1. PURPOSE

To provide a report on the analysis of the toxicity of Nano Solution on human lung cells (BEAS-2B).

2. MATERIALS AND EQUIPMENT

2.1 Samples

Sample	Details
Nano Solution	Batch # unknown (new batch)
Acrylic	Provided by Nano Medical Technology

2.2 Materials

Material	Details
Non-cancerous human bronchial epithelial cell line	BEAS-2B, American Type Culture Collection
Dulbecco's Modified Eagle's Medium	DMEM, Sigma-Aldrich
Antibiotic-Antimycotic	Thermo Fisher Scientific
Foetal Bovine Serum	FBS
Phosphate Buffered Saline	PBS, Sigma-Aldrich
TrypLE Select	Thermo Fisher Scientific
Trypan Blue (0.4%)	Invitrogen
96 Well Cell Culture Plate	Costar, Sigma-Aldrich
Pipettes	
Pipette tips	

Falcon tubes	
Eppendorf tubes	

2.3 Equipments

Equipment	Details
Incucyte ZOOM	Live cell analysis system
Centrifuge	

3. PREPARATION OF REAGENTS AND SAMPLE

3.1 Reagents

Prepare the following reagents according to the details given below:

Reagent	Details
Complete media	10% FBS, 1% antibiotic-antimycotic, DMEM
FBS free media	1% antibiotic-antimycotic, DMEM

3.2 Samples

Prepare the following stock solutions of the samples to be used for analysis, according to the details given below:

Sample Stock Solution	Details
Nano Solution	Dilute in serum free media to achieve desired concentrations

4. METHODS

Day 1

1. Take out media
2. Wash with 10 mL PBS X2
3. Add 3 mL of TrypLE
4. Incubate for 5 min
5. Stop trypsinisation with 3 mL media (to deactivate TrypLE - 1:1)
6. Transfer to falcon tube
7. Centrifuge at 1.2 rpm for 2 min
8. Tip out and get rid of excess media

9. Resuspend cells in 2 mL of media
10. Count cells:
    1. Mix 20 µL trypan blue in an eppendorf with 20 µL of resuspended cells (1:1)
    2. Leave to incubate for a few minutes (~5 min)
    3. Put 10-15 µL into a cell counter slide; adjust focus
11. Adjust cell concentration accordingly
12. Add 100 µL media per well
13. Add 100 µL cells
14. Place in incucyte overnight

Day 2

1. Remove media from each well and discard
2. Add treatment in FBS free media (new Nano Solution)
3. Place in incucyte for 24 h

Day 3

1. Remove media from each well and discard
2. 1× PBS wash
3. Replace with fresh complete media
4. Place in incucyte

5. RESULTS

Dose response toxicity on BEAS-2B was observed. Toxicity was not observed at dissolved Ti ≤5 ppm. At dissolved Ti 12.5 and 25 ppm, cellular toxicity and particle agglomeration was observed, forming a layer that sedimented and covered the cells. Visually higher number of surviving cells at dissolved Ti 12.5 ppm than 25 ppm.

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