New concepts in Remotely-Powered Telemetry of the Human Metabolism
Different outcomes for different patients

<table>
<thead>
<tr>
<th>Therapeutic area</th>
<th>Rate of efficacy with standard drug treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer (all types)</td>
<td>25%</td>
</tr>
<tr>
<td>Alzheimer's disease</td>
<td>30%</td>
</tr>
<tr>
<td>Incontinence</td>
<td>40%</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>47%</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>48%</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>50%</td>
</tr>
<tr>
<td>Migraine (prophylaxis)</td>
<td>50%</td>
</tr>
<tr>
<td>Migraine (acute)</td>
<td>52%</td>
</tr>
<tr>
<td>Diabetes</td>
<td>57%</td>
</tr>
<tr>
<td>Asthma</td>
<td>60%</td>
</tr>
<tr>
<td>Cardiac arrhythmias</td>
<td>60%</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>60%</td>
</tr>
<tr>
<td>Depression</td>
<td>62%</td>
</tr>
</tbody>
</table>

For depression, the data apply specifically to the drug class known as selective serotonin reuptake inhibitors.

The Development of new Implantable Medical Devices is a key-factor for succeeding in Personalized therapy.

- **Drug/marker detection**
- **Drug dispensing**
- **Data Analysis**

Glucose by Menarini

Insulin by Medtronic
## New development for the Nano-Bio-Sensors

<table>
<thead>
<tr>
<th>Probe Enzymes</th>
<th>Endogenous metabolites</th>
<th>Exogenous metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose Oxidase</td>
<td>Glucose</td>
<td></td>
</tr>
<tr>
<td>Lactate Oxidase</td>
<td>ATP</td>
<td></td>
</tr>
<tr>
<td>Glutamate Oxidase</td>
<td>Lactate</td>
<td></td>
</tr>
<tr>
<td>P450 11A1</td>
<td>Cholesterol</td>
<td></td>
</tr>
<tr>
<td>P450 2B4</td>
<td></td>
<td>Benzphetamine</td>
</tr>
<tr>
<td>P450 3A4</td>
<td></td>
<td>Dextrometorphane</td>
</tr>
<tr>
<td>P450 3A4</td>
<td></td>
<td>Cyclophosphamide</td>
</tr>
<tr>
<td>P450 2C9</td>
<td></td>
<td>Flurbiprofene</td>
</tr>
<tr>
<td>P450 2C9</td>
<td></td>
<td>Naproxene</td>
</tr>
</tbody>
</table>

S.Carrara, EPFL Lausanne (Switzerland)
Oxidases for Biomarkers detection

Glucose, or Lactate, or Cholesterol, etc ...

Oxygen

Hydrogen peroxide

Oxidase

Product

Amperometric Detection !!!!!
Cytochromes P450 for Drugs Detection

RH (e.g. benzphetamine)

From electrode

Drugs detection!

Cytochrome P450 2B4

more soluble then faster secreted

NADP

O₂

H₂O₂

2e⁻

R-OH

Oxidized form

H-O-H

NADP⁺
Problems on Detection Limits

Detection of verapamil by 3A4, an antihypertensive drug, was from 400 µM to 3mM while its therapeutic range is below 0.3 µM.
Nano-Bio-Sensors Macro-Assembly

BARE ELECTRODE

CARBON NANOTUBES

CNTs + PROBE ENZYMES

10.3 ± 1.14 nm

19.9 ± 3.38 nm

3.6 nm

4.9 nm

5.2 nm

Boero et al. / IEEE PRIME 2009
Boero et al. / IEEE ICME 2010
Carrara et al. / Biosensors and Bioelectronics 2011

S.Carrara, EPFL Lausanne
(Switzerland)
Nano-Bio-Sensors Micro-Spotting

Carbon Nanotubes + Nafion

S.Carrara, EPFL Lausanne
(Switzerland)
New Challenges on CNT integration directly onto Silicon chips

Taurino et al. / Electrochem. Comm./2011 submitted
Taurino et al. / Sensors and Actuators B, 2011 submitted

S.Carrara, EPFL Lausanne
(Switzerland)
Carbon Nanotubes contribute to Redox Reactions Efficiency

Nernst equation

\[ E = E^0 - \frac{RT}{nF} \ln \left( \frac{C_k(0,t)}{C_0(0,t)} \right) \]

Randles-Sevcik equation

\[ i(0,t) \propto nFAD \left( \frac{nF \nu D}{RT} \right)^{1/2} C(0,t) \]

Cottrell equation

\[ i(x,t) = \frac{nFAD^{1/2} C(x,t)}{\pi^{1/2} t^{1/2}} \]
Nernst Effect

Benzphetamine detection by means of P450 2B4 immobilized onto Single Walled Carbon Nanotubes

S.Carrara, EPFL Lausanne
(Switzerland)
Cholesterol detection by means of P450 11A1 immobilized onto Multi Walled Carbon Nanotubes

S.Carrara, EPFL Lausanne
(Switzerland)
Improved Detection Limit in Drugs detection

Cyclophosphamide (CP), an anti-cancer agent, detected by P450 3A4 onto MWCNT
Improved Sensitivity in Peroxide Based Detections

Oxidases onto MWCNT for Glucose and Lactate

~ 7.5 times more!!

C. Boero, S. Carrara et al., IEEE Prime, 2009

S. Carrara, EPFL Lausanne (Switzerland)
### Drugs for treating Breast Cancer

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Pharmacological range</th>
<th>Enzymes involved in drug metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide&lt;sup&gt;(2),(3)&lt;/sup&gt;</td>
<td>2.68-76.6 µM</td>
<td></td>
</tr>
<tr>
<td>Etoposide&lt;sup&gt;(4),(5)&lt;/sup&gt;</td>
<td>33.98-101.94 µM</td>
<td>3A4, 1A2 (-)</td>
</tr>
<tr>
<td>Ifosfamide&lt;sup&gt;(2)&lt;/sup&gt;</td>
<td>10-160 µM</td>
<td>3A4, 2B6</td>
</tr>
<tr>
<td>Mitoxantrone&lt;sup&gt;(6)&lt;/sup&gt;</td>
<td>1.84-3.31 µM</td>
<td>3A4, 1B1 (-)</td>
</tr>
<tr>
<td>Tegafur&lt;sup&gt;(7)&lt;/sup&gt; (contain Fluorouracil)</td>
<td>1 µM-10 µM</td>
<td>1A2, 2A6</td>
</tr>
</tbody>
</table>

Good concentration ranges for the sensitivity of our technology!

The CYP in the table are sorted according to their importance in the drug metabolism. The symbol (-) means that the CYP isoform is involved as the minor enzymatic components in the drug metabolic pathway.
## Measurement in Serum!

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Pharmacologic range (μM)</th>
<th>P450 enzyme</th>
<th>Sensitivity (nA/μM*mm²)</th>
<th>Detection limit (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PBS</td>
<td>Serum</td>
<td>PBS</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>2.68-76.6</td>
<td>2B6</td>
<td>1.021</td>
<td>0.279</td>
</tr>
<tr>
<td>Ifosfamide</td>
<td>10-160</td>
<td>3A4</td>
<td>1.602</td>
<td>0.430</td>
</tr>
<tr>
<td>Ftorafur</td>
<td>1-10</td>
<td>1A2</td>
<td>8.832</td>
<td>3.469</td>
</tr>
<tr>
<td>Etoposide</td>
<td>33.98-101.94</td>
<td>-</td>
<td>73.73</td>
<td>9.142</td>
</tr>
</tbody>
</table>
Breast cancer drugs cocktail

- cyclophosphamide, methotrexate, and fluorouracil (CMF)\(^8\)(11); 
- fluorouracil, doxorubicin, and cyclophosphamide (FAC)\(^8\); 
- cyclophosphamide, doxorubicin and 5-fluorouracil (CAF)\(^9\); 
- fluorouracil, epirubicin, and cyclophosphamide (FEC)\(^8\)(11)(12); 
- fluorouracil, doxorubicin, and cyclophosphamide \(^{(11)(12)}\); 
- ifosfamide, Carboplatin, Etoposide (ICE)\(^9\); 
- ifosfamide, methotrexate and 5-fluorouracil (IMF)\(^9\); 
- cyclophosphamide, mitoxantrone, and etoposide\(^{12}\).

GABRIELN. HORTOBAGYI, M.D.
A.Y. Chang, L. Hui, R. Asbury, L. Boros, G. Garrow, J. Rubins
Manfred Kaufmann, Gunter von Minckwitz, Roy Smith, Vicente Valero, et al
Different Drugs give peaks in different positions

<table>
<thead>
<tr>
<th>Substrate/inhibitor of CYP2C9</th>
<th>$K_m$ (µM)</th>
<th>$K_i$ (µM)</th>
<th>CYP2C9 (mV)</th>
<th>$E_{mid}$ CYP2C9 + substrate (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torsemide (s)</td>
<td>11.4</td>
<td></td>
<td>−41</td>
<td>−19</td>
</tr>
<tr>
<td>Diclofenac (s)</td>
<td>6.8</td>
<td></td>
<td>−41</td>
<td>−41</td>
</tr>
<tr>
<td>Tolbutamide (s)</td>
<td>120&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>−41</td>
<td>−37</td>
</tr>
<tr>
<td>S-Warfarin (s)</td>
<td>6&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>−41</td>
<td>−36</td>
</tr>
<tr>
<td>Sulfaphenazole (i)</td>
<td></td>
<td>0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>−41</td>
<td>−41</td>
</tr>
<tr>
<td>CO&lt;sub&gt;(g)&lt;/sub&gt;</td>
<td></td>
<td></td>
<td>−41</td>
<td>8</td>
</tr>
</tbody>
</table>

The cytochrome P450 2C9 presents peak shifts in the range of tens of mV by changing drug substrates.

D.L. Johnson et al. / Biochemical Pharmacology 69 (2005) 1533–1541

\[
i(V) = i_c(V) + \sum_{\forall k} A_k e^{-\frac{(V-V_k)^2}{\sigma_k^2}}
\]

Charging current

Faradic currents
The Hetero-tropic Kinetics

- **Enzyme ACTIVATION**
- **Enzyme INHIBITION**
Multiple drugs detection: CYP3A4

Cyclophosphamide (CP) and Dextromethorphan (DX) detection by P450 3A4 onto MWCNT

S. Carrara, EPFL Lausanne (Switzerland)
Multiple drugs detection: CYP2C9

CYP2C9 + Flurbiprofen 200 M
Peak variation upon naproxen addiction

Activation of FL detection

Naproxen (NP) and Flurbiprofen (FL) detection
by P450 2C9 onto MWCNT

S.Carrara, EPFL Lausanne (Switzerland)
Peaks Amplitude is affected by the other drugs.

The Gaussian decomposition in cytochrome P450 based detection has to account for the heterotropic kinetics.

<table>
<thead>
<tr>
<th>Substrate/inhibitor of CYP2C9</th>
<th>$K_m$ (µM)</th>
<th>$K_i$ (µM)</th>
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<th>$E_{mid}$ CYP2C9 + substrate (mV)</th>
</tr>
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<tbody>
<tr>
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<td>11.4</td>
<td>-</td>
<td>-41</td>
<td>-19</td>
</tr>
<tr>
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<td>6.8</td>
<td>-</td>
<td>-41</td>
<td>-41</td>
</tr>
<tr>
<td>Tolbutamide (s)</td>
<td>120(^a)</td>
<td>-</td>
<td>-41</td>
<td>-37</td>
</tr>
<tr>
<td>S-Warfarin (s)</td>
<td>6(^b)</td>
<td>-</td>
<td>-41</td>
<td>-36</td>
</tr>
<tr>
<td>Sulfaphenazole (i)</td>
<td>-</td>
<td>0.1(^c)</td>
<td>-41</td>
<td>-41</td>
</tr>
<tr>
<td>CO(_{(g)})</td>
<td>-</td>
<td>-</td>
<td>-41</td>
<td>-41</td>
</tr>
</tbody>
</table>

Dependence from the other drug concentrations

\[ i(V) = i_C(V) + \sum_{\forall k} \prod_{\forall j \neq k} A_k\left( [C_j] \right) \]

Charging current

Faradic currents

The Gaussian decomposition in cytochrome P450 based detection has to account for the heterotropic kinetics.
The Problem of multi-panel arrays response
Developing of the full system

Building-block diagram for a biosensing platform

G. De Micheli et al. / DATE 2011

S. Carrara, EPFL Lausanne
(Switzerland)
A reliable full system requires:

1. Precise Current measurements
2. Multiplexing for different molecules
3. Reliability in Temperature and pH
4. Multiplexing Molecular Detection with T and pH
5. Reliable in sweeping the Voltage
6. Security
7. Privacy
1. Precise Current measurements

Current-to-frequency converter

\[ I_{WE} \rightarrow f_{counter} \]
2. Multiplexing Molecular Detection

Different working electrodes are multiplexed to the current-to-frequency converter

S.Carrara, EPFL Lausanne (Switzerland)
3. Reliability in Temperature & pH

$$E = E^0 - \frac{RT}{nF} \ln \left( \frac{C_r}{C_o} \right) - \frac{RT}{F} \text{pH}$$

$i \propto nFAD \left( \frac{nFvD}{RT} \right)^{1/2} C_r$

Figure 2. Peak Potential shift versus pH

Figure 3. Peak current variation induced by temperature
4. Multiplexing Molecular detection with T and pH

The switches also multiplex the T and pH measure
5. Reliable for Sweep in Voltage

Sweeping the voltage is definitely required to distinguish each single drug contribution in the Voltammogram.

S.Carrara, EPFL Lausanne
(Switzerland)
The Direct Digital Synthesis (DDS) method to generate the triangular voltage waveform and based on Capacitor charging/discharging Method
6. Security

On line reliability by novel collaborative monitoring frame-work to track circuit level performance degradation

B. Datta & W. Burleson, GLSVLSI 2010

S. Carrara, EPFL Lausanne
(Switzerland)
7. Privacy

Lightweight identity-based encryption system

C.C. Tan et al., WiSec 2008
Conclusions

- P450 Cytochromes are required to detect Exogenous metabolites (Drugs)
- Oxidases are required to detect endogenous metabolites (bio-markers)
- Carbon Nanotubes are required to improve sensitivity of electrochemical detection
- Data analysis is required to improve specificity on exogenous compounds
- New CMOS design is required to develop Dedicated systems for molecular detection
- New system architectures are required to improve and assure Security and Privacy
Thanks to:

- Andrea Cavallini
- Camilla Bai-Rossi
- Cristina Boero
- Sara Ghoreishizadeh
- Daniel Torre
- Daniela De Venuto
- Jacopo Olivo
- Irene Taurino
- Dino Giuseppe Albini
- Victor Erokhin
- Giovanni De Micheli
Thanks to my Sponsors

S.Carrara, EPFL Lausanne
(Switzerland)
Thank you for your attention!

Coordinates

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email: sandro.carrara@epfl.ch

Related References: