

An Updated Report of the Ultrashort
Pulsed Laser Technology and its
Perspectives in Biomedical ApplicationsShaw-Wei D. Tsen¹ and Kong-Thon Tsen^{2*}¹Department of Radiology, Washington University School of Medicine, St Louis, MO 63110²Department of Physics and Center for Biophysics, Arizona State University, Tempe, AZ 85287-1504

Article Information

Received date: Nov 16, 2015

Accepted date: Nov 17, 2015

Published date: Nov 23, 2015

*Corresponding author

Kong-Thon Tsen, Department of Physics
and Center for Biophysics, Arizona State
University, Tempe, AZ 85287-1504, USA,
Email: tsen@asu.edu

Distributed under Creative Commons
CC-BY 4.0

Recent reports by BBC News [1], New Scientist [2], Popular Mechanics Magazine [3] and Discover Magazine [4] on the invention of a new Ultra Short Pulsed (USP) laser technology by a U.S. group led by Kong-Thon Tsen of Arizona State University and Shaw-Wei D. Tsen of Washington University have revealed the great potential of USP lasers in biomedicine. This article provides an update on the current status of this USP laser technology and its perspectives in biomedical applications.

Pathogens including viruses and bacteria represent one of the greatest threats to human health worldwide. New pathogens will continue to emerge for the foreseeable future, highlighting the need for preventative measures such as pathogen inactivation of blood products, as well as a system for rapid vaccine development, to address the initial stages of an outbreak or epidemic.

The USP laser pathogen inactivation technology is a promising solution to these challenges. In a paradigm shift from traditional pathogen inactivation methods, Tsen and colleagues made use of the mechanical vibrations excited by the USP laser irradiation and demonstrated that visible wavelength USP lasers could be used to efficiently inactivate a broad spectrum of pathogens, including viruses and bacteria, while preserving desirable materials such as mammalian cells and proteins [5-12]. The USP laser treatment does not cause heating effects or covalent structural changes in biological systems, and the process does not involve adding any chemical or biological reagents. Therefore, USP laser treatment is a promising pathogen inactivation technology with applications in disinfection of therapeutics and in the production of inactivated vaccines with minimal clinical side effects.

The mechanism by which the USP laser treatment inactivates pathogens has been recently elucidated. Both enveloped and non-enveloped viruses are inactivated by the visible USP laser irradiation through Impulsive Stimulated Raman Scattering (ISRS) processes, leading to structural damage to the viral capsid [5-7]. Data suggest that inactivation of bacteria by the visible USP laser irradiation is due to protein aggregation, also induced by ISRS processes [8]. Therefore, it is concluded that the ISRS process is the primary mechanism responsible for the inactivation of pathogens such as viruses and bacteria. Tsen and colleagues have also found evidence that USP laser-inactivated viruses are functionally impaired at the level of capsid disassembly/uncoating during the infection of cells (Figure 1) [11].

To date, the USP laser technology has been demonstrated successfully in several biomedical applications including (1) the disinfection of human plasma [9]; (2) the disinfection of cell cultures [12]; and (3) the production of a potentially safer, higher potency influenza vaccine [10].

In 2014, the group published a proof-of-concept for the use of visible USP laser to inactivate pathogens with good preservation of human coagulation factor function [9]. Table 1 displays the percent retention of coagulation factor activity of relevant proteins in human plasma after USP laser irradiation. Table 2 shows the inactivation efficacy of USP laser treatment against representative pathogens in human plasma. Efforts are underway to both make the pathogen inactivation by the USP laser irradiation more efficient and extend the technology towards disinfection of other blood products such as platelets, red blood cells, and even whole blood. A pathogen inactivation system with minimal side effects to safeguard all blood products from existing as well as emerging pathogens would represent a "holy grail" in transfusion medicine.

More recently, in 2015, the group demonstrated that visible USP laser-inactivated influenza virus could be used as a safer, more potent influenza vaccine relative to conventionally inactivated influenza vaccines [10]. Figure 2 shows the protection of the USP laser-inactivated influenza vaccine against lethal influenza challenge in a mouse model. Table 3 shows data indicating that the hemagglutinin antigen of influenza virus is preserved after USP laser irradiation, a likely reason for the improved potency of the laser-inactivated influenza vaccine. Figure 3 depicts the generation

Table 1: Retention of coagulation factor activity for USP laser-treated plasma [9].

Coagulation factor	Control plasma	Laser-irradiated plasma	Normal reference range	% Retention after treatment
fibrinogen (mg/dl)	218 ± 4	158.5 ± 2.5	170 - 400	73
factor II (IU/ml)	0.82 ± 0.02	0.98 ± 0.03	0.75 - 1.30	120
factor V (IU/ml)	0.84 ± 0.01	0.80 ± 0.06	0.50 - 1.25	95
factor VII (IU/ml)	0.92 ± 0.01	2.22 ± 0.23	0.50 - 1.75	241
factor VIII (IU/ml)	0.72 ± 0.01	0.49 ± 0.01	0.50 - 1.60	68
factor IX (IU/ml)	1.01 ± 0.01	0.96 ± 0.09	0.55 - 1.60	95
factor X (IU/ml)	1.02 ± 0.03	1.04 ± 0.03	0.60 - 1.60	102
factor XI (IU/ml)	1.01 ± 0.01	0.55 ± 0.09	0.60 - 1.40	54
factor XII (IU/ml)	0.90 ± 0.00	0.69 ± 0.02	0.45 - 1.70	77

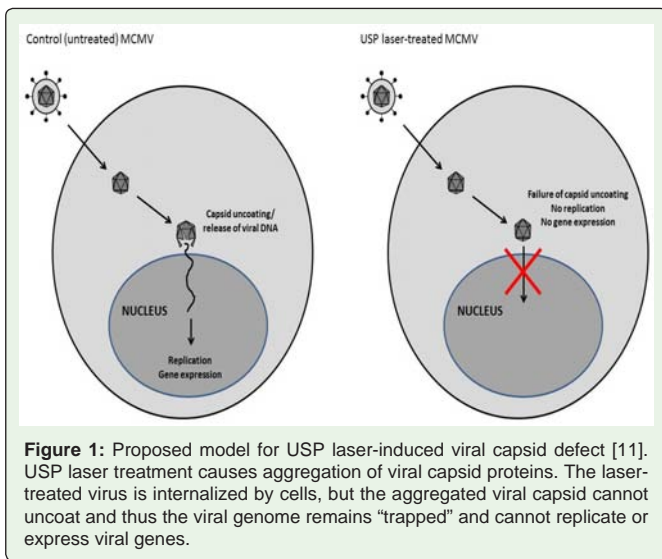


Figure 1: Proposed model for USP laser-induced viral capsid defect [11]. USP laser treatment causes aggregation of viral capsid proteins. The laser-treated virus is internalized by cells, but the aggregated viral capsid cannot uncoat and thus the viral genome remains “trapped” and cannot replicate or express viral genes.

Table 2: Load reduction value for viruses spiked in human plasma after the USP laser treatment [9].

Microorganism	Load reduction value
HIV	$(1.2 \pm 0.1) \times 10^2$
HAV	$(1.1 \pm 0.1) \times 10^1$
MCMV	$(1.1 \pm 0.1) \times 10^3$

Table 3: Haemagglutination activity of live and the USP laser-inactivated influenza virus with A/PR/8/34 strain [10].

Strain	Method of Inactivation	HAU/ml
A/PR/8/34	Original live stock	$(2.3 \pm 0.2) \times 10^5$
	USP laser inactivation	$(2.1 \pm 0.2) \times 10^5$

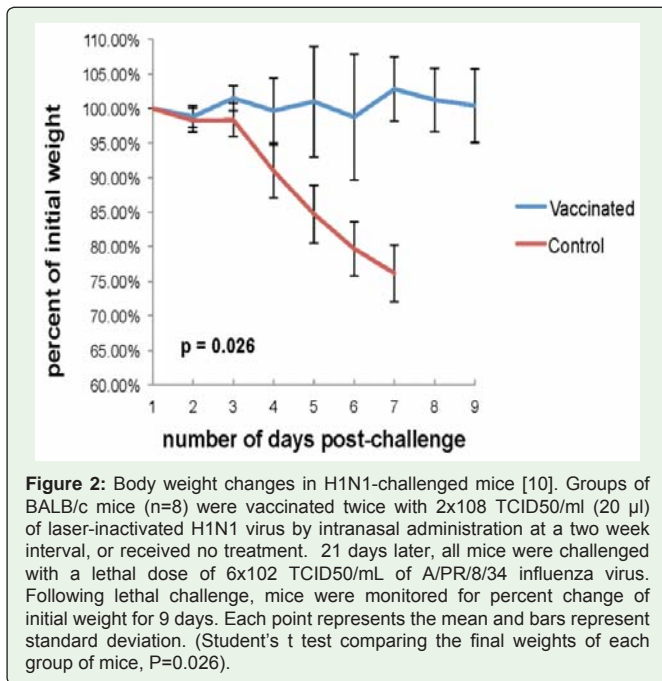


Figure 2: Body weight changes in H1N1-challenged mice [10]. Groups of BALB/c mice (n=8) were vaccinated twice with 2×10^8 TCID50/ml (20 μ l) of laser-inactivated H1N1 virus by intranasal administration at a two week interval, or received no treatment. 21 days later, all mice were challenged with a lethal dose of 6×10^2 TCID50/mL of A/PR/8/34 influenza virus. Following lethal challenge, mice were monitored for percent change of initial weight for 9 days. Each point represents the mean and bars represent standard deviation. (Student's t test comparing the final weights of each group of mice, $P=0.026$).

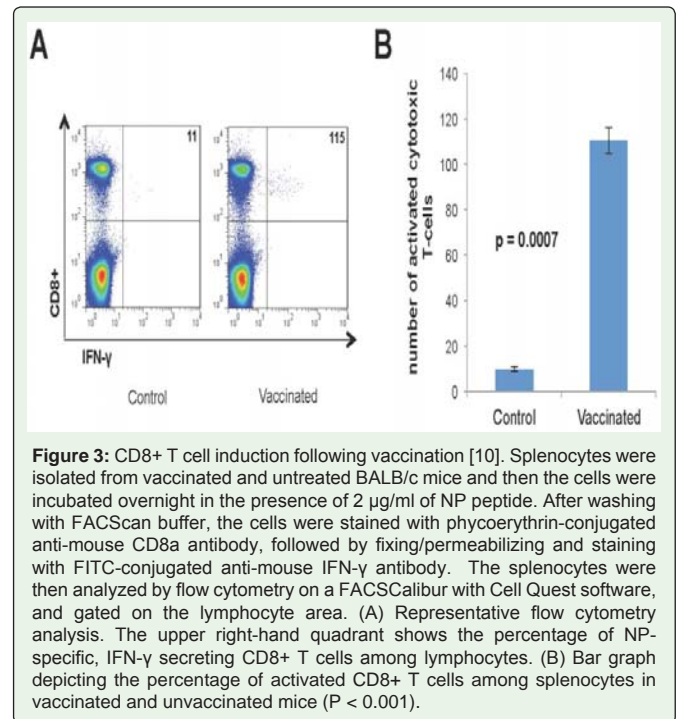


Figure 3: CD8+ T cell induction following vaccination [10]. Splenocytes were isolated from vaccinated and untreated BALB/c mice and then the cells were incubated overnight in the presence of 2 μ g/ml of NP peptide. After washing with FACSscan buffer, the cells were stained with phycoerythrin-conjugated anti-mouse CD8a antibody, followed by fixing/permeabilizing and staining with FITC-conjugated anti-mouse IFN- γ antibody. The splenocytes were then analyzed by flow cytometry on a FACSCalibur with Cell Quest software, and gated on the lymphocyte area. (A) Representative flow cytometry analysis. The upper right-hand quadrant shows the percentage of NP-specific, IFN- γ secreting CD8+ T cells among lymphocytes. (B) Bar graph depicting the percentage of activated CD8+ T cells among splenocytes in vaccinated and unvaccinated mice ($P < 0.001$).

of influenza-specific T cell responses in mice after vaccination. Such T cell responses can lead to cross-protection against other strains of influenza. Furthermore, as mentioned previously, no chemicals are added in the production process of vaccine by the USP laser irradiation. In these regards, the USP laser treatment is a novel method to generate inactivated vaccines that are safer, more potent, and broadly protective against varied strains of pathogens relative to conventional inactivation techniques. In the near future, the technology will be readily applicable to the breakthrough production of vaccines against existing pathogens such as Respiratory Syncytial Virus (RSV), Human Immunodeficiency Virus (HIV), Severe Acute Respiratory Syndrome (SARS) virus, West Nile Virus (WNV), Middle East Respiratory Syndrome (MERS) virus, avian flu virus, and possibly Tuberculosis (TB) and malaria as well, which hitherto have no available safe and effective vaccines.

The USP laser technology represents a new and unique method to protect against both existing and emerging pathogens. The research team welcomes inquiries and collaborative proposals to speed up the development of this potentially life-saving technology. The group can be contacted at tsen@asu.edu or tsens@wusm.wustl.edu.

References

1. Laser treatment 'could kill HIV'. 2007.
2. Visible light pulses knock out viruses in blood. 2007.
3. 10 High-Tech Health Breakthroughs Coming Soon to Your Body. 2009.
4. Zapping HIV with Lasers. 2008.
5. Shaw-Wei D Tsen, Travis Chapa, Wandy Beatty, Kong-Thon Tsen, Dong Yu, Samuel Achilefu. Inactivation of enveloped virus by laser-driven protein aggregation. *Journal of biomedical optics*. 2012; 17: 128002.
6. Shaw-Wei D Tsen, David H Kingsley, Christian Poweleit, Samuel Achilefu, Douglas S Soroka, Kong-Thon Tsen, et al. Studies of inactivation mechanism of non-enveloped icosahedral virus by a visible ultrashort pulsed laser. *Virology journal*. 2014; 11: 20.
7. KT Tsen, Shaw-Wei D Tsen, Q Fu, SM Lindsay, Li Zhe, Stephanie Cope, et al. Studies of inactivation of encephalomyocarditis virus, M13 bacteriophage, and *Salmonella typhimurium* by using a visible femtosecond laser: insight into the possible inactivation mechanisms. *Journal of biomedical optics*. 2011; 16: 078003.
8. Chieh-Han Lu, Kung-Hsuan Lin, Yung-Yuan Hsu, Kong-Thon Tsen, Yung-Shu Kuan. Inhibition of *Escherichia Coli* respiratory enzymes by short visible femtosecond laser irradiation. *Journal of Physics D: Applied Physics*. 2014; 47: 315402.
9. Shaw-Wei D Tsen, David H Kingsley, Karen Kibler, Bert Jacobs, Sara Sizemore, Sara M Vaiana, et al. Pathogen Reduction in Human Plasma using an Ultrashort Pulsed Laser. *PLOS one*. 2014; e111673.
10. Shaw-Wei David Tsen, Nisha Donthi, Victor La, Wen-Han Hsieh, Yen-Der Li, Jayne Knoff, et al. Chemical-free inactivated whole influenza virus vaccine prepared by ultrashort pulsed laser treatment. *J. Biomedical Optics*. 2015; 20: 051008.
11. Shaw-Wei D Tsen, Travis Chapa, Wandy Beatty, Baogang Xu, Kong-Thon Tsen, Samuel Achilefu. Ultrashort pulsed laser treatment inactivates viruses by inhibiting viral replication and transcription in the host nucleus. *Antiviral Research*. 2014; 110: 70-76.
12. Shaw-Wei D Tsen, Karen Kibler, Bert Jacobs, Justin C Fay, NP Podolnikova, TP Ugarova, et al. Selective photonic disinfection of cell culture using a visible ultrashort pulsed laser. *Biophotonics issue of Journal of Special Topics in Quantum Electronics*. 2016.