Investigation of temporal vascular effects induced by focused ultrasound treatment with speckle-variance optical coherence tomography

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Abstract: Focused ultrasound (FUS) can be used to locally and temporally enhance vascular permeability, improving the efficiency of drug delivery from the blood vessels into the surrounding tissue. However, it is difficult to evaluate in real time the effect induced by FUS and to noninvasively observe the permeability enhancement. In this study, speckle-variance optical coherence tomography (SVOCT) was implemented for the investigation of temporal effects on vessels induced by FUS treatment. With OCT scanning, the dynamic change in vessels during FUS exposure can be observed and studied. Moreover, the vascular effects induced by FUS treatment with and without the presence of microbubbles were investigated and quantitatively compared. Additionally, 2D and 3D speckle-variance images were used for quantitative observation of blood leakage from vessels due to the permeability enhancement caused by FUS, which could be an indicator that can be used to determine the influence of FUS power exposure. In conclusion, SVOCT can be a useful tool for monitoring FUS treatment in real time, facilitating the dynamic observation of temporal effects and helping to determine the optimal FUS power.

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1. Introduction

The endothelium is one thin layer composed of endothelial cells that lines the interior surface of a vessel, forming an interface between the blood and the rest of the vessel wall [1]. Such a structure becomes a physical barrier between the interior of the vessel and the surrounding tissue, and also limits drug delivery to intended targets. Focused ultrasound (FUS) can concentrate the ultrasound energy on a target without damaging the surrounding tissue, enabling disruption of the barriers and enhancement of the vascular permeability to increase

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the efficiency of drug delivery. Thus, FUS has become a potential tool to affect the endothelium for enhancement of vascular permeability [2,3], treatment of thrombolysis [4,5], and transient blood-brain barrier (BBB) opening [6,7]. Previous studies have demonstrated that FUS can enhance enzymatic thrombolysis by increasing the transport of thrombolytic agents into clots. In addition, the previous results have shown that FUS is promising for tumor chemotherapy due to improved nanodrug delivery. Moreover, to further improve the efficiency of FUS treatment, microbubbles have been employed during FUS exposure to facilitate thrombolysis even without the administration of thrombolytic agents. In addition, BBB disruption using FUS in the presence of microbubbles can increase the efficiency of localized chemotherapeutic drug delivery for brain tumor treatment [8]. Also, when the acoustic wave interacts with microbubbles, the local tissue motion contributed from microbubble oscillation may be enhanced, and physiological effects on blood vessels have been observed [9,10]. Although FUS treatment has become a novel method for enhancing drug delivery, it is difficult to investigate the dynamic and temporal effects on blood vessels noninvasively. Moreover, the local motion/vibration caused by FUS exposure is difficult to observe and is poorly understood, owing to strict spatial and temporal requirements. Also, the effect of FUS exposure can be influenced by acoustic parameters, such as acoustic pressure, exposure duration, or exposure mode (continuous or pulsed waves). Therefore, an imaging technique with high-resolution and high-temporal resolution is required to monitor and evaluate the effects of FUS treatment in a more detailed way.

Currently, the permeability enhancement due to FUS exposure can be visualized by using a fluorescent dye as a contrast agent under fluorescence microscopy [11,12]. However, with fluorescence microscopy, only superficial effects can be observed, and it is difficult to observe the permeability enhancement in the deeper structures. In addition, magnetic resonance imaging (MRI) [13–16] has been utilized to observe the effects induced by FUS in animal models. However, it is difficult to image the microvascular structures with MRI, due to the resolution limit. Moreover, to obtain high-resolution anatomical images, histology is the gold standard for realizing the morphological changes induced by FUS [6,17]. Although histology can accurately identify the permeability enhancement and the structural changes due to FUS exposure, it is a time-consuming and invasive method. Moreover, both MRI and histology are difficult to use for real-time monitoring of the dynamic changes of vessels. Currently, FUS-induced morphological changes to biological tissue and vessels are poorly understood as is the relationship between the morphological changes and permeability.

In 1991, optical coherence tomography (OCT) was invented to noninvasively probe the internal microstructures of tissue; this technique has been widely used for various biomedical applications due to its noninvasive, label-free, high-speed, and high-resolution imaging characteristics [18,19]. Based on an interferometer configuration, the microstructure of a sample can be obtained. Before the development of Fourier-domain OCT (FD-OCT) [20,21], the standard technique was time-domain OCT (TD-OCT) [22,23], in which mechanical scanning in the reference arm of an interferometer was required to modulate the optical path difference between the reference and sample arms. However, such a mechanism suffers from a limited scanning range and speed. Thus, to improve the imaging speed of OCT systems, FD-OCT was developed, including spectral-domain OCT (SD-OCT) [24–26] and swept-source OCT (SS-OCT) [27–29]. In FD-OCT systems, the amplitude and echo time delay of the backscattered light can be resolved through a fast Fourier transform of the interference spectrum without any mechanical scanning in the reference arm. For an SD-OCT system, a wavelength-resolved interference signal can be detected by a spectrometer composed of a grating and a line scan camera. In contrast, in an SS-OCT system, a high-speed swept source is employed to induce the wavelength-resolved (time-resolved) interference signal, which can be detected by a balanced detector. Because of the improvements that have been made to line scan cameras and swept sources, either SD-OCT or SS-OCT can provide a superior imaging speed and sensitivity, making the real-time reconstruction of microstructures of biological
tissue feasible. Moreover, compared to TD-OCT, previous reports have demonstrated that FD-OCT has superior performance in terms of the sensitivity and the imaging speed over those of TD-OCT systems [30,31].

Aside from three-dimensionally reconstructing the microstructures of biological tissue, there has been intensive development of functional imaging abilities, such as angiography [32–36,38–44], birefringence [45,46], and spectroscopic properties [47,48]. In the past decade, numerous groups have focused on the development of angiography techniques based on OCT, including phase-resolved Doppler OCT (PRDOCT) [32,33], scattering optical coherence angiography [34,35], speckle variance [36,38–40], optical micro-angiography [41,42], and correlation mapping method [43,44]. Based on the calculation of the Doppler frequency shift between adjacent A-scans, the flow velocity can be estimated. Recently, OCT has also become a novel method for intravascular imaging for detecting cardiac diseases [49,50]. Furthermore, birefringence of biological tissue can be measured with polarization-sensitive OCT (PS-OCT). Previous research showed that PS-OCT can be implemented for diagnosis of sun damage, burn depth, or skin aging. Moreover, some groups proposed that multispectral OCT can be used to investigate the spectroscopic properties of biological tissue, such as absorption, scattering, and attenuation.

In this study, we propose to use speckle-variance OCT (SVOCT) for investigation of temporal and local effects on biological tissue and vessels due to FUS exposure. With OCT scanning, the dynamic changes during FUS exposure are studied. Moreover, to further characterize the occurrence of blood leakage resulting from the enhancement of vascular permeability, the speckle variance of OCT images is estimated to acquire microvascular images and identify blood leakage based on the increase in speckle variance. The treatment effects induced by FUS with and without microbubbles are also demonstrated and compared.

2. System setup and experimental methods

2.1 OCT and FUS systems setup

In this study, we demonstrated an SS-OCT system with a MEMS-based swept source for investigation of vascular effects induced by FUS treatment, as shown in Fig. 1(a). The center wavelength of the swept source (HSL-20, Santec Corp., Japan) is located at 1310 nm with a scanning range of 105 nm. The scanning rate and the output power can achieve values of 100 kHz and 30 mW, respectively. The light source was connected to a Mach–Zehnder interferometer, consisting of two circulators and two couplers. Ninety percent of the laser output power was connected to the sample arm, and an objective lens (LSM02, Thorlabs) was used in the sample arm to provide a lateral resolution of ~10 μm. To resample the interference spectrum, a k-clock signal generated from the light source was utilized as an external clock.

In our OCT system, the physical area of OCT imaging is approximately 2 × 2 × 3 mm³, corresponding to 1000 × 500 × 600 voxels. With an A-scan rate of 100 kHz, the frame rate of our OCT system can achieve 100 frames/s, in which each frame consists of 1000 A-scans. In Fig. 1(b), a high-intensity FUS transducer (Imasonics, Besancon, France; diameter: 60 mm, radius of curvature: 80 mm, frequency: 400 kHz, electric-to-acoustic efficiency: 70%) was driven by a function generator (33220A, Agilent, Santa Clara, CA) to generate the acoustic wave. Before feeding the driving signal into the FUS transducer, the FUS excitation signal was amplified by a radio-frequency power amplifier (150A100B, Amplifier Research, Souderton, PA) and was monitored by a power meter (Model 4421, Bird, Atlanta). Then, the acoustic wave was transmitted through a homemade water tank and focused on the ear of a mouse, which was mounted on a transparent plastic plate. In our experimental setup, the acoustic wave was focused on the bottom surface of the sample, and the optical beam was incident on the top surface of the sample.
2.2 Experimental method

Before FUS exposure, the mice (C57 wild-type; male; 7-to-8-weeks old) were anesthetized with isoflurane, and the ears were mounted on a plastic plate. Ultrasound gel was swabbed on both sides of the ear to provide acoustic path coupling. To investigate the effects induced by FUS with microbubbles, an intravenous catheter was then inserted into the tail to allow tail-vein injections of microbubbles (Sonovue, Bracco, Italy). For the FUS exposures, a burst-mode wave was delivered (burst length: 40 ms, pulse repetition frequency: 10 Hz, duration: 120 s). The FUS powers delivered from the ultrasound transducer were set to be 1, 5, 10, and 15 W, which are equivalent to the rarefactive peak pressures of 128, 253, 310, and 408 kPa, respectively. However, to reduce the damage accumulated on a given mouse ear and to better observe the relationship between vascular leakage/deformation and the power that the tissue is exposed to, only four different powers were used in our experiments. When the mouse ear was exposed to ultrasonic pressure, the exposed region was simultaneously scanned by the OCT system. The experiment was repeated, exposing the same location to various FUS powers. To simultaneously record the dynamic changes in biological tissue during FUS exposure, the OCT system was synchronized with the function generator. Because the burst length was set to 40 ms and the frame rate of the OCT system was 100 frames/s, four sequential OCT B-scans could be obtained during each exposure length. The animal testing in this study was approved by the Laboratory Animal Center, Chang Gung University.

![Platform setup for OCT scanning and FUS exposure](image)

Fig. 1. Platform setup for OCT scanning and FUS exposure. (a) Schematic diagram of the SS-OCT system. (b) Setup for FUS exposure. FC: fiber coupler; CIR: circulator; G: galvanometer; M: mirror, and SL: scan lens. The cone was filled with deionized and degassed water to facilitate the transmission of acoustic waves. The acoustic wave was focused on the bottom surface of the sample, and the optical beam was incident on the top surface of the sample. The physical area of OCT imaging is approximately $2 \times 2 \times 3$ mm$^3$.

3. 2D OCT scanning results

3.1 Dynamic observation with and without microbubbles

Figure 2 shows the sequential B-scan results taken with the OCT system at the location on the mouse ear that was sequentially exposed to FUS powers of 1, 5, 10, and 15 W without...
microbubbles. The four sequential images were taken with a temporal spacing of 10 ms. Figures 2(a)–2(d) represent sequential B-scan results before the FUS exposure. Figures 2(e)–2(t) show the sequential B-scan results obtained during exposures with various powers of 1 W (Figs. 2(e)–2(h)), 5 W (Figs. 2(i)–2(l)), 10 W (Figs. 2(m)–2(p)), and 15 W (Figs. 2(q)–2(t)). The black area inside the rectangular region bounded by the dashed lines represents the vessel area; a 3 × magnified version of the vessel area is shown in the lower right corner of each part of the figure. The white arrows in the magnified images represent the interwall separation of the vessel, the value of which can be estimated from OCT images, as shown in the figure. The results show that when the ear was exposed to the higher powers of 10 W and 15 W, a change in the vascular area could be observed, as shown in Figs. 2(m)–2(t), which demonstrate a significant lumen formation and intravascular space change. Moreover, the magnified images show that the vascular area expanded and contracted with the acoustic wave.

Fig. 2. Sequential B-scan OCT images of the mouse ear without microbubbles, which were acquired before the FUS exposure ((a)–(d)) and during the exposures to FUS with the various powers of 1 W ((e)–(h)), 5 W ((i)–(l)), 10 W ((m)–(p)), and 15 W ((q)–(t)). The vascular area within the rectangular region bounded by the white dashed lines in (a) was magnified by a factor of 3; the magnified version of each vascular area is shown in the lower right corner of each panel in the figure. The four sequential images in each row were taken with a temporal interval of 10 ms. The white arrows in the magnified images represent the interwall separation of the vessel, the value of which can be estimated from the OCT images, as shown in the figure.
To investigate the morphological changes induced by FUS in the presence of microbubbles, a second set of scans was taken. Figures 3(a)–3(d) represent sequential B-scan results of the same ear location, obtained before FUS exposure and Figs. 3(e)–3(h), 3(i)–3(l), 3(m)–3(p), and 3(q)–3(t) represent the corresponding scan results taken during FUS exposures with powers of 1 W, 5 W, 10 W, and 15 W, respectively. The four sequential images were taken with a temporal interval of 10 ms. Here, all panels in Fig. 3 use the same intensity scale. Again, the black area inside the box marked by the dashed lines represents the vessel area; a 3 × magnified version of each vessel area is shown in the lower right of the corresponding panel. Before FUS exposure, no significant change in the vascular morphology can be found, even during the FUS exposure with the lowest power of 1 W. As the power was increased to more than 5 W, the vascular area became larger than that at the lower powers. In addition, the backscattered intensity of the OCT image decreased with increasing power, especially in the area surrounding the vessel. This was probably due to blood leakage from the vessel into the surrounding tissue, causing an increase in optical absorption.

Fig. 3. Sequential B-scan OCT images of another mouse ear, which were acquired before the FUS exposure ((a)–(d)) and during the FUS exposures in the presence of microbubbles at powers of 1 W ((e)–(h)), 5 W ((i)–(l)), 10 W ((m)–(p)), and 15 W ((q)–(t)). The area bounded by the box drawn in dashed lines in (a) is magnified by a factor of 3 and shown in the lower right corner for each panel. The black region represents the vessel structure. The four sequential images in each row were taken with a time interval of 10 ms.
To understand the superficial difference between the states before and after exposure to various power levels in the presence or absence of microbubbles, we consider Fig. 4, which shows photographs taken of the mouse ear before and after being exposed to each power level in the experiments of Figs. 2 and 3. The corresponding scanning locations of Figs. 2 and 3 are marked as the red lines in Fig. 4. In Fig. 4(e), the red spot, highlighted by the black arrow, was due to blood leakage when a higher FUS power of 15 W was applied. In contrast, in the presence of microbubbles, blood leakage could be found when the applied power attained a level of 10 W. Thus, the occurrence of permeability enhancement can be found from the photographs, as indicated by the black arrows.

Fig. 4. (a)–(e) Photographs taken of the mouse ear before and after being exposed to each power level in the experiment of Fig. 2. (f)–(j) Photographs taken before and after being exposed to each power level in the experiment of Fig. 3. The red lines indicate the corresponding scanning locations of Figs. 2 and 3.

3.2 Quantitative analysis of changes in vascular areas

Figures 2 and 3 show sequential B-scan images obtained before and during FUS exposures at various powers in the absence and presence of microbubbles. To quantitatively analyze the results of Figs. 2 and 3, the vascular area was estimated, based on the image-processing algorithm proposed in our previous study [51]. First, to investigate the relationship between the change in the vascular area and the ultrasound power, the vascular areas during FUS exposures were compared with that of the same vessel before FUS exposure. Thus, the ratio, 

\[ R = \frac{A_m}{A_o} \]

is defined for use in evaluating the change in the vascular area induced by FUS, where \( A_m \) is the mean vascular area of each exposure length. Here, since four sequential B-scans can be obtained during each exposure length, \( A_m \) was obtained by averaging the areas of the same vessel in the four sequential B-scans. Subsequently, \( A_o \) was defined as the mean vascular area of the same vessel before FUS exposure, which was obtained by averaging the areas of the same vessel in the twenty sequential B-scans obtained before FUS exposure. In our experiments, the mouse ear was exposed with a FUS exposure duration of 120 s for each power level, and the repetition frequency was set to be 10 Hz. For statistical analysis of \( R \) values, the last twenty exposure lengths were chosen to estimate the mean and standard deviation of \( R \) values for each power level. Figure 5(a) shows the means and standard deviations of \( R \) values at powers of 1, 5, 10, and 15 W. Figure 5(a) shows that the mean of the \( R \) values (gray column) gradually increased with increasing power when microbubbles were not used. In contrast, when microbubbles were used, the mean of the \( R \) values (orange columns) significantly increased with power, as shown in Fig. 5(a), illustrating how the mean vascular area increased with increasing power. In particular, when the exposure power exceeded 10 W, the vascular area obviously became significantly larger when using microbubbles. This phenomenon indicates that the mechanical vibration induced by FUS can be enhanced by microbubbles.

Subsequently, to further investigate the deviation of the vascular area during FUS exposure, we define the parameter, \( A_{d_s} \), such that
\[ A_d = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (A_i - A_\text{avg})^2 / A_0} \]  

where \( N \) is the total number of sequential B-scan images used for evaluation. In this study, \( N \) is equal to 4 for each exposure length. For the statistical analysis of the deviation of \( A_d \) values, the last twenty exposure lengths were chosen for evaluating the mean and standard deviation of the \( A_d \) values. Figure 5(b) shows the statistical results for the \( A_d \) values estimated from Figs. 2 and 3. In Fig. 5(b), the yellow columns represent the statistical result of the \( A_d \) values for different FUS powers in the absence of microbubbles. The mean of the estimated \( A_d \) value increased with power. However, when the power was increased to 15 W, the deviation of the vascular area became smaller than that of 10 W. That might be due to the endothelium damage induced by the FUS. In contrast, the red columns represent the means of the estimated \( A_d \) values at various powers in the presence of microbubbles. Compared with the result of the yellow columns, the mean of the \( A_d \) values became smaller when the FUS power exceeded 5 W. Similarly, this phenomenon might be due to the endothelium damage induced by FUS exposure. Therefore, the statistical evaluation of \( R \) and \( A_d \) values can be effective for determining the optimal power to use, based on the occurrence of decreases in the deviation of the vascular area (\( A_d \)).

3.3 Estimation of speckle variance

To further characterize blood leakage due to permeability enhancement by FUS, the speckle variance of the OCT images was estimated. Here, the estimation of the speckle variance of the OCT images enables not only the reconstruction of the depth-resolved microvascular structures, which is independent of the blood velocity or the imaging angle, but also the revealing of the blood leakage from the vessel region. The speckle-variance image can be obtained by calculating the intensity variance of the interframes. The estimation of the speckle variance (\( SV_{ijk} \)) can be written as

\[ SV_{ijk} = \frac{1}{N} \sum_{i=1}^{N} \left( I_{ijk} - \frac{1}{N} \sum_{k=1}^{N} I_{ijk} \right)^2 \]  

where \( i \) and \( j \) represent the transverse and depth indices of each frame [36]. \( N \) is the total number of B-scan frames used for speckle-variance estimation. Normally, the occurrence of speckle variance results from moving particles in biological tissue, such as blood flow [36,38–40] or nanoparticle diffusion [37]. Since no extraneous particles were used in our
experiment, the speckle variance was due to contributions from red blood cell (RBC) extravasations. The speckle-variance images of Figs. 2 and 3 are shown in Fig. 6. Figures 6(a)–6(e) show the speckle-variance images of Fig. 2, which were obtained from the mouse ear during FUS exposure in the absence of microbubbles. Again, the speckle variance of Fig. 3 was also calculated, as shown in Figs. 6(f)–6(j). From Figs. 6(a)–6(e), the intensity and distribution of speckle variance did not show a significant increase until the FUS power attained a level of 15 W. However, the results show that both the intensity and distribution of the speckle variance increased with increasing FUS power when the microbubbles were used.

Fig. 6. Speckle variance estimated from Figs. 2 and 3. Speckle-variance images, which were estimated from the OCT images obtained (a) before FUS exposure and during exposure to various FUS powers of (b) 1 W, (c) 5 W, (d) 10 W, and (e) 15 W in the absence of microbubbles. Speckle-variance images, which were estimated from the OCT images obtained (f) before FUS exposure and during exposure to various FUS powers of (g) 1 W, (h) 5 W, (i) 10 W, and (j) 15 W in the presence of microbubbles.

Fig. 7. Estimation of the vascular areas of Fig. 6. Curves I and II plot the relationship between the estimated vascular area and the FUS power, estimated from Figs. 6(a)–6(e). Curves III and IV show the relationship between the estimated vascular area and the FUS power, estimated from Figs. 6(f)–6(j).

Subsequently, the change of speckle-variance images was also estimated to study the temporal effects, such as blood leakage, resulting from the permeability enhancement. First, the speckle-variance image was digitized using a threshold value to reject the background signal. Then, the intensity of the selected region was integrated over the entire region prior to multiplying by the pixel area. Two regions in each frame were chosen for estimating the changes in the vascular areas, marked by the rectangular regions bounded by the red dashed lines in Fig. 6. To obtain the results of the exposures in the absence of microbubbles, regions I...
and II were chosen for estimating the changes in vascular areas. In contrast, regions III and IV were chosen for the case in which microbubbles were present during the FUS exposure. The results for regions I–IV are shown in Fig. 7. From curves I and II, a significant increase in the estimated areas of regions I and II can be found when a 15-W exposure was used. Here, the increase in the area of the speckle variance was due to the blood leakage. Furthermore, compared with curves I and II, curves III and IV show a significant increase in the vascular area when the power of the FUS exposure only exceeded 5 W. From Fig. 7, the result illustrates that the FUS power required to induce blood leakage due to permeability enhancement can be effectively reduced in the presence of microbubbles.

4. 3D scanning results

Aside from observation of the temporal effects induced by FUS with 2D OCT imaging, 3D imaging was also performed to investigate the FUS-induced vascular effects. Similarly, a mouse ear was sequentially exposed to various FUS powers. The microbubbles were injected into the mouse through tail-vein injections before the FUS exposure. Then, the burst-mode wave was delivered with the same exposure parameters (burst length: 40 ms, pulse repetition frequency: 10 Hz, duration: 120 s). The FUS powers from the ultrasound transducer were set to be 1, 5, 10, and 15 W, respectively. Then, the same marked region of the mouse ear was sequentially exposed to FUS, using various FUS powers, beginning from 1 W and increasing to 15 W. After being exposed to each power level, the same region was scanned with the OCT system to acquire 3D images. For obtaining 3D speckle-variance images, the scan protocol samples the same lateral location four times, resulting in the recording of four sequential B-scans of the same location to obtain a 2D speckle-variance image. Then, a physical area of 2 mm × 2 mm (xy) was scanned with a pixel size of 1000 × 500 (xy). Finally, the same procedure was employed to obtain speckle-variance images, mentioned in Section 3.3. Figure 8 shows the projection view of the 3D speckle-variance images, obtained before and after exposures with various powers. This result shows that no significant change in the vascular areas can be found when a lower FUS power was applied. However, the vascular area significantly increased when the power exceeded 5 W. Again, for quantitatively evaluating the change in the distribution of speckle variance, three regions, I, II, and, III,
indicated by the rectangular regions bounded by dashed lines, were chosen for estimation of the change in vascular area. Subsequently, the distributions of speckle variance in the three regions were estimated, based on the processing procedure of Fig. 7, as shown in Fig. 9. The result shows that the distribution of speckle variance in the three regions increased when the FUS power exceeded 5 W. The increase in the distribution of speckle variance resulted from the blood leakage induced by the FUS. In addition, the blood leakage was evidence of the permeability enhancement induced by the FUS. Thus, based on the 3D speckle-variance images, the area of the blood leakage can be spatially identified. The speckle-variance results shows that the temporal effects and permeability enhancement induced by FUS can be non-invasively observed and quantitatively evaluated with SVOCT, making SVOCT a feasible method for real-time monitoring of the treatment outcomes and temporal effects of FUS.

![Fig. 9. Estimation of the distribution of the speckle variance in Fig. 8. The estimated regions, I, II, and III, are indicated by the rectangular regions bounded by dashed lines in Fig. 8.](image)

5. Discussion and conclusions

From Fig. 5, it can be observed that the mean vascular area increased with increasing FUS power, either in the presence or absence of microbubbles. However, the mean vascular area became quite significant as the FUS power was increased to 15 W in combination with microbubbles. Moreover, the estimated $A_r$ values also show a trend where the deviation of the vascular area increased when the area was exposed to a lower power. As the power was increased to above 5 W in the presence of microbubbles, the deviation of the vascular area decreased, which probably resulted from the occurrence of endothelium damage. It was presumably that, from the analysis of the change in the vascular area during FUS exposure, the results demonstrate that the FUS-induced vascular morphological change can be enhanced by the presence of microbubbles. In addition, based on the evaluation of the speckle variance in sequential OCT B-scans, not only can the vascular images be obtained, but also the blood leakage can be observed. Thus, in Fig. 7, the distribution of the speckle variance began to become larger when the FUS power was greater than 5 W when combined with the presence of microbubbles. In contrast, without the microbubbles, the distribution of speckle variance became significantly larger until a power of 15 W was applied. Again, the result also implies that the vascular effects induced by FUS can be enhanced by microbubbles. Also, such information from estimation of speckle variance is consistent with the results of Figs. 4 and 5. Furthermore, the increase in the distribution of speckle variance was due to the blood leakage induced by the permeability enhancement after FUS exposure. Thus, from the results of vascular area estimation and speckle variance, the optimal power of FUS exposure can be determined to be 10 W when microbubbles were used, and significant RBC extravasations can be prevented.
In conclusion, FUS has become a novel method to temporally and locally enhance vascular permeability, enabling an improvement in the efficiency of drug delivery. However, when limited to the microvascular size and dynamic effects, it is difficult to noninvasively monitor the vascular effects in real time. In this study, we demonstrated an implementation of OCT that allowed dynamic observation of the morphological change in vessels during FUS exposure and investigation of the relationship between the morphological change in vessels and the FUS power. In addition, the effects induced by FUS exposure with and without microbubbles were compared. From the results, it can be observed that the morphological change in vessels can be enhanced by FUS alone but was more profound when the FUS was combined with the use of microbubbles. It is notable that, to quantitatively observe the blood leakage due to the permeability enhancement induced by FUS, SVOCT was implemented for the calculation of speckle variance due to blood flow and blood leakage, which cannot be detected by using OCT images. The result showed that the permeability can be enhanced by using microbubbles with a lower power of 10 W. Therefore, this study has established the feasibility of this methodology as a means of real-time monitoring of FUS treatments.

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