Cytotoxicity and Inflammatory Effect of Silver Nanoparticles in Human Cells

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1. Approaches to practical toxicology tests to assess nanoparticles

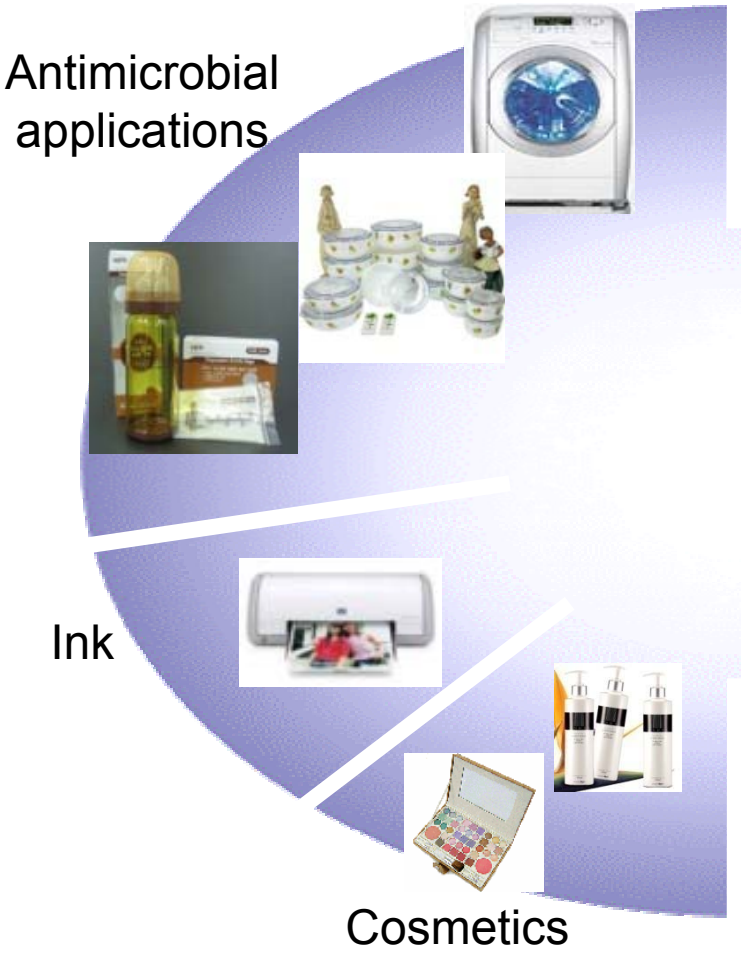
2. Cytotoxicity and inflammatory effects of silver nanoparticles

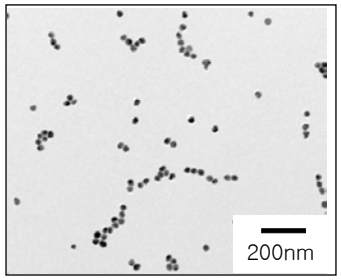
Nanoparticles and toxicity assay

- The rapidly developing field of nanotechnology will result in exposure of nanoparticles to humans via several routes (e.g., inhalation, ingestion, skin, etc.). Nanoparticles can translocate from the route of exposure to other vital organs and penetrate cells.
- Toxicity studies to determine the deleterious effects of nanoparticles on living cells are required.
- Due to the nanosize and the nature of agglomeration, simple standard methods to characterize the biological effects of nanoparticles are currently unavailable.
- In this study, practical information regarding the optimal *in vitro* tests for nanotoxicity were evaluated.

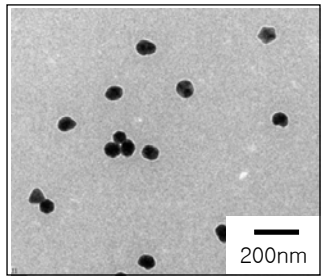
Silver nanoparticles

- Antimicrobial reagents, detergents, water purificants, wall paints, textiles

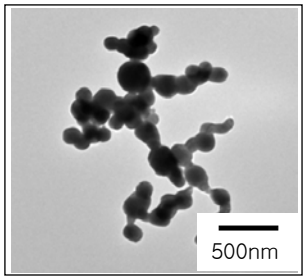




20 nm (synthetic)



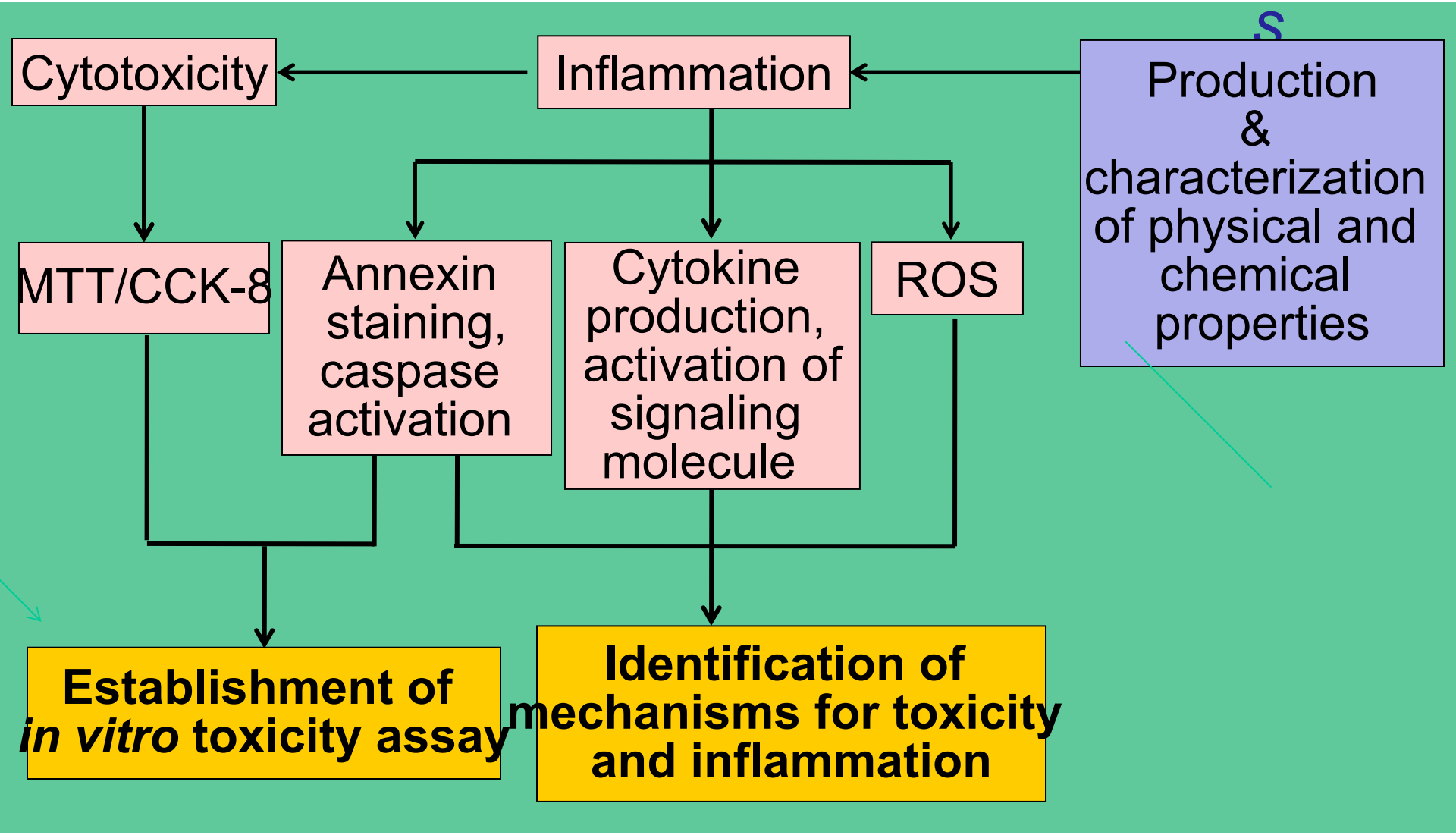
80 nm (synthetic)



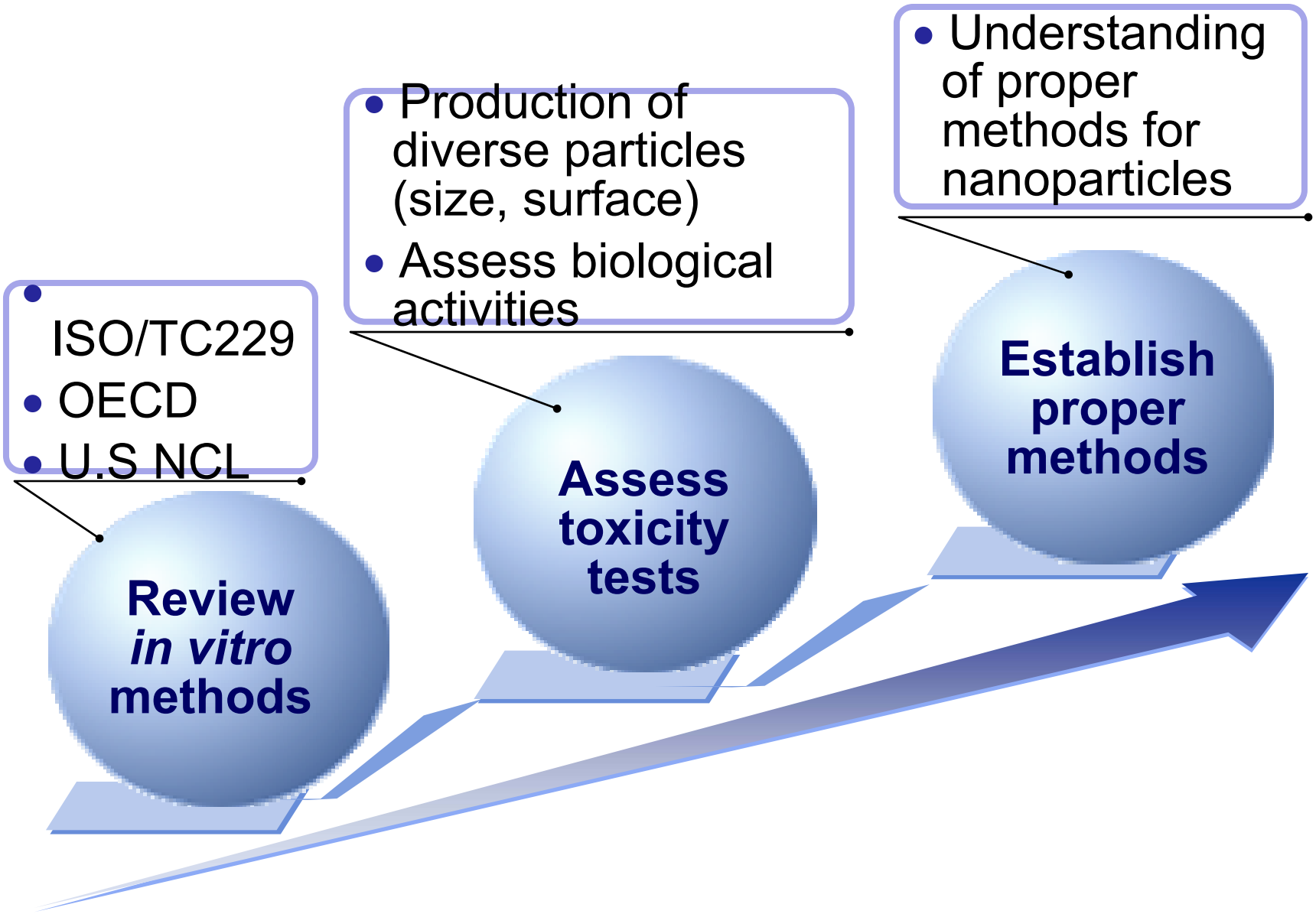
180 nm (commercial, Aldrich)

Biological tests

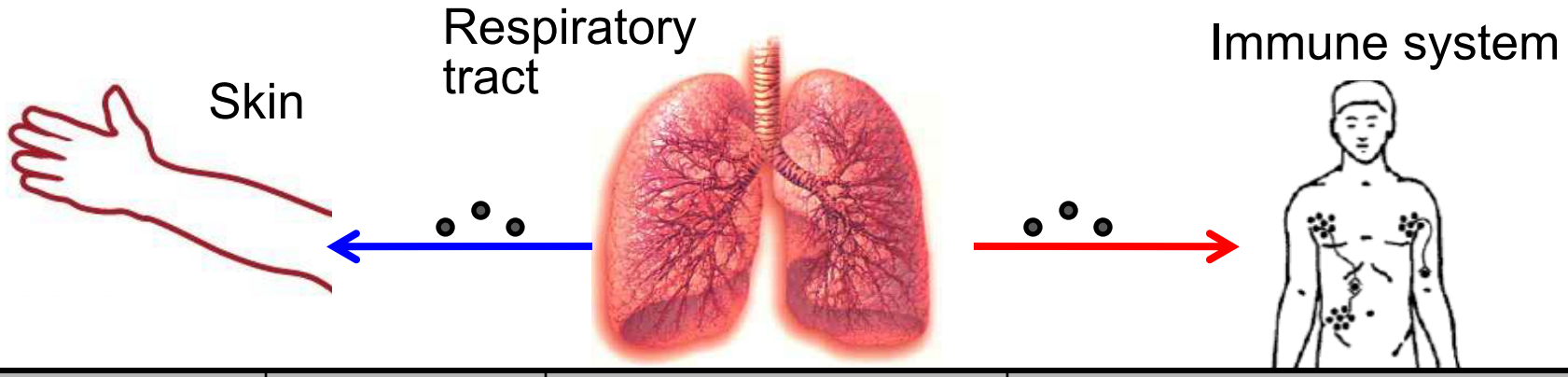
Synthesi



In vitro tests for nanoparticles



Exposure routes of nanomaterials



	Cell line	Origin	Characteristics
Respiratory	A549	Lung epithelial	Proper for cytotoxicity
	BEAS-2B	Bronchial epithelial	Proper for cytokine production
Immune	U937	Macrophage	Proper for cytotoxicity and cytokine production
Skin	SK-Mel	Skin epithelial	Proper for cytotoxicity and cytokine production
	A375	Skin epithelial	Too fast growing

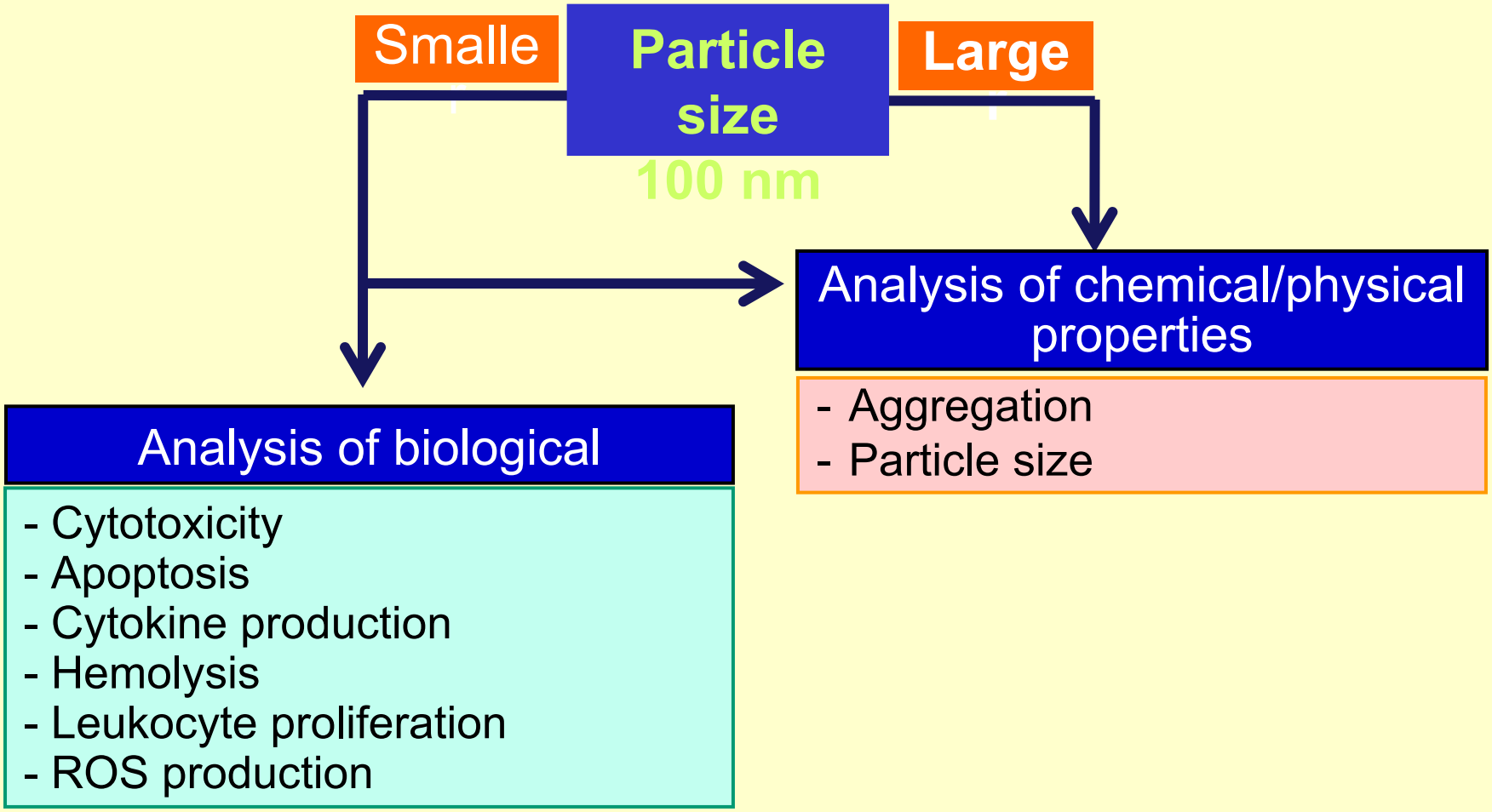
Standard toxicology tests and silver nanoparticles

Category	Tests	Mechanism	Method	Suggestion
In Vitro Immunology (Blood contact Properties)	Hemolysis	Release of hemoglobin	Standard	Proper
	Complement activation	Activation of C3 complement	Standard	Inappropriate
In Vitro Immunology (Cell-based assays)	Leukocyte proliferation	Leukocyte proliferation with mitogen stimulation	Standard	CCK-8
	Phagocytosis	Zymosan assay	Standard	Proper
	Cytokine induction	Cytokine production	Standard	Proper
Toxicity	Oxidative stress	Detection of ROS	Standard	Proper
	Cytotoxicity (necrosis)	Cell viability and mitochondrial integrity	Standard	CCK-8
	Cytotoxicity (apoptosis)	Activation of caspase 3	Standard	Annexin-V
Targeting	Cell binding/internalization	N/S	N/S	TEM, confocal microscope or other methods

Characteristics specific to metal nanomaterials

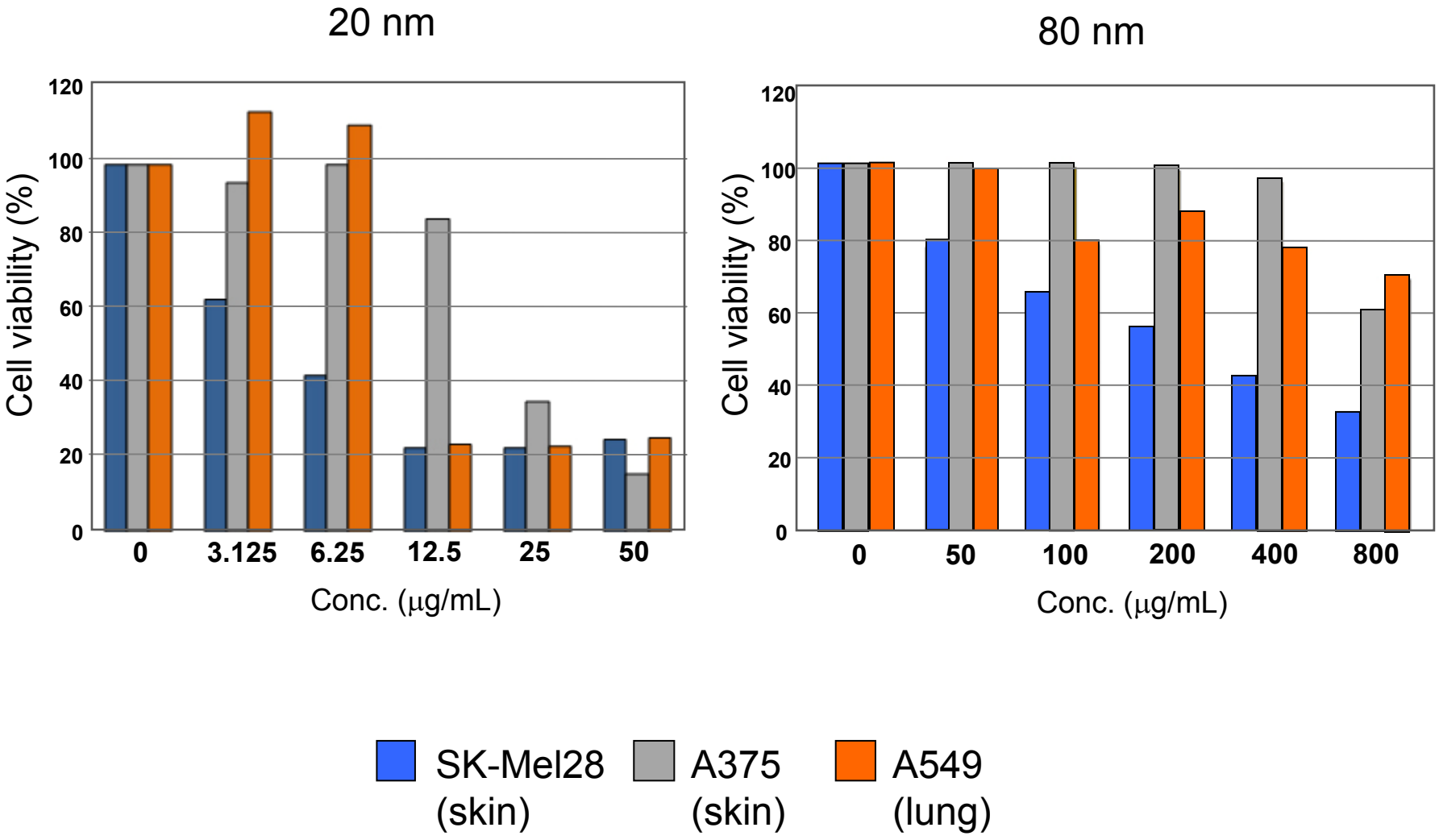
- Nanoparticles larger than 100 nm tend to aggregate relatively quickly *in vitro* when compared to nanoparticles smaller than 100 nm. Fresh samples within two weeks after synthesis is recommended for tests.
- Each standard toxicology method must be verified before use. (ex. interference with a specific wavelength, electrophoresis)

Flow chart for nanotoxicity tests

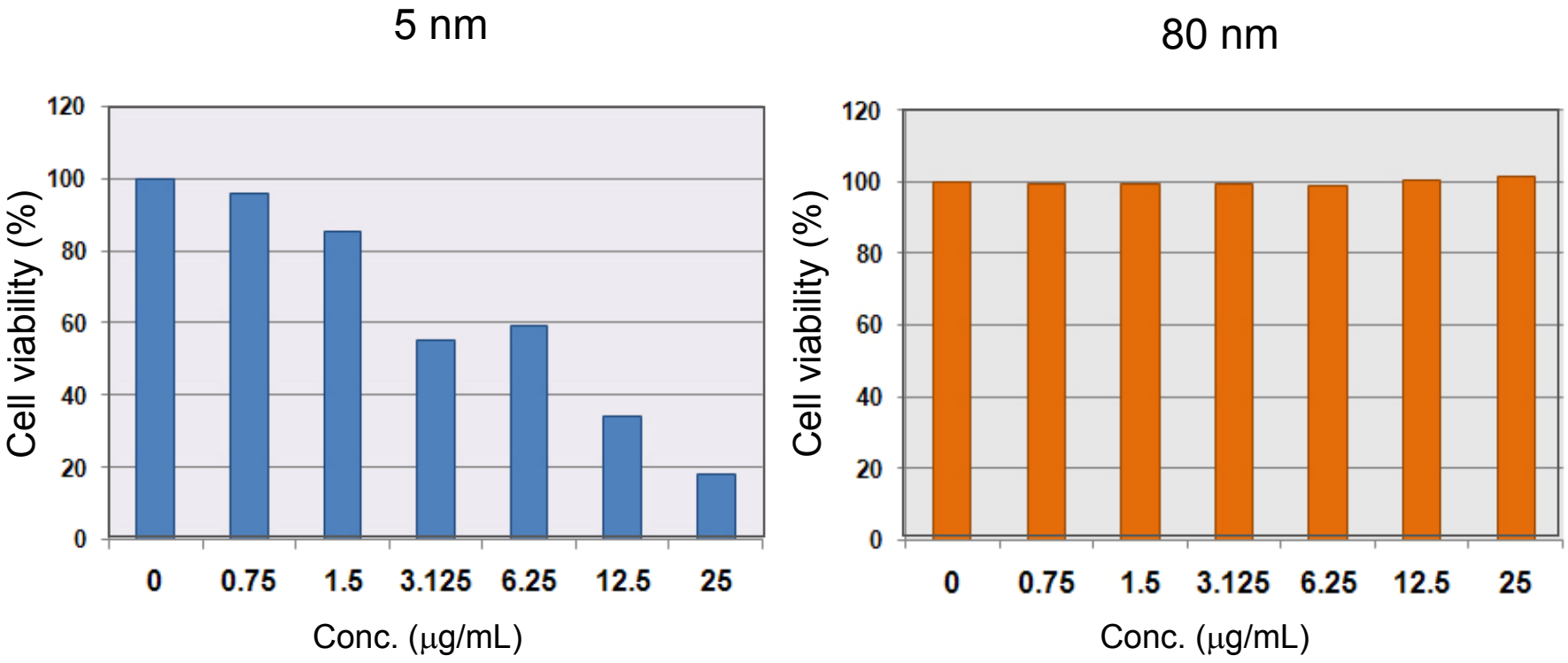


Biological reactivity of silver nanoparticles

Cytotoxicity of silver nanoparticles



Cytotoxicity of silver nanoparticles

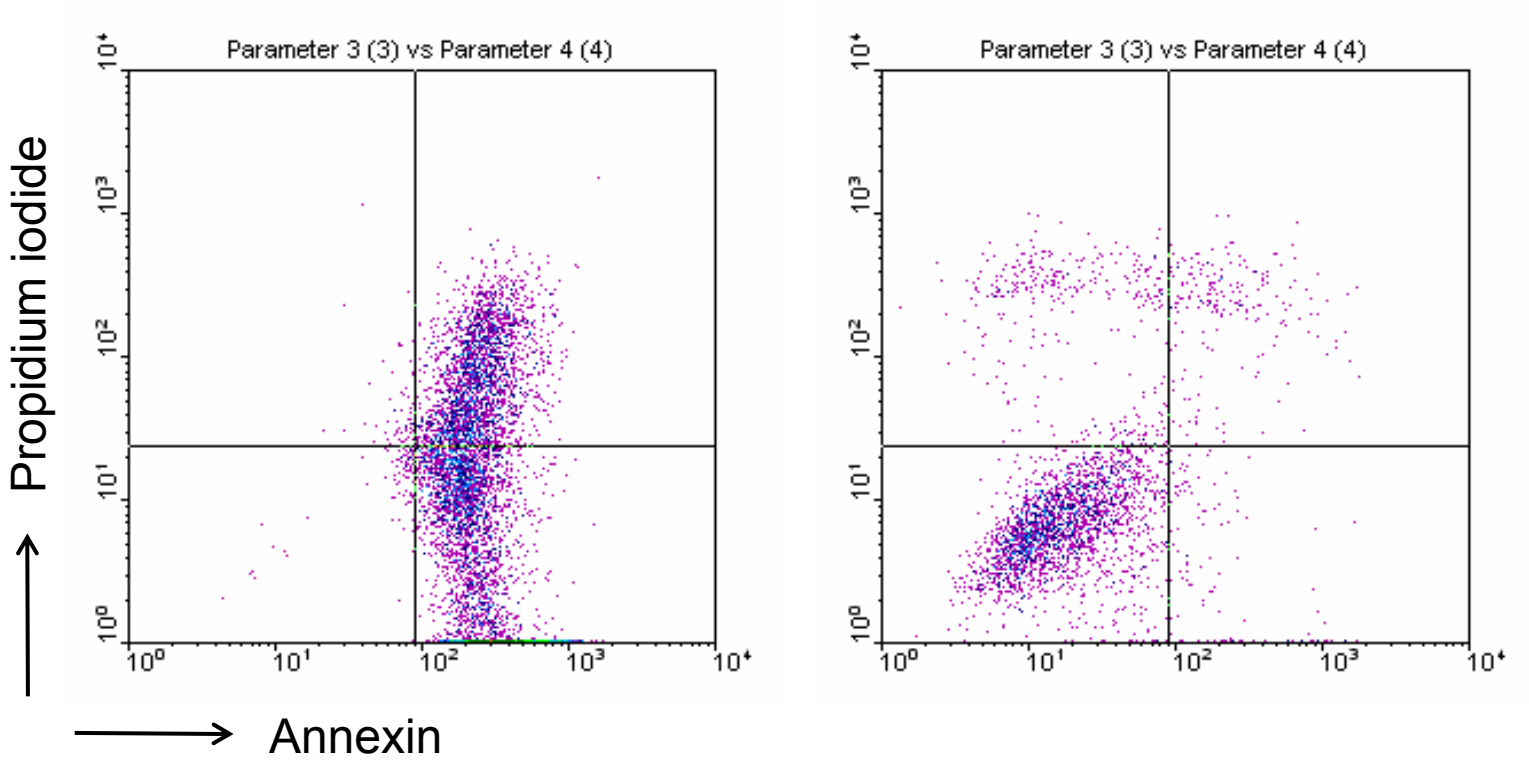


U937 cells (macrophage)

Induction of apoptosis by silver nanoparticles

20 nm

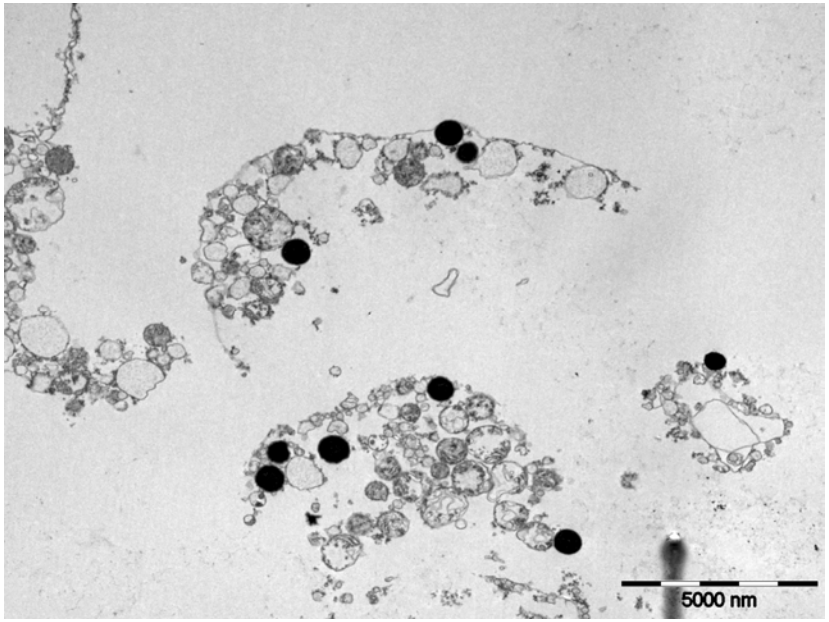
80 nm

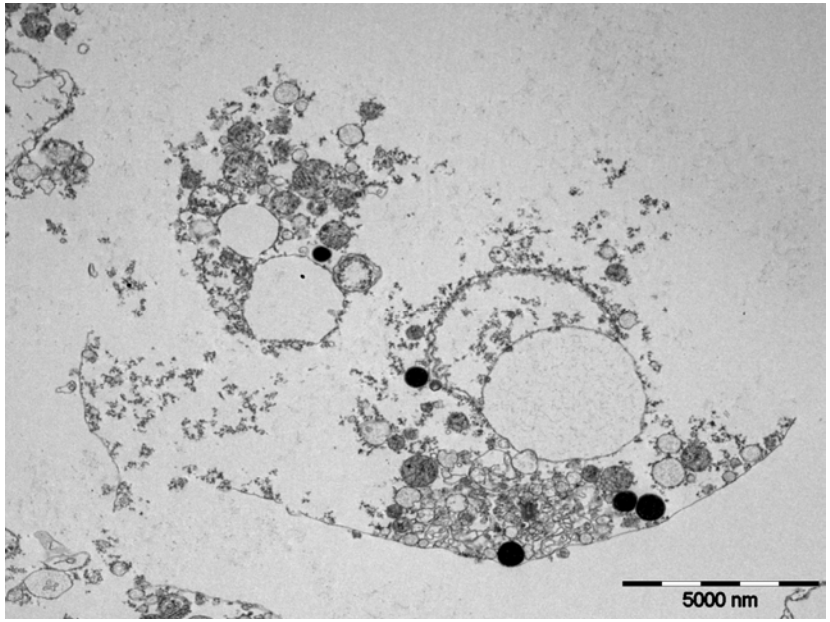


: U937 cells (macrophage)

: 25 μg/mL for 15 hrs

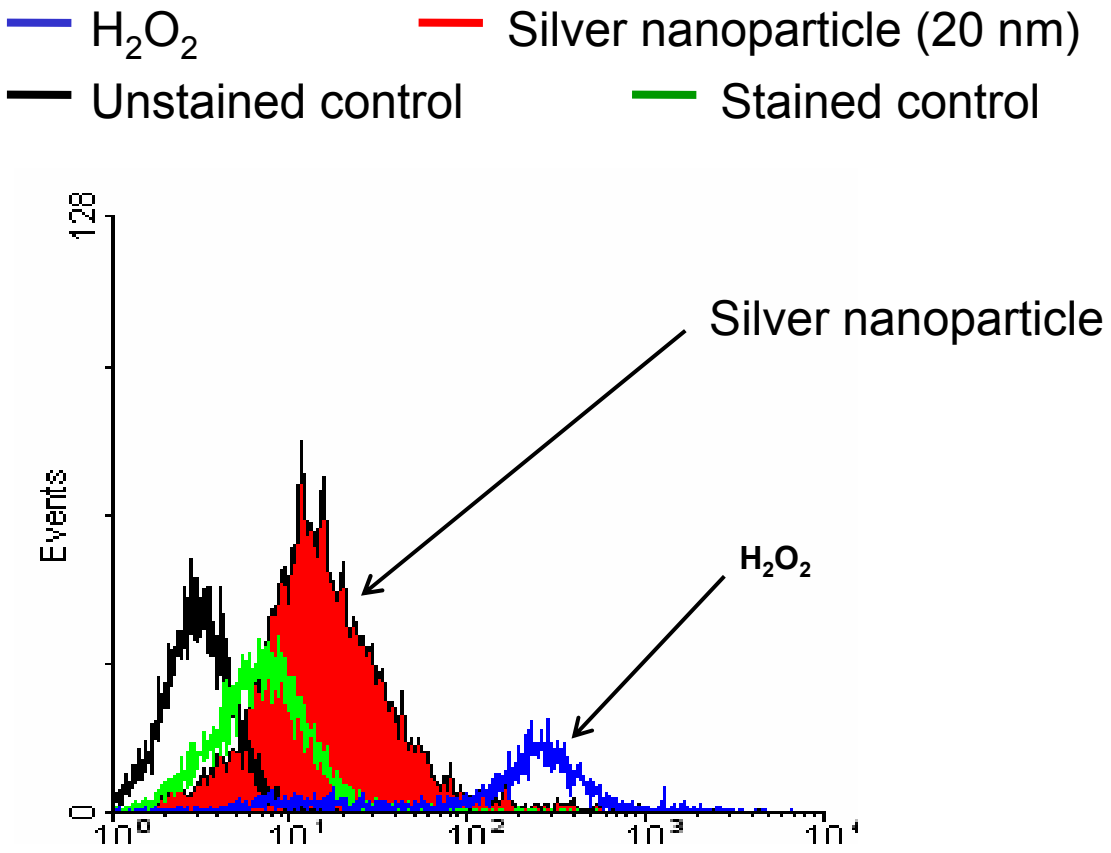
Lysosomal aggregation by silver nanoparticles





: U937 cells (macrophage)
: 20 nm, 25 μg/mL for 24 hrs

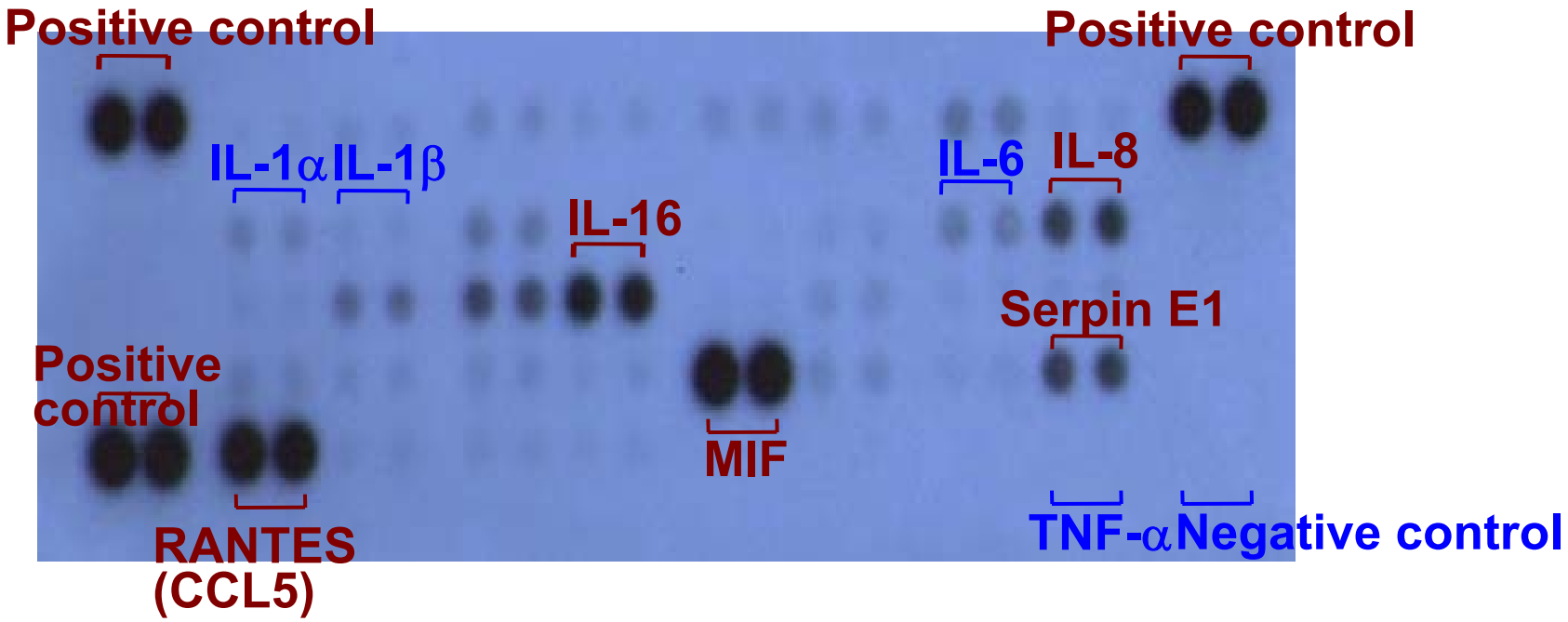
ROS production by silver nanoparticles



: BEAS-2B (lung)
: 20 nm, 30 μg/mL, for 3 hrs
: stained with CM-H_2DCFDA

Cytokine production by silver nanoparticles

- Cytokine array

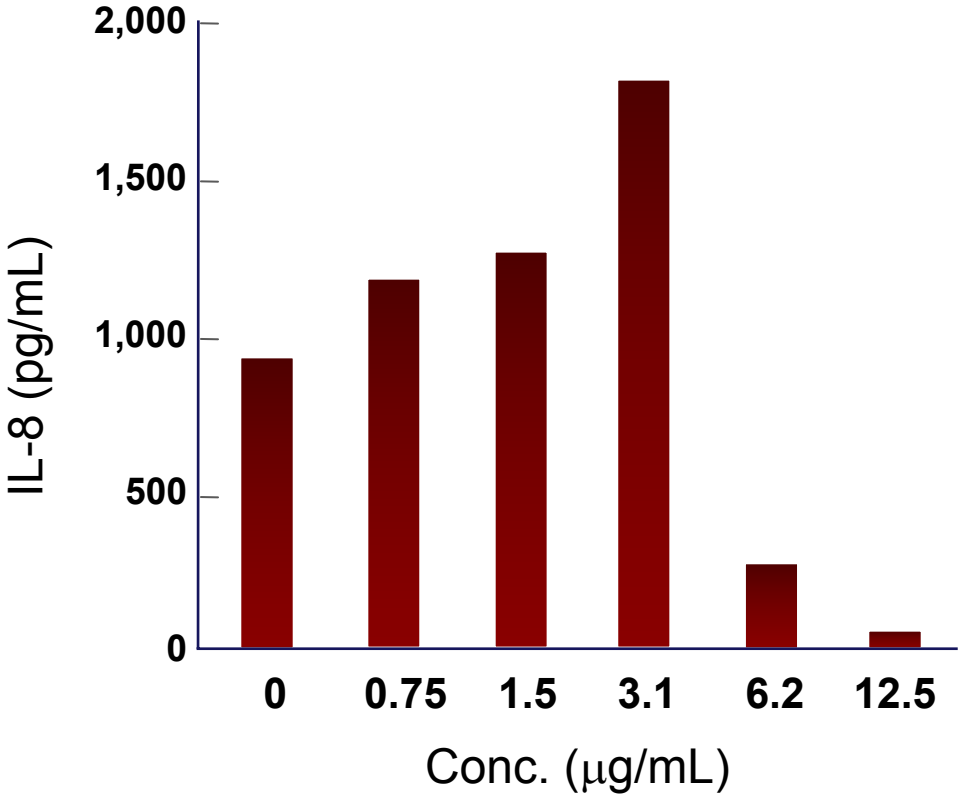


Positive: chemokines (IL-8, MIF, RANTES), Serpin E1, IL-16

Negative: TNF-α, IL-6, IL-1

Cytokine production by silver nanoparticles

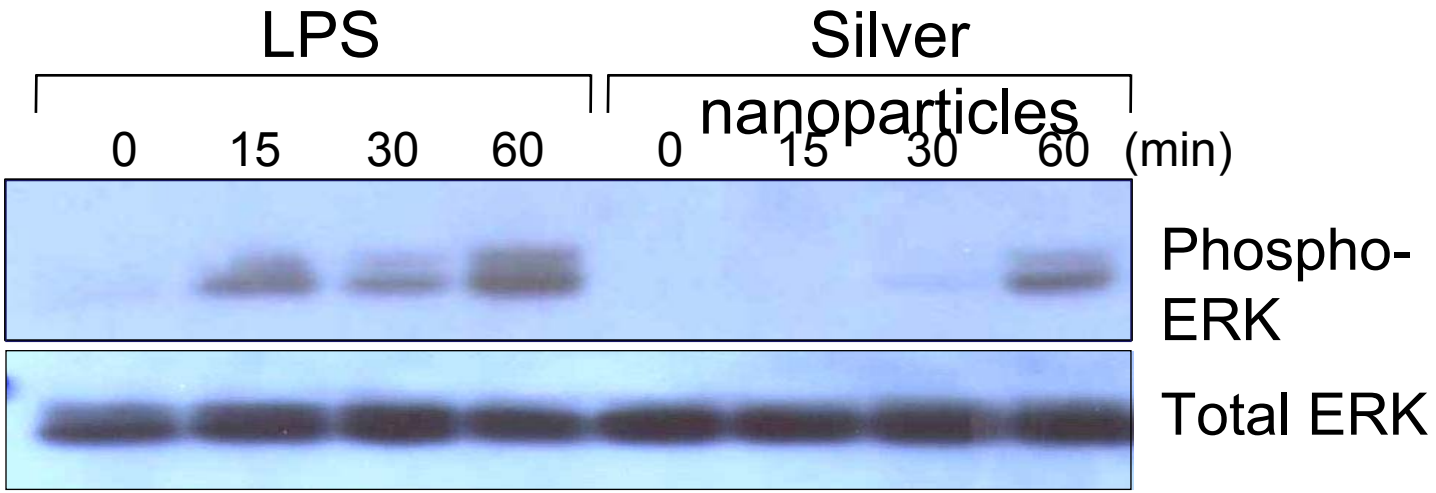
- ELISA (IL-8)



: U937 cells
(macrophages)
: 20 nm for 24 hrs

Activation of signaling molecule by silver nanoparticles

- MAP kinase (ex. ERK) activation



: Protein 30 μg loading
: LPS (*E. coli* lipopolysaccharide) 50 ng/mL
: 5 nm silver nanoparticles, 1.5 μg/mL

Summary

- In human cells, epithelial cells from skin or lung, and macrophages, 5 nm and 20 nm silver particles induced stronger cytotoxicity and ROS synthesis than 80 nm particles did.
- 5 nm and 20 nm silver particles induced chemokine production, mainly IL-8, MIF and RANTES, while proinflammatory cytokines, IL-1, IL-6 and TNF-α were not induced significantly in the same conditions.
- Some MAP kinase signaling pathways were activated during exposure to silver nanoparticles at lower concentrations which do not induce cytotoxicity.

Conclusion

- The toxicity and inflammatory effects of nanoparticles are dependent on their size. In silver nanoparticles smaller than 20 nm induce cytotoxicity significantly *in vitro*.
- Nanoparticles induce inflammatory immune responses at lower concentrations and chemokines are the major cytokines induced at early stages of exposure to silver nanoparticles.

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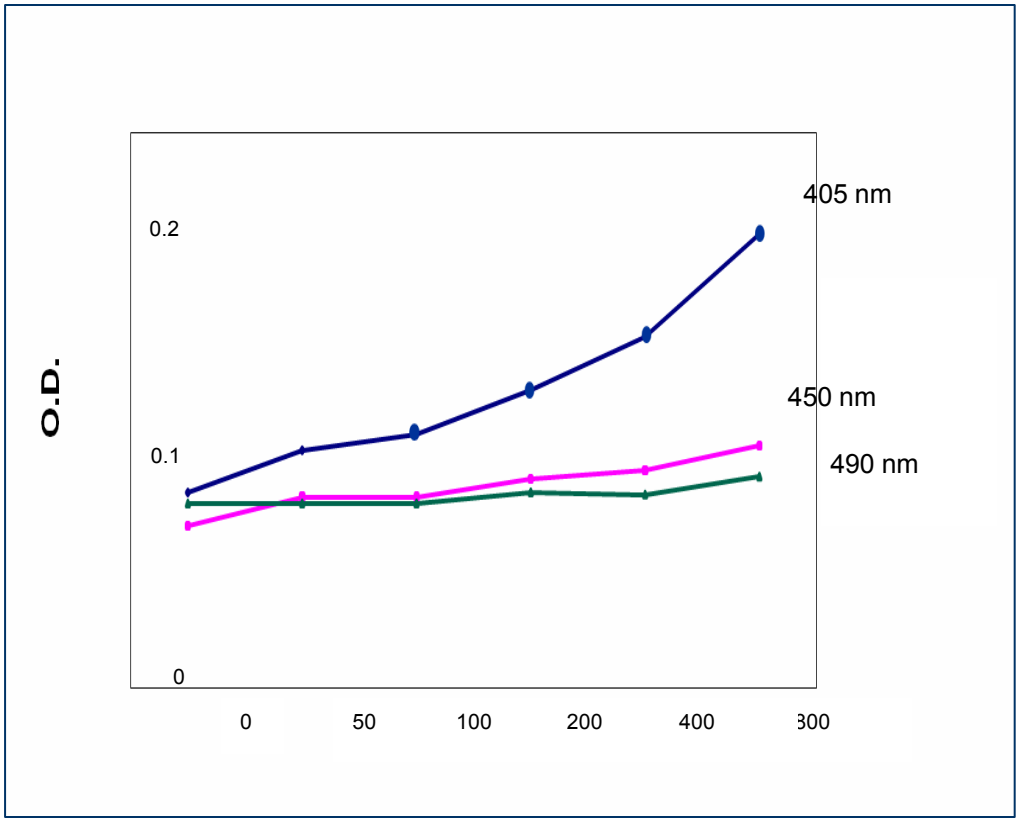
O.D.
0.2
0.1
0
0
50
100
200
400
300
405 nm
450 nm
490 nm