From Bench to Brain
In Situ Tissue Engineering for Brain Aneurysms

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Director, New England Center for Stroke Research

SIMI 2016 – 25th Anniversary; Buenos Aires
Disclosures

• Research Grants (last 12 months):
  – NINDS, NIBIB, NIA, NCI
  – Philips Healthcare
  – MicroVention/Terumo
  – Stryker Neurovascular
  – Codman Neurovascular
  – eV3 Neurovascular / Covidien
  – InNeuroCo Inc
  – Blockade Medical
  – CereVasc LLC
  – Gentuity
  – Cook Medical
  – Neuronal Protection Systems LLC
  – Spineology Inc
  – Silk Road
  – Wyss Institute
  – Neuravi

• Consulting (fee-per-hour, last 12 months):
  – Stryker Neurovascular
  – Codman Neurovascular

• Investment (Stocks)
  – InNeuroCo Inc
Patient-Specific Hemodynamic Analysis and Treatment Efficacy (Flow Diversion)
Flow Mechanics

Flow driven by $\Delta P$

Momentum Transfer

Fundamental Goal: Design technology that can disrupt momentum transfer into the aneurysm producing exclusion from the circulation without occluding perforators/ jailed vessels
In a Word(s)...

- BETTER – in situ tissue engineering

<table>
<thead>
<tr>
<th>Submission Deadline</th>
<th>Project Start Date</th>
<th>Project End Date</th>
<th>Campus</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/1/03</td>
<td>12/1/03</td>
<td>11/30/08</td>
<td>Medical</td>
</tr>
</tbody>
</table>

**Principal Investigator:** Baruch Barry Lieber, PhD

**Co-Principal Investigator:** Ajay Kumar Wakhloo, MD, PhD

**School:** College of Engineering

**Department:** Biomedical Engineering

**School:** Medicine

**Department:** Radiology

**Project Title:** Flow Divertors to Cure Cerebral Aneurysms

**Sponsoring Agency:** National Institutes of Health

**Joint Proposal:** % distribution between Depts/Div's:

- Radiology 50%
- Biomedical Engineering 50%

**Type of Project:** [ ] Research  [ ] Clinical Trial  [ ] Training  [ ] Construction  [ ] Other

**Type of Proposal:** [ ] New  [ ] Revision  [ ] Supplemental  [ ] Competitive Renewal  [ ] Non-Competitive Renewal

**Budget Summary** (details attached)
The Observation

*Neuroradiology 1992, AJNR 1994, 1995*
Fig. 7. Longitudinal section of a formaldehyde-fixated common carotid artery with thrombosed and organized lateral aneurysm (short arrow). The vessel (original diameter approximately 3.8 mm) was harvested 6 months after implantation of a heat-treated self-expanding nitinol stent (5-mm diameter in fully expanded state). A thin intimal fibrocellular tissue is covering the struts (curved black arrow). There is a markedly thickened vessel wall in the stented portion including the ostium of the aneurysm because of intimal proliferation. (Note the artificial reduction of the nonstented vessel segment after resection and fixation compared with the treated rigid part, curved white arrow).

B, Scanning electron photomicrograph of a carotid artery harvested 6 months after nitinol stent placement demonstrates the flow-induced macroscopic architecture of the neointima (original magnification ×20).

C, Transverse section of a common carotid artery 6 months after implantation of a nitinol stent (hematoxylin and eosin stain, original magnification ×25). I indicates intima; M, media; A, adventitia; S, empty space corresponding to stent filaments. Thickness of intima covering the filaments is approximately 80 μm and between the

Wakhloo et al. AJNR 1994
Mean Circulation: Function of FD Design

Sadasivan and Lieber, Stroke 2010
Porosity and Mesh Density

- Porosity = Mesh Density

50% Metal Coverage
- 2 pores per diamond

50% Metal Coverage
- 32 pores per diamond
Braid angle 109°

Braid angle 150°

96 wires

48 wires

0 Velocity [m/s] > 0.25

96 wires

48 wires

0 WSS [Pa] > 2

Courtesy of Matthieu De Beule, FEops
Braid angle 109°

72 wires

Braid angle 150°

96 wires

48 wires

72 wires

Courtesy of Matthieu De Beule, FEops
Mean Circulation: Function of FD Design

Fate of Perforators/ Jailed Arteries

Figure 11: Mean flow rate in the vertebral artery before and after implantation of flow divertors.

Do Engineering Models Translate to In Vivo
• Hypothesis: FDs with high/uniform pore density accelerate cell growth (formation of the neointima).
• Goal: to demonstrate formation of the basement membrane and subsequent endothelization rates after FD implant
Rabbit Aneurysm Model

Pre

Post

FU – 1 wk
Angiographic Aneurysm Occlusion at Different Time Points

<table>
<thead>
<tr>
<th>Porosity</th>
<th>Pore Density (pores/mm$^2$)</th>
<th>21 days</th>
<th>90 days</th>
<th>180 days</th>
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<tbody>
<tr>
<td>70%</td>
<td>11.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>65%</td>
<td>13.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70%</td>
<td>18.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sadasivan, Cesar, Seong, Rakian, Hao, Tio, Wakhloo, Lieber  Stroke 2009
Tissue Engineering: A Function of FD Design?
In Situ Tissue Engineering

- Canine, side-wall aneurysm – 7 days post FD implant

Porosity ~ 70%
48 wires
In Situ Tissue Engineering

Porosity ~ 70%
72 wires
The objective of this study:

- to demonstrate formation of the basement membrane and subsequent endothelialization rates after flow diverter stent implant.
Methods

*Rabbit Elastase Induced Aneurysm Model*

- 24 extracranial (innominate artery) aneurysm

  - **Efficacy:**
    - FD endothelial coverage – histology, SEM
    - Aneurysm occlusion rate – DSA, MR

  - **Safety** (complications)
    - Local: FD occlusion, stenosis

- 2 different types of FD:
  - 48-Wire Device
  - 72-Wire Device

- **Periprocedural medication** (based on literature review)
  - 10mg/kg clopidogrel and
  - 1mg/kg ASA
## Study Design

<table>
<thead>
<tr>
<th>Animal Grouping</th>
<th>Number of 72-Wire FDs</th>
<th>Number of 48-Wire FDs</th>
<th>FD Implant Procedure</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>10 (± 1) days</td>
</tr>
<tr>
<td>Group 2</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>20 (± 2) days</td>
</tr>
<tr>
<td>Group 3</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>30 (± 2) days</td>
</tr>
<tr>
<td>Group 4</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>60 (± 2) days</td>
</tr>
<tr>
<td>Totals</td>
<td>8</td>
<td>8</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>
Grouping of aneurysm was based on:
- aneurysm morphology
- Vessel diameter proximal and distal to the aneurysm
- Length of proximal segment of the vessel – landing zone!!

<table>
<thead>
<tr>
<th></th>
<th>48-wire</th>
<th>72-wire</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>aneurysm height</td>
<td>6.9 ±1.8</td>
<td>7.1 ±1.6</td>
<td>0.86</td>
</tr>
<tr>
<td>aneurysm width</td>
<td>5.5 ±2.3</td>
<td>5.0 ±1.9</td>
<td>0.64</td>
</tr>
<tr>
<td>aneurysm neck</td>
<td>5.3 ±1.9</td>
<td>4.6 ±1.4</td>
<td>0.47</td>
</tr>
<tr>
<td>aspect ratio</td>
<td>1.4 ±0.5</td>
<td>1.6 ±0.4</td>
<td>0.42</td>
</tr>
<tr>
<td>parent vessel diameter 5mm distal the aneurysm</td>
<td>4.6 ±1.0</td>
<td>4.4 ±0.6</td>
<td>0.64</td>
</tr>
</tbody>
</table>
A.) Pre-procedural DSA, frontal view

B.) Post-implant angiography, FD is not apposed at the proximal site;

C.) angioplasty

D-E.) VasoCT, distal end of FD slightly compressed (deployed into a 2.5mm vessel), part bad apposition proximally

F.) after 2 attempt of angioplasty DSA showed improved apposition (arrow-head)
A.) DSA prior FD implant shows a small neck aneurysm with a distally dilated parent-vessel
B.) After NEG implant, some contrast inflow is still present on DSA (arrow),
C.) 30 days follow up DSA indicates complete aneurysm occlusion.

Aneurysm occlusion rate in two compared FD groups

- Complete occlusion
- More than 50%
- Less than 50%
- No occlusion

48-wire: [Graph]
72-wire: [Graph]
Basement Membrane

• Important first step, forms substrate for endothelialization

**Device-1**

10-day

[Image A]

60-day

[Image C]

[Image D]

**Device-2**

Time dependent tissue coverage of flow diveters

- Device-1
- Device-2

<table>
<thead>
<tr>
<th>Time-points</th>
<th>Device-1</th>
<th>Device-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>30</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>60</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
Basement Membrane

- Important first step, forms substrate for endothelialization
<table>
<thead>
<tr>
<th>Score</th>
<th>Coverage of Struts</th>
<th>Description of Coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0%</td>
<td>No coverage</td>
</tr>
<tr>
<td>1</td>
<td>1-25%</td>
<td>Contains EPCs, inflammatory cells, red blood cells, proteins, and other components such as fibrin and collagen</td>
</tr>
<tr>
<td>2</td>
<td>26-50%</td>
<td>Contains EPCs, inflammatory cells, red blood cells, proteins, and other components such as fibrin and collagen for the beginning of the basement membrane</td>
</tr>
<tr>
<td>3</td>
<td>51-75%</td>
<td>Contains EPCs, inflammatory cells, red blood cells, proteins, and other components such as fibrin and collagen creating the basement membrane</td>
</tr>
<tr>
<td>4</td>
<td>76-99%</td>
<td>Contains EPCs and/or endothelial cells along with the components of the basement membrane</td>
</tr>
<tr>
<td>5</td>
<td>100%</td>
<td>Fully Endothelialized</td>
</tr>
</tbody>
</table>

Table 1. Scoring system for assessing the rate of flow diverter endothelialization (S-FDE)
48-Wire (Device-1): EC scores related to location ($p=0.083$)

72-Wire (Device-2): EC scores are function of time ($p=0.013$)
500x mag.
A.) center of the aneurysm neck, partial coverage of struts
B.) 2mm proximal to image A, disorganized cell network on the surface of basal membrane
C.) 5mm proximal to image A, endothelial cells are evenly distributed
Immuno-gold labeling for SEM

CD-34 antibody

CD-34 antigen

Endothelial progenitor cell

- biotin
- streptavidin
- 60nm gold particle
A.) 500x, image of the inner surface of the NEG implant, 10 days after implantation
B.) 10,000x, the immuno-gold labeling on the surface of the cell (white arrows)
C.) manually zoom of the image B for better visualization of the gold nanoparticles
Preliminary results – anti-platelet drugs activity tests and APD (anti-platelet drug) dosing strategy

- sample collection: femoral artery
- timing: prior terminal angiography
- test: clopidogrel and aspirin activity – VerifyNow (PRU-P2Y12 Reaction Units)
- data interpretation according HUMAN studies:
  - P2Y12 Reaction Units (PRU) result of ≥208 were at a significantly increased risk of cardiovascular events
  - and patients with a PRU of < 95 were receiving virtually no additional protection from cardiovascular events, but at a significantly increased risk of bleeding

<table>
<thead>
<tr>
<th></th>
<th>PRU (Clopidogrel test)</th>
<th>ARU (Aspirin Test)</th>
</tr>
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<tbody>
<tr>
<td>N=16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>results</td>
<td>102 (61-129)</td>
<td>652 (636-664)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>In-stent stenosis</th>
<th>In-stent thrombosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>results</td>
<td>0/16 (0%)</td>
<td>0/16 (0%)</td>
</tr>
</tbody>
</table>
Flow Diversion: Summary

- Evidence: curative treatment of brain aneurysms
  - Treats diseased segment of the blood vessel
  - Endoluminal reconstruction is ideal
- Engineer construct and surface properties to promote rapid endothelialization
- Need to remove dependency on dual antiplatelet medication
- Need imaging tools developed specifically for technology to ensure proper deployment
• **UMass Collaborations**
  – Marc Fisher, MD
  – Neil Aronin, MD
  – Alexei Bogdanov, PhD
  – Greg Hendricks, PhD
  – Guanping Gao, PhD
  – Miguel Esteves, PhD
  – Linda Ding, PhD
  – Srinivasan Vedantham, PhD
  – John Weaver, MD

• **Collaborations**
  – Alex Norbash, MD – BU
  – Thanh Nguyen, MD - BU
  – Italo Linfante, MD - Baptist
  – Guilherme Dabus, MD - Baptist
  – Don Ingber, PhD – Harvard
  – Netanel Korin, PhD - Technion
  – Johannes Boltze, MD, PhD – Fraunhofer Institute
  – Raul Nogueira, MD - Emory

• **NECStR**
  – Ajay Wakhloo, MD, PhD
  – Ajit Puri, MD
  – Juyu Chueh, PhD
  – Miklos Marosfoi, MD
  – Martijn van der Bom, PhD
  – Kajo van der Marel, PhD
  – Anna Kühn, MD, PhD
  – Ivan Lylyk, MD
  – Frédéric Clarençon, MD, PhD
  – Bo Hong, MD
  – Mary Howk, MS, CRC
  – Thomas Flood, MD, PhD
  – Erin Langan, BS
  – Olivia Brooks
  – Conrad Bzura, BS
  – Chris Brooks, PA
  – Mary Perras, NP
  – Shaokuan Zheng, PhD
Mean Rate of Angiographic Aneurysm Occlusion

Sadasivan, Cesar, Seong, Rakian, Hao, Tio, Wakhloo, Lieber  Stroke 2009
Histology – Progressive Occlusion – Rabbit Elastase Aneurysm Model

Amorphous clot - Organizing clot  Collagen formation and Endothelialization

21 days  90 days  180 days

Sadasivan, Cesar, Seong, Rakian, Hao, Tio, Wakhloo, Lieber  Stroke 2009
Perforators

- Large struts that cover approximately >50% of the ostium increase resistance to flow and can lead to perforator thrombosis
Perforators/ Jailed Arteries

- Model: Rabbit Aorta w/ covered Lumbar Arteries and Renal Arteries
  - Test propensity to shed emboli to kidney – both with single and double FD coverage
  - Test risk of perforator occlusion

Gounis and Wakhloo, in preparation 2015
Study Design

- 45 Animals: 5 Timepoints – 7, 28, 90, 180 and 365 days
  - Per Timepoint: 6 animals for histology, 2 animals for SEM, 1 Naïve Control
  - Antiplatelet: ASA (10mg) and Clopidogrel (10mg) 4 days prior to implant, continued for 30 days

- Endpoints:
  - Vascular Response to Implants
  - Kidney histopathology
  - Perforator (lumbar arteries) patency
Thromboembolic Events

- Kidneys bread-loafed, 1 section each from cranial, mid and caudal aspects analyzed by light microscopy for ischemic changes
- 0 ischemic events
Perforator Patency

- All lumbar arteries remained patent (angio, SEM, H&E)
Vascular Response

- Pathology report:
  - “Histomorphometric analysis showed neointimal proliferation to be negligible at all five timepoints.”
  - “Inflammation, injury, and neointimal fibrin was overall minimal to mild in the Endograft group”
  - “showed acceptable vascular healing and produced a minimal tissue response”

- “There was complete or nearly complete endothelialization and neointimal maturation at the 28-day time point.”
Step 1: Aneurysm Thrombosis

- Patient-Specific Hemodynamics is **ONE-THIRD** of Aneurysm Thrombosis
In-vitro thrombogenicity assessment of flow diversion and aneurysm bridging devices

Gaurav Girdhar¹ · Junwei Li² · Larisa Kostousov³ · John Wainwri
Wayne L. Chandler⁴
After FD implantation

Baseline

After FD implantation

Average flow (projected cm/s)

Average flow (projected cm/s)

Average flow (projected cm/s)
Apposition – Assumed!

- All models assumed device apposition to wall
- Non-binned, small FOV CE-CBCT

Flood et al JNIS 2014
Intravascular Imaging

K van der Marel, et al., JNIS
Accepted
Intravascular Imaging

K van der Marel, et al, JNIS Accepted