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ORIGINAL ARTICLE

In vitro experiment using porcine artery for evaluation of ultrasonic measurement of arterial luminal surface profile

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Abstract
Introduction In early-stage atherosclerosis, the luminal surface of the arterial wall becomes rough because of detachment of endothelial cells and degeneration of the internal elastic layer. Therefore, it would be useful if minute luminal surface roughness of the carotid arterial wall, which occurs in the early stage of atherosclerosis, could be measured noninvasively with ultrasound. The injured luminal surface is believed to have roughness of a few hundred micrometers. However, in conventional ultrasonography, the axial resolution of a B-mode image depends on the ultrasonic wavelength (150 μm at ultrasonic center frequency of 10 MHz) because a B-mode image is constructed using the amplitude of the RF echo signal. Therefore, such surface roughness cannot be measured accurately from a conventional B-mode image. Recently, we successfully measured such minute surface profile transcutaneously using the phase shift of an ultrasonic echo from the carotid arterial wall. In our previous validation experiment, a silicone phantom with minute surface roughness of 10–20 μm was measured. However, the feasibility of our proposed method has never been validated using biological tissues.
Materials and methods In the present study, luminal surface roughness of a porcine artery was measured and the result was evaluated by comparing it with the result measured using a stylus profilometer.
Results and conclusion The root mean squared difference between the surface roughness measured by ultrasound and the stylus profilometer was 10.5 μm. This result proves that our proposed method can be used to measure minute surface roughness of biological tissue.

Keywords Luminal surface of arterial wall · Roughness · Porcine artery · Atherosclerosis

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Introduction

Circulatory diseases are considered to be mainly caused by atherosclerosis, and early diagnosis or prevention of atherosclerosis is important. Medical ultrasound is widely used for diagnosis and treatment in clinical settings. Diagnostic ultrasound is noninvasive and, thus, can be repeatedly employed. Such properties of diagnostic ultrasound are suitable for diagnosis of atherosclerosis because the progress of atherosclerosis needs to be followed for a long term [1–4].

The luminal surface of a clinically healthy artery is covered by endothelial cells and is smooth. However, in the early stage of atherosclerosis, endothelial cells and the internal elastic layer are damaged, and endothelial cells are detached from the luminal surface [5, 6]. As a result, the luminal surface of the arterial wall will have roughness measuring a few hundred micrometers [7–9]. Therefore, it would be useful to measure such minute surface roughness of the carotid arterial wall for early diagnosis of atherosclerosis. The measurement of such minute surface roughness requires a high spatial resolution. However, in conventional

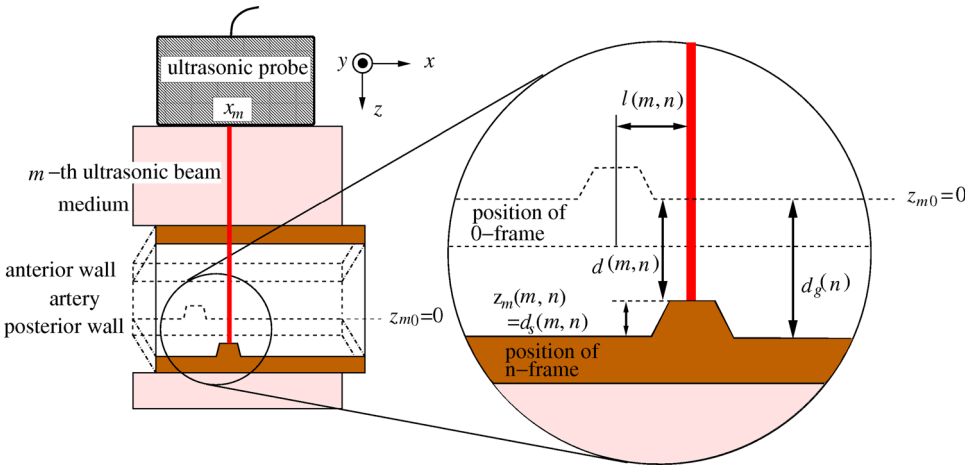

Fig. 1 Illustration of displacement of the arterial wall between the 0-th frame and *n*-th frame

ultrasonic diagnostic equipment, the spatial resolution in the axial direction depends on the ultrasonic wavelength (150 μm at ultrasonic center frequency of 10 MHz) because a B-mode image is constructed using the amplitude of the ultrasonic RF echo. Arihara et al. [10] measured the micron-order surface profile of an object made of silicone rubber using the phase shift of RF echoes during sweeping of an ultrasonic beam. However, in this method, the luminal surface cannot be measured accurately in vivo because the sound speed in the propagation medium between the skin surface and the carotid artery is inhomogeneous and the estimation of the distance from an ultrasonic probe to the surface (corresponding to the surface profile) is significantly affected by such inhomogeneity of the speed of sound.

In recent years, owing to significant improvements in the performance of ultrasonic diagnostic equipment, the movement of the arterial wall not only in the arterial radial direction but also in the longitudinal direction has come to be observed in a conventional B-mode image [11]. This longitudinal movement is considered to be caused by contraction of the heart. Cinthio et al. showed that minute luminal surface roughness could be measured using the arterial wall movement in the arterial longitudinal direction without sweeping an ultrasonic beam [12, 13]. In this method, the phased-tracking and block-matching methods were used to measure displacements of the luminal surface caused by roughness and pulsation of the arterial diameter during a cardiac cycle. In their validation experiments, the surface profiles of silicone phantoms with surface roughness of 13–33 μm were measured, and minute surface roughness was shown to be measured by ultrasound. However, the movement of the arterial wall in the longitudinal direction is small (less than 1 mm) and, thus, the length of a region measured by a fixed ultrasonic beam is limited. Therefore, Kitamura et al. [14] expanded the measurable region by combining multiple surface profiles measured by multiple ultrasonic beams.

In our series of studies, our method was validated by phantoms made of silicone rubber and has never been validated using biological tissues. Therefore, in the present study, we measured a porcine artery in a water tank and evaluated our proposed method by comparing the results to the results of measurement with a stylus profilometer.

Materials and methods

Measurement of surface roughness of arterial wall [14]

Figure 1 illustrates the displacement of the arterial wall during a cardiac cycle. In a fixed ultrasonic beam, the axial (z-axis) displacement would be detected when the luminal surface is rough and the arterial wall moves in the arterial longitudinal (lateral) direction. In addition, the arterial wall also moves in the arterial radial (axial) direction owing to expansion of the artery caused by an increment of blood pressure during a cardiac cycle. The sum of these axial displacements can be estimated by the ultrasonic phased-tracking method [15–17].

In Fig. 1, the lateral position of the m-th ultrasonic beam is denoted by x_m ($x_m = m{\cdot}\delta x$, where δx is the spacing between ultrasonic beams). The axial (z-axis) displacement of the posterior wall surface $\Delta d(x_m, n) \equiv \Delta d(m, n)$ between the n-th frame [t (s)] and the $(n + 1)$-th frame [$(t + \Delta T)$ (s)] (ΔT frame interval) obtained by the phased-tracking method is expressed by the difference between the axial positions $z(x_m, n) \equiv z(m, n)$ and $z(x_m, n + 1) \equiv z(m, n + 1)$ of the surface in the n-th and $(n + 1)$-th frames as follows:

$$\Delta d(m, n) = z(m, n + 1) - z(m, n). \quad (1)$$

In the present study, the displacement $d(m, n)$ was measured by the phased-tracking method, and the measured displacement $\hat{d}(m, n)$ contains both the global

displacement of the arterial wall $d_g(n)$ due to expansion of the artery and the displacement $d_s(m,n)$ owing to surface roughness, where ^ denotes the estimate. The measured displacement $\hat{d}(m,n)$ is expressed as follows:

$$\hat{d}(m,n) = d_g(n) + d_s(m,n). \tag{2}$$

The width of the measured region (about 8 mm) is much smaller than the wavelength of the pulse wave (several meters at 1 Hz) propagating along the carotid artery. Therefore, the global wall motion between the n-th and $(n+1)$-th frames at positions $\{x_m\}$ $(m = 0, 1, 2, \ldots, M-1)$ is assumed to be the same. The axial global displacement $d_g(n)$ caused by global wall motion can be estimated by averaging the estimated axial instantaneous displacements $\{\Delta\hat{d}(m,n)\}$ as follows:

$$\hat{d}_g(n) = \sum_{i=0}^{n-1} \Delta\hat{d}_g(i), \tag{3}$$

$$\Delta\hat{d}_g(n) = \frac{1}{M}\sum_{m=0}^{M-1} \Delta\hat{d}(m,n), \tag{4}$$

where M is the number of ultrasonic beams.

By defining the lateral (arterial longitudinal) displacement measured in the m-th ultrasonic beam in the n-th frame by $l(m, n)$, the lateral and axial positions of the luminal surface measured by the m-th ultrasonic beam in the n-th frame are $x = x_m + l(m, n)$ and $z(m, n)$, respectively. Therefore, the height of the surface (= surface profile) $h_m(x; h_{m0})$ measured by the m-th ultrasonic beam can be obtained as a function of the lateral position $x = x_m + l(m, n)$ as follows:

$$\begin{aligned}\hat{h}_m(x; h_{m0}) &\equiv \hat{h}_m(x_m + \hat{l}(m,n); h_{m0}) = \hat{z}(m,n) \\ &= h_{m0} + \sum_{i=0}^{n} \Delta d_s(m,i),\end{aligned} \tag{5}$$

where

$$\Delta\hat{d}_s(m,n) = \Delta\hat{d}(m,n) - \Delta\hat{d}_g(n), \tag{6}$$

and h_{m0} is the initial height.

The arterial longitudinal displacement $l(m, n)$ needs to be estimated by 2D tracking methods, e.g., block-matching technique [18] with reconstructive interpolation [19], to obtain the surface profile $h_m(x; h_{m0})$ as a function of lateral position $x = x_m + l(m,n)$. Furthermore, surface profiles $\{h_m(x; h_{m0})\}$ were measured in multiple ultrasonic beams $\{m\}$ and were combined to obtain a surface profile $\hat{h}(x)$ in a wider region.

The above-mentioned procedure of measurement of surface roughness of the carotid arterial wall is shown in Fig. 2.

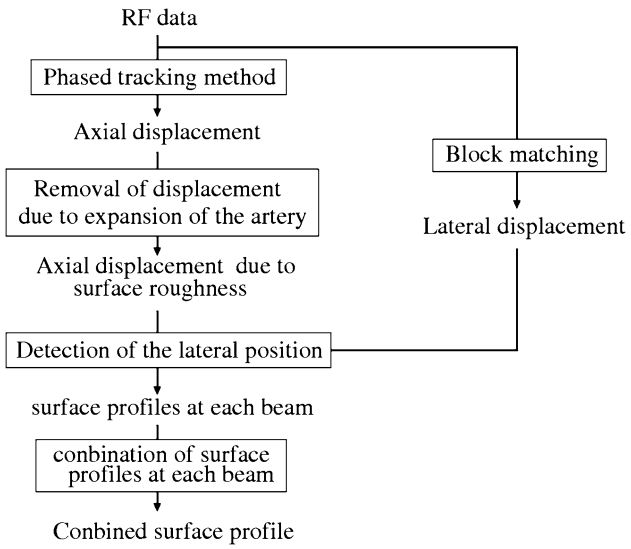

Fig. 2 Procedure for measurement of surface roughness

Evaluation of measured surface profile

In the present paper, the difference between the surface profiles, e.g., surface profiles $\hat{h}_a(x)$ and $\hat{h}_b(x)$, obtained from different measurements was evaluated using the root mean squared difference (RMSD) $\varepsilon_{\mathrm{RMSD}}$ defined as follows:

$$\varepsilon_{\mathrm{RMSD}} = \sqrt{\frac{1}{K}\sum_{i=0}^{K-1}\left\{\hat{h}_a(x_i) - \hat{h}_b(x_i)\right\}^2}, \tag{7}$$

where K is the number of positions where the surface profile was measured.

Also, the root mean squared roughness (Rq) was used to show the average roughness. The Rq is defined as follows:

$$Rq = \sqrt{\frac{1}{K}\sum_{i=0}^{K-1}\left\{\hat{h}(x_i) - \bar{h}\right\}^2}, \tag{8}$$

where $\bar{h}$ is the average of $\{\hat{h}(x_i)\}$.

In vitro experiments

Experimental system

Figure 3a shows a healthy porcine descending aorta used in experiments, and the aorta was cut opened in measurements, as shown in Fig. 3b. Figure 4 shows a picture of the luminal surface of the porcine artery observed with an optical microscope (BX40, Olympus Corp., Japan). Measurements were conducted with an ultrasound system (ULA-OP, Firenze Univ., Italy) and a stylus profilometer

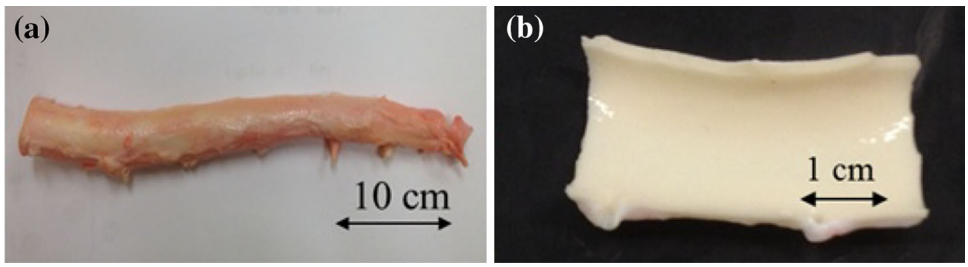

Fig. 3 **a** Picture of the healthy porcine descending aorta used in the present study. **b** Picture of the cut-open porcine descending aorta without tissue fixation

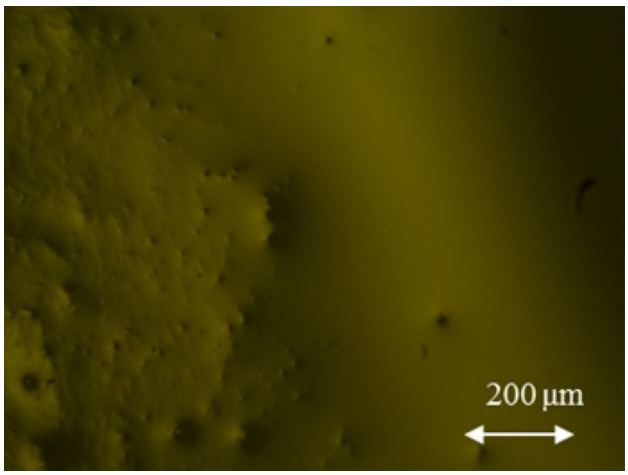

Fig. 4 Picture of the luminal surface of the porcine artery without tissue fixation observed using an optical microscope

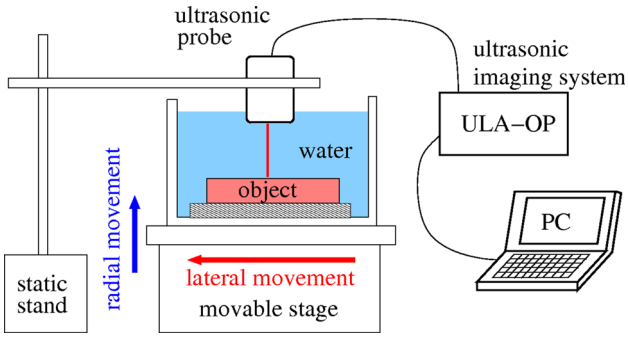

Fig. 5 Illustration of the experimental system. The porcine artery was moved using a movable stage to simulate displacement of carotid arterial wall

(XP-1, Ambios technology Inc., USA) to compare the results obtained by these systems and validate the accuracy of the ultrasonic measurement.

In the measurements with the stylus profilometer, the luminal surface of the porcine artery might be deformed due to pressure of the stylus. Therefore, to reduce the effect of pressure of the stylus, a pretreatment was applied to the porcine artery before measurements. To increase the toughness and to fix the surface profile, the porcine arteries were fixed using formalin. The concentration was 19 %, and the time for tissue fixation was about 8 h. After the tissue fixation, the porcine artery was fixed on a rubber plate using needles. Also, markers (needles) specifying the measured section were put on the luminal surface.

First, ultrasonic measurements were conducted. Porcine arteries were measured in a water tank and were moved using a movable stage, as shown in Figure 5, to simulate the displacement of the carotid artery. The velocities in the lateral and axial directions were 1.4 and 0.7 mm/s, respectively. The ultrasonic probe was fixed using a fixed stand. After ultrasonic measurements, the porcine artery was measured with a stylus profilometer. The resolution of the stylus profilometer was 1.5 nm in the axial direction, and the stylus force was 5.0 mg (50 µN).

Experimental results

Figure 6a shows a B-mode image of the porcine artery. The RF data were acquired using a 9.38-MHz linear-type probe, and the frame rate was set at 160 Hz. Figure 6b shows surface profiles measured by ultrasound. The measurements were performed three times to confirm the reproducibility of the proposed method. A minute surface profile was found to be measured reproducibly by our proposed method, as shown in Fig. 6b.

Figure 6c shows the surface profiles measured by ultrasound (red line) and a stylus profilometer (blue line). The surface profile measured by ultrasound in Fig. 6c is the average of the surface profiles in Fig. 6b.

In measurement using the stylus profilometer, the same position of the porcine artery identified by markers was measured. The surface profile measured by a stylus profilometer was also averaged for three times. As shown in Fig. 6c, overall, the surface profile measured by ultrasound agreed well with that measured by the profilometer, and the root mean squared difference ε_{RMSD} between these two profiles was 10.5 µm. Slightly larger errors around the lateral positions of 2–5 mm might be caused by the disagreement of the regions measured by ultrasound and the profilometer because the width of the ultrasound beam of 0.5 mm (width at half maximum) was larger than the diameter of the stylus of 2 µm.

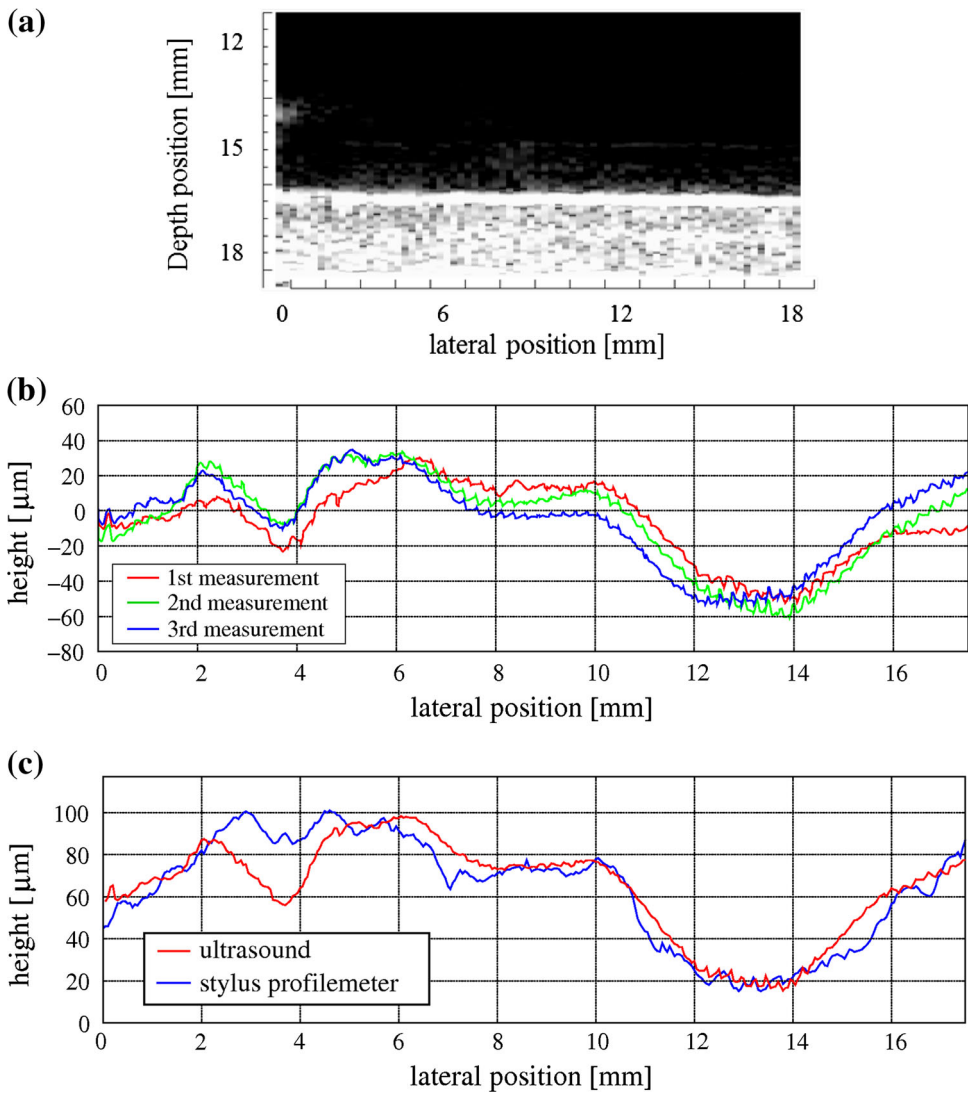

Fig. 6 **a** B-mode image of the porcine artery. **b** Surface profiles measured three times by the proposed ultrasonic method. **c** Averaged surface profile of the porcine artery measured by the proposed method (*red line*) and stylus profilometer (*blue line*)

In actual in vivo measurement, there is a medium with non-uniform sound velocity distribution between the skin and the anterior wall of the carotid artery. To investigate the effect of the propagation medium, the same porcine artery was measured under the condition that a porcine cutlet simulating the medium with non-uniform sound velocity distribution was inserted between the porcine artery and the ultrasonic probe. The thickness of the porcine cutlet was about 8 mm. Figure 7 shows the measured surface profiles with (green line) and without (red line) the porcine cutlet. Both surface profiles were measured by ultrasound. RMSD between the two surface profiles was 6.2 μm. Although the difference was slightly larger around the lateral position of 2 mm, the overall shapes of the surface profiles measured with and without the porcine cutlet were very similar to each other. From these results, it was shown that minute surface roughness of several tens of micrometers could be measured under a condition simulating in vivo measurement.

Discussion

We proposed an ultrasonic method for measurement of minute surface roughness of the carotid arterial wall occurring in the early stage of atherosclerosis. In our previous studies, the accuracy of our proposed method was validated using phantoms made of silicone rubber, and the feasibility of our method for the measurement of biological tissue had not been shown. Therefore, in the present study, the accuracy of our proposed method was validated using porcine arteries. To compare the surface profile measured by ultrasound with that measured with a stylus profilometer, the porcine arteries were fixed with formalin to

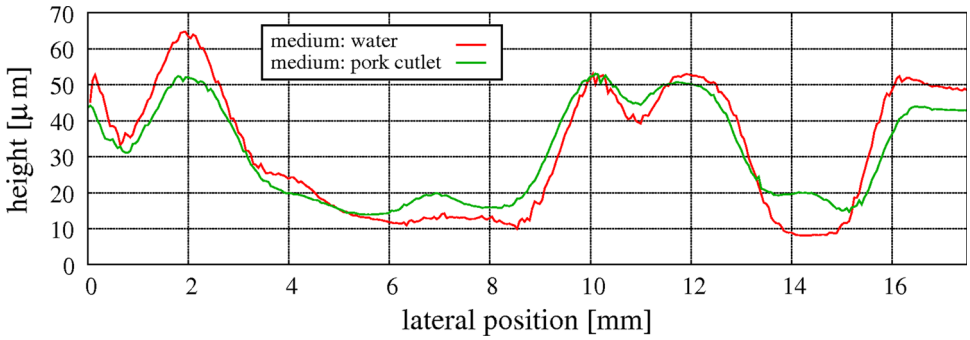

Fig. 7 Surface profiles of the porcine artery measured with (*green line*) and without (*red line*) the porcine cutlet between the porcine artery and ultrasonic probe

Table 1 Root mean squared roughness *Rq* of fresh porcine arteries

Porcine artery	*Rq* (μm)
Sample 1	18
Sample 2	14
Sample 3	22
Average	18

Table 2 Root mean squared roughness *Rq* of porcine arteries with tissue fixation

Porcine artery	*Rq* (μm)
Sample 1	23
Sample 2	20
Sample 3	17
Average	20

increase the hardness because the stylus deformed the surface profile when a fresh porcine artery was used. However, the surface roughness of a porcine artery may be changed by formalin fixation. Therefore, to examine the influence of tissue fixation on surface roughness, luminal surface profiles of six arteries (three fresh, three fixed) from the same pig were measured by ultrasound. For evaluation of the change in surface roughness, root mean squared roughness (*Rq*) was used. Tables 1 and 2 show the measured {*Rq*}. The average *Rq* of the fresh porcine arteries was 18 μm, and that of the porcine arteries with tissue fixation was 20 μm. The difference in {*Rq*} was 2 μm, and this small difference indicates that the effect of tissue fixation can be ignored.

Conclusions

In the present study, the luminal surface profile of a porcine descending aorta was measured by our proposed methods and the result was evaluated by comparing it with the result from measurement with a stylus profilometer. From these results, it was confirmed that the luminal surface profile, which has roughness of tens of micrometer, can be measured reproducibly and accurately by our proposed method. Furthermore, the medium with non-uniform sound velocity distribution between the skin and anterior wall of the carotid artery was simulated by inserting a porcine cutlet between the porcine artery and the ultrasonic probe. From this measurement, it was also confirmed that the minute luminal surface profile can be measured under the condition very similar to in vivo measurement.

Conflict of interest None.

Ethical standard In this study, no laboratory animals were used; only an excised porcine artery obtained from slaughterhouse waste was used.

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