

# Rapid Delivery of Gold Nanoparticles into Colon Cancer HT-29 Cells by Electroporation: In-vitro Study

Arab-Bafrani Z.<sup>1</sup>, Shahbazi-Gahrouei D.<sup>2\*</sup>, Abbasian M.<sup>3</sup>

## ABSTRACT

**Background:** Electroporation has become a routine technique for rapid drug delivery for the treatment of cancer. Because of its simplicity and wide range of application, it has been applied for the transfer of gold-nanoparticles and can facilitate entry into target cancer cells.

**Objective:** The aim of this study is finding optimal conditions in order to obtain high GNPs- uptake and cell viability by means of electroporation.

**Materials and Methods:** In this in vitro study, exponential electrical pulse with electric field intensity ranging from 0.2 -2 kV/cm, pulse length of 100  $\mu$ s and the pulse number of 2 was used. Electroporated cell viability was investigated using MTS assay and GNPs-cellular uptake was assayed using graphite furnace atomic absorption spectrometry (GFAAS). Finally, electroporation results were compared with passive method.

**Results:** The maximum uptake occurred at 1.2 to 2 kV/cm and passive method happened. The cell viability of 1.2 kV/cm and passive method was about 90%, while the cell viability in 2 kV/cm drastically decreased to 50%. The findings showed that using two pulses of 1.2 kV/cm and 100  $\mu$ s is a convenient way and surrogate of passive method for internalizing GNPs into cells.

**Conclusion:** It is concluded that the electroporation-GNPs method could create an opportunistic context for colon cancer therapy. This type of treatment is especially attractive for highly immunogenic types of cancers in patients who are otherwise not surgical candidates or whose tumors are unresectable.

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## Keywords

Gold Nanoparticles; Electroporation; Drug Delivery; HT29 Cells; Cell Survival

## Introduction

Colon cancer has recently become a common malignancy in Asian countries, and is the third most common cancer in the world [1]. Surgery and chemotherapy are widely used as the gold standard for cure of colorectal cancer, and radiation therapy is the complementary therapy. However, despite all recent developments in cancer therapy, colon cancer recurrence (50%) remains a major problem. Therefore, new approaches are needed to enhance the contrast of colorectal cancer and the radiotherapeutic efficiency of these cells [2-4].

Application of radio-sensitizers is one of the strategies to enhance

<sup>1</sup>PhD, Stem Cell Research Center and Department of Medical Physics-Clinical Biochemistry, School of Medicine, Golestan University of Medical Sciences, Gorgan, Iran  
<sup>2</sup>PhD, Department of Medical Physics, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran  
<sup>3</sup>PhD, Department of Biotechnology, College of Agriculture, Isfahan University of Technology, Isfahan, Iran

\*Corresponding author:  
 D. Shahbazi-Gahrouei  
 Department of Medical Physics, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran  
 E-mail: shahbazi@med.mui.ac.ir

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the radiation efficiency without exceeding the maximum tolerated dose of normal tissues. Today, gold nanoparticles (GNPs) are established as good radio sensitizers due to their high bio-compatibility and high penetration rate into cancer cells [5-8]. Moreover, GNPs are emerging as a good contrast agents for computerized tomography (CT) and can enhance contrast of the tumor tissues [9]. Therefore, it seems GNPs are a good option for dual-mode enhancement of computed tomography (CT) imaging and radiation therapy.

Previous studies have shown that production of much higher GNPs, cellular uptake is a prerequisite to achieve significant radio-sensitization and tumor tissues contrast in imaging [5, 6]. Despite GNPs, advantages mentioned, fast and high internalization of GNPs are not easy. GNPs passively diffuse into the cell [6]. In the passive uptake, cells should be incubated with GNPs about 24 hr prior to experiment and therefore is time-consuming, and is a poor optimal method for certain application such as disease monitoring. On the other hand, the distribution of nanoparticles inside the cells cannot be controlled.

Electroporation is a physical method that uses short and intense electric pulses to increase membrane permeability and therefore, increases the uptake of molecules such as DNA, antibodies and drugs into the cells [10,11]. This method has been utilized in the laboratory for many years to transfer macromolecules into the target cells. According to recently published reports, transfer of various anti-tumor genes via electroporation led to tumor regression in most cell lines [12,13]. The diameters ranging of the electroporation pores are 20 to 200 nm; therefore, particles with smaller size rather than the pores can pass through the cell membrane and enter cells. Recently, it has been demonstrated that transient electroporation is much faster and more efficient than passive uptake for delivering silver nanoparticles into a cell [14]. Cell viability and percentages of cell permeabilization are con-

trolled with amplitude, duration and number of pulses [15]. Consequently, to achieve high cell viability and high cell permeabilization, optimization of three mentioned parameters is necessary [16].

Afterwards, due to the role of GNPs in diagnosis and treatment of cancer and electroporation advantages in drug delivery, the aim of the present study is to optimize electroporation protocol for delivering GNPs into colorectal cancer cells (HT-29).

## Material and Methods

In this in vitro study, colorectal (HT-29) cell line was purchased from Pasteur Institute, Tehran, Iran. Cells were cultured in RPMI 1640 medium (Gibco-Invitrogen) supplied by 10% fetal bovine serum, (Gibco-Invitrogen), 1% antibiotic mixture containing penicillin (Sigma-Aldrich) and streptomycin (Sigma-Aldrich). These cells were stored at humidified atmosphere at 37 °C with 5% CO<sub>2</sub>. The medium of cells was changed every two days, and when they reached more than 80% confluency, they were split with 0.05% Trypsin/0.02% EDTA and sub-cultured for more passages.

## Gold Nanoparticle Uptake

### Passive Uptake

When cells reached more than 80% confluency, they were trypsinized and seeded in 24-well plate (4000 cells/well) for 48 hr (in logarithmic phase). Consequently, the cells were exposed to 60 μM of 50 nm-gold nanoparticle (Sigma-Aldrich), and stored at humidified atmosphere at 37 °C with 5% CO<sub>2</sub> for 24 hr. When the incubation period passed, the culture medium was removed, washed three times in PBS and cells were detached with Trypsin-EDTA, and the number of cells was counted with a hemocytometer. The amount of 1 ml HCl (5 M) was added in each sample for lyses of the cells. The concentration of GPNs was measured by graphite furnace atomic absorption spectrometry (GFAAS) analysis. The

uptake of nanoparticles by each cell was calculated using the following equation (1):

$$\text{number of GNPs per cell} = \frac{\text{number of GNPs in the lysis}}{\text{number of cells}}$$

#### Active Uptake: Electroporation Delivery

$4 \times 10^6$  cell /ml HT-29 colon cancer cells were trypsinized, centrifuged and suspended in the RPMI 1640 medium. Then, 400  $\mu$ l cell suspensions incubated with 60  $\mu$ M of GNPs and was added into a sterile electroporation cuvette (Eppendorf, Netherlands) with a 4-mm gap between the electrodes.

The cell/GNPs mixture of the cuvette was incubated for 10 min at 4 °C on ice and then transferred to an electroporation chamber. Number of four electric pulses of different voltages 200, 400, 600, 800, 1000 and 1200 with duration of 100  $\mu$ s and pulse number of 2 were delivered using an Electroporator (Eppendorf). Field amplitudes higher than 1200 V/cm are not normally tested as it is known that they are highly toxic for the cells (induction of irreversible electroporation and, thus, of cell death). Immediately after electroporation, the cuvette was taken out from the chamber and incubated for 2 min at 4 °C on ice and then cells were stored at humidified atmosphere at 37 °C with 5% CO<sub>2</sub> for 3-4 hr before viability and uptake assay processes using GFAAS analysis as described above.

#### Viability Assay

For the assessment of cell viability in passive uptake, cells were seeded at  $4 \times 10^3$  per well of a 96-well tissue culture plate. After 48 hr, 50 nm-spherical GNPs (Sigma Aldrich) (60  $\mu$ M) were added to the cells. Cell viability was investigated using MTS assay, the Cell Titer 96 Aqueous One Solution Cell Proliferation (ProMega), after 24 and 48 hr. Optical density (OD) was recorded at 490 nm in a 96-well plate reader (Biorad). Cell survival was evaluated using the following equation (2) [17]:

$$\text{Survival fraction} = \frac{(\text{mean OD in test wells} - \text{mean OD in cell free wells})}{(\text{mean OD in control wells} - \text{mean OD in cell free wells})}$$

To evaluate cell death of electroporated cell suspension, after electroporation process,  $4 \times 10^3$  cells were seeded in each well of a 96-well tissue culture plate. After 48 hr, MTS assay was done as already mentioned.

#### Results

In this work, delivery of GNPs into colon cancer HT-29 cells using the electroporation method was studied. Figure 1 illustrates HT-29 cells growth at first (a), fifth (b) and seventh (c) day post-culture. Figure 2 shows the results of using electroporation to deliver GNPs into HT-29 cells. Figure 3 shows the cell viability due to using electroporation with different protocols. Although the highest uptake occurred at 2 kV/cm, cell viability in this electrical intensity was very low (approximately 50%).

#### Discussion

As mentioned before, the cells were first mixed with GNPs in RPMI 1640 medium. As can be seen from Figure 2, due to the cell membrane barrier, GNPs could hardly enter the interior of cells without electroporation; while, by using electroporation the process entails rapid, localized structural rearrangements within the membrane resulting in membrane permeability enhancement and formation of pores perforating the membrane. This caused the GNPs transfer into the interior of the cell through the openings on the membrane. As the electrical impulse fades, the membrane recovers its integrity and functions normally as a barrier again. The maximum uptake occurred at 1.2 and 2 kV/cm and method (5.432,  $5.84 \times 10^4$  respectively). It should be noted that this procedure was completed in a very short period of time (less than one minute) and makes it very convenient and fast for GNPs delivery into living cells.

Herein, the GNPs with 60 nm diameter were used. Since the main mechanism of entrance is believed to be due to 20–200 nm transient pores produced on the cell membrane, impermeable particles of less than this size could

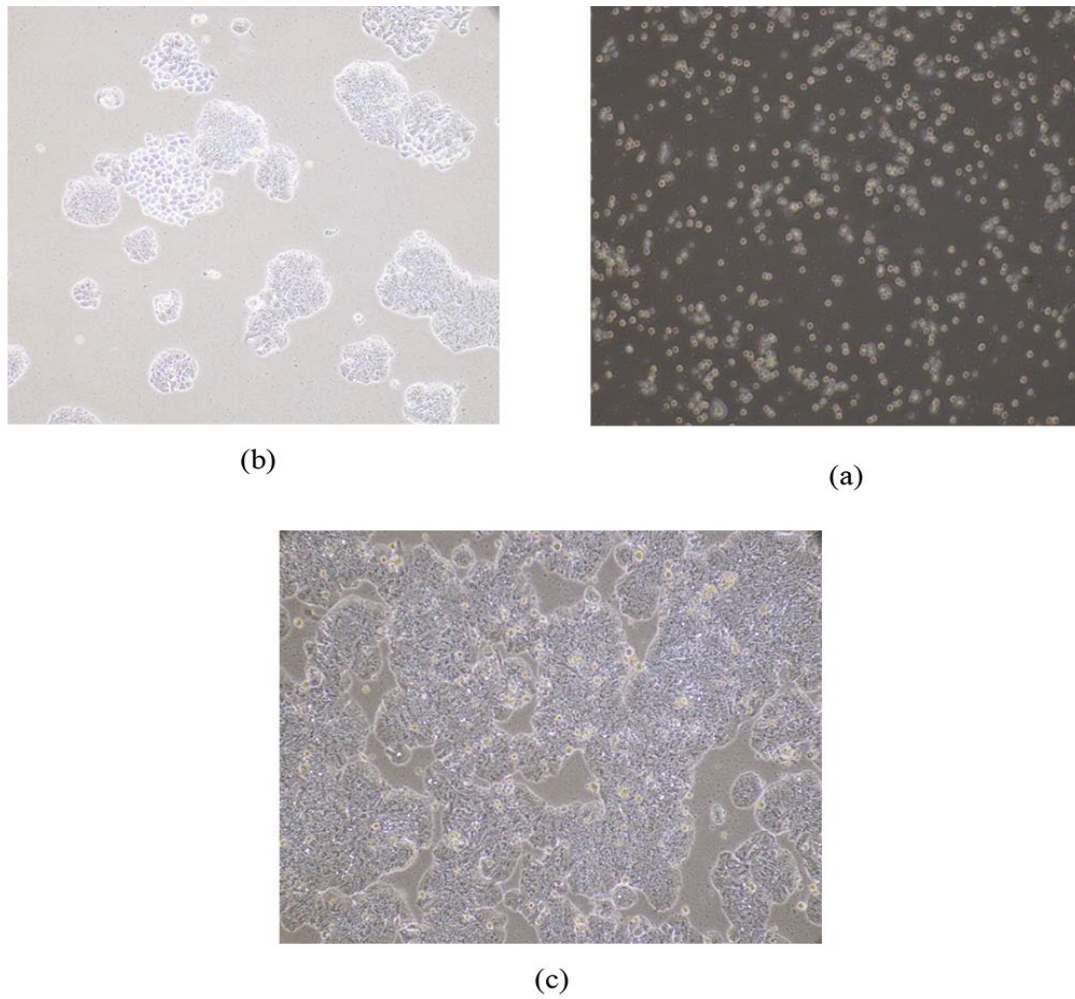


Figure 1: HT-29 cells growth at first (a), fifth (b) and seventh (c) day.

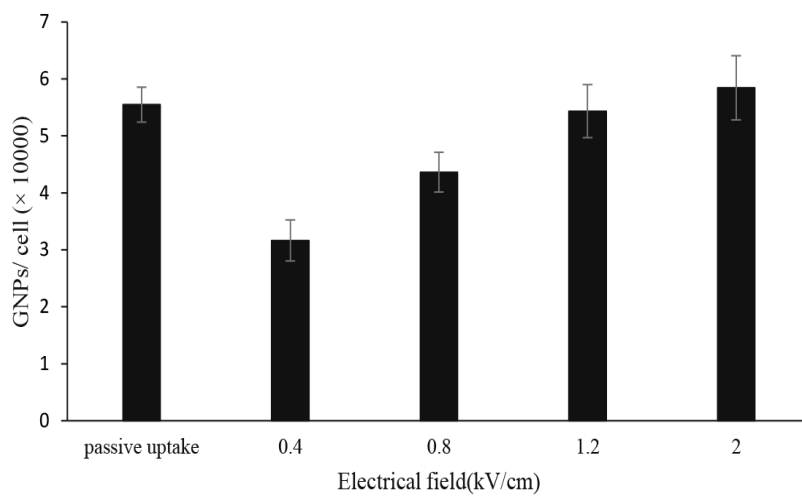
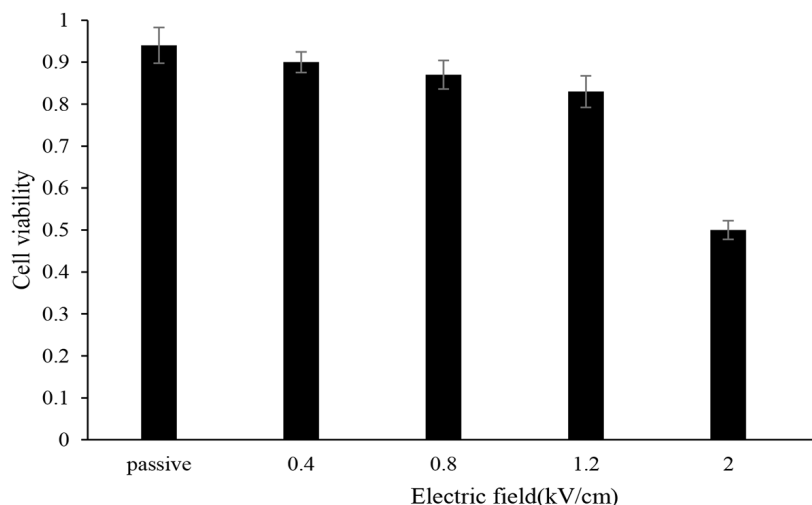


Figure 2: Results of using electroporation to deliver GNPs into HT-29 cells.



**Figure 3:** Cell's viability due to using passive and active (electroporation) methods.

freely diffuse into the cells [18]. The size of used GNPs was remarkably smaller than the diameter of the pores on cell membranes produced by electroporation. Consequently, considerable amount of 60 nm GNPs size could go through these 20–200 nm pores.

As indicated in Figure 3, cell viability and cellular uptake at 1.2 kV/cm were high, and there was no significant difference between this method and the passive method ( $P > 0.05$ ). Nonetheless, it seems 1.2 kV/cm can act as such a passive method but in a very short time.

Passive uptake (PU) was also followed in this study. Passive uptake is referred to a mechanism in which the GNPs entered the cells via the receptor-mediated endocytosis pathway (RME) [19,20]. In Chithrani et al. study, about 70% decrease in the uptake of the GNPs was observed at low temperature, 4 °C instead of 37 °C [19,20]. In the present work, to make sure that GNPs are delivered by electroporation instead of uptake via RME, the cells and the GNPs were mixed and incubated for 10 min at 4 °C on ice prior to electroporation. Drug transfer through cell membrane using the GNPs and electroporation is an attractive technique of interest for multiple reasons. It is simple, does not require any viral vectors and

is not limited to dividing cells. Compared with other methods, electroporation-GNPs drug transfer requires only a small volume and a low infusion rate that does not require outflow obstruction, thus conferring a low risk of acute problems.

## Conclusion

The electroporation-GNPs method could create an opportunistic context for colon cancer therapy. This type of treatment is especially attractive for highly immunogenic types of cancers in patients who are otherwise not surgical candidates or whose tumors are unresectable.

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## Conflict of Interest

There are no conflicts of interest.

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