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Characterization of erythrocyte aggregation with ultrasound

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1. Ultrasound scattering in blood

Ultrasound is a sound wave with frequencies above the audible range. When an acoustic wave propagates through an inhomogeneous media such as biological tissues, it is diverted by reflection and refraction from tissue interfaces, by scattering if the size of the interfering particles is much smaller than the ultrasound wavelength (λ), or absorbed by the medium and converted into heat. Scattering of ultrasound by blood is almost entirely due to the erythrocytes because they are much more numerous than the slightly larger leukocytes, and significantly larger and more numerous than platelets. With most clinical instruments, the frequency of the acoustic waves is in a range of 2 to 30 MHz. The wavelengths corresponding to these frequencies are smaller than the size of a red blood cell (RBC). When an incident ultrasound beam is scattered by RBCs, the scattered power can be measured at any scattering angle between 0 and 360° [10]. Scattering measurements are made with one transducer for backscattering at 180° or by two transducers for other angular measurements [11].

2. Ultrasound backscattered power and RBC aggregation

Several research groups have shown that the intensity of the ultrasound signal backscattered by blood depends on the presence of RBC aggregates [3]. According to the literature, a correlation exists between the size of the aggregates and the ultrasonic backscattered power.

When the blood sample is in motion, the shearing effects of the flow cause the RBC aggregates to break apart, which lead to smaller aggregates and weaker backscattered power. Conversely, when the flow is ceased or reduced, the shearing effects decay accordingly and consequently, the RBCs reaggregate, which is reflected by a stronger backscattered power. For blood samples with little or no aggregates, such as bovine whole blood or RBCs suspended in a saline solution, the backscattered power is independent of the shearing effects of the flow under laminar conditions.

Based on the Rayleigh scattering theory, the power backscattered by one particle (backscattering cross-section) depends on the fourth power of the ultrasound transmitted frequency, and the square of the

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particle volume [11]. However, for a high number of particles at a constant volume concentration (hematocrit), the ultrasound backscattered power is proportional to the mean scatterer's volume [3,7] when they are much smaller than the wavelength of the ultrasound transmitted signal and monodisperse in size. Rayleigh scattering implies that the dimension (diameter) of the scatterers in the direction of propagation of the ultrasonic waves is less than one tenth of the wavelength [11]. For example, at 10 MHz, by considering a speed of sound in blood at 1570 m/s, the dimension of the scatterers should be less than 15.7 μm for this condition to be met. According to this theory, doubling the mean size of monodisperse RBC aggregates, at a constant hematocrit, should produce similar effect on the backscattered power, as long as scatterers remain smaller than $\lambda/10$. It should be kept in mind, however, that other factors can influence the backscattered power. Although it can be difficult to assess their impact, the number (hematocrit), shape, variance in size, and packing organization of the aggregates can be of major importance.

3. *In vitro* and *in vivo* results

Figure 1 shows backscattered power measurements performed in a flow model with equine blood samples modified to obtain a wide range of RBC aggregation [4]. As shown, exponentially decaying relationships were observed for all AI values (aggregation index measured by light reflectometry). The power was maximum at low shear rates and dropped as the shear rate was increased. At a given shear rate, the power was proportional to the level of aggregation. Based on these results, a feasibility study was conducted to quantify the level of RBC aggregation, *in vivo*, in the common femoral artery and vein of normal subjects and patients with hyperlipidemia [5]. The hypotheses of this study were: (i) the backscattered power should be higher in veins than in arteries because smaller shear rates are present in the former vessels compared to the latter, and (ii) the power should be higher in patients with hyperlipidemia because of the enhancement of RBC aggregation in this group. The vessel was identified using ultrasound B-mode imaging and the backscattered power was measured in Doppler mode at the center of each vessel to optimize the chance of detecting RBC aggregates. Figure 2 shows the results obtained. Based on an analysis of variance, the backscattered power was significantly higher in veins compared to arteries ($p < 0.05$), and significantly higher in patients with hyperlipidemia compared to controls ($p < 0.001$).

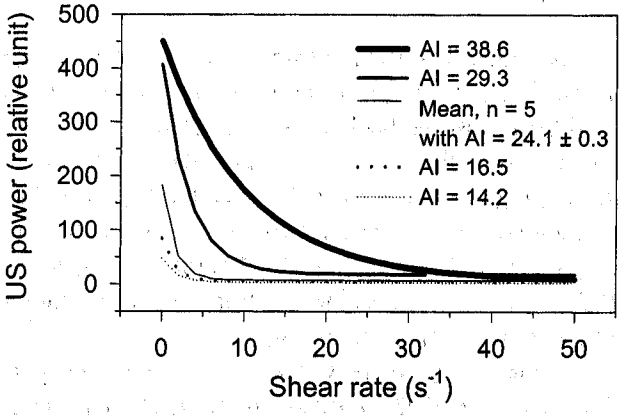

Fig. 1. Shear rate dependence of the ultrasound (US) backscattered power for horse blood models characterized by different RBC aggregability. The parameter AI reflects the level of aggregation measured by laser reflectometry. Figure reproduced from [4].

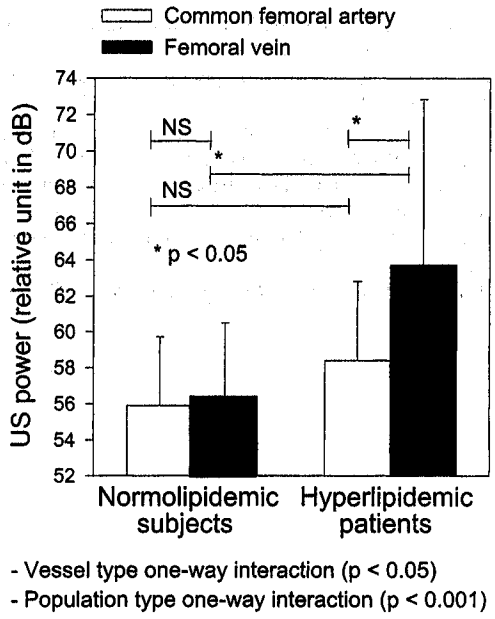

Fig. 2. Backscattered power in decibel (dB) for recordings performed in the femoral artery and vein of normal subjects and patients with hyperlipidemia. Figure reproduced from [5].

4. Conclusions

With the exception of ultrasound backscattering and intravital microscopy, no other method can assess the level of aggregation *in situ* in animal or human vessels. The visualization of erythrocyte aggregates with intravital microscope is limited to vessels smaller than about 10 μm, and this technique was mainly used for animal studies [1,6,9]. Ultrasound backscattering was used to measure erythrocyte aggregation in human veins and arteries [5,8]. Recent developments suggest that this technique may be applicable to study erythrocyte aggregation in vessels as small as 50 μm [2]. The development of ultrasound backscattering may stimulate clinical studies aimed at elucidating the role of erythrocyte aggregation in cardiovascular diseases. This technique also has the potential to improve our basic understanding of the relationship between the hemodynamics of the circulation and erythrocyte aggregation in animal and human vessels.

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