Plasma Medicine, 5(1): 49–55 (2015)

Inactivation of Hepatitis C Virus Cells Using Gliding Arc Discharge

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ABSTRACT: Hepatitis C virus (HCV) is a positive-strand RNA virus that causes severe liver diseases, such as cirrhosis and hepatocellular carcinoma. In this study, we investigated viral inactivation by DC and pulsed gliding arc discharge devices. Virus inactivation efficiency of HCV in human blood cells is about 67% within 2 min of the plasma exposure, using a non-equilibrium DC gliding arc discharge device.

KEY WORDS: hepatitis C virus, gliding arc discharge, inactivation effect, plasma exposure

I. INTRODUCTION

A promising advanced oxidation process for sterilization, called gliding arc discharge (GAD) plasma, has been used by researchers since Czernichowski[1] initially proposed the device for environmental control. The GAD device is a simple and inexpensive way to generate nonequilibrium plasmas. They are highly reactive and often have a high selectivity for chemical processes. The GAD is usually generated between two diverging electrodes, typically in a gas flow.[2] The discharge ignites at the shortest distance between the electrodes (1 mm). Typical breakdown voltages are 2–6 kV.[3] The formation of hot quasi-thermal plasma corresponds with a decrease in voltage and strong increase in current.[3] Owing to the gas flow, the discharge moves downward, and the length of the plasma column increases. This increasing length amplifies heat loss in the column, which exceeds the input energy of the power supply.[3] The quasi-thermal plasma converts into nonthermal plasma; this change corresponds to the decrease in current and the increase in voltage due to the increasing resistivity of the plasma. Eventually, the plasma extinguishes because the power supply cannot maintain such a long plasma column. At this point, plasma recombination starts and the discharge reignites at the minimum distance between the electrodes. This causes the self-pulsing nature of GADs, which is always clearly visible in current–voltage waveforms and typically occurs on a 10-ms timescale.[3,4]

Persistent infection with hepatitis C virus (HCV) is the predominant cause of chronic liver disease in the United States, and the World Health Organization recently estimated that 3% of the world's population has been infected with this pathogen.[5] The most effective therapy for HCV infection involves inhibiting an HCV-encoded enzyme.[6] However, the HCV genome rapidly acquires mutations that render the virus drug resistant because of the low fidelity of the viral replication machinery.[7] Thus, these inhibitors must be combined with interferon to significantly improve treatment outcome of HCV infection.

Because of the expense and severe side effects that accompany interferon treatment,[8] the search for new strategies to treat HCV infection is merited.

HCV is an enveloped RNA virus, which causes most non–hepatitis B virus infections that are transmitted parenterally (i.e., by injection, transfusion, or other contact with body fluids). HCV is a member of the Flaviviridae family[9–11] of viruses and is about 50 nm in diameter. The positive-sense RNA genome codes for production of a polyprotein; enzymes produced by the virus and the host cell then cleave the polyprotein into the smaller structural and nonstructural proteins that make up the mature virus particle. The structural proteins, which are incorporated into the viral envelope, consist of the core (nucleocapsid) protein and two envelope glycoproteins (E1 and E2).

The aim of this work is to examine the use of a GAD treatment system and the ability of pulsed plasma arc discharges to directly inactivate HCV.

A. Material and Methods

1. Characteristics of the gliding arc discharge (GAD)

The gliding arc experiment consists of two identical diverging electrodes made of copper, each with a length of 44 mm, a width of 26 mm, a thickness of 15 mm), arc (deviation of electrode) angle of 120º, and a gap between two electrodes of 1 mm. The electrodes are connected to a DC power supply (6 kV) via resistor (R = 200 kΩ) to avoid high current. The input voltage was controlled with a variac transformer. The flow rate of the nitrogen used for the experiment was maintained at 10–40 SCFH by the pressure regulator. Discharge voltage was controlled by the voltage slide regulator, and then high voltage was applied to the electrodes. Discharge current was measured using both a digital clamp meter (for digital value) and a Rogowski coil connected to an oscilloscope (to obtain the current oscillogram).

The temperature distribution of the DC gliding arc plasma in the axial direction was measured by a noncontact infrared thermometer. The gas temperature of the plasma at a site 1 cm from the opening of the device was only 300 K when the applied voltage was 6 kV and the nitrogen flow rate was 20 SCFH. Thus, the thermal effect of HCV cell inactivation appears to be rather insignificant.

The gap between the two electrodes is 1 mm. One electrode was connected to a DC power supply and the other was connected to ground. The gas was injected from a narrow tube placed in the narrow gap between the two electrodes. The gas flow can be controlled via flow meter. The two electrodes and the gas pipe were located inside a wood block. Figure 1 shows a photograph of the GAD and its electrical circuit.

A LeCroy 200 MS/sec four-channel digital storage oscilloscope (model 9304C) records voltage and current waveforms via a high-voltage probe and a pulse-current transformer, respectively. Figure 2 shows the current and voltage waveforms measured as a function of time at the maximum applied voltage of 6 kV.

Blood samples were collected from healthy donors at the blood bank; the amount of blood drawn was 5–7 mL in heparin tubes. The heparinized blood was divided into two groups; three samples were processed (n = 3) for each exposure time, with a total

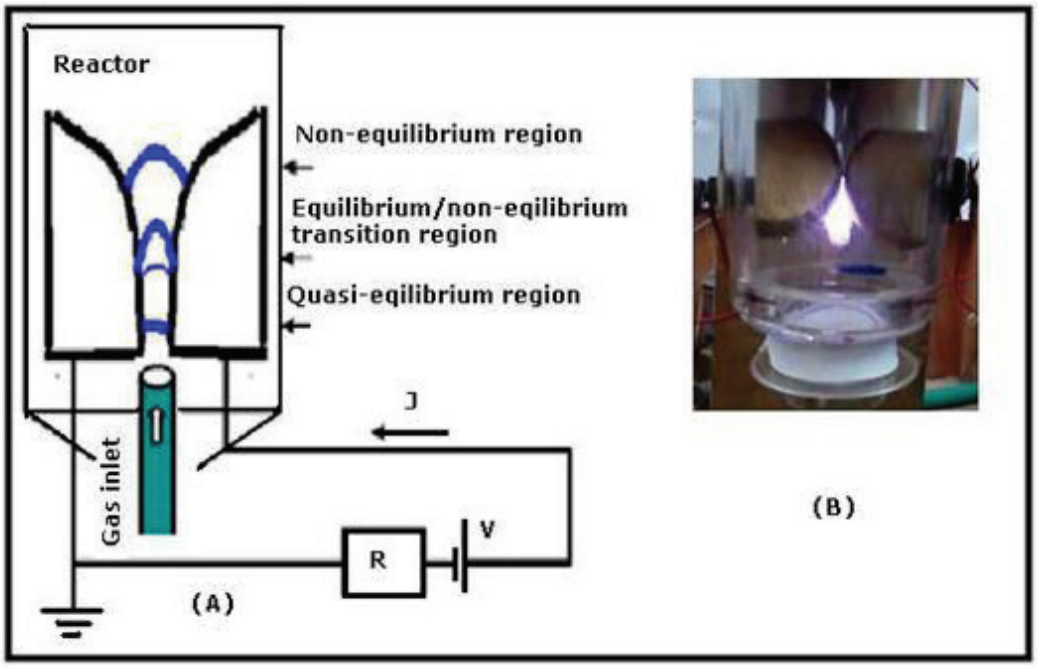

FIG. 1: **(a)** Schematic diagram of gliding arc discharge. **(b)** Image of gliding arc discharge with nitrogen gas

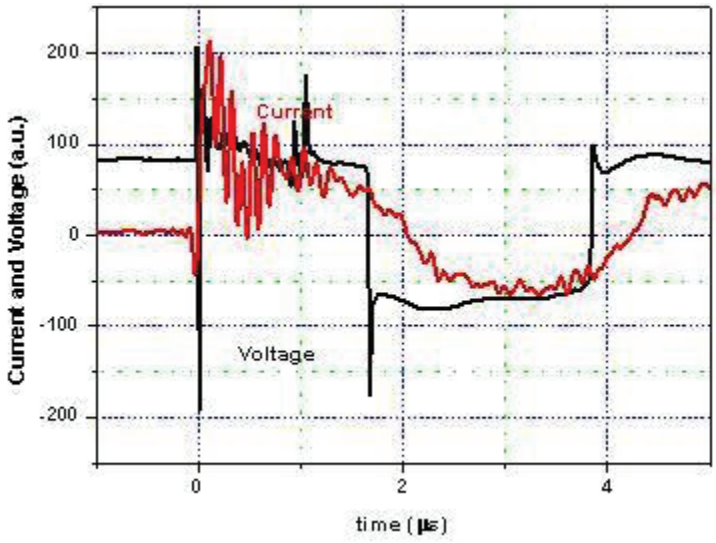

FIG. 2: Typical discharge current and the voltage waveforms

of 25 samples. The GAD plasma was applied directly to the heparinized blood samples (four groups) at a distance of 5 cm from the blood surface in each tube, at the four exposure periods selected for study (30 sec, 60 sec, 90 sec, and 120 sec). One unexposed sample was reserved to represent the control group. In these experiments, samples were examined to test the effect of GAD plasma on viral load by using real–time quantitative polymerase chain reaction (PCR) with a fluorescent reporter. A blood sample is taken and the amount of HCV RNA in 1 mL of blood is measured.

2. Results and Discussion

The mechanisms by which low-temperature atmospheric pressure plasmas affect viruses, bacteria, and eukaryotic cells are based on the synergy of several biologically active plasma components. The most important are plasma-generated ultraviolet (UV) radiation and reactive species (RS), including free radicals and some ground state molecules such as peroxides and ozone.[17] Under certain conditions, heat, charged particles, and metastable-state molecules and atoms produced by plasmas may also play important roles in the interaction between plasma and biological systems.

Burlica et al.[12] used a 250-mW pulsed GAD reactor to inactivate colonies of *Escherichia coli* grown on the surface of an agar substrate. Their reactor successfully reduced the surviving numbers of bacterial colonies by up to 4 logarithmic units (about 60.2%) after 2 min of treatment with either air- or argon-containing water spray that passed through the active plasma. They also showed that the plasma with water spray caused bacterial inactivation of one to two orders of magnitude beyond those of spraying H_2O_2 alone.

Kamgang-Youbi et al.[13] treated *Hafnia alvei* CIP 5731 bacteria with GAD plasma using humid air as the carrier gas. For a 2-min treatment, bacterial cultures were reduced by 3.7 decimal logarithm units (about 56.82%) in 20 min after application to cells. Abatement greater than 7 decimal logarithm units (about 84.51%) resulted from the same contact time with water activated with plasma for 10 min. They also proved that activated water treated with GAD plasma can also be used for sterilization.

GAD with a high-velocity carrier gas flows through the channel between the electrodes connecting to high voltage, and a plasma plume is generated. Due to the formation of chemically active species, including short-lived active species and long-lived active molecules (such as H_2O_2, ozone and other excited state neutral molecules),[12] the plasma regions have high reaction activity.[14] These species have relatively high oxidative power and can damage bacteria and viruses effectively.

Sterilization effect of gliding arc discharge is generally considered as a result of several factors, whereas all of them are acting essentially through oxidative mechanisms.[15] These species can enhance permeability or even partially destroy the outer membranes of the microorganisms, which leads to the effective protein denaturation, lipid peroxidation, as well as major changes in the cell cytoplasm and nucleus.[17,18]

As mentioned previously, HCV consist of the core (nucleocapsid) protein and single-stranded RNA. The plasma-generated reactive species thus first damage the core, which mainly consists of proteins. After etching away the protective protein layer, the reactive

species could directly act on the RNA of the virus, thereby destroying the HCV genetic material. Also, the UV radiation generated by the GAD has a lethal effect on bacteria and viruses.[19] The main mechanism of the dramatic reduction of HCV cells is related to either the damage to the virus core (nucleocapsid) protein, destruction of the RNA, or both. The second effect may be likely due to the structural damages (e.g., oxidation) to the HCV RNA during the plasma treatment.[20]

The results of the two groups of data (pulsed and DC, respectively) are shown in Figures 3 and 4. The inactivation efficiency of HCV by DC GAD is about 67%, and by pulsed GAD, it is 62%. So the sterilization ability of DC GAD plasma is higher than that of pulsed GAD for the active species that the GAD plasma generates.

Gliding arc dischage devices are expected to have quite similar effect on a range of other viral pathogens thereby significantly expanding the options and improving the therapeutic efficacy of pre-emptive anti-viral treatments. Virus inactivation using gliding arc discharge is rapid and effective, the inactivation efficiency of HCV was about 67%, and 62% by DC, and pulsed gliding arc discharge respectively.

In conclusion, successful HCV virus inactivation in cold atmospheric-pressure plasmas generated by gliding arc dischage device is demonstrated. More extensive study is needed for the possible use of gliding arc discharge device to improve the inactivation HCV virus.

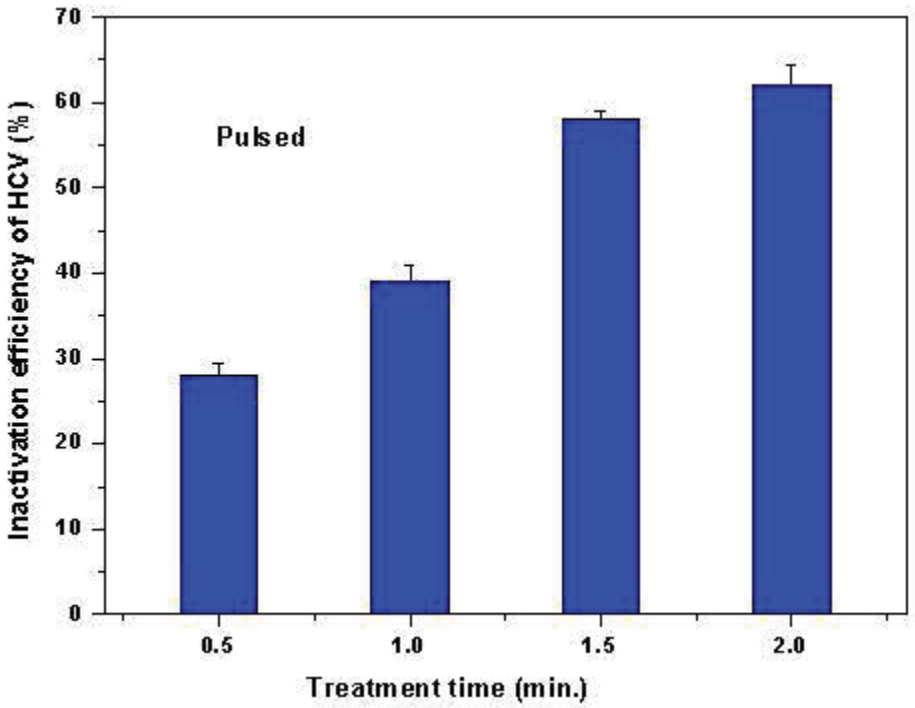

FIG. 3: Inactivation efficiency of HCV using pulsed gliding arc discharge

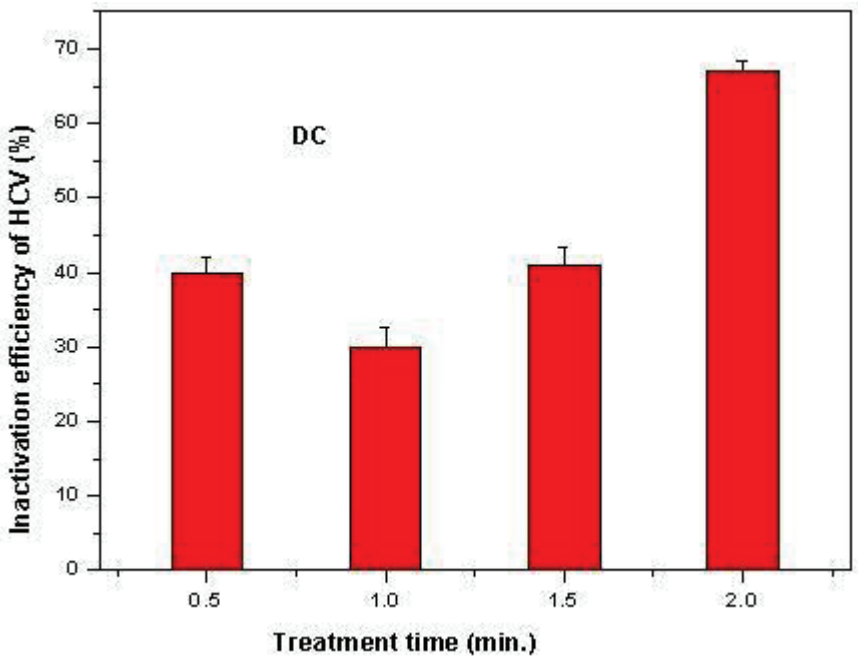

FIG. 4: Inactivation efficiency of HCV using DC gliding arc discharge

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