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FAST TRACK COMMUNICATION

Selective inactivation of human immunodeficiency virus with subpicosecond near-infrared laser pulses

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Abstract
We demonstrate for the first time that human immunodeficiency virus (HIV) can be inactivated by irradiation with subpicosecond near-infrared laser pulses at a moderate laser power density. By comparing the threshold laser power density for the inactivation of HIV with those of human red blood cells and mouse dendritic cells, we conclude that it is plausible to use the ultrashort pulsed laser to selectively inactivate blood-borne pathogens such as HIV while leaving sensitive materials like human red blood cells unharmed. This finding has important implications in the development of a new laser technology for disinfection of viral pathogens in blood products and in the clinic.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

It is now clear that the biochemical and pharmaceutical methods currently used for the inactivation of viral particles, although quite successful, encounter problems of drug resistance in the target virus. In addition, they also have clinical side effects. Ultraviolet disinfection is effective; however, it not only kills the unwanted microorganisms but also raises concerns of damaging sensitive materials such as human cells and protein components. In other words, it lacks selectivity. A new method is therefore necessary to efficiently inactivate the viral particles, while minimizing the side effects and the possibility of generating mutated strains of the target virus. One such technique has recently been developed by Tsen *et al* [1–4], in which an ultrashort visible/near-infrared (NIR) laser has been employed to coherently excite the mechanical vibrations of the protein capsid of target viral particles, leading to their damage/disintegration and resulting in the loss of viral infectivity. Here, impulsive stimulated Raman scattering (ISRS) [5–8] has been demonstrated to be the physical mechanism that inactivates the M13 bacteriophages [1–4].

M13 bacteriophage has been shown to be inactivated by a low-power-intensity ultrashort visible laser [1]. The selectivity of this new laser technology has also been shown in a variety of

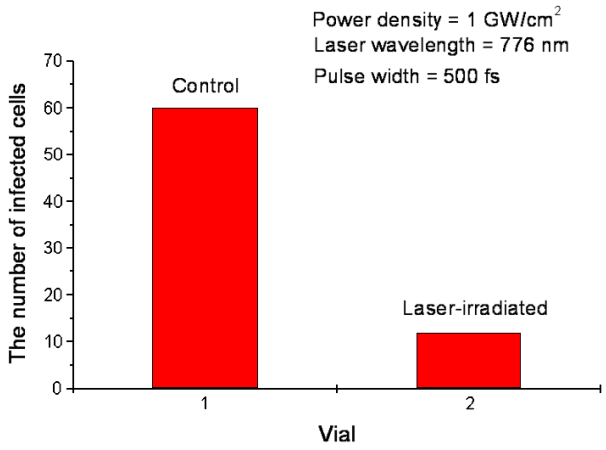

Figure 1. The number of infected cells for the control and laser-irradiated HIV samples. The viral concentration is about 60 per well. The laser-irradiation time is 2 h.

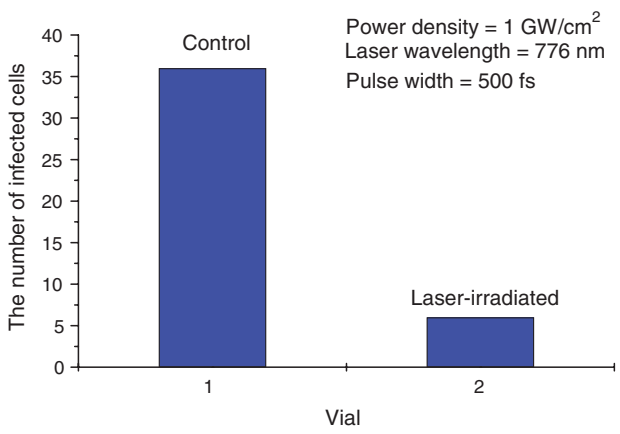

Figure 2. The number of infected cells for the control and laser-irradiated HIV samples. The viral concentration is about 36 per well. The laser-irradiation time is 2 h.

microorganisms [3]. Because the wavelength of the laser used was either in the visible or NIR spectral range, the concerns of damaging nucleic acid genomes or protein components are expected to be minimal. Since this laser technology targets the intrinsic mechanical (vibrational) properties of protein capsids and membrane structures, it is relatively insensitive (perhaps completely immune) to genetic mutation in the target microorganisms. This is because vibrational modes of low frequency characteristically have long wavelengths; as a result, they are most influenced by global structure rather than by specific details in local structure. Thus it is highly unlikely that target microorganisms can avoid destruction through genetic mutation.

However, we notice that all the previous work of inactivation of microorganisms with ultrashort pulsed lasers has emphasized non-pathogenic microorganisms. In this paper, we report the first successful use of this technique in the load reduction of an extremely important human pathogen—human immunodeficiency virus (HIV)—*in vitro*. We show that HIV can be inactivated by a subpicosecond NIR laser with a moderate laser power density. By comparing this threshold laser power density with those of human red blood cells and mouse dendritic cells, we conclude that the ultrashort laser technique, if appropriately manipulated, can be used to selectively inactivate HIV and other pathogens while leaving useful materials such as human cells unharmed.

2. Samples and experimental method

HIV stocks (NL4-3, provided by NIH) were diluted to 1×10^5 cpm ml^{-1} in DMEM (with no phenol red) for the laser-irradiation experiments.

U373-MAGI-CXCR4$_{CEM}$ cells (NIH AIDS Research & Reference Reagent Program) were seeded at 6×10^4 cells/well in 24-well plates. In the assaying of the infectivity of HIV, these cells were infected with samples, either laser-irradiated or not irradiated (control), at indicated viral concentrations. Following 48 h incubation, cells were fixed with a 1% paraformaldehyde, 0.2% gluteraldehyde solution prepared in phosphate-buffered saline (PBS). Fixed cells were washed twice with PBS and stained with a solution containing 0.4 mM potassium ferrocyanide, 2.0 mM $MgCl_2$, and 0.4 mg ml^{-1} X-gal. Cells positive for β-galactosidase activity were counted manually (sum of 10 fields, duplicate samples).

The HIV samples were irradiated with the second harmonic output (SHO) of a fiber laser (with seed and amplifier, from Raydiance Inc.). The fiber laser, which has a wavelength of 1.55 μm, was chosen to operate at a repetition rate of 500 kHz and 5 μJ/laser pulse. The SHO of the fiber laser used in the laser-irradiation experiments has a wavelength of 776 nm, about 1.4 μJ/laser pulse, a pulse width of about 500 fs and a spectral width of about 100 cm^{-1}. The details of the estimation of the laser power density used in the experiments have been published elsewhere [4]. All the laser-irradiation experiments were carried out at room temperature. Because of bio-safety considerations, polyethylene vials were used to hold the HIV samples. A magnetic stirrer was used to stir the sample so as to facilitate the interaction of the laser with the HIV particles. We have found that a sample of HIV loses about 30% of its infectivity after being left at room temperature for 2 h even without laser irradiation. Therefore, we have limited the duration of the laser irradiation to 2 h in all of our experiments. This limitation, as will be shown later, manifests itself in the efficiency of the load reduction observed in our experiments.

3. Experimental results

Figure 1 shows the number of infected cells for the control and laser-irradiated HIV samples. The laser power density was 1.1 ± 0.1 GW cm^{-2}. The control infected 60 ± 8 cells; whereas the laser-irradiated sample infected 12 ± 4. A reduction of viral infectivity of about 80% was observed. Figure 2 shows the result for a similar experiment with a lower virus concentration and a load reduction of about 84% was revealed. These experiments were repeated several times, and a viral load reduction of similar percentage was observed. Therefore, we conclude that the ultrashort laser pulses can significantly reduce the viral load of HIV *in vitro*.

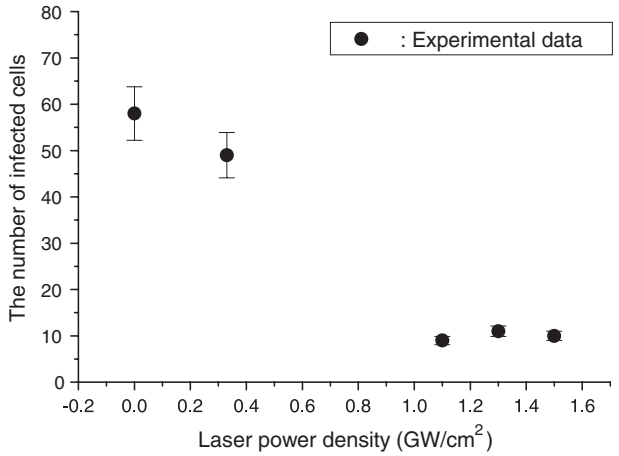

Figure 3. The number of infected cells as a function of laser power density for an HIV sample with 58 viral particles per well. The HIV virus has been found to lose its infectivity at a laser power density of about 1.1 GW cm^{-2}.

Figure 3 shows the number of infected cells as a function of laser power density for HIV samples with about 60 viral particles. The data at the zero power density is the control. We have found that HIV remains infectious when irradiated with laser power density which is smaller than about 500 MW cm^{-2}. However, as the laser power density increases to or beyond 1.1 GW cm^{-2}, HIV loses its infectivity.

We have carried out similar experiments with human red blood cells [9] as well as the mouse dendritic cells [9]. Both the human red blood cells and mouse dendritic cells were found to have a significantly higher inactivation threshold than that of HIV. All the experimental results are summarized in table 1. The results from table 1 indicate that there exists a window in laser power density that enables us to kill HIV while leaving useful materials such as human red blood cells and mouse dendritic cells unharmed. It is therefore plausible that the ultrashort pulsed laser, if appropriately manipulated, can be used to selectively kill blood-borne pathogens such as HIV with minimal damage to sensitive materials.

4. Discussions

Our experiments show that ultrashort laser pulses can reduce about 80% of the viral load in HIV samples. This is obviously not sufficient by clinical standards for therapeutic purposes. However, we notice that the relatively small viral load reduction observed in our experiments is actually a manifestation of the use of a magnetic stirrer as well as the limitation imposed on the duration of the laser-irradiation time as discussed before. We have found that, in the inactivation of other viral systems such as M13 bacteriophages and tobacco mosaic virus (TMV), by using a syringe pump-capillary-micro-tube setup instead of the magnetic stirrer, one can improve the efficiency of the viral reduction by almost two orders of magnitude [9]. Experiments are currently planned to use the syringe pump setup to greatly reduce the load of HIV.

At this moment, we think that the most probable reason for the inactivation of HIV by the subpicosecond laser pulses has something to do with the laser-induced damage of the capsid through ISRS. We believe that the reason why there exists a window in laser power density that allows the selective inactivation of HIV as demonstrated in table 1 is most likely due to the size effects. HIV is an enveloped virus with a capsid and is about 0.1 μm in diameter, whereas the shape of a human red blood cell is like a donut, about 10 μm in diameter, 2 μm in thickness. The mouse dendritic cell is about 10 μm in diameter. Since these microorganisms are embedded in water, the water molecules play a crucial role in the damping of the vibrations excited by the laser. The relatively large size of either the human red blood cell or the mouse dendritic cell as compared with that of HIV means that there are more water molecules surrounding the red blood cells and dendritic cells than HIV; in other words, the damping associated with the coherent excitation created by the laser is lower for HIV than for red blood cells or dendritic cells. As a result, the amplitude of vibrations created by a given laser power density can be much higher for HIV than for red blood cells or mouse dendritic cells.

Table 1. Threshold laser power density for the inactivation of a variety of microorganisms.

	Microorganisms		
Laser power density	HIV	Human red blood cell	Mouse dendritic cell
For threshold inactivation (GW cm^{-2})	1.1	$\geqslant$15	$\geqslant$10

5. Conclusion

We have demonstrated for the first time that an ultrashort pulsed laser can be used to reduce the viral load of HIV *in vitro*. Comparison of the HIV results with those of human red blood cells as well as mouse dendritic cells suggests that it is plausible to use an ultrashort pulsed laser to selectively inactivate target pathogens without damaging the useful materials such as red blood cells and dendritic cells. Therefore, this finding is an important step toward the development of a new technology for disinfection of viral pathogens in blood products and in the clinic.

Acknowledgments

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