Lecture 10: Bioengineering applications of hydrogels: Molecular Imprinting and Drug Delivery

Last Day: polyelectrolyte gels  
Polyelectrolyte complexes and multilayers  
Applications in bioengineering  
Theory of ionic gel swelling

Today: Molecular imprinting  
Hydrogels in drug delivery

Supplementary Reading:  

Molecular Imprinting\textsuperscript{1,2}

Concepts of molecular imprinting

- Molecular imprinting is the design of polymer networks that can recognize a given target molecule and bind it preferentially in the presence of an excess of irrelevant molecules, some of which may have very similar molecular structures  
  - Seeks to mimic specificity in biological recognition obtained through protein-protein interactions  
- Steps to the preparation of molecularly-imprinted networks:
  1. mixing of binding monomers and target molecule  
     - target can be mixed directly with liquid monomers in bulk or co-dissolved in a non-interfering solvent  
     - monomers bind target  
       - covalent interactions  
       - non-covalent bonding  
       - metal coordination  
     - mixture usually at high concentration (e.g. 50% w/vol solutions): enforces close interactions of target with binding monomers and leads to a tight network that holds the position of functional groups in position of template binding  
  2. polymerization of monomers in place  
     - usually photopolymerization (rapidly ‘trap’ structure)  
  3. washing for removal of target molecule from network pockets
• types of target molecules:
  o small-molecule drugs
  o steroids
  o nucleic acids
  o amino acids
  o metal ions
  o proteins

Structure of Molecularly-Imprinted Networks

• structure of molecularly-imprinted networks
  o imprinted networks can be confined to a thin surface layer or prepared in bulk
    o surface networks usually perform better for capture of large molecules like proteins

• simple synthetic components for recognition networks
  o monomers:
    o methacrylic acid
    o itaconic acid
    o acrylamides
    o 4-vinyl pyrrolidone
    o β-cyclodextrin
    o other designed monomers
  o cross-linkers
    o ethylene glycol dimethacrylate
    o PEG dimethacrylate
  o ‘chain effect’
    o binding of monomers to macromolecular templates causes a reduction in chain termination and thus an overall increase in reaction rate

• Example of molecular recognition: molecular imprinting of D-glucose (Peppas)
  o Monomers chosen as analogs of the amino acid residues that bind to glucose in vivo:
    o WHAT RECEPTORS BIND GLUCOSE?
      • Aspartate
• Glutamate
• Asparagines
• Serine
  o Draw structures on board
  o Simple synthetic monomers chosen to mimic the bonding interactions of these amino acids:

![Structures](image)

Specificity of binding:

![Graph](image)

**Issues:**

Tightly cross-linked networks hold functional group positions for better recognition but restrict entry of target into network

Limited complexity in recognition units

- Improving recognition by surface templating (Ratner\textsuperscript{4,5})
  o Protein adsorbed to mica surface, coated with disaccharide, then coated with C\textsubscript{3}F\textsubscript{6} film by radiofrequency glow-discharge plasma treatment
  o Sugar coating protects protein from denaturation on dehydration
**Trehalose (disaccharide)**

**Resulting recognition:**

- LSZ = lysozyme
- LSZ in solution can exchange with imprinted LSZ, but Rnase cannot displace LSZ on surface

**Utilizing in-situ formability of photopolymerized hydrogels for lab-on-a-chip applications**

**Photopolymerized Bulk templates (Peppas):**

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**Figure 1:** Protocol for template imprinting of proteins. (See refs. 18 and 20 for details. a) Template protein was adsorbed onto a freshly cleaved mica in ethanol-propanol-water solution (96:24:2, v/v/v) at 4°C for 1 h. b) Imprint was spin-coated from a 10% (w/v) polymer solution. The sample was put into the in-grow chamber of a 200MHz R-64 reservoir. Plasma deposition of OPA was conducted at 60°C for 5 min, forming a 100-nm-thick plasma polymer film. The resulting plasma film was used to grip the protein onto the imprint surface. c) A tapping-mode AFM image of the surface of a fibrinogen imprint, together with a drawing of fibrinogen. d) Mechanism for the specific protein recognition in protein-imprinted surfaces. A specificity-bound template protein is prevented from exchange with other protein molecules in the solution because of specific interactions and overall strong interaction. The latter is due to many cooperative van der Waals forces and hydrophilic interactions for example.
Plasma-deposited surface templates patterned by microcontact printing (Ratner):

Figure 4: Visual demonstration of template recognition by imprints of protein mixtures patterned with microcontact printing. a. BSA-anti-BSA (SA) in PBS buffer (pH 7.4) was adsorbed onto a poly(dimethylsiloxane) (PDMS) stamp that has micrometre-scale circular indentations, followed by a buffer and water rinse and drying under a stream of nitrogen. The stamp was printed onto a mica surface for ~5 s, transferring a monolayer of streptavidin to mica only in the contacted regions. The mica surface was then exposed to antibody (BSA) in PBS.

This adhesion block the uncovered surface area. A template imprint of the SA-BSA patterned surface was prepared according to Fig. A. Binary protein adsorption onto the imprint was performed from a SA-BSA (1:1) solution in PBS for 5 s. The imprint was incubated in a solution of biotin-BSA labeled with 10 nm colloidal gold to reveal the surface distribution of adsorbed streptavidin with AFM.
Hydrogels in drug delivery

- What

**Control of drug release kinetics by hydrogel structure**

- Release from stable hydrogels is controlled by diffusion of solute through the network
- Diffusion is described by Fick’s second law:

\[
\frac{\partial C}{\partial t} = D_{gel} \frac{\partial^2 C}{\partial x^2}
\]

- Recall the solution to Fick’s second law for a semi-infinite slab contacting a perfect sink:

\[
\frac{c_0 - c(x)}{c_0} = 1 - \text{erf} \left( \frac{x}{2\sqrt{D}t} \right)
\]

- Diffusion of drugs through a network is controlled by the mesh size (ζ)
The mesh size is related to the network swelling $Q$ and the end-to-end distance between cross-links:

$$<r_0^2>^{1/2} = N_c^{1/2}a$$

Eqn 3

- ...assuming a polymer chain that has 2 carbon-carbon bonds per repeat unit
- derived from random walk chain statistics
  - Where $l$ is the bond length in the polymer backbone
  - $M_c$ is the molecular weight between cross-links
  - $M_0$ is the molecular weight per repeat unit
  - Where $C_n$ is the characteristic ratio for the polymer chain

Eqn 4

$$\xi = \left(\frac{r_0^2}{\phi_{2,3}}\right)^{1/3} = Q^{1/3}(<f_0^2>)^{1/2} = C_n^{1/2}Q^{1/3}N^{1/2}l$$

- $Q$ is the degree of swelling = $V_{\text{dry polymer}}/V_{\text{swollen polymer}}$
- $N$ is the degree of polymerization between cross-links
- The mesh size is related to the diffusion constant of a solute in the network
- Eyring theory of diffusion:

Eqn 5

$$D = T\nu \frac{\Delta G^*}{kT} = T\nu \frac{\Delta H^*}{kT} e^{-\frac{\Delta S^*}{k}}$$
- Where $\Delta G^*$ is the activation energy, $\Delta H^*$ is activation enthalpy, and $\Delta S^*$ is activation entropy
- $N = \text{translational oscillating frequency of solute molecule (jump rate!)}$
- $T = \text{temperature}$
- $k = \text{Boltzman constant}$

The ratio of diffusion constant in the gel to that in solution is:

\[
\hat{D} = \frac{D_{\text{gel}}}{D_0} = \frac{e^{\frac{\Delta S^*_{\text{gel}}}{k}}}{e^{\frac{\Delta S^*_{\text{gel}}}{k}}}
\]

- Where $\Delta S^*_{\text{gel}}$ is the activation entropy for diffusion in the gel and $\Delta S^*_{0}$ is the activation entropy in for diffusion in the solvent
- This assumes the activation enthalpy and oscillation frequencies for diffusion are approximately the same in the gel and pure solvent (reasonable for dilute and chemically inert systems)

The activation entropies are:

\[
\Delta S^*_{\text{gel}} = k \ln P^* - k \ln P_0
\]

\[
\Delta S^*_{0} = k \ln P^*_0 - k \ln P_0
\]

\[
\hat{D} = \frac{P^*_{\text{gel,opening}}}{P^*_{0,\text{volume}}} = \frac{P^*_{\text{gel,opening}}}{P^*_{0,\text{volume}}}
\]

- Where $P^*_{\text{gel,opening}}$ is the probability that a solute-sized gap to jump through
- $P^*_{0,\text{volume}}$ is the probability that a solute-sized volume of free space exists to jump into

\[
P^*_{\text{gel,opening}} = \frac{\xi - r}{\xi} = 1 - \frac{r}{\xi}
\]

- Where $r$ is the radius of the solute (drug) and $\xi$ is the network mesh size

The probability of a volume to jump into is an exponential of the ratio of the solute size to the available free volume per mole:

\[
P^*_{\text{gel, volume}} \sim e^{-v^*_{\text{free, gel}}}
\]

\[
P^*_{0,\text{volume}} \sim e^{-v^*_{\text{free}}}
\]

- Where $v_{\text{free}}$ is the specific free volume and $v^*$ is the volume of the solute (drug)
- Refs for free volume theory applied here:
Now:

\[ \frac{P_{gel, volume}^*}{P_{0, volume}^*} = e^{\left( \frac{v^*}{v_{free,1}^*} - \frac{v^*}{v_{free,1}} \right)} \]

The free volume in a swollen gel is approximately \( v_{free,1} \) since the free volume contribution from polymer is extremely low (2.5% even in solid polymers at 25°C)

\[ v_{free,1} = \phi_1 v_{free,1} + \phi_2 v_{free,2} \]

Therefore:

\[ v_{free,1} \approx (1-\phi_2) v_{free,1} = (1-1/Q) v_{free,1} \]

Where \( Q \) is the swelling degree = \( V_{swollen gel} / V_{dry gel} = 1/\phi_2 \)

\[ \frac{P_{gel, volume}^*}{P_{0, volume}^*} = e^{\left( \frac{v^*}{(1-\phi_2) v_{free,1}} - \frac{v^*}{v_{free,1}} \right)} = e^{\left( \frac{1}{(1-\phi_2)} \right) \left( \frac{1}{Q-1} \right)} \approx e^{\left( \frac{1}{Q-1} \right)} \]

\[ v^*/v_{free,1} \approx 1 \text{ for most polymers, experimentally} \]

Therefore:

\[ \hat{D} \approx \left( 1 - \frac{r}{\xi} \right) e^{-\frac{1}{(Q-1)}} \]

And thus finally:

\[ D_{gel} \approx D_0 \left( 1 - \frac{r}{\xi} \right) e^{-\frac{1}{(Q-1)}} \]

\[ \text{Insulin: MW} \approx 5900 \text{ g/mole; hydrodynamic radius} = 16 \text{ Å} \]

**Design of glucose-responsive drug delivery microgels for treatment of diabetes**

- Work by Podual and Peppas
- Immobilized glucose oxidase enzyme within pH-responsive polyelectrolyte gel network along with encapsulated insulin
  - Network composed of DEAEM, PEGMA, and TEGDMA
  - GOD covalently tethered to network
  - Insulin entrapped in network
  - Polymerized gels as microspheres
• Synthesis of microgels?

- Fast variation in swelling due to microgel dimensions
- Mesh size responds in a similar manner, using theory described above:

  - The gels thus designed respond to concentrations of glucose in the surrounding medium, dynamically:
Fig. 3. Change in the pH of glucose solutions due to the oxidation of glucose to gluconic acid by GOD immobilized in PDEAEM-g-EG) gels. The reduction in pH is shown for 200 (●), 100 (■) and 50 mg/dl (☆) of glucose.

Fig. 6. Variation of the volume swelling ratio of 160-μm particles with time due to a pulsatile concentration of glucose in the swelling medium. These particles have a nominal crosslinking ratio, Χ = 0.02, and enzyme loading, $E = 3.3 \times 10^{-4}$ g/g of polymer. The concentrations of glucose used in this study are shown on top of the plot.

Fig. 5. Normalized insulin diffusion coefficient through PDEAEM-g-EG) at 37°C as a function of the volume swelling ratio normalized with respect to the maximum volume swelling ratio. The diffusion behavior is for gels with initial crosslinking ratio of $\chi = 0.005$ (---), $\chi = 0.01$ (—), $\chi = 0.02$ (-----) and $\chi = 0.04$ (☆). The data are shown for hydrogels with $\chi = 0.02$ (●), $\chi = 0.03$ (■) and $\chi = 0.04$ (☆).

Fig. 11. Variation of the diffusion coefficient of insulin in PDEAEM-g-EG) microspheres of particle size = 160 μm, $E = 3.3 \times 10^{-4}$ g/g polymer, and $M_{\text{EG}} = 200$ to alternate pH changes between pH 7.4 and 3.2. The diffusion coefficient is normalized against the diffusion coefficient of insulin in water, which was calculated to be $1.41 \times 10^{-9}$ cm$^2$/s. The data are shown for hydrogels with $\chi = 0.02$ (●), $\chi = 0.03$ (■) and $\chi = 0.04$ (☆).
References


