MR Standards for Tissue Engineered Cartilage Characterization

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Magnetic Resonance Imaging and Spectroscopy

Magnetic Resonance Spectroscopy

- Tissue structure: Atomic Scale $\rightarrow$ mm
- Information: Metabolites, ECM quantity and dynamics, cells proliferation, phenotype

Magnetic Resonance Imaging

- Tissue structure: $\mu$m $\rightarrow$ mm
- Information: Porosity, tissue microstructure, ECM composition

- Wealth of information.
- Variety of pulse sequences/probes to investigate any kind of material.
- Suitable for in vitro/ex-vivo investigations.

- Localized information.
- Best soft tissue contrast
- Non-invasive and non-ionizing.
- Suitable for in vitro, ex vivo and in vivo investigations.
History of correlating MR properties with native cartilage parameters

High Resolution MRI of Bovine Articular Cartilage

Yin et al, ICMRM 2011
Engineered cartilage tissue: Different approaches

A. Human Mesenchymal Stem Cells (HMSCs) + Collagen/Chitosan scaffold

B. Bovine Chondrocytes + Microfuge tube + Culture media + Centrifuge → Cell pellets

C. Bovine Chondrocytes + 1.25% Alginate Solution → Precipitation In CaCl₂

(a)

1. chondrocytes
2. MSCs + growth factors

Centrifuge

Cell Pellets

scaffold

scaffold + cells

Vertical bore MRI

Typical sample probe: 5/10/20/30 mm diameter

(b)

1. Scaffold-free Pellets
2. scaffold + stem cells

2 weeks 4 weeks 6 weeks

Small Animal MRI

Typically suitable for rats and mice

Application of NMR/MRI in tissue engineering

- As cell-biomaterial interaction progresses within the tissue engineered constructs, changes in ECM, water content, lipid ratio occur, which are reflected in MR parameters.

**MR parameters that matter**

- Proton Density
- Chemical Shift
- Spin-Lattice Relaxation Time ($T_1$)
- Spin-Spin Relaxation Time ($T_2$)
- Apparent Diffusion Coefficient (ADC)
- Spin-Lattice Relaxation Time in Rotating frame ($T_{1\rho}$)
- Magnetization Transfer Ratio (MT)
- Double-Quantum and Triple Quantum Coherence Spectra (DQ, TQ)
- And many more…
<table>
<thead>
<tr>
<th>Current Methods</th>
<th>Parameter</th>
<th>Macromolecule probed</th>
<th>nature of correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnetic Resonance Spectroscopy (MRS)</td>
<td>Chemical shift</td>
<td>Proteoglycans, Collagen, Cell density and phenotype</td>
<td>Quantitative</td>
</tr>
<tr>
<td>((^1)H, (^13)C, (^23)Na)</td>
<td>Line integral and broadening</td>
<td>Proteoglycans, Collagen</td>
<td>Quantitative</td>
</tr>
<tr>
<td>Magnetic Resonance Imaging (MRI)</td>
<td>Site specific relaxation rates</td>
<td>Proteoglycans (HRMAS), Collagen (CPMAS)</td>
<td>Site specific motion</td>
</tr>
<tr>
<td></td>
<td>Multiple-quantum coherence spectroscopy</td>
<td>Proteoglycans, Collagen order</td>
<td>Molecular motion and tissue anisotropy</td>
</tr>
<tr>
<td>Image contrast</td>
<td></td>
<td>Proteoglycans, Collagen, Cell density for SPIO labeled cells</td>
<td>Non-specific, qualitative</td>
</tr>
<tr>
<td>T(_1) relaxation time</td>
<td></td>
<td>Proteoglycans, Tissue Water</td>
<td>Primarily proteoglycans, but all ECM component contribute</td>
</tr>
<tr>
<td>T(_2) relaxation time</td>
<td></td>
<td>Proteoglycans, Collagen, Tissue water</td>
<td>Depends on the scaffold/cells, Primarily collagen but all ECM component contribute</td>
</tr>
<tr>
<td>Apparent Diffusion Coefficient (ADC)</td>
<td></td>
<td>Proteoglycans, Collagen, Tissue Water</td>
<td>For short observation time - water content and for long observation time - ECM component contribute</td>
</tr>
<tr>
<td>Magnetization Transfer Ratio (MT)</td>
<td></td>
<td>Proteoglycans, Collagen, Tissue Water</td>
<td>Primarily proteoglycans, both ECM component can contribute depending upon experimental conditions</td>
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MR characterization of engineered cartilage (chondrocytes seeded in alginate beads)

An example of MRI assessment of human chondrocytes seeded in alginate beads. (a) $T_1$ (b) $T_2$ and (c) ADC values over the period of four weeks. $T_1$ decreased by 6%, $T_2$ increased by 8% and ADC values decreased by 5%.

Kotecha et al. Tissue Science, 2013
Issues currently faced

• Current standard biochemical and immunohistochemistry characterization techniques for tissue engineered cartilage relies on the production of collagen and proteoglycans as biomarkers for success while ignoring their composition which affects the tissue functionality. Mechanical testing addresses this issue, but MR techniques has potential to solve this problem.

• Current standard MR characterization techniques (proton relaxation and diffusion measurements) uses the benchmark of native cartilage tissue for correlating MR properties with ECM components of engineered tissues while ignoring the contribution of scaffolds, cells and exact composition of ECM components.
Native vs Engineered

Future Direction

• We need to find strategies to separate out contribution from scaffolds, cells and ECM components in MR parameters of scaffold-based engineered cartilage.

• Tissue functionality (molecular dynamics, tissue anisotropy) is an important parameter, and this information is only available through NMR/MRI, so tissue engineering community should be encouraged to include these parameters when evaluating engineered tissue.

• Currently there is no MR standard for ECM quantification. This need to be addressed.

• ECM specific new MR biomarkers need to be developed, which will get away from ambiguity.
Future directions (New Biomarkers)

Kotecha et al, NMR in Biomedicine, 24(6), 709-717, 2013.
Thank You!!!