



UNIVERSITY  
*of* VIRGINIA

ORTHOPAEDIC SURGERY

# Antioxidative Fullerol for Orthopaedic Diseases

Xinlin Yang, PhD

Assistant Professor of Orthopaedics

May 9, 2015





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

**Yang X**, Li CJ, Wan Y, Smith P, Shang G, Cui Q.

[Antioxidative fullerol promotes osteogenesis of human adipose-derived stem cells.](#) Int J Nanomedicine. 2014 Aug 20;9:4023-31.

**Yang X**, Jin L, Yao L, Shen FH, Shimer AL, Li X.

[Antioxidative nanofullerol prevents intervertebral disk degeneration.](#) Int J Nanomedicine. 2014 May 15;9:2419-30.

**Yang X**, Ebrahimi A, Li J, Cui Q.

[Fullerene-biomolecule conjugates and their biomedical applications.](#) Int J Nanomedicine. 2014;9:77-92. Review.

Liu H, **Yang X**, Zhang Y, Dighe A, Li X, Cui Q.

[Fullerol antagonizes dexamethasone-induced oxidative stress and adipogenesis while enhancing osteogenesis in a cloned bone marrow mesenchymal stem cell.](#) J Orthop Res. 2012 Jul;30(7):1051-7.

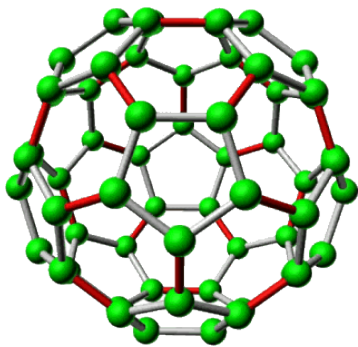




# Fullerene C<sub>60</sub>

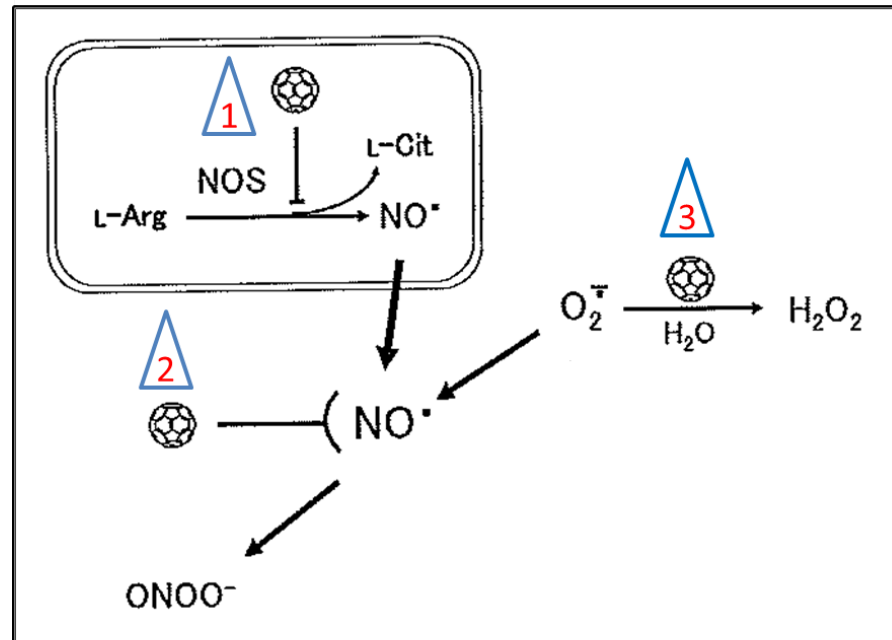
➤ **Drs. Smalley, Curl and Kroto, 1985/1996**

**Molecular model of Fullerene C<sub>60</sub>**



➤ **Its antioxidative capacity hundreds times higher than...**

## Fullerene-ROS interactions (Sato and Takayanagi, 2006)



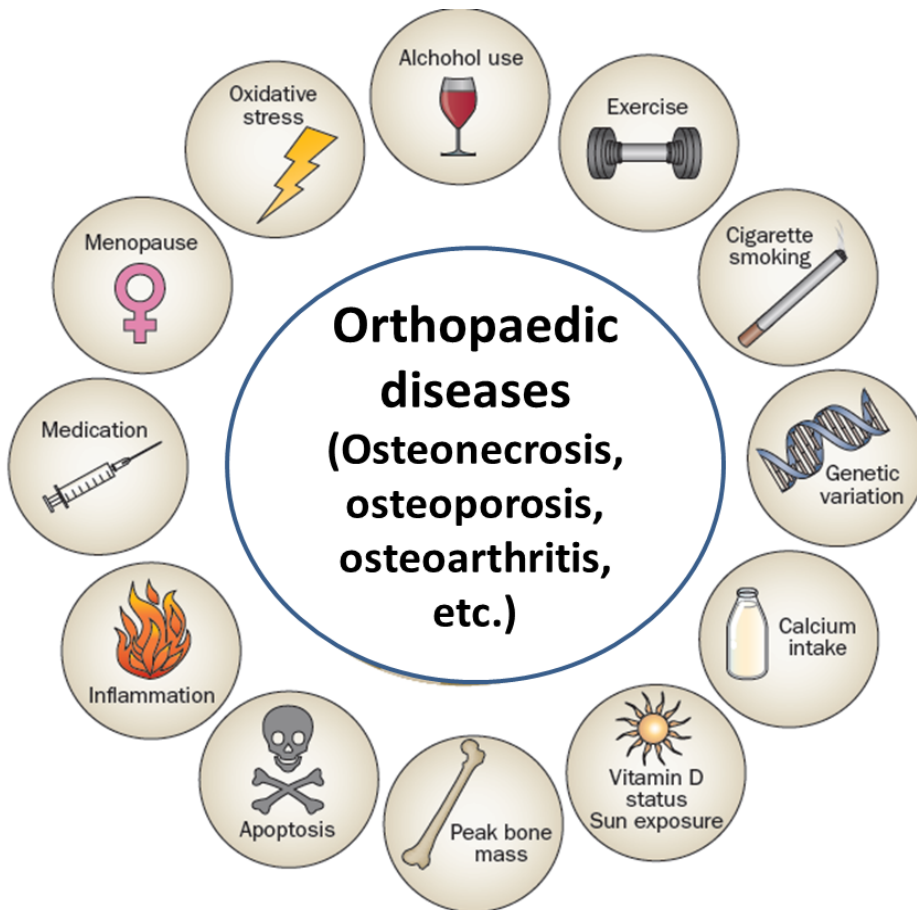
NOS: nitric oxide synthase

**1** NOS inhibition; **2** NO scavenging

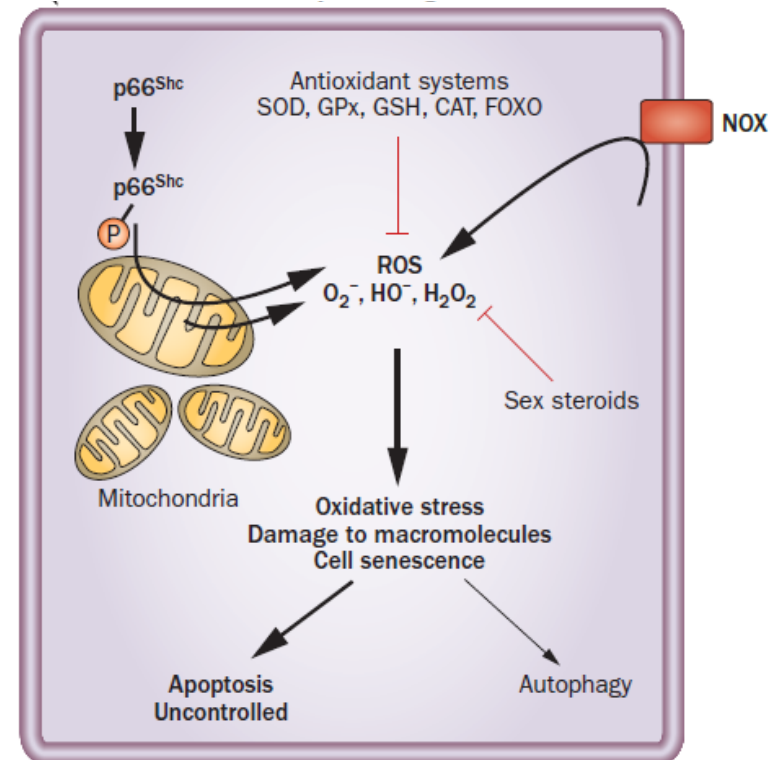
**3** Superoxide dismutase mimic



## ➤ Multifactorial diseases



## ➤ Oxidative stress (OS)

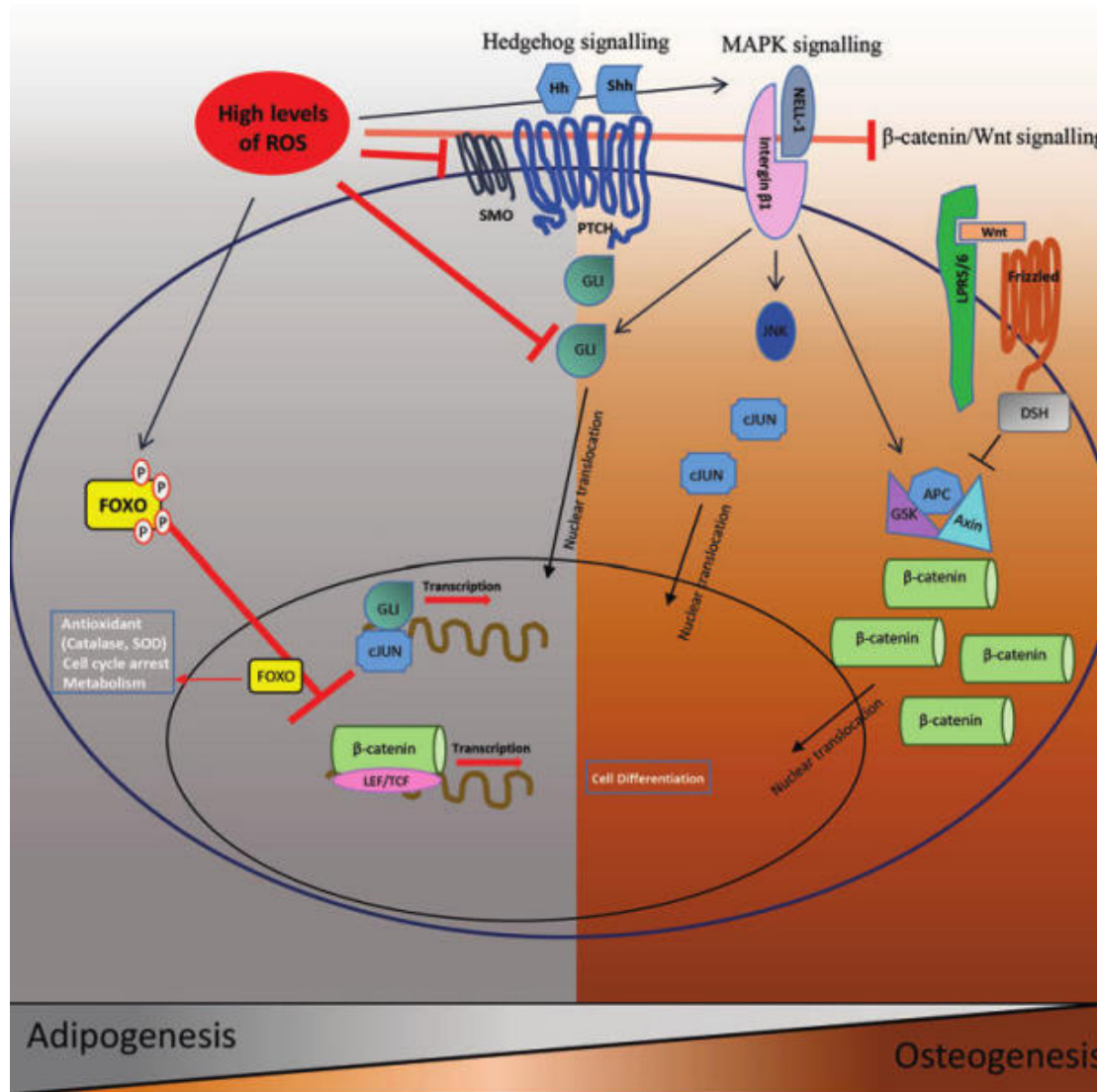


(Hendrickx et al., 2015)





# OS/adipo/osteo/pathways

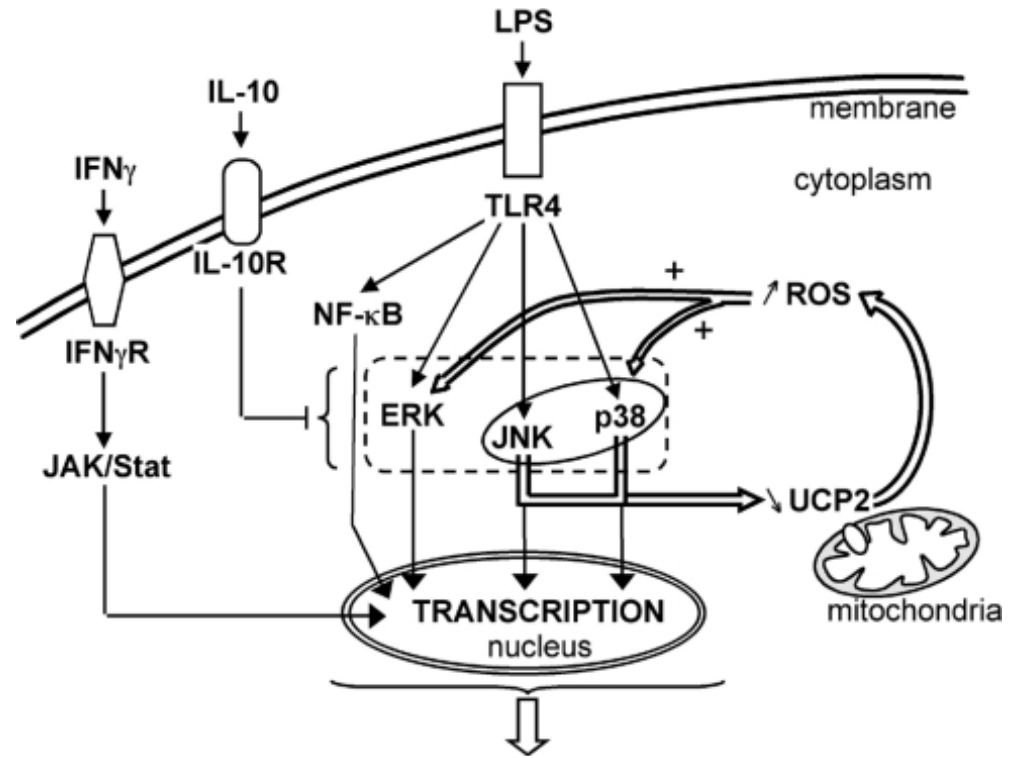
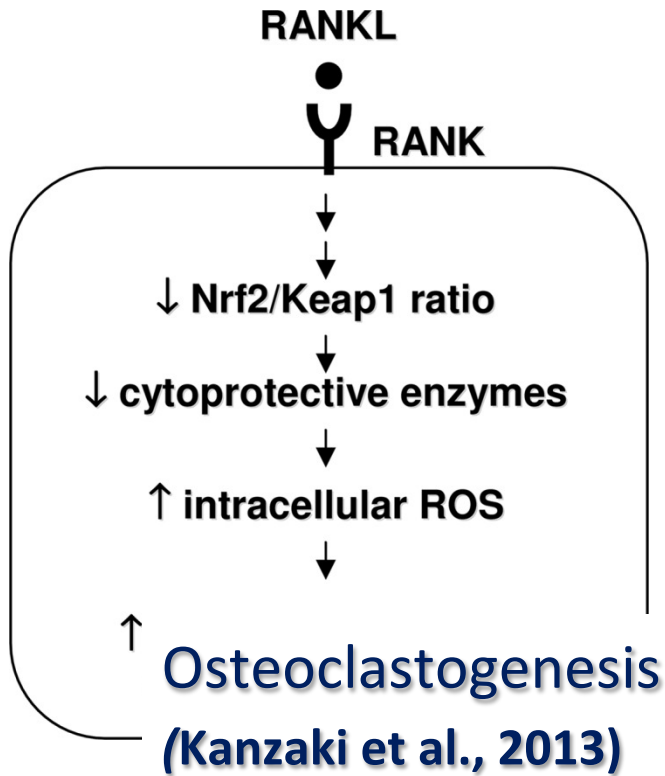


- Oxidative stress ↑  
Adipogenesis ↑
- Oxidative stress ↑  
Osteogenesis ↓





# OS/OC/inflammation/pathways



Inflammation (Emre et al., 2007)

➤ Oxidative stress ↑  
Osteoclastogenesis ↑

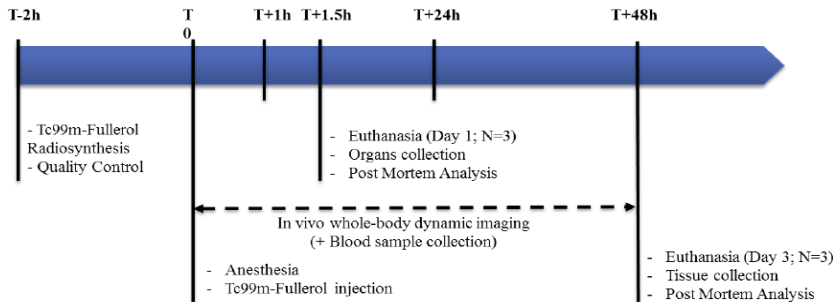
➤ Oxidative stress ↑  
Inflammation ↑



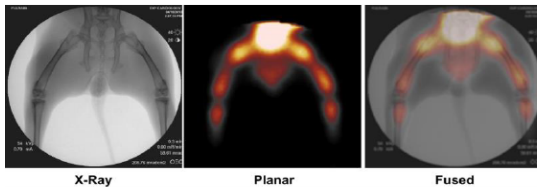


# Biodistribution/Blood clearance

## A: Experimental protocol

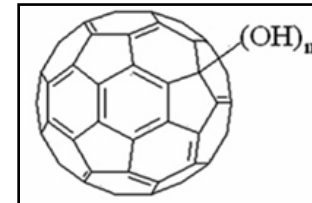
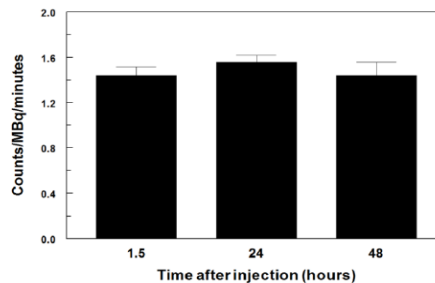


## B: SPECT imaging



1.5 h post injection

## C: Imaging quantification

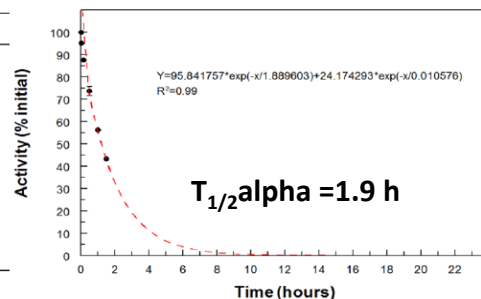


## D: Organ distribution

	Time after injection	
	1.5h	48h
Liver	0.262 ± 0.020	0.309 ± 0.048
Kidney	0.097 ± 0.014	0.083 ± 0.011
Spleen	0.735 ± 0.061	0.910 ± 0.097
Cortical bone	0.013 ± 0.002	0.004 ± 0.002*
Femoral bone marrow	0.767 ± 0.107	0.472 ± 0.145
Belly fat	0.003 ± 0.000	0.001 ± 0.000*
Articular cartilage	0.042 ± 0.019	0.018 ± 0.009

\*p<0.05 vs. 1.5h  
Data are expressed as a percent of injected dose per gram of tissue (%ID/g)

## E: Blood clearance



- ❖ rabbit
- ❖ TC99m-Fullerol
- ❖ SPECT/
- ❖  $\gamma$  counter

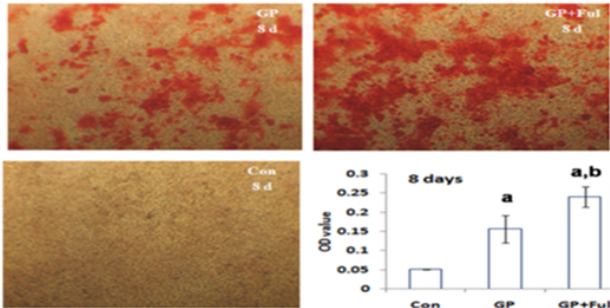
- Retained in bone marrow for up to 48 h
- Half time of 1.9 h in blood



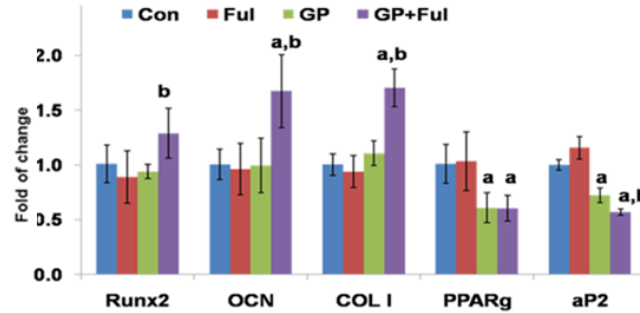


# Osteonecrosis

A: ARS/mouse D1

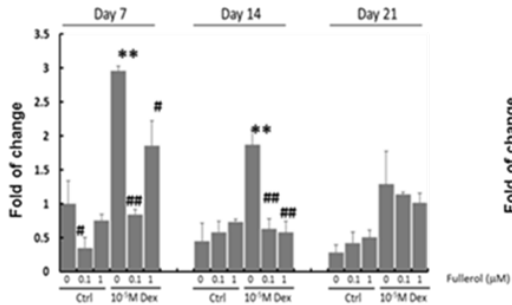


B: Gene expression/mouse D1

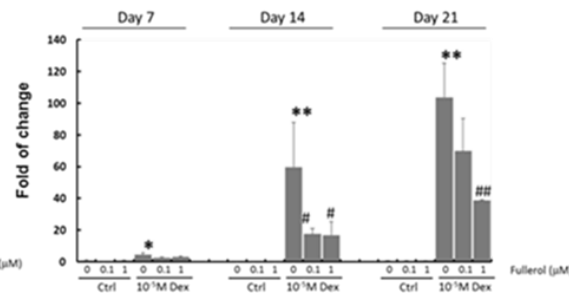


- ❖ D1 & hBMSC
- ❖ Fullerol
- ❖ ARS staining
- ❖ ORO staining
- ❖ qRT-PCR
- ❖ Rabbit
- ❖ MPSL
- ❖ H & E staining

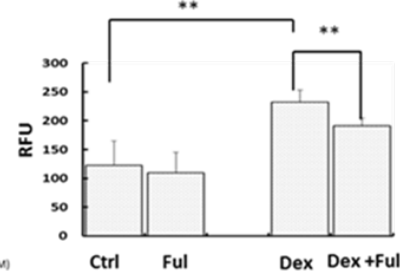
C: PPARγ/hBMSCs



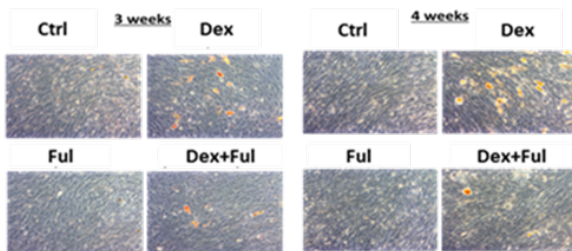
D: aP2/hBMSCs



E: ROS assay/ hBMSCs



F: Oil Red O/hBMSCs



G: Rabbit ONFH model (data is pending)

Group	P	F	S	S+F
MPSL	NO	NO	YES	YES
Nano-Fullerene	NO	Fullerol	NO	Fullerol
Animal number	9	9	11	11

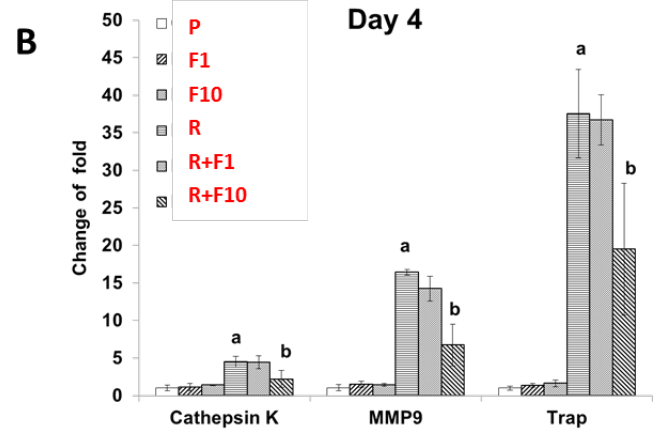
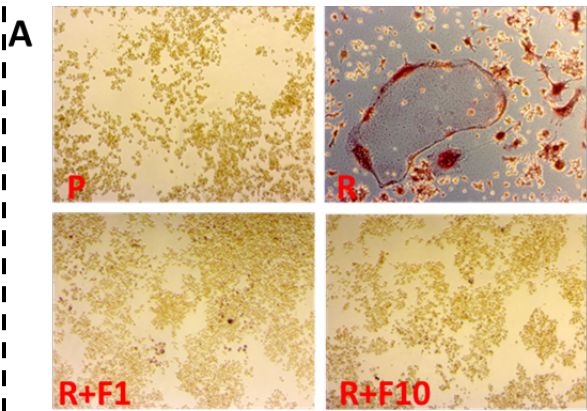
- ROS ↓
- Osteo ↑
- Adipo ↓
- Pending histological data of FH



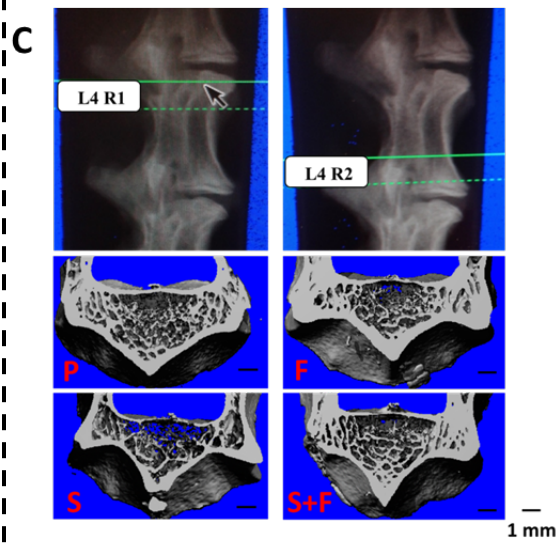




# Osteoporosis

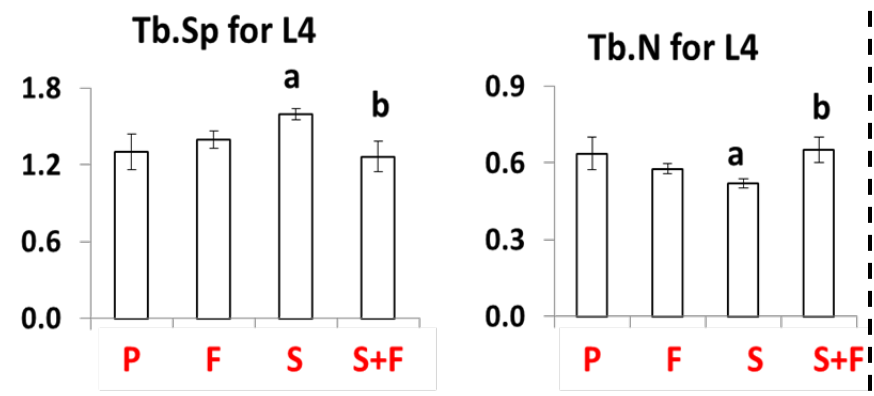


- ❖ RAW264.7
- ❖ RANKL
- ❖ Fullerol
- ❖ Trap staining
- ❖ qRT-PCR
- ❖ Rabbit
- ❖ MPSL
- ❖ Micro-CT



**D**

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

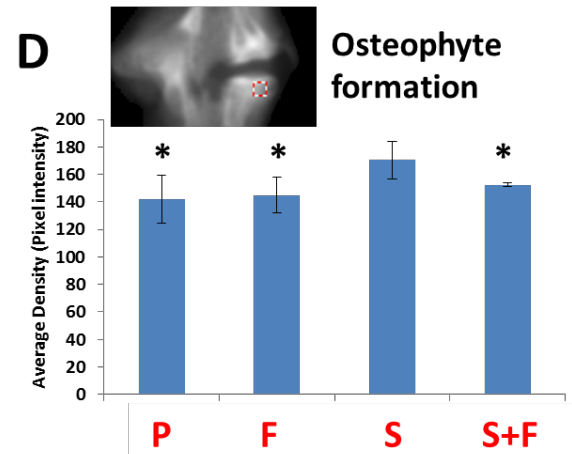
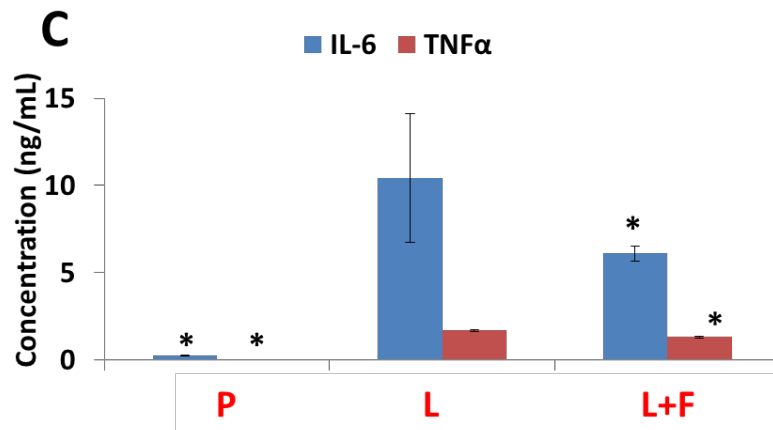
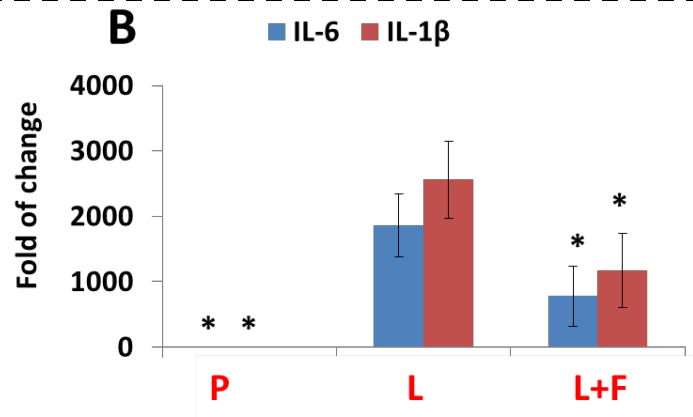
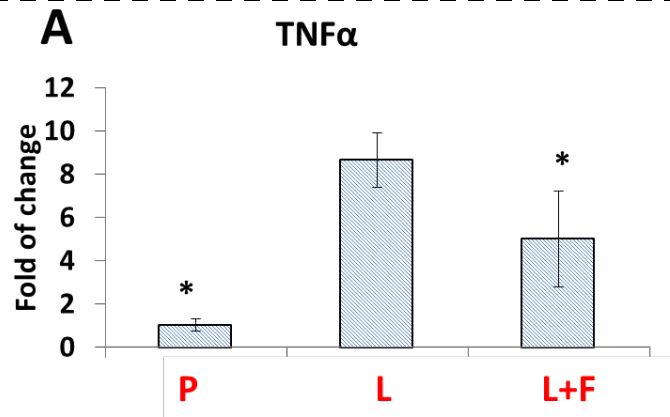


- OC ↓
- VB Bone loss ↓





# Disc degeneration



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

➤ Inflammation ↓

➤ IVDD ↓





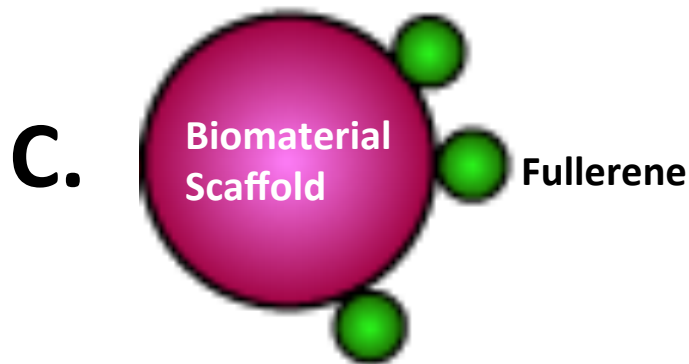
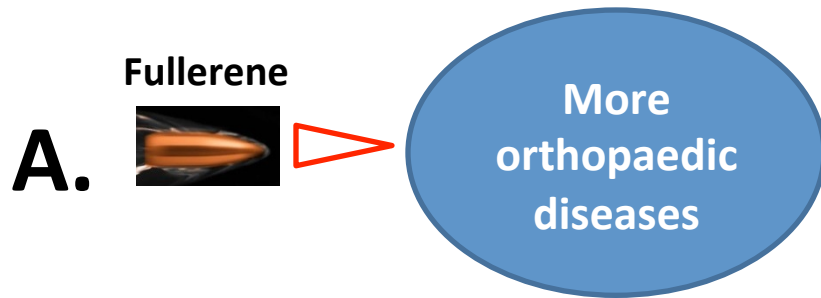
# Summary

- **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



**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)**
- **NIH/NIAMS R21 AR062732 (Dr. Cui)**

