3rd International Summit on
Toxicology & Applied Pharmacology
October 20-22, 2014 Chicago, USA

Assessment of ZnO and SiO_2 nanoparticle permeability through blood–brain barrier and their toxicity using Evans blue and TEM

Organization of research team for nano-associated safety assessment in effort to study nanotoxicology of zinc oxide and silica nanoparticles

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Needs: assessing toxicity of nanoparticles

Diverse commercial products with nanoparticles on the market

 (나노처리된 강황을 코팅한 강황쌀)	 (나노 칼슘제)	 (나노기술 적용한 두통약)	
Nanoparticle curcumin rice	Nanoparticle Calcium supplement	Nanoparticle Pain medicine	
 (나노기술 화장품 '나노시스')	 (나노 샴푸)	 (나노 실버 젖병)	 (나노 치약)
Nanoparticle cosmetics	Nanoparticle shampoo	Nanoparticle Baby bottle	Nanoparticle Tooth paste

Objectives

ZnO & SiO_2 nanoparticles

Characterization of Chemical, Physical & Surface properties

Toxicity effect from Chemical, Physical & Surface properties of NPs

Overcoming the current methods of their toxicity evaluations

Suggesting novel methods of toxicity evaluations

Evaluating NPs with newly developed in vitro & in vivo methods

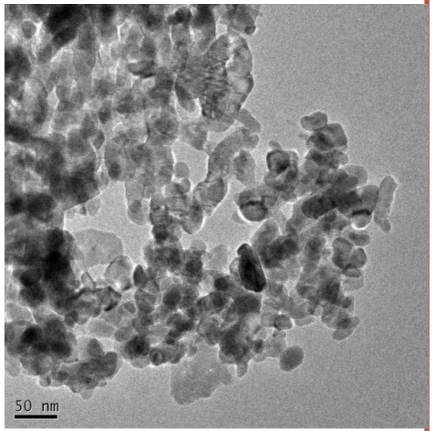
ZnO & SiO_2 nanoparticles

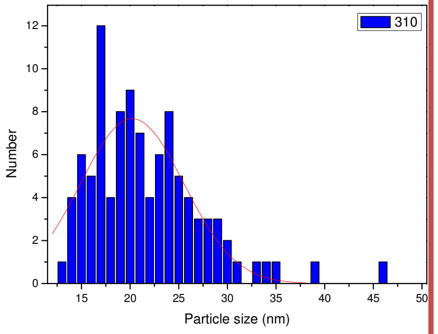
ZnO & SiO_2 NPs
20nm (+) charged
20nm (-) charged
100nm (+) charged
100nm (-) charged

Characterization of NPs

- ➢Selection of NPs by TEM images

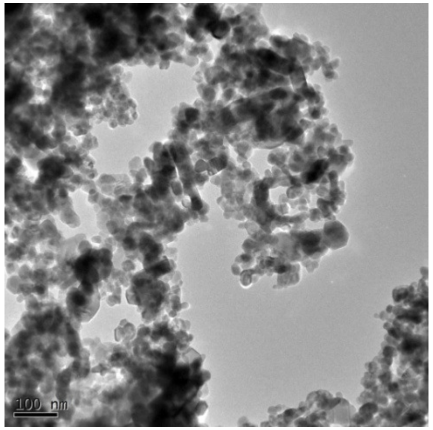
Sumitomo 310

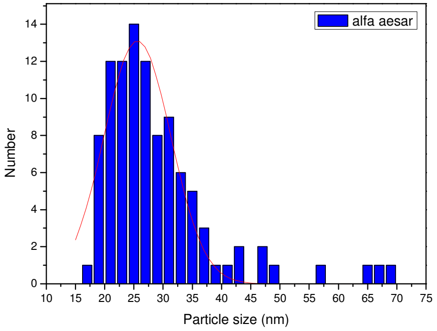




21.20±5.72nm

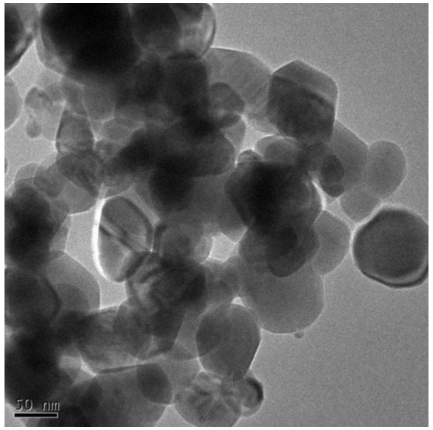
AA 20

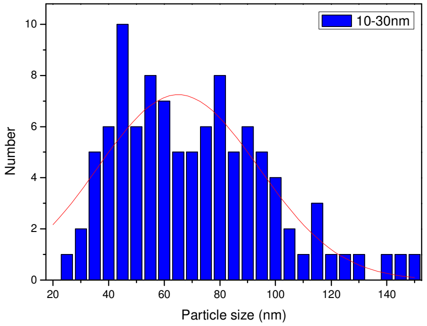




28.03±9.83nm

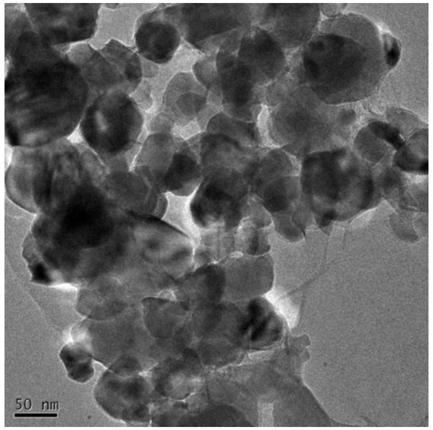
AE 20

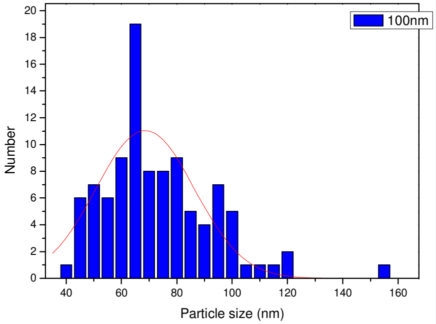




68.90±27.67nm

AE 100





70.60±19.45nm

Preparation of NPs

- Preparing (-)charge NPs

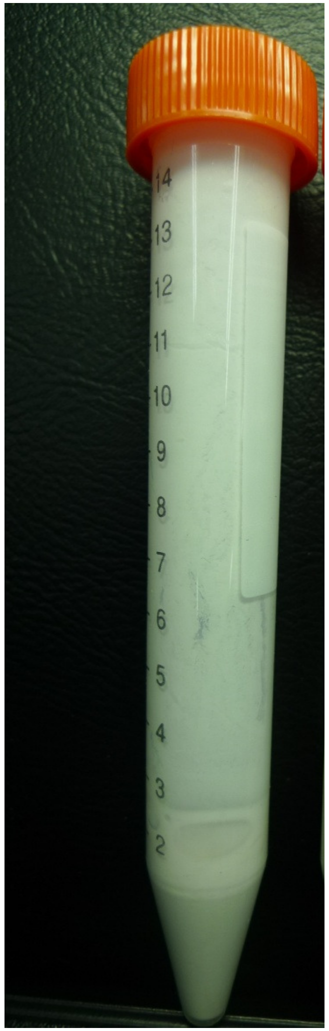
Candidates for (-)charge preparation:
PAA [Poly (acrylic acid)], **Citrate** [Sodium citrate]

pH selection :
pH6, pH6.5, pH7

Concentrations of Citrate :
Citrate buffer 1%, 2%, ZnO concentrations 10%, 20%, 100%

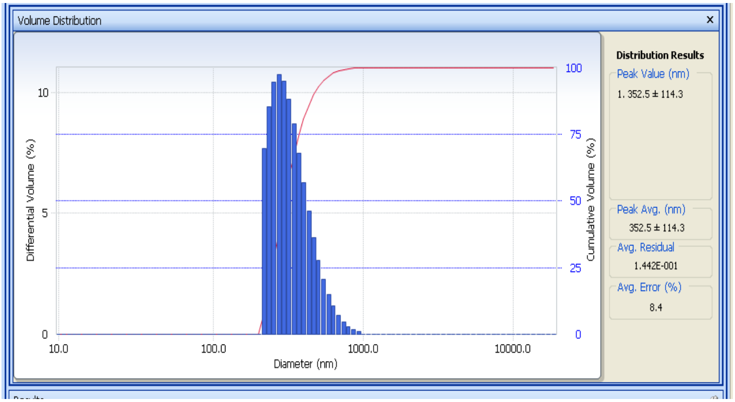
Dispersion, Distribution and Z-test

- ➢ Dispersion of Serine treated
- ➢ ZnO NPs (HEPES pH6)


14
13
12
11
10
9
8
7
6
5
4
3
2

No ligand

352.5±114 nm


Volume Distribution
×
Differential Volume (%)
Cumulative Volume (%)
10
5
0
100
75
50
25
0
10.0
100.0
1000.0
10000.0
Diameter (nm)

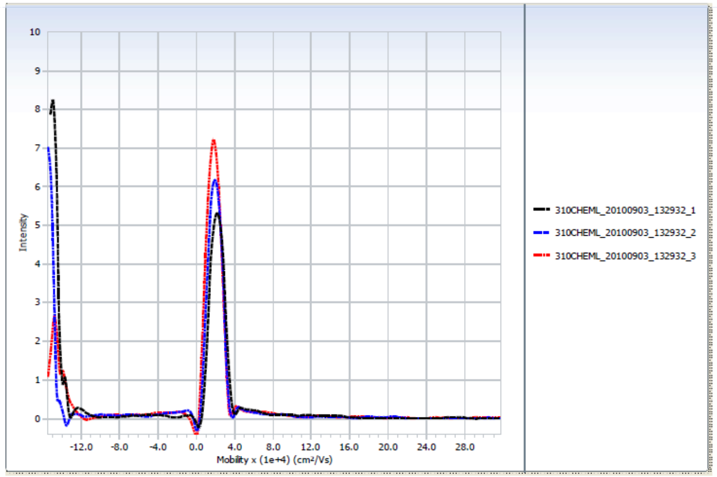
Distribution Results

Peak Value (nm)
1. 352.5 ± 114.3

Peak Avg. (nm)
352.5 ± 114.3

Avg. Residual
1.442E-001

Avg. Error (%)
8.4



No	File Name	Repet. No	MeasTime	pH	Zeta Potential (mV)	Mobility (cm²/Vs)	E. Field (V/cm)
1	310CHEML_20100903_132932	1	13:29:32	NA	28.03	2.186e-004	16.30
2	310CHEML_20100903_132932	2	13:29:32	NA	25.03	1.952e-004	16.30
3	310CHEML_20100903_132932	3	13:29:32	NA	23.33	1.820e-004	16.30
Average					25.46	1.986e-004	16.30

+ 25.46 mV

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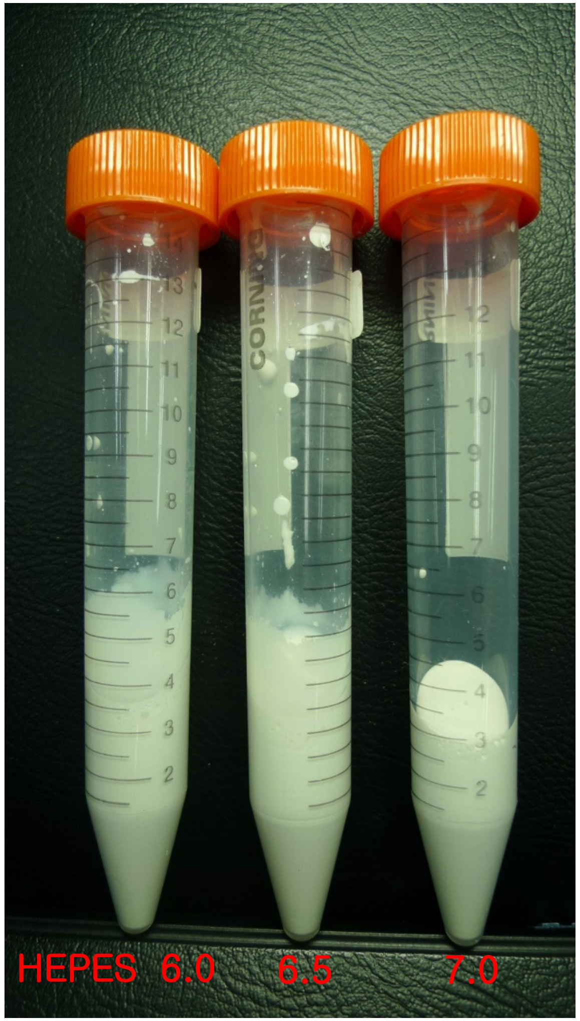
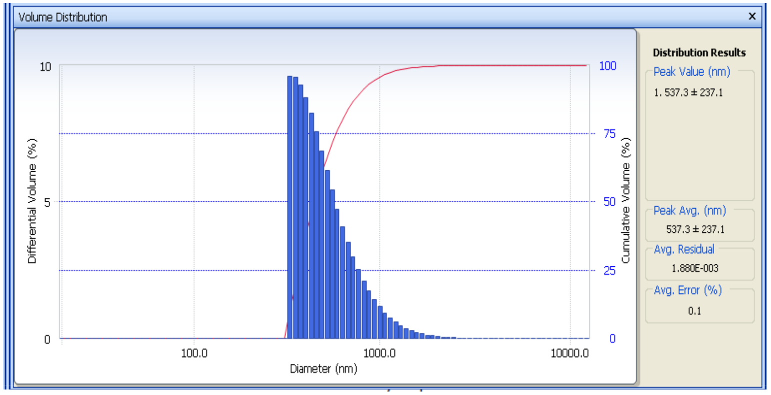
Dispersion, Distribution and Z-test

- ZnO: citrate(10:1), ddH2O, voltex 30s, No sonication,10min for settlement


HEPES 6.0
6.5
7.0

537.3±237 nm


Volume Distribution
10
5
0
Differential Volume (%)
100
75
50
25
0
Cumulative Volume (%)
100.0
1000.0
10000.0
Diameter (nm)

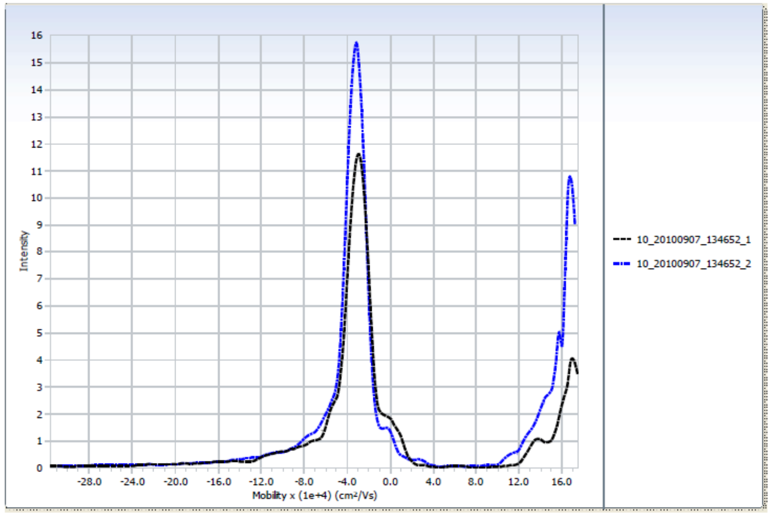
Distribution Results

Peak Value (nm)
1. 537.3 ± 237.1

Peak Avg. (nm)
537.3 ± 237.1

Avg. Residual
1.880E-003

Avg. Error (%)
0.1



No	File Name	Repet. No	MeasTime	pH	Zeta Potential (mV)	Mobility (cm²/Vs)	E. Field (V/cm)
1	10_20100907_134652	1	13:46:52	NA	-38.19	-2.978e-004	-16.31
2	10_20100907_134652	2	13:46:52	NA	-41.09	-3.204e-004	-16.31
Average					-39.64	-3.091e-004	-16.31

−39.64 mV

Preparation protocol

- (+)charged NPs

Candidates for (+)charging:

No ligand,

TREN [Tris (2-aminoethyl) amine], **PAH,** [Poly (allylamine hydrochloride)], **PDAC**, **DETA** [Diethylenetriamine], **Thiamine, Arginine, Lysine, Serine**

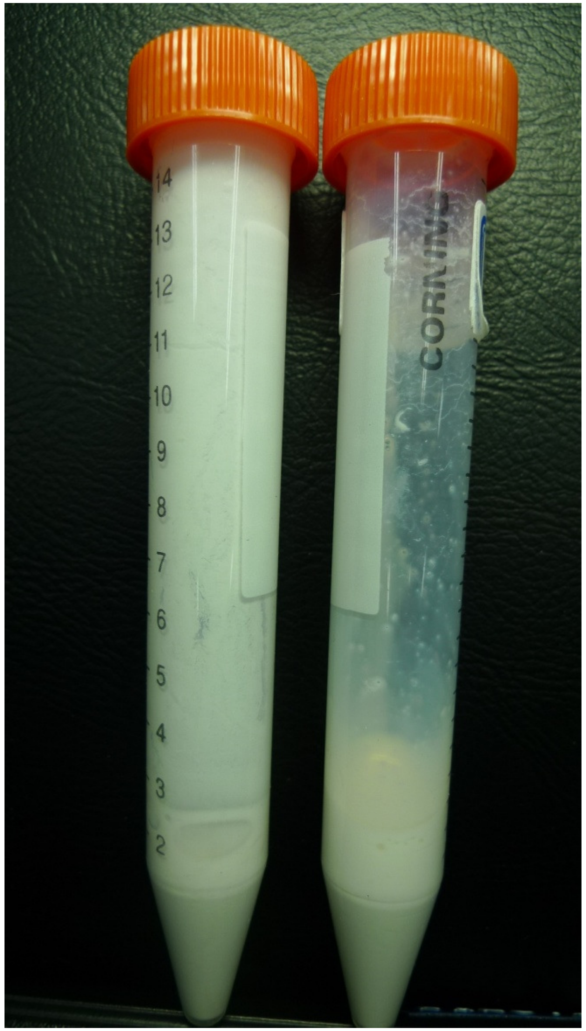
pH selection:
pH6, pH6.5, pH7

Concentrations of Serine:
Serine buffer: 1%, 2%, ZnO concentrations: 10%, 20%, 100%

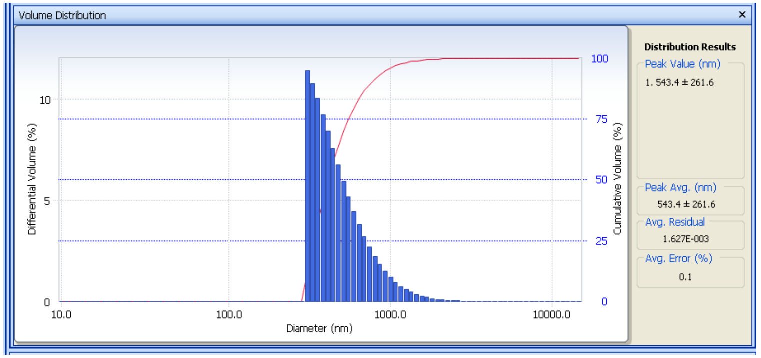
Dispersion, Distribution and Z-test

1% serine HEPES pH6

+ Serine


CORNING
14
13
12
11
10
9
8
7
6
5
4
3
2

543.4±261 nm

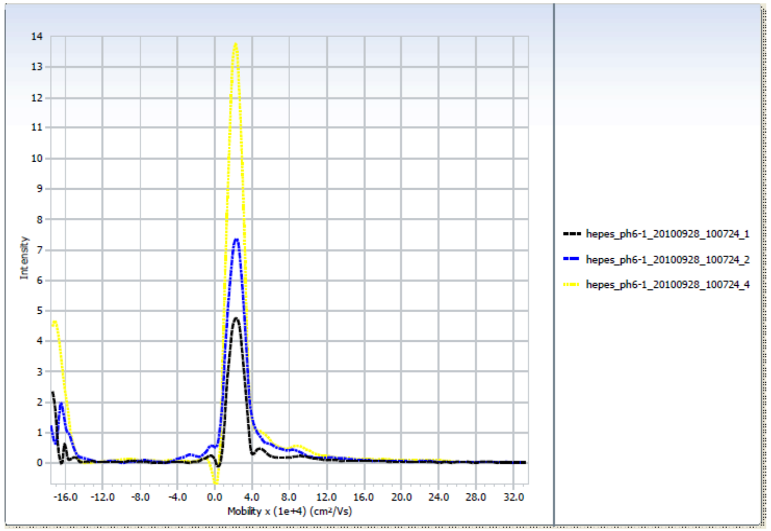

Volume Distribution
×
Differential Volume (%)
10
5
0
10.0
100.0
1000.0
10000.0
Diameter (nm)
Cumulative Volume (%)
100
75
50
25
0
Distribution Results

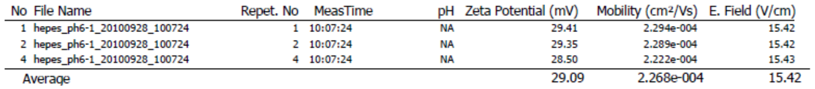
Peak Value (nm)
1. 543.4 ± 261.6

Peak Avg. (nm)
543.4 ± 261.6

Avg. Residual
1.627E-003

Avg. Error (%)
0.1

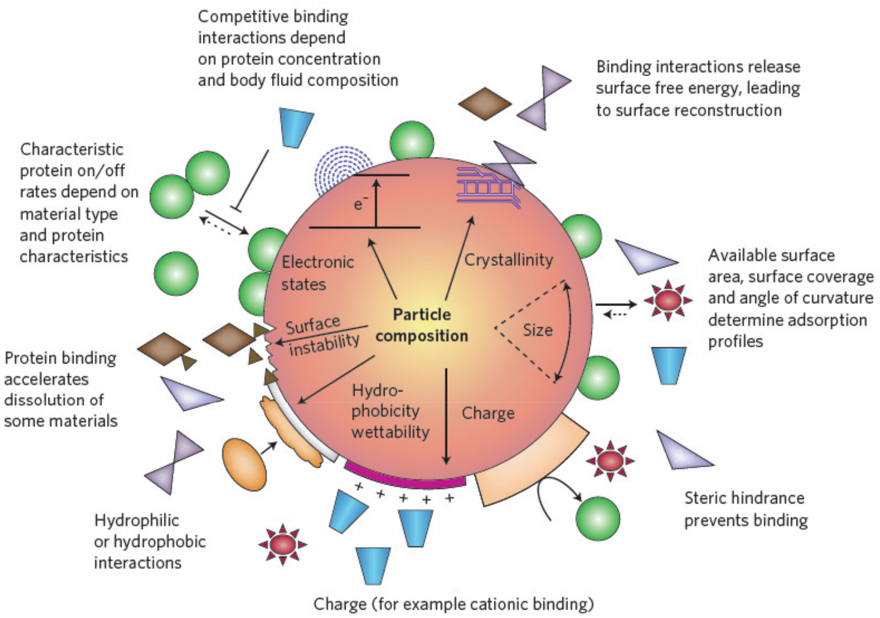


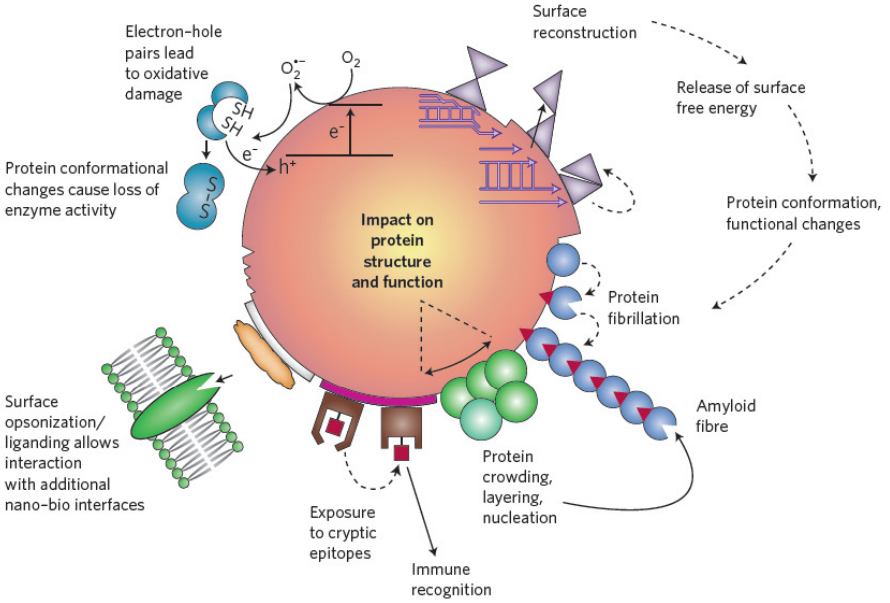


No	File Name	Repet. No	MeasTime	pH	Zeta Potential (mV)	Mobility (cm²/Vs)	E. Field (V/cm)
1	hepes_ph6-1_20100928_100724	1	10:07:24	NA	29.41	2.294e-004	15.42
2	hepes_ph6-1_20100928_100724	2	10:07:24	NA	29.35	2.289e-004	15.42
4	hepes_ph6-1_20100928_100724	4	10:07:24	NA	28.50	2.222e-004	15.43
Average					29.09	2.268e-004	15.42

+ 29.09 mV

Nanoparticles & Protein Corona


Competitive binding interactions depend on protein concentration and body fluid composition
Binding interactions release surface free energy, leading to surface reconstruction
Characteristic protein on/off rates depend on material type and protein characteristics
Electronic states
Crystallinity
e^-
Surface instability
Particle composition
Size
Hydro-phobicity wettability
Charge
Available surface area, surface coverage and angle of curvature determine adsorption profiles
Protein binding accelerates dissolution of some materials
Steric hindrance prevents binding
Hydrophilic or hydrophobic interactions
+ + + + +
Charge (for example cationic binding)


Electron-hole pairs lead to oxidative damage
$O_2^{\bullet-}$
O_2
Surface reconstruction
Release of surface free energy
SH
SH
e^-
h^+
e^-
S
S
Protein conformational changes cause loss of enzyme activity
Impact on protein structure and function
Protein conformation, functional changes
Protein fibrillation
Amyloid fibre
Surface opsonization/ liganding allows interaction with additional nano-bio interfaces
Protein crowding, layering, nucleation
Exposure to cryptic epitopes
Immune recognition

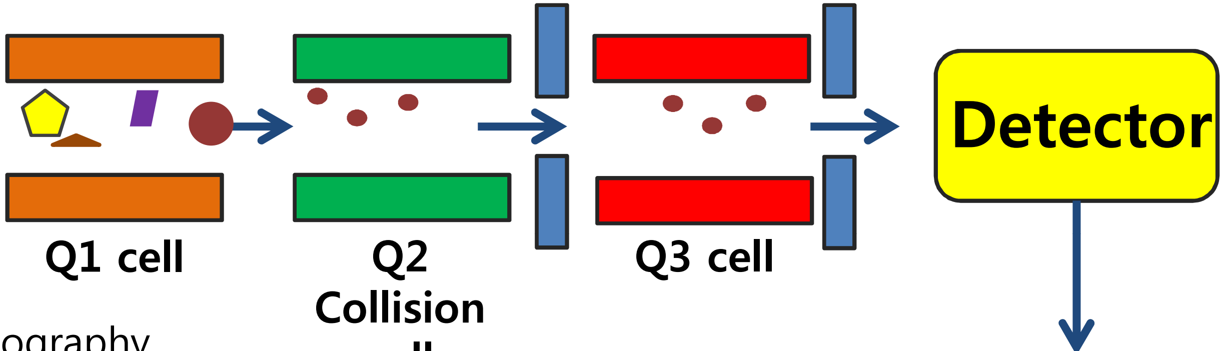
- Protein corona is likely to determine the fate of the nanomaterials *in vivo* (Cedervall *et al*, 2007).
- proteins can potentially interact with a nanomaterial to control its access to specific compartments, mark it for efficient removal by tissue-resident macrophages, and promote undesirable inflammation, thrombosis, and anaphylaxis (Peppas *et al*, 2006).

LC-MS/MS method

Sample
After trypsin digestion

SDS-PAGE & LC column

① Physical separation of samples

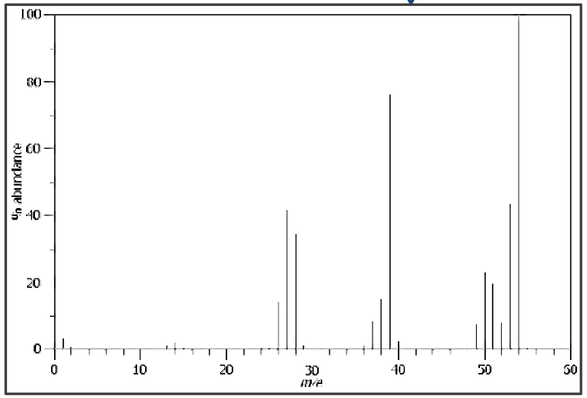


Ionization

② Mass analysis

1. SDS-PAGE & Liquid Chromatography
   -Separate digested peptides for high resolution
2. Ionization
   -Convert solution phase ions into the gas phase
3. Mass Analyze
   -Measures the mass-to-charge ratio of charged particles
   -Determining the elemental composition of a sample
4. Detect & Data Collection
   -Elucidating the chemical structure of molecule
   -Qualitative and quantitative data

MS/MS Spectrum



③ Data collection

Protein corona SiO_2& ZnO NPs (– 20/100 nm)

Samples	Total (ZnO)	Total (SiO_2)	Common
20 nm in plasma	58	48	9
100 nm in plasma	44	36	9
20 nm in BH	294	125	48
100 nm in BH	339	145	77

Unique plasma and BH proteins between SiO_2 20 & 100 NPs

Plasma		Brain Homogenate	
20 nm	100 nm	20 nm	100 nm
hemopexin precursor, C4b-binding protein alpha chain, lipopolysaccharide-binding protein precursor, glyceraldehyde-3-phosphate dehydrogenase, inter-alpha-trypsin inhibitor heavy chain H3 precursor, T-kininogen 2 precursor, alpha-1-inhibitor 3 precursor, Serine protease inhibitor, ceruloplasmin precursor, Kallistatin, mast cell protease 9, serine protease inhibitor A3N, protein AMBP precursor, complement component C8 beta chain	Plasminogen, selenoprotein P precursor, vitronectin, alpha-1-antiproteinase precursor, plasma kallikrein precursor, kininogen-1 isoform 1, plasma protease C1 inhibitor precursor, apolipoprotein C-I precursor	calcium/calmodulin-dependent protein kinase, serine/threonine-protein phosphatase, histidine-rich glycoprotein, ATP-dependent RNA helicase A	pre-mRNA-processing-splicing factor 8, AP-2 complex subunit alpha-1, band 4.1-like protein 3, polyadenylate-binding protein 4, ADP-ribosylation factor 1, cytoplasmic dynein 1 heavy chain 1, kinesin heavy chain isoform 5C nucleolin

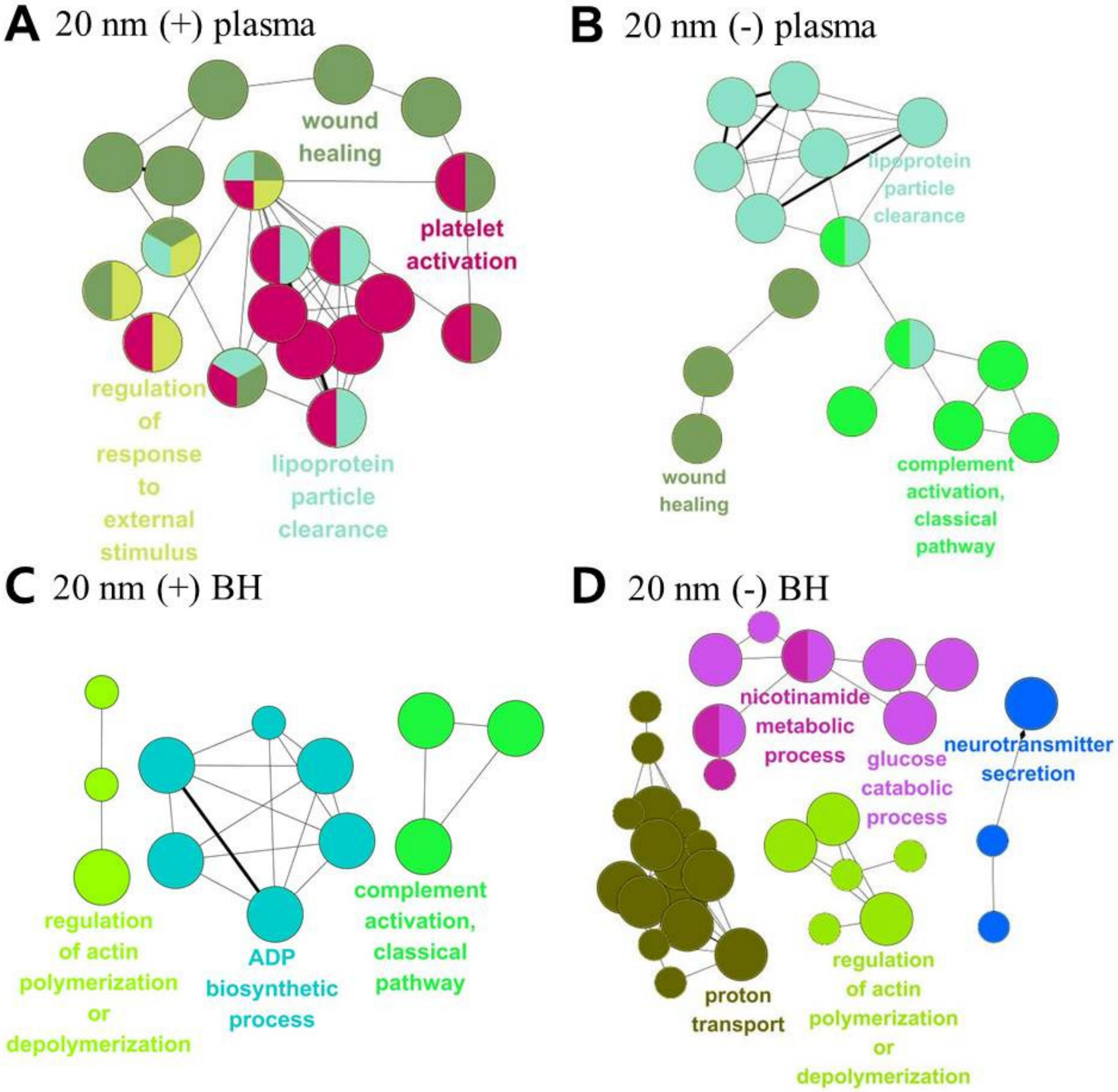
Correlated plasma and BH proteins between SiO_2&ZnO NPs

Plasma		Brain Homogenate	
20 nm	100 nm	20 nm	100 nm
apolipoprotein B-100 precursor complement C3 complement component C9 apolipoprotein E precursor complement C4 precursor fibronectin precursor PREDICTED: histidine-rich glycoprotein-like PREDICTED: hypothetical protein PREDICTED: complement component 5 anionic trypsin-1 precursor PREDICTED: histidine-rich glycoprotein-like	apolipoprotein B-100 precursor complement C3 apolipoprotein E precursor PREDICTED: histidine-rich glycoprotein-like complement C4 precursor inter-alpha-inhibitor H4 heavy chain vitronectin fibronectin precursor plasma protease C1 inhibitor precursor PREDICTED: complement component 5 PREDICTED: histidine-rich glycoprotein-like	tubulin beta-3 chain, tubulin alpha-1A chain, clathrin heavy chain 1, synapsin-1 isoform b, glyceraldehyde-3-phosphate dehydrogenase, tubulin beta-2B chain, ATP synthase subunit alpha, mitochondrial precursor, dynamin-1, ATP synthase subunit beta, mitochondrial precursor, actin, aortic smooth muscle, heterogeneous nuclear ribonucleoprotein K, vesicle-fusing ATPase, elongation factor 1-alpha 2, V-type proton ATPase catalytic subunit A, actin, cytoplasmic 2, AP-2 complex subunit beta	tubulin beta-3 chain, tubulin alpha-1A chain, syntaxin-binding protein 1, vesicle-fusing ATPase, clathrin heavy chain 1, synapsin-1 isoform b, tubulin beta-2B chain, microtubule-associated protein 1B, glyceraldehyde-3-phosphate, ehydrogenase, ATP synthase subunit alpha, mitochondrial precursor, actin, aortic smooth muscle, 6-phosphofructokinase type C, 6-phosphofructokinase, muscle type, elongation factor 1-alpha 2, 2',3'-cyclic-nucleotide 3'-phosphodiesterase, ATP synthase subunit beta, mitochondrial precursor

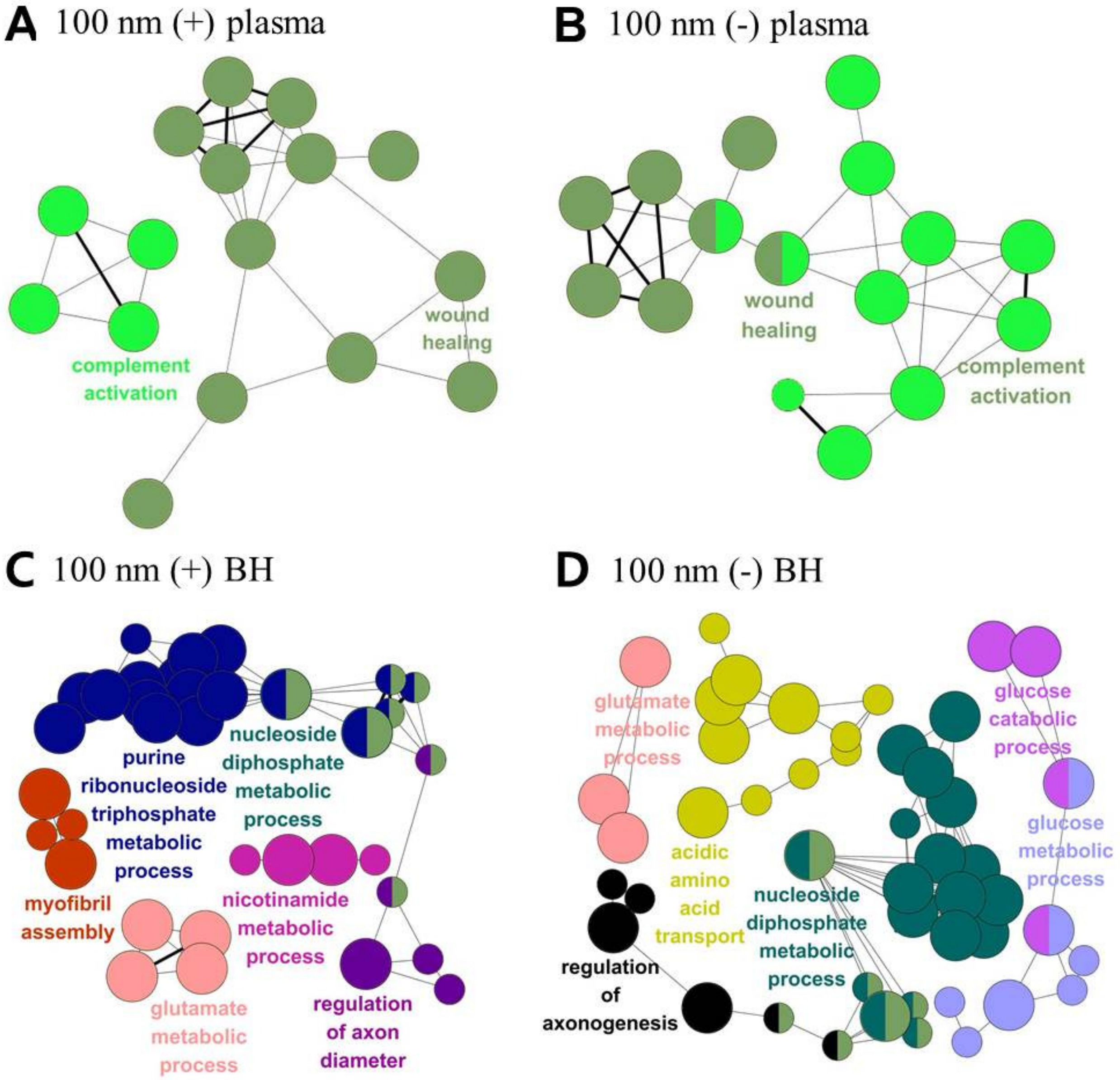
Unique plasma and BH proteins between SiO_2&ZnO NPs

Plasma		Brain Homogenate	
SiO2	ZnO	SiO2	ZnO
serum albumin precursor, histidine-rich glycoprotein, kininogen-1 isoform 2, apolipoprotein A-I preproprotein	fibrinogen gamma chain, fibrinogen beta chain precursor, gelsolin precursor	T-complex protein 1, 6-phosphofructokinase	pyruvate kinase isozymes M1/M2, alpha-internexin, phosphoglycerate kinase 1

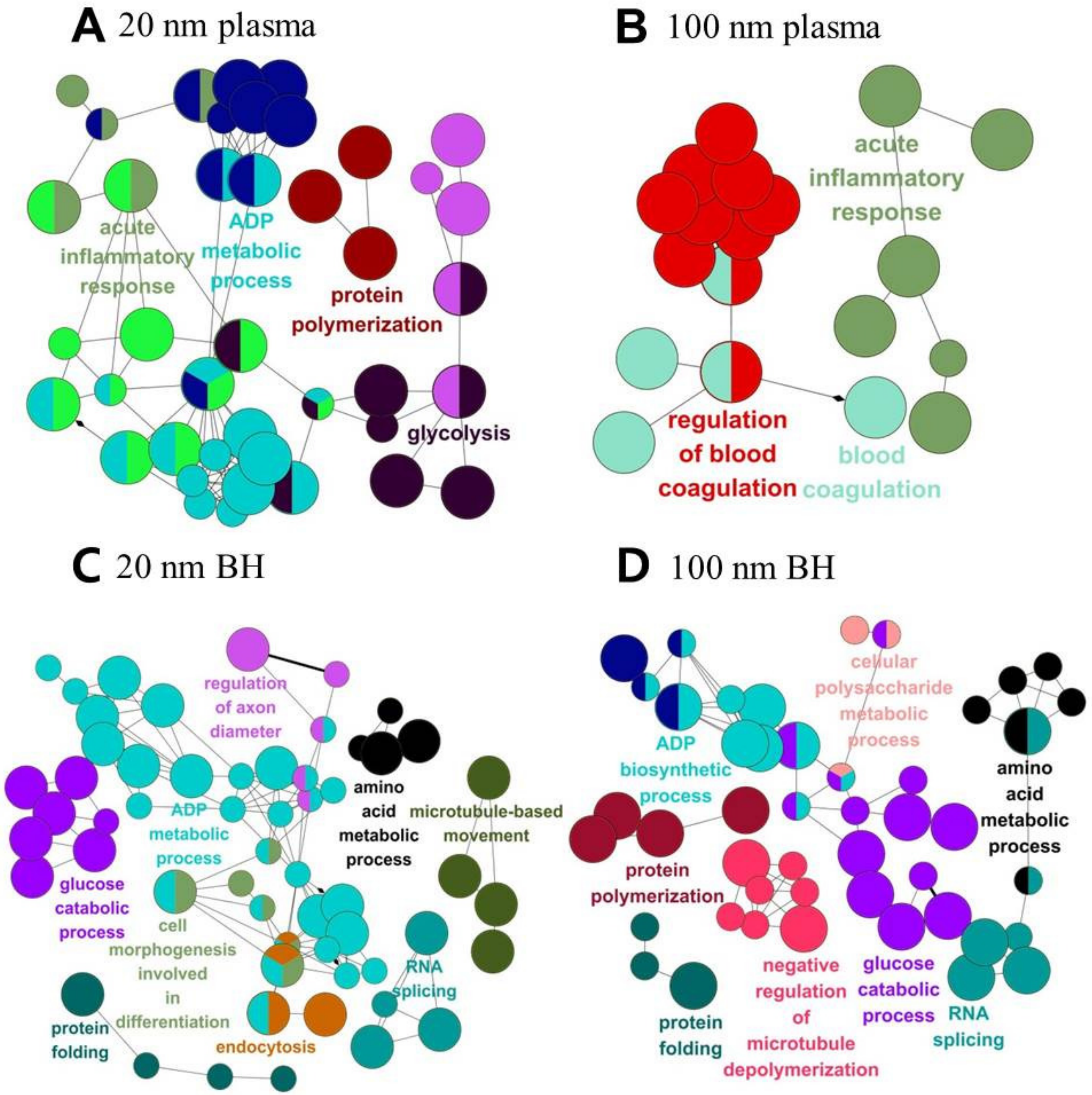
Implicated biological processes by ZnO


A 20 nm (+) plasma
wound healing
platelet activation
regulation of response to external stimulus
lipoprotein particle clearance
B 20 nm (-) plasma
lipoprotein particle clearance
wound healing
complement activation, classical pathway
C 20 nm (+) BH
regulation of actin polymerization or depolymerization
ADP biosynthetic process
complement activation, classical pathway
D 20 nm (-) BH
nicotinamide metabolic process
glucose catabolic process
neurotransmitter secretion
proton transport
regulation of actin polymerization or depolymerization

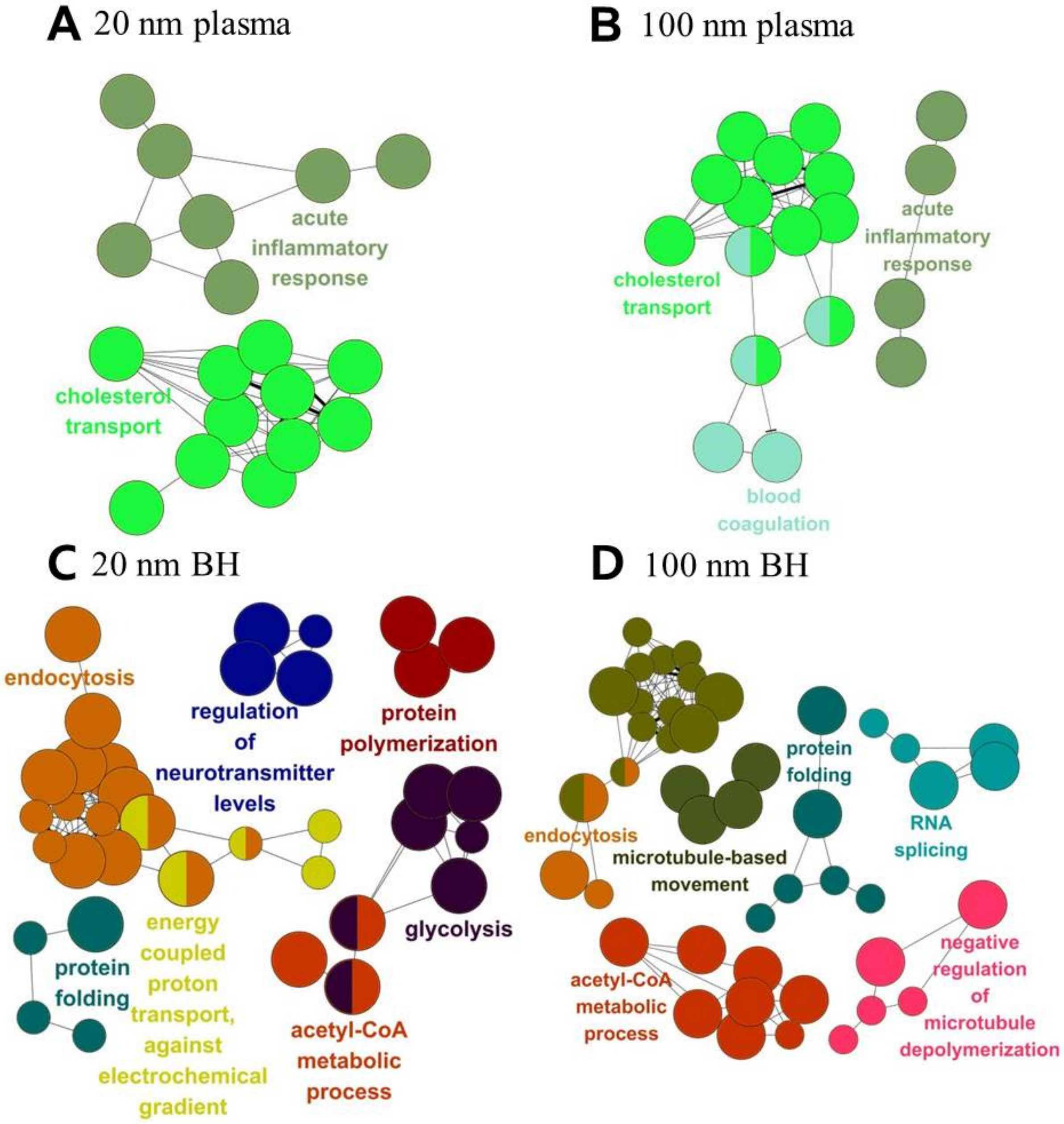
Implicated biological processes by ZnO


A
100 nm (+) plasma
wound healing
complement activation
B
100 nm (-) plasma
wound healing
complement activation
C
100 nm (+) BH
purine ribonucleoside triphosphate metabolic process
nucleoside diphosphate metabolic process
myofibril assembly
nicotinamide metabolic process
glutamate metabolic process
regulation of axon diameter
D
100 nm (-) BH
glutamate metabolic process
glucose catabolic process
glucose metabolic process
acidic amino acid transport
nucleoside diphosphate metabolic process
regulation of axonogenesis

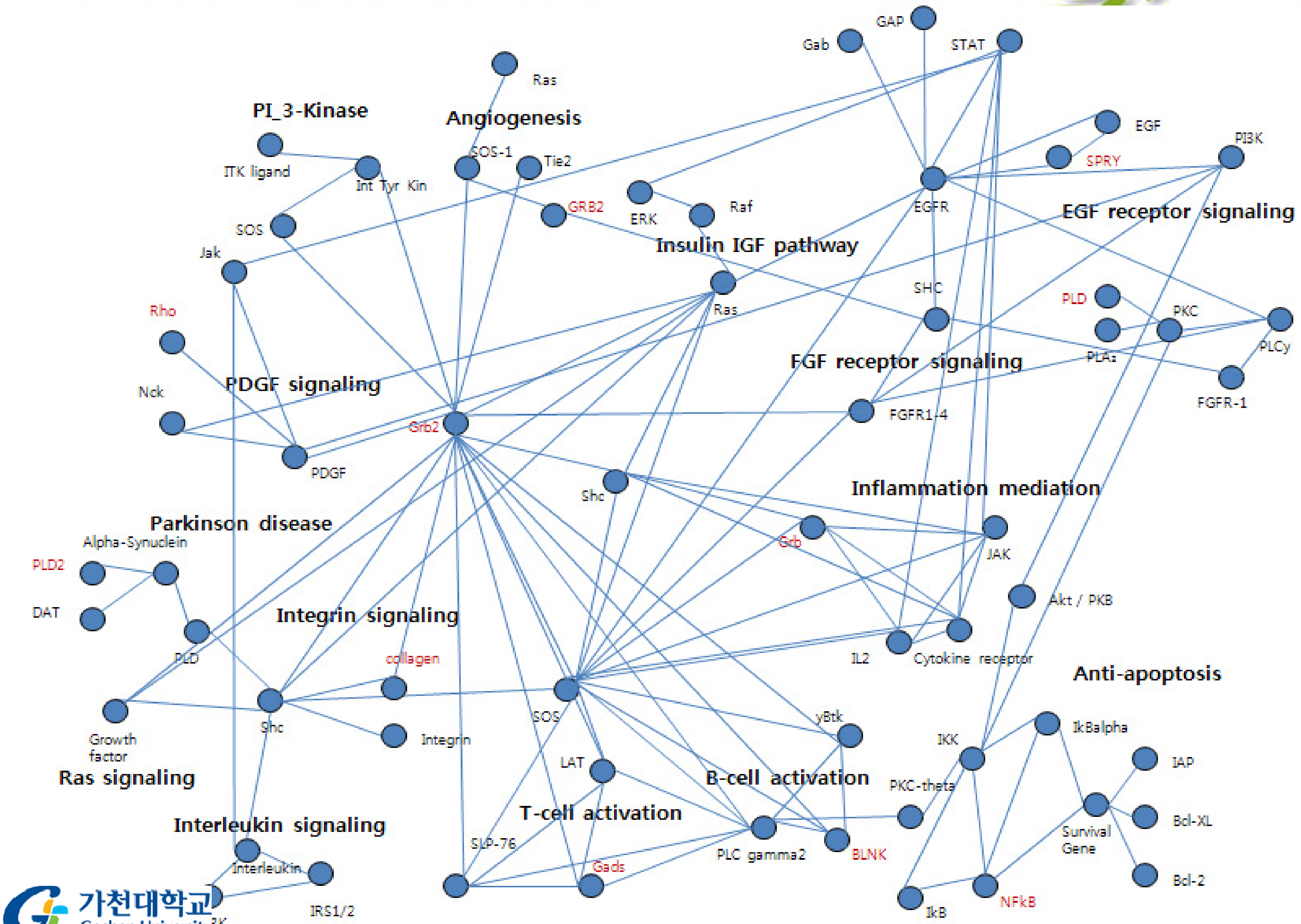
Implicated biological processes by SiO_2 (+)


A 20 nm plasma
acute inflammatory response
ADP metabolic process
protein polymerization
glycolysis
B 100 nm plasma
acute inflammatory response
regulation of blood coagulation
blood coagulation
C 20 nm BH
regulation of axon diameter
amino acid metabolic process
microtubule-based movement
ADP metabolic process
glucose catabolic process
cell morphogenesis involved in differentiation
RNA splicing
protein folding
endocytosis
D 100 nm BH
cellular polysaccharide metabolic process
ADP biosynthetic process
amino acid metabolic process
protein polymerization
protein folding
negative regulation of microtubule depolymerization
glucose catabolic process
RNA splicing

Implicated biological processes by SiO_2 (-)


A 20 nm plasma
acute inflammatory response
cholesterol transport
B 100 nm plasma
cholesterol transport
acute inflammatory response
blood coagulation
C 20 nm BH
endocytosis
regulation of neurotransmitter levels
protein polymerization
glycolysis
energy coupled proton transport, against electrochemical gradient
protein folding
acetyl-CoA metabolic process
D 100 nm BH
protein folding
RNA splicing
endocytosis
microtubule-based movement
acetyl-CoA metabolic process
negative regulation of microtubule depolymerization

NF-κB implicated network


GAP
Gab
STAT
Ras
PI_3-Kinase
Angiogenesis
EGF
PI3K
SOS-1
Tie2
SPRY
ITK ligand
Int Tyr Kin
GRB2
ERK
Raf
EGFR
EGF receptor signaling
SOS
Jak
Insulin IGF pathway
SHC
Ras
PLD
PKC
Rho
PLA₂
PLCγ
FGF receptor signaling
Nck
PDGF signaling
FGFR-1
Grb2
FGFR1-4
PDGF
Shc
Inflammation mediation
Parkinson disease
Alpha-Synuclein
Grb
JAK
PLD2
DAT
Akt / PKB
Integrin signaling
PLD
collagen
IL2
Cytokine receptor
Anti-apoptosis
SOS
Growth factor
Shc
Integrin
yBtk
IKK
IkBalpha
Ras signaling
LAT
B-cell activation
IAP
PKC-theta
T-cell activation
Interleukin signaling
Survival Gene
Bcl-XL
SLP-76
PLC gamma2
BLNK
Interleukin
Gads
Bcl-2
IRS1/2
IkB
NFkB

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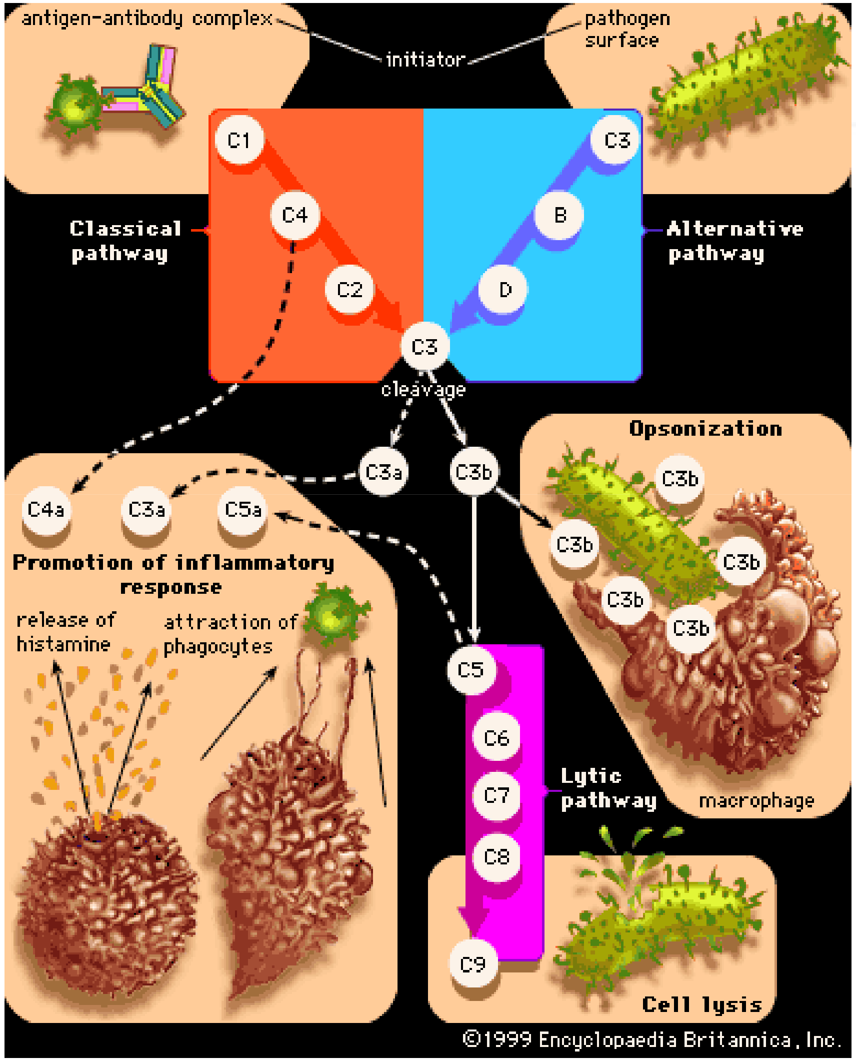
Complement component


antigen-antibody complex
initiator
pathogen surface
C1
C4
C2
C3
B
D
Classical pathway
Alternative pathway
C3
cleavage
C3a
C3b
Opsonization
C3b
C3b
C3b
C3b
C3b
C4a
C3a
C5a
Promotion of inflammatory response
release of histamine
attraction of phagocytes
C5
C6
C7
C8
C9
Lytic pathway
macrophage
Cell lysis

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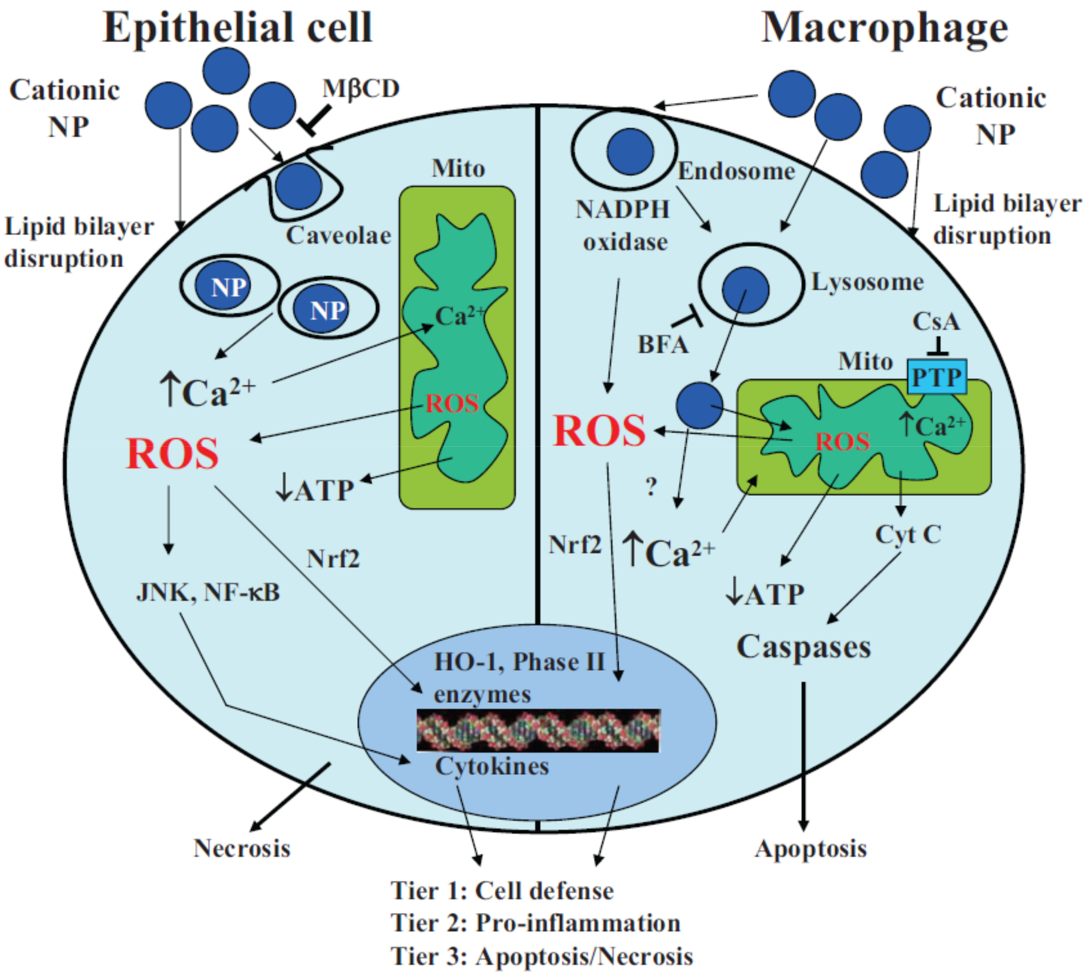
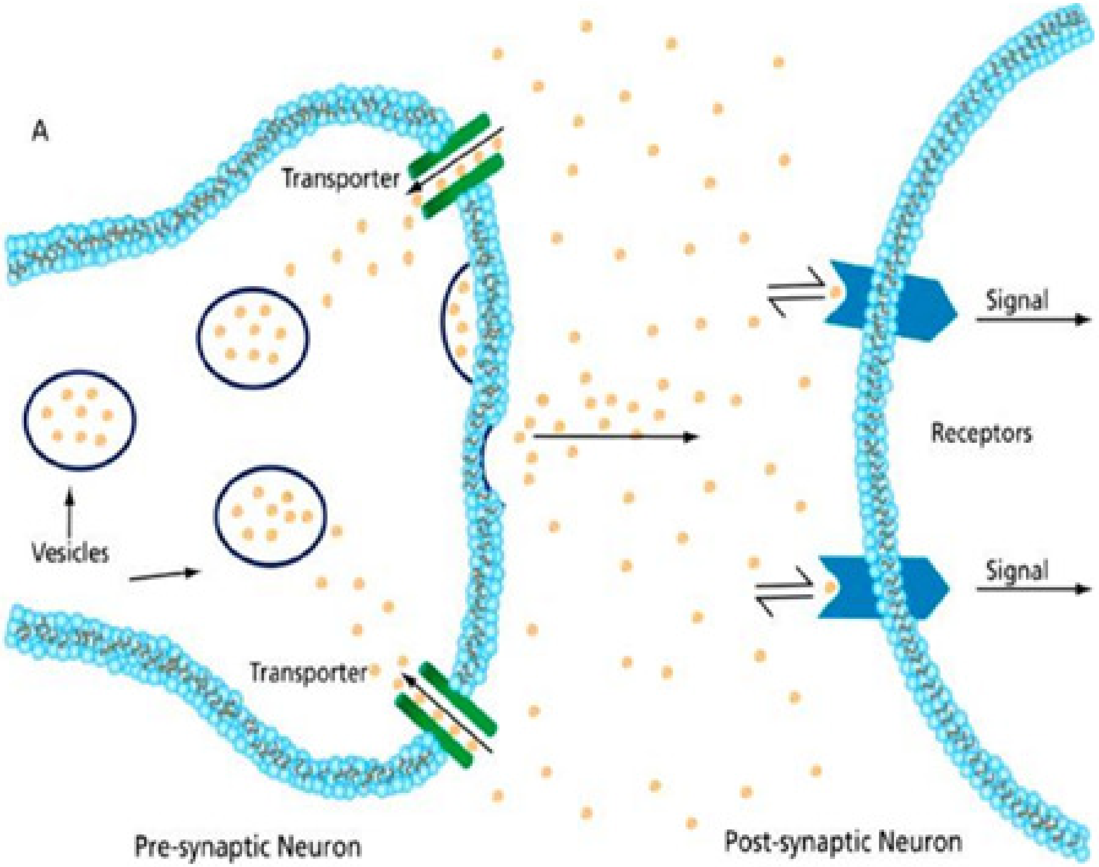
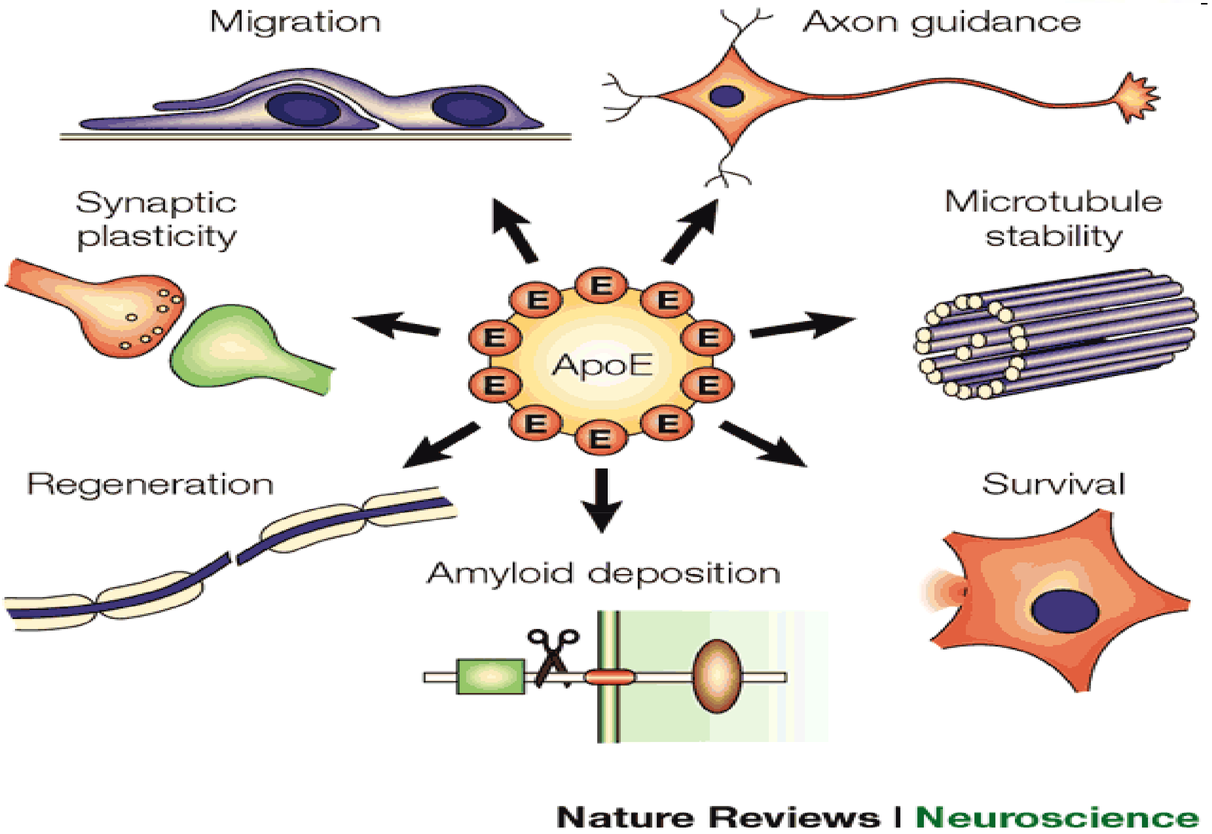
Nanotoxocity based mitochondria dysfunction


Epithelial cell
Macrophage
Cationic NP
MβCD
Caveolae
Mito
Lipid bilayer disruption
NP
NP
Ca^{2+}
↑Ca^{2+}
ROS
ROS
↓ATP
JNK, NF-κB
Nrf2
Endosome
NADPH oxidase
Cationic NP
Lipid bilayer disruption
Lysosome
BFA
CsA
Mito
PTP
ROS
ROS
↑Ca^{2+}
?
Nrf2
↑Ca^{2+}
Cyt C
↓ATP
Caspases
HO-1, Phase II enzymes
Cytokines
Necrosis
Apoptosis
Tier 1: Cell defense
Tier 2: Pro-inflammation
Tier 3: Apoptosis/Necrosis

Nanotoxocity based neurotransmitter


A
Transporter
Vesicles
Transporter
Signal
Receptors
Signal
Pre-synaptic Neuron
Post-synaptic Neuron

APOE and brain development


Migration
Axon guidance
Synaptic plasticity
Microtubule stability
ApoE
E
Regeneration
Survival
Amyloid deposition

Nature Reviews | Neuroscience

↓

Abnormal function

↓

INCREASED RISK FOR DEMENTIA

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Gachon University

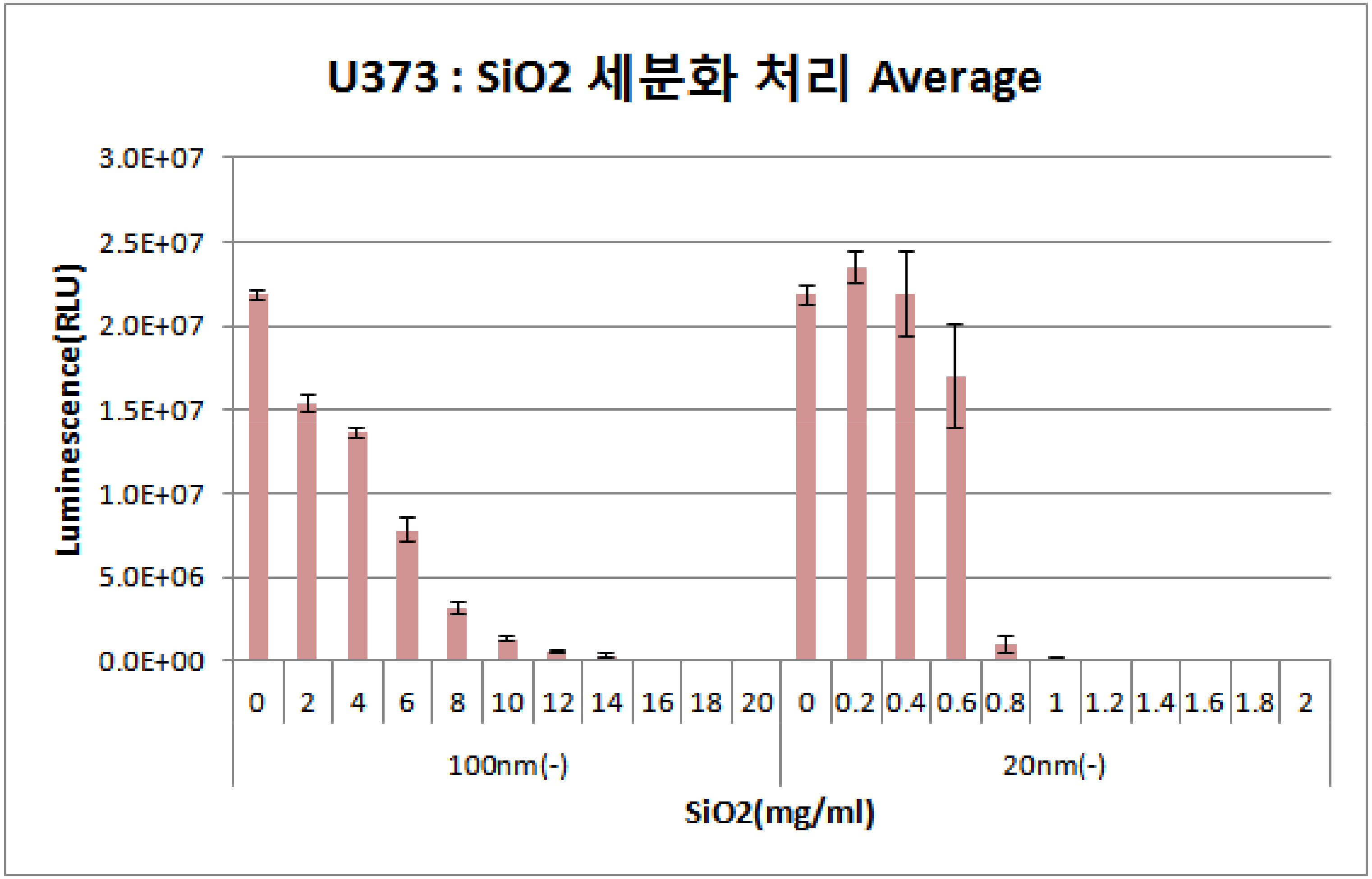
in vitro studies

SiO_2, 20/100 nm, (-) in vitro Study

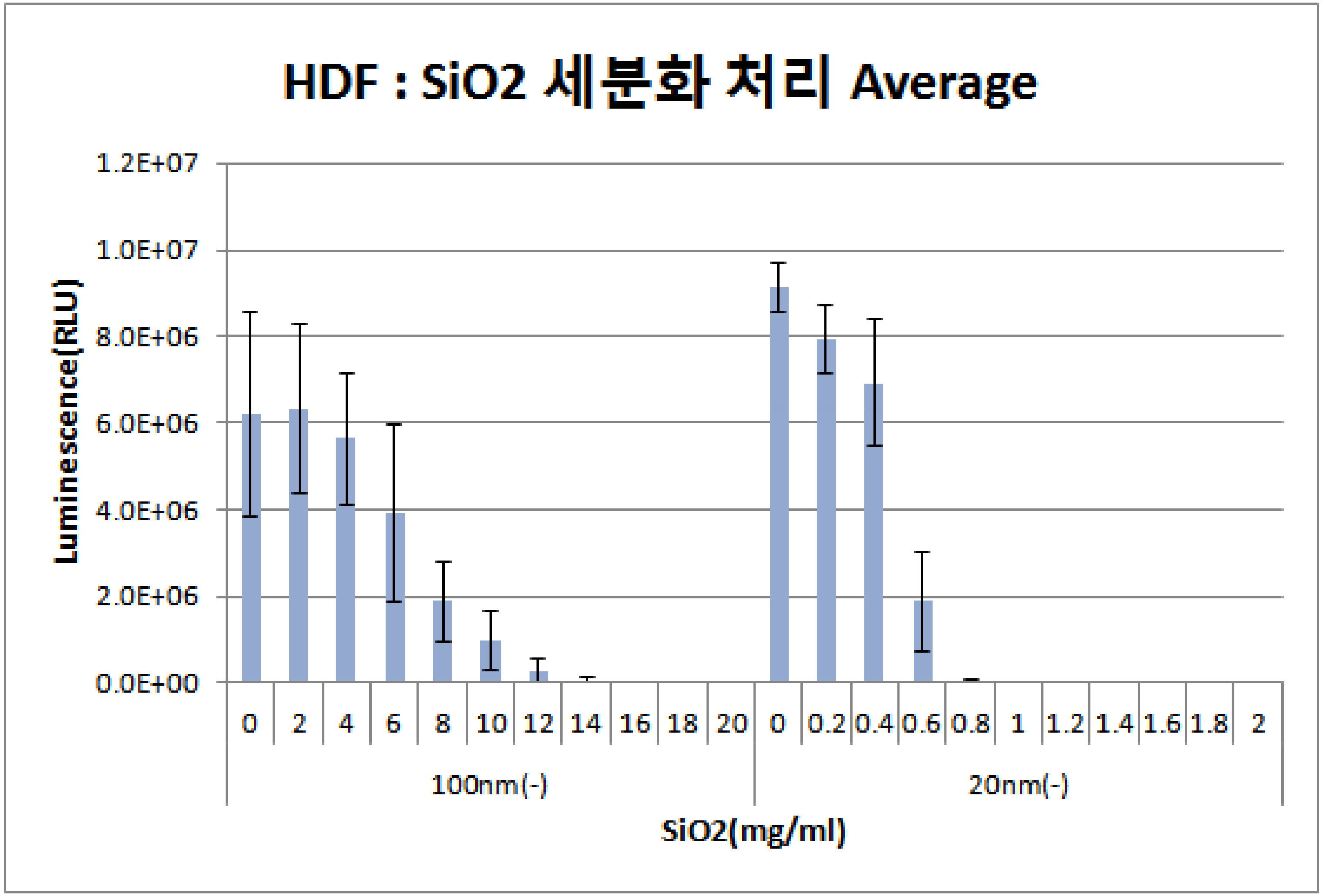
- **Cytotoxicity of SiO_2 with cell types as below**
  - in vitro cellular models for Neuron, skin, GI tract were selected.
  - skin model: HDF human Dermal Fibroblast
  - neuronal model: U373 human astrocyte/glioblastoma cell line
  - GI tract model: HCT116 human colon carcinoma cell-line

- **Method**
  - Measuring cell viability after 24 hrs from treating cell with varying concentrations of $SiO_2^{20,(-)}$ & $SiO^{100,(-)}$
  - Concentrations of ATP were measured for cell viability with luminescence-based assay, Cell-titerglo assay (Promega).

Cellular viability of Neuronal model



Cellular viability of skin model



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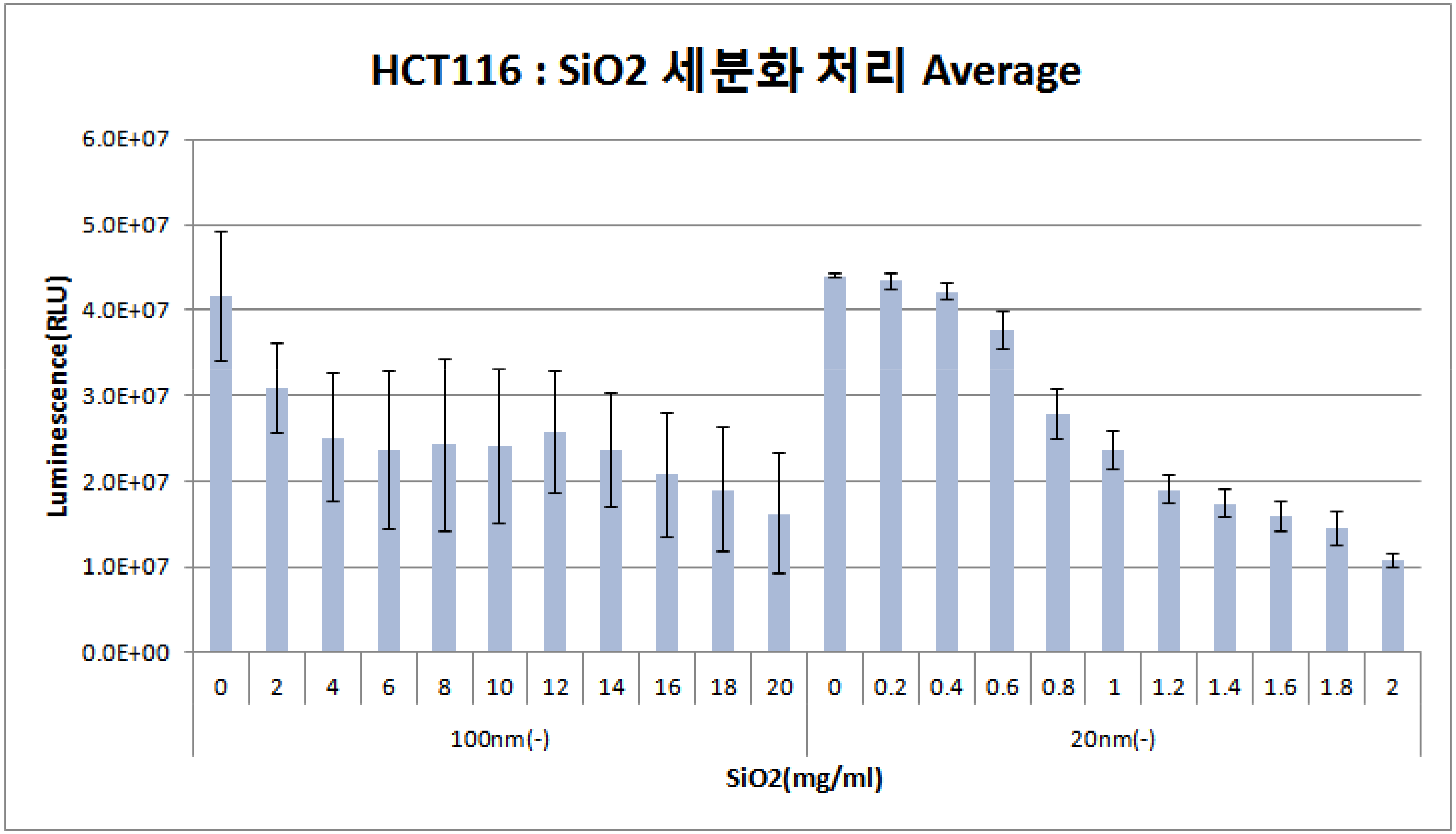
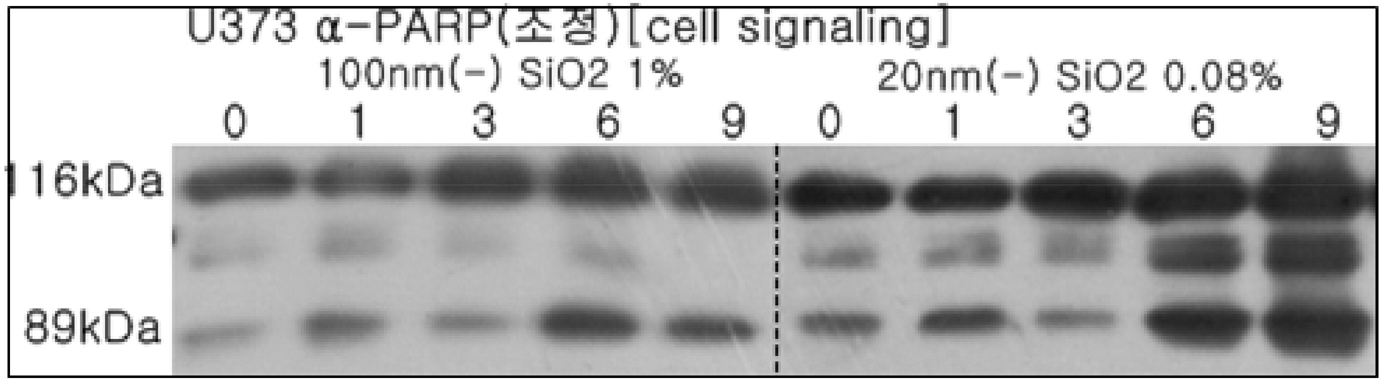
Cellular viability of GI tract model

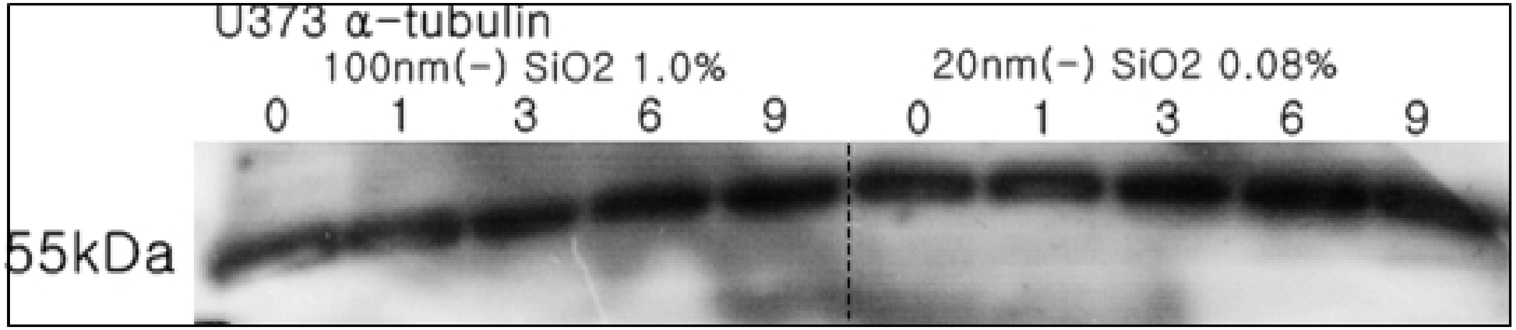


in vitro analysis of apoptosis

Poly (ADP-ribose) polymerase (PARP) cleavage

- Based on the results from celltiter-glo assay, predetermined and administered $SiO_2^{100,(-)}$ 및 $SiO_2^{20,(-)}$ and measured the cleavage of PARP from full-length 116kD to 89kDa (or 85kDa) at 0, 1, 3, 6, 9 hrs, with Anti-PARP antibody(cell signaling, Promega). Western Blot analyisis


U373 α-PARP(조정)[cell signaling]
100nm(-) SiO2 1%
20nm(-) SiO2 0.08%
0
1
3
6
9
0
1
3
6
9
116kDa
89kDa

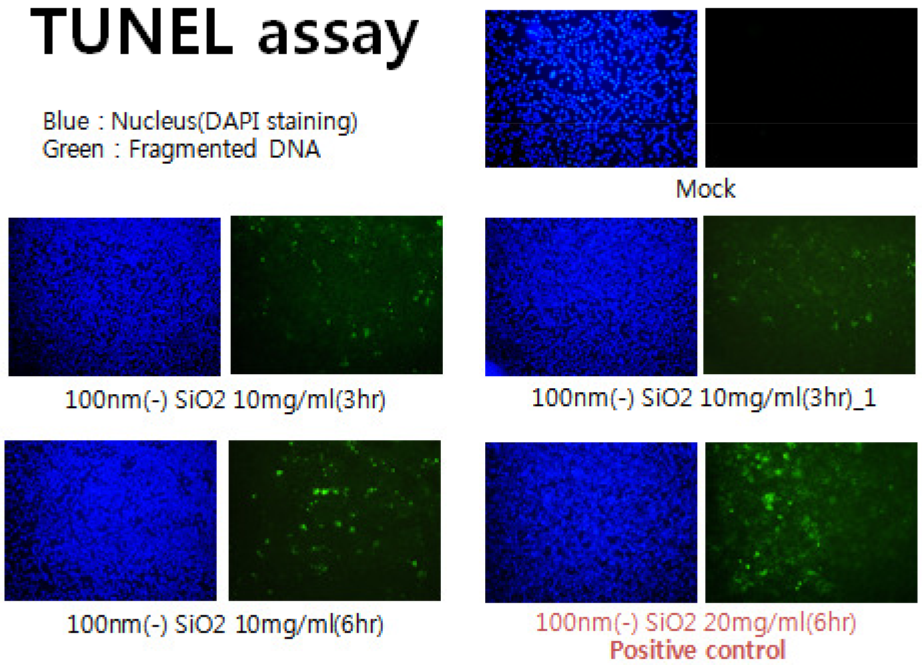

U373 α-tubulin
100nm(-) SiO2 1.0%
20nm(-) SiO2 0.08%
0
1
3
6
9
0
1
3
6
9
55kDa

in vitro analysis of apoptosis

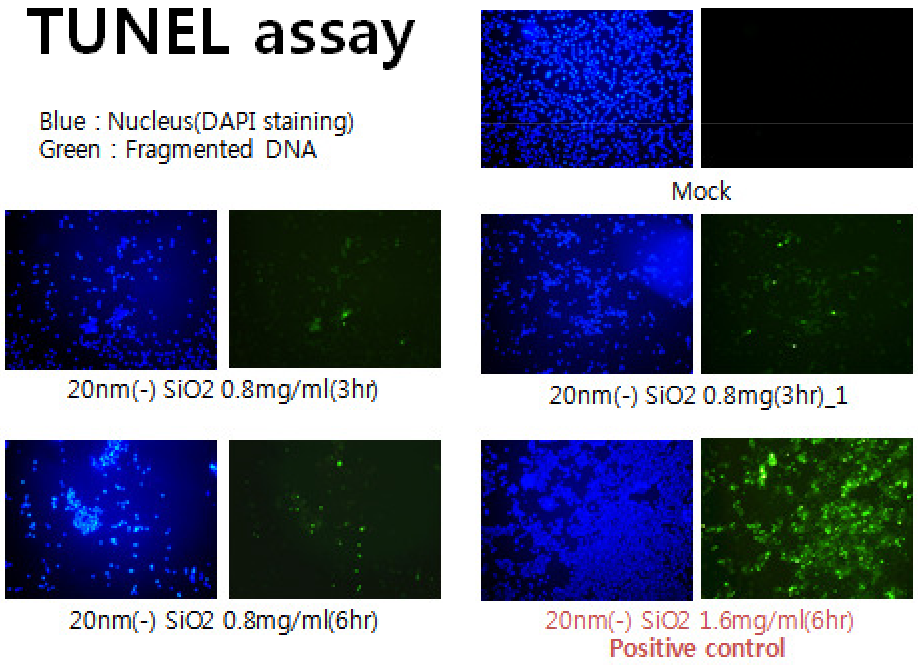
DNA fragmentation : U373

- DNA fragmentations were analyzed by labeling fluorescence-labeled dUTP to the end of DNA with Terminal deoxynucleotidyl transferase.

- Method: DeadEnd™ Fluorometric TUNEL System assay kit (Promega)

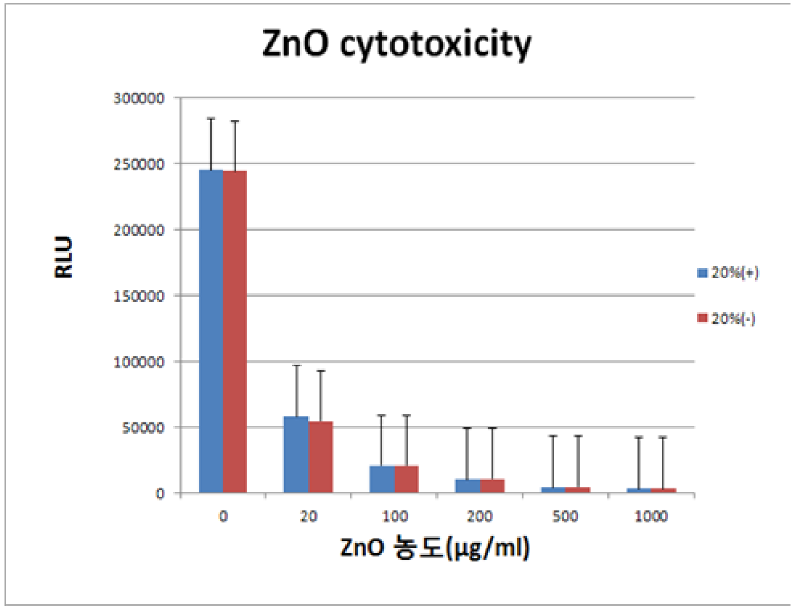

TUNEL assay
Blue : Nucleus(DAPI staining)
Green : Fragmented DNA
Mock
100nm(-) SiO2 10mg/ml(3hr)
100nm(-) SiO2 10mg/ml(3hr)_1
100nm(-) SiO2 10mg/ml(6hr)
100nm(-) SiO2 20mg/ml(6hr)
Positive control

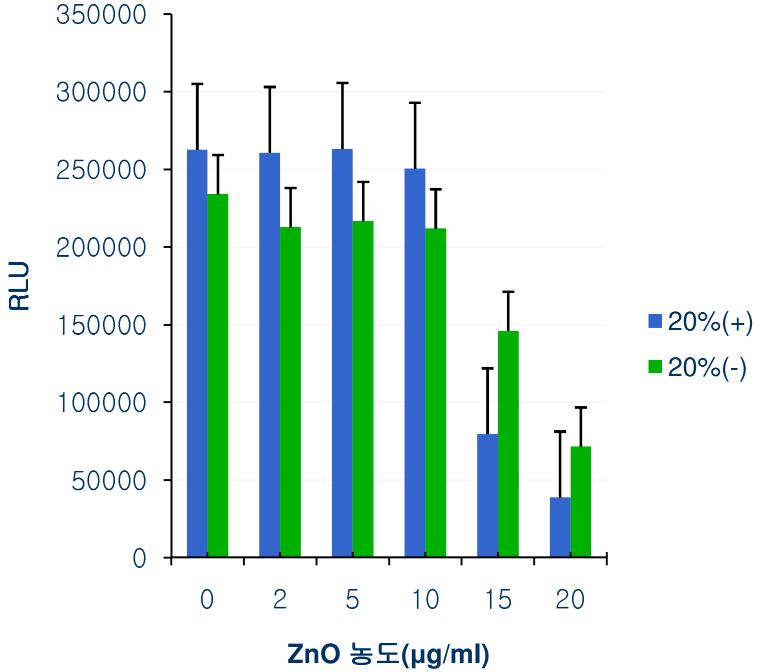
DNA fragmentation analysis by $SiO_2^{100,(-)}$에


TUNEL assay
Blue : Nucleus(DAPI staining)
Green : Fragmented DNA
Mock
20nm(-) SiO2 0.8mg/ml(3hr)
20nm(-) SiO2 0.8mg(3hr)_1
20nm(-) SiO2 0.8mg/ml(6hr)
20nm(-) SiO2 1.6mg/ml(6hr)
Positive control

DNA fragmentation analysis by $SiO_2^{20,(-)}$

in vitro study





Neuroblastoma U373 cell viability was measured after 48 hrs from treatment with cell-titerglo assay.

80% of cell death were observed at 20ug/ml.

농도의 처리시 각 70%, 40% of cell death were observed from 15 ug/ml of ZnO (+) 또는 ZnO (-), respectively.

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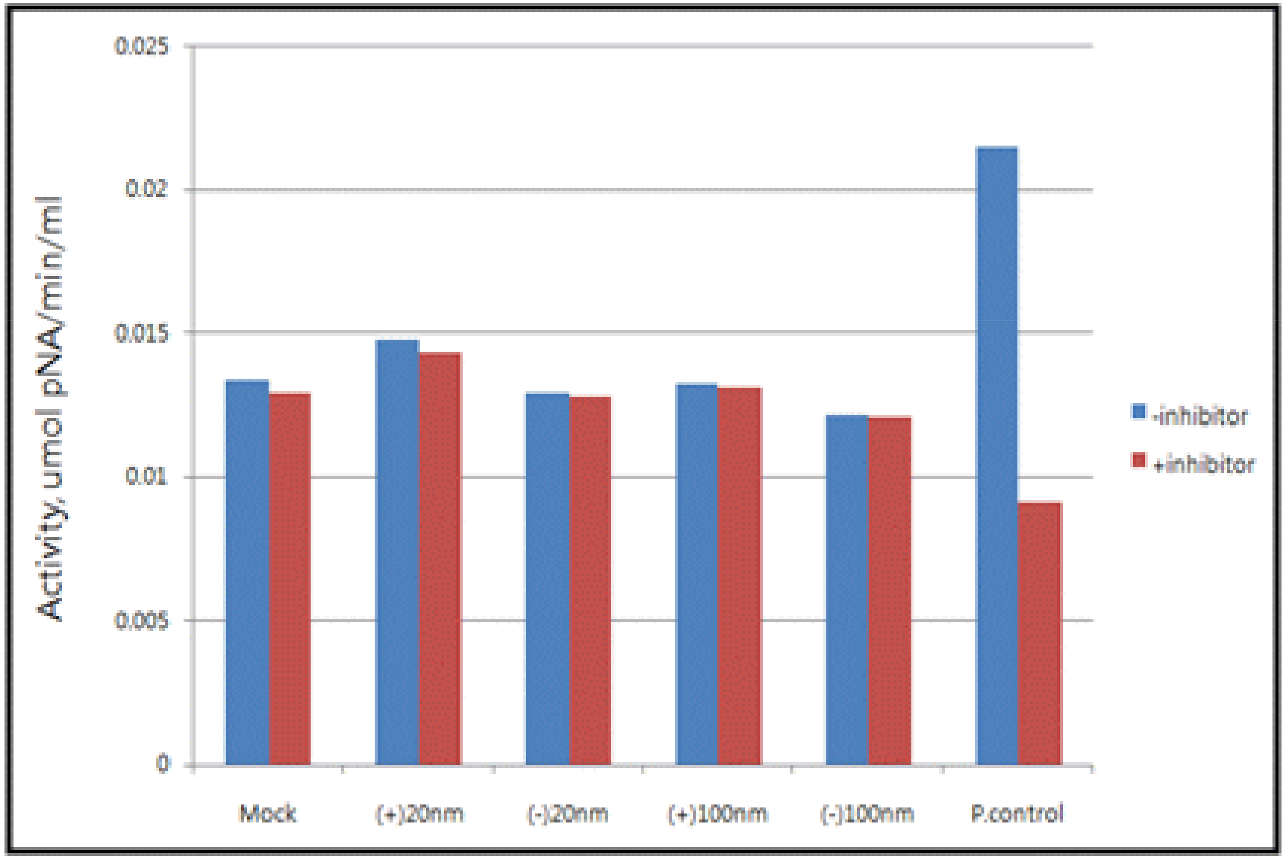
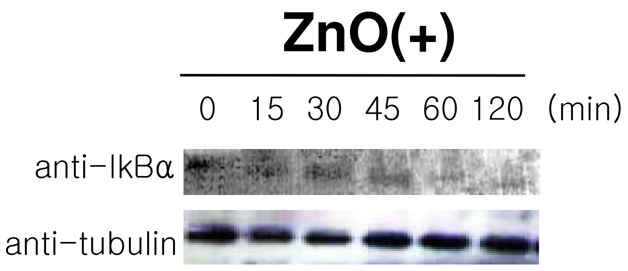
in vitro study

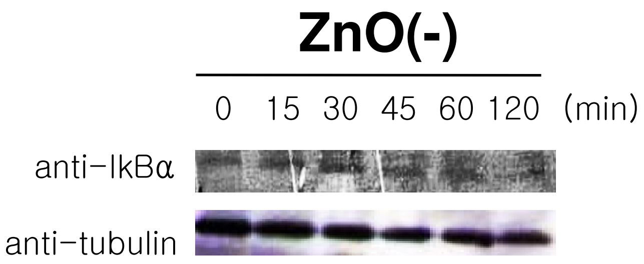
Caspase 3 after ZnO exposure to U373



in vitro study

Activation of NF-κB by ZnO


ZnO(+)
0 15 30 45 60 120 (min)
anti-IkBα
anti-tubulin


ZnO(-)
0 15 30 45 60 120 (min)
anti-IkBα
anti-tubulin

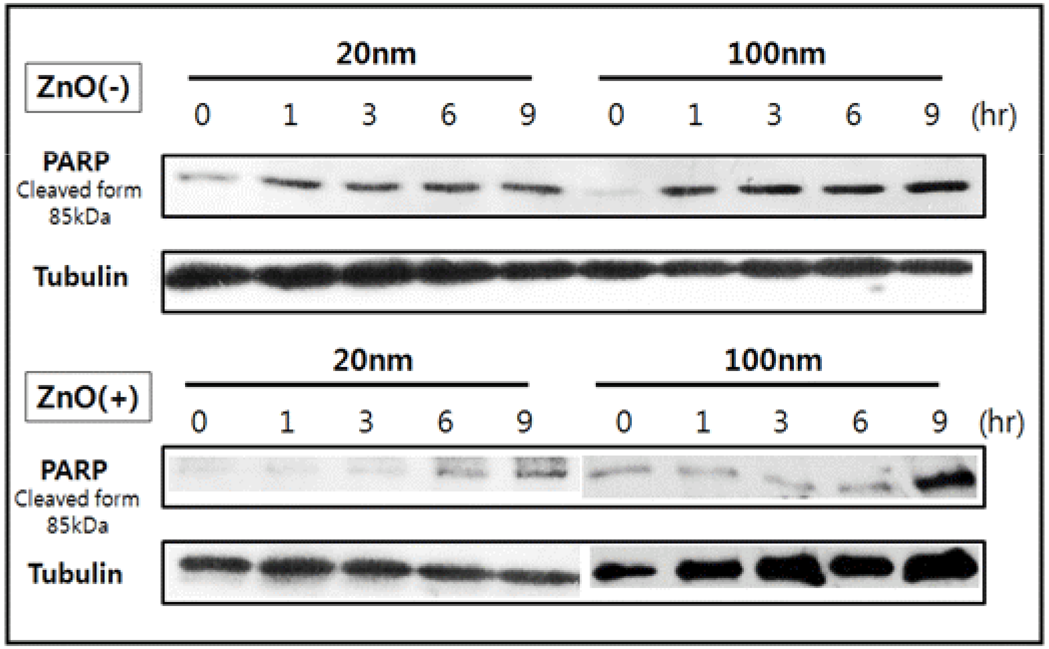
After treating U373 cells with 20% (+) charged ZnO or 20% (−) charged ZnO. western blot analysis was performed to see the activation of NF-κB through IκBα

After 15-30 min from treatment with ZnO (+), activation of IKK cause the lysis of IκBα.

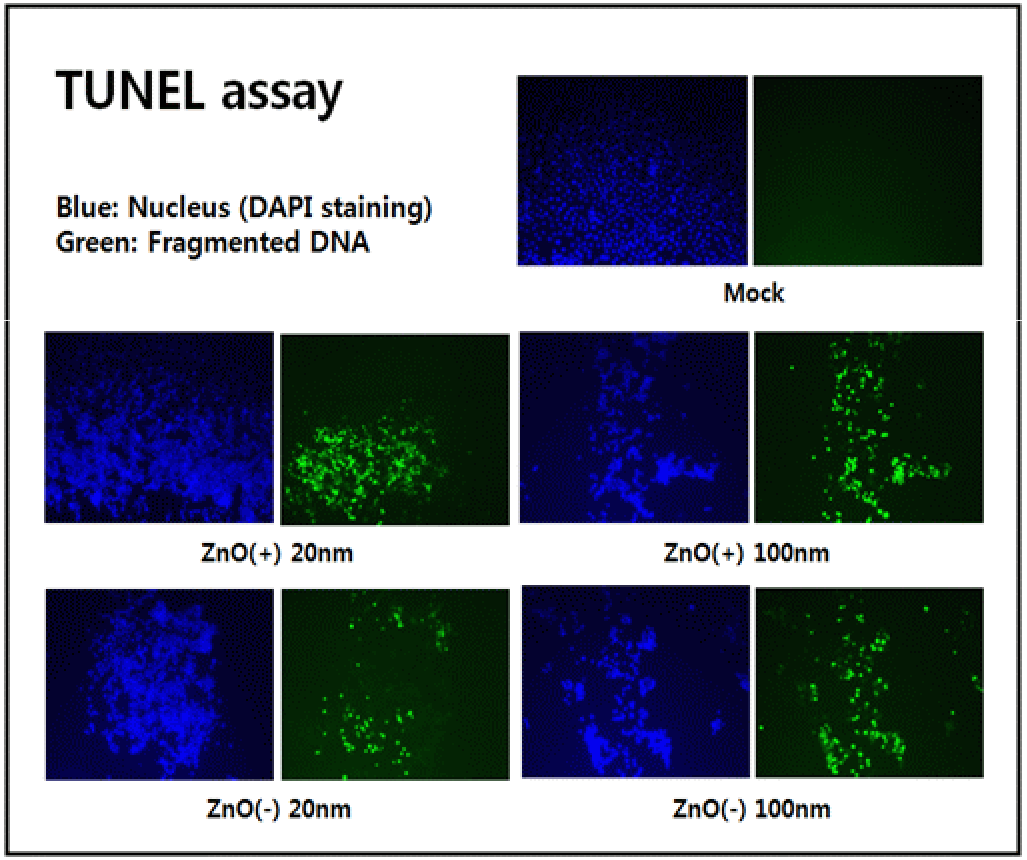
45 min for ZnO (−) treatment.

in vitro study

Poly (ADP-ribose) polymerase (PARP) cleavage analysis after ZnO treatment on U373 cells


ZnO(-)
20nm
100nm
0 1 3 6 9 0 1 3 6 9 (hr)
PARP
Cleaved form
85kDa
Tubulin
ZnO(+)
20nm
100nm
0 1 3 6 9 0 1 3 6 9 (hr)
PARP
Cleaved form
85kDa
Tubulin

in vitro study


TUNEL assay
Blue: Nucleus (DAPI staining)
Green: Fragmented DNA
Mock
ZnO(+) 20nm
ZnO(+) 100nm
ZnO(-) 20nm
ZnO(-) 100nm

DNA fragmentations in U373 cells from ZnO treatment

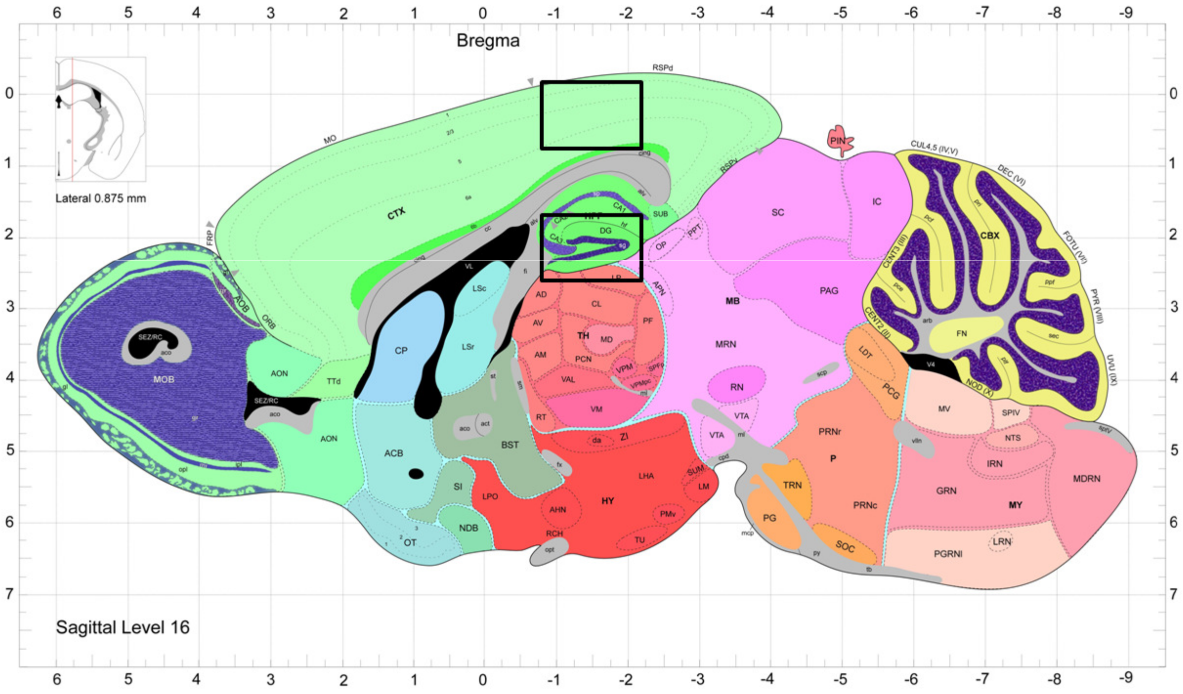
in vivo study

28 days of repeated oral administration ($ZnO^{20,(+)}$, $ZnO^{20,(-)}$, $SiO_2^{20,(-)}$, $SiO_2^{20,Arg}$)

samples	NPs	sex	# animals	disage (mg/kg)	Conc. (mL/kg)
1 control	ddH2O	female	5	0	10
2. Buffer control	HEPES-Citrate Buffer	female	5	0	10
3 buffer control	HEPES-Serine Buffer	female	5	0	10
4 A.A control	L-arginine	female	5	0	10
5 High Conc.	$ZnO^{20,(-)}$ (20nm, - charge)	female	5	500	10
6 High Conc.	$ZnO^{20,(+)}$ (20nm, + charge)	female	5	500	10
7 High Conc.	$SiO_2^{20,(-)}$ (20nm, - charge)	female	5	2000	10
8 High Conc.	$SiO_2^{20,Arg}$ (20nm, - charge)	female	5	1000	10

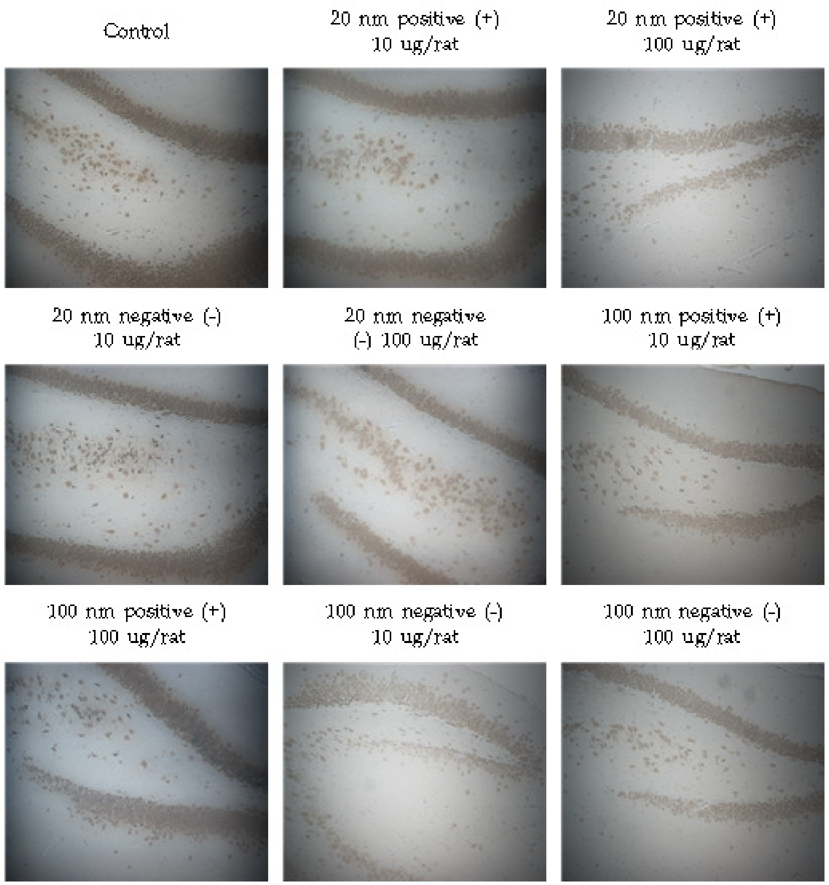
in vivo study

NeuN expression in Rat brain


Bregma
Lateral 0.875 mm
Sagittal Level 16
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
0
1
2
3
4
5
6
7
RSPd
MO
CTX
FRP
AOB
ORB
MOB
SEZ/RC
aco
AON
TTd
CP
LSc
LSr
VL
ACB
SI
NDB
OT
BST
ac
HPF
CA1
CA3
DG
SUB
OP
PPT
APN
AD
AV
AM
CL
TH
MD
PCN
VAL
VPM
PF
LP
RT
VM
ZI
LPO
AHN
HY
LHA
SUM
LM
PMv
TU
RCH
SC
IC
PAG
MB
MRN
RN
VTA
PIN
CBX
CUL4,5 (IV,V)
DEC (VI)
FOTU (VII)
PYR (VIII)
UVU (IX)
NOD (X)
CENT3 (III)
CENT2 (II)
FN
V4
LDT
PCG
MV
SPIV
NTS
IRN
GRN
MY
MDRN
LRN
PGRNl
PRNr
P
PRNc
TRN
PG
SOC

in vivo study: Neurotoxicity

Hippocampus: staining for NeuN, DAB$^+$



Control

20 nm positive (+) 10 ug/rat

20 nm positive (+) 100 ug/rat

20 nm negative (-) 10 ug/rat

20 nm negative (-) 100 ug/rat

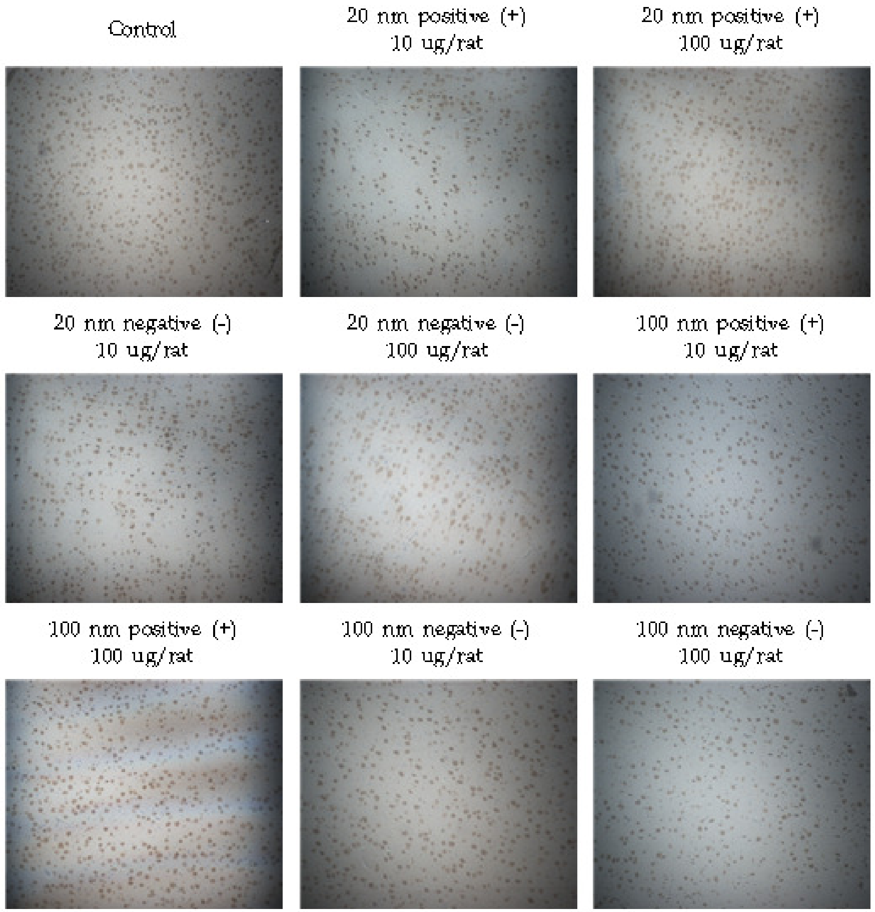
100 nm positive (+) 10 ug/rat

100 nm positive (+) 100 ug/rat

100 nm negative (-) 10 ug/rat

100 nm negative (-) 100 ug/rat

in vivo study: Neurotoxicity

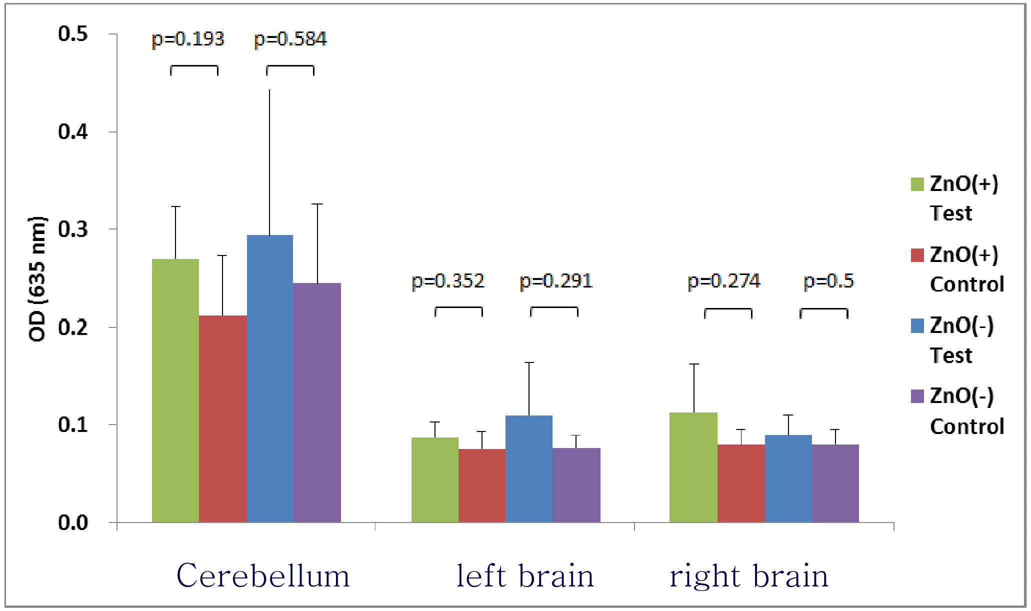

Control
20 nm positive (+) 10 ug/rat
20 nm positive (+) 100 ug/rat
20 nm negative (-) 10 ug/rat
20 nm negative (-) 100 ug/rat
100 nm positive (+) 10 ug/rat
100 nm positive (+) 100 ug/rat
100 nm negative (-) 10 ug/rat
100 nm negative (-) 100 ug/rat

Cortex: Stained for NeuN, DAB$^+$

in vivo study: Neurotoxicity

Evans Blue analysis after 28days of $ZnO^{20,(+)}$, $ZnO^{20,(-)}$ treatment

- After administration of 20 nm ZnO + , − for 28 days, Rt, Lt brains and cerebellum were separated and evans blue (ug/g) was measured.
- Increased Evans blue was seen in all brain without significance.



in vivo study: Neurotoxicity

Evans Blue analysis after 28days of ZnO$^{20,(+)}$, ZnO$^{20,(-)}$ treatment

- After administration of 20 nm ZnO + , − for 28 days, Rt, Lt and cerebellum were separated and evans blue (ug/g) was measured.
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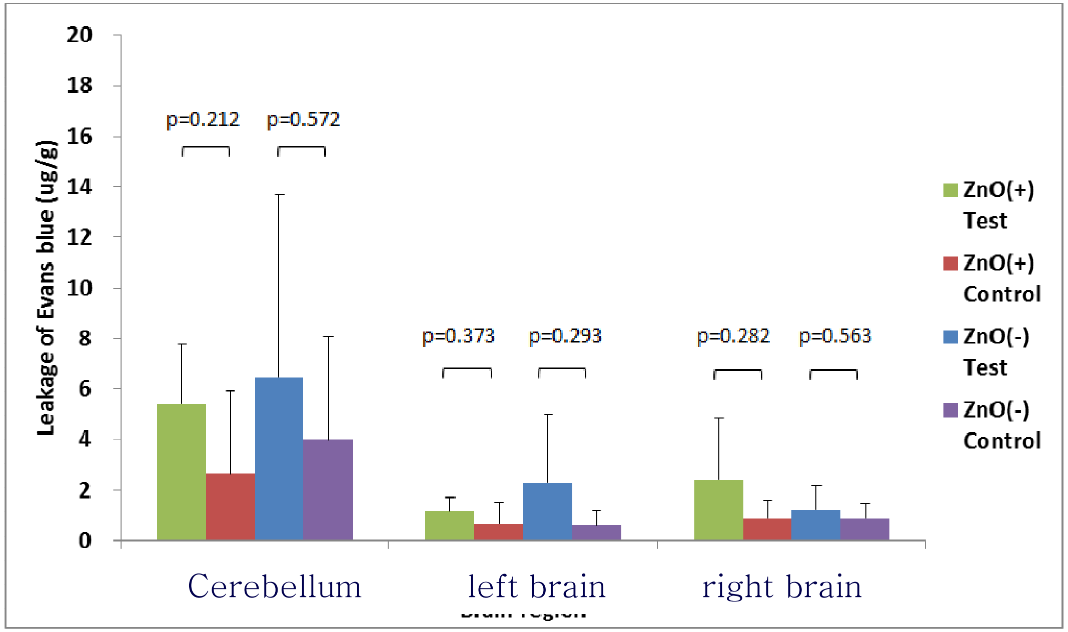


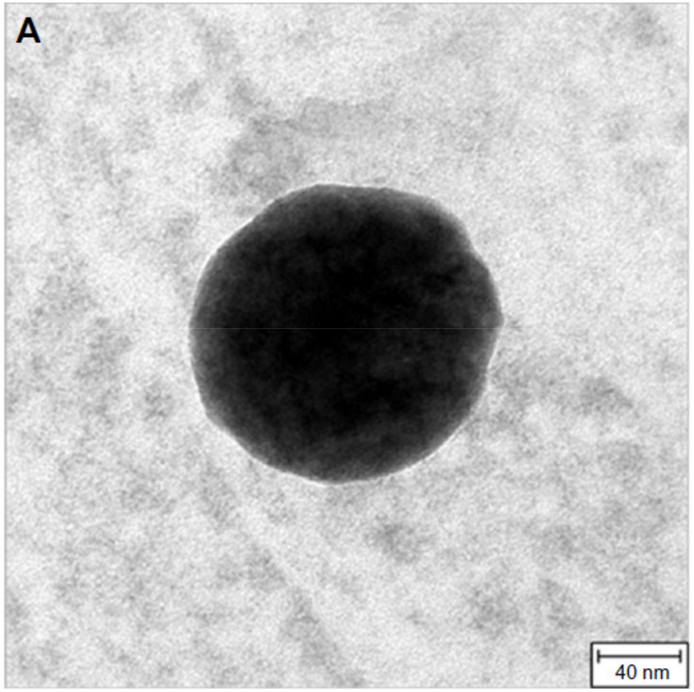
in vivo study: Neurotoxicity

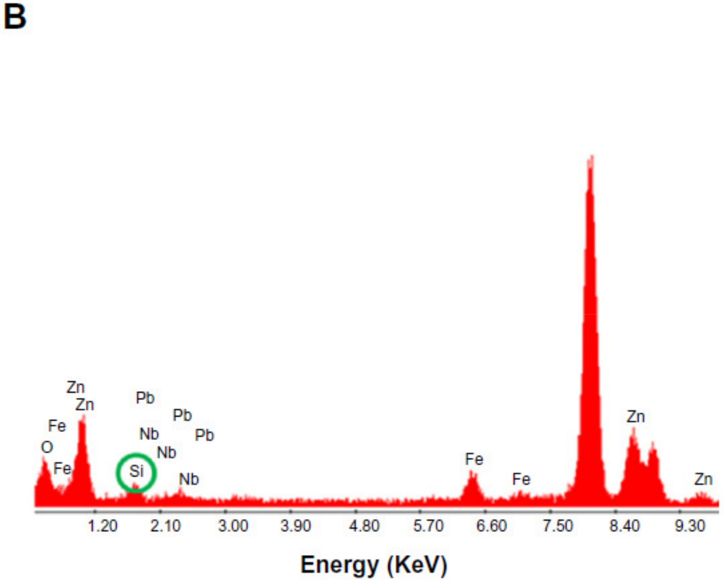
After 90 days of repeated treatments

route	NPs	disage (mg/kg)	Conc. (mL/kg)
oral	$SiO_2^{20,Arg}$ (20nm, negative charge)	1000	10
	$SiO_2^{100,Arg}$ (100nm, negative charge)	1000	10
dermal	$SiO_2^{20,(-)}$ (20nm, negative charge)	2000	10
	$SiO_2^{100,(-)}$ (100nm, negative charge)	2000	10

in vivo study: Neurotoxicity

TEM analysis: control



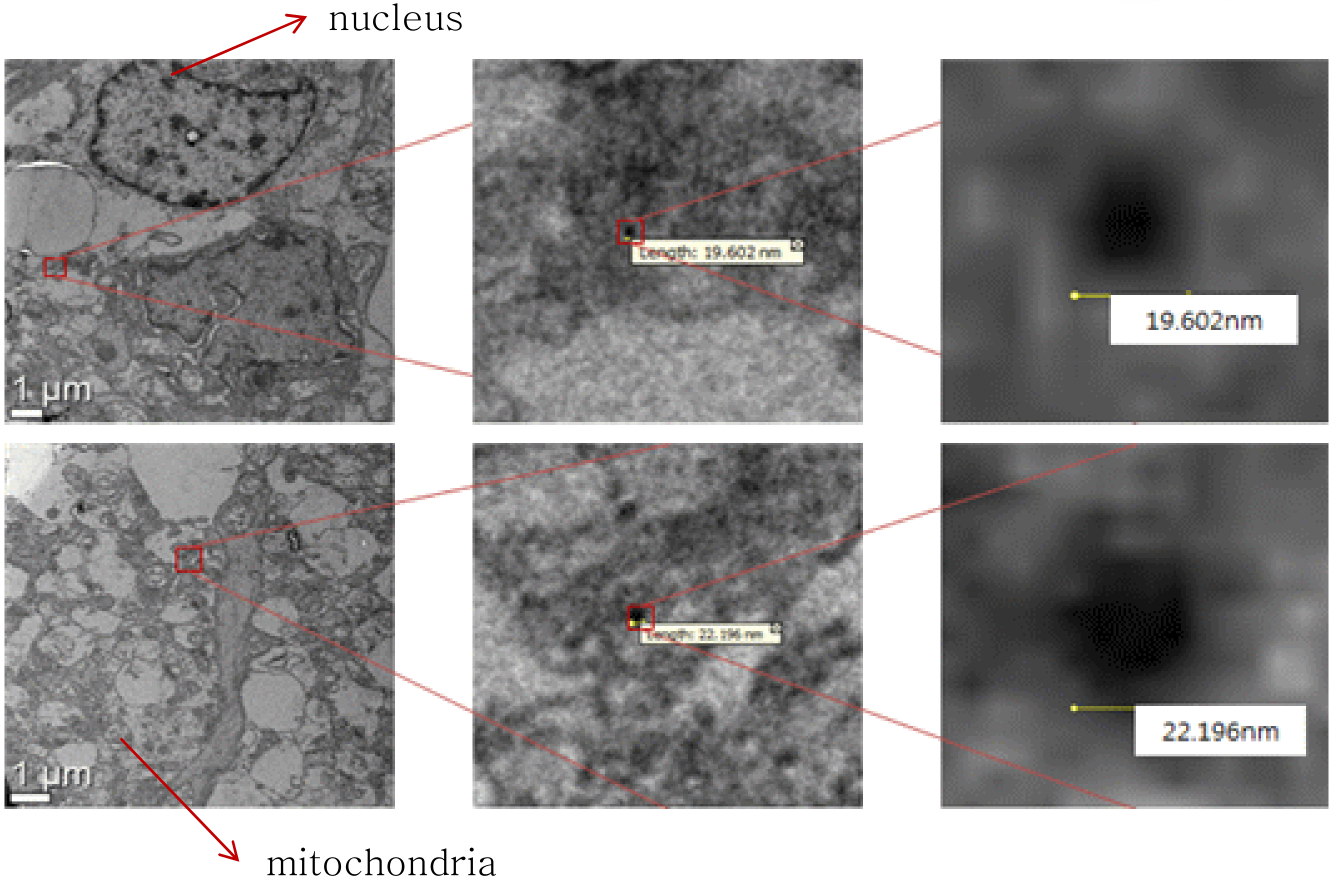


(A) SH-SY5Y neuroblastoma cells treated with SiO2ENB100,(–)

(B) graph of energy-dispersive X-ray spectroscope analysis.

in vivo study: Neurotoxicity

TEM analysis after 90 days of repeated $SiO_2^{20,arg}$ [Cerebellum]


1 μm
Length: 19.602 nm
19.602nm
1 μm
Length: 22.196 nm
22.196nm

nucleus

Cerebellum 1

Cerebellum 2

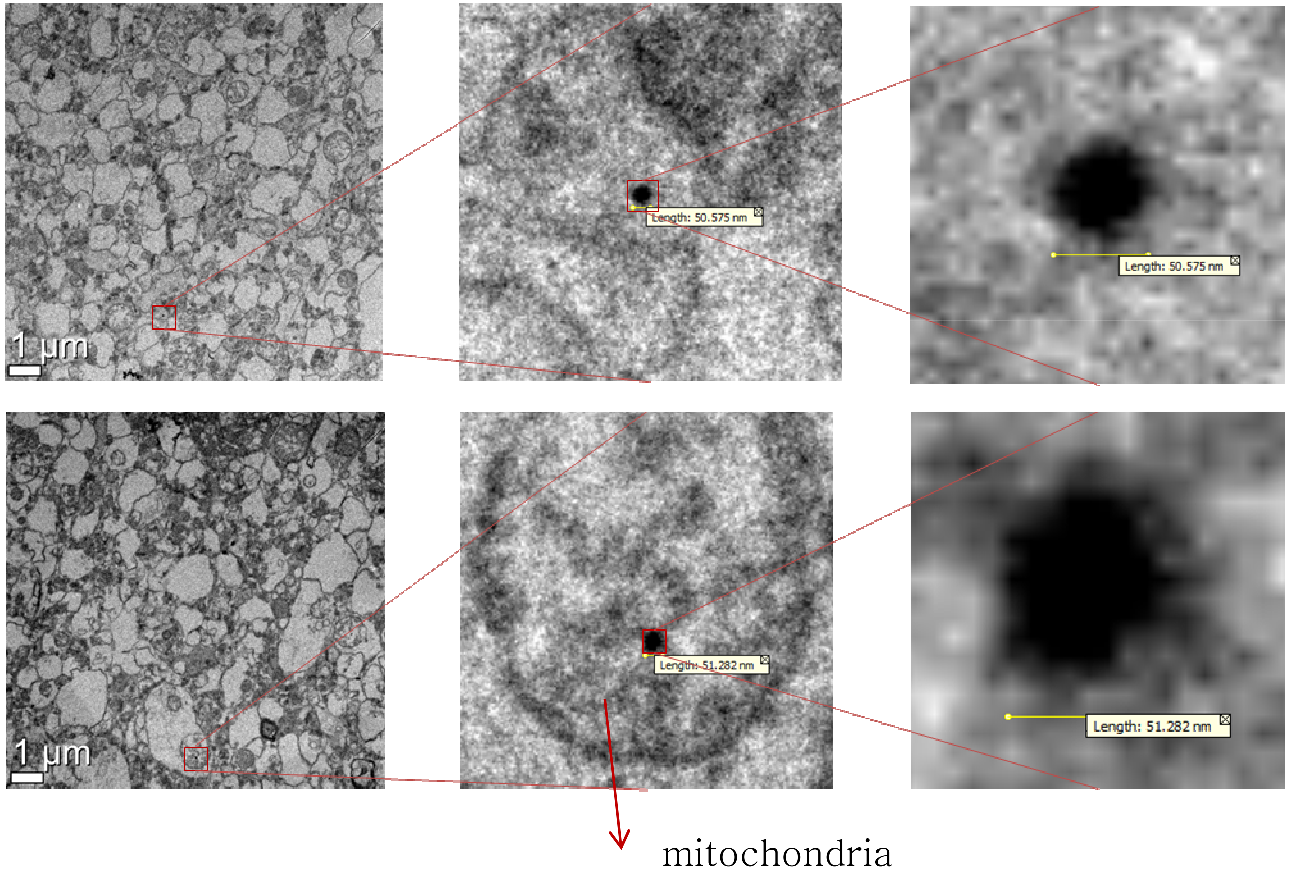
mitochondria

in vivo study: Neurotoxicity

TEM analysis after 90 days of repeated $SiO_2^{20,arg}$ [Hippocampus]

Hippocampus 1

Hippocampus 2


1 μm
Length: 50.575 nm
Length: 50.575 nm
1 μm
Length: 51.282 nm
Length: 51.282 nm

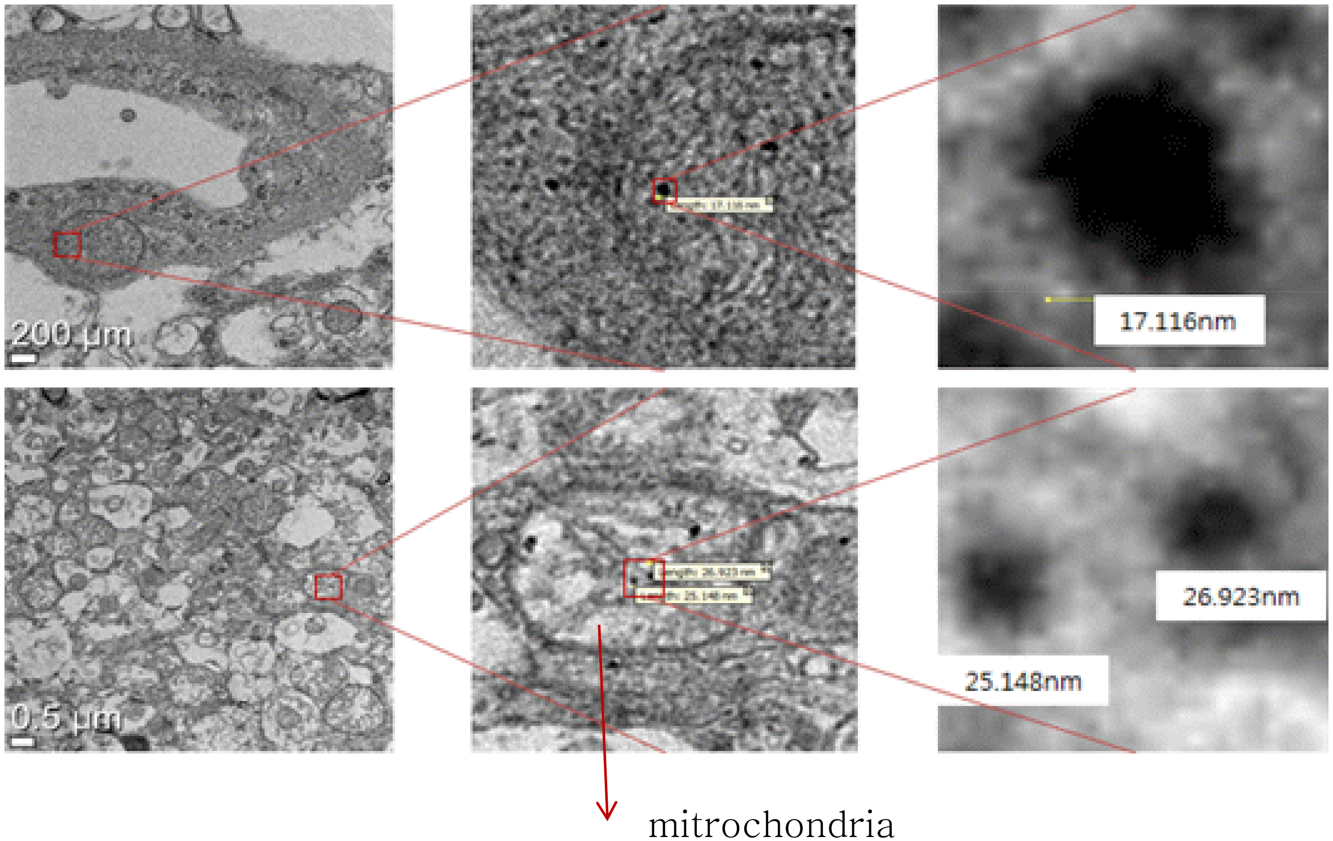
mitochondria

in vivo study: Neurotoxicity

TEM analysis after 90 days of repeated $SiO_2^{20,arg}$ [Striatum]

Striatum 1

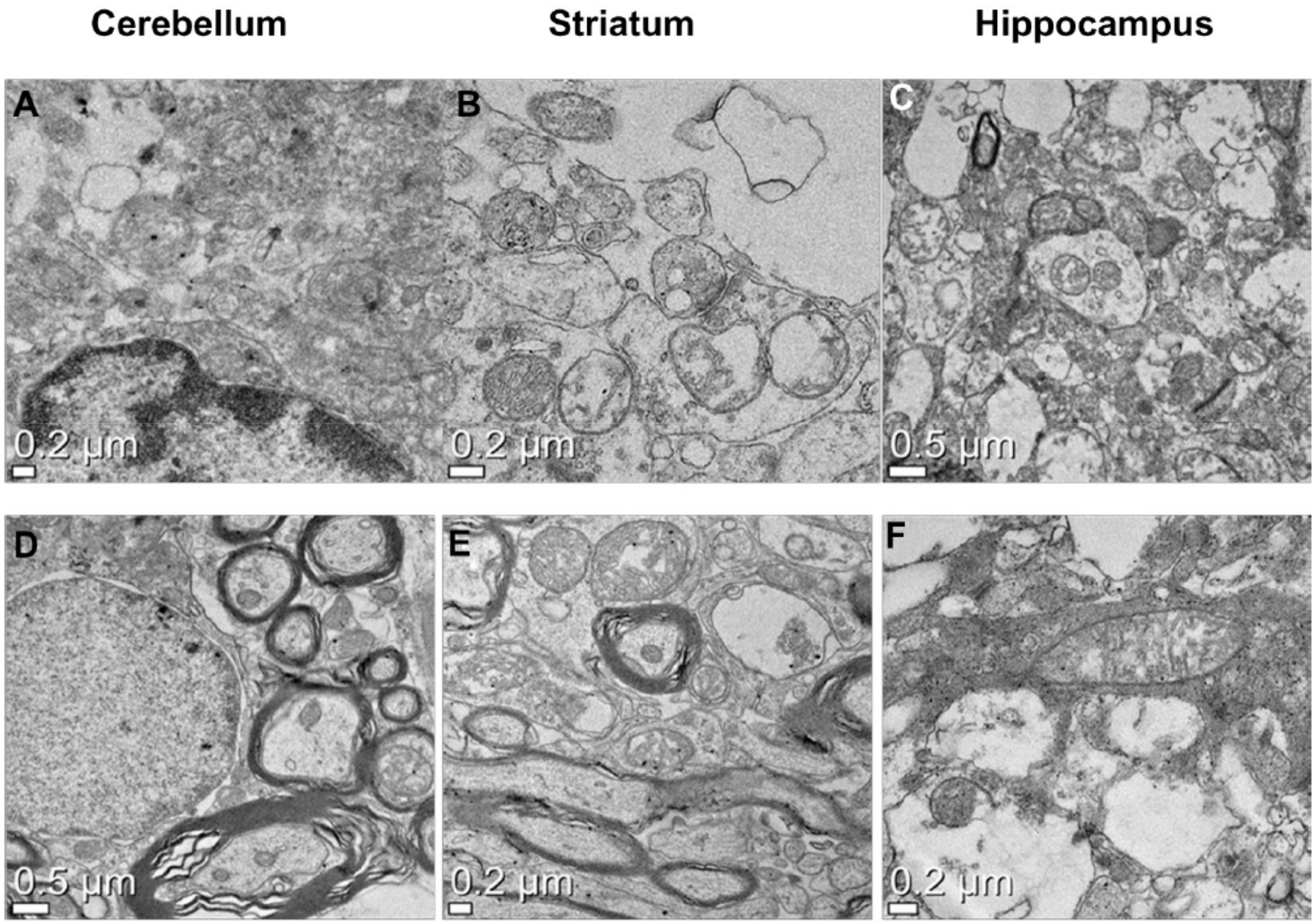
Striatum 2


200 μm
17.116nm
0.5 μm
26.923nm
25.148nm

mitrochondria

in vivo study: Neurotoxicity

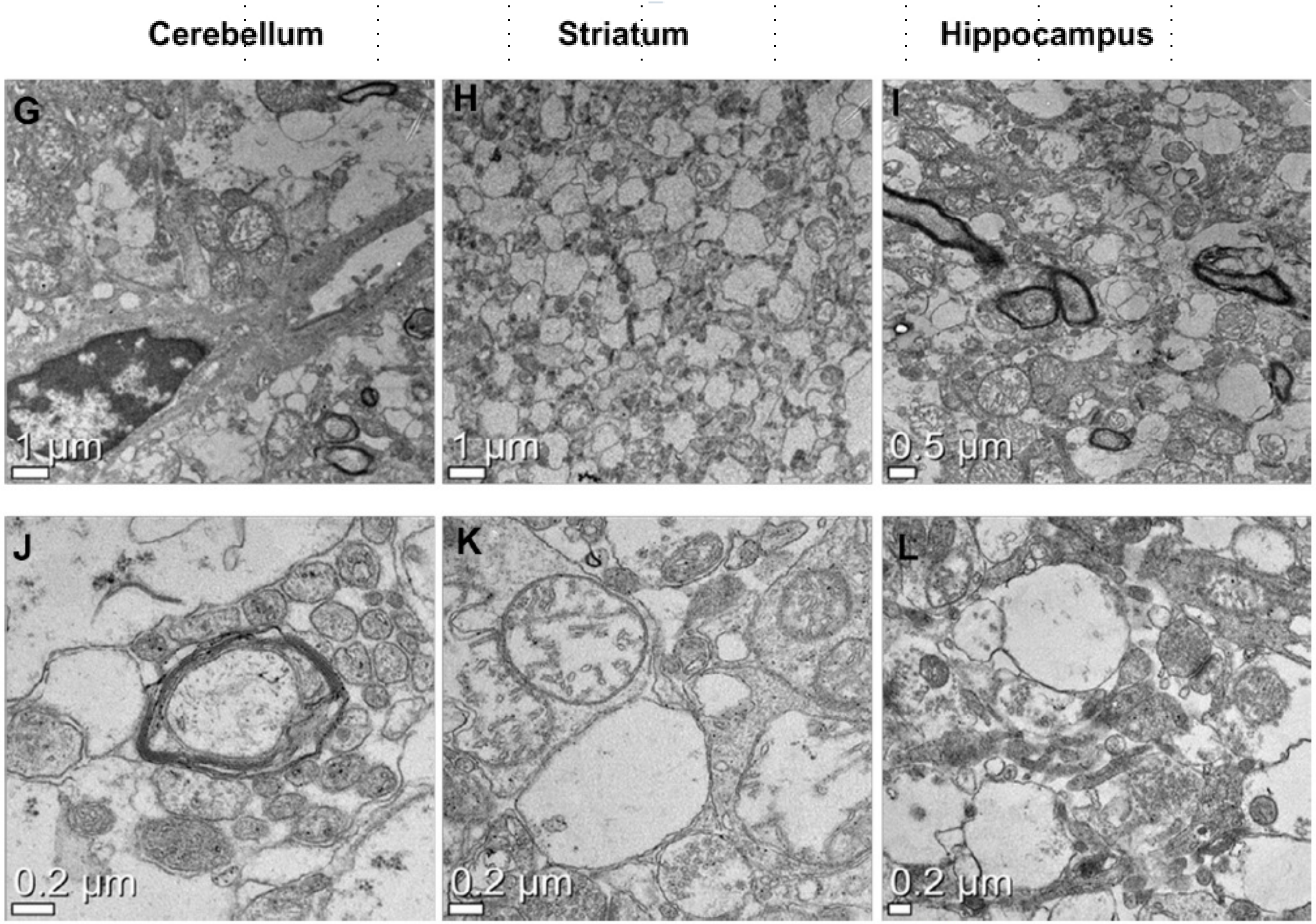
TEM analysis


Cerebellum
Striatum
Hippocampus
A
0.2 µm
B
0.2 µm
C
0.5 µm
D
0.5 µm
E
0.2 µm
F
0.2 µm

(A–C) Dermally administered SiO_2EN20(–)
(D–F) dermally administered SiO_2EN100(–)

in vivo study: Neurotoxicity

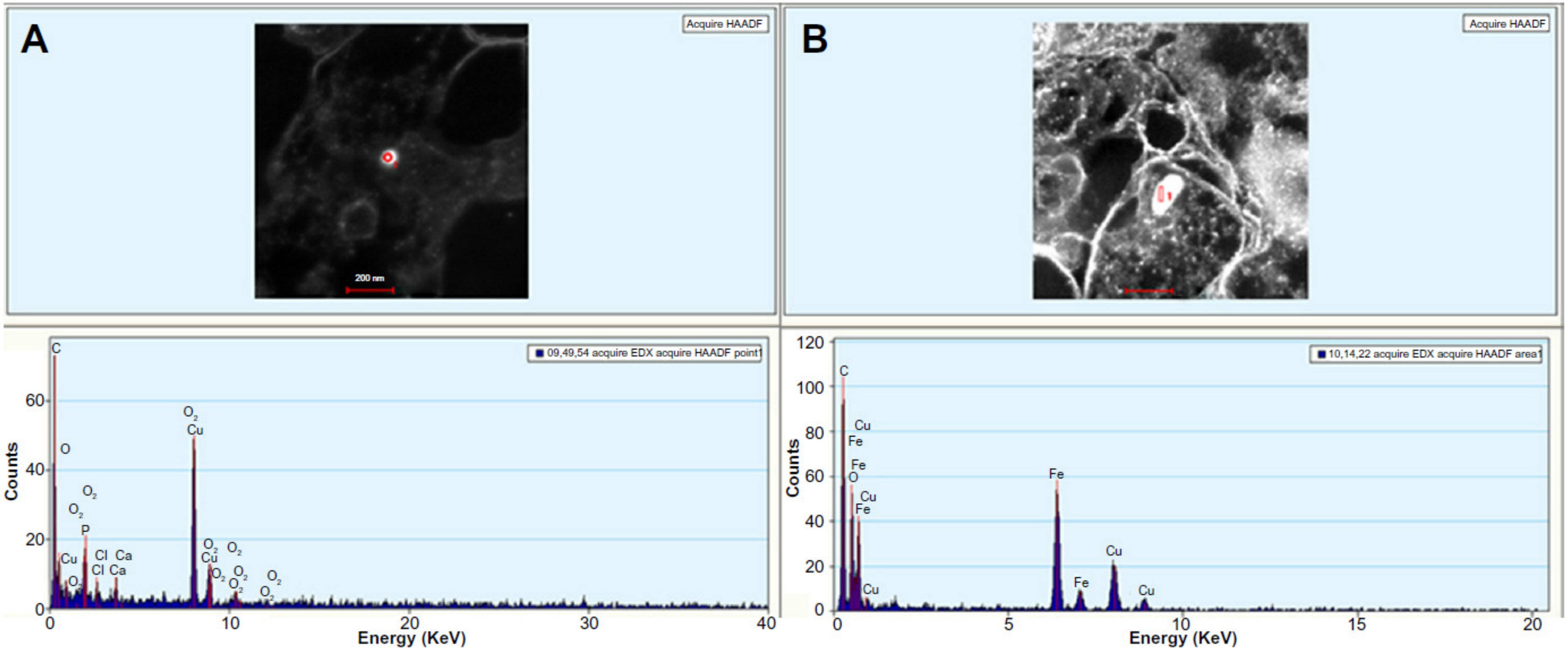
TEM analysis


Cerebellum
Striatum
Hippocampus
G
1 µm
H
1 µm
I
0.5 µm
J
0.2 µm
K
0.2 µm
L
0.2 µm

(G–I) orally administered SiO_2EN20(R)
(J–L) orally administered SiO_2EN100(R).

in vivo study: Neurotoxicity

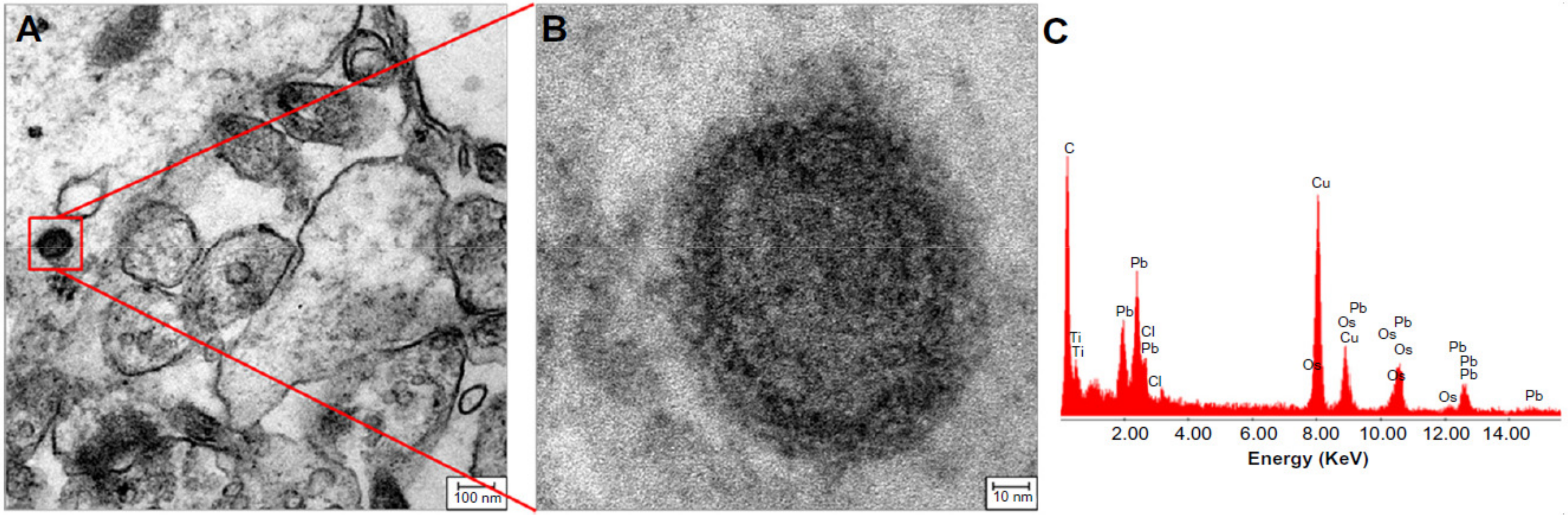
TEM analysis



The results of energy-dispersive X-ray spectroscope analysis of SiO_2EN20(.)-treated rat hippocampus via dermal administration.

in vivo study: Neurotoxicity

TEM analysis


A
100 nm
B
10 nm
C
C
Ti
Ti
Pb
Pb
Cl
Pb
Cl
Cu
Os
Pb
Os
Cu
Os
Pb
Os
Os
Pb
Pb
Pb
Os
Pb
2.00
4.00
6.00
8.00
10.00
12.00
14.00
Energy (KeV)

(**A**) Transmission electron microscope image of hippocampus in $SiO_2$100(–) dermal administration group
(**B**) enlarged image of suspected substance
(**C**) graph of energy-dispersive X-ray spectroscope analysis.

Conclusion

- NPs need to be characterized prior to any experiments
- Diverse proteins were found in protein corona on NPs
- Smaller NPs revealed higher toxicity than the larger NPs
- NPs could cause apoptosis
- TEM imaging were used to search NPs in

  brain, hippocampus, striatum, and cerebellum
- BBB seemed to be intact.

Q & A

THANK YOU!