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EFFECTS OF ULTRASOUND ACTION ON CELLULAR MEMBRANES

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The classification of possible mechanisms of acoustical action on cells is reported. New criterion based on solution of model problem considering an influence of incident monochromatic acoustical wave upon spherical shell is substantiated to theoretically predict possible membrane rapture. This new criterion takes into account the local relative area change of each cellular membrane element and predicts the rupture when local area change exceeds a threshold value 2-3% in contrast to standard criterion concerning the change of total membrane area. Nonlinear dynamics of circular hole in elastic flat membrane (as a model of cellular membrane pore) under external straining stress is reported.

It is well known that the necessary condition of medical ultrasound diagnostics is the passive (without any consequences) ultrasound action on the biological tissues and organs. Last years developed high intensity focused ultrasound surgery method is based on high temperature destruction (albumen denaturation, thermal necrosis at 80-100 ^{0}C) of local pathological regions in biological tissues or organs. This process can be characterized as the "gross" action of ultrasound on biological structures. The "delicate" bioeffects of ultrasound due nonthermal mechanisms (ultrasound therapy) seems to be very effective for special proposes. These nonthermal effects include alterations and damages at both the cellular and tissue levels, for example permeability changes due opening and forming new membrane pores to facilitate drug delivery in cells and gene transfer. These facts make the study of ultrasound influence at cellular level very perspective and applicable. Classification of possible ultrasound influence mechanisms on biological tissues and single cells is shown in figure 1.

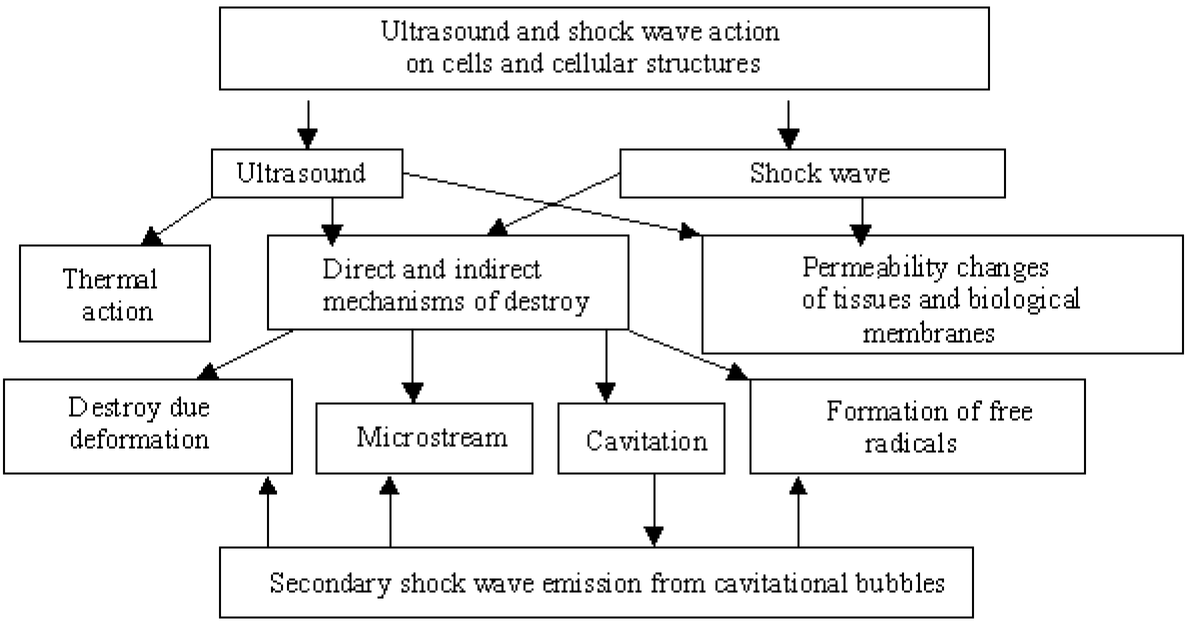

Fig.1 Possible mechanisms of acoustical action on biological objects.

Possibility or impossibility of cellular membrane rupture by ultrasound is important information to predict some resulting effects during acoustical treatment. Traditionally the rupture of cellular membrane is concerned to situation when the relative change of total membrane area exceeds the experimentally determined threshold that is equal to 2-3 % [1,2]. But this integral criterion for total cellular surface area change can give incorrect theoretically predictions of the membrane destruction. We will show it by considering of incident ultrasound monochromatic wave upon the fluid-filled and submerged spherical shell (fig.2) and confirm that the use of more accurate criterion for the local area (for the elements of membrane surface) change is necessary to make mentioned above evaluations.

We will use the mathematical model of tiny shells theory [3] to analyze deformation of the membrane elements. Since wave pressure is symmetric with respect to the *z*-axis the problem is independent of the azimuth angle φ (fig. 2). Calculated radial u_r and tangent u_ϑ displacements of membrane points can be expanded in the infinite series:

$$u_r = u_0 + \sum_{n=1}^{\infty} W_n P_n(\eta);\; u_\vartheta = \sum_{n=1}^{\infty} U_n \left(1-\eta^2\right)^{1/2} \frac{dP_n(\eta)}{d\eta}, \quad (1)$$

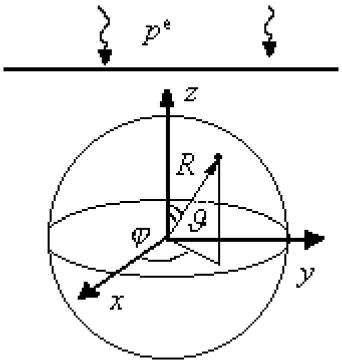

Fig.2 Model problem geometry.

where $P_n(\eta)$ is Legendre polynomial of the first kind of order n, $\eta = \cos\vartheta$, U_n and W_n are the expansion coefficients that must satisfy boundary conditions, u_0 is a radial displacement of surface points during uniform expansion. Hence using relationship for calculation of deformed surface area [4] we can estimate area strain defined as $((S - S_0)/S_0)\cdot 100\%$:

$$S = 4\pi R_b^2 + \frac{1}{2}\sum_{n=1}^{\infty}\left(n^2+n+2\right)W_n^2, \quad (2)$$

where $R_b = R_0 + u_0$ is the average radius; R_0, S_0 are radius and area of initial sphere respectively.

Our numerical calculation based on the above expressions have shown that relative total area change of membrane in used frequency range at intensity 10 W/cm^2 is less than 0.1%. This value does not exceeds critical threshold (2-3%) of cellular surface area strain and consequently criterion for membrane rupture mentioned above does not predict possible destroy of cell. But experiments have shown [5] that ultrasound intensity of 10 W/cm^2 causes cell lyses.

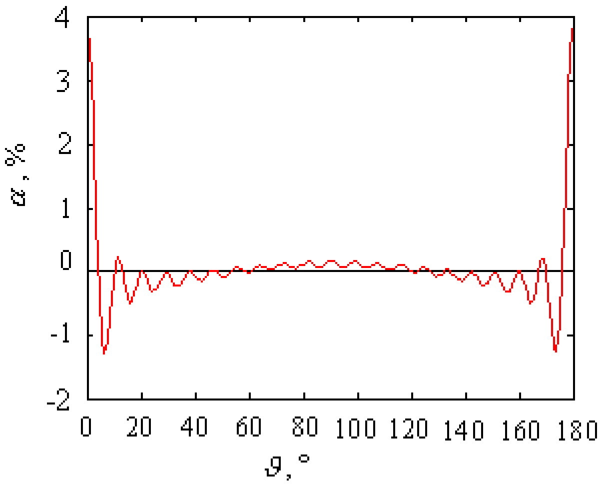

Fig.3 dependence of relative area strain amplitude α upon ϑ, frequency 1 MHz.

For the more accurate analysis of the membrane area change we must take into account the spatial heterogeneity of incident wave acting on membrane elements due their different orientation relative direction of wave propagation (fig. 2). Hence we will analyze relative area change of membrane surface element depending on angle ϑ (variable η). This change is defined by the following obtained expression:

$$\frac{dS - dS_0}{dS_0} = \frac{1}{R_b}\sum_{n=1}^{\infty} P_n(\eta)\left(n(n+1)U_n + 2W_n\right) + 2\frac{u_0}{R_b}, \quad (3)$$

where dS_0 and dS are surface element before and after deformation respectively. Calculated dependence of $\alpha = 100\%\cdot\left(dS - dS_0\right)/dS_0$ is shown in figure 3. It indicates that the area strain of membrane elements that are disposed approximately perpendicularly relative to direction of wave propagation can attain and exceed critical area strain threshold and thus the membrane could be destroyed in the region of these elements. Consequently we have shown that the differential criterion for the local area strain of different membrane elements is more applicable to predict possibility of the membrane rapture during acoustical action.

Another interesting problem is associated with permeability change of biological membrane due ultrasound and shock wave treatment (action). This phenomenon can be induced by size alteration of membrane pores [1, 6]. To analyze this problem the mathematical model of nonlinear circular hole dynamics in flat membrane element is determined and the dynamics of this hole due the various kinds of mechanical force is investigated. This problem is applicable to study the dynamics of the typical constructive elements such as plates with holes. It should be noted that nonlinear dynamics of hole in membrane could be considered as two-dimensional analogy of cavitation.

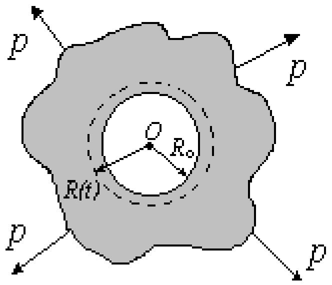

Fig.4 Element of membrane with tear.

Element of the flat elastic membrane with circular hole was considered (fig.4). To describe the nonlinear dynamics of through hole radius R we have used a model of two-dimension stress in Euler's description. Biological membranes cannot considerably change the area surface due their structure [1, 2]. In connection with this membrane property we assume that surface element area is constant, though displacements of membrane elements can be significant. To analyze this problem we have introduced polar coordinates with center in point O (fig. 4) and considered the model to be independent of polar angle φ. Plate thickness h is assumed to be constant. Let us suppose that external stress exerting the membrane don't induce any shear. Then equation of motion in case of flat tension is following:

$$\rho_0 \frac{d^2 u_r}{dt^2} = \frac{\partial p}{\partial r} + \frac{\partial \sigma_{rr}}{\partial r} + \frac{\sigma_{rr} - \sigma_{\varphi\varphi}}{r}, \qquad (4)$$

where p is external radial stress, σ_{ik} is inner membrane stress caused by deformation of surface element; ρ_0 is the mass density of membrane and u_r is radial displacement of membrane points. It should be noted that on the left hand side of equation (4) there are significant nonlinear terms to be a part of total time derivative and nonlinear terms on the right hand side in this equation are induced by connection of σ_{ik} with nonlinear deformation tensor. By integrating of equation (4) with lower $r = R$ and upper $r = mR$ limits respectively, where $m >> 1$ is arbitrary value and R is transient pore radius, we have eliminated dependence upon radial coordinate r and gotten an equation of motion with dependence upon variable R only. Integration must be made on total membrane surface, but in

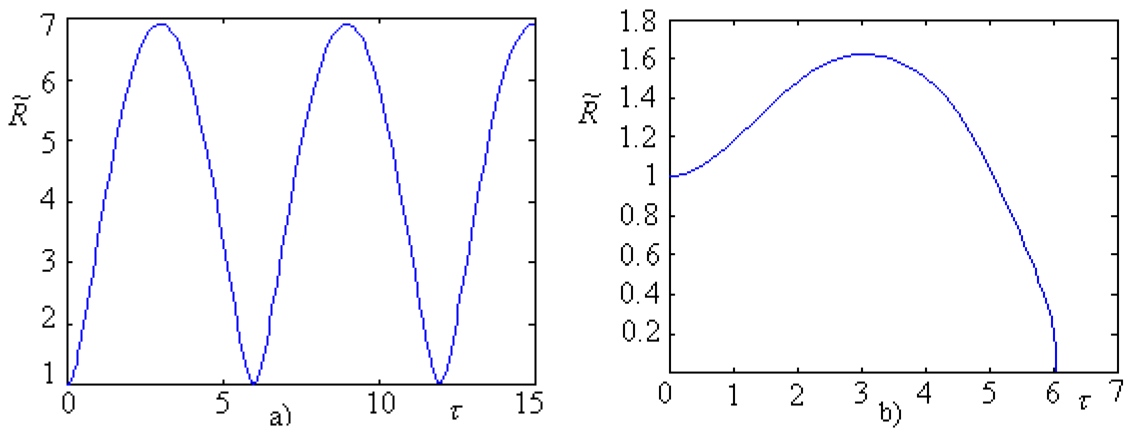

Fig.5 Pore radius dynamic a) induced by constant external stress $\tilde{p} = 10$;
b) cased by step-pulse with duration $T = 4$ and amplitude $\tilde{p} = 0.4$.

two-dimensional case there is the logarithmic divergence at infinite r due existing point source [7]. In connection with this upper infinite limit of integration was replaced by arbitrary limit mR; this enable us to avoid formal integration with infinite upper limit and consequently eliminate divergence mentioned above. Connection of σ_{ik} with nonlinear deformation tensor was chosen in form of linear Hooke low. Using this relation and integrating equation (4) as mentioned above we have gotten the equation of motion relative transient pore radius R. In dimensionless form it is given by:

$$\tilde{R}\tilde{R}'' + \tilde{R}'^2 - \frac{\tilde{R}'^2}{2}\frac{m^2-1}{m^2\ln m} = \tilde{p} + \frac{1-\tilde{R}^2}{2}\left(1-\frac{\nu}{\tilde{R}^2}\right) + \frac{1-\nu}{4}\left(\ln\frac{m^2}{(m^2-1)\tilde{R}^2+1} + \left(1-\tilde{R}^2\right)\frac{m^2-1}{m^2\tilde{R}^2}\right), \qquad (5)$$

where $\tilde{R} = R/R_0$, $\tau = t/T_0$, $T_0 = R_0\sqrt{\rho_0\left(1-\nu^2\right)\ln m/E}$, $\tilde{R}' = d\tilde{R}/d\tau$ and $\tilde{p} = p\left(1-\nu^2\right)/E$. In figure 5 dependence of pore radius based on equation 4 during external constant exerting stress (fig. 5(a)) and impulse is shown (fig. 5(b)). In last case the hole is collapsed.

To make numerical calculations following parameters $R_0 = 10nm$, Young's modulus $E = 7\cdot 10^7\ N/m^2$, Poisson's ratio $\nu = 0.49$ and mass density of membrane $\rho_0 = 0.8\,g/cm^3$ and initial conditions $\tilde{R}\big|_{t=0} = 1$, $\dot{\tilde{R}}\big|_{t=0} = 0$ were used. With elevation of $\tilde{p}$ tension phase will increase and pressing phase will decrease respectively. Under step-impulse amplitude of induced pore oscillations significantly depends upon its duration.

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