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In vitro monitoring of oxidative processes with self-aggregating gold nanoparticles using all-optical photoacoustic spectroscopy

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ABSTRACT

In this work, the assembly of gold nanoparticles of (AuNPs) is used to detect the presence of the biomolecule glutathione (GSH) using a novel technique called "all-optical photoacoustic spectroscopy" (AOPAS). The AOPAS technique coupled with AuNPs forms the basis of a biosensing technique capable of probing the dynamic evolution of nano-bio interfaces within a microscopic volume. Dynamic Light Scattering (DLS) and ultraviolet–visible (UV–vis) spectra were measured to describe the kinetics governing the interparticle interactions by monitoring the AuNPs assembly and evolution of the surface plasmon resonance (SPR) band. A comparison of the same dynamic evolution of AuNPs assembly was performed using the AOPAS technique to confirm the validity of this method. The fundamental study is complemented by a demonstration of the performance of this biosensing technique in the presence of cell culture medium containing fetal bovine serum (FBS), which forms a protein corona on the surface of the AuNPs. This work demonstrates that the *in vitro* monitoring capabilities of the AOPAS provides sensitive measurement at the microscopic level and low nanoparticle concentrations without the artifacts limiting the use of conventional biosensing methods, such as fluorescent indicators. The AOPAS technique not only provides a facile approach for *in vitro* biosensing, but also shed a light on the real-time detection of thiol containing oxidative stress biomarkers in live systems using AuNPs.

1. Introduction

The interface between thiol-containing amino acids and gold nanoparticles (AuNPs) has become a useful tool in bioanalytical applications due to the distinct properties of the nanoparticles (Elghanian et al., 1997; Rosi and Mirkin, 2005; Voliani et al., 2012; Wang et al., 2005). The challenge in biological applications is to develop highly sensitive and selective agents that can overcome the deficiencies of conventional sensing technologies while utilizing practical and robust means of detection. Among the various nanoparticle compositions, nanoparticles composed of gold and silver have successfully been implemented into assays to monitor biomolecular interactions by providing information *via* optical, electric or chemical signals (Alivisatos, 2004; Maswadi et al., 2011; Medintz et al., 2003; Rosi and Mirkin, 2005; Wang et al., 2004). Amid these selective sensing mechanisms, molecularly mediated processing and assembly of AuNPs has emerged as an important strategy (Lim et al., 2006, 2008; Lim and Zhong, 2009). The ability to manipulate size, shape, composition, and interparticle properties of nanoparticles confers considerable flexibility in the design of sensitive and selective nanomaterial-based sensors.

Thiol-containing amino acids such as glutathione (GSH), homocysteine (Hcys) and cysteine (Cys), have a significant role in this type of interparticle interaction *via* amino acid linkages (Zakaria et al., 2013; Zhang et al., 2007). These biomolecules are also recognized as oxidative stress biomarkers (OSB) and are utilized in epidemiological studies to provide a better understanding of the role of reactive oxygen species (ROS) in the pathogenesis and progression of diseases (Dalle-Donne et al., 2006; Griffiths et al., 2002). Molecules such as GSH have important roles as biomarkers for oxidative stress and also have critical roles in oxidative detoxification, especially the removal of peroxidation products

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(Muller et al., 2007; Sohal and Orr, 2012). Existing measures of oxidative stress in biological systems, while sensitive, are often complex and labor-intensive, *e.g.* the western blot immunoassay for protein carbonyl adducts (Shacter et al., 1994). By exploiting the interfacial interactions, several studies have recently described the reactivity of biomolecules in the presence of AuNPs (Basu et al., 2007; Gerdon et al., 2004; Schaaff and Whetten, 2000; Sudeep et al., 2005; Zakaria et al., 2013; Zhang et al., 2007). This interaction often results in an assembly of AuNPs mediated by the biomolecule that can be monitored by the separation-dependent dielectric environmental change *via* surface plasmon resonance (SPR) and band shifts (Alvarez et al., 1997; Jain et al., 2006; Lee and El-Sayed, 2006). This assembly ultimately results in the AuNPs suspension experiencing a colorimetric change. While this strategy is sufficient, it is also well known that, once AuNPs are introduced to biological environments, proteins have an affinity for the nanoparticles and form a nano-bio interface termed the protein corona at various time scales and degrees of penetrability (Miclăuş et al., 2014). This corona is known to influence the size, shape, and surface chemistry of the AuNPs affecting their interaction with biomolecules and cells. As a result, understanding the physicochemical properties of the corona is important while implementing AuNPs in biosensing applications.

The most common approach to study AuNPs SPR shifts induced by nanoparticles′ agglomeration has been carried out by UV–vis spectroscopy, although the limited sensitivity of this technique renders it increasingly difficult for measuring nanoparticles at lower concentrations. To overcome this limitation, various groups have attempted different strategies to amplify the signal (Nam et al., 2004); However, these strategies are time-consuming and processed in multi-step procedures. A potentially more sensitive approach for nanoparticle monitoring is photoacoustic spectroscopy (PAS). Photoacoustic spectroscopy is based on the direct optical absorption of pulsed light by a material in which the absorbed light converts into heat resulting in a thermal expansion of the material. Due to this thermal expansion, an ultrasonic wave arises and propagates through the surrounding medium (*e.g.* water). Liu and coworkers introduced PAS (Gonzalez et al., 2010; Liu et al., 2012) to monitor AuNPs assembly in the presence of biomolecules. By using this technique, they measured the degree of AuNPs aggregation and achieved a much higher sensitivity than traditional UV–vis spectrophotometry. One drawback to this approach is the use of a piezoelectric transducer (PZT) for detecting acoustic waves. These types of sensors do not lend themselves readily to *in vitro* biosensing applications as they must be well-coupled to the environment being probed to achieve the greatest sensitivity and the transducer may be subject to contamination due to contact with biological agents. In addition, they tend to be fragile and are easily damaged by physical stress and shocks.

An additional limitation relates to the ultrasonic frequencies emitted by smaller scale materials during the generation of photoacoustic signals. For example, nanoscale range materials are expected to generate higher frequency (>100 MHz) signals, requiring larger or multi-element transducers to detect these frequencies. Once these higher frequency acoustic signals are generated they attenuate faster in the medium. To overcome these limitations, our approach expands upon traditional PAS techniques by introducing a novel all-optical photoacoustic spectroscopy (AOPAS) technique based on the Probe Beam Deflection Technique (PBDT) which measures the PA response using a continuous wave probing laser beam (Barnes et al., 2014; Jackson et al., 1981). This method provides the ability to obtain *in situ* PA measurements through a non-contact sensor with a smaller footprint and reduced geometric constraints when compared to other probing techniques.

In this work, a systematic characterization of the self-assembly of AuNPs mediated by GSH at various nanoparticle and analyte concentrations using UV–vis spectroscopy, electron microscopy and dynamic light scattering was performed. These results are compared to the AOPAS technique by probing the evolution of the plasmon resonance shift. Finally, the same model is used in the presence of cell culture medium containing proteins having a strong affinity to the AuNPs to demonstrate the feasibility of sensing reactive oxidative species in the presence of potentially interfering macromolecules, which supports the development of future nanoparticle-based biosensor applications.

2. Materials and methods

2.1. Materials

Hydrogen tetrachloroaurate ($HAuCl_4$, 99%), trisodium citrate dihydrated (Cit), ferric chloride ($FeCl_3$), sodium hydroxide (NaOH, 98%), hydrochloric acid (HCl, $\geq 37\%$) and L-glutathione (reduced) (L-GSH, 98%) were purchased from Sigma Aldrich (St. Louis, MO, USA). Cell culture medium Dulbecco's Modified Eagle Medium Nutrient Mixture F-12 (DMEM/F12) containing L-Glutamine, and 15 mM HEPES without Phenol Red was obtained from Life Technologies Corporation (Grand Island, NY, USA). The media was completed by adding 1% fetal bovine serum purchased from Life Technologies Corporation (Grand Island, NY, USA).

2.2. Synthesis

The citrate-capped gold nanoparticles are synthesized by a well-known procedure (Frens, 1973; Grabar et al., 1995). Briefly, 30 ml of 20.40 mM sodium citrate solution are added to 600 ml of 0.29 mM $HAuCl_4$ solution upon boiling. After 45 min of heating 15 nm AuNPs are formed as indicated by a ruby red color. The estimated concentration of AuNPs stock solution is 2.54×10^{17} NPs/ml (Lewis et al., 2006). The pH of the AuNPs is then adjusted to 6.4 using NaOH solution to disperse the NPs in the solvent.

2.3. Aggregation Procedure

To initiate the aggregation procedure, 55 μL of 10 mM $FeCl_3$ was added to 1 ml of AuNPs solution at pH 2.79 and stirred for 15 min. This reaction increased the ion concentration surrounding the AuNPs which accelerated the attachment of the GSH to the citrate capping initially on the surface of the nanoparticles (Bard and Faulkner, 2000; Lim et al., 2006). Following incubation, 55 μL of 1 mM glutathione (GSH) at pH 3.98 was added to the AuNPs solution. A ligand exchange process (Stobiecka et al., 2010) occurred resulting in GSH capping the AuNPs, which facilitated H-bonding between AuNPs leading to the agglomeration of the AuNPs and changed the solution from red to blue. This model was tested with six different dilutions of AuNPs and by controlling reaction conditions (*i.e.* pH and surface charge). The pH of all the diluted AuNPs and $FeCl_3$ solution were adjusted to 6.4 and 2.79, respectively, using NaOH solution. The pH of the diluted GSH solution was adjusted to 3.98 using HCl solution. This adjustment of pH for all diluted AuNPs, $FeCl_3$ and GSH solutions maintained similar ion concentration in all dilutions before and after the mixing, and helped to maintain a similar GSH-mediated AuNPs assembly for all concentrations. The optical characterizations using AOPAS and UV–vis were done for all concentrations. In the experiments involving the cell culture medium, in place of deionized water, 0.5 ml DMEM F-12 culture media containing 1% fetal bovine serum (FBS) was mixed with 5.5 ml AuNPs stock solution to obtain