Generator and Setup for Emulating Exposures of Biological Samples to Lightning Strokes

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***Abstract*—*Goal:* We aimed to develop a system for controlled exposure of biological samples to conditions they experience when lightning strikes their habitats. *Methods:* We based the generator on a capacitor charged via a bridge rectifier and a dc–dc converter, and discharged via a relay, delivering arcs similar to natural lightning strokes in electric current waveform and similarly accompanied by acoustic shock waves. We coupled the generator to our exposure chamber described previously, measured electrical and acoustic properties of arc discharges delivered, and assessed their ability to inactivate bacterial spores. *Results:* Submicrosecond discharges descended vertically from the conical emitting electrode across the air gap, entering the sample centrally and dissipating radially toward the ring-shaped receiving electrode. In contrast, longer discharges tended to short-circuit the electrodes. Recording at 341 000 FPS with Vision Research Phantom v2010 camera revealed that initial arc descent was still vertical, but became accompanied by arcs leaning increasingly sideways; after 8–12 μs, as the first of these arcs formed direct contact with the receiving electrode, it evolved into a channel of plasmified air and short-circuited the electrodes. We eliminated this artefact by incorporating an insulating cylinder concentrically between the electrodes, precluding short-circuiting between them. While bacterial spores are highly resistant to electric pulses delivered through direct contact, we showed that with arc discharges accompanied by an acoustic shock wave, spore inactivation is readily obtained. *Conclusion:* The presented system allows scientific investigation of effects of arc discharges on biological samples. *Significance*: This system will allow realistic experimental studies of lightning-triggered horizontal gene transfer and assessment of its role in evolution.**

***Index Terms*—Electroporation, electrotransformation, evolution, exposure system, horizontal gene transfer (HGT), lightning.**

I. INTRODUCTION

NATURAL lightning strokes are unpredictable, uncontrollable, and impossible to ignore; they turn sand into glassy mineral tubes (fulgurites), damage electrical equipment, and harm living organisms.

A number of emulators and standards have been developed for testing the robustness of devices that can get exposed to lightning strokes in their operating environment [1]–[3]. Realistic testing of such robustness is particularly important in aircraft industry, and modern lightning emulators designed for such testing can reproduce the electric currents of natural lightning strokes, with peak amplitudes of up to 200 kA, and with waveforms emulating those in lightning strokes: zero-to-peak time of several microseconds and decay time in tens to hundreds of microseconds [4], [5]. This allows us to emulate the effect of the stroke current proceeding in its entirety through the device, which has to withstand this without any loss of functionality.

While lightning also strikes natural habitats of many living organisms, scientific interest in studying the effects of such exposures has until recently been scarce. A likely contributor to this was the prevailing view that a stroke can only harm the organisms, and the mechanisms of inflicted damage—thermal (degradation of biomolecules), electrical (electroporation of cells' membranes and resulting leakage of intracellular material), and mechanical (pressure shock due to the strong acoustic waves accompanying a stroke)—are generally rather well understood.

Cell membrane electroporation caused by high-voltage short-duration electric pulses can be irreversible, killing the cells, but it can also be reversible, with the exposed cells remaining viable, which is increasingly exploited to introduce into living cells various active substances that cannot permeate an intact membrane, particularly anticancer drugs and genetic material [6], [7]. Taking the latter fact into account, there were several studies focusing on—or at least touching upon—the possibility of electroporation-based gene transfer triggered naturally by lightning strokes [8]–[10]. Still, the exposures used in these experiments were produced by electric pulse generators in which neither the current's peak nor its time course parameters (zero-to-peak time, peak-to-half time) were similar to those of lightning strokes. Moreover, the current was always delivered through electrodes in direct contact with the sample, thus lacking the acoustic shock wave characteristic of strokes and more generally of electric arcs.

In the last two decades, sequencing of genomes gradually started revealing that biological evolution, particularly in unicellular organisms, is influenced significantly by horizontal gene transfer (HGT)—uptake of genetic material from their environment or other organisms, and its integration into the new host's genome [11]. The well-recognized biochemical mechanism of uptake from the environment is termed natural competence (or natural transformation), and it was recently demonstrated that

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this mechanism functions even with highly fragmented and damaged DNA [12]. Still, as natural competence is based on a complex system of proteins [13], it must itself have developed during a certain stage of evolution, and how DNA uptake from the environment could have proceeded before this is an open question.

Furthermore, natural competence is found in some bacteria and in rare archaea [14], but there are no known naturally competent eukaryotes (organisms whose cells' genetic material is contained in a nucleus), yet during the last five years the scientists are increasingly acknowledging the evolutionary importance of HGT also in eukaryotes [15]. The currently recognized mechanisms of HGT in eukaryotes are gene transfer from their intracellular symbionts [16] and from infecting viruses [17], but it has been demonstrated that the most important genes transferred into eukaryotes by HGT stem from archaea [18], and whether these can all be attributed to transfer from endosymbionts (which are overwhelmingly bacteria, not archaea) or viruses is highly questionable.

We have recently posited that a possible answer to both early-life and modern gene transfer into organisms lacking natural competence lies in physical (as opposed to biochemical) mechanisms of DNA release and uptake, which could have functioned from the very beginning of cell-based life, and which also function in eukaryotes. We have argued that a particularly promising such mechanism, with both theoretical and indirect empirical support, is lightning-triggered HGT—DNA transfer enabled by lighting-induced cell membrane electroporation, and possibly augmented by lightning-driven electrophoretic motion of DNA [19].

For realistic experimental studies of the feasibility of lightning-triggered HGT, the conditions have to be as close to natural ones as possible. Organisms exposed should be chosen among those living in habitats accessible to lightning strokes, the lab exposure environment should emulate that habitat (e.g., marine bacteria should be exposed in a medium resembling seawater), and only natural DNA should be used (in biotechnological applications, DNA molecules are often artificially modified to increase their stability and transferability). At least as important is a realistic emulation of the lightning stroke; the current should be delivered through an actual electric arc proceeding from the emitting electrode through air into the sample, and the time course of the current should resemble that of a typical lightning stroke.

With tests of aircraft robustness, where the whole lightning stroke current can proceed through one of its devices, both the current amplitude and its waveform delivered in testing have to be equal to those of actual strokes. Partly in contrast, in exposure systems designed for studying lightning-triggered HGT, the electric current waveform should also equal to that of a stroke with respect to the time scale (i.e., it should have similar zero-to-peak and decay times), yet its amplitude can be downscaled without any essential loss of emulation consistency. Namely, upon its entry from the air into the ground, the lightning stroke's current always dissipates, and thus, a downscaling of current amplitude in exposure systems merely reduces the size of concentric areas subjected to various ranges of current density and resulting effects on exposed sample. Obviously, the very highest current densities of lightning strokes are absent in downscaled exposure systems, but those are lethal to all living organisms, while the conditions of reversible electroporation, and thus favourable for HGT, are reached even with downscaling of the stroke current by a factor of 10^4 [20].

We have previously reported on our design of an exposure system that provides easy sample insertion and removal, protection from airborne particles, observability during the exposure, accurate discharge positioning, and delivery into the sample through an arc with an adjustable air gap [21]. In its initial testing, we connected the system to a taser gun confirming that for discharges lasting $\sim$0.5 μs, the conical emitting electrode delivers the current across an air gap centrally downward into a disk-shaped sample, and that the ring-shaped receiving electrode ensures radial current dissipation through the sample [21]. Here, we describe our design and testing of a generator delivering discharges similar to those of natural strokes both in its duration and its waveform of the electric current (zero-to-peak time of $\sim$5 μs, peak-to-half time of $\sim$75 μs), and also similarly accompanied by an acoustic shock wave.

We first describe the design of the generator circuit for delivery of arc discharges. We then show how with the exposure system outlined above a transition from short discharges (up to several microseconds) to those with durations characteristic of lightning strokes (tens or hundreds of microseconds) alters the current flow radically, with eventual short-circuiting between the electrodes proceeding through the air plasmified by the discharge current. We present the details of this artefact through photographs taken at 341 thousand frames per second with Vision Research Phantom v2010, one of the fastest currently commercially available cameras, and we describe a modification of the exposure system that eliminates this artefact. We tested our generator and setup by exposing the spores of *Bacillus pumilus* to the discharges, attaining consistent and reproducible irreversible electroporation of spores; in contrast, no such effect was obtained with classical-type exposure in which the discharges were delivered through electrodes in direct contact with the sample so that an arc and the accompanying acoustic shock wave were absent.

II. MATERIALS AND METHODS

A. Generator Design and Functioning

The schematic diagram of the pulse generator we developed is shown in Fig. 1. We opted for a capacitor discharge circuit [22] as the waveform of its output current is very similar to those of lightning strokes [20]. For safety reasons and to minimize leakage current, we isolated the output of the generator from the line voltage by an isolation transformer (T; 18 V, 60 VA, ELMA, Slovenia). A high-voltage direct current converter (HV dc–dc; RB60-6P, Matsusada, Japan) was used to supply the output stage of the generator. The output voltage of the converter was set by a potentiometer (P) in the range from 0 to 5 kV, and monitored by a voltmeter (V). We used two 200-kΩ resistors (R) to separate the converter from the output. The 1-μF capacitor (C; MOP105-5MN, Condenser Products, USA) stored the

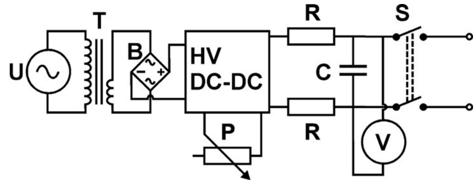

Fig. 1. Schematic diagram of the pulse generator for lightning emulation. U: grid power supply. T: isolation transformer. B: bridge rectifier. HV dc–dc: high-voltage dc–dc converter. P: linear potentiometer. R: resistors (200 kΩ each). C: capacitor (1 μF, 5 kV). S: high-voltage relay. V: digital voltmeter.

energy for the pulse during the charging phase, and the high-voltage relay (S; RL 42, SPS electronic, Germany) released this energy into the load during discharge phase. As the impedance of the exposure system was approximately 100 Ω, the 1-μF capacitance of the capacitor C was chosen so that its discharge generated an arc current with a time constant of exponential decay of $\sim$100 μs, similar to those of natural lightning strokes.

During the first batch of our experiments, the discharge generator was connected to the exposure system as described previously [21]. Later, as we discovered that discharges longer than 8–12 μs gradually deviate from a vertical path into a diagonal one and short-circuit the electrodes (see Section III-A), we modified the setup by enclosing the emitting electrode with an inner concentric Plexiglas cylinder reaching down to the sample surface, thus forcing the discharge current to flow through the sample and thus avoiding the short-circuiting.

Capacitor C was charged to 5 kV and discharged through the high-voltage relay S, proceeding from the conical emitting electrode vertically downward into the sample, and hence, horizontally radially through the sample into the ring-shaped receiving electrode, thus emulating the exposure of the sample to a lightning stroke.

B. Monitoring and Measurements

Evolution of the discharge arc was imaged by a high-speed camera (Phantom v2010, Vision Research, USA) with fixed focal length lens (EF 50 mm f/1.8, Canon, Japan), either with or without a welding protection glass (90 × 110 mm Shade 11, Technolit, Germany) placed between the arc and the camera lens. During the experiment, the image frames were being acquired continuously by the high-speed camera with 256×128 resolution and 12 bits per pixel at 341 000 frames per second (2.93 μs per frame, consisting of 2.55-μs-exposure time and 0.38-μs-interframe delay). Acquisition was triggered by the oscilloscope as it detected the onset of the electric current, with the first frame marked as time zero (0 μs).

Time courses of voltage and electric current of the discharge were measured by a voltage probe, a current probe, and an oscilloscope (PPE6kV, AP015, and WavePro 7300A; all from LeCroy, USA) isolated from the line voltage by an isolation transformer KDVP-23499 (Elma TT, Slovenia). In cases of short-circuiting between the electrodes, current was measured by CWT 150B Mini current probe (Powertek, U.K.).

Acoustic shock wave was measured with a sound pressure level (SPL) meter with an attenuator (2230 and ZF 0020; both from Brüel & Kjær, Denmark), and by a microphone with a preamplifier and an amplifier (4191, 2669, and Nexus; all from Brüel & Kjær, Denmark), and a sound card (E-MU 0404 USB, Creative, USA). Measurement system was calibrated with a pistophone (124 dB). The measurements were taken at 1-m distance from the exposure system, both with and without the outer Plexiglas cylinder enclosing the exposure system, and both with and without the inner Plexiglas cylinder used to prevent short-circuiting between the electrodes. The frequency spectrum of the acoustic shock wave was computed using the fast Fourier transform in MATLAB R2014 (MathWorks, Natick, USA).

C. Experiments

Sporulation of *Bacillus pumilus* (ATCC 27142) was performed for five days at 37 °C in Difco sporulation medium [23]. Residual vegetative bacteria were removed by heat shock (80 °C for 20 min) and lysozyme digestion (50 μg/ml of lysozyme in 50-mM Tris-HCl at pH 6.2; for 1 h at 37 °C). Spores were purified by centrifugation (5 min, 10 000 g) and cleaning (by 0.05% SDS, then three times by deionized water), and spread onto agar on 90-mm petri dishes. Inactivation was performed by delivering an arc discharge centrally into the petri dish. Inactivation rate was evaluated by comparison between the number of colonies in the area of interest before and after exposure to electric arcs.

III. RESULTS AND DISCUSSION

A. Design and Testing of the Generator and Setup

The most challenging choice we had to make in the design of the generator was that of the most appropriate output switch. The options considered were an insulated-gate bipolar transistor (IGBT), a spark gap, and a relay [24], [25]. All these options are commercially available for 5 kV as the maximum output voltage we opted for, but IGBTs are rather limited in their maximal current and their rise times, prone to irreparable damage, and furthermore, they are the most expensive of the considered options. We, thus, opted for a commercial relay, with the air gap between the emitting electrode and the sample in our exposure system forming the spark gap to which this relay delivers a discharge.

In our initial tests, we connected the generator shown in Fig. 1 to the exposure chamber that we have previously described and tested with short discharges ($\sim$0.5 μs) delivered by a taser gun [21]. For such short discharges, the arc of the discharge always descends roughly vertically from the tip of the conical emitting electrode into the sample, and then dissipates through the sample radially toward the ring-shaped receiving electrode (see e.g., [21, Figs. 3(a) and 4(a)]).

In contrast, as shown in Fig. 2, when we delivered a discharge designed to decay exponentially with a time constant of $\sim$100 μs, during the first $\sim$0.5 μs the discharge and its arc behaved as predicted, with the electric current rising to $\sim$50 A and voltage gradually decreasing from the initial $\sim$5 kV, but then the current kept rising, first at a decreasing rate, yet after $\sim$8 μs increasing very rapidly to almost 1400 A, thus almost

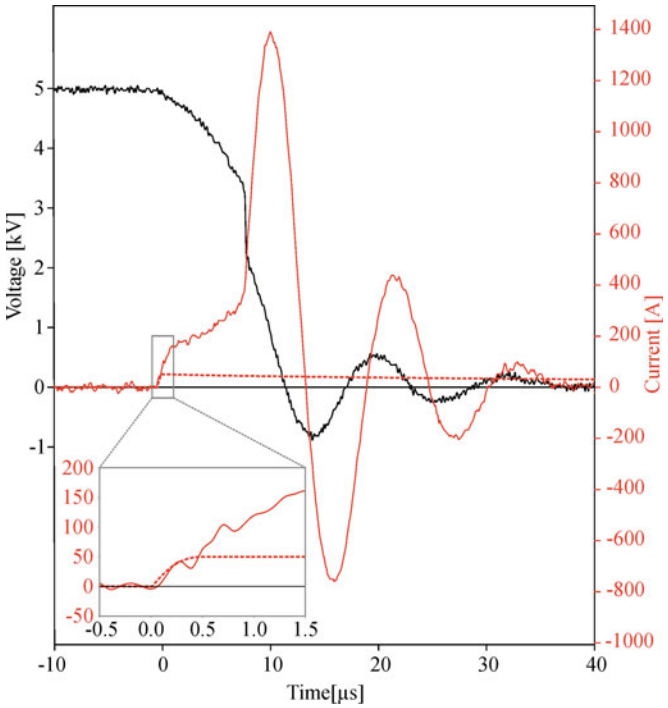

Fig. 2. Time course of voltage (black) and current (red) of the discharge delivered by the generator shown in Fig. 1 connected to our exposure chamber described and illustrated in detail in [21]. The dashed curve sketches the time course of the electric current expected of a discharge without short-circuiting between the electrodes (see Fig. 5).

instantaneously discharging the capacitor, followed by further $\sim$35 μs of strong current and voltage oscillations, likely attributable to the inductance of the wires connected to the generator's rapidly discharging capacitor.

Visually, we observed a very bright arc formed along the shortest path between the tip of the conical emitting electrode and the ring-shaped receiving electrode, proceeding entirely through plasmified air, thus short-circuiting the electrodes and evading the sample. Still, it was clear that due to the extreme brightness of this arc, the transient phenomenon preceding this short-circuiting was concealed from the naked eye. To investigate this transient phenomenon, we utilized the Vision Research Phantom v2010, the fastest currently commercially available camera kindly provided by Vision Research Europe, and recorded the events at 341 000 FPS (one frame per 2.93 μs), with the relevant frames shown in Fig. 3.

As Fig. 3 shows, the arc first descended vertically into the sample. Within $\sim$3 μs, the vertical arc became accompanied by several radial arcs proceeding partly through the sample, but gradually ascending into the plasmified air above it. After $\sim$9 μs (in this case, and generally 8–12 μs), a significant initial segment of the radial arcs was already above the sample, and their visible endpoints, still in the sample, were rapidly approaching the receiving electrode at sample's outer edge. As one of these arcs came into direct contact with the receiving electrode, it transformed into a channel of plasmified air short-circuiting the electrodes, drawing almost all current of the discharge through this channel and thus enhancing its plasmification, glowing so

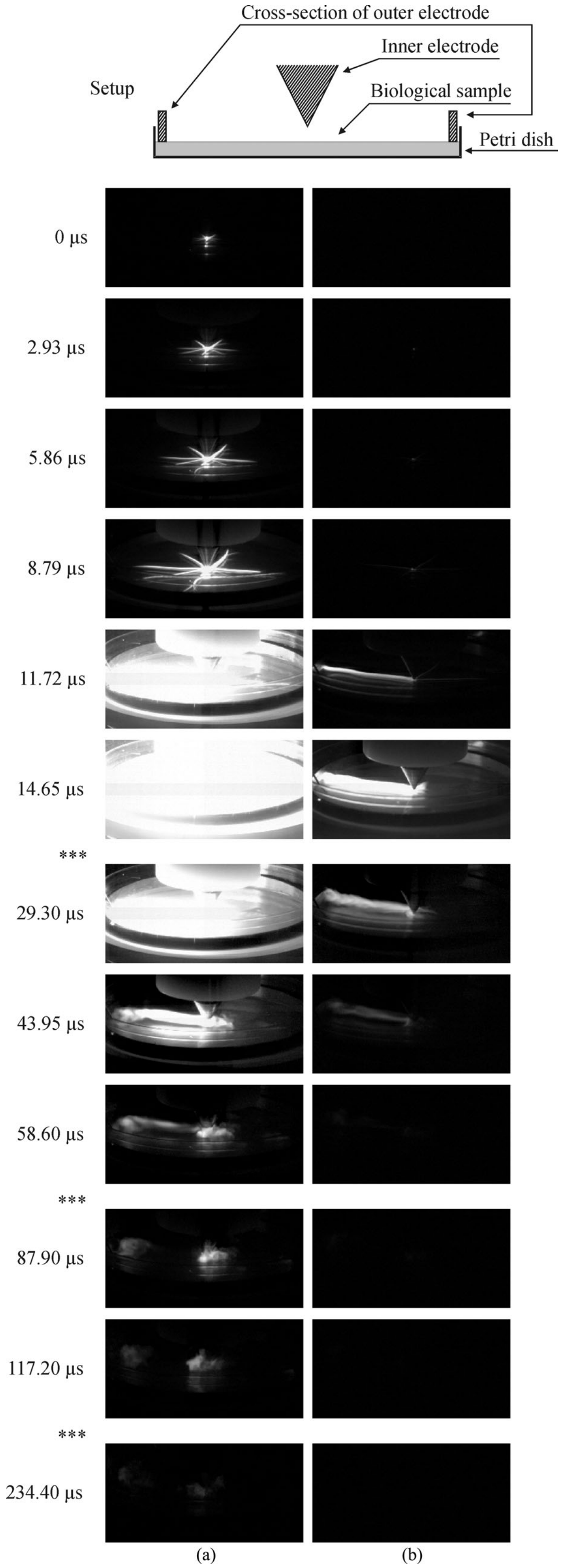

Fig. 3. Evolution of the discharge arc delivered by the generator shown in Fig. 1 connected to our exposure chamber described and illustrated in [21]; the frame timing is at the left, with the arc formation serving as the trigger. (a) Frames taken without a filter. (b) Frames taken through a welding protection glass. The three asterisks (***) indicate skipped frames.

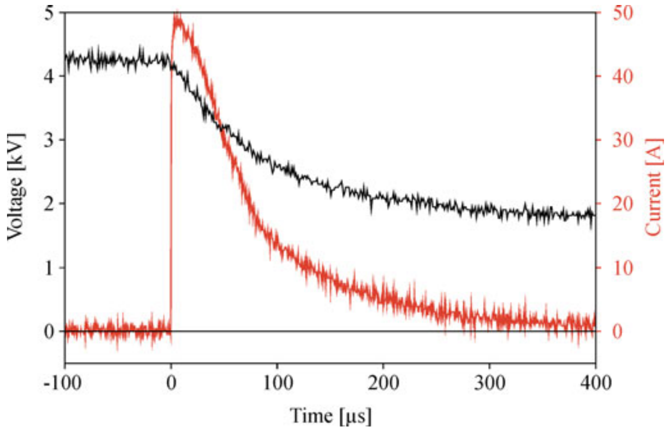

Fig. 4. Time course of current (black) and voltage (red) of the discharge delivered by the generator shown in Fig. 1 connected to our exposure chamber modified by addition of an inner insulating cylinder preventing the short-circuiting and formation of a plasma channel between the electrodes.

bright as to saturate the frames unless they were photographed through a welding protection glass (see the frames in Fig. 3 from 11.72 μs onward). The frames at the bottom of the left row of Fig. 3 show that even after the electric current ceased, the plasmified air continued to glow visibly for further ~200 μs.

In natural lightning strokes, there is no equivalent to the discrete receiving electrode of our exposure system, so there is also no counterpart to the short-circuiting effect described and depicted above, which thus represents an artefact resulting from system's design. As long as the current dissipates through the sample roughly radially outward into the receiving electrode, this is an adequate emulation of the stroke current's dissipation in the ground [26], but after radial arcs emerge largely into the air above the sample, and particularly as one of these arcs short-circuits the metallic electrodes, such an "emulation" loses all resemblance to lightning strokes.

To eliminate this artefact, we modified the exposure system, incorporating an additional, inner Plexiglas cylinder placed concentrically between the emitting electrode and the receiving electrode so as to form a tight contact with the surface of the sample, thus precluding the discharge from evading the sample from above and short-circuiting the electrodes. We first tested this solution with a cylinder almost as wide as the sample (inner and outer radius of 35 and 40 mm, respectively, while the receiving electrode had an inner radius of 41 mm; see the top of Fig. 5). As Fig. 4 shows, this resulted in the discharge current evolving largely as initially envisaged, reaching the peak of ~50 A in ~5 μs and then decaying exponentially with a peak-to-half time of ~65 μs. As revealed by Fig. 5, the radial rays still evolved sideways, but more gradually and only until reaching the inner edge of the newly incorporated insulating cylinder.

Inside the cylinder, the rays still gradually emerged into the air above the sample, reaching their maximal luminosity after 15–25 μs and then gradually fading, with only a central glow persisting for further ~100 μs (in later experiments, we used narrower cylinders to force the discharge current to flow almost entirely through the sample—see e.g., Fig. 8).

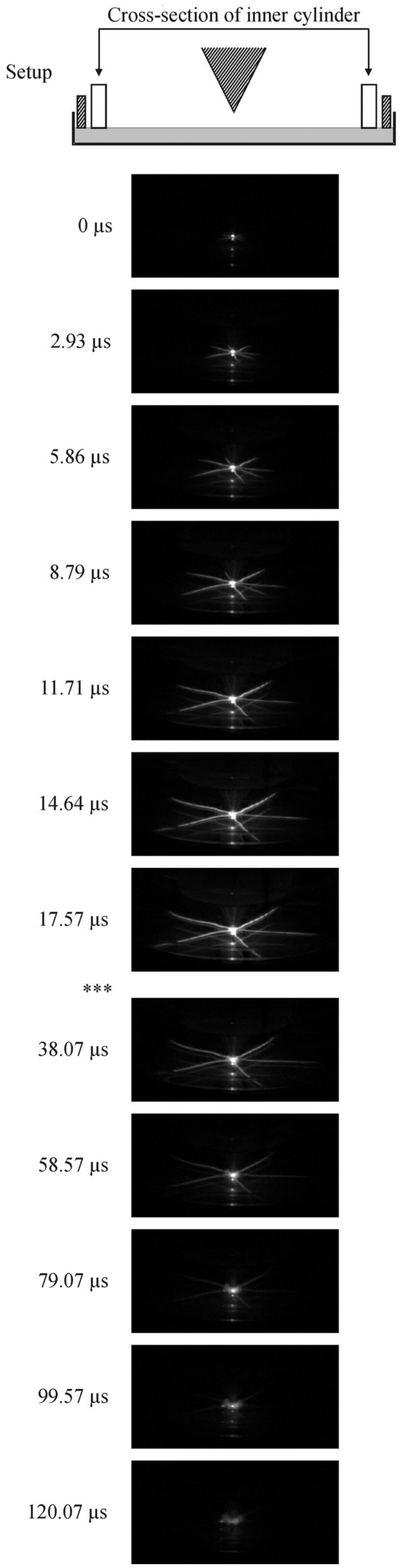

Fig. 5. Evolution of the discharge arc delivered and recorded as in Fig. 3(a), but with an inner insulating cylinder preventing the short-circuiting and formation of a plasma channel between the electrodes. Frame timing is at the left, and three asterisks (***) indicate skipped frames.

Here, we should address a possible concern regarding the considerable downscaling in the electric current of a discharge delivered by our exposure system (e.g., as shown in Fig. 4) with respect to lightning strokes; while their waveforms are very similar, the amplitudes differ by a factor of $\sim$600 (peak current of $\sim$50 A as opposed to $\sim$30 kA). Yet both with the lightning stroke and with the arc discharge delivered by our system, once the electric current enters from the air into a conductive medium, it dissipates through this medium roughly radially away from its point of entry. Thus, in both cases, as the distance r from the point of discharge's entry increases, the current density J and the electric field E it induces decrease in a monotonic and continuous manner. As a consequence, the same values of J and E caused in a given medium by a lightning stroke's current at a given r_1 are also caused in this same medium by a discharge current downscaled in amplitude at some $r_2 > r_1$. For example, for a lightning stroke current of 30 kA dissipating three-dimensionally in the ground, $J = 50$ A/cm^2 is reached at $r = 9.77$ cm, while for a discharge with a peak current of 50 A dissipating two-dimensionally in a disk-shaped sample 5-mm thick, the same value of J (and, if the medium is the same, also the same value of E) is reached at $r = 3.18$ mm.

It is true that the very highest values of J and E caused by lightning strokes do not occur in downscaled exposure systems, yet under such extreme conditions, all living matter is damaged lethally and through mechanisms that are now rather well understood: resistive heating, irreversible electroporation of cell membranes, and irreversible electroconformational changes in membrane proteins [27], [28], as well as electrolytic contamination and degradation, including formation of gas bubbles in cells and tissues [29].

These considerations show that with the exception of the most extreme (and destructive) conditions, the setup as described above—necessarily with an inner cylinder installed as to prevent short-circuiting between the electrodes—can emulate the electrical conditions of an exposure to a lightning stroke current quite adequately. While most natural lightning strokes are unipolar, there are also bipolar strokes, exhibiting polarity reversals during a single flash [30], and our setup could, with a suitable polarity switch or an adapted generator, also be used to deliver bipolar discharges. With respect to unipolar discharges, bipolar ones of the same amplitude and duration do not differ in the degree of resistive heating, but cause a detectably increased extent of cell membrane electroporation [31] and decreased extent of electrolysis [32], [33].

In lightning strokes, electric current's exponential decay from the peak amplitude often slows down (after $\sim$7–40 μs) into a much more gradual descent that continues for tens of hundreds of milliseconds, with the current of up to several hundred amperes; this is attributed to a persistence of some electric charge in the cloud and some electric conductivity of the lightning channel between the cloud and the ground [34], [35]. This level of current is of two orders of magnitude lower than its preceding peak value; hence, so is the electric field it induces, which can thus cause electroporation of membranes and electroconformational changes in proteins only in a very limited volume that has already been subjected to much more intense effects of this kind in the earlier stage of the stroke's current. In contrast, resistive heating and electrolysis can still be prominent, as this phase of the current lasts much longer than the preceding rapid decay, but as stated above, these effects are rather well understood both theoretically and empirically. The generator presented in this paper does not emulate the transition of the electric current from the exponential decay into a slow descent, but could be adapted to also emulate the latter phase, e.g., by incorporating another capacitor in series with a resistor of sufficiently high impedance, and connecting this *RC* element to the electrodes at the desired time during the fading discharge of the original capacitor; if the conductivity of the air channel would prove insufficient to sustain this current, it could also be injected directly into the sample below from a programmable current generator.

Lightning strokes are accompanied by thunder, and the arc discharges delivered by our system were also accompanied by a loud sound, so we analyzed the similarities of the acoustic shock waves generating them.

A high-pressure shock wave is formed in plasmified air during the lightning stroke, which travels faster than the speed of sound [36]. The shock wave expands roughly cylindrically away from typical plasma channels of lightning strokes, while for downscaled laboratory arc discharges the expansion is roughly spherical [37]. With expansion, the shock wave slows down, and upon reaching the speed of sound, it transforms into an acoustic wave (known as thunder in lightning strokes) with a characteristic frequency spectrum. The distance at which this occurs is referred to as the relaxation radius r_{R} of the shock wave, and for typical lightning strokes it is at $\sim$40 cm, where the peak sound pressure is $\sim$200 kPa (200 dB) [36]. At $r > r_{\mathrm{R}}$, the acoustic wave of a stroke (the thunder) has a distinctive frequency spectrum with two most prominent acoustic frequencies at $\sim$200 and $\sim$800 Hz. Close to r_{R}, the two peaks are of similar magnitude, but the higher frequency is attenuated more rapidly, so that farther away the frequency at $\sim$200 Hz prevails in the spectrum, and it characterizes the sound heard most dominantly in a typical thunder [38].

The arc discharge delivered by our setup is also consistently accompanied by a shock wave. To estimate its strength and relaxation radius, we measured its actual sound pressure (see Fig. 6) and frequency spectrum (see Fig. 7) with the outer Plexiglas cylinder of our system removed, as it would attenuate the pressure and distort the spectrum. Measured at 1 m from the arc, the peak sound pressure was 255 Pa (142 dB) and the peak frequency $\sim$3400 Hz when the short-circuiting plasma channel was formed (i.e., with also the inner Plexiglas cylinder absent), and 63 Pa (130 dB) with peak frequency of $\sim$1800 Hz when short-circuiting was prevented by adding the inner cylinder. Using the equations from [36] and [39], the relaxation radius of our arc discharge without short-circuiting is at $\sim$15 cm, where the peak sound pressure is in the range between $\sim$420 Pa (146 dB) and $\sim$2.8 kPa (163 dB), with the lower bound corresponding to cylindrical, and the upper bound to spherical expansion of the shock wave.

With the outer cylinder reinstalled, the SPL at 1 m was attenuated to 23.8 Pa (121.5 dB) and 6.47 Pa (110.2 dB) with the inner cylinder absent and present, respectively.

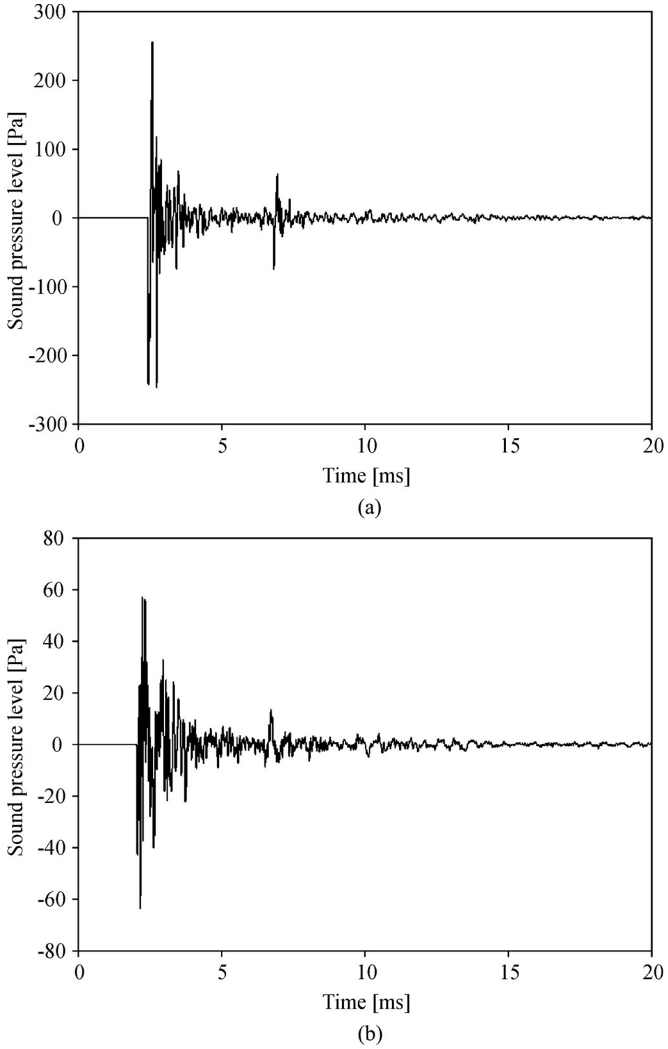

Fig. 6. Acoustic shock wave (time course of the SPL) accompanying the discharge arc delivered by our setup, as measured 1 m from the arc, without the outer Plexiglas cylinder enclosing the exposure system. (a) Without the inner Plexiglas cylinder added to prevent short-circuiting between the electrodes; (b) with this inner cylinder.

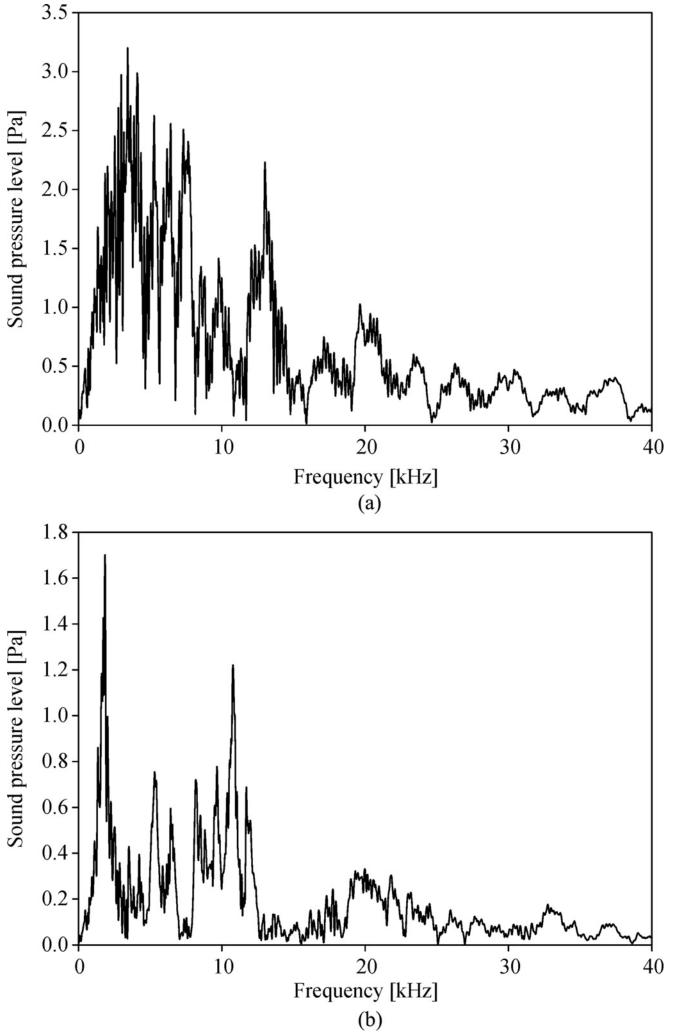

Fig. 7. Frequency spectrum of the acoustic shock wave shown in Fig. 6. (a) Without the inner Plexiglas cylinder; (b) with this inner cylinder.

These measurements show that compared to natural lightning strokes, the arc discharges delivered by our setup generate acoustic shock waves at roughly the same order of magnitude of downscaling in the sound pressure as in the total electric current, electric current density, and electric field. Still, the acoustic spectrum of the setup's arcs differs from that of a lightning stroke, in particular it is characterized by a higher peak frequency (1800 Hz, as opposed to 200 and 800 Hz in natural strokes), and a potential importance of this difference remains to be investigated.

B. Application in Biological Experiments

We tested the use of our generator and setup by delivering various discharges to a sample of *Bacillus pumilus* spores. Bacterial spores are known to be highly resistant to "classical" electroporation protocols in which the discharges are delivered through electrodes in direct contact with the sample, where the electric arc does not proceed through the air, and the accompanying acoustic shock wave is thus absent [40]. In Fig. 8, we demonstrate successful killing of these spores by discharges delivered into the spore-containing sample as arcs through an air gap and thus accompanied by an acoustic shock wave. Panels (b) and (c) clearly illustrate the difference between the effect of pulses short enough (0.5 μs) for the discharge arc to remain vertically descending, and pulses long enough (20 μs) for the arc to start and complete its migration from the vertical path to the diagonal path between the electrodes, finally evading the sample. Comparison of panels (d) and (e) shows that consecutive discharges tend to form plasma channels in directions proximate to the direction of the first discharge, yet gradually scattering. Panel (f) demonstrates that spore killing is also significant when short-circuiting between the electrodes and, thus, plasmification of the air above the spores is prevented.

In this paper, we included the experiment on bacterial spores primarily as an example of our system's use, but in experiments aimed at elucidating the (bio)physical, (bio)chemical, and biological effects of arc discharges on living matter, considerable

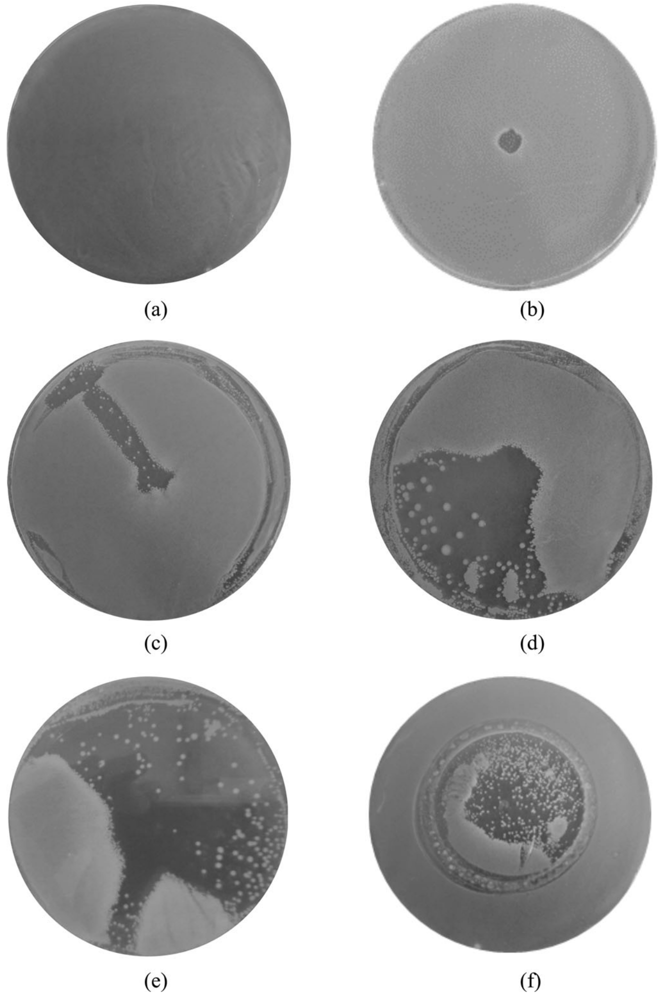

Fig. 8. Areas of *Bacillus pumilus* spore killing with various number, type, and duration of arc discharges delivered with our setup into petri dishes with a radius of 45 mm. (a) Control (no discharges delivered). (b)–(e) Discharges delivered without the inner Plexiglas cylinder added to prevent short-circuiting between the electrodes: (b) 200 discharges of 12 kV, 0.5 μs; (c) 1 discharge of 5 kV, 20 μs; (d) 20 discharges of 5 kV, 20 μs; (e) 50 discharges of 5 kV, 20 μs. (f) 50 discharges of 5 kV, 100-μs time constant, with the inner cylinder present (its inner radius was 22 mm, outer radius 26 mm, and its position is revealed by the ring-shaped imprint in the agar at the outer rim of the inactivation area).

attention will have to be devoted to distinguish between the effects of the electric field and those of the phenomena that accompany this field when it is delivered through a discharge arc: the mechanical (acoustic) pressure wave [41], possible cavitation due to this pressure [42], as well as formation of plasma near the arc and its rays [43], and the ultraviolet light emitted by the arc [44].

IV. CONCLUSION

We have developed and tested a discharge generator that can be used with the previously described exposure system to expose biological samples to controlled and reproducible arc discharges, thus allowing to study the effects of such discharges on living organisms. In particular, we have demonstrated that such arcs, in which the electric field is accompanied by an acoustic shock wave, can cause killing of the generally extremely resilient bacterial spores.

Moreover, as outlined in Section I, there is ample motivation for systematic studies of lightning-triggered HGT among microorganisms, with potential implications for their evolution—both early and modern. The tests of our generator and the exposure system described here, together with the calculated estimates presented, show that this setup is adequate for emulation of lightning strokes entering natural habitats, and should thus act as an essential piece of equipment for realistic experimental studies of the feasibility of lightning-triggered HGT.

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