Gold nanoparticles as a tool to detect UTI and their cytotoxic effects in DU-145 cells

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ABSTRACT

Urinary tract infection (UTI) is a predominant condition in prostrate cancer patients. *E. coli* ORN178 (EC 178) is the uropathogen that causes recurrent infection by binding specifically to adhesins of DU-145 cells. Gold nanoparticles (GNPs) have been used in biodiagnosis of pathogens. In this study we have investigated the binding time of EC 178 to DU-145 cells and the cytotoxicity of plain and mannose functionalized GNPs (Mn-GNPs). It was seen that EC 178 binds to the cells by 3 h of incubation with the cell line, plain 20 nm GNPs decrease the percentage of viable cells in 48 and 72 h, and, Mn-GNPs along with EC 178 exhibit cytotoxic effects at the end of 3 h of incubation with DU-145 cells.

Keywords: gold nanoparticles, *E. coli*, DU-145 cells, urinary tract infection, cytotoxicity, biodiagnostic

1 INTRODUCTION

The estimated risk of prostrate cancer is 21% and the lifetime risk of death is 2-5%. Even though it can be diagnosed early and therapy can be started immediately, patients developing metastatic conditions die. Apart from the potent issue of cancer, recurrent UTI is one of the most prevalent symptoms [1]. EC 178 is the most common cause of UTI in humans. Various urovirulence factors of EC 178 have been identified such as molecular biology of surface receptors of the urothelial cells of the urinary tract, the adhesin specificity and primarily and the Type 1 fimbriae associated with the organism [2]. Detection, diagnosis and treatment of UTI play an important role in prostrate cancer. One of the novel tools currently vastly studied are nanoparticles. Nanoparticles continue to be used as carriers for localized drug diffusion to treat and detect infections and diseases like cancer [3]. Owing to their *nano* size it is easy for these particles to diffuse into the cells and effect desired responses in treatment of diseases. However, the size, type and surface charges of the particles play a vital role. Of the different kind of nanoparticles GNPs have been extensively studied in this regard. It has been shown that EC 178 binds specifically to D-mannose, which is an integral part of the glycoproteins that are a part of the adhesive domain on host cells [4]. The fimbriae of the uropathogenic *E. coli* bind to the urolapkins on the surface of urothelial cells of the human bladder. We have demonstrated D-mannose functionalized 200 nm GNPs bind specifically to EC 178 [5]. To continue in the same direction in this study we attempt to see if GNPs can be used as specific biodiagnostic tool to detect and treat UTI in prostrate cancer cells (DU-145). This study investigates the binding time of EC 178 and *E. coli* ORN208 (EC 208) to DU-145 cells. It has been shown that EC 178 binds specifically to D-mannose only and EC 208 serves as a negative control as it has Type 1 pili that fail to bind to D-mannose. The cytotoxicity tests of plain and functionalized 20 nm and 200 nm GNPs to DU-145 was performed to study their biocompatibility *in vitro*.

2 MATERIALS AND METHODS

The strains EC 178 and 208 were provided by Dr. Chu-Cheng Lin, Department of Zoology, National Taiwan Normal University and were transformed with plasmid pGREEN by electroporation. Two different GNPs:20 nm and 200 nm were purchased from Ted Pella Inc., USA. The concentration of 20 nm GNPs was 7×10^{11} particles/ml and 200 nm GNPs was 7×10^{8} particles/ml. The D-mannan (Mn) was purchased from VWR (USA). The sugar were dissolved in 0.3 M sodium phosphate buffer. Surface functionalization of GNPs with the Mn was carried out by a modified multistep procedure [5-6]. All chemicals required for funcationalizing GNPs were purchased from VWR, USA. Human prostate carcinoma cell line DU-145 was graciously given by Dr Arun Sreekumar, Baylor College of Medicine, Houston, Texas. Dulbecco's modified Eagle's medium (DMEM) was modified to contain Earles Balanced Salt Solution, non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate, and 1500 mg/L sodium bicarbonate. It was supplemented with fetal bovine serum to a final concentration of 10%, 100 UI/ml penicillin G, and 100 µg/ml streptomycin in a humidified incubator with 5% CO2 in the air at 37°C. All the media components were purchased from Promega, USA.

2.1 Binding of *E. coli* to DU-145 cells

Two 8 well chamber slides were used to perform the cell adhesion assay. A total of 0.5 ml of DU 145 cells (1.5 x 10^6cells/ml) were seeded in each well. The chamber slides were incubated at 37°C overnight for attachment and fresh media was added. EC 178 and 208 were cultured overnight in Tryptic Soy Broth with ampicillin (50 µg/ml). The cultures were washed in sterile PBS and resuspended in sterile PBS. A 100 µl (3 x 10^8 cells/ml) aliquot of EC 178 and 208 was added to all wells in the first and second chamber slides. The slides were incubated for 1, 2 and 3 h.

At the 3 different time periods wells were washed with with PBS and images were taken by a fluorescent scope (Zeiss LSM-510).

2.2 Cytotoxicity of GNPs to DU-145 cells

The cytotoxicity of plain GNPs was tested by CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS) purchased from Promega, USA. It is a colorimetric method for determining the percentage of viable cells that are proliferating. Briefly, the MTS tetrazolium is bioreduced by the viable cells into a colored formazan product which is stable and can be measured at an absorbance of 490 nm. A 100 µl aliquot of DU-145 cells (1.5 x 10^6 cells per well) were seeded in a 96 well plate. Plain GNPs were added as listed in Table 1.

Table 1. Final concentration of 20 nm and 200 nm GNPs in the wells

20 nm		200 nm	
Amount	Particles	Amount	Particles
1 µl	7 x 10^8	1µl	7.8 x 10^5
2 µl	1.4 x 10^9	2 µl	1.4 x 10^6
4 µl	2.8 x 10^9	4 µl	3.1 x 10^6
8 µl	5.6 x 10^9	8 µl	6.1 x 10^6

The plates were incubated for 24, 48 and 72 h. MTS assay was performed and the percentage of cell viability was determined.

2.3 Cytotoxicity of functionalized GNPs to DU-145 cells and *E.coli* ORN178 and 208

The cytotoxicity of functionalized Mn-GNP was carried out by adding 200 µl of DU-145 cells (1.5 x 10^6 cell per well) into a 96 well plate. After incubation at 37°C for 24 h, the cells were treated with 20 nm and 200 nm GNPs, GNPs with bacteria EC 178 and 208 (Table 2). A total of 50µl of 20 nm and 200 nm was added. For the preincubated mix, 1ml of the GNPs and 1 ml of the microorganisms were incubated at 37°C with shaking for 1 h. The solution was centrifuged and washed with sterile PBS and resuspended in sterile PBS before adding to the 96 well plate. Three different plates were set up for 1, 2 and 3 h time periods. The MTS assay was performed.

Table 2. Different treatments performed on DU-145 cells

Control - DU145
A - DU145+NP (200 nm) then added EC after 1hr
B- DU145+NP (20 nm) then added EC after 1hr
C- DU145+NP+EC all together (200 nm)
D- DU145+NP+EC all together (20 nm)
E- DU145+NP+EC preincubated mixture (200 nm)
F- DU145+NP+EC preincubated mixture (20 nm)
G - DU145+NP 200 nm
H - DU145+NP 20 nm
I - DU145 and EC only

2.4 Statistical analysis

All experiments were carried out in triplicates with results expressed as mean ±standard error (SE). Statistically significant differences were calculated using the two-tailed unpaired t-test or one-way analysis of variance (ANOVA) with a p value of ≤0.05 considered significant using Prism 5.0 (GraphPad Software, CA).

3 RESULTS

3.1 Binding of *E. coli* to DU-145 cells

The binding of *E. coli* ORN178 and ORN208 to DU-145was studied for 1, 2 and 3 h. Phase contrast images were taken as shown in Figure 1. It was seen that the EC 208 cells did not bind to DU-145 cells at all during all the three time periods which is expected as it serves as a negative control (Fig. 1B). On the contrary the EC 178 cells tend to bind to the cell wall of the DU-145 cells at 2 h time period (Fig. 1D). After the 3 h time period they appear to be inside the DU-145 cell line (Fig. 1E). Due to the wash step after time periods it is seen that only few cells manage to bind and enter the cell line between 2-3 hours.

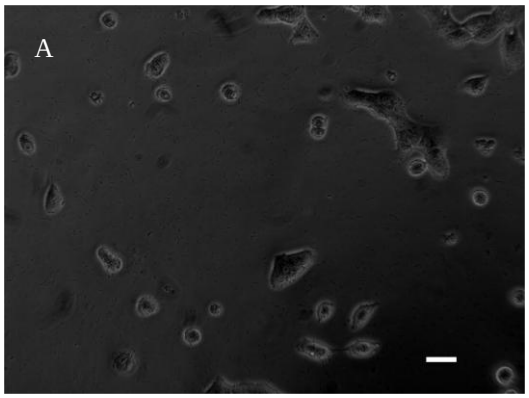


Figure 1.A. DU-145 cells with EC 178 at 0 min, B. DU-145 cells with EC 208, C. DU-145 cells with EC 178 at 1h, D. Bright field flourescent image of DU-145 cells with EC 178 at 2h, E. DU-145 cells with EC 178 at 3h, F. Control DU-145 cells. 200X magnification. Scare bar – 10 um

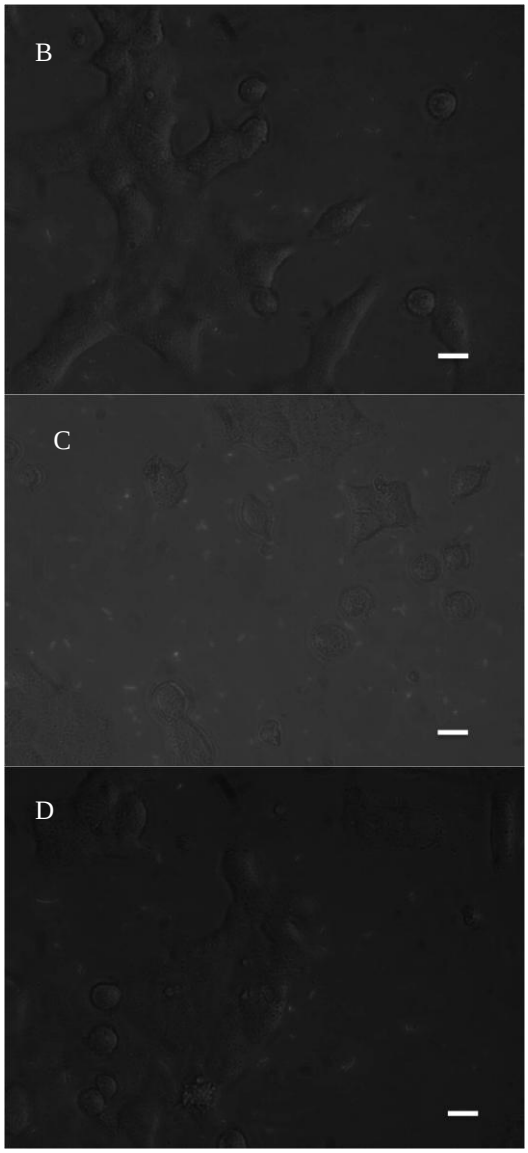
3.2 Cytotoxicity of GNPs to DU-145 cells

The cytotoxicity of 20 nm and 200 nm GNPs at three time points is shown in Fig. 2. It is seen that the 20 nm GNPs seem to have a significant effect on the DU-145 cells only after 48 and 72 h of incubation compared to the 200 nm GNPs. Not much significant change in viablity is observed by 200 nm GNPs. The 20 nm GNPs exhibit significant decrease in the viable cells at 1 and 2 µl concentrations in 48 and 72h samples.

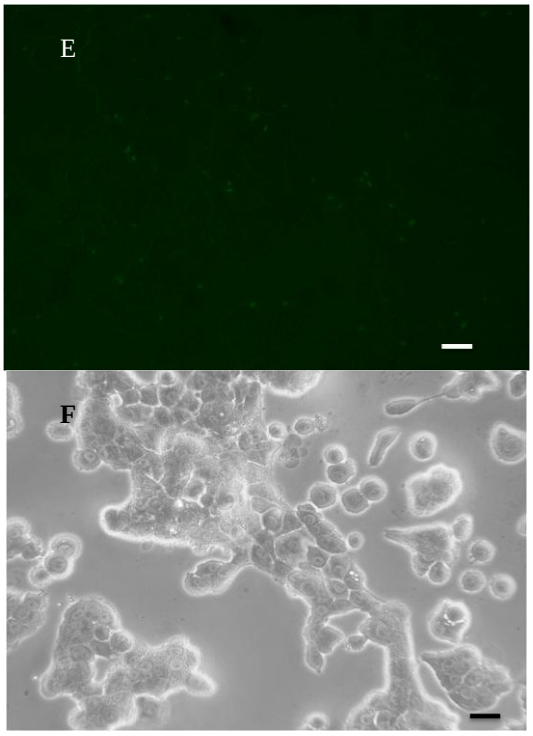
3.3 Cytotoxicity of functionalized GNPs to DU-145 cells and EC 178 and 208

Figure 3 shows the cytotoxicity of functionalized GNPs to the DU-145 cells at 1, 2 and 3h time points. Since a higher concentration of GNPs (50 µl) was used in this assay the

cytotoxicity profile was expected to be different than the previous assay. After various binding assays, it was seen that 50 µl is the minimum amount of GNPs is required to effect binding. At 1 h, significant cytotoxicity was observed in samples G and H when compared ot the control (Fig 3.i).



After 2 h, GNPs again showed significant reduction in the number of viable cells in sample B, C, D and E (Fig. 3.ii) when compared to the samples containing EC 208. Significant reduction of cells observed in samples B, E and H containing EC 178. Over all this assay was done to assess the interaction between *E. coli* and Mn-GNPs and their uptake in the DU-145 cells. The cytotoxicity profiles indicate that as time of incubation increases the cytotoxicity



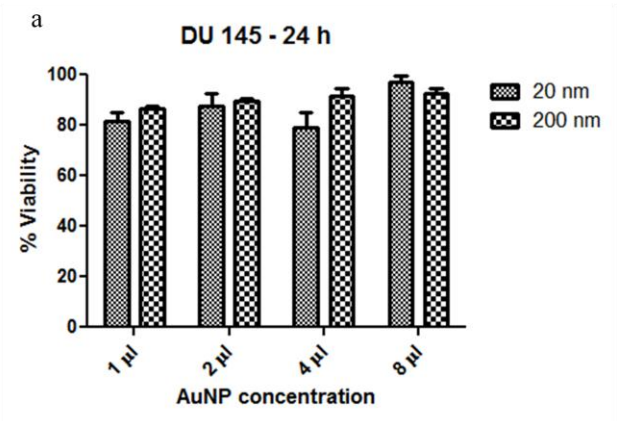
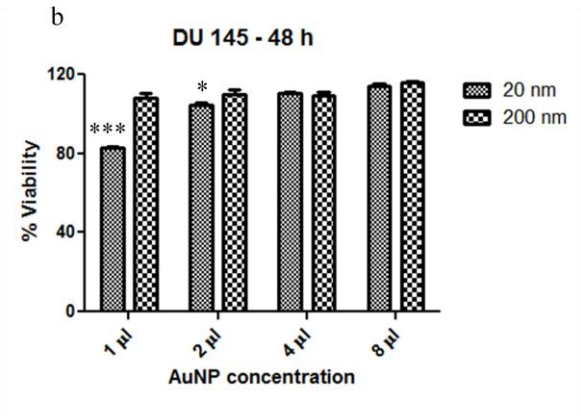
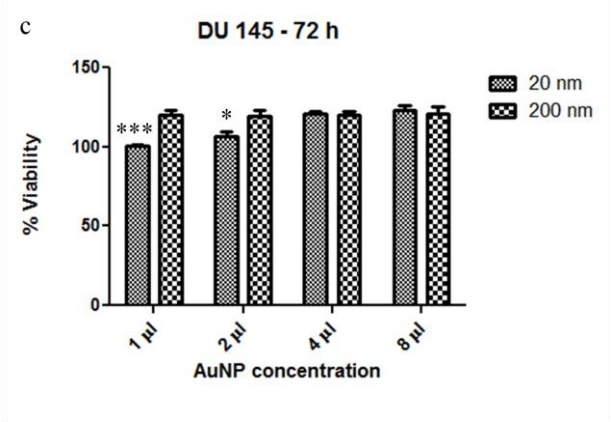


Figure 2. a, b and c show the cytotoxicity of GNPs when added to DU-145 cells at 24, 48 and 72h.
*p-Value ≤0.05, ***p-Value ≤0.001





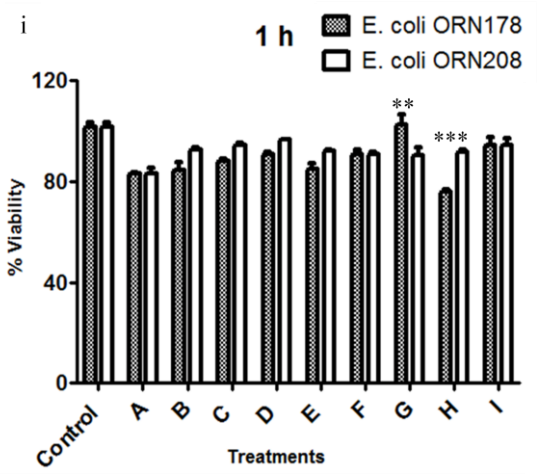
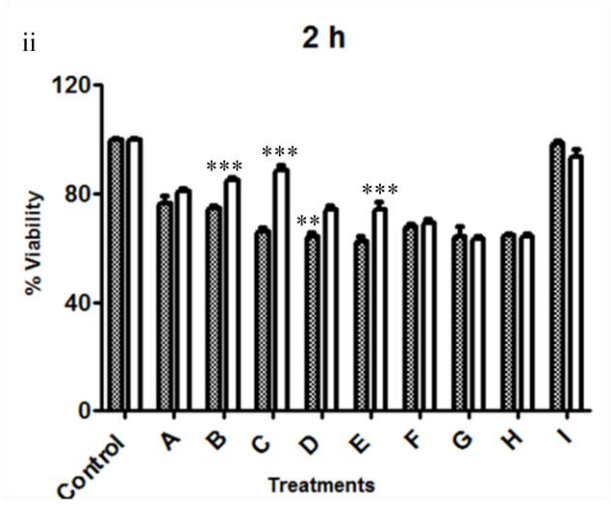
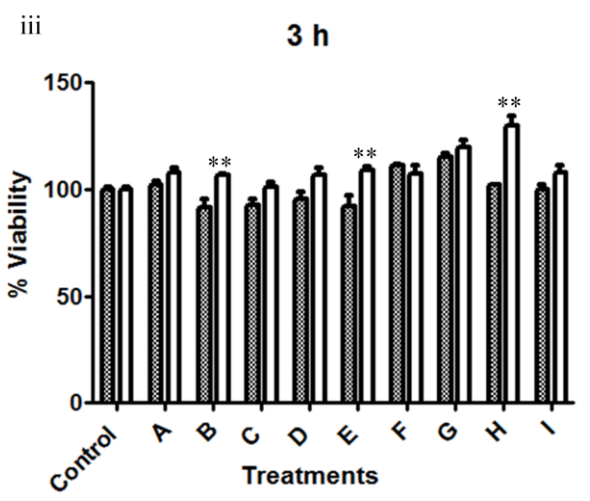


Figure 3. i, ii and iii show the cytotoxicity of Mn-GNPs when added to DU-145 cells at with both the E. coli strains for 1, 2 and 3h.**p-Value ≤0.01, ***p-Value ≤0.001



of the preincubated mix of 200 nm Mn-GNPs cause cytotoxicity along with EC 178 (Fig 3.iii).



4 CONCLUSION

It has been shown previously in our investigation [5] that GNPs when functionalized with D-mannose bind specifically to only EC 178. Based on fact this investigation attempted to carry out cytotoxicity assays with plain and Mn-GNPs it is seen that 20 and 200 nm GNPs cause cytotoxic effects by decrease in viability of prostrate cancer cells significantly by 48h of incubation. It has also been shown that GNPs of different sizes between 20-100 nm have shown to alter signalling pathways in cells and mediate biological processes [7], based on which they can be used for targeted drug delivery as well as for detection purposes. Lastly, the binding of EC 178 shows that competitive binding to Mn-GNP can be done to avoid binding of the bacteria to the DU-145 cells and hence, this mechanism can be further developed to prevent recurrent UTI in prostrate cancer cells.

REFERENCES

1. Klein, E.A. and I.M. Thompson, *Chemoprevention of prostate cancer: an updated view.* World J Urol, 2012. **30**(2): p. 189-94.
2. Sokurenko, E.V., et al., *Diversity of the Escherichia coli type 1 fimbrial lectin. Differential binding to mannosides and uroepithelial cells.* J Biol Chem, 1997. **272**(28): p. 17880-6.
3. P.C. Chen, S.C. Mwakwari, and A.K. Oyelere, *Gold nanoparticles: from nanomedicine to nanosensing.* Nanotech. Sci. Appl., 2008. **1**: p. 45–66.
4. Sharon, N., *Carbohydrates as future anti-adhesion drugs for infectious diseases.* Biochimica et Biophysica Acta (BBA) - General Subjects, 2006. **1760**(4): p. 527-537.
5. Vedantam, P., et al., *Binding of Escherichia coli to Functionalized Gold Nanoparticles.* Plasmonics: p. 1-8.
6. Aslan, K., J.R. Lakowicz, and C.D. Geddes, *Tunable plasmonic glucose sensing based on the dissociation of Con A-aggregated dextran-coated gold colloids.* Analytica Chimica Acta, 2004. **517**(1-2): p. 139-144.

7. Jiang, W., et al., *Nanoparticle-mediated cellular response is size-dependent.* Nat Nano, 2008. **3**(3): p. 145-150.