Supporting Information

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SI Text

Section S1. Dynamics of a BLS Surrounded by Water in an US Field (Model I). A simplified BLS model was constructed (shown schematically in Fig. 1*A*) with a circular, axisymmetric, piece of a bilayer membrane, made of two monolayer leaflets. At the reference case, before US is applied, water saturated with air surrounds the leaflets from outside the membrane and a thin air layer compartment lies in between the two parallel leaflets (S_0 in Fig. 1*A*). The thickness of the air layer at the reference case is determined by a force balance between the atmospheric pressure in the water, the air pressure in the air layer, and the molecular attraction/repulsion force per area (pressure) between the leaflets. For water saturated with air (as in our simulation) the air pressure in the gas layer is atmospheric and the force between the leaflets is zero. However, for degassed water and reduced dissolved air in the water, less air will accumulate between the leaflets, the air pressure will decrease, and the repulsion pressure between them will increase. While the two leaflets are forced to separate, they move opposite to each other in a symmetric way. For simplicity, only one leaflet's dynamics (the upper leaflet in Fig. 1*A*) are modeled while the other leaflet (the lower leaflet in Fig. 1*A*) is kept fixed at the symmetry plane and cannot move. The rims of the leaflets are connected at radius a by a circumferential support that prevents any in-plane motion. Uniform acoustic pressure (P_A) is applied from above the upper leaflet while attraction/repulsion force per area (pressure) between the two leaflets is applied from below. This force is parallel but not uniform. It is obtained by integration over a distributed force that varies with the radial coordinate (r) and depends on the local distance between the two leaflets. In addition, the pressure in the gas compartment acts from below the leaflet. Due to force imbalance on the upper leaflet it deforms perpendicular to the plane and acquires a dome shape as shown in Fig. 1*A*. When the deviation of the dome center from the initial planar position (H) is small ($|H| \leq H_{min}$), the mechanical response (e.g., acceleration) of the upper leaflet and the liquid above it is negligible and the equilibrium equation takes the form

$$P_{ar} + P_{in} - P_0 + P_A \sin \omega t = 0. \qquad \textbf{[S1.1]}$$

Here P_A is the acoustic pressure, ω is the angular frequency of ultrasound, and P_{ar} is attraction/repulsion pressure,

$$P_{ar} = \frac{2}{(a^2 + H^2)} \int_0^a f(r) r dr, \qquad \textbf{[S1.2]}$$

that is attributed to the attraction/repulsion forces $f(r)$

$$f(r) = A_r \left[\left(\frac{\Delta}{h(r) + \Delta} \right)^m - \left(\frac{\Delta}{h(r) + \Delta} \right)^n \right]. \qquad \textbf{[S1.3]}$$

Here, Δ is an initial gap between the upper and lower leaflets and $h(r)$ is the local deviation of the leaflet from the initial position. Values of $A_r = 10^5$ Pa, $\Delta = 1.4$ nm, $m = 5$, and $n = 3.3$ are chosen to correspond to experimental data for repulsion/attraction forces between two bilayers assuming that they are about the same as between leaflets of the same membrane (1). The maximal separating pressure required to separate the two leaflets of the bilayer membrane is calculated thereafter to be $\sim 1.48 \times 10^4$ Pa at $h = 0.49$ nm. This separating pressure is in accord with Craig's measurements of the force between two surfactant-coated silica surfaces (2). Upon separation, the attraction force on short distances <10 nm was not more than $\sim 10^{-8}$ N, from which an attraction pressure of 10^5 Pa can be estimated on the basis of the estimated area of contact of $\sim 10^{-13}$ m^2. However, because the monolayers in Craig's experiment attract each other even from relatively large distances up to several hundred nanometers, it seems that much greater areas account for attraction force $<< 10^5$ Pa. How does this pressure compare with the pressure required to inflate a tiny spherical nanobubble? The acoustic pressure (p) that is required to expand the bubble should overcome the inward, contracting surface forces $p \sim 2\sigma/r$, where σ is the surface tension (~ 0.07 $N \cdot m^{-1}$ for air/water interfaces) and r is the bubble radius. When $r = 1$ nm, the required pressure amplitude must exceed 1.4×10^8 Pa; and indeed it is much easier to inflate the bilayer membrane rather than a nanometric spherical bubble.

The local deviation $h(r)$ may be expressed as

$$h = \sqrt{R^2 - r^2} - R + H, \qquad \textbf{[S1.4]}$$

where R is the instantaneous radius of curvature of the membrane,

$$R = \frac{a^2 + H^2}{2H}, \qquad \textbf{[S1.5]}$$

assumed to be constant over the membrane surface.

The gas pressure between the membrane and a solid P_{in} is determined by the shape of the membrane. Assuming that initially $P_{in} = P_0$, and depending on the value of H, P_{in} may be expressed as

$$P_{in} = P_0 \left[1 + \frac{H}{6\Delta} \left(3 + \frac{H^2}{a^2} \right) \right]^{-\kappa}. \qquad \textbf{[S1.6]}$$

Here κ is a polytropic constant, being dependent on the value of the gas volume falling in the range between 1 and the ratio of the gas-specific heats. Taking into account a very small volume of the gas, it may be assumed that the compression/expansion of the gas is isotropic and that $\kappa = 1$ (3). It is also assumed that in the initial moment $t = 0$, when $H = 0$ and $\Delta = s$, the membrane is in equilibrium; namely, $P_{ar} = 0$.

Finally, Eq. **S1.2** divided by Eq. **S1.6** is substituted into Eq. **S1.1** to provide a transcendental, quasi-steady equation that can be solved for $H(t)$. When H increases, the mechanical response of the leaflet, and the liquid above it, can no longer be neglected and is taken into account by using the following equation (4):

For $H > H_{min}$:

$$\frac{d^2H}{dt^2} + \frac{3}{2R}\left(\frac{dH}{dt}\right)^2 = \frac{1}{\rho_l R}\left[P_{in} + P_{ar} - P_0 + P_A \sin \omega t - P_s(R) - \frac{4}{R}\frac{dH}{dt}\left(\frac{3\delta_0 \mu_s}{R} + \mu_l\right)\right]. \qquad \textbf{[S1.7a]}$$

For $H < -H_{min}$:

$$\frac{d^2|H|}{dt^2} + \frac{3}{2R}\left(\frac{dH}{dt}\right)^2 = \frac{1}{\rho_l R}\left[-P_{in} - P_{ar} + P_0 - P_A \sin \omega t - P_s(R) - \frac{4}{R}\frac{d|H|}{dt}\left(\frac{3\delta_0 \mu_s}{R} + \mu_l\right)\right]. \qquad \textbf{[S1.7b]}$$

Here ρ_l is the density of surrounding liquid; μ_l is the dynamic viscosity of the liquid; μ_s is the dynamic viscosity of the membrane; and δ_0 is the initial thickness of the membrane. Note that Eq. **S1.7** is based on the RP equation that was derived originally for a spherical bubble; nevertheless, it is applicable to any curved surface that is a part of a sphere as well, as in the case of the leaflet under consideration.

The pressure P_s attributed to the circumferential tension per unit length (T') in the membrane may be found from the force-balance equation

$$T' = P_s \frac{a^2 + H^2}{2H}, \tag{S1.8}$$

where

$$T' = k_s \left(\frac{H}{a}\right)^2, \tag{S1.9}$$

and where k_s is the area compression modulus, and the membrane surface is

$$S = \pi(a^2 + H^2);\ S_0 = \pi a^2, \tag{S1.10}$$

and the pressure is

$$P_s = \frac{2k_s H^3}{a^2(a^2 + H^2)}. \tag{S1.11}$$

The area compression modulus (area stiffness) varies over a wide range of values, from $<k_s = 0.06\ \mathrm{N \cdot m^{-1}}$, an overestimated average value for a highly nonlinear curve of $T' - S$ typical of undulated membrane at low tension (5, 6), and $k_s = 0.24\ \mathrm{N \cdot m^{-1}}$, for a stretched bilayer membrane, already flattened (7). At low projected areal strain (below ~10%), the leaflet is wavy and undulated (8). Stretching the leaflet in this case primarily flattens it, overcoming bending resistance, where the bending stiffness of a bilayer membrane is $\sim 0.08\ \mathrm{N \cdot m^{-1}}$ ($20k_B T$, where k_B is the Boltzmann constant), being $0.01\ \mathrm{N \cdot m^{-1}}$ for a half-thickness leaflet, because of the bending stiffness $\sim \delta_0^3$. An upper limit for leaflet stretching stiffness that accounts both for stretching and for bending was set to the stretching stiffness of a bilayer membrane, namely $0.24\ \mathrm{N \cdot m^{-1}}$ or $60k_B T$ (7).

The diffusion of dissolved gas in the water is controlled by Fick's equation:

$$\frac{\partial C_a}{\partial t} = D_a \nabla^2 C_a. \tag{S1.12}$$

Here C_a is the mole concentration of the air in the surrounding liquid, and D_a is the diffusion constant. The membrane is assumed as a flat and fixed disk on a plane with radius a that bounds the space filled with water. No air diffuses through the plane and spherical symmetry is assumed. So basically we solve the diffusion problem in a semi-infinite space above a plane where the initial and boundary conditions for the air concentration on that plane are

$$C_a(\xi, 0) = C_{ia}; \tag{S1.13}$$

$$C_a(a, \tau) = C_s;\quad \tau > 0. \tag{S1.14}$$

According to Henry's law,

$$C_s = \frac{P_{in}}{k_a}, \tag{S1.15}$$

where k_a is the Henry's constant and the internal pressure, P_{in}, may be expressed by

$$P_{in} = \frac{n_a R_g T}{V_a}. \tag{S1.16}$$

Here, R_g is the universal gas constant, T is the absolute temperature, and V_a is the air volume under the leaflet:

$$V_a = \pi a^2 \Delta \left[1 + \frac{H}{6\Delta}\left(3 + \frac{H^2}{a^2}\right)\right]. \tag{S1.17}$$

The air molar content n_a is determined by

$$\frac{dn_a}{dt} = S D_a \left(\frac{\partial C_a}{\partial r}\right)_{r=a}, \tag{S1.18}$$

where the initial condition is

$$n_a|_{t=0} = \frac{P_0 V_a}{R_g T}. \tag{S1.19}$$

Section S2. Pressure Amplification by a Pulsating Gas Bubble (Model III). The following model was developed to demonstrate how a bubble that pulsates steadily near a wall in an ultrasonic field [see also our study (ref. 9) for a similar approach] acts as an amplifier of the acoustic pressure pulse. In fact, the bubble amplifies the pressure pulse even without being near a wall. The model is simple in the sense that the bubble dynamics equation is with spherical symmetry, despite the presence of the wall near the bubble. Consider a spherical bubble in infinite space subjected to an ultrasound field. The pulsations of the bubble are described by the following equation for bubble dynamics (10),

$$\left(1 - \frac{\dot{R}}{C_l}\right) R\ddot{R} + \frac{3\dot{R}^2}{2}\left(1 - \frac{\dot{R}}{3C_l}\right) = \left(1 + \frac{\dot{R}}{C_l}\right)\frac{P}{\rho_L} + \frac{R}{C_l}\frac{1}{\rho_L}\frac{dP}{d\tau}, \tag{S2.1}$$

with the initial condition

$$R|_{\tau=0} = R_0. \tag{S2.2}$$

Here

$$P = P_L - P_\infty - \frac{2\sigma}{R} - \frac{4\mu\dot{R}}{R} \tag{S2.3}$$

and is the pressure at infinity, oscillating with time:

$$P_\infty = P_0[1 + A\sin(\omega\tau + \beta_0)];\quad \omega = 2\pi f. \tag{S2.4}$$

In the adiabatic case the pressure inside the bubble P_L may be represented as

$$P_L = \left(P_0 + \frac{2\sigma}{R_0}\right)\left(\frac{R_0}{R}\right)^{3\kappa}. \tag{S2.5}$$

Here τ is time, R is the bubble radius, and R_0 is the initial radius,

$$\dot{R} \equiv \frac{dR}{d\tau},\quad \ddot{R} \equiv \frac{d^2R}{d\tau^2},$$

where P_0 is the initial pressure of the gas inside the bubble, P_L is the pressure inside the bubble, σ is the surface tension, κ is the gas ratio of specific heats, μ is the dynamic viscosity of the liquid, ρ_L is the liquid density, C_l is the velocity of sound in the liquid, and f is the US frequency.

The pressure distribution along the z axis is derived from the energy conservation (Bernoulli) equation along a streamline of a noncompressible nonviscous liquid,

$$\frac{p}{\rho_L} + \frac{\partial \theta}{\partial \tau} + \frac{v^2}{2} = \text{const}, \qquad \textbf{[S2.6]}$$

where θ is the velocity potential.

If we denote the pressure at the bubble's external surface as P_s, the pressure at the wall may be expressed as

$$p_w = P_s - \rho_L \int_{H-R}^{0} \left[\frac{\partial \theta}{\partial \tau} + \frac{1}{2}\left(\frac{\partial \theta}{\partial z}\right)^2 \right] dz, \qquad \textbf{[S2.7]}$$

where the pressure at the bubble surface is

$$P_s = P_L - \frac{2\sigma}{R}. \qquad \textbf{[S2.8]}$$

A potential flow solution is then obtained around a gas bubble that pulsates near a rigid wall in a nonviscous liquid. The equation for the velocity potential θ at time t may be written as

$$\nabla^2 \theta \equiv \frac{1}{x}\frac{\partial}{\partial x}\left(x \frac{\partial \theta}{\partial x}\right) + \frac{\partial^2 \theta}{\partial z^2} = 0 \qquad \textbf{[S2.9]}$$

$$z \geq 0; \; -\infty < x < \infty; \; (z - H)^2 + x^2 > R^2$$

with the boundary conditions

$$\frac{\partial \theta}{\partial z} = 0 \; \text{ at } z = 0 \qquad \textbf{[S2.10]}$$

$$\frac{\partial \theta}{\partial n} = \dot{R}(t) \text{ at the bubble surface.} \qquad \textbf{[S2.11]}$$

n is external normal to the bubble surface and $R(t)$ is a solution of the bubble dynamic equation:

$$\theta \to 0 \text{ at } x \to \pm\infty \; \text{ and/or } z \to \infty. \qquad \textbf{[S2.12]}$$

In vivo experiments. In vivo experiments that were carried out using a multilayered epithelium model and that we previously used for characterizing ultrasound induced bioeffects (11–13) were thoroughly reexamined. All animal work was carried out under an approved protocol and according to institutional guidelines. The epidermis of fish lacks the stratum corneum of terrestrial vertebrates and instead resembles their mucous membranes, being similarly composed of multiple layers of all live cells. Fish epidermis, located exterior to their scales, also contains mucous-secreting cells (analogous to goblet cells) that migrate to the epidermal surface where they then release their contents.

Common goldfish (4–5 cm in length) were obtained from a nearby commercial fish farm, maintained in filtered fresh water at room temperature (20 °C), and fed ad libidum. Following an acclimation period of at least 1 wk, treatments were carried out individually using the following procedure. Fish were placed in a small (1 L) holding tank containing the anesthetic benzocaine at a concentration of 0.25 $g \cdot L^{-1}$. Once they stopped swimming, they were removed from the tank and a 12-mm-wide band of foam rubber was secured around their midsection. This band was then used to fasten the fish to the bottom of a larger (12 L) tank filled with fresh tap water, also at room temperature.

Ultrasound exposures were carried out using a standard physical therapy device (Sonicator 720; Mettler Electronics). The transducer was inserted into the tank, just below the water line, where the active region (10 cm^2) was positioned directly over the head of the fish and parallel to the space between the fish's eyes, at a distance of ~15 cm. Exposures were carried out in continuous mode at 1 and 3 MHz and at a range of intensities (1.0–2.2 $W \cdot cm^{-2}$) and durations (30–120 s). Exposures at 1 MHz, at all of the intensities (MI in the range 0.17–0.26), generated stable cavitation in the fluid medium between the transducer and the treated surface (11). On the other hand, exposures at 3 MHz (MI in the range 0.10–0.15) did not generate cavitation, even at the highest intensity used, which was still below the cavitation threshold (12). The presence or lack thereof of stable cavitation during the exposures was validated by the increase in the intensity of the backscattered signal when calibrating the exposures and the increased attenuation of the propagating beam using diagnostic ultrasound, where both these phenomena are characteristic of a bubble cloud.

Immediately after the exposures, the fish were taken out of the tank and a scalpel was used to remove a 3×3-mm section (0.5 mm thick) of the epidermis from the intereye region. Samples were fixed in glutaric dialdehyde (3% vol/vol), postfixed in osmium tetroxide (1% vol/vol), both in sodium cacodylate buffer (0.1 M, pH 7.3), dehydrated in increasing concentrations of ethanol (50–100%), cleared with propylene oxide, and embedded in Epon (45% Agar 100 resin, 26.7% methyl nadia anhydride, 26.7% dodecenyl succinic anhydride, 1.6% benzyldimethylamine vol/vol). Sections from the hardened blocks were cut perpendicular to the skin surface, mounted on copper grids, and then stained with both uranyl acetate and lead citrate. Representative transmission electron micrographs of control and treated tissues were taken in black and white at magnifications ranging from 2,000× to 50,000×, using a transmission electron microscope (JEM-100S; JOEL). These micrographs were subsequently scanned and saved digitally in JPEG format.

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Table S1. Parameters for the simulation runs of models I and II

Parameter	Symbol	Unit	Value	Source
Thickness of the leaflet	δ_0	nm	2	(5)
Initial gap between the two leaflets	Δ	nm	1.4	(1)
Dynamic viscosity of the leaflet	μ_s	$Pa \cdot s^{-1}$	0.05	A guess
Attraction/repulsion pressure coefficient	A_r	Pa	10^5	(1)
Exponent in the repulsion term	m	—	5	(1)
Exponent in the attraction term	n	—	3.3	(1)
Diffusion coefficient of air in water	D_a	$m^2 \cdot s^{-1}$	$2 \cdot 10^{-9}$	(14)
Density of the liquid	ρ_l	$kg \cdot m^{-3}$	1,056	(15)
Dynamic viscosity of the water	μ_l	$Pa \cdot s^{-1}$	10^{-3}	(15)
Initial air molar concentration in water	C_i	$mol \cdot m^{-3}$	0.69	
Air polytropical constant	κ	—	1	
Speed of sound in the water	C_l	$m \cdot s^{-1}$	1,500	(15)
Henry's constant	k_a	$Pa \cdot m^3 \cdot mol^{-1}$	$1.46 \cdot 10^5$	
Static pressure in the water	P_0	MPa	0.1	
Air/ water surface tension	σ	$N \cdot m^{-1}$	0.06	
Initial phase	β_0	Radian	3.141	

Parameters are based on refs. 1, 5, 14, and 15.