Controlled Degradation and Mechanical Behavior of Photopolymerized Hyaluronic Acid Hydrogel Networks

Jason A. Burdick¹,², Cindy Chung¹, Xinqiao Jia¹, Mark A. Randolph², and Robert Langer¹

¹Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA

²Division of Plastic Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA

Biomacromolecules 6 (2005) 386-391

Presented by Sudhir Khetan
Wednesday, April 10, 2013
Updates

- Paper for 1st LBL (next Friday) posted on website
  - I recommend saving and opening in Adobe – the browser view is somewhat distorted
  - Updated LBL guidelines – short assignment for non-presenters
- First homework assigned today, due Wednesday (see syllabus)
  - Due date extended since protein-biomaterial interactions not covered until Friday (Professor Currey)
"...focuses on interdisciplinary investigations exploring the interactions of macromolecules with biological systems and their environments as well as biological approaches to the design of polymeric materials".

- Covers topics including, but not limited to:
  - Sustainable chemistry
  - Monomers and polymers based on natural resources
  - Biomimetics
  - Metabolism of polymers and polymer degradation products

- Covers applications including, but not limited to:
  - Packing and consumer products
  - Biomedical polymers, polymeric drugs
  - Tissue engineering
  - Polymers for electronics or photonics applications
Authors

- Jason A. Burdick, Associate Professor, UPenn
  Ph.D. University of Colorado 2002
  - Tissue engineering, biomaterials, drug delivery
  Cindy Chung

- Robert Langer, Institute Professor, MIT
  Sc.D., Massachusetts Institute of Technology, 1974
  - Cell and tissue engineering, biomaterials, drug delivery
  Xinqiao Jia

- Mark A. Randolph, Instructor in Surgery, HMS
  B.S., University of Maine, 1979
  - Education in surgery, laboratory management
A **hydrogel** is a water-swollen, crosslinked polymer network!

**Hydrogel formation:**

- **MeHA:** methacrylated hyaluronic acid
- **MeHA solution**
- **Gelation**
- **other polymers:** polyethylene glycol (PEG) alginate

**Formation of a physical or covalent bond**

**crosslinks** the chains

Guvendiren M, Burdick JA. *Nature Communications* 2012
Cell encapsulation within hydrogels

- Most studies of cell behavior have been performed in 2D
  - After fabrication of a hydrogel, cells are seeded atop the surface
- However, 3D encapsulation within hydrogels may better mimic the native cellular microenvironment
- 3D encapsulation into hydrogels is only ~10-15 years “old”
Cell encapsulation within hydrogels

One of the first examples used photopolymerization of PEODM:

PEODM
poly(ethylene oxide) dimethacrylate

Background: hyaluronic acid

- biological significance
  - found in many body tissues including:
    - skin
    - cartilage
    - synovial fluid
  - roles in angiogenesis, wound healing, development

- synthetic versatility
  - acrylated
  - methacrylated

Background: articular cartilage

- The target application of the hydrogels in this study was articular cartilage regeneration.

The middle-deep zones are typically the regenerative targets for cartilage engineering.
Methods: general outline

• MeHA macromer synthesis and polymerization into hydrogels
• Hydrogel characterization
  – Volumetric swelling ratio
  – Mechanical properties
  – Proteolytic degradation
• Chondryocyte isolation and photoencapsulation
  – Auricular cartilage harvest from 3 – 6 month old swine
• Subcutaneous implantation and assessment of \textit{in vivo} tissue formation
  – Implantation into nude mice (4, 6, 8 week timepoints)
  – Histological analysis of explants for cartilage markers
  – Quantitative biochemical assays
Methods: MeHA and hydrogel syntheses

Figure 1

Methacrylated HA (MeHA) – synthesized as reported previously (Smeds J Biomed Mater Res 2001)

**Discussion**: what concerns (material or biological) could the use of photo initiation bring up?

radical initiation (UV light exposure)
365 nM, ~4 mW/cm²

polymerization
Methods: MeHA hydrogel characterization

• Volumetric swelling ratio
  – Ratio of hydrogel wet to dry weight

• Compressive modulus
  – Parallel plate compression at 10% strain
    • Using an Instron 5542 mechanical tester
    – $E = \text{slope of stress strain curve at } <20\% \text{ strain}$

• Degradation analysis
  – Incubation of hydrogels in PBS containing either 10 or 100 U/mL hyaluronidase
  – Uronic acid (a breakdown component of MeHA) assay used to measure degradation
Methods: chondrocyte isolation and photoencapsulation

• Chondrocyte isolation
  – 3 – 6 month old swine were used
  – Cartilage tissue was harvested in a sterile manner
  – Procedure to isolate chondrocytes from cartilage tissue:
    • 18 h digestion in 0.1% collagenase → filtration to remove undigested cartilage and centrifugation → 2x washing and counting with a hemacytometer

• Chondrocyte photoencapsulation
  – 2 wt%, 350 kDa MeHA hydrogels were prepared using the same procedure described earlier
    • Prior to light exposure, the MeHA/initiator solution was used to re-suspend a cell pellet containing the number of cells for a final concentration in the gels of 40 million cells/mL
  – **Note:** photoencapsulation was first performed with an established cell line (NIH 3T3 fibroblasts) to assess cytocompatibility of the procedure.
Methods: subcutaneous implantation and assessment of *in vivo* tissue formation

- **Subcutaneous implantation**
  - 4 hydrogels with photoencapsulated chondrocytes placed into subcutaneous dorsum of nude mice (4 implants per mouse)
  - After 4, 6, or 8 weeks, animals euthanized and explants harvested

- **Histological analysis**
  - Constructs fixed for 24 h in 10% formalin, embedded in paraffin, and sectioned into ~10 μm sections.
  - Sections stained with *Safrinin O*, for glycosaminoglycans (GAGs)

- **Biochemical analysis**
  - Constructs digested for 15 h in a papain solution
  - Total DNA content within the hydrogels measured using a previously reported fluorescent dye assay ([Kim YJ et al. Anal Biochem 1998](#))
  - Total GAG content determined using the dimethylmethylene blue method ([Taylor KB and Jeffree GM Histochem J 1969](#)) and normalized to cell number
    - Cell number determined using a factor of 7.7 pg of DNA per chondrocyte
Results and discussion: network synthesis

• All MeHA macromers obtained through the same techniques (concentrations of HA and methacrylic anhydride)
  – Different extent of methacrylation attributed to decreased viscosity (i.e., higher mobility and reactivity) of the low MW (50 kDa) HA relative to the mid (350 kDa) and high (1100 kDa) MW

• Macromer concentrations for gel synthesis (last column) chosen based on the highest MeHA concentrations that could be pipetted (i.e., below a threshold viscosity)
  – Goal was to obtain a range of different network properties

Table 1

<table>
<thead>
<tr>
<th>MW of macromer (kDa)</th>
<th>methacrylation (%)</th>
<th>macromer in precursor solution (wt %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1100</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>350</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>50</td>
<td>12</td>
<td>2, 5, 10, 20</td>
</tr>
</tbody>
</table>

note: 1 wt% = 1 cg/mL
Results and discussion: hydrogel characterization – swelling ratio

• For each MW, a decrease in $Q_v$ was observed with increasing HA concentration (as expected)
  – * indicates statistically significant differences with $p < 0.05$ (two-tailed t-test)
• However, no differences between MWs at the same HA concentration of 2 wt%
• “Using Flory-Rehner calculations, the network mesh size and the cross-linking density, which are important when explaining mechanics and degradation, are directly correlated to $Q_v$”

![Figure 2](image)
Results and discussion: hydrogel characterization – mechanical properties

- (A) representative stress-strain curves of 10% (solid) and 5 wt% (dotted) 50 kDa MeHA hydrogels
  - “the general slope is linear at low strains (<20%) and the increases with an increase in strain”
- (B) compressive modulus of various HA network formulations
  - Moduli were significantly different between macromer concentrations for each MeHA MW
  - “Overall, the modulus (i.e., slope of stress versus strain curve at low strains) correlates well with the network cross-linking density (i.e., swelling)”

Figure 3
Results and discussion: hydrogel characterization – degradation

• Bars represent time until complete degradation in 100 U/mL hyaluronidase/PBS
  – HA’ase refreshed every other day during the study
• Good correlation was again observed between degradation time and crosslinking density
  – Post-degradation NMR revealed minimal double-bond presence, indicating that their conversion to kinetic chains in crosslinking was ~100%
• Study only shows relative degradation time for one type of HA’ase in vitro

Discussion: What does the claim about ~100% crosslinking efficiency assume? (hint: if crosslinking wasn’t 100% efficient, what else could explain there being no double bonds present by the time NMR was performed?) Do you think this is a valid concern?
Results and discussion: hydrogel characterization – degradation (cont.)

• (A) cumulative percentage of uronic acid released from hydrogels synthesized from 2 (●), 5 (▪), or 10 (▲) wt% 50 kDa MeHA in 100 U/mL HA’ase

• (B) cumulative percentage of uronic acid released from hydrogels synthesized from 5 wt%, 350 kDa MeHA in 100 (●) or 10 (▪) U/mL HA’ase
Results and discussion: 3T3 fibroblast encapsulation and viability

• Initial proof of concept study to assess cytocompatibility of encapsulation procedure
• Fibroblasts were encapsulated at a density of 40 million cells/mL and they hydrogels maintained in culture for 1 week
• Absorbance in Figure 6 (right) is indicative of encapsulated cell mitochondrial activity, and thus, viability
• For all MW’s, a decrease in viability was observed with increasing MeHA concentration; possible causes:
  – increased radical concentrations in the higher concentrations groups
  – Increased crosslinking density causing decreased nutrient and waste transport, reducing cell viability

Figure 6

“Overall, these results indicate that...the higher macromer hydrogels application as cell carriers is limited due to low viability...”

both these conditions afforded >95% viability from live/dead staining (data not shown)
Results and discussion: neocartilage formation

- 2 wt%, 350 kDa MeHA hydrogel condition chosen from the previous study due to optimal cell viability
- Polyethylene dimethacrylate (PEGDM) used as a control
  - PEG is synthetic, and thus, lacks the assumed pro-cartilage properties of HA
- (A) staining of histological sections for glycosaminoglycans (GAGs). Scale bars = 100 μm.
- (B) GAG content (ng chondroitin sulfate/chondrocyte) at 0 (black), 4 (grey) or 8 (white) weeks of encapsulated hydrogel culture in mice
- MeHA hydrogel explants with encapsulated chondrocytes exhibited a shiny white cartilage-like appearance relative to without cells (data not shown)
Results and discussion: neocartilage formation

• Specific comments for parts A and B
  • (A)
    – Light background staining observed in implanted acellular hydrogels (data not shown)
    – Darker staining in PEGDM gels limited to pericellular (immediately adjacent to the cells) regions
      • Possibly due to the non-degradability of PEG relative to HA preventing matrix distribution
  • (B)
    – At 12 w (white bars), GAG levels reached 75% of those in native articular cartilage
    – Little difference in GAG quantity produced in PEGDM versus MeHA hydrogels (though distribution is different)
    – Minimal GAG detected in hydrogels immediately after hydrogel encapsulation

Discussion: This finding is surprising given the staining results. What possible explanations are there and what additional control groups would have possibly helped clarify (try to think of at least 1 for each)
Conclusions

• Photocrosslinking of MeHA is a versatile platform to fabricate hydrogels that promote high encapsulated cell viability

• The range of material properties (e.g., mechanics, degradation) possible with this system may render them valuable for clinical and noninvasive implantation for regenerative applications
General tips for your LBLs

• Make discussion questions that promote **critical thinking**
  – “What did the authors conclude was the main takeaway from Figure 1”, for example, is a **bad question**! We know what the authors are telling us; our job is to try to find the holes (or at least, the dents) in their work!

• In the intro slides, try to explain techniques/concepts that the paper assumed was background knowledge of the reader (e.g., free radical crosslinking here)

• **Not nearly all** of the methods details needed in your slides/presentations (use your judgement)

• When doing your background section, remember we may have covered the same topics in class in the preceding weeks (so as in-depth an introduction might not be necessary)

• You can introduce your own visual content (e.g., my free radical mechanism schematic on slide XX) or figures from other paper/reviews for further clarification