Ultrasound and the physics of mechanobiology

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The basic question here is: How can low intensity ultrasound (LIUS) affect cell cultures? We cover briefly the basics of ultrasound interaction with biological material, the physics of adherent cells and current molecular models of mechanotransduction. LIUS falls outside the frequency range and distance range of deformation gradients of all models of mechanotransduction. It is therefore difficult to see how the molecular mechanisms described can be triggered by LIUS with wavelengths much larger than a cell. The remaining hypothesis of intramembrane cavitation does not seem credible, but deserves thorough, critical analysis. Failing to find a clear hypothesis of a clear mechanism of mechanotransduction of LIUS one needs to critically appraise studies claiming to correlate LIUS with measurable biological changes in cell cultures.

I. ULTRASOUND–BIOPHYSICS MECHANISMS

O'Brien 2007[1]

> Commenting on diagnostic devices in 1990, Williams [503] notes that "the time-averaged intensities emitted by many devices in certain operating modes can exceed the time-averaged intensities to which patients are being subjected during typical physiotherapeutic treatments. We are consequently in a state of confusion where the physiotherapists are convinced (albeit based on tenuous or anecdotal evidence) that their treatments change the functioning of tissues and are therefore efficacious, while the clinical users are equally convinced (based on even less evidence) that their diagnostic investigations are not changing the tissues at all. Clearly both groups of users cannot be correct in their beliefs." (Leighton) (need to fix the original reference)

Cell cultures: The contrast at the glass where cells adhere represent the only steep gradient in deformation/stress compared to the size of a cell. We should compare what the difference in local deformations between Thomas' device and Kurashina[2].

The interaction between acoustic waves and biological materials is not obvious, this has always to do with the fact that biological are usually mainly composed of water. This has two main consequences on the physics point of view.

First, the speed of sound in water under normal conditions is around 1540 m/s, meaning that the wavelength of a sound at a frequency of 100 kHz will be around 15 mm, for a frequency of 10 MHz (which is the of the order of magnitude of the upper frequency used for US diagnosis or treatment) it will be around 150 μm. For both cases, this is orders of magnitude bigger than a typical cell size meaning that there should not be any gradients of pressure at the cell level: from the point of view of the cell, an acoustic wave will be mainly a constant pressure change. Except for the case of sub-wavelength oscillators (we will come back to it), one does not expect a big interaction between a wave and an object which is way smaller than its own size.

Another point resides in the very small impedance contrast between water and biological materials, and the very big acoustic impedance of water based materials. The acoustic impedance of a material is defined as (for a plane wave):

$$Z_{\text{ac}} = \rho c = \frac{p}{v} \tag{1}$$

with ρ being the density of the material, c the speed of sound in this material, p the acoustic pressure (in Pa) and v the velocity of particles in the media. An acoustic wave is a pressure wave travelling in a media. The pressure change is associated with a local motion of the particles composing the media. There is no global motion of the media: its particles move back and forth at the passage of the wave (this is actually a constituent of the acoustic wave). The acoustic impedance links the ratio between these two quantities.

1. *Particle motion in an acoustic field*

To give some ideas, let us calculate the motion of a water fluid particle undergoing a sound wave. First, let us calculate the acoustic impedance of water, which is a good approximation of a biological material:

$$Z_{\text{water}} = 1000 \times 1500 = 1.5\,10^6\,\text{Pa s/m} \tag{2}$$

That leads, if one consider a acoustical pressure of $1\,\text{bar} = 1\,10^5$ Pa to a local motion speed of:

$$v = \frac{p}{Z_{\text{water}}} = \frac{1\,10^5}{1.5\,10^6} = 0.066\,\text{m/s} \tag{3}$$

Which is very small. Considering the frequency of the wave, one can easily calculate the motion of the particles. In all cases, this is a global motion at the scale of the particles as the wavelength is usually great compared to the distinct biological objects.

2. Impedance contrast

At the interface between two materials of impedances Z_1 and Z_2 one can calculate the reflection coefficient of the acoustic *energy* as:

$$R = \left(\frac{Z_2 - Z_1}{Z_1 + Z_2}\right)^2 \tag{4}$$

Because the surrounding media and cells are mainly constituted of water, the impedance contrast between these two materials is very small, leading to a poor deposition of energy to cells: the impedances of water, human grease and blood are respectively $1.5\,10^6\,\mathrm{Pa\,s/m}$, $1.38\,10^6\,\mathrm{Pa\,s/m}$ and $1.62\,10^6\,\mathrm{Pa\,s/m}$. This is the reason with echographic measurements usually show poor contrast. For *in vitro* experiments however, as the cells are resting on a solid material (the petri dish usually - higher impedance than water) itself resting on an air layer (lower impedance). What is happening at this interface is not trivial, this is likely a fixed pressure boundary condition, but we can try to figure it out.

That said, there are things going on at the biological scale when ultrasound are going through biological materials. The phenomenons can mainly be divided into two groups: thermal mechanisms and non thermal mechanisms.

A. Thermal mechanisms

1. General principle

In general, an ultrasonic beam wave propagating in a material will lose part of its energy along the way. In absence of exotic events[29] this loss is caused by two distinct mechanisms: scattering where the wave will bounce on scattering objects and part of its energy will change direction which is conservative mechanism, and attenuation in which the material will convert part of the acoustic energy into heat. This is mechanism we are interested in in this section.

2. Calculation of heat creation in a tissue

The rate of heat generation in a tissue can be expressed as [3]:

$$\dot{Q} = 2\alpha I_{\mathrm{TA}} = \frac{\alpha p p^*}{\rho c} \tag{5}$$

where α is the ultrasonic amplitude absorption coefficient, p and p^* are the instantaneous acoustic pressure (and its complex conjugate), ρ is the material density and c the speed of sound is the considered material. Note that the product pp^* is actually the acoustic pressure amplitude *squared*, thus the rate of heat generation $\dot{Q}$ can be calculated as a time averaged quantity.

Neglecting the heat dissipation, thus for short time exposure, one can calculate the maximum temperature increase at a given point of the tissue [4]:

$$\Delta T_{\max} = \frac{\dot{Q}\Delta t}{C_v} \tag{6}$$

where Δt is the time exposure and C_v is the medium heat capacity per unit volume. Let us do a rough numerical estimate: the maximal acoustic intensity considered to have no effect on living tissue is $I_{\mathrm{IPTA}} = 100\,\mathrm{mW/cm^2}$. As biological tissues are mainly constituted of water, one can considerate a good approximation to use the heat capacity of water for tissues: $C_v \approx 4.18\,\mathrm{J/cm^{-3}/K}$. Remains the attenuation coefficient α.

a. Evaluation of the attenuation coefficient in living tissues The common (and empirical) value for the attenuation rate of US in a living tissue by physicians (also called the *derating rate*) is an attenuation of

$$0.3\,\mathrm{dB\,cm^{-1}MHz^{-1}} \tag{7}$$

That says that, during a propagation of 1 cm in living tissue of a ultrasonic wave which frequency is (say) 5 MHz, the ratio of intensity between the resulting wave and the incoming wave can be expressed as

$$\frac{P_1}{P_0} = 10^{-0.3\cdot 5/10} \approx 70\,\% \tag{8}$$

leading to an attenuation coefficient $\alpha = 1 - P_1/P_0 \approx 0.3/\mathrm{cm}$.

b. Heat generation rate From 5 one can calculate the heat generation rate $\dot{Q} = 0.06\,\mathrm{J/cm^{-3}}$. This leads, using equation 6 to a maximum temperature increase in the tissue of:

$$\frac{\Delta T_{\max}}{\Delta t} \approx 0.014\,\mathrm{K/s} \tag{9}$$

Such a change of temperature is not expected to have any effect. The power used here is very small however, considering powerful medicine equipment (up to $500\,\mathrm{W/cm^2}$), this rate can go as high as

$$\frac{\Delta T_{\max}}{\Delta t} \approx 60\,\mathrm{K/s} \tag{10}$$

which, for long exposure, is largely enough to kill living tissues.

3. General case

For long time exposures, one cannot neglect heat dissipation, due by diffusion and convection[30], and the complete bioheat transfer equation needs to be considered [5]:

$$\frac{\partial T}{\partial t} = \underbrace{\kappa \nabla^2 T}_{\text{diffusion}} - \underbrace{\frac{\Delta T}{\tau}}_{\text{perfusion}} + \frac{\dot{Q}}{C_v} \tag{11}$$

where κ is the thermal diffusivity, and τ is the perfusion time constant.

B. Non-thermal mechanisms

In medicine, a quantity commonly used to dose the consequences of an ultrasound wave on a biological material is the Mechanical Index:

$$\mathrm{MI} = \frac{p_{r,3}}{\sqrt{f}} \tag{12}$$

where $p_{r,3}$ is the *derated* rarefactional peak pressure in MPa and f is the frequency of the excitation in MHz. Obviously, using SI units for both gives the same result (what a weird unit). The derated pressure is usually calculated using equation 7.

To think about: is there a theoritical justification for this?

1. Radiation pressure

I have to dig more into this but as this is a time-averaged phenomenon. As a sound can usually be considered periodical, this is a second-order phenomenon[31] that does carry way less energy that the initial and periodical pressure wave.

> Other than (thoses) responses related to macrostreaming, there is a limited association, possibly only speculative, between the response and radiation force. [1]

2. Inertial cavitation

3. Ultrasonic contrast agent, a few considerations

Ultrasonic contrast agents have commonly used for ultrasound imaging. As they consist of micro-bubbles that can be injected in blood vessels, they create good scatterers for sound wave to bounce on, hence boosting the echo used for ultrasound imaging. Bubble dynamic under a pressure wave is highly non-linear and ruled by the Rayleigh-Plesset equation:

$$R\ddot{R} + \frac{3}{2}\dot{R}^2 + 4\frac{\nu_L}{R}\dot{R} + \frac{2\gamma}{\rho_L R} + \frac{\Delta P(t)}{\rho_L} = 0 \tag{13}$$

where R is the radius of the bubble, $\Delta P(t) = P_\infty(t) - P_B(t)$ with $P_B(t)$ the pressure inside the bubble, ν_L and ρ_L being the viscosity and the density of the fluid, respectively. This non-linearity is actually used in imaging devices: sending a pulse at a frequency and listening only to the response at the harmonic frequencies improve the image quality by keeping the bubble response only.

II. CONTINUUM VS MOLECULAR PERSPECTIVE

Humphrey 2001[6]

"It is widely accepted that numerous cell types respond to mechani- cal stimuli, yet there is no general agreement as to whether particular cells respond directly to stress, strain, strain-rate, strain-energy, or other mechanical quantities."

... "there are two important, yet different, aspects of quantification in mechanobiology: quantification of actual mechanisms and identification of reliable correlates that predict responses under prescribed conditions."...

"Hookes work ... is to seek to correlate cellular responses with coordinate invariant quantities, including the principal invariants of a stress or strain tensor or its magnitude. Such correlations appear to have received little attention in the cell mechanics literature."

"Many are pursuing the underlying mechanisms of mechanotransduction, which, if found, will provide the deepest and most valuable level of understanding. This must remain our fundamental goal." ...

"Descriptions of molecular and cellular mechanisms should be based on more fundamental quantities, as, for example, conformational changes of molecules that result from changes in force at the atomic or molecular level."

Molecular perspective (too much now, but important for later): Bustamante 2004[7]

III. PHYSICS OF ADHERENT CELLS

Schwartz 2013[8]

IV. MOLECULAR LEVEL MECHANOSENSING/TRANSDUCTION

Make overview of the proposed mechanisms. Dufort 2011[9], Janmey 2011[10], Argentati: lists of things[11], Vogel and Sheetz[12], Jansen[13] Reviews from Nature 2017-2019[14–27] Mechanosensitive channels:[28]

V. INTRAMEMBRANE CAVITATION

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[29] such as cavitation

[30] also called perfusion in a medical context

[31] ie, non-linear?