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Determining the Size Dependence of Colloidal Gold Nanoparticle Uptake in a Tumor-like Interface (Hypoxic)

Mehrnoosh Neshatian [a], Stephen Chung [b], Darren Yohan [a], Celina Yang [a], Devika B. Chithrani [a,c,*]

[a] Department of Physics, Ryerson University, 350 Victoria Street, Toronto, ON M5B 2K3, Canada
[b] Ontario Cancer Institute, Toronto Medical Discovery Tower, Toronto, ON M5G 1L7, Canada
[c] Keenan Research Centre, Li Ka Shing Knowledge Institute, St. Michael's Hospital, 30 Bond Street, Toronto, ON M5B 1W8, Canada

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ABSTRACT

Colloidal gold nanoparticles (GNPs) are being used as drug delivery vehicles and radiation dose enhancers in cancer therapy. Oxygen concentration in human tumours is highly heterogeneous with many regions at very low levels of oxygen (hypoxia). A majority of tumours contain regions with oxygen pressure values of less than 0.7% in the gas phase. The purpose of this study was to investigate how the size of the NPs affects their uptake process in a tumour-like hypoxic environment. We used GNPs of diameter 15, 50, and 74 nm, and carried out our experiment under 0.2% (hypoxic) and 21% (normoxic) oxygen levels using MCF-7 and HeLa cells. Our results showed that NPs of size 50 nm had the highest uptake following prolonged exposure to hypoxia. There was no significant toxicity introduced by NPs under hypoxic conditions. These findings will play a vital role in the optimization of GNP-based therapeutics in cancer treatment.

The ultimate or fundamental goal of nanoparticle (NP) based platforms is the successful targeted delivery and monitoring of therapeutics to tumors, while causing minimal damage to normal tissue and side effects to the patient. Among other NPs, colloidal gold NPs (GNPs) are being explored as a model NP-system for cancer research due to their ability to act as both a radiosensitizer and drug carrier in cancer therapy [1–3]. Previous studies have shown that the size of the NP matters [4–6]. For example, GNPs of size 50 nm showed the highest radiation dose enhancement among NPs of sizes between 14 and 74 nm [4]. Most of these studies were performed with properly oxygenated (normoxic) cells. However, if we were to use these NPs effectively for cancer therapeutic applications, it is essential to understand their uptake behavior in a tumor-like environment, such as hypoxia.

It has been shown that low levels of oxygenation (hypoxia), commonly present in solid tumors, protect cells from death by irradiation [7]. For example, damage to DNA is created via direct ionization from radiation, or is induced by the interaction with free radicals (e.g. hydroxyl radical) formed by the ionization of water surrounding the DNA. If oxygen is available, it can react with the broken ends of DNA, thereby creating stable organic peroxides. This type of DNA damage cannot be easily repaired. However, the damage is more readily repairable in the absence of molecular oxygen which would lead to less damage following radiation or chemotherapy [8,9]. One of the primary reasons for cancer recurrence is that these hypoxic cells can survive the treatment. GNPs are being explored to overcome the resistance by these hypoxic cells since they can be used in combined therapeutics of radiation therapy and chemotherapy [2]. However, it is not known how the GNP-based therapeutic response would modulate in a real tumor where hypoxia is present. If we were to use GNPs for improved cancer therapeutics, it is necessary to understand their behavior under hypoxia.

A majority of solid tumors contain regions with O_2 pressure values of less than 0.7% O_2 in the gas phase, while the partial pressure of oxygen of normal tissues is about 4–7% O_2 in the gas phase [10–12]. We conducted our experiments under 0.2% O_2 level. The effect of the size of colloidal GNPs on their cellular uptake is known under normoxic conditions [4]. For example, colloidal GNPs of size 50 nm have the highest cell uptake among the size range 14–74 nm. However, most of the cancer cells in the solid tumor are hypoxic. It is not known yet how the size of colloidal GNPs affects their cellular uptake in a real tumor-like environment (hypoxic). Hence, the goal of this study is to investigate how the size of colloidal GNPs affects their cellular uptake in a

* Corresponding author at: Department of Physics, Ryerson University, 350 Victoria street, Toronto, ON M5B 2K3, Canada. Tel.: +1 416 979 5000x4115; fax: +1 416 979 5000.
E-mail address: devika.chithrani@ryerson.ca (D.B. Chithrani).

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Diameter of GNPs (nm)	As made gold		GNPs in tissue culture media (incubated in normoxic chamber for 24 hours)		GNPs in tissue culture media (incubated in hypoxic chamber for 24 hours)	
	Hydrodynamic diameter (nm)	UV visible wavelength (nm)	Hydrodynamic diameter (nm)	UV visible wavelength (nm)	Hydrodynamic diameter (nm)	UV visible wavelength (nm)
15	18 .3 ± 4.3	519	23.2 ± 3.1	523	24.1 ± 4.6	524
50	55.2 ± 5.5	535	61.8 ± 6.1	540	61.2 ± 5.6	539
70	76.4 ± 7.2	550	80.7 ± 6.8	554	81.2 ± 7.3	555

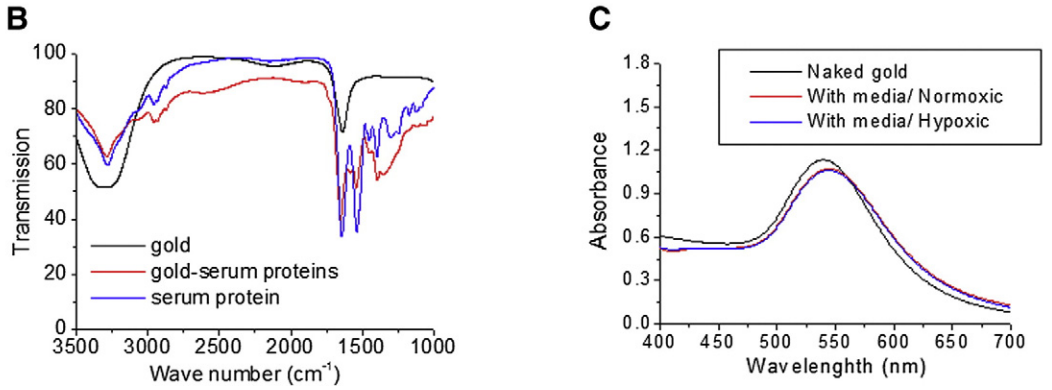

Fig. 1. Characterization of colloidal GNPs. A) Hydrodynamic diameter and UV visible peak wavelength of as-made GNPs and GNPs incubated with FBS supplemented media for duration of 24 h under normoxic and hypoxic (0.2% O_2) conditions, respectively. B–C) FTIR spectra and UV visible spectra of naked 50 nm GNPs and GNPs incubated with FBS supplemented media for duration of 24 h in normoxic and hypoxic (0.2% O_2) conditions, respectively.

hypoxic tumor environment to improve the bio-nano interface. If there is an optimum NP size, we can use that particular size for an improved outcome in therapeutic applications. For example, colloidal GNPs are being explored as radiation sensitizers in radiation therapy and drug carriers in chemotherapy [13]. Hence, it is important to evaluate the size-dependent uptake of colloidal GNPs in a real tumor-like environment, if we were to use them for such therapeutic applications. This article demonstrates how colloidal GNPs can be used to optimize the bio-nano interface in a real tumor-like environment (hypoxic), since their size and surface properties can be tailored easily.

GNPs of diameter 15, 50, and 70 nm were synthesized using the citrate reduction method. Colloidal GNPs were characterized by UV–vis spectroscopy, Dynamic Light Scattering (DLS), and Transmission Electron Microscopy (TEM) imaging. To study the effect of the hypoxic environment on the stability of NPs, they were kept in a hypoxia chamber for 24 h. UV–vis spectroscopy and DLS measurements were performed to investigate the changing characteristics of the NPs. GNPs were also incubated in the tissue culture media supplemented with FBS (Fetal Bovine Serum) under hypoxic and normoxic conditions for 24 h. The medium can have a profound influence on particle uptake

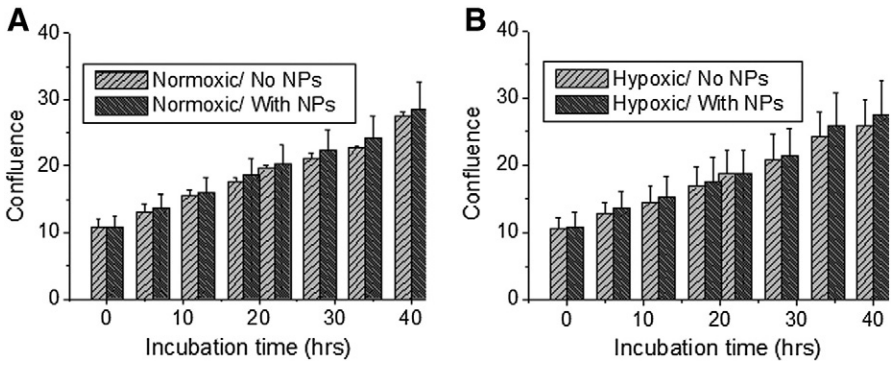

Fig. 2. Evaluation of the toxicity of GNPs. A–B) The toxicity induced by NPs was measured by monitoring cell proliferation for MCF-7 cells in normoxic and hypoxic conditions, respectively. The concentration of NPs used was 0.6 nmol. All the results are the mean of three independent experiments ± SE.