Interaction of ultrasound with microporous polyethylene scaffolds

Zhao, Liuxian; Lam, Yee Cheong; Lai, Chang Quan

2019

Zhao, L., Lam, Y. C., & Lai, C. Q. (2019). Interaction of ultrasound with microporous polyethylene scaffolds. *Applied Acoustics*, 153, 102-109.
doi:10.1016/j.apacoust.2019.04.007

<https://hdl.handle.net/10356/143228>

<https://doi.org/10.1016/j.apacoust.2019.04.007>

© 2019 Elsevier Ltd. All rights reserved. This paper was published in *Applied Acoustics* and is made available with permission of Elsevier Ltd.

Downloaded on 26 Apr 2023 22:06:36 SGT

Published in Appl. Acoust. 153, 102–109 (2019).
DOI: <https://doi.org/10.1016/j.apacoust.2019.04.007>

This Article Has Been Accepted and Published in

Appl. Acoust. 153, 102–109 (2019).

DOI: <https://doi.org/10.1016/j.apacoust.2019.04.007>

Interaction of Ultrasound with Microporous Polyethylene Scaffolds

Liuxian Zhao¹, Yee Cheong Lam², Chang Quan Lai^{1*}

¹*Temasek Laboratories, Nanyang Technological University, 50 Nanyang Drive, Singapore 637553*

²*Mechanical and Aerospace Engineering, Nanyang Technological University, 50 Nanyang Ave, Singapore, 639798*

* cqlai@ntu.edu.sg

Keywords: Porous Polyethylene, Scaffolds, Low Intensity Pulsed Ultrasound, Mie scattering, Microporous, Colloids, Powder Compaction

ABSTRACT

Ultrasound - stimulated healing in porous scaffolds has generated much interest in recent years. Most of the studies attempting to uncover the mechanisms behind this process, however, have mainly focused on the biochemical aspects and neglected the mechanical interaction between the ultrasonic waves and porous scaffolds. Such interactions are important, as they determine the distribution of ultrasonic waves around and within the scaffolds, which affects the local physical environment that, in turn, stimulates the biological responses. Here, we aim to identify the physical mechanism behind this interaction and elucidate the factors involved. To achieve this, microporous scaffolds were first fabricated by sintering monodisperse Polyethylene (PE) colloids of different sizes. The transmission and reflection coefficients of these scaffolds were experimentally determined in an immersion setup, over the clinically relevant range of 1 – 30 MHz and compared with trends obtained from an analytical model and finite element simulations. The experimental and simulation data were found to agree well with the analytical solutions of Mie scattering, and the absorption coefficient was observed to depend more strongly on particle size than the exact shape or arrangement of the individual particles. Nonlinear fluctuations of the absorption coefficient with frequency were also predicted by the analytical model and observed experimentally. Furthermore, finite element simulations showed that the ultrasonic waves have poor penetration depth and scattering takes place mainly at the surface of the microporous scaffolds. Our results indicate that lower frequencies, smaller scaffold characteristic lengths, thinner scaffolds and orientations that maximize surface exposure of scaffolds to ultrasonic waves will enhance the penetration of ultrasound into the structures and potentially improve the rate of ultrasound-stimulated healing processes.

1. INTRODUCTION

Ultrasound is an indispensable modern medical tool with many applications. As an imaging device, it is used to safely examine fetuses, soft tissues, tumors, bones and implants up to 4 dimensions (3 spatial dimensions over time) [1-8]. More than just images, the use of elastography techniques and Doppler effect enables ultrasound to measure mechanical properties of biological tissues [9] and the rate and direction of blood flow [10] for diagnostic purposes as well. As a therapeutic tool, it can be used to deliver genes and drugs into cells for the treatment of cancers [11-14], induce arterial contraction [15, 16] or function as a surgical tool for noninvasive removal of tumors through thermal ablation or cavitation [17, 18]. In addition, ultrasonic waves have also been shown to promote healing in nerves [19, 20], bones [21, 22], skin [23] and cartilage [24] through direct stimulation of biological cells and chemicals (*e.g.* growth factors, cytokines) [21, 22, 24], encouraging cell proliferation and nutrient distribution through fluid transport [22], as well as modulation of the physical cell environment through mechanical stresses and heat deposition [25, 26].

Along with ultrasonic stimulation, microporous or granular materials are often used as scaffolds at injury sites to enhance tissue regeneration and accelerate the healing process [27, 28]. Several studies have shown that the combination of low-intensity pulsed ultrasound (LIPUS) and scaffolds, for instance, encourages bone and cartilage to regenerate faster and more extensively than if either technique was used alone [28-31]. This may, in part, be due to the improved cell adhesion induced by ultrasound [31] which, together with the increased surface area provided by scaffolds, facilitates cell proliferation.

To date, most of the studies examining the effect of ultrasound on osseointegration of scaffolds have focused only on the biochemical aspects, such as gene expressions and stem cell differentiation [22, 29, 31], and neglected the mechanical interaction between ultrasound and scaffold geometry. This is important, as the scattering of ultrasonic waves on the surface and

within the scaffolds can amplify the ultrasound at some regions and attenuate them at others, leading to different biological responses at different parts of the scaffold [22].

By establishing the influence of the scaffold geometry on the distribution of ultrasonic waves around the scaffold and drawing the link between the biological responses at each specific location to this distribution, a clearer understanding of cell response to ultrasonic stimulation is expected to emerge. Using this knowledge, it would be then be possible to design and optimize scaffold geometries that can provide the fastest healing rate for patients.

As a first step towards this goal, the current study aims to investigate the interaction of ultrasound with microporous scaffolds and ascertain the scattering mechanism. To this end, the absorption coefficients of ultrasonic propagation through sintered microporous polyethylene (PE) colloids will be experimentally determined and compared with quantitative trends derived from finite element simulations and analytical equations.

2. METHODS

2.1 Fabrication of Microporous PE Scaffolds

The scaffold employed for the ultrasonic investigations in this study is microporous PE, which is commonly used for maxillofacial reconstruction [32-34]. The fabrication process involved placing monodisperse PE colloids (Cospheric LLC, USA) in a stainless steel holder of inner diameter ~ 4 mm and height ~ 4 mm, on top of a 25 mm $\times$ 25 mm glass slide. The average diameter of the colloids varied from 36 ± 13 μm to 275 ± 35 μm . These diameters were selected based on commercial availability and 36 ± 13 μm was the minimum diameter that the PE colloids were found to exist as free flowing powder, amenable to molding and sintering, rather than clumps.

The samples were then sintered at 110 °C for 120 minutes in an oven (Heratherm Oven, Thermo Scientific), after which they were removed and allowed to cool to room temperature under ambient conditions. The size of the pores in the sintered PE scaffolds ranged from a few micrometers to hundreds of micrometers (Figure 1), which is consistent with the dimensions reported for scaffolds in the literature [29, 30]. Following this, the microporous PE scaffolds, together with the stainless steel holders, were detached from the glass slides.

For ultrasound tests, the sintered PE colloidal blocks remained in the stainless steel molds, which act as holders. However, for mass measurements, the sintered PE colloidal blocks were removed from the molds. The PE colloidal blocks were weighed and the masses were divided by the volume of the blocks to obtain the effective mass densities. The solid fraction can then be calculated by dividing the effective mass density of each colloidal block with the mass density of the PE material ($= 960 \text{ kg/m}^3$) provided by the manufacturer. The solid fraction of each sample type was calculated from the average mass density of 3 separate microporous PE scaffolds (Table 1).

Table 1: Physical properties of the PE colloidal blocks.

Original Colloidal Diameter (a)	$36 \pm 13 \mu\text{m}$	$69 \pm 8 \mu\text{m}$	$138 \pm 18 \mu\text{m}$	$275 \pm 35 \mu\text{m}$
Solid Fraction (%)	93 ± 6	87 ± 14	84 ± 12	70 ± 3
Sample Dimensions	4 mm (diameter) x 2 mm (height)			

Figure 1: SEM micrographs of microporous PE scaffolds with average unit diameter: (a) $36 \pm 13 \mu\text{m}$, (b) $69 \pm 8 \mu\text{m}$, (c) $138 \pm 18 \mu\text{m}$, and (d) $275 \pm 35 \mu\text{m}$. (e) Top view of microporous scaffolds in a stainless steel holder. (f) Side view of the microporous scaffold after the stainless steel holder has been removed.

2.2 Ultrasonic Transmission and Reflection Measurements

Figure 2 shows the schematic illustrations of the experimental setups used to measure the ultrasonic transmission and reflection characteristics of the PE scaffolds. These immersion setups are commonly used [35, 36] and had also been shown to give good measurements of ultrasonic transmission through structures of similar pore sizes and overall dimensions as the ones used in this study [36]. The emitter was a non-focused ultrasonic transducer (diameter = 3 mm) (Olympus V316) connected to a power amplifier (E&I 325LA) and the waveform was

generated by an ultra-high frequency lock-in amplifier (UHFLI, Zurich Instruments) (Figure 2a). The frequencies were swept through the clinically relevant range of $f = 1$ MHz to 30 MHz [11], in intervals of 0.133 MHz. Ultrasonic waves were emitted in tone bursts consisting of 5 cycles at each frequency. A settling time of 2×10^{-5} s was included between each tone burst to allow a signal to dissipate before the next measurement. The transmitted power, as well as the glancing angle reflected power, were measured using a needle hydrophone (tip diameter = 0.2 mm; calibrated for frequency range of 1 – 30 MHz) (Precision Acoustics) attached to a hydrophone preamplifier (Precision Acoustics). The power measured was relayed to the UHFLI (Figure 2a), which averaged it over 5 full cycles of the tone burst for each frequency to minimize background noise.

The ultrasonic transducer and hydrophone receiver were placed at a distance of 10 mm and 2 mm from the sample respectively. By moving the 2-axis micrometer translation stage (Thor Labs Inc., USA) that the needle hydrophone was connected to, measurements were taken at six different locations for each sintered PE colloidal block sample and then averaged. Prior to the experiments, the sintered PE colloidal blocks were wetted with Isopropyl Alcohol (IPA) and then immersed in water to ensure that air bubbles were removed and water would successfully infiltrate the pores of the colloidal blocks [36].

To obtain the absorption coefficient, the transmitted and reflected power were first normalized with respect to the incident wave power and then subtracted from 1. The incident wave power was obtained by measuring the power of the wave generated by the emitter before it interacted with a sample. As such, the absorption coefficient may be written as,

$$A = 1 - T - R \quad (1)$$

where T and R refer to the transmission and reflection coefficients respectively. Note that in this case, the absorption coefficient takes into account the energy lost through both scattering and conversion to other forms.

Figure 2: (a) Schematic diagram of the connections between the Ultra High Frequency Lock-In (UHFLI) amplifier and the emitter and receiver. (b) Image and (c) Schematic diagram of the immersion setup for transmission measurements. (d) Image and (e) Schematic diagram of the immersion setup for glancing angle reflection measurements. Note that the sample was placed in the through-hole of the holder so that no solid material was present between the emitter and the sample or between the receiver and the sample.

2.3 Finite Element Simulations

Finite element simulations were performed on COMSOL Multiphysics 5.3a [37] to model the interaction of ultrasound waves with the sintered PE colloidal blocks underwater. As a first order approximation, the sintered PE colloidal blocks were modelled with perfect spheres arranged in a Face-Centered Cubic (FCC) geometry. The density, Young's modulus and Poisson's ratio used for PE were $\rho = 970 \text{ kg/m}^3$, $E = 200 \text{ MPa}$ and $\nu = 0.47$ respectively [38].

This sintered PE colloidal blocks were sandwiched by two fluid domains with physical properties equivalent to that of water (Figure 3). A Perfectly Matched Layer (PML) was added to one of the fluid domains to prevent reflected waves from interfering with the transmitted wave measurements. This is a fair reflection of the experimental setup, where the closest water-air and water-solid boundaries were at least 150 colloidal diameters away. Floquet periodicity was defined for the sides of this stack to avoid the influence of lateral edge effects. Finally, an incident ultrasonic wave normal to the stack surface was added to initiate the numerical computations.

Figure 3: Schematic of the water-scaffold arrangement.

3. ANALYTICAL MODEL

Since the average diameter of the PE colloids was varied from $36 \pm 13 \mu\text{m}$ to $275 \pm 35 \mu\text{m}$ and the frequency range investigated was 1 MHz to 30 MHz (wavelength in water $\sim 50 - 500 \mu\text{m}$), it is most likely that the ultrasound will undergo Mie scattering in the microporous scaffolds, as the wavelength of the ultrasound and dimensions of the colloids were similar (Figure 4) [39]. For this reason, analytical equations describing Mie scattering are employed to obtain the theoretical transmission and reflection coefficients of the microporous PE scaffolds.

Figure 4: The scattering regimes for a given wavelength and particle size [39]. The ultrasonic wavelength and particle sizes used in this study is indicated with solid lines.

Based on the model presented previously in section 2, we first consider a layer of scattering particles arranged in a row inside a non-viscous fluid (water) and a wave traveling from the fluid toward the layer was considered, as shown in Figure 5. The scattering layer has a thickness of $2h$ and the scatterers have identical geometrical (spherical) and elastic properties, and they

are distributed in the fluid such that the number concentration of scatterers is n_0 . The thickness of the layer is $2h$.

Figure 5: Acoustic wave scattering through layers of scatters. (gray color is microporous PE scaffolds and blue color is water).

As the incident wave interacts with the particles, it is scattered multiple times inside the layer due to the difference in acoustic impedance of the medium and particle. This scattering eventually leads the wave to exit the particle layer at both ends as reflected and transmitted waves. Aristegui and Angel [40, 41] found that the wave system in Figure 5 is one-dimensional after averaging the scatterings. The amplitude of reflection coefficient, R , and transmission coefficient, T , can be given as [40, 41]

$$R = -\frac{Qe^{-2jh}}{D}(1 - e^{4jKh}), \quad (2)$$

$$T = \frac{1-Q^2}{D}e^{2j(K-k_0)h}, \quad (3)$$

where

$$D = 1 - Q^2e^{4jKh}, \quad (4)$$

$$Q = \frac{1-\sigma}{1+\sigma}, \quad (5)$$

$$\sigma = \frac{k_0}{K} \left(1 + \frac{2\pi n_0}{k_0^2} (f(0) - f(\pi)) \right), \quad (6)$$

j is the imaginary number, K is the complex-value of wave number and k_0 is the wave number of the ultrasound propagating through the fluid, n_0 is the number concentration of the scatters, $f(0)$ and $f(\pi)$ are the angular shape functions at the angle of 0 rad and π rad respectively.

4. RESULTS

4.1 Experimental Results

Figure 6 shows the experimental results of acoustic transmission, T , and absorption coefficient, α , measured at different frequencies, f , for the porous PE scaffolds with average particle diameters, $a = 36 \pm 13 \mu\text{m}$, $a = 69 \pm 8 \mu\text{m}$, $a = 138 \pm 18 \mu\text{m}$ and $a = 275 \pm 35 \mu\text{m}$. The attenuation coefficient, C_A , is also given in Fig. 6a. C_A is related to the transmission coefficient for power, T , through the Beer-Lambert law, which can be given by [42]

$$C_A = -\frac{20 \log_{10} T}{2h}, \quad (7)$$

where C_A is in dB per unit length and $2h$ refers to the thickness of the sample. From Fig. 6a, it is observed that the attenuation coefficient of the microporous samples measured in this study can be as high as 100 dB/cm. This is comparable to the Alderson *et. al.*'s previous observation that microporous PE samples gave an attenuation coefficient of 47 dB/cm at 10 MHz [42]. In addition, the transmission coefficient for a given frequency was generally higher for samples with smaller particle diameters, although these values were negligible above ~ 8 MHz for samples with colloidal diameters $\geq 69 \mu\text{m}$. In other words, ultrasound can generally penetrate deeper into scaffolds formed from smaller particle diameters.

From Fig. 6b, it can be observed that the absorption coefficient rose rapidly within a narrow range of frequency (~ 5 MHz) and stabilized at a value near unity after that. There were irregular dips in the general trends of α vs. f , however, implying that scattering was not as

effective at certain frequencies. These fluctuations appear to be more numerous for decreasing particle sizes.

Figure 6: (a) Transmission coefficient of the microporous scaffolds fabricated from different colloids of different diameters. The values in the brackets show the corresponding attenuation coefficient in units of dB/cm. (b) Experimental measurements of the acoustic absorption coefficient.

4.2 Comparison with Analytical and Simulation Results

Comparing the experimental values of absorption coefficient with those obtained analytically and through finite element simulations shows that there is good agreement between the trendlines (Figure 7), indicating that Mie scattering was indeed the dominant mechanism behind the interaction of the ultrasonic waves with the porous scaffolds. This agreement was achieved in spite of the fact that the colloids within the scaffolds were not actually arranged in an orderly FCC geometry, and the shape of the colloids after sintering were not exactly spherical as well (Figure 1). Furthermore, the solid fraction within the scaffolds varied from 0.7 to 0.93 (Table 1), depending on the particle size, but they were assumed to be constant in the finite element simulations. While these inconsistencies certainly contribute to the discrepancies found between the analytical, numerical and experimental trendlines, the

differences are relatively minor. This suggests that particle shape, arrangement and number density do not influence the scattering process as much as the particle size and ultrasonic frequency.

Figure 7: Comparison of acoustic absorption performance obtained experimentally, analytically and numerically with diameters (a) $36 \pm 13 \mu\text{m}$, (b) $69 \pm 8 \mu\text{m}$, (c) $138 \pm 18 \mu\text{m}$, and (d) $275 \pm 35 \mu\text{m}$. Legend: Expt – experimental; Ana – analytical; Num – numerical.

In addition, it was observed that the analytical solutions could predict the α vs. f relationship better than the finite element simulations, which were only able to describe the general trends but not the detailed fluctuations of α , which is an important characteristic of Mie scattering [43]. This may be due to insufficient resolution of the finite element mesh used, but

finer mesh sizes tend to require excessive computational resources that may not be practically available *i.e.* there is a tradeoff between the accuracy and practicality.

Despite this limitation, the finite element simulations are nevertheless useful for providing insights into the approximate spatial distribution of ultrasonic pressure waves in the scaffolds. Figure 8 shows that the ultrasonic waves were effectively attenuated within the first 2 layers of particles at 30 MHz. The waves propagate through more layers for smaller particle sizes, but the reduced particle diameter meant that the absolute penetration depth remained shallow. This was observed across most particle sizes and frequencies, implying that the ultrasound waves were mainly scattered or converted to other forms of energy by the microporous scaffold near its surface. This is consistent with the low transmission coefficients and high absorption coefficients obtained experimentally (Figure 6).

Figure 8: Representative pressure distribution at $f = 30$ MHz in immersed microporous PE scaffolds consisting of particles with average diameters (a) $36\ \mu\text{m}$, (b) $69\ \mu\text{m}$, (c) $138\ \mu\text{m}$, and (d) $275\ \mu\text{m}$. The color scale bar indicates the normalized pressure.

5. DISCUSSION

Based on the results presented, it is clear that Mie scattering is the main mechanism behind the interaction of ultrasound and microporous scaffolds in a fluid medium. The good agreement between the experimental data and analytical solutions suggests that the most dominant geometric parameter in ultrasonic propagation through the scaffolds is the original particle size. The packing arrangement of the particles and slight shape change due to the merger of particles after sintering are second order factors.

It was also found that the majority of the scattering took place within the first few layers of particles, and most of the high frequency ultrasonic waves (> 8 MHz for scaffolds used in this study) were unable to penetrate into the scaffolds. The implication of this is that only biological cells on the surface of a microporous scaffold facing the incoming ultrasound will receive appreciable levels of ultrasonic stimulation. Any cell that proliferates into the scaffold subsequently will be shielded from such excitation, potentially slowing down the ultrasound-stimulated healing process. To circumvent this, microporous scaffolds should be made thinner or oriented to maximize surface exposure to ultrasonic stimulation.

Alternatively, as Fig. 6a indicates, low frequency ultrasound and/ or microporous scaffolds made from smaller particles can be employed to enhance ultrasound penetration into the scaffold. It should be noted, however, that the transmission of ultrasound through microporous scaffolds at low ultrasound frequency (3 – 8 MHz for scaffolds used in this study) can be highly sensitive to the ultrasonic frequency, depending on the characteristic length of the scaffold. Therefore, Mie scattering calculations outlined in this study are helpful in informing the exact extent of ultrasonic propagation into microporous structures, which would in turn, influence the effectiveness of ultrasound-stimulated healing.

Since the ultrasonic pressure field and possibly, thermal field, surrounding porous media in the body, such as scaffolds and trabecular bone, are highly dependent on the characteristic size and geometry of these structures, slight differences may result in completely different biological responses. For instance, LIPUS treatment on the trabecular bone of osteoporotic mice was shown to promote bone formation [44], but similar treatment in osteopenia rats resulted in no discernible effects [45]. One possible reason for this discrepancy could be the difference in pore size and density in the trabecular bones between these animal models, which interacted with the ultrasound in different ways. Another, more direct, example can be found in Aliabouzar *et. al.*'s recent study, which showed that lattices with square pores improved mesenchymal stem cell proliferation and differentiation over lattices with hexagonal pores under LIPUS treatment [29]. The implication of this is that the optimum frequency for ultrasound stimulated healing may be patient-, location- and scaffold- specific, and future studies should aim to ascertain this by varying the ultrasonic frequencies used, as current LIPUS treatment generally tend to rely on a narrow range of frequencies between 1 – 3 MHz [46].

6. CONCLUSIONS

Microporous scaffolds with different characteristic lengths were fabricated by sintering monodisperse PE colloids of different sizes. The transmission and reflection coefficients of these scaffolds were experimentally determined and compared to trends obtained from analytical models and finite element simulations. The experimental and simulation data were found to agree well with the analytical solutions of Mie scattering, where the absorption coefficient is highly dependent on particle size and frequency. The results of this study indicate that low ultrasound frequencies, scaffolds with smaller characteristic lengths, thinner scaffolds and scaffold orientations that maximize surface exposure to ultrasonic waves would better

Published in Appl. Acoust. 153, 102–109 (2019).
DOI: <https://doi.org/10.1016/j.apacoust.2019.04.007>

subject biological cells to ultrasound-stimulated healing. Computations based on Mie scattering are useful for informing the exact penetration extent of ultrasonic waves into scaffolds of a given characteristic length, which allows for the judicious selection of ultrasonic frequencies and scaffold design for accelerating the healing processes.

ACKNOWLEDGEMENTS

The authors would like to acknowledge funding for this project by the Temasek Research Fellowship.

References

- [1] Culjat MO, Choi M, Singh RS, White SN. Ultrasound imaging of dental implants. 2012 Annual International Conference of the IEEE Engineering in Medicine and Biology Society 2012. p. 456-9.
- [2] Guillaume R, Pieter K, Didier C, Pascal L. In vivo ultrasound imaging of the bone cortex. *Physics in Medicine & Biology*. 2018;63:125010.
- [3] Lai CQ, Jamil M, Yap CH, Lim GL, Mattar CNZ, Biswas A. Fluid mechanics of blood flow in human fetal left ventricles based on patient-specific 4D ultrasound scans. *Biomechanics and Modeling in Mechanobiology*. 2016;15:1159-72.
- [4] Wiputra H, Lai CQ, Lim GL, Heng JJW, Guo L, Soomar SM, et al. Fluid mechanics of human fetal right ventricles from image-based computational fluid dynamics using 4D clinical ultrasound scans. *American Journal of Physiology-Heart and Circulatory Physiology*. 2016;311:H1498-H508.
- [5] Edmonds PD, Abramowicz JS, Carson PL, Carstensen EL, Sandstrom KL. Guidelines for Journal of Ultrasound in Medicine Authors and Reviewers on Measurement and Reporting of Acoustic Output and Exposure. *Journal of Ultrasound in Medicine*. 2005;24:1171-9.
- [6] Wells PNT, Liang H-D. Medical ultrasound: imaging of soft tissue strain and elasticity. *Journal of the Royal Society, Interface*. 2011;8:1521-49.
- [7] Henderson REA, Walker BF, Young KJ. The accuracy of diagnostic ultrasound imaging for musculoskeletal soft tissue pathology of the extremities: a comprehensive review of the literature. *Chiropractic & manual therapies*. 2015;23:31-.
- [8] Gielen JC, R.; van Holsbeeck, M. . *Ultrasound of Soft Tissue Tumors*. Berlin, Heidelberg: Springer; 2006.
- [9] Sigrist RMS, Liao J, Kaffas AE, Chammas MC, Willmann JK. Ultrasound Elastography: Review of Techniques and Clinical Applications. *Theranostics*. 2017;7:1303-29.
- [10] Chang C-H, Chang F-M, Yu C-H, Liang R-I, Ko H-C, Chen H-Y. Systemic assessment of fetal hemodynamics by Doppler ultrasound. *Ultrasound in Medicine and Biology*. 2000;26:777-85.
- [11] Karimi M, Ghasemi A, Sahandi Zangabad P, Rahighi R, Moosavi Basri SM, Mirshekari H, et al. Smart micro/nanoparticles in stimulus-responsive drug/gene delivery systems. *Chemical Society reviews*. 2016;45:1457-501.
- [12] Kotopoulis S, Dimceviski G, Helge Gilja O, Hoem D, Postema M. Treatment of human pancreatic cancer using combined ultrasound, microbubbles, and gemcitabine: A clinical case study. *Medical Physics*. 2013;40:072902.
- [13] Postema M, Gilja O. *Ultrasound-Directed Drug Delivery* 2008.
- [14] Delalande A, Postema M, Mignet N, Midoux P, Pichon C. Ultrasound and microbubble-assisted gene delivery: recent advances and ongoing challenges. *Therapeutic Delivery*. 2012;3:1199-215.
- [15] Martin E, A Duck F, Winlove C. Thermally-Mediated Ultrasound-Induced Contraction of Equine Muscular Arteries In Vitro and an Investigation of the Associated Cellular Mechanisms 2011.
- [16] Martin E, A Duck F, E Ellis R, Winlove C. Ultrasound-induced Contraction of the Carotid Artery in vitro 2009.
- [17] Martin E, Jeanmonod D, Morel A, Zadicario E, Werner B. High-intensity focused ultrasound for noninvasive functional neurosurgery. *Annals of Neurology*. 2009;66:858-61.
- [18] Kennedy JE, ter Haar GR, Cranston D. High intensity focused ultrasound: surgery of the future? *The British Journal of Radiology*. 2003;76:590-9.
- [19] Sato M, Motoyoshi M, Shinoda M, Iwata K, Shimizu N. Low-intensity pulsed ultrasound accelerates nerve regeneration following inferior alveolar nerve transection in rats. *European Journal of Oral Sciences*. 2016;124:246-50.
- [20] Daeschler SC, Harhaus L, Schoenle P, Boecker A, Kneser U, Bergmeister KD. Ultrasound and shock-wave stimulation to promote axonal regeneration following nerve surgery: a systematic review and meta-analysis of preclinical studies. *Scientific Reports*. 2018;8:3168.

- [21] Claes L, Willie B. The enhancement of bone regeneration by ultrasound. *Progress in Biophysics and Molecular Biology*. 2007;93:384-98.
- [22] Padilla F, Puts R, Vico L, Raum K. Stimulation of bone repair with ultrasound: A review of the possible mechanic effects. *Ultrasonics*. 2014;54:1125-45.
- [23] Dalecki D, Comeau ES, Raeman CH, Child SZ, Hobbs L, Hocking DC. Guiding tissue regeneration with ultrasound in vitro and in vivo: SPIE; 2015.
- [24] Cui JH, Park SR, Park K, Choi BH, Min B-h. Preconditioning of Mesenchymal Stem Cells with Low-Intensity Ultrasound for Cartilage Formation In Vivo. *Tissue Engineering*. 2007;13:351-60.
- [25] Kruse DE, Mackanos MA, Connell-Rodwell CEO, Contag CH, Ferrara KW. Short-duration-focused ultrasound stimulation of Hsp70 expression in vivo. *Physics in Medicine & Biology*. 2008;53:3641.
- [26] Martin E. The Cellular Bioeffects of Low Intensity Ultrasound. *Ultrasound*. 2009;17:214-9.
- [27] Lei Y, Rai B, Ho KH, Teoh SH. In vitro degradation of novel bioactive polycaprolactone—20% tricalcium phosphate composite scaffolds for bone engineering. *Materials Science and Engineering: C*. 2007;27:293-8.
- [28] Wang Y, Qiu Y, Li J, Zhao C, Song J. Low-intensity pulsed ultrasound promotes alveolar bone regeneration in a periodontal injury model. *Ultrasonics*. 2018;90:166-72.
- [29] Aliabouzar M, Lee S-j, Zhou X, Zhang GL, Sarkar K. Effects of scaffold microstructure and low intensity pulsed ultrasound on chondrogenic differentiation of human mesenchymal stem cells. *Biotechnology and Bioengineering*. 2017;115:495-506.
- [30] Tanzer M, Harvey E, Kay A, Morton P, Bobyn JD. Effect of noninvasive low intensity ultrasound on bone growth into porous-coated implants. *Journal of Orthopaedic Research*. 1996;14:901-6.
- [31] An Y, Song Y, Wang Z, Wang J, Wu G, Zhu G, et al. Effect of low-intensity pulsed ultrasound on the biological behaviors of bone marrow mesenchymal stem cells on titanium with different surface topographies. *American journal of translational research*. 2018;10:67-76.
- [32] Lee S, Maronian N, Most SP, et al. Porous high-density polyethylene for orbital reconstruction. *Archives of Otolaryngology–Head & Neck Surgery*. 2005;131:446-50.
- [33] Sevin K, Askar I, Saray A, Yormuk E. Exposure of high-density porous polyethylene (Medpor®) used for contour restoration and treatment. *British Journal of Oral and Maxillofacial Surgery*. 2000;38:44-9.
- [34] Suwanprateeb J, Suvannapruk W, Wasoontararat K, Leelapatranurak K, Wanumkarng N, Sintuwong S. Preparation and comparative study of a new porous polyethylene ocular implant using powder printing technology. *Journal of Bioactive and Compatible Polymers*. 2011;26:317-31.
- [35] Secomski W, Bilmin K, Kujawska T, Nowicki A, Grieb P, Lewin PA. In vitro ultrasound experiments: Standing wave and multiple reflections influence on the outcome. *Ultrasonics*. 2017;77:203-13.
- [36] Krodel S, Daraio C. Microlattice Metamaterials for Tailoring Ultrasonic Transmission with Elastoacoustic Hybridization. *Physical Review Applied*. 2016;6:064005 (9 pp.).
- [37] COMSOL Multiphysics version 5.3a-Structural and Acoustics Module.
- [38] Warfield RW, Barnet FR. Elastic constants of bulk polymers (II). *Die Angewandte Makromolekulare Chemie*. 1975;44:181-4.
- [39] Caballero R. Atmospheric radiation. *Physics of the Atmosphere: IOP Publishing*; 2014. p. 5-1-5-35.
- [40] Aristegui C, Angel YC. Effective mass density and stiffness derived from P-wave multiple scattering. *Wave Motion*. 2007;44:153-64.
- [41] Angel YC, Aristegui C. Analysis of sound propagation in a fluid through a screen of scatterers. *Journal of the Acoustical Society of America*. 2005;118:72-82.
- [42] Alderson KL, Webber RS, Mohammed UF, Murphy E, Evans KE. An experimental study of ultrasonic attenuation in microporous polyethylene. *Applied Acoustics*. 1997;50:23-33.

- [43] Hans M, John AO. Parameterization of the Aerosol Upscatter Fraction as Function of the Backscatter Fraction and Their Relationships to the Asymmetry Parameter for Radiative Transfer Calculations. *Atmosphere*, Vol 8, Iss 8, p 133 (2017). 2017:133.
- [44] Yoshida A, Sasaki H, Furuya Y, Yoshinari M, Yajima Y. Effect of Low-Intensity Pulsed Ultrasound on Bone-Healing Process in Murine Low-Turnover Osteoporosis Model. *Journal of Hard Tissue Biology*. 2013;22:301-10.
- [45] Kawano T, Miyakoshi N, Kasukawa Y, Hongo M, Tsuchie H, Sato C, et al. Effects of combined therapy of alendronate and low-intensity pulsed ultrasound on metaphyseal bone repair after osteotomy in the proximal tibia of glucocorticoid-induced osteopenia rats. *Osteoporosis and Sarcopenia*. 2017;3:185-91.
- [46] Yang M-H, Lim K-T, Choung P-H, Cho C-S, Chung JH. Application of ultrasound stimulation in bone tissue engineering. *International journal of stem cells*. 2010;3:74-9.