Cell mediated, heat-based therapy for treating HIV infection

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ABSTRACT

Although several treatment strategies have developed to control HIV infections, most of them have major drawbacks. Hence, HIV infection control still remains a major medical challenge and unconventional therapies to combat the disease have started to emerge. This work investigates remote heating of internalized nanoparticle as a potential method for targeted killing of HIV infected macrophages, one of the host cells for virus multiplication. First, radio frequency heating (RF) of 1.4 nm gold nanoparticle in solutions is evaluated employing fluorescence thermometry and quantum dots as temperature sensors. It is found that the particles are capable of generating sufficient temperature increase to cause cell apoptosis. Then THP-1 cells are exposed to gold nanoparticle solutions of different concentration, left to incubate for 24 hours and after which were subjected to RF electromagnetic field. Significant cell death was observed in specimen exposed to higher particle concentration and higher power of electromagnetic field.

1. INTRODUCTION

Acquired Immune Deficiency Syndrome (AIDS) is caused by human immunodeficiency virus (HIV). Since the first cases were reported, about a quarter century ago, HIV has become one of the deadliest pandemics in human history, killing more than 25 million people around the world. Despite intensive research at present there is no cure for HIV infection, though it can now be treated as a chronic illness. Furthermore available treatments have major drawbacks preventing some infected people from treatment. First of all, their effectiveness decreases over time as the virus accumulates mutations becoming drug-resistant. At present ~50% of patients receiving antiretroviral therapy are infected with viruses that express resistance to at least one of the available antiretroviral drugs [1]. Moreover, in time a drug typically loses its potency because viruses evolve naturally and develop resistance to it. In addition, current antiretroviral therapy requires patients to follow a strict medication routine. This makes it harder for the patients to adhere to the therapy, and lapse in the routine contributes to increasing the occurrence of HIV drug-resistance [2]. Hence there is an increased interest in developing alternative therapies to combat this deadly disease. For example, employing *in-vivo* miniaturized filtration devices was shown to be a promising approach for controlling HIV when implemented in the beginning stages of the disease [3,4]. More recently targeted killing of HIV infected cells achieved via radiolabeled antibodies have been shown to be an effective method for controlling the infection [5].

This work investigates remote heating of internalized nanoparticle as a platform for targeted killing of HIV infected macrophages. Macrophages are a very important viral reservoir serving as one of the host cells for HIV multiplication [6,7]. They have been used previous as carriers in antiretroviral drug delivery to enhance drug distributions in areas of active HIV replications [8]. The vision behind present work is that macrophages can be collected from blood, multiplied and modified (i.e. loaded with nanoparticle) *ex vivo* [9,10] after which they can be reintroduced into the circulatory system. Following an incubation period to allow the cells to become infected, they could be stimulated into apoptosis by remote heating of internalized nanoparticles.

Present work focuses on two critical aspects of the proposed therapy. In the first part, the temperature rise of 1.4 nm gold nanoparticles solutions subjected to radio frequency (RF) electromagnetic field is measured employing fluorescence thermometry. This is necessary in order to ensure that particles are capable of generating sufficient heating to cause cell apoptosis. In the second part, THP-1 cells are exposed to gold nanoparticle solutions of different concentration. Then RF electromagnetic field of 800 and respectively 900 kHz is applied to both nanoparticle exposed cells as well as unmodified cells for control. The effects of nanoparticle concentration and electromagnetic heating on the cell cultures are assessed by visual inspection.

2. PROBING REMOTE HEATING OF GOLD NANOPARTICLES

The heating generated by gold nanoparticles exposed to RF electromagnetic field was evaluated employing fluorescent thermometry and quantum dots as temperature sensors. Gold nanoparticles, 1.4 nm in diameter, functionalized with primary amine groups were procured from Nanoprobes Incorporated (2022). Fluorescent quantum dots (Qdot 525 ITK carboxyl quantum dots 8 μM solution) were purchased from Invitrogen Corporation. A solution of gold nanoparticles to quantum dots in 3:1 ratio was then prepared according to the following protocol. First 10 mM borate buffer was prepared from boric acid and borate and its

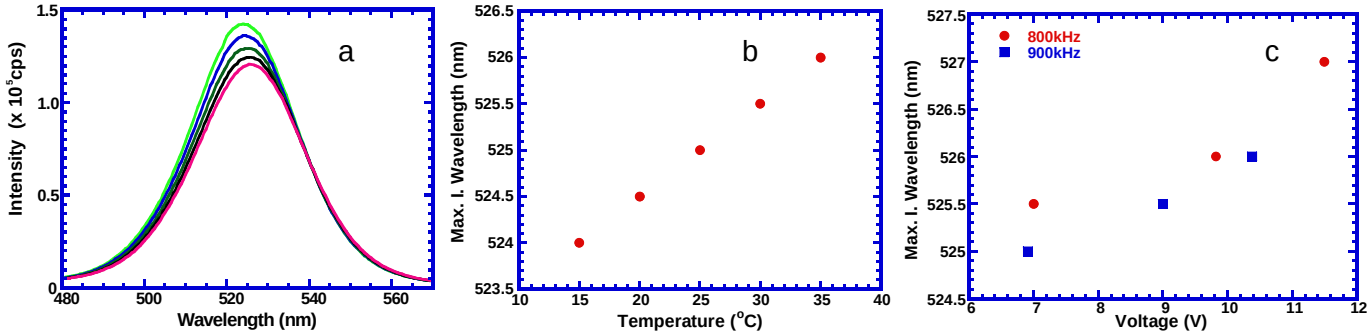

Figure 1: Temperature dependent fluorescence properties of quantum dots in 3:1 gold nanoparticles to quantum dots suspensions. (a) Fluorescent intensity as function of emission wavelength for temperatures increasing from 15° C to 35°C; (b) Wavelength of maximum intensity as function of temperature; (c) Wavelength of maximum intensity as function of the AC voltage applied to the coil at 800 and respectively 900 kHz.

pH was adjusted to 7.6 using sodium hydroxide (NaOH). Then 18.75 µL of an 8 µM stock solution of quantum dots was added to 123.8 µL of borate buffer. Next 100 µL of borate buffer were added to 6 nano-moles of gold nanoparticles to create a 60 µM stock solution of nanoparticles suspension. From this suspension, 7.5 µL were taken and mixed with the quantum dots in borate buffer solution to ensure a 3:1 molar ratio of gold nanoparticles to quantum dots. The solution was then placed in a quartz cuvette fitted in a special holder that allowed a copper wire to be wrapped around it to form a coil. The coil was later used to apply an RF electromagnetic field to the nanoparticle-quantum dots solution.

Before assessing the remote heating of gold nanoparticles, a calibration experiment was first performed. A Fluorolog-3 (Horiba Jobin Yvon) equipped with a Peltier module for temperature control was used to measure the emission spectrum as function of temperature of the fluorescent dots. During calibration, the temperature of the cuvette was increased from 15ºC to 35ºC in 5ºC increments. Once thermal equilibrium was achieved (approximately 45 min. after Peltier module reached the temperature set-point) the emission spectrum of the quantum dots was collected for an excitation wavelength of 390nm. Figure 1 (a) shows the fluorescence intensity versus emission wavelength of the quantum dots at each temperature set-point. It is observed that the intensity of the fluorescence emission decreases and its peak shift towards red at higher temperatures, as previously reported [11]. The wavelength corresponding to maximum intensity was determined using a commercial program (PeakFit, SPSS). Figure 1(b) shows the wavelength corresponding to maximum emission intensity as function of temperature.

Following calibration, the sample was immediately returned to 15ºC and maintained for 45 minutes to reach equilibrium. Then alternative current (AC) of 800 kHz and respectively 900 kHz was applied to the coil surrounding the cuvette. The AC signal was generated with an HP 1Hz-20MHz pulse/function generator (model 8111A) and was amplified by an RF power amplifier directly connected to the coil. A digital voltmeter was used to measure the voltage drop across the coil. Figure 1(c) shows the wavelength of maximum intensity of emission as function of the AC voltage applied to the coil. Based on these calibration and heating data, shown in Fig. 1(b) and respectively Fig. 1(c), the temperature increase above ambient (kept at 15 C) due to gold nanoparticle heating was estimated to be 30 degree at 800 kHz and the highest voltage applied to the coil.

In a separate experiment it was determined that heating does not occur in quantum dots based solution (without gold) under similar conditions. These indicate that gold nanoparticles exposed to RF electromagnetic field can generate significant heating which is in agreement with previous work [12], although the mechanisms responsible for heat generation are not well understood [13]. At present more investigations are being carried out to understand underlying physical phenomena causing heating and to measure local temperature rise in the vicinity of gold nanoparticle since both play a critical role when employing nanoparticles for targeted killing of cells.

3. TARGET KILLING OF NANOPARTICLE LOADED THP-1 CELLS

THP-1 cells, a human monocytic leukemia cell line that can be differentiated into macrophage-like cells, were used for proof-of-concept experiments of targeted killing via remotely heated gold nanoparticles. THP-1 cells were maintained in RPMI cell culture media at 37ºC, 5% CO_2 and 70% relative humidity. To prepare the cells for experiments, the media was exchanged and replaced with media containing phorbol 12-myristate 13-acetate (PMA, 10^{-8} M). To confirm proper differentiation and that they were able to internalize nanoparticles, the cells were first exposed to fluorescent latex beads 100 nm in diameter. For increased phagocytosis, 24 hours was allowed for incubation. Figure 2 is a digital picture (taken under microscope) of the THP-1 cells with internalized beads at the end of the incubation period. A significant particle uptake was observed and future experimentation also used 24 hours for incubation.

Next the cells were plated on two 12 mm circular coverslips in each of 12 separate wells and were allowed to differentiate for 24 hours in the presence of standard 10^{-8} M PMA solution. After this period, nanoparticles were added to

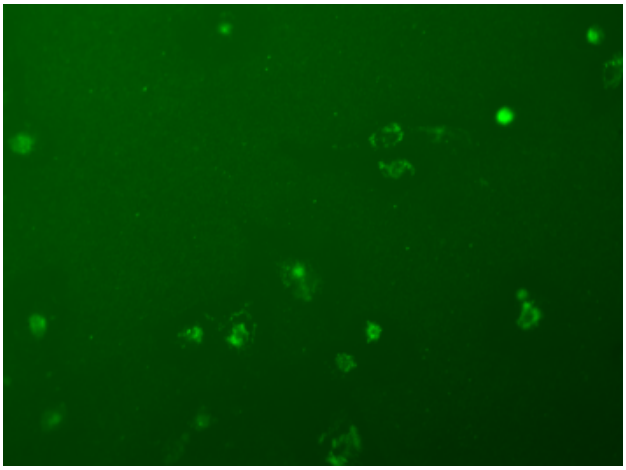

Figure 2: Differentiated THP-1 cells after 24 hours of incubation with 100 nm diameter fluorescent latex beads.

9 out of 12 of the wells in 5 different nanoparticles to cells ratios. The concentrations were estimated assuming that each well held approximately 250,000 cells. The ratio tested were 10^2:1, 10^3:1, 10^4:1, 10^5:1 and respectively 10^6:1. To prepare these concentrations, first a 6 μM stock solution of gold was prepared by adding 1 mL to 6 nmoles of gold nanoparticles. The most concentrated solution (needed for 10^6:1 ratio), was then prepared taking 1.7 μL of the 6 μM stock solution and mixing it mixed with 98.3 μL of phosphate buffered saline (PBS). This dilution created a 0.1038 μM solution, from which 4 μL were taken and added to two wells. For each subsequent concentration, 10 μL of previous dilution was diluted by 10 fold mixing it with 90 μL of PBS. This allowed each well to have 4 μL of PBS with different concentration of nanoparticles.

The particles and cells were left to incubate for 24 hours. After this period, specimens of nanoparticle loaded and mock-treated THP-1 cells were exposed to alternating electromagnetic field of 800 and respectively 900 kHz (not absorbed by the tissue) and variable power. This field was delivered employing a similar setup as the one used in Section 2 for probing remote heating of gold nanoparticles. A number of 22 specimens were tested under the conditions shown in Table 1. The time of exposure to electromagnetic field was fixed at 2 min. during all tests. In addition, each control coverslip was also removed from storing media and placed in the same ambient for 2 min. to ensure they were exposed to the same conditions. 24 hours after the test were completed, the specimens were exposed to a blue dye. This was done to help visualize the cellular death since the dye is only absorbed by dead cells. The cells were then inspected visually to assess viability after the treatment. Several examples of dye stained specimens are shown in Fig. 3 (a) to (f). The death cell percentage for all specimens is listed in Table 1. Less then 15% of cells in unheated specimens underwent apoptosis, with exception of the specimen exposed to highly concentrated nanoparticle solution (10^6:1) were cell death increased to 27%. For heated specimens exposed to nanoparticle solutions below 10^2:1 ratio there were no visible

Sample	Gold:cell ratio	Frequency	Applied voltage (function generator)	% dead cells
1A	0	0	0	14%
1B	0	900 KHz	0.634	10%
2A	0	800 KHz	0.314	15%
2B	0	800 KHz	0.731	0%
3A	10^2:1	0	0	0%
3B	10^2:1	900 KHz	0.594	12%
4A	10^2:1	800 KHz	0.315	12%
4B	10^2:1	800 KHz	0.734	7%
5A	10^3:1	0	0	8%
5B	10^3:1	900 KHz	0.598	44%
6A	10^3:1	800 KHz	0.318	25%
6B	10^3:1	800 KHz	0.735	26%
7A	10^4:1	0	0	8%
7B	10^4:1	900 KHz	0.594	0%
8A	10^4:1	800 KHz	0.316	16%
8B	10^4:1	800 KHz	0.733	27%
9A	10^5:1	0	0	6%
9B	10^5:1	900 KHz	0.597	13%
10A	10^5:1	800 KHz	0.314	14%
10B	10^5:1	800 KHz	0.732	73%
11A	10^6:1	0	0	27%
11B	10^6:1	800 KHz	0.733	62%

Table 1: Testing conditions and death rates for cell cultures.

differences between cell death in the heated and unheated samples. However, for specimens exposed to higher nanoparticle concentration, less cells were found to survive after exposure to electromagnetic field. Furthermore, in these specimens the number of dead cells increased as the voltage applied to the coil was increased. For example at 10^4:1 ratio, the percent of dead particles for the lowest applied voltage is similar to that in the control group (unheated samples). However when the voltage was raised, the percentage of dead cells increased to 27%. Similarly, at 10^5:1 ratio there is an increase from 14% at low voltage to 73% death at high voltage (Fig. 3b and c). For specimens exposed to the highest nanoparticle concentration (10^6:1 ratio) the number of dead cells in unheated sample was also significantly less than that in heated sample. However, at 10^6:1 ratio, the unheated specimen experienced up to two fold increase in dead cells than all other unheated samples. This may suggest that gold nanoparticles could be toxic to the cells at very high concentrations, though more investigations are needed in order to confirm this hypothesis. Nevertheless, these results indicate that targeted killing of cells via RF heated, ultra-small gold nanoparticles has potential as an alternative therapy for HIV infection control.

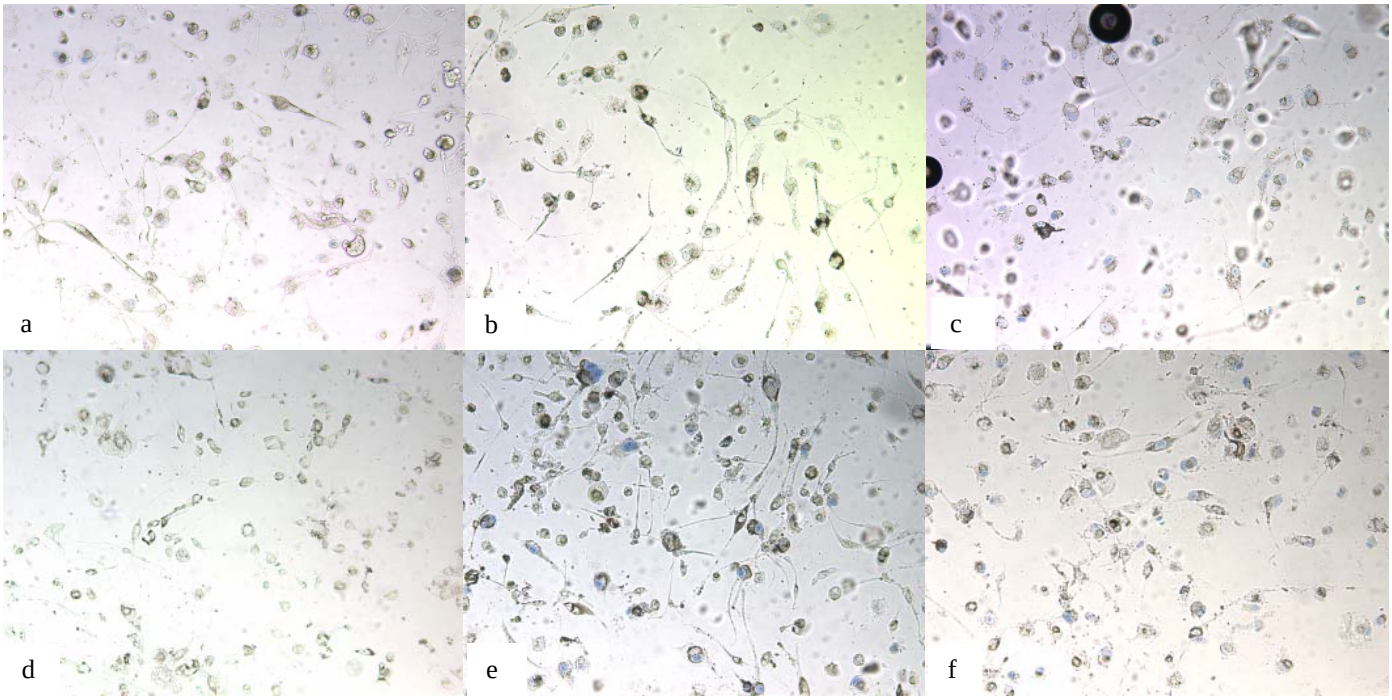

Figure 3: Digital picture of stained cells. Dead cells uptake the dye becoming blue. (a) Specimen 9A (from Table 1). (b) Specimen 10 A. (c) Specimen 10B. (d) Specimen 2B. (e) Specimen 11 A and (f) Specimen 11 B.

4. SUMMARY

Targeted killing of nanoparticle loaded cells via remote RF heating is reported. The nanoparticle used in this study, 1.4 nm diameter gold particles, were shown to be produce up to 30 C increase in bulk temperature when exposed to RF electromagnetic field of 800 kHz. THP-1 cells were used for proof-of-concept experiments of targeted killing via remotely heated gold nanoparticles. Preliminary results suggest that a ratio of 10^5:1 of gold nanoparticle to cells and a RF field of 800 kHz have optimum effects resulting in 73% cell death.

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