Research article

The feasibility study of the transmission mode photoacoustic measurement of human calcaneus bone \textit{in vivo}

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\textbf{A B S T R A C T}

The photoacoustic (PA) technique is uniquely positioned for biomedical applications primarily due to its ability to visualize optical absorption contrast in deep tissue at ultrasound resolution. In this work, via both three-dimensional (3D) numerical simulations and \textit{in vivo} experiments on human subjects, we investigated the possibility of PA measurement of human calcaneus bones \textit{in vivo} in a non-invasive manner, as well as its feasibility to differentiate osteoporosis patients from normal subjects. The results from the simulations and the experiments both demonstrated that, when one side of the heel is illuminated by laser with light fluence under the ANSI safety limit, the PA signal generated in the human calcaneus bone can be detected by an ultrasonic transducer at the other side of the heel (i.e. transmission mode). Quantitative power spectral analyses of the calcaneus bone PA signals were also conducted, demonstrating that the microarchitectural changes in calcaneus bone due to osteoporosis can be detected, as reflected by enhanced high frequency components in detected PA bone signal. Further statistical analysis of the experimental results from 10 osteoporosis patients and 10 healthy volunteers showed that the \textit{weighted frequency} as a quantified PA spectral parameter can differentiate the two subject groups with statistical significance.

\section{1. Introduction}

Osteoporosis has been defined as a systemic skeletal disease that is characterized by low bone mineral density (BMD) and bone microarchitectural (BMA) deterioration, with a consequent increase in bone fragility and susceptibility to fracture [1]. Clinically available diagnostic technologies for osteoporosis rely on either X-rays or ultrasound (US) [2–13]. Dual-energy X-ray absorptiometry (DEXA), commonly considered as the gold standard diagnostic technology for osteoporosis, incompletely describes the fracture risk with limited accuracy. This is because DEXA, as an areal-based measurement of bone mineral, provides only a measure of BMD; while other essential parameters, such as BMA and material properties, are not assessed [1]. In addition, X-ray based techniques involve ionizing radiation which is a concern especially for repeated use in long-term longitudinal monitoring. Quantitative ultrasound (QUS) techniques provide a practical and low-cost substitute for DEXA, and have already led to clinical instruments [14, 15]. QUS assessment of bone structure and strength is mainly based on the measurement of speed of sound (SOS) and broadband ultrasonic attenuation (BUA), both strongly correlated with BMD but less reflective of BMA and bone chemical properties [16–18].

Recent developments in magnetic resonance imaging (MRI) have demonstrated the feasibility and diagnostic relevance of characterizing not only BMA but also bone metabolic processes at the molecular level [19]. MRI has the potential to investigate several aspects of bone physiology that are not captured by DEXA, including marrow fat content, perfusion, and molecular diffusion [20–22]. Bone marrow adipose tissue (BMAT) has been proposed as a biomarker predictive of osteoporosis-associated bone loss and even skeletal fragility [23]. Besides BMAT, bone perfusion is another physiological biomarker reflecting bone health. Several studies have demonstrated that the perfusion indices from MRI are significantly decreased in osteoporotic subjects compared with osteopenic subjects or those with normal bone density [20,21]. While metabolic processes in bone can be measured

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using MRI, the high cost, limited availability and accessibility of MRI preclude this technique from point-of-care implementation.

Biomedical photoacoustic (PA) technologies have drawn considerable attention in the last decades, and have been explored extensively for many preclinical and clinical applications [24–31]. In prior studies, the method of detecting backscattered PA signals has been explored for assessing ex vivo bone structure and density [32,33]. Researchers characterized the PA waves in the frequency domain with multiple optical wavelengths, which allowed assessment of the gross biochemical composition in the cortical bone, the SOS dispersion, and the BUA [34, 35]. In a study based on leukemic mouse models, it was confirmed that the PA imaging could reliably assess the changes in bone marrow oxygenation which is an emerging imaging biomarker for understanding many hematological diseases manifested as bone marrow failure [36]. Through a study on a rat model of estrogen-deficient bone loss and drug-induced preservation, our group investigated the feasibility of evaluating bone chemical properties using thermal photoacoustic measurement [37]. In another study on the same animal model, we found that, by performing photoacoustic spectrum analysis (PASA), the dynamic changes of BMA can be evaluated [38]. Recently, in a study in human subjects by using a dual-modality multispectral PA system, in vivo measurement of the blood/fat ratio in the marrow of the long bone was demonstrated [39]. As suggested by all these prior studies, PA techniques can measure not only the parameters associated with non-organic minerals, such as BMD and BMA, but also the contents and distributions of organic components such as collagen, hemoglobin, and lipid in bone. Based on the highly sensitive optical absorption contrast, PA techniques, with costs much lower than MRI, hold great potential to assess the metabolic processes in bone at the molecular level.

In this study, to pave the road toward clinical applications, we investigated the feasibility of transmission mode PA measurement of human calcaneus bone in vivo in a non-invasive manner. Understanding of the PA signal from the calcaneus bone was investigated via three-dimensional (3D) numerical simulations which considered light distribution, PA signal generation, and PA signal propagation in both soft tissue and bone. In parallel, we also conducted experiments on human calcaneus bone in vivo, and measured two groups of subjects including 10 osteoporosis patients and 10 healthy volunteers as the control.

2. Theory

2.1. Light fluence in bone

Light diffusion in an optically scattering medium such as bone can be simply calculated using the diffusion approximation [40],

\[ Q(r) = \mu_r(r)\Phi(r) - \nabla \cdot [D(r)\nabla \Phi(r)] \]

where \( Q \) denotes the source, \( \mu_r \) denotes the absorption coefficient, and \( \Phi(r) \) denotes the fluence. When \( \mu_s \gg \mu_a \) and in a nearly isotropic medium [40], the diffusion coefficient \( D \) is given by

\[ D = \frac{1}{3\mu_s} \mu_s (1 - g) \]

where \( \mu_s \) is the scattering coefficient, \( \mu_r \) is the reduced scattering coefficient, and \( g \) is the anisotropy factor. If the scale of the optical microheterogeneities is smaller than a few photon transport mean free paths, light fluence is smooth and can be characterized by a macrohomogeneous medium model. For a homogeneous medium [i.e. \( D = D_0 \), \( \mu_s = \mu_s_0 \), \( \mu_a = \mu_a_0 \) and a point source [i.e. \( Q(r) = \delta(r - r') \)], the solution of the diffusion equation using Green’s function is given as

\[ \Phi(r) = \frac{1}{4\pi D_0 \| r - r' \|} \exp(-\mu_{\text{eff}} \| r - r' \|) \]

where \( \mu_{\text{eff}} = \sqrt{3\mu_s \mu_a} \) is the effective attenuation coefficient, and \( r' \) is the spatial position of the source.

When the boundary between medium I and II is refractive index matched and the source to medium I is located at the interface, the boundary condition is expressed as

\[ \Phi(r) - 2D \frac{\partial \Phi(r)}{\partial z} = 0 \]  

When the boundary between medium I and II is refractive index mismatched, the boundary condition is expressed as

\[ \Phi(r) - 2D \frac{1 + R_s \Phi(r)}{1 - R_s} \frac{\partial \Phi(r)}{\partial z} = 0 \]

where \( R_s = \frac{\omega}{\omega_0} 2 \sin^2 \cos\theta R_0 \text{d} \Phi, \) and \( R_0 = \frac{\omega_0^2}{\omega} 2 \sin^2 \cos\theta R \text{d} \Phi \) [40].

2.2. Photoacoustic signal generation in bone

The initial pressure \( P_0 \) generated in the soft tissue/bone matrix induced by the absorption of optical radiation under the conditions of both thermal confinement and stress confinement can be written as Eqs. (6) and (7) [37]:

\[ P_0(r) \approx \Gamma \mu_a \Phi(r), \]

for soft tissue,

\[ P_0(r) \approx \frac{r}{(1 + \frac{3}{\lambda_0})} \mu_a \Phi(r), \]

for bone matrix.

Eq. (7) considers the difference in acoustic impedance between the hard tissue and the coupling medium. In Eqs. (6) and (7), \( \Gamma \) is the Gruneisen parameter and can be written as \( \Gamma = \frac{\beta c_0}{\omega_0^2 \rho}, \) where \( \beta \) is the isobaric volume expansion coefficient in \( K^{-1} \), \( c_0 \) is the heat capacity per unit mass in \( J \cdot K^{-1} \cdot kg^{-1} \), \( \rho \) is the density of the material in \( m/s \), \( \mu_a \) is the absorption coefficient in \( cm^{-1} \), \( \Phi(r) \) is the local light fluence in \( J/cm^2 \), \( c_0 \) and \( c_s \) are the SOS of the bone matrix and the coupling medium, respectively; \( \rho_a \) and \( \rho_b \) are the density of the bone matrix and the coupling medium, respectively.

2.3. Photoacoustic signal propagation in bone

The calcaneus bone is a complex elastic medium that combines both soft tissue and hard tissue. A cortical shell surrounds trabecular bone which is an anisotropic, heterogeneous and two-phase medium made of a complex network of interconnected solid plates or trabeculae saturated by marrow. In biological tissue, the laser induced PA pressure acts as an acoustic source and initiates further acoustic waves combining both compressional and shear waves propagation in a 3D space. In soft tissue, such as skin and marrow, ultrasound shear waves are usually neglected because shear waves are highly attenuated at ultrasonic frequencies. However, in hard tissue, such as cortical bone and trabeculae, both the compressional and shear waves must be considered. Therefore, in our simulation of PA signal propagation in human calcaneus bone, both the compressional and the shear waves were taken into consideration.

2.4. Photoacoustic spectral analysis (PASA)

The PASA method [41,42] used in this study is similar to our previous work based on a small-animal rat model [38], both studying the power spectra density \( PSD(f) \) of the PA signal \( p(t) \) acquired from bone tissue as shown in Eq. (8):

\[ PSD(f) = (\text{FFT}(p(t)))^2 \]

In our previous work, after getting the power spectra density of the PA signal, a linear fit was performed which led to a quantified spectral
parameter slope. In this study in human subject, a different spectral parameter, weighted frequency, is generated for the PA signal from each calcaneus bone by using the following equation:

\[ W = \int_{f_1}^{f_2} PSD(f) \cdot f \cdot df \]  

(9)

where \( f_1 \) and \( f_2 \) give the frequency range for integration.

3. 3D numerical simulations and experiments

3.1. Digital samples of human calcaneus bone

A calcaneus bone specimen from a cadaver donor with unknown age and gender was used in this study. The bone specimen was degassed and immersed in distilled water, and scanned by a micro-CT (Skyscan 1076, Skyscan, Antwerp, Belgium) for imaging the microstructures. The bone specimen was scanned using a 1-mm aluminum filter with the following settings: voxel size 18 \( \mu \text{m} \), voltage 75 kV, and current 130 \( \mu \text{A} \). Data were recorded every 0.8° of rotation up to 180°. Reconstruction was done using NRecon software (version1.6.9.8, Skyscan, Antwerp, Belgium) with attenuation data translated into 8-bit grayscale. A cuboid volume of interest (approximately 50 mm in each dimension) within the center of the bone specimen was delineated for data analysis. Fig. 1(a) shows the 3D microarchitecture of the calcaneous bone specimen reconstructed by the ImageJ software, and Fig. 1(b) shows the superior region [43] used for analyzing the parameter of bone volume/total volume (BV/TV). To simulate the light distribution in the digital bone specimen and the soft tissue, we used the NIRFAST-MATLAB 9.1 toolbox (Dartmouth College, Univ. of Birmingham and Kitware Inc.) [44], [45]. The digital samples of human calcaneus bone were imported into the MATLAB workplace, and then the images were downsampled to decrease the digital size (or numbers of pixels) for each image without changing their physical sizes. The digital 3D model for ultrasound simulation had a grid size of 50 × 50 × 50 mm^3, where each grid cube represents 0.016 mm^3. The model contained three components which were water, soft tissue, and bone (combining both bone matrix and bone marrow). At 690-nm laser wavelength, \( \mu_a \) and \( \mu_s \) were set as 10 cm\(^{-1}\) and 0.001 cm\(^{-1}\) for PML, 0.06 cm\(^{-1}\) and 15.0 cm\(^{-1}\) for the 20–30 years old bone [46], 0.09 cm\(^{-1}\) and 13.00 cm\(^{-1}\) for >50 years old bone [46], and 0.12 cm\(^{-1}\) and 14.8 cm\(^{-1}\) for soft tissue [47], as shown in Table 1. The optical source was set as a disk field source with a diameter of 15 mm at the center of the x-y plane.

The 3D numerical simulation of the light distribution in bone is shown in Fig. 5. Fig. 5(a) shows the 3D light distribution in a projection of another binarized 3D image using a threshold of 55 which leads to a BV/TV at the superior region of 18.76 %, consistent with the previously obtained BV/TV of human calcaneus bones at ages of >50 years [43].

The digital bone specimens in Fig. 1 were used in numerical simulations. The whole bone thickness along the Z direction shown in Fig. 1 was approximately 30 mm. In simulations, we also added 7-mm thick soft tissue at each side to cover the bone entirely, as shown in Fig. 2(a). In addition, we added bone marrow component to fill the pores in the trabecular bone in simulations.

3.2. 3D modeling

In this study, PA signal measurement was in the transmission mode. The light source and transducer were at opposite sides of the bone, as shown in Fig. 2(a). PML represents the perfectly matched layer. The process of the 3D numerical simulation is shown in Fig. 2(c).

3.3. 3D simulation of light distribution in bone

To simulate the light distribution in the digital bone specimen and the soft tissue, we used the NIRFAST-MATLAB 9.1 toolbox (Dartmouth College, Univ. of Birmingham and Kitware Inc.) [44], [45]. The digital samples of human calcaneus bone were imported into the MATLAB workplace, and then the images were downsampled to decrease the digital size (or numbers of pixels) for each image without changing their physical sizes. The digital 3D model for ultrasound simulation had a grid size of 50 × 50 × 50 mm^3, where each grid cube represents 0.016 mm^3. The model contained three components which were water, soft tissue, and bone (combining both bone matrix and bone marrow). At 690-nm laser wavelength, \( \mu_a \) and \( \mu_s \) were set as 10 cm\(^{-1}\) and 0.001 cm\(^{-1}\) for PML, 0.06 cm\(^{-1}\) and 15.0 cm\(^{-1}\) for the 20–30 years old bone [46], 0.09 cm\(^{-1}\) and 13.00 cm\(^{-1}\) for >50 years old bone [46], and 0.12 cm\(^{-1}\) and 14.8 cm\(^{-1}\) for soft tissue [47], as shown in Table 1. The optical source was set as a disk field source with a diameter of 15 mm at the center of the x-y plane.

The 3D numerical simulation of the light distribution in bone is shown in Fig. 5. Fig. 5(a) shows the 3D light distribution in a projection...
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Fig. 3 (b) shows the 2D cross-sectional view of the light distribution. Fig. 3 (c) shows 1D light distribution as a function of depth in the bone. The light fluence has -8.5 dB attenuation through the 7-mm thick soft tissue, and then has -28.7 dB to -31.6 dB attenuation when passing through the 30-mm thick bone.

3.4. 3D simulations of PA signal generation and propagation

In 3D simulations of PA signal generation and propagation, we used the elastic wave model provided by the open-source k-Wave MATLAB toolbox [48]. The propagation medium was isotropic and homogeneous with different propagation speeds, densities, and coefficients of ultrasound attenuation for water, soft tissue, and bone, respectively, as shown in Table 2 [49–51]. The three-dimensional grid was made of 200, 200, and 400 points along the x (elevation), y (azimuth), and z (depth) directions, respectively. The spatial step size was 0.25 mm in all three directions. Hence, the grid had a dimension of 50 mm along x-axis, 50 mm along y-axis, and 100 mm along z-axis. The transducer was set as 24.5 mm in diameter, placed at z = 68.3 mm and centered in the corresponding x-y plane. In addition, a PML was set around the computational domain, with 20 points thick (5 mm) along each of the x, y, and z directions. This PML ensured that no signal was reflected from the domain boundaries. The simulated PA signal was a 1-cycle sine wave of frequency 3 MHz, weighted by a Gaussian window. The signal

![Fig. 2. (a) 3D numerical simulation modeling. PML represents the perfectly matched layer. (b) The section view of the modeling geometry. (c) The process of the 3D numerical simulation.](image)

![Table 1](image)

| Material parameters used in simulations of light distribution. |
|---------------------------------------------------------------|
| Material parameters used in simulations of light distribution. |
|---------------------------------------------------------------|
| 20–30 years old | > 50 years old | Soft tissue | Perfectly matched layer (PML) |
| μa (cm⁻¹) | 0.06 | 0.09 | 0.12 | 10 |
| μs (cm⁻¹) | 15.00 | 13.00 | 14.80 | 0 |
| Cuboid size (x mm, y mm, z mm) | 50 × 50 × 50 | 50 × 50 × 50 | 50 × 50 × 50 | 50 × 50 × 50 |

![Fig. 3. 3D simulation of the light distribution in the bone and the covering soft tissue. (a) Projection view of the light distribution. (b) The light distribution along the center cross section. (c) Optical attenuation in the bone and the covering soft tissue as a function of depth. The results from the 20-30 years old bone and the >50 years old bone are compared.](image)
propagation lasted for 60 μs in simulation, which allowed the signal to travel through and beyond the specified domain.

The results of the laser-induced compressional wave and shear wave propagation maps along different views are shown in Fig. 4(a)–(c) and (e)–(g), respectively. This result is a snapshot of the compressional wave and the shear wave at the time of 35 μs after laser illumination. The whole PA signal profile combining both the compressional wave and the shear wave received by the transducer is shown in the Fig. 4(d). The signal from the bone, marked as region of interest (ROI), is enlarged and shown in Fig. 4(h).

Although the PA signal from a human calcaneus bone received by the transducer in the transmission mode can contain both compressional wave and shear wave, the contribution from the shear wave is minimal. This is mainly due to the fact that the shear wave attenuation in trabecular bone is very high, at approximately 17 dB/mm at 1 MHz according to a previous study [49]. Hence, the shear wave propagation in human calcaneus, as shown in Fig. 4(e)–(g), is mostly along the cortical layer. Human calcaneus bone has a thin cortical layer about 1 mm [52, 53]. As a result, the shear wave propagation along the cortical layer not only is inefficient but also leads to a delay in arrival time when compared to the compressional wave.

3.5. In-vivo experiments on human subjects

The experimental data analyzed in this work were from our clinical study on 20 human subjects that has been previously reported [54]. The objective of our previous paper was to demonstrate the feasibility of PA measurement, when aided by QUS, for assessment of human bone healthy. With a focus on characterizing lipid and marrow clusters in bone, PASA were performed at 930-nm and 800-nm wavelengths, respectively. In this work, the main purpose of analyzing the experimental data is to confirm the findings from the 3D modeling and validate that non-invasive PA measurement of human calcaneus bone in the transmission mode is feasible. The PASA in this work was conducted at 690-nm wavelength. According to our previous studies [37, 55], both hydroxyapatite and hemoglobin contribute to the optical absorption of trabecular bone at this wavelength. Hence, PASA at 690 nm reflects the bone microarchitecture formed by both trabeculae and bone marrow. To differentiate their contributions, multi-wavelength PA measurement powered by spectral unmixing methods may be utilized in the future.

In the control group, 10 young, healthy female volunteers aged 20–30 years old were enrolled. The osteoporosis group including 10 female subjects aged 50 years or older were recruited through the University of Michigan Department of Orthopaedic Surgery Fragility Fracture Clinic. All subjects in the osteoporosis group were diagnosed with osteopenia or osteoporosis, as confirmed by DEXA [54]. Subjects in the control group also received DEXA. In our simulation study, the micro-CT images served as the ground truth for bone microstructures. Micro-CT, however, is not a clinically available tool for osteoporotic patients. Therefore, DEXA was involved in our experimental study as the clinical standard to validate the pathologic conditions of the osteoporosis patients, and to confirm the difference between the two subject groups involved. All procedures in this study were approved by the Institutional Review Board of the University of Michigan School of Medicine.

| Table 2 |
| --- |
| Material parameters using in simulations of PA signal generation and propagation. |
| Cortical bone | Trabeculae | Bone marrow | Soft tissue | Water |
| Density (g/cm³) | 1900 | 1800 | 900 | 900 | 1000 |
| SOS of compression wave (m/s) | 4000 | 2000 | 1540 | 1540 | 1500 |
| SOS of shear wave (m/s) | 2000 | 1500 | 0 | 0 | 0 |
| Coefficient of compression wave | 10 | 10 | 0.02 | 0.02 | 0.02 |
| Coefficient of shear wave | 12 | 17 | 0 | 0 | 0 |
| Cuboid size (x mm, y mm, z mm) | 50 × 50 × 100 | 50 × 50 × 100 | 50 × 50 × 100 | 50 × 50 × 100 | 50 × 50 × 100 |

![Fig. 4. 3D simulation of the PA signal propagation in a human calcaneus bone. (a-c) The compressional wave in the x-y plane, x-z plane, and y-z plane, respectively, at 35 μs after laser illumination. (e-g) The shear wave in the x-y plane, x-z plane, and y-z plane, respectively, at 35 μs after laser illumination. (d) 3D simulation result of the PA signal, including both compressional and shear wave, generated in the bone and received by the ultrasound transducer. (h) Enlarged PA signal amplitude to better show the profile of the signal generated in the bone as marked by ROI.](image-url)
Medicine (IRB: HUM00105987, Assessment of bone health with light and sound. PI: Kozloff). The experimental setup is shown in Fig. 5(a)–(c). Using the transmission mode, the light illumination was from the lateral side of the heel, and the ultrasound transducer received the PA signal from the medial side.

In the PA measurement, an Nd:YAG laser pumped OPO (Phocus MOBILE, OPOTEK Inc.) was used to provide laser pulses with a repetition rate of 10 Hz and a pulse width of 5 ns. The laser beam was approximately 1.5 cm in diameter, with the pulse energy less than 45 mJ, which led to a laser light fluence of about 19 mJ/cm² on the skin surface, well below the ANSI safety limit of 20 mJ/cm² at 690 nm. The ultrasound transducer (Olympus, V301-SU) used for PA signal detection was unfocused, with 0.5-MHz central frequency, 1-inch diameter. Despite the fact that the initial pressure generated by the light absorption in bone tissue is broadband, due to the strong acoustic attenuation in the bone especially in the high frequency range, most of the PA signals received by the transducer are lower than 1 MHz. Hence, using a low frequency transducer for signal detection, as the one used in this study, can enhance the signal-to-noise ratio (SNR). After the PA signal was received by the transducer, it was amplified first with a 46-dB low-noise signal preamplifier (NF, SA-220F5) and then with another 40-dB amplifier (Pulse/receiver 5072PR) before being digitized and saved by an oscilloscope (Tektronix MSO54). A signal averaging over 300 laser pulses was conducted to further improve the SNR.

![Experimental setup and system calibration](image)

**Fig. 5.** The experimental setup and the system calibration for the in vivo study on human subjects. (a)-(c) The experimental setup for PA measurement of a human calcaneus bone. By measuring the laser-induced PA signal from a 200-μm microbead, as shown in (d), the frequency response of the PA detection system was calibrated, as shown in (e).
3.6. Calibration of the PA bone measurement system

We calibrated the piezoelectric conversion coefficient of the transducer used in our PA system by using a calibrated broadband Onda hydrophone (Onda Corporation, Sunnyvale, CA, USA). The piezoelectric conversion coefficient of the transducer was measured and calculated to be $2.2 \times 10^{-3}$ V/Pa at central frequency of 0.5 MHz. In addition, we calibrated the frequency response of the transducer by measuring the PA signal generated from a microbead with a diameter of 200 μm, as shown in Fig. 5(d). The diameter of microbead was much smaller than the resolution of the utilized transducer working at a central frequency of 0.5 MHz. In this case, the microbead can be considered as a point object, and the frequency spectrum of the PA signal generated by the microbead and detected by the transducer was mainly determined by the frequency response of the transducer. The calibrated frequency response of the transducer is shown in Fig. 5(e).

4. Results and discussions

Based on the 3D modeling of light distribution and PA signal propagation, we simulated the PA signal reaching to the position of the transducer. Then, we applied the transducer response to the PA signal obtained in 3D modeling to simulate the PA signal detected by the transducer, as the results shown in Fig. 6(a). The lateral soft tissue near the light source has a very large signal amplitude, while the medial bone and the soft tissue near the ultrasound transducer have a much lower signal amplitude. Working in the transmission mode, the peak-to-peak value (PPV) of PA signal from the calcaneus bone was in the range of 0.11–0.15 Pa when reaching at the transducer surface. Hence, as demonstrated by the simulation results, even penetrating through the soft tissue and the whole calcaneus bone, the light is still strong enough to generate detectable PA signal in a human calcaneus bone. We also calculated the power spectrum density (PSD) of the PA signal marked as ROI (400 time points which is about 8 cycles) in Fig. 6(a), as well as the calibrated PSD after removing the frequency response of the transducer. The PSD curves before and after calibration from the two different digital bone samples (i.e. simulated 20–30 years old and simulated >50 years old) are shown in Fig. 6(b) and (c), respectively, both normalized at 0.1 MHz.

The PA signals acquired in vivo from the heels of a randomly selected healthy volunteer and an osteoporosis patient are shown in Fig. 6(d). The PA signal profiles observed in the experiments are similar to that from the simulations. Due to the strong light attenuation, the PA signal generated by the soft tissue at the light illumination side was very strong, while the PA signal generated in the bone, as marked in the grey box, and the PA signal generated in the soft tissue at the transducer...
the center frequencies of the PSD and calibrated PSD from the calcaneus demonstrated that the PSD, both before and after calibration, of the PA results. In addition, both the simulation and the experimental results shown in Fig. 6 (e) and (f), respectively. In these experimental results, the center frequencies of the PSD and calibrated PSD from the calcaneus bones were 0.2–0.5 MHz which is also consistent with the simulation results. In addition, both the simulation and the experimental results demonstrated that the PSD, both before and after calibration, of the PA signals from the osteoporosis calcaneus bones with lower BV/TV had stronger high frequency components when compared to the results from the healthy bones with higher BV/TV.

To understand whether this observed difference in PSD can differentiate the two subject groups, we further quantitatively analyzed the PSD curves. For each normalized PSD curve, either before or after calibration, a quantified spectral parameter namely weighted frequency was acquired via an integration of the spectral power over the range of 0.1–0.74 MHz, as marked in Fig. 6(e)(f). Setting the cut-off frequency at 0.1 MHz avoided the strong low frequency noise, while the upper limit of 0.74 MHz was the -20 dB cut-off frequency of the transducer used in the experiments. For each subject, the weighted frequencies quantified from the PA measurements of the two feet were averaged first, leading to a single data point for each patient. Then with the weighted frequencies measured from all the involved subjects, the mean and the standard deviation for the healthy control group are compared to those for the osteoporosis group, as shown in Fig. 7(a) and (b). An unpaired two-tailed independent samples t-test (with Welch’s correction in cases of unequal variances) was conducted by using the GraphPad Prism 7.0 software to analyze the results in Fig. 7(a) (from non-calibrated PSD curves) and Fig. 7(b) (from calibrated PSD curves) individually. Both t-tests led to p < 0.01, indicating that the weighted frequencies as a quantified spectral parameter can differentiate the two groups with statistical significant difference. In addition, we also examined if there is any difference in PA signal pressure as quantified by the PPV between the two subject groups, as shown in Fig. 7(c). Although the PA signal pressure from the calcaneus bone in the osteoporosis group seems higher than that in the healthy control group, the unpaired two-tailed independent samples t-test did not lead to statistically significant difference.

To further confirm the pathological conditions of the osteoporosis group and its difference in bone quality compared to the healthy control group, a T-score value was derived from the DEXA images of each subject. Fig. 8 shows the ages and T-score values for all the involved human subjects, including the control data marked in blue and the osteoporosis data marked in red. An unpaired two-tailed independent samples t-test comparing the T-scores from the two subject groups was conducted, as shown in Fig. 8(b), leading to p < 0.001. In addition, we also studied the correlation between the weighted frequency and the T-score results, as shown in Fig. 8(c). The quantified Spearman’s Rank Correlation Coefficient $R_s = -0.57$ suggests a moderate correlation between the weighted frequencies from the PA measurements and the T-score results from the DEXA of the femur bones involved in this study.

5. Conclusions and discussions

In this study, the feasibility of non-invasive PA measurement of human calcaneus bone in vivo in the transmission mode was investigated through both 3D numerical simulations and the experiments on human subjects. In simulations, the light distribution in the heel, the PA signal generation and propagation, and the transducer’s response were considered. In experiments, PA measurements were conducted on total of 20 human subjects, including 10 healthy volunteers with ages in 20–30 years old and 10 osteoporosis patients with ages >50 years old. The results from the simulations and the experiments have a good match, as reflected by the similarities in PA signal profiles in both time and frequency domains. Both results suggest that, when working in the transmission mode using light fluence within the ANSI safety limit, the light can penetrate through the covering soft tissue and generate detectable PA signal in a human calcaneus bone.

In addition, the results from both simulations and experiments suggest that the PA signals from the calcaneus bones in the osteoporosis group contain stronger high frequency components. To examine whether this difference noticed in PA power spectrum can be used as a new parameter to assess the change in BMA resulting from osteoporosis, quantitative analyses of the PSD of the PA signals from the two subject groups were later conducted. The results from the 20 subjects demonstrated that the quantified PA spectral parameter weighted frequency can differentiate the control group and the osteoporosis group with statistical significant difference.

The concept used in this study on human subjects is similar to that in our previous work in small-animal rat model [38]. Both of these two studies aim at evaluating osteoporosis related changes in bone micro-architecture by performing spectral analysis of photoacoustic signals from bone. The detailed methods to achieve PASA of human calcaneus bones and PASA of rat femur bones, however, are different. This is mainly because that both the micro-size and the macro-size of human calcaneus bone are different from those of rat femur bone. First, human bones have relatively larger trabeculae and pores than rat bones. These optically absorbing structures with larger micro-sizes, under the illumination of laser pulses, generate lower frequency PA signals. To match the power spectrum of the generated PA signals from bone, the PASA in
this study of human subjects was performed over a low frequency range (i.e. 0–1 MHz) instead of the high frequency range (i.e. 0–20 MHz) utilized for our previous study in rat model. Second, human calcaneus bones as well as the covering soft tissues also have much larger macro-sizes compared to those of the rats. The larger tissue sizes especially the bone size lead to stronger ultrasound attenuation. Because the ultrasound attenuation in bone is highly dependent on the ultrasound frequency, after propagating through the human calcaneus bone, the PA signals detected in the transmission mode are mostly low frequency and narrow in bandwidth. Hence, a low frequency transducer, such as the one used in this study, can provide optimal sensitivity for PA signal detection.

Besides the difference in detecting transducers, the methods for PASA were also different. In our previous study in the rat model, the PASA was conducted by performing a linear fit of the PA signal power spectrum, which then led a quantified spectral parameter slope. This slope reflects the ratio between the high frequency and the low frequency components in the PA signal. Hence, its accuracy is relevant to the frequency range of the detected PA signal. This is not a problem for our previous study in the rat mode where a large detection frequency range of 0–20 MHz was realized, but could be concern for this study in human subjects where the detection frequency was 0–1 MHz. Therefore, in this study, a different PA spectral parameter, weighted frequency, was quantified from the PA measurement of each human calcaneus bone. Compared with the spectral parameter slope, weighted frequency is more suitable for analyzing PA power spectrum with a narrow frequency range.

As shown in Figs. 7 and 8, the p value achieved by PASA in differentiating the two subject groups is not as good as that achieved by the T-score from DEXA (p = 0.0086 vs. p < 0.001). DEXA, however, is solely based on the measurement of BMD; while BMA, as another important parameter reflecting bone strength, is not assessed. PASA, as demonstrated in this study, offers a new method to characterize BMA. Working in the same transmission mode, PA measurement can be easily combined with the existing QUS to assess multiple physical properties of human calcaneus bone simultaneously. Besides bone physical properties, PA measurement of bone can also be performed at multiple laser wavelengths to characterize additional chemical and molecular properties. All these diagnostic information are highly valuable for understanding bone healthy and underlying physiological conditions.

With the capability to assess human bone health at a low cost, in a non-invasive and non-ionizing manner, PA technique can be developed into a point-of-care tool for clinical diagnosis and treatment assessment of osteoporosis and other bone conditions. Before clinical applications can be possible, however, some further developments are necessary. First, the sensitivity of the PA bone measurement system should be further improved to enhance the SNR, especially at the high frequency range. This can be realized by optimizing the laser illumination pattern and light fluence on the skin surface, utilizing a custom-designed transducer with further improve receiving sensitivity and frequency range, and designing dedicated signal amplification and digitization circuit. With the SNR and the frequency range of the PA signal from human calcaneus bone improved, the sensitivity and specificity of PA technique in characterizing bone microarchitecture and possible chemical and molecular properties can be further elevated. Second, the impact of the difference in the overlying soft tissue thickness on PASA of bone microarchitecture was ignored in 3D numerical simulations. Considering that the ultrasound attenuation in soft tissue is low at the frequency range of 0–1 MHz, this impact caused by the overlying soft tissue should not be high. In the future, further studies will be conducted through both numerical simulations and experiments to investigate the impact from the overlying soft tissue such as thickness and skin color. Third, the performance and limitations of the PA technique should be further evaluated via clinical studies on larger numbers of populations. In this initial study, a limited number of subjects with two extreme conditions (osteoporosis vs. healthy) were involved. In the future, additional clinical data should be collected and analyzed on a larger cohort of subjects with a broad age range so that population-based differences in PA parameters across a wide range of ages can be assessed.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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