A Translational Platform for Unmet Needs in Peripheral Arterial Disease

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Atlanta VA Medical Center, Atlanta, USA
Pandemic of Arterial Disease

- Cardiovascular diseases are the world’s largest killers, claiming 17.1 million lives a year.
- Over 80% of cardiovascular disease deaths take place in low-and middle-income countries and occur almost equally in men and women.

Allan Callow MD, PhD

http://www.who.int/cardiovascular_diseases/en/
Peripheral Arterial Disease

• What is it?
  • PAD is defined as the narrowing of arteries outside the brain and heart (neck to feet)
  • Spectrum of Disease
    – Asymptomatic
    – Symptomatic
      » Claudication
      » Critical Limb Ischemia
        • Rest pain
        • Tissue loss

• Why is it Important?
  – Affects 12-20% of the population over 65.
  – Doubles Cardiovascular mortality and event rate
# Ankle (or Toe) Brachial Index

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Intermittent Claudication</th>
<th>Rest Pain</th>
<th>Impending Gangrene</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of limbs</td>
<td>50</td>
<td>213</td>
<td>77</td>
<td>36</td>
</tr>
<tr>
<td>Mean</td>
<td>1.11</td>
<td>0.59</td>
<td>0.26</td>
<td>0.05</td>
</tr>
<tr>
<td>S.D.</td>
<td>0.10</td>
<td>0.15</td>
<td>0.13</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Highly significant (better than 0.01 percent)

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James T. Yao, MD, PhD
Critical Limb Ischemia

- Between 1-10% of patients progress to critical limb ischemia annually
  - most common in patients who smoke or have diabetes
- 120,000 amputations annually in US
- Amputation is common
  - Between 1/3 and ½ of CLI patients do not have surgical options
  - Non-operatively managed patients
    - 6 months 50% death or amputation
- There is a need for alternative limb salvage therapies
  - Preserving targets
  - Vascular and tissue regeneration
# Chronic Limb Ischemia

<table>
<thead>
<tr>
<th>Fontaine</th>
<th>Rutherford</th>
<th>Grade</th>
<th>Category</th>
<th>Clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage</td>
<td>Clinical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Asymptomatic</td>
<td>0</td>
<td>0</td>
<td>Asymptomatic</td>
</tr>
<tr>
<td>IIa</td>
<td>Mild claudication</td>
<td>I</td>
<td>1</td>
<td>Mild claudication</td>
</tr>
<tr>
<td>IIb</td>
<td>Moderate to severe claudication</td>
<td>I</td>
<td>2</td>
<td>Moderate claudication</td>
</tr>
<tr>
<td>III</td>
<td>Ischemic rest pain</td>
<td>II</td>
<td>4</td>
<td>Ischemic rest pain</td>
</tr>
<tr>
<td>IV</td>
<td>Ulceration or gangrene</td>
<td>III</td>
<td>5</td>
<td>Minor tissue loss</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III</td>
<td>6</td>
<td>Major tissue loss</td>
</tr>
</tbody>
</table>
PAD is a Disease of Aging

- Diabetic (and Dialysis) patients have much earlier age of onset

Are Your Arteries Older Than Your Age?


Department of Surgery, Loyola University Medical Center, Maywood, IL 60153-3304, USA
Demographics of Persons 65+

Population 65+ by Age: 1900-2050

Number of Persons 65+

Administration on Aging, Profile of Older Americans: 2008
Veteran Demographics (>65)

• 40% of Veteran Population 65 or older
• 2,272,000 Living WW II Veterans
• 850 WW II Veterans Pass Away Per Day
• >931,000 Veterans over age 85
Patients are also Living Longer...With Cardiovascular Disease
Exercise is an Important Part of PAD Therapy

- Peak Walking Time (PWT)
- Mean time to claudication (COT)

Improved with both Revascularization and walking

Supervised Exercise, Stent Revascularization, or Medical Therapy for Claudication Due to Aortoiliac Peripheral Artery Disease

The CLEVER Study

Timothy P. Murphy, MD,* Donald E. Cutlip, MD,†† Judith G. Regensteiner, PhD,‡‡ Emile R. Mohler III, MD,∥
David J. Cohen, MD, MSc,¶ Matthew R. Reynolds, MD,¶ Joseph M. Massaro, PhD,¶# Beth A. Lewis, PhD,**
Joselyn Cerezo, MD,* Niki C. Oldenburg, DnPH,‡ Claudia C. Thum, MA,‡ Michael R. Jaff, DO,††
Anthony J. Comerota, MD,§§ Michael W. Steffes, MD,†† Ingrid H. Abrahamsen, MS,∥ Suzanne Goldberg, MSN,∥∥
Alan T. Hirsch, MD††

Stenosis and Circulation

- Arterial stenoses are a part of a complex hemodynamic circuit
  - Compensatory dilation of resistance vessels in peripheral bed
  - Development of collaterals
    - Increased pressure gradient across the midzone bed
    - Likely mediated by NO

It would take 10000 collaterals with a diameter of 1.0 mm to reduce the segmental resistance to that of a major vessel with a diameter of 10mm
Hemodynamics of Multilevel Obstruction

Exercise increases blood flow to thigh, reduces flow to calf and decreases peripheral blood pressure
Revascularization is the Gold Standard, but it doesn’t Cure everyone

Open versus Endovascular Intervention for Critical Limb Ischemia: A Population-Based Study

David L Cull, MD, FACS, Eugene M Langan, MD, FACS, Bruce H Gray, DO, Brent Johnson, MS, Spence M Taylor, MD, FACS

CONCLUSIONS: Although there has been an absolute increase in the number of revascularization procedures for CLI, with a clear shift toward endovascular therapy, the amputation rates for these patients have not changed. However, the shift to endovascular interventions has increased the number of secondary procedures required to maintain limb-salvage rates equivalent to those of the pre-endovascular era. (J Am Coll Surg 2010;210:555–563. © 2010 by the American College of Surgeons)

Comparison of Interventional Outcomes According to Preoperative Indication: A Single Center Analysis of 2,240 Limb Revascularizations

Spence M Taylor, MD, FACS, David L Cull, MD, FACS, Corey A Kalbaugh, MS, Herman F Senter, PhD, Eugene M Langan III, MD, FACS, Christopher G Carsten III, MD, FACS, John W York, MD, FACS, Bruce A Snyder, MD, FACS, Bruce H Gray, DO, Mark P Androes, MD, FACS, Dawn W Blackhurst, DnPH

CONCLUSIONS: There is a declining spectrum of outcomes performance from claudication to rest pain to tissue loss. These findings question the accuracy of all previously published data for critical limb ischemia, for which rest pain and tissue loss are usually blended and reported as a single outcomes value. (J Am Coll Surg 2009;208:770–780. © 2009 by the American College of Surgeons)
The Persistent Problem

- Restenosis is common
  - Femoral angioplasty 25 - 40%
  - Femoral-popliteal bypass
    - prosthetic grafts 40-65%
    - vein grafts 30-50%

- Failure is the norm
End Organ Disease

Clinical Specimen of Patient with Critical Limb Ischemia (CLI)
Results: No significant differences in demographic, preoperative, or anesthetic variables were found between the matched, high-risk amputation or bypass groups (792 and 780 patients, respectively). **Bypass was associated with a lower 30-day postoperative mortality than amputation (6.54% vs 9.97%; \( P = .0147 \)).** Amputation was associated with higher rates of pulmonary embolism (0.9% vs 0% for amputation vs bypass groups, respectively; \( P = .009 \)) and urinary tract infection (5.2% vs 2.7%; \( P = .01 \)), while bypass was associated with higher rates of return to the operating room (14.1% vs 27.6%; \( P < .001 \)) and a trend toward higher postoperative transfusion requirements (0.9% vs 2.1%; \( P = .054 \)). The postoperative time to discharge did not differ between the two groups.

Infrainguinal bypass is associated with lower perioperative mortality than major amputation in high-risk surgical candidates


**FIGURE 1.** Forest plot representing crude and adjusted odds ratios for mortality based on propensity score modeling
Research Platform

• **Tissue Research objective:**
  • To promote regeneration and function in ischemic limbs

• **Clinical Goal is to Limit:**
  – Limit Major Amputation
  – Promote Functional Recovery

• **Funding:**
  – AHA IRG; DOD/OPORP
  – Departmental Support
  – Seed grants
MSC Therapy to Direct Angiogenic Response to Limb Ischemia

1. Arteriogram of patient demonstrating proximal arterial flow and collateralization developing from proximal to distal, and deep to superficial.

2. Stimulating or delivering MSCs to integrate cellular networks (below MSCs in fibrin gel) distally and superficially may extend collateral network and limit tissue ischemia.

3. Improved perfusion through angiogenic extension of collateral vessels via MSCs.
Current Limitations to Translation

- Therapeutic Benefit of Cell Therapy in Phase 3 Trials is still lacking

- Mesenchymal Stem Cells Hold Promise

<table>
<thead>
<tr>
<th>Lead Author</th>
<th>Fresh vs. thawed cells</th>
<th>% Diabetes Sample Size (Treatment / Control)</th>
<th>Helpful for:</th>
<th>Trial characteristics</th>
<th>Total cells Mode of Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Dash</td>
<td>Fresh cells</td>
<td>25%/25% 12/12</td>
<td>Pain free walking distance;</td>
<td>Not blinded; not placebo-controlled</td>
<td>&gt;1 x10⁶/cm² IM + topical</td>
</tr>
<tr>
<td>2. Lu</td>
<td>Fresh cells</td>
<td>100%/100% 20 Limbs/41 Limbs</td>
<td>Amputation; ulcer healing; ? improved perfusion</td>
<td>Double blind; 5-10% lost to follow up; Placebo control was ipsilateral limb *0.1 gain in ABI within error of the test</td>
<td>9.6 x 10⁸ IM 3 weeks to expand cells</td>
</tr>
<tr>
<td>3. Debin</td>
<td>Fresh cells</td>
<td>100%/100% 22/23</td>
<td>Amputation ? perfusion</td>
<td>Not blinded; no placebo *10% dropout from clinical worsening or failure of MSC growth</td>
<td>7.3-56 x 10⁸ IM+ subcutaneous</td>
</tr>
</tbody>
</table>

- But Should we Get them from the Patient, the Young... or The Wise
Rejuvenation and Expansion of Mesenchymal Stem Cells from Amputated Limbs

Not Dead Yet
Healthy

Ischemic

Ischemic + Diabetic

Fold Change over Isotype

Healthy

Ischemic

Ischemic + Diabetic

Fold Change over Isotype

CD34 CD45 CD73 CD105 CD90 HLA-I

FBS

fdPL

Fold Change over Isotype

CD34 CD45 CD73 CD105 CD90 HLA-I

FBS

fdPL

Fold Change over Isotype

CD34 CD45 CD73 CD105 CD90 HLA-I

FBS

fdPL

Fold Change over Isotype

CD34 CD45 CD73 CD105 CD90 HLA-I

FBS

fdPL
Comparing Human Platelet Lysate (2%) with Fetal Bovine Serum (5%) On Human MSC Doubling time
Table I. Bone marrow mesenchymal stem cell (MSC) donor characteristics\textsuperscript{a}

<table>
<thead>
<tr>
<th>Patient groups</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>22-year-old female</td>
<td>26-year-old male</td>
<td>34-year-old male</td>
<td>28-year-old male</td>
</tr>
<tr>
<td></td>
<td>Nonsmoker</td>
<td>Nonsmoker</td>
<td>Nonsmoker</td>
<td>Nonsmoker</td>
</tr>
<tr>
<td>ISC</td>
<td>76-year-old male with CLI.</td>
<td>46-year-old male with CLI.</td>
<td>81-year-old female with CLI.</td>
<td>85-year-old female with CLI.</td>
</tr>
<tr>
<td></td>
<td>Comorbidities: hypertension,</td>
<td>Comorbidities: Hypertension,</td>
<td>Comorbidities: Hypertension,</td>
<td>Comorbidities: hypertension,</td>
</tr>
<tr>
<td></td>
<td>atrial fibrillation, dyslipidemia.</td>
<td>chronic obstructive pulmonary disease, obesity.</td>
<td>chronic obstructive pulmonary disease, obesity.</td>
<td>congestive heart failure,</td>
</tr>
<tr>
<td></td>
<td>Smoker</td>
<td>Smoker</td>
<td>Smoker</td>
<td>coronary artery disease.</td>
</tr>
<tr>
<td>ISC+DM</td>
<td>85-year-old diabetic female</td>
<td>67-year-old diabetic female</td>
<td>69-year-old diabetic male with</td>
<td>81-year-old diabetic male with</td>
</tr>
<tr>
<td></td>
<td>with CLI.</td>
<td>with CLI.</td>
<td>with CLI.</td>
<td>with CLI.</td>
</tr>
<tr>
<td></td>
<td>Comorbidities: hypertension,</td>
<td>Comorbidities: Hypertension,</td>
<td>Comorbidities: Hypertension,</td>
<td>Comorbidities: hypertension,</td>
</tr>
<tr>
<td></td>
<td>prior stroke, prior distal leg bypass.</td>
<td>chronic obstructive pulmonary disease, congestive heart failure, dyslipidemia.</td>
<td>chronic obstructive pulmonary disease, congestive heart failure, dyslipidemia.</td>
<td>congestive heart failure,</td>
</tr>
<tr>
<td></td>
<td>Nonsmoker</td>
<td>Nonsmoker</td>
<td>Nonsmoker</td>
<td>dyslipidemia.</td>
</tr>
</tbody>
</table>

\textit{CLI}, Critical limb ischemia; \textit{DM}, diabetes mellitus; \textit{ISC}, ischemia.

\textsuperscript{a}Four persons were included in each group. Groups were labeled as healthy, ISC, or ISC+DM. Bone marrow from healthy donors was harvested from the iliac crest. Bone marrow from ISC and ISC+DM donors was harvested from their tibia after major amputation. Ages, sex, comorbidities, and smoking status are included. Of note, only 2 of 12 patients were active smokers at time of harvest, and both were in the ISC group.

Table II. Estimated fold expansion benefits of platelet lysate (PL) over fetal bovine serum (FBS)\textsuperscript{a}

<table>
<thead>
<tr>
<th>PL vs FBS</th>
<th>Healthy</th>
<th>ISC</th>
<th>ISC+DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL</td>
<td>134 million-fold expansion</td>
<td>4 million-fold expansion</td>
<td>500,000-fold expansion</td>
</tr>
<tr>
<td>FBS</td>
<td>65,000-fold expansion</td>
<td>2000-fold expansion</td>
<td>2000-fold expansion</td>
</tr>
</tbody>
</table>

\textit{ISC}, Ischemia; \textit{ISC+DM}, ischemia and diabetes mellitus.

\textsuperscript{a}Based on the calculated cumulative population doubling times for PL and FBS, we could estimate that for a similar culture duration time, culturing healthy mesenchymal stem cells (MSCs) in PL would allow for a 134-million fold expansion, whereas the fold expansion for FBS would only be 65,000. MSCs from ISC and ISC+DM donors have reduced expansion potential compared with MSCs from healthy donors, but can still yield clinically relevant cell numbers that are superior with PL compared with FBS.
MSCs Provide Stromal Support for Endothelial Cells
Summary 1

Expansion and angiogenic potential of mesenchymal stem cells from patients with critical limb ischemia

Luke Brewster, MD, PhD, a,b,c Scott Robinson, MD, PhD, a Ruoya Wang, PhD, a Sarah Griffiths, PhD, c Haiyan Li, MD, a Alexandra Peister, PhD, d Ian Copland, PhD, c,e,f and Todd McDevitt, PhD, c,f Atlanta, Ga

• The MSCs from these patients are usable (Angiogenic)
• and can be cultured to sufficient numbers for cellular therapy
Current Limitations to Translation

- Therapeutic Benefit of Cell Therapy in Phase 3 Trials is still lacking
- Cell Survival after Transplantation is Awful
  - 1-10% after 1 hour after IM
  - Less after vascular delivery
- Fertile Grounds for Bioengineering Solutions
A novel platelet lysate hydrogel for endothelial cell and mesenchymal stem cell-directed neovascularization

Scott T. Robinson a, Alison M. Douglas b, Tatiana Chadid a, Katie Kuo a, Ajai Rajabalan a, Haiyan Li a, Ian B. Copland c, Thomas H. Barker b, Jacques Galipeau c, Luke P. Brewster a,b,d,*

a Emory University, Department of Surgery, Atlanta, GA 30322, USA
b Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA 30322, USA
c Emory University, Department of Hematology and Oncology, Winship Cancer Institute, Atlanta, GA 30322, USA
d Atlanta Veterans Affairs Medical Center, Surgical and Research Services, Decatur, GA 30030, USA

Fig. 1. PL hydrogels self-assemble with thrombin activation. (A) Fibrinogen rich platelet lysate (PL) was generated by exposing human to sequential rounds of freeze-thaw cycles with a rapid warming phase. Immediately prior to hydrogel formation, frozen PL aliquots were rapidly warmed to 37°C, centrifuged and filtered. The addition of thrombin led to self-assembly of 3D hydrogels. (B) Alexa Fluor 488 conjugated fibrinogen was incorporated into 50% PL and fibrin hydrogels (5% labeled fibrinogen by weight) and imaged with confocal microscopy. Representative maximum intensity projections from a 10 μm stack are shown for each condition at 63X. Scale bars = 20 μm. (C) Scanning electron microscopy performed on 50% PL and 1.0 mg/mL and 2.5 mg/mL fibrin hydrogels. Representative images are shown for each condition at 20,000X. Scale bar = 1 μm. Despite a fibrin concentration of ~0.250 mg/mL, PL gel has an intermediary appearance between the 1 and 2.5 mg/mL fibrin gels in both confocal and electron microscopy.
Summary 2

• PL gel provides a rich nutritive environment for MSCs that may be exploited as a cell delivery vehicle
But Mice Aren’t People
And People Have Clinical Problems
Large Animal Models May Answer The Questions Humans Cannot
Current Steps

• In vivo MSC Delivery
  – Porcine MSCs into gastrocnemius muscle in pig model of CLI

• Quantify
  – MSC retention
  – MSC effect of perfusion
  – MSC effect on myopathy
Research Team

- Brewster laboratory:
  - Past:
    - Roy Wang
    - Scott Robinson
  - Current:
    - Haiyan Li
    - Katie Kuo
    - Tatiana Chadid Santamaria
    - Summer students: Mason Griffin

- Ian Copland and Jacques Galipeau
  - PL and FACS and IND preparation

- Todd McDevitt and Sarah Griffiths at GTEC
  - Secretome ELISA
  - Spheroid culture system

- Alex Peister and her lab at Morehouse
  - Human MSC differentiation

- David Lefer: LSU
  - David Polhemus
  - Traci Goodchild

- Bob Taylor and his laboratory
  - Laser Doppler

- Iraklis Pipinos: University of Nebraska

- Division of Vascular Surgery
  - Emory University Hospital
  - Atlanta VA Medical Center
  - My partners and patients

- Parker H. Petit IBB @ GTEC

- Walter Reed Medical Center
  - Major Kyle Potter MD and Katie Cilwa (contractor)
Gratitude

1) Emory University
   Dean Larsen and BME
2) Emory Department of Surgery
   Dr. John Sweeney
3) Emory Division of Vascular Surgery and Endovascular Therapy
   Faculty at Emory University Hospital, Atlanta VA, CHOA, Grady Memorial, and St. Joseph’s Hospital
4) Funding:
   REM: Supported in part by PHS Grant UL1TR000454 from the Clinical and Translational Science Award Program, National Institutes of Health, National Center for Advancing Translational Sciences
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   DOD; OPORP