

Toxicity and biodistribution of surface chemically modified Ag nanoparticles

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Application of AgNPs



Battery



Antibiotic coating on medical devices



Bone cement



Textile



Application Area



Wound dressings

The total product amount of AgNPs reached up to **55** ton in 2012.

Risk of AgNPs?



AgNPs



Release



Risks?

Hazard: AgNPs can migrate to liver, spleen, lungs, kidneys and brain and induce **toxicity in vivo** as well as induced apoptosis, membrane damage, inflammation and DNA damage **in vitro**.

Surface Modification

With the advance in material science, AgNPs with **different surface coating** were exploited to develop faster electronic, brighter displays, and more sensitive diagnostic agents for medical imaging.

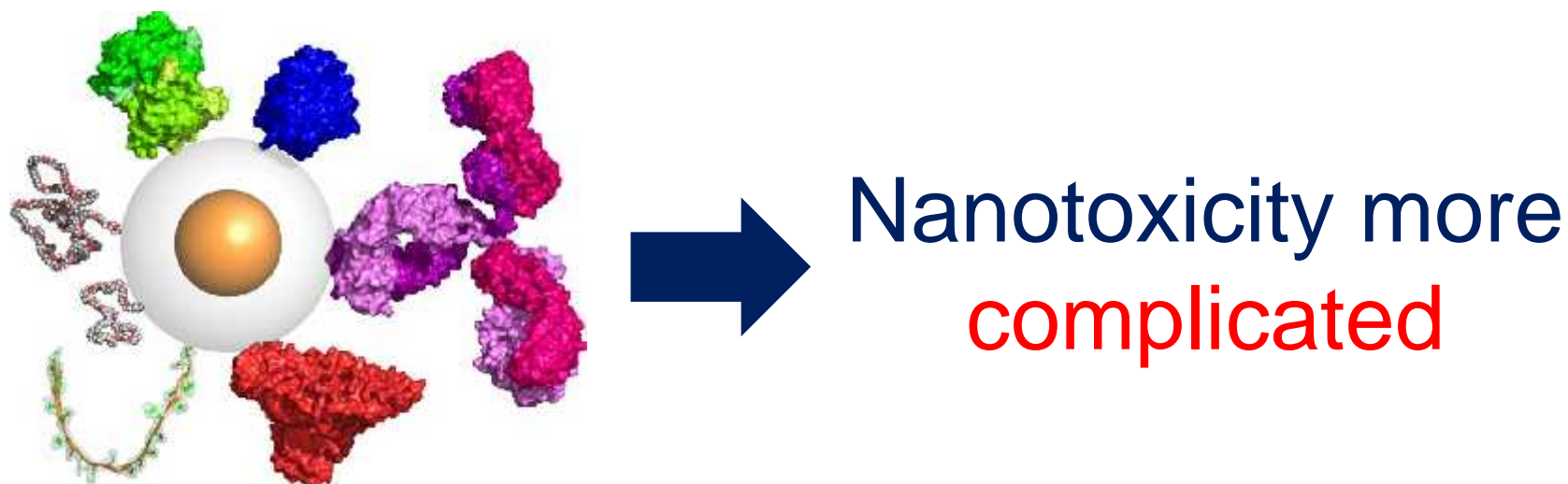
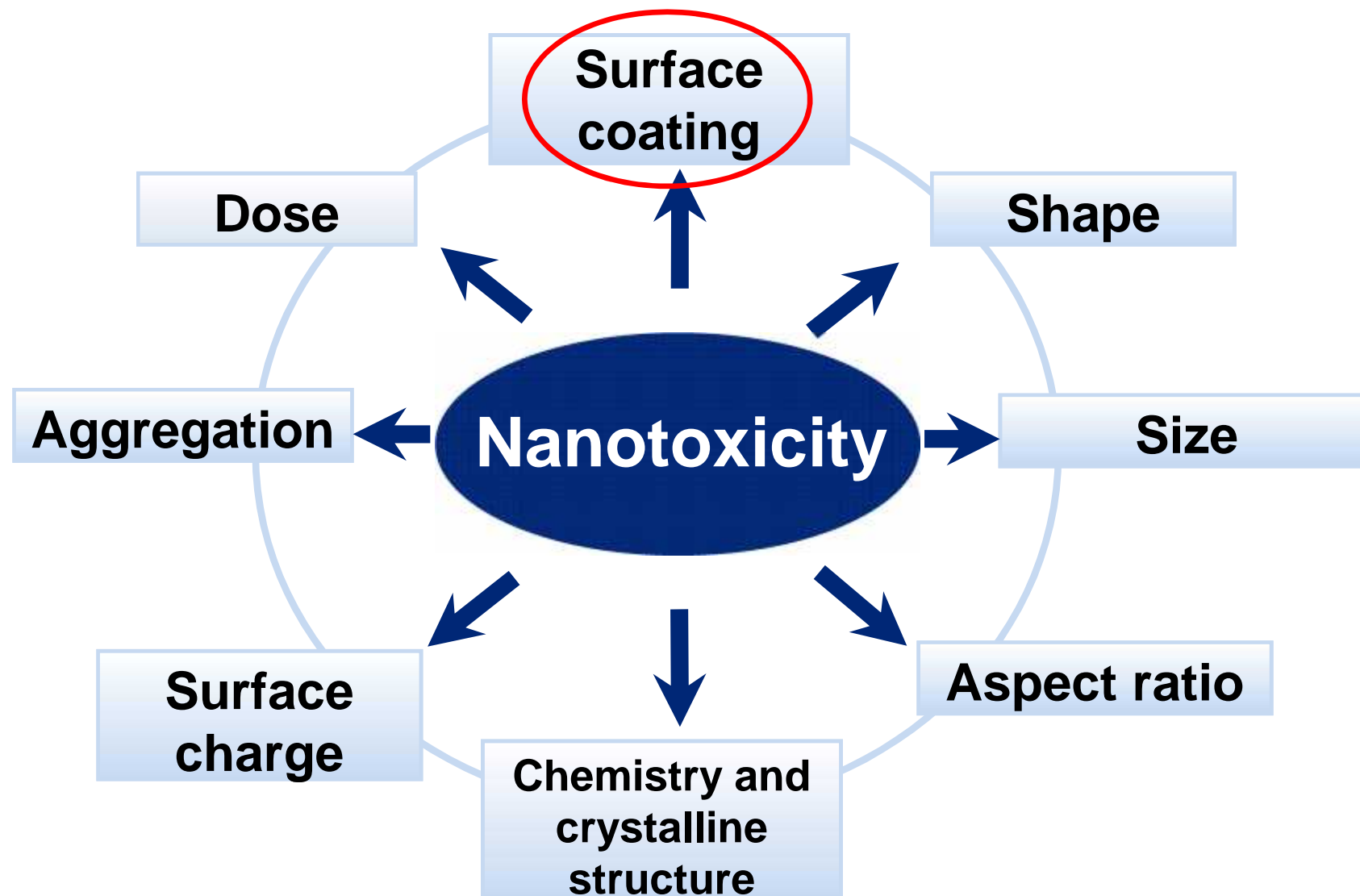


Figure: Relative size of nanoparticles and biomolecules, drawn to scale. Schematic representation of a nanoparticle with 5 nm core diameter, 10 nm shell diameter, with PEG molecules of 2000 and 5000 g mol⁻¹ (on the left, light grey), streptavidin (green), transferrin (blue), antibody (IgG, purple), albumin (red), single-stranded DNA (20mer, cartoon and space filling). Proteins are crystal structures taken from the Protein Data Bank (<http://www.rcsb.org>) and displayed as surfaces; PEG and DNA have been modelled from their chemical structure and space filling. (Source from Sperling & Parak, 2010)

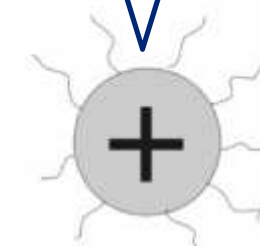
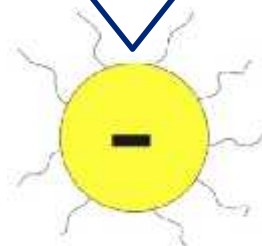
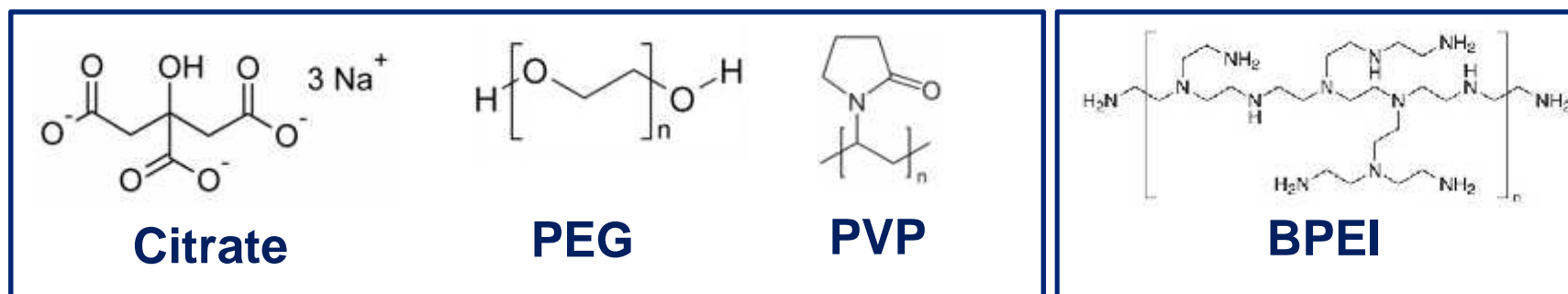
Nanotoxicity & Properties



Aim

Test Chemicals

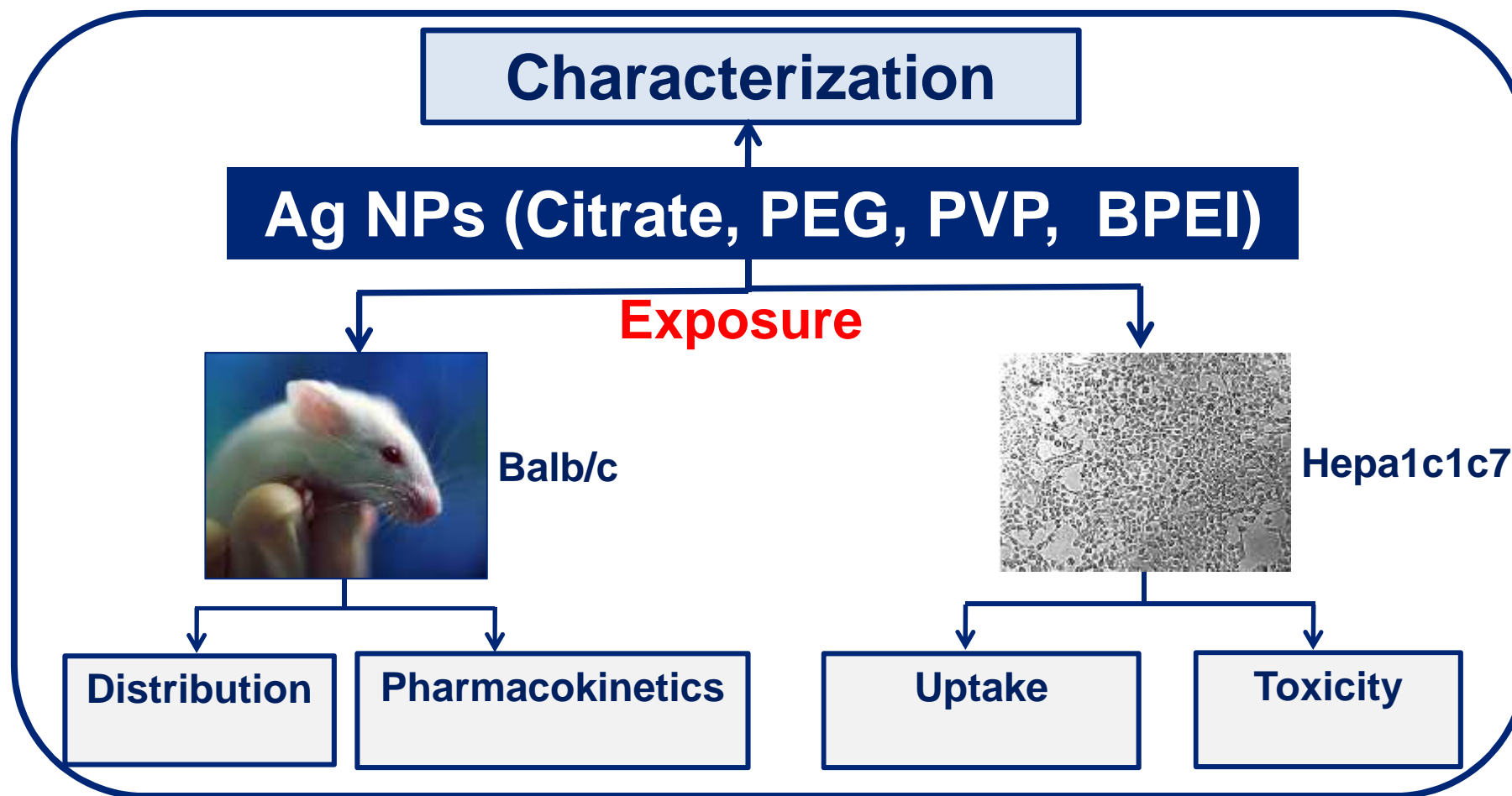
Ag NPs



Different toxicity



Experimental Set Up



Toxicological data to risk assessment of AgNPs

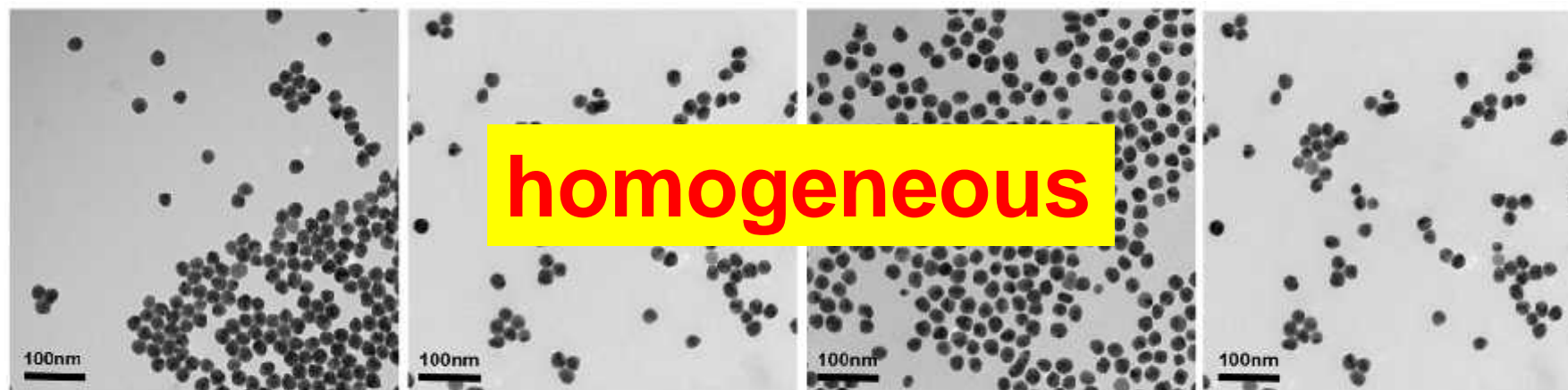
Results and Discussion

Characterization of AgNPs in stock suspension

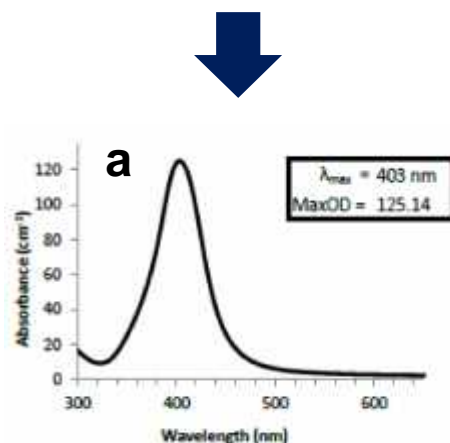
Name	TEM Diameter (nm)(SD)	Hydrodynamic Diameter (nm)	Zeta Potential (mv)	Mass Conc. (mg/ml)	Ag ⁺ Conc. (µg/L)
Citrate-AgNPs	28.7(3.6)	35.8	-22.9	1.14	0.43(0.10)
PEG-AgNPs	32.9(3.2)	54.6	-16.2	1.08	0.45(0.03)
PVP-AgNPs	28.7(2.1)	36.2	-22.1	1.07	0.58(0.10)
BPEI-AgNPs	30.0(3.0)	63.0	46.5	0.49	0.80(0.07)

Results and Discussion

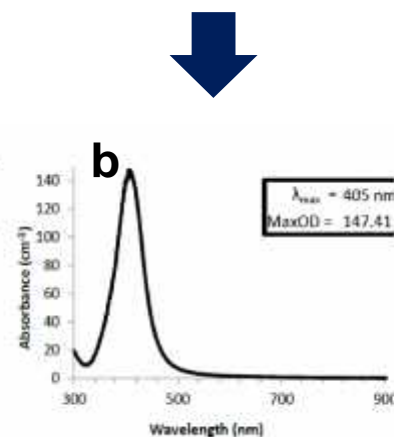
TEM image



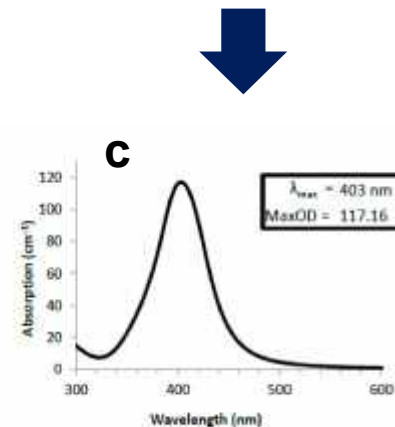
Citrate AgNPs(N)



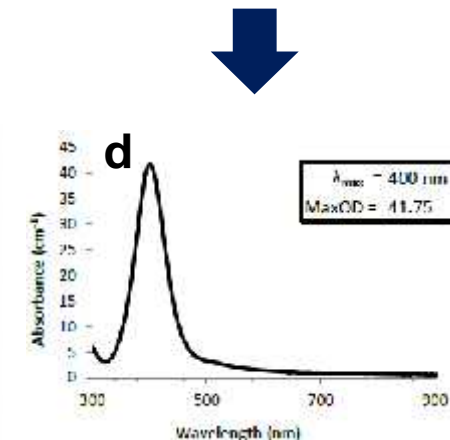
PVP AgNPs(N)



PEG AgNPs (N)

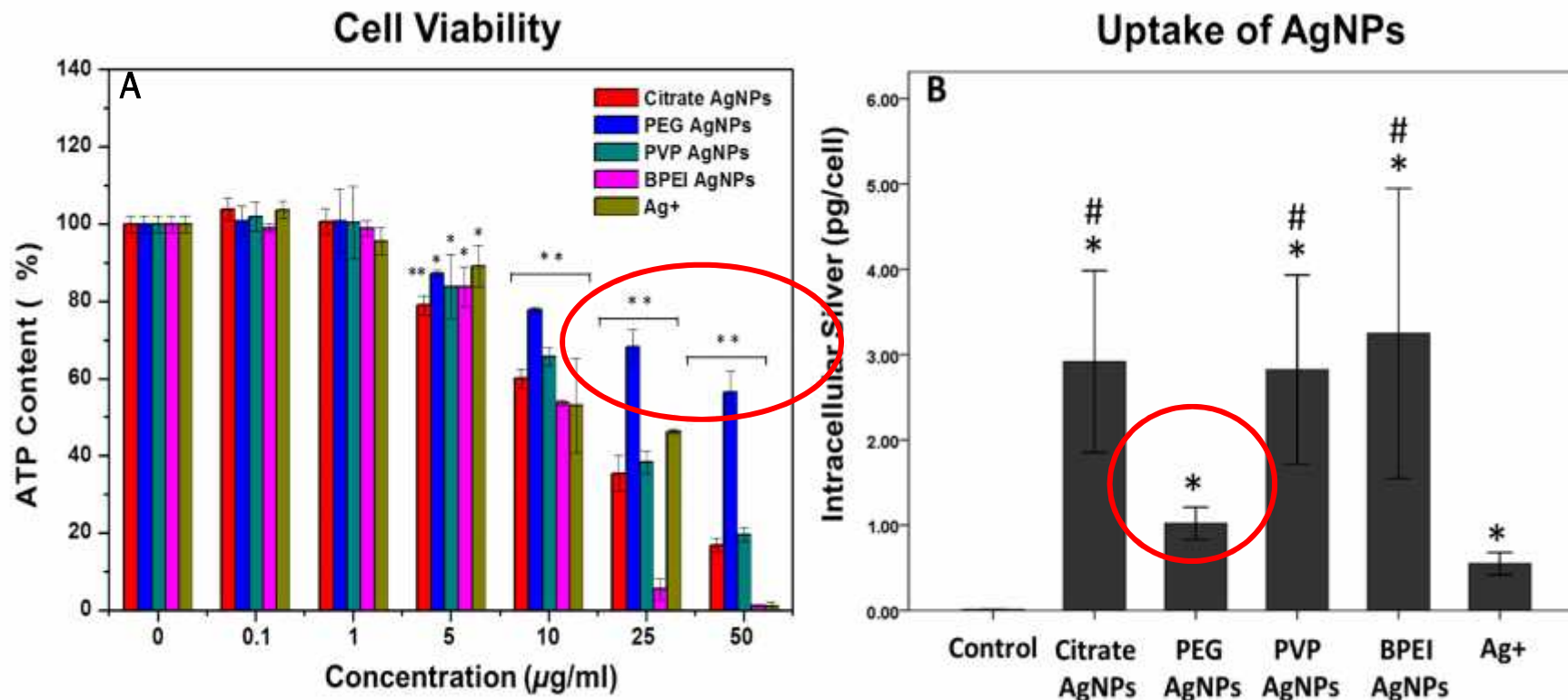


BPEI AgNPs (P)



Results and Discussion in vitro

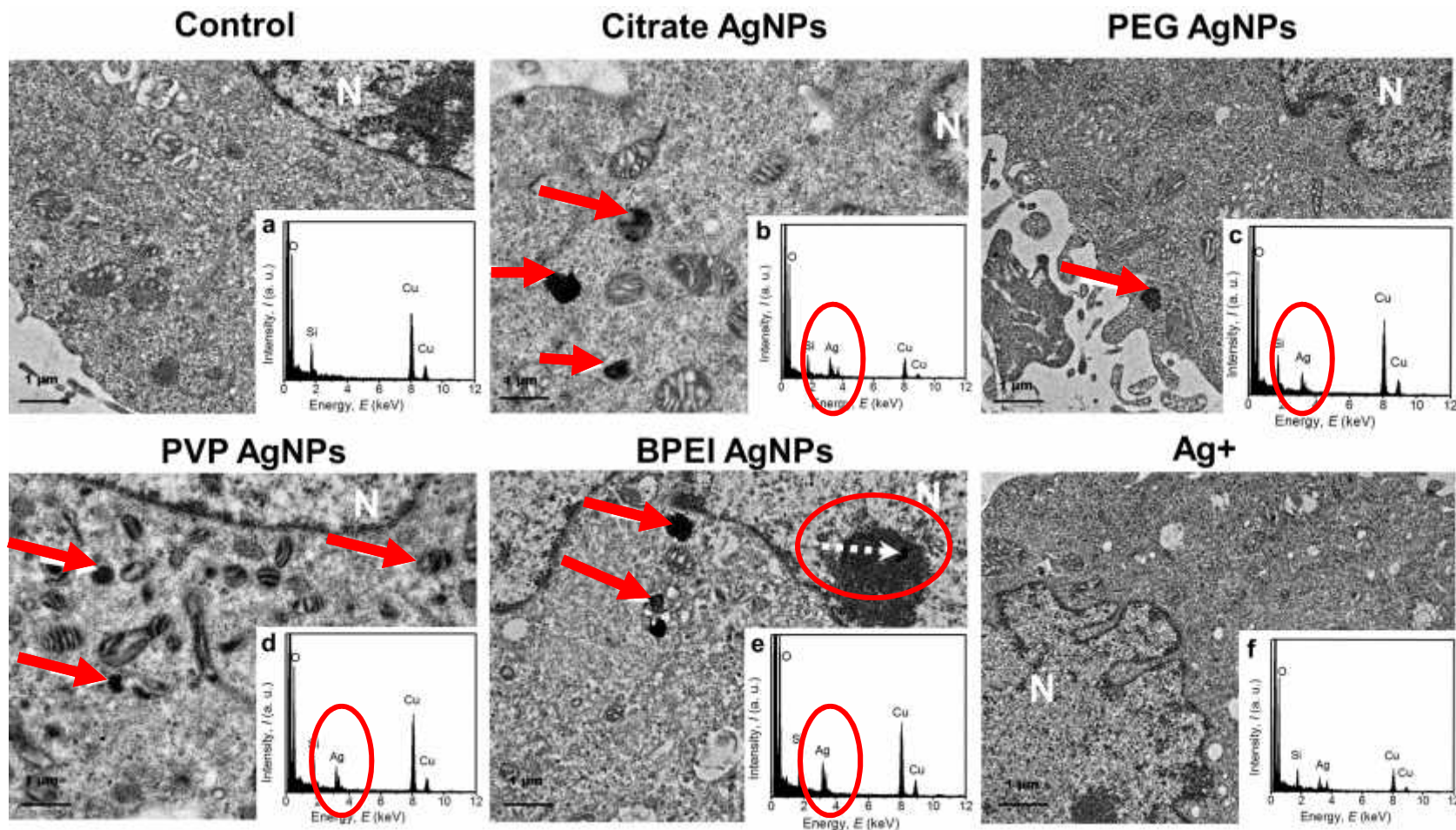
Effects and uptake of AgNPs (Hepa1c1c7, 24h)



- BPEI AgNPs treated cells showed the high toxicity to cells ($p < 0.001$).
- PEG AgNPs treated cells showed the lowest toxicity ($p < 0.05$).
- The small amount AgNPs uptake by the cells showed the lower toxicity.

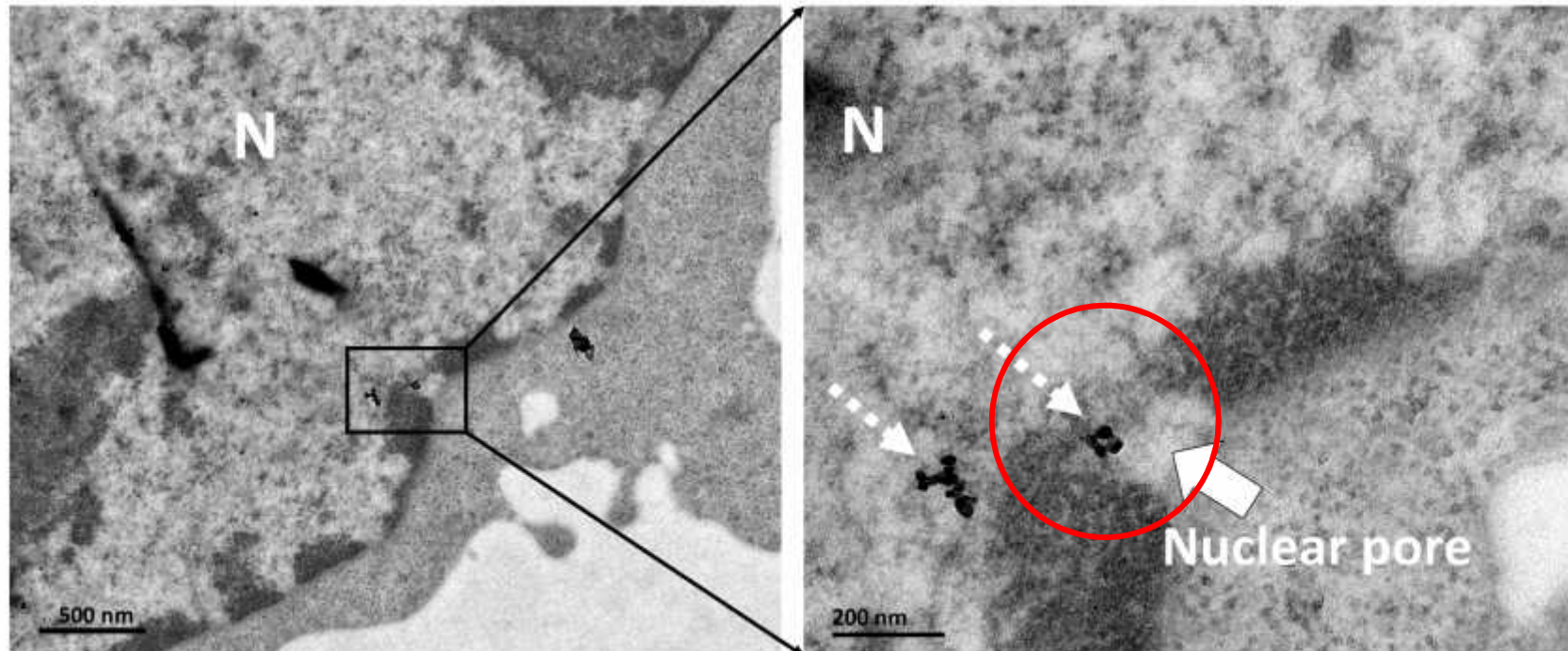
Results and Discussion in vitro

TEM-EDX analysis of AgNPs in cells (Hepa1c1c7, 24h)



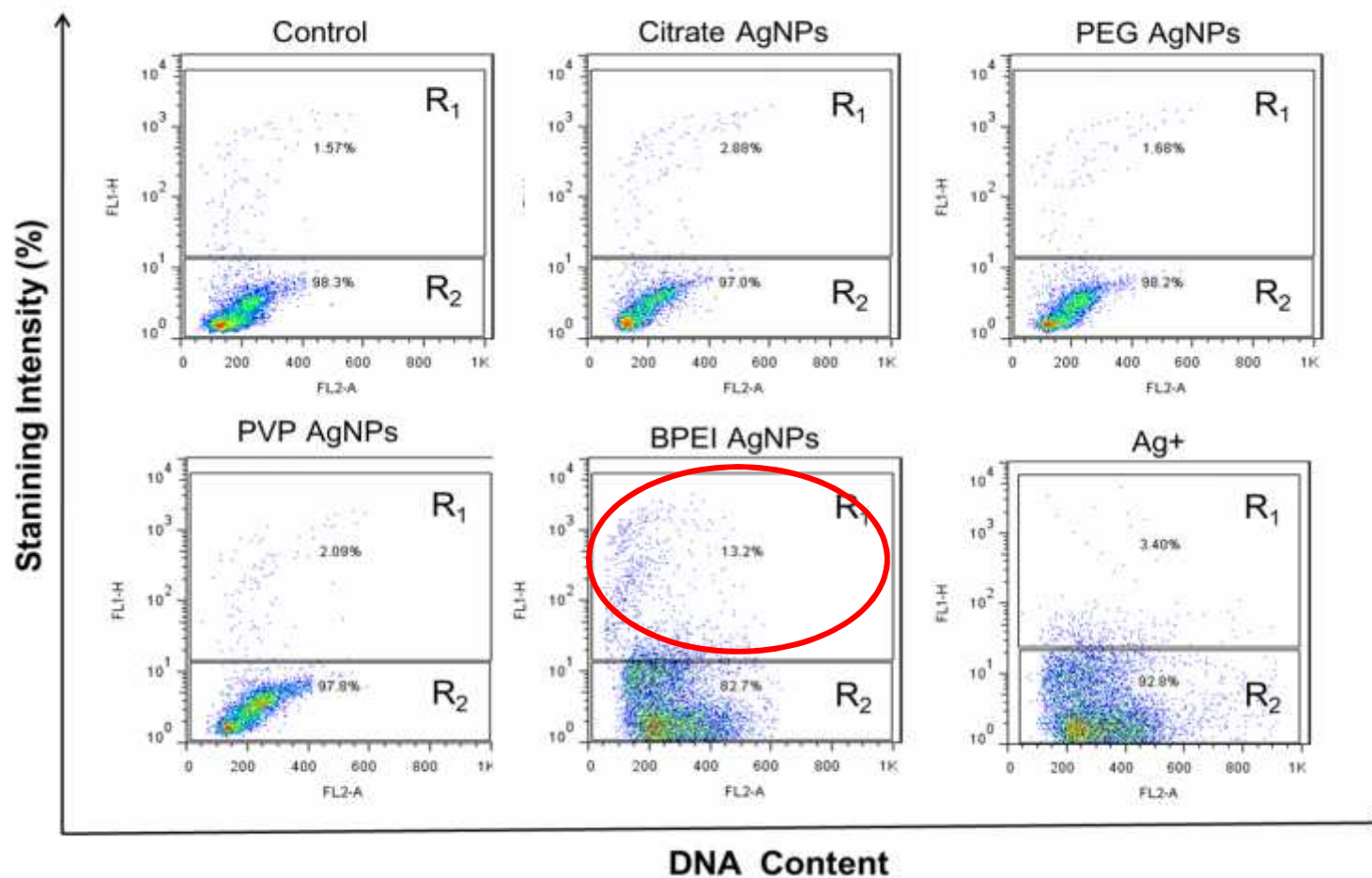
Results and Discussion in vitro

BPEI AgNPs entered into the nucleus (Hepa1c1c7, 24h)



Results and Discussion in vitro

DNA fragmentation



- Only BPEI AgNPs treated cells showed DNA fragmentation (**13.2%**)

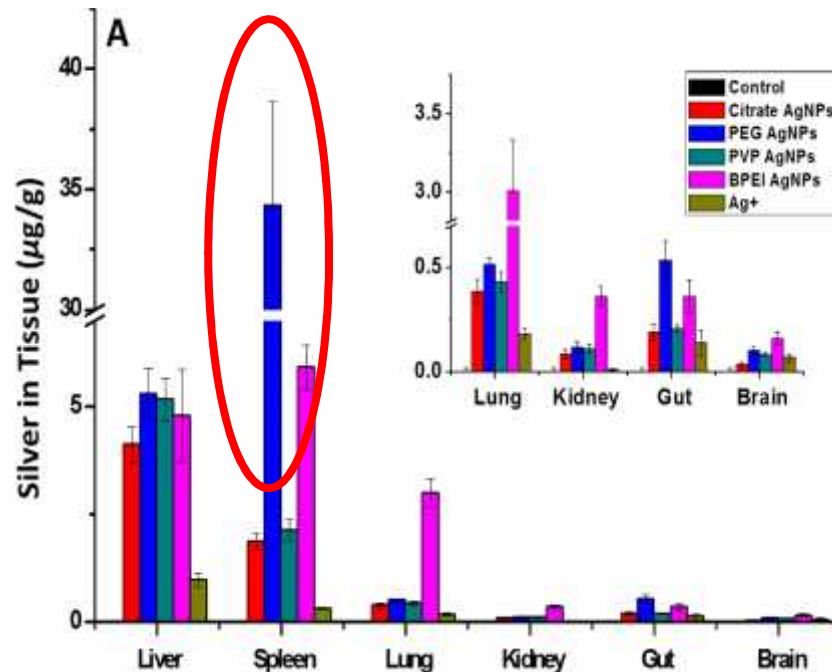
Results and Discussion in vivo

Biodistribution

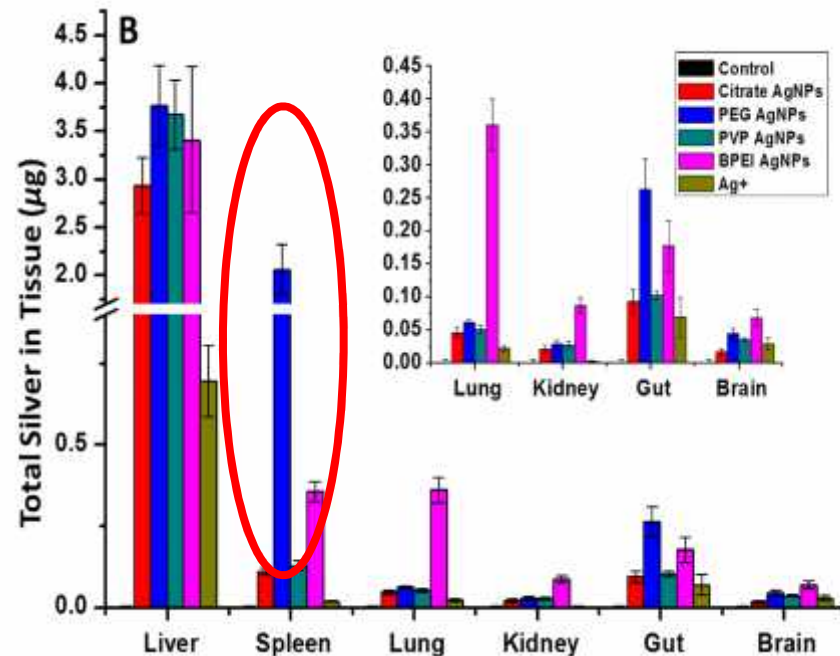
Dose: 1mg/kg mice
(Balb/c, 24h exposure)



Silver Con. in Tissue



Total Silver in Tissue



- The silver levels were higher **in spleen and liver** followed in a decreasing order by lungs, gut, kidneys and brain after intravenous injection.
- PEG AgNPs was highly uptaken by **spleen** of mice.

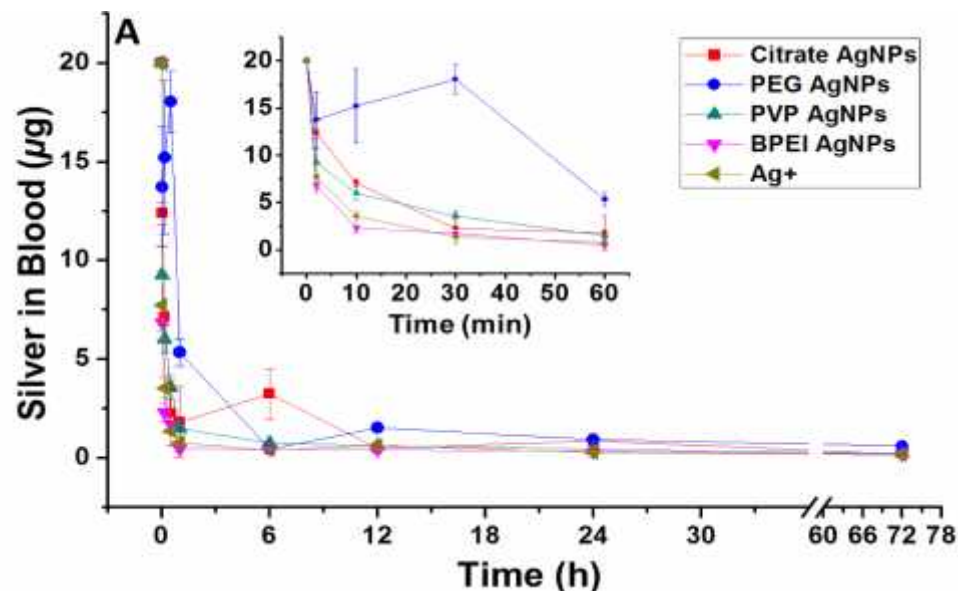
Results and Discussion in vivo

The evaluation of histopathological changes

	TEM Image of Liver	Liver	Spleen	Lung	Kidney	Liver	Spleen	Lung	Kidney
Control						-	-	-	-
Citrate AgNPs						+++	-	+	++
PEG AgNPs						+	-	+	+
PVP AgNPs						-	-	+	+++
BPEI AgNPs						+	-	-	++
Ag+						-	-	-	++

Results and Discussion in vivo

Pharmacokinetics of AgNPs in mice



➤ The elimination half-life of PEG AgNPs was much higher than other AgNPs and Ag⁺ treatments

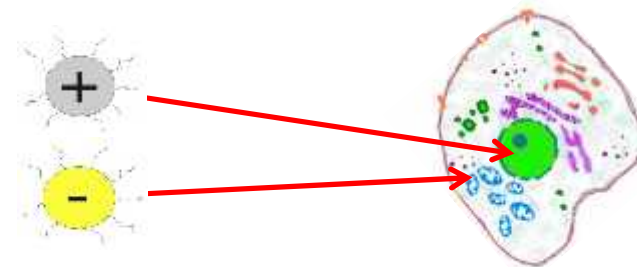
Parameters	Two compartment model				Non compartment model
	Citrate AgNPs	PVP AgNPs	BPEI AgNPs	Ag ⁺	PEG AgNPs
AUC (h·µg/g)	39.18	9.45	7.90	13.91	59.13
t _{1/2} (h)	0.11	0.02	0.05	0.10	51.65
t _{1/2} ^B (h)	17.81	3.03	6.76	15.79	—
CL (g/h)	0.51	2.12	2.53	1.44	0.23
CLD2(g/h)	10.14	37.90	30.72	19.20	—
V _{ss} (g)	12.66	8.86	23.24	30.87	14.18
V1(g)	2.03	1.02	3.10	3.14	—
V2 (g)	10.63	7.84	20.14	27.74	—

Conclusions

- Toxicity of AgNPs were **surface chemistry** dependent significantly:

BPEI AgNPs > Citrate AgNPs = PVP AgNPs > PEG AgNPs

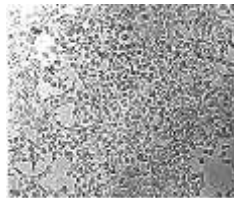
- The **surface charge** played an important role: Positive surface charge (**BPEI**) AgNPs showed higher toxicity because of the **strong interplay** between the higher positive surface charge and the membrane of nucleus.



- The PEG AgNPs showed higher bioaccumulation and **low toxicity** in **spleen**, and long **half-life** in blood indicated a high potential application in drug delivery.



← AgNPs →



- The toxicity of AgNPs *in vitro* was consistent with its *in vivo* which suggested an ***in vitro* model using Hepa1c1c7 cell line** connection with uncertainty factor in the risk assessment of AgNPs.

Acknowledgements

- National Science Foundation of China (Grant No. 21277168, 21321004, 21407162),



- The China Postdoctoral Science Foundation Project (No. 2013M541056)



- The European Union Seventh Framework Programme (Grant No. 604305, SUN).



- We thank Molecular and Environmental Toxicology Group (RCEES, CAS) help for technical support.





Thank you for your attention!

Adsorption BSA ability of AgNPs

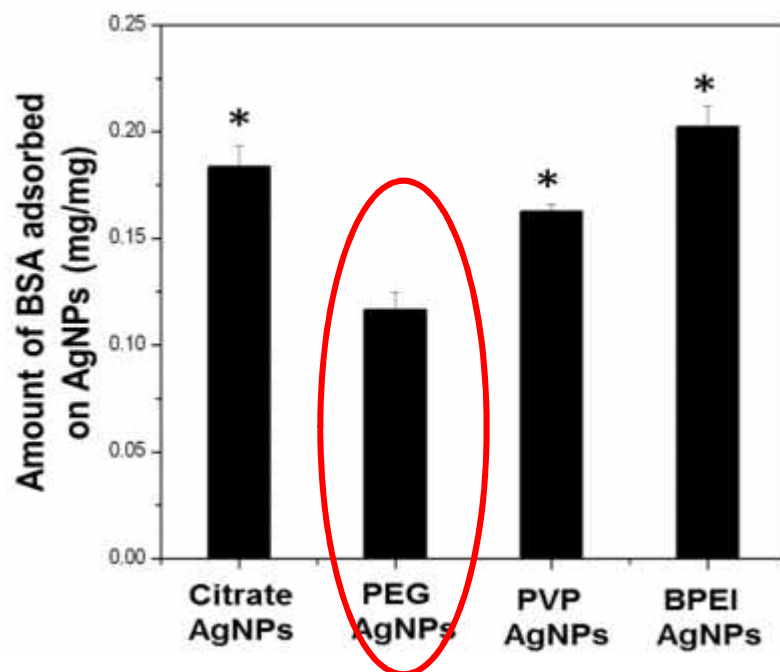
Ag NPs (Citrate, PVP, PEG, BPEI)

BSA(Bovine Serum Albumin)

BCA Protein Assay Kit
Centrifugation



$$q = \frac{(C_0 - C_1)V}{m}$$

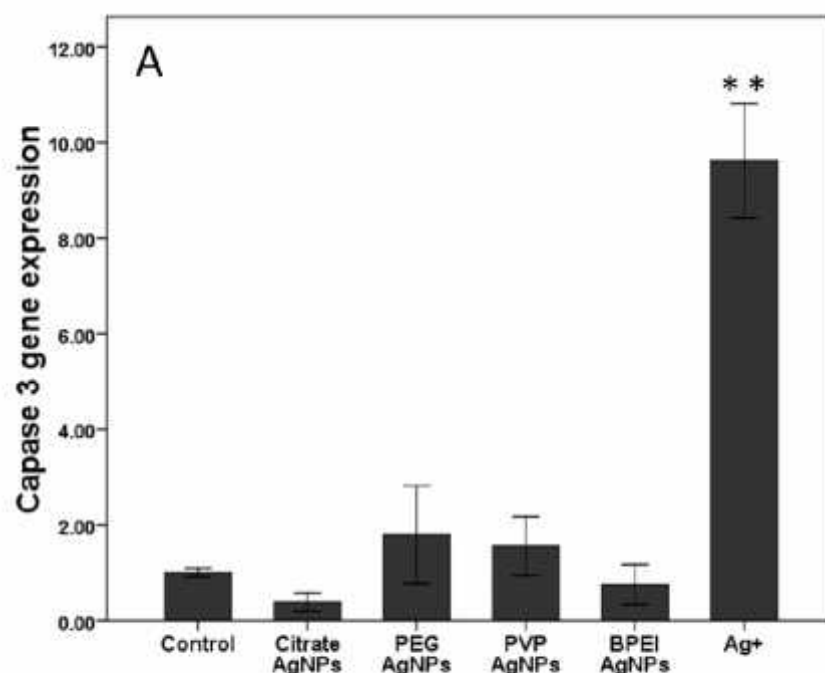


- PEG AgNPs showed lower protein adsorption than other AgNPs particles ($p < 0.05$).
- The surface coating of PEG on AgNPs can diminish opsonization and therefore heavily reduced uptake of AgNPs into cells.

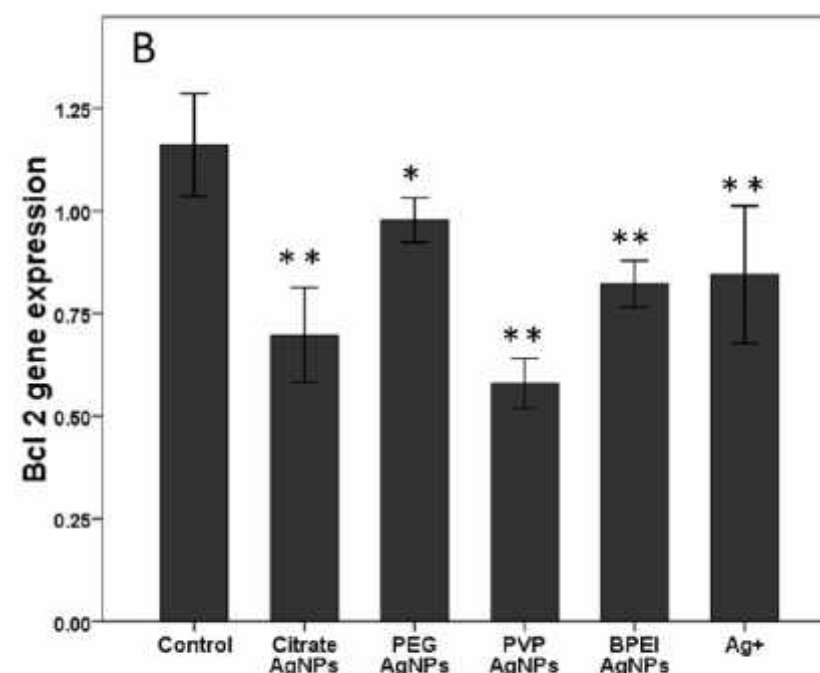
Results and Discussion in vitro

Apoptosis: Gene expressions (Hepa1c1c7, 5 μ g/ml, 24h)

Caspase 3

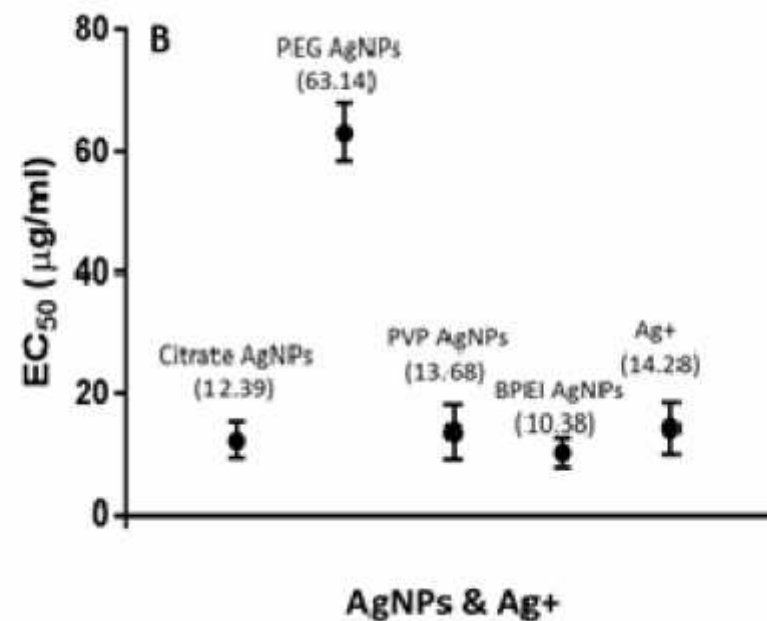
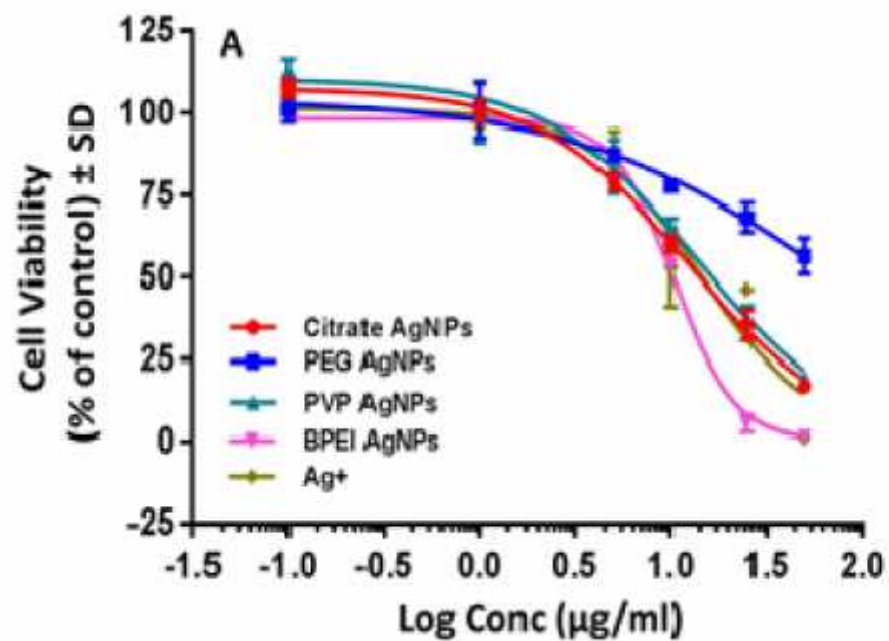


Bcl2



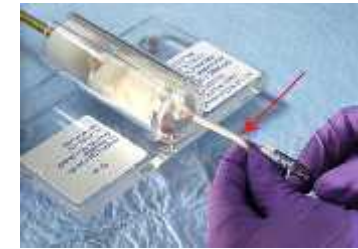
- The expression of Caspase 3 gene in Ag+ treatment was significant up-regulated ($p < 0.05$).
- Bcl 2 gene expression showed significant low in Citrate AgNPs, PVP AgNPs, and BPEI AgNPs treatments than its in control ($p < 0.05$)

Results and Discussion in vitro

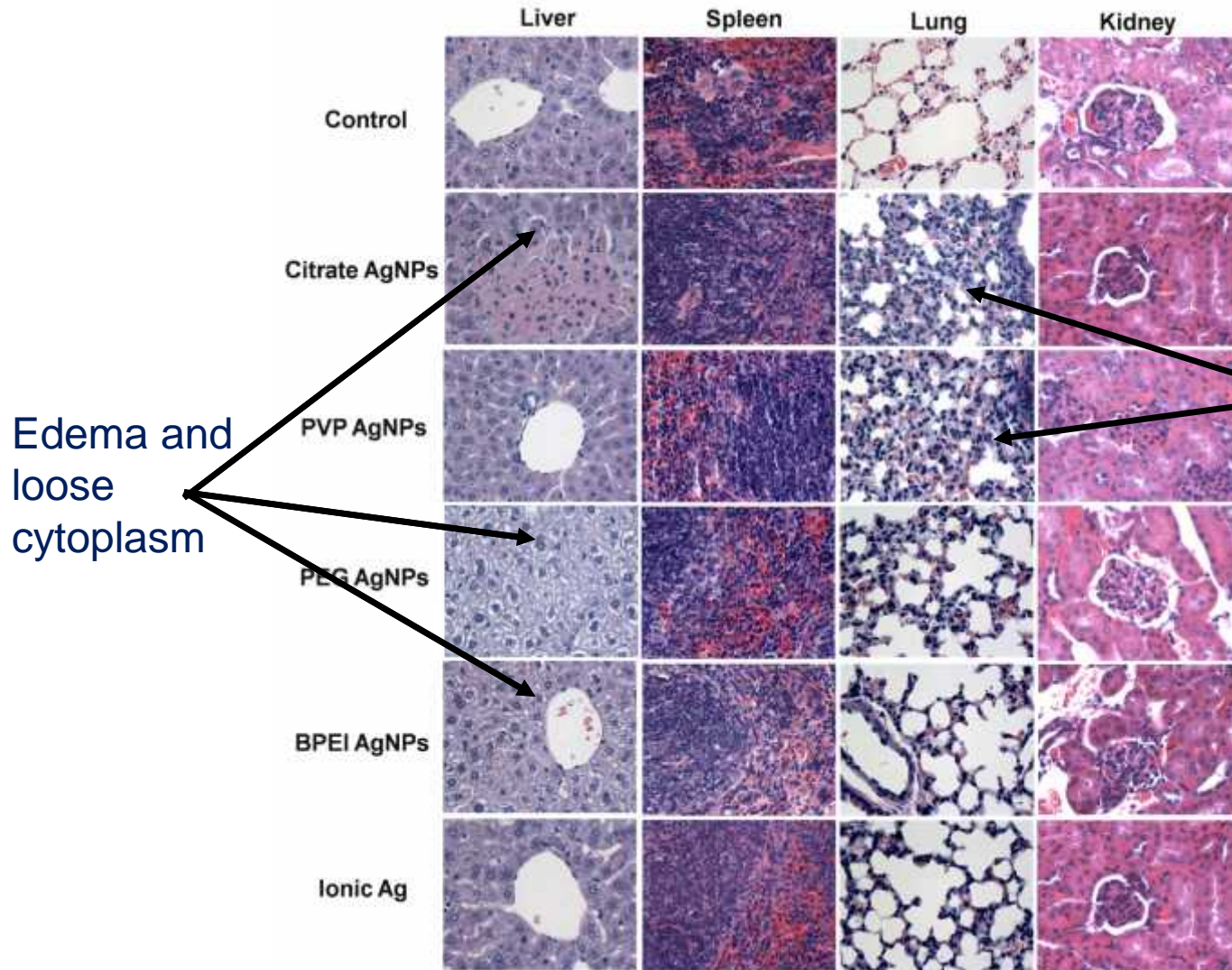


Results and Discussion in vivo

Histopathology (AgNPs exposure 24h)

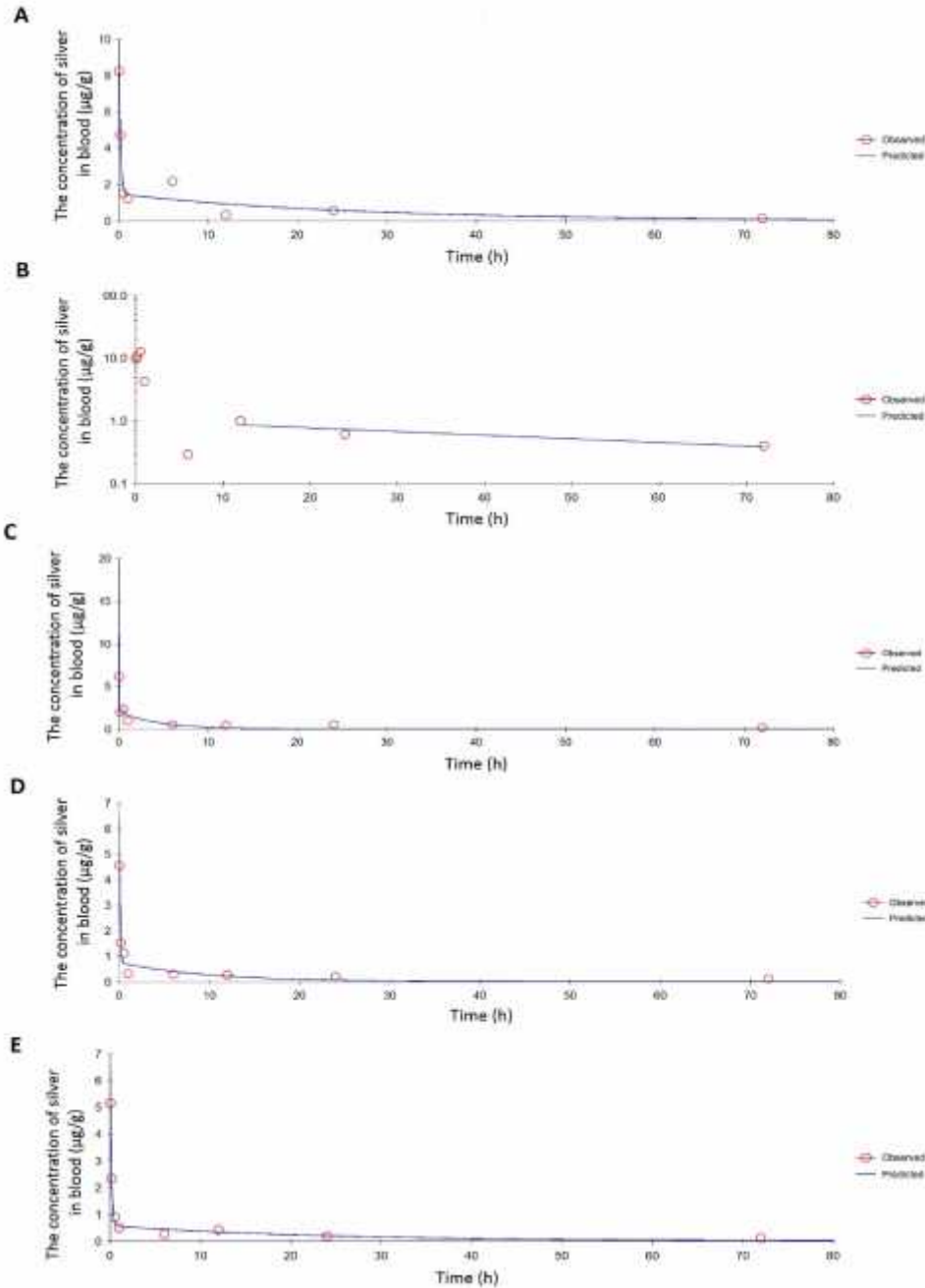


Dose: 1mg/kg mice
(Balb/c, 24 exposure)



Edema and loose cytoplasm

Thickened alveolar walls and infiltration of focal inflammatory cells



Time course of the mean blood silver levels (Observed) and the PBPK model values (Predicted) were obtained after a single injection of various AgNPs to mice. A. Citrated AgNPs; B. PEG AgNPs; C. PVP AgNPs; D. BPEI AgNPs; E. Ag^+ .