Outline

• Biocompatibility
• Surface Modification
• Biosensors (Ligand assays)
• Immobilization
  – Surface-Enhanced Laser Desorption Ionization
  – Surface Plasmon Resonance
  – Nanomechanical Cantilever Array
  – ...
• ...
• ...
Abstract

MEMS devices are manufactured using similar microfabrication techniques as those used to create integrated circuits. They often, however, have moving components that allow physical or analytical functions to be performed by the device. Although MEMS can be aseptically fabricated and hermetically sealed, biocompatibility of the component materials is a key issue for MEMS used in vivo. Interest in MEMS for biological applications (BioMEMS) is growing rapidly, with opportunities in areas such as biosensors, pacemakers, immunoisolation capsules, and drug delivery. The key to many of these applications lies in the leveraging of features unique to MEMS (for example, analyte sensitivity, electrical responsiveness, temporal control, and feature sizes similar to cells and organelles) for maximum impact. In this paper, we focus on how the biological integration of MEMS and other implantable devices can be improved through the application of microfabrication technology and concepts. Innovative approaches for improved physical and chemical integration of systems with the body are reviewed. An untapped potential for MEMS may lie in the area of nervous and endocrine system actuation, whereby the ability of MEMS to deliver potent drugs or hormones, combined with their precise temporal control, may provide new treatments for disorders of these systems.
Contents

• Implantable MEMS
  – Biosensors, Stents, Immunoisolation devices, Drug delivery systems
• Injectable MEMS
  – Microneedles, Injectable micromodules
• Biocompatibility and preservation of tissue function
  – MEMS material biocompatibility, Surface modification, MEMS technology for tissue engineering

Immunoisolation Devices

• Traditionally, polymer-based capsules are used for implantation of cells in an attempt to prevent immunorejection
• Semipermeable immunoisolation capsules are used, for example, to isolate implanted islet cells from the surrounding biological environment, while allowing the cells to remain viable and secrete insulin for treatment of diabetes
• Polymer-based capsules suffer from several disadvantages, including inadequate mechanical strength and a broad pore size distribution
• These factors can cause mechanical failure of the capsule and immunorejection due to diffusion of antibodies (usually IgG or IgM) and complement components through the membrane
Nano-Porous Silicon Membrane

Biocompatibility

- The biocompatibility requirements vary considerably depending on the device function and design
- Biocompatibility is defined as the ability of a material to perform with an appropriate host response in a specific application
- Biocompatibility is a surface-mediated property, and the biocompatibility of a device depends only on those materials in contact with tissue
On a macroscopic level, the surface of a metal implant may appear to be smooth, uniform, and inert. On the microscopic level, such a surface probably varies in chemical composition and topology and is the location of a number of dynamic molecule-surface interactions. A number of these molecule-surface interactions can have far-reaching physiological effects (initiating the process of coagulation, for example) that are relevant to the wound healing process and to the long-term viability of the implant.

**Examples**

- The performance of sensors (glucose, pH, etc.), for example, is limited by bio-fouling and isolation of the sensor surface.
- However, neural electrodes must remain in intimate contact with the neurons that they are stimulating or recording.
- The ISO 10993 standards outline minimum tests of material characterization, toxicity, and biodegradation that may be augmented depending on actual device usage.
ISO 10993

- The ISO 10993 series entail a series of standards for evaluating the biocompatibility of an implant prior to a clinical study.
- 1: Evaluation and testing
- 2: Animal welfare requirements
- 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity
- 4: Selection of tests for interactions with blood
- 5: Tests for in vitro cytotoxicity
- 6: Tests for local effects after implantation
- 7: Ethylene oxide sterilization residuals
- 8: Selection and qualification of reference materials for biological tests
- 9: Framework for identification and quantification of potential degradation products
ISO 10993

- 10: Tests for irritation and delayed-type hypersensitivity
- 11: Tests for systemic toxicity
- 12: Sample preparation and reference materials
- 13: Identification and quantification of degradation products from polymeric medical devices
- 14: Identification and quantification of degradation products from ceramics
- 15: Identification and quantification of degradation products from metals and alloys
- 16: Toxicokinetic study design for degradation products and leachables
- 17: Establishment of allowable limits for leachable substances
- 18: Chemical characterization of materials

Evaluated Materials

- Single crystal silicon (Si)
- Polycrystalline silicon (poly-Si), CVD
- Oxide (SiO₂), thermal oxidation of Si
- Silicon nitride (Si₃N₄), CVD
- Single crystal cubic silicon carbide (3C-SiC or β-SiC), CVD
- Titanium (Ti), PVD
- SU-8 epoxy photoresist, spin coating
Test Methods

• Subtle differences in processing and constituents can change the outcome of biocompatibility tests
• It is important to unambiguously characterize the material tested so that others can duplicate favorable outcomes
• This characterization can be accomplished by clearly describing the fabrication and handling of the test materials and by characterization of the specimens produced

Test Methods

• Materials characterization tests
• Aqueous physiochemical tests
• Biocompatibility tests
• Cytotoxicity
• Implantation
• Scanning electron microscopy
Types of Tests

- In vitro assays include leaching of material, corrosion testing, protein adsorption testing, and cell culturing on material samples
- In vivo biocompatibility assays typically involve the implantation of material or a device at the eventual site of use
- In vitro assays are easier to perform and provide more quantitative results, but in vivo assays are more relevant and can capture systemic effects
- The local and systemic responses are evaluated over days, weeks, or months
- In vivo tests can also exhibit variation due to implant shape, surface texture, and size

Results

- Only silicon nitride and SU-8 leached detectable nonvolatile residues in aqueous physiochemical tests, and only SU-8 leached detectable nonvolatile residues in isopropyl alcohol
- None of the materials were found to be cytotoxic in vitro using mouse fibroblasts
- All seven materials were classified as nonirritants based on 1- and 12-week rabbit muscle implantations
- There were few concerns about using any of these materials for implanted devices
PARYLENE FLEXIBLE NEURAL PROBE WITH MICRO FLUIDIC CHANNEL

COMPACT, SEAMLESS INTEGRATION OF ACTIVE DOSING AND ACTUATION WITH MICRONEEDLES FOR TRANSDERMAL DRUG DELIVERY
DETACHABLE SILICON MICRONEEDLE STAMPS FOR ALLERGY SKIN PRICK TESTING

Biodegradable Polymer Microneedles

[Images and graphs related to biodegradable polymer microneedles]
Surface Modification

• Although biocompatibility and tissue function preservation are of vital importance, the ultimate utility of many implantable MEMS devices may be limited by another device - biological environment issue - namely, biofouling
• The adsorption of biomolecules (peptides and proteins) followed by cells frequently leads to device fouling and failure
• Major research efforts are dedicated toward developing methods to substantially reduce the phenomenon and produce devices that do not promote bio-fouling, yet retain their bio-compatibility

Surface Modification

• This effort often takes the form of surface chemical modification
• Chemical modification to reduce biofouling falls into one of two general methods
• The first method uses surface immobilized polymers that reduce adsorption of biological materials
• The second method relies on the self-assembly process to passivate the MEMS device surface
Protein-Surface Interactions

TABLE 3.1. Properties of Proteins That Affect Their Interaction With Surfaces

<table>
<thead>
<tr>
<th>Property</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>Larger molecules can have more sites of contact with the surface</td>
</tr>
<tr>
<td>Charge</td>
<td>Molecules near their isoelectric point generally adsorb more readily</td>
</tr>
<tr>
<td>Structure</td>
<td></td>
</tr>
<tr>
<td>Stability</td>
<td>Less stable proteins, such as those with less intramolecular cross-linking, can unfold to a greater extent and form more contact points with the surface</td>
</tr>
<tr>
<td>Unfolding rate</td>
<td>Molecules that rapidly unfold can form contacts with the surface more quickly</td>
</tr>
</tbody>
</table>

TABLE 3.2. Properties of Surfaces That Affect Their Interaction With Proteins

<table>
<thead>
<tr>
<th>Feature</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topography</td>
<td>Greater texture exposes more surface area for interaction with proteins</td>
</tr>
<tr>
<td>Composition</td>
<td>Chemical makeup of a surface will determine the types of intermolecular forces governing interaction with proteins</td>
</tr>
<tr>
<td>Hydrophobicity</td>
<td>Hydrophobic surfaces tend to bind more protein</td>
</tr>
<tr>
<td>Heterogeneity</td>
<td>Nonuniformity of surface characteristics results in domains that can interact differently with proteins</td>
</tr>
<tr>
<td>Potential</td>
<td>Surface potential will influence the distribution of ions in solution and interaction with proteins</td>
</tr>
</tbody>
</table>
Blood-Surface Interactions

• The actual hierarchy of blood proteins on surfaces, however, is more complicated than this simplified, diffusion-limited example.

• Because of their affinity or additional kinetic factors (e.g., convection), molecules other than those with the highest concentration will also bind to the surface.

• Adsorption of proteins involved in blood clotting, such as fibrinogen and factor XII, has great importance for determining tissue-implant interactions.

### TABLE 3.3. Plasma Proteins With the Highest Concentration

<table>
<thead>
<tr>
<th>Protein</th>
<th>Concentration (mg/ml)</th>
<th>Molecular Weight</th>
<th>Diffusion Coefficient (10⁻⁷ cm²/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>40</td>
<td>66,000</td>
<td>6.1</td>
</tr>
<tr>
<td>IgG</td>
<td>15</td>
<td>150,000</td>
<td>4.0</td>
</tr>
<tr>
<td>α₂-Antitrypsin</td>
<td>3</td>
<td>54,000</td>
<td>5.2</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>3</td>
<td>340,000</td>
<td>2.0</td>
</tr>
<tr>
<td>Low-density lipoprotein (LDL)</td>
<td>3</td>
<td>5,000,000</td>
<td>5.4</td>
</tr>
<tr>
<td>α₂-Macroglobulin</td>
<td>3</td>
<td>725,000</td>
<td>2.4</td>
</tr>
<tr>
<td>Transferrin</td>
<td>2.6</td>
<td>77,000</td>
<td>5.0</td>
</tr>
<tr>
<td>IgA</td>
<td>2.3</td>
<td>162,000</td>
<td>3.4</td>
</tr>
<tr>
<td>α₂-Haptoglobins</td>
<td>2</td>
<td>100,000</td>
<td>4.7</td>
</tr>
<tr>
<td>High-density lipoprotein (HDL)</td>
<td>2</td>
<td>195,000</td>
<td>4.6</td>
</tr>
<tr>
<td>Complement 3</td>
<td>1.6</td>
<td>180,000</td>
<td>4.5</td>
</tr>
</tbody>
</table>

### TABLE 3.4. Rate of Arrival of the Proteins Listed in Table 3.3, Based on Diffusion-Limited Mass Transport

<table>
<thead>
<tr>
<th>Protein</th>
<th>C (µM)</th>
<th>D (10⁻⁷ cm²/s)</th>
<th>C/√D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>606</td>
<td>6.1</td>
<td>1,497</td>
</tr>
<tr>
<td>IgG</td>
<td>100</td>
<td>4</td>
<td>200</td>
</tr>
<tr>
<td>α₂-Antitrypsin</td>
<td>56</td>
<td>5.2</td>
<td>127</td>
</tr>
<tr>
<td>Transferrin</td>
<td>34</td>
<td>5</td>
<td>76</td>
</tr>
<tr>
<td>α₂-Haptoglobins</td>
<td>20</td>
<td>4.7</td>
<td>43</td>
</tr>
<tr>
<td>IgA</td>
<td>14</td>
<td>3.4</td>
<td>26</td>
</tr>
<tr>
<td>High-density lipoprotein (HDL)</td>
<td>10</td>
<td>4.6</td>
<td>22</td>
</tr>
<tr>
<td>Complement 3</td>
<td>9</td>
<td>4.5</td>
<td>19</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>9</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>α₂-Macroglobulin</td>
<td>4</td>
<td>2.4</td>
<td>6</td>
</tr>
<tr>
<td>Low-density lipoprotein (LDL)</td>
<td>1</td>
<td>5.4</td>
<td>1</td>
</tr>
</tbody>
</table>

### TABLE 3.5. Exchange Hierarchy of Plasma Proteins on Glass and Metal Oxide Surfaces

<table>
<thead>
<tr>
<th>Protein</th>
<th>Adsorbs First</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen</td>
<td></td>
</tr>
<tr>
<td>Fibronectin</td>
<td></td>
</tr>
<tr>
<td>Factor XII</td>
<td></td>
</tr>
<tr>
<td>High-molecular-weight kininogen</td>
<td></td>
</tr>
</tbody>
</table>
Blood Compatibility of Polyethylene Oxide Surfaces


Protein Resistance

- Possible mechanisms
  - Hydrophilicity
  - Steric stabilization effect
  - Chain mobility
**Steric Stabilization Effect**

- The repulsive forces by the adsorbed PEO chain are generated by the loss of possible chain conformations, as the volume available to the adsorbed chains is reduced between approaching surfaces.
- PEO shows a large excluded volume in water and thus is very effective for steric repulsion.

**Chain Mobility**

- For an irreversible adhesion, proteins should be in contact with a foreign surface more than a certain measure of time.
- PEO shows high mobility and hydration in water.
- Rapidly moving hydrated PEO chains on a surface will effectively prevent stagnation of the proteins on the surface, probably because the contact time is shortened.
- The mobility of the hydrated PEO chains increases with their chain length up to about 100; hence the long PEO chains are supposed to suppress the adsorption of proteins more effectively than shorter chains.
A Precision Technology for Controlling Protein Adsorption and Cell Adhesion in BioMEMS

K.F. Böhringer’s Group, U. Washington
MEMS 2001

Abstract

A surface coating technique is investigated to enhance device biocompatibility by eliminating biofouling, the strong but nonspecific affinity of proteins and cells to attach to surfaces. This coating is a conformal, thin poly(ethylene glycol)-like film deposited in a glow discharge of tetraglyme. Substrates with different chemistries are successfully modified, and exhibit ultralow protein adsorption and cell attachment with the coating. This stealth or non-fouling coating can also be faithfully patterned using standard photolithography processes. The interaction of proteins and cells with patterned surfaces is limited only to the protein-adhesive domains, thus creating heterogeneous patterns of proteins and cell cultures on the surface. The potential benefits of our technique to applications such as cell-based assays and micro-electrodes are discussed.
Plasma Polymerization

Results

Au  PEG-like pp4G

SiO₂

SiO₂
Surface Modification and Modulation in Microstructures: Controlling Protein Adsorption, Monolayer Desorption, and Micro-Self-Assembly

K.F. Böhringer’s Group, U. Washington
J. Micromech. Microeng., 2003

Abstract

The surface-to-volume ratio increases with decreasing scale, thus, controlling and changing the surface properties of microstructures can be a powerful tool in the design, fabrication and use of microsystems. This paper overviews several recent projects that utilize the modulation of surfaces from hydrophobic to hydrophilic and vice versa, or from protein adsorbing to non-fouling, with applications in biomedical microdevices and self-assembling MEMS.
Programmable Surfaces

- Bio-fouling: the tendency of biological substances (proteins, cells, bio-films, etc) to attach to exposed surfaces
- Poly-N-isopropylacryl-amide (pNIPAM): a transition from hydrophilic and non-fouling behavior at room temperature to hydrophobic and fouling behavior above its lower critical solution temperature (LCST)

Principles

- Microheaters on a glass slide coated with ppNIPAM
- Active heater turns the surface hydrophobic and fouling
- Selective protein adsorption occurs exclusively on heated areas
Ligand Assay

• Ligand
  – Small molecule that binds to a site on a macromolecule's surface by intermolecular forces
  – Principle of complementarity
• Specific binding between
  – Probe: known
  – Target: undetermined
  – Antibody-antigen
  – Drug target – drug
• Ligand assay
  – rely on observation of the **product(s)** of the binding reaction

Ligand Assay

• Immobilization
• Miniaturization
• Parallelization
• Sampling
• Detection
Ligand Assay

Planar Arrays
- GHI
- DEF
- ABC

Bead Based Arrays
- ID of Capture Agent
- ID of Analyte

Data & Analysis
- Fluorescence
- Radioactivity

DNA Microarray

1. Photolithographic DNA patterning

2. Ink jet DNA patterning
Nanoparticle-Based DNA Detection

By studying the materials properties of nanoparticles functionalized with DNA, it is feasible to identify many novel structures, which are proving useful in new biodetection schemes.

Protein Immobilization

- **Solid foundations**
- **Do not interfere with its binding activity**
- ** Orientated so that binding sites are accessible to interacting partners**
- The optimal immobilization strategy is one in which the ligand is presented to its binding partners in a form that allows the conditions of the interaction to closely mimic the in vivo microenvironment.
Amino Acids

• All 20 of the common amino acids are α-amino acids
• They have a carboxyl group and an amino group bonded to the same carbon atom
• They differ from each other in their side chains, or R groups, which vary in structure, size, and electric charge, and which influence the solubility of the amino acids in water

\[
\begin{align*}
\text{H}_3\text{N} & \text{- C - H} \\
\text{R} & \\
\text{COO}^- & 
\end{align*}
\]

Peptides and Proteins

• Polymers of amino acids
• Biologically occurring polypeptides range in size from small to very large, consisting of two or three to thousands of linked amino acid residues
• Two amino acid molecules can be covalently joined through a substituted amide linkage, termed a peptide bond, which is formed by removal of the elements of water (dehydration) from the -carboxyl group of one amino acid and the –amino group of another

\[
\begin{align*}
\text{H}_3\text{N} - \text{CH}\text{C}-\text{OH} & \overset{\text{H}_2\text{O}}{\longrightarrow} \text{H}_2\text{O} \\
\text{H}_3\text{N} - \text{CH}\text{C}-\text{NH} - \text{CH} - \text{COO}^- \\
\text{R}^1 & \text{H} \text{ R}^2 \\
\text{R}^1 & \text{ R}^2 \\
\end{align*}
\]
Proteins

Antibody and Antigen

• The proteins at the heart of the humoral immune response are antibodies
• Any molecule or pathogen capable of eliciting an immune response is called an antigen
Antibody Structures

- Antibodies are immune system related proteins called immunoglobulins.
- Each antibody consists of four polypeptides: two heavy chains and two light chains joined to form a "Y" shaped molecule.

Enzyme-Linked ImmunoSorbent Assay (ELISA)

1. Coat surface with sample (antigens).
2. Block unoccupied sites with nonspecific protein.
3. Incubate with primary antibody against specific antigen.
4. Incubate with antibody-enzyme complex that binds primary antibody.
5. Add substrate.
6. Formation of colored product indicates presence of specific antigen.
Immobilization of Oriented Protein Molecules on Poly(Ethylene Glycol)-Coated Si(111)

X.Y. Zhu’s Group, U. Minnesota Proteomics, 2004

Abstract

A high-density poly(ethylene glycol) (PEG)-coated Si(111) surface is used for the immobilization of polyhistidine-tagged protein molecules. This process features a number of properties that are highly desirable for protein microarray technology: (i) minimal nonspecific protein adsorption; (ii) highly uniform surface functionality; (iii) controlled protein orientation; and (iv) highly specific immobilization reaction without the need of protein purification. The high-density PEG-coated silicon surface is obtained from the reaction of a multi-arm PEG (mPEG) molecule with a chlorine terminated Si(111) surface to give a mPEG film with thickness of 5.2 nm. Four out of the eight arms on each immobilized mPEG molecule are accessible for linking to the chelating iminodiacetic acid (IDA) groups for the binding of Cu21 ions. The resulting Cu21-IDA-mPEG-Si(111) surface is shown to specifically bind 6x histidinetagged protein molecules, including green fluorescent protein (GFP) and sulfotransferase (ST), but otherwise retains its inertness towards nonspecific protein adsorption. We demonstrate a particular advantage of this strategy: the possibility of protein im-mobilization without the need of pre-purification ...
Reaction Scheme

(a) 40% NH$_4$F, 10 min, 25°C (b) Cl$_2$ gas, 20 min, 100°C (c) mPEG, overnight, vacuum, 15°C (d) DSC, DMAP, DMF, 6 h, 25°C (e) DEIDA, DMAP, DMF, overnight, 25°C (f) BBTO, diethyl ether, 6 h, 25°C (g) CuSO$_4$, ethanol, 20 min, 25°C (h) 6x His-tagged protein incubation

Surface-Enhanced Laser Desorption Ionization

Ciphergen Biosystems, Inc.
Process Flow

- **Choosing an array**
- Arrays are available with different chromatographic properties, including hydrophobic, hydrophilic, anion exchange, cation exchange, and immobilized-metal affinity surfaces
- Other arrays with pre-activated surfaces are available for covalently coupling protein, DNA, RNA or other bait molecules by the user

Process Flow

- **Sample application**
- Crude biological samples such as serum, cell lysates or other protein preparations, including those with high salt or detergent concentrations, can be applied directly
- Application can be done manually by pipetting or by employing laboratory automation station
- The arrays are formatted with robot-friendly spot spacing and a bioprocessor rack of 12 arrays forming a standard microplate footprint
**Process Flow**

- **Removal of unbound components**
- After a short incubation period, unbound proteins are washed off the surface of the array
- Only proteins interacting with the chemistry of the array surface are retained for analysis
- After washing, energy absorbing molecules are applied to the array as a final step

**Process Flow**

- **Analysis in the reader**
- Arrays are then analyzed in the reader, a time-of-flight mass spectrometer
- The mass values and signal intensities for the detected proteins and peptides can be viewed in several formats and then transferred to software suites for further in-depth analysis
Principles

Surface Plasmon Resonance

Biacore International AB
Surface Plasmon Resonance

- An electron charge density wave phenomenon that arises at the surface of a metallic film when light is reflected at the film under specific conditions
- The resonance is a result of energy and momentum being transformed from incident photons into surface plasmons, and is sensitive to the refractive index of the medium on the opposite side of the film from the reflected light
- Used to monitor interactions occurring in a bio-specific surface on a metal layer by measuring changes in the solute concentration at this surface as a result of the interactions

金屬可視為由自由電子與不能移動的正離子晶格所組成的電漿系統，因此整體的電子會往左右的來回振盪。
Total Internal Reflection (TIR)

- Occurs at an interface between non-absorbing media
- When a light beam propagating in a medium of higher refractive index meets an interface at a medium of lower refractive index at an angle of incidence above a critical angle, the light is totally reflected at the interface and propagates back into the high refractive index medium.

Evanescent Field Wave

- Although the fully reflected beam does not lose any net energy across the TIR interface, the light beam leaks an electrical field intensity called an evanescent field wave into the low refractive index medium.
- The amplitude of this evanescent field wave decreases exponentially with distance from the interface, decaying over a distance of about one light wavelength from the surface.
1. 光波以適當角度($\theta$)入射時，可在玻璃與金屬薄膜介面引發Total Internal Reflection現象，產生可傳入金屬薄膜的Evanescent Field Wave (Kx)
2. 當共振(resonance)發生時，入射光被金屬所吸收，使反射光能量急劇下降，介面的全反射條件將被破壞，呈現衰減全反射現象

Principles

- An increased sample concentration in the surface coating of the sensor chip causes a corresponding increase in **refractive index** which alters the angle of incidence required to create the SPR phenomenon (the SPR angle)
- This SPR angle is monitored as a change in the detector position for the reflected intensity dip (from I to II)
角度：固定波長，改變角度（較常用）
波長：調節角度，改變波長

Example: A monolayer of cytochrom c leads to an angular shift of ~0.5 Deg. The corresponding mass coverage is ~3000 pg/mm². For an angular sensitivity of 0.1 mDeg, the corresponding mass sensitivity is 0.6 pg/mm² or 0.6 RU.

Sensorgram

• By monitoring the SPR-angle as a function of time the kinetic events in the surface are displayed in a sensorgram
Advantages and Disadvantages

- Allows to study interactions between biomolecules in real-time
- No need for labeling
  - Time saving
  - No change of the native condition of the molecule
- Molecules is not destroyed, can be collected and analyzed by other techniques
- Expensive
- Sensitivity depends on optical thickness of absorbed layer, small molecules cannot be measured in low concentration
- Only interactions predictions possible, no information of the 3D structure

Quartz Crystal Microbalance

- Extreme sensitivity of piezoelectric devices towards mass changes at the surface of QCM electrodes
  \[ \Delta f = - C_f \cdot \Delta m \]
- \( \Delta f \): the observed frequency change, in Hz
- \( \Delta m \): the change in mass per unit area, in g/cm\(^2\)
- \( C_f \): the sensitivity factor for the crystal used (i.e. 56.6 Hz µg\(^{-1}\) cm\(^2\) for a 5MHz AT-cut quartz crystal at room temperature)
Abstract

We report the specific transduction, via surface stress changes, of DNA hybridization and receptor-ligand binding into a direct nanomechanical response of microfabricated cantilevers. Cantilevers in an array were functionalized with a selection of biomolecules. The differential deflection of the cantilevers was found to provide a true molecular recognition signal despite large nonspecific responses of individual cantilevers. Hybridization of complementary oligonucleotides shows that a single base mismatch between two 12-mer oligonucleotides is clearly detectable. Similar experiments on protein A-immunoglobulin interactions demonstrate the wide-ranging applicability of nanomechanical transduction to detect biomolecular recognition.
Procedure

- Synthetic 5’ thio-modified oligonucleotides with different base sequences were **covalently** immobilized on the **gold-covered** side of the cantilevers.
- The complementary 16-mer oligonucleotide solution was injected into the liquid cell followed by injection of complementary 12-mer oligonucleotide solution.
- This results in a **difference in surface stress** between the functionalized gold and the nonfunctionalized Si surface, which bends the cantilever.
Results

• Signals from individual cantilevers displayed drifts of several tens of nm during equilibration.
• Injections of liquid resulted in spikes of up to 100 nm in amplitude, which are ascribable to turbulences.
• Followed by an immediate increase of the signal by ~50 nm and by a fast relaxation process.

Results

• These large, unspecific signals from individual cantilevers can be removed by extracting the differential signal from two cantilevers.
• Because all cantilevers of an array are physically identical, the differential signal is sensitive only to the individual cantilevers’ ability to recognize complementary oligonucleotides.
• Unspecific binding bends the cantilevers in parallel, leading to no overall differential signal.
Results

- Differential signal of a hybridization experiment, showing detection of a single base mismatch in 12-mer oligonucleotides

Multiple Label-Free Biodetection and Quantitative DNA-Binding Assays on a Nanomechanical Cantilever Array

R. McKendry, et al., IBM Research
PNAS, 2002
Abstract

We report a microarray of cantilevers to detect multiple unlabeled biomolecules simultaneously at nanomolar concentrations within minutes. Ligand-receptor binding interactions such as DNA hybridization or protein recognition occurring on microfabricated silicon cantilevers generate nanomechanical bending, which is detected optically in situ. Differential measurements including reference cantilevers on an array of eight sensors can sequence-specifically detect unlabeled DNA targets in 80-fold excess of nonmatching DNA as a background and discriminate 3 and 5 overhangs. Our experiments suggest that the nanomechanical motion originates from predominantly steric hindrance effects and depends on the concentration of DNA molecules in solution. We show that cantilever arrays can be used to investigate the thermodynamics of biomolecular interactions mechanically, and we have found that the specificity of the reaction on a cantilever is consistent with solution data. Hence cantilever arrays permit multiple binding assays in parallel and can detect femtomoles of DNA on the cantilever at a DNA concentration in solution of 75 nM.

Methods

- Microfabricated arrays of eight identical silicon cantilevers with 250-μm pitch and a spring constant of 0.02 N/m
- Coated on one side with a 2-nm titanium adhesion layer followed by 20 nm of gold
- The functionalization of each cantilever with a different thiolated probe sequence was performed in parallel and under identical conditions by using microcapillaries
Results

• The cantilevers are deflected but will regain their equilibrium within minutes
• Washing the array with dehybridization agents dehybridized the specifically interacting oligos, and then the array is ready for the next cycle
• The experiments demonstrate that the differential bending is clearly sequence-specific and provides an unambiguous yes or no response
NANOMECHANICAL PROTEIN DETECTORS
USING ELECTROTHERMAL NANO-GAP ACTUATORS