Fabrication, Microstructure and Mechanical Properties of an Osteochondral Scaffold

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Collaborators

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• BWH: M Spector, H-P Hsu

• Cambridge: W Bonfield, AK Lynn, S Best, RE Cameron
Outline

• Cartilage and Current Treatments
• Design Considerations for Osteochondral Scaffolds
• Collagen-GAG Scaffold
• Mineralized Collagen-GAG Scaffold
• Osteochondral Scaffold: Fabrication, Microstructure, Animal Studies
Articular Cartilage

Bone: Type I collagen
Cartilage: Type II collagen

Greene WB (2006)
Netter’s Orthopaedics

Image by MIT OpenCourseWare.
Articular Cartilage

• Avascular: no blood supply
• Low density of chondrocytes
• Damage: sports injuries, osteoarthritis
• Poor capacity for self-repair
Current Treatments: Marrow Stimulation

- Subchondral bone plate punctured to induce bleeding from marrow cavity
- Marrow derived stem cells entrapped in blood clot
- Stem cells form repair tissue throughout defect
- > 75,000 procedures in the US/year

POOR QUALITY OF REPAIR

Courtesy of Andrew Lynn. Used with permission.

Lynn (2005)
Current Treatments: Osteochondral Autograft

- Plugs of bone and cartilage harvested from non load-bearing sites
- Plugs used to fill defect site in a mosaic pattern (mosaicplasty)
- < 2,000 procedures in the US/year

DONOR SITE MORBIDITY

Lynn (2005)
Current Treatments: Autologous Chondrocyte Implantation

- Cartilage cells harvested from non load-bearing site
- Cells isolated and cultured 2-3 weeks
- Cells re-implanted
- < 2,000 procedures in the US/year

**TWO SURGERIES,**

**COST OF CELL CULTURE**

(Dara Torres age 45; Olympic swimmer 84, 88, 92, 00, 08 – 3 silvers)

Courtesy of Andrew Lynn. Used with permission.

Lynn (2005)
Osteochondral Scaffolds: Design Considerations

• Use healthy articular joint as a model for scaffold structure and composition
• Bone layer allows access to mesenchymal stem cells
• Control of scaffold parameters (e.g. mineral content, pore size)
• Use materials appropriate for rapid regulatory compliance
Osteochondral Scaffold

- Unmineralized type II collagen
- Interdiffusion region
- Mineralized type I collagen

Courtesy of Andrew Lynn. Used with permission.
Collagen-GAG Scaffold: Fabrication

Production of CG Suspension

Collagen: Type II

0.05M Acetic Acid
pH ~3.2

GAG: Chondroitin-6-sulfate

Mix

Overhead blender

Degas under vacuum

Store at 4°C

Refrigerant-cooled flask (4C)

CG Suspension

Courtesy of Brendan Harley. Used with permission.
CG Scaffold: Fabrication

Place CG suspension into stainless steel pan (12.5 x 12.5 cm)

Ice crystals surrounded by collagen and GAG fibers

Sublimation:
- $P=75$ mTorr, $T=0^\circ$C
- Removess ice content

Porous, CG scaffold

Yannas, Harley

Courtesy of Brendan Harley. Used with permission.
CG Scaffold: Microstructure


Pek et al., 2004  
O’Brien, Harley et al., 2004

Relative density = 0.005
CG Scaffold: Pore Size


Harley and Flemings: solidification model
CG Scaffold: Compression (Dry)

Harley et al., 2007

CG Scaffold: Mechanical Properties

<table>
<thead>
<tr>
<th></th>
<th>$E^*$ (Pa)</th>
<th>$\sigma^*_{el}$ (Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry</td>
<td>30,000</td>
<td>5,150</td>
</tr>
<tr>
<td>Wet</td>
<td>208</td>
<td>21</td>
</tr>
</tbody>
</table>
Mineralized CG Scaffolds: Fabrication

Collagen: type I, bovine tendon
GAG: chondroitin 6-sulfate, shark cartilage
Calcium ($Ca^{2+}$): $Ca(NO_3)_2\cdot4H_2O$ & $Ca(OH)_2$
Phosphoric Acid: 0.05M, pH ~3.2

Mix CGCaP Suspension:
Overhead blender: 15,000 rpm
Combine
FREEZE-DRY

T = 4°C

EDAC CROSSLINK

Courtesy of Brendan Harley. Used with permission.

Harley, Lynn, Kanungo
Mineralized CG Scaffolds: Fabrication

- Mineral: brushite (CaHPO$_4$.2H$_2$O)
- Can control mass fraction of brushite between 0 and 80 wt% by controlling the molar ratio of the calcium nitrate hydrate and calcium hydroxide used and the molarity of the phosphoric acid
- Brushite then converted to octacalcium phosphate and then to apatite by hydrolytic conversion
Mineralized CG Scaffold: Microstructure


Harley et al., 2010

Pore size 50-1000 μm depending on freezing conditions

Pore size for bone regeneration: 100-500 μm
Mineralized CG Scaffold: $\mu$CT

Uniform distribution of mineral

Harley et al., 2010
Mineralized CG Scaffold: EDX


1mm Uniform distribution of mineral

Harley et al., 2010
Mineralized CG Scaffold: Compression

<table>
<thead>
<tr>
<th>Scaffold</th>
<th>$E^*$ (kPa)</th>
<th>$\sigma^*$ (kPa)</th>
<th>$\varepsilon^*$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry</td>
<td>762</td>
<td>85</td>
<td>11</td>
</tr>
<tr>
<td>Wet</td>
<td>4.12</td>
<td>0.29</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Harley et al., 2008

Mineralized CG Scaffold: Compression

• Mineralized scaffold can be manually compressed
• On unloading and hydration, recovers all deformation
• Interested in increasing mechanical properties of the mineralized scaffold for improved handling during surgery
Mineralized CG Scaffold: Compression

- Cross-linked collagen of osteoid has a hydrated modulus in the range of 25-40 kPa
- Engler et al. (2006) show that differentiation of MSCs depends on substrate stiffness
- Substrate stiffness similar to collagen of osteoid leads to differentiation to osteoblast-like morphology
- Interest in increasing stiffness of mineralized scaffold to mimic osteoid stiffness
Cellular Solids Modelling

\[ \frac{E^*}{E_s} = \left( \frac{\rho^*}{\rho_s} \right)^2 \]
\[ \frac{\sigma^*}{\sigma_s} = 0.3 \left( \frac{\rho^*}{\rho_s} \right)^{3/2} \]

Foam properties depend on:
- solid properties: \( E_s, \sigma_s \)
- relative density: \( \rho^*/\rho_s \)
- geometrical factor: 1, 0.3
Increase Mineral Content

Increase Mineral Content

<table>
<thead>
<tr>
<th>Scaffold</th>
<th>$E^*$ (kPa)</th>
<th>$\sigma^*$ (kPa)</th>
<th>$\varepsilon^*$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 wt.%</td>
<td>780</td>
<td>90</td>
<td>12</td>
</tr>
<tr>
<td>75 wt.%</td>
<td>370</td>
<td>35</td>
<td>10</td>
</tr>
</tbody>
</table>

Scaffolds of higher mineral content have increased defects: e.g. voids in cell walls, disconnected walls

Kanungo et al., 2008
Increase Mineral Content

Increase Relative Density

\[ \frac{\rho}{\rho_s} = 0.045 \]
\[ d = 311 \mu m \]

\[ \frac{\rho}{\rho_s} = 0.098 \]
\[ d = 196 \mu m \]

\[ \frac{\rho}{\rho_s} = 0.137 \]
\[ d = 159 \mu m \]

\[ \frac{\rho}{\rho_s} = 0.187 \]
\[ d = 136 \mu m \]

Kanungo and Gibson, 2009

Increase Relative Density

<table>
<thead>
<tr>
<th>$\rho^*/\rho_s$ (-)</th>
<th>E (dry) (kPa)</th>
<th>E(wet) (kPa)</th>
<th>$\sigma^*$ (dry) (kPa)</th>
<th>$\sigma^*$ (wet) (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.045</td>
<td>780</td>
<td>6.44</td>
<td>39</td>
<td>0.55</td>
</tr>
<tr>
<td>0.098</td>
<td>3156</td>
<td>17.8</td>
<td>132</td>
<td>0.83</td>
</tr>
<tr>
<td>0.137</td>
<td>6500</td>
<td>34.8</td>
<td>242</td>
<td>2.12</td>
</tr>
<tr>
<td>0.187</td>
<td>3660</td>
<td>38.8</td>
<td>275</td>
<td>1.79</td>
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</tbody>
</table>
Increase Cross-linking
($\rho^*/\rho_s = 0.137$, wet)

<table>
<thead>
<tr>
<th></th>
<th>$E^*$ (kPa)</th>
<th>$\sigma^*$ (kPa)</th>
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</thead>
<tbody>
<tr>
<td>Non-cross-linked</td>
<td>34.8</td>
<td>2.12</td>
</tr>
<tr>
<td>DHT</td>
<td>56.0</td>
<td>3.26</td>
</tr>
<tr>
<td>EDAC</td>
<td>91.7</td>
<td>4.15</td>
</tr>
</tbody>
</table>
Increase Relative Density

<table>
<thead>
<tr>
<th>$\rho^*/\rho_s$</th>
<th>E (kPa) (no x-link)</th>
<th>E (kPa) (DHT)</th>
<th>E (kPa) (EDAC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)</td>
<td>6.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.045</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.098</td>
<td>17.8</td>
<td></td>
<td></td>
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<tr>
<td>0.137</td>
<td>34.8</td>
<td>56.0</td>
<td>91.7</td>
</tr>
<tr>
<td>0.187</td>
<td>38.8</td>
<td></td>
<td></td>
</tr>
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</table>

Can obtain E (wet) in range of 25-40kPa for MSC differentiation to osteoblast-like cells
Mineralized CG Scaffold: Strut Properties

MCG scaffold: \( E_s = 7.34 + 3.73 \text{ GPa (dry)} \)

Nanoindentation: \( \sigma_s = 201 + 52 \text{ MPa (dry)} \)

Trabecular bone: \( E_s = 18 \text{ GPa}, \sigma_s = 182 \text{ MPa} \)
Cellular Solids Models

\[
\left( \frac{E^*}{E_S} \right) \approx 0.047 \left( \frac{\rho^*}{\rho_S} \right)^2
\]

\( E_S = 7.34 \text{ GPa} \)


Kanungo and Gibson, 2009
Cellular Solids Models

\[
\left( \frac{\sigma^*}{\sigma_S} \right) \approx 0.023 \left( \frac{\rho^*}{\rho_S} \right)^{1.5}
\]

\[\sigma_S = 201 \text{ MPa}\]


Osteochondral Scaffolds: Design Considerations

- Use healthy articular joint as a model for scaffold structure and composition
- Control of scaffold parameters (e.g. mineral content, pore size)
- Use materials appropriate for rapid regulatory compliance
Osteochondral Scaffold: Fabrication

• Liquid-phase co-synthesis
• Pour mineralized CG slurry into mold, then CG slurry into mold
• Allow the two slurries to interdiffuse for 30 minutes at room temperature
• Place the mold containing the slurries into the freeze drier
• Cross-link with EDAC
Osteochondral Scaffold

Mineralized CG scaffold
Type II collagen scaffold
Osteochondral Scaffold: Micro-CT

# Osteochondral Scaffold: Microstructure

<table>
<thead>
<tr>
<th>Scaffold</th>
<th>Porosity</th>
<th>Pore size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen-GAG</td>
<td>98.3%</td>
<td>653</td>
</tr>
<tr>
<td>Mineralized CG</td>
<td>95.5%</td>
<td>419</td>
</tr>
</tbody>
</table>
Osteochondral Scaffold: Gradual Interface

Collagen-GAG

Mineralized Collagen-GAG

Energy-dispersive X-ray (EDX)

Harley et al., 2010

Osteochondral Scaffold: Goat Model

- Initial animal studies indicate bone and cartilage regeneration (Vickers, 2007)
- Longer term (52 week) animal studies ongoing (Lynn)


Vickers, 2007
Osteochondral Scaffold: Clinical Use

- CE Mark approval for clinical use in Europe obtained January 2009
- First clinical use February 2009 in backfill of mosaicplasty donor site
- Currently using for primary sites
- As of April 2012: roughly 200 patients treated
Conclusions

• Fabricated bilayer osteochondral scaffold with gradient interface
• Structure and composition mimic osteochondral tissues
• Range of mineral content, porosity, pore sizes possible by control of the process
• Used materials already approved for medical devices
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