

Hyperthermia Treatment of Cervical Cancer Cells using Carbon Nanotubes Excited with Near Infrared and Radiofrequency Radiation

Giovanni Caro¹, Kenny García¹, André Marra¹, Barbara Avalos¹, Rafael Velázquez², Abelardo Colón³, Darinel Ortiz⁴, Javier Avalos^{5,7}, Gerardo Morell^{2,7} and Brad R. Weiner^{6,7}

¹Department of Biology, University of Puerto Rico, San Juan, PR 00936, USA

²Department of Physics, University of Puerto Rico, San Juan, PR 00936, USA

³Department of Environmental Sciences, University of Puerto Rico, San Juan, PR 00936, USA

⁴Department of Biology, University of Puerto Rico, Bayamón, PR 00959, USA

⁵Department of Physics, University of Puerto Rico, Bayamón, PR 00959, USA

⁶Department of Chemistry, University of Puerto Rico, San Juan, PR 00936, USA

⁷Institute for Functional Nanomaterials, University of Puerto Rico, San Juan, PR 00931, USA

ABSTRACT

Current treatments for cancer include surgery, chemotherapy, and radiation, as well as combinations of these. Although widely utilized, these treatments are not entirely effective. Our primary aim is to develop a non-invasive cancer therapy using localized heat with the aid of nanotechnology. We demonstrate *in vitro* the use of carbon nanotubes (CNTs) for the selective hyperthermia treatment of cervical cancer cells (HeLa). The results show that phototherapy using CNTs excited with near infrared (NIR) and radiofrequency (RF) radiation is a promising method against cancer cells, which can be combined with CNT targeted drug delivery for more effective cancer treatments.

Keywords: carbon nanotubes, hyperthermia, near infrared photothermal therapy, cancer treatment

1 INTRODUCTION

Single wall carbon nanotubes (CNTs) offer new ways to treat malignant cells. Studies show that functionalized CNTs can be effectively used for targeted drug delivery against cancer [1]. Moreover, CNTs present favorable characteristics for photothermal therapy as they are good transducers of electromagnetic radiation, such as radiofrequency (RF) and near-infrared (NIR), into heat.

Previous reports [2,3] showed that RF irradiation of CNT-free cancerous tissues presented incomplete tumor destruction and damage to both malignant and normal tissues. As such, RF treatment alone is regarded as an invasive and limited type of treatment. By comparison, studies that have utilized RF-irradiated CNTs, both *in vitro* and *in vivo*, have

shown a strong reduction in the number of cancer cells [2,3,4].

In this study, we show *in vitro* the effectiveness of RF- and NIR-irradiated CNTs against HeLa cells. NIR has the advantage over RF of not being harmful to healthy tissue at the intensities required to obtain therapeutic effects. RF and NIR experiments were done in parallel for comparison purposes.

2 MATERIALS AND METHODS

2.1 Single Wall Carbon Nanotubes (CNTs) Solution Preparation and Deposition

The gold-coated Kapton substrates were thoroughly rinsed with distilled water and 95% ethanol before CNTs (www.cheaptubes.com) deposition. After drying, a CNTs solution was prepared by mixing 2 mL of benzene with the CNTs powder and sonicating for approximately 10 minutes. Around 15 μ L of the CNTs solution were deposited on the substrate making sure the area was covered. The covered substrates were then left to dry over a hot plate at 40°C for 30 minutes, to allow benzene to evaporate from the solution.

2.2 Human Cell Lines

HeLa cells (ATCC-CCL-2) were cultured in EMEM medium with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin antibiotic solution at 37°C under standard atmospheric conditions. In order to evaluate RF and NIR photothermal therapy cytotoxicity, cells were grown overnight in 60 mm glass culture dishes and later exposed to the treatment.

2.3 RF Therapy Implementation

A total of four glass culture dishes were prepared and divided into two major groups: irradiated and non-irradiated. The non-irradiated groups served as control groups in order to observe if there was any difference in the number of cells contained in the glass culture dish that was exposed to the treatment. Two major subgroups were implemented: (1) glass culture dish with cells and medium; and (2) glass culture dish with cells, medium, gold-coated Kapton substrates, and CNTs. Cells were incubated for 24 hours before the RF therapy implementation, which was long enough for the cells to adhere onto the surface of the biosensor.

Glass culture dishes labeled to be irradiated were transported to the Agilent 8712ES RF Network Analyzer (10 mW) to receive RF treatment. The frequency was set to 13.56 MHz with a 10-minute irradiation time. The glass culture dishes were placed halfway between two antennas, an emitter and a receiver. After irradiation was completed, the glass culture dishes were brought back to the cell culture room for cell counting in order to compare with the data obtained from cell counting the non-irradiated group.

2.4 NIR Therapy Implementation

A total of four glass culture dishes were prepared and divided into the two major groups, as described above for the RF experiments. Cells were incubated for 24 hours before the NIR (1070 nm) therapy implementation, which was long enough for the cells to adhere onto the surface of the biosensor.

The NIR source consisted of a 1070 nm, 7.5 mW epoxy-encased light emitting diode (LED) and a 1000Ω resistor connected to a circuit board, and powered by 9V New Leader Carbon-Zinc battery. The source's power is 5 Watts per square centimeter. The circuit was placed inside a sealed plastic chamber that contained the samples to be irradiated.

The glass culture dishes to be treated were placed inside the chamber, together with the NIR circuit, to be irradiated for 10-min each (individually). After the designated treatment time had elapsed, we proceeded to the cell culture room where both groups' cell concentrations, irradiated and non-irradiated, were estimated through cell counting protocol.

3 RESULTS AND DISCUSSION

Figure 1 shows the effect of RF irradiation on the cell concentration of HeLa. For control experiments, we studied the HeLa cell viability on Petri dish, gold, and CNTs/gold substrates without the application of RF irradiation. In these control cases, the HeLa concentrations remained stable at values between $3.8\text{-}5.4 \times 10^4 \text{ cm}^{-2}$. Application of RF on HeLa cells kept on Petri dishes and on gold substrates appears to produce some variations in cell count, but these changes remained within the experimental uncertainty. Remarkably, the RF irradiation on the CNTs/Gold substrates produced a $42 \pm 7 \%$ reduction in HeLa cells in just 10 min.

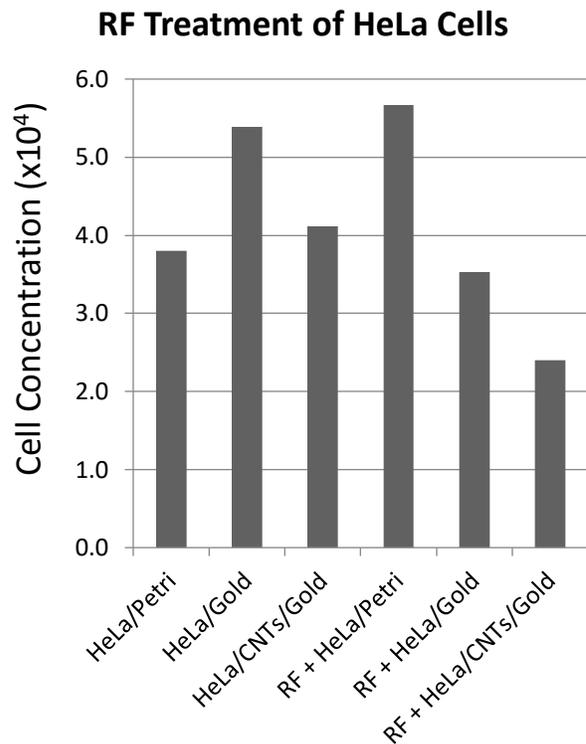


Figure 1: HeLa cell viability on various types of substrates with and without RF irradiation.

The results indicate that the CNTs likely act as RF-heat energy transducers leading to HeLa cell death due to hyperthermia.

NIR Treatment of HeLa Cells

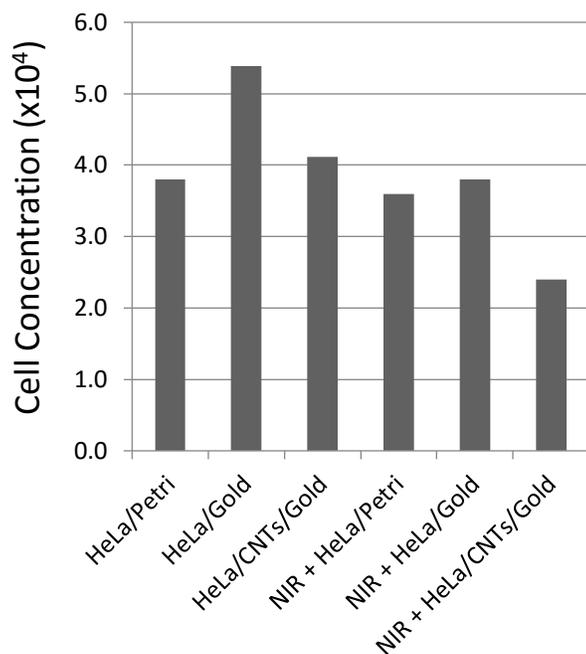


Figure 2: HeLa cell viability on various types of substrates with and without NIR irradiation.

Figure 2 shows the effect of NIR irradiation on the cell concentration of HeLa. Similar to the experiments with RF irradiation, we performed control experiments by studying the HeLa cell viability on Petri dishes, gold, and CNTs/gold substrates without the application of NIR irradiation. The HeLa concentrations for the control experiments remained stable. However, the experiments involving the application of NIR on HeLa cells deposited on Petri dishes, gold, and CNTs/gold resulted in a decrease in cell concentration of $5 \pm 1\%$, $29 \pm 6\%$, and $42 \pm 7\%$, respectively. This reduction in HeLa cells on CNTs in just 10 min is significant beyond the experimental uncertainties. The results suggest that the presence of CNTs may serve as NIR-heat energy transducers leading to HeLa cell death due to hyperthermia.

4 CONCLUSION

We have successfully shown the effectiveness of NIR and RF irradiation for the reduction of HeLa cells *in vitro*. HeLa cell reduction of $42 \pm 7\%$ took place under both NIR and RF after only 10 min of irradiation. These results show that phototherapy using CNTs excited with near infrared (NIR) or radiofrequency (RF) radiation holds promise as a treatment method against cancer cells. CNT phototherapy combined with CNT targeted drug delivery can result in more effective cancer treatments.

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