

ACOUSTICS AND MOLECULAR PHYSICS
DEFORMATIONS OF LIPIDE MEMBRANES
UNDER THE EFFECT OF ULTRASOUND AND LOCAL
CRITERION OF THEIR DISINTEGRATION

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Possible mechanisms of acoustic effect on cells are classified. The solution of a model problem on the effect of a plane ultrasonic wave on a spherical biomembrane and analysis of the resultant deformation substantiate the local significance of the criterion of damage the membrane suffers when its area is increased in excess of the permissible value.

The various problems that are now being solved in medicine with the aid of ultrasound are oriented to the manifestation of some or other effects subsequent to its action on biostructures. Most of the works available in the literature deal with the effect of ultrasound at the macroscopic level, i. e., on individual organs and areas of biotissues [1, 2]. However, ever increasing attention is being recently paid to the effects of ultrasound on individual cells as elementary independently functioning structural components [3, 4]. For example, much promise is offered by the possibility of altering by means of ultrasound the permeability of cellular membranes on account of activation of the membrane pores and formation of fresh pores (sonic pore formation [4]) for the purpose of “target” introduction of drugs or desirable genes into cells. Numerous experimental studies into the action of ultrasound at the cellular level are characterized by a wide spread of the ultrasound treatment conditions and parameters (ultrasound frequency and intensity) used and, correspondingly, of the effects obtained, which makes it difficult to establish cause-and-effect relations and predict further results. For this reason, we have tried to systematize the data available on the sonic action on cells (Fig. 1).

By using various acoustic radiation parameters, one can in different ways affect the life activity of cells or cause their disintegration. Ultrasonic action occurs as a result of nonthermal (acoustomechanical) factors, cavitation, and thermal effects due to the absorption of ultrasound in the biotissue exposed to it. The basic mechanisms underlying the sonic action on cells and biomembranes, as well as the interrelations of these mechanisms, are illustrated in the scheme suggested by us (Fig. 2).

Of key importance in studying and practically using some or other ultrasonic effects is the possibility or impossibility of disintegration of cellular biomembranes. The theoretical excess of the relative change of the total area of the cellular membrane, due to the external action on it, over the permissible value of 3–5% is frequently used as a membrane disintegration criterion [5, 6]. However, in the case of certain types of external actions this widely used integral criterion can lead to incorrect predictive estimates and conclusions. Let us demonstrate this statement by an example of calculation of the effect of a plane ultrasonic wave on a liquid-filled spherical shell of radius R and thickness $h \ll R$ located in a liquid (Fig. 3), which simulates the ultrasound effect on a cellular membrane. In this example, we will disregard the viscosities of the external and internal liquids (cellular contents and intercellular substance), as well as the viscosity

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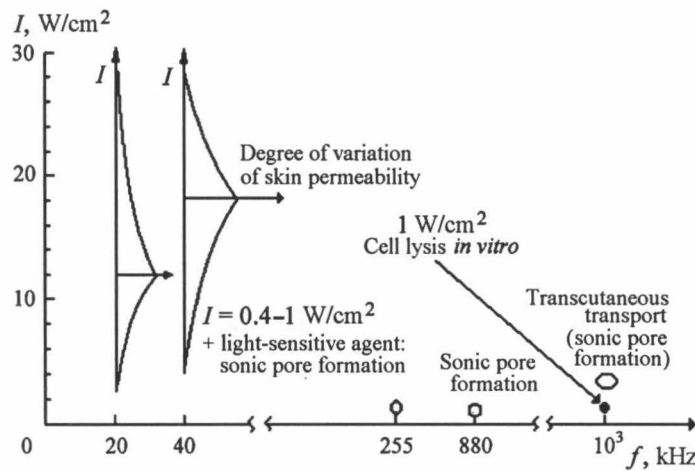


Fig. 1

Parameters of ultrasound and the effects observed under its action.

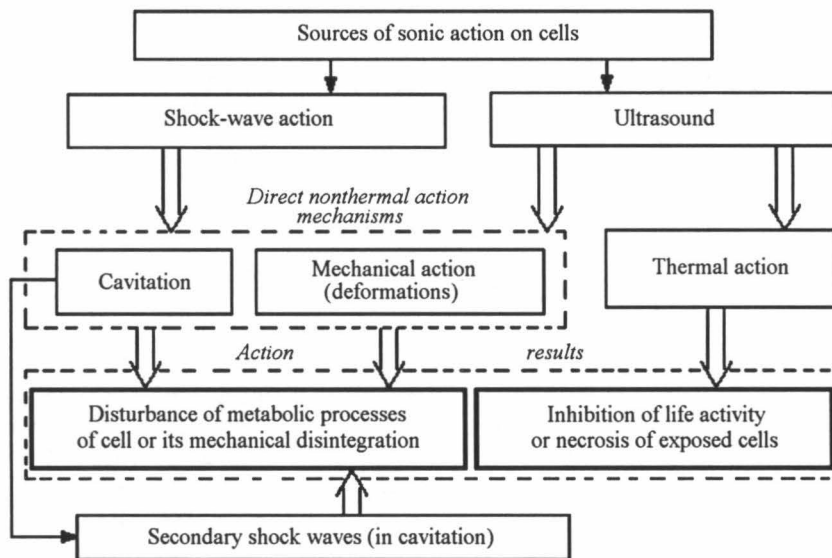


Fig. 2

Sonic action factors.

of the shell material (lipide bilayer). Proceeding from our analysis of this problem, we will substantiate the need for a more exact criterion of differential membrane disintegration based on local changes in the biomembrane area.

The Hamilton variational principle, the use of a spherical coordinate system, and the symmetry of the problem about the angle φ lead to the following equations of motion of the elements of the thin elastic shell [7, 8]:

$$\frac{1-\nu^2}{E} \rho_s R^2 \frac{\partial^2 u}{\partial t^2} L_{uu} u + L_{uw} w + = 0, \quad (1)$$

$$\frac{1-\nu^2}{E} \rho_s R^2 \frac{\partial^2 w}{\partial t^2} + L_{wu} u + L_{ww} w = \frac{1-\nu^2}{Eh} R^2 \delta f, \quad (2)$$

where δf is the possible external force acting in the radial direction on a unit surface area of the membrane and u and w are the meridional (tangential) and radial components of the shell element displacement vector, respectively. The differential operators entering into the system of equations (1) and (2) have the

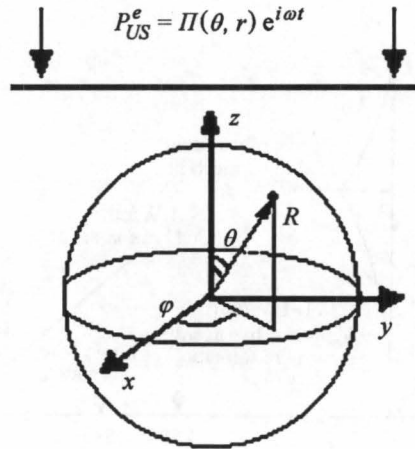


Fig. 3

Geometry of the model problem of the plane harmonic wave effect on a membrane.

form $e = \frac{1}{12}(h/R)^2$ and $\eta = \cos \theta$, respectively,

$$\begin{aligned} L_{uu} &= -(1+e) \left\{ (1-\eta^2)^{1/2} \frac{d^2}{d\eta^2} (1-\eta^2)^{1/2} + (1-\nu) \right\}, \\ L_{uw} &= (1-\eta^2)^{1/2} \left\{ [e(1-\nu) - (1+\nu)] \frac{d}{d\eta} + e \frac{d}{d\eta} \Delta \right\}, \\ L_{ww} &= e\Delta^2 + e(1-\nu)\Delta + 2(1+\nu), \\ L_{wu} &= - \left\{ [e(1-\nu) - (1+\nu)] \frac{d}{d\eta} (1-\eta^2)^{1/2} + e \frac{d}{d\eta} \Delta \frac{d}{d\eta} (1-\eta^2)^{1/2} \right\}, \end{aligned}$$

where $\Delta = \frac{d}{d\eta}(1-\eta^2)\frac{d}{d\eta}$ is the Laplacean operator. In equations (1) and (2), we will use the following parameters characteristic of cells: the membrane density $\rho_s = 0.8 \text{ g/cm}^3$, Young's modulus $E = 7 \times 10^3 \text{ N/cm}^2$, Poisson coefficient $\nu = 0.49$, membrane thickness $h = 6 \text{ nm}$, and average cell radius $R = 15 \text{ }\mu\text{m}$.

By virtue of harmonic time dependence, we put $\delta f = \delta F e^{i\omega t}$, $u = U(\eta) e^{i\omega t}$. To define the external force δf due to the ultrasonic action of the incident plane wave, it is convenient to introduce the velocity potentials φ^e and φ^i in the external and internal (with respect to the spherical membrane) regions of the liquid, respectively, and due to the harmonic time dependence, $\varphi^{i,e} = \Phi^{i,e}(r, \theta) e^{i\omega t}$. Since the velocity potentials are the solutions of the wave equation in the spherical (for the given problem) coordinate system with the zero asymptotic for φ^i at the origin and the condition of emission at infinity for φ^e , we have the following representation:

$$\Phi^i = \sum_{n=0}^{\infty} B_n j_n(k^i r) P_n(\eta), \quad \Phi^e = \sum_{n=0}^{\infty} A_n h_n^{(2)}(k^e r) P_n(\eta), \quad (3)$$

where $P_n(\eta)$ is a Legendre polynomial of order n ; j_n is a spherical Bessel function of order n ; $h_n^{(2)}$ is a spherical Hankel function of second kind of order n ; and $k^{i,e} = \omega/c^{i,e}$ are the wave numbers in the external and internal media, respectively. The relationship between the "intrinsic" pressures $p^{i,e}$ in the external and internal liquids and the respective velocity potentials $\varphi^{i,e}$ for the harmonic processes of interest is

$$p^{i,e} = -i\omega \rho^{i,e} \varphi^{i,e}(r, \theta). \quad (4)$$

If a harmonically varying pressure $p_{US}^e = \Pi(\theta, r) e^{i\omega t}$ is produced in the external liquid as a result of an external ultrasonic action, the amplitude of the force acting on a unit surface area of the membrane in the radial direction is expressed (with due regard for relations (4)) as follows:

$$\delta F = -i\omega (\rho^i \Phi^i(\theta, R) - \rho^e \Phi^e(\theta, R)) - \Pi(\theta, R).$$

Let us expand $w(\theta, t)$, $u(\theta, t)$, and $\Pi(\theta, r)$ into series in Legendre polynomials,

$$W(\eta) = \sum_{n=0}^{\infty} W_n P_n(\eta), \quad U(\eta) = \sum_{n=1}^{\infty} U_n (1 - \eta^2)^{1/2} \frac{dP_n(\eta)}{d\eta}, \quad \Pi(r, \eta) = \sum_{n=0}^{\infty} \Pi_n(r) P_n(\eta), \quad (5)$$

where $\Pi_n = \frac{2n+1}{2} \int_{-1}^1 \Pi(\eta, r) P_n(\eta) d\eta$, W_n , and U_n are unknown serial expansion coefficients. The coefficients A_n and B_n in (3) will be found proceeding from the boundary conditions: the radial displacement of the internal liquid particles, ($s^i = \frac{1}{i\omega} \frac{\partial \Phi^i(r, \eta)}{\partial r} \Big|_{r=R} e^{i\omega t}$), and of the external liquid particles, ($s^e = \frac{1}{i\omega} \frac{\partial \Phi_{\text{tot}}^e(r, \eta)}{\partial r} \Big|_{r=R} e^{i\omega t}$), adjacent to the membrane should be equal to the radial displacement of the membrane element, $w = W e^{i\omega t}$. Here $\Phi_{\text{tot}}^e(r, \theta, t) = \sum_{n=0}^{\infty} \left[i \Pi_n(r) / (\rho^e \omega) \Pi_n(r) + A_n h_n^{(2)}(k^e R) \right] P_n(\eta)$ is the amplitude of the velocity potential of the external liquid, which is the superposition of two potentials: φ^e and the term due to the presence of the harmonic wave, whose explicit expression can be obtained using the relationship similar to (4) between the velocity potential and the ultrasonic wave pressure p_{US}^e . Hence we get the following boundary conditions:

$$B_n = i \frac{c^i}{j_n'(k^i R)} W_n, \quad A_n = i \frac{c^e}{h_n^{(2)'(k^e R)} W_n - i \frac{b_n}{\rho^e \omega k^e h_n^{(2)'(k^e R)}}, \quad (6)$$

where $b_n = (2n + 1)/2 \int_{-1}^1 (\partial \Pi(r, \eta) / \partial r) \Big|_{r=R} P_n(\eta) d\eta$, $h_n^{(2)'(k^e R)} = (dh_n^{(2)}(z) / dz) \Big|_{z=k^e R}$.

Substituting into equations of motion (1) and (2) the expressions for the displacement components and the force δf , with due regard for expansions (3) and (5), and using the differential operators entering equations (1) and (2), we arrive at an inhomogeneous system of $2n$ equations in W_n , U_n and A_n , B_n . Boundary conditions (6) allow the problem to be reduced to a system of equations in the unknown coefficients W_n and U_n , with the known external forces,

$$\begin{aligned} & - [\Omega^2 + (1 + e)(1 - \nu - \lambda_n)] U_n + [e(1 - \nu - \lambda_n) - (1 + \nu)] W_n = 0, \\ & \lambda_n [e(\nu + \lambda_n - 1) + (1 + \nu)] U_n \\ & + \left[\Omega^2 - \lambda_n e(\lambda_n + \nu - 1) - 2(1 + \nu) + \omega \frac{1 - \nu^2}{Eh} R^2 \left(\rho^i c^i \frac{j_n(k^i R)}{j_n'(k^i R)} - \rho^e c^e \frac{h_n^{(2)}(k^e R)}{h_n^{(2)'(k^e R)}} \right) \right] W_n \quad (7) \\ & = \frac{1 - \nu^2}{Eh} R^2 \left(\Pi_n - \frac{h_n^{(2)}(k^e R) b_n}{h_n^{(2)'(k^e R) k^e} \right), \end{aligned}$$

where $\Omega^2 = \omega^2 R^2 \rho_s (1 - \nu^2) / E$ is dimensionless frequency.

Let us specify the expression for $\Pi(\theta, r)$. As already noted above, the problem considers a shell exposed to a plane harmonic wave of constant amplitude (see Fig. 3). The amplitude action along the normal to the shell (the wave pressure) will depend on the spherical coordinates θ and r ,

$$\Pi = \Pi_0 e^{i[\omega t - k^e (R - r \cos \theta)]}. \quad (8)$$

On expanding $\Pi(\theta, r)$ in (8) into a series in Legendre polynomials, we substitute the coefficients Π_n into determining system (7). Equations of motion (7) were solved numerically by means of a Matlab 6.0 program. The expansions of W and U in Legendre polynomials with the coefficients determined by system (7) are convergent series that can be truncated starting with $n = 50$ without distorting in any substantial way the results obtained. With W_n and U_n found, we can determine the displacements (see (5)) of the elements of the sphere at each point of its surface and get an idea of the deformation of the initially spherical surface of the membrane under the action of the ultrasonic wave. Figure 4 shows the resultant shape of the membrane surface (scaled up for obviousness purposes) deformed under the action of a plane harmonic ultrasonic wave with a frequency of 500 kHz and intensity $I = 10 \text{ W/cm}^2$. Figure 4 illustrates the extent of the membrane deformation as a function of angle θ . Analysis also shows that the extent of the membrane deformation depends on the frequency of the incident wave as well.

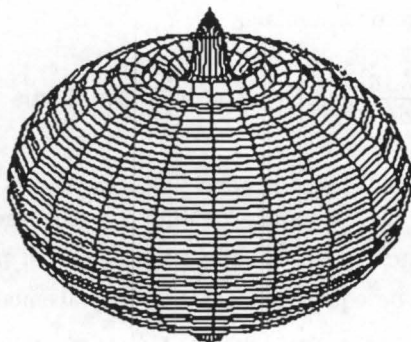


Fig. 4

Surface of the membrane deformed under the action of ultrasound ($f = 500$ kHz).

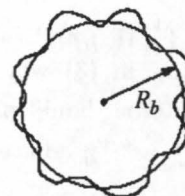


Fig. 5

Distortion of the spherical shape of the membrane relative to its average radius R_b .

We consider the qualitative characteristics of the surface area variation of the initially spherical membrane upon its deformation under the action of the supersonic wave. We will estimate the relative change of the total area of the deformed membrane surface, i. e., find the characteristic lying at the root of the biomembrane disintegration criterion used. We introduce the following designations: $\bar{R} = R_b + w$, $R_b = R + w_0$ is the average radius of the sphere over the angles θ and φ , R is the radius of the undeformed sphere, $w_0 = W_0 e^{i\omega t}$ is the radial displacement due to the zero harmonic, and $w(\eta, t) = e^{i\omega t} \sum_{n=1}^{\infty} W_n P_n(\eta)$ is the radial displacement of the liquid particles less the zero-mode displacement relative to the sphere of radius R_b . Thus, u and w describe deviations from the surface of the sphere (Fig. 5). According to the known formulas of differential geometry [9], we represent the deformed surface element, with due regard for the quadratic terms, as

$$dS = R_b^2 \left(1 + \frac{1}{R_b} \frac{\partial u}{\partial \theta} + \frac{u}{R_b} \cot \theta + 2 \frac{w}{R_b} + \frac{1}{2} \frac{u^2}{R_b^2} + \frac{1}{2R_b^2} \left(\frac{\partial w}{\partial \theta} \right)^2 + \frac{w}{R_b^2} \frac{\partial u}{\partial \theta} + \frac{1}{R_b} \frac{u}{R_b} \frac{\partial u}{\partial \theta} \cot \theta + \frac{w}{R_b} \frac{u}{R_b} \cot \theta + \left(\frac{w}{R_b} \right)^2 \right) \sin \theta d\theta d\varphi. \quad (9)$$

If we use only a linearized version of expression (9) in integrating over surface to calculate the total surface area of the membrane, we will then obtain $S = 4\pi R_b^2$ that reflects the change of the membrane surface area as a result of its general symmetrical expansion and contraction. Since $W_0 \ll R$ and $W_0 \ll W_n$ at $n < 30$, of prime interest is the change of the surface area of the membrane on account of the formation of "folds" on its surface (see Fig. 4) against the background of the small-scale general expansion-contraction. This change is defined by the quadratic terms in expression (9). To simplify subsequent calculations, note that from expression (7) there follows $U_n/W_n \cong 1\lambda_n$ at $n \geq 3$, and so we can assume that $u(\theta) = 0$. Using this approximation and also expansion (5) for W in expression (9), we get the following simple expression for the surface area of the deformed membrane:

$$S = 4\pi R_b^2 + 2\pi e^{2i\omega t} \sum_{n=1}^{\infty} \frac{n^2 + n + 2}{2n + 1} W_n^2.$$

The maximum relative change of the total surface area of the membrane, $\beta = 100\% \times (S - S_0)/S_0$ (S_0 and S are the surface areas of the undeformed and deformed membrane, respectively), calculated on the basis of the above formulas for the frequency range used (500 kHz–2 MHz) at an incident wave intensity of 10 W/cm^2 did not exceed a few tenths of a percent, which is much below the threshold value of 3–5% used as a biomembrane disintegration criterion. At the same time, experiments show [10] that waves of so high an intensity destruct cellular membranes. To eliminate this contradiction, let us take into account the nonuniform deformation of the membrane (see Fig. 4) subject to the supersonic action under consideration and determine the local relative variations of the area of the spherical surface element as a function of its location as regards the angle θ . Restricting ourselves to the terms linear in w/R_0 and u/R_0 in (9) and

substituting expansions (5) for w and u , we arrive at the following expression for the maximal relative change of the area of the surface element:

$$(dS - dS_0)/dS_0 = 1/R \sum_{n=1}^{\infty} P_n(\eta)(\lambda_n U_n + 2W_n) + 2W_0/R, \quad (10)$$

where $dS_0 = R^2 \sin \theta d\theta d\varphi$ and dS stand for the surface element prior to and after deformation, respectively. In expression (10), R_b is replaced by R , for $W_0 \ll R$. The series in expression (10) describes the relative change of the surface element area due to the formation of folds (local maxima and minima) on the deformed spherical surface, while the second term reflects the change of the surface element area caused by the variation of the radius of the sphere, i. e., the effect of the general expansion-contraction of the sphere. The theoretical dependence of the relative change of the surface element area, $\alpha = 100\% \times (dS - dS_0)/dS_0$, on the angle θ is illustrated in Fig. 6 hence one can see that the change of the area of the surface elements for which the ultrasonic wave incidence is practically normal amounts to a few percent and can exceed the critical value. This points to a possible rupture of the membrane in these regions. The shape of the deformed surface presented in Fig. 4, where considerable "folding" is manifest in the same regions, bears out the reality of such a rupture.

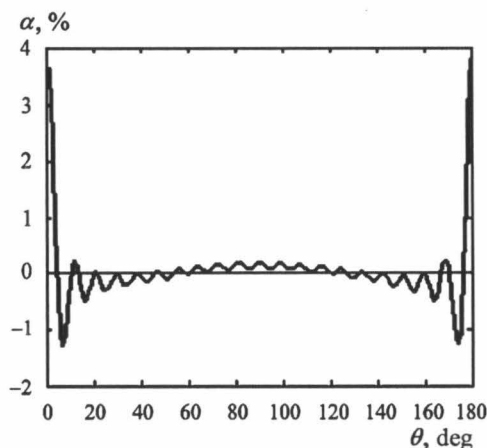


Fig. 6

Maximum relative change α of the area of the membrane surface element as a function of the angle θ . Frequency $f = 500$ kHz.

The main contribution to the change dS in expression (10) comes from the terms entering the series. Hence, we can conclude that substantial size variations dS are associated with the presence of folds due to the superposition of harmonics. At the same time, the total change of S amounts to a few fractions of a percent. This can mathematically be substantiated by calculating the above integrals which indicate that the change of the total surface area of the sphere will be affected by the terms of the second order of smallness making an insignificant contribution.

Thus, analysis of the deformation of a cellular membrane under the action of a plane harmonic ultrasonic wave of sufficiently high intensity (10 W/cm^2) has shown that the relative change of the total surface area of the membrane as a result of this process amounts to a few fractions of a percent and is substantially below the generally accepted threshold value (3–5%) used at present as a biomembrane disintegration criterion. At the same time, experiments demonstrate indisputable destruction of biomembranes by ultrasound of so high an intensity [10]. The value of α defined in this work exceeds the above-mentioned 3–5% in certain areas of the membrane, where its deformations are maximal (see Fig. 4), which means that the disintegration criterion holds true locally and agrees with the actual experimental outcome—disintegration of biomembranes under such conditions. Hence the conclusion that to predict the possible damage of biomembranes under the effect of external factors causing their deformation accompanied by an increase of

their surface area, the local (differential) criterion for the relative change of the area of individual elements of their deformed surface proves more exact and correct.

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