Next Generation Hemostatic Devices

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Disclosures:
None

Disclaimer:
Not a trauma surgeon
Not a vascular surgeon

Content available: www.nebraskasurgicalresearch.com
Overview

• Currently available hemostatic aids & devices
• Description of Univ Nebr hemostasis research
• And, a completely unrelated topic in General Surgery
The Military Experience

- 15-20% of combat deaths are potentially preventable
- Rapid battlefield evacuation has resulted in more soldiers dying at medical treatment facilities
- About half of the deaths from penetrating trauma are due to hemorrhage
- Hemorrhage is the #1 cause of preventable death

Impetus to improve treatment of exsanguinating hemorrhage
Clinical Problem: exsanguinating hemorrhage

Fig. 4. Mechanism of death in ground combat, Vietnam War.\textsuperscript{1,10} KIA, killed in action; DOW, died of wounds.

FIGURE 3. Sixteen potential causes of death in the 12 potentially survivable casualties.

\textit{Ann Surg} 2007; 245: 986-991

\textit{J Trauma} 2003; 54: S13-S19
Current Hemostatic Devices

Distinction:

*locally-applied (topical)* vs. systemic agents

Dry Fibrin Sealant Dressing
(American Red Cross)

NovoSeven
(Novo Nordisk)
Coagulation Cascade

**TISSUE FACTOR PATHWAY**
(Extrinsic Pathway)
"Tissue Damage"

**CONTACT FACTOR PATHWAY**
(Intrinsic Pathway)

**Protein Concentrations**

<table>
<thead>
<tr>
<th>Component</th>
<th>Molecular Weight</th>
<th>Plasma Concentration μg/ml</th>
<th>Plasma Concentration μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen (I)</td>
<td>350,000</td>
<td>3000</td>
<td>9.09</td>
</tr>
<tr>
<td>Prothrombin (II)</td>
<td>72,000</td>
<td>90</td>
<td>1.388</td>
</tr>
<tr>
<td>Factor V</td>
<td>330,000</td>
<td>10</td>
<td>0.03</td>
</tr>
<tr>
<td>Factor VII</td>
<td>50,000</td>
<td>0.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>350,000</td>
<td>0.1</td>
<td>0.0003</td>
</tr>
<tr>
<td>Factor IX</td>
<td>50,000</td>
<td>5</td>
<td>0.08928</td>
</tr>
<tr>
<td>Factor X</td>
<td>58,800</td>
<td>8</td>
<td>0.13605</td>
</tr>
<tr>
<td>Factor XI</td>
<td>160,000</td>
<td>5</td>
<td>0.031</td>
</tr>
<tr>
<td>Factor XII</td>
<td>80,000</td>
<td>30</td>
<td>0.375</td>
</tr>
<tr>
<td>Factor XIII</td>
<td>320,000</td>
<td>10</td>
<td>0.3125</td>
</tr>
<tr>
<td>Protein C</td>
<td>62,000</td>
<td>4</td>
<td>0.0645</td>
</tr>
<tr>
<td>Protein Z</td>
<td>62,000</td>
<td>2.2</td>
<td>0.0355</td>
</tr>
<tr>
<td>Prekallikrein</td>
<td>86,000</td>
<td>50</td>
<td>0.5814</td>
</tr>
<tr>
<td>HK</td>
<td>110,000</td>
<td>70</td>
<td>0.6563</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>450,000</td>
<td>300</td>
<td>0.6667</td>
</tr>
<tr>
<td>Antithrombin III</td>
<td>58,000</td>
<td>290</td>
<td>5</td>
</tr>
<tr>
<td>Plasminogen</td>
<td>90,000</td>
<td>216</td>
<td>2.4</td>
</tr>
<tr>
<td>Urokinase</td>
<td>53,000</td>
<td>0.1</td>
<td>0.001887</td>
</tr>
<tr>
<td>Heparin Cofactor II</td>
<td>66,000</td>
<td>99</td>
<td>1.3636</td>
</tr>
<tr>
<td>Alpha2 Antiplasmin</td>
<td>65,000</td>
<td>60</td>
<td>0.9524</td>
</tr>
<tr>
<td>Protein C Inhibitor</td>
<td>57,000</td>
<td>4</td>
<td>0.0702</td>
</tr>
<tr>
<td>Alpha2 Macroglobulin</td>
<td>725,000</td>
<td>2100</td>
<td>2.8966</td>
</tr>
</tbody>
</table>

**FIBRINOLYSIS**

- Plasminogen
- UK, SK tPA
- PAI-1
- Plasmin
- FXDP FDP
- IIa, TAFI

**Inhibitors in black**
**Fibrinogen**
**gene mutation**
The Ideal Hemostatic Device

1. *Efficacy*
2. Biocompatibility
3. Safety
4. Pliability
5. Simplicity
6. Stability
7. Affordability
8. Scalability
9. FDA compatibility
State of the Art:
Locally-Applied Hemostatic Devices

Categories

1. Occlusion/tamponade agents
2. Scaffolding agents
3. Chitin-based agents
4. Mineral agents
5. Thrombin-based agents
6. Sealants
7. Experimental treatments

...a Growth Industry
1. Occlusion & Tamponade Agents

<table>
<thead>
<tr>
<th>Agent</th>
<th>composition</th>
<th>mechanism</th>
<th>advantages</th>
<th>disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone wax (Ethicon)</td>
<td>beeswax, paraffin</td>
<td>occlusion &amp; tamponade</td>
<td>cheap; rapid; not dependent on patient</td>
<td>nonabsorbable; inhibits union; limited to bone</td>
</tr>
<tr>
<td>Ostene (Ceremed)</td>
<td>alkylene oxide copolymer</td>
<td>occlusion &amp; tamponade</td>
<td>rapid; not dependent on patient; slowly eliminated</td>
<td>limited to bone; local effects?</td>
</tr>
</tbody>
</table>

Indication: bleeding from cut surface of bone

Sternotomies
2. Scaffolding Agents

<table>
<thead>
<tr>
<th>Agent</th>
<th>Composition</th>
<th>Mechanism</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avitene (Davol)</td>
<td>microfibrillar collagen (multiple formulations)</td>
<td>clotting scaffold; platelet activation</td>
<td>biodegradable; pH neutrality; versatility; no effect by heparin; no swelling</td>
<td>less effective with thrombocytopenia; handling issue with powder form</td>
</tr>
<tr>
<td>Helistat (Integra)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Instat (Ethicon)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GelFoam (Pharmacia &amp; Upjohn)</td>
<td>gelatin foam (hydrolyzed collagen)</td>
<td>clotting scaffold; tamponade</td>
<td>biodegradable; pH neutrality; combinable with thrombin</td>
<td>swelling brings risk of compression</td>
</tr>
<tr>
<td>Surgicel (Ethicon)</td>
<td>oxidized regenerated cellulose (wood pulp)</td>
<td>clotting scaffold</td>
<td>absorbable; pliable; versatile; antimicrobial</td>
<td>acidic degradation; possible long-term complications</td>
</tr>
</tbody>
</table>

Indication: raw surface bleeding; small vessel/orifice bleeding
### 3. Chitin-based agents

<table>
<thead>
<tr>
<th>Agent</th>
<th>composition</th>
<th>mechanism</th>
<th>advantages</th>
<th>disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>HemCon (HemCon Medical Technologies)</td>
<td>polymers of ( N )-acetyl-glucosamine (chitin, chitosan)</td>
<td>scaffolding agent; vasoconstrictor; clotting activation</td>
<td>absorbable; antimicrobial; versatile; stable; gauze-based products are more efficacious than regular gauze</td>
<td>allergy risk; relies on patient's clotting cascade; gauze-based products are nonabsorbable</td>
</tr>
<tr>
<td>Celox (Celox Medical)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>modified Rapid Deployment Hemostat, mRDH (Marine Polymer Technologies)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TraumaStat (Ore-Medix)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Indication: severe traumatic injuries; field use; elective procedures
4. Mineral agents

<table>
<thead>
<tr>
<th>Agent</th>
<th>Composition</th>
<th>Mechanism</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>QuickClot, Combat Gauze (Z-Medica)</td>
<td>mineral zeolite (microporous aluminosilicate)</td>
<td>absorbent; increases local concentration of patient's clotting factors</td>
<td>field use; simple; newer bandage forms available with prehydration</td>
<td>exothermic reaction; foreign body reaction &amp; fibrosis; relies on patient's clotting</td>
</tr>
<tr>
<td>WoundStat (TraumaCure)</td>
<td>clay (silicate) -based granules (bentonite, kaolin, smectite)</td>
<td>tamponade; sealant; scaffold</td>
<td>field use; simple; no exothermia</td>
<td>foreign body; relies on patient; *Army halted use in 2009</td>
</tr>
</tbody>
</table>

Indication: severe traumatic injuries; field use
All Army Activity Message, 17 April 2009:

"The risk inherent in WS (WoundStat) use outweighs its benefits as a back-up hemostatic agent to combat gauze.

"CG (combat gauze) remains the recommended hemostatic agent for current combat operations."

Figure 7. Embolized WS residues and associated arterial thrombosis in the lung. The hematoxylin and eosin stained tissue as seen under normal light (A) and under polarized light (B), which identifies the WS residue clearly.

J Trauma 2010; 68: 269-278
5. Thrombin agents

<table>
<thead>
<tr>
<th>Agent</th>
<th>composition</th>
<th>mechanism</th>
<th>advantages</th>
<th>disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombin JMI (King Pharmaceuticals)</td>
<td>bovine thrombin</td>
<td>activated thrombin</td>
<td></td>
<td>requires patient clotting factors; allergenic</td>
</tr>
<tr>
<td>Evithrom (Ethicon)</td>
<td>human plasma-derived thrombin</td>
<td></td>
<td>simple to use</td>
<td>requires patient clotting factors; plasma-derived</td>
</tr>
<tr>
<td>Recothrom (Zymogenetics)</td>
<td>human recombinant thrombin</td>
<td></td>
<td></td>
<td>requires patient clotting factors</td>
</tr>
<tr>
<td>FloSeal (Baxter)</td>
<td>human plasma-derived thrombin + gelatin</td>
<td>activated thrombin + scaffold</td>
<td>swelling + scaffold effect</td>
<td>requires patient clotting factors; plasma-derived; risk of swelling complications</td>
</tr>
</tbody>
</table>

Indication: treatment of raw surface ooze; low pressure/small orifice bleeding (scaffold combinations)
# 6. Sealants

<table>
<thead>
<tr>
<th>Agent</th>
<th>Composition</th>
<th>Mechanism</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>BioGlue (CryoLife)</td>
<td>glutaraldehyde-crosslinked albumin</td>
<td>wound attachment; sealant; clot scaffold</td>
<td>cross-links to tissue; rapid effect; not affected by temperature or liquid</td>
<td>risk of local nerve complications</td>
</tr>
<tr>
<td>Coseal (Baxter)</td>
<td>polyethylene glycol polymers</td>
<td>wound attachment; sealant</td>
<td>cross-links to tissue; rapid effect; minimal inflammation</td>
<td>possible swelling risk</td>
</tr>
<tr>
<td>Crosseal (Ethicon)</td>
<td>human plasma-derived fibrinogen and thrombin + plasminogen inhibitor</td>
<td>true fibrin sealant</td>
<td>not dependent on patient’s clotting</td>
<td>plasma-derived; not useful in the field nor for arterial bleeding; very little Factor XIII</td>
</tr>
<tr>
<td>Evicel (Ethicon)</td>
<td>human plasma-derived fibrinogen and thrombin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tisseel (Baxter)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitagel (Orthovita)</td>
<td>microfibrillar collagen + plasma-derived thrombin + patient’s plasma</td>
<td>scaffold; fibrin sealant; platelet gel</td>
<td>combines fibrin sealant &amp; platelet activities</td>
<td>complex preparation includes harvest of patient plasma</td>
</tr>
</tbody>
</table>

Indication: sealing of raw surface ooze and/or small orifice bleeding; tissue “welding”
### 7. Experimental Treatments

<table>
<thead>
<tr>
<th>Agent</th>
<th>composition</th>
<th>mechanism</th>
<th>advantages</th>
<th>disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Fibrin Sealant Dressing, DFSD</td>
<td>freeze dried fibrinogen + thrombin on polyglactin mesh</td>
<td>formation of human clot in an absorbable scaffold</td>
<td>little dependence on human factors; anecdotal battlefield success stories (DFSD); absorbable</td>
<td>expensive; brittle handling; plasma-derived; minimal Factor XIII activity</td>
</tr>
<tr>
<td>(American Red Cross)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tachosil (Nycomed)</td>
<td>collagen sponge + fibrinogen + thrombin</td>
<td></td>
<td>little dependence on human factors; anecdotal battlefield success stories</td>
<td>plasma-derived; minimal Factor XIII activity</td>
</tr>
<tr>
<td>Recombinant Hemostatic Devices</td>
<td>recombinant human fibrinogen, thrombin, and Factor XIII; ± polylactide mesh</td>
<td>formation of cross-linked human clot in an absorbable scaffold</td>
<td>most likely the fastest &amp; strongest clotting; no dependence on patient factors; fully recombinant; absorbable; pliable; versatile; optimizable</td>
<td>complex</td>
</tr>
<tr>
<td>(University of Nebraska)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Indication:** varied, including exsanguinating trauma in the field
8. Others

1. **Hemostase** (CryoLife): plant protein; scaffold/activation mechanism

2. **Haempatch** (Venomics): prothrombinase (Factor Xa mimetic) from snake venom in a collagen scaffold (compare to FloSeal, thrombin-soaked GelFoam)

3. **Amylopectin** powder (Hemostasis LLC)

4. **Arista AH; TraumaDex** (Medafor): microporous polysaccharide spheres
Hemostasis Research at the University of Nebraska:

Recombinant clotting factors in a variety of formulations
Why is our research “Next Generation”?

1. Recombinant source of all clotting proteins
2. Factor XIII
3. Biodegradable, nanoengineered matrix
4. Treatment can be optimized/configured

Ideal hemostatic device:

1. Efficacy
2. Biocompatibility
3. Safety
4. Pliability
5. Simplicity
6. Stability
7. Affordability
8. Scalability
9. FDA compatibility and
10. Versatility
Simplified Coagulation Cascade

RECOMBINANT VERSION:

- Some have access to this
- Everyone has access to this
- *Only NU has access to this*

1. fibrinogen (Factor I)
2. thrombin (Factor II)
3. fibrin monomer
4. fibrin polymer
5. Factor XIII (crosslinking factor)
6. fibrin clot
Expression of Human Proteins in Animal Vectors

Transgenic pigs produce functional human factor VIII in milk


Plasma Derivatives and Biopharmaceuticals Department, National Institutes of Health, Bethesda, MD 20892. *Chemical Engineering and Dyer Science Departments, Virginia Polytechnic and State University, Blacksburg, VA 24061. "Departments of Biochemistry, Genetics, and Biochemistry, George Washington University, Washington, DC 20037. *Corresponding author (e-mail: Lubon@nih.gov).

Received 24 April 1997; accepted 26 July 1997

Transgenic pigs produce functional human factor VIII in milk

1. Insert gene + mammary specific-promoter into embryo
2. Screen offspring for gene insertion
3. Harvest milk, extract/purify protein product
4. Fibrinogen, thrombin, Factors VII, VIII, IX, XIII, and more

National Geographic 1999; 196, No. 4

Nature Biotechnol 1997; 15: 971-975

Bos taurus

Sus scrofa domesticus

Pichia pastoris
Thromboelastography (TEG)

- **In vitro** performance of recombinant clotting factors
  - (clot “strength,” not adherence)

- Normal human blood
  - High [fibrinogen] + IIa + XIIIa
  - Low [fibrinogen] + IIa + XIIIa

- *Graph showing the amplitudes of different blood samples over time.*
Polylactic acid (PLA) polymer

(or gelatin, chitosan, elastin, composites, etc.)
Preclinical Studies

- Swine (~35 kg)
- Femoral arteriotomy (4-6 mm)
- Stellate liver injury, central region
- Major hepatic resection

Endpoints:

a) hemostasis
b) blood loss
c) survival
d) vessel patency
e) long-term effects
Figure 4: the swine femoral arteriotomy model.
(A) The femoral artery (FA) is exposed through a groin incision (right side shown), and controlled with vessel loops. A 4 mm arteriotomy is made with a punch instrument between the loops. A dressing (microporous PLA in this image) then is applied, finger pressure is held for 5 min, and then the site is inspected for bleeding. (B) Hemostatic result after application of microporous PLA in 2 plies, containing thrombin and recombinant Factor XIII in the inner ply, and recombinant fibrinogen in the outer ply. Doppler flow was present in the distal artery. L = lateral; M = medial; prox = proximal; dist = distal. (C) Hemostatic result after application of the macroporous ("woolly") PLA in 2 layers with the same distribution of clotting factors as in B. Doppler flow was present in the distal artery. (D) Appearance of a macroporous PLA bandage containing all 3 clotting factors post-removal from an arteriotomy site. Note formation of a prominent vessel groove (arrowheads) in the dressing material. This used bandage was quite firm to the touch.

Femoral Arteriotomy
Treatment of femoral arteriotomy with microporous PLA wet with recombinant fibrinogen, thrombin, and XIIIa

[go to video file “Vid1_microporous.mov”]
Treatment of femoral arteriotomy with macroporous PLA wet with recombinant fibrinogen, thrombin, and XIIIa
Blood-letting through femoral arteriotomy with flash of proximal clamp (MAP > 100 mm Hg)

[go to video file “Vid3_bloodletting.mov”]
Treatment of central liver injury with soluble recombinant factors (fibrinogen, thrombin, XIIIa)
treatment of central liver injury with liquid recombinant factors (fibrinogen, thrombin, XIIIa) delivered with aerosol propulsion

[go to video file “Vid4_centralinjury.mov”]
Hemostasis of major hepatic resections with recombinant clotting factors
Major liver resection treated with recombinant fibrinogen, thrombin, and XIIIa

[go to video file “Vid5_majorresection.mov”]
Quantification of efficacy:

Aerosolized clotting factors & small hepatic wedge cuts

Hemostasis obtained with [fibrinogen] < 10% of that contained in Tisseel
Application of recombinant factors with dual-reservoir aerosol gun
Small hepatic wedge excisions treated with aerosolized fibrinogen, thrombin, and XIIIa
Planned Experiments in Swine

1. Trial of our hemostatic bandage vs. competitor bandage in swine femoral arteriotomy and/or aortotomy models

2. Trial of our fibrin sealant vs. competitor fibrin sealant in a swine liver resection model (normothermic/noncoagulopathic)

3. Trial of our fibrin sealant vs. competitor fibrin sealant in a swine liver injury model (hypothermic/coagulopathic)

4. Investigational studies of recombinant factors as systemic adjunctive treatment of coagulopathic hemorrhage (e.g., treatment of liver fracture in cold/coagulopathic swine with intravenous recombinant fibrinogen)

5. Investigational studies of recombinant factors + absorbable scaffold for treatment of incompressible hemorrhagic injuries
Opinions

1. Variety of topical hemostatic agents with reasonable efficacy
2. Treatment options for exsanguinating hemorrhage in the field have improved, but still need to be better
3. Hemostatic devices composed of recombinant fibrinogen, thrombin, and Factor XIIIa have great potential