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Enhanced single strand breaks of supercoiled DNA in a matrix of gold nanotubes under X-ray irradiation

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ABSTRACT

Single-strand-breaks (SSBs) of supercoiled DNA (scDNA) molecules were used to probe the enhancement of X-ray radiation effect on scDNA mixed with gold nanotubes (AuNTs) in water. The amounts of measured enhancements using SSBs were significantly lower than the expected increase in energy deposition in water by AuNTs under hard X-ray irradiation. Three factors were identified to negatively affect the enhancement: (1) Attenuation of kinetic energies carried by electrons escaped from AuNTs, (2) Scavenging of OH radicals (·OH) by the surface of bare AuNTs, and (3) Steric effect due to soluble scDNA molecules away from the surface of AuNTs. Benefits and limits of using gold nanomaterials as radiation enhancers and contrast agents are discussed.

1. Introduction

Nanomaterials have been used to increase the absorption of electromagnetic radiation in X-ray, optical, and microwave wavelength regions [1–6]. In the region of visible or near IR optical wavelength, the absorbed radiation is suggested to be converted to heat that leads to a temperature jump in the immediate medium such as water surrounding the nanomaterials. Interactions of X-rays with nanomaterials, however, give rise to outcomes quite different from those stated above, and investigations to date have uncovered at least three important mechanisms that are critical to developing new materials, devices, and methodologies for energy production, cancer treatment, and 3-D imaging.

The most surprising mechanism involving X-ray irradiation of nanomaterials is a process named chemical enhancement (CE) [7]. It is the first example in which the surface of X-ray activated nanomaterials is found to be responsible for increasing the yield of a chemical reaction. Such an enhancement is claimed to be ubiquitous, and it is expected that many other reactions may be similarly enhanced when the surface of nanomaterials is made suitable for catalyzing these reactions.

A more readily perceived enhancement of X-ray effects by nanomaterials is through physical processes that do not rely on the chemical properties of the surface of nanomaterials. In one demonstration of such interactions, which is of nanoscale, small gold nanoparticles (3-nm dia.) were found to enhance the effect of hard X-rays through releasing low-energy electrons that deposit their energy within nanometers of the surface of the absorbing nanoparticles [6]. Such a local energy absorption and deposition (denoted as type 2 physical enhancement or T2PE) can be used to excite fluorophores within nanometer distances of the nanoparticles, to create chemically active species in a volume of nanometer dimensions, or to generate geometry enhancement when nanostructures are arranged in geometries favoring the highest enhancement [8]. One application of employing T2PE is the creation of nanomachines that can utilize low-energy electrons released from nanoparticles to produce reactive oxygen species (ROS) such as hydroxyl radicals (·OH) within a small volume around the nanoparticles. One of the early demonstrations of such mechanisms was that ·OH generated from these low-energy electrons released from gold nanoparticles caused damage to biological targets such as DNA molecules directly bound to these nanoparticles [5,6]. Such an externally triggered process occurring on the nanometer scale may be useful for local initiation of chemical reactions. The local effect has been demonstrated to create more single-strand-breaks (SSBs) when small gold nanoparticles chemically conjugated to supercoiled DNA molecules

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(scDNA) were radiated with X-rays [6], and the enhancement was as high as 300% for approximately ten 3-nm diameter gold nanoparticles intercalated to a ~5376 base-pair DNA molecule.

However, in order to use CE or T2PE, the nanomaterials need to be placed within nanometers of the target. For instance for cancer treatment where nuclear DNA is targeted, nanoparticles would be required to be placed within nanometers of the nuclear DNA, which can be challenging. In practice, trafficking nanomaterials into the cell has been realized under many different conditions, but delivering nanoparticles to nuclear DNA is still difficult [9–13].

A second type of enhancement of physical nature is termed remote or average effect (here denoted as type 1 physical enhancement or T1PE) that results from energy deposition by energetic electrons released from gold nanoparticles with many keV kinetic energy. T1PE leads to the deposition of additional energy in water over a volume of the order of at least microns in any direction. Most reports published to date cited T1PE as the main cause to explain their observed effects. For example, preliminary animal work using small gold nanoparticles in animal radiation therapy was conducted, and it was found that these nanoparticles may cause enhanced toxicity to tumor tissues in cancer-bearing mice [14]. The exact mechanisms of the enhanced damage were unknown, but it was speculated that the observed therapeutic results were caused by T1PE.

In light of the new developments in the area of nanomaterials synthesis and a tremendous interest in using nanomaterials to improve traditional X-ray applications, one may wonder to what extent nanostructures can be employed to enhance the absorption of ionizing radiation and generate enhanced damage to biological targets if only T1PE is supposed to be the dominating mechanism. At the first glance, it seems that greater densities or percentage weight of nanostructures should lead to more absorption of ionizing radiation and, therefore, greater damage to the target. However, this may not necessarily be true because several additional processes may complicate this simple and straightforward physical picture. For instance, in order to effectively generate ·OH in water, a majority of the absorbed electromagnetic energy must escape the nanostructures. If the physical dimension of the structure is too large, then the absorbed X-ray energy cannot be completely released in the form of kinetic energy of the escaping electrons. Another requirement is that the added nanomaterials should not scavenge ROS such as ·OH. In many cases, surfactants and even nanomaterials themselves scavenge ·OH, reducing enhancement or even causing anti-enhancement [7]. This is an effect opposite to CE mentioned above: CE enhances X-ray effect through "catalytic processes", whereas scavenging attenuates enhancement, and both processes rely on gold surface. Yet another requirement is that the distance between nanomaterials and the target should be optimized. For example, the distance between nanostructures should not be too large so that the targets can still be effectively damaged by the electrons released from the nanostructures and radicals generated by these electrons.

In order to explore the upper limit of T1PE, we employed gold nanotubes (AuNTs) that not only effectively absorb hard X-rays but also can be made in dimensions favoring the release of energetic electrons of up to a few keV. These nanotubes also possess relatively large surface areas, are self-supported (even though they are not soluble) in water without being highly soluble, and are separated from each other by hundreds of nanometers in average distance. These characteristics seem to suggest that these nanotubes may create extremely high enhancements. In this report, we wish to demonstrate that these AuNTs can indeed increase the radiation damage to DNA molecules in water. However, because we employed chemical probes of DNA SSBs that did not directly measure the energy deposition enhancement, the experimentally observed damage in the form of SSBs was much lower than the expected values derived from the increased X-ray absorption and subsequent energy deposition due to AuNTs. In the following, we will present the experimental results of using AuNTs to enhance SSBs of scDNA molecules under hard X-ray irradiation, followed by theoretical discussions. Among many benefits, the results obtained here can provide guidance to future nanomaterial-based therapeutic studies, and a brief discussion regarding using nanomaterials to enhance energy deposition in cells is presented.

2. Experimental and theoretical methods

2.1. Gold nanotubes synthesis & gold nanotube–DNA mixing

Gold nanotubes (AuNTs) were synthesized as previously reported [15]. To prepare smooth-surface nanotubes for scDNA irradiation experiments, 0.5 ml aliquots of the AuNTs (containing nearly 60 mg AuNTs, weighed with a microbalance) suspended in water were transferred to Smith process vials and sealed with Teflon septums and aluminum caps. The filled vials were then heated in an Emrys Optimizer™ (Personal Chemistry, 2.45 GHz) for 10 min at 150 °C and 3.8 bars above ambient pressure to smooth the surface. The nanotubes were then collected by centrifugation and redispersed by vortexing in autoclaved 18 MΩ H_2O. 1.0 ml aliquots of the suspended nanotube solution containing 18 ± 1 mg of nanotubes were placed in homemade ½ in. diameter shallow plastic vials for radiation experiments. The nanotubes were allowed to settle out of solution to form a AuNT matrix, and water in it was removed by repeated micropipetting. The dry form of AuNTs in the home-made plastic vials was inspected with scanning electron microscopy (SEM, FEI XL30 SFEG). After no more water could be removed from the vials, 100 ng of ϕX174 scDNA molecules (Invitrogen) in 20 μl water with the required Tris concentration was then injected into the resulting gold nanotubes matrix in the vials. It was observed that the thickness of the dry AuNT matrix matched that of water in the vials, and the total thickness of the AuNT aqueous samples in the vials was less than a few hundred microns, allowing uniform radiation throughout the whole sample.

2.2. Radiation and detection of SSBs of scDNA

The samples were then irradiated with X-rays (HP Faxitron 43855A, operated at 110 kV and 3 mA) for specified times and at specified dose rates. After irradiation, the DNA solution was removed from the AuNT matrix with a micropipette and placed in an agarose gel for analysis (Invitrogen, 0.8% Agarose). The gel electrophoresis was used to determine the percentage of the scDNA with one SSB in the entire scDNA molecule. The percentage of circular DNA with one SSB was kept between 20% and 50% in all experiments to guarantee that the amount of damage was linearly dependent on the X-ray dose. The enhancement was determined by the ratio of slopes of damage as a function of X-ray dose within the said limits for two samples, with and without AuNTs. This method was much more accurate than measuring damage at a single X-ray dose. More than 10 measurements at different X-ray doses were performed at each Tris concentration to obtain the slopes, and at least three sets of measurements of the slope were carried out to obtain the reported enhancement at each Tris concentration. The average enhancement and standard deviation were calculated for each Tris concentration.

2.3. Modeling methods

A detailed description of the Monte-Carlo method is given in Supplementary Material. In brief, each of the following steps was modeled. First, X-rays from a 100-kVp tungsten target were