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Noninvasive Micromanipulation of Live HIV-1 Infected Cells via Laser Light*

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Abstract. Live mammalian cells from various tissues of origin can be aseptically and noninvasively micromanipulated via lasers of different regimes. Laser-driven techniques are therefore paving a path toward the advancement of human immunodeficiency virus (HIV-1) investigations. Studies aimed at the interaction of laser light, nanomaterials, and biological materials can also lead to an understanding of a wealth of disease conditions and result in photonics-based therapies and diagnostic tools. Thus, in our research, both continuous wave and pulsed lasers operated at varying wavelengths are employed, as they possess special properties that allow classical biomedical applications. This paper discusses photo-translocation of antiretroviral drugs into HIV-1 permissive cells and preliminary results of low-level laser therapy (LLLT) in HIV-1 infected cells.

INTRODUCTION

Intracellular delivery of genetic materials and other macromolecules has a crucial role in medicine and biology. Some applications involve introduction of potentially therapeutic material, including proteins, oligonucleotides, ribonucleic acid (RNA), and deoxyribonucleic acid (DNA) [1]. There are numerous means of promoting the inclusion of foreign genes and other macromolecules into mammalian cells. These range from chemical (cationic polymers and lipids) to viral to physical approaches [2]. Each of these systems has both advantages and disadvantages, and effective alternatives are still being sought. For protocols performed *in vitro* and *in vivo*, the goal is a gene, drug, and/or vaccine delivery technology that has minimum cytotoxicity and immunogenic response, can be applied under sterile tissue culture protocols, and can also offer targeted treatment of individual cells, organelles and organs [3]. Optical cell translocation satisfies these criteria. A variety of lasers, ranging from continuous wave visible sources [4–8] to pulsed infrared [9–12] sources, have been applied for efficient introduction of biomaterials into mammalian cells.

To capitalize on the targeted delivery of biomaterials using laser pulses, our studies examined selective delivery of the antiretroviral (ARV) drugs tenofovir, nevirapine, and efavirenz into live TZM-bl cells. Highly active antiretroviral therapy (HAART), which is used to treat human immunodeficiency virus type-1 (HIV-1), has contributed positively to the decline of deaths related to acquired immunodeficiency syndrome (AIDS) [13]. As a result of oral administration and in high concentrations, HAART has been reported to cause unwanted side effects [14]. The literature has suggested drug delivery systems that deposit drugs directly into infected or diseased sites can reduce drug-related toxicities [15]. On the other hand, low-level laser therapy (LLLT) has been a method of choice for its photostimulatory effect on slow-healing wounds [16]. For example, a number of monochromatic radiation sources, such as gas-state lasers (argon-ion at 488 nm, HeNe at 633 nm), solid-state lasers (neodymium: yttrium–aluminum–garnet at 1064–1320 nm), and laser diodes (gallium–aluminum–arsenide at 980 nm, gallium-arsenide at 904 nm) have been identified as producing a beneficial biological effect known as "photostimulation" or "biostimulation," a process reported to generally depend on wavelength and dose of laser irradiation [16]. Because lasers and light-emitting diodes (LEDs) have a wide application in wound-healing research, our research sought to investigate potential applications of LLLT in the treatment of HIV-1 *in vitro*.

* See also Patience Mthunzi, "Could We Cure HIV with Lasers?" TED2015, Vancouver, Canada, March 16–20, published as a TED Talk video August 14, 2015, https://www.youtube.com/watch?v=yMWlkJAqKYU. —*Eds.*

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EXPERIMENTAL PROCEDURE

Cell Cultures

In our research cells were cultured in a 37°C, 5% carbon dioxide (CO_2), and 85% humid incubator (optimum growth conditions). As described by Mthunzi *et al.* (2010) [3], cells were routinely grown in T25 vented-top culture flasks (Nunc™), subcultured twice weekly at a ratio 1:4. The TZM-bl cells, also called JC57BL-13, used in this study were obtained from the National Institutes of Health (NIH) AIDS Research and Reference Program (catalogue no. 8129). TZM-bl cells are a HeLa derived, genetically engineered cell line that expresses endogenous CXCR4 and transgenic CD4 and CCR5 on their cell membrane. The HIV-1 permissive cell line also contains Tat-responsive reporter genes for firefly luciferase (*Luc*) and *Escherichia coli* (*E. coli*) β-galactosidase under the control of an HIV-1 long terminal repeat (LTR). These cells are highly permissive to infection by various HIV-1 strains, including primary and molecularly cloned *Env*-pseudotyped viruses [17]. TZM-bl cells were grown in general medium (GM) containing DMEM and supplemented with 1% PEST.

Optical Setup

The optical setup (Fig. 1) was constructed using a femtosecond laser that emitted light at 1064 nm, 80 MHz, and 200 fs, with an average power at the beam focus of 60 mW. The beam was magnified through two lenses to perfectly fit the back aperture of a 60X Nikon objective lens of numerical aperture (NA) 0.8. At the sample plane a focused laser spot of 1.1 μm diameter was measured, and a mechanical shutter (Newport, SA) located in the beam path was employed to regulate the time of beam exposure at the cell sample plane. The sample was moved via a manual *xyz* stage (Newport, SA) and lighted through Koehler illumination. The cell sample was imaged by a long working distance objective onto a Watec color camera (WAT-250D) placed below the sample stage.

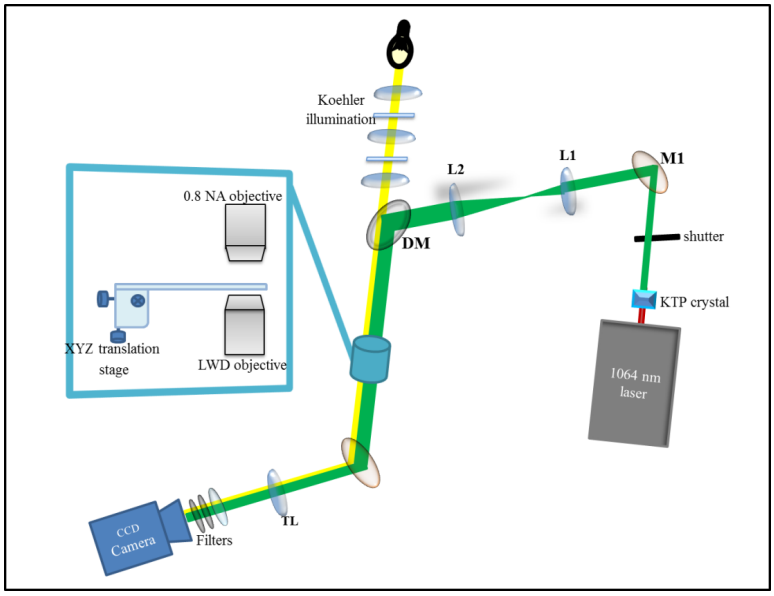

FIGURE 1. Optical setup employed during targeted optical anti-HIV-1 drug delivery experiments.

Phototranslocation of Antiretroviral Drugs

The TZM-bl cells were irradiated at 1064 nm in the presence of tenofovir, nevirapin, and efivarenz. Briefly, a sample of TZM-bl cells (at 2×10^3) was seeded, then the cell monolayer was submerged in 20 μL of neat GM that contained a final concentration of 20, 10, and 14 μg/mL of tenofovir, nevirapine, or efavirenz, respectively. To differentiate between laser-assisted drug delivery and the spontaneous uptake of the drugs by the cells, control sample dishes were prepared. After three doses of laser irradiation using 60 mW and 50 ms as the laser-to-cell exposure

time, the monolayer was washed with 1 × PBS. The cell monolayer was supplemented with 200 µL of GM and incubated overnight.

Low-level Laser Therapy in HIV-1 Infected Cells

The TZM-bl cells were infected using the *Env*-pseudotyped virus through adding the cells to all wells of the 96-well plate at 10,000 cells/100 µL in growth media containing DEAE dextran (Sigma Aldrich, SA) to a final concentration of 20 µg/mL per well. The plate was incubated for 48 hours in a 37°C, 5% CO_2, and 85% humidity environment. Bright Glo luciferase substrate (Promega) was used as the detection system for infection. Here the infectivity of the *Env*-pseudotyped virus was measured according to the expression of the *Luc* reporter gene in the TZM-bl cells after a single round of viral infection while the substrate functioned to lyse the cells and cause luminescence of successfully infected cells. The relative luminescence units (RLU) were quantified using the Tecan Infinite F500 microplate reader. Before laser treatment, approximately 6 x 10^5 cells in 3 mL complete medium were seeded from 75 cm^2 flasks into sterile 3.5 cm diameter culture plates and incubated overnight in optimum growth condition. These cells reached 98–100% confluence and were irradiated using a diode laser at 632.8 nm with a beam radius expanded to 5 cm using a two-lens telescope (Fig. 2).

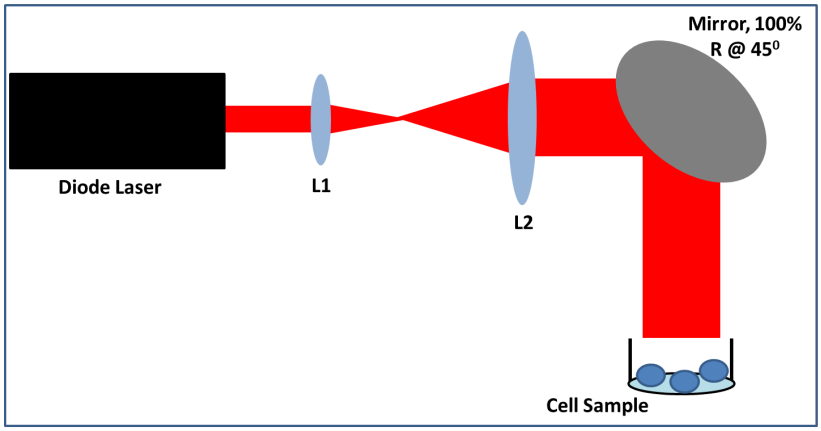

FIGURE 2. A simple two-lens system was used to complete LLLT studies. The laser beam was reflected to the cell sample by using a 100% reflective mirror.

Cells were irradiated at doses of 2 J/cm^2 to 10 J/cm^2 in 800 µL culture supernatant at room temperature in a dark room. The time of exposure for the different average energy density values (0.2–1 J) applied was calculated using the following formula:

$$t = \frac{\phi A}{P}, \tag{1}$$

where t is the time in minutes, Φ is the energy per unit area (specified) in J/cm^2, A is the area (measured – used that of the culture petri dish) in cm^2, and P is the laser power output (measured using a Coherent Labmaster power meter and the Coherent LM-3 HTD detector) in mW.

RESULTS

Phototranslocation of Antiretroviral Drugs

ARV drugs cause unwanted side effects, mainly because orally administered drugs are circulated in the blood stream through the gastrointestinal tract before reaching the target site. The drug is therefore carried to areas of the body that do not need it. Some of the drug is lost during transit to the target site, which means that high dosages of the drugs must be administered to ensure adequate bioavailability. A solution could be to use phototranslocation to deposit the drug directly at the target sites. For example, our data (Fig. 3) show that cells treated with translocation of the drug tenofovir show a significantly lowered rate of infectivity as measured by RLU.

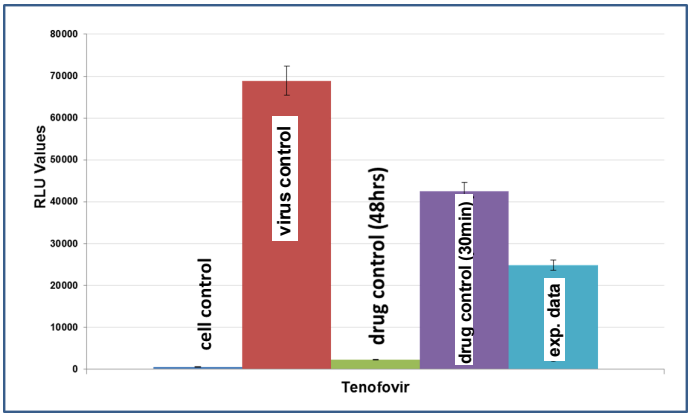

FIGURE 3. Presented here are the cell control (no laser treatment/no virus/no drug), the virus control (infected cells/no drug/no laser), drug controls (infected cells treated with tenofovir over 48 hours or 30 minutes) and the experimental data (infected cells irradiated with a 1064 nm pulsed laser at 60 mW and 50 ms of exposure).

Low-level Laser Therapy in HIV-1 Infected Cells

Although there is not much literature on the interaction of light and HIV infected tissue, there is reported evidence of $CD4^{+}T$ cell proliferation with a so-called intranasal light therapy. Explained briefly, the mechanism behind any laser–tissue interactions entails oxygen molecules being raised to a higher energy state upon photon absorption. The excited molecule then experiences a change through having an unpaired electron, turning it into singlet oxygen, which then sends a "redox signal" to the body to counterbalance the free radical. Essentially, dosages induced during LLLT are large enough to stimulate certain bioprocesses but not usually sufficient to evoke cytotoxicity. Figure 4 shows our results of LLLT with HIV-1 infected cells. Infection rates, as measured by RLU values, are lowest at a dosage of 10 J/cm^2.

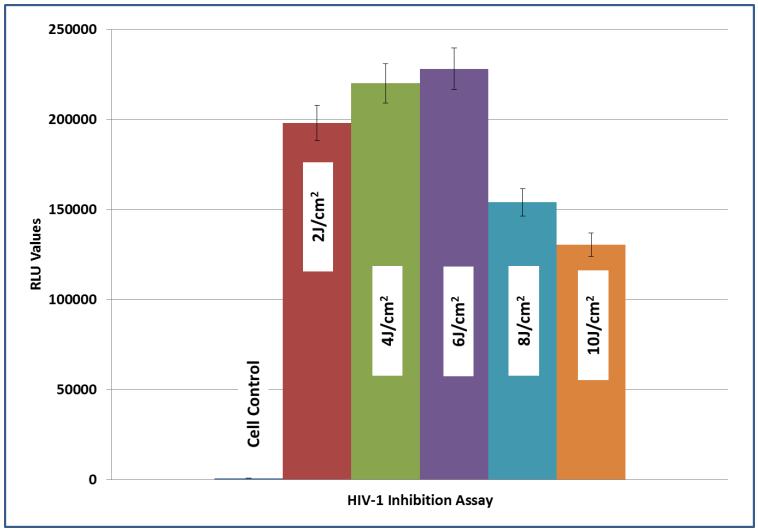

FIGURE 4. Infected TZM-bl cells dosed at 2–10 J/cm^2 using a 632.8 nm diode laser compared to the non-irradiated, uninfected cell control.

CONCLUSION

We showed that targeted photo-translocation of antiretroviral drugs into HIV-1 permissive cells can decrease cell infectivity. Studies aiming at optimizing *in vitro* parameters prior to the commencement of *in vivo* experiments are also underway. Finally, the preliminary results of low-level laser therapy in HIV-1 infected cells indicate that at higher laser fluences it might be possible to reduce viral infectivity. Tests looking into cell viability and different enzyme assays for use after treatment are currently being completed.

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Biography. *Patience Mthunzi holds a PhD in Physics (Biophotonics–Optical Tweezers Area) from the University of St. Andrews, Scotland, UK. She is the first known person in South Africa to qualify for a PhD in this field of study. She joined the National Laser Centre (NLC) in the Council for Scientific and Industrial Research (CSIR) in the Biophotonics Group in October 2004. During November 2005 to April 2006 she set up a fully functional cell culture facility at the NLC. She is currently a senior scientist researcher at the NLC in the Biophotonics Group, leading single-cell and/or molecule projects. She recently received one of the country's highest orders, the Order of Mapungubwe, for her contribution in the field of biophotonics.*