POTENTIAL CYTOTOXICITY AND PHOTOACTIVE EFFECT OF GOLD NANORODS ONTO COLORECTAL CANCER CELLS

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Abstract

Cancer is still one of the main death causes worldwide. Recently the use of nanotechnology in nanobiomedicine is considered as one of the most promising research applications, nanotechnology is providing an exceptional opportunity to improve the treatment of various diseases particularly cancer. This is highly due to the unique chemical and physical properties that are observed at the nanoscale. Gold nanorods (GNRs) in specific show a surface plasmon resonance (SPR) band at the near infra-red (NIR) region for this reason they have been widely studied in biomedical research and are of great interest for the development of nanomedicine precisely phototherapy of cancer and drug delivery. The goal of the current study was to investigate the stability of GNRs in biological media and subsequently assesse their cytotoxic properties and photo killing ability on colorectal cancer (CaCo-2) cells. In this study The GNRs were firstly characterised by, Zeta potential (ζ-potential), ultraviolet–visible (UV–Vis) spectroscopy, and transmission electron microscopy (TEM). The potential cytotoxic effects of the GNRs onto CaCo-2 cell lines were assessed using inverted light microscopy for morphological changes, Trypan blue exclusion assay and (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) for viability, adenosine triphosphate luminescence and cell impedance studies for proliferation, and lactate dehydrogenase membrane integrity assay for potential cell damage. The characterisation results showed that the physicochemical properties of the GNRs in Dulbecco's Modified Eagle's medium **(**DMEM**)** were maintained when compared to GNRs suspended in DI water. The GNRs showed a slight increase in size through aggregation but retained their charge. Upon Irradiation at a wavelength of 660 nm, GNRs decreased cell viability and proliferation, increased cell damage. In conclusion, this work clearly demonstrates that GNRs activated at 660 nm significantly increase cytotoxicity induced in CaCo-2 cells.

Keywords**:** Nanomedicine, gold nanorods, cytotoxicity, colorectal cancer

Introduction

Cancer is one of the main death causes worldwide. Cancer is the third leading cause of death after stroke and heart disease in developed countries (Cai et al., 2008). The extensive rise in cancer incidences has lead to an increased demand for safe and effective materials for its treatment. Cancer is defined as an unregulated genetic life threatening disease associated with uncontrollable cell growth (Bhandare, 2014, Andreeff, 2000), it is primarily caused by environmental factors classified as carcinogens (air, water, food, chemicals and sunlight) that people are exposed to (Alison, 2001). Cancer of the colon is the third most prevalent cancer affecting both man and woman. Statistics estimated 93,830 new cases of colon and 40,000 new cases of rectal cancer arising in both man and woman in 2014 (American cancer society, 2014). Colorectal cancer (bowel cancer) is the uninhibited growth of abnormal cells inside the colon and or the rectum. In the present year, 49,700 deaths are expected to occur due to colon cancer along with 93,090 new cases of colon cancer and 39,610 new cases of rectal cancer are expected to be diagnosed this directly signifies a fast growing health problem (American cancer society, 2013, American cancer society, 2015, Prados et al., 2014, Thomas et al., 2013). There are a number of conventional therapies associated with colorectal cancer treatment such as surgery, radiation, or chemotherapy. These conventional therapies have drawbacks such as

Low selectivity for cancerous cells combined with their high toxicity to non-targeted cells in the body and frequently cause side effects which can be more unbearable than the disease at that set moment (American Cancer Society., 2014**,** Prados, et al, 2014, Kralova et al., 2008, Marsh et al., 2009 and Evans et al., 2003). Hence there was an urgent need to introduce more effective alternative conventional approaches like photodynamic therapy for the treatment of colorectal cancer patients (Prados, et al, 2014).Photodynamic therapy (PDT) yielded better clinical results when compared to the conventional cancer therapies, had fewer adverse side effects and was also accompanied with higher selectivity (Bugaj et al., 2007; Kralova et al., 2008). PDT has developed as one of the essential therapeutic alternatives in controlling other diseases and cancer (Chatterjee et al., 2008). Although photodynamic therapy is said to be a low traumatic method which significantly improved the quality of life and life expectancy of patients with cancer, further improvements in the therapeutic efficacy are still required to overcome various side effects related to conventional PDT (Gumelia et al.2010, Lim et al .,2013). Medicine and many other fields in science have been revolutionised by nanomedicine, advances in these fields have led to the development of different useful noble metal nanoparticles (Hauck et al.,2008, Ulatowska-Jarza et al., 2011, Moghimi et al., 2005). The growing interest in nanomaterials is due to their properties which are different from both the bulk solids and the molecule (atom) materials (Jisen et al., 2004, Khan et al., 2013). Nanoparticles are said to be so special because they have unique optical, physical; chemical and electronic properties (Liu and Bando, 2003). In the past researchers have frequently described gold as a healthy adjuvant solely because of the unique properties that it possesses. Gold nanoparticles have attracted interest because of their properties, they have interesting optical absorption cross-section, they are easy to synthesis, resistant to corrosion, enhanced permeability and retention in tumor tissue, they inert and they biocompatible these properties makes gold nanoparticles very useful and attractive

to researchers mainly focused on the biomedical applications (Lounis et al.,2012,Khan et al,2013, Demberelnyamba et al.,2010,Johnson, 2010).

Among various shapes of gold nanoparticles synthesised, gold nanorods optical properties are of scientific interest (Lounis et al.2013).GNR have two kinds of absorption: one due to the short axis(transverse plasmon), and the other due to the long axis (longitudinal plasmon) and they can also scatter near infrared (NIR (650-1200 nm) light resonantly upon excitation of surface plasmon, this allows for deep tissue penetration and the background fluorescence is low which is an advantage over uv and visible excitation light which have very poor tissue penetration depths (Vankayala et al.2013, Khan et al,2013, Lounis et al.2013, Lasagna-Reeves et al, 2010). The longitudinal plasmon bands that gold nanorods show in the visible and near-infrared (NIR) region offers many opportunities for sensing, imaging, and photonics applications (Lounis et al. 2013). However Studies on the interaction between the physical strictures of nanoparticles and cell biology have just began and an inordinate deal is still mysterious also the possible toxicity which may result (Hauck et al. 2008, Grabinski et al., 2011). First and foremost fundamental understanding is of great importance before we can intricately apply the GNRs (Zhang et al., 2012). Photodynamic therapy (PDT) is an alternative method for the treatment of a variety of diseases that require the eradication of pathological cells (cancer, infectious microorganisms and other diseases), it involves the administration of photosensitizer (PS); co localization of light of an appropriate wavelength for the PS; molecular oxygen to produce cytotoxic reactive oxygen species (ROS) to achieve photo cytotoxicity (Lim et al., 2013; Saczko et al., 2008; Huang et al., 2012).This current study aimed to asses or rather evaluate the physicochemical properties and consequently investigate the potential toxicity of gold nanorods onto colorectal cancer cells.

Material and methods

Preparation of GNRs: Gold nanorods (GNRs) were purchased from Mintek, SA, with an axial diameter of about 20 nm with a localized surface plasma resonance (LSPR) absorption maximum at ~ 655 nm. As received CTAC Au NRs were washed thoroughly using DI water to remove the unbound CTAC (hexadecyltrimethylammonium chloride) via centrifugation, and then re-dispersed in DI water.

Characterization of the physicochemical properties of the GNRs: The physicochemical properties of the GNRs in DI water and cell culture medium (DMEM) were determined. For the stability of GNRss in culture medium, the nanorods suspended in DI H_2O, were centrifuged at 13 000 x g for about 30 min and in turn re-suspended in DMEM culture medium. For further Characterization both GNRs suspended in Di H20 and in culture medium were used. To study the peak absorption band, Gold nanorods were characterized by UV-Vis absorption spectroscopy using Shimadzu UV-2450 PC dual-beam spectrophotometer using 1 cm path length quartz cuvettes. Spectra were collected for the aqueous solutions within the 400–800 nm spectral range. FTIR measurements of the GNRss were recorded on an FT-IR spectrophotometer(Perkin Elmer spectrum 100) equipped with a universal ATR sampling accessory. Spectra were obtained in transmission mode over the 4000 cm−1 to 550 cm−1 wave-number region at a resolution of 4 cm−1 averaging 16 scans. Dynamic Light Scattering (DLS) was used to determine the GNRs hydrodynamic radius a He-Ne laser (633 nm, 4 mW) and avalanche photodiode detector (APD) equipped Zetasizer Nano ZS was used .TEM images of GNRs were collected on on a Tecnai G2 Spirit TEM instrument operating voltage range was at 120 kV. Pictures were collected on a Gatan digital imaging system using the Power Mac 8600 computer Digital Micrograph software. The TEM samples were prepared by placing small drops of the GNRs solution onto a carbon coated TEM copper grid and allowed to dry prior to analysis.Zeta potential (ζ-potential) measurements of the GNRs were done using Malvern Instruments' Zetasizer Nano ZS whereas pH measurements were performed at 37°C using the CyberScan pH 6500 instrument.

Evaluation of the cytotoxic effects of GNRs

Cell Culture, Materials and Reagents: CaCo-2 cells were grown in (DMEM, Sigma-Aldrich, D6429) supplemented with 10% foetal bovine serum (FBS, Gibco, 306.00301) 1% antibiotic (penicillin-streptomycin, Gibco, 15140), and 1% antifungal (amphotericin-B, Gibco, 104813).Cultures were maintained in a 85% humidified atmosphere containing 5% CO_2 37∘C. Once cells reached 80–90% confluence, they were harvested and seeded at a density of 5×10^4 in 3mL media into sterile culture dishes, with a diameter of 3.4 cm. Cells were allowed to attach overnight. Once cells have reached confluency the monolayer was washed with Hank's Balanced Salt Solution (HBSS) then harvested by momentarily incubating in trypsin/ EDTA solution. Cell culture reagents were purchased from Sigma Aldrich.

Cellular morphology: Morphology photographs of cells treated with GNRs were taken after 24 h incubation using Wirsam, Olympus CKX41 inverted microscope, and a comparison of the images was done. Untreated control (group 1) was compared with the GNRs treated (group2). As soon as the images were recorded, TrypLE Express was used to trypsinise the cells and resuspend in HBSS (unless stated otherwise) for further assays.

Cell proliferation: MTS Assay (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay: Caco-2 cells were grown using normal tissue culture techniques. The cells (5 x 105 cells/ml) were incubated in 96 well plates at 37°C overnight, with the subsequent addition of the GNRs sample supplied, in 4 concentration ranges (table 2). The

cells were incubated for 4 days, after which MTS (5µl) was added to the cells. The absorbance values were measured at 490 nm after 1h, 2h and 4 hour incubation periods (Panyam et al .,2003).

Real time Monitoring of Cell Maturation and Cytotoxic Responses: xCELLigence Caco-2 colorectal adenocarcinoma cells were grown using standard tissue culture techniques. Cells (5 x 105 cells/ml) were incubated at 37°C overnight (24h), in a gold electrode coated 96 well E-plate, with subsequent addition of GNRs sample (Table 1). Cells were incubated for 4 days, with impedance measurements taken at various time points during the course of the incubation period. The data were retrieved and a graphic representation of the toxicity was constructed.

Evaluation of the photoactive effect of GNRs
CaCo-2 cells were divided into four groups that is three control groups and one experimental group. The control groups included cells receiving GNRs at a concentration of 1.5 x 10^{11} np/ml only, untreated cells those are cells that neither received GNRs nor irradiation these cells were sham irradiated , cells receiving laser irradiation only (5 J/cm^2 and 10 J/cm^2). The experimental group received both the GNRs (1.5 x 10^{11} np/ml) and irradiation (5 J/cm2 / 10 J/cm2 fluence). Before the addition of the GNRs or irradiation the cells were allowed to attach onto the plate surface, rinsed with Hank's Balanced Salt Solution (HBSS, Invitrogen, 10-543F) and flooded with 1mL fresh media. Cells were irradiated in the dark from above in an open culture dish, using a continuous diode laser emitting light at a wavelength of 660 nm; cells were irradiated with a fluence of 5 J/cm2 and 10 J/cm2. Laser irradiation parameters used are tabulated in Table 1 in the table section. Biological responses were evaluated after further incubation for 24 hours.

Cell Viability: Trypan blue exclusion assay: Trypan blue exclusion assay: Cell viability percentage was determined using trypan blue exclusion method: trypan blue reagent (20µl) and cell suspension in HBSS (20µl) were incubated at room temperature for about 5 minutes. In this protocol, non-viable (dead) cells have a blue cytoplasm whereas viable cells remain transparent they had a clear cytoplasm (Strober, 2001; Ho and Pal, 2005)

Cell proliferation: (ATP) assay: Adenosine Triphosphate Luminescence (ATP) assay: Cell Titer-Glo Luminescence cell viability assay was used to determine the number of viable (live) cells. Detection was based on luciferace reaction to quantify the amount of ATP from viable cells. Upon addition of Cell Titer-Glo ® reagents three things occurred: cell lysis to release ATP, inhibition of endogenous ATPase's and lastly the provision of other reagents necessary in measuring ATP.A luminometer was be used to measure luminescent signal, which is proportional to the amount of ATP present (Promega, G7573, Mvula et al., 2008).

Cytotoxicity Lactate dehydrogenase (LDH) membrane integrity assay: Caco-2 Cells were grown using standard tissue culture techniques. The cells (5 x 10^5 cells/ml) were incubated in 3.4 cm^3 plates at 37°C overnight, with subsequent addition of GNRs sample at 0, 1, 2 and 5 np/ml in fresh supplemented media followed by incubation for 1 hour. Cytotoxicity was assessed using CytoTox-ONE™ Homogeneous Membrane Integrity Assay based on the amount of LDH released from non-viable cells. 96 well plates were used. Then 50 µl CytoTox-ONE™ Reagent was added to each well and then 50 ul of medium from the plates was added, including a background well with just medium .The plate was covered with foil and incubated at room temperature for 30 minutes, followed by the addition of 50 µl stop solution to each well. Fluorescence was recorded at excitation/emission wavelengths of 560/590 nm using the FLx 800 fluorescence Microplate plate reader.

Statistics

Each set of experiments was repeated four times (number = 4), whereas each Biochemical assay was done in duplicate all in all the results were used in their averaged form. Statistical calculations such as the standard deviation, mean and the significant variations were calculated. In the graphs on the results section the dispersion bars represent the standard error and the statistical differences are shown as $(*)$ $P < 0.05$, $(**)$ $P < 0.01$, and $(***)$ $P < 0.001$.

Results and Discussion

Characterization of The physicochemical properties of the GNRs

GNRs were characterised using UV-vis spectroscopy, TEM, and zeta potential. The UV-Vis absorbance spectrum of the GNRs provides information into modifications in their properties if any; such modifications can be observed when the GNRs were re-suspended, from DI water, into DMEM. The GNRs UV-vis spectra in all solutions showed one transversal surface plasma peak at 520 nm and longitudinal ones at 600-800 nm (Table 1 and Figure 1) (Xu *et al*.,2006, Kanjanawarut *et al*.,2013).TEM was used to determine the morphology and size distribution of the GNRs and to also evaluate if the size, morphology and state of aggregation of the GNRs were changed when re suspended in DMEM (Table 1 and Figure 2). Data obtained from TEM 1for all GNRs samples was observed to be rod-like with a small amount of spheres. Zeta potential measurements were used help characterise the surface of nanoparticles and predict their behavior in different surroundings. Zeta potentials of the GNRs were measured using Zetasizer Nano ZS(Table 1).The Zeta potential measurements obtained for the GNRs have indicated a net positive charge in both suspensions tested for (Di water ,DMEM). The ζ-potential remained more or less the same upon their suspension changes. (Table 1) (Barratt, 1999). All in all the GNRs were neutral, which has been observed by other scientists which is attributed to be due to the amino acids in the media most likely from the FBS adsorbing to the particles (Grabinski *et al*., 2011), leading to all GNRs samples (GNRs-MEM and GNRs DMEM) bearing the same effective charge, irrespective of the original GNRs (GNRs-DI) surface charge (Alkilany *et al*., 2009).The pH of the GNRs suspended in DI water and DMEM was measured at 37°C and compared to that of DMEM. The pH measurements did not show any alarming decrease nor increase (Table 1). The pH of the culture medium decreased slightly upon addition of the GNRs (Table 1) though the observed decrease was not biologically noteworthy.

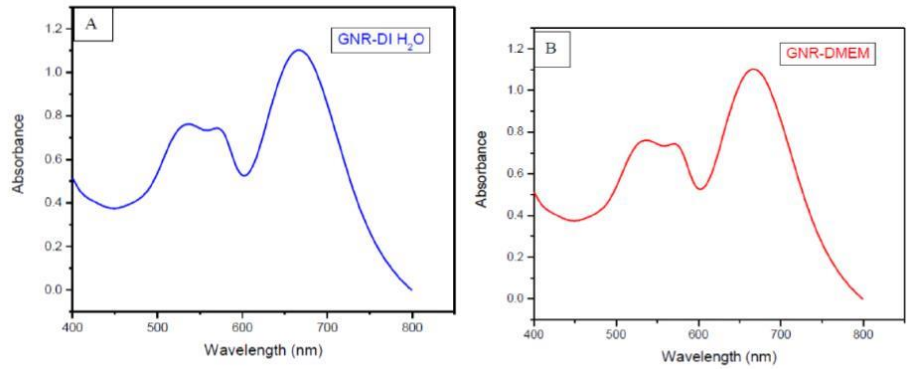

Figure 1 The absorption spectrum of GNRs in: (A) GNRs in DI water (B) GNRs in DMEM.
Note: The GNRs UV-vis spectra in all solutions showed one transversal surface plasma peak at 520 nm and longitudinal ones at 650 nm.

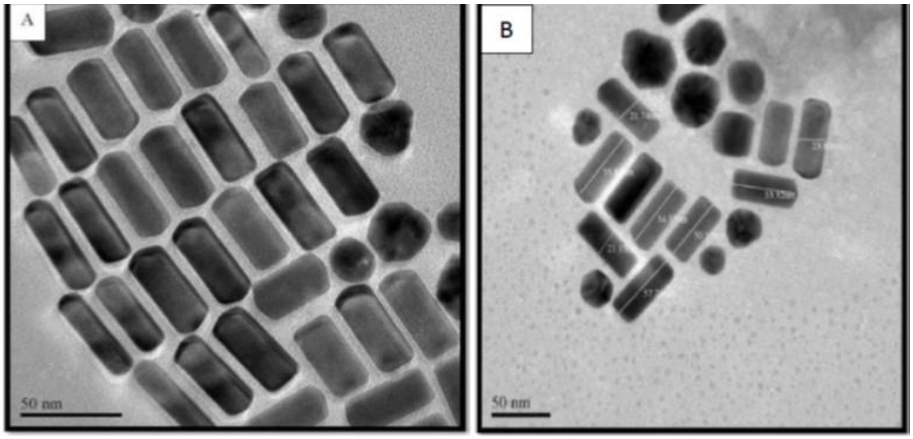

Figure 2 Transmission electron microscopy of the GNRs. (A) GNRs in DI water (B) GNRs in DI water
Notes: GNRs have a rod shape (B) with with a small amount of spheres, all in all the shape of the GNRs did not change in different solutions.

Cytotoxic effects of GNRs

An assessment of the potential cytotoxic effects of the GNRs was executed. Following treatment with GNRs the cellular morphology of the CaCo-2 cancer cells was assessed, and the morphological features of cells treated with low concentrations of GNRs (1.5 x10 ^{11}np/ml, 2.5 x 10 ^{11}np/ml) were found to be similar in characteristics to the untreated control cells Figure A, B, C. High concentrations of GNRs 5 x 10 ^{11} np/ml showed an increase in the number of free-floating cells (Figure 1D) and cytodamage was observed.

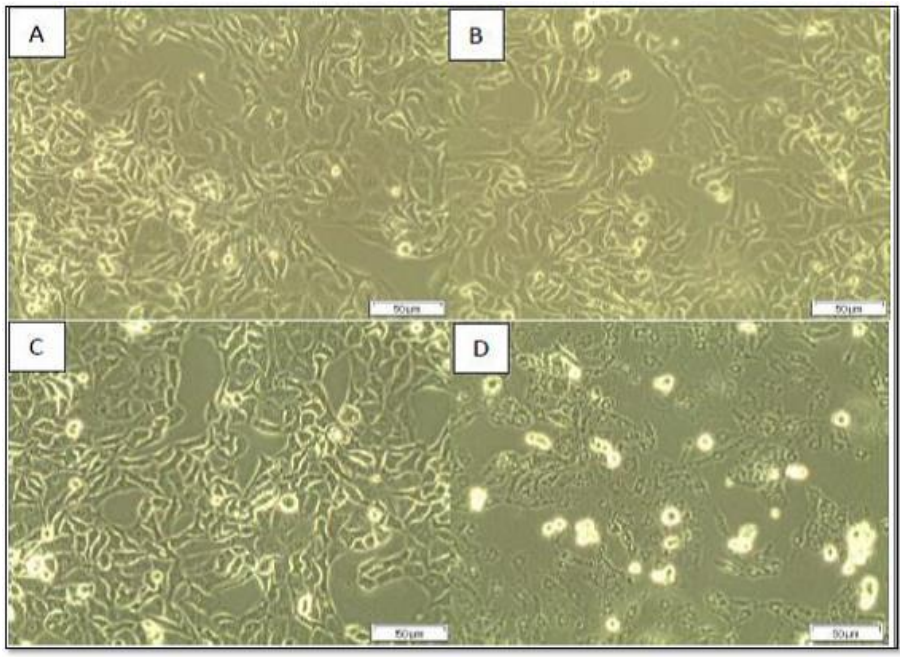

Figure 3 Cellular morphology after the treatment of CaCo-2 cancer cells. A) untreated cells (Cells only) B) 1.5 x10 ^{11}np/ml C) 2.5 x 10 ^{11}np/ml D) 5 x 10 ^{11} np/ml
Notes: Cells treated with either 1.5 x10 ^{11}np/ml C) 2.5 x 10 ^{11}np/ml of GNRs did not morphologically differ from the untreated cells (cells only) only 5 x 10 ^{11} np/ml appeared to be different, with an increased number of free-floating cells.

An MTS (3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium) *in vitro* cytotoxicity assay is conducted to determine change in cell viability, through the use of a color change. MTS assays were performed to determine the effect of 24 h exposure to GNRs on the cell proliferation of CaCo-2 cells, after a 24 h exposure to GNRs, Figure 2A illustrated the toxicity of the GNRs sample, at the high concentration ranges, and no toxicity was observed at low concentrations Figure 2C.The xCELLigence system is a real time cell based analysis system that uses impedance as a measure of cell viability. The system uses gold electrode coated 96-well plates to measure current across the base of a single well. A living cell with active metabolic function affords resistance and so produces a visible graph. This growth pattern is specific to the type of cell line used. As long as the cells remain viable, there will be current impedance and a graph to match. When the cells are no longer viable, there will be no impedance and therefore no impedance graph (Sergent *et al*., 2012, Şeker *et al*., 2014).The GNRs sample was added after 24 hours, as indicated by the spike in cell index observed at this time. The blank affords no resistance and was observed as the baseline with a cell index of around 0. The normal cell growth curve was observed for the 0.00 nps/ml labelled sample, clearly visible in Figure 2B. Figure 2D showed concentration dependant toxicity for the high GNRs concentrations (from 1.00 x 10^{11} nps/ml down to 2.50 x 10^{9} nps/ml) added. Our results, generated with the RTCA technology Figure 2B and Figure 2D positively correlated with those generated with the conventionally used MTS-based toxicity assay Figure 2A and Figure 2C.

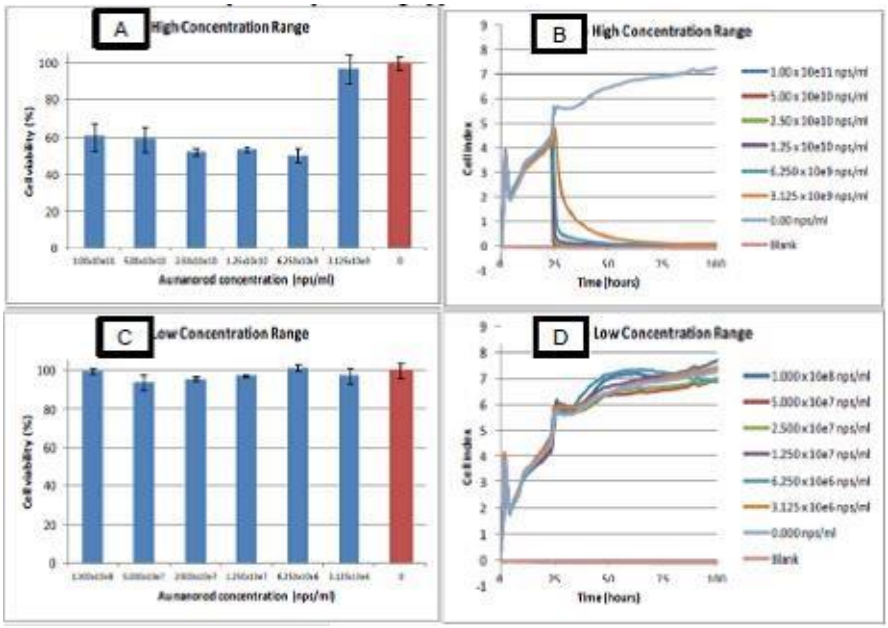

Figure 4 Toxicity studies using conventional assays (MTS) and unconventional assay (Xcelligence). A) High GNRs concentration MTS assay B) High concentration GNRs cell impedance readings C) Low GNRs concentration MTS assay D) Low concentration GNRs cell impedance readings
Notes: Cells treated with low concentration of GNRs showed no effect on cell index same as the untreated cells (cells only) .the cell index of the high concentrations of the GNRs appeared to be different showing a drastic decline suggesting cell death had occurred.

Photoactive effect of GNRs

The three control cells did not show any changes morphologically after 24 h incubation period as observed in (Figure).Following incubation, the morphology of experimental group CaCo-2 cells appeared altered as compared to all the three control cells. The experimental group had cells that had detached from the plate surface, cells appeared less uniform with loss of membrane integrity. The morphological differences were distinct when comparing with the control cells.
Quantitative assays were also performed to establish the extent of cell damage. Figure demonstrates that that the individual effects of either the laser or the GNRs did not cause a decrease in the viability percentage. Though, the viability percentage dropped drastically in the experimental groups (5 and 10 J/cm^2).The cell proliferation of the Caco-2 cells did not decrease when laser irradiation alone and GNRs alone were applied; though, when both laser and GNRs were combined, their effects led to a decrease in proliferation of the CaC0-2 cells for both fluencies (5 and 10 J/cm^2 respectively).laser irradiation by itself, as well as GNRs alone, showed decrease levels of LDH, but more cytodamage and substantial LDH release were observed at the same degree in both fluencies (5 and 10 J/cm^2 respectively) (Figures 5B, respectively).

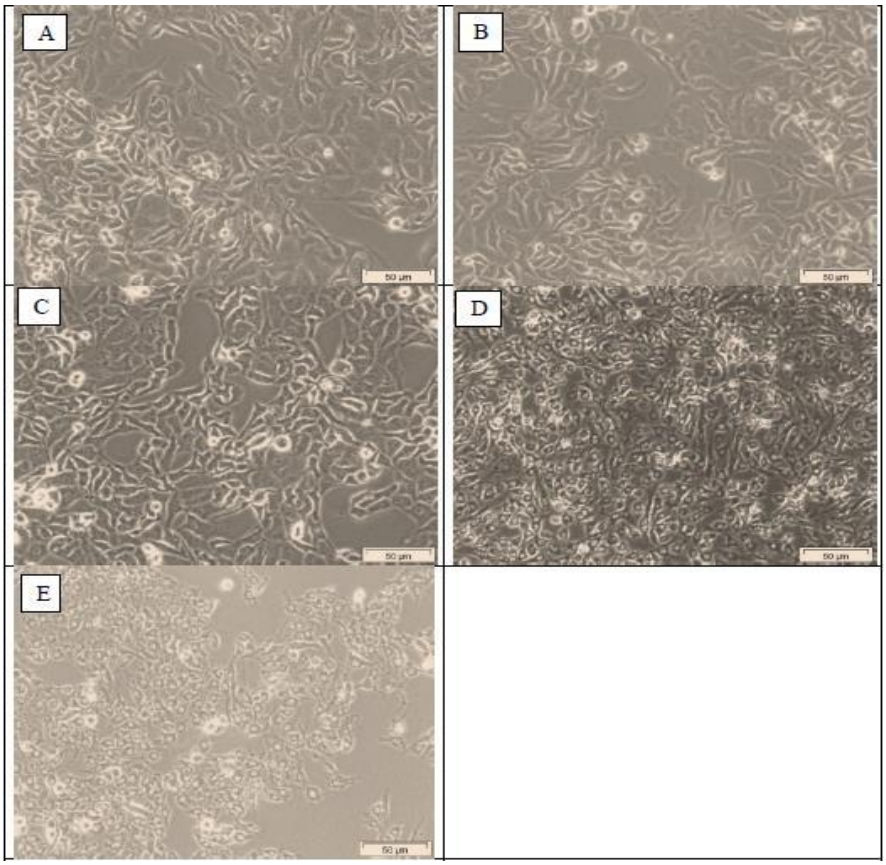

Figure 5 Cellular morphology after treatment of CaCo-2 cancer cells. A) Untreated cells (Cells only) B) 10 J/cm^2 Laser only C) GNRs 2.5 x10 ^{11}np/ml D) GNRs 2.5 x10 ^{11}np/ml + 5 J/cm^2 and E) GNRs 2.5 x10 ^{11}np/ml + 10 J/cm^2.CaCo-2 cells treated with either GNRs or 10 J/cm^2 laser irradiation only did not show any morphology differences to the untreated cells (cells only).
Notes: Following treatment with GNRs and laser irradiation at both fluences 5 J/cm^2 and 10 J/cm^2 cells changed in appearance, an increase with free-floating cells was observed.

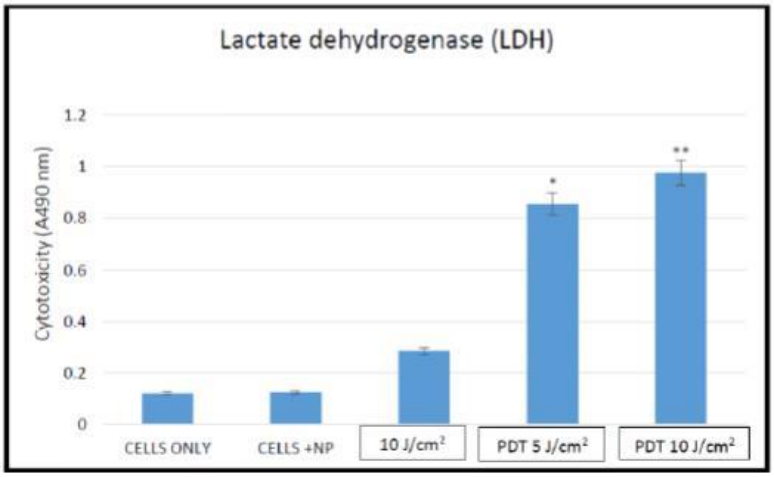

Figure 6: Cytotoxicity (LDH) of three control groups and two experimental groups of CaCo-2 cancer cells: Three control groups include cells alone, cells with 1.5 x 10 ^{11} ng/mL GNRs, cells with laser alone, and experimental group includes 1.5 x 10 ^{11} ng/mL of GNRs and 5 J/cm^2 and 10 J/cm^2 laser irradiation.
Notes: Upon Irradiation at a wavelength of 660 nm, GNRs increased cell damage significantly 5 J/cm^2 ($P < 0.005$) and 10 J/cm^2 ($P < 0.001$).

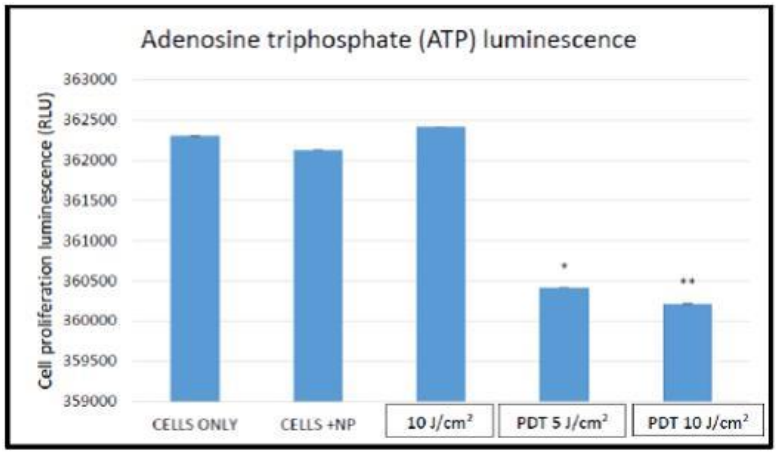

Figure 7: ATP Luminescence cell proliferation of three control groups and two experimental of CaCo-2 cancer cells: Three control groups include cells alone, cells with 1.5 x 10 ^{11} ng/mL GNRs, cells with laser alone, and experimental group includes 1.5 x 10 ^{11} ng/mL of GNRs and 5 J/cm^2 and 10 J/cm^2 laser irradiation.
Notes: Upon Irradiation at a wavelength of 660 nm, GNRs decreased proliferation significantly 5 J/cm^2 ($P < 0.005$) and 10 J/cm^2 ($P < 0.001$).

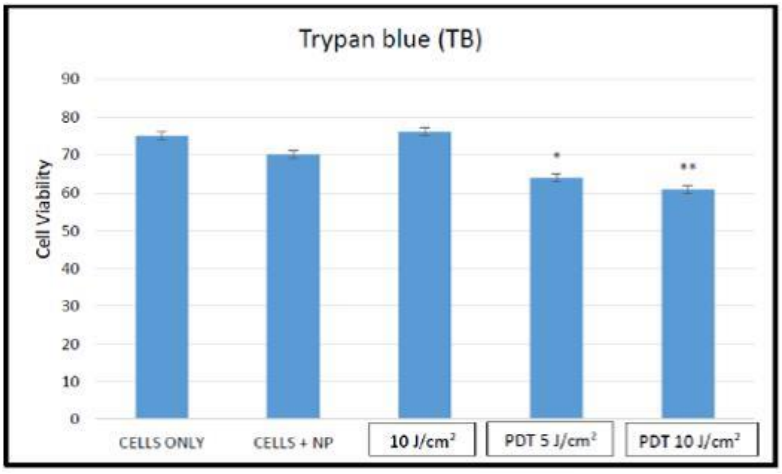

Figure 8: Cell viability (Trypan blue) of three control groups and two experimental of CaCo-2 cancer cells: Three control groups include cells alone, cells with 1.5 x 10 ^{11} ng/mL GNRs, cells with laser alone, and experimental group includes 1.5 x 10 ^{11} ng/mL of GNRs and 5 J/cm^2 and 10 J/cm^2 laser irradiation.
Notes: Upon Irradiation at a wavelength of 660 nm, GNRs decreased cell viability significantly 5 J/cm^2 ($P < 0.005$) and 10 J/cm^2 ($P < 0.001$).

Conclusions

In conclusion, the GNRs in solution were characterized by UV-visible spectroscopy and Transmission electron microscope. In addition the in vitro stabilities of the GNRs stored at room temperature in media were studied using UV-visible spectroscopy, measuring the zeta potential and hydrodynamic size using the Zetersizer and by measuring size using Transmission electron microscope. These particles were found to be easily dispersed in water and stable across the various experimental conditions that were adopted. The findings of the study lead to the conclusion that the GNRs can be effectively used in biomedical application as both chemotherapeutic agent and Photo active agent in the treatment of CaCo-2 cells.

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Disclosure

This article has neither been published, nor is being considered elsewhere for publication.
There is no conflict of interest.

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Table 1: [Concentration ranges]

Main Concentration range(nps/ml)				
Serial dilutions	1.00×10^{11}	1.00×10^{10}	1.00×10^{9}	1.00×10^{8}
	5.00×10^{11}	5.00×10^{10}	5.00×10^{9}	5.00×10^{8}
	2.50×10^{11}	2.50×10^{10}	2.50×10^{9}	2.50×10^{8}
	1.25×10^{11}	1.25×10^{10}	1.25×10^{9}	1.25×10^{8}
	6.25×10^{11}	6.25×10^{10}	6.25×10^{9}	6.25×10^{8}
	3.125×10^{11}	3.125×10^{10}	3.125×10^{9}	3.125×10^{8}
Control sample	0.00 nps /ml, Cells only			
Blank	No cells, Media only			

Table 2 [Physicochemical properties of gold nanorods in Di water and DMEM]

Sample	Average Particle size by TEM average	ζ-Potential (mV)	UV-vis	Average Particle size by(DLS)	pH at 37 °C
GNR in DI water	21+/-	0.110	654	53;9	1.55
GNR in DMEM	21+/-	0.101	654	124;17	7.06