R&D of Biosensors

- High Sensitive Immunochromatography (PCT/JP2006/323617)
- DEP-Chip Series for Electrochemical Biosensors

Gold–Linked Electrochemical Immuno Assay (GLEIA, PCT/JP2007/56992)

- Compact potentiostat “BDTminiSTAT100”
- Compact electrochemical luminescence device “BDTeCL-P100”

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# Products and Service List

<table>
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<th>Name</th>
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<tr>
<td>Printed Electrodes for Biosensors</td>
<td>DEP-Chip series</td>
</tr>
<tr>
<td></td>
<td>SP-N, SP-P</td>
</tr>
<tr>
<td></td>
<td>SR-N, SR-P</td>
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<tr>
<td></td>
<td>ER-N, ER-P, ER-PP</td>
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<tr>
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<td>TG-1, TG-3</td>
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<td></td>
<td>PCR-P01, PCR-P03</td>
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<tr>
<td>Compact Potentiostat</td>
<td>BDTminiSTAT100</td>
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<tr>
<td></td>
<td>BDTminiSTAT400 (4 channel)</td>
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<td></td>
<td>BDTminiSTATSR-6 (6 channel)</td>
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<td></td>
<td>BDTminiSTAT100-BTR(blue tooth connection)</td>
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<tr>
<td>Electrochemical Luminescence Device</td>
<td>BDTeCL-P100</td>
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<tr>
<td>Trial Manufacture of Immunochromatography</td>
<td>NEW</td>
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<tr>
<td>Manufacture of customized printed electrodes on assignment</td>
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<td>Contract research on biosensors</td>
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</table>
Compact Potentiostat “BDTminiSTAT100”

- Five measurement modes of CA, CV, LSV, DPV, SWV
- Compact, Light weight and Easy to carry
- Power supplied with USB connection to PC
- Easy to use with the proprietary software
- Optional software for Automatic peak detection and determination

Compact Potentiostat BDTminiSTAT100

Light weight, Power supplied with USB connection to PC, Easy to use with a proprietary software. Use from research to measurement outdoors.
Wireless Potentiostat “BDTminiSTAT100-BTR”

- Five measurement modes of CA, CV, LSV, DPV, SWV
- Compact, Light weight (120 g) and Easy to carry
- Power supplied by two AAA size batteries
- Easy to use with the proprietary software
- Wireless connection to computer by Bluetooth
Compact Multi-channel Potentiostat
BDTminiSTAT400, BDTminiSTATSR-6

• 5 measurement modes of CV, LSV, CA, DPV, SWV
• Compact size: 130 x 100 x 40 (mm) 300 g
• The applied electromotive force: -2.0 ~ +2.0 V, resolution: 2 mV
• The measurement range: 6 range (1 nA ~ 100 μA), resolution: 0.1%
• Easily input measuring conditions (measurement mode, voltage range, time and scan rate, etc.)
• On-line graphical representation of measurements
• Automatic safe-keeping of measured data (CSV file)

Mail to
postmaster@biodevicetech.com
Electrochemical Luminescence Device “BDTeCL-P100”

- Compact ECL device
- Simple and rapid measurement enabled by its dedicated software.
- Highly sensitive and selective measurement enabled

**BDTeCL-P100**

<table>
<thead>
<tr>
<th>PC OS</th>
<th>Windows Vista, 7, 8 (32 bit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size (mm)/weight (Kg)</td>
<td>115(L) x 125(W) x 185(H)/ 4</td>
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System
Printed Electrodes “DEP-Chip series”

Square working electrode
- SP-N, SP-P (carbon)
- SR-N (gold)

Round working electrode
- EP-N (carbon)
- EP-PP (carbon, WE with ring and dam)
- ER-N (gold)

One drop test

mass production/disposal/low cost
Possible customized fabrication

PCR-P01

Adapted size for PCR tube

Printed Electrodes “DEP-Chip series”

Square working electrode
- SP-N, SP-P (carbon)
- SR-N (gold)

Round working electrode
- EP-N (carbon)
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One drop test

mass production/disposal/low cost
Possible customized fabrication

PCR-P01

Adapted size for PCR tube
Advantage of DEP-Chips

*DEP-Chip series is a trade name of our original printed electrodes which were developed for fabrication of various biosensors.

- Disposable
- No contamination
- Easy to use
- Mass productive and low cost
- High quality CV < 5%
- Small amount of reagents and samples 1-2 μL
- Various applications for electrochemical determination with BDTminiSTATs and for electrochemical luminescence determination with BDTeCL-P100

- Especially suitable for biosensors
Biosensor printed electrodes “DEP-Chip series” are evaluated worldwide

Genetic testing at a snip

12 April 2007

A cheap, rapid and portable point-of-care genetic test could be a step closer thanks to a team of scientists from Japan and Thailand.

Single nucleotide polymorphisms (SNPs, pronounced snips) are genetic variations in a person’s DNA sequence. They can indicate a susceptibility to disease and could be useful in predicting a patient’s response to therapy. Now Eiichi Tamiya at the Japan Advanced Institute of Science and Technology in Nomi City and his team have developed a chip that can detect SNPs and used it to find the variations in a gene linked to Alzheimer’s disease.

“The sensors have the potential to meet the need for inexpensive, rapid and hand-held systems for genetic analysis, diagnosing cancer predisposition, identifying genetically modified organisms, and reducing recent threats of bioterrorism.”
- Eiichi Tamiya

The group used disposable printed chips to analyse DNA from human volunteers. Before analysis, the DNA sample is amplified with a segment of DNA corresponding to the SNP of interest. The DNA is then added to the chip, where it binds to a redox molecule. If the SNP is present, then the amplification step is successful and so a smaller current is measured by voltammetry. Importantly, the method does not require the redox molecule to be immobilised on the sensor. This helps make the chip system simpler and cheaper than existing SNP tests. The group went on to apply the method to detect SNPs linked to

Detection of HIV-1 protease inhibition
K. Kerman, Chem. Commun., 2007, 3829

Printed electrode in RT-PCR flow chip for Flu virus detection
1. Total viable bacterial sensor
2. DNA sensor
3. Immunochemical sensor
4. Enzyme sensor
5. Heavy metal sensor
6. Residual agricultural chemical sensor
Total viable bacterial sensor

Count of viable bacteria numbers

Respiratory activity (consumption of oxygen)

Determination of dissolved oxygen decrease by the electrochemical method

CV (Cyclic Voltammetry) 50mV/sec
Printed carbon electrode (DEP-Chip SP-P)

DEP-Chips: Low cost/Easy to use
miniSTAT100: compact and light weight

The smallest potentiostat in the world
Original principle of our DNA sensor
(Japanese patent No. 364257)

This method is suitable for detection of DNA amplification after PCR or LAMP.
An example of DNA sensor system

Procedure (RT-LAMP)
Analyst 2011, DOI: 0.1039/c1an15638a

Primer mixture + Methylene Blue
+ influenza virus + dried enzyme + buffer reagents
(Loopamp®) total volume 50 μL

Printed electrode was inserted in the micro tube and the tube was incubated at 63°C for 40 min.

During incubation, electrochemical measurements (SWV) were performed every 5 min.

(A) Photograph of the screen-printed electrodes. a: screen-printed electrode, b: screen-printed electrode inserted into the 200 mL micro tube.
(B) Illustration of the semi-real time monitoring system using the screen-printed electrode with a USB powered portable potentiostat. a: heat block, b: screen-printed electrode inserted into the micro tube, c: USB powered portable potentiostat, d: laptop computer.
(C) Photographs of the USB powered portable potentiostat and screen-printed electrode inserted into the micro tube with heating unit. a: USB powered portable potentiostat (BDTmini-STAT100, Bio device technology Co., Ltd., Japan).
Example of detection of the food poisoning infectious organism gene

【Electrochemical determination】

Ipo (negative)  
Without salmonellas

Ipa (positive)  
With salmonellas
1. Pathogenic microbe
   - Salmonella, E. coli O–157, MRSA (nosocomial infection),
   - Periodontal disease bacteria, Anthrax

2. Infectious virus
   - Influenza, Hepatitis virus B

3. Food
   - Determination of species of meat (pork, beef, chicken),
   - genetically modified food (GMO corn, GMO soybean etc.)

4. SNPs
   - drug resistant gene, Alzheimer’s disease–associated gene
Gold nanoparticle redox signal enhancement for detection of antigens

After the recognition reaction between the surface-immobilized primary antibody and hCG, the captured antigen was sandwiched with a secondary antibody that was labeled with gold-nanoparticles. Then the amount of gold nanoparticles was determined by its redox signal.

The primary antibody was immobilized directly on the working electrode, and a sandwich-type immuno-reaction was performed.

A high potential was applied in HCl for the oxidation of gold nanoparticles.

The voltammetric measurement was performed.

**Reference:**
### Comparative table of GLEIA and ELISA, CLEIA

<table>
<thead>
<tr>
<th></th>
<th>GLEIA</th>
<th>ELISA, CLEIA</th>
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</thead>
<tbody>
<tr>
<td><strong>Labeling</strong></td>
<td>Gold nanoparticle</td>
<td>Enzyme</td>
</tr>
<tr>
<td><strong>Detection method</strong></td>
<td>Electrochemical measurement</td>
<td>Optical measurement</td>
</tr>
<tr>
<td></td>
<td>(reduction current of gold particle)</td>
<td>(coloring or fluorescence by enzyme activity)</td>
</tr>
<tr>
<td><strong>Measurement time</strong></td>
<td>Within 60 min (within 15 min by combination with micro flow channels)</td>
<td>2 – 3 hours</td>
</tr>
<tr>
<td><strong>Threshold</strong></td>
<td>pg/mL ~ ng/mL</td>
<td>pg/mL ~ ng/mL</td>
</tr>
<tr>
<td><strong>Sample volume</strong></td>
<td>1 μL</td>
<td>A few tens of μL</td>
</tr>
<tr>
<td><strong>Cost</strong></td>
<td>Less than 800 JPY/test</td>
<td>800～1600 JPY/test</td>
</tr>
<tr>
<td><strong>Device</strong></td>
<td>inexpensive • compact</td>
<td>expensive • large sized</td>
</tr>
<tr>
<td><strong>Purpose</strong></td>
<td>Medical/foods/environmental</td>
<td>Medical/foods/environmental</td>
</tr>
<tr>
<td></td>
<td>(For point of care testing at bed-side, clinic, manufacturing floor)</td>
<td>(For facilities for medical examination, Laboratories, stationary/batch processing)</td>
</tr>
<tr>
<td><strong>Application</strong></td>
<td>hCG, insulin in human serum, IgA, hemoglobin, albumin in human urine, DNA detection after PCR with FITC and biotin labeled primer</td>
<td></td>
</tr>
</tbody>
</table>
Application of Gold-Linked Electrochemical Immuno-Assay I

Typical differential pulse voltammograms of GLEIA.

Human Chorionic Gonadotropin Hormone standard curves constructed with buffer (●) and with serum (●).

Sample volume: 1 – 1.5μL
Lower detection limit: 62.5 pg/mL

Current (μA)

Potential (V)

hCG concn. (pg/mL)

control1 \( R^2 = 0.9996 \)

serum \( R^2 = 0.9971 \)
Glucose sensor with Glucose Dehydrogenase using DEP-Chips (Chrono-amperometry)
**Heavy metal determination (DPV)**

### Pb conc. (ppb) vs Peak Height (μA)

<table>
<thead>
<tr>
<th>Pb conc (ppb)</th>
<th>Peak Height (μA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.150</td>
</tr>
<tr>
<td>10</td>
<td>0.385</td>
</tr>
<tr>
<td>100</td>
<td>0.624</td>
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**DEP-Chip SP-N SPKI13**

Dilution with 0.1N

### Cd conc. (ppb) vs Peak Height (μA)

<table>
<thead>
<tr>
<th>Cd conc (ppb)</th>
<th>Peak Height (μA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>0.433</td>
</tr>
<tr>
<td>50</td>
<td>1.046</td>
</tr>
<tr>
<td>100</td>
<td>1.628</td>
</tr>
</tbody>
</table>

**DEP-Chip SR-N SRJR10**

Dilution with 0.1N

**Pb**

Pb 赤：100、緑：10、青：0 （ppb）

**Cd**

Cd 赤：100、緑：50、青：20、桃：0（ppb）
Detection of residual agricultural chemicals (RAC) using inhibition of Acetylcholine esterase activity (Electrochemical method)

Acetylcholine esterase (AChE) mediates the following reaction. Agricultural chemicals inhibit the activity of AChE and inhibit formation of thiocholine (TCh)

Principle of reaction

\[
\begin{align*}
(\text{CH}_3)\text{N}^+\text{CH}_2\text{CH}_2\text{SCOCH}_3 + \text{H}_2\text{O} & \quad \xrightarrow{\text{AChE}} \quad (\text{CH}_3)\text{N}^+\text{CH}_2\text{CH}_2\text{SH} + \text{CH}_3\text{COOH} \\
\text{ATCh} & \quad \xrightarrow{\text{AChE} + \text{RAC}} \quad \text{TCh}
\end{align*}
\]

AChE converts ATCh to TCh. After 10 min reaction, the current became larger by the increased TCh. When the activity of AChE was inhibited by RAC, TCh was not formed and the increase of the current was also inhibited.

Blue line: 2 min after ACTh addition, Red line: 10 min after ACTh addition
Direct fabrication of catalytic metal nanoparticles onto the surface of a screen-printed carbon electrode.
*Electrochemistry Communications, 8, 1375, 2006*

An electrochemical on-field sensor system for the detection of compost maturity
M. Chikae, K. Kerman, N. Nagatani, Y. Takamura, E. Tamiya
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Label-free electrical sensing of small-molecule inhibition on tyrosine phosphorylation
K. Kerman, M. Vestergaard, E. Tamiya
*Anal. Chem., 79, 6881, 2007*

Electrochemical DNA biosensor using a disposable electrochemical printed (DEP) chip for the detection of SNPs from unpurified PCR amplicons
M. U. Ahmed, K. Idegami, M. Chikae, K. Kerman, P. Chaumpluk, S. Yamamura, E. Tamiya
*Analyst, 132, 431, 2007*

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K. Idegami, M. Chikae, K. Kerman, N. Nagatani, T. Yuhi, T. Enco, E. Tamiya
*Electroanalysis, 20, 14, 2008*

Electrochemical Biosensors for Medical and Food Applications
M. U. Ahmed, M. M. Hossain, E. Tamiya
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Level-free Electrochemical Detection for Food Allergen using Screen Printed Carbon Electrode
Masato SAITO, Masaaki KITSUNAI, Mihnaz Uddin AHMED, Shigeru SUGIYAMA, and Eiichi TAMIYA,
*Electrochemistry, 76(8), 606-609, 2008*

Electrochemical genosensor for the rapid detection of GMO using loop-mediated isothermal amplification
*Analyst, 134, 966, 2009*
Rapid, Sensitive, and Label-Free Impedimetric Detection of a Single-Nucleotide Polymorphism Correlated to Kidney Disease
Alessandra Bonanni, Martin Pumera, and Yuji Miyahara
*Anal. Chem.* 2010, 82, 3772–3779

Meat species identification based on the loop mediated isothermal amplification and electrochemical DNA sensor
*Food Control*, 21, 599, 2010

Highly Sensitive Method for Electrochemical Detection of Silver Nanoparticle Labels in Metalloimmunoassay with Preoxidation/Reduction Signal Enhancement,
Miyuki CHIKAE, Koutarou IDEGAMI, Naoki NAGATANI, Eiichi TAMIYA and Yuzuru TAKAMURA,
*Electrochemistry*, vol.78 No.9 SEP. 2010, P.748-753

Rapid detection for primary screening of influenza A virus: microfluidic RT-PCR chip and electrochemical DNA sensor
*Analyst*, 136, 2064, 2011

Semi-real time electrochemical monitoring for influenza virus RNA by reverse transcription loop-mediated isothermal amplification using a USB powered portable potentiostat
*Analyst*, 136, 5143, 2011

Semi-quantitative detection of gene expression using bisbenzimide dye
P. Kittimongkolsuk, T. Tencomnao2, R. Santiyanont

Development of Label-Free Impedimetric Hcg-Immunosensor Using Screen-Printed Electrode
Truong TN Lien, Nguyen Xuan Viet, Miyuki Chikae, Yoshiaki Ukita and Yuzuru Takamura
Electrochemical detection of specific DNA and respiratory activity of Escherichia coli
Electrochimica Acta, 82, 132, 2012

A thiophene-containing compound as a matrix for matrix-assisted laser desorption/ionization mass spectrometry and the electrical conductivity of matrix crystals
A. Yasuda, T. Ishimaru, S. Nishihara, M. Sakai, H. Kawasaki, R. Arakawa, and Y. Shigeria,

Gold-linked electrochemical immunoassay on single-walled carbon nanotube for highly sensitive detection of human chorionic gonadotropin hormone
Nguyen Xuan Viet, Miyuki Chikae, Yoshiaki Ukita, Kenzo Maehashi, Kazuhiko Matsumoto, Eiichi Tamiya, Pham Hung, Yuzuru Takamura,
Biosensors and Bioelectronics, 42, 592-597, 2013

Quantitative detection for Porphyromonas gingivalis in tooth pocket and saliva by portable electrochemical DNA sensor linked with PCR,
K. Yamanaka, S. Sekine, T. Uenoyama, M. Wada, T. Ikeuchi, M. Saito, Y. Yamaguchi, E. Tamiya,
Electroanalysis, 2686-2692, 2014

総説類

民谷栄一: ポイントオブケア型バイオセンサーの開発とその展開, 臨床化学, 44, 126-134 (2015)
民谷栄一: プリンタブル技術とバイオセンサー-開発, 化学工業, 65(10) 40-50 (2014)
民谷栄一: プリンタブルバイオセンサーの開発, 日本印刷学会誌, 51(1) 2-10 (2014)