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Contribution to the biophysics of the lethal effects of electric field on microorganisms

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Abstract

The proposed model assumes that the criteria leading to the lethal breakdown of microorganisms suspended in a continuous medium depend on two parameters: (a) the applied electric field must exceed the critical field of membrane to create holes and (b) the Joule energy (deposited in the membrane) must exceed the minimum value beyond which the cell can not recover. The first parameter initiates (reversible) breakdown and the second one, the completion of the (irreversible) electrical breakdown leading to death of the cell. The number of cells surviving the electric field treatment is related to statistical distribution of cell size. Comparison between theory and the experimental results of Kinosita and Tsong (1977); Hülsheger et al. (1980, 1981, 1983); Rosemberg and Korenstein (1990) and others is given.

Keywords: Electrical breakdown; Cell electrodynamics; Cell membrane; Electroporation; Electric field; Lethal effect

1. Introduction

The object of this paper is to elucidate the consequences of pulsed current flow in a conducting continuous medium in which cells are suspended. The cells are assumed to be spherical as defined by Sale and Hamilton [1]. Cases considered are: (1) the membrane is impermeable to diffusion and electric current (which passes around the cell after the membrane capacitance is fully charged). (2) The membrane resembles a thin 'oil film' with small pores, through which electric current enters the cell and permits the core to assume a bulk conductivity. The pores are considered small enough not to interfere with the biological functions of the cell. (3) The current load is sufficient to damage the region around a single pore. In this case the detailed geometry of the current passage through the pores must be considered. An account of the experiments by different authors and comparison with the theory will be made. The current phenomenon has been extensively studied and reported in the literature (cf. the two excellent recent reviews [2,3]). Theoretical approaches to this problem exist but most restrict themselves to the case of a plane membrane [3,4]. We propose to derive phenomenological criteria for cell death in electrical discharges.

2. Formulation

2.1. Membrane as an impermeable structure in an electrical field

We define Ψ as the current flow (stream) function, E the electric field vector and V the potential. For a spherical co-ordinate system these quantities are related in the following fashion [5]:

$$E_\theta = \frac{1}{r \cdot \sin\theta} \cdot \frac{\partial \Psi}{\partial r} = -\frac{1}{r} \cdot \frac{\partial V}{\partial \theta}$$

$$E_r = -\frac{1}{r^2 \sin\theta} \cdot \frac{\partial \Psi}{\partial \theta} = -\frac{\partial V}{\partial r} \qquad (1)$$

where Θ is the angle between the radius vector (originating at the cell centre) and the direction of the field line at

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infinity. Here, a is the radius of the cell and E, the field far from the cell giving the stream function

$$\Psi = \frac{1}{2} \cdot Er^2 \cdot \left[\left(1 - (a/r)^3\right]\sin\theta \right. \quad (2)$$

Introducing $u = (a/r)^3$, the Joule heating around the cell becomes:

$$W = \sigma_1\left(E_\theta^2 + E_r^2\right)$$

$$= \sigma_1 E^2\left[(1-u)^2\cos^2\theta + \left(1 + \frac{u}{2}\right)^2 \sin^2\theta\right] \quad (3)$$

If $A = (1-u)$, $B = (1 + u/2)$ and w, the normalized power density, $(= W/(\sigma_1 E^2))$ we get,

$$w = A + B + (A - B) \cdot \cos 2\theta \quad (4)$$

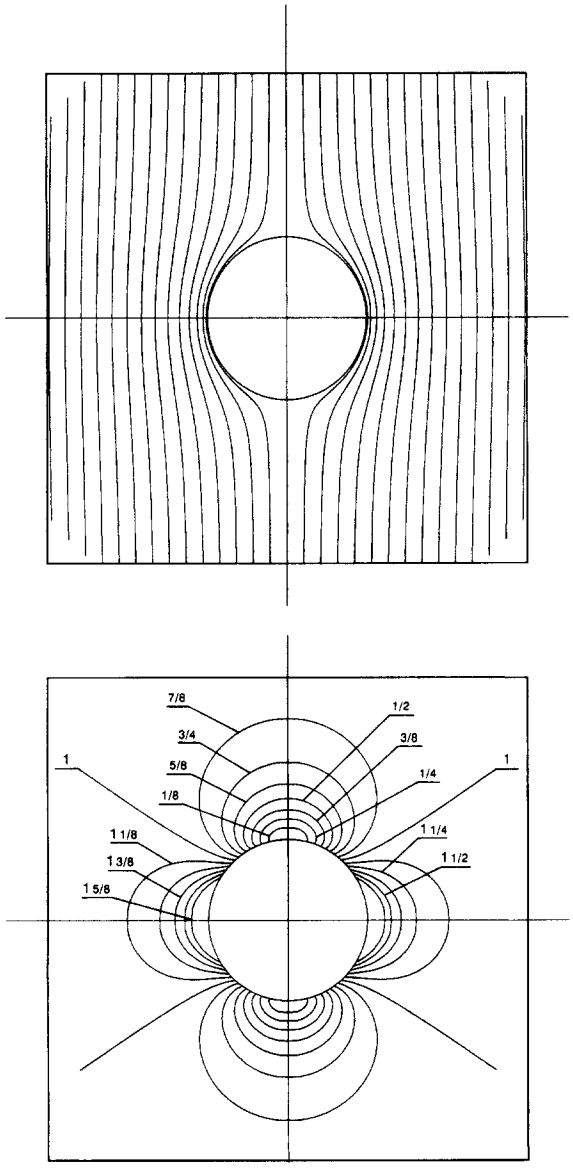

Fig. 1. Current lines (top) in a conducting suspension, when the membrane is impermeable to electric current. Bottom – power density lines.

Eq. (4) gives the constant normalized power density lines and Eq. (2), the current lines (Fig. 1). At the surface of the cell, $u = 1$, Eq. (4) becomes $w = (9/4)\sin^2\Theta$ making the heating rate 9/4 times larger at the equator of the cell than in the liquid far from the cell. The heating falls in a continuous fashion from equator to the poles via the $\sin^2\Theta$-law. There is no heating at the poles.

An estimate of the effect of local departure from thermodynamic equilibrium could be obtained from the following diffusion expression:

$$\Delta x = \sqrt{Dt}$$

Here, D is the diffusion coefficient ($2.5 \cdot 10^{-5}$ cm^2/s in water at 25°C and atmospheric pressure) and t is the duration of the applied voltage impulse. For $t = 10$ μs, Δx becomes 0.158 μm. This suggests that, if the cell diameter is 1 μm, the equilibrium in temperature between the equator and the poles will not occur during the electrical pulse.

An alternative mechanism is based on the rate at which local pressure perturbations are equilibrated throughout the region, i.e., the speed of sound. The appropriate time constant is in the order of tens of nanoseconds (and equal to the ratio of the size of the cell and the velocity of sound).

Experimental findings [1,6–9] indicate that the temperature increases 3 to 5°C in the liquid in which microorganisms experience lethal effects. From Eq. (4) it follows that the temperature rise at the equator is 9/4 times larger, i.e., it is 6.75 to 11.25°C, while the temperature at the poles remain unaffected.

Temperature changes arise from the conversion of Joule heating to internal energy of the liquid in accordance with the current line distribution around the cell. However, our underlying conditions seem to rule out a mechanism of this kind to lead to distortion, rupture and lysis of the membrane by thermomechanical forces alone.

2.2. *Membrane as a penetrable barrier in an electrical field*

We assume that a membrane behaves like a thin 'oil film' composed of no more than two layers of molecules, pierced by numerous pores through which the electric current crosses into the cytoplasm. After initial charging of the membrane, we can assume that the current short circuits the membrane as it passes through the numerous pores without distorting them.

From such assumptions we estimate the average rise in temperature. If the electrical conductivity of the core is σ_3 and of the suspension σ_1, a general solution for the current lines of Eq. (2) is:

$$\Psi_1 = \left(\frac{A}{r} - Br^2\right)\sin^2\theta$$

$$\Psi_3 = -Cr^2\sin^2\theta \quad (6)$$

The constants A, B and C are determined from the following conditions: (a) the field far away from the suspended cell is E, (b) the current continuity is preserved at $r = a$ = radius of the cell, and (c) the tangential components of the field at $r = a$ must be in balance. We find that if $\sigma_3 \gg \sigma_1$ at the surface of the cell, the normalized power density, w is $9\cos^2\Theta$ and the core of the cell receives a negligible amount of energy. The heating is maximal at the poles and falls towards the equator via $\cos^2\Theta$-law. The power density at the equator is not affected by the passage of current and the temperature maintains its initial value. This analysis suggests that, if the time constant for achieving thermodynamic equilibration is very long, the poles's temperature for the experimental conditions [1,6–9] would reach between 27 and 45°C.

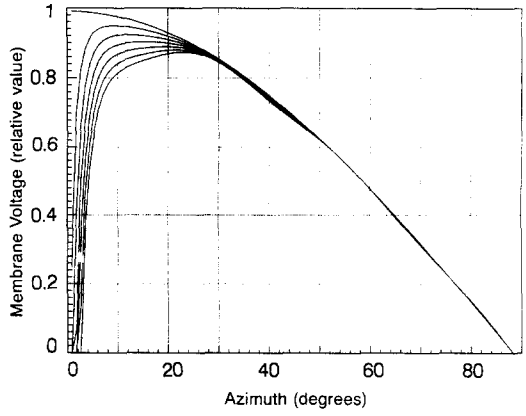

Fig. 3. Voltage across the membrane as a function of polar angle. Without holes, the peak voltage is one. Otherwise, the maximum voltage value is shifted towards 30 degrees.

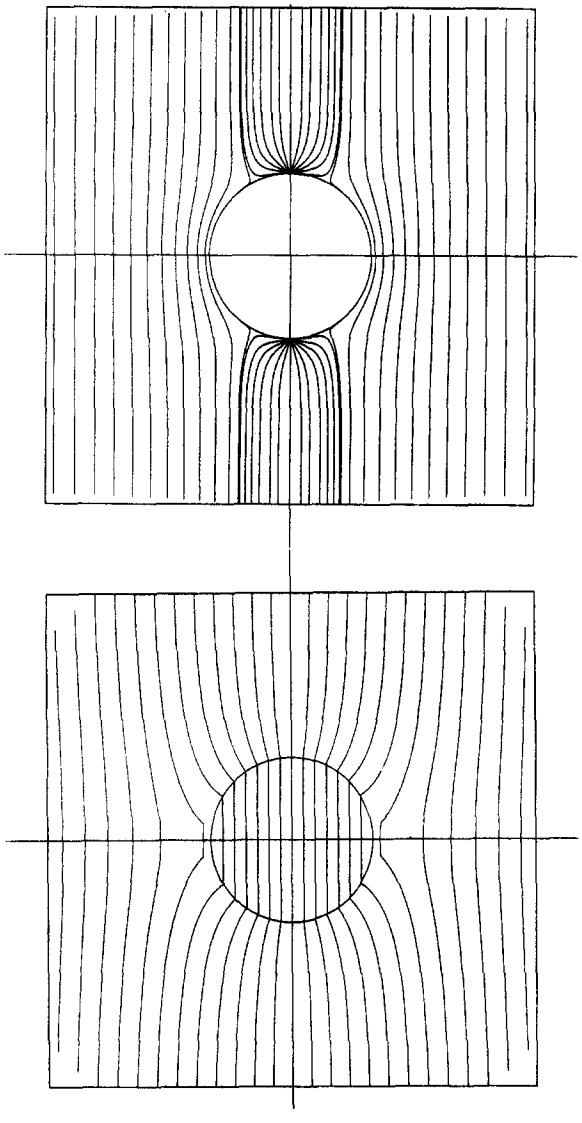

Fig. 2. Current lines around the cell when the membrane is totally permeable to electric current (bottom) and with a pair of holes through the membrane at the poles (top). To depict current concentration at the poles, a fine mesh of current lines is used.

If the surface tension of the membrane would experience a sharp drop in its value due to a rise in temperature, this mechanism on its own could be responsible for the rupture of the membrane. However, further examination of this model shows that the threshold level can not be reached due to insufficient current concentration at the membrane surface.

2.3. Membrane as a semi-permeable barrier in an electrical field

We suggest that reversible breakdown is caused by the electric field exceeding the critical field of the membrane to create holes. It is described by the relationship between the potential difference, V across the membrane and the radius, b of the cell:

$$V = 1.5bE\cos\theta \tag{7}$$

implying $b \sim (E)^{-1}$ where V is about 1 V.

Eq. (7) suggests that the field strength needed to damage a particular cell is inversely proportional to the radius of that cell. See Zimmermann et al. [6]. Since this voltage V reaches maximum at the poles ($\cos\theta = 1$), breakdown will occur here first and current continuity demands that a pair of holes, one at each pole (north and south) is created. The course of the current lines around the cell with a pair of holes is given in Fig. 2. As indicated in Appendix A, the holes relieve this voltage at the poles but it builds up some distance from the pole to a value smaller than the original one (in the unperforated condition). The holes shift the maximum electric field (stagnation point) from the pole ($\cos\Theta = 1$) to a region where the unperturbed electric field is smaller. It follows that further strikes become less probable after the first dielectric breakdown (perforation) (see Fig. 3). However, if the applied electric field E exceeds the inception field E_0, a more or less regular pattern of holes will develop around the apex, each separated from its neighbour by a region of voltage relief.

From Fig. 2 we conclude that the total current entering the cell will not change materially if the single hole is replaced by a cluster of holes. The course of current lines ahead of the cell is not greatly affected by the exact distribution of the cluster around the poles.

Experimental evidence on the number of holes or pores appears inconclusive: studies involving the migration of a fluorescent dye by Dimitrov and Sowers [10] indicate an asymmetric picture, suggesting migration through a single hole, where current continuity is preserved through the capacitance of the membrane. Conversely, the photographs presented by Tsong [2] are symmetrical with respect to the electrodes and the dye interface is straight, indicating that the discharge current (and hence the dye) enters through an array of pores placed symmetrically about the equatorial plane in a form of 'narrow bright bands'. At any rate, the membrane voltage relief near the hole is a local phenomena and is relatively unaffected by holes in the opposite hemisphere.

Chang and Rees [11] have detected these electrically generated holes by means of electron microscopy and have conjectured that their density may be a function of the applied field.

For simplicity, we consider a single (equivalent) hole of a small diameter forming a long cylinder. Current conservation demands that the electric field inside the hole is $E_m \sim (\sigma_l/\sigma_m)(E - E_o)b^2/r^2 \sim (E - E_o)b^2/r^2$. The Joule energy density ($= \sigma_m E_m^2 \Delta t$) is dissipated in the breakdown-created hole of average conductivity σ_m, the field E_m and the pulse width Δt and raises the temperature inside the hole. A part of this energy is spent to erode the material inside the holes, thereby increasing their diameter, and a part to increase the pressure inside the cell above the osmotic level. This leads to swelling of the cell as the pressure from the cylinder is transferred to the cytoplasm. Therefore, $\sigma_m E_m^2 \Delta t$ can be expressed in terms of the excess force F acting on the surface area of the cell ($= 4\pi b^2$). If F exceeds the critical force F_o (where F_o = constant), an irreversible electrical breakdown condition is reached where the useful material of the cytoplasm (: cytoplasmic macromolecules) is discharged into the suspension. The membrane behaves as if it is being 'ruptured'. Hence:

$$r^2 \sim b^3(E - E_o)(\Delta t)^{1/2} \tag{8}$$

Rosemberg and Korenstein [12] confirmed Eq. (8) by measuring the relative membrane conductance ($\sim \sigma_m r^2$). For constant pulse duration Δt they obtained $r^2 \sim (E - E_o)$. By re-plotting their data on a log-log scale for constant field strength (of 1 kV/cm), we find that $r^2 \sim (\Delta t)^{0.50}$, as one expects from Eq. (8). However at a field of 1.8 kV/cm, we find r^2 is $\sim (\Delta t)^{0.73}$. As the exponent of Δt approaches unity, it can be shown that the 'thin sheet' approximation of field distribution applies. Here, the length of the cylinder is small compared to its diameter.

The criterion of critical force could be expressed in terms of the Joule energy per unit length which results in membrane swelling. For a given species, this length representing radial expansion follows from the theory of elasticity. Hence, the total Joule energy ($= V^2 \Delta t/(R)$ = constant) received by the hole is related to the critical force and cell death. Here V is defined by Eq. (7), the resistance of the hole, R by $\delta/(\pi r^2 \sigma_m)$, r^2 by Eq. (8) and δ is the thickness of the membrane. Therefore:

$$b \sim \left((E - E_o)^2 \Delta t\right)^{-3/10} \tag{9a}$$

If experimental work is done with only a single specie of microorganisms, cell destruction follows the law: $f(E, \Delta t) = (E^2 \Delta t)^{3/10}$ = constant, if $E \gg E_o$ (see Eq. (9a)). It is assumed in Eq. (8) that the Joule energy is dissipated in the hole and transferred to the cytoplasm neglecting energy loss to suspension. This implies that Eq. (9a) is only valid for pulses having short widths and high amplitudes. For long duration pulses, the heat loss and the detailed evaluation of Joule heat generation must be considered, e.g., $\int (V^2/R)dt$ = constant. For the initial value of V given by Eq. (7), the voltage across the hole will start to fall exponentially in time (as it is known in electrical discharges). The integration shows that for a limited time range, $f(E, \Delta t)$ can be approximated as $((E - E_o)^2 \Delta t^n)^{3/10}$ = constant, where n is less than one.

On the other hand, Carslaw and Jaeger [13] give a solution of the heat conduction problem of a conducting sphere separated from infinitive medium by a thin layer with contact resistance. If this layer is thought to represent the cell membrane, the relevant equation for early times (on p. 350, Eq. (22)) shows that the temperature change (the cooling rate) is proportional to the ratio of surface area to mass of the sphere (= well known 'square-cube' law). It could be argued (due to the analogy between the heat conduction and diffusion) that the leakage rate of cytoplasmic molecules also follows the square-cube law. With surface area being $4\pi b^2$ and with the cone of cytoplasmic volume (or mass) ($\sim \pi r^2 b/3$) discharged into suspension, we have from Eq. (8) that:

$$b \sim \left((E - E_o)^2 \Delta t\right)^{-1/4} \tag{9b}$$

Eq. (9a) corresponds to lethal damage to the microorganism's surface (e.g., a burn) independently of volume of the body. Yet, Eq. (9b) demands that the cell looses its interior at a constant rate. However, the dependence of b on E appears to be small (see Fig. 4).

It would be useful to estimate the error caused by assuming a single hole at the pole stated by Eq. (9a). Breakdown starts at the poles and could spread to occupy the area of a spherical cap ($= 2\pi b^2(1 - \cos\Theta)$) with assumed uniform pore density α. Its edge is characterized by the condition that the applied field E must be equal to the inception field E_0. Also, the total area of the holes: nr^2 can be expressed as $b^2(V - V_o)(\Delta t)^{1/2}$ where, n is the

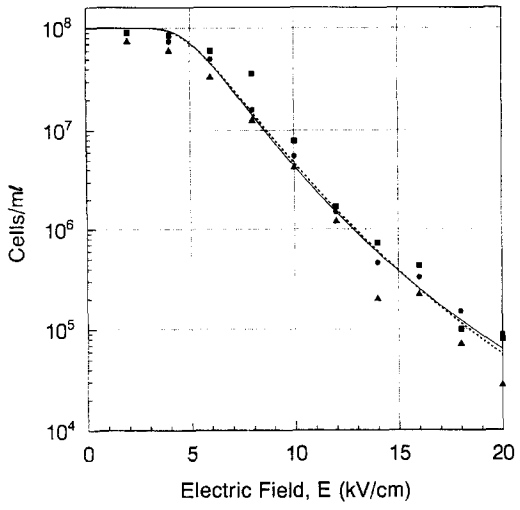

Fig. 4. Survival rate as a function of electric field strength for *E. coli* K12. Experimental points after Hülsheger and Niemann [7] with the following electrolyte suspension: ▲ for NaCl, ■ for S_2O_3 and ● for PO_4. To get agreement with experiments, the theoretical curve (solid line) based on Eq. (9a) is: $S = 0.5 \cdot 10^8 (1 + \mathrm{Erf}(4.508(-0.965 + 2.773/E^{0.6})))$. If Eq. (9b) is used, the dotted curve also provides good fit: $S = 0.5 \cdot 10^8 (1 + \mathrm{Erf}(5.184(-0.965 + 2.329/E^{0.5})))$. E is in kV/cm.

number of holes (see Eq. (8)). Hence, the constant Joule (dosage) criterion: $D = \int (V^2/R)\Delta t$ becomes

$$D = (2\pi b^2 \alpha)(1.5Eb)^2(\sigma_m/\delta)(\Delta t)^{3/2} \int \cos^2\Theta (V - V_0) \times \sin\Theta \, d\Theta$$

Defining $x = 1.5Eb/V_0$ where V_0 corresponds to the inception voltage, we get

$$D = (2\pi b^2 \alpha)(1.5Eb)^2(\sigma_m/\delta V_0)(\Delta t)^{3/2} \times \left[-(1/3)(1 - 1/x^3) + (x/4)(1 - 1/x^4)\right] \quad (9c)$$

For $E \gg E_0$, $x \gg 1$ and constant D, we recover Eq. (9a). Eq. (9c) becomes useful if we know the value for E_0. Effect of multi-shot exposure on Eq. (9c) is discussed in Appendix B.

3. Experimental evidence and discussions

We assume the radius of the cell to be subject to a statistical distribution of Gaussian type:

$$dN = \gamma(2\pi)^{-1/2} \exp\left(-(\gamma(b - b_0))^2/2\right) db$$

N is the relative population number, b the radius of the cell, γ a constant associated with the variance and b_0 the population's mean radius. To obtain the number of cells, this expression must be integrated over the domain governed by Eq. (7). The relative number subjected to lysis, $L = 0.5(1 - \mathrm{Erf}(2^{-1/2}\gamma(b - b_0)))$, and the relative number surviving, $S = 0.5(1 + \mathrm{Erf}(2^{-1/2}\gamma(b - b_0)))$. Here, b is related to the criterion for cell death by Eq. (9).

A suitable choice of disposable parameters allows the

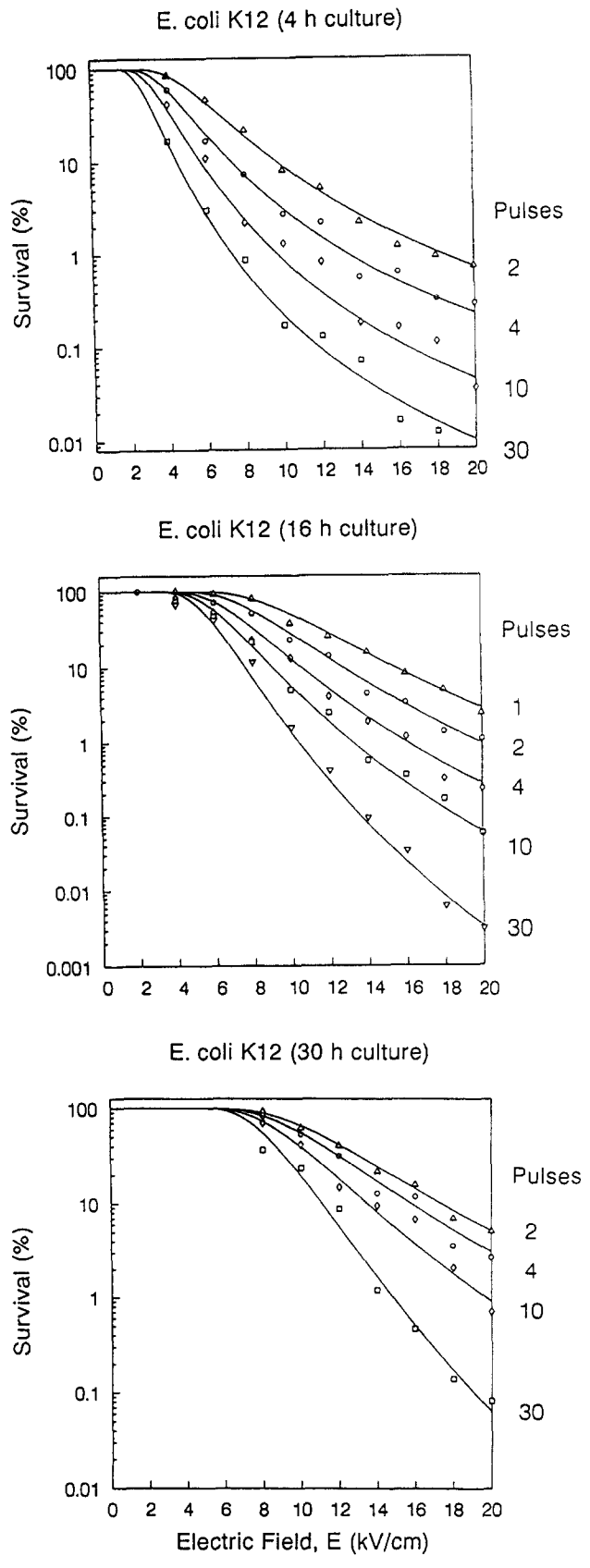

Fig. 5. Relationship between the surviving cell and electric field E for *E. coli* K12 with varying growth phase. Experimental points are after Hülsheger et al. [8,9]. Parameters used to plot the survival curves are given in Table 1.

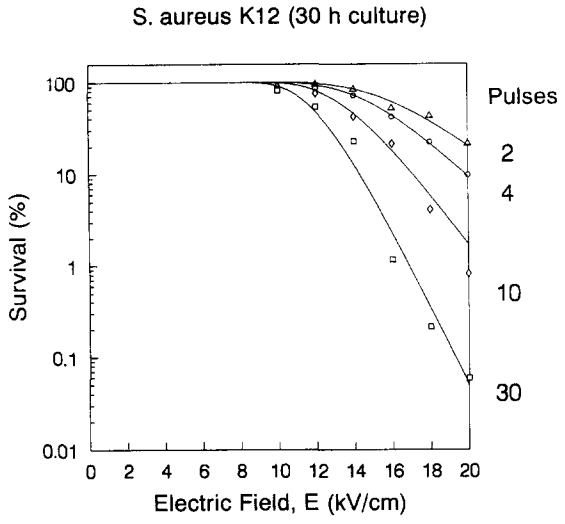

Fig. 6. As in Fig. 5 but for *S. aureus*.

theoretical curves to fit the experimental data obtained by Hülsheger et al. [7–9] resulting in Figs. 4–6, Table 1 and Fig. 7 give the parameters used to obtain Figs. 5 and 6. In these curves the width of electrical pulses is constant. Hence, if we use Eq. (9a), $b \sim E^{-0.6}$.

For single shot exposure, the analysis is straightforward. The value for b_0 can be obtained from the mean volume of the species (given by Hülsheger et al [7–9]) by assuming the cells are spherical. The value for γ is obtained from curves describing the cell volume distribution. Unfortunately, the local experimental conditions (: selection process/method, temperature, and growth medium, strain differences) play an important role. Uncritical choice of γ from literature may miss an important local condition of the experiment. However if we assume that the cell volume distribution curves for rapidly growing culture of bacteria are congruent with each other, (see Kubitschek [14]) it can be seen that the product of $\gamma/2^{0.5}$ (given in Table 1) times b_0^2 converges to a constant. This justifies presenting Table 1 with the view of providing a base for future analysis.

However, if the cell volume distribution could be measured before and after electric pulse treatment, the measured survival curves permit us to choose correctly the criteria for cell death (: Eq. (9a) or Eq. (9b)) From Fig. 4 we see that both cases appear possible and the choice must be made on 10% deviation of γ.

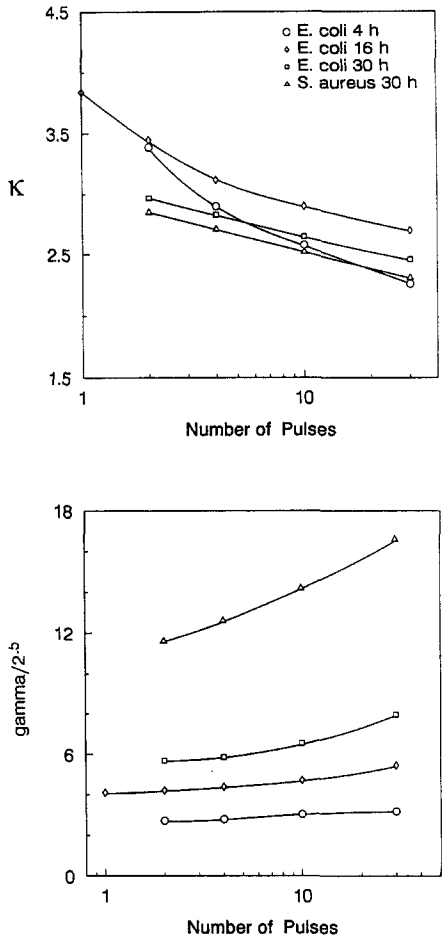

Fig. 7. The plot of parameters given in Table 1.

We now introduce the parameter K, which according to Eq. (9a) is the proportionality constant between b and $((E-E_0)^2\Delta t)^{-3/10}$. If K is constant, it follows that the membranes of different cells have the same features: i.e., the same Joule dosage is required to 'burn' the same thickness of membrane enveloping different cells.

Table 1
Parameters used to plot the survival curves given in Figs. 5 and 6

Type of cell	b_0	Parameter	Number of pulses				
			1	2	4	10	30
E. coli K12 4 h culture	1.20	$\gamma/2^{.5}$	–	2.72	2.80	3.05	3.19
		K	–	3.39	2.90	2.58	2.26
E. coli K12 16 h culture	0.965	$\gamma/2^{.5}$	4.10	4.21	4.39	4.72	5.45
		K	3.84	3.45	3.12	2.90	2.70
E. coli K12 30 h culture	0.694	$\gamma/2^{.5}$	–	5.68	5.86	6.55	7.96
		K	–	2.97	2.83	2.65	2.46
S. aureus 30 h culture	0.523	$\gamma/2^{.5}$	–	11.6	12.6	14.2	16.6
		K	–	2.85	2.71	2.52	2.31

The curves are defined as: $S = 0.5(1 + \mathrm{Erf}(2^{-1/2}\gamma(-b_0 + K/E^{0.6})))$. E is in kV/cm, b_0 in μm, γ in $(\mu\mathrm{m})^{-1}$, and K in $(\mathrm{kV/cm})^{0.6}$.

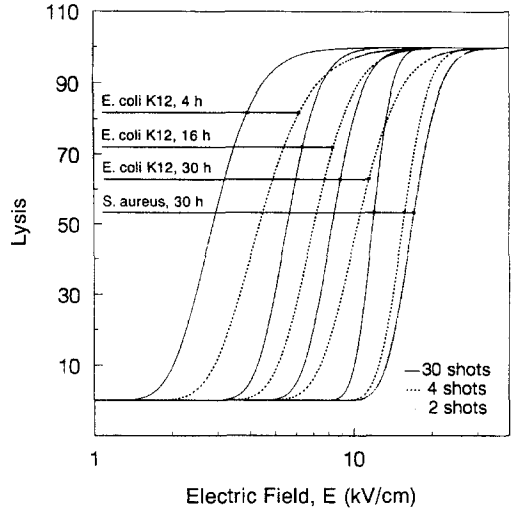

Fig. 8. Theoretical (survival) curves given in Figs. 5 and 6 are reevaluated to yield the lysis curves.

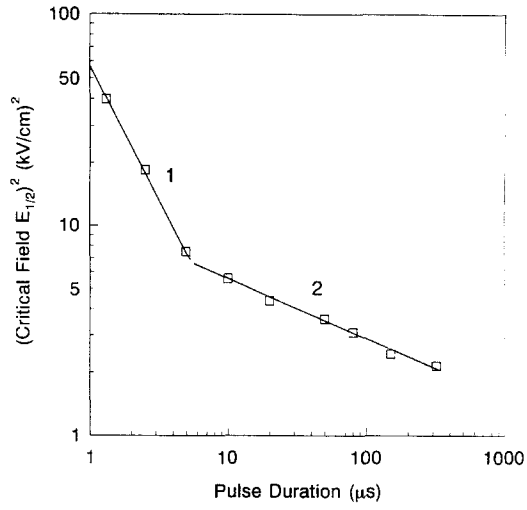

Fig. 9. Critical field $E_{1/2}$ vs. pulse duration. The points are after Kinosita and Tsong [15], and the solid lines are the regression curves: 1 for $E^2_{1/2}\Delta t^{1.12}$ = constant and 2 for $E^2_{1/2}\Delta t^{0.27}$ = constant.

The remaining task concerns the results of multiple-shot exposure on cell distribution. Each shot alters the distribution of healthy cells from Gaussian, as it has killed cells of large diameter. The next electrical pulse now faces this modified Gaussian curve. If $x = \gamma(b - b_0)$ and $x > 0$, the integral of Gaussian probability function equals $0.5 + 0.5(1 - \exp(-2x^2/\pi)^{1/2} \approx 1 - (1/4)\exp(-2x^2/\pi)$. The result is again approximately Gaussian, where in zero order approximation γ has to be multiplied by $4/\pi$. This fact indicates that $\gamma/2^{1/2}$ in Table 1 rises with the number of shots.

By re-plotting the survival curves of Hülsheger et al. the lysis curves, Fig. 8 and Table 2 are obtained. These results draw attention to the work of Sale and Hamilton [1]. The present work supports their two main claims: (a) an approximately inverse relationship exists between cell diameter and electric field and (b) that the critical potential difference across the membrane can be estimated by the product of cell diameter and electric field, $E_{1/2}$ at which 50% lysis occurred. However, examination based on Hülsheger et al.'s work suggests that the parameters of Sale and Hamilton are also functions of the number of shots used (see Table 2).

To enable comparison with the work by Kinosta and Tsong [15], we write the lysis, L as a function of $f(E,\Delta t)$ thus:

$$L = 0.5(1 - \mathrm{Erf}(-9.96 + \mathrm{constant}/f(E,\Delta t)) \qquad (10)$$

The value 9.96 was derived from data on human red blood cells given by Ponder [16]. The volume of the cell is $(88 \pm 6)\ \mu m^3$. If approximated by a sphere, the mean radius is $(2.759 \pm 0.197)\ \mu m$. Since two-thirds of the diameters lie within this volume range, we find that $(2)^{-1/2}\gamma(b - b_0) = 0.69$ (as Erf(0.69) ≈ 0.667). Therefore, $b_0 - b = 0.197\ \mu m$ and $(2)^{-1/2}\gamma b_0 = 9.66$.

The experimental points of Fig. 5 given by Kinosita and Tsong [15] and describing the critical field, $E_{1/2}$ vs. the pulse duration Δt was re-plotted on a log-log scale, resulting in Fig. 9. For pulses of short (1.3, 2.5 and 5 μs) duration, we obtain the following experimental results: $(E^2_{1/2}(\Delta t)^{1.12}) = \mathrm{constant}$. To get an agreement between their measured points (of haemolysis) and Eq. (10) (see

Table 2
The critical potential difference across the membrane, V was estimated as $1.5b_0E_{1/2}$

Type of cell	Parameter	Number of pulses				
		1	2	4	10	30
E. coli K12 4 h culture	$E_{1/2}$ (kV/cm)	–	5.65	4.34	3.58	2.88
	V (V)	–	1.017	0.782	0.644	0.518
E. coli K12 16 h culture	$E_{1/2}$(kV/cm)	9.96	8.34	7.05	6.24	5.56
	V (V)	1.442	1.208	1.021	0.903	0.805
E. coli K12 30 h culture	$E_{1/2}$ (kV/cm)	–	11.31	10.41	9.37	8.29
	V (V)	–	1.178	1.084	0.976	0.863
S. aureus 30 h culture	$E_{1/2}$ (kV/cm)	–	16.87	15.53	13.73	11.90
	V (V)	–	1.324	1.218	1.077	0.934

Here, $E_{1/2}$ is the field at 50% lysis.

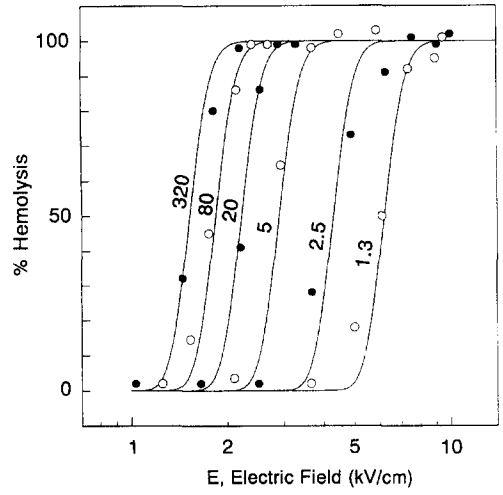

Fig. 10. Extent of haemolysis at different pulse duration (in μs). Theoretical curves are solid lines and the points are taken after Kinosita and Tsong [15].

Fig. 10), the experimental data: $f(E,\Delta t) = (E^2_{1/2}(\Delta t)^{1.12})^{3/10}$ was used. For E in kV/cm and t in μs, the constant in Eq. (10) has the value of 32.5. For pulses of long (20, 80, and 320 μs) duration, we find that the experimental curve (obtained from Kinosita and Tsong' points) is $f(E,\Delta t) = (E^2_{1/2}(\Delta t)^{0.27})^{3/10} = \text{constant}$. For this class of pulses, the constant in Eq. (10) has the value of 20.5. To get a better fit, it may be necessary to retain $(E_{1/2} - E_0)^2$ instead of the measured value of $E^2_{1/2}$. With $E_0 = 0$, the lysis' curves (plotted on a logarithmic scale of E) are congruent as suggested by Kinosita and Tsong and shown in Fig. 10. However, the calculations suggest that E_0 decreases the steepness of the lysis curves at higher fields (with shorter pulses) relative to the those obtained with smaller field (and longer pulses).

4. Conclusions

To explain the survival curves, we must assume the formation of a relatively regular pattern of holes around two loci in the membrane facing the electrodes. Once a hole is established, the local high current concentration in the hole produces sufficient Joule heating to cause local high pressure (due to temperature rise and ion concentration) which drives fluid into the cytoplasm. Later, the interior of the cell driven by the pressure, is forced out. Consequently the interior is replaced by the inorganic matter in which the cells are suspended, to the extent that biological activity is inhibited. We confirm the mechanism of cell lysis proposed by Kinosita and Tsong [14]. Cell death occurs, not through primary leakage of cell material across electroporetically induced holes, but rather through increase in osmotic pressure. To appreciate this mechanism, the following properties of water should be noted: a temperature jump from 0 to 45°C leads to a density change of 1% while a pressure of 200 atmospheres is also required to increase the density by 1% [17].

We suggest that the lethal effect at small intensity fields at multiple shot exposures is due to the Gaussian nature of biological cell distribution (cell size, age of the cells, etc). Detailed knowledge of this distribution throughout treatment is required, if we are to appreciate the multiple-shots experiments.

Derivation of the theory points to a complete analogy with the electrical breakdown in some gases and solids [18]. The lethal effects on micro-organisms depend on (a) the applied electric field exceeding the critical field of membrane breakdown to form holes and (b) the Joule energy (deposited in the membrane) exceeding a minimum value beyond which the cell can not recover. In electrical discharge phenomena, breakdown is dependant on these same parameters. However, the second one is modified to read Joule energy per length of the dielectric (per thickness of the 'membrane' ranging from a few μm to tens of metres).

An objection to our approach has been raised, to the extent that the effect of shape distribution may be equally or more important than the linear or volumetric size of the cells. This may indeed be the case. However, as our criteria are integral relations (see Eq. (9a)), we are confident that the effect of shape will be of second order in magnitude. We intend to discuss this in a subsequent paper. Here, we are only attempting to show that the functional relations derived, fit the measurements of a number of workers in the field. This would be convincing proof if the constants K and γ had been independent of the pulse number (Fig. 7). Although this dependence is weak and within the limits of experimental scatter, it shows the requirement for theoretical refinement.

Our views essentially confirm the pictures of electroporation delineated by previous workers and extends them to the statistical distribution of cell parameters. In the future we propose extending the treatment while considering the thermal widening of pores combined with healing mechanisms. This will necessitate detailed consideration of current flow in and around the membrane perforation as carried out by Pastushenko [19]. We have made a distinction between reversible and irreversible breakdown, as was discussed by Glaser et al. [20], as this depends strongly on the surface chemistry of the interfaces involved, although as demonstrated by Chernomordik et al. [21], lysis is invariably associated with irreversible discharges.

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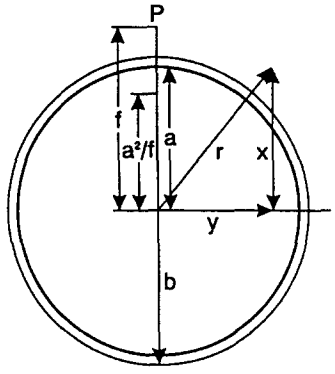

Scheme 1. Schematic for current flow past a sphere with radical holes.

Appendix A

A.1. Current lines around a sphere with two radial holes

We assume that most current passes around the sphere (i.e., the membrane resistance is high), and that some travels through two diametrically opposite holes with their connecting lines parallel to the applied field. Also, the holes can be represented electrically by a point source and sink with equal but opposite strength. The hydrodynamic analogy of a source at distance f (see Milne-Thompson [5] and Scheme 1) has the image of another source of strength ma/f where m is the original source strength at a point a. The distance is a^2/f from the centre, i.e., the inverse point plus a line source of strength, $-m/a$, extending from the inverse point to the centre. The stream function Ψ at A on the sphere, the source and its image are given by:

$$\Psi = ky^2\left[1-(a/r)^3\right] + \frac{m(x-f)}{\sqrt{(x-f)^2+y^2}} + \frac{ma(x-a^2/f)}{\sqrt{(x-a^2/f)^2+y^2}} - (m/a)\left[r-\sqrt{(x-a^2/f)^2+y^2}\right] \quad \text{(A1)}$$

where $r=(x^2+y^2)^{1/2}$. To complete the solution, this stream function is applied to the source of strength m at $f=a$ and the sink of strength $-m$ at $f=-a$, giving:

$$\Psi = ky^2\left[1-(a/r)^3\right] + \Psi_1 + \Psi_2$$

where

$$\Psi_1 \frac{2m(x-a)}{\sqrt{(x-a)^2+y^2}} - (m/a)\left[r-\sqrt{(x-a)^2+y^2}\right]$$

$$\Psi_2 \frac{2m(x+a)}{\sqrt{(x+a)^2+y^2}} + (m/a)\left[r-\sqrt{(x+a)^2+y^2}\right]$$

Here, $k=E/2$ where E is the electric field strength at infinity, and m depends on the hole's diameter.

Derivation of the voltage across the membrane as a function of the polar angle, θ requires spherical coordinates (r,θ). The potential outside the sphere, v_0, is

$$v_0 = E\cdot\cos\theta + m/\sqrt{f^2+r^2-2fr\cdot\cos\theta}$$

Similarly, the potentials v_1, v_2 and v_3 can be determined for the three media. The last term can be expanded into a Legendre polynomial series:

$$mf\sum_n (r/f)^n P_n(\cos\theta)$$

where $P_n(\cos\Theta)$ are Legendre polynomials. If the radius of the inner sphere is b, i.e., $a-b=$ membrane thickness, then the boundary conditions are:

$$r=a,\quad v_1+v_0=v_2,\quad (v_1+v_0)'=G_1'\cdot v_2'$$

$$r=b,\quad v_2=v_3,\quad v_2'=G_2\cdot v_3'$$

where G_1 and G_2 are conductivity ratios and the primes denote differentiation with respect to r (partial). The potentials, v_1, v_2 and v_3 can be expressed in terms of Legendre series:

$$v_1 = \sum_n A_n.r^{-n-1}P_n$$

$$v_2 = \sum_n \left(B_n.r^{-n-1}+C_n r^n\right)P_n$$

$$v_3 = \sum_n D_n r^n P_n$$

where A_n, B_n and D_n are constants to be determined by substitution into the boundary conditions and equating terms of equal order n. The final membrane potential V is then also given by a Legendre series,

$$V = \sum_n E_n.P_n(\cos\theta)$$

where each term E_n is expressed as:

$$E_n = m\cdot(b/f)^n C_n \times\left[\frac{n(1-G_2)}{1+n+G_2 n}\left(1-(b/a)^{n+1}\right)+1-(a/b)^n\right]$$

where

$$C_n = \frac{(n+2)/(1+n+nG_1)}{1+\dfrac{n(n+1)(1-G_1)(1-G_2)(b/a)^{2n+1}}{(1+n+nG_2)(1+m+nG_1)}}$$

This expression for V is identical with the one given in Eq. (7) in the main text, provided $m=0$. The course of this function is shown in Fig. 3.

The solution above applies to a single point source at distance f from the centre. To obtain the final solution for two holes at opposite ends of a diameter, one must put $f=a$ and add a second source of strength $-m$ at $f=-a$. This results in the suppression of all even order terms in the Legendry series.

The Appendix proves that if the membrane is overvolted, the voltage near the hole is lessened ('relieved') but builds up again further away from the hole. This supports our contention that a pattern of holes will develop around the apex of the cell.

It is hoped that the current derivation will offer theoretical support for the observed fluorescence changes of two narrow bright bands appearing around two loci facing the electrodes [2], and that numerous (> 1000) discrete electropores are induced in the membranes [12], when the electric field increases. These results [12] are in accordance with the observation found in the classical breakdown phenomena in gases [18].

Appendix B

B.1. Lysis by multiple-shot exposure

If the cells are shot repeatedly, the right hand side of Eq. (9a) must be multiplied by a factor reflecting the increased membrane hole area. After n shots, the fraction of surface area covered by holes is a_n. We can not assume that $a_n = na_1$, as some holes will land in previously exposed areas and inflict no further damage. This is apparent, when the suspension is not stirred between shots, because no further cells die when the survival curve ranges about $10^{-2}\%$. Hence, the probability that shot $n+1$ will strike an unexposed surface is reduced by the factor $1 - a_n$. This leads to the relation:

$$a_{n+1} = a_n + a_1(1 - a_n)$$

This recurrence relation defines a set of (non-orthogonal) polynomials in a_1. These are geometrical progressions in powers of $1 - a_1$ which can be summed using:

$$a_n = 1 - (1 - a_1)^{n+1}$$

This shows that $a_1 = 1 - 1/x$, where x is defined by Eq. (9c) in the main text. To account for exposure to n shots, it follows that the right hand side of Eq. (9c) must be multiplied by:

$$(1 - 1/x^n)/(1 - 1/x)$$

The second bracket ensures that the expression will reduce to unity when $n = 1$. If the value of cell radius b for constant Joule dosage is required, we get:

$$b \sim \left((E - E_o)^2 \Delta t\right)^{-3/10} \left[(1 - 1/x)/(1 - 1/x^n)\right]^{1/5} \quad (9d)$$

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