2021 43rd Annual International Conference of the
IEEE Engineering in Medicine & Biology Society (EMBC)
Oct 31 - Nov 4, 2021. Virtual Conference

Contactless Cell Permeabilization by Time-Varying Magnetic fields: Modelling Transmembrane Potential and Mechanical Stress in in-vitro Experimental Set-Up

E. Chiaramello, S. Fiocchi, M. Bonato, S. Gallucci, M. Benini, G. Tognola, P. Ravazzani, *Member, IEEE* and M. Parazzini, *Member, IEEE*

Abstract— **The feasibility of using time-varying magnetic field as a contactless cells permeabilization method was demonstrated by experimental results, but the underlying mechanism is still poorly understood. In this study a numerical analysis of the transmembrane potential (TMP) at cell membranes during permeabilization by time-varying magnetic fields was proposed, and a first quantification of mechanical stress induced by the magnetic and electric fields and hypothesized to play an important role in the permeabilization mechanism was carried out. TMP values induced by typical in-vitro experimental conditions were far below the values needed for membrane permeabilization, with a strong dependence on distance of the cell from the coil. The preliminary assessment of the mechanical pressure and potential deformation of cells showed that stress values evaluated in conditions in which TMP values were too low to cause membrane permeabilization were comparable to those known to influence the pore opening mechanisms.**

Clinical Relevance— **Results represent a significant step towards a better comprehension of the mechanism underlying cell membrane permeabilization by time-varying magnetic fields.**

I. Introduction

Increasing cell membrane permeability and enhancing molecular transmembrane transport mechanisms is of fundamental importance for enabling innovative delivery of drugs, antibodies and genetic material into cells, thus offering a wide range of application, including delivery chemotherapeutic drugs [1-2], immunotherapy of cancer and neurodegenerative diseases [3], and DNA vaccine delivery [4-5]. The best known and most used technique for creating pores in biological membranes is based on the use of high amplitude pulsed electric fields and it is usually referred as electroporation [6]. Although many advantages and the continuous evolution of the electroporation protocols towards better effectiveness and efficiency, a direct contact between the electrodes and the tissues is always required, involving serious drawbacks [7-8], and making difficult to use the technique for wide range treatments, such as vaccination campaigns.

Recently, the use of time-varying magnetic fields (Pulsed ElectroMagnetic Fields (PEMF)) was proposed as a completely non-invasive membrane permeabilization method. Both in vitro [9-13] and in vivo [14-15] studies showed that, even if the efficiency is lower than the one achieved by classical electroporation approach, the use of PEMF induced permeabilization of cells.

The main hypothesis about the mechanism of permeabilization is that the electric fields induced by the time varying magnetic field cause a change in transmembrane voltage, similarly to the mechanism achieved by classical electroporation protocols, but when estimating the amplitude of the electric field they were found to be 100–1000 times lower from those commonly obtained by conventional electroporation [16].

This suggests that the mechanism of membrane permeabilization by time varying magnetic field could be influenced by other physical phenomena. A recent hypothesis towards this direction suggested that PEMF cell permeabilization could be influenced by hydrostatic pressure induced by the gradient magnetic fields [17-19]. This mechanical stress, additional to radial pressure on cell membrane due to the interaction between surface charges and the induced electrical field, could influence the stretch-activated gating of ion channels, and potentially affect the pore opening energetic balance [17-19]. To the best of our knowledge, the mechanical stress of cell membrane was never quantified in realistic *in vitro* experimental conditions.

In this study a numerical analysis of the time-dependent TransMembrane Potential (TMP) at cell membranes due to an externally applied PEMF is proposed. The *in vitro* experimental conditions described in [11] were simulated taking into account cell models with different geometrical characteristics and placed at different distances from the magnetic field source. The two components of the mechanical pressure on cell membrane due to the interaction between free charges and induced electric field and due to the gradient of the magnetic field were quantified.

II. Materials and Methods

A. Experimental Set up

The simulated experimental set-up was the one described in [11]: the biological cell was located in a region surrounded by 66 wound coils consisting of 11 windings stacked in six layers, with a cylindrical hole at the core. The average radius of the coils from the axial center was equal to 3.75 mm, and

All authors are with the Institute of Electronics, Computer and Telecommunication Engineering, CNR, Milano, Italy (phone: +39 02 23993697; e-mail: E.C. emma.chiaramello@ieiit.cnr.it; S.F serena.fiocchi@ieiit.cnr.it; M.Bo. marta.bonato@ieiit.cnr.it; S.G. silvia.gallucci@ieiit.cnr.it; M.Be. martina.benini@ieiit.cnr.it; M. P. marta.parazzini@ieiit.cnr.it).

S. Gallucci and M. Benini are also with the Department of Electronics, Information and Bioengineering (DEIB), Politecnico di Milano, Italy.

the cell was supposed to be placed in a range of positions with distances from the innermost radius of the coil in the range 0.01 - 4 mm. The magnetic pulse was approximated as a half period sinusoid with frequency equal to 33 kHz and amplitude equal to 5.5 T.

The cell was modeled by a three-layered spherical model, composed by three homogenous, isotropic regions: the extracellular medium (0#), the membrane (1#), and the intracellular cytoplasm (2#). To assess the influence on the TMP of the modeled geometrical characteristics of the cell, the external cell radius and the membrane thickness were hypothesized to vary in the ranges reported in Table I. The dielectric permittivities and conductivities in the three regions ε_0, ε_1, ε_2 and σ_0, σ_1, σ_2, respectively, are reported in Table I.

B. Evaluation of transmembrane potential

The electric field induced by the time-varying magnetic field in the biological media is:

$$E = -\partial A/\partial t - \nabla V \quad (1)$$

where A is the magnetic vector potential induced by the current in the coil. In spherical coordinates (r, θ, φ) centered in the coil the two components $A_r = A_\theta = 0$, while the third component is [20]:

$$A_\varphi = (\mu_0 N I(t) a)/4\pi \int (cos(\varphi)\, d\varphi)/\sqrt{(r^2+a^2-2aRsin(\theta)cos(\varphi))} \quad (2)$$

where a is the coil radius, $I(t)$ the current flowing in the coil, N the number of turns of the coil, μ_0 is vacuum permeability, and R is the radial distance between a field point and a point on the ring. By converting A to a Cartesian frame centered at the coil, translating to the cell center, and converting to spherical coordinates (r', θ', φ') centered in the cell center, it is possible to obtain the $A_{r'}$, $A_{\theta'}$ and $A_{\varphi'}$ components.

The potential V was the electric scalar potential due to charge accumulation that appears from the application of a time-varying magnetic field [21]. In charge-free regions, V was obtained by solving Laplace's equation $\nabla^2 V = 0$. In spherical coordinates (r', θ', φ'), the solution for Laplace's equation can be written as:

$$V_n = (C_n r'^n + D_n r'^{-(n+1)}) sin\theta' cos\varphi' \quad (3)$$

where C_n, D_n were unknown coefficients (n = 0,1,2). The unknown coefficients were obtained by considering the conditions of continuity of the electric potential, and the normal component of the electric current density at each of boundary between cell regions, the finiteness of the electric potential inside the cell and the uniformity of the electric field at an infinite distance from the cell.

C. Mechanical pressure

Two contributions to the mechanical pressure on cell membrane were quantified: the "electric stress" component, due to the interaction between free charges and induced electric field [21] and the "magnetic stress" component, due to the gradient of the magnetic field [18].

As to the "electric stress", it was quantified by the approach described in [21]: the free charges densities at the medium/membrane and at the membrane/cytoplasm interfaces ($\rho s_{01}(r,\theta,\varphi)$ and $\rho s_{12}(r,\theta,\varphi)$) were evaluated as

$$\begin{aligned} \rho s_{01}(r', \theta, \varphi) &= \varepsilon_1 E_{1r'} - \varepsilon_0 E_{0r'} \quad \text{for } r' = R^+ \\ \rho s_{12}(r', \theta, \varphi) &= \varepsilon_2 E_{2r'} - \varepsilon_1 E_{1r'} \text{ for } r' = R^- \end{aligned} \quad (4)$$

where $E_{nr'}$ is the radial component of the electric field in each layer. The radial component of the stress was [21]:

$$\begin{aligned} Pr'_{01} &= \tfrac{1}{2} (E_{1r'} + E_{0r'}) \rho s_{01} \quad \text{for } r' = R^+ \\ Pr'_{12} &= \tfrac{1}{2} (E_{1r'} + E_{2r'}) \rho s_{12} \quad \text{for } r' = R^- \end{aligned} \quad (5)$$

For the thin, uncompressible membrane, the net radial electric stress was defined as $PEr' = Pr'_{01} + Pr'_{12}$.

As to the component of the stress due to the gradient of the magnetic field, it was calculated as defined by [19]:

$$PM = B\, grad(B)\, (\chi_c - \chi_m)\, Volume / (2\mu_0\, Surface) \quad (6)$$

where χ_c and χ_m are the magnetic susceptibility of the cell and the extracellular medium, respectively, μ_0 is vacuum permeability, *Volume* is the volume of the cell, *Surface* is the cell cross-sectional area parallel to the magnetic gradient vector, and B and *grad*(*B*) are amplitude and spatial gradient of the magnetic field. *Surface* was defined as the membrane cross-sectional area, discarding the contribution of the cross-sectional areas of the cytoskeleton [19], while the difference of magnetic susceptibility was fixed equal to $9 * 10^{-6}$.

TABLE I
DIELECTRIC PROPERTIES AND GEOMETRICAL CHARACTERISTICS OF CELL MODEL

Dielectric properties of cell layers		
	ε (F/m)	σ (S/m)
Extracellular medium	$5.93*10^{-10}$	0.55
Membrane	$1.035*10^{-10}$	$1.26 * 10^{-7}$
Cytoplasm	$5.93*10^{-10}$	0.55
Geometrical characteristics		
	min	max
External radius of the cell	5 μm	25 μm
Membrane thickness	3 nm	10 nm

III. RESULTS

Fig. 1 shows, as an example, the temporal behavior of TMP in the point of maximum polarization (fig.1a) and the distribution of the module of TMP along the cell surface in the instant of maximum polarization. The cell was modeled considering external radius and membrane thickness equal to 10 μm and 10 nm, respectively, and a distance from the cell center to the innermost radius of the coil equal to 0.75 mm. The polarization of the cell membrane is dependent on the geometry, and the maximum absolute values of TMP were equal to 0.0367 V.

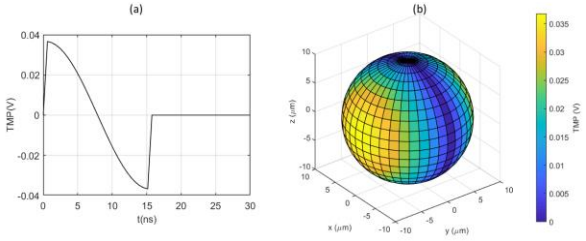

Figure 1. Temporal behavior of TMP in the point of maximum polarization (a) and regional values of TMP of the spherical cell membrane in the instant of maximum polarization (b).

Fig. 2a shows the dependence of the maxima TMP values from the external radius and membrane thickness of the spherical cell model, when the cell is placed at a very small distance, equal to 0.1 mm, from the innermost radius of the

coil. As a comparison, the TMP critical value equal to 1.032 V at which the phenomenon of pores creation is assumed to be activated [22] is reported. For all the modeled cell dimensions the TMP were far below the critical value for pores opening. The cell model with larger radius showed higher TMP values, while the membrane thickness appeared to be very slightly influential. Fig. 2b shows maxima TMP values when considering cells with different radii and membrane thickness equal to 10 nm placed at different distance from the innermost radius of the coil. Whatever the cell could be placed, TMP values were always far below the critical value for pores opening.

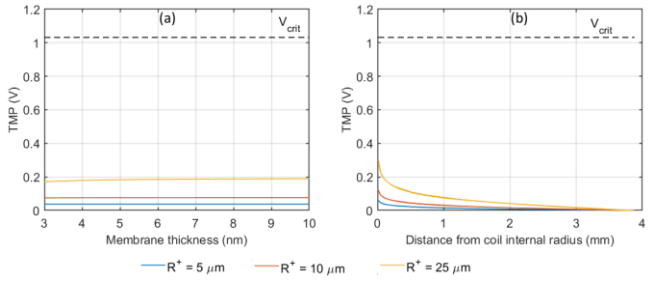

Figure 2. Dependence of TMP on cell dimensions (external radius and membrane thickness) (a) and on distance from the coil internal radius (b).

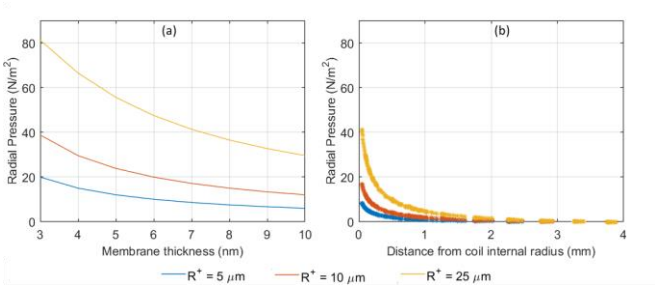

Figure 3. Dependence of "electric stress" on cell dimensions (external radius and membrane thickness) (a) and on distance from the coil internal radius (b).

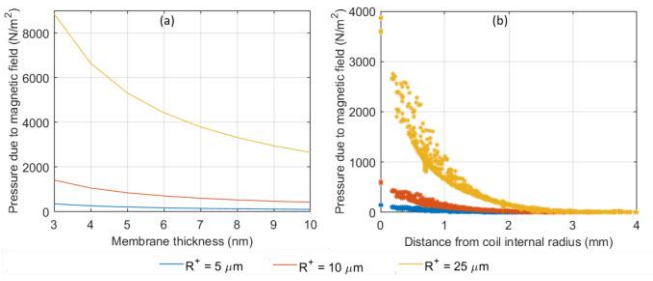

Figure 4. Dependence of "magnetic stress" on cell dimensions (external radius and membrane thickness) (a) and on distance from the coil internal radius (b).

Fig. 3a shows the "electric stress" values for a cell placed at very small distance equal to 0.1 mm from the innermost radius of the coil (corresponding to TMP values shown in fig. 2(a)) and their dependence on the cell radius and membrane thickness. Cell models with larger radius and smaller membrane thickness showed higher pressure levels, with the highest value equal to 81.19 N/m^2 for a cell with radius and membrane thickness equal to 25 μm and 3 nm, respectively. Fig. 3(b) shows the "electric stress" values corresponding to TMP values shown in fig. 2(b) when considering cells with different radii and fixed membrane thickness, equal to 10 nm, placed at different distance from the innermost radius of the coil. The "electric stress" showed a behavior similar to the TMP values, with higher values for cells placed at a short distance from the coil. The highest observed "electric stress" value was equal to 41.8 N/m^2, for a cell with radius equal to 25 μm, placed at a distance of 0.05 mm from the coil.

Fig. 4a shows the "magnetic stress" values for a cell placed at very small distance equal to 0.1 mm from the innermost radius of the coil (corresponding to TMP values shown in fig. 2(a) and to "electric stress" values shown in fig. 3(a)) and their dependence on the cell radius and membrane thickness. Cell models with larger radius and smaller membrane thickness values showed higher pressure levels, with the highest value equal to 8856 N/m^2 for a cell with radius and membrane thickness equal to 25 μm and 3 nm, respectively. Fig. 3(b) shows the "magnetic stress" values corresponding to TMP values shown in fig. 3(b) when considering cells with different radii and membrane thickness equal to 10 nm, placed at different distance from the innermost radius of the coil. The "magnetic stress" showed higher values for cells placed at a short distance from the coil, with and highest observed "magnetic stress" value equal to 3868 N/m^2, for a cell with radius equal to 25 μm placed at a distance of 0.05 mm from the coil.

IV. Discussion

This study focused on the assessment of the time-dependent cell transmembrane potential occurring when applying Pulsed Electromagnetic Field to achieve the cell membrane permeabilization. TMP was assessed simulating the *in vitro* experimental conditions described in [11]. Results showed that the TMP values were far below the critical value needed to enable pore openings.

As to the influence of cell geometric parameters (i.e., membrane thickness and cell radius) on TMP, results showed that variations in cell radius have a strong influence on TMP, while membrane thickness was slightly influential.

As expected, a strong dependence of the TMP values on distance of the cell from the coil was observed, but whatever the position of the cell the TMP values were found to be well below the critical value needed for the pore creation. These findings are coherent with results by previous modelling studies [23-24]: in [23] the authors found that with a waveform with frequency equal to 25 kHz and amplitude equal to 5.5 T, the maximum TMP value was found equal to 0.5 V, far below the critical value for pores opening, while for a faster signal with rise time equal to 10 ns (and thus higher frequency components) it was possible to reach TMP values higher enough to enable pore opening. Similarly, also in [24] it was found that signals with higher frequency contents caused higher TMP values, but still all the signals investigated by the authors did not reached TMP values high enough for assuring pore openings.

Despite these modeling findings, in literature several *in-vitro* studies found that, although with a lower efficiency compared to conventional electroporation approach, the use

of PEMF induced permeabilization of cells [9-13, 16]. In [11] PEMF with amplitude 5.5 T, duration of 15 ms and dB/dt equal to 10^6 T/s, similar to the pulse modelled in this study, was used to increase membrane permeability of Chinese Hamster Ovary (CHO) cells, finding a percentage up to 70% of permeabilized cells. Similarly, in [9] CHO cells exposed to magnetic pulses of amplitude 2.2 T and duration 250 μs, showed an increase of transmembrane molecular transport.

A possible explanation about this discrepancy between modeling and experimental results could be the presence of other physical phenomena influencing the permeabilization mechanism. To investigate the effect due to mechanical stress on cell membrane, both induced by the spatial gradient of the time varying magnetic field and by the concentration of surface charge on the membrane and its interaction with the induced electric field, both the "electric stress" and the "magnetic stress" were quantified. Both the components showed a strong influence on cell geometrical characteristics and distance from the coil. At the same conditions, the "magnetic stress" showed higher values compared to the "electric stress". This suggest that the gradient of the magnetic field could play a fundamental role in enhancing the probability of pore openings in realistic *in-vitro* conditions. The total levels of mechanical stress observed when considering pulses similar to the ones used *in-vitro* studies may be sufficient to affect the cell functionality through stretch-activated gating of ion channels [25] and to influence the energy balance governing pore opening [21]. In future studies it would be interesting to simulate actual deformation of the cell membrane, to quantify the effective influence of stretch-activated channels on pore opening mechanisms.

V. Conclusion

This study focused on the quantification of the TMP occurring when using time varying magnetic fields to permeabilize cell membrane. A preliminary assessment of the mechanical pressure on cells during *in vitro* PEMF permeabilization suggests that the stress values could influence the pore opening mechanisms. This was the first attempt towards a quantification of these mechanical contributions hypothesized to be responsible of the apparent discrepancy between modelling and experimental data. In future study it would be interesting to include these components in the modelling of pore opening dynamics, to contribute to a better comprehension of the phenomena underlying the mechanism of cell membrane permeabilization by time varying magnetic field.

References

[1] D. Miklavcic and V. D. Rafael, "Electrochemotherapy (ECT) and irreversible electroporation (IRE)-advanced techniques for treating deep-seated tumors based on electroporation," *BioMedical Engineering OnLine*, vol. 14, no. 3, 2015.

[2] M. Marty, et al., "Electrochemotherapy – An easy, highly effective and safe treatment of cutaneous and subcutaneous metastases: Results of ESOPE (European Standard Operating Procedures of Electrochemotherapy) study," *European Journal of Cancer Supplements*, vol. 4(11), pp. 3-13, 2006.

[3] D. Fioretti, S. Iurescia, V. Michele Fazio, and M. Rinaldi, M. "In vivo DNA electrotransfer for immunotherapy of cancer and neurodegenerative diseases." *Current drug metabolism*, vol. 14(3) pp. 279-290, 2013.

[4] L. Lambricht, A. Lopes, S. Kos, G. Sersa, V. Préat, and G. Vandermeulen, "Clinical potential of electroporation for gene therapy and DNA vaccine delivery," *Expert Opin. Drug Deliv*. vol, 13, pp.295–310, 2016.

[5] N.Y. Sardesai and D.B. Weiner, "Electroporation delivery of DNA vaccines: Prospects for success," *Curr. Opin. Immunol.* vol. 23, pp. 421–429, 2011.

[6] L.Rems and D. Miklavcic, "Tutorial: Electroporation of cells in complex materials and tissue." *Journal of Applied Physics*, vol. 119(20) pp. 201101, May 2016.

[7] S. Mahnič-Kalamiza, and D. Miklavčič, "Scratching the electrode surface: Insights into a high-voltage pulsed-field application from in vitro & in silico studies in indifferent fluid," *Electrochimica Acta*, vol. 363, pp. 137187, 2020.

[8] E. Guenther, N. Klein, P. Mikus, M.K. Stehling, and B. Rubinsky, "Electrical breakdown in tissue electroporation," *Biochemical and biophysical research communications*, vol. 467(4), pp. 736-741, 2015.

[9] L. Towhidi, S.M.P. Firoozabadi, H. Mozdarani, and D. Miklavcic, "Lucifer Yellow uptake by CHO cells exposed to magnetic and electric pulses," *Radiol Oncol* vol. 46, pp. 119–125, 2012.

[10] Z. Shankayi, Z., S.M.P. Firoozabadi and M.G. Mansurian, "The Effect of Pulsed Magnetic Field on the Molecular Uptake and Medium Conductivity of Leukemia Cell," *Cell Biochem. Biophys,* vol. 65, pp. 211–216, 2013.

[11] V. Novickij, J. Dermol, A. Grainys, M. Kranjc, and D Miklavčič, " Membrane permeabilization of mammalian cells using bursts of high magnetic field pulses," *PeerJ*, vol. 5, pp. e3267, 2017.

[12] V. Novickij, et al. "Pulsed electromagnetic field assisted in vitro electroporation: a pilot study," *Scientific reports*, vol. 6(1), pp. 1-10, 2016.

[13] V. Novickij, et al. "Reversible permeabilization of cancer cells by high sub-microsecond magnetic field," *IEEE Transactions on Magnetics*, vol. 53(11), pp. 1-4, 2017.

[14] T.J. Kardos, and D.P. Rabussay, D. P. "Contactless magneto-permeabilization for intracellular plasmid DNA delivery in-vivo," *Human vaccines & immunotherapeutics*, vol. 8(11), pp. 1707-1713, 2012.

[15] S. Kranjc, M. Kranjc, J. Scancar, J. Jelenc, G. Sersa, and D Miklavcic, "Electrochemotherapy by pulsed electromagnetic field treatment (PEMF) in mouse melanoma B16F10 in vivo," *Radiology and oncology*, vol. 50(1), pp. 39-48, 2016.

[16] D. Miklavcic, V. Novickij, M. Kranjc, T. Polajzer, S.H. Meglic, T.B Napotnik, and D. Lisjak, "Contactless electroporation induced by high intensity pulsed electromagnetic fields via distributed nanoelectrodes," *Bioelectrochemistry,* vol. 132, pp. 107440, 2020.

[17] T. Polyakova, V. Zablotskii, and A. Dejneka, "Cell membrane pore formation and change in ion channel activity in high-gradient magnetic fields," *IEEE Magn. Lett* vol. 8, pp. 1-5, 2017.

[18] V. Zablotskii, T. Polyakova, O. Lunov, and A. Dejneka, "How a high-gradient magnetic field could affect cell life," *Sci. Rep*. vol. 6(1), pp. 1-16, 2016

[19] V. Zablotskii, O. Lunov, S. Kubinova, T. Polyakova, E. Sykova, and A. Dejneka, "Effects of high-gradient magnetic fields on living cell machinery," *J. Phys. D: Appl. Phys,* vol.49 (49), pp. 493003, 2016.

[20] R.A. Schill, "General relation for the vector magnetic field of a circular current loop: a closer look," *IEEE Transactions on Magnetics*, vol. 39(2), pp. 961-967, 2003.

[21] H. Ye, and A. Curcuru, A. "Biomechanics of cell membrane under low-frequency time-varying magnetic field: a shell model," *Medical & biological engineering & computing*, vol. 54(12), pp. 1871-1881, 2016.

[22] J.C. Neu and W. Krassowska, "Asympototic model of electroporation," *Phys. Rev. E*, vol. 59, no. 3, pp. 3471–3482, Mar. 1999.

[23] A. Lucinskis, et al. "Modelling the cell transmembrane potential dependence on the structure of the pulsed magnetic field coils," *Elektronika ir Elektrotechnika*, vol. 20(8), pp. 9-12, 2014.

[24] Q. Hu, R.P. Joshi, and D. Miklavčič, "Calculations of Cell Transmembrane Voltage Induced by Time-Varying Magnetic Fields," *IEEE Transactions on Plasma Science*, vol. 48(4), pp. 1088-95, 2020.

[25] R. Bouffanais, J. Sun and D.K. Yue, "Physical limits on cellular directional mechanosensing," *Phys. Rev. E*, vol. 87, 052716, 2013.