

Cytotoxicity Studies of Functionalized Gold Nanoparticles Using Yeast Comet Assay

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Abstract

In the present study gold nanoparticles and glucose capped gold nanoparticles are synthesized by chemical route method and characterized using UV-SPR, FTIR and TEM analysis. Single cell gel electrophoresis (SCGE) assay was used to study DNA damage. Studies show that glucose capped gold nanoparticles are less toxic as compare to gold nanoparticles at DNA level. Somewhat larger gold nanoparticle used to monitor endocytosis in log-phase *S. cerevisiae* spheroplasts at 10 to 30 μM was not reported to cause growth inhibition. It shows that glucose capped gold nanoparticles are nontoxic to yeast strain D7. DNA damage was observed by using standard method called Yeast comet assay, which provides a very sensitive method for detecting strand breaks and repair kinetics in single cells. Studies showed that 5 μM -30 μM having very less sign of DNA damage in case of Glucose capped gold nanoparticles and it also shows toxic effect for without glucose capped gold nanoparticles. OTM for different concentration as shown in the image and OTM with respect to different concentration shows the DNA damage, these studies also correlated with survival studies.

Keywords: Gold nanoparticles; Glucose capped gold nanoparticles; Yeast Comet assay; DNA damage

Introduction

In recent years, most of the metal nanoparticles, particularly gold nanoparticles are very attractive from their property such as bio-applications, therapeutics and diagnostics. Synthesis of nanoscale structures of inert metals like gold is of great interest for the current day researchers as gold possess certain physical properties, which are suitable for several biomedical applications. Thus gold nanoparticles (AuNps) having unique physicochemical features show significant future promise in the fields of biomedical imaging and therapy [1-5]. Studies also lay special emphasis on the uniqueness of the gold nanoparticles for treatment of life threatening diseases like cancer [6]. Available reports show potential of gold nanoparticles as explored in the field of photodynamic therapy due to their ability of producing heat to kill the tumors [7]. However although gold is biologically inert and thus shows much less toxicity, as compared to other metal nanoparticles, gold has a relatively lower rate of clearance from circulation and hence can pose serious deleterious effects on health[8]. Recent studies showed them AuNPs could cross the blood-brain barrier, interact with the DNA and even produce genotoxic effects [9]. As such surface modifications of AuNps are gaining attention in current day research programs where attaching a ligand, capping of the AuNps could help making the particles more biocompatible so as to achieve specific targeting of diseased cells and tissues [10-14]. Synthesizing AuNps with lesser toxicity is now one of the primary interests in the field of nanotechnology for its applicability in biomedical sciences. Based on this perspective the present work has been designed to formulate a synthesis of a novel glucose capped gold nanoparticle as a better theranostic candidate. The glucose analog 2-deoxy-D-glucose(2DG),an inhibitor of glycolytic ATP production and

glucose transport is the most widely reported, metabolic inhibitor for targeting glucose metabolism . 2-deoxy-D-glucose labeled gold nanoparticles have been shown to provide high-resolution metabolic and anatomic information of tumor in a single CT scan [15,16]. Studies showed that glucose capped gold nanoparticles has been specially choosen to target cancer cells as such capped nanoparticles show faster cellular uptake in cancer cells. Additionally, larger number of glucose molecules are internalized via glucose transporter (GLUT) receptors present on the cancer cell surface [17]. These types of gold nanoparticles are incorporated into numerous technologies and applications widely used in case of Therapeutics and biomedical because of their conjugation with various bio molecules due to the presence of 6s free electrons in conduction band of nano-Gold [18,19]. Moreover, Yeast Comet assay is a new, fast and easily developing assay for detection of environmental genotoxic agents without using higher organisms. *Saccharomyces cerevisiae*, which is a fast growing organism and easy to handle. This simple eukaryote possesses homologues or functional analogues of almost all factors involved in these processes. *S. cerevisiae* is one of the most thoroughly studied model systems whose full genome sequence is now available. For these reasons, the yeast has become a valuable tool for studying the eukaryotic cell and it has also been used as a test organism for estimating the mutagenic potential of different chemicals [20,23]. Yeast comet assay also used to study DNA damage in case of food additives. Zymolose-20T did not open the cell wall, therefore zymolose-100T used to achieve degradation of cell wall [24]. DNA damaging agent was used as Glucose-gold nanoparticles. Gold nanoparticles having tremendous properties in the field of biomedical application due their wider range of properties. Bio molecular capping gold nanoparticles shows very less toxicity, Because of their metabolic properties. Main Purpose of the studies is showing level of DNA damage caused due to gold nanoparticles. These studies are more compatible for biomedical application due to their easy availability.

Materials and Methods

Chemicals

HAuCl₄.3H₂O (Sigma), β-D glucose, Sodium hydroxide, Agarose normal and low melting powder form, EDTA sodium salt, Tris base, Triton X – 100, Di methyl sulphoxide, Ethidium bromide, Sodium chloride, Methanol were procured from Alpha.

Synthesis of gold nanoparticles

Gold nanoparticles are synthesized by using citrate reduction method and Glucose-capped AuNPs were synthesized by chemical route method [18] using HAuCl₄.3H₂O and β-D-Glucose. The aqueous solution of 0.05M HAuCl₄.3H₂O was added to β-D-glucose (0.03M) and stirred for 30 minutes. Subsequently, 0.5 M sodium hydroxide (NaOH) was added for completing reduction of gold salt. This resulted in a red colored solution of Glu-AuNPs. β-D-glucose acted as both reducing as well as capping agent in the AuNp synthesis. Capping was confirmed by FTIR and TEM analysis.

Characterization of Glucose capped gold nanoparticles

Synthesis of glucose-capped gold nanoparticles and gold nanoparticles are observed by UV-visible absorption spectrometer (model HU-1090) which shows a surface plasma resonance (SPR) at a wavelength 540 nm FTIR analysis was carried out to study the elemental chemical bonding, and Transmission electronic microscopic (TEM) analysis shows dimension of gold nanoparticles.

Samples preparation

A mutant type diploid yeast strain, *S. cerevisiae* D7 was used for the present study. The single cell stationary-phase cultures were obtained by growing the cells in Yeast extract: Peptone: Dextrose (YEPD) (1:2:2%) medium for several generations in stationary phase to a density of approximately 3×10^8 cells/ml. Cells were washed thrice by centrifugation (2000 for 5 min) using double distilled water and re-suspended to a cell concentration of 1000 cells/ml (by counting in hemocytometer) in a polypropylene vial for treatment with gold nanoparticles and without capped gold nanoparticles for 1hr.

Survival assay

Treated and untreated samples were suitably diluted and plated in quadruplicate on YEPD agar medium. Plates were incubated for 2-3 days at 30°C in dark and normal atmospheric conditions and the colonies were counted. The data points in all figures in the survival results are the means from at least three independent experiments. The error bars in all figures indicate the standard error of the mean. The statistical analysis was carried out using origin 8.0 software.

Yeast comet assay

Yeast comet assay was performed by using *Saccharomyces cerevisiae* D7 strains. Five milliliters of YEPD (Yeast extract: Peptone : Dextrose) (1:2:2%) are inoculated with D7 strain and incubated overnight at 30°C, 200 rpm. At 1×10^6 cells were harvested by centrifugation at 4°C, after washing, the pellet was re suspended in 10 ml ice-cold S buffer (1 M sorbitol, 25 mM KH₂PO₄, pH-6.5). Cells were distributed by aliquots containing 4×10^6 cells and centrifuged at 4°C, 18000 rpm. These cells are treated with nanoparticles for 1 h in the concentration

range of 5 μM-30 μM. The cells was re suspended in 80 μl of 1.5% (w/v) LMA (Low Melting Agarose) containing 2 mg/ml zymolyase 100 T at 35°C (LMA was previously melted with S buffer at 50°C, cooled to 35°C and zymolyase was added, mixed and maintained at 35°C until use). The suspension was transferred to a glass slide and covered immediately with the cover slip before solidification. Glass slides were incubated at 30°C for 60-90 min and cover slips were removed gently. All subsequent manipulations were done at 4°C. The toxic was removed by incubating the glass slides in S buffer for 5 mins and cells were lysed with lysis buffer (30 mM NaOH, 1 M NaCl, 0.05%, w/v, laurylsarcosine, 50 mM EDTA, 10 mM Tris-HCl, pH 10) for 20 min. After lysis, cells were washed three times with electrophoresis buffer (30 mM NaOH, 10 mM EDTA, 10 mM Tris-Hcl, pH10) for 20 min. Glass slides were placed in an electrophoresis tank immersed in electrophoresis buffer and electrophoresis was done at 0.7 V/cm for 10 min. After electrophoresis cells were neutralized with incubation in 10 mM Tris-Hcl, pH 7.4 for 10 min and treated with 76% ethanol 10 min and, subsequently with 96% ethanol for 10 min. DNA was stained with 10 μg/ml ethidium bromide (10 μl), covering the area of the cells in the glass slide, without the cover slip, and overlaying with a new one and visualization was done immediately or after several days (slides were stored at 4°C). Observation of comets was done with a fluorescence microscope with 100x magnification. Comets were analyses using the Comet score software.

Gold nanoparticles are treated with Yeast strains: Yeast strain D7 was treated with different concentration of with and without capped gold nanoparticles of the range of 10 μM, 20 μM, 30 μM and 40 μM. D7 strains are treated with different concentration of nanoparticles for 1hr.

Result and Discussions

UV-Visible spectroscopy shows absorbance peak at 545 nm as shown in the Figure 1, FTIR spectrum shows the –OH stretching at 3332.5 cm⁻¹ due to the presence of glucose on the surface of gold nanoparticles (Figure 2).

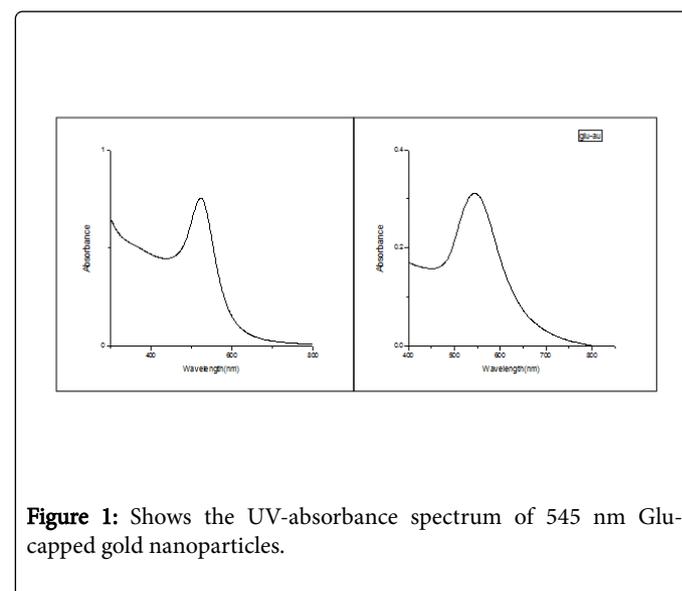


Figure 1: Shows the UV-absorbance spectrum of 545 nm Glu-capped gold nanoparticles.

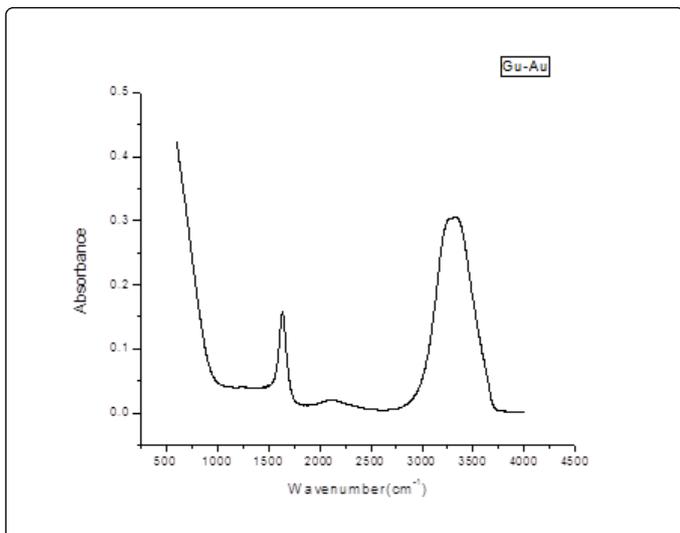


Figure 2: Shows the FTIR Spectrum of Glucose-capped gold nanoparticles.

Morphology of gold nanoparticles is analyzed from TEM analysis. It shows the triangular shape of the gold nanoparticles and also shows the size distribution plot as shown in Figures 3 and 4.

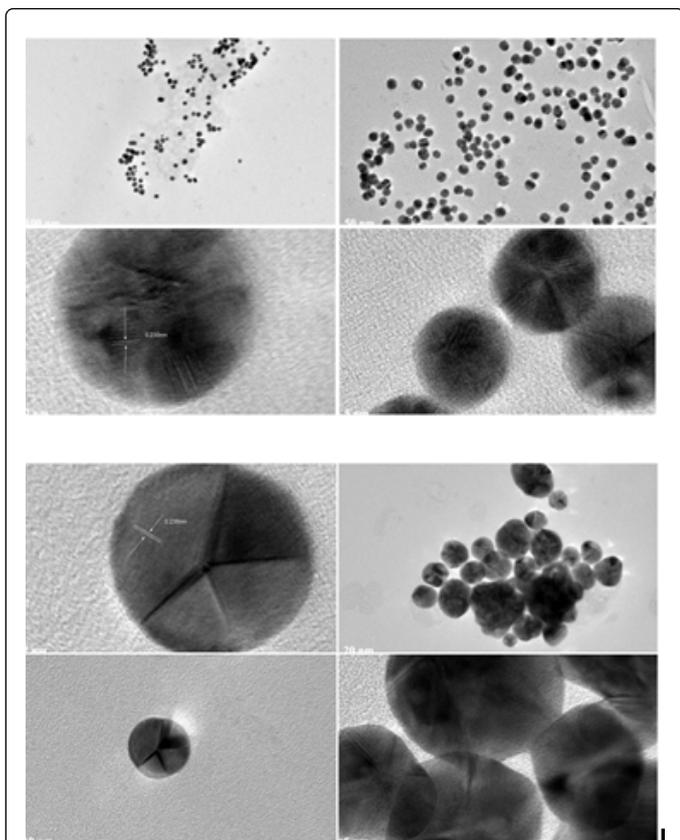


Figure 3: Shows the TEM image of glucose-capped gold nanoparticles: i) d spacing 0.236nm was calculated by using J-image software. ii) Shows the images of glucose-capped gold nanoparticle

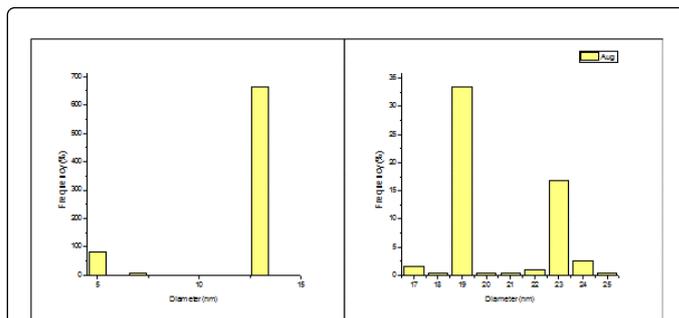


Figure 4: Shows that diameter of glucose-capped gold nano particles are 19.04 ± 1.13 nm.

Glucose capped gold nanoparticles are treated yeast strain D7 with different concentration in the range of $10 \mu\text{M}$ - $30 \mu\text{M}$. The increasing use of nanoparticles in industrial processes and commercial products has generated a need for systematic assessment of potential biological and environmental risks. A related but somewhat larger gold nanoparticle used to monitor endocytosis in log-phase *S. cerevisiae* spheroplasts at 10 to $30 \mu\text{M}$ was not reported to cause growth inhibition Survival plot as shown in the Figure 5.

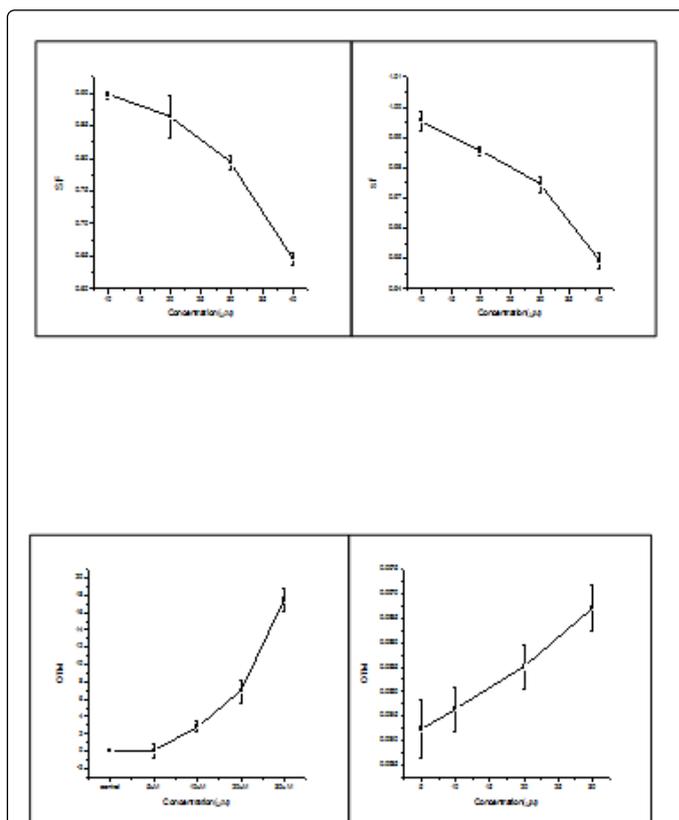


Figure 5: Shows OTM increases with increase in concentration of Glu-Au.

A related but somewhat larger gold nanoparticle used to monitor endocytosis in log-phase *S. cerevisiae* spheroplasts at 10 to 30 μM was not reported to cause growth inhibition, from the graph it shows that 90% of the viability at concentration 10 μM and upon increasing the concentration to 30 μM , viability was reduced to 70 %. It shows that glucose capped gold nanoparticles are nontoxic to yeast strain D7. DNA damage was observed by using standard method called Yeast comet assay, OTM for different concentration as shown in the image and OTM with respect to different concentration shows the DNA damage. Correlation plot between OTM and Survival Fraction gives R 2 value is about -0.99 as shown in the Figure 6. Results shows that gold nanoparticles are toxic as compare to glucose capped gold nanoparticles, Because of the metabolic properties (Figure 7).

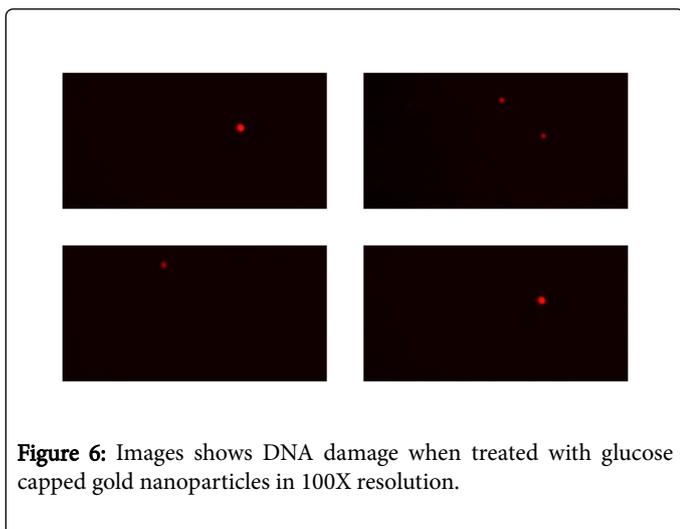


Figure 6: Images shows DNA damage when treated with glucose capped gold nanoparticles in 100X resolution.

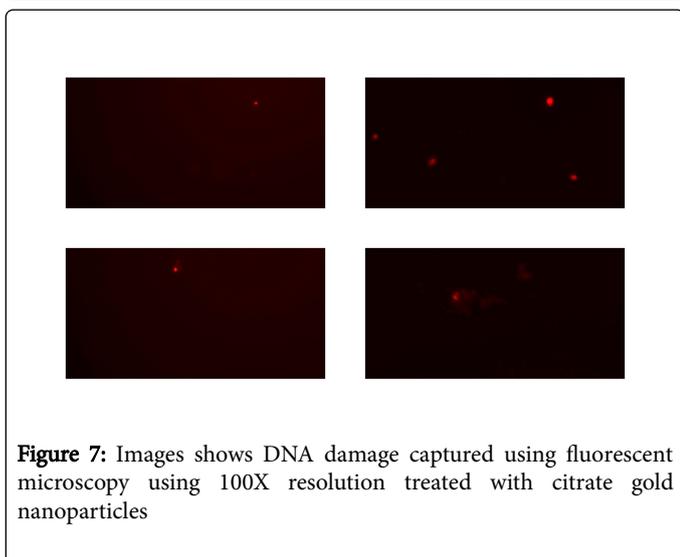


Figure 7: Images shows DNA damage captured using fluorescent microscopy using 100X resolution treated with citrate gold nanoparticles

Conclusion

Concluded that, Gold nanoparticles and Glucose capped gold nanoparticles are characterized by using UV, FTIR and TEM analysis. Yeast strain D7, are treated with different concentration of with and without capped gold nanoparticles, to check the DNA damage using Single-cell gel electrophoresis (yeast comet assay), which provides a very sensitive method for detecting strand breaks and repair kinetics in

single cells. Studies showed that 5 μM -30 μM having very less sign of DNA damage in case of Glucose capped gold nanoparticles and it also shows toxic effect for without glucose capped gold nanoparticles.

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