<td><em>Bacillus anthracis</em></td>
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<tr>
<td><em>Helicobacter pylori</em></td>
<td>Cytomegalovirus</td>
<td><em>Trypanosoma</em> cruzi</td>
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<tr>
<td><em>Mycobacterium tuberculosis</em></td>
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<tr>
<td><em>Neisseria meningitidis</em></td>
<td>Cytomegalovirus</td>
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<tr>
<td><em>Streptococcus pneumoniae</em></td>
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<tr>
<td><em>Listeria monocytogenes</em></td>
<td>Dengue Virus</td>
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<tr>
<td></td>
<td>HPV</td>
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<td></td>
<td>West Nile virus</td>
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<tr>
<td></td>
<td>Yellow fever virus</td>
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