HYDROGELS FOR PROTEIN DELIVERY

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3rd Long-Acting Injectables and Implantables Conference, February 6th 2020, San Diego, USA
Why hydrogels for release of proteins?

Biocompatibility
Degradability/selection of building block
Possibilities to tailor releases (water content/crosslink density/degradation kinetics)
Different geometries (macroscopic/microspheres/nanoparticles)

Crosslinking might affect protein stability/integrity
approach: post loading; protein friendly crosslinking chemistry
Complex coacervation-based protein loading and tunable release from monodisperse glycosaminoglycan microgels
Complex coacervates

Van der Gucht et al. Journal of Colloid and Interface Science 2011
Charged materials - Hypothesis

<table>
<thead>
<tr>
<th>Protein</th>
<th>Isoelectric point pH(I)</th>
<th>Molecular weight in kDa</th>
<th>Charge at pH=7.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>hTGF-β3</td>
<td>8.8*</td>
<td>44341*</td>
<td>8.0 °H, SO₂Na</td>
</tr>
<tr>
<td>hTGF-β3</td>
<td>8.3*</td>
<td>47328*</td>
<td>4.6 °H, SO₂Na</td>
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<tr>
<td>hBMP-2</td>
<td>9.2*</td>
<td>44702*</td>
<td>10.5 °H, SO₂Na</td>
</tr>
<tr>
<td>hBMP-7/OP-1</td>
<td>7.7*</td>
<td>49313*</td>
<td>2.6 °H, SO₂Na</td>
</tr>
<tr>
<td>hBMP-14/hGDF-5</td>
<td>9.8*</td>
<td>55411*</td>
<td>27.9 °H, SO₂Na</td>
</tr>
<tr>
<td>hIGF-I</td>
<td>9.8*</td>
<td>21841*</td>
<td>19.4 °H, SO₂Na</td>
</tr>
<tr>
<td>hIGF-2</td>
<td>11.2*</td>
<td>30770*</td>
<td>31.8 °H, SO₂Na</td>
</tr>
<tr>
<td>hIGF-18</td>
<td>9.9*</td>
<td>23989*</td>
<td>18.9 °H, SO₂Na</td>
</tr>
<tr>
<td>hPDGF-BB</td>
<td>9.4*</td>
<td>27283*</td>
<td>11.8 °H, SO₂Na</td>
</tr>
</tbody>
</table>

*Calculated using sequence from www.uniprot.org and inserted into isoelectric point tool. Please note: This is an approximation for unfolded protein. The estimate assumes all residues have pKa values that are equivalent to the isolated residues.

Lysozyme:
- 128 residues
- 14.3 kDa
- +8 charge (pH 4)

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Preparation of monodisperse HA/CS microgels

Fig. 1 Schematic representation of a custom-built microfluidic device for generation of monodisperse aqueous HAMA and CSMA droplets.
Fabrication of HAMA and CSMA microgels

A. [Graph showing the relationship between continuous flow rate and droplet diameter for different concentrations of HAMA and CSMA.]

B. [Image of microgels with scale bar = 500 µm.]

Soft Matter, 2018, 14, 6327-6341
Loading of CS/HA microgels with lysozyme in low ionic strength buffer of pH 7.4

Results:
Quantitative loading
High loading capacity (3-4 mg protein per 1 mg HA/CS)
Deswelling of the microspheres
Lysozyme release from microgels

HAMA (1 COO⁻)

CSMA (1 COO⁻ and 1 SO₄⁻)
Lysozyme release from CSMA microgels in scaffolds

- **A. Control**
- **B. Blend**
- **C. Microcomposite**

### Graphs

- **D.** Cumulative release (%) vs. Time (days) for 170 mM
- **E.** Cumulative release (%) vs. Time (days) for 500 mM

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**Soft Matter**, 2018, **14**, 6327-6341
Release from scaffolds

A. Control

B. Blend

C. Microcomposite

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$M_n$ in kDa</th>
<th>PDI</th>
<th>DM in %</th>
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</thead>
<tbody>
<tr>
<td>HAMA</td>
<td>57</td>
<td>nd.</td>
<td>12.6</td>
</tr>
<tr>
<td>CSMA</td>
<td>27 (94 wt%)</td>
<td>1.4</td>
<td>11.8</td>
</tr>
<tr>
<td>Thermopolymer</td>
<td></td>
<td>1.3</td>
<td></td>
</tr>
</tbody>
</table>

Thermopolymer
methacrylated poly[N-2hydroxypropyl)methacrylamide
mono/dilactate]-PEG triblock co-polymer
Release from scaffolds

A. Control  B. Blend  C. Microcomposite

D. Cumulative release (%) over time (days) for 170 mM

E. Cumulative release (%) over time (days) for 500 mM
THANK YOU FOR YOUR ATTENTION!