

# **In Vivo and In Vitro Toxicity of AuNPs**

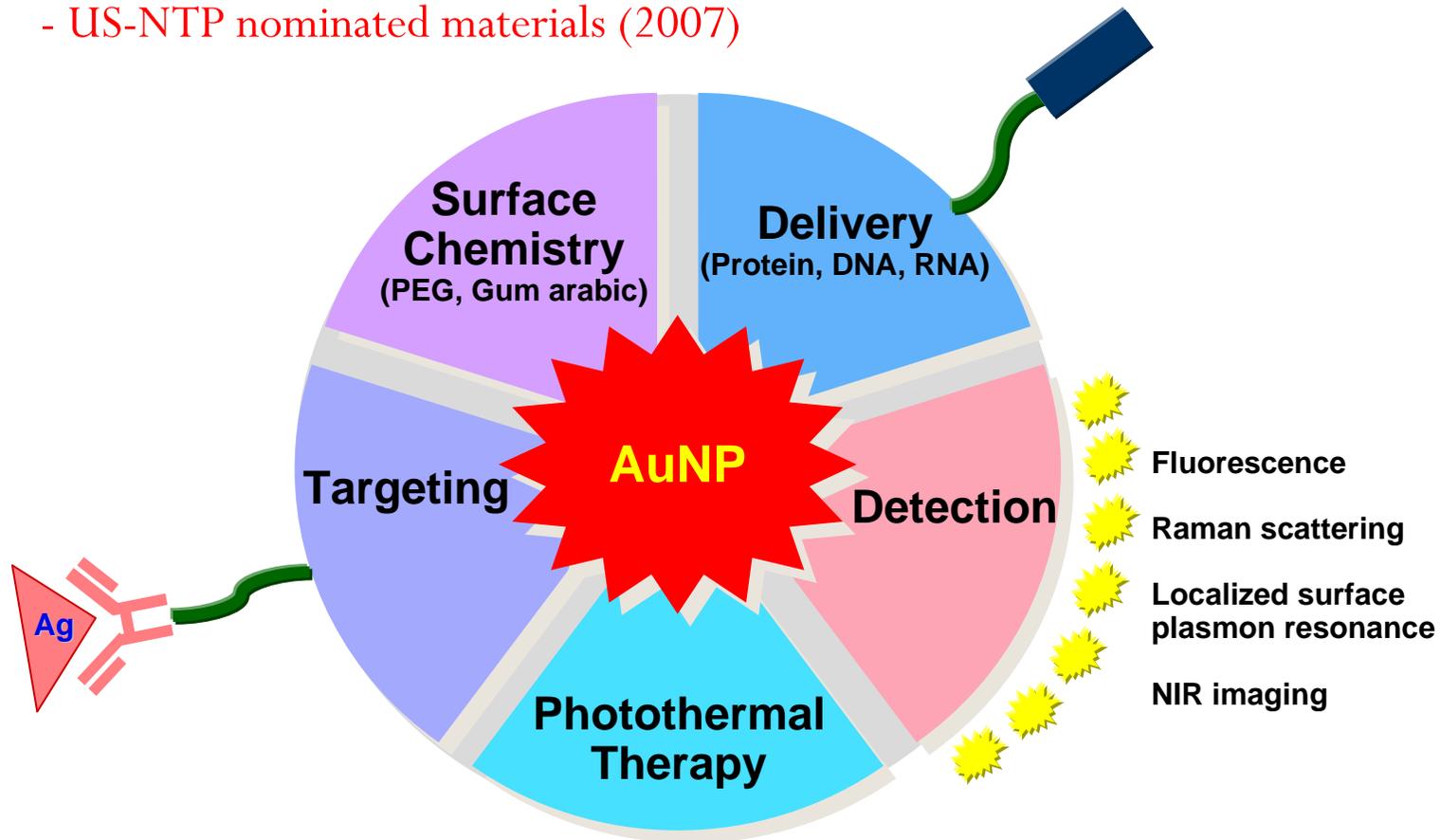
**Division of Toxicological Research  
National Institute of Toxicological Research  
Wan-Seob Cho, DVM, PhD.**

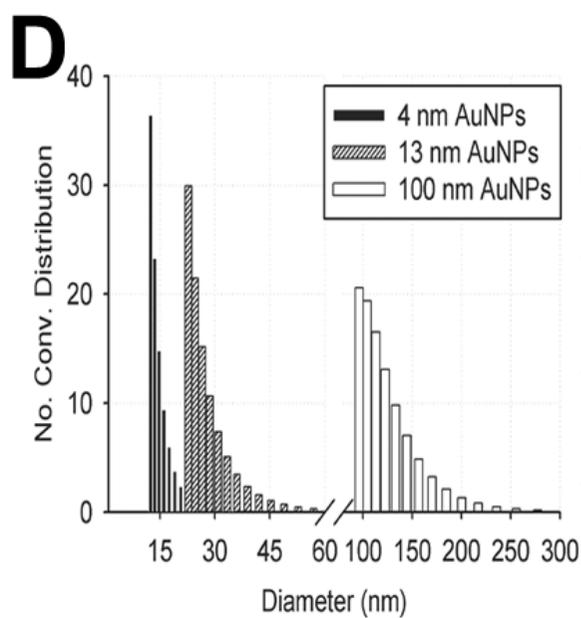
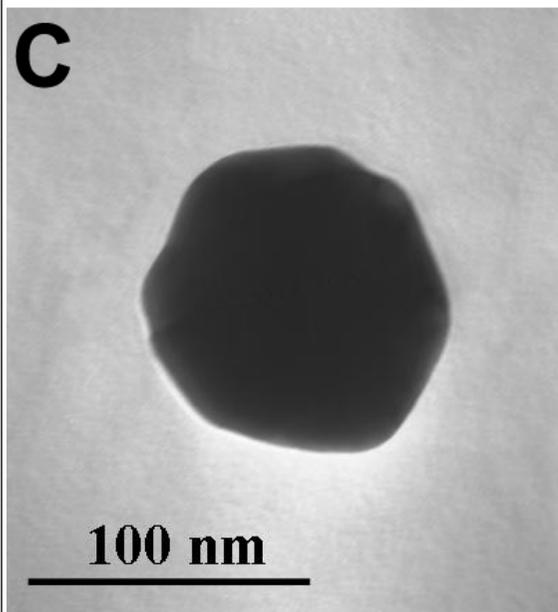
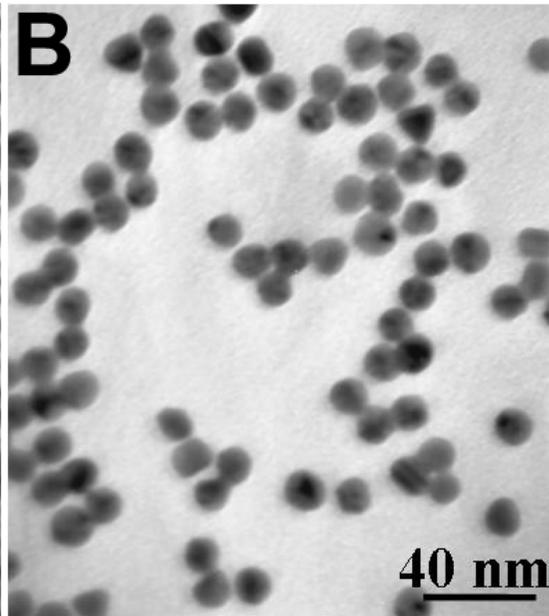
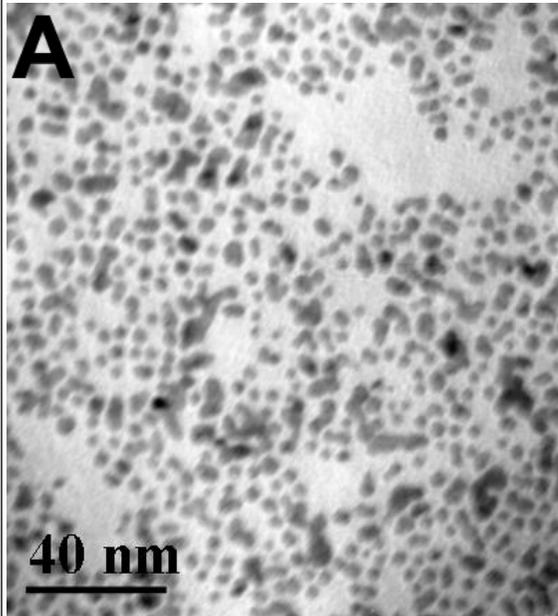
# Contents

- Synthesis and Stability of Gold Nanoparticles
  - Three different sizes (4, 13, or 100 nm)
  - Size confirmation by TEM and DLS
- Single Dose Toxicity Study with Tail Vein Injection
  - Tissue distribution and pharmacokinetics
  - Histopathology and TUNEL assay
  - Adhesion molecules, chemokines, and cytokines expression
- In vitro Toxicity Study of Gold Nanoparticles
  - Cytotoxicity and chemotaxis assay
  - Cytokines expression

# Synthesis and Stability of Gold Nanoparticles

- Background for selection of gold nanoparticles
  - US-NTP nominated materials (2007)





TEM

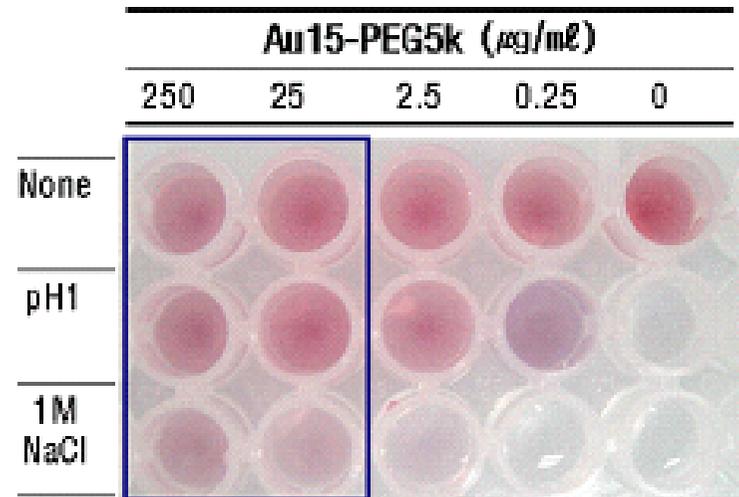
-(A) 4 nm

-(B) 13 nm

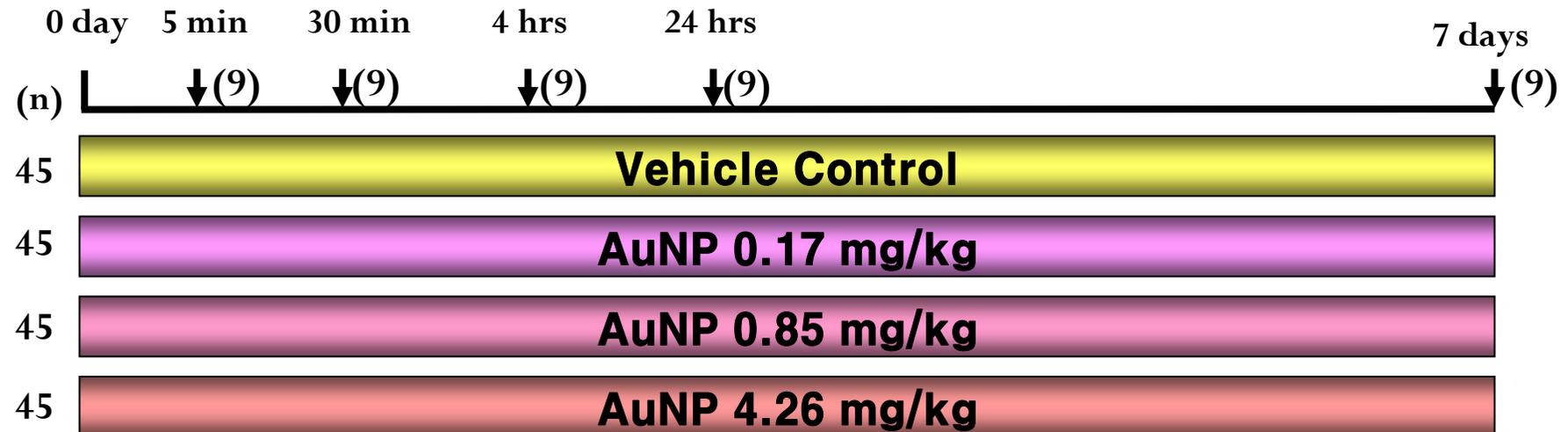
-(C) 100 nm

DLS

-PEG-coated AuNPs



# In Vivo Toxicity Study



Animal : Male BALB/c mice

AuNP: PEG coated AuNPs

## Check Lists

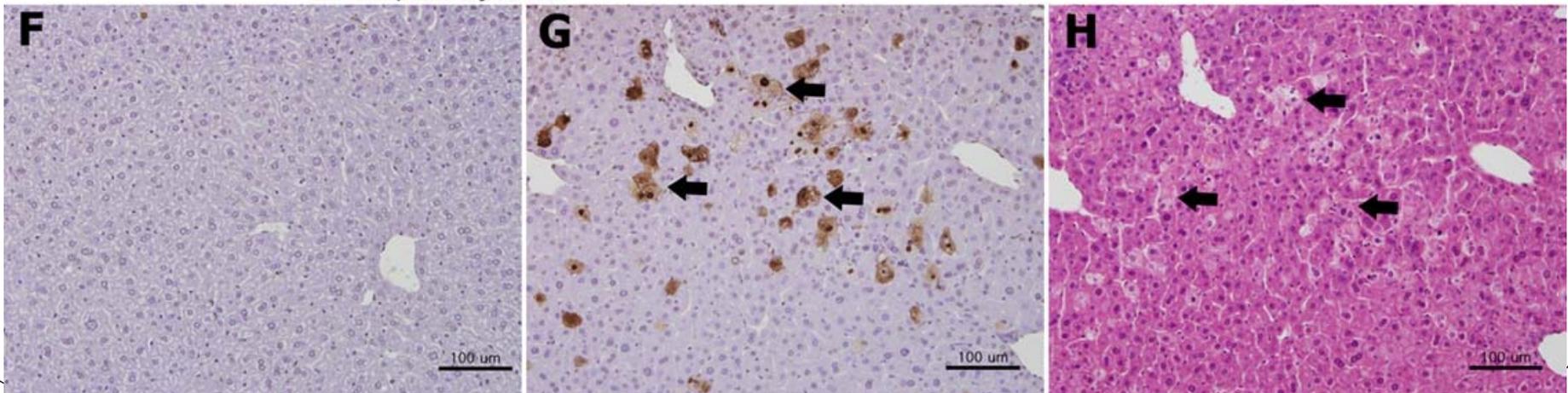
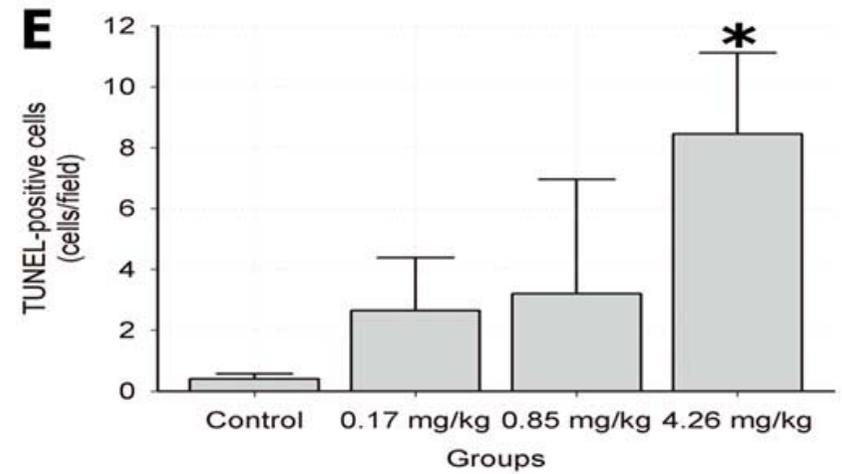
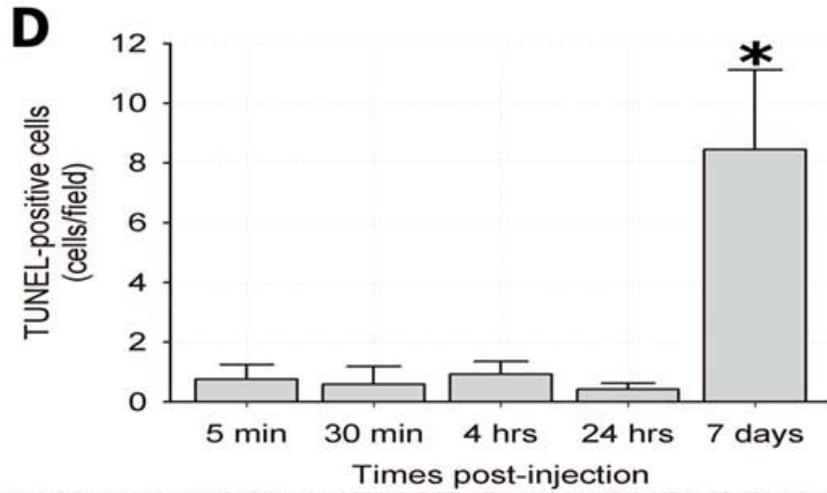
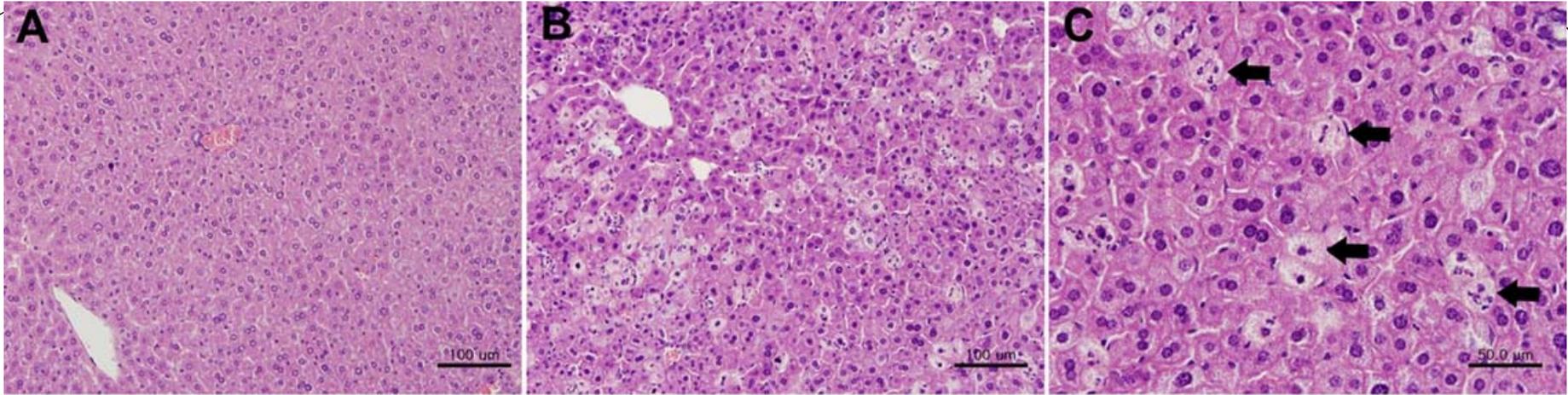
1. Histopathology
2. TUNEL assay
3. Tissue distribution and pharmacokinetics analysis
4. Cellular localization by TEM
5. Adhesion molecules, chemokines, and cytokines expression in the liver

# Histopathology

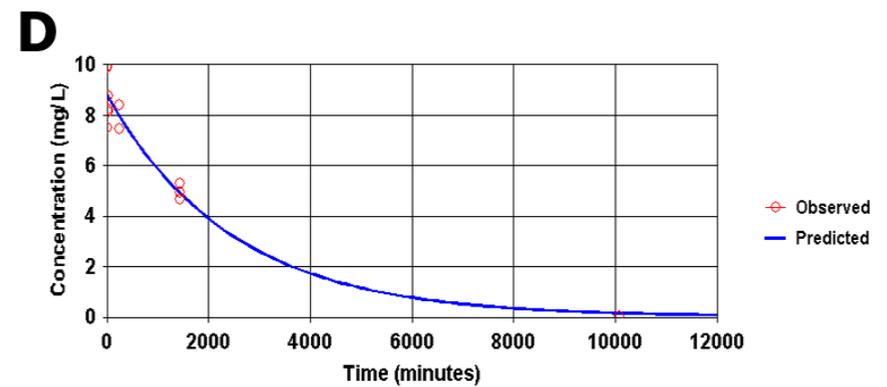
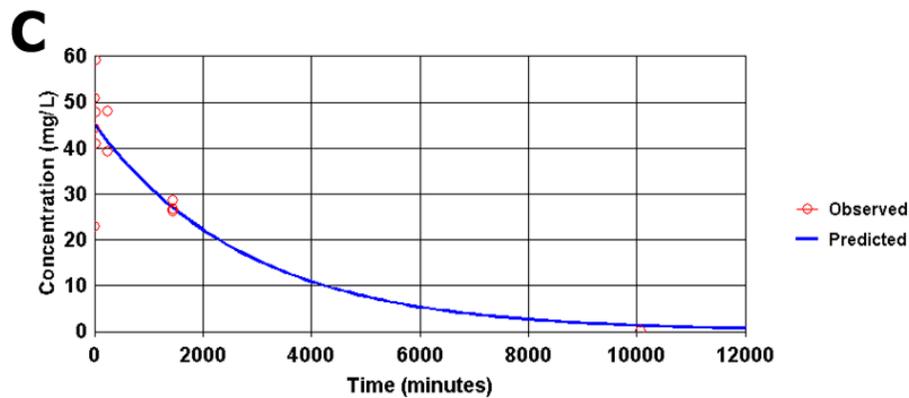
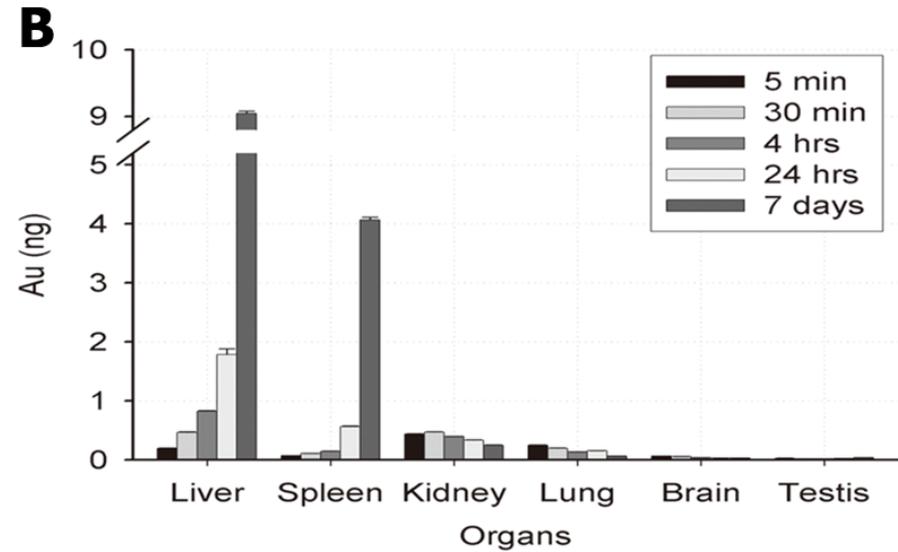
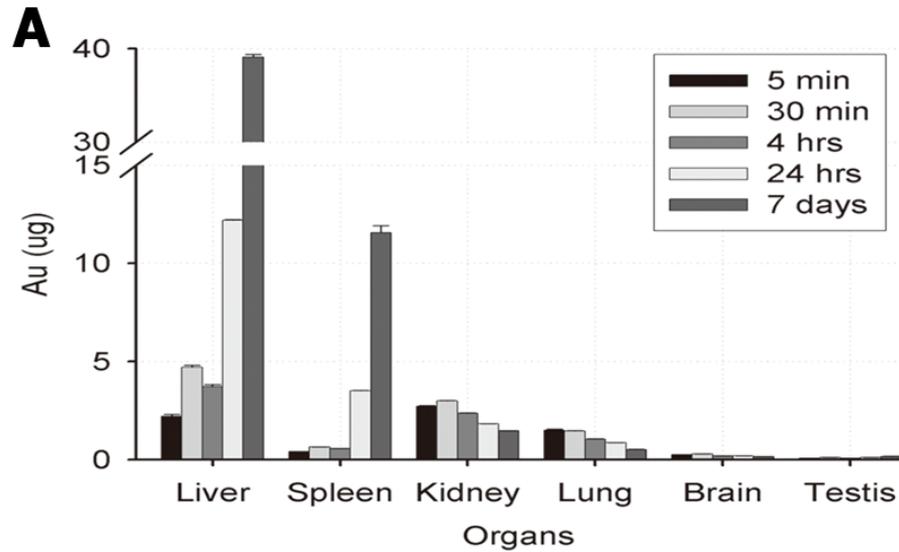
Diagnosis	Groups	Time after injection				
		5 min	30 min	4 hrs	24 hrs	7 days
Apoptotic necrosis	Vehicle control	0 <sup>a</sup>	0	0	0	0
	0.17 mg/kg	1	0	0	0	2
	0.85 mg/kg	0	0	0	1	5*
	4.26 mg/kg	2	0	0	0	6*
Inflammation	Vehicle control	0	0	1	0	0
	0.17 mg/kg	4*	0	0	2	3
	0.85 mg/kg	2	0	3	2	5*
	4.26 mg/kg	2	0	3	1	4*

a, number of animals

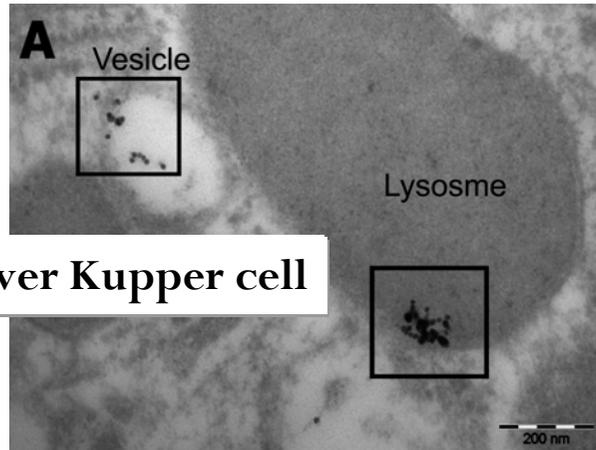
\*, Significantly increased compared with vehicle control group by Chi-square analysis ( $p < 0.05$ )



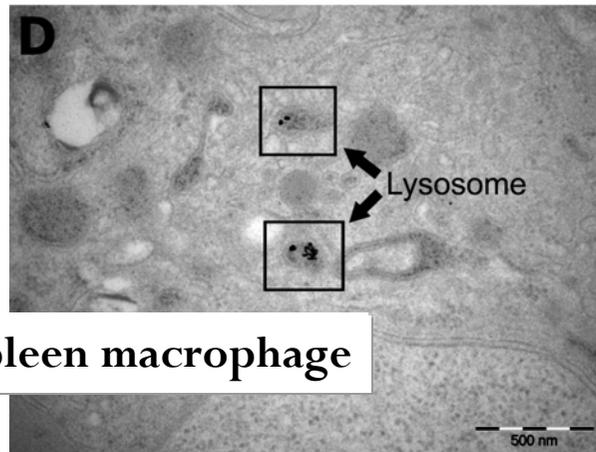
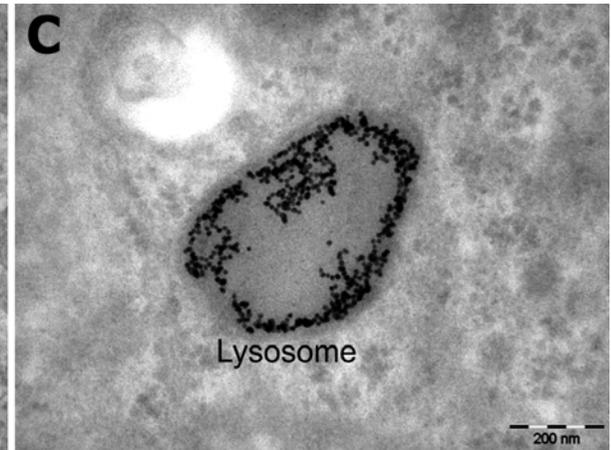
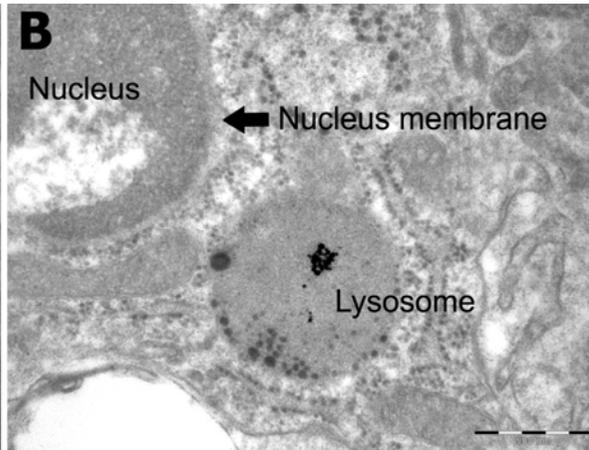
# Tissue Distribution and Pharmacokinetics



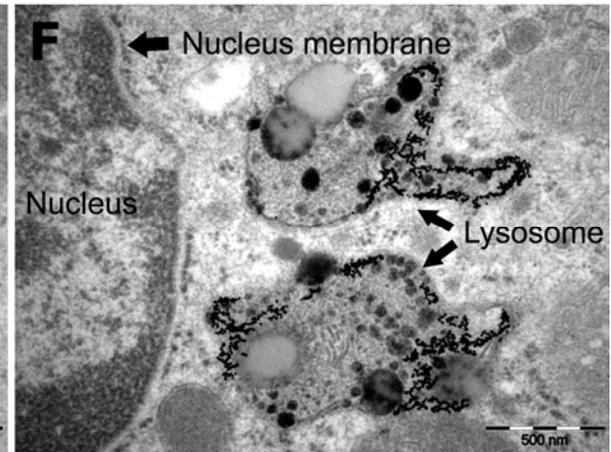
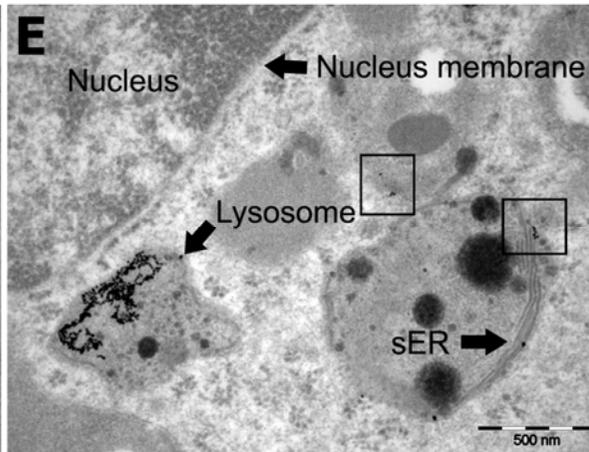
# Cellular Localization by TEM



Liver Kupper cell



Spleen macrophage



24 h

7 days

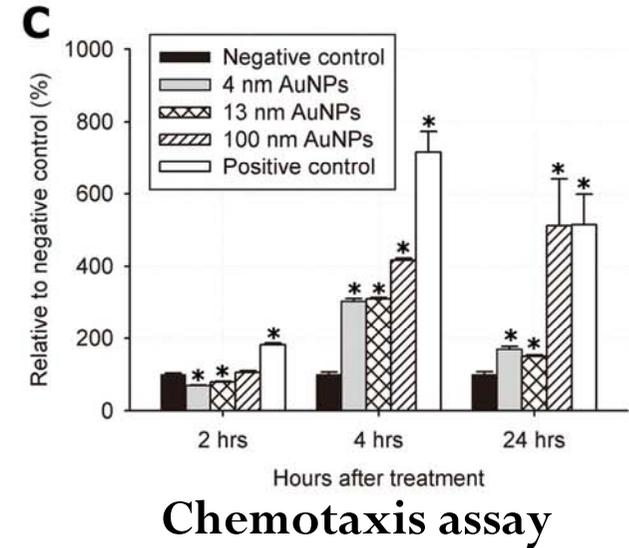
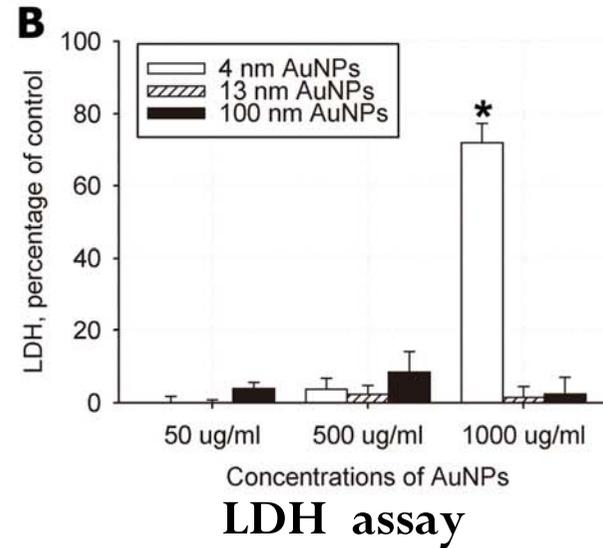
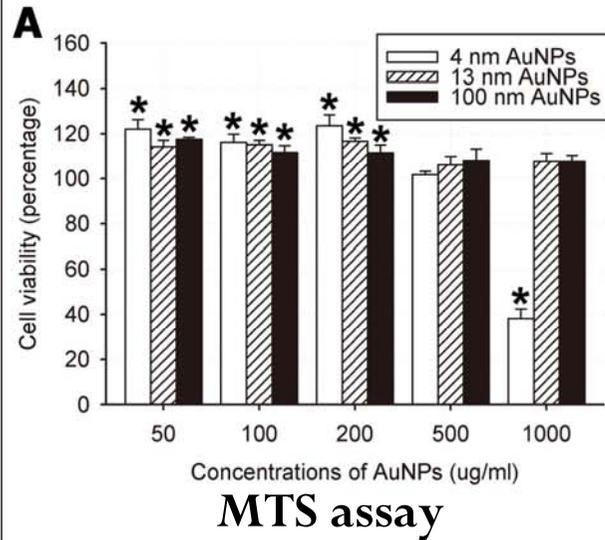
7 days

Group	Gene name	Expression time					Dose dependency
		5 min	30 min	4 hrs	24 hrs	7 days	
Adhesion molecules	ICAM-1	X	X	O	X	X	O
	E-selectin	X	X	O	X	X	O
	VCAM-1	X	X	X	O	X	X
	VEGF	X	X	X	X	X	X
	PECAM-1	X	X	X	X	X	X
Chemokine	MCP-1 / CCL-2	X	X	O	X	X	O
	MIP-1 $\alpha$ / CCL-3	X	O	X	X	X	O
	MIP-1 $\beta$ / CCL-4	X	O	X	X	X	O
	RANTES / CCL-5	X	X	O	O	X	O
	MCP-5 / CCL-12	X	X	X	X	X	X

Group	Gene name	Expression time					Dose dependency
		5 min	30 min	4 hrs	24 hrs	7 days	
Cytokines	IL-1 $\beta$	X	O	X	X	X	O
	IL-6	X	O	X	X	X	O
	IL-10	X	O	X	X	X	O
	IL-12	X	X	O	X	X	O
	TNF- $\alpha$	X	O	O	X	X	O
	IL-8	X	X	X	X	X	X
	IFN- $\gamma$	X	X	X	X	X	X
	GM-CSF	X	X	X	X	X	X
ETC	iNOS	X	X	X	X	X	X
	COX-2	X	X	X	X	X	X

# In Vitro Toxicity Study

- Cell line : Raw 264.7 (mouse macrophage cell line)  
HL-60 (human macrophage cell line)
- Measurements
  - Cell proliferation (MTS assay)
  - Cytotoxicity (LDH release)
  - Chemotaxis assay
  - Cytokine expression assay by realtime PCR and ELISA



- 4 nm PEG-coated AuNPs caused reductions in cell proliferation after 16 h incubation at 1,000 ug/ml
- As particle sizes reduced, cell proliferation was increased
- 4 nm PEG-coated AuNPs at only 1,000 ug/ml produced significant increases in LDH levels
- Cell migration of PEG-coated AuNPs increased in the order 100 nm > 13 nm > 4 nm

# Conclusions

- 13 nm PEG-coated AuNPs induced acute inflammation, apoptotic necrosis
- 13 nm PEG-coated AuNPs were found to accumulate in liver and spleen for up to 7 days after injection and to have long blood circulation times
- 13 nm PEG-coated AuNPs transiently induced adhesion molecules, chemokines, and inflammatory cytokines in the mouse liver
- 4 nm AuNPs were cytotoxic at 1,000 ug/ml by MTS and LDH assay
- TNF- $\alpha$  is a sensitive marker, and that it could be used to evaluate the toxicities of PEG-coated AuNPs

# Acknowledgements

- **AuNPs synthesis:**

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- Korea Research Institute of Bioscience and Biotechnology
- Dr. Bonghyun Chung / Dr. Jinyoung Jeong / Dr. Yong Taik Lim

- **In vivo and In vitro toxicity study:**

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- National Institute of Toxicological Research,
- Korea Food and Drug Administration
- Dr. Jayoung Jeong / Dr. Beom Seok Han / Dr. Minjung Cho  
/ Dr. Wan-Seob Cho

