Measuring Lactate to Evaluate Ex-vivo Donor Heart Functionality

BMEN 5151
Intro to BioMEMs
Spring 2018
Heart Failure Prevalence

Projected US Heart Failure Prevalence and Direct Cost

Nationally

Leading cause of death in the US.
Over 600,000 associated deaths/year.

Globally

Impacts 15 million people across Europe.
Affects nearly 26 million people worldwide.

Photo courtesy of: Konstam, Marvin A. Circulation 125.6 (2012): 820-827.
A Patient’s Path to a Heart Transplant

1. Diagnosis
2. Diet & Lifestyle Changes
3. Medication
4. Electrical Therapies
   - Defibrillators
5. Circulatory Assist Devices
   - Left Ventricular Assist Devices (LVADs)
6. Heart Transplant
Heart Transplantation

- Waiting list exceeds the number of available hearts for donation
  - 49 heart/lung transplant candidates on the waitlist as of April 12, 2018
- Explanted organs quality and function can decrease, preventing transplantation

\[
\begin{array}{l}
\text{Table 6.1(i) Deceased donors / hearts in Eurotransplant from 2012 to 2016} \\
\hline
\begin{array}{l}
\text{Donors} \\
\text{All donors reported} \\
\text{Non-heart donors} \\
\text{Heart donors reported} \\
\text{Heart donors not used} \\
\text{Total heart donors used} \\
\end{array}
\begin{array}{l}
\text{2012} \\
2421 \\
1515 \\
906 \\
299 \\
607 \\
\end{array}
\begin{array}{l}
\text{2013} \\
2302 \\
1404 \\
898 \\
309 \\
589 \\
\end{array}
\begin{array}{l}
\text{2014} \\
2299 \\
1367 \\
932 \\
298 \\
634 \\
\end{array}
\begin{array}{l}
\text{2015} \\
2317 \\
1431 \\
886 \\
281 \\
605 \\
\end{array}
\begin{array}{l}
\text{2016} \\
2306 \\
1428 \\
878 \\
291 \\
587 \\
\end{array}
\begin{array}{l}
\text{2015/2016} \\
-0.8\% \\
-0.8\% \\
-0.9\% \\
0.8\% \\
-3.1\% \\
\end{array}
\end{array}
\hline
\begin{array}{l}
\text{Hearts} \\
\text{Reported} \\
\text{Offered} \\
\text{Accepted} \\
\text{Transplanted} \\
\end{array}
\begin{array}{l}
\text{2012} \\
906 \\
901 \\
708 \\
607 \\
\end{array}
\begin{array}{l}
\text{2013} \\
898 \\
895 \\
685 \\
589 \\
\end{array}
\begin{array}{l}
\text{2014} \\
932 \\
925 \\
738 \\
634 \\
\end{array}
\begin{array}{l}
\text{2015} \\
886 \\
882 \\
690 \\
605 \\
\end{array}
\begin{array}{l}
\text{2016} \\
878 \\
876 \\
699 \\
587 \\
\end{array}
\begin{array}{l}
\text{2015/2016} \\
-0.9\% \\
-0.7\% \\
1.3\% \\
-3.1\% \\
\end{array}
\end{array}
\]

1. Eurotransplant, 2016 Annual Report
2. Eurotransplant, 2010 Annual Report
OCS Heart

- Portable heart perfusion system
  - Pumps oxygenated, nutrient-enriched blood through the heart to preserve and monitor organ function
OCS Heart

Advantages of OCS Heart

- Increases number of hearts available for transplants.
- Improves a potential transplant range.
- Substantially reduces cold ischemic time.
- Provides ex-vivo evaluation of physiologic function.
- Pre-transplant assessment tool for the prediction of post-transplant outcome.
- Decreases the risk for primary allograft failure.
- Leads to higher postoperative survival rates.
Current Measure of Heart Functionality

- Abbott’s blood gas analyzer: i-STAT system

- Limitations
  - Requires manual collection of blood into syringe, onto cartridge, and transfer to external machine
  - Incremental, not continuous
  - Collection of extraneous blood gas information
Importance of Blood Lactate

- Markers established to monitor tissue perfusion
- Lactate level correlates with mortality in extracorporeal systems
- Lactate Inflection Point (LIP): blood lactate exponentially increases

If a *rise in lactate* can be detected, factors responsible for inadequate perfusion may be identified and *function can be better assessed.*


https://metsperformance.com/blog/maximising-functional-capacity-delay-your-lactate-inflection-point
Proposed Technology

Incorporated continuous blood lactate analyzer

- Located on oxygenated and deoxygenated sides
- Blood lactate sensor
  - Measure lactate production/use in perfused heart
  - Enzymatic amperometric sensors
- Membrane-based bioMEM
  - Flexible

The Annals of Thoracic Surgery 2014 98, 2099-2106 DOI: (10.1016/j.athoracsur.2014.06.098)
Current Lactate Biosensors

- **Blood sampling**
  - Oxygen content during disease
  - Understanding anaerobic/aerobic exercise
  - High altitude exercise

- **Wearable sweat monitors**
  - Continuous monitoring
  - Flexible, thin

http://sportscience.sportsci.org/2009/ss.htm

https://www.bsxinsight.com/
## Variety of Potential Enzymes

<table>
<thead>
<tr>
<th>Enzyme; origin; amount used</th>
<th>Immobilization matrix; Working electrode material</th>
<th>Type of transducer; immobilization method</th>
<th>Sensitivity</th>
<th>Detection limit</th>
<th>Linearity</th>
<th>Applications/inhibitors</th>
<th>Response time (s)</th>
<th>Storage stability (days)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-LDH (no EC given); rabbit muscle; 5/ 10/20U</td>
<td>CA membrane/Meldola blue, NADP; carbon, screen printing ink electrode</td>
<td>Amperometric; NR</td>
<td>24.38 nM mmol⁻¹ L⁻¹</td>
<td>0.5–8 mmol L⁻¹</td>
<td>1–20 mmol L⁻¹</td>
<td>NR; ascorbate, pyruvate</td>
<td>NR</td>
<td>3</td>
<td>[73]</td>
</tr>
<tr>
<td>LOD (EC 1.1.3.2); Pedicoccus sp.; 0.15U</td>
<td>Polypropylene (PPyox) membrane; Pt electrode</td>
<td>Amperometric; entrapment</td>
<td>72 ± 0.1 mA Mmol⁻¹ L⁻¹</td>
<td>NR</td>
<td>NR</td>
<td>Tomato juice; aceta-minginen, ascorbate, urate</td>
<td>NR</td>
<td>60</td>
<td>[75]</td>
</tr>
<tr>
<td>LOD (no EC given); Aerococcus viridans; 22U/mL</td>
<td>Semi-permeable membrane using a SIRE technology; Pt electrode</td>
<td>Amperometric; NR</td>
<td>0.05 mmol L⁻¹</td>
<td>0–0.1 mmol L⁻¹</td>
<td>NR</td>
<td>Tomato paste, baby food</td>
<td>NR</td>
<td>70</td>
<td>[76]</td>
</tr>
<tr>
<td>L-LDH (EC 1.1.3.7); rabbit muscle; 3.34U/mL; pyruvate oxidase (EC 1.2.3.2); Aerococcus viridans; 1.40 U mL⁻¹; salicylate hydroxylase (EC 1.14.13.1); Pseudomonas sp; 0.05 U mg⁻¹</td>
<td>Poly(carboxymethyl) sulfamate (PCS) hydrogel on a Teflon membrane/Pt</td>
<td>Amperometric; NR</td>
<td>3.05–270.35 mA mmol⁻¹ L⁻¹</td>
<td>4.3 mmol L⁻¹</td>
<td>0–400 μM</td>
<td>Healthy supplements, soda, sport drinks, yo-gurt milk, electroactive substances</td>
<td>2</td>
<td>11</td>
<td>[78]</td>
</tr>
<tr>
<td>LOD (no Ec given); Pedicoccus species 20–40 units/mg</td>
<td>Poly/DVB/EVB-coated composite membranes; Au coated polymer support</td>
<td>Amperometric; cross-linking with glutaraldehyde</td>
<td>FIA/amperometric</td>
<td>NR</td>
<td>NR</td>
<td>Blood samples; NR</td>
<td>1</td>
<td>21</td>
<td>[72]</td>
</tr>
<tr>
<td>LDH (EC 1.1.3.7); Porcine heart; 257 U/mg</td>
<td>Amucil/albioniin hydrogel matrix</td>
<td>Amperometric; NR</td>
<td>0.537 ± 0.007 μM M⁻¹</td>
<td>0.8 μM</td>
<td>2–1000 μM</td>
<td>Blood sample; NR</td>
<td>150</td>
<td>[70]</td>
<td></td>
</tr>
<tr>
<td>LOD (no EC given); NADH</td>
<td>Mesoporous silica/screen-printed Prussian Blue (PB)/ hydrophilic porous membrane; NADH</td>
<td>Electrochemical;</td>
<td>150–11 mM</td>
<td>NR</td>
<td>NR</td>
<td></td>
<td>NR</td>
<td>NR</td>
<td>[71]</td>
</tr>
<tr>
<td>LOD (no EC given); HRP (no EC given);</td>
<td>CNT/polyaniline membrane; screen printed electrode</td>
<td>Amperometric; NR</td>
<td>0.05 mg L⁻¹</td>
<td>0.1–5 mg L⁻¹</td>
<td>NR</td>
<td>Wine and beer samples; NR</td>
<td>NR</td>
<td>NR</td>
<td>[74]</td>
</tr>
</tbody>
</table>

CA: Cellulose acetate.  
Pt: Platinum.  
SRE: Sensors based on injection of the recognition element.  
DVB: Divinylbenzene.  
EVB: Ethyvinylbenzene.  
MBRS-SPE: Meldola’s Blue-Reinkecke salt- screen-printed carbon electrode.  
CNT: Carbon nano tube.  
NR: Not reported.

Design
Enzymatic Amperometric Lactate Sensor

- Membrane based enzyme biosensor
- 2 electrode system
  - Working and reference
- Electrodes placed proximal to PA and distal to the Aorta
- Electrochemical transducer to convert Lactate levels into current output

How to machine

Simple 5 step fabrication process

- Electron-beam deposition
- Photolithography and Wet-etching
- Second photolithography
- Deposition of protective Membrane
- Enzyme deposition

# Safety

<table>
<thead>
<tr>
<th>Device Categories</th>
<th>Biological Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body Contact</strong></td>
<td>Contact Duration</td>
</tr>
<tr>
<td></td>
<td>A = Limited (≤24 Hours)</td>
</tr>
<tr>
<td></td>
<td>B = Prolonged (24 Hours – 30 Days)</td>
</tr>
<tr>
<td></td>
<td>C = Permanent (&gt;30 Days)</td>
</tr>
<tr>
<td><strong>Surface Devices</strong></td>
<td>Cytotoxicity</td>
</tr>
<tr>
<td>Skin</td>
<td>x</td>
</tr>
<tr>
<td>B</td>
<td>x</td>
</tr>
<tr>
<td>C</td>
<td>x</td>
</tr>
<tr>
<td><strong>Mucosal Membrane</strong></td>
<td>Sensitization</td>
</tr>
<tr>
<td>A</td>
<td>x</td>
</tr>
<tr>
<td>B</td>
<td>x</td>
</tr>
<tr>
<td>C</td>
<td>x</td>
</tr>
<tr>
<td><strong>Breached or Compromised Surfaces</strong></td>
<td>Acute Systemic Toxicity</td>
</tr>
<tr>
<td>A</td>
<td>x</td>
</tr>
<tr>
<td>B</td>
<td>x</td>
</tr>
<tr>
<td>C</td>
<td>x</td>
</tr>
<tr>
<td><strong>Blood Path, Indirect</strong></td>
<td>Genotoxicity</td>
</tr>
<tr>
<td>A</td>
<td>x</td>
</tr>
<tr>
<td>B</td>
<td>x</td>
</tr>
<tr>
<td>C</td>
<td>x</td>
</tr>
<tr>
<td><strong>Externally Communicating Devices</strong></td>
<td>Implantation</td>
</tr>
<tr>
<td><strong>Tissue/Bone/ Dentin</strong></td>
<td>Hemocompatibility</td>
</tr>
<tr>
<td>A</td>
<td>x</td>
</tr>
<tr>
<td>B</td>
<td>x</td>
</tr>
<tr>
<td>C</td>
<td>x</td>
</tr>
<tr>
<td><strong>Circulating Blood</strong></td>
<td>Genotoxicity</td>
</tr>
<tr>
<td>B</td>
<td>x</td>
</tr>
<tr>
<td>C</td>
<td>x</td>
</tr>
<tr>
<td><strong>Implant Devices</strong></td>
<td>Hemocompatibility</td>
</tr>
<tr>
<td><strong>Tissue/Bone</strong></td>
<td>x</td>
</tr>
<tr>
<td>A</td>
<td>x</td>
</tr>
<tr>
<td>B</td>
<td>x</td>
</tr>
<tr>
<td>C</td>
<td>x</td>
</tr>
<tr>
<td><strong>Blood</strong></td>
<td>x</td>
</tr>
<tr>
<td>A</td>
<td>x</td>
</tr>
<tr>
<td>B</td>
<td>x</td>
</tr>
<tr>
<td>C</td>
<td>x</td>
</tr>
</tbody>
</table>

### Impact

<table>
<thead>
<tr>
<th>Improved Patient Safety</th>
<th>Quality Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>● Continuous monitoring of function</td>
<td>● Automated sensing procedure</td>
</tr>
<tr>
<td>● Better indications for viability and mortality</td>
<td>● No need for manual sampling</td>
</tr>
<tr>
<td>● Potential for immediate intervention</td>
<td>● Finer control over lactate measurements</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Membrane-Based Enzyme Sensing</th>
<th>Enhanced Sensor Capability</th>
</tr>
</thead>
<tbody>
<tr>
<td>● Highly selective to analyte</td>
<td>● Direct enzymatic immobilization</td>
</tr>
<tr>
<td>● Sensitive and accurate signal</td>
<td>● Prevents loss of enzyme</td>
</tr>
<tr>
<td>● Inexpensive and disposable</td>
<td>● Reduces enzymatic response time</td>
</tr>
</tbody>
</table>
Limitations

Fabrication

Reproducing enzyme layer

L-LDH stability of about 11 days

Interfacing with existing tubing

Functional

Single-use device (blood-contacting)

Accuracy of calibration
Thank you!
Questions?
References

17. Xuesong Yang., et al. "Lactate Sensors on Flexible Substrates" MDPI: Biosensors, 21 September 2016, 6, 48