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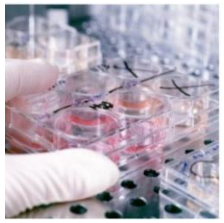
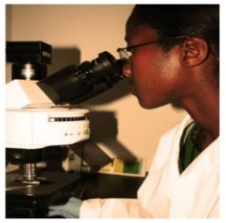


INTRACELLULAR UPTAKE AND TOXICITY OF GOLD NANOPARTICLES (AuNPs) IN REPRESENTATIVE CELL LINES

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Tshwane University of Technology
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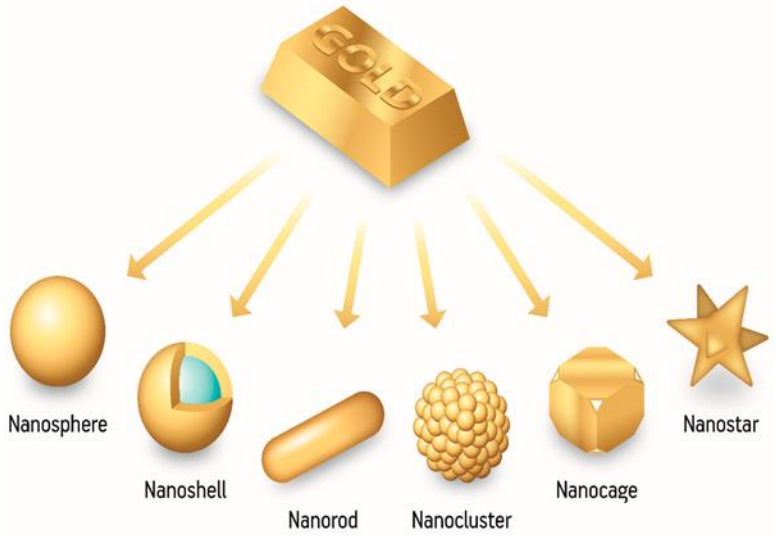
BACKGROUND

What are NPs?

- Nanoparticles (NPs) or Engineered nanomaterials (ENM) - are microscopic entities having at least one dimension 1-100 nm in size.

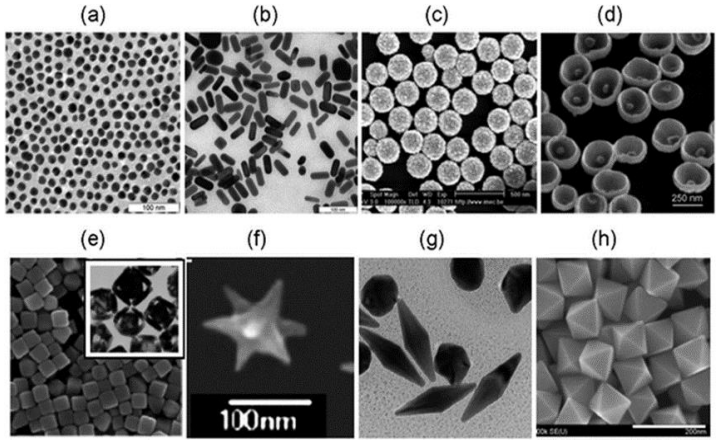
- The most commonly used nanoparticles (NPs) are titanium dioxide (TiO_2), zinc oxide (ZnO), silver (Ag), carbon nanotubes (CNTs), silicon dioxide (SiO_2) and gold (Au).



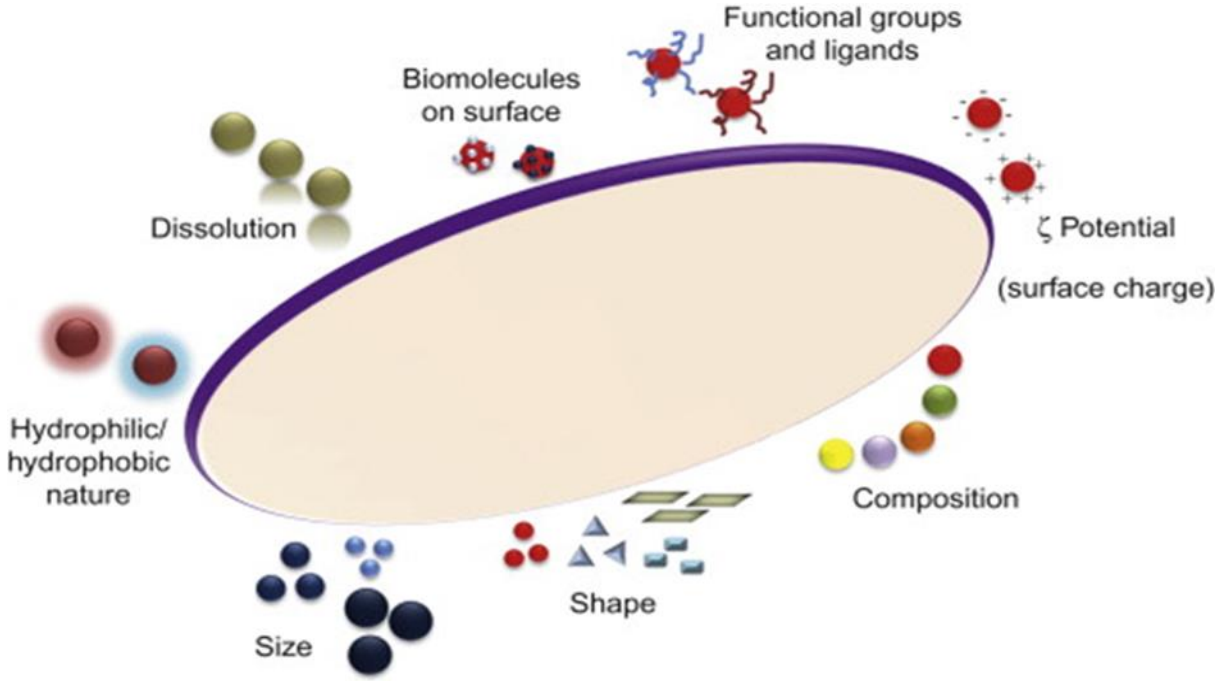
BACKGROUND

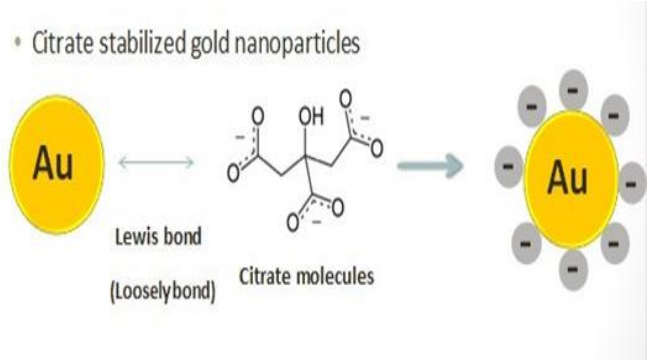
Characteristics of AuNPs

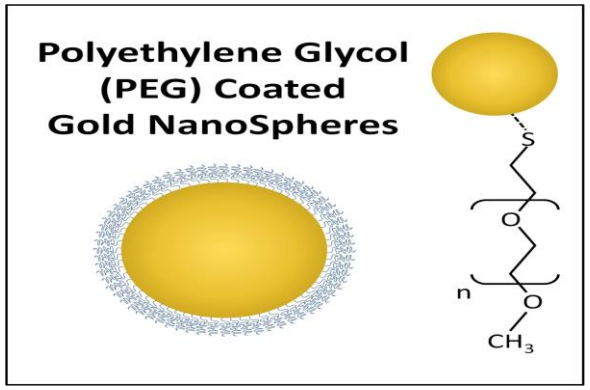
- NPs do not behave like their bulk counterparts
  - posses unique properties



Physiochemical properties of AuNPs



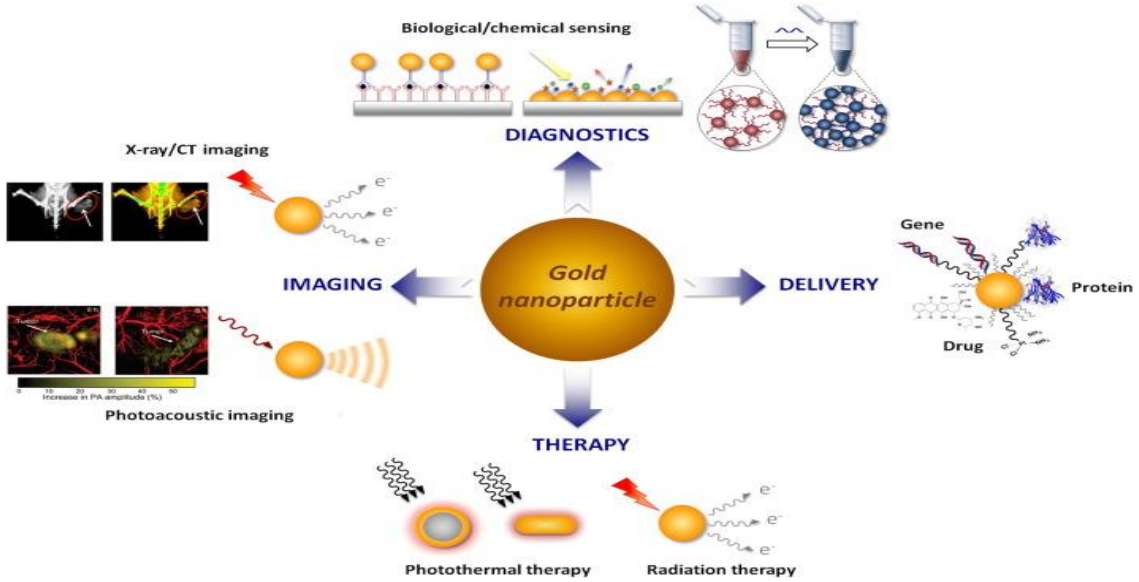




BACKGROUND

Applications of AuNPs

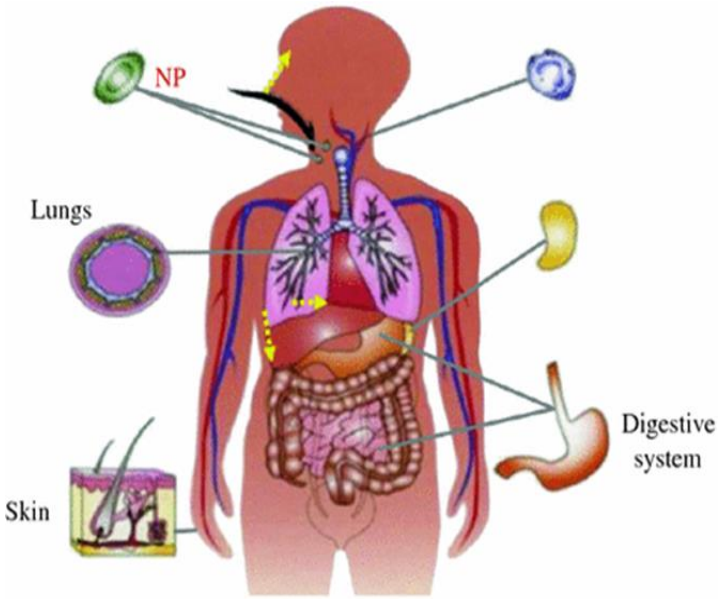
- Modified AuNPs can be used in bio- applications such as:



- Some NPs readily travel throughout the body, deposit in target organs, penetrate cell membranes and may prompt injurious responses.

BACKGROUND

Routes of NP exposure



- Common routes of exposure
  - inhalation
  - intravenous injection
  - **ingestion**
  - **transdermal delivery**

- Human skin keratinocyte (HaCaT) cells are used to investigate the effects of NPs on skin

- The boundary to AuNPs entering the body through direct ingestion is the gastrointestinal (GI) system
  - human epithelial colorectal adenocarcinoma (Caco-2) cells can been used as a general *in vitro* model for intestinal cells.

- Exposure of NPs to various cell lines may lead to uptake of these nanomaterials.

AIM and OBJECTIVES

Aim

- The purpose of this study was to investigate the effects of different functional groups, present on ligands bound to the surface of AuNPs, on their intracellular uptake, cytotoxicity and genotoxicity in the human epithelial colorectal adenocarcinoma cells (Caco-2) and the human skin keratinocyte cells (HaCaT) cells.

Objectives

- To assess the uptake of 14 nm citrate-stabilized (CITR14) AuNPs and 14 nm PEG-liganded amine (PEG-$NH_2$14) AuNPs using the CytoViva darkfield hyperspectral imaging (HSI) system.

- To assess the cytotoxicity of CITR14 AuNPs and PEG-$NH_2$14 AuNPs using the xCELLigence system.

- To assess the genotoxicity of CITR14 AuNPs and PEG-$NH_2$14 AuNPs using the *in vitro* micronucleus assay.

METHODOLOGY

NP Uptake

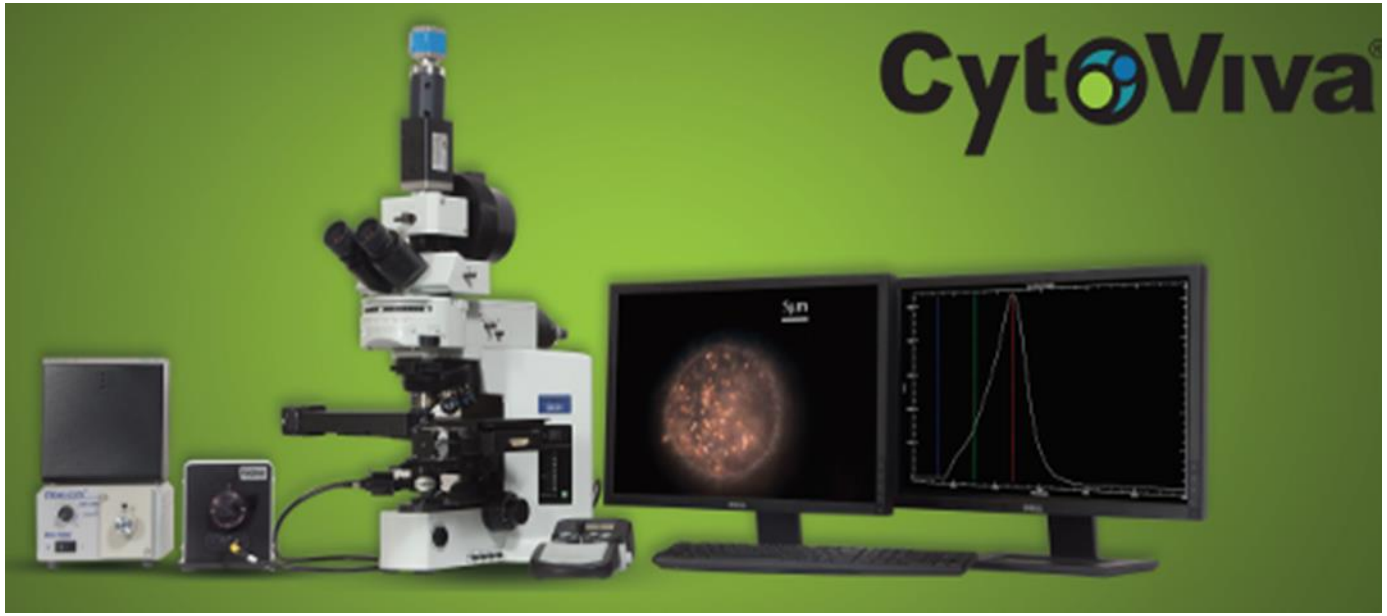
Cell culture

- Caco-2 and HaCaT cells supplemented with 10% fetal bovine serum and 1% penicillin streptomycin

- The CytoViva hyperspectral imaging (HSI) system is a technique used for assessing NP intracellular distribution and it can also confirm the presence of NPs within the cells

NP Internalization
CytoViva HSI

Cells treated with
- CITR14 AuNPs
- PEG-$NH_2$14 AuNPs
- Concentrations -
- 1 nM
- 5 nM



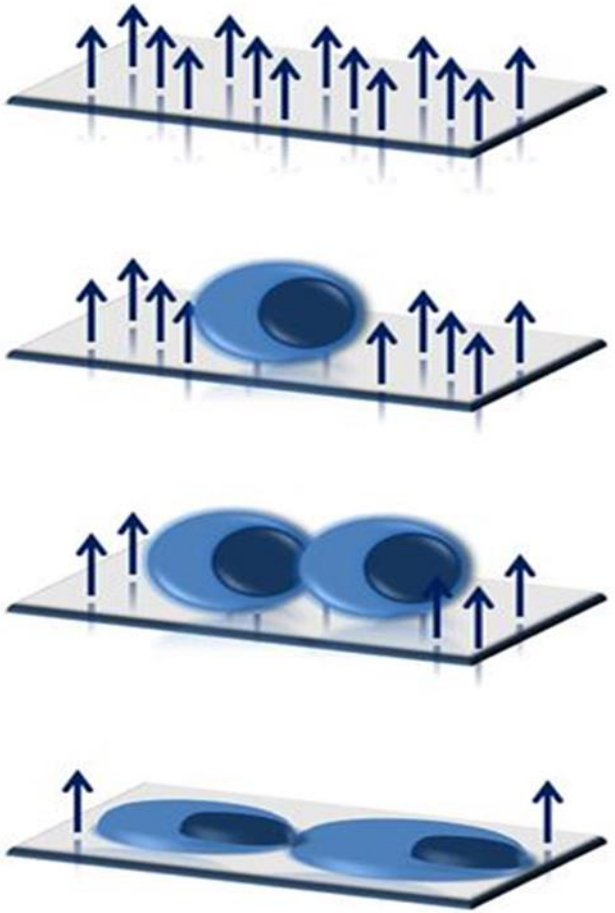
METHODOLOGY

Cytotoxicity

Cytotoxicity
xCELLigence RTCA

Cells treated with AuNPs (in E-plate) – 0.5, 1, 2 & 5 nM

- Assess cell proliferation
- Adhesion
- Cell viability



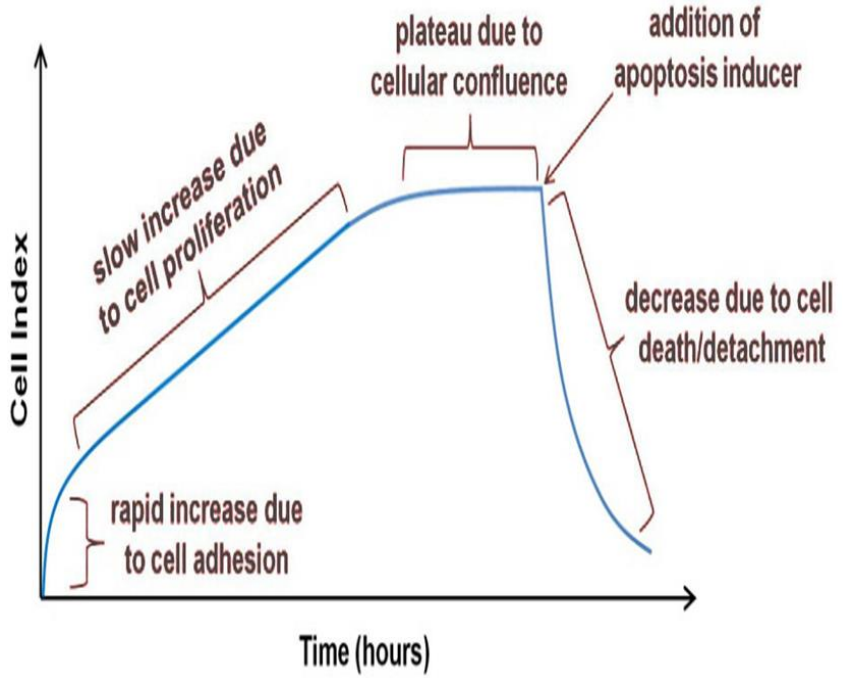


Fig. 1. Principles of the xCELLigence system (Tampa *et al.*, 2016)

METHODOLOGY

Genotoxicity

Micronucleus (MN) assay

- MN assay quantifies subnuclear bodies- micronuclei
  - detects chromosome breakage and changes in chromosome number
- Treated cells were grown for 20 hrs to allow chromosome or spindle damage
  - leading to formation of micronuclei (MNi), nucleoplasmic bridges (NPBs) and nuclear buds (NBuds)

Genotoxicity
Micronucleus assay

- Cells treated with AuNPs
- Cells fixed on microscope slides
- Micronuclei examined by light microscope

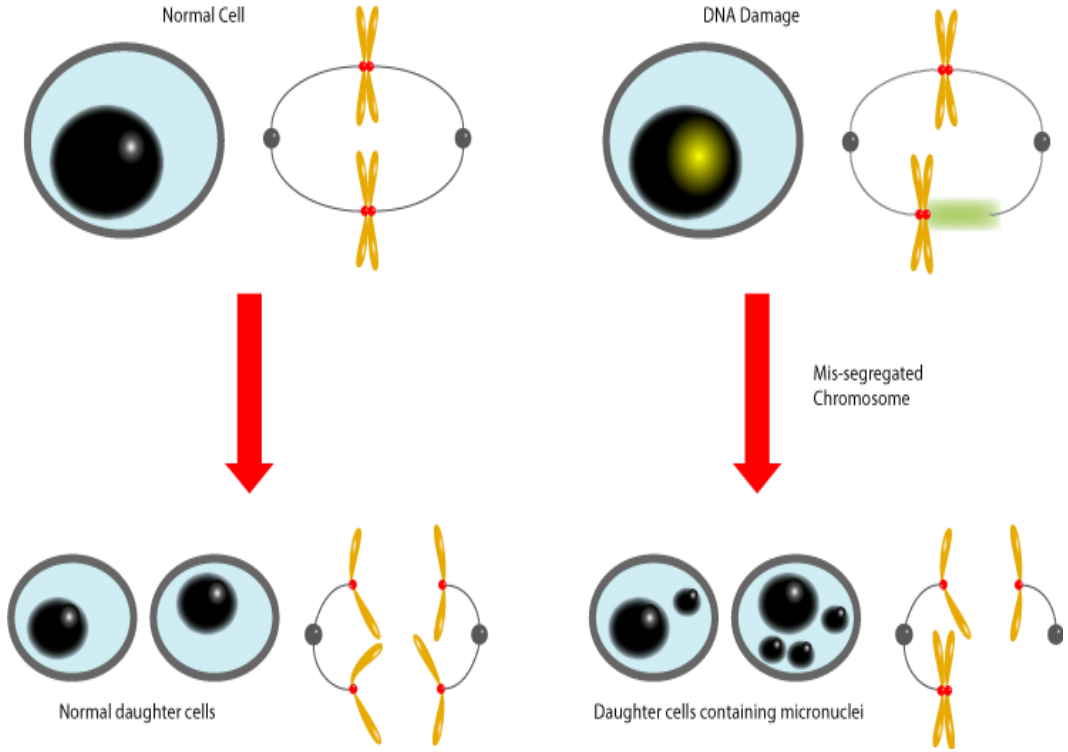


Fig. 2. Principles of the MN assay (Fenech *et al.*, 2007)

RESULTS AND DISCUSSIONS

AuNP uptake

AuNP uptake by Caco-2 cells

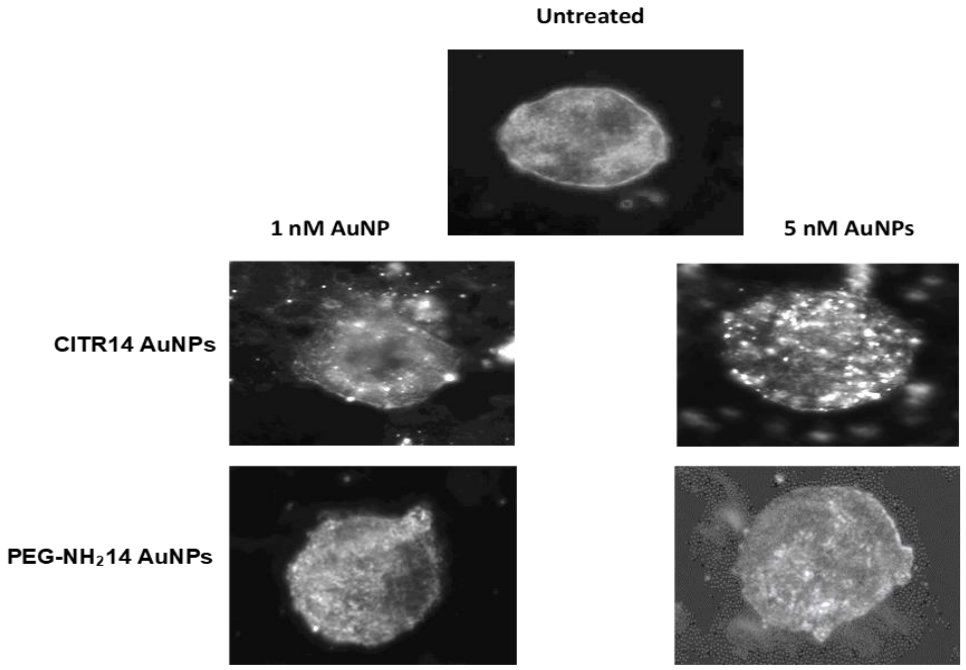


Fig. 3. Caco-2 cells treated with CITR14 AuNPs and PEG-$NH_2$14 AuNPs at concentrations of 1 nM (left panel) and 5 nM (right panel) and for the negative control, cells were not exposed to AuNPs. Cells were exposed to AuNPs for 24 hrs.

AuNP uptake by HaCaT cells

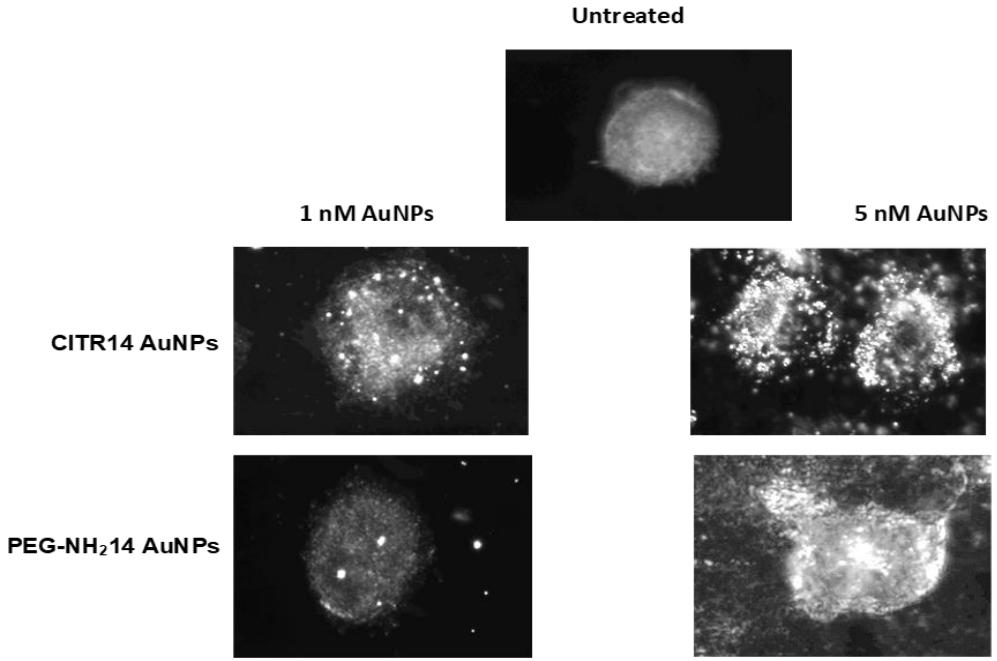


Fig. 4. HaCaT cells treated with CITR14 AuNPs and PEG-$NH_2$14 AuNPs at concentrations of 1 nM (left panel) and 5 nM (right panel) and for the negative control, cells were not exposed to AuNPs. Cells were exposed to AuNPs for 24 hrs.

RESULTS AND DISCUSSIONS

Cytotoxicity of AuNPs

Cytotoxic effects of CITR14 AuNPs in Caco-2 cells

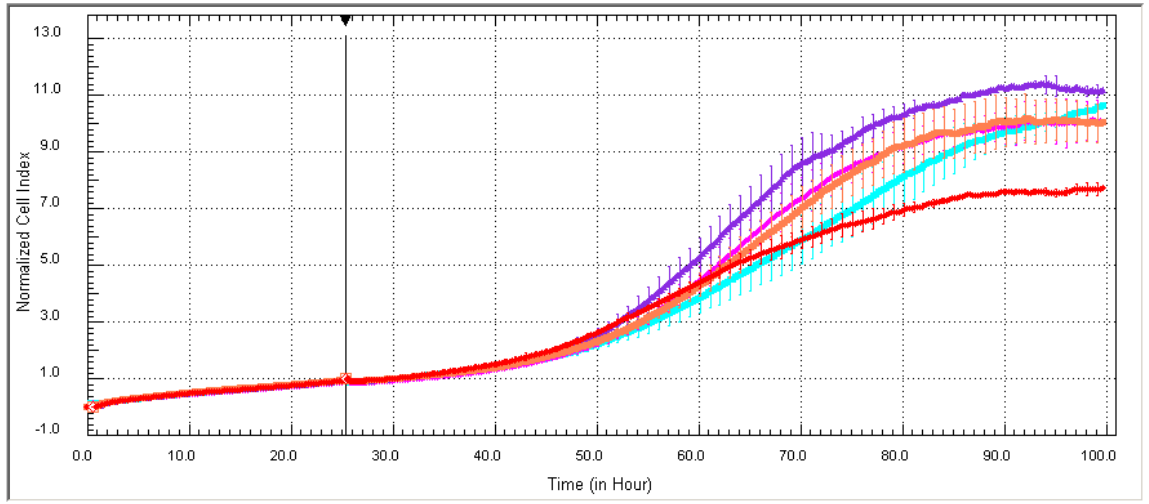


Fig. 5. The cytotoxicity of CITR14 AuNPs in the Caco-2 cells indicated by the normalized CI over a period of 100 hours. Error bars represents SD between the replicates.

- A similar CI for cells treated with 0.5 nM, 1 nM, 2 nM CITR14 AuNPs and the untreated cells, while 5 nM induced significant ($p < 0.01$) CI decrease

Cytotoxic effects of CITR14 AuNPs in HaCaT cells

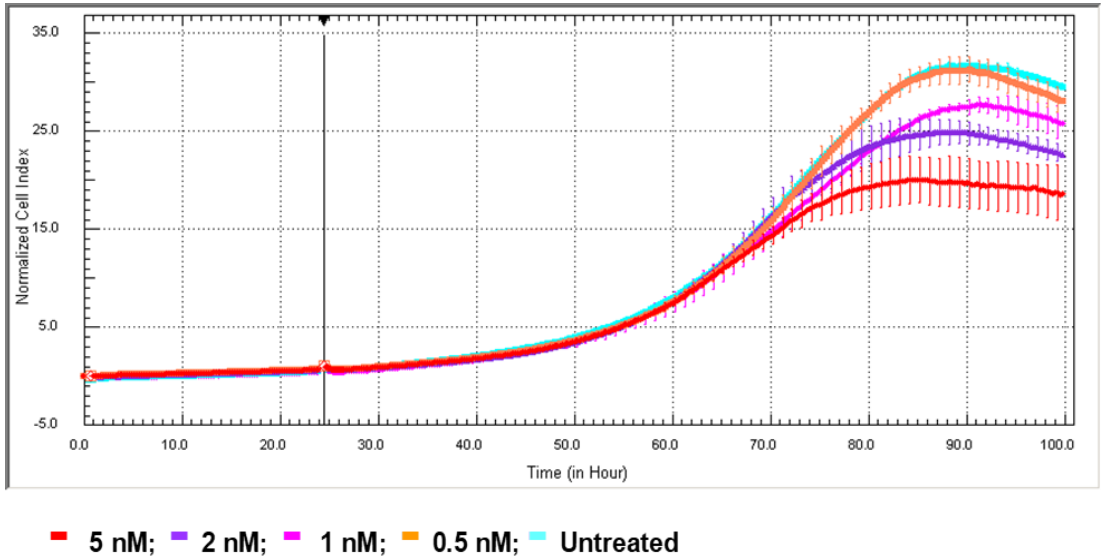


Fig. 6. The cytotoxicity of CITR14 AuNPs in the HaCaT cells indicated by the normalized CI over a period of 100 hours. Error bars represents SD between the replicates.

- 5 nM reduced CI the most and showed a statistically significant difference ($p < 0.001$) from the untreated control.
- No significant difference ($p > 0.05$) between other treatments and the control.

RESULTS AND DISCUSSIONS

Cytotoxicity of AuNPs

Cytotoxic effects of PEG-$NH_2$14 AuNPs in Caco-2 cells

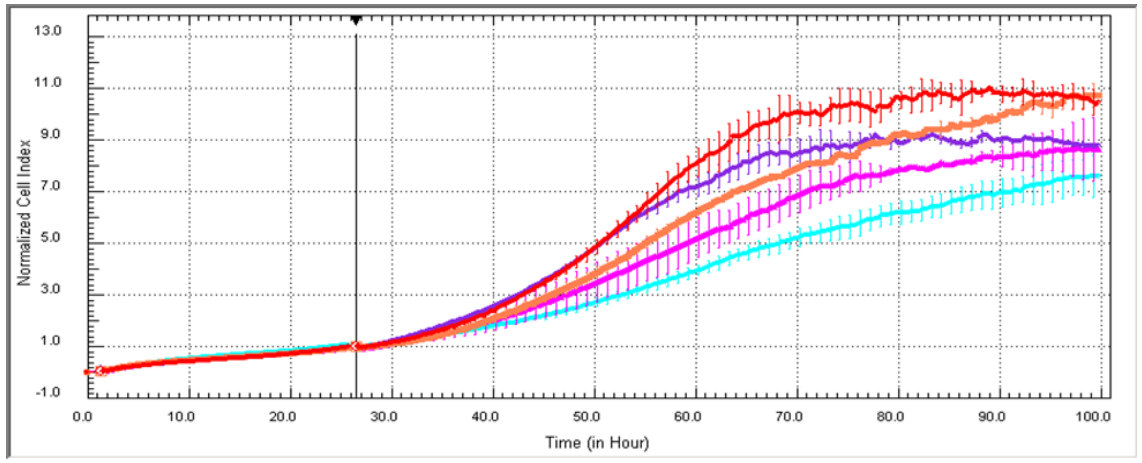


Fig. 7. The cytotoxicity of PAM14G AuNPs in the Caco-2 cells indicated by the normalized CI over a period of 100 hours. Error bars represents SD between the replicates.

- No significant difference was observed between the cells treated with PEG-$NH_2$14 AuNPs and the untreated control ($p > 0.05$)

Cytotoxic effects of PEG-$NH_2$14 AuNPs in HaCaT cells

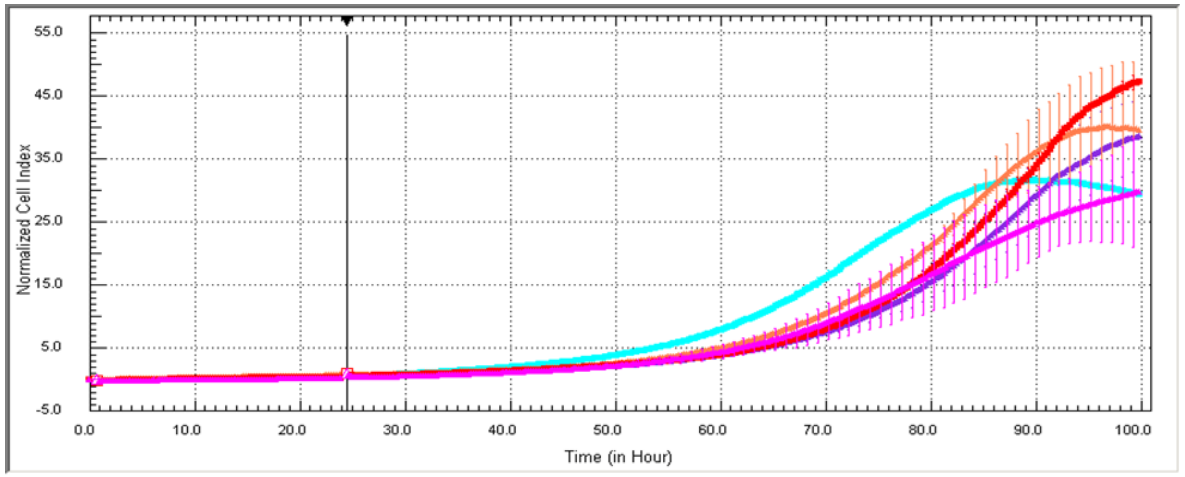


Fig. 8. The cytotoxicity of PEG-$NH_2$14AuNPs in the HaCaT cells indicated by the normalized CI over a period of 100 hours. Error bars represents SD between the replicates.

- All concentrations increased CI, there was no statistical difference ($p > 0.05$) between the treatments and the untreated control.

RESULTS AND DISCUSSIONS

Genotoxicity of AuNPs

- Different types of chromosome aberrations
- Visualized and scored using the white light microscope at 100 X magnification

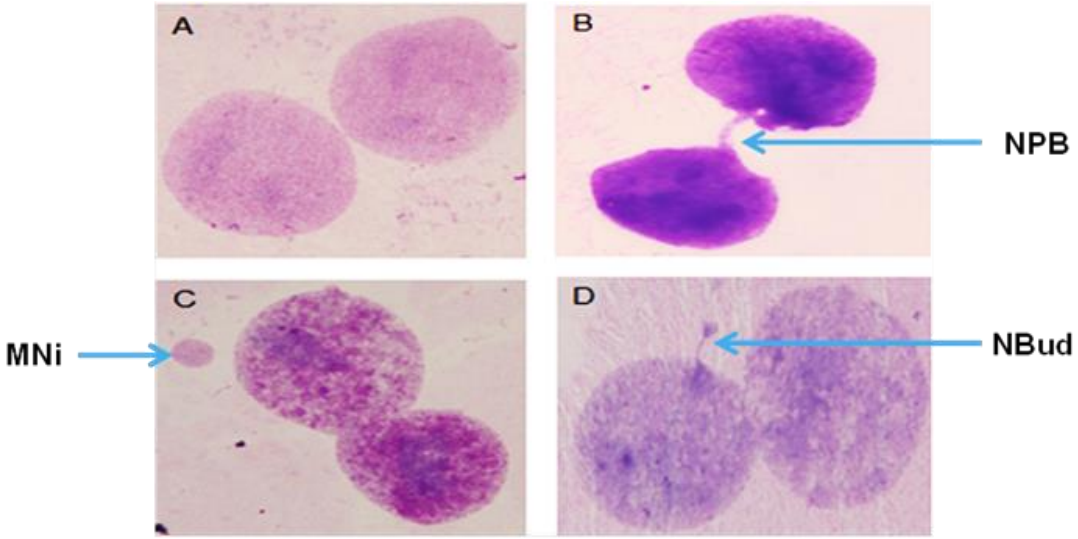


Fig. 4.19. Different types of chromosome aberrations formed in the Caco-2 and HaCaT cells using the MN assay. (A) Untreated binucleated Caco-2 cells with no aberrations, (B) binucleated cells with a NPB, (C) binucleated cell with a MNi and (D) binucleated cell with a NBud

RESULTS AND DISCUSSIONS

Genotoxicity of AuNPs

Genotoxic effects of CITR14 AuNPs in Caco-2 cells

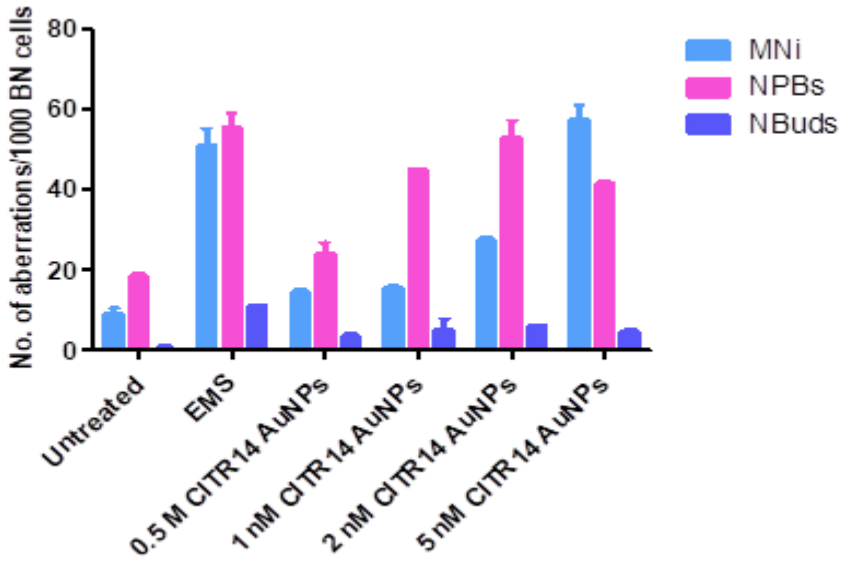


Fig. 10. Genotoxicity of CITR14 AuNPs at various concentrations. Ethyl methanesulfonate (EMS) was used as a positive control. Error bars represents mean SD.

- 2 nM and 5 nM AuNPs induced statistically significant ($p < 0.001$) MNi
- NPBs 1 nM and 5 nM ($p < 0.01$) and 2 nM ($p < 0.001$)
- All treatments induced significant nuclear buds ($p < 0.05$).

Genotoxic effects of CITR14 AuNPs in HaCaT cells

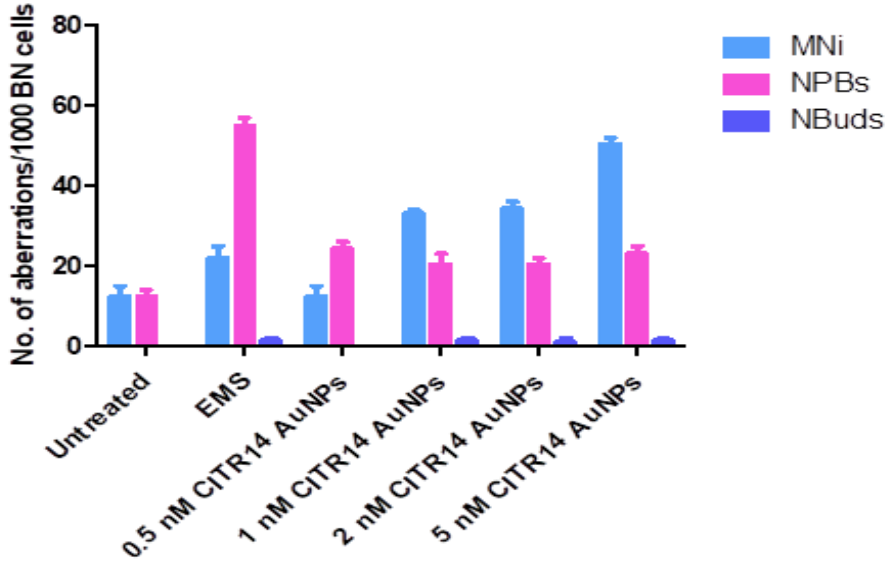


Fig. 11. Genotoxicity of CITR14 AuNPs at various concentrations. EMS was used as a positive control. Error bars represents mean SD.

- MNi - 1 nM ($P < 0.01$), 2 nM and 5 nM ($P < 0.001$)
- NPBs - all treatments ($p < 0.05$)
- NBuds - all treatments ($p > 0.05$)

RESULTS AND DISCUSSIONS

Genotoxicity of AuNPs

Genotoxic effects of PEG-$NH_2$14 AuNPs in CaCo-2 cells

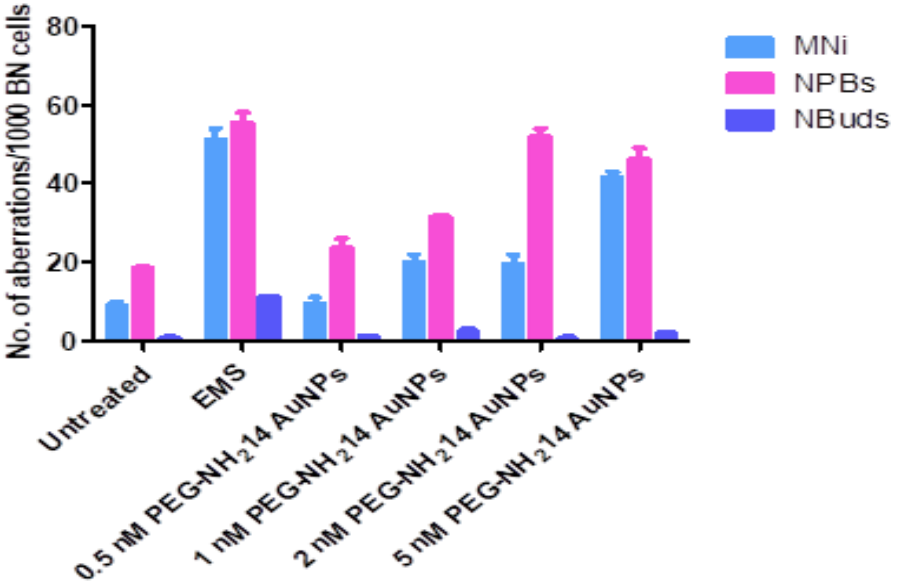


Fig. 12. Genotoxicity of PEG-$NH_2$14 AuNPs at various concentrations. Ethyl methanesulfonate (EMS) was used as a positive control. Error bars represents mean SD.

- MNi - 1 nM and 2 nM ($p < 0.05$) and 5 nM ($p < 0.001$)
- NPBs - 2 nM and 5 nM ($p < 0.01$)
- Buds - all treatments ($p > 0.05$)

Genotoxic effects of PEG-$NH_2$14 AuNPs in HaCaT cells

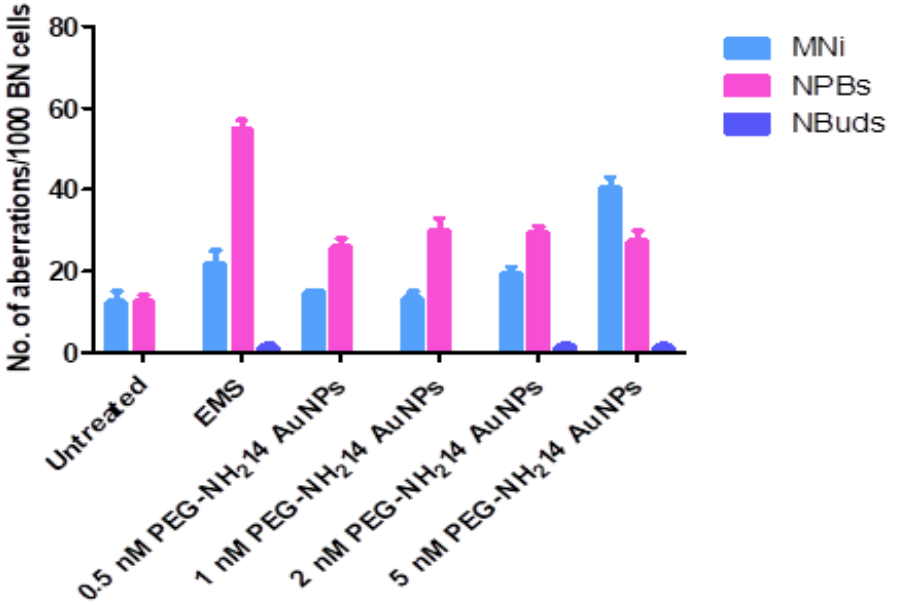


Fig. 13. Genotoxicity of PEG-$NH_2$14 AuNPs at various concentrations. EMS was used as a positive control. Error bars represents mean SD.

- MNi - 5 nM ($p < 0.001$)
- NPBs- all treatments ($p > 0.05$).
- Buds - 2 nM and 5 nM ($p < 0.01$)

CONCLUSIONS

- The citrate-stabilized AuNPs entered both the Caco-2 and HaCaT cell lines at a higher level compared to PEGylated AuNPs.
  - This suggests that PEGylation discourages AuNP uptake
- CITR14 AuNPs showed a significant reduction in CI at the highest concentration of 5 nM in Caco-2 and HaCaT cells, therefore indicating cytotoxicity.
- In contrast, PEG-$NH_2$14 AuNP induced cell proliferation indicating that the amine functional group possibly enhance cell proliferation.
- All types of chromosomal aberrations (MNi, NPBs and NBuds) were formed by both the AuNPs in both cell lines.
  - NPBs abundant
- Therefore, non-cytotoxic concentrations have the ability to induce genotoxic effects in Caco-2 and HaCaT cell lines.
- Suggesting that DNA damage was due to indirect mechanisms, such as the production of reactive oxygen species (ROS).

FUTURE WORK

- Gene expression profiles of AuNP treated cells should be determined to show the regulation of genes and growth factors that affect cell proliferation.

- Effects of different chemical functional groups, upon cell entry and toxicity must be investigated.

- ROS generation must be investigated to ascertain the possibility of oxidative DNA damage being the cause of the genotoxicity seen in this study.

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