Comparison of Nanoparticle Toxicity to Yeast Cells and Human Lung Epithelial Cells

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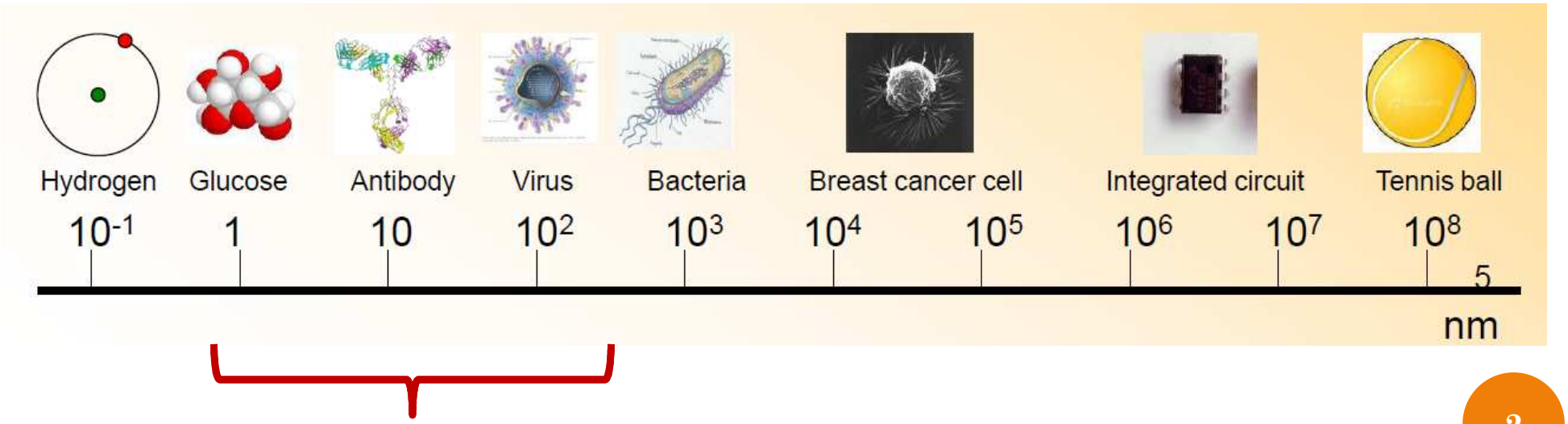
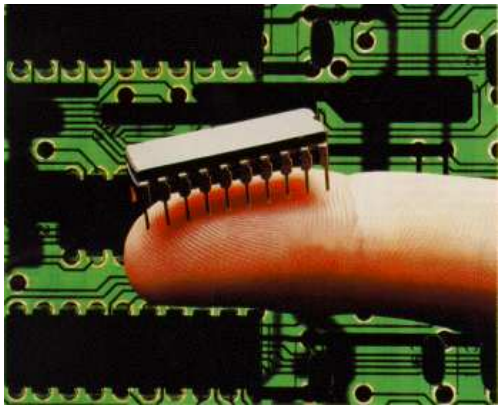
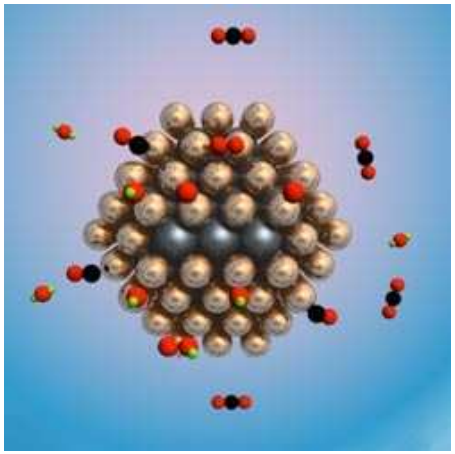
INTRODUCTION

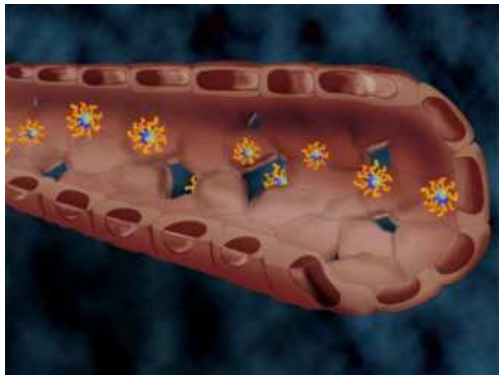
- Nanoparticles (NPs) are particles sized in less than 100 nm.

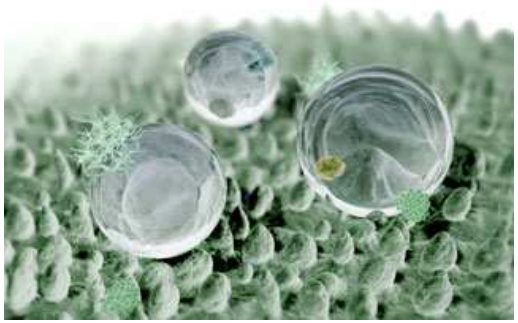


NPs – Applications





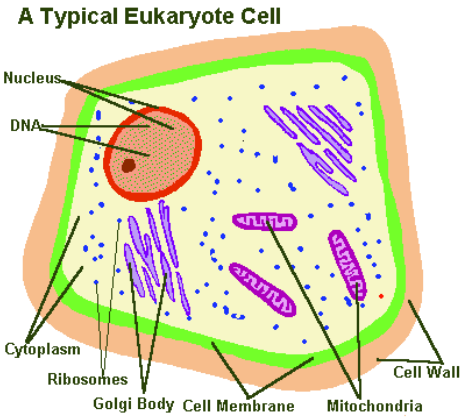


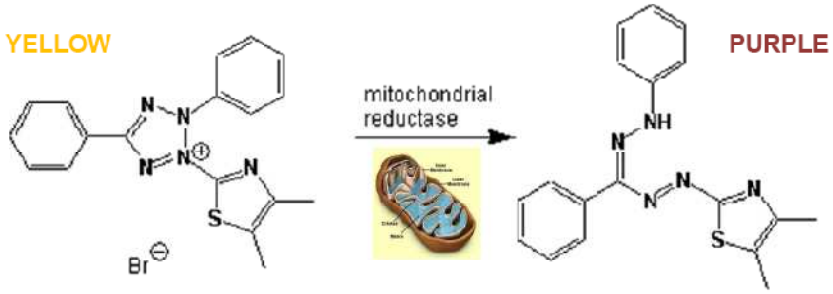


Unusual Physicochemical Properties

- Small size
- High specific surface area
- Surface structure
- Shape
- Aggregation

Problems Assessing NPs Toxicity





- **Interference in classical methods** dependent on colorimetric or fluorimetric measurements.

  e.g. Mitochondrial Toxicity Test (MTT)

- **Agglomeration →** most studies do not include characterization of NPs in biological medium.

OBJECTIVES

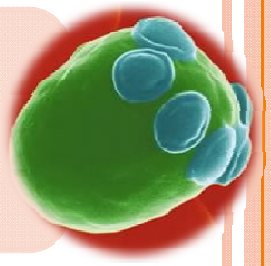
- Assessing the toxicity of some NPs using tests which do not suffer from interference in the measurement technique.

- Compare the toxicity of NPs to *S. cerevisiae* and human lung epithelial cells.

Materials & Methods

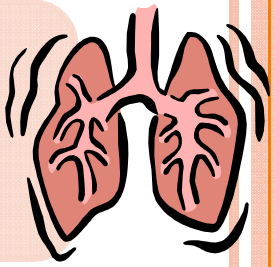
Yeast cells (*Saccharomyces cerevisiae*)

- O_2 uptake
- Cell membrane integrity



Human lung epithelial cells (16 HBE)

- Impedance based assay: xCELLigence system (Roche)



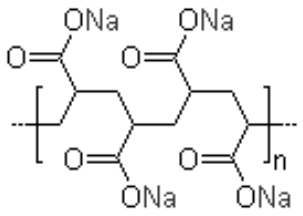
Materials & Methods

- Preparation of NPs suspensions

SiO_2
HfO_2
Al_2O_3
CeO_2
Mn_2O_3
ZnO
TiO_2
ZVI
Fe_2O_3
ZrO_2
Ag

dispersant
or no
dispersant

pH adjusted to ≈ 7



Dispex (sodium polyacrylate)

Materials & Methods

- O_2 uptake assay

Saccharomyces cerevisiae

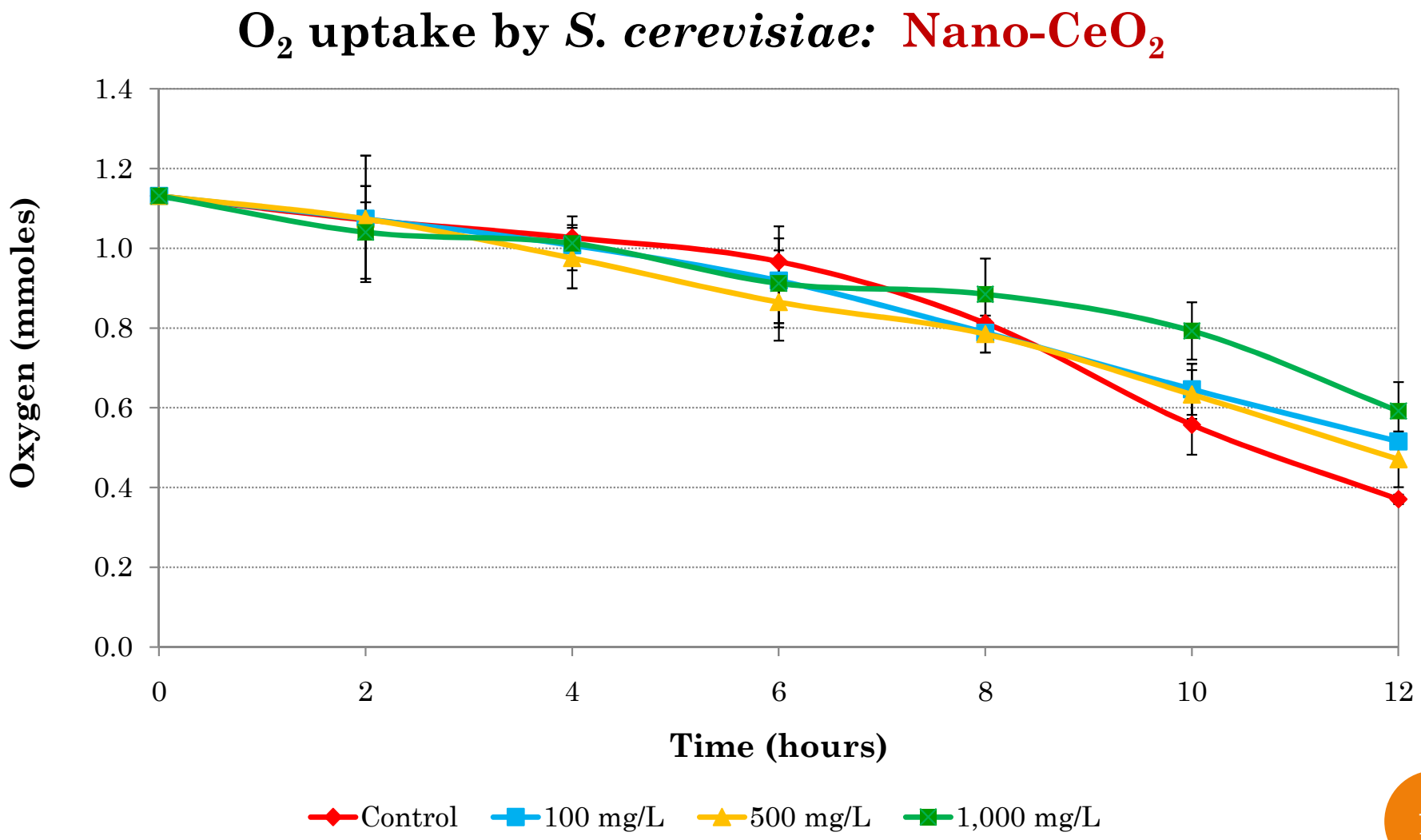
Yeast culture
- YEPD[1] medium
- NPs suspension
- Yeast inoculum

Incubation
- 30°C
- 200 rpm
- 10 hours

O_2 measure
- GC

[1]Yeast Extract Peptone Dextrose

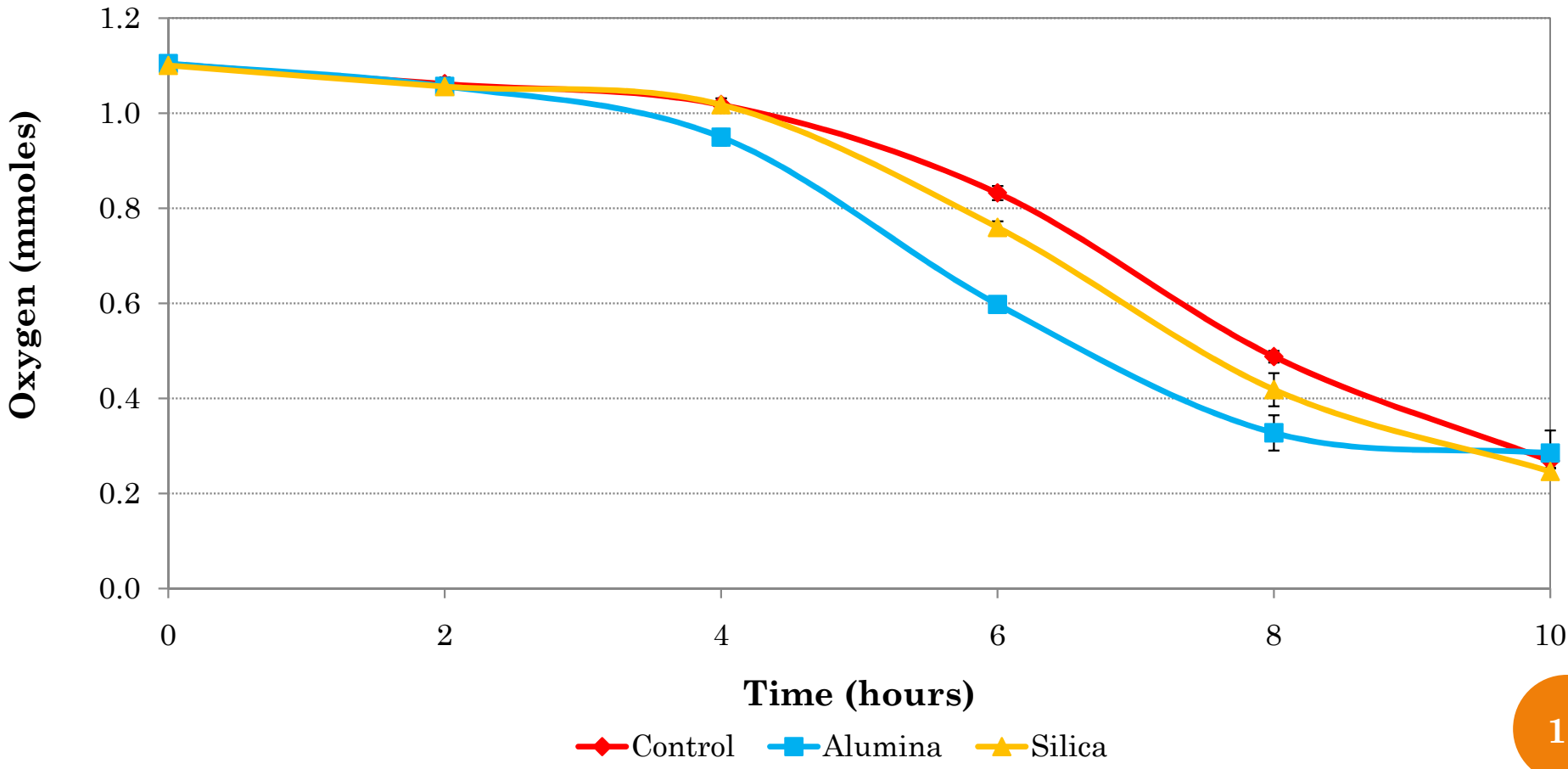
RESULTS


O2 uptake by S. cerevisiae: Nano-CeO2
Oxygen (mmoles)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
Time (hours)
Control
100 mg/L
500 mg/L
1,000 mg/L

RESULTS

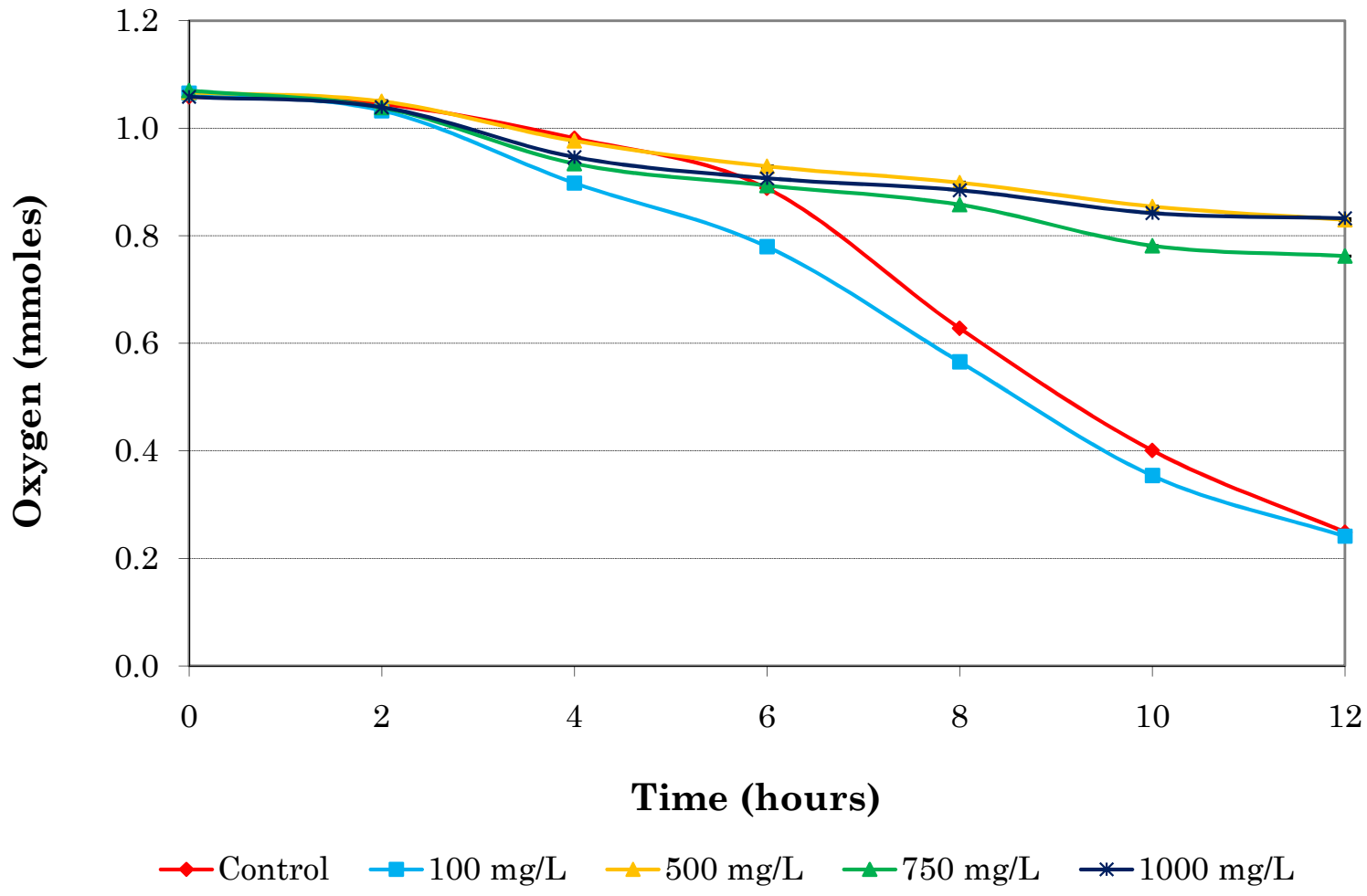
O_2 uptake by *S. cerevisiae:*

Alumina & Silica at 1,000 mg/L

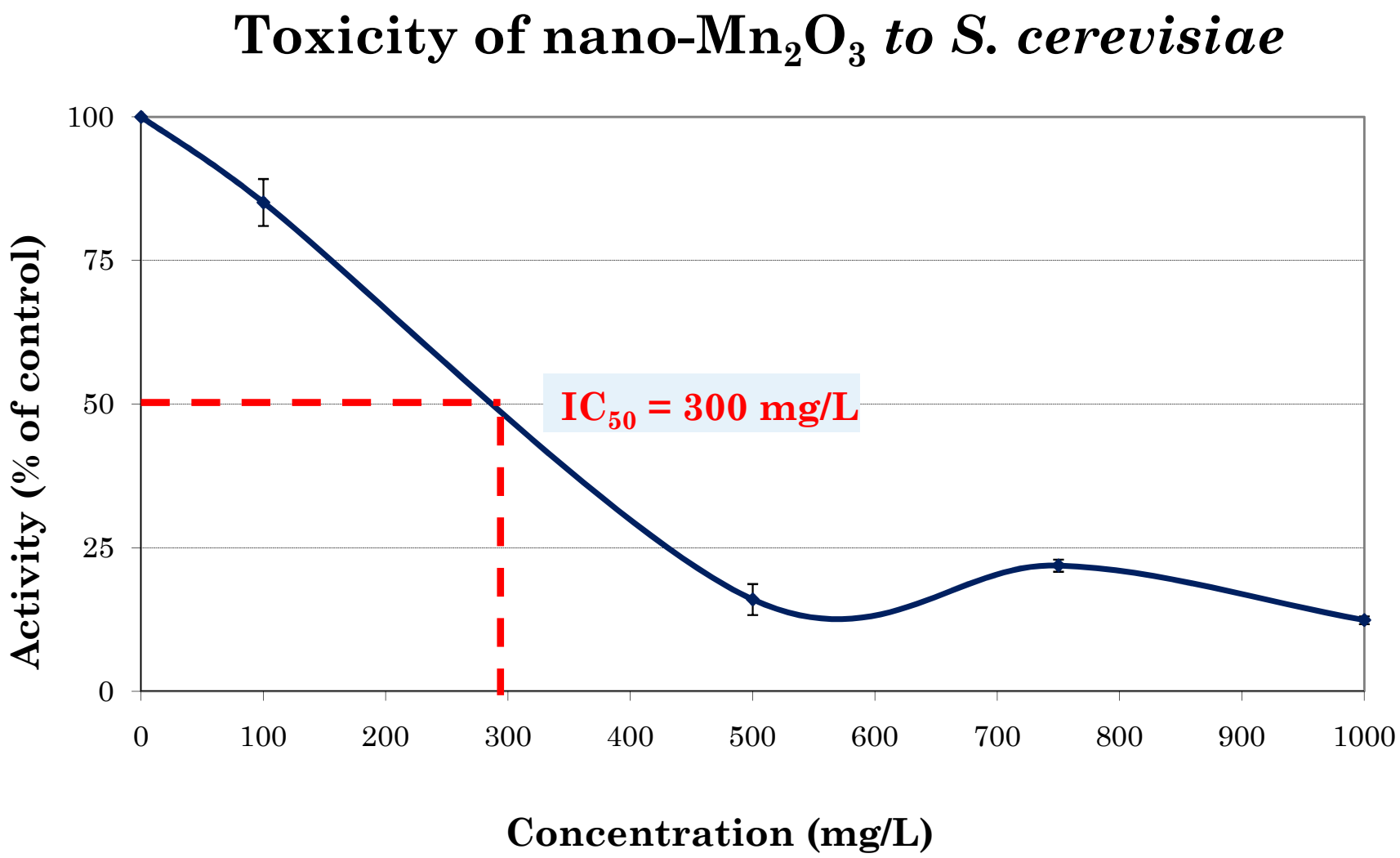


RESULTS

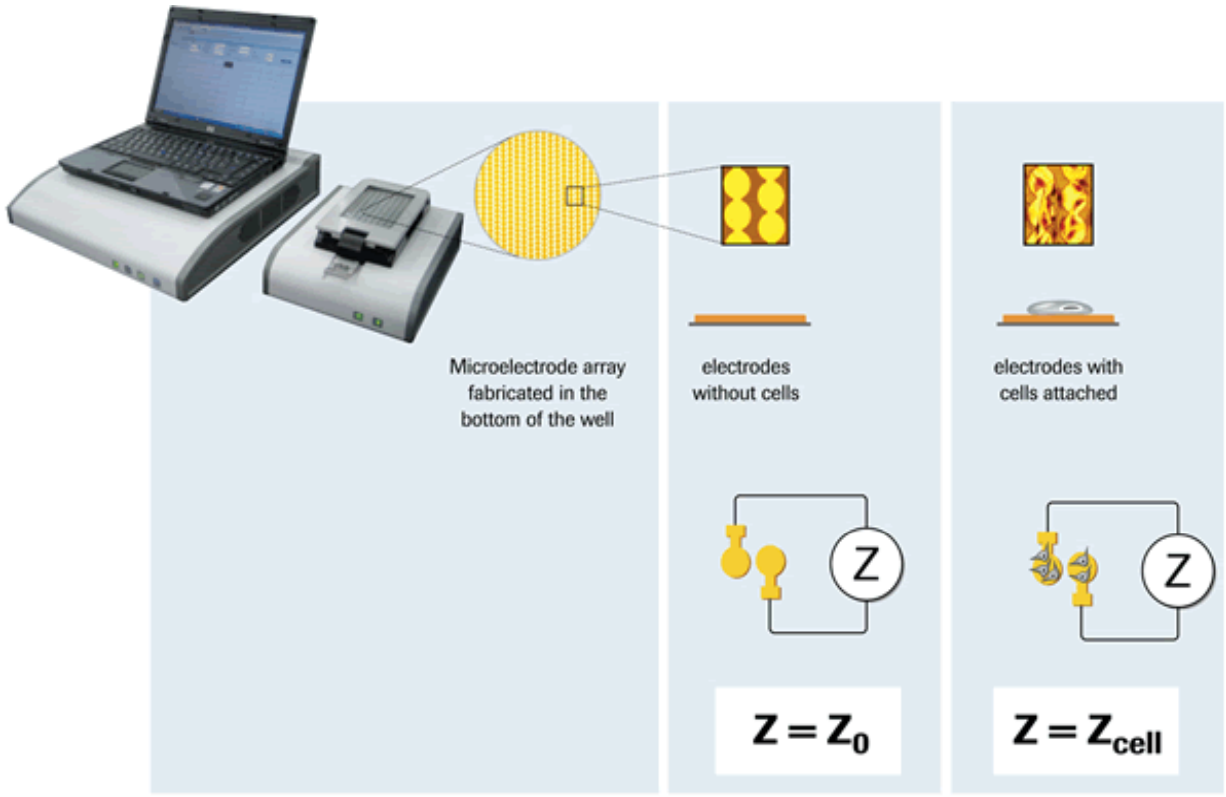
O_2 uptake by *S. cerevisiae:* nano-Mn_2O_3



RESULTS


Toxicity of nano-Mn_2O_3 *to S. cerevisiae*
Activity (% of control)
100
75
50
25
0
IC_{50} = 300 mg/L
0
100
200
300
400
500
600
700
800
900
1000
Concentration (mg/L)

Impedance Based Assay: xCELLigence

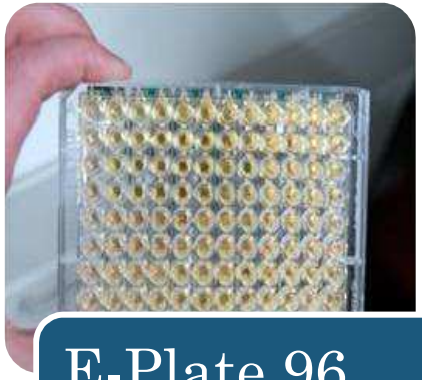


- The xCELLigence System measures **electrical impedance** across interdigitated micro-electrodes integrated on the bottom of tissue culture E-Plates.
- The impedance provides information about the **biological status** of the cells.
- The xCELLigence system does **not** need **fluorescent labels**.

Materials & Methods

- xCELLigence system- Lung epithelial cells: 16 HBE

16 HBE culture
- MEM[1] (10% FBS[2])
- 37°C



E-Plate 96
- MEM (5% FBS)
- 37°C

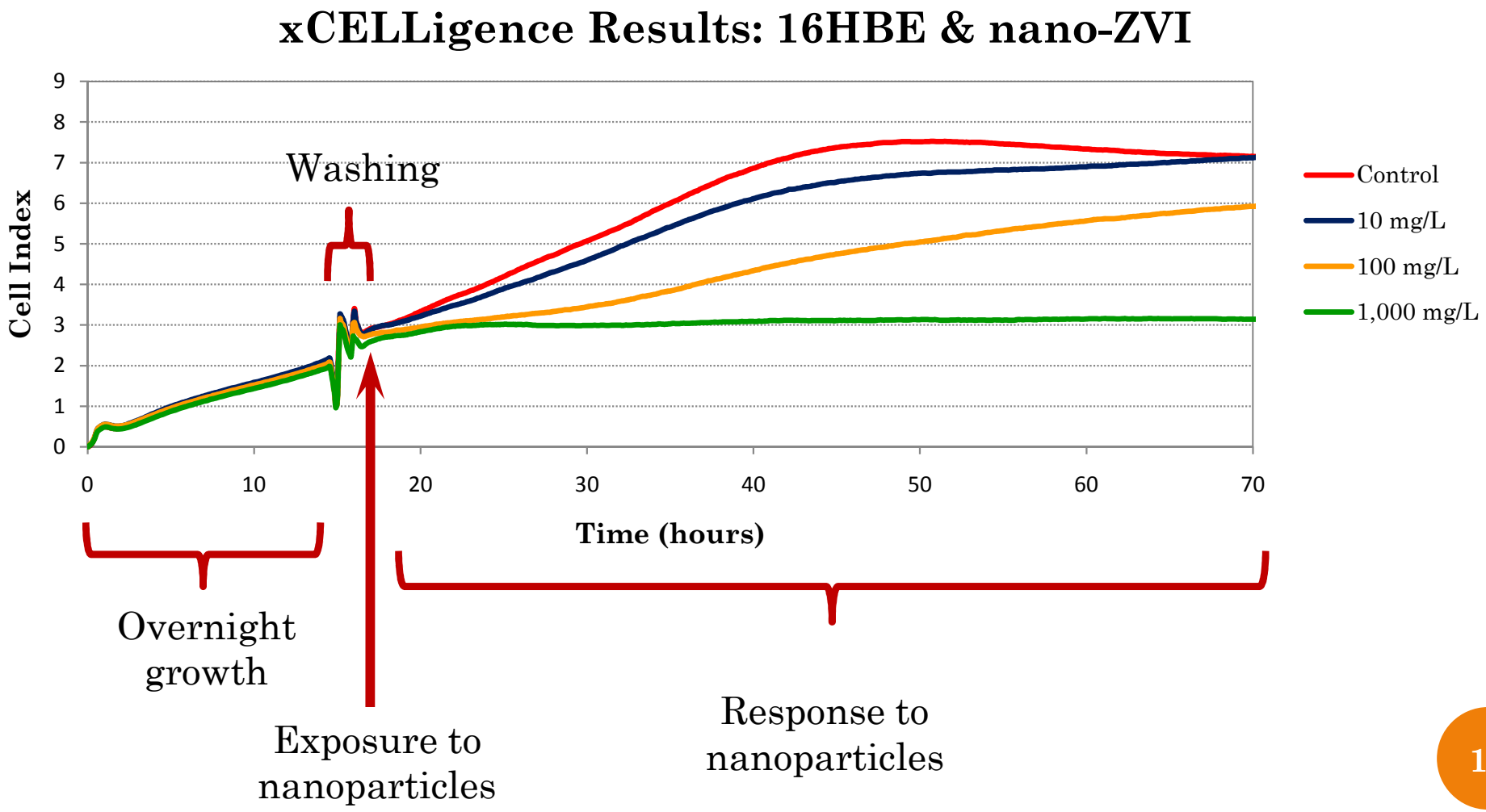
xCELLigence
- 37°C

[1] Minimum Essential Medium

[2] Fetal Bovine Serum

Impedance Based Assay: xCELLigence

- Experiment stages



Optimization of xCELLigence assays

Cell number per well

- 50,000
- 75,000
- 100,000

Medium

- HBSS[1]
- MEM

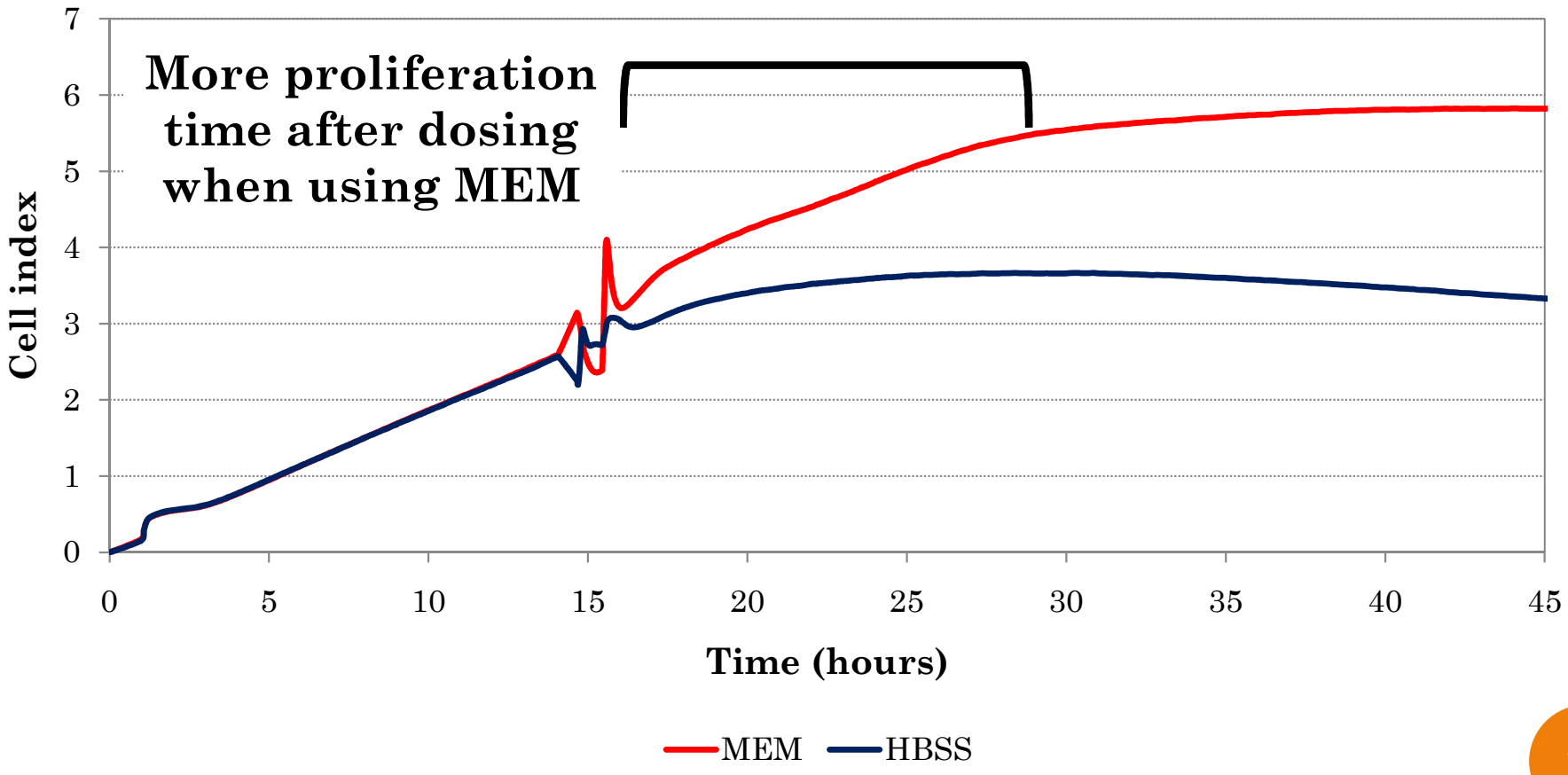
% of FBS

- 0% FBS
- 2% FBS
- 5% FBS
- 10% FBS

[1]Hank's Buffered Salt Solution

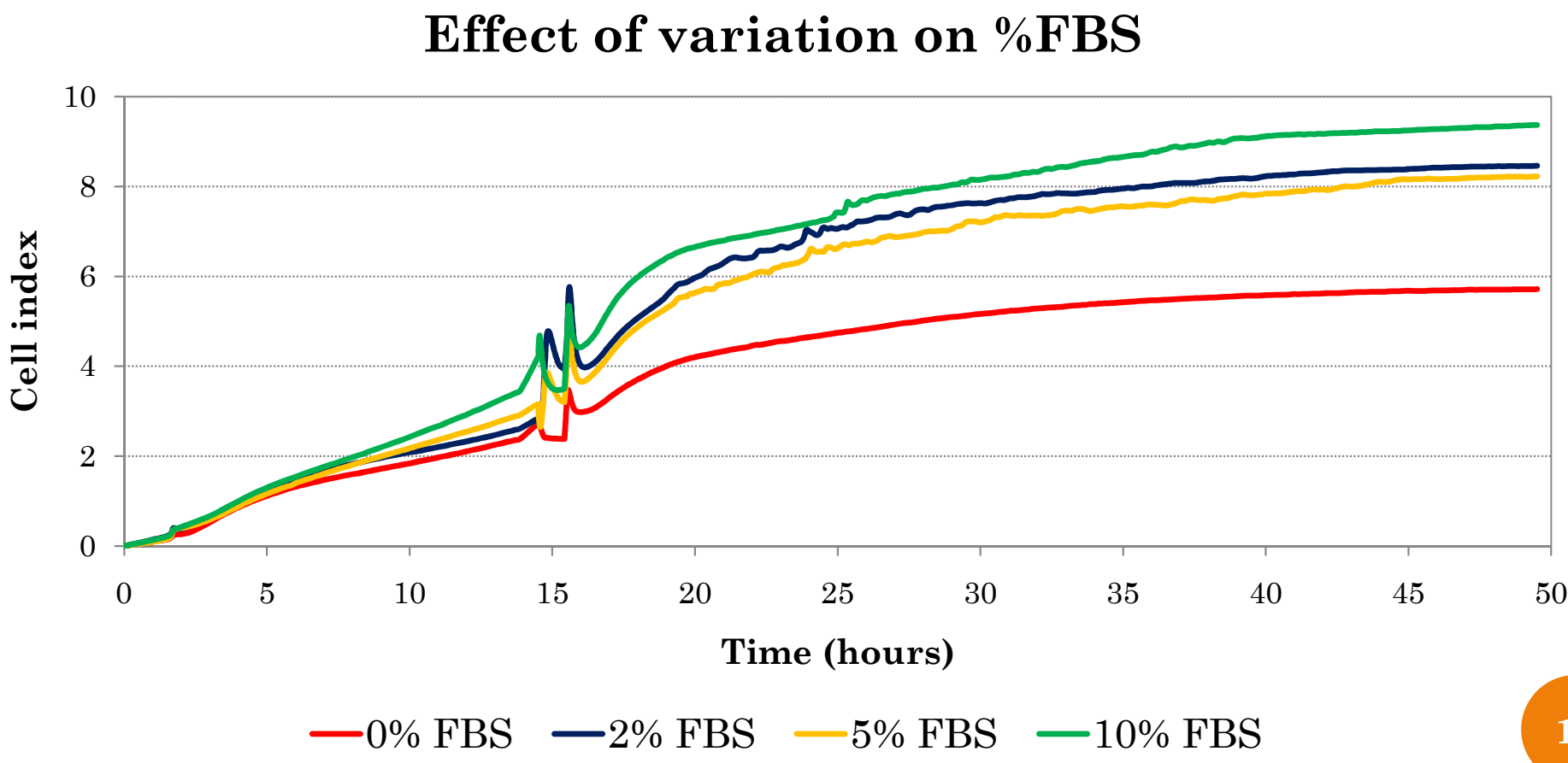
Selection of the assay medium

- Testing of MEM and HBSS



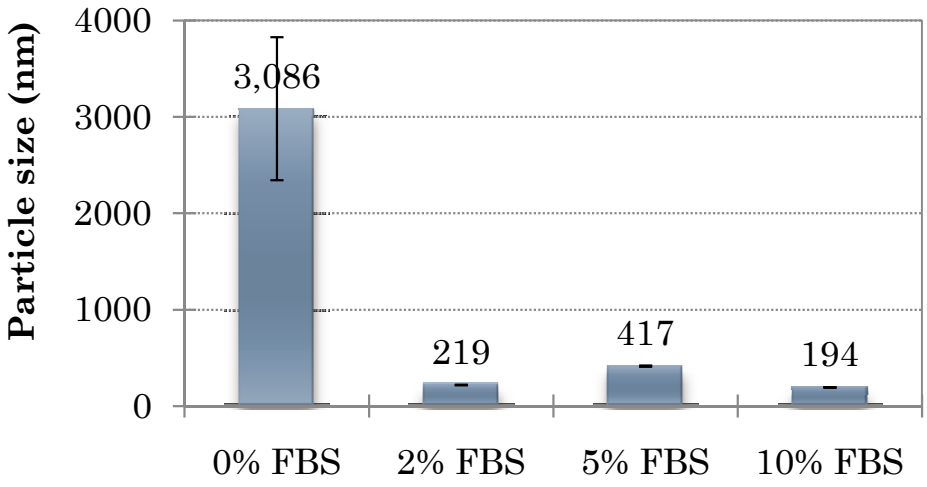
Selection of Protein (FBS) Content in the Medium

- Testing of MEM with different FBS levels

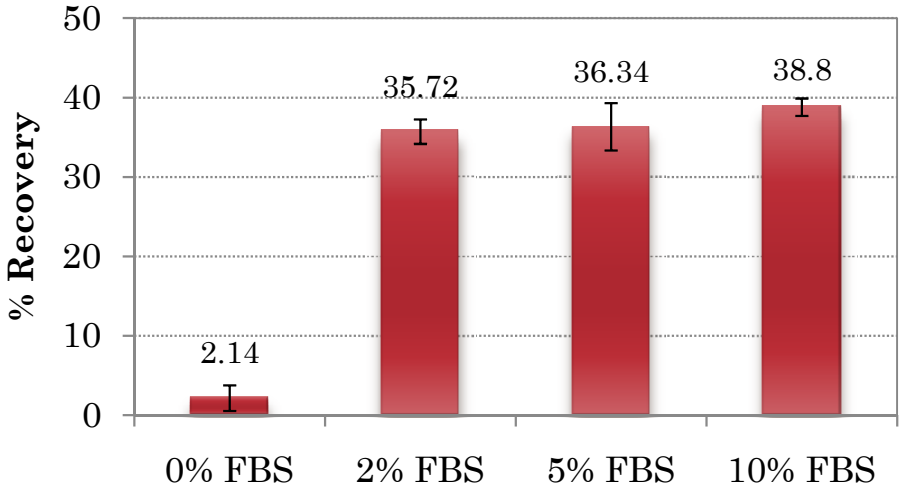


Effect of %FBS on stability of Al_2O_3

Particle size of Al_2O_3 in MEM at different %FBS



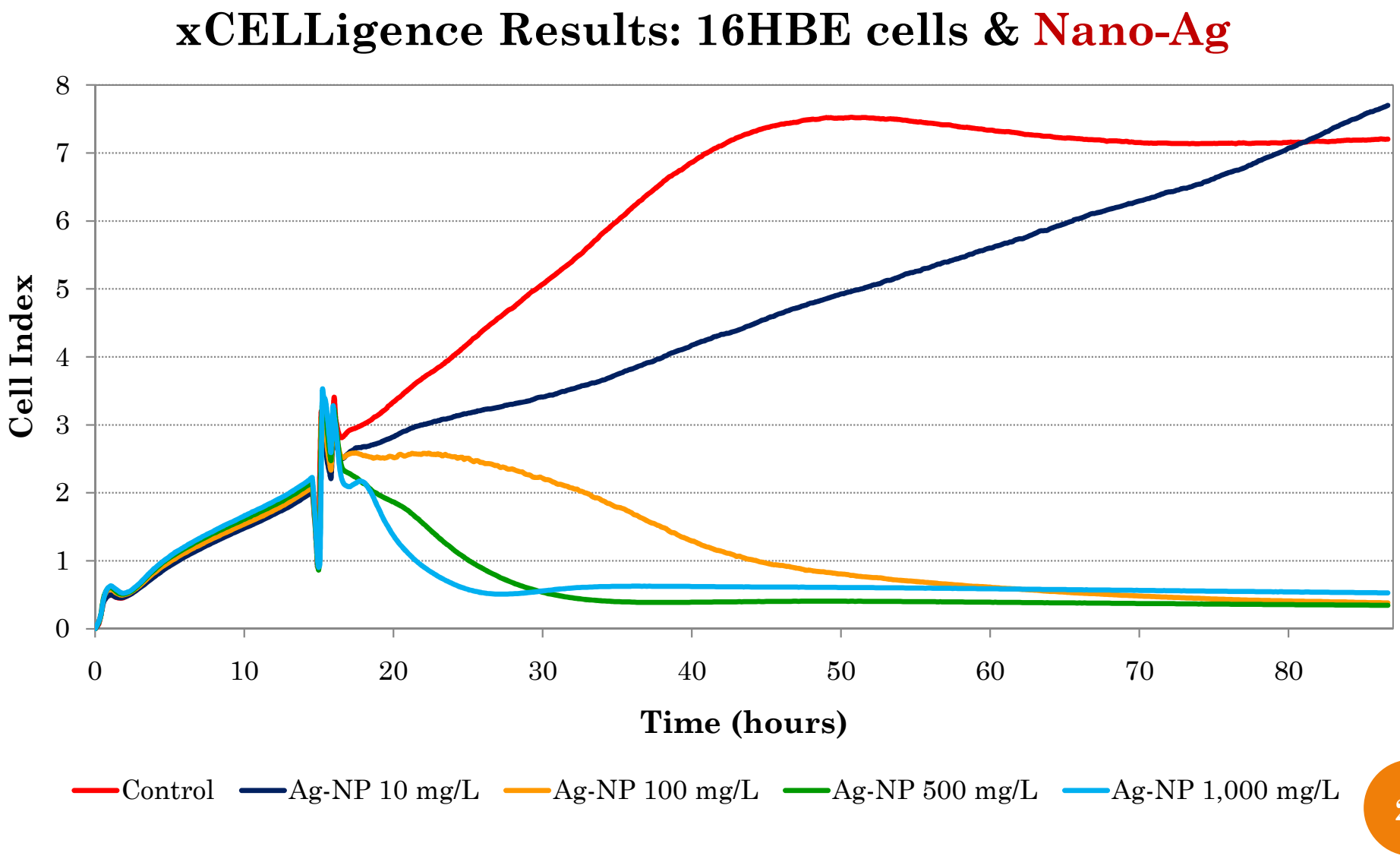
Percentage of Al_2O_3 recovered from the supernatant



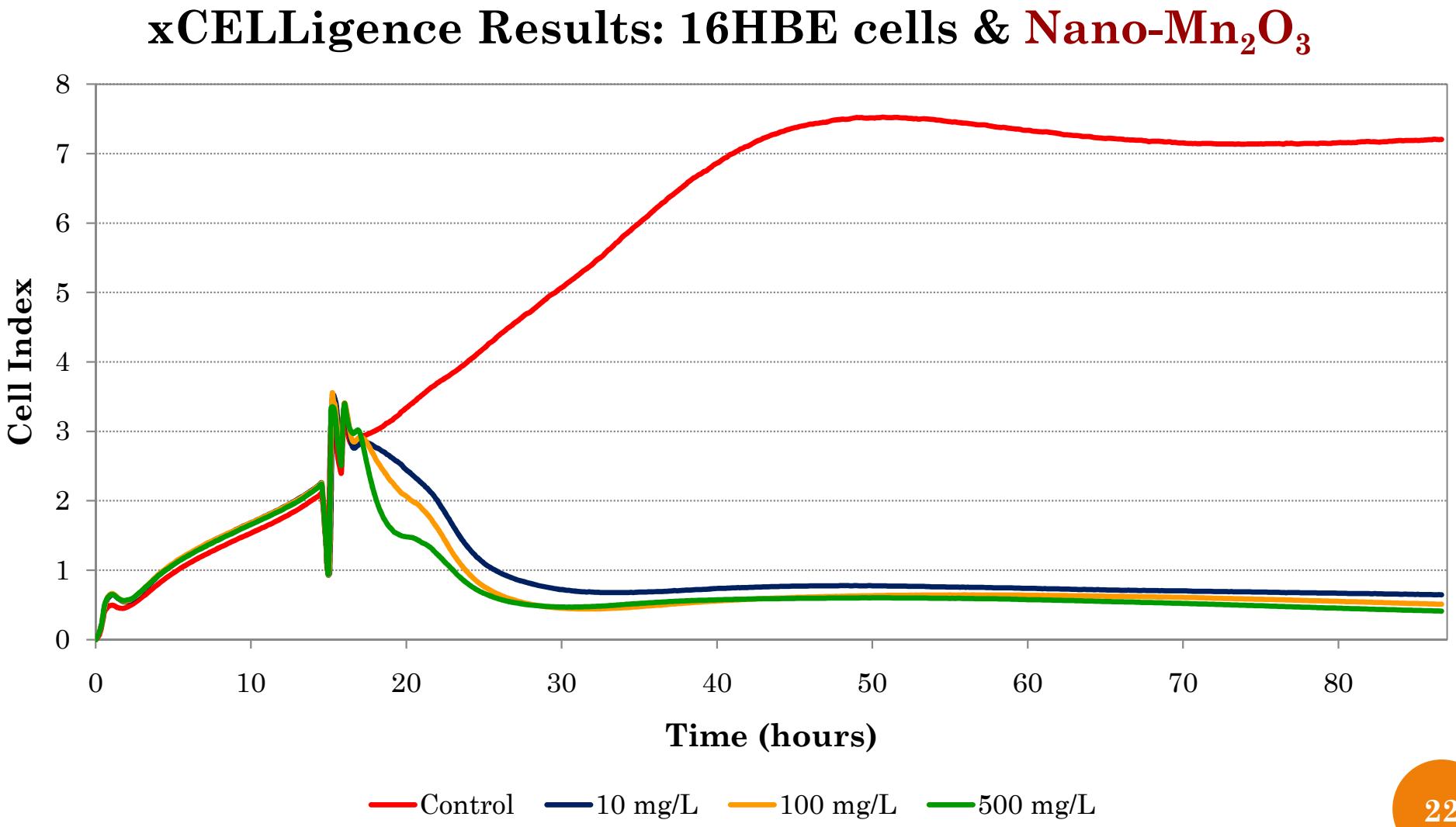
The presence of proteins in the medium seems to **stabilize NPs**

Stabilization by proteins is reflected in particle size and in concentration of nanoparticles in the supernatant.

RESULTS


xCELLigence Results: 16HBE cells & Nano-Ag
Cell Index
0
1
2
3
4
5
6
7
8
0
10
20
30
40
50
60
70
80
Time (hours)
Control
Ag-NP 10 mg/L
Ag-NP 100 mg/L
Ag-NP 500 mg/L
Ag-NP 1,000 mg/L

RESULTS


xCELLigence Results: 16HBE cells & Nano-Mn2O3
Cell Index
0
1
2
3
4
5
6
7
8
0
10
20
30
40
50
60
70
80
Time (hours)
Control
10 mg/L
100 mg/L
500 mg/L

RESULTS

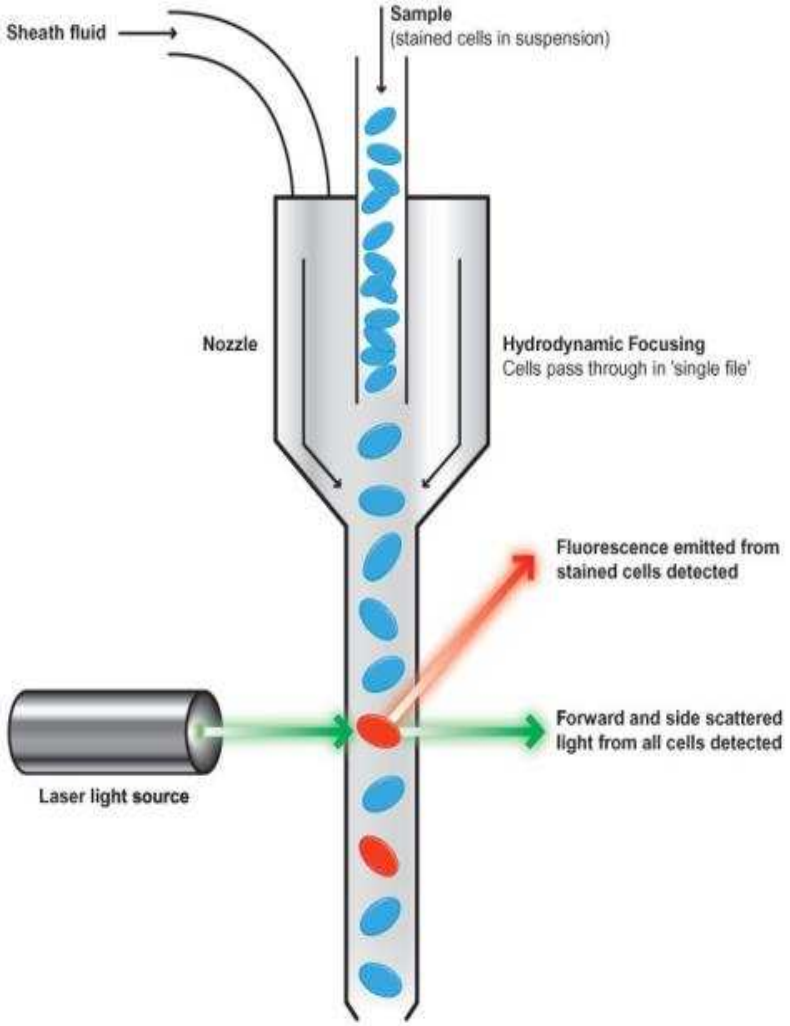
Summary of IC_{50} obtained for *S. cerevisiae* and for human lung epithelial cells (16 HBE)

Nanoparticle	IC_{50} for *S. cerevisiae* (mg/L)	IC_{50} for 16 HBE (mg/L)
ZnO	70	13
Mn_2O_3	300	<10
ZVI	1,000	85
Ag	-	21
Al_2O_3	>1,000	>250

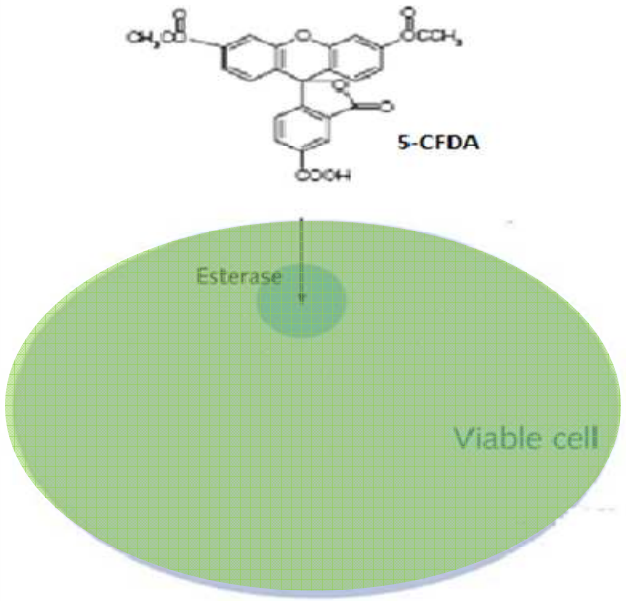
Toxicity Mechanisms

- Membrane damage
- Solubilization of toxic metal species
- Reactive oxygen species (ROS)

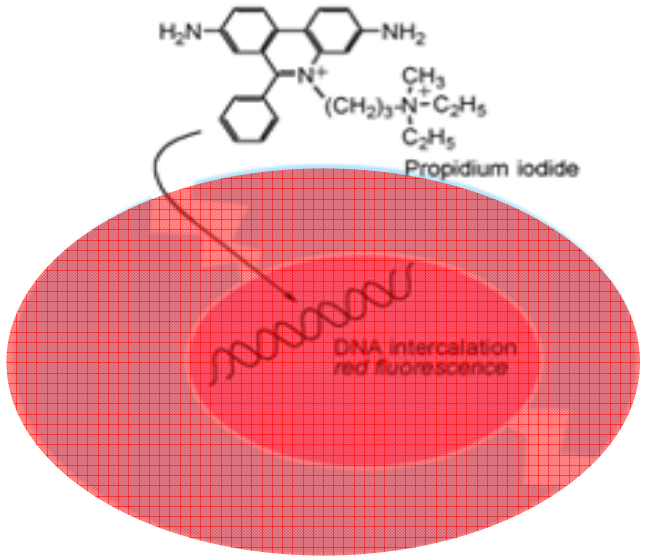
Flow Cytometry



- CFDA



- PI



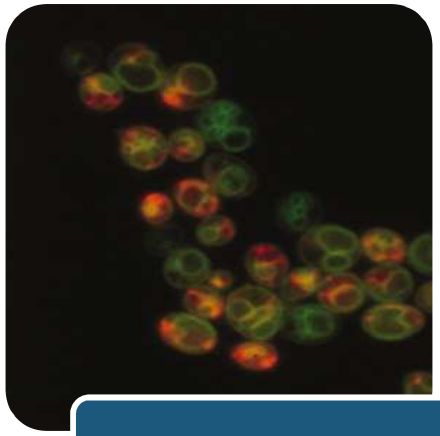
Materials & Methods

- Cell membrane integrity assay

Saccharomyces cerevisiae

  - Same conditions as in O_2 uptake assays

Sampling



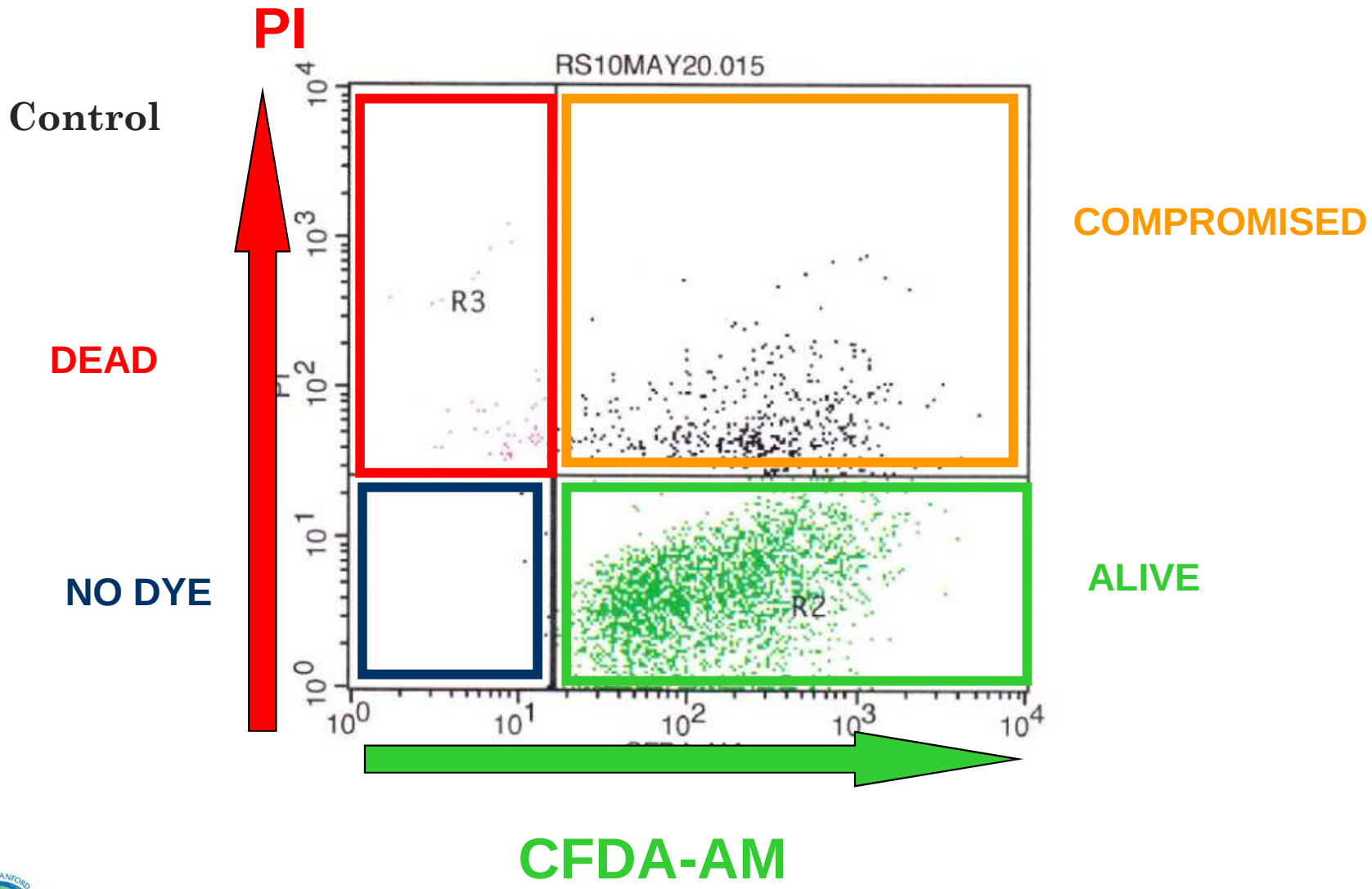
Staining

Flow Cytometry

FungaLight™ CFDA,AM/PI Yeast Vitality Kit

RESULTS

- Flow Cytometry



RESULTS

- Cell membrane damage by Mn_2O_3

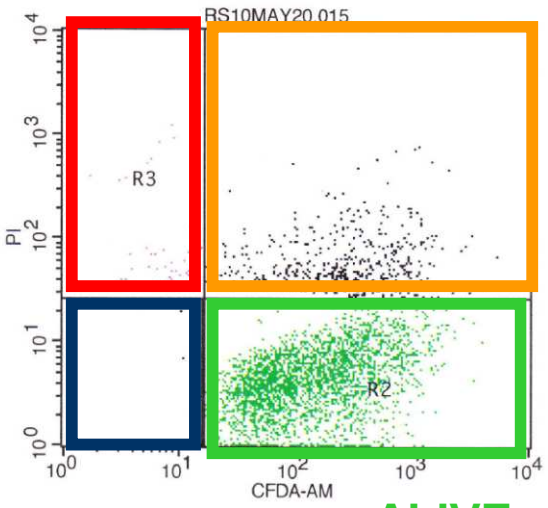
COMPROMISED

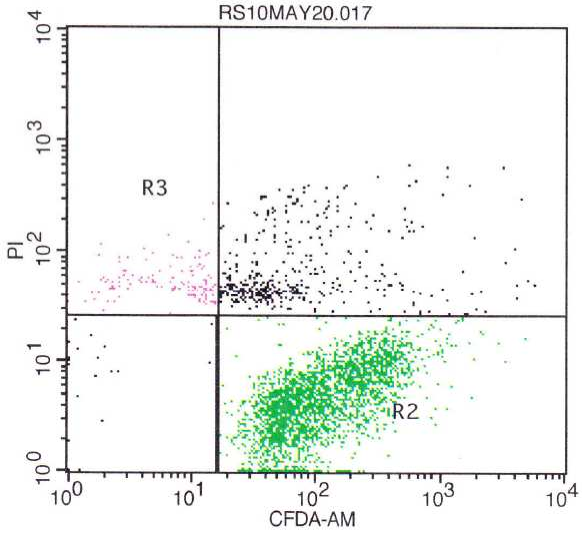
Control

DEAD

NO DYE

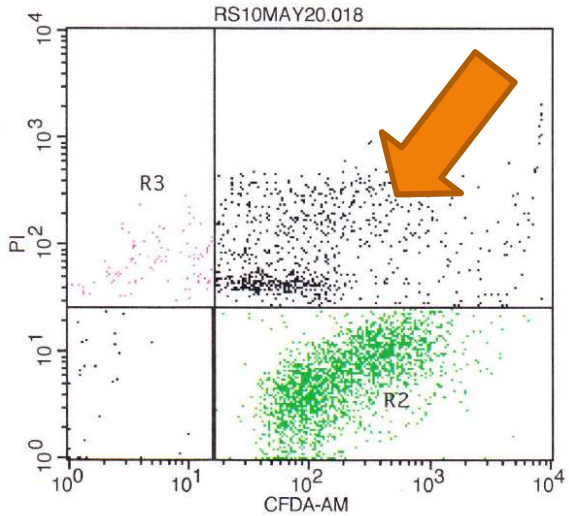
ALIVE

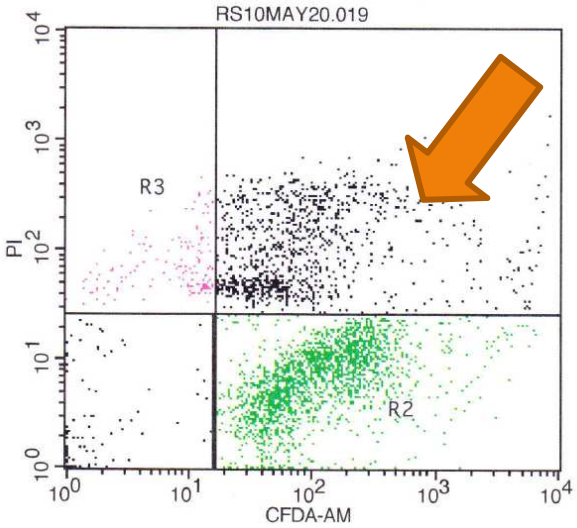




Mn_2O_3 300 mg/L

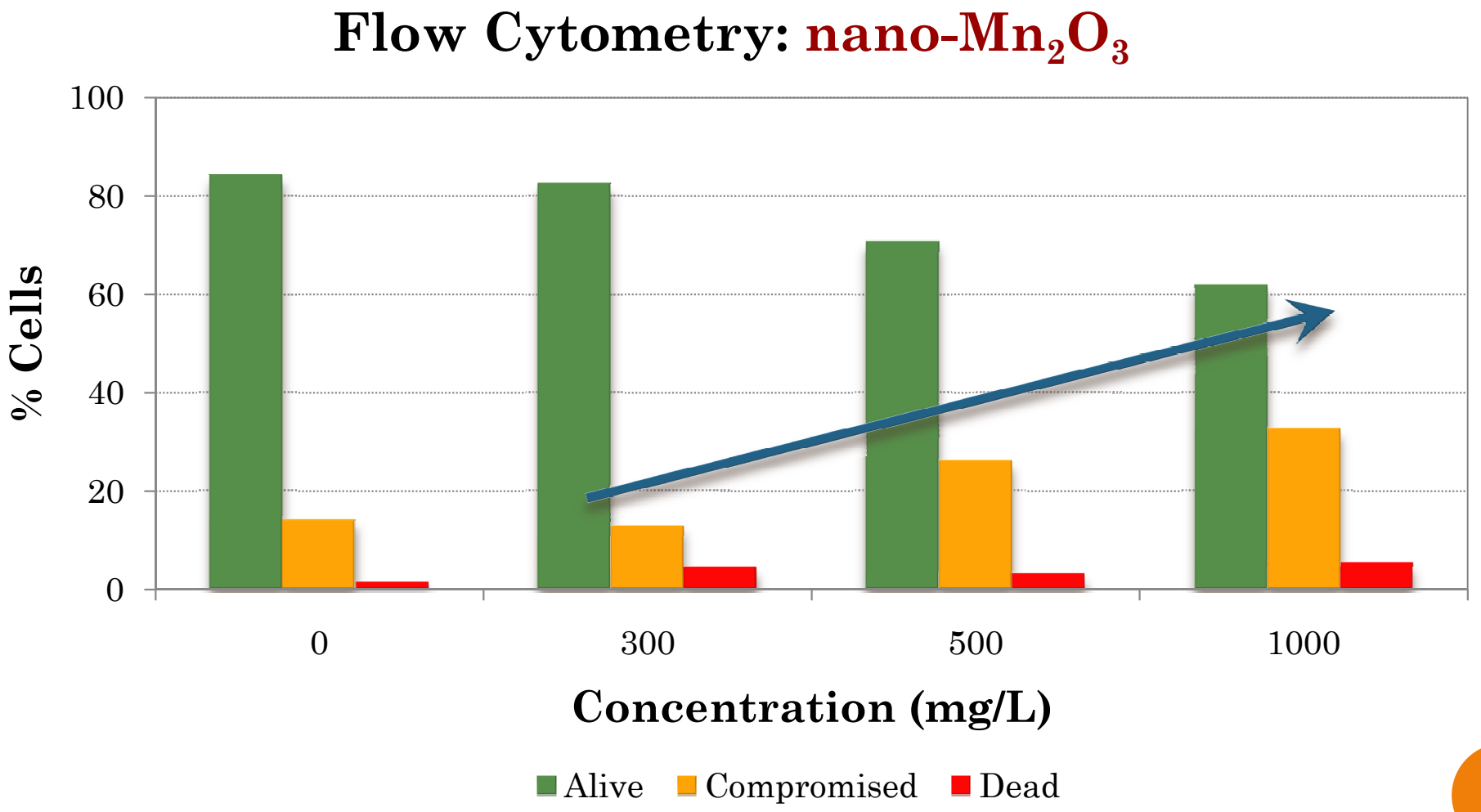
Mn_2O_3 500 mg/L





Mn_2O_3 1,000 mg/L

RESULTS


Flow Cytometry: nano-Mn_2O_3
100
80
60
40
20
0
% Cells
0
300
500
1000
Concentration (mg/L)
Alive
Compromised
Dead

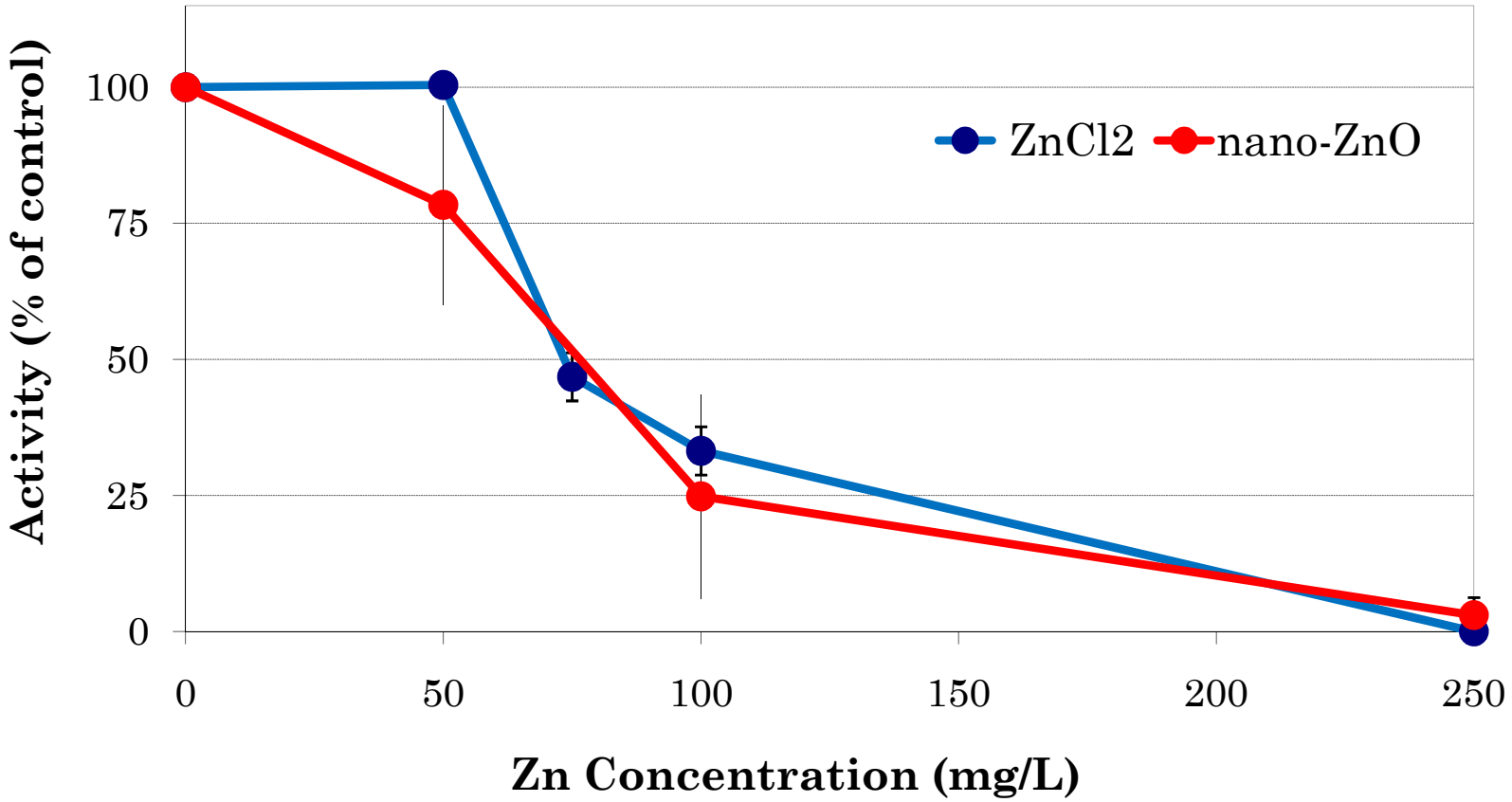
RESULTS

Summary of remaining activity in O_2 uptake test and live cells in membrane integrity test at 1,000 mg/L

Nanoparticle	Remaining activity in O_2 uptake test (% of control)	Live cells in membrane integrity test (% of control)
ZnO	**0**	**9**
Mn_2O_3	**12**	**73**
ZVI	**56**	94
CeO_2	**53**	97
SiO_2	100	**79**
Al_2O_3	100	**85**
Fe_2O_3	100	97
HfO_2	100	100
ZrO_2	100	100
TiO_2	100	100

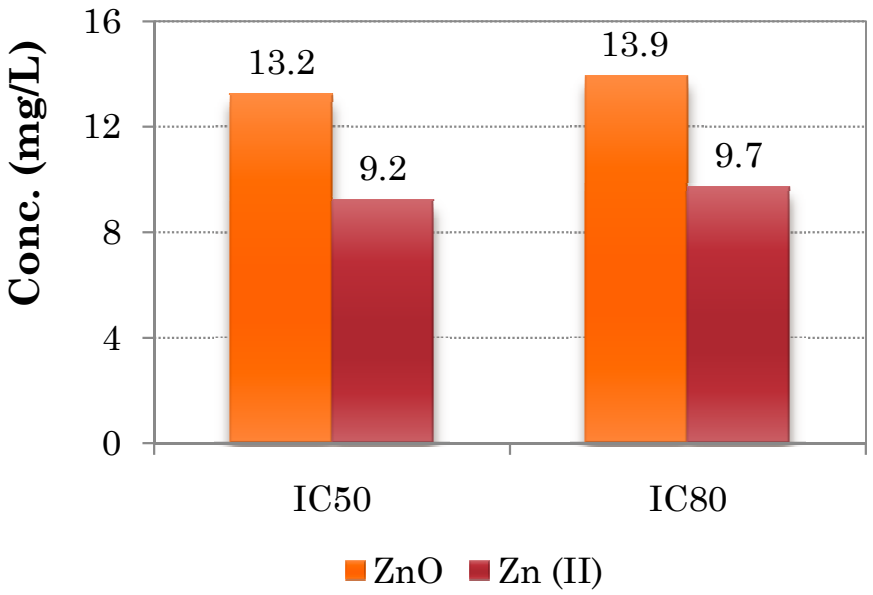
Toxicity of nano-ZnO *vs.* soluble Zn(II) in yeast assay

Results indicate that dissolution of ZnO to Zn(II) is the main cause of toxicity

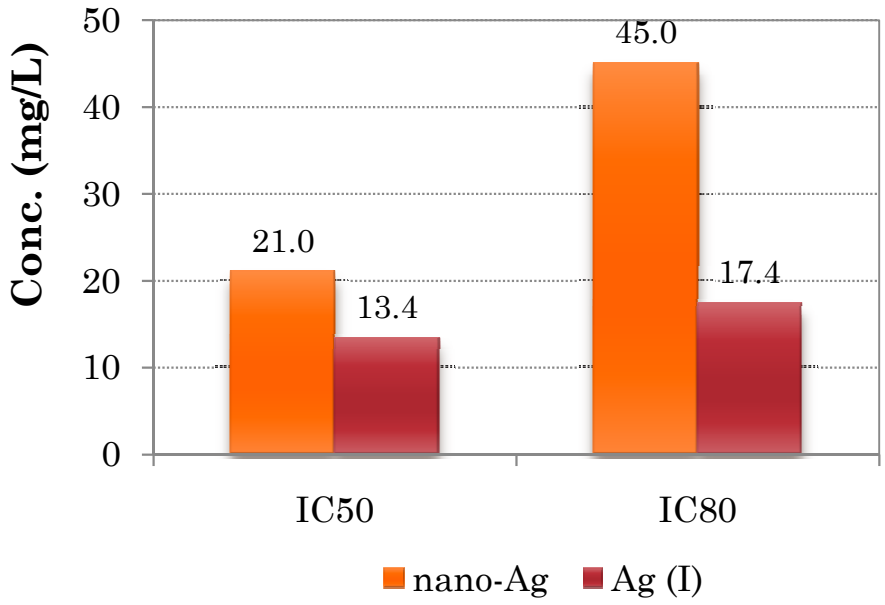


Toxicity of NPs *vs.* soluble metal species in xCELLigence assays

Comparison of toxicity of nano-ZnO & Zn (II)



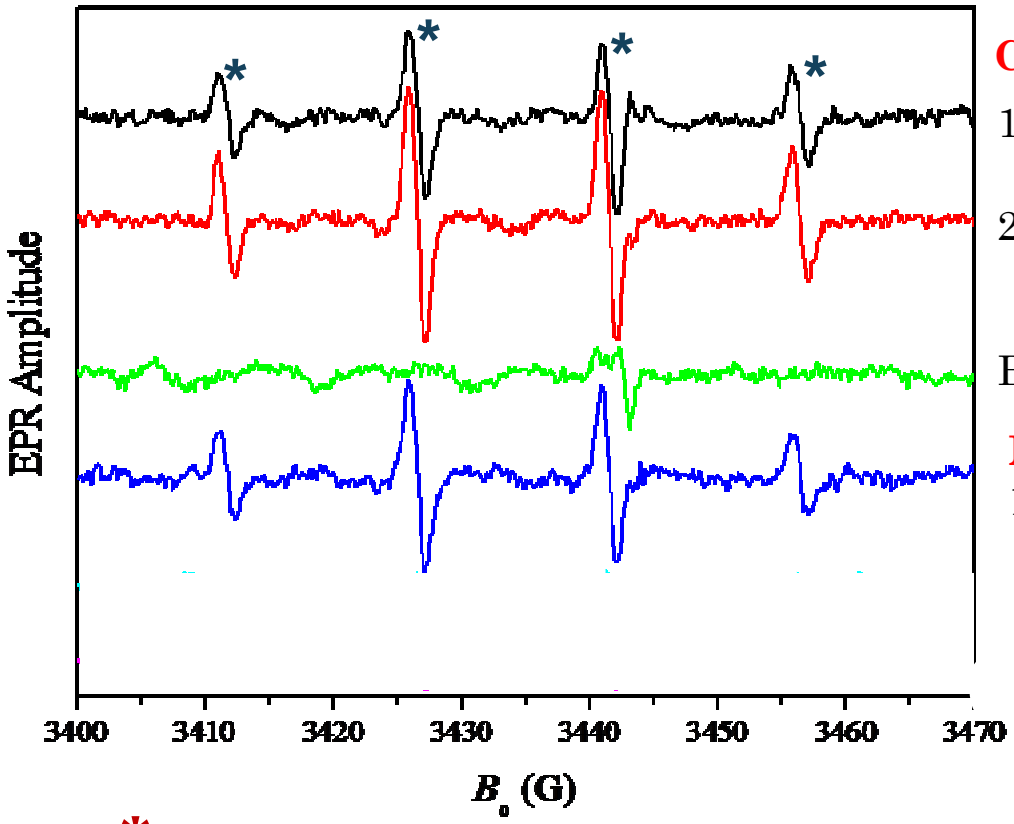
Comparison of toxicity of nano-Ag & Ag (I)



Soluble and nanoparticle toxic concentrations were correlated suggesting a role of soluble species in toxicity.

ROS Generation by Nanoparticles

DMPO+OH spin adducts



"*" denote lines of the DMPO+OH spin adduct.

CeO_2 and Mn_2O_3 produce $OH^{\cdot}$ radicals in water

CONCLUSIONS

- Yeast test was developed to assess the toxicity of NPs. The test relies on measurement of O_2-respiration, avoiding interference problems often associated with colorimetric or fluorimetric measurements

- Most NPs did not show toxicity to *S. cerevisiae* in the O_2 uptake assay. Only CeO_2, ZVI, Mn_2O_3 and ZnO NPs were toxic. ZnO was the most toxic compound tested.

- A new impedance based system called xCELLigence, was optimized and is being used to assess the toxicity of NPs to human lung epithelial cells (16 HBE).

Conclusions

- All NPs tested showed higher toxicity to 16 HBE cells than to yeast.
- Nano-sized ZnO, Mn_2O_3, SiO_2 and Al_2O_3 also caused cell membrane damage.
- Results showed that ZnO and silver NP toxicity is related wholly or in part to their dissolution, respectively.
- Mn_2O_3 and CeO_2 were shown to form ROS species in water.

ACKNOWLEDGMENTS

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