Biochip-Technologies (2)

T. Brandstetter
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- Materials and surface modifications (24.04.15)
- Manufacturing of Biochips (22.05.15)
- Biochip technologies – Between research and routine diagnostics (state of the art, 19.06.15)
- Nucleic acid based techniques (26.06.15)
- Biochips for protein analytics (03.07.15)
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Core competences of CPI

- Tailor made surfaces
- Characterization of surfaces
  - by SPR, ellipsometry, FTIR
- Polymer synthesis
- Characterization by
  - NMR, GPC, XPS, titration
- Functionalization of surfaces
- Microstructuring of surfaces
- Bioanalytical surfaces (Dr. T. Brandstetter)
Bioanalytical Surfaces @ CPI

Who we are

Bioanalytical Surfaces - Dr. Thomas Brandstetter

Bioanalytical Surfaces
http://www.cpi.uni-freiburg.de/bioanalytical-surfaces-dr-brandstetter
Manufacturing of Biochips
Manufacturing of Biochips - overview

**Capabilities**

1. Rapid prototyping using Micromilling, Laser Ablation
2. Metal Mold Fabrication (>1000 replicates)
3. Emboss/Injection mold in a variety of materials (ceramics, metal alloys, plastics)
4. HARMS (Aspect ratios 50:1)
5. Pattern sub-micron features
6. Fabricate 3D structures
Fig. 1. This scheme shows the interrelationship of various technologies that contribute to clinical nanodiagnosics. These technologies also contribute to development of nanomedicine under the concept of personalized medicine.
Fig. 2. Block diagram of the proposed architecture.

Architecture of a Portable System Based on a Biochip for DNA Recognition
M. Piedade, L. Sousa, J. Germano, J. Lemos, B. Costa, INESC-ID/IST, R. Alves Redol, 9, 1000-029, Lisboa, Portugal
Figure: flowchart of the microarray fabrication process, including an artist’s view of the crosslinking reaction and a molecular scheme of the photoreaction of the photoactive benzophenone moieties contained in the polymer.

Simple One-Step Process for Immobilization of Biomolecules on Polymer Substrates Based on Surface-Attached Polymer Networks
Rendl et al., 2011; Langmuir, Article ASAP, DOI: 10.1021/la1050833, Publication Date (Web): April 14, 2011

www.imtek.de/cpi T. Brandstetter/ 22.05.2015 / slide 9
# Biochip materials

## Microscope slide of glass
- Silica (SiO$_2$)
- Sodium carbonate (Na$_2$CO$_3$)
- Limestone (CaCO$_3$)
- Magnesium Carbonate (MgCO$_3$)

## Microscope slides of plastic
- PMMA (Polymethymethacrylate)
- Polystyrene (PS)
- Polycarbonate (PC)
- Cyclin olefin copolymers (COC)
- Polypropylene (PP)
## Biochip coatings

**directly**

- *In situ* synthesis on glass
- Silanized probes on unmodified glass
- Photocrosslinking on unmodified plastic

**chemically modified surfaces**

- activated glass by poly-carbodiimide, aminosilane, aldehyde
- graft coating polymers on silicon (glass)
- plastic-based DNA microarrays using carbodiimide chemistry
- amine-modified PMMA substrates
- activated polystyrene, polypropylene, polycarbonate (PC)
PCR-ON-A-CHIP, THE DIFFERENT CHIP TYPES

Chip injection molded in PC in different variants

Concept 1:
Fixed number of PCR cycles on chip
Chips with different cycle numbers available:
15, 36, 41 cycles

Concept 2:
Arbitrary number of PCR cycles controlled by pumping
Chip injection molded in PC in different variants

Concept 1:
Fixed number of PCR cycles on chip
OFF-THE-SHELF PRODUCTS

Micro mixer

Extractor

Micro mixer array

Nanotiterplate

Extractor

Luer Lok compatible adapters
Figure 5: Signals measured after hybridization of the microarrays with biotinylated PCR amplicons of HPV 16 specific sequences and staining with Cy5-conjugated streptavidin. Shown are the specific signals for HPV 16, the unspecific discriminated signals for HPV 6 and the negative control for different support materials. Displayed is the average intensity of 8 dots per parameter.

Simple One-Step Process for Immobilization of Biomolecules on Polymer Substrates Based on Surface-Attached Polymer Networks Rendl et al., 2011; Langmuir, Article ASAP, DOI: 10.1021/la1050833, Publication Date (Web): April 14, 2011
Figure 6: Signals measured after incubation with serum containing TPO specific antibodies and labeling with Cy5-conjugated anti-human detection antibodies. Shown are the specific signals for the parameter TPO, the unspecific discriminated signal for Jo-1 and the negative control for different substrate materials. Displayed is the average intensity of 8 dots per parameter.

Simple One-Step Process for Immobilization of Biomolecules on Polymer Substrates Based on Surface-Attached Polymer Networks Rendl et al., 2011; Langmuir, Article ASAP, DOI: 10.1021/la1050833, Publication Date (Web): April 14, 2011
Manufacturing of biochips – in general (1)

1. Untreated slide
   mixed analyte solution

2. Microarray printing

3. Immobilisation
I. Contact printing

http://www.anopoli.com/

Print pins

http://www.anopoli.com/
Microstructuring in biochip technologies, contact printing

- Omnigrid from GeneMachine®
  - Contact printing procedure
  - 65% humidity, RT
  - Steel or tungsten needle with reservoir
  - droplet volume 400 – 600 pl
  - droplet diameter 140 – 200 µm
  - Process variance > 10%
Pin heads make the difference.

**Split pin**
- Spot diameters: 75µm to 215 µm
- Uptake volumes: 0.25µl to 0.64 µl

**Solid pin**
- Spot diameters: 75µm to 450 µm

http://www.anopoli.com/
II. Contactless printing/Piezo Electric Dispenser
II. Piezo Electric dispenser

- Piezo Electric dispenser (Scienion AG®)
  - Contactless printing procedure
  - 65% humidity, RT
  - droplet volume 410 pl,
  - droplet diameter 175 µm
  - droplet volume and diameter is adjustable
  - Process variance < 10%
Microstructuring in biochip technologies, contactless printing

2D = printing with PBS without polymer
3D = printing with PBS 1 mg/ml PDMAA-co-5%MABP-co-2,5%VPA

Photos after print
Microstructuring in biochip technologies
Micronas Biochip technology

- Piezo Electric dispenser (Scienion AG®)
  - Contactless printing procedure
  - 80% humidity, RT
  - droplet volume 390 pl,
  - photodiode diameter 180 µm
  - printing on structured surfaces
  - Process variance < 10%
Microstructuring in biochip technologies
Micronas Biochip technology

- Piezo Electric dispenser (Scienion AG®)
  - printing directly on a photodiode

π 180 µm
Microstructuring in biochip technologies

Micronas Biochip technology

- Piezo Electric dispenser (Scienion AG®)
  - printing directly on a photodiode
  - pattern matching using a software
Affymetrix biochip technology

![Diagram of photolithographic synthesis](image)

**Fig. 1** - The mask only allows light to pass to specific features on the chip

**Fig. 3** - Silanation – each “Si” is a starting point

*GeneChip® Microarray Curriculum – 2005 Version*
Affymetrix biochip technology

Fig. 5 - Deprotection of Feature #3 and 4

Fig. 6 - Addition of Adenine (A) Nucleotides
Affymetrix biochip technology

Fig. 7 - 2nd mask (deprotecting Feature 1 and 4)

Fig. 8 - Addition of the 2nd nucleotide (C)
Affymetrix biochip technology

Fig. 9 - 3rd Mask (Deprotection of Feature 2 & 3)

Fig 10 - 3rd nucleotide (addition of Guanine to Features #2 and #3)
Affymetrix biochip technology

Fig. 11 - Adding of the capping agent

Fig. 12 - Finished Probes

Fig. 13 - Final Results after removal of protection molecule and capping agent
Affymetrix biochip technology

Fig. 15 - Packaging the chip

Fig. 16 - Picking and Placing Chips into a Package
Detection methods

- Imaging SPR
- Imaging Ellipsometry
- Microbalance
- SERS
- Conductivity
- Enzymatic redox reaction
- Fluorescence
- Scanners
- Polarization
- Evanescent field
- Life-time
- Chemoluminescence
- Silver colloids

**Optical**

**Electrical**

**Radioactivity**

**Label-free**

- Imaging SPR
- Imaging Ellipsometry
- Microbalance
- SERS

**Optical**

**Electrical**

**Radioactivity**

**Label-free**
Optical methods

Fluorescence detection

- Most commonly used: Cy3 and Cy5 fluorescent dyes
- Wavelength of emitted light is higher than excitation wavelength (*Stokes shift*)
- Detection by optical bandpass filters
- Used in fluorescence microscopes, confocal microarray scanners or other microarray readers (e.g. GeneScan BioDetect)
Optical methods

Polarisation detection

• Emitted fluorescent light is randomly polarized
• D. Klotzkin, SPIE News 2007
  DOI: 10.1117/2.1200705.0748
Optical methods

Fluorescence detection in TIRF / ATR – Setup

- Excitation of fluorescent dyes by evanescent field
- Several setups to couple light in a waveguide (edge, grating)

Setup by Duveneck et al. (2002), *Analytica Chimica Acta*, 469, 49-61
Optical methods

Fluorescence life-time

- Light emitted by fluorescent dyes decays within nanoseconds
- Quantum dots as fluorescent dye show decay times of milliseconds
- Decay ("lifetime") is specific for each fluorophore
- Commercial setups available ("FLIM") DOI: 10.1080/10408349208051651

Nagl et al. (2005), Microchimica Acta, 151, 121
Optical methods

Chemical or enzymatic reactions

- Chemoluminescence (e.g. HRP)
- Silver ion crystallisation on Au-nanoparticles by hydroquinone

Conductivity

- Silver crystallisation can be used to short-circuit gap between electrodes

Electrical readout

Enzymatic potential generation

• DNA probe molecules immobilised on interdigital electrodes
• Redox cycling

Elsholz B. et al. (2006), *Anal Chem*, 78, 4794-4802
Label-free methods

iSPR – imaging Surface Plasmon Resonance

Steiner G. (2004), *Anal Bioanal Chem*, 379, 328-331
Other label-free methods

• iElli – imaging Ellipsometry
• SERS – surface enhanced Raman scattering
• Nanocantilever-array

McKendry et al. (2002), Proc Natl Acad Sci U S A, 99, 9783-9788
Digital microfluidics-based biochips

Figure 1: Schematic of a digital microfluidic biochip: (a) basic cell; (b) Top view of microfluidic array.

Figure 2: Microfluidic array used for multiplexed bioassays.

Krishnendu Chakrabarty and Fei Su, Design Automation Challenges for Microfluidics, TIMA Labs/DTIP 2005
NanoDetection technology

(www.nanodetectiontechnology.com)
Programmable Bio-sorting Microsystem Chip using Enhanced DEP Array

- To separate, sort out, purify and detect target biological particles/molecules like DNA, proteins, viruses, and cells

- adaptive biochips with the features of the high effective and adaptive separation, sorting, and purification of target bio-molecules for low concentration bio-sample solution.
Programmable Bio-sorting Microsystem Chip using Enhanced DEP Array
Programmable Bio-sorting Microsystem Chip using Enhanced DEP Array

Fig. 2 SEM pictures of micro-pyramid DEP traps with metal deposition and wires.

Fig. 3 The trapping /releasing pictures for the probe bead via micro-pyramid DEP trap
Photonic wires to detect pathogens

- sensors to quickly and reliably detect contaminants in food or infectious agents in human tissue

- photonic-wire evanescent field (PWEF) sensor technology would replace fluorescent labelling

- analyze a biological sample to determine the presence of a target molecule, such as a piece of DNA or a marker molecule that betrays the presence of a specific pathogen

www.nrc-cnrc.gc.ca/ highlights/2008/0810ims_e.html
Integrated Biochip for Clinical Disease detection

- Optical biosensor, including high sensitivity, convenient, and high precision
- Specific chemistry enzyme reaction to induce light emitting, and CMOS photo-diode
- Optical tweezers is to use laser or optical beam to manipulate nano/micro particles/bacteria
Manufacturing in biochip technologies, summary

- Piezo Electric dispenser (Scienion AG®)
  - Contactless printing procedure
  - Process variance < 10%

- Omnigrid from GeneMachine®
  - Contact printing procedure
  - Steel or tungsten needle with reservoir
  - Process variance > 10%

- Affymetrix biochip technology®
  - Photolithography

- Different microfluidic devices
  - electrical
  - optical
Thank you for your attention!
Literature

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