Investigation of gold nanoparticle cytotoxicity and uptake in kidney cells *in vitro*

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Introduction

As nanoscience and nanotechnology advances, the importance of a detailed knowledge of interactions between nanomaterials, organisms, tissues and cells is becoming increasingly important. Nanoparticles can enter the human body through ingestion, inhalation and through skin absorption and proceed to interact with various biomolecules such as proteins, sugars and lipids found in the interstitial fluid between blood, lymph, and cells such as kidney cells. Previous studies demonstrated the sensitivity of kidney cells to nanoparticles hence they were used in this study. Studies have shown that nanotechnology can greatly revolutionize the way we diagnose and treat diseases such as cancer, but the ability to regulate how the engineered nanoparticles interact within our internal environment remains an area of large focus The aim of this study was to investigate the cytotoxicity and uptake of 14nm polyethylene glycol carboxyl (PEG-COOH) gold nanoparticles in human embryonic kidney (HEK 293) cells *in vitro*.

AuNPs cytotoxicity

1. Effects of AuNPs (2nM, 5nM, 10nM) on cell proliferation in HEK 293 cell line using xCELLigence real time cell analyser (RTCA)

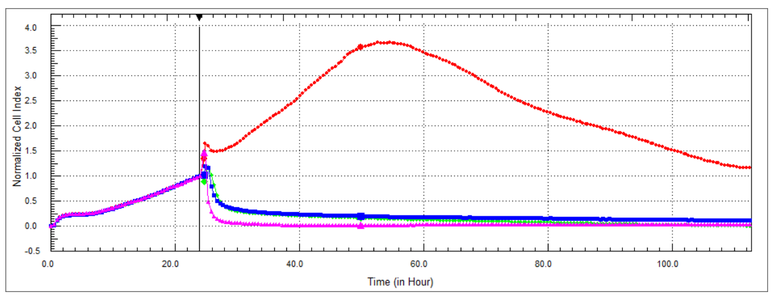

Figure 1 : Dynamic monitoring of cell proliferation. Cells were seeded in the E-Plate 96, and continuously monitored by measuring cell index (CI) to investigate the toxicity of the AuNPs in the concentration range :(2nM, 5nM, 10nM) Cells were seeded to allow for attachment and after 24 hours they were treated with the AuNPs at concentrations listed above. The adhesion, spreading, and proliferation of HEK 293 cells were dynamically monitored every 15 minutes for 100 hours using the RTCA SP instrument. Colored curves indicate the different AuNPs concentrations (nM) seeded per well in an E-Plate 96 : green: 2nM; dark blue: 5nM; pink:10nM; red: untreated cells.

2. Effects of AuNPs (0.13nM , 0.25nM, 0.5 nM, 1nM and 2nM) on cell proliferation in HEK 293 cell line using xCELLigence real time cell analyser (RTCA)

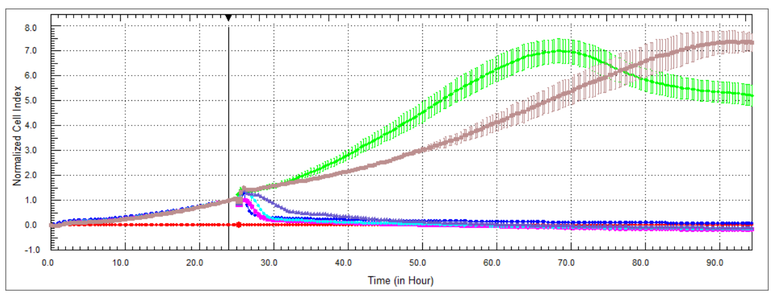

Figure 2 : Dynamic monitoring of cell proliferation. Cells were seeded in the E-Plate 96 and continuously monitored by measuring cell index (CI) to investigate the toxicity of the AuNPs in the concentration range :(0.13nM , 0.25nM, 0.5 nM, 1nM and 2nM). Cells were seeded to allow for attachment and after 24 hours they were treated with the AuNPs at concentrations listed above The adhesion, spreading, and proliferation of HEK 293 cells were dynamically monitored every 15 minutes for 90 hours using the RTCA SP instrument. Colored curves indicate the different AuNPs concentrations (nM) seeded per well in an E-Plate 96 : **red:** no cells; **green:** untreated cells; **dark blue:** 2nM; **pink:** 1nM; **Turquoise blue**: 0.5nM; **purple:** 0.25; **brown:** 0.13 nM.

AuNPs Spectral imaging

3. Spectral profile of AuNPs on HEK 293 cell lines using Hyper spectral imaging (HIS)

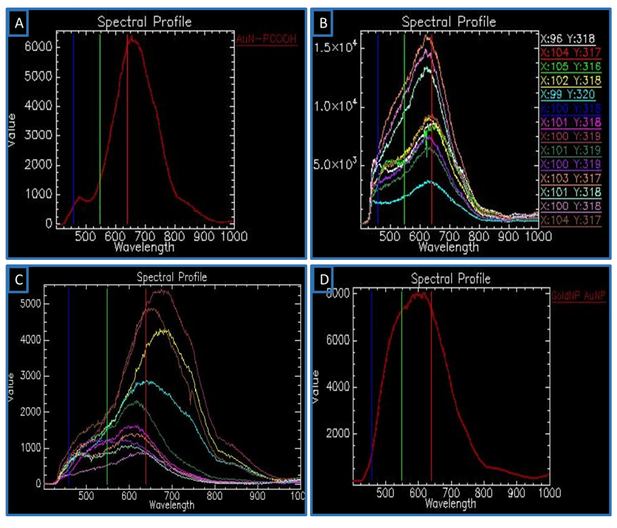

Figure 3 : Spectral profile showing wavelength versus intensity value of nanoparticles. COOH-PEG-AuNPs showing a cross-sectional scan of nanoparticles from the gold nanoparticle only control slide. **A** and **D =** Singe nanoparticles**. B and C =** Different nanoparticles. Spectral profile **A** was then used to create a spectral library and for spectral angle mapping.

Methods

1. Human embryonic kidney (HEK 293) cells were seeded at a density of 10 000 cells/cm² to allow for attachment.

2. Morphology and size of the nanoparticles was analysed using transmission electron microscopy (TEM)

3. Attached cells were incubated with 14 nm PEG-COOH gold nanoparticles (AuNPs) [0.13nM-10nM] for at least 24 hours.

4. Cytotoxicity was assessed using xCELLigence real time cell analyser.

6. AuNPs cellular distribution was assessed using spectral angle mapping features of the Cytoviva hyperspectral imaging.

5. AuNPs uptake was analysed using darkfield microscoy features of the Cytoviva hyperspectral imaging.

Results and Conclusion

HEK 293 cells exposed to gold nanoparticles at a concentration of 0.5 nM, 1nM, 2nM and 10 nM induced significant cytotoxicity after 24 hours of exposure when compared to the untreated cells. These concentrations induced an 8%, 32%, 54% and 96% statistically significant (p<0.05) decrease in cell proliferation after 24 hours of exposure respectively. Cells were subsequently treated with gold nanoparticles at a concentration of 0.13nM and 0,25nM, in order to access cytotoxicity at a lower concentration range. The 0.13nM concentration did not affect cell proliferation while the 0.25nM induced a statistically significant (p<0.05) decrease in cell proliferation.

Dark field microscopy using Cytoviva hyperspectral imaging micrographs showed that the gold nanoparticles, at a sub-lethal concentration of 0.03nM were internalised by the cells at 24 hours, while they were only embedded on the surface membrane at 18 hours and not taken up by the cells at 4 hours.

Spectral angle mapping using Cytoviva further confirmed the cellular internalization of the gold nanoparticles disbursed throughout the cells.

These findings illustrate that the gold nanoparticles are taken up by the cells and induce cytotoxicity. A previous study by Liu *et el* (2014) found that the growth of A549 cells was inhibited after treatment with 5-nm gold nanoparticles.

Future studies will investigate the possible induction of autophagy and lysosomal membrane permeabilisation by the internalised nanoparticles and assess the interference of these AuNPs with the biological assays to be employed.

AuNPs cellular uptake

4. Uptake of AuNPs by HEK 293 cell line using darkfield microscopy

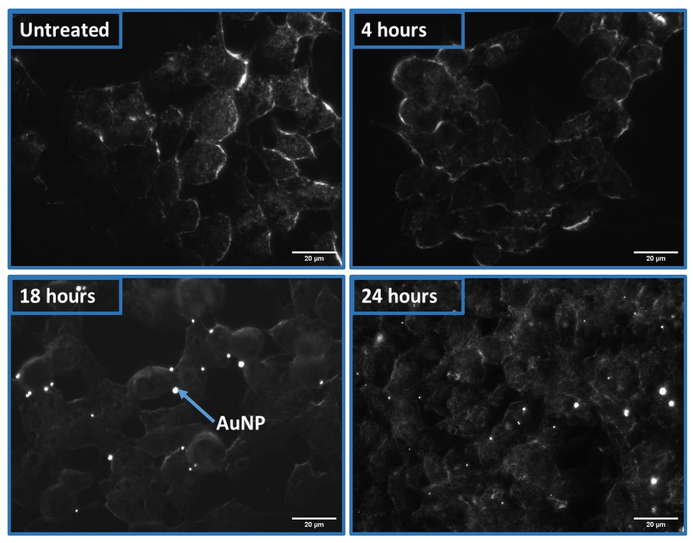

Figure 4: Uptake of gold nanoparticles using darkfield microscopy. HEK 293 cells were incubated with PEG-COOH AuNPs for 4 hours, 18 hours and 24 hours and uptake of the AuNPs was visualised using the darkfield microscopy feature of Cytoviva hyperspectral imaging at 60 x magnification. The gold nanoparticles can be distinguished in the images as bright figures resulting from their intense ligh-tscattering ability as labelled in the 18 hour image as AuNP. (Scale bar 20µm)

5. Uptake of AuNPs on HEK 293 cell lines using Spectral angle mapping

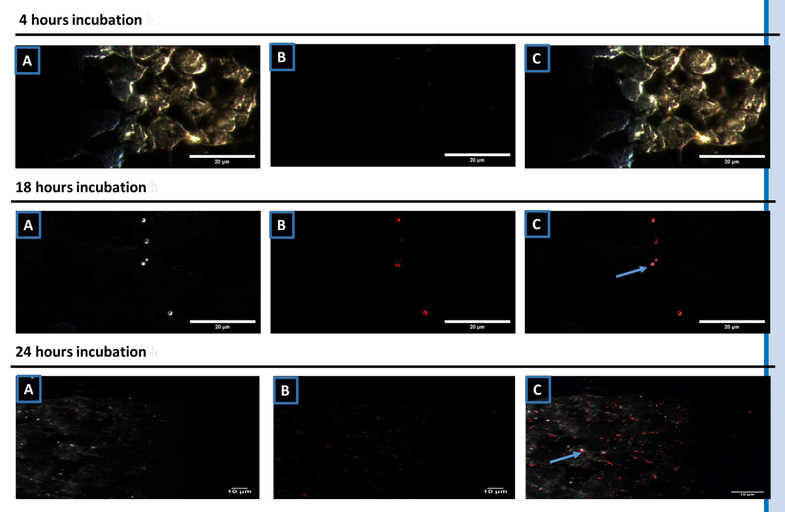

Figure 5: Uptake of gold nanoparticles using spectral angle mapping. HEK 293 cells were incubated with PEG-COOH AuNPs for 4 hours, 18 hours and 24 hours respectively and uptake of the AuNPs were visualised using the spectral angle mapping feature of Cytoviva hyperspectral imaging at 60 x magnification. The spectral profile created in **figure 3** was used to identify the gold nanoparticles as red pixels from hyperspectral images (HIS) as indicated in **5(B).** The pixels were then mapped onto the HIS images as indicated in **5(C).** The arrows in **5(C)** show the individual nanoparticles mapped with the red pixels. 5 (A) shows the HSI scan, **5 (B)** the gold nanoparticle pixels in red and **5 (C)** the mapped HIS scan with the red gold nanoparticle pixels. (Scale bar 20µm and 10µm)

6. Characterisation of AuNPs (morphology)

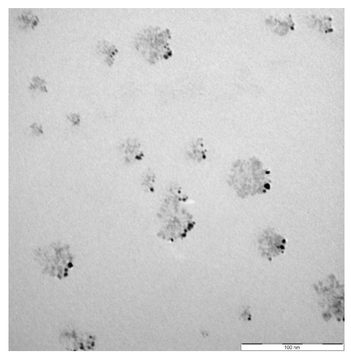

Figure 6: AuNPs suspended in MilliQ water. 14nm polyethylene glycol carboxyl (PEG-COOH) gold nanoparticles suspended in milliQ water as provided by Mintek. Nanoparticles were analysed using JOEL transmission electron microscopy (TEM) at 50x magnification. (Scale bar 100nm)

References

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