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Bioelectrorheological Model of the Cell. 4. Analysis of the Extensil Deformation of Cellular Membrane in Alternating Electric Field

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ABSTRACT Analysis of the angular distribution of extensil mechanical stress, σ^e, generated in cytoplasmic membranes by an external oscillating electric field, is presented. Theoretical considerations show that σ^e is directly proportional to the local relative increase in membrane area and/or to the local relative decrease in its thickness. The magnitude of this stress depends on the position of the analyzed point of the membrane in relation to field direction. The maximal value, σ_0^e, is reached at the cell "poles." The magnitude of σ_0^e depends on electric and geometric parameters (in particular on field frequency) of the system studied.

The foregoing analysis can be applied to quantitatively describe the destabilizing effects of the electric field on the cellular membrane, leading to its poration, fusion, and destruction.

INTRODUCTION

An external electric field acting upon artificial lipid vesicles or cells causes an alteration in membrane potential and generates different types of mechanical stress in the membrane (1). Theoretical analysis suggests that, within a definite range of conditions, the integrity of the membrane may be disturbed. This is supported by experiments, in which destabilization of the membrane manifests itself either by a transient increase in its permeability (2, 3) or by its irreversible breakdown (4). Both processes have been extensively investigated in connection with the widespread use of electroporation and electrofusion in molecular and cellular biology (5). High intensity rectangular pulses (6), exponentially decaying pulses (7), and a superposed oscillating field with rectangular pulses (8) have been applied in these experiments.

Though several theoretical treatments of the reversible electrical breakdown of the membrane have been proposed, this complex process is not yet well understood. Previously published theoretical models have considered various mechanisms: (i) electromechanical compression of a continuous elastic body, resulting in membrane defects (9–12); (ii) destabilization and widening of defects of the membrane (13, 14); (iii) redistribution and reorientation of the molecular constituents of the membrane (15, 16).

Recently, a local sonicative effect of an oscillating electric field on charged membrane molecules has been assumed (but not quantitatively discussed) to be a destabilizing factor (8, 17).

In this study theoretical analysis of the angular distribution of extensil stress in the membrane, modeled as a thin spherical shell subjected to alternating electric field, is proposed. The extensil stress is defined as the difference between two components of total stress corresponding to extension and compression of the membrane at a given point (Fig. 1). The model considers Maxwell stress developing at the surfaces of the shell, averaged over time. Complex notation enables the description of the dissipation effects in the system (dielectric losses, conducting media). The shell is regarded as an elastic body. Pressure alterations in the internal medium, resulting from cell deformation, are accounted for.

Rigorous considerations of the mechanical properties of the membrane lead to respective constitutive equations

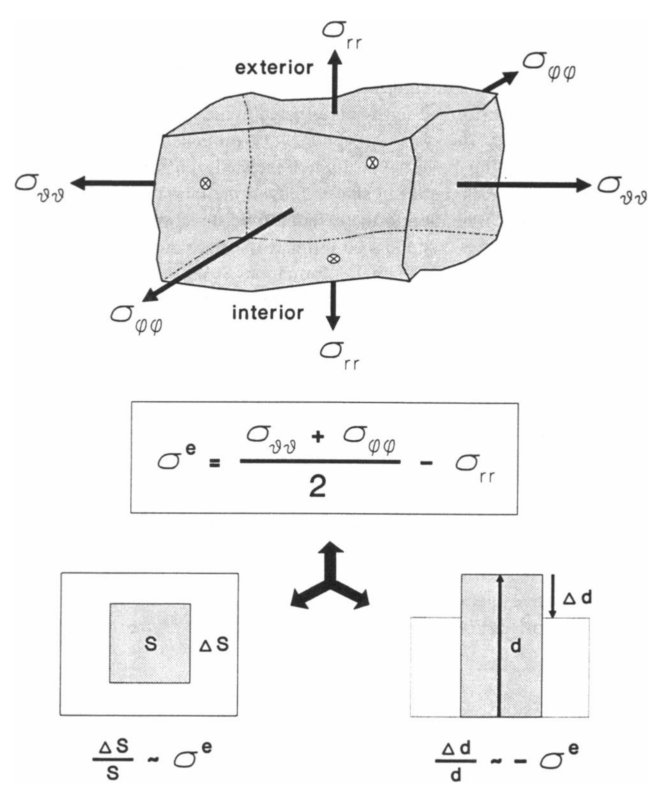

FIGURE 1 Graphic presentation of extensil stress, σ^e, acting on an imaginary sector of the membrane. σ_{ij} is the components of stress tensor in the spherical co-ordinates system (r, ϑ, φ).

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which relate relative local alterations of both area and thickness of the membrane to extensil stress. It is assumed that under definite conditions the development of the extensil stress in the cellular membrane may result in its reversible or irreversible damage. Thus, it is suggested that the proposed analysis may be useful for quantitatively describing processes such as electroporation, electrofusion, and electrodestruction.

An analysis of angular distribution of stress, based on the equilibrium equation for a thin spherical shell, is performed. The value of stress depends on electric field amplitude and frequency, and on the electric and geometric parameters of the system.

THEORETICAL ANALYSIS OF EXTENSIL STRESS

Electromechanical model

The electromechanical model considers local isothermal variations of the area and thickness of a thin spherical shell separating two different media, which is subjected to external homogeneous alternating electric field. The increments of both stress and deformation are calculated in relation to the initial state of the shell. The shell is described as a homogeneous elastic body with a noncompressible volume; both its area and thickness are assumed to be weakly compressible. The elastic properties of the shell are locally isotropic in a plane tangential to the shell surface. Internal and external media are regarded as homogeneous, isotropic, and not compressible; they are also nonviscous liquids.

From the electric point of view all media are generally considered to be homogeneous, isotropic liquids with some dielectric and conductive properties (relaxation is admissible). The mechanism of Maxwell-Wagner polarization is taken into consideration, and the resulting Maxwell stress is calculated. A zero volumetric density of free charges is assumed. Additional effects related to change in the dielectric permittivity (due to the deformation) as well as to the magnetic component of stress are neglected.

Analysis of stress and deformation, both averaged over time (period of electric field), is performed for points situated in the middle of the shell, at its initial thickness. The system of spherical co-ordinates (r, ϑ, ψ) is used. The surface including the initial position of the analyzed points is described for $r = R$, where R is the mean shell radius in the initial state of shell. ϑ denotes the angle in relation to the external field direction. The following analysis does not depend on the ψ co-ordinate due to rotatory symmetry. An approximation of the infinitesimal deformations is used.

The constitutive equations relating the mechanical stress tensor in the shell, σ_{ij}, to the deformation tensor, ϵ^{ij}, in directions of the local reference system corresponding to versors of the spherical co-ordinates $\hat{r}$, $\hat{\sigma}$, *and* $\hat{\varphi}$ may be presented in matrix form:

$$\begin{bmatrix} \epsilon_{rr} \\ \epsilon_{\vartheta\vartheta} \\ \epsilon_{\varphi\varphi} \end{bmatrix} = \begin{bmatrix} A & B & B \\ B & C & D \\ B & D & C \end{bmatrix} \begin{bmatrix} \sigma_{rr} \\ \sigma_{\vartheta\vartheta} \\ \sigma_{\varphi\varphi} \end{bmatrix} \quad (1)$$

where A, B, C, and D, are components of the tensor of the compressibility coefficients. They are constant when the local reference system rotates around the $\hat{r}$ axis.

Local relative alterations of shell thickness, $\Delta d/d$, of shell area, $\Delta S/S$, and of shell volume, $\Delta V/V$, are expressed as components of the ϵ_{ij} tensor as follows.

$$\frac{\Delta d}{d} = \epsilon_{rr} \quad (2)$$

$$\frac{\Delta S}{S} = \epsilon_{\vartheta\vartheta} + \epsilon_{\varphi\varphi} \quad (3)$$

$$\frac{\Delta V}{V} = \epsilon_{rr} + \epsilon_{\vartheta\vartheta} + \epsilon_{\varphi\varphi} \quad (4)$$

In addition, $\Delta V/V$ is zero for each stress, σ_{ij}, developed in a volumetrically not compressible shell. This condition, together with Eqs. 1 and 4), leads to following relationships for A, B, C, and D coefficients as follows.

$$A + 2B = 0 \quad (5)$$

$$B + C + D = 0 \quad (6)$$

Using Eqs. 1, 5, and 6, $\Delta d/d$ and $\Delta S/S$ may be expressed as

$$\frac{\Delta d}{d} = -\frac{\Delta S}{S} \quad (7)$$

$$\frac{\Delta S}{S} = \frac{1}{K_{be}}\sigma^{e} \quad (8)$$

where K_{be}, biaxial extensional elasticity, is defined as $K_{be} = 1/A$, and the mechanical extensil stress, σ^e, is defined as follows.

$$\sigma^{e} = \tfrac{1}{2}(\sigma_{\vartheta\vartheta} + \sigma_{\varphi\varphi}) - \sigma_{rr} \quad (9)$$

As shown below (Appendix 1), in an oscillating uniform external electric field the angular distribution of σ^e assumes the form:

$$\sigma^{e} = \tfrac{1}{2}\sigma_0^{e}(3\cos^2\vartheta - 1) \quad (10)$$

where σ_0^e is the maximal value of the extensil stress, depending on both strength and frequency of the electric field and on the electric and geometric parameters of the system.

Stress, σ^e, attains extreme values, amounting to σ_0^e and $-0.5\ \sigma_0^e$, at the poles ($\vartheta = 0°, 180°$) and at the equator ($\vartheta = 90°$) of the shell, respectively. The change in the sign of σ^e reflects variations of the character of stress, as a function of the position of the analyzed point, in relation to the field direction (compression versus extension). Stress, σ_0^e, is bound to the radial increments of the mechanical normal stress $(\Delta\sigma_{rr})^{var}$ and to the mechanical tangential stress, $\sigma_{r\vartheta}^{var}$:

$$\sigma_0^{e} = 2\left[\frac{1}{6}\frac{1+K}{1-K}(\Delta\sigma_{rr})^{var} + \sigma_{r\vartheta}^{var}\right] \quad (11)$$

where K is the geometric parameter of the shell equaling the ratio of the internal to external radius (see Appendix 2, Eq. A53).

Stresses $(\Delta\sigma_{rr})^{var}$, $\sigma_{r\vartheta}^{var}$ are generated by Maxwell stress at the surface of the shell. Consequently, they depend on the amplitude of the electric field in square, field frequency, and on electric and geometric parameters of the system.

Connotation "var," implies that these components describe the variations of stress with changes in angular co-ordinate ϑ. As a consequence of the assumed noncompressibility of the internal medium, the constant element of stress, "con," has no share in Eq. 11.

According to Eqs. 7, 8, and 10, extensil deformations $\Delta d/d$ and $\Delta S/S$ are related to σ_0^e and to the ϑ position:

$$\frac{\Delta S}{S} = -\frac{\Delta d}{d} = \frac{1}{2K_{be}}\sigma_0^{e}(3\cos^2\vartheta - 1) \quad (12)$$

The values corresponding to variations of $\Delta d/d$ and $\Delta S/S$ may be essential when the integrity of the shell is considered. Accordingly, when electroporation and electrodestruction of cells are discussed on the basis of the shell model, it is necessary to find σ_0^e, together with its dependence on field frequency and on other experimentally controlled physical parameters.

As an example of application of the foregoing theoretical analysis (Appendixes 1 and 2), the numerical values of σ_0^e as a function of field frequency were calculated for *Neurospora crassa* cells at different conductivities of the external medium and constant field strength (Fig. 2).

Stress, σ_0^e, assumes positive values within the whole range of the investigated field frequencies. In accordance with Eq. 12, maximal extensil stress, σ_0^e, represents thickness compression and area extension of the membrane at a cell poles. At low field frequencies σ_0^e assumes a 1high constant value. Under these conditions the membrane behaves as a dielectric placed between the capacitor conducting plates. The membrane is compressed by

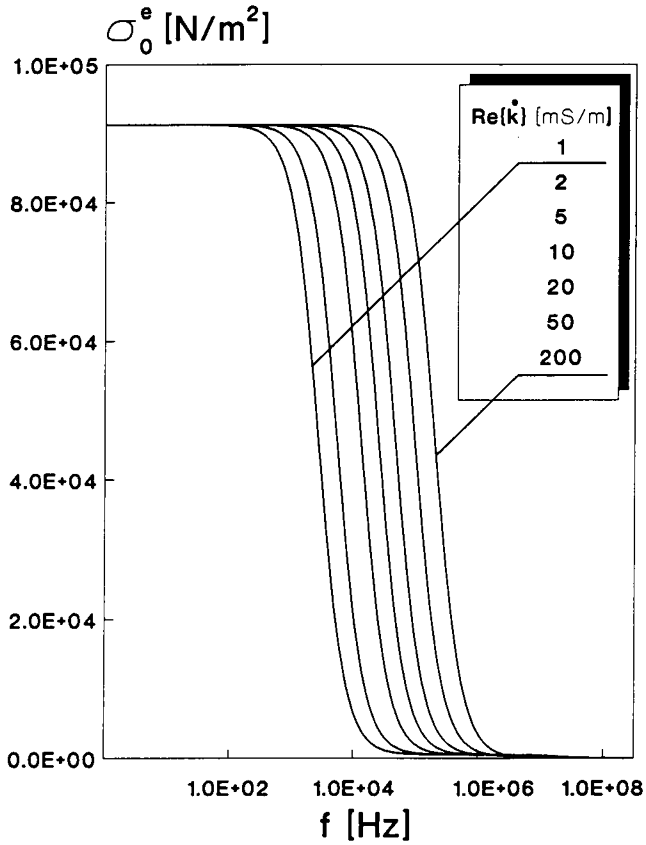

FIGURE 2 Calculations of maximal extensil stress, σ_0^e, developed in the membrane of a *N. crassa* cell at its "pole," as a function of field frequency, *f*, for various conductivities of the external medium, Re[k]. Field strength E_0 = 25 kV/m; other parameters are as in Appendix 2 of Ref. 22.

the charges accumulated at the surfaces of the plates. Thus, the magnitude of stress depends on the membrane dielectric properties (real part of dielectric permittivity), membrane thickness, and cell radius.

The region of a constant stress value is limited to the low-frequency range. It expands with an increase in the external medium conductivity. With a further increase in frequency, the curves illustrating the values of the maximal extensil stress, σ_0^e, decline sigmoidally, owing to relaxation of Maxwell-Wagner polarization. For a given set of physical parameters, at high field frequencies ($f \geq 10^5$ Hz), the curves representing σ_0^e as a function of field frequency proceed asymptotically toward relatively low values. For these frequencies, Maxwell-Wagner polarization becomes negligible when compared to the polarization of ideal dielectrics.

DISCUSSION

This paper is a successive contribution to the cycle of publications (1, 18–20) aimed at a full, general theoretical and experimental analysis of mechanical stress generated in cell and cellular membrane by an alternating electric field within the range of field frequencies $f = 10^2$–10^7 Hz. This work is limited to the frequency range where the previously analyzed shearing forces (20) decrease and extensil forces increase (1). The presented analysis may provide better insight into the basic mechanisms, control, and regulation of the effects such as electroporation, electrofusion, and electrodestruction. These phenomena have so far been discussed mostly in terms of electric models.

The present analysis shows that mechanical stress in cellular membranes is generated by an alternating electric field as a result of the action of Maxwell stress on membrane areas facing internal and external media. It is suggested that extensil stress may substantially contribute to generation of the above mentioned effects.

Mechanical stress was theoretically evaluated by including into calculations the dielectric permittivity and electric conductivity of the external and internal medium and the membrane, nonzero magnitude of field frequency, variations of cytoplasmic pressure (bound to cell deformation), and curvature of cell surface. These factors have not been taken into account when electroporation was previously explained in terms of an electromechanical model (10).

The calculated values of extensil stress in the membrane represent increments of this stress, relative to the initial state of the cell being in equilibrium with the medium, in the absence of external electric field. It remains unclear how this initial state, which may differ for various cells, influences their reaction to the external field and to the stress created by this field.

The analysis presented in this work permitted determination of the extensil stress in the shell considered in the "Electromechanical model." If K_{be} is known from independent studies, it could be used in the determination of the extensil deformation.

This analysis could be applied in predictions of the effects of variations in electric and geometric parameters on stress values (i.e., Fig. 2). However, predictions concerning how this stress would modify the system, if a given stress is sufficient for cell disruption, and how K_{be} depends on other parameters are not allowed.

The above analysis was applied to the more complex case of a cell surrounded by a cellular membrane. Then, the mechanical parameter, K_{be} became an effective one, its value resulted from different contributions of all considered media, their heterogeneity included (22).

APPENDIX 1: EXTENSIL MECHANICAL STRESS, σ^e, IN THE SHELL, AVERAGED OVER THE ELECTRIC FIELD PERIOD

Stress distribution averaged over the field period, within a thin spherical shell, was analyzed to determine the temporal mean of extensil stress, σ^e.

When the mass acceleration effects are neglected, Newton's Second Law can be formulated in tensor form for the forces acting on a shell subjected to an alternating electric field:

$$\sum_{j=1}^{3} \frac{\partial}{\partial x_j} T_{ij} + f_i^{el} = 0 \quad (i = 1, 2, 3) \tag{A1}$$

where T_{ij} is the temporal mean of the symmetric tensor of mechanical stress in the shell, f_i^{el} is the temporal mean of the volumetric density of electric forces, x_j is the Cartesian co-ordinate of the deformed shell.

Assumptions about the electric properties of the system (see Text) allow for neglecting volumetric density of the electric forces ($f_i^{el} = 0$). Then, under approximation for infinitesimal deformations, and for points situated in the middle of the shell thickness ($r = R$), Eq. A1, formulated using spherical co-ordinates r, ϑ, and φ, for the force balance in the direction of versor $\hat{r}$, assumes the following form.

$$\frac{\partial}{\partial r}T_{rr} + \frac{1}{R}\frac{\partial}{\partial \vartheta}T_{r\vartheta} + \frac{1}{R}[2T_{rr} - (T_{\vartheta\vartheta} + T_{\varphi\varphi}) + T_{r\vartheta}\mathrm{ctg}(\vartheta)] = 0 \quad \text{(A2)}$$

When the system is in its natural orientation the description of the system does not depend on the angular co-ordinate φ (see text).

Extensil stress T can be formulated as follows.

$$T^e = \tfrac{1}{2}(T_{\vartheta\vartheta} + T_{\varphi\varphi}) - T_{rr} \quad \text{(A3)}$$

It was assumed that in the initial state (nat), in the absence of external electric field, stress is distributed with spherical symmetry. In this case, equation A2. is of the following form.

$$\frac{\partial}{\partial r}T_{rr}(\mathrm{nat}) - \frac{2}{R}T^e(\mathrm{nat}) = 0 \quad \text{(A4)}$$

Subtracting equations A2 and A4 gives:

$$\frac{\partial}{\partial r}\sigma_{rr} + \frac{1}{R}\frac{\partial}{\partial \vartheta}\sigma_{r\vartheta} - \frac{2}{R}\left[\sigma^e - \frac{1}{2}\sigma_{r\vartheta}\,\mathrm{ctg}(\vartheta)\right] = 0 \quad \text{(A5)}$$

where $\sigma = T - T(\mathrm{nat})$ expresses the variations of the stress values in relation to the initial state, and as such it is to be analyzed in the following calculations. For the sake of brevity, σ is referred to as stress.

Extensil stress, σ^e, can be calculated by solving the Eq. A5 when the constituent $\sigma_{r\vartheta}$ and radial gradient $(\partial/\partial_r)\sigma_{rr}$ are known.

In the case of a thin spherical shell, the unknown constituent and the gradient can be substituted, respectively, by:

$$\sigma_{r\vartheta} = \frac{\sigma_{r\vartheta}^{\mathrm{int}} + \sigma_{r\vartheta}^{\mathrm{ext}}}{2} \quad \text{(A6)}$$

$$\frac{\partial}{\partial r}\sigma_{rr} = \frac{\sigma_{rr}^{\mathrm{ext}} - \sigma_{rr}^{\mathrm{int}}}{d} \quad \text{(A7)}$$

where the upper indices denote points on the internal (int) and external (ext) surfaces of the shell, respectively, at a fixed angular co-ordinate ϑ.

By definition, the stresses on shell surface represent the respective constituents of the area density of forces, $\vec{f}^{\,\mathrm{int}}, \vec{f}^{\,\mathrm{ext}}$, acting on the internal and external shell surfaces, respectively.

$$\sigma_{rr}^{\mathrm{ext}} = \vec{f}^{\,\mathrm{ext}} \cdot \vec{e}_r \quad \text{(A8)}$$

$$\sigma_{r\vartheta}^{\mathrm{ext}} = \vec{f}^{\,\mathrm{ext}} \cdot \vec{e}_\vartheta \quad \text{(A9)}$$

$$\sigma_{rr}^{\mathrm{int}} = -\vec{f}^{\,\mathrm{int}} \cdot \vec{e}_r \quad \text{(A10)}$$

$$\sigma_{r\vartheta}^{\mathrm{int}} = -\vec{f}^{\,\mathrm{int}} \cdot \vec{e}_\vartheta \quad \text{(A11)}$$

In the presence of an electric field, the conditions required for attainment of force equilibrium on boundary surfaces ca be expressed as follows.

$$\vec{f}^{\,\mathrm{int}} = (\overset{s}{\Pi}_m - \overset{i}{\Pi}_m + \overset{i}{p}\delta) \cdot \vec{e}_r \quad \text{(A12)}$$

$$\vec{f}^{\,\mathrm{ext}} = (\overset{e}{\Pi}_m - \overset{s}{\Pi}_m - \overset{e}{p}\delta) \cdot \vec{e}_r \quad \text{(A13)}$$

where Π_m is the Maxwell electric stress tensor, p is constant pressure (21), δ is the unit tensor, i, s, and e are the upper indices denoting internal medium, shell and external medium, respectively (all values represent variations averaged over time, in relation to the initial state).

Maxwell stress is proportional to squared field strength. It was assumed that the resulting electric field consists of both constant field occurring in the initial state and the external oscillating electric field $\mathrm{Re}[\tilde{E}\exp(i_u\omega t)]$. In this case Maxwell stress increments averaged over time represent contributions from the external field only.

In complex notation, where the induction of the electric field $\tilde{D}$ is $\mathrm{Re}[\epsilon\tilde{E}\exp(i_u\omega t)]$, the constituents of the Maxwell stress tensor (averaged over the field period) can be expressed as:

$$(\Pi_m)_{ij} = \frac{1}{4}\mathrm{Re}[\epsilon]\left[E_i^*E_j + E_iE_j^* - \left(\sum_{k=1}^{3}E_kE_k^*\right)\delta_{ij}\right] \quad (i, j = 1, 2, 3) \quad \text{(A14)}$$

where * denotes complex conjugation.

When the distribution of the additional electric field in a shell and in the surrounding medium is known (see Appendix 2), Eqs. A8–A14 lead to the following relationships:

$$\frac{\sigma_{r\vartheta}^{\mathrm{int}} + \sigma_{r\vartheta}^{\mathrm{ext}}}{2} = 2\sigma_{r\vartheta}^{\mathrm{var}}\sin(\vartheta)\cos(\vartheta) \quad \text{(A15)}$$

$$\sigma_{rr}^{\mathrm{ext}} - \sigma_{rr}^{\mathrm{int}} = (\Delta\sigma_{rr})^{\mathrm{con}} + 2\,(\Delta\sigma_{rr})^{\mathrm{var}}\cos^2(\vartheta) \quad \text{(A16)}$$

where

$$\sigma_{r\vartheta}^{\mathrm{var}} = -\tfrac{1}{4}(\alpha_1 + \alpha_2)E_0^2 \quad \text{(A17)}$$

$$(\Delta\sigma_{rr})^{\mathrm{con}} = [\Delta\sigma_{rr}(p)]^{\mathrm{con}} + [\Delta\sigma_{rr}(\mathrm{el})]^{\mathrm{con}} \quad \text{(A18)}$$

$$[\Delta\sigma_{rr}(p)]^{\mathrm{con}} = \overset{i}{p} - \overset{e}{p} \quad \text{(A19)}$$

$$[\Delta\sigma_{rr}(\mathrm{el})]^{\mathrm{con}} = (\beta_2 - \beta_1)E_0^2 \quad \text{(A20)}$$

$$(\Delta\sigma_{rr})^{\mathrm{var}} = \tfrac{1}{2}(\alpha_2 - \alpha_1 + \gamma_2 - \gamma_1)E_0^2 \quad \text{(A21)}$$

and where

$$\alpha_1 = \tfrac{1}{2}\mathrm{Re}[\overset{i}{\epsilon}]|\overset{i}{m}|^2 - \tfrac{1}{2}\mathrm{Re}[\overset{s}{\epsilon}](|\overset{s}{m}|^2 + \mathrm{Re}[\overset{s}{m}\overset{s}{n}^*] - 2|\overset{s}{n}|^2) \quad \text{(A22)}$$

$$\beta_1 = \tfrac{1}{4}\mathrm{Re}[\overset{s}{\epsilon}]|\overset{s}{m} - \overset{s}{n}|^2 - \tfrac{1}{4}\mathrm{Re}[\overset{i}{\epsilon}]|\overset{i}{m}|^2 \quad \text{(A23)}$$

$$\gamma_1 = -\tfrac{9}{4}\mathrm{Re}[\overset{s}{\epsilon}]|\overset{s}{n}|^2 \quad \text{(A24)}$$

$$\alpha_2 = \tfrac{1}{2}\mathrm{Re}[\overset{e}{\epsilon}](1 + \mathrm{Re}[\overset{e}{n}] - 2|\overset{e}{n}|^2) - \tfrac{1}{2}\mathrm{Re}[\overset{s}{\epsilon}](|\overset{s}{m}|^2 + \mathrm{Re}[\overset{s}{m}\overset{s}{n}^*]K^3 - 2|\overset{s}{n}|^2K^6) \quad \text{(A25)}$$

$$\beta_2 = \tfrac{1}{4}\mathrm{Re}[\overset{s}{\epsilon}]|\overset{s}{m} - \overset{s}{n}K^3|^2 - \tfrac{1}{4}\mathrm{Re}[\overset{e}{\epsilon}]|1 - \overset{e}{n}|^2 \quad \text{(A26)}$$

$$\gamma_2 = \tfrac{9}{4}\mathrm{Re}[\overset{e}{\epsilon}]|\overset{e}{n}|^2 - \tfrac{9}{4}\mathrm{Re}[\overset{s}{\epsilon}]|\overset{s}{n}|^2K^6 \quad \text{(A27)}$$

where E_0 is the real amplitude of the external electric field, K is the geometric parameter of a shell, | | is the complex modulus. Complex numbers n and m with upper indices are coefficients of field distribution, which depend on its frequency, on electric parameters of the system and on K. For details see Appendix 2.

In Eqs. A15 and A16, the constant part (con) and the part varying with angular coordinate ϑ (var) (for $\vartheta = 45°$) were separated from the mean stress and from the radial increment. Additionally, in Eqs. A18–A20 the elements related to mechanical pressure (p) and to the electric field (el) were separated in part con of radial increment. Taking into account the relative volume variations $\Delta V/V$ of the internal medium we can write:

$$[\Delta\sigma_{rr}(p)]^{\mathrm{con}} = -\overset{i}{K}_v\frac{\Delta\overset{i}{V}}{\overset{i}{V}} \quad \text{(A28)}$$

where $\overset{i}{K}_v$ is the volumetric compressibility modulus of the internal medium.

Use is made of Eqs. (A5, A6, A7, A15, A16, A18, and A28) to obtain:

$$\sigma^{e} = \left\{\frac{1}{4}\left(\frac{1+K}{1-K}\right)\left[-\overset{i}{K}_{v}\left(\frac{\Delta \overset{i}{V}}{\overset{i}{V}}\right) + [\Delta\sigma_{rr}(\mathrm{el})]^{\mathrm{con}} + \frac{2}{3}\Delta\sigma_{rr}^{\mathrm{var}}\right] + \left[\frac{1}{6}\left(\frac{1+K}{1-K}\right)\Delta\sigma_{rr}^{\mathrm{var}} + \sigma_{r\vartheta}^{\mathrm{var}}\right](3\cos^{2}\vartheta - 1)\right\} \quad (A29)$$

In the description of the electromechanical model, it was shown that stress σ^{e} is associated with the relative local variations of shell surface $\Delta \overset{i}{S}/\overset{i}{S}$. As a result of volumetric noncompressibility of the internal medium ($\Delta V/V = 0$) and of the shell ($\Delta V/V = 0$), the global surface of the shell remains unchanged. For the medium surrounded by a shell of the radius, R, surface, S_R, and volume, V_R, we obtain:

$$\iiint_{r\le R} \sum_{k=1}^{3} \frac{\partial u_k}{\partial x_k}\, dV_R = 0 \quad (A30)$$

and

$$\iiint_{r\le R} \sum_{k=1}^{3} \frac{\partial u_k}{\partial x_k}\, dV_R = \iint_{r=R} \sum_{k=1}^{3} u_k n_k\, dS_R = \frac{1}{2} R \iint_{r=R} \frac{\Delta S}{S}\, dS_R \quad (A31)$$

where u_k is the displacement vector.

Use was made of Gauss' theorem and of the fact that the local relative variations of shell surface are approximately proportional to the displacement vector. Precisely, the u_ϑ component does not contribute to the surface integral.

$$\frac{\Delta S}{S} = 2\frac{u_r}{R} + \ldots \quad (A32)$$

Using Eq. 8 (see Electromechanical Model section) and Eqs. A30 and A31 one gets:

$$\int_{0}^{\pi} \sigma^{e} \sin\vartheta\, d\vartheta = 0. \quad (A33)$$

Application of the A33 condition in Eq. A29 results in the following relationships:

$$\overset{i}{K}_{v} \frac{\Delta \overset{i}{V}}{\overset{i}{V}} = [\Delta\sigma_{rr}(\mathrm{el})]^{\mathrm{con}} + \frac{2}{3}\Delta\sigma_{rr}^{\mathrm{var}} \quad (A34)$$

and

$$\sigma^{e} = \tfrac{1}{2}\sigma_{0}^{e}(3\cos^{2}\vartheta - 1) \quad (A35)$$

where

$$\sigma_{0}^{e} = 2\left[\frac{1}{6}\frac{1+K}{1-K}\Delta\sigma_{rr}^{\mathrm{var}} + \sigma_{r\vartheta}^{\mathrm{var}}\right] \quad (A36)$$

Stress σ_0^e is the maximal extensil stress on cell poles ($\vartheta = 0°, 180°$).

APPENDIX 2: ELECTRIC FIELD DISTRIBUTION

In the description of alternating electric field in lossy media, by applying complex notation the induction of the electric field, $\vec{D}$, assumes the form:

$$\vec{D} = \mathrm{Re}[\epsilon \vec{E} \exp(i_u \omega t)] \quad (A37)$$

where $\vec{E}$ is the complex electric field amplitude, ϵ is the complex dielectric permittivity of the medium, $(\omega/2\pi)$ is the field frequency, t is time, i_u is an imaginary unit, and $\mathrm{Re}[\cdot]$ is the real component of the complex number.

In the electric model it is assumed that all media are homogeneous, isotropic, and neural. According to Maxwell's laws, for complex values we can write:

$$\mathrm{div}(\epsilon \vec{E}) = 0 \quad (A38)$$

$$\mathrm{div}[(k + i_u \omega\, \epsilon)\vec{E}] = 0 \quad (A39)$$

where k is the complex conductivity of the medium

$$\mathrm{div} = \sum_{j=1}^{3} \frac{\partial}{\partial x_j}.$$

Equation A39 shows the boundary conditions at the boundary between medium a and medium b:

$$\overset{a}{\xi}\, \overset{a}{\vec{E}} \cdot \vec{n} = \overset{b}{\xi}\, \overset{b}{\vec{E}} \cdot \vec{n} \quad (A40)$$

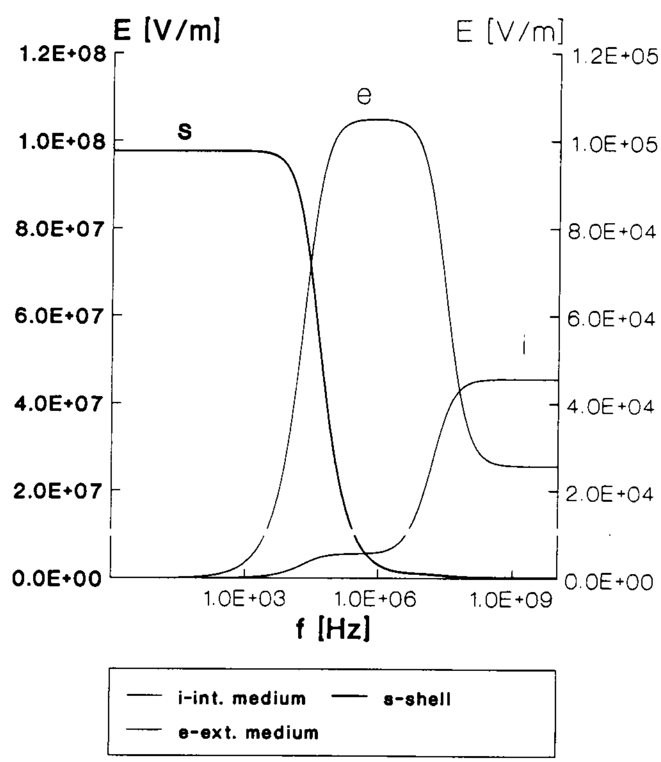

FIGURE 3 Theoretical relationship between the distribution of field amplitude $(E \cdot E^{*})^{1/2}$ in cytoplasm, inside the cellular membrane and at the external cell surface, ($\vartheta = 0°, 180°$), and the external field frequency, f. Field strength $E_0 = 25\mathrm{kV/m}$. Electric and geometric parameters for *N. crassa* cells taken from Appendix 2 of Ref. 22.

where

$$\xi = \epsilon - i_u\left(\frac{k}{\omega}\right) \quad (A41)$$

where $\vec{n}$ is the vector normal to the boundary surface.

If the assumption is made that the external electric field $\vec{E}_0$ is homogeneous either far from the cell or that no cell is present, and there is zero phase displacement, the complex field amplitude $\vec{E}_0$ is of the form:

$$\vec{E}_0 = E_0\vec{e}_f \quad (A42)$$

where $\vec{E}_0$ is the real amplitude of the external electric field, $\vec{e}_f$ is the versor of the field direction.

In the system of spherical co-ordinates, r, ϑ, φ, (cf. text under Electromechanical Model section), solving of the equation A38 with consideration of the boundary conditions A40 and of the condition at infinity A42 affords the description of the distribution of the complex electric field amplitude in a shell and in the surrounding media in vectorial form:

$$\overset{\alpha}{\vec{E}} = \left(\overset{\alpha}{m} - \frac{\overset{\alpha}{N}}{r^3}\right)E_0\vec{e}_f + 3\frac{\overset{\alpha}{N}}{r^3}\cos(\vartheta)E_0\vec{e}_r \quad (A43)$$

where the upper index α denotes the respective medium.

Factors of field distribution, m, N, are of the following forms:

(i) Internal medium ($r < R - d/2$):

$$\overset{i}{m} = \frac{9}{(b_{is} + 3)(b_{se} + 3) + 2b_{is}b_{se}K^3} \quad (A44)$$

$$\overset{i}{N} = 0 \quad (A45)$$

(ii) Shell ($R - d/2 < r < R + d/2$):

$$\overset{s}{m} = \tfrac{1}{3}(b_{is} + 3)\overset{i}{m} \quad (A46)$$

$$\overset{s}{N} = \overset{s}{n}\left(R - \frac{d}{2}\right)^3 \quad (A47)$$

$$\overset{s}{n} = \tfrac{1}{3}b_{is}\overset{i}{m} \quad (A48)$$

(iii) External medium ($r > R + d/2$):

$$\overset{e}{m} = 1 \quad (A49)$$

$$\overset{e}{N} = \overset{e}{n}\left(R + \frac{d}{2}\right)^3 \quad (A50)$$

$$\overset{e}{n} = \tfrac{1}{9}\left[(b_{is} + 3)b_{se} + b_{is}(2b_{se} + 3)K^3\right]\overset{i}{m} \quad (A51)$$

where

$$b_{\alpha\beta} = \frac{\overset{\alpha}{\xi} - \overset{\beta}{\xi}}{\overset{\beta}{\xi}} \quad (A52)$$

and the geometric parameter of the shell, K, is the ratio of the internal to external radius:

$$K = \frac{R - d/2}{R + d/2} \quad (A53)$$

The final results of the above analysis are depicted in Fig. 3.

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