Assessment of Red Blood Cell Aggregation Using Normalized Power Spectrum of High Frequency Ultrasound

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Abstract—Red blood cell (RBC) aggregation, which is one of the induces showing blood viscosity, plays an important role in blood rheology. RBC aggregation is formed by adhesion of RBCs because electrostatic repulsion between RBCs weakens as protein and saturated fatty acid in blood increase. Excessive RBC aggregation promotes various circulatory diseases in the clinical situation. The purpose of this study is to establish a noninvasive and quantitative method for assessment of RBC aggregation. The spectrum of nonaggregating RBCs presents Rayleigh behavior, which means that the power of scattered wave is in proportion to the fourth power of frequency. By dividing the measured power spectrum by that of echo from a silicone plate, the frequency responses of transmitting and receiving transducer are removed from the measured power spectrum. The normalized power spectrum changes linearly with respect to logarithmic frequency. In non-Rayleigh scattering, on the other hand, the spectral slope decreases. This is derived that a larger scatterer also behaves as a reflector and on echo from a reflector does not show frequency dependence. As a result, the influence of Rayleigh scattering is getting weak. Therefore, it is possible to assess the RBC aggregation from the spectral slope value. The spectral slope of the normalized power spectrum of echoes from the lumen of the vein in dorsum manus of 24-year-old healthy male was close to that from microspheres with diameter of 11 µm, and the standard RBC diameter is similar to this value. These results show the possibility of the proposed method for the noninvasive assessment of RBC aggregation.

I. INTRODUCTION

Medical ultrasound is used to make a diagnosis for various organs in the clinical situation because ultrasound is noninvasive and little stressful for a patient and it is possible to make repetitive diagnosis to confirm time-dependent changes of diseases. For diagnosis of blood vessel, ultrasound is used to evaluate the shape and the motion with recognizable in conventional B-mode images. Ultrasound B-mode images are not used for the condition of the blood in the blood vessel, because red blood cells (RBC), which are the main components of blood, are much smaller than the wavelength of ultrasound and the variation of acoustic impedance between blood and RBC is much small. However, the condition of blood could be a source of causes various diseases, and the evaluation and diagnosis of the condition of blood is important to detect the earlier diseases.

Fig. 1. Microscopic images of RBCs. (a) Healthy RBCs. (b) Aggregated RBCs.

Fig. 2. Microchannel of MC-FAN method.

Red blood cell aggregation, as one of the determinants of blood viscosity, plays an important role in blood rheology. Figure 1 shows the microscopic images of healthy RBCs and aggregated RBCs [1]. Healthy RBCs are charged with negative electricity in adventitia and will not adhere each other by electrostatic repulsion. However, due to the increases of protein and saturated fatty acid in blood, electrostatic repulsion between RBCs is gradually weakened and RBC aggregation is formed by overlap of RBCs each other. The main function of blood is to transport nutrients, oxygen, and constitutional
element to tissues and to remove metabolic products such as carbon dioxide and lactic acid produced by those tissues [2]. However, RBC aggregation degrades this function completely because of the decrease of the superficial area used to transport materials. Excessive RBC aggregation may promote various circulatory diseases in the clinical situation, such as atherosclerosis, hypercholesterolemia, diabetes, thrombosis and so on [3], [4]. Therefore, assessment of RBC aggregation is highly required. Figure 2 shows MC-FAN method, which is a technique for measurement of RBC aggregation developed recently. MC-FAN method observes the flowing red blood cells between silicone substrates simulating blood capillaries. However, this method is invasive and is not quantitative. The purpose of this study is to establish a noninvasive and quantitative method for assessment of RBC aggregation.

II. METHODS

RBC is a very small ultrasonic scatterer whose radius is 8 µm at most, and amplitudes of scattered RF echoes are very small. To assess the level of RBC aggregation, the power spectrum is calculated using the fast Fourier transform (FFT) and the scattering properties of RF echoes are evaluated in the frequency domain. In this study, it is assumed that the diameter of a scatterer increases depending on the degree of RBC aggregation. Echoes from scatterers with diameters sufficiently smaller than the ultrasonic wavelength show Rayleigh behavior, i.e. the power of scattered wave is proportional to the forth power of frequency [6], [7]. Echoes from scatterers with larger diameters include the components of reflection, which have no frequency dependence. Therefore, the spectral slope will be decreased when the sizes of scatterers increase and the components of reflection is included in echoes.

The measured power spectrum of received ultrasonic echo signal contains both properties, the scattering property from a microscopic sphere and the frequency response of transmitting and receiving transducer. Therefore, by normalizing the measured power spectrum by the power spectrum of echo from the silicone plate, the frequency response of transmitting and receiving transducer are removed from the measured power spectrum [8].

Figure 3 shows a system to measure an echo from a silicone plate. The distance between the probe and the silicone plate is equal to the distance between the probe and the middle part of the intravascular lumen in in vivo measurement. Figure 4 shows the power spectrum from a silicone plate that is obtained by averaging over 1000 power spectra of RF signals from a silicone plate. The frequency range for used normalization was determined because the ultrasonic pulse used in this study has a finite frequency bandwidth. In the present study, the width at -10 dB from maximum in the power spectrum of echo from a silicone plate was used. It is assumed that the echoes due to reflection do not show frequency dependence, only the scattering property of a target remain in the normalized power spectrum.

In Rayleigh scattering, the normalized power spectrum changes linearly with respect to the logarithmic frequency and scatterer’s effective diameter can be estimated from the intercept. In non-Rayleigh scattering, on the other hand, the spectral slope decreases as the scatterer’s diameter increases. Therefore, it is possible to estimate the scatterer’s diameter and to assess RBC aggregation from the spectral slope and intercept value.

III. BASIC EXPERIMENT USING MICROSPHERES

The ultrasound diagnostic equipment (UD-1000, Tomey, Japan) equips a mechanical scan probe at a center frequency of 40 MHz (wavelength is about 40 µm). RF echoes were acquired at a sampling frequency of 1 GHz and their power spectra were obtained by Fourier transform with a Hanning window of 1.024 µs. To reduce the influence of random noise, 1000 power spectra were averaged. Table 1 shows the information of measured objects which have different diameters. Microspheres with different diameters simulated RBCs and aggregated RBCs. They were mixed with water at a concentration of 3.00 g/l. RF echoes at the focal point were acquired as in the measurement of a silicone plate.

Figure 5(a) shows the averaged power spectra of echoes from the microspheres. Figure 5(b) shows the normalized power spectrum. The number is corresponding to the microspheres’ numbers in Table 1. The spectral slope and intercept values were shown in Table 1. Figure 6 shows mean and the standard deviation of spectral slope for about five times measurements for each microsphere. In Fig. 6, the slope
<table>
<thead>
<tr>
<th>No.</th>
<th>particle diameter [µm]</th>
<th>slope [1/\log_{10} f]</th>
<th>intercept [no dimension]</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>5 ± 2</td>
<td>3.73</td>
<td>-29.1</td>
</tr>
<tr>
<td>(2)</td>
<td>11 ± 3</td>
<td>2.14</td>
<td>-17.3</td>
</tr>
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<td>(3)</td>
<td>15 ± 5</td>
<td>1.78</td>
<td>-14.2</td>
</tr>
<tr>
<td>(4)</td>
<td>30 ± 10</td>
<td>0.17</td>
<td>-2.10</td>
</tr>
</tbody>
</table>

value of the normalized power spectrum of a larger scatterer shows smaller that of a smaller scatterer. This result shows that the slope value of normalized power spectrum is related to scatterer’s diameter.

IV. *In vivo* EXPERIMENT

Figure 7 shows a M-mode image from the vein at dorsum manus of a 24-year-old healthy male. It is possible to reduce the attenuation of the high frequency ultrasound because that the vein at dorsum manus is a superficial blood vessel. In addition, the vein at dorsum manus measured in this study has a large vessel diameter and fast blood flow, which affect RBC aggregation. Therefore, the measurement is from the non-aggregated RBCs.

Figure 8(a) shows the the power spectrum of echo from the lumen of the vein at dorsum manus of a 24-year-old healthy male and Fig. 8(b) shows the normalized power spectrum and the least-mean-squared regression line. The spectral slope and intercept were determined to be 2.12 and -17.7, respectively.

V. DISCUSSION

From the basic experiments using microspheres, it is found that the increase of the scatterer diameter causes the decrease of the slope of normalized power spectrum. This is derived that components of Rayleigh scattering in echoes are dominant in the case that scatterer diameter is small. However, the larger scatterer diameter is, more dominant the components of reflection are. Components of reflection show no frequency dependence, and the spectral slope value is decrease.

In *in vivo* measurement, the slope value of normalized power spectrum of echoes from RBCs is close to that of microsphere (2). RBC diameter is 8 µm at most and this value of diameter is between those of microspheres (1) and (2). However, the measured spectral slope was close to that of microsphere (2) whose diameter (11 µm) was slightly larger than those of RBCs. This is expected to be caused by white blood cells (diameter: 7-25 µm) and other impurities, whose sizes are larger than RBC and affect the slope value. Although the estimated RBC diameter did not exactly match the real RBC diameter, these results showed that the slope of normalized power spectrum changes with scatterer’s diameter.
VI. CONCLUSION

The effective diameter of nonaggregating RBC was estimated in vivo using spectral parameters. These results show the possibility of the proposed method for the assessment of RBC aggregation.

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REFERENCES