Antioxidative Fullerol for Orthopaedic Diseases

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Research interests

- **Fullerene C<sub>60</sub> and orthopaedic diseases**
- **Fullerene C<sub>60</sub> and cell differentiation/function**


Fullerene $C_{60}$

- **Drs. Smalley, Curl and Kroto, 1985/1996**

Molecular model of Fullerene $C_{60}$

- Its antioxidative capacity hundreds times higher than...

Fullerene-ROS interactions
(Satoh and Takayanagi, 2006)

![Diagram of Fullerene-ROS interactions]

- NOS: nitric oxide synthase
- NOS inhibition; NO scavenging
- Superoxide dismutase mimic
Orthopaedic diseases/OS

- Multifactorial diseases
  - Oxidative stress
  - Alcohol use
  - Exercise
  - Cigarette smoking
  - Menopause
  - Medication
  - Inflammation
  - Apoptosis
  - Peak bone mass
  - Sun exposure
  - Calcium intake
  - Genetic variation

Orthopaedic diseases (Osteonecrosis, osteoporosis, osteoarthritis, etc.)

- Oxidative stress (OS)
  - Antioxidant systems (SOD, GPx, GSH, CAT, FOXO)
  - p66Shc
  - ROS
  - O$_2^-$, HO-, H$_2$O$_2$
  - Mitochondria
  - Oxidative stress
  - Damage to macromolecules
  - Cell senescence
  - Apoptosis
  - Uncontrolled
  - Autophagy
  - Sex steroids

(Hendrickx et al., 2015)
OS/adipo/osteo/pathways

High levels of ROS

Hedgehog signalling
MAPK signalling

β-catenin/Wnt signalling

Adipogenesis

Oxidative stress
Adipogenesis

Osteogenesis

Oxidative stress
Osteogenesis

(Atashi et al., 2015)
**OS/OC/inflammation/pathways**

- **Osteoclastogenesis**
  - (Kanzaki et al., 2013)

  - Oxidative stress
  - Oxidative stress

- **Inflammation**
  - (Emre et al., 2007)

  - Oxidative stress
  - Oxidative stress

**Diagram Details:**
- **RANKL**
  - **RANK**
    - ↓ Nrf2/Keap1 ratio
    - ↓ cytoprotective enzymes
    - ↑ intracellular ROS
    - ↑ Osteoclastogenesis

- **Inflammation**
  - LPS
  - IL-10
  - IFNγ
  - TLR4
  - NF-κB
  - ERK
  - JNK
  - p38
  - UCP2
  - membrane
  - cytoplasm
  - mitochondria

- **Transcription**
  - Nucleus

**University of Virginia Orthopaedic Surgery**
Biodistribution/Blood clearance

A: Experimental protocol

- Tc99m-Fullerol Radiosynthesis
- Quality Control
- Euthanasia (Day 1: N=3)
- Organs collection
- Post Mortem Analysis
- Anesthesia
- Tc99m-Fullerol injection
- Euthanasia (Day 3; N=3)
- Tissue collection
- Post Mortem Analysis

In vivo whole-body dynamic imaging (+ Blood sample collection)

B: SPECT imaging

1.5 h post injection

X-Ray
Planar
Fused

C: Imaging quantification

Counts/Min/Region

1.5h 24h 48h

Activity(% initial)

D: Organ distribution

<table>
<thead>
<tr>
<th></th>
<th>Time after injection</th>
<th>1.5h</th>
<th>48h</th>
</tr>
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<tbody>
<tr>
<td>Liver</td>
<td></td>
<td>0.262</td>
<td>0.309</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td>0.097</td>
<td>0.083</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td>0.756</td>
<td>0.910</td>
</tr>
<tr>
<td>Cortical bone</td>
<td></td>
<td>0.013</td>
<td>0.048</td>
</tr>
<tr>
<td>Femoral bone marrow</td>
<td></td>
<td>0.767</td>
<td>0.145</td>
</tr>
<tr>
<td>Biliary fat</td>
<td></td>
<td>0.003</td>
<td>0.001</td>
</tr>
<tr>
<td>Articular cartilage</td>
<td></td>
<td>0.042</td>
<td>0.018</td>
</tr>
</tbody>
</table>

E: Blood clearance

T1/2alpha = 1.9 h

- rabbit
- TC99m-Fullerol
- SPECT/
- γ counter

Retained in bone marrow for up to 48 h

Half time of 1.9 h in blood
Osteonecrosis

A: ARS/mouse D1

B: Gene expression/mouse D1

C: PPARγ/hBMSCs

D: aP2/hBMSCs

E: ROS assay/ hBMSCs

F: Oil Red O/hBMSCs

G: Rabbit ONFH model (data is pending)

<table>
<thead>
<tr>
<th>Group</th>
<th>P</th>
<th>F</th>
<th>S</th>
<th>S+F</th>
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<tbody>
<tr>
<td>MPSL</td>
<td>NO</td>
<td>NO</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Nano-Fullerene</td>
<td>NO</td>
<td>Fullerol</td>
<td>NO</td>
<td>Fullerol</td>
</tr>
<tr>
<td>Animal number</td>
<td>9</td>
<td>9</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

- D1 & hBMSC
- Fullerol
- ARS staining
- ORO staining
- qRT-PCR
- Rabbit
- MPSL
- H & E staining
- ROS
- Osteo
- Adipo
- Pending histological data of FH
Osteoporosis

- RAW264.7
- RANKL
- Fullerol
- Trap staining
- qRT-PCR
- Rabbit
- MPSL
- Micro-CT

- OC
- VB Bone loss

![Images of osteoporosis studies with results for different treatments and staining methods.](attachment:osteoporosis_images.png)

- Diagram showing changes in fold change for different treatments.
- Graphs showing Tb.Sp for L4 and Tb.N for L4 with significance levels indicated.

a, P<0.05 vs group A; b, P<0.05 vs group C
Disc degeneration

- RAW264.7
- LPS
- Fullerol
- qRT-PCR
- ELISA
- Rabbit
- MPSL
- Micro-CT

- Inflammation
- IVDD
Summary

- Fullerol is enriched in the femoral bone marrow.
- Fullerol regulates differentiation of bone marrow stem cells and monocytic progenitors.
- Fullerene as a powerful antioxidant could potentially be used for treatment of osteonecrosis, osteoporosis and intervertebral disc degeneration.
Future Plans

A. Fullerene → More orthopaedic diseases

B. Fullerene → Drug
   Targeted Drug Delivery Systems for bone marrow disorders

C. Biomaterial Scaffold
   Fullerene

Novel antioxidative biomaterials

➢ Our R21 on the topic of fullerene-PLGA for bone repair was scored 22. Release profile is being prepared.
Funding Sources

• Orthopaedic Research and Education Foundation/Zachary B. Friedenberg Clinician Scientist Award (Dr. Cui)

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Thank you for your attention! Questions?