Ultrasound in Med. & Biol., Vol. 39, No. 4, pp. 670–680, 2013

0301-5629/$ - see front matter

http://dx.doi.org/10.1016/j.ultrasmedbio.2012.10.018

● *Original Contribution*

THE EFFECT OF FLOW ACCELERATION ON THE CYCLIC VARIATION OF BLOOD ECHOGENICITY UNDER PULSATILE FLOW

CHIH-CHUNG HUANG,* CHEN-CHIH LIAO,* PO-YANG LEE,*† and CHO-CHIANG SHIH*†

*Department of Electrical Engineering, Fu Jen Catholic University, New Taipei City, Taiwan; and †Graduate Institute of Applied Science and Engineering, Fu Jen Catholic University, New Taipei City, Taiwan

(*Received* 26 *June* 2012; *revised* 22 *September* 2012; *in final form* 28 *October* 2012)

Abstract—It has been shown that the echogenicity of blood varies during a flow cycle under pulsatile flow both *in vitro* and *in vivo*. In general, the echogenicity of flowing whole blood increases during the early systole phase and then reduces to a minimum at late diastole. While it has been postulated that this cyclic variation is associated with the dynamics of erythrocyte aggregation, the mechanisms underlying this increasing echogenicity with flow velocity remain uncertain. The effect of flow acceleration has also been proposed as an explanation for this phenomenon, but no specific experiments have been conducted to test this hypothesis. In addition, the influence of ultrasonic attenuation on the cyclic variation of echogenicity requires clarification. In the present study, a Couette flow system was designed to simulate blood flowing with different acceleration patterns, and the flow velocity, attenuation, and backscattering coefficient were measured synchronously from 20%- and 40%-hematocrit porcine whole blood and erythrocyte suspensions using 35-MHz ultrasound transducers. The results showed ultrasonic attenuation exerted only minor effects on the echogenicity of blood under pulsatile flow conditions. Cyclic variations of echogenicity were clearly observed for whole blood with a hematocrit of 40%, but no variations were apparent for erythrocyte suspensions. The echogenicity did not appear to be enhanced when instantaneous acceleration was applied to flowing blood in any case. These findings show that flow acceleration does not promote erythrocyte aggregation, even when a higher peak velocity is applied to the blood. Comparison of the results obtained with different accelerations revealed that the cyclic variation in echogenicity observed during pulsatile blood flow may be jointly attributable to the effect of shear rate and the distribution of erythrocyte on aggregation.
(E-mail: j648816n@ms23.hinet.net)

Key Words: **Erythrocyte aggregation, Pulsatile flow, Cyclic variation, Ultrasonic attenuation, Echogenicity.**

INTRODUCTION

Erythrocyte aggregation in flowing blood is a reversible physiologic phenomenon. Many studies have indicated that abnormal erythrocyte aggregation can occur in some pathologies and diseases, such as vascular thrombosis and cardiovascular diseases (Chabanel et al. 1994; Demiroglu et al. 1996; Hahn et al. 1989), hypercholesterolemia (Bosch et al. 2001), diabetes (Cloutier et al. 2008; Le Devehat et al. 1990; Schmid-Schönbein and Volger 1976), hypertension (Razavian et al. 1992), hyperlipidemia (Cloutier et al. 1997; Razavian et al. 1994), morbid obesity (Samocha-Bonet et al. 2004) and heavy smoking (Li et al. 2011). This makes it crucial to characterize erythrocyte aggregation in flowing blood in clinical applications. Both the *in vitro* and *in vivo* properties of erythrocyte aggregation under steady flow conditions have been widely studied using ultrasound techniques because of their real-time and noninvasive capabilities (Shung and Thieme 1993). Because the ultrasonic backscatter signal is strongly related to the size of the rouleaux, the level of erythrocyte aggregation can be evaluated by measuring the echogenicity of blood. This has led to many studies demonstrating that erythrocyte aggregation in steadily flowing blood is dependent mainly on the hematocrit, turbulence, shear rate, plasma fibrinogen concentration, vessel-wall compliance and flow disturbance (Cloutier et al. 1996; Huang et al. 2009; Huang and Wang 2007; Yuan and Shung 1988a, 1988b). Some studies have also characterized erythrocyte aggregation under pulsatile flow conditions using ultrasound. However, the relationship between erythrocyte aggregation and ultrasonic backscatter signals from pulsatile flowing blood is not fully understood.

Address correspondence to: Chih-Chung Huang, Department of Electrical Engineering, Fu Jen Catholic University, 510 Chung Cheng Rd, Hsin Chuang District, New Taipei City 24205, Taiwan. E-mail: j648816n@ms23.hinet.net

Under steady flow, the effect of shear rate has been suggested as a crucial hemodynamic factor influencing erythrocyte aggregation. An increase in shear rate can break up the formation of rouleaux and reduce the intensity of the echogenicity of flowing blood. In contrast to steady flow, some studies have shown the existence of a cyclic variation in the echogenicity of pulsatile flowing blood both *in vitro* and *in vivo* (Cloutier and Shung 1993; De Kroon et al. 1991; Missaridis and Shung 1999; Paeng and Shung 2003; Paeng et al. 2001, 2010; Huang 2009, 2011; Thompson et al. 1985; Wu and Shung 1996). In general, the cyclic variations in the ultrasonic backscatter signal or Doppler power have been clearly observed for blood with a higher hematocrit, lower stroke rate, and lower peak flow velocity, and when using high-frequency ultrasound. It was proposed that the shear rate was responsible for the cyclic variation in the echogenicity of blood under pulsatile flow conditions in a tube when measured with spatial and temporal variations in ultrasonic Doppler signals. Because the shear rate acting on the erythrocyte aggregates across the tube varies with the time during a flow cycle, cyclic variations in echogenicity might be related to the dynamics of erythrocyte aggregation (Lin and Shung 1999). However, recent results indicate that the echogenicity of flowing blood is minimized in the late diastole phase and increases during the early systole phase, reaching a maximum before peak systole. It subsequently decreases again, reaching a minimum at late diastole (Huang 2009, 2011; Paeng et al. 2001). This observation opposes the previously held opinion that the increase in shear rate should disrupt erythrocyte aggregation and reduce the echogenicity of blood; however, it was found that the echogenicity increased during the acceleration phase in pulsatile flow. Consequently, some studies indicate that the higher echogenicity of blood during early systole cannot be explained by shear-rate analysis alone, which led to the hypothesis that flow acceleration underlies the increase in echogenicity of blood during the acceleration phase. Flow acceleration might promote rouleaux formation, because when the flow is accelerating there will be more opportunities for erythrocytes to interact to form larger rouleaux (Cao et al. 2001; Paeng et al. 2001, 2004). Although the cyclic variation in echogenicity was explained by combined effects on erythrocyte aggregates, the contribution of each factor to the echogenicity of pulsatile flowing blood remains unclear because it is difficult to estimate the effect of these two hemodynamic factors independently. In other words, the interpretation of the effects of these mechanisms on erythrocyte aggregates needs further detailed inspection to obtain a better understanding of their influence. In addition, in most previous studies the Doppler power or backscatter power was measured to represent the level of erythrocyte aggregation; however, the effect of attenuation compensation on backscatter measurements was not considered. Because the attenuation of ultrasound in blood depends on the shear rate (Huang and Chang 2011), the effect of attenuation on the cyclic variation of echogenicity should also be considered under conditions of pulsatile flow.

The purpose of this study was to determine quantitatively how flow acceleration affects the cyclic variation of blood echogenicity under pulsatile flow conditions. To simplify the complicated kinetics of blood flowing in a circular tube, a Couette flow apparatus was designed to simulate the pulsatile flow condition (Nguyen et al. 2008). Fresh porcine whole blood and erythrocyte suspensions with hematocrit values of 20% and 40%, respectively, were circulated in the flow phantom at different flow acceleration settings. High-frequency ultrasound is more sensitive for detecting erythrocyte aggregates (Huang 2009); therefore, the ultrasonic backscattering coefficient and flow velocity of flowing blood were measured synchronously using a 35-MHz ultrasonic transducer and a 35-MHz pulsed-wave Doppler flowmeter, respectively. To measure the backscattering coefficients accurately, backscatter data were compensated according to the shear-rate–dependent attenuation. This study focused on how blood flow acceleration affects erythrocyte aggregates and the echogenicity during the acceleration phase under pulsatile flow conditions.

MATERIALS AND METHODS

Blood samples

All experiments were performed on porcine blood within 24 h of its collection (from a local slaughterhouse). Whole blood (1 L, to which 30 mL of the anticoagulant ethylenediaminetetraacetic acid was added at a concentration of 11 gm/dL) was passed through a sponge to filter out impurities such as fatty tissue and hair. It was then centrifuged and washed twice using a saline buffer solution to separate the erythrocytes from the plasma and other cells. The concentrated erythrocytes and plasma were stored in a refrigerator, and the desired hematocrit values for the whole-blood experiments (20% and 40%) were obtained by mixing the appropriate amount of concentrated erythrocytes with plasma. Erythrocyte suspensions with hematocrit values of 6% and 40% in 0.9% saline solution were also prepared. The erythrocyte suspension with a 6% hematocrit was used as a reference medium for measuring the backscattering coefficient. In total, 10 porcine blood samples from 10 different animals were used in the present study.

Couette flow system

A Couette flow system was designed for this study to simulate the blood flowing under pulsatile flow