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Optical nanobiosensor: A new analytical tool for monitoring carboplatin–DNA interaction *in vitro*

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ABSTRACT

The interaction of DNA and Carboplatin was studied with DNA labeled gold nanoparticles (AuNPs) based optical nanobiosensor. Carboplatin, a cytotoxic drug, is responsible for producing nephrotoxicity at effective dose. Thus, we have developed optical nanobiosensor for monitoring carboplatin–DNA interaction based on Fluorescence Resonance Energy Transfer (FRET) phenomenon. Paracetamol, an analgesic agent, was used as controlled drug in this study. The DNA labeled AuNPs, exposed to carboplatin, a binding event among the DNA and carboplatin takes place, resulting in a conformational change within the biosensor complex which decreases the distance among the fluorescent molecules or the fluorescent/quencher molecules. As the carboplatin interact with DNA, an increase in fluorescence intensity was observed. So, the major difference in increased fluorescence intensity between carboplatin–DNA and paracetamol–DNA interaction shows significant observations. Results have demonstrated that Optical sensor is able to rapidly and effectively monitor carboplatin–DNA interaction with a detection limit up to 0.45 μg/ml. This suggests that the developed optical nanobiosensor was ideal for monitoring Drug–DNA interaction studies while performing combinatorial synthesis for new drug development.

1. Introduction

Recently, much attention has been concentrated on the study of the interaction between the biological macromolecules, such as DNA–DNA, DNA–protein, and protein–protein. Especially for DNA damage which leads to loss of some functions of DNA, even to apoptosis of the cell and further induces cancer [1]. Tremendous amount of work has been done to investigate the relationship between DNA damage and cancer using different spectroscopic and chromatographic techniques and cell culture assays. Several papers have been reported on biosensor monitoring Drug–dsDNA, Drug–ssDNA, heavy metal–DNA and Drug–RNA interactions [2,3]. But, till date no such nanobiosensor has been reported which can monitor drug–DNA interaction. Here, we have developed an optical nanobiosensor based on gold nanoparticles labeled with DNA. Carboplatin is a second generation cytotoxic drug exhibiting significant anti-cancer activity. The anti-tumor effect is due to the interaction with DNA via intrastrand and interstrand cross-links. This leads to DNA damage and adduct formation, which may contribute to toxic effects [1]. Paracetamol, an analgesic drug, was used as controlled drug in this study. It is a toxicity free drug and does not damage DNA. Our study shows significant observations in fluorescent intensity amongst carboplatin and paracetamol.

Nanotechnology is playing an increasingly important role in the advancement of biosensors. The performance of biosensors is being improved by using nanomaterials for their construction [4]. Nanoparticles play a key role in adsorption of biomolecules due to their large specific surface area and high surface free energy. Gold nnoparticles and quantum dots have been widely used due to their optical properties. Recently, regulation of protein–DNA interaction was reported by Jun Fang and her coworkers [5]. Xinbing Zuo and his team developed different DNA probes on AuNPs to compare single stranded DNA and hybridized DNA interaction with Hg^{2+} using both absorption and fluorescence detection [7]. Numerous research work has been done to study DNA detection and DNA hybridization assays using gold nanoparticles and quantum dots [6–13]. Thus, combination of nanomaterials and biomolecules is of considerable interest in nanobiotechnology. Fig. 1 shows the schematic representation of development of optical nanobiosensor. DNA labeled AuNPs when exposed to drug solution show decreased FRET.

Fluorescence resonance energy transfer (FRET) assays are often used to identify the interaction of two molecules. One molecule is labeled with a fluorescence acceptor, which is excited only when a molecule—usually a binding partner—bearing a fluorescence donor is in the vicinity. In general, the energy transfers from the

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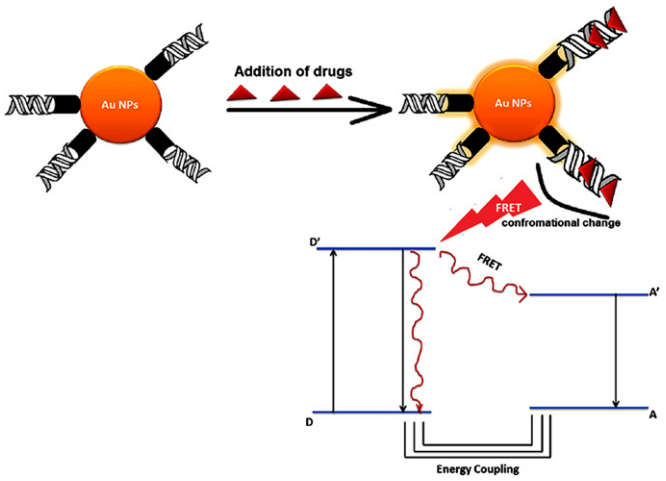

Fig. 1. Schematic representation of optical nanobiosensor. AuNPs were labeled with DNA and exposed to drug solution. Fluorescence enhancement observed on binding of DNA to drug via FRET. Solid and wavy arrows indicate the radiative and nonradiative processes, respectively.

donor to the acceptor [14]. In 1948, Theodor Förster observed that when the resonating dipole moments of two molecules are identical, which in this case are D and A, an energy coupling between the two occurred resulting in the photon-less transfer of energy. In addition, the excited acceptor molecule (A′) returns to the ground state (A) by losing its energy via photon emission (in case, acceptor is a fluorophore), i.e., fluorescence (Fig. 1) [15]. Irrespective of the photo-physical characteristic of the acceptor, i.e., whether it is a chromophore or fluorophore, the energy transfer process is called as Förster resonance energy transfer colloquially referred to as fluorescence resonance energy transfer. When the acceptor molecule is non-emitting then the fluorescence intensity is solely due to the donor's fluorescence, as in the case of this experiment.

2. Experimental

2.1. Chemicals

Highly polymerized calf thymus DNA (MP Biomedicals, US) was used in this study. DNA dilutions were prepared in phosphate buffer pH 7. Phosphate buffer was prepared by dissolving 0.1 M disodium hydrogen phosphate in water and adjusting the pH by adding 0.1 M HCl. $HAuCl_4$ and Tri-sodium citrate were used to prepare AuNPs. All chemicals were purchased from E-Merck (Mumbai, India), SRL (Mumbai, India) and were all analytical reagent grade. Carboplatin was obtained from Cipla Ltd. and used without purification. Paracetamol was obtained from Sun Pharmaceuticals. All aqueous solutions were prepared in Milli-Q water from a Millipore purification system and all experiments were done at room temperature.

2.2. Characterization and measurement

Fluorescence intensity was measured by Jasco FP-6500 spectroflurometer (Jasco, Japan) at a scan rate of 200 nm/min. An excitation and emission bandwidth of 10 nm and 5 nm was used respectively. Excitation and emission wavelength of 450 nm and 480 nm was used respectively. UV spectra were obtained on a JASCO V-670 spectrophotometer (Jasco, Japan). Particle size of AuNPs was carried out by Malvern Zeta-sizer (Model—The Zetasizer Nano ZS, UK).

2.3. Citrate-capped AuNPs synthesis and modification

AuNPs were prepared by citrate reduction of $HAuCl_4$ according to documented methods with slight modifications [16–17]. A 25 ml aqueous solution containing of 1 mM $HAuCl_4$ was brought to a vigorous boil with stirring in a round bottom flask; a 2.5 ml, 38.8 mM trisodium citrate solution was then added rapidly to the above solution. The mixture solution was heated for another 20 min and color of the solution changed from pale yellow to deep red. Subsequently, the solution was cooled to room temperature and stirred continuously. Finally, a deep red, monodisperse "naked" AuNPs was obtained and this solution was used as the stock solution. The sizes of the AuNPs were verified by Malvern Zeta-sizer.

2.4. DNA labeled AuNPs

Citrate capped AuNPs were labeled with dsDNA (double stranded DNA). Twenty mg/ml DNA solution was prepared in phosphate buffer having pH 7. AuNPs solution formerly prepared was diluted (by double-distilled water) to 2×2 ml of diluted AuNPs was added to 2 ml of DNA solution, and the solution was stirred at 150 rpm for 3 min, which was then used for carboplatin–DNA and paracetamol–DNA interaction studies.

3. Results and discussion

3.1. Characterization of gold nanoparticles

Fig. 2 shows the SEM image of AuNPs. The particles were predominantly spherical in shape with diameter ranging 20 ± 5 nm. Larger particles with diameter 40 ± 10 nm were also obtained.

The particle size characterization was determined by Malvern zeta-sizer. This instrument allows the measurement of particle size distributions in the range 0.6 nm–10 μm. The average particle size of AuNPs was 14 nm. Fig. 3(A) shows the particle size distribution of AuNPs. Particles were in range of 25.92 nm (83.7%), 0.7579 nm (10%), and 3.039 nm (3.3%). From Malvern zeta sizer and SEM analysis, it is clear that most of the particles were ranging 20 ± 5 nm and suggested as donor particles in the experiment.

3.2. Evaluation of DNA labeled AuNPs

dsDNA has a stable double-helix geometry that always presents the negatively charged phosphate backbone [7]. So they have

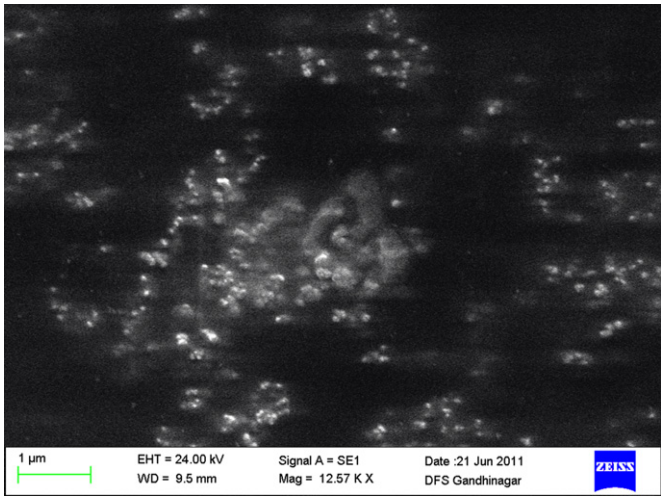

Fig. 2. SEM image of AuNPs.