Study of Effects of Microgravity on Disc Height and Compositional Analysis

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Disc Herniation and Back Pain

- Commonly occurs in astronauts
- Increases the injury likelihood and inhibits daily life
- Underlying factor: intervertebral disc (IVD) degeneration (NASA)
- Endplates
  - Separates disc and vertebral body
  - Structural and compositional changes during space travel
    - Spinal elongation
    - Imbalance of collagen-proteoglycan ratio (Pedrini-Mille)
Gravity Effects on Spine

• Spine compressed during return to Earth
• Intervertebral discs compressed and may cause disc herniation (Maynard)
• Compositional and structural changes in trabecular bone (LeBlanc)
Objectives

• To investigate structural and compositional changes of mice intervertebral discs under microgravity by examining
  – Disc heights
  – Trabecular analyses

• Methods
  – Micro computed tomography (μCT)
  – Tri 3D Bon software and CT Analyser software
  – Mimics 13.0 and 13.1

• Analyses parameters
  – Bone volume ratio (BV/TV)
  – Trabecular number (Tb.N)
  – Trabecular thickness (Tb.Th)
  – Bone mineral density (BMD)
  – Disc height
Samples

NASA Discovery STS-131 mission mice
Strain: C57Bl/6
CD45.1 congenic mice
Weigh 25g
Image Acquisition

Shimadzu SMX-160CTS

Chamber design

separator
Micro CT Scanning: High Resolution Imaging

• Conditions
  – 1.59 microns isotropic
  – source-to-object distance (SOD) of 3.5mm
  – Voltage: 65 kV – 72 kV
  – Reconstruction matrix size 512 x 512
  – Field of view (FOV XY) approximately 0.8 mm

• Limitation: incomplete view of endplate
Image Reconstruction

1. Tri/3D Bon (Ratoc System Engineering)
   - Import raw data from µCT
   - Noise reduction and filter
   - Generate 3D model
   - Export files as bitmap

2. Mimics 13.1 ® (Materialise)
   - Import bitmap files
   - Threshold and segment
   - Generate reconstruction 3D model
   - Export point cloud (with Mimics 13.0)
Disc Height Calculation

1. Eraser Program

- Open point cloud data from Mimics
- Erase unnecessary regions
- Generate endplate
- Save files as .txt

2. DHD Program

- Open caudal and cranial erased text files
- Calculate disc height
Trabecular Analysis

1. Tri/3D Bon (Ratoc System Engineering)
   • Import raw data from μCT
   • Noise reduction and filter
   • Generate 3D model
   • Export files as bmp

2. CT Analyser Software
   • Import reconstructed data
   • Select region of interest (ROI)
   • Calculate trabecular parameters
   • Export analyses as text file
Results: Disc Height Practice

Resolution level 2
X/Y: 2 x 0.009 (mm)
Z: 1 x 0.009 (mm)

Resolution level 3
X/Y: 3 x 0.009 (mm)
Z: 1 x 0.009 (mm)

Resolution level 4
X/Y: 4 x 0.009 (mm)
Z: 1 x 0.009 (mm)

Resolution level 5
X/Y: 5 x 0.009 (mm)
Z: 1 x 0.009 (mm)
X-Y resolution reduction by 2-5x appears appropriate for estimating IVD height in mice

<table>
<thead>
<tr>
<th>resolution</th>
<th>mean</th>
<th>minimum</th>
<th>maximum</th>
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<td>n/a</td>
<td>n/a</td>
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<tr>
<td>2</td>
<td>0.2887</td>
<td>0.1383</td>
<td>0.5271</td>
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<td>3</td>
<td>0.284</td>
<td>0.1364</td>
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<td>4</td>
<td>0.2849</td>
<td>0.136</td>
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<td>5</td>
<td>0.2898</td>
<td>0.136</td>
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<td>STDEV</td>
<td>0.00283</td>
<td>0.00110</td>
<td>0.0288</td>
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Table 03. Disc heights of L45 of mice spine sample 1 at various resolutions
Results: Trabecular Bone Analysis

<table>
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<tr>
<th>#</th>
<th>Date</th>
<th>type</th>
<th>voltage (kV)</th>
<th>brightness (kV)</th>
<th>FOV XY (mm)</th>
<th>FOV Z (mm)</th>
<th>SOD (mm)</th>
<th>Resolution (microns)</th>
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<td>cancellous bone</td>
<td>62</td>
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<td>3.098</td>
<td>1.895</td>
<td>8.6</td>
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<td>cancellous bone</td>
<td>60</td>
<td>0</td>
<td>0.873</td>
<td>0.825</td>
<td>3.9</td>
<td>512; (1.705)</td>
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• Trabecular bone images of the fourth vertebrate at two different resolutions.

Scan #1 Scan #2
Figure 01. Bone volume ratio of different aged mice spines of twelve days, twenty-four days, and one month over the six lumbar levels.
Figure 02. Bone surface density of different aged mice spines of twelve days, twenty-four days, and one month over the six lumbar levels.
Results: Trabecular Bone Analysis

Figure 03. Trabecular thickness of different aged mice spines of twelve days, twenty-four days, and one month over the six lumbar levels.
Results: Trabecular Bone Analysis

Figure 04. Trabecular number of different aged mice spines of twelve days, twenty-four days, and one month over the six lumbar levels.
Results: Endplate Imaging

<table>
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<th>#</th>
<th>date</th>
<th>voltage (kV)</th>
<th>brightness (power)</th>
<th>FOV XY (mm)</th>
<th>FOV Z (mm)</th>
<th>resolution (microns)</th>
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<td>512; (1.465)</td>
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Table 04. micro CT scanning results of endplates at various voltage-power settings with a source-to-object distance (SOD) of 3.5 mm and a matrix of 512 x 512.
Table 05. micro CT scanning results of endplates at various voltage-power settings with a source-to-object distance (SOD) of 3.5 mm and a matrix of 512 x 512.
Discussion

• Accomplished/Established:
  – Trabecular and endplate high resolution scanning protocol
  – Mimics software protocol
  – Micro CT scanning and reconstruction practice

• Future Goals
  – Space mice disc heights calculation and analysis
  – Further assessment of parameters of trabecular analyses
  – Biochemical analyses of collagen-proteoglycan ratio
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