Bioconjugate Materials: Nanopatterns of Biomolecules on Surfaces

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Topic of Today’s Lecture

This lecture will focus on nanomaterials research, specifically combining NANO and BIO on surfaces
UCLA
Department of Chemistry and Biochemistry provides exciting opportunities for graduates and postdocs for collaborative research at the interface of chemistry and materials
California NanoSystems Institute

Established by the State of California in 2000

Interdisciplinary research and education focused on nanotechnology

Joint Institute between UCLA and UCSB
This lecture will focus on nanomaterials research, specifically combining NANO and BIO on surfaces.
What is Nano?

• Nanoscience is the study of objects measured in nanometers
  – 1-billionth of a meter
  – ~80,000 times smaller than the diameter of a single human hair
Closer Look at a Human Hair

Width of this line is 100 nm

http://www.aber.ac.uk/bioimage/image/uwbl-0411-w.jpg
What is Nano?

• Nanoscience is the study of objects measured in nanometers
  – 1-billionth of a meter
  – ~80,000 times smaller than the diameter of a single human hair
  – New properties emerge at the nanoscale
    • Size and shape matter
Super-Repellent Nano-Materials

http://cjmems.seas.ucla.edu/members/changhwan/main.html
http://www.engineer.ucla.edu/magazine/fall06/noslip.html
Geckos Walk on Walls
Nano-Finger Tips Allow Geckos to Stick

http://robotics.eecs.berkeley.edu/~ronf/Gecko/index.html
Man-Made Geckos
Super Adhesive Nano-Materials

Synthetic nano-materials can exhibit strong adhesion similar to gecko fingers

Topic of Today’s Lecture

This lecture will focus on nanomaterials research, specifically combining NANO and BIO on surfaces.
Protein
Protein comes from Greek word proteios meaning primary.

Proteins serve many different functions:

- **Hemoglobin** carries oxygen through the body.
- **Melanin** gives skin pigmentation and the iris color.
- **Keratin** provides structure of hair and nails.
- **Serum Albumin** maintains blood pressure.
- **Alcohol Dehydrogenase** breaks down alcohol in the liver.

Examples:

http://en.wikipedia.org/wiki/Protein
Why Nano and Bio on Surfaces

Diagnostics

– Achieve greater sensitivity
– Simultaneous detection of multiple disease markers

~10 nm  ~1 µm

Biomaterials

– Better mimicry of extracellular matrix
   (control of cell differentiation and behavior)
How to Pattern and Critical Features

Diagnostics, biomaterials, tissue engineering and most applications require bioactive proteins on the surface

Fully active proteins are especially important for nanoscale patterns of proteins

Chemoselective reactions that occur under mild, aqueous conditions with out the addition of reagents are important

Many Techniques to pattern:
- scanning probe techniques
- stamping
- self assembly
- lithography:
  e-beam, photolithography

Outline

• Overview of techniques to pattern biomolecules at the nanoscale

• Example 1: Multiprotein patterns by e-beam lithography

• Example 2: Cell adhesive materials
Outline

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Introduction to AFM

A feedback system to control the vertical position of the tip

A coarse positioning system to bring the tip into the general vicinity of the sample

A probe tip

A piezoelectric scanner which moves the sample under the tip (or the tip over the sample) in a raster pattern

A computer system that drives the scanner, measures data and converts the data into an image.
SAMS (Self Assembled Monolayers)

Alkane thiol: $\text{HS}(-\text{CH}_2)_9\text{X}$
AFM, Nanografting
Nuraje, et al. JACS, 2004, 126, 8088-8089
AFM, Dip Pen Lithography

MHA = mercapto-hexadecanoic acid
Au = gold
SA = streptavidin
R = BSA
EG$_3$-SH = 11-mercaptop-undecyltri(ethylene glycol)
Why Patterns of Streptavidin

Biotin-streptavidin complex
Freitag, S. et al., *Protein Science* 1997, 6, 1157

- Streptavidin binds four biotins with high affinity ($K_a = 10^{15}$)
- Used as adapter molecule for many applications

Patterns of streptavidin are an excellent platform for further elaboration because many biotinylated molecules are available
Bovine Serum Albumin (BSA) as a Model Protein

- Conjugation to BSA
  - Most common protein in blood
  - One free cysteine

Carter & Ho, *Protein Chem.* 1994, 45, 153-204
Electron Beam (E-beam) Lithography
NanoStamping
Coyer, S. R. et al.  

---

**a)** planar elastomer inked with proteins

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<table>
<thead>
<tr>
<th>Elastomer</th>
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<tbody>
<tr>
<td>Nanotemplate</td>
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**b)** contact and release generates pattern by subtraction

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**c)** contact and release prints protein pattern to substrate

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<table>
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<tr>
<td>Substrate</td>
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**d)**

![Image](image_url)

Clean and reuse nanotemplate

10 μm
Self Assembly - DNA
Yan, et al. Science 2003, 301, 1882-1884
Self Assembly - Proteins
Outline

• Overview of techniques to pattern biomolecules at the nanoscale

• Example 1: Multiprotein patterns by e-beam lithography

• Example 2: Cell adhesive materials
Experimental Approach


**Our approach:**
-Synthesize 8-arm star PEGs with groups that can bind to specific sites on proteins and cross-link to the surface using electron beams.
Site Specific Conjugations

**Biotin – Streptavidin:**

(Freitag, S. et al., *Protein Science* 1997, 6, 1157)

- Streptavidin binds four biotins with high affinity

**Maleimide – Free Cysteines**

- Maleimide reacts selectively with cysteines not in disulfide bonds
More Site-Specific Conjugations

Ketone - Aminooxy

- N-terminal α-oxoamide protein binds to aminooxy to form oxime bond

NTA-Ni$^{2+}$ - Histidine

Proteins modified with His-Tags

- Histidine tagged proteins bind to Ni$^{2+}$ - NTA
Polymers for Site Specific Protein Conjugation

**Biotin Star**

\[
\text{PEG Star} \quad \overset{\text{Sulfo-NHS-LC-Biotin}}{\longrightarrow} \quad \text{Biotin Star}
\]

\(\text{Mn} = 10,000; 8 \text{ arms}\)

91% substitution by NMR


**Maleimide Star**

\[
\text{PEG Star} \quad \overset{\text{Sulfo-SMCC}}{\longrightarrow} \quad \text{Maleimide Star}
\]

\(\text{Mn} = 10,000; 8 \text{ arms}\)

100% substitution by NMR

**Aminooxy Star**

\[
\text{PEG Star} \quad \overset{\text{PPh}_3, \text{DIAD}}{\longrightarrow} \quad \text{Aminooxy Star}
\]

\(\text{Mn} = 20,000; 8 \text{ arms}\)

97% substitution by NMR

Polymers for Site Specific Protein Conjugation

NTA Star

PEG Star

\[ \text{maleimido-C3-NTA} \]

\[ \text{H}_2\text{O}:\text{MeOH (3:1)} \]

\[ 23^\circ \text{C}, 12\text{h} \]

Mn = 10,000; 8 arms

100% substitution by NMR
Experimental Approach

E-beam: accelerating voltage 30kV, current 4.5 pA, dose 1.1 - 140 µC/cm²

-E-beam induced cross-linking produces patterns of functional groups
Micron-Sized Arrays of Single Proteins

- SAv bound by ligand binding sites (biotin)
- BSA Michael addition of free thiol to maleimide
- α-glyoxylamide-modified myoglobin binds via oxime bond formation
- Histidine-tagged calmodulin binds to nickel (II) surface (top - SEM before protein adsorption)

All reactions are under mild aqueous solutions and do not require additional reagents that can lead to protein denaturation or reduced activity
Multicomponent Nanopatterns

For many desired applications, multiple proteins are required

Yet this is difficult to achieve

Can we utilize e-beam lithography to achieve this? With e-beams, nanoscale spacings are possible.

Pattern PEGs with orthogonal reactivity side-by-side
Multiple Proteins by E-beam Lithography

E-beam-induced cross-linking of biotin-PEG and maleimide-PEG, followed by modification with SAv and BSA proteins

Simultaneous immobilization of multiple proteins from mixtures at the micron and nanometer scale

Dimension 3100 (Digital Instruments) AFM in tapping mode: silicon cantilever, spring constant = ~ 40 N/m, tip radius = < 10 nm, scan rate = 1.5 Hz
Multilayer Three-Dimensional Patterning

PEG can be cross-linked to itself

Can we use this strategy to prepare 3D multilayer patterns of multiple proteins?

This would be interesting to produce multiplexed biomolecules in three-dimensional multilayer formats for a wide variety of applications such as site-isolation enzyme cascades, “nanoscale factories,” mimic natural complex structures such as protein-signaling assembles and viral capsids, present chemical and topographical cues to study and control cell adhesion
Simultaneous immobilization of SAv and BSA from a mixture
Other Proteins

Range of multicomponent, multilayer nanoscale patterns are possible

Modified with SAv & α-glyoxylamide-myoglobin
Three-Component Structures

Modified with Sav, BSA, & α-glyoxylamide- myoglobin

Complex patterns with multiple proteins and different topographies are readily prepared

Start to explore biological questions utilizing these strategies

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• Overview of techniques to pattern biomolecules at the nanoscale

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• Example 2: Cell adhesive materials
Protein adsorption

• Results in bioactive surfaces that mediate cell attachment

• Causes attachment of cells
  – Sometimes advantageous: osteoblasts in a bone implant
  – Sometimes disadvantageous: platelets on the lining of an vascular graft

• How and why do cells attach to these surfaces?
Cell Attachment

- Adsorbed adhesion proteins such as fibronectin, fibrinogen, and vitronectin

- Cells attach to adsorbed proteins as they do to native extracellular matrix (ECM) proteins
There are five principal classes of cell-adhesion molecules (CAMs)

Figure 22-2
Integrins

A family of membrane glycoproteins that bind to collagen, laminin, fibronectin and other ECM components.

Cell Surface Receptors for ECM Constituents

Involved in cell adhesion, migration, survival, growth, differentiation, and gene expression
Structure of Integrins

Each consists of two different transmembrane polypeptides, α and β subunits.

Extracellular binding sites recognize RGD and other parts of glycoproteins.

The intracellular portions of integrins have the binding sites for cytoskeleton molecules.

Intracellular cytoskeleton and extracellular matrix are integrated by integrins.
Receptors

- Specific amino acid sequences in ECM molecules bind to cell surface receptors (integrins)
  
  - arginine-glycine-aspartic acid (RGD) tripeptide: 1\textsuperscript{st} discovered in fibronectin (Pierschbacher and Ruoslahti, 1984)
Fibronectin

Soluble plasma and fibrillar ECM protein

Fibrin – blood clotting protein

Heparin – anti-clotting protein

RGD binding sequence

There are separate domains for Type I, II, and III collagen
The influence between cytoskeleton and ECM is mutual.

By binding to integrin, fibronectin can trigger the reorganization of cytoskeleton inside the cell, which affects cell shape and motility.

Intracellular cytoskeleton can also influence the attachment and orientation of ECM.
Cell Adhesion to Surfaces via Integrins

Focal Adhesion Formation – Integrin Clustering

Petit & Thiery, Biology of the Cell, 92 (2000), 477-494
Focal Adhesions & Stress Fibers

Petit & Thiery, Biology of the Cell, 92 (2000), 477-494
Bioengineering Surfaces

- Coat surfaces with ECM molecules (for example, fibronectin)

- Design ligands and ligand-bearing surfaces to optimize attachment (and/or cell function) by mimicking the ECM
Integrated Implant Materials

Integrated implant - elicit cells to adhere

Adhesion factor

An inert surface allows one to control the biological response
RGD-Promotes Cell Adhesion

- Soluble peptide inhibits cell adhesion to fibronectin
- Surfaces coated with RGD peptide promote cell attachment and spreading
  - Utilized in numerous biomaterials

\[
\text{Gly-Arg-Gly-Asp} \\
\text{GRGD}
\]
Relevant Scales of Cell Adhesion

Nanoscale presentation of ligands is critical for cell adhesion – yet few examples
Self Assembly - Polymers

Au nanoparticle (3-8 nm)
Table 1. *Surface-pattern and cell-adhesion characteristics.*

<table>
<thead>
<tr>
<th>PS(x)-b-P2 VP(y)[^{[a]}]</th>
<th>Au dot diameter [^{[b]}][nm]</th>
<th>Au dot separation [^{[c]}][nm]</th>
<th>Au dot density [^{[d]}][dots/ m²]</th>
<th>Cell spreading[^{[e]}]</th>
<th>Focal adhesion formation[^{[e]}]</th>
<th>Actin fiber formation[^{[e]}]</th>
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<td>90[^{[f]}]</td>
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Self Assembly or E-beam lithography – Polymers to probe cell adhesion at the nanoscale
Cell Adhesion to Surfaces via Integrins


Pattern components of the extra cellular matrix (ECM): peptide RGD and polysaccharide heparin
Fibronectin—Primary Component of ECM

- RGD peptide binds to cells via cellular integrins
- Heparin is polysaccharide found in the ECM and on cell surfaces that binds to growth factors such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF)
Stable Heparin Mimics by RAFT

M:CTA:I = 100:2:1, DMF:H₂O = 1:1

PSS:PEGMA
Feed: 2:1
Polymer: 2.2:1

Mₙ (GPC) = 24,000
PDI = 1.17

Polymer readily synthesized and reduced to free thiol
Sulfonated Polymer Binds bFGF & VEGF

Modify gold surfaces with polymer for surface plasmon resonance (SPR) studies

SPR results demonstrate that both bFGF and VEGF bind to polystyrene sulfonate via the heparin binding domain
Pattern Heparin Mimic Polymer

Utilize e-beam radiation to cross-link heparin mimic polymer to surface at the micron scale:

dose of 1100 $\mu$C/cm$^2$
beam current 4.8 pA

Features = 5 x 5 $\mu$m$^2$
VEGF and bFGF can be patterned at the micron and nanometer scale using e-beam lithography on a heparin mimicking polymer.

Topic of Today’s Lecture

Combining NANO and BIO on surfaces provides exciting opportunities for engineering development, as well as application
Acknowledgements

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