Cancer therapy system based on Gold Nanoparticle / Cold plasma via stimulated singlet Oxygen production

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Abstract. Gold nanoparticles have unique physical/chemical properties which let them very useful in several applications. In addition, to their biocompatibility which is very important for biomedical applications. Recently gold nanoparticles and reactive species of cold plasma have been successfully used for tumor cells and cancer treatment. This method depends on the type of reactive ionized species or the size of gold nanoparticles which it produces and its directed effect on the cell cycle. Our proposed novel designed system which combines both gold nanoparticles and reactive species of nitrogen and argon cold plasma is obtained. Therefore, generating singlet oxygen and reactive species with confirming gold nanoparticles stability as well as to its exposure to cold plasma at different conditions and time periods is accomplished. The analysis of this system will be done using Raman spectroscopy technique. This procedure will enhance better, direct, effective and selective targeting for different kinds of bio-threats such as tumor cells, bacteria, fungi, and virus. This innovative system may be used as a new sterilized technique for different fields such as medical and biological sector, and more promise as cancer therapy selective technique.

1. Introduction

Plasma medicine is an emerging field leading to multi-disciplinary research works attracting researchers from different fields. The emerging field of plasma sustained at atmospheric pressure is highlighted as extremely promising, opening many new horizons for applications e.g. in plasma healthcare. Plasma jets operate in open air at room temperature, direct contact and have highly selective plasma chemistry which induces specific response and modifications with tissue without denture the tissue [1-14]. More than plasma jet can be designed individually for desired application by adding different gases, catalysts or known medically active agents. This combination of characteristics makes them ideal for many applications in healthcare ranging from wound healing up to cancer therapy [1-6]. Reactive species and active agents of plasma jet can be applied on a molecular level to the tissue selectively and fast. At least Non-equilibrium plasmas have shown the ability to trigger complex biochemical processes resulting in modifications to biological systems.

Gold nanoparticles have been used recently in biomedical applications, including the delivery of drugs or genes, phototherapy Imaging, sensor [15-20]. The gold nanoparticles (NPs) have been proposed to be extremely useful factors in phototherapy due to its unique size and optical characteristics dependent on the shape, high absorption coefficients, ease of synthesis, compatibility with life, and ability to hold a variety of ligands [19-21]. It is known that Gold NPs are stabilized by cells and remain in vesicles between cells [22]. Furthermore, targeting specific sites inside the cell by functional surface with the cell peptides penetrate or peptides containing nuclear settlement has been reported [23]. Alternatively, to selectively target to specific cell types, gold NPs can be adjusted with appropriate antibodies. This approach has been studied for potential use in Photothermal treatment of cancer, where
cancer cells overexpressing human epidermal growth factor receptor (HER2) or epithelial growth factor (EGFR) were incubated with gold NPs conjugated with an anti-HER2 [24] or anti-EGFR [25-27] antibodies, respectively. The NPs were then irradiated within their plasmon resonance absorbance band to heat the cells to temperatures leading to cell death [26,27].

In our study, we generate selectively reactive argon, nitrogen ion species, and photons from a capacitive discharge plasma and plasma jets [2-9]. The interaction of plasma with gold nanoparticles and detecting different reactive species such as singlet oxygen \( ^1\text{O}_2 \) and reactive ions species with gold nanoparticle is determined [10,11]. Moreover, the investigating of a suitable time for exposure to the reactive species of plasma to generate effective singlet reactive oxygen is discussed. Finally, the output results of reactive species of plasma with additive reactive singlet oxygen will be more effective in killing the cancer cells without damaging normal cells or using in sterilization [11].

2. Cold plasma: as a medical tool in the future

In our study we apply two different system, the capacitive coupled plasma discharge (CCPs) which is confined within a container known as a chamber or reactor and plasma jets experiment. CCPs device is comprised of heavy ions and light electrons. The mass ratio \( m_i/m_e \) equals at least 1836 (for hydrogen) but lies more often around \( 10^5 \) (e.g. \( 7.3 \times 10^4 \) for nitrogen or argon). Due to their higher mobility, the electrons will initially lose to the grounded walls at a much faster rate than the heavier ions. This results in a small layer close to the walls dominated by positive ions, forming a positive space charge region known as a sheath. As the sheath is positive along the wall, the electric field which forms is directed from the plasma to the walls, therefore repelling further electrons from walls while simultaneously accelerating the ions towards the walls. The equilibrium state occurs when the electron and ion fluxes to the wall are equal, i.e., the phase average of the electron current is equal to the current of the ions at the walls. Finally, the bulk plasma fills most of the discharge volume \( V \) of size \( L \), while the sheath forms a thin layer of average thickness \( S \), a few Debye lengths, we adopt length scales where \( \lambda_D \ll S \ll L \). That is why that the plasma reactor can be separated into regions: the boundary layers at the walls, which turn out to be sheaths of space charge; the bulk plasma, where the net space charge is
almost zero and the quasi-neutrality condition prevails. All reactive ions species are activated in this region of plasma boundary sheath. In the following example for a plasma processing discharge, we have a fixed parameter of a more fundamental model, namely a self-consistent one-dimensional fluid sheath model [6,13]. The pressure was $p = 770 \text{ mTorr}$, $T_G = 300 \text{ K}$. The applied voltage was $V(t) = 200\sin(\omega_{rf}t)$, with $\omega_{rf} = 2\pi \times 13.56 \text{ MHz}$, the ion current as $e\psi_i = 1.27 \text{ mA m}^{-2}$. Furthermore, the power $P \approx 50 \text{ Watt}$.

3. Materials and methods

In our study, we use CCPs and plasma jets experimental [5,6] devices to generates reactive species by different techniques and different gas. CCPs generate reactive species of nitrogen under vacuum (770 mTorr) and plasma jet generate reactive species of argon with direct contact with a sample at normal atmospheric pressure.

| Type of used gas | Time of exposure | Type of device | Pressure   | Power  |
|-----------------|-----------------|---------------|------------|--------|
| Nitrogen        | 2 min           | CCPs          | 770 mtorr  | 50 Watt|
| Argon           | 30 sec          | Plasma jets   | Atmospheric| 6.6 Watt|

4. Spherical Gold Nanoparticles Synthesis

Gold nanoparticles have been prepared by chemical reduction method as reported by Turkevich [28] with slightly modification. A solution of HAuCl$_4$ has been used as Au$^{3+}$ ions precursor. The sodium citrate has been used as reducing and PEG as stabilizing agent. The color of the solution slowly turned into faint pink color, indicating the reduction of the Au$^{3+}$ ions to Au nanoparticles.

**Optical Properties:** UV-Vis absorption spectra were obtained on an Ocean Optics USB2000 + VIS-NIR Fiber optics spectrophotometer.

**Size & Shape:** TEM were performed on JEOL JEM-2100 high resolution transmission electron microscope at an accelerating voltage of 200 kV, respectively.

5. Results and discussion

The UV–vis absorption spectra of gold nanoparticles AuNPs are shown in Fig. 3a. The absorption peak of AuNPs is about 520 nm. The TEM image of AuNPs is shown in Fig. 3b. The measured average size of AuNPs is about $18 \pm 0.5 \text{ nm}$. To understand the nature of the coating layer of the nanoparticles, SERS measurements were performed to ensure the complexation present between PEG and AuNPs. Fig. 4x shows the SERS spectra of the PEG-AuNPs colloidal samples.
The spectrum of PEG-AuNPs has characteristic absorption bands of the PEG polymer [29]. The peaks in 461 and 387 cm\(^{-1}\) related to AuNPs banding with oxygen from hydroxyl groups of PEG chains. The most intense features are the bands at 850 cm\(^{-1}\) caused by ethers C—O–C and the bands 1060 and 1140 cm\(^{-1}\) caused by the C—O stretching vibrations. Apart from the PEG characteristic peaks, the 1440 cm\(^{-1}\) and 1480 cm\(^{-1}\) peaks corresponding to the C—H bending vibrations. However, the peaks appear at 2800–2950 cm\(^{-1}\) corresponding to C—H stretching vibrations.

Fig. 3 (a) The optical absorption of collide gold nanoparticles (b) The TEM image of AuNPs

Fig. 4 Raman spectra of PEG as surfactant for colloidal gold nanoparticles
Our results show that the reactive species at two different gases (Ar and N): Reactive Argon Species (RAS) and Reactive Nitrogen Species (RNS) of plasma ions interact with PEG-AuNPs leads to the production of Singlet Oxygen Group (SOG). The two bands appear at 1480 cm\(^{-1}\) and 1580 cm\(^{-1}\) corresponding to the generation of SOG \(^1\)O\(_2\) and triplet oxygen \(^3\)O\(_2\) respectively. The band appear at 3050 cm\(^{-1}\) corresponding to OH group. Irradiation of spherical gold NPs at 532 nm leads to the excitation of their plasmon resonance. Since the photon energy is close to the minimum energy required for direct excitation of d-band electrons into the conduction band of gold. The excited electrons initially have a nonthermal energy distribution, and often are referred to as “primary hot electrons”. Hot electrons have been shown to cause gold–PEG bond dissociation at the surface of gold NPs and have been suggested to be responsible for the creation of SOG \(^1\)O\(_2\) by irradiation of gold NPs laser source.

6. **Conclusions**

To shift a new criterion of cancer cell lines treatment we utilize a combination effect of reactive plasma species of different gases with gold nanoparticles. The study of (gold nanoparticles /plasma) is focused to optimize several factors such as suitable reactive gas, time exposure and the exact dose of the effective reactive species for generates singlet oxygen. Moreover, two different technique with two varying gases (Nitrogen and Argon) are applied to achieve the results. The first gold nanoparticles sample is treated by CCPs with applying nitrogen gas under a vacuum of pressure \(p = 770\) mTorr, sustain the energy of plasma \(P = 50\) Watt and is exposed to reactive species of nitrogen (RNS) at a period of time (3 min). The second one is treated by plasma jet with applying argon gas under atmospheric pressure, sustain the energy \(P = 6.6\) Watt and exposed to reactive species of argon (RAS) at a period of time (30 sec). The analysis using Raman spectroscopy show that the singlet oxygen SOG \(^1\)O\(_2\) is obtained using CCPs and plasma jet experiments. In addition, the group triplet oxygen \(^3\)O\(_2\) and OH group are found. Finally, the formation of singlet oxygen (SOG \(^1\)O\(_2\)) and OH group have been generated which is sufficient for tumor cell lines therapy.

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