Membrane Technologies for Therapeutic Plasma Separation and Online Plasma Treatment

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Outline of Presentation

- Efficient plasma separation by membranes
- Temperature control in online plasma filtration for therapeutics
- Thoughts on apheresis technologies
Membrane Technology

- Well-established therapeutic medical technology
- Hemodialysis
- ECMO, hemofiltration
- Therapeutic apheresis
Plasma Separation From Whole Blood

CENTRIFUGAL PLASMA SEPARATION

CELL FREE PLASMA

CENTRIFUGAL FORCE SEPARATES CELLS FROM PLASMA

MEMBRANE PLASMA SEPARATION

CELL FREE PLASMA

SHEAR FLOW CAUSES CELL MIGRATION AWAY FROM WALL
Filtration Flux as a Function of Transmembrane Pressure

For plasma separators, and ultrafiltration by hemofilters and dialyzers

Sieving as a Function of Molecular Diameter

For membranes used in plasma separation, hemofiltration (RP-69) and dialysis (Cuprophan PT-150)

Schematic of Hollow Fiber Membrane Plasma Separation
Plasma flow is regulated by the pump in the plasma circuit. Pi is the inlet pressure to the plasma separator, PF is the plasma separator filtrate-side pressure, Ps is the inlet pressure to the plasma processing chamber, and Po is the pressure measured prior to blood return to the patient.
Experimental in vitro plasma flow (QF) varying results for the Kuraray 301-K module (polyvinyl alcohol membrane) at a blood flow (QB) of 100 mL/min studied with normal bovine blood of a mean hematocrit of 35%. The maximum plasma flow is determined to be 42.5 mL/min. The maximal experimental plasma flow is taken as the highest plasma flow for which the transmembrane pressure is stable in time and for which no increase in hemolysis occurs.
Maximum plasma flux as a function of the average wall shear rate. Testing was done in vitro with normal bovine blood.
Scanning electron microscopic pictures at the same magnification of the blood contacting surfaces of the membranes made from polymethylmethacrylate (left), polyethylene (middle), and polyvinyl alcohol (right).
Scanning electron microscopic pictures at the same magnification of the blood contacting surfaces of polymethylmethacrylate membrane prior to (upper) and following (lower) whole blood perfusion with no plasma pumping.
SEM Images of PE Membrane

Scanning electron microscopic pictures at the same magnification of the inner surface of a polyethylene membrane: Native state (left) and following plasma contact (right).
Membrane Properties for Select Plasma Separator Modules

<table>
<thead>
<tr>
<th>Module</th>
<th>Nominal Surface Area (m²)</th>
<th>Porosity*</th>
<th>Effective Surface Area (m²)</th>
<th>Pore Density by Visual Count (pore # / cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE-MH</td>
<td>0.65</td>
<td>0.65</td>
<td>0.42</td>
<td>1.43 x 10^8</td>
</tr>
<tr>
<td>PVA-KS</td>
<td>0.63</td>
<td>0.50</td>
<td>0.32</td>
<td>1.96 x 10^8</td>
</tr>
<tr>
<td>PMMA-TP</td>
<td>0.50</td>
<td>0.75</td>
<td>0.38</td>
<td>1.14 x 10^8</td>
</tr>
</tbody>
</table>

* From manufacturer, personal communication.
Theories of Two Phase Flow With Filtration

The modified lift theory predicts, and experimental evidence has shown, the presence of a plasma skimming layer at the filtering wall under stable filtration conditions.

Photographs Through Differential Interference Contrast Optics

Hematocrit 20% (half fiber in view)
Applications of Membrane Plasma Separation

• Various commercial membrane devices available (most notably hollow fiber technology)
• High solute sieving, no blood cell loss, operation at low transmembrane pressures
• Use more dominant in countries manufacturing/marketing devices
• Used predominantly in “dialysis-like” setting versus blood banking
Rationale for Extracorporeal Therapies

• Abnormal chemistries in disease states
• Excess solutes may be detrimental
• Removal of excesses will improve symptomology and possibly effect a cure
• Define removal means
Example of Renal Failure

• Abnormal chemistries
  – Toxins, electrolytes, water
  Excess solutes?

• Removal means
  – Forced diuresis, sauna bath treatment, edible sorbents, microorganisms, blood exchange, dialysis (GI, peritoneal, hemo)
  – Dialysis “most practical”
Extrapolation to Other Diseases

• Standard dialysis has molecular size removal limits
• Many disease states (metabolic, immunologic) exhibit abnormalities of higher molecular weight solutes or protein-bound solutes
• Identification of appropriate separation/removal means (i.e. sorption, plasma exchange, blood/plasma treatment) is necessary
## Immunological Disorders

<table>
<thead>
<tr>
<th>Disease</th>
<th>Increased factor(s) or abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myasthenia gravis</td>
<td>Anti-acetylcholine receptor Ab, ganglioside Ab, anti-skeletal muscle Ab</td>
</tr>
<tr>
<td>Guillan-Barré syndrome</td>
<td>Anti-myelin Ab, anti-skeletal muscle Ab</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>Anti-myelin Ab, anti-skeletal muscle Ab</td>
</tr>
<tr>
<td>Goodpasture syndrome/Renal transplant rejection</td>
<td>Antibody to glomerular and lung basement membranes</td>
</tr>
<tr>
<td>Rhesus incompatibility</td>
<td>Anti-D Ab</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>Anti-DNA Ab, anti-nuclear Ab, immune complexes of DNA, macroglobulins, cryoglobulins</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>Immune complexes or auto-Ab, fibrinogen, complement</td>
</tr>
<tr>
<td>Waldenstrom's macroglobulinemia</td>
<td>Increased IgM and hyperviscosity</td>
</tr>
<tr>
<td>Red cell immunization</td>
<td>Anti-Rh Ab (put together w/ Rhesus and HA, TTP??)</td>
</tr>
<tr>
<td>Autoimmune hemolytic anemia</td>
<td>Antibody to RBCs</td>
</tr>
<tr>
<td>ABO-incompatible kidney Tx</td>
<td>Anti-A or B Ab, anti-lymphocyte Ab</td>
</tr>
</tbody>
</table>

### Immunological Disorders, continued

<table>
<thead>
<tr>
<th>Disease</th>
<th>Increased factor(s) or abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pemphigus vulgaris</td>
<td>Increased IgG Ab, anti-epidermal cell membrane glycoprotein Ab</td>
</tr>
<tr>
<td>Asthma bronchitis</td>
<td>Increased IgE</td>
</tr>
<tr>
<td>Myeloma</td>
<td>Increased myeloma globulin</td>
</tr>
<tr>
<td>Raynaud's disease</td>
<td>Increased macroglobulin, increased viscosity</td>
</tr>
<tr>
<td>Thrombocytopenic purpura</td>
<td>Increased immune complexes, anti-platelet Ab</td>
</tr>
<tr>
<td>Cancers</td>
<td>α-1, α-2 globulins, β-globulins, α1-antitrypsin, ceruloplasmin, orosomucoid, haptoglobin, IgA, mucopolysaccharides (Sezary)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Increased circulating immune complex</td>
</tr>
<tr>
<td>Polyneuropathy</td>
<td>Increased antibodies to myelin</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Immune complexes, rheumatoid factor, anti-RANA Ab, macroglobulins, cryoglobulins</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Autoantibodies to insulin receptor</td>
</tr>
</tbody>
</table>

*International Center for Artificial Organs and Transplantation*
## Metabolic Disorders

<table>
<thead>
<tr>
<th>Disease</th>
<th>Increased factor(s) or abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypercholesterolemia</td>
<td>Cholesterol forms including LDL, VLDL, Lp(a)</td>
</tr>
<tr>
<td>Hypertriglyceridemia</td>
<td>Triglycerides and hyperviscosity</td>
</tr>
<tr>
<td>Hypercoagulability/ Hyperviscosity Syndrome</td>
<td>Fibrinogen, fibronectin</td>
</tr>
<tr>
<td>Waldenstrom's macroglobulinemia</td>
<td>Increased IgM and hyperviscosity</td>
</tr>
<tr>
<td>Chylomicronemia</td>
<td>Chylomicrons</td>
</tr>
<tr>
<td>Hepatic coma and insufficiency</td>
<td>Metabolic factors</td>
</tr>
<tr>
<td>Refsum Disease</td>
<td>Phytanic acid (bound to lipoproteins)</td>
</tr>
<tr>
<td>Poisonings</td>
<td>Protein-bound drug</td>
</tr>
<tr>
<td>Dialysis dementia</td>
<td>Protein-bound aluminum</td>
</tr>
<tr>
<td>Amyotrophic lateral sclerosis (?)</td>
<td>Cytotoxic factors, immune complexes suspected</td>
</tr>
</tbody>
</table>

Apheresis Technology

• Plasma separation
  – Exchange
  – Treatment
    • Sorbent
    • Membrane
    • Other physio-chemical means
Disincentives of Plasma Exchange
(Centrifugal or Membrane)

• Requirement for plasma products for infusion (availability?)
  – Possible contamination of infusion solution or reactions thereof (viruses, prions, foreign proteins)
• Loss of plasma solutes and cells
• Loss of essential plasma constituents
Selection of Solute(s) Removal Means

- Safe
  - Biocompatible, releases no detrimental agent, requires no biological infusion products
- Clinically effective
- Not too selective (broad selectivity)
- Versatile
- Cost effective
Renal Failure: Lessons Learned

- Non-selective (dialysis) approach; although imperfect proves to be life sustaining
- Cost effective
Severe Sepsis: Lessons Learned

• Major cause of mortality
• Leads to multiorgan failure
• Causes homeostasis imbalance
• Shows increase in pro- and anti-inflammatory mediators
  – Single causative mediator unlikely

**• Non-selective extracorporeal removal methods perform better**

• Continuous methods perform better
• Treatment dose important
• Early intervention is beneficial

Malchesky PS. Plasma Solute Removal in Critical Care. Presented at the 5th World Congress of the ISFA. May 5-8, 2005 in Rostock, Germany.
Nonbiologic Semiselective Plasma Treatment

- Eliminates requirement for plasma replacement products
- Spares normal plasma constituents
- Treatment modules are safe
- General applicability
- Cost-effective
Schematic of Circuitry for Nonbiologic Plasma Treatment
Why Membrane Plasma Filtration?

- Disease states marker solutes are of a molecular weight greater than that of albumin and generally greater than 100,000 daltons
- Molecular cut offs of membranes not very selective
- Improve selectivity by augmenting molecular separation (i.e. temperature)
  - Cryofiltration for removal of cold aggregating solutes
  - Thermofiltration for removal of lipids and higher molecular weight solutes
Membrane Plasma Filtration

• Based on size differential
• More selective than plasma exchange
• Cost effective compared to other on-line treatments
• Temperature dependent
• Unique membranes available
• Broad range of module designs
Cryoprecipitable Proteins in Patient Plasmas With Various Autoimmune Diseases

From the right: normal control, rapidly progressing glomerulonephritis, rheumatoid arthritis, myasthenia gravis and Sezary syndrome
Schematic of Temperature Regulated Membrane Plasma Filtration
Scheme of Cryofiltration

Diseases Successfully Treated by Cryofiltration

- Cryoglobulinemia
  - With renal impairment
  - Peripheral neuropathy
  - Vasculitis
- Cryofibrinogenemia
- Cold autoimmune hemolytic anemia
- Cold IgM with cryopositive agglutinin disease
- Hepatitis C virus infection
- B-cell lymphoma
- Chronic renal failure
  - Waldenström’s macroglobulinemia
- Hypertension
- Ischemic heart disease
- B-cell lymphoma

- Rheumatoid arthritis
- Rheumatoid vasculitis
- Sjögren’s syndrome
- Polyarteritis nodosa
- Polymyositis
- Myasthenia gravis
- Guillain-Barré
- Lupus nephritis
- SLE with vasculitis
- TTP
- Peripheral vascular disease
- Non-healing leg ulcers, gangrene
- Sepsis
- Monoclonal gammopathy
- ABO-incompatible transplants

Siami GA, Siami FS. *Ther Apher* 1998; 2:228-35 and various Cleveland Clinic Foundation and Japanese Investigators.
Studies on Cryofiltration

- Initial application on 62 y.o. female with Stage IV rheumatoid arthritis
- Other diseases treated: RA, SLE, lupus nephritis, cold agglutinin hemolytic anemia, dermatomyositis, etc., etc.
- Thousands of procedures performed, no significant side effects; select studies showed improvements in cellular functions; removal of pathologic molecules followed single pool model
- Multicenter (4) trials involving over 60 patients with rheumatoid arthritis demonstrated favorable results
- Double-blind, randomized control two center trial performed showing significant improvement in RA patients
Treatment Costs for a Rheumatoid Arthritis Patient

(Excluding drugs, 62 y.o. female, functional class IV)

Size of Lipid Fractions
Schematic of Temperature Regulated Membrane Plasma Filtration
Studies on Thermofiltration

- Allows larger plasma volumes to be processed with removal of pathologic lipids (LDL-VLDL fraction) and other macromolecules and preservation of HDL and albumin; temperature control at 35-42°C
- Demonstrated slowing or stopping the progression of atherosclerosis in homozygous rabbit model
- Well tolerated with significant removal of LDL and sparing of HDL in humans
Aortic Atherosclerotic Plaque Reduction

15.0% vs. 44.2% in a rabbit study
Application of Membrane Plasma Filtration

• Double membrane filtration schemes eliminate need for foreign protein replacement products
• Avoids potential biological contamination
• Prevents loss of essential plasma solutes
• Filter and temperature selection provide selectivity
• Cost-effective and safe
• Offer design options for new clinical applications
Apheresis Influences

• Technically driven
• Product availability
• Physician training
• Federal regulations
• Reimbursement
• Safety and cost effectiveness
• Research funding: governmental and commercial
### Medicare Approved Procedures for Apheresis

As of 2015 (from ASFA)

<table>
<thead>
<tr>
<th>Disease</th>
<th>Methodology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myasthenia gravis, acquired</td>
<td>Plasma exchange</td>
</tr>
<tr>
<td>Leukemia</td>
<td>Leukopheresis</td>
</tr>
<tr>
<td>Macroglobulinemia, primary</td>
<td>Plasmapheresis</td>
</tr>
<tr>
<td>Hyperglobulinemia as cryoglobulemia, multiple myeloma, hyperviscosity syndromes</td>
<td>Apheresis</td>
</tr>
<tr>
<td>TTP, life threatening</td>
<td>Plasmapheresis, Plasma Exchange</td>
</tr>
<tr>
<td>Rheumatoid vasculitis, life threatening</td>
<td>Plasmapheresis, Plasma Exchange</td>
</tr>
<tr>
<td>Pruritis</td>
<td>Plasma Perfusion (charcoal)</td>
</tr>
<tr>
<td>Goodpasture syndrome</td>
<td>Plasma Exchange</td>
</tr>
<tr>
<td>Glomerulonephritis, advanced</td>
<td>Plasma Exchange</td>
</tr>
<tr>
<td>Polyneuropathy, chronic relapsing</td>
<td>Plasmapheresis</td>
</tr>
<tr>
<td>Scleroderma, life threatening</td>
<td>Plasmapheresis</td>
</tr>
<tr>
<td>Polymyositis, life threatening</td>
<td>Plasmapheresis</td>
</tr>
<tr>
<td>Guillain-Barré</td>
<td>Apheresis</td>
</tr>
<tr>
<td>Systemic lupus erythematosus, life threatening</td>
<td>Apheresis</td>
</tr>
<tr>
<td>Cutaneous T-cell lymphoma, skin lesions</td>
<td>Extracorporeal Photopheresis</td>
</tr>
<tr>
<td>Familial hypercholesterolemia</td>
<td>LDL Apheresis (Liposorber, HELP)</td>
</tr>
<tr>
<td>Chronic refractory ITP, rheumatoid arthritis</td>
<td>Immunosorption (Prosurba); not available</td>
</tr>
<tr>
<td>Transplantation in leukemias, etc.</td>
<td>Stem Cell Collections</td>
</tr>
</tbody>
</table>
# Macromolecule Removal in Disorders Approved by Medicare for Treatment

<table>
<thead>
<tr>
<th>Disease</th>
<th>Macromolecule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myasthenia gravis</td>
<td>Acetylcholine receptor antibody</td>
</tr>
<tr>
<td>Hyperproteinemia</td>
<td>IgM paraprotein, Ig monoclonals, elevated protein concentrations, cryoglobulins</td>
</tr>
<tr>
<td>Hyperproteinemia, Macroglobulinemia, Globulinemia, Cryoglobulinemia</td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>Antibodies?, aggregating factor?</td>
</tr>
<tr>
<td>Rheumatoid vasculitis/arthritis</td>
<td>Immune complexes, cryoglobulins</td>
</tr>
<tr>
<td>Goodpasture’s syndrome</td>
<td>Anti-GBM antibody, fibrinogen</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>Antibodies</td>
</tr>
<tr>
<td>Polyneuropathies</td>
<td>Antibodies, immune complexes</td>
</tr>
<tr>
<td>Scleroderma</td>
<td>Antibodies</td>
</tr>
<tr>
<td>Polymyositis</td>
<td>Antibodies?, paraproteins</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>Anti-DNA antibodies, immune complexes</td>
</tr>
<tr>
<td>Cutaneous T-cell lymphoma, skin lesions</td>
<td>Mucopolysaccharides, cryoglobulins</td>
</tr>
<tr>
<td>Familial hypercholesterolemia</td>
<td>Cholesterol forms as LDL, VLDL, Lp(a)</td>
</tr>
</tbody>
</table>
Plasma Treatment Costs (US$)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Japan*</th>
<th>US*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL adsorption column</td>
<td>725 + 275</td>
<td></td>
</tr>
<tr>
<td>Cryofiltration column</td>
<td>230 + 275</td>
<td></td>
</tr>
<tr>
<td>Endotoxin column</td>
<td>3042</td>
<td></td>
</tr>
<tr>
<td>Adsorption/filtration</td>
<td></td>
<td>1790</td>
</tr>
<tr>
<td>FFP/albumin</td>
<td>2295</td>
<td>545</td>
</tr>
</tbody>
</table>

*Kawamura A. Ther Apher 2003;7:497
*Therapeutic Apheresis: A guide to billing and securing appropriate reimbursement; 2015, ASFA
Limitation to Achieving Potential

- Therapeutic efficacy not proven
  - Lack of clinical trials
- Cost of therapy
- Limited reimbursement
- Experience and education of clinicians
- Commercial push
Therapeutic Artificial Organs

• Early intensive therapy
• Less frequent maintenance treatments
• Wean off treatment