Comment on "Deformation of biological cells in the acoustic field of an oscillating bubble"

Youngsoo Choi and Won-Suk Ohm*
School of Mechanical Engineering, Yonsei University, Seoul, Korea

Yong-Tae Kim
Korea Research Institute of Standards and Science, Daejeon, Korea

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A recent paper by Zinin *et al.* [Phys. Rev. E **79**, 021910 (2009)] regarding the dynamics of biological cells in an acoustic field draws conclusions that we find open to debate. The present paper examines these conclusions and addresses some findings.

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I. INTRODUCTION

From promoting the differentiation of mesenchymal stem cells [1] to aiding intracellular drug delivery [2], ultrasound is shown to be an effective tool for manipulating and healing cells. The exact mechanism by which ultrasound affects metabolic activities of cells is yet to be found. The consensus is summed up as "sonoporation" in which ultrasound induces mechanical deformation of the plasma membrane, leading to the change in membrane permeability to ions, proteins, and drugs. Deeper understanding of the cellular-level bioeffects of ultrasound thus requires a model describing the dynamics of cells under sonication.

A theoretical framework given by Zinin *et al.* [3,4] is a welcome development in this regard. In their "shell model," the cell wall is modeled as a thin elastic spherical shell, which contains the cytoplasm (or the inner fluid) and is suspended in the surrounding (or outer) fluid. The deformation of the shell is assumed to result primarily from the quadruple mode of vibration. Among the conclusions in their latest paper [4] (hereafter referred to as the 2009 paper) are: (a) the quadruple mode has only one natural frequency; (b) cells can be classified according to the quality factor Q of free quadruple oscillation; and (c) the quadruple mode has resonance at characteristic frequency $\omega_K/2\pi$ based on the area compression modulus K_A of the shell. Although the shell model seems to provide a sound theoretical framework, we find the aforementioned conclusions in the 2009 paper debatable. The purpose of our paper is to revisit them within the context of the shell model. Specifically, three major points of contention exist in the following areas: (a) the possibility of the second natural frequency; (b) the validity of the cell classification scheme based on the quality factor; and (c) the accuracy of $\omega_K/2\pi$ as an estimate of the resonance frequency.

II. NATURAL FREQUENCIES

A complete description of the shell model is given in two papers by Zinin *et al.* [3,4]. Natural frequencies of a cell are given by the roots of the dispersion relation

$$d^n(\omega) = 0, \tag{1}$$

where ω is the angular frequency, n is the mode number, and $d^n(\omega)$ is the determinant of the system matrix for Eqs. (38)–(41) in the 2009 paper [5,6]. Note that the roots ω_n of the dispersion relation [Eq. (1)] are in general complex

$$\omega_n = \Omega_n - i\alpha_n, \tag{2}$$

where Ω_n denotes the natural frequency of free oscillation and α_n the rate of its decay. The quality factor of free oscillation is then given by

$$Q_n = \frac{\Omega_n}{2\alpha_n}. \tag{3}$$

From a numerical standpoint, finding roots of the dispersion relation [Eq. (1)] requires minimizing the determinant $d^n(\omega)$ in the $\Omega-\alpha$ space. An alternative approach is to calculate the condition number of the system matrix for a range of (Ω,α) and perform a two-dimensional search to locate (Ω_n,α_n), where the condition number attains its local maxima. (Note that the larger the condition number is, the more singular the system matrix is [7].) We find this alternative method more convenient than solving Eq. (1) directly, because peaks of the condition number are much more pronounced than the troughs of the determinant $d^n(\omega)$ when plotted in the $\Omega-\alpha$ space.

For computation of natural frequencies and quality factors, material properties of cells must be determined. Throughout our calculations the following material properties of fresh water at 20 °C are used for the inner (cytoplasm) and outer (surrounding) fluids regardless of the cell type: $\rho_{i,o}=1000$ kg/m^3; $c_{i,o}=1481$ m/s; and $\eta_{i,o}=10^{-3}$ Pa s. The variables $\rho_{i,o}$, $c_{i,o}$, and $\eta_{i,o}$ are densities, sound speeds, and dynamic viscosities of the inner and outer fluids, respectively. Cell-specific properties are the cell radius a, the area compression modulus K_A, the shear modulus μ, and the constant surface tension T_0 of the shell. Properties of five different types of cells are listed in Table I. To facilitate comparison with the analysis in the 2009 paper, we adopt the same procedure and parameters used in Ref. [3] to estimate the cell properties. For example, the moduli K_A and μ for *E. coli*, B. yeast, and *N. tabacum* are calculated using Eqs. (45) and (46) of Ref. [3] with the known values of Young's modulus E, Poisson's ratio ν, and the thickness h of

*Corresponding author; ohm@yonsei.ac.kr

TABLE I. Cell-specific properties for different types of cells.

Cell type	a (μm)	K_A (N/m)	μ (N/m)	T_0 (N/m)
E. coli[a]	0.5	8.93×10^{-2}	6.47×10^{-2}	7.50×10^{-2}
B. yeast[b]	4.5	6.02×10^{-2}	2.00×10^{-2}	2.10×10^{-2}
B. emersonii[c]	10	64.0	21.9	32.0
D. carota[d]	30	90.0	30.8	45.0
N. tabacum (protoplast)[e]	20	1.56×10^{-5}	5.2×10^{-6}	7.8×10^{-6}

[a]References [3,7].
[b]Reference [3].
[c]Reference [3].
[d]Reference [3].
[e]References [8,9].

the cell wall. For *B. emersonii* and *D. carota*, the modulus K_A is estimated from the surface tension T_0 via $K_A=2T_0$ and the modulus μ is then calculated using Eqs. (45) and (46) of Ref. [3]. The surface tension T_0 for *E. coli*, *B. emersonii*, and *D. carota* is obtained via Laplace's law $T_0=P_T a/2$, where P_T is the known intracellular (or turgor) pressure. The turgor pressure for B. yeast and *N. tabacum* is hard to obtain, and therefore the values of T_0 for these two types of cells in Table I are our best guesses. Note that Zinin *et al.* listed an incorrect value of the constant surface tension ($T_0=7.5\times10^{-3}$ N/m) for *E. coli* in Refs. [3,4]. (See Table I in the 2009 paper.) The correct value computed using Laplace's law with $P_T=0.3$ MPa [8] is $T_0=7.5\times10^{-2}$ N/m. The elastic constants K_A, μ, and T_0 for *N. tabacum* are based on values $E=2.6$ kPa and $\nu=0.5$ for a mesophyll protoplast of *N. tabacum* [9]. The radius and the thickness of *N. tabacum* are estimated to be $a=20$ μm and $h=6$ nm [10].

Natural frequencies $\Omega_2/2\pi$ and the associated quality factors Q_2 for the quadruple mode ($n=2$) of vibration are computed and compiled in Table II. As stated earlier, we look for points in the $\Omega-\alpha$ space, where the condition number of the system matrix has its local maxima. Figure 1(a) shows the condition number vs. ($\Omega/2\pi$, $\alpha/2\pi$) plot for the case of *E. coli*. In Fig. 1(a), two peaks are clearly identified within the frequency range of $0\leq\Omega/2\pi\leq20$ MHz. The lowest of the two natural frequencies is 7.2 MHz with $Q_2=1.2$. Zinin *et al.* obtained $\Omega_2/2\pi=4.58$ MHz and $Q_2=0.8$ for *E. coli* in Refs. [3,4]. These values seem erroneous, which may be attributed to the use of the incorrect surface tension $T_0=7.5\times10^{-3}$ N/m as previously pointed out.

What is striking in Fig. 1(a) is the presence of the second natural frequency at 14.4 MHz. Zinin *et al.* [3,4] assume that the dispersion relation [Eq. (1)] has only one root (hence only one natural frequency), citing the work of Prosperetti [11]. However, we do not find any remark to that effect in Ref. [11]. One may argue that the second natural frequency occurs only for bacteria with $T_0\sim K_A$; for most cells with $T_0\ll K_A$ the dynamics is dictated by K_A and the dispersion relation may have only one root as assumed by Zinin *et al.* To see whether the dominance of K_A rules out the possibility of the second root, we investigate two cases for *E. coli* where $T_0=0.1K_A$ and $T_0=0$ with all other parameters the same as in Fig. 1(a). As illustrated in Figs. 1(b) and 1(c), even K_A-dominant cells have the second resonance frequency. Furthermore, it is the first natural frequency that is more influenced by the reduction in T_0, while the second natural frequency is very robust to a change in T_0.

B. emersonii also has two natural frequencies, the lowest of which, $\Omega_2/2\pi=2.24$ MHz with $Q_2=16.0$ (Table II), agrees well with the result in the 2009 paper. Note that the second natural frequency $\Omega_2/2\pi=24.2$ MHz has the quality factor $Q_2=0.9$, which is an order-of-magnitude lower than that of the first natural frequency. A similar trend exist for *D. carota*. This leads us to conclude that a cell can have multiple natural frequencies (and resonances) with dramatically different quality factors. The protoplast of *N. tabacum* is a cell that has no natural frequency. Because the cell membrane has very low elasticity, it exhibits an aperiodic relaxational response.

III. AREA DEFORMATION OF CELLS

Given the right kind of excitation, a cell with a second natural frequency will show a corresponding resonant peak in its frequency response. To demonstrate this, we solve the full equations of motion [Eqs. (38)–(41) in the 2009 paper] for the quadruple deformation of a cell driven by an adjacent

TABLE II. Natural frequencies $\Omega_2/2\pi$, quality factors Q_2, resonance frequencies $f_{\max}$, and characteristic frequencies $\omega_K/2\pi$ and $\omega_T/2\pi$ of the quadruple ($n=2$) oscillation for different types of cells.

Cell type	$\Omega_2/2\pi$ (MHz)	Q_2	$f_{\max}$ (MHz)	$\omega_K/2\pi$ (MHz)	$\omega_T/2\pi$ (MHz)
E. coli	7.2	1.2		4.7	8.5
	14.4	0.5	12.7		
B. yeast	0.16	1.6	0.19	0.14	0.17
	0.584	0.6	0.488		
B. emersonii	2.24	16.0	2.25	1.39	1.97
	24.2	0.9			
D. carota	0.517	23.5	0.517	0.32	0.45
	7.0	0.9			
N. tabacum (protoplast)	None		None	0.24×10^{-3}	0.34×10^{-3}

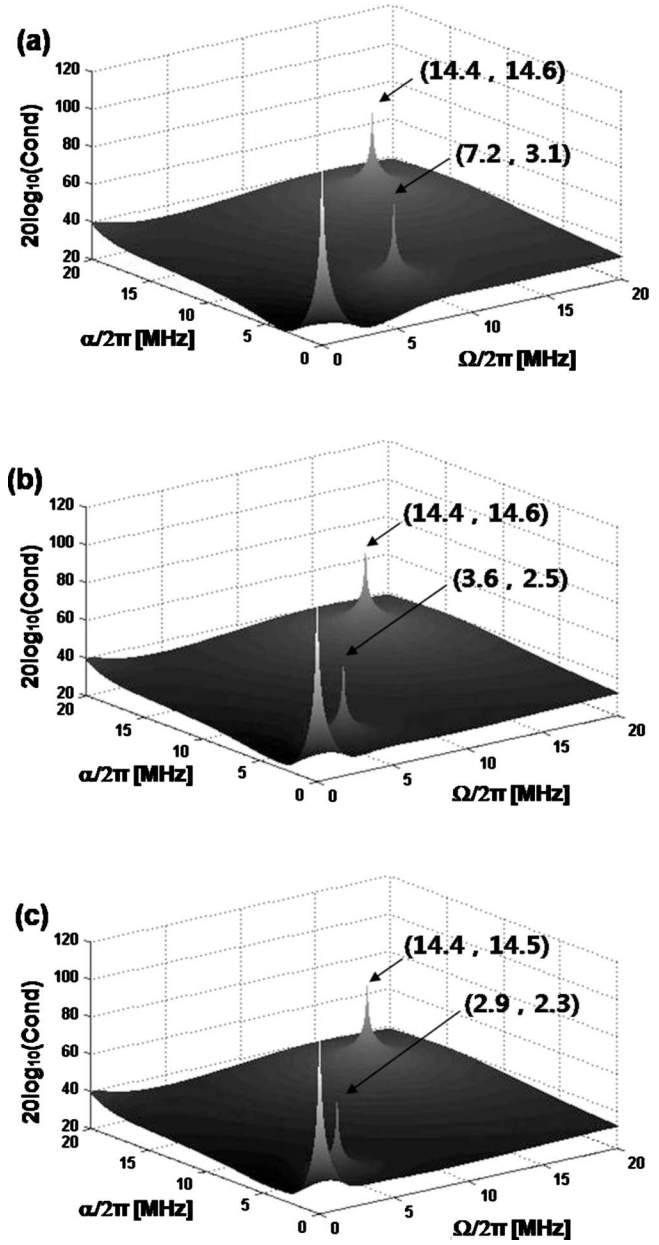

FIG. 1. Condition number of the system matrix as a function of frequency $\Omega/2\pi$ and decay rate $\alpha/2\pi$ for the case of *E. coli*: (a) $T_0=7.50\times10^{-2}$ N/m, (b) $T_0=0.1K_A$, and (c) $T_0=0$.

pulsating bubble, using the same procedure and parameters in the 2009 paper. Figure 2 shows the relative area deformation $\Delta S/S$ of *E. coli* as a function of frequency. (Note that the curve in Fig. 2 does not agree with the counterpart labeled "$a=0.5$ μm" in Fig. 5 of the 2009 paper. The reason for the discrepancy is not clear, although it may be partly due to the use of a surface tension $\sigma_{ST}=0.0725$ N/cm in the 2009 paper, instead of the correct value which is 0.0725 N/m.) The deformation has the maximum at $f_{\max}=12.7$ MHz, which is unequivocally closer to the second natural frequency (14.4 MHz) than the first (7.2 MHz). The resonant peak expected at the first natural frequency does not appear, perhaps because two low-quality resonances in close proximity have merged into one resonant peak. Figure 3 shows the case of B.

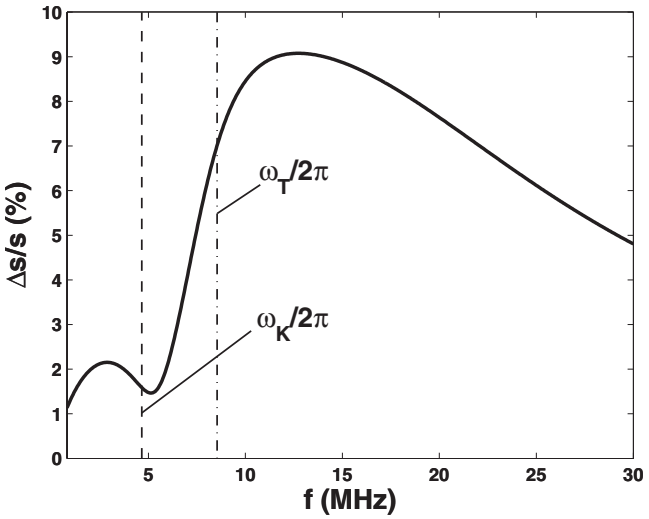

FIG. 2. Relative area deformation $\Delta S/S$ of *E. coli* as a function of frequency. Locations of characteristic frequencies given by Eqs. (4) and (5) are marked with dashed ($\omega_K/2\pi$) and dash-dot ($\omega_T/2\pi$) lines.

yeast, where the frequency response exhibits two resonant peaks. The first peak occurs at 0.19 MHz, close to the first natural frequency (0.16 MHz). The second resonant peak at 0.488 MHz is the testament to the existence of the second root of the dispersion relation [Eq. (1)], hence the second natural frequency at 0.584 MHz. The second peak is relatively broad because of the low-quality factor ($Q_2=0.6$).

In Table II, characteristic frequencies $\omega_K/2\pi$ and $\omega_T/2\pi$ are judged against resonance frequencies $f_{\max}$ for their validity. Here, $\omega_K/2\pi$ and $\omega_T/2\pi$ are defined by

$$\omega_{Kn}^2=\frac{n(n+1)K_A}{\rho_* a^3}, \tag{4}$$

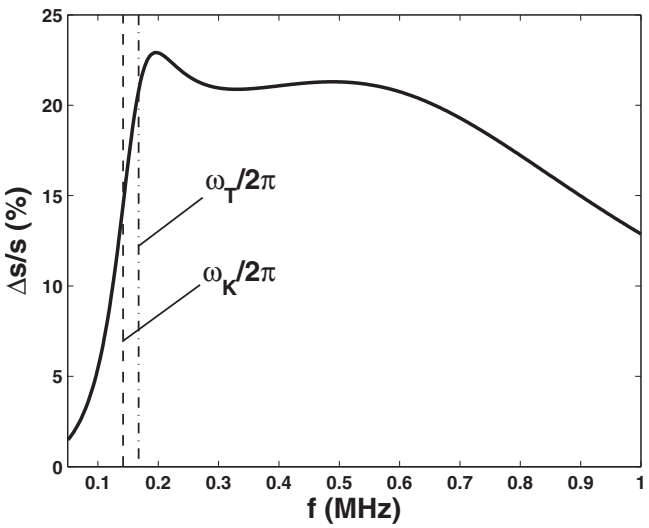

FIG. 3. Relative area deformation $\Delta S/S$ of B. yeast as a function of frequency. Locations of characteristic frequencies given by Eqs. (4) and (5) are marked with dashed ($\omega_K/2\pi$) and dash-dot ($\omega_T/2\pi$) lines.

and

$$\omega_{Tn}^2 = \frac{(n-1)n(n+1)(n+2)T_0}{\rho_* a^3}, \tag{5}$$

where $\rho_* = (n+1)\rho_i + n\rho_o$ is the normalized density and $n=2$ for the quadruple mode. Note that, contrary to the conclusion in the 2009 paper, $\omega_T/2\pi$ consistently provides better estimates of resonance frequency than $\omega_K/2\pi$ (see Fig. 3 for example). The exceptions are *E. coli* (see Fig. 2) and *N. tabacum* (no resonance frequency) for which neither $\omega_K/2\pi$ nor $\omega_T/2\pi$ can be used as a reliable predictor of resonance frequency. One should exercise caution when using these characteristic frequencies because they can be misleading particularly for cells with no resonance.

IV. CONCLUSIONS

In light of our computation of natural frequencies and the area deformation of cells for the quadruple mode of vibration, we make the following observations contrary to Zinin and Allen (2009). First, there are more than one natural frequency associated with the quadruple mode. Second, the dichotomy of cells based on the quality factor is not warranted because a cell can have multiple resonances with both high ($Q>1$) and low ($Q<1$) quality factors. Third, the quadruple mode shows resonance more closely at the characteristic frequency $\omega_T/2\pi$ than at $\omega_K/2\pi$.

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[5] Equation (39) in the 2009 paper has typographical errors, where $\mathcal{H}_n(\xi_i)$ should be replaced with $\mathcal{H}_n(\xi_o)$.

[6] The iteration relation for $\mathcal{J}_n(\xi_i)$ [Eq. (B3) in the 2009 paper] has a typographical error, where $\mathcal{J}_n(\xi_i)$ should be replaced with $\xi_i^2\mathcal{J}_n(\xi_i)$.

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