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# Ultrasound Mechanical Index as a Parameter Affecting of the Ability of Proliferation of Cells

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***Abstract*—**Mechanical index (MI) is used for quantifying acoustic cavitation and the relationship between acoustic pressure and the frequency. In this study, modeling of the MI was applied to provide treatment protocol and to understand the effective physical processes on reproducibility of stem cells. The acoustic pressure and MI equations are modeled and solved to estimate optimal MI for 28, 40, 150 kHz and 1 MHz frequencies. Radial and axial acoustic pressure distribution was extracted. To validate the results of the modeling, the acoustic pressure in the water and near field depth was measured by a piston hydrophone. Results of modeling and experiments show that the model is consistent well to experimental results with 0.91 and 0.90 correlation of coefficient ($p<0.05$) for 1 MHz and 40 kHz. Low intensity ultrasound with 0.40 MI is more effective on the proliferation rate of the spermatogonial stem cells during the seven days of culture, in contrast, high MI has a harmful effect on the spermatogonial stem cells. This model provides proper treatment planning *in vitro* and *in vivo* by estimating the cavitation phenomenon.

***Keywords*—**Ultrasound, mechanical index, modeling, stem cell.

## I. Introduction

NOWADAYS, new studies were done based on the application of bio-stimulation such as low intensity laser, the electromagnetic fields and low intensity ultrasound (LIUS) *in vitro* for more proliferation and differentiation of stem cells [1]. Mechanical stimulation is necessary to create condition for proliferation and differentiation of stem cells. And loss of mechanical stress reduces the differentiation ability of stem cells [2]. LIUS is an acoustic pressure wave that can produce a localized mechanical stimulation of cells to conduct activity of tensile receptors of membrane cells, ion channels and integrins (extracellular messages) [3]. However, the effect of acoustic interactions on cell growth and the signal transduction mechanisms induced by this type of mechanical stimulation is not well understood [4]. LIUS is an oscillating pressure wave that can enhance acoustic cavitation. During subsequent pressure oscillations, these bubbles can grow, oscillate and collapse, which can have benefit and violent effects on cells, but cavitation in low-intensity ultrasound radiation does not damage the cells [5]. Sonoporation is one of the effects of ultrasonic cavitation that it can be effective for modifying the permeability of the cell membrane and transfer the extracellular between cells in culture [6]. So, the effects of LIUS required specific exposure conditions with the following important characteristics: Physical or chemical interaction between bubbles and cells is required for a bioeffect to occur

Acoustic cavitation is quantified by MI that establishes the relationship between sound pressure and frequency. To extract the MI, in this study, ultrasound waves should be modeled on the biological environment. Therefore, in this study, the minimum acoustic pressure is distributed in the environment and ultimately the MI distribution at each frequency according to the desired intensity should be determined by solving the equation of wave propagation and determination of pressure profile. In this study, with frequency and intensity changes, MI distribution was modeled and extracted with 0.7, less. To calculate a treatment plan, the integral equation Rayleigh was used to extract the distribution of mechanical pressure. Finally, the effect of the LIUS based on the MI in limits of 0.7, lower and higher than it, were investigated on stem cells proliferation.

## II. Materials and Methods

- Acoustic linear diffusion equation: Assuming a thermo-viscous and tissue homogenization, the relationship between acoustic pressures with density can be achieved through low pressure Taylor expansion around equilibrium density of $\rho_\circ$ [7]:

$$P = c_\circ^2(\rho - \rho_\circ) + \frac{c_\circ^2}{\rho_\circ}\frac{B}{2A}(\rho - \rho_\circ)^2 + \cdots \quad (1)$$

$C_0$ is acoustic propagation velocity, $\rho$ the density and the coefficient B/2A parameter nonlinear acoustic propagation in the environment. In this study, since acoustic intensity maximum of 1.34 W/cm$^2$ is used, according to the results of previous studies [8], the effects of wave nonlinear propagation can be ignored. Based on the relationship, the linear wave propagation equation in soft tissue for acoustic waves with limited range of pressure and assumption of thermo-viscous environment is defined as:

$$\nabla^2 P - \frac{1}{C^2}\frac{\partial^2 P}{\partial t^2} + \frac{\delta}{C^4}\frac{\partial^3 P}{\partial t^3} = 0 \quad (2)$$

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In this study (Fig. 1), it is assumed that each small element of ds crystal surface fluctuates continuously perpendicular to the surface with the same speed u=u.exp(iωt). Acoustic diffusion coefficient (δ) is also heat-related pressure range reduction and viscosity:

$$\delta = \frac{2c^3.\alpha}{\omega^3} \quad (3)$$

That α is the acoustic absorption coefficient, ω is angular frequency and c is the speed of acoustics propagation. Then the integral equation is transformed as [3]:

$$P(r') = ikc\rho_t \iint_s \frac{u}{2\pi r'} exp^{-(\alpha+ik)r'}\, dS \quad (4)$$

Based on [10], (4) is solved and acoustic pressure in cylindrical coordinates is extracted to plan the MI according to the intended frequency (f):

$$\mathrm{MI} = \frac{\mathrm{P(min)}}{\sqrt{\mathrm{f}}} \quad (5)$$

Acoustics variables including frequency (40 kHz), acoustics intensity (zero to 1.34 W/cm$^2$) in continuous mode, the effective radiation area (5 cm$^2$) and effective target radius (1.8 cm) (Ultrasound Laboratory, Medical Physics department, Tarbiat Modares University) are used as inputs variables and also water [9] is placed between transducer and the target environment. Acoustic calibration was carried out by the hydrophone method in water of 32 °C (PA124, Precision Acoustics Ltd, Dorchester, UK, sensor diameter of 25 mm).

Modeling results were compared to experimental results. The calculation is done in water A similar model parameters, to perform the results validation of the numerical calculations. To measure the pressure with a hydrophone, distilled water was used to remove the effects of water impurities. To achieve equivalent cell studies using heaters (electric heaters RENA, France), the ambient temperature was brought to 32 °C [10]. Recorded acoustic signals were done in different irradiation situations on the field of ultrasonic of 40 kHz in continuous mode and at the intensity of 0.28, 0.34, 0.39, 0.45, 0.48, 0.56, 0.72, 0.96, 1.17, 1.26 and 1.34 W/cm$^2$ at 0.5, 1.0 and 1.5 cm from the transducer area. To reduce errors, acoustic signal amplitude measurement was repeated 5 times in each of the irradiation conditions (Fig. 1). The correlation between experimental and modeling approaches was carried out and the linear regression function and Pearson correlation coefficient (r) were presented ($p<0.05$).

Irradiation of LIUS on spermatogonial stem cells (SSCs): To separate cells, neonatal mice (NMRI, National Medical Research Institute) were used for 3-6 days. The testicles were extracted in 10 minutes and after one wash, according to specified protocol its medium containing enzyme (Sigma Co., Germany). Thus, the cell suspension containing autologous Sertoli and spermatogonia cells was prepared and incubated at 37 °C [11].

Male mice lacking expression of PLZF, a DNA sequence-specific transcriptional repressor, show progressive germ cell depletion due to exhaustion of the spermatogonial stem cell population.

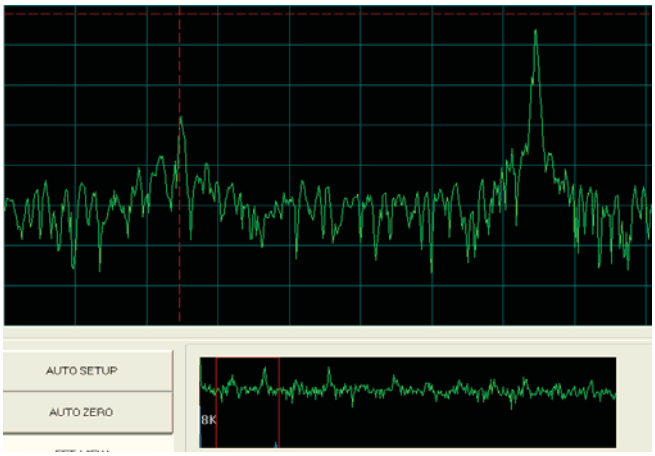


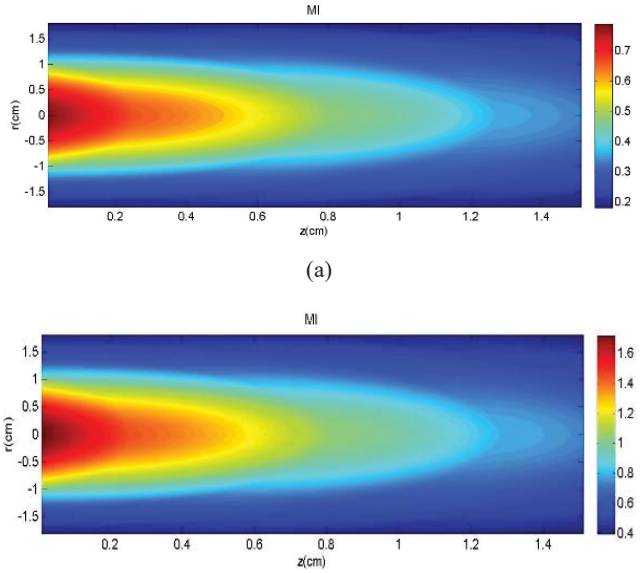

Fig. 1 A sample of the spectrum of 40 kHz with an intensity of 1.34 W/cm$^2$ in 0.5 cm distance recorded by Spectrum Analyzers; the horizontal axis shows frequency (Hz) and the vertical axis is acoustics pressure (MPa)

Immunofluorescence of PLZF staining (Promyelocytic Leukaemia Zinc Finger) was used for qualitative study of protein markers expression. PLZF and of Oct4 against cell surface proteins (Santa Cruz Biotechnology, USA). Adult germline stem cells are capable of self-renewal, tissue regeneration and production of large numbers of differentiated progeny. Secondary antibodies, FITC and RT (Santa Cruz Biotechnology, USA), DAPI (4',6-Diamifino-2-Phenylindole) and PI (Propidium iodide) (Santa Cruz Biotechnology, USA), were used to stain the nucleus [12]. The exposure method was selected according to elements similarity and hypothesis of research based on previous studies [9], and the initial parameters of the MI model. In order to evaluate the effect of MI of LIUS on proliferation, 6 cell groups with 500 thousand cells were placed at a distance of 0.5 cm from the surface of the 40 kHz ultrasonic transducer with MI average of 0.40, 0.51, 0.75 and 0.89 that were compared with control and sham groups. Ultrasound stimulation applied to spermatogonial cells cultured in an enclosed strerile 3.5 cm tissue culture plate in an incubator (32 °C, 5.3% $CO_2$). The exposure time was selected under the hyperthermia limit (± 1 °C).

The data of proliferation rate of ultrasound stimulated cells are presented as mean±standard deviation. The one-way ANOVA test used to analyze differences between groups, at a significant level of 0.05 ($p<0.05$) (SPSS/PC Inc., Chicago, IL).

## III. Results

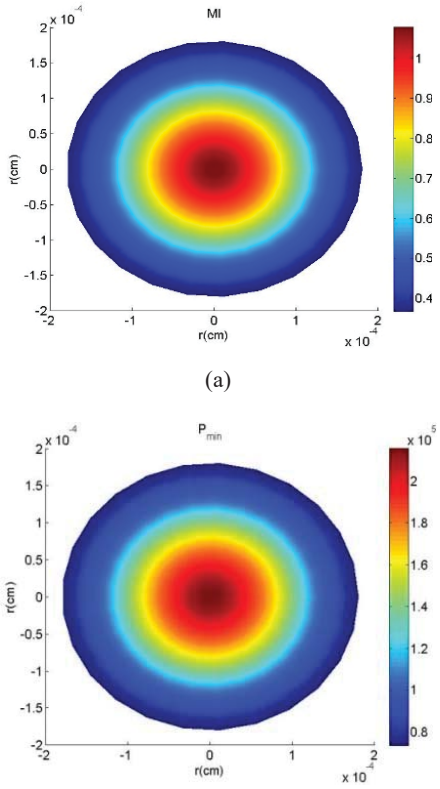
The results of numerical calculation are shown in Fig. 2. As increasing intensity of ultrasound waves from 0.28 to 1.34 W/cm$^2$on the target surface with radius of 1.8 cm, the increase of waves MI well have been observed. According to the mentioned conditions, ultrasound MI is ranged from 0.27 (1.5 cm, from the surface of the ultrasonic transducer) to 0.96 (0.3 cm).




(a)

(b)

Fig. 2 Ultrasound MI contour of 40 kHz in the r-z, intensity of (a) 0.28 W/cm$^2$, (b) 1.34 W/cm$^2$: The vertical axis (r) and the horizontal axis (z) show the radial and axial lengths in cm; Color map from blue to red indicates increase in MI




(a)

(b)

Fig. 3 A sample of counter maps of acoustics pressure and MI of 40 kHz in 0.5 cm from the transducer; Vertical and horizontal axes of radial distance (r) is in terms of cm; The color map represents the MI range (a) and the minimum acoustics pressure (b), respectively; 0.75, 0.96 W/cm$^2$

Pressure changes contour and the MI in transverse cross-section show 40 kHz ultrasound transducer in the range of induced cavitation threshold, 0.7, more or less than the threshold (Fig. 3). Plotted contours show the minimum pressure changes in terms of MPa according to transducer frequency and selected intensities. Color changes from blue to red indicates increase in the minimum acoustics pressure from $0.55\times10^2$ to $1.92\times10^2$ kPa and ultrasound MI from 0.50 ± 0.27 to 0.35 ± 0.96.

The correlation between measured pressure and calculated pressure was performed by Pearson correlation analysis (Fig. 4). There is a significant correlation between the measured pressure and calculated pressure for 40 kHz ($r=0.90$) ($p<0.05$).

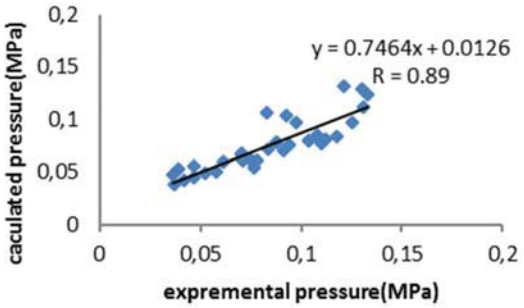



Fig. 4 The regression curve between calculated and measured pressure (kPa), 40 kHz, 1.26 cm radius

Proliferation of stem cells: Obtained colonies were spherical or elliptical shape with defined boundaries (Fig. 5).

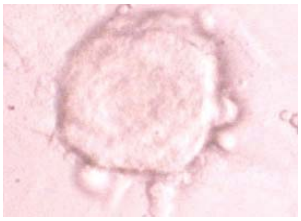


Fig. 5 Spermatogonia and Sertoli cells forming a colony, ×400

Qualitative study by immunoflurecent staining was done to establish the nature of obtained SSCs and qualitative evaluation of specific protein markers of SSCs. Spermatogonial stem cell pluripotent activity is expressed. The effect of ultrasound was studied in culture medium containing 10% FBS, 500 thousand SSCs exposed 40 kHz ultrasound with MI of 0.40, 0.51, 0.75 and 0.89, the energy density of 20.16, 18.90, 32.64, 32.16 J/cm$^2$, exposure time of 72, 42, 34 and 24 s, for 5 consecutive days and to comparison control and sham groups. At the end of the passage cells, proliferation of SSCs was examined (Fig. 6).

Increasing reproducibility in different groups for the initial cell number showed that in the group of 0.40 MI with the 2.89±0.07 times and 0.51 MI with the 2.17±0.10 times had the maximum reproducible and 0.89 MI group with 1.34 ± 0.03 times had the least reproducibility compared to control group (Fig. 6). Also, there is a significant difference among 0.40 and 0.51 MI groups with other groups ($P<0.05$).

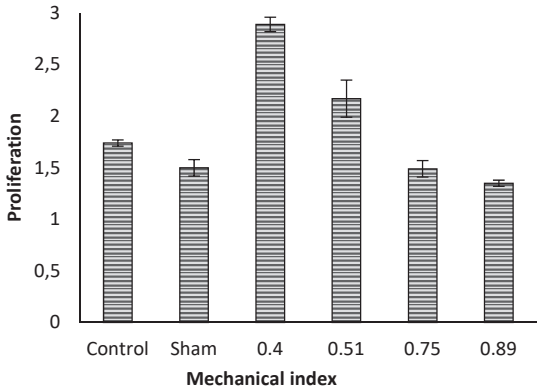



Fig. 6 Comparison of the proliferation of cultured cells after the first passage compare to the initial cell numbers in control, sham groups and 0.40, 0.51, 0.75, 0.89 MI groups

## IV. Discussion

In the report of British Medical Ultrasound Society [3], cavitation threshold is by MI higher than 0.7 as this amount is related to cavitation threshold in the tissue. In recent studies, to control the risks of diagnostic and clinical applications of ultrasound, the MI have been introduced for certain organs. In [13], to avoid the dangers and harm to the baby in clinical trials for the diagnosis and treatment of baby's lung, MI less than 0.3 and to prevent damage to the intestinal the MI between 0.3 to 0.7 were proposed, and MI above 0.7 increases the risk of damage.

In [14], the MI of 0.23 for eye applications was introduced in a report of the Food and Drug Administration. According to studies and concerning MI range of cavitation threshold, the MI in water is less than 0.7, because the eye tissue is made of water-equivalent material. Thus, considering cavitation threshold of different materials is necessary to find effective MI ranges on proliferation for the used frequencies.

According to the model results presented in this study, in order to solve the pressure equation and sound propagation and comparing these results with those obtained from experimental measurements with a hydrophone, it is observed that the results of this model well agree with the experimental values. Therefore, the model can be used to calculate the distribution of ultrasonic waves MI in various tissue and treatment plans in culture laboratory. On the other hand, the results in 0.40 MI parameter show increase in stem cells proliferation. Thus, considering cavitation threshold of different materials is necessary to find effective MI ranges on proliferation for the used frequencies.

## V. Conclusion

This acoustic propagation model and ultrasound MI assessments can be used with acceptable accuracy, for the extraction special arrangement of acoustic exposure used in biological condition *in vitro*.

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## References

[1] H. Wu, K. Ren, W. Zhao, GE Baojian, S. Peng, "Effect of electromagnetic fields on proliferation and differentiation of cultured mouse bone marrow mesenchymal stem cells," *J. Huazhong. Univ. Sci. Technol. Med. Sci.*, vol. 25, pp. 185-187, 2005

[2] J. L. Ruan, Y. N. Wang, A. C. Lawrence, B. M. Stuart, "Ultrasound generated mechanical induction of mesenchymal stem cells," *J. Acoust. Soc. Am.,* vol. 127, pp. 1763- 1766, 2010.

[3] A. H. Barati, M. Mokhtari-Dizaji, H. Mozdarani, S. Z. Bathaie, Z. M. Hassan, "Treatment of murine tumors using dual-frequency ultrasound in an experimental in vivo model," *Ultrasound Med. Boil*., vol. 35, pp. 756-763, 2009.

[4] S. H. Zhou, A. Schmelz, T. H. Seufferlein, Y. Li, J. Zhao, "Molecular mechanisms of low intensity pulsed ultrasound in human skin fibroblasts," *J. Biol. Chem*., vol. 279, pp. 54463- 54469, 2004.

[5] K. Keyhani, H. R. Guzman, A. Parsons, T. N. Lewis, M. R. Prausnitz, "Intracellular drug delivery using low-frequency ultrasound: quantification of molecular uptake and cell viability," *Pharm. Res*. vol. 18, pp. 1514-1520, 2001.

[6] C. Church, C. Labuda, K. Nightingale, "A theoretical study of inertial cavitation from acoustic radiation force impulse (ARFI) imaging and implications for the mechanical index," *Ultrasound Med. Biol*. vol. 41, pp. 472-485, 2015.

[7] M. F. Hamilton, D. T. Blackstock, "*Nonlinear acoustics*, "Academic Press, Boston, 1998; Chap 1-3, pp. 1-62.

[8] P. Hariharan, M. R. Myers, R. K. Banerjee, "HIFU procedures at moderate intensities: Effect of large blood vessels," *Phys. Med. Biol*., vol. 52, pp. 3493-3513, 2007.

[9] A. P. Sarvazyan, M. W. Urban, J. F. Greenleaf, "Acoustic waves in medical imaging and diagnostics," *Ultrasound Med. Biol*., vol. 39, pp. 1133-1146, 2013.

[10] M. E. Ravari, M. Mokhtari-Dizaji, S. H. Momeni-Masuleh, S. Motiee, "Estimation of ultrasound pressure distribution due to 1 MHz ultrasonic transducer for ultrasonic treatment planning in hyperthermia methods," *J. Acoust. Eng. Soc. Iran,* vol. 3, pp. 46-57, 2015.

[11] M. Mohaqiq, M. Movahedin, M. Mokhtari Dizaji, Z. Mazaheri, "The effect of low-intensity pulsed ultrasound stimulation on neonate mouse spermatogonial stem cells," *MJMS: Pathobiology.* vol. 16, 85-94, 2013.

[12] A. A. Ketkar, K. V. R. Reddy, "Expression pattern of OCT-4 and PLZF transcription factors during the early events of spermatogenesis in mice," *J. Cell Sci. Ther.,* vol. 3, pp. 1-6, 2012.

[13] S. Kotopulis, "*Biomedical ultrasounic, caviatation and sonoporation*", Thesis for the degree of doctor philosophy; Hull University, Yorkshire, England; 2011: Chap 1, pp. 11-84.

[14] Z. Hlinomazova, I. Hrazdira, "Alara: Principle and safety problems of diagnostic ultrasound," *SCRIPTA Medica (BRNO)*, vol. 78, pp. 341-346, 2005.