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Comparative study between the photodynamic ability of gold and silver nanoparticles in mediating cell death in breast and lung cancer cell lines

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ABSTRACT

Cancer is one of the dreadest diseases once diagnosed and has severe impacts on health, social and economic global aspects. Nanomedicine is considered an emerging approach for early cancer diagnosis and treatment. The multifunctional effects of silver and gold nanoparticles (Ag and Au NPs) have rendered them to be potent candidates for biomedical applications. The current work presents a comparative study between Au NPs and Ag NPs as possible potent photosensitizers (PS) in photodynamic therapy (PDT). Transmission electron microscopy (TEM) was used to identify and characterize the shape, size, and cellular localization of Au NPs; the absorption properties of Au NPs were determined using ultraviolet–visible spectroscopy (UV–Vis) and zeta potential was used to identify surface charge. Inverted light microscopy (LM), Trypan blue exclusion assay, adenosine triphosphate luminescence (ATP), and lactate dehydrogenase membrane integrity assays (LDH) were used for investigating the photodynamic ability of these nanostructures on breast (MCF-7) and lung (A549) cancer cell lines. Flow cytometry using Annexin V and propidium iodide (PI) dyes was used to determine the cell death pathway induced. The average size of the synthesized Au NPs was 50 nm, having an absorption peak at 540 nm with −7.85 mV surface net charge. MCF-7 and A549 cells were able to absorb the Au NPs. The latter, when irradiated with laser light in the phototherapeutic window, promoted cytotoxicity and a significant reduction in cell viability and proliferation were observed. The photodynamic activity that was observed in both cancer cell lines was found to be less eminent than that observed in case of the Ag NPs when compared to Au NPs. The present study is the first that compares the photodynamic ability of two different nanoparticles, silver and gold, as photosensitizers without any further functionalization. This study extends the possibilities of using such nanostructures in PDT within the therapeutic window wavelength, yet through the conjugation of Au NPs with other photosensitizers to synergize its effect.

1. Introduction

In Africa the quadruple burden of non-communicable diseases, of which cancer is a major contributor, have crippled the health, social and economic systems of most countries. According to a survey performed by the international agency for research on cancer (IARC), an estimated 542,000 cancer deaths and 715,000 new cancer cases are recorded in Africa annually. The agency expects these figures to double and reach 1.28 million new cancer cases and 970,000 cancer deaths by 2030. They based their estimates on the rapid growth of the population as well as aging rate [1].

The current work focused on two major cancer types—breast and lung cancer since the incidence of these cancers is high in Africa and rapidly increasing yearly. Breast cancer is the leading cause and most diagnosed type of cancer in women and accounts for 4500 and 14,600 deaths as well as 9000 and 28,000 newly diagnosed cases in the southern and northern parts of Africa respectively. The fact that the highest incident rate of breast cancer was found to be in women from the southern part of the continent can be justified by the demographic analysis, where the white population constructs a big portion of the population map. This population is characterized by early menarche and late child bearing age which are considered high breast cancer risk factors [2]. Tobacco epidemic accounts for the wide dispersion of lung cancer on the whole continent, but with more severity in the southern parts. Sixty five percent of lung cancer could be attributed to smoking. Lung cancer accounts for 9600 deaths and 10,400 newly diagnosed cases in the northern parts of the continent [3–5].

Despite the emerging need of PDT in the field of cancer treatment, there are still many therapeutic limitations that include the absence of sound protocols and the relatively poor cure rate when compared to other non-discriminative chemo- or radio therapeutic modalities. Such limitations are challenging PDT development as a diagnostic and a therapeutic tool. Recently, the interests increased in using nanoparticles in

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various applications, one of which could be PDT. Nano structures have two distinct characteristics which render them suitable candidates as they have high surface area to volume ratio and possess different physical and optical properties than those when the element in the bulk state.

Au NPs have been studied more intensively in the medical applications due to numerous characteristics. They are highly permeable with a high safety profile, can be used as an imaging enhancing tool, a drug vehicle delivery system as well as targeting certain cells upon conjugation with antibodies. Nevertheless, the latter has the disadvantage of the poor orientation of the antibody recognition portion and consequently non-specific binding [6]. Various shapes of Au NPs have been shown to be photothermal agents upon illumination with light wavelength at 700–800 nm [7]. Yet, there are many drawbacks in adopting the photothermal regime where the cancer cells are killed by the heat generated by the NPs upon irradiation with an external source of very high power density that can reach to 40 J/cm^2 with poor selectivity. Moreover, Au NPs based on having high atomic number have been used to increase the efficiency of radiotherapy regime by destroying the DNA of cancer cells through tumor-localized photoelectron and Auger electron ejection [8]. Other studies are focusing on conjugating Au NPs with well-known photosensitizers to enhance the quantum yield of singlet oxygen and thus more killing efficiency and subsequent decrease of the cancer cells [9, 10]. In parallel to the recent growing interests of developing functionalized and conjugated NPs with PS drugs to enhance the PDT efficacy and singlet oxygen yield, the current work shed light on the ability of Au NPs on their own in killing cancer cells and comparing its potential activity with the PDT effect of Ag NPs that were previously investigated. The approach aims for a better understanding of the possible actions for such NPs that can be added as the next step for functionalizing and conjugating with suitable drugs. Eventually, we are aiming in developing a potent and highly selective photosensitizer to enhance the PDT efficiency that can conquer cancer disease.

2. Materials and Methods

Cancer cells were selected to be the same cell lines that have been used in our earlier work with Ag NPs for the comparative aim of this manuscript. Breast (MCF-7) and lung (A549) cancer cell lines were used from the American Type Cell Culture (ATCC; Manassas, VA, USA) (ATCC® HTB-22 and ATCC® CCL-185, respectively). All culture conditions, media and kits used for investigation were to be exactly the same as the previous work with Ag NPs; for instance the media used for cell culture is manufactured by Gibco® (Thermo Fisher Scientific, Waltham, MA, USA) and the cultural plates are incubated in the same incubators with the same incubation conditions. The assays used in the current cellular investigations; Trypan blue exclusion, Annexin V and propidium iodide (PI) are all obtained from Sigma-Aldrich Co. (St Louis, MO, USA).

The CellTiter-Glo® Luminescent Cell Viability Assay and CytoTox 96® assay were supplied from Promega (Promega Corporation, Madison, WI, USA). The measurements of the flow cytometry assay were performed using the BD FACSAriaTM (642226; BD Biosciences, San Jose, Ca, USA) while Victor3 Multilabel Plate Reader (PerkinElmer Inc., Waltham, MA, USA) was used in cellular assays measurements. The Au NPs were synthesized according to the synthesis described by Nombana et al. [11] In summary, $HAuCl_4 \cdot 3(H2O)_3$ (0.019 g, 25 mM) was stirred in 4 ml toluene followed by a drop wise addition of $NaBH_4$ (0.002 g, 36 mM) dissolved in 3 ml of water to the brownish solution of the gold till eventually the solution became purple and left stirring for 40 minutes. The excess reducing agent was washed out with water several times leaving Au NPs of an estimated concentration of $4.99 \times 10^{-5}\ M^{-1}L^{-1}$. The Au NPs were stored in a minimum amount of toluene, where toluene, $HAuCl_4 \cdot 3(H2O)_3$, $NaBH_4$ were obtained from Sigma Aldrich—South Africa.

Table 1
Parameters of the lasers used in this study [12].

Parameters	Diode laser
Manufacturer	Oriel Corporation
Wavelength	636 nm
Wave emission	Continuous
Spot size	9.1 cm^2
Output power	88 mW
Power density	10.288 mW/cm^2
Light doses	10 J/cm^2
	15 J/cm^2
	20 J/cm^2
Irradiation times	16 min, 11 s
	24 min, 17 s
	32 min, 23 s

Cell lines were irradiated by the same laser device that has been provided by the National Laser Centre of South Africa, Pretoria, South Africa with the same parameters that were used in the earlier work with Ag NPs. The irradiation wavelength was 636 nm with a power density of 10.288 mW/cm^2 and the samples were irradiated after calculating the required time to get a final light dose of 10 J/cm^2, 15 J/cm^2, and 20 J/cm^2 respectively. The laser parameters are summarized in Table 1.

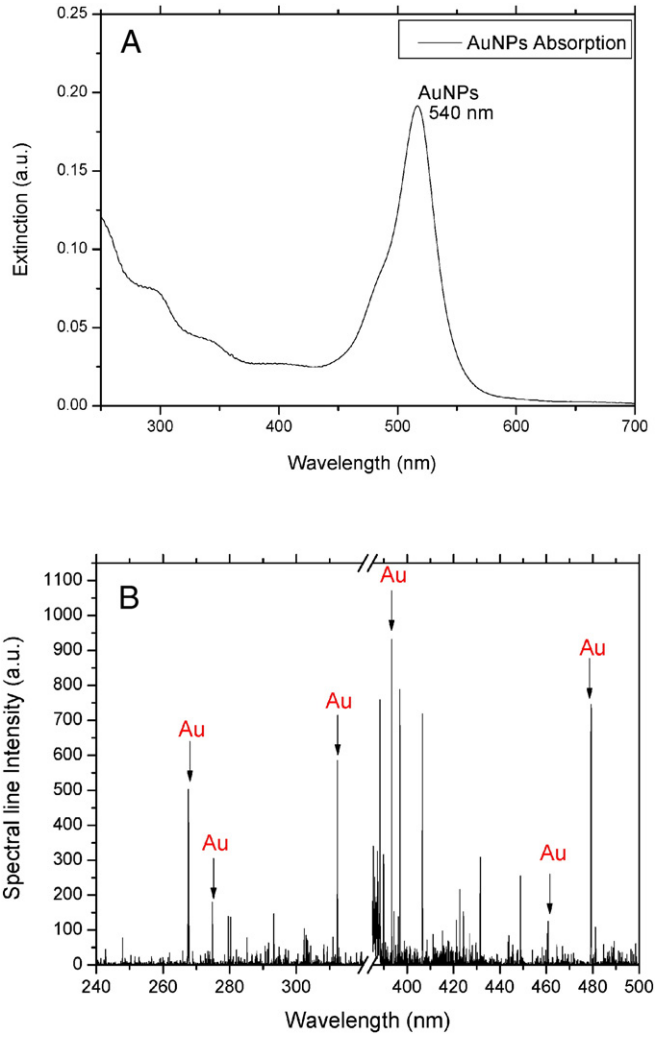

Fig. 1. (A) UV–Vis absorption spectrum shows the absorption peak of Au NPs to be at 540 nm and (B) LIBS spectrum for the synthesized nanoparticles confirming the presence of gold.