Fabrication of functionalized nanoceria: A nanotheranostic avenue towards cancer therapy

Sriparna De,[1*] Arijita Basu[2], Arpita Adhikari[2], Animesh Dey[1], Anal Mandal[1], Suresh K Verma[3]

[1]Dept. of Allied Health Sciences, Brainware University, Kolkata-700125
[2]Department of Polymer Science and Technology, University of Calcutta, 92, A.P.C. Road, Kolkata 700 009, India
[3]Dept. of Biotechnology, KIIT University, Bhubaneswar, Odhisa-751024

***Abstract*— Exploration of nanomaterials delivers good synergism between nanoscience and nanotechnology endeavors new frontiers in plethora of biomedical applications. Multifunctional nanoparticles are now widely exploited and proven their versatility as drug delivery conveyance in cancer therapeutics area. Herein in this proposed work a synthesis of folic acid (FA) functionalized rare earth metal oxide has been carried out to as nanotherapeutics in cancer therapy and diagnostic field. Functionalization of FA onto cerium oxide (CeO_2) enables enhanced cellular uptake in cancer cells confirmed from DAPI micrographs. The cytomorphological architecture and cell migration assays of the cancer cells has also been monitored using scanning electron and optical micrographs respectively. The findings implying the inhibition of cancerous cells migration upon treatment. Further the nanotherapeutic modality of these nanoparticles was evaluated and screened in human colorectal carcinoma cells signifying the anticancer efficacy of the nanoparticles.**

***Key words*— Nanoparticle, folic acid, nanoceria, cytomorphology, apoptosis, cancer therapy**

I. INTRODUCTION

Over the past few years nanostructured cerium oxide has acquired unprecedent ate recognition due to its versatile characteristic phenomenon. Remarkable research has been underway to explore the therapeutic efficacy in diverse application area including antioxidant therapy, neuroprotection, radioprotection, and ocular protection [1-5].

Owing to profound antioxidant therapy, inflammatory response and good catalytic ability and reactivity with reactive oxygen species (ROS) nanoceria are being cultivated. The facile cyclic oxidation states between trivalent (Ce^{3+}) and tetravalent (Ce^{4+}) promoting them to protect cells against various ROS [6]. Catalase and super oxide dismutase provide the shielding effect upon excess release of ROS in intracellular level to some extent. Intracellular ROS production is influenced by oxidase enzymes thereby leads to the formation of oxidative stress ultimately results cell and tissues damage [7-9]. Conventional chemotherapeutic agents showing drawbacks towards specific targeting of cancer cells. However typical adverse side-effects from chemotherapy are well-known. This can be addressed by targeted nanomedicine endeavors a course to deliver cancer therapeutics to tumor site of interest without any alteration of healthy one. Folic acid (FA) known as folate, is actively devoted as a targeting ligand due to their high affinity binding towards folate receptors (FR) [10]. FRs are recognized as significant therapeutic tumor markers overexpressed on the surface of almost nearly 40% of cancer cells including lung, breast, kidney, ovarian, endometrial and renal cancer whereas its expression in healthy cells is highly confined. FA offers versatile characteristics properties including non-immunogenicity, small molecular size, good stability and cost effective [11]. These modulating features instigate facile cellular internalization within cancer cells penetrating through the cell membrane. In this study a good synergistic methodology was adopted utilizing nanoceria and folic acid wherein folic acid functionalized nanoceria was synthesized. In-vitro anti-cancer efficacy was assessed using cytomorphological study and evaluated by cellular uptake.

II. Materials & Method

Analytical grade ceric ammonium nitrate and folic acid was procured from Sigma Aldrich. To carry out the synthesis scheme and monitor the characterizations other reagents were purchased from Merck & SRL.

Synthesis and Fabrication of FA-CeO_2 nanoparticles

Briefly, Cerium oxide nanoparticles (CeO_2) were synthesized by precursor ceric ammonium nitrate $(NH_4)_2Ce(NO_3)_6$ (99.999 % pure) in deionized water followed by hydrolysis using NH_4OH in basic medium [12]. Afterwards thorough washing was carried out to neutralize the precipate followed by vaccum drying. Surface functionalization upon CeO_2 with FA was performed using amminopropyl triethoxy silane (APTMS) as a linker agent. Conjugation was achieved using 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) and N-hydroxysulfosuccinimide coupling chemistry [13].

Cell culture and *In-vitro* cellular study:

The human mammary carcinoma cell line, MDAMB-231 has been procured from National Centre for Cell Science, Pune, India and hervested in Dulbecco's Modified Eagle's Medium or DMEM containing10% Fetal Bovin was incoporated followed by incubation at 37°C with 5%CO_2 [14].

To examine the apoptotic feature DAPI staining were performed, which gets agglomerated and appears round apoptotic bodies along with clustered morphology upon treatment. Further cell migration assay was studied to monitor the anti-migratory behaviour in ~80-90% confluent cell medium by creating scratch using a sterile tip. Moreover, cytomorphological architecture of MDA-MB231 was scrutinized using scanning electron microscopy (SEM) after treatment with synthesized nanoparticles. Further

apoptosis assay in human colon cancer (HCT116) cells was evaluated by fluorescent microscopy adopting of acridine orange (AO)/EtBr staining protocol.

III. Results & Discussion

Synthesis of folic acid functionslized nanoceria (FA-CeO_2)

Synthesis of FA-CeO_2 was confirmed using FTIR spectroscopy. As shown in Fig.1 the characteristics peak at 1700 cm^{-1} is ascribed to carbonyl stretching vibration of amide and acidic groups. Two distinct peaks were found at 1600cm^{-1} and 1520cm^{-1} due to stretching vibration and benzoic vibration in the folic acid justifying the formation of FA conjugated nanoceria [15]. The size distribution pattern was also corroborated using transmission electron micrographs and dynamic light scattering (DLS). The findings showed the particle size of ~10-20 nm whereas DLS spectra exhibited hydrodyanmic size of ~299nm possibly caused by the prominent aggregation of agglomerated particles in its hydrated state [16].

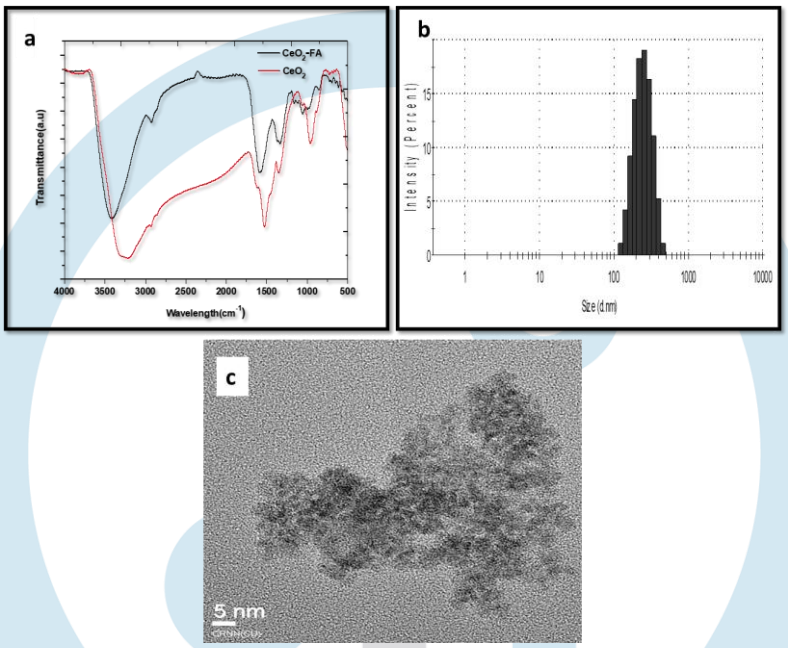

Figure 1: Physicochemical characterization of synthesized FA-CeO_2 nanoparticle using a) FTIR spectra b) DLS spectra c) TEM micrograph

Study of anti-cancer efficacy of FA-CeO_2

To understand the behavior of the synthesized nanoparticle in cell culture medium and their toxicity profile can be monitored through cellular bio-imaging studies. As evident from Fig 2, after 24 h of exposure to FA-CeO_2 the cell blebbing was noted and the cells appeared to have round shaped apoptotic morphology. This conveys the nano-formulation seems to trigger nuclear morphology by inducing cell membrane association and chromatin marginalization.

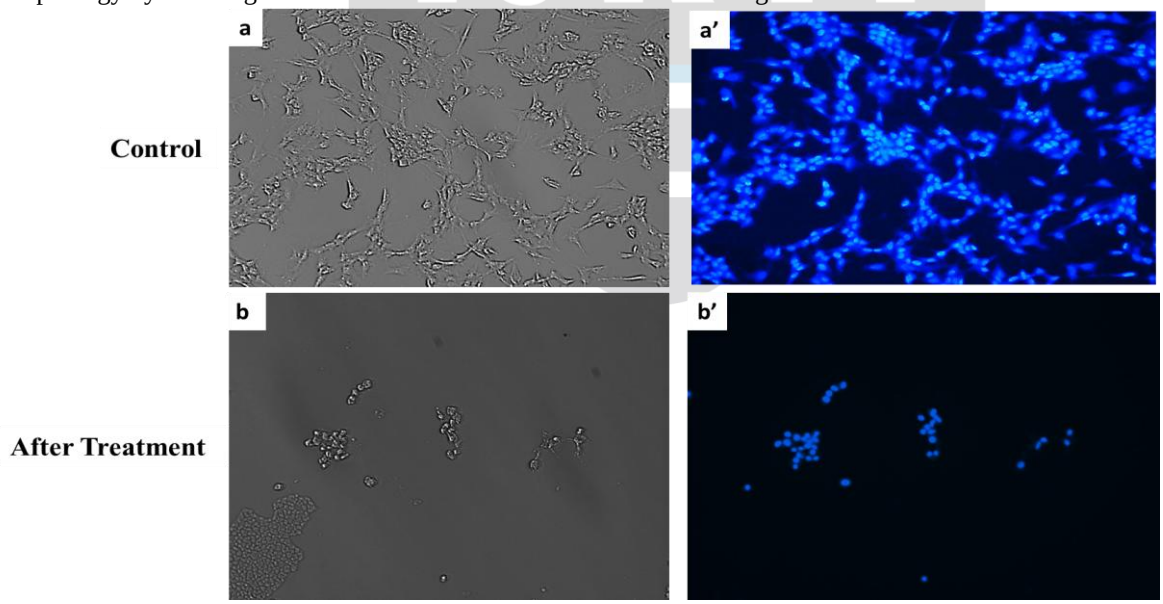

Figure 2: DAPI staining micrographs of control (a-optical, a'-DAPI stained) and treated MDA-MB-231 cells (b-optical & b'-DAPI stained)

Whereas control cells showed cell clumping with confluent cell aggregates wherein no such significant alteration in cellular integrity was observed magnifying clustered cellular morphology [17].
Further to reinstate the findings we had channelized the study towards cellular morphology analysis by SEM micrographs. FA-CeO_2. As observed upon treatment with nanoparticle a significant membrane blebbing, distinct feature of apoptotic cells as compared to the intact cellular architecture of untreated one thereby indicating cellular apoptosis which ultimately induced cell death in MDA-MB231 cells [18].

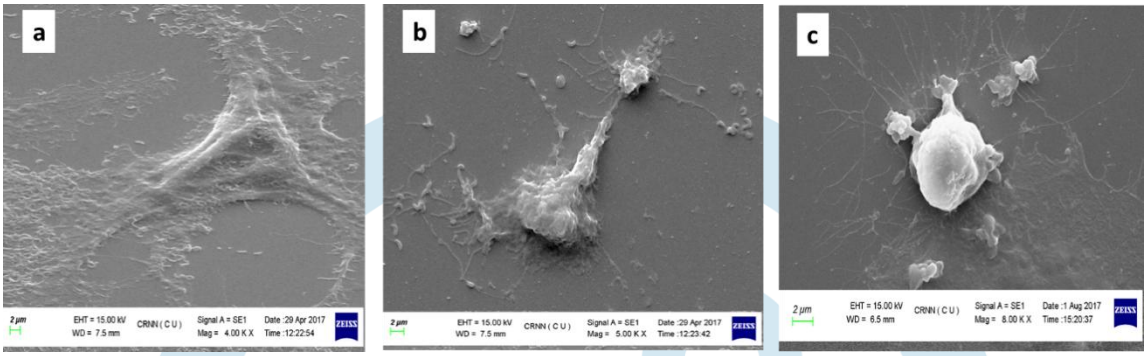

Figure 3: SEM micrographs depicting the alteration of cellular morphology a) control b) & c) upon treatment with nanoparticles at 12 h and 24 h respectively.

Study of anti-migratory efficiency of cancer cells
In order to explore the anti-migratory behavior of synthesized FA-CeO_2 nanoparticles, primarily bidirectional wound healing assay in MDAMB-231, signified that nanoparticle impeded cancer cell migration by inhibiting the extension of lamellipodia in a time dependent fashion elucidated in Fig. 3.

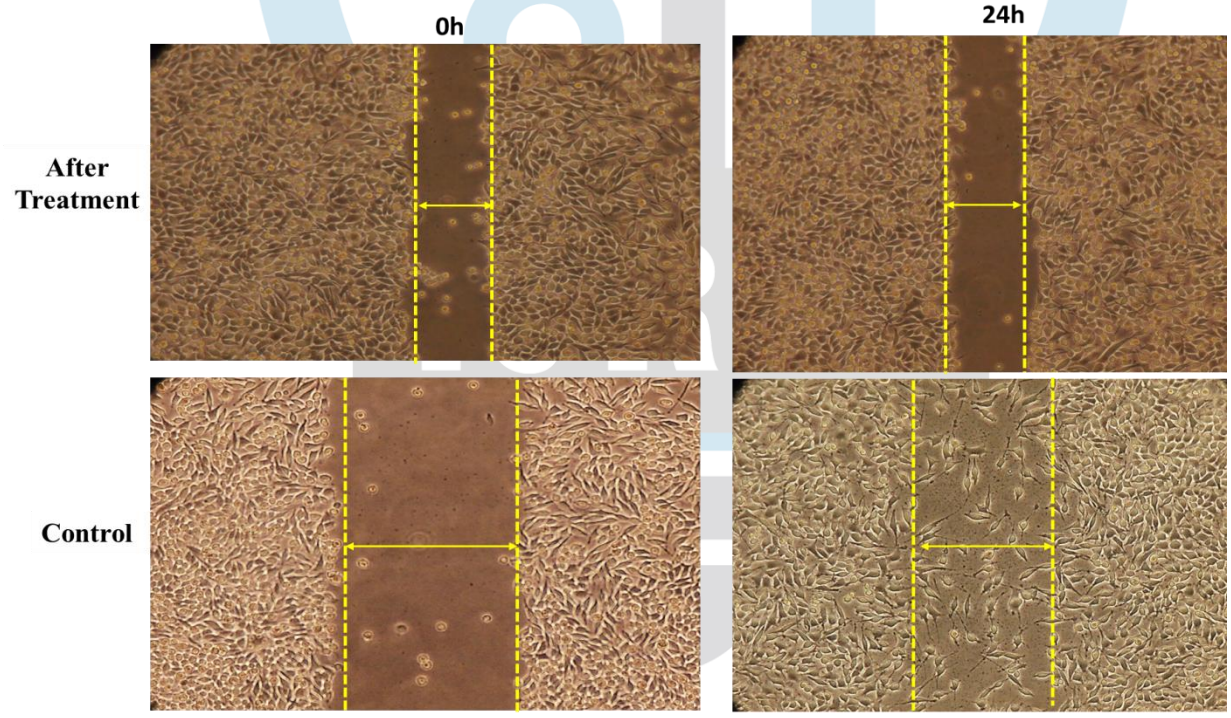

Figure 4: FA-CeO_2 nanoparticle treatment favor in the attenuation of cell migration through depolymerization of actin cytoskeleton

In contrast no such significant alteration in cellular morphology was observed in control cells corroborating the anti-migratory potency of nanoparticles towards breast cancer cells [19].

Evaluation of anti-cancer efficacy of nanoparticles
Further to scrutinize the apoptosis scheme, human colon carcinoma (HCT116) cells was adopted to perform the fluorescent micrographs using the standard protocol of acridine orange (AO) staining.

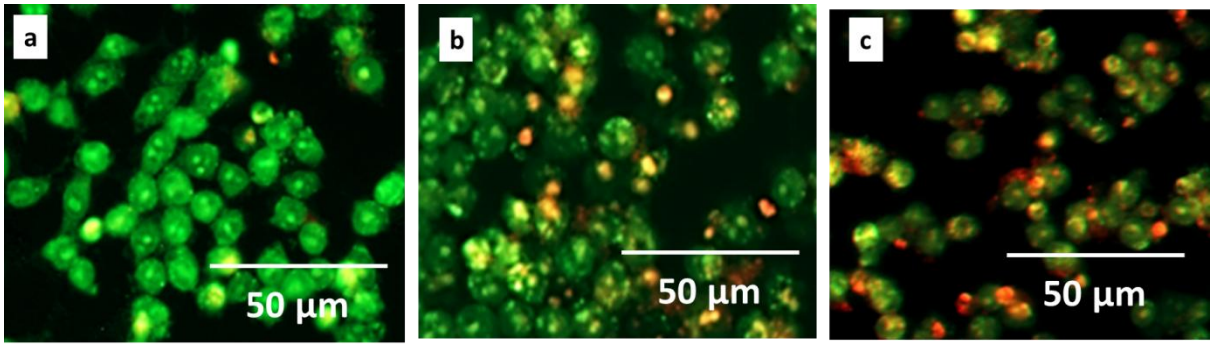

Figure 5: Fluorescent micrographs of HCT116 cells stained with AO/EtBr treated with a) control b) 20µg/ml c) 50 µg/ml of Fe-CeO_2 nanoparticles

As shown in Fig. 5, cells were found to be in the late apoptotic phase, whereas the population of dead cells with lost membrane integrity and fragmented DNA was enhanced in concentration dependent manner. From the above findings it is clearly visible that after treatment with Fe-CeO_2 (50 µg/ml) most of the cells, stained orange distinctive feature of apoptotic cells undergoing program cell death mechanism. [20]

IV. Conclusion

In summary we have elucidated the facile syntheis of folic acid conjugated nanoceria and fundamentally explored its anti-cancer efficacy towards breast cancer cell line. From DAPI micrographs and cell migration assay, it was inferred that loss in cell membrane integrity and constriction of cell proliferation eliciting the potent anti-cancer efficacy towards cancer cells. Further the cytomorphological micrographs validated the above findings illustrating the inhibition of cellular protrusion aiding disrupt cellular morphology. The late apoptosis pathway was also substantiated using AO/EtBr staining method. Henceforth, it is imperative that these functionalized nanoceria spectacle promising applications in plethora of therapeutic and diagnosis area.

V. Acknowledgment

We hereby acknowledge DST-SERB NPDF (Sanction no. PDF/2016/000880), Government of India, for the financial support to carry out the research and the central facility of Centre for Research in Nanoscience and Nanotechnology, University of Calcutta. We thank Dr. Arghya Adhikari (University of Calcutta) for availing those research facilities and immense guidance.

References

[1] C.K. Sen, G.M. Gordillo, S. Roy, R. Kirsner, L. Lambert, T.K. Hunt, et al., Human skin wounds: a major and snowballing threat to public health and the economy, Wound Repair Regen. 17, 2009, pp. 763-771.

[2] R.J. Hay, N.E. Johns, H.C. Williams, I.W. Bolliger, R.P. Dellavalle, D.J. Margolis, et al., The global burden of skin disease in 2010: an analysis of the prevalence and impact of skin conditions, J. Invest. Dermatol. 134 ,2014, pp.1527-1534.

[3] R. Krishnan, S.D. MacNeil, M.S. Malvankar-Mehta, Comparing sutures versus staples for skin closure after orthopaedic surgery: systematic review and meta-analysis, BMJ Open 6, 2016, pp.e009257.

[4] J.J. Blaker, S.N. Nazhat, A.R. Boccaccini, Development and characterisation of silver-doped bioactive glasscoated sutures for tissue engineering and wound healing applications, Biomaterials 25, 2004, pp.1319-1329.

[5] Ma, X.; Tao, H.; Yang, K.; Feng, L.; Cheng, L.; Shi, X.; Li, Y.; Guo, L.; Liu, Z. A functionalized graphene oxide-iron oxide nanocomposite for magnetically targeted drug delivery, photothermal therapy, and magnetic resonance imaging. Nano Res. 2012, 5, pp. 199−212

[6] Vassie, James A; Whitelock, John M and Lord, Megan S. Targeted delivery and redox activity of folic acid-functionalized nanoceria in tumor cells, DOI: 10.1021/acs.molpharmaceut.7b00920.

[7] Wu, H.; Li, F.; Wang, S.; Lu, J.; Li, J.; Du, Y.; Ceria nanocrystals decorated mesoporous silica nanoparticle based ROS Scavenging tissue adhesive for highly efficient regenerative wound healing 10.1016/j.biomaterials.2017.10.018

[8] Hirst, S. M.; Karakoti, A. S.; Tyler, R. D.; Sriranganathan, N.; Seal, S.; Reilly, C. M. Anti-Inflammatory Properties of Cerium Oxide Nanoparticles. Small 2009, 5, pp. 2848–2856.

[9] Xia, T.; Kovochich, M.; Liong, M.; Madler, L.; Gilbert, B.; Shi, H.; Yeh, J. I.; Zink, J. I.; Nel, A. E. Comparison of the Mechanism of Toxicity of Zinc Oxide and Cerium Oxide Nanoparticles Based on Dissolution and Oxidative Stress Properties. ACS Nano 2008, 2, pp. 2121–2134.

[10] Naumann RW, Coleman RL, Burger RA, Sausville EA, Kutarska E, Ghamande SA, Gabrail NY, Depasquale SE, Nowara E, Gilbert L, et al. PRECEDENT: a randomized phase II trial comparing vintafolide (EC145) and pegylated liposomal doxorubicin (PLD) in combination versus PLD alone in patients with platinum-resistant ovarian cancer. J Clin Oncol. 31(35), 2013, pp. 4400–6.
[11] Elnakat H, Ratnam M. Role of folate receptor genes in reproduction and related cancers. Front Biosci. 11, 2006, pp.506–19

[12] Das S, Singh S, Dowding JM, Oommen S, Kumar A, Sayle TXT, Saraf S, Patra CR, Vlahakis NE, Sayle DC, et al. The induction of angiogenesis by cerium oxide nanoparticles through the modulation of oxygen in intracellular environments. Biomaterials. 33(31),2012, pp.7746–55.

[13] Cimini A, D'Angelo B, Das S, Gentile R, Benedetti E, Singh V, Monaco AM, Santucci S, Seal S. Antibody-conjugated PEGylated cerium oxide nanoparticles for specific targeting of Aβ aggregates modulate neuronal survival pathways. Acta Biomater. 2012;8(6) pp. 2056–67

[14] Arijita Basu, Priyanka Upadhyay, Avijit Ghosh, Aparajita Bose, Payal Gupta, Sreya Chattopadhyay, Dipankar Chattopadhyay, Arghya Adhikary, Hyaluronic acid engrafted metformin loaded graphene oxide nanoparticle as CD44 targeted anti-cancer therapy for triple negative breast cancer BBA-General Subjects 1865 ,2021, pp.129841

[15] Berthomieu C, Hienerwadel AER. Fourier transform infrared (FTIR) spectroscopy. Photosynth Res. 2009;101, pp.157–170.

[16] Sriparna De, Kartick Patra, Debatri Ghosh, Koushik Dutta, Aditi Dey, Gunjan Sarkar, Dipankar Chattopadhyay, "Tailoring the Efficacy of Multifunctional Biopolymeric Graphene Oxide Quantum Dot-Based Nanomaterial as Nanocargo in Cancer Therapeutic Application" *ACS Biomater. Sci. Eng.* 2018, 4, 2, pp. 514–531

[17] R U Jänicke , M L Sprengart, M R Wati, A G Porter, Caspase-3 is required for DNA fragmentation and morphological changes associated with apoptosis, J Biol Chem. 1998 Apr 17;273, 16, pp. 9357-60, doi: 10.1074/jbc.273.16.9357

[18] Shadia Al-Bahlani,1 Buthaina Al-Dhahli,1 Kawther Al-Adawi,2 Abdurahman Al-Nabhani,2 and Mohamed Al-Kindi, Platinum-Based Drugs Differentially Affect the Ultrastructure of Breast Cancer Cell Types, BioMed Research International Volume 2017, Article ID 3178794, 13 pages https://doi.org/10.1155/2017/3178794.

[19] Hui Chen and Josephine Nalbantoglu, Ring cell migration assay identifies distinct effects of extracellular matrix proteins on cancer cell migration, Chen and Nalbantoglu BMC Research Notes 2014, 7:183 http://www.biomedcentral.com/1756-0500/7/183

[20] Suresh K. Verma, Ealisha Jha, Pritam Kumar Panda, Arun Thirumurugan, S. K. S. Parashar, Shubhransu Patro, and Mrutyunjay Suar Mechanistic Insight into Size-Dependent Enhanced Cytotoxicity of Industrial Antibacterial Titanium Oxide Nanoparticles on Colon Cells Because of Reactive Oxygen Species Quenching and Neutral Lipid Alteration, ACS Omega 2018, 3, pp.1244−1262