Study on the Antifungal Effect of Atmospheric-Pressure Microplasma Excited by High-Repetition-Rate Inductive Energy Storage

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Abstract—Atmospheric microplasma in a plane-parallel configuration was excited by high–repetition-rate inductive energy storage. We studied (1) the homogeneity of the microplasma device and (2) the inactivation efficacy for *Aspergillus brasiliensis* ATCC 9642 and *Aspergillus niger* NBRC 6341 when exposed to microplasma. Filamentary images during breakdown and afterglow were captured using a high-performance intensified CCD camera. Microorganisms of different species, *Aspergillus brasiliensis* ATCC 9642 and *Aspergillus niger* NBRC 6341, were used as biological indicators. Our work recorded inactivation time as a function of gas mixture. These experimental results suggest microplasma can strongly enhance antibiotic effects through UV radiation, a high electric field, and plasma radicals. Our experimental results have shown that plasma with a higher nitrogen ratio and a small percentage of oxygen has better inactivation properties for microorganisms with high UV tolerance. The small percentage of oxygen is needed for the excitation process and oxidation.

Keywords—Atmospheric plasma, microplasma, disinfection, *Aspergillus niger*, *Aspergillus brasiliensis*

I. INTRODUCTION

Maintaining homogeneous discharge at atmospheric pressure without using an inert gas such as helium is difficult. It is possible to achieve homogeneous breakdown by means of exciting the gas with the generation of plasma discharges that are limited to high-voltage short pulses of several hundred nanoseconds. This can also be used to prevent plasma from becoming arc discharge. The method reduces the chemical and physical effects of the plasma, however. Other than helium gas, plasma can also be generated using other type of inert gas, such as argon and reactive gases such as oxygen and nitrogen.

Microdischarge devices are characterized by plasma dimensions below 1 mm and operating pressures up to and exceeding 1 atm. Scaling down the size would lower energy and gas consumption, while operating at atmospheric pressure offers advantages in comparison to low-pressure plasma. These features appeal to the manufacturing industry. Just like plasma, microplasma can be characterized by reactive, irradiative, conductive, and dielectric properties. These features enable a wide variety of applications, including microbial inactivation, surface cleaning, surface coating, and gas treatment. In a microplasma environment, notably homogeneous discharge can be created at the expense of a reduced amount of helium or without helium insertion. Also, excitation can be realized at low frequency with a high-repetition pulse. More about microplasma and its application can be found here [1–6].

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In this work, microdischarge was generated by an inductive energy storage (IES) pulse generator. In contrast to conventional switches that are slow and unsuitable for short pulses of high current and to supply high voltage, the use of a static induction thyristor (SI-Thy) in an IES circuit has made it possible to generate a voltage pulse with an extremely high rising rate [7–9].

We studied the homogeneity of microdischarges generated by high-voltage-rise pulse power. The microplasma discharge was generated at 1 atm pressure using a DBD configuration and air as the working gas. The applied voltage originated from a power source that drove trains of pulse signals with a pulse width of 100 ns, providing approximately 20 kW of energy per pulse at a frequency of 500 Hz.

Studies on microbial inactivation using atmospheric-pressure plasma processes have received heightened interest in scientific research, and development. This is because plasma produces active species, including strong oxidizing agents, free radicals, and ultraviolet photons that have properties to inactivate microorganisms [10–20].

A common biological indicator used for evaluating plasma inactivation includes *Escherichia coli* (*E. coli*), *Bacillus subtilis*, *Geobacillus stearothermophilus*, etc. Few works have been dedicated to the inactivation of *Aspergillus niger*, a mold spore with high UV tolerance [6, 19–22].

The main mechanisms of inactivation by plasma are chemically reactive species, UV radiation, charged particles, and local heat, depending on the process gas used [13, 14, 18]. Each of the single mechanisms mentioned is germicidal, but they always occur in combinations in the gas plasma and can enforce the sterilizing effect synergistically. The main sterilizing agents in normal dielectric-barrier discharge plasma are UV radiation, reactive species, and charged particles,

TABLE I
BACTERIA TYPE AND DOSAGE REQUIRED FOR DISINFECTION

Bacteria		Ultraviolet dosage required for 99.9% destruction ($\mu Ws/cm^2$ at 254 nm)
Gram-negative strain	*Eschericha coli*	5.4
	Pseudomanonas aeruginosa	10.5
Gram-positive strain	*Staphylococcus aereus*	9.3
	Bacillus subtilis Sawamura (spores)	33.3
Mold	*Aspergillus niger*	264

*Ultraviolet sterilization system catalogue, Iwasaki Electric

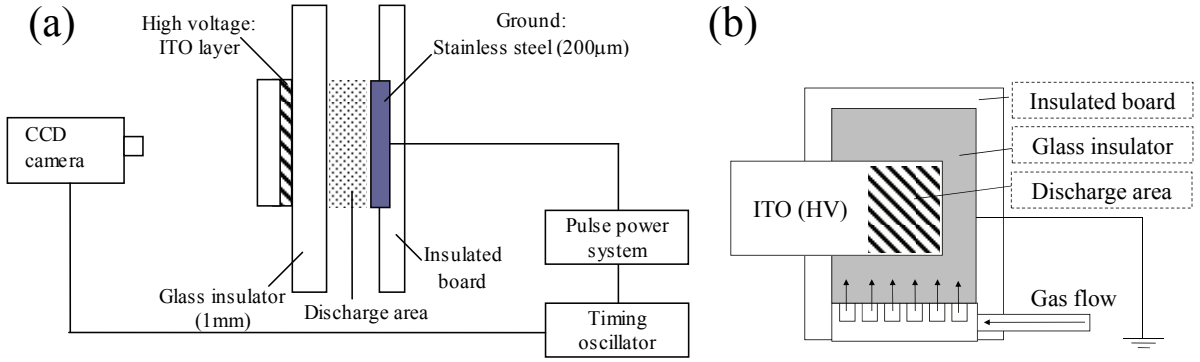

Fig. 1. Diagram of a microplasma discharge device.
(a) Diagram of the experiment, side view (b) structure of the microplasma discharge device.

which can be influenced by the power applied, the process gas and the flow rate.

UV radiation damages the genetic material of cells by causing lesions of the DNA. The dimerization of thymine bases in the DNA strands inhibits the bacteria's ability to replicate properly [13]. The absorption maximum of DNA is located at a wavelength of 260 nm. The dosage needed for inactivation varies by type of bacteria (Table 1). Furthermore, the effect of UV may differ with different species.

Further inactivation mechanisms are reactive species. In air, there are reactive oxygen and nitrogen species like O, O_3, OH, NO, and NO_2, which could affect membrane lipids and proteins of the cell [13].

Our work is a preliminary experiment on the inactivation efficacy for *Aspergillus brasiliensis* ATCC 9642 and *Aspergillus niger* NBRC 6341 using a microplasma apparatus.

II. EXPERIMENTAL PART

A. Observation of the homogeneity of microplasma

The experimental system for the homogeneity experiment is shown in Fig. 1(a). It consists of a high-performance intensified CCD camera (PI-MAX, Princeton Inst.), a microplasma device, and a pulse power unit. The microplasma device configuration consists of plane-parallel electrodes. The anode is transparent indium tin oxide (ITO) that is deposited onto a glass plate, and the cathode is a stainless steel sheet (SUS) of 200 μm thickness. The 1 mm glass insulator plate works as the dielectric barrier. The distance between the electrodes was varied at 600 μm and 1050 μm. The electrodes were driven with a pulsed high-voltage power supply of 6 kV and 8 kV. A high-voltage probe (P3000, Tektronix), a current-viewing probe (TCP312, Tektronix), and an amplifier (TCPA300, Tektronix) were used to obtain the applied voltage, the discharge current and to synchronize with the camera trigger.

Filamentary microplasma images were taken at 100 ns intervals for evaluation of homogeneity. The images of discharges were produced in an area of 11 mm × 12 mm. Experiments were conducted for three conditions: (i) 600 μm gap distance and 6 kV voltage; (ii) 1050 μm gap distance and 6 kV voltage; and (iii) 1050 μm gap distance and 8 kV voltage. The homogeneous properties of discharges were observed.

B. Inactivation of biological indicator

Our work is a preliminary experiment on the inactivation efficacy for *Aspergillus brasiliensis* ATCC 9642 and *Aspergillus niger* NBRC 6341. For the purpose of inactivation, microplasma discharges were ignited using mixtures of helium, nitrogen and oxygen. These

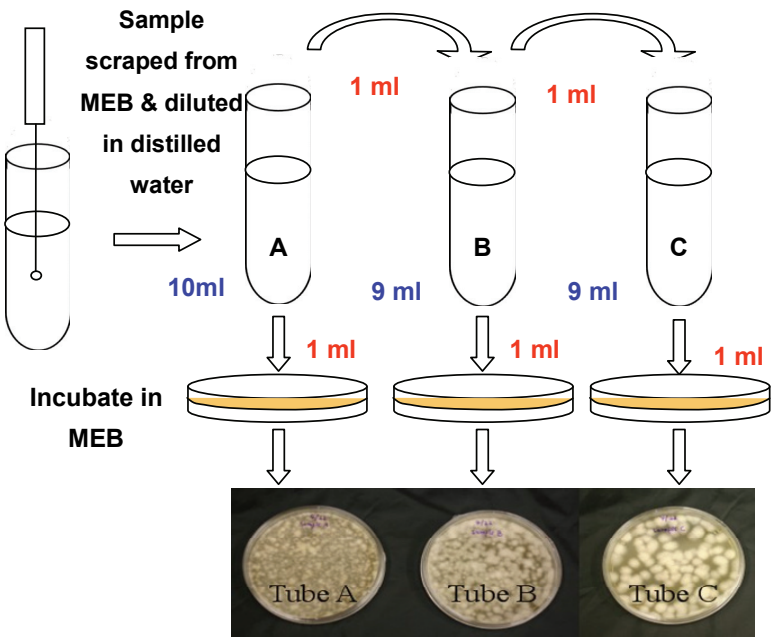

Fig. 2. Preparation of biological indicator.

gases are excellent sources of reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as atomic oxygen (O), ozone (O_3), hydroxyl (OH), NO, NO_2, etc. [13]. As the reactive species mentioned above have inactivation effects on the cells of microorganisms, we recorded inactivation time as a function of gas mixtures.

The density of the indicator was adjusted in the following way (see Fig. 2). The microorganisms were incubated in malt extract broth (MEB) agar culture. A serial dilution of 10 mL of distilled water was prepared in test tube A, and 9 mL in test tubes B and C. Spores were scraped from incubated sample using a wire and then diluted in test tube A. An amount of 1 mL was taken from test tube A and diluted in test tube B. Then, from test tube B, another 1 mL was taken and diluted in test tube C.

After that, from test tube C, 1 mL of the suspension was taken and cultured on MEB culture medium. After 48 hours, approximately 103 colony forming units (CFU) were formed in a Petri dish that originated from test tube C. Fig. 2 shows cultured samples of the 1 mL suspension taken from test tubes A, B, and C. Samples cultured from tube A are the densest followed by tube B and tube C. CFU in tube A and B were so dense that it is impossible to see the boundary between the colonies moreover count the CFUs.

We only counted CFU inoculated from test tube C because samples from test tubes A and B were too dense. For confirmation, the experiment was repeated 4 times. Again, we only counted CFU cultured from test tube C. Results indicate that between 1 and 2 × 10^2 CFU/mL were cultured.

One CFU with MEB underneath was cut and applied to a sterilized cover glass, 18 mm × 18 mm in size and 150 μm in thickness. The sample was placed on a dielectric barrier covering cathode for sterilization treatment. It is to be noted that the test samples were colony forming units with nutrients inside the agar underneath them with sizes of approximately 10 mm × 10 mm.

The microplasma device from the homogeneity experiment was improved for the inactivation process experiment in different gases. A nozzle for gas flow was added, and gap distance was increased to 10 mm to accommodate the test sample. This made the gap distance for discharge less than 1 mm. A schematic diagram of the microplasma device for the inactivation process is shown in Fig. 1(b).

A 200 μm-thick nickel was used as the cathode electrode. At the cathode, a dielectric barrier in the form of a glass insulator, 150 μm in thickness, was used. The biological indicator was inoculated over a thin solid agar layer on the glass plate described above. The glass plate worked as both carrier, and cathode insulator. A working gas mixture of helium, nitrogen and oxygen was supplied from an array of apertures of 1.5 mm in diameter. Gas mixture and flow were controlled with a mass flow controller. Plasma was supplied with a series of short pulses with a width of 100 ns and at a repetition rate of 500 Hz. The pulse signal had a maximum voltage of 8 kV, a maximum current of 2 A, and a maximum power of 20 kW. Gas mixtures and treatment times for microbial inactivation were recorded.

After microplasma treatment, the glass with the test samples on it was immersed in MEB culture medium. The samples were then incubated at 28 ^{o}C. Inactivation evaluation was based on observation of fungal growth in the culture medium after 7 days. Disinfection was achieved when no sign of propagation was observed.

III. Experimental Results

A. Homogeneity

Fig. 3 shows the trigger-voltage-current-power characteristics of the atmospheric air microplasma. Pulse train width was 100 ns. Maximum voltage was 6 kV. Maximum current was 4.4 A. Maximum power was 24 kW. It is to be noted that the pulse at maximum current may be shorter than shown because of the latest measuring equipment for current has a minimum measuring capacity of 12 ns.

Using the microplasma antifungal apparatus (Fig. 1(b)) in He gas, filamentary images during breakdown were observed, as shown in Fig. 4. The images show that

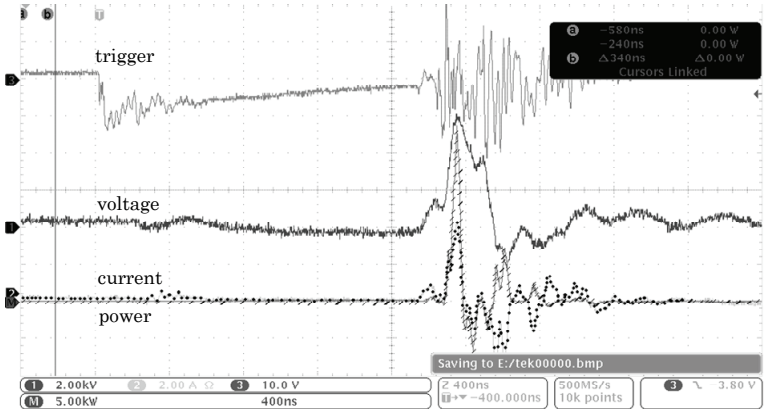

Fig. 3. Trigger-voltage-current-power characteristics of atmospheric-air microplasma.
first line: trigger signal (V); second line: voltage (kV); filled symbol (●): current (A); slash symbol (/): power (kW); Y-axis: time (40ns)

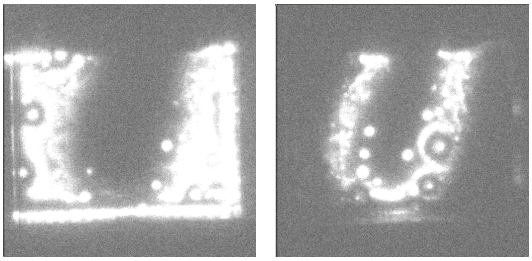

Fig. 4. Sequential frame images during breakdown phase at 30 ns intervals.

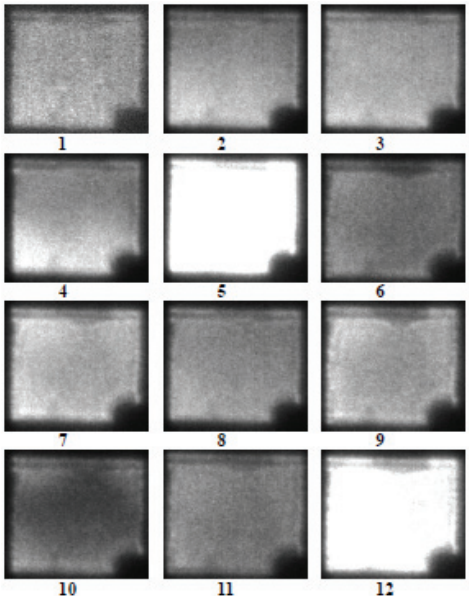

Fig. 5. Images of homogeneity during condition (i) 600 μm gap distance and 6 kV voltage. Numbers indicate the consecutive order of time after the camera was triggered.

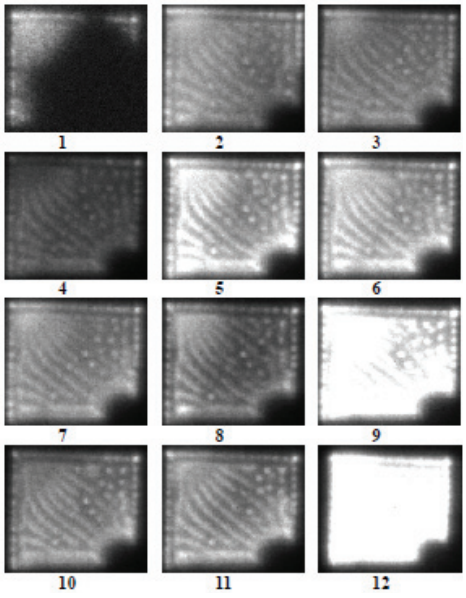

Fig. 6. Images of homogeneity during condition (ii) 1050 μm gap distance and 6 kV voltage. Numbers indicate the consecutive order of time after the camera was triggered.

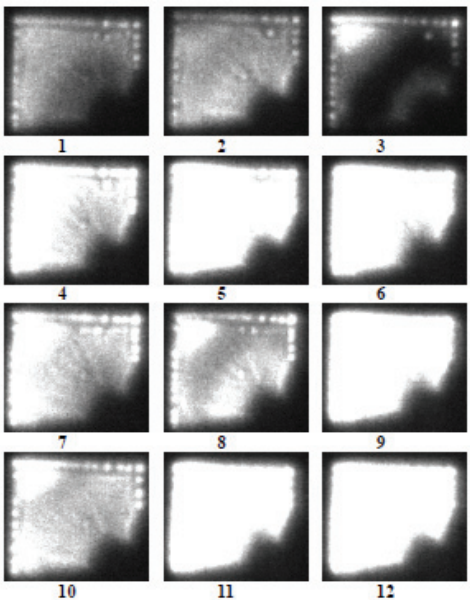

Fig. 7. Images of homogeneity during condition (ii) 1050 μm gap distance and 8 kV voltage. Numbers indicate the consecutive order of time after the camera was triggered.

plasma starts at the side of the electrode moving inwards in an avalanche-like movement. The experiment was repeated three times and the results were identical.

The filamentary images in Figs. 5, 6, and 7 were afterglow plasma observed in the microplasma homogeneity apparatus 10 μs after breakdown. Digital images for conditions (i), (ii), and (iii) are shown in Figs. 5, 6, and 7 respectively. Each image is taken consecutively every 100 ns. The numbers beneath each image in Figs. 5, 6, and 7 indicate the consecutive order of time after the camera was triggered. The camera trigger was set 10 μs after the breakdown.

Fig. 5 shows digital images during condition (i), where gap distance is 600 μm and voltage is 6 kV. Fig. 6 shows digital images during condition (ii), where gap distance is 1050 μm and voltage is 6 kV. Fig. 7 shows digital images during condition (iii), where gap distance is 1050 μm and voltage is 8 kV.

Diagonal bright stripes were observed during condition (ii) and (iii). The stripes became more distinctive as the applied voltage increased. From observation it is suggested that discharges were not stationary. At this point the direction of movement, which is either moving from the lower right corner toward the upper left corner or the opposite, has not yet been confirmed.

From sequential images, we think that the distinctive diagonal lines were not caused by the glass barrier configuration. We observed changing positions of the lines, suggesting they were not static and that there was line movement. There is also the possibility that the effect of air flow caused this phenomenon. Verification of air flow as the cause of this phenomenon was done by inserting helium gas to flow into the discharge unit. The device was in the same configuration as the one used in the experiment on microbial inactivation.

TABLE II
INACTIVATION RESULTS FOR *ASPERGILLUS BRASILIENSIS* ATCC 9632 (HE 200 ML/MIN)

Symbols: (-) inactivation failed; (+) inactivation achieved

Flow rate (ml/min)		Exposure time (min)					
nitrogen	oxygen	0	1	2	3	4	5
70	30	-	-	-	-	+	+
80	20	-	-	-	-	+	+
90	10	-	-	-	+	+	+

TABLE III
INACTIVATION RESULTS FOR *ASPERGILLUS NIGER* NBRC 6341 (HE 200 ML/MIN)

Symbols: (-) inactivation failed; (+) inactivation achieved

Flow rate (ml/min)		Exposure time (min)			
nitrogen	oxygen	0	10	20	30
70	30	-	-	-	-
80	20	-	-	-	-
90	10	-	-	-	+
180	20	-	-	+	+

B. Inactivation of biological indicator

Test for *Aspergillus* fungal inactivation were carried out. Colonies from *Aspergillus brasiliensis* ATCC 9642 and *Aspergillus niger* NBRC 6341 were tested separately. Test samples were put onto glass of 150 μm thickness and then were irradiated with microplasma. Helium gas flow was fixed at 200 mL/min. The gas flow of oxygen and nitrogen was varied. After irradiation, the samples were incubated in MEB, and survival after 7 days was recorded.

Tables 2 and 3 show the results of inactivation for *Aspergillus brasiliensis* ATCC 9642 and *Aspergillus niger* NBRC 6341. Samples that showed no propagation (inactivation achieved) were marked with a positive (+) symbol. Samples that survived (inactivation failed) were marked with a negative (-) symbol.

Aspergillus brasiliensis ATCC 9642 required exposure for 4 minutes for inactivation. Conditions with higher nitrogen flow showed the shortest exposure time at 3 minutes. *Aspergillus niger* NBRC 6341 required exposure for 30 minutes for inactivation only when nitrogen gas flow was at the highest. When 190 mL/min nitrogen and 20 mL/min oxygen were supplied, the inactivation effect was observed after 20 minutes of exposure.

IV. DISCUSSION

In this work, we have shown homogeneity in filamentary microplasma at 600 μm gap distance when 6 kV was applied to the electrodes. Increasing the gap distance and applied voltage resulted in striation. The

striation became more distinctive as gap distance increased. Microplasma intensity also increased when supplied voltage was increased.

Helium was used because its low breakdown voltage has a principal effect of exciting plasma at atmospheric pressure. The energy transfers from excitation by high energy metastable helium atoms are responsible for the expansion of the microdischarges and excitation of oxygen, nitrogen mixed molecules, and free radicals. It is reported that the synergistic effect of plasma that contributes to inactivation efficacy and the debate on which plays the main role is still under debate, as in the reference to sterilization mechanisms and synergistic effects in, Moisan *et al.* [14]. However, these experimental results suggest, microplasma can strongly enhance these triple effects—UV radiation, high electric field, and plasma radicals—when the discharge is excited by microplasma electrodes. Other findings indicate that higher concentrations of oxygen-based reactive species enhance the ability of cold plasmas to inactivate bacteria [14, 20, 25].

The excitation of nitrogen gas produces high atomic energy in the N atom, N_2^+ ions, NO radicals, and also N_3^- (azide). Excited N_2^+ and NO radicals produce UV-C, which is a known disinfectant. Azide inactivates microorganisms by binding with enzymes that inhibit the enzyme from its function. A high electric field causes ion migration in the microorganism membrane, thus resulting in rupture and death. All of the above synergistic reactions contribute to the inactivation of *Aspergillus brasiliensis* ATCC 9642 and *Aspergillus niger* NBRC 6341.

Comparing the effectiveness of microplasma inactivation between *Aspergillus brasiliensis* ATCC 9642 and *Aspergillus niger* NBRC 6341, *Aspergillus brasiliensis* ATCC 9642 needed only 3 minutes to achieve complete inactivation. Depending on the working gas mixtures, *Aspergillus niger* NBRC 6341 needed at least 20 minutes to achieve complete inactivation.

Our experimental results have shown that plasmas with higher nitrogen ratio have better inactivation properties for microorganisms with high UV tolerance, although a small percentage of oxygen is still needed for excitation process and oxidation.

V. Conclusion

New findings in this experiment are

(1) Previously assumed same species of *Aspergillus brasiliensis* ATCC 9642 and *Aspergillus niger* NBRC 6341 have different inactivation properties. This work shows experimental method of stress free for bioburden testing.

(2) Present understanding of low frequency excitation of microplasma is that the filamentary discharge movement is randomly flowing at the axis. Our work observed that nanosecond pulsed discharge starts at the side of the electrode moving inwards in an avalanche-like movement during breakdown and homogeneously during afterglow.

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