A Combinatorial Hydrogel Platform to Probe Stem Cell Chondrogenesis in 3D

Sebastián L. Vega, Kwang Hoon Song, Chao Wang, Lin Han, Jason A. Burdick

Monday October 24, 2016, 2:05PM
The Stem Cell Microenvironment

Challenges in deciphering cell-ECM interactions:
• Tedious to evaluate many conditions
• Unable to decouple mechanical and biochemical cues

Need for novel materials and high-throughput techniques to probe cell-ECM interactions

Guvenriden et al., Curr Opin Biotechnol, 2014.
Techniques to probe cell-biomaterial interactions

**ECM Mimetic Arrays**
- Complex DOE
- Expensive equipment not readily available

**1st Generation Combinatorial Hydrogels**
- Mostly explored in 2D contexts
- 2D does not represent 3D environments

---

Combinatorial hydrogels to screen 3D cell-ECM interactions

**Design Criteria:**
- 3D cell encapsulation
- Cytocompatible crosslinking
- Ability to vary biochemical and mechanical signals
- Rapid single cell imaging possible

**Thiol-ene reactions between norbornene and thiols**

**Scheme for fabricating combinatorial hydrogels**

Gramlich et al., Biomaterials, 2013.
RGD gradients to probe effects on chondrogenesis

**Chondrogenic Differentiation**

MSCs → Chondroprogenitors → Chondrocytes

- Sox9
- N-cadherin
- Fibronectin
- Aggrecan
- Col II
- TGF-β3

0 → 1-3 days → 7-14 days → >21 days

**Experimental Design**

NorHA: 4wt%, ~50% mod
Crosslinker: DTT, \( X_{DTT} \) 0.2
MSCs: 5E6/ml
Chemically-defined media + TGFβ3 (10ng/ml)

Hydrogel characterization
RGD gradient, mechanics

Cellular studies
Viability (+7d), Sox9 (+1d), aggrecan (+7d)

Confocal microscopy of 5x5 mm hydrogels

Develop whole-hydrogel imaging protocol
Tile-scan (6x6) image

Develop Sox9 and aggrecan image analysis protocols

Sox9 (+1 day)

Aggrecan (+7 days)

\[ \frac{\sum \text{Pixel Intensity}}{V_{\text{Nucleus}}} \]
Combinatorial hydrogel characterization

- Tile-scan confocal imaging
- Measure horizontal fluorescence (x1 → x10)
- 5 horizontal regions (n=3)
- Irradiate gels, measure fluorescence of known RGD concentrations

**Graphs:**

1. **RGD Gradient:**
   - X-axis: RGD concentration (mM)
   - Y-axis: Fluorescence
   - Data points: 0, 1, 3, 5 mM RGD

2. **Fluorescence vs. X-Position:**
   - X-axis: X-position
   - Y-axis: Fluorescence
   - Data points: 0, 1, 3, 5, 7, 10 mM RGD
## Combinatorial hydrogel characterization

### Elastic moduli (5 – 9 kPa)

<table>
<thead>
<tr>
<th>7.8</th>
<th>8.4</th>
<th>8.6</th>
<th>8.6</th>
<th>8.0</th>
<th>8.9</th>
<th>7.9</th>
<th>7.9</th>
<th>8.5</th>
<th>6.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.2</td>
<td>8.9</td>
<td>8.6</td>
<td>7.1</td>
<td>8.6</td>
<td>8.9</td>
<td>8.3</td>
<td>8.9</td>
<td>8.6</td>
<td>8.6</td>
</tr>
<tr>
<td>8.1</td>
<td>8.4</td>
<td>8.4</td>
<td>7.9</td>
<td>8.8</td>
<td>8.5</td>
<td>8.2</td>
<td>6.6</td>
<td>6.2</td>
<td>5.7</td>
</tr>
<tr>
<td>7.8</td>
<td>7.6</td>
<td>7.6</td>
<td>7.9</td>
<td>7.6</td>
<td>8.1</td>
<td>6.5</td>
<td>5.8</td>
<td>5.8</td>
<td>6.0</td>
</tr>
<tr>
<td>6.9</td>
<td>7.9</td>
<td>7.9</td>
<td>7.4</td>
<td>8.0</td>
<td>6.7</td>
<td>6.3</td>
<td>5.8</td>
<td>6.7</td>
<td>5.6</td>
</tr>
<tr>
<td>7.8</td>
<td>7.9</td>
<td>8.5</td>
<td>6.7</td>
<td>6.0</td>
<td>6.0</td>
<td>5.7</td>
<td>6.8</td>
<td>5.3</td>
<td>5.6</td>
</tr>
<tr>
<td>7.8</td>
<td>7.2</td>
<td>5.9</td>
<td>7.1</td>
<td>7.6</td>
<td>6.5</td>
<td>6.5</td>
<td>5.8</td>
<td>5.7</td>
<td>5.6</td>
</tr>
<tr>
<td>5.9</td>
<td>7.2</td>
<td>7.2</td>
<td>7.5</td>
<td>7.2</td>
<td>7.6</td>
<td>6.3</td>
<td>6.3</td>
<td>5.8</td>
<td>5.6</td>
</tr>
<tr>
<td>7.2</td>
<td>6.9</td>
<td>7.5</td>
<td>7.8</td>
<td>6.5</td>
<td>6.3</td>
<td>6.4</td>
<td>6.2</td>
<td>5.6</td>
<td>5.5</td>
</tr>
<tr>
<td>7.4</td>
<td>7.9</td>
<td>6.7</td>
<td>7.2</td>
<td>7.5</td>
<td>7.0</td>
<td>6.2</td>
<td>6.0</td>
<td>5.2</td>
<td>5.6</td>
</tr>
</tbody>
</table>

### Variation from mean (< 30%)

<table>
<thead>
<tr>
<th>0.09</th>
<th>0.17</th>
<th>0.21</th>
<th>0.20</th>
<th>0.12</th>
<th>0.25</th>
<th>0.11</th>
<th>0.11</th>
<th>0.19</th>
<th>0.07</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.14</td>
<td>0.25</td>
<td>0.21</td>
<td>0.01</td>
<td>0.20</td>
<td>0.25</td>
<td>0.16</td>
<td>0.25</td>
<td>0.21</td>
<td>0.21</td>
</tr>
<tr>
<td>0.14</td>
<td>0.17</td>
<td>0.18</td>
<td>0.10</td>
<td>0.23</td>
<td>0.19</td>
<td>0.15</td>
<td>0.08</td>
<td>0.13</td>
<td>0.20</td>
</tr>
<tr>
<td>0.10</td>
<td>0.06</td>
<td>0.07</td>
<td>0.11</td>
<td>0.06</td>
<td>0.14</td>
<td>0.10</td>
<td>0.19</td>
<td>0.19</td>
<td>0.15</td>
</tr>
<tr>
<td>0.03</td>
<td>0.11</td>
<td>0.11</td>
<td>0.04</td>
<td>0.12</td>
<td>0.06</td>
<td>0.12</td>
<td>0.19</td>
<td>0.07</td>
<td>0.22</td>
</tr>
<tr>
<td>0.09</td>
<td>0.10</td>
<td>0.19</td>
<td>0.06</td>
<td>0.15</td>
<td>0.15</td>
<td>0.21</td>
<td>0.05</td>
<td>0.26</td>
<td>0.21</td>
</tr>
<tr>
<td>0.10</td>
<td>0.00</td>
<td>0.17</td>
<td>0.00</td>
<td>0.06</td>
<td>0.09</td>
<td>0.08</td>
<td>0.18</td>
<td>0.21</td>
<td>0.21</td>
</tr>
<tr>
<td>0.17</td>
<td>0.01</td>
<td>0.01</td>
<td>0.05</td>
<td>0.01</td>
<td>0.06</td>
<td>0.11</td>
<td>0.12</td>
<td>0.19</td>
<td>0.22</td>
</tr>
<tr>
<td>0.01</td>
<td>0.03</td>
<td>0.05</td>
<td>0.10</td>
<td>0.09</td>
<td>0.12</td>
<td>0.10</td>
<td>0.14</td>
<td>0.21</td>
<td>0.23</td>
</tr>
<tr>
<td>0.04</td>
<td>0.11</td>
<td>0.06</td>
<td>0.01</td>
<td>0.04</td>
<td>0.02</td>
<td>0.13</td>
<td>0.16</td>
<td>0.27</td>
<td>0.22</td>
</tr>
</tbody>
</table>

### Hertz indentation model

\[
F = \frac{4}{3} \cdot \frac{E_s}{1 - \mu^2} \cdot \sqrt{r \cdot \sigma^2}
\]

- \(E_s\) = hydrogel modulus
- \(\mu\) = Poisson’s ratio
- \(r\) = probe radius

10x10 array, 15 measurements/position

7.1 ± 1 kPa
Assessment of MSCs encapsulated in combinatorial hydrogels

**Cellular Studies**

- Divide gel into 10 vertical regions (x1 → x10)
- Divide each region into 10 squares
- For each square:
  - **Viability**: count live/dead cells
  - **Sox9**: nuclear fluorescence
  - **Aggrecan**: secreted volume

**Viability**

- High MSC viability for at least 7 days in combinatorial hydrogels
- Rapid imaging & image analysis developed (>300 cells analyzed per position)
Assessment of MSCs encapsulated in combinatorial hydrogels

**Nuclear Sox9 fluorescence (+1d)**
- No RGD (x10) → highest nuclear Sox9

**Aggrecan production (+7d)**
- Best: x8 (~1mM RGD), x10 (no RGD)
Summary

Developed combinatorial hydrogel system

- High viability
- Wide range of biochemical cues (0 → 5mM)
- No significant change in elastic moduli

Rapid confocal imaging and image analysis

Significant differences in chondrogenic markers

Can add additional biochemical cues

\[ \sum \text{Pixel Intensity} \]

\[ \frac{V_{\text{Nucleus}}}{N} \]
Acknowledgments and questions

Polymeric Biomaterials Lab

Dr. Jason A. Burdick
Dr. Kwang Hoon Song
Sara Trujillo-Muñoz
John Bricker
Michelle Kwon
Dr. Claudia Loebel
Renata Lang Sala
Dr. Chris Highley
Dr. Yi-Cheun Yeh
Dr. Adrianne Rosales
Dr. Dawn Pedrotty
Dr. Su Chin Heo

Dr. Steven Caliari
Dr. Kalil Abdullah
Dr. Chris Rodell
Andrew Rouff
Minna Chen
Josh Mealy
Leo Wang
Liliang Ouyang
James Howard
Jonathan Galarraga
Mu-Huan Lee

NanoBiomechanics Lab

Dr. Lin Han
Chao Wang