Cold plasma accelerates the uptake of gold nanoparticles into glioblastoma cells

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Abstract: The gold nanoparticles and cold plasma have been widely investigated separately in cancer therapy. In this paper, the two agents were combined in order to achieve a more profound understanding of their interactions in glioblastoma and normal human astrocytes. The plasma accelerates the uptake of the AuNPs in cancer cells while the normal cells are unaffected. The synergistic effect of the two agents can be correlated in order to maximize the efficiency.

Keywords: gold nanoparticles, cold plasma, uptake, glioblastoma

1. Introduction

Gold nanoparticles (AuNPs) has been investigated as an anti-cancer reagent in various fields for decades, such as drug delivery agent [1], radio sensitizer [2], photothermal reagent [3] and so forth. These applications imply that the uptake of AuNPs contributes an important part in the investigation. It has been demonstrated that the uptake of AuNPs dependence on the cell line [4, 5], the physics properties of the particles such as size and shape [6], and the chemical properties of the particles such as the coated ligands or polymers [7]. In the meantime, cold plasma has shown exquisite selectivity towards cancer therapy [8, 9]. However, the synergistic effect of AuNPs and cold plasma has been barely studied. Kim \textit{et al.} [10] were the first to publish their work in this interdisciplinary area, showing that a five-fold increase in melanoma cell death over the case of the cold plasma alone by using plasma with AuNPs bound to anti-FAK antibodies.

In this work, the uptake of glioblastoma U87 cell line and immortalized normal human astrocytes E6/E7 cell line was studied. 100 nm AuNPs were used in combination with cold plasma to enhance the therapeutic effect on U87, while keeping the normal cells unharmed.

2. Methods

The cold plasma device created at the George Washington University [11] has a configuration of central powered electrode of 1 mm diameter coating with 2 mm ceramic layer and a grounded outer electrode wrapped around the outside of a 4.5 mm diameter quartz tube. The electrodes were connected to a secondary of high voltage resonant transformer (voltage up to 10 kV, frequency 30 kHz). The plasma discharge was driven by AC high voltage. The output voltage was set to 3.16 kV. The feeding gas helium (Airgas, USA) was set at a flow rate of 4.7 l/min.

Glioblastoma cells, U87, and immortalized normal human astrocytes, E6/E7, were used in this study. Both cell lines were cultured in Dulbecco’s Modified Eagle Medium (Life Technologies) supplemented with 10% (v/v) fetal bovine serum (Atlantic Biologicals) and 1% (v/v) Penicillin and Streptomycin (Life Technologies). Cultures were maintained at 37 °C in a humidified incubator containing 5% (v/v) CO\(_2\).

In order to track the uptake of AuNPs into cells before/after plasma treatment as a function of time, Zeiss 510 confocal microscope (60x magnification) was used to image the cells. Each cell line was plated in nine 35 mm glass bottom dishes at a cell confluence of 70,000/dish. After 24 hr incubation, the culture media was replaced with 2 ml of fresh culture media with AuNPs at a concentration of \(\sim 5.7 \times 10^8\) /ml. After 15 min incubation with the AuNPs, all 9 dishes of the cells were treated with cold plasma for 30 s for each dish, and then put back into incubator. In the following 45 min (15 - 60 min after AuNPs addition), dishes of the cells were taken out one by one at every 5 min time point and fixed by 0.5% glutaraldehyde. To briefly describe the cell fixation procedure: cells were rinsed 3 times with phosphate buffer saline (PBS, Lonza, USA) to wash out the unabsorbed AuNPs and unhealthy cells, fixed with 0.5% Glutaraldehyde in PBS for 5 min, and then again washed with PBS for 3 times. After 60 min, all the 9 dishes of cells were fixed and taken to confocal microscope. The whole set of experiment was repeated three times.

Atomic force microscope (AFM, Asylum, MFP-3D, California) was used to characterize the surface topography of the cell samples. Images were recorded in PBS at room temperature. All measurements were done in a contact mode using sharpened silicon nitride probes TR400PSA (Veeco-OTR4, Olympus, Japan) with two sets of two triangular levers. Resonance frequency of the shorter, 100 µm lever is 21 - 52 kHz and has a force (spring) constant of 0.02 - 0.23 N m\(^{-1}\); for the longer, 200 µm lever the resonant frequency is 7 - 15 kHz and has a force constant of 0.01 - 0.05 N m\(^{-1}\). The image data
were collected in the deflection mode with the loading force of 1 to 5 nN at scan rate of, typically, 0.9 Hz. The images obtained in the deflection mode enhance structural details.

Cell viability was monitored using the MTT assay (Sigma-Aldrich, M2128), which is a colorimetric assay for measuring the activity of mitochondria and cellular dehydrogenase enzymes that reduce 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide, MTT, to its insoluble formazan, giving a purple color. The detailed procedure can be found elsewhere [8]. Briefly, cells were plated at a confluence of 20,000 ml⁻¹, and then incubated for one day to ensure a proper cell adherence and stability. Before treatment, cells were replaced with fresh media, and treated with cold plasma followed by an additional incubation at 37 °C for 24 h. On the third day, 100 µl of MTT solution per well (7 mg Thiazolyl Blue Tetrazolium Blue in 10 ml medium for one plate) was added into each well. Reactions were maintained for 3 h at 37 °C. The MTT solution was aspirated and 100 ul of MTT solvent (0.4% (v/v) HCl in anhydrous isopropanol) was added to each well to dissolve formazan crystals. Reactions were monitored by the Synergy H1 Hybrid Multi-Mode Microplate Reader at 570 nm. The entire set of experiments was repeated three times in duplicates.

3. Results and discussion

Reflection images of U87 (Panel A and B) and E6/E7 (Panel C and D) cells with 60 min AuNP incubation taken by confocal microscope are shown in Fig. 1. After 60 min incubation, the penetration of AuNPs into U87 without plasma treatment (Panel A) is evidently lower than those with 30 s plasma treatment (Panel B). However, the number of AuNPs in E6/E7 cells shows no significant difference with or without 30 s plasma treatment (Panel C and D).

Fig. 1. Reflection image of cells with 60 min AuNPs incubation: (A) U87 without plasma treatment, (B) U87 with 30 s treatment, (C) E6/E7 without plasma treatment, and (D) E6/E7 with 30 s plasma treatment.

More detailed data of AuNPs uptake ratio into cells as a function of incubation time is plot in Fig. 2. Every data point was averaged by the number of AuNPs of 10 cells. The average number of AuNPs absorbed by U87 and E6/E7 cells within the first 60 min of AuNP addition occurred in a time-dependent manner for both cell lines. After 30 min incubation, E6/E7 cells endo-cytosed significantly fewer AuNPs than U87 cells. The different uptake of AuNPs can be explained as the different electrostatic properties of the membrane of individual cell line [5], giving that the AuNPs were not coated with any ligands or polymers, and the culture media for the two cell lines are the same. The cell membrane strongly affects the internalization process, which can be either direct endocytosis of the cell membrane, or receptor mediated endocytosis [12].

Fig. 2 also shows that the plasma accelerates the AuNPs uptake after certain time of incubation. One can see that there is a clear increase in the amount of nanoparticles absorbed in the U87 cell line when the cells are treated with plasma. This increase begins to occur after

40 minutes of incubation between the time the gold nanoparticles are administered and the time that it is treated. On the other hand E6/E7 shows no significant
difference in the amount of nanoparticles absorbed when treated with plasma. Two aspects should be taken into account while attempting to explain the acceleration effect: plasma reaction with the AuNPs, and plasma stimulation on the cell membrane. As described above, the AuNPs purchased from Nanopartz were not coated with any ligands or polymers. It is unlikely that the internalization rate is increased due to the AuNPs and plasma reaction. The possible explanation to this is that the plasma reacts with the cell membrane.

To further illustrate the plasma effect on the cell membrane, atomic force microscope (AFM) is used to capture the detailed membrane morphology. The resulting pictures are shown in Fig. 3. The left column of the figure shows the overall view of cell membrane, while the right column depicts the detailed membrane morphology. Obvious differences can be seen from the U87 and E6/E7 cells. It is straightforward to see the U87 cell membrane was smooth and had no granular elevations before plasma treatment (Panel A). After treatment, however, the membrane became uneven and developed small pores on surface (Panel B). E6/E7 cells differ because they had many small protrusions before plasma treatment, believed to be the microvilli. After plasma treatment there was no significant change in the texture of the cell morphology (Panel C and D).

![Fig. 3. AFM images of U87 and E6/E7 cells under low and high magnification: (A) U87 cells before plasma treatment, (B) U87 cells after plasma treatment, (C) E6/E7 cells before plasma treatment, (D) E6/E7 cells after plasma treatment.](image-url)
In Fig. 4, the combination effect of AuNPs with 30 s plasma treatment in E6/E7 and U87 cells is a parabolic curve as a function of AuNP concentration, showing that the effect of cold plasma is strengthened by AuNPs, and this strengthen effect varies with AuNP concentration. The E6/E7 cells were the most sensitive at the AuNPs concentration of 1 ul/ml (~5.7 × 10^8 /ml). The optimal therapy dosage of AuNPs for the U87 while keeping the E6/E7 unharmed is 0.5 ul/ml (~2.85 × 10^8 /ml). This is important because AuNPs help to maximize efficiency of the plasma jet at proper dosage. These results are in agreement with the data of U87 that was previously studied [13, 14].

Fig. 4. Cell viability of E6/E7 cells with 24 h incubation of various AuNP concentration 0 – 2 µl/ml after plasma treatment.

4. Conclusions

Gold nanoparticles were endocytosed at an accelerated rate in the U87 cell membrane due to the plasma treatment. This caused many divots across the cell membrane which made it more porous. This differs from the E6/E7 cell membrane as there was no significant difference for penetration of gold nanoparticles or the cell membrane structure. As proven in previous experiments the addition of the nanoparticles would decrease cell viability, allowing the cold plasma to be more efficient.

5. References