原　著

Generation of spontaneous echo contrast using non-human blood

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Key words：ultrasound, spontaneous echo contrast (SEC), non-human blood, the solution for artificial SEC

Abstract A spontaneous echo contrast (SEC) is often seen in intravenous ultrasound examinations of the lower extremities. The SEC shows a thrombus preparatory state and implies the possibility of a symptom of an embolic disease. Although the SEC is reproducible in vitro using human blood, it is difficult to keep its condition constant. The generation of artificial SECs may contribute to the diagnostic decision-making process and assessment of the treatment effects. In this study, we attempted to produce artificial SECs using various types of solutions other than human blood. Several types of solutions, such as tomato juice, canned sugar - free coffee, and instant café au lait were used for the experiment. We observed ultrasound images of these solutions flowing through two types of tubes (5 mm and 8 mm in diameter) and compared these with clinical SEC images. As a result, we found that a triple concentration solution of instant café au lait could be used instead of human blood regardless of the diameter of the tubes. Moreover, the SEC was able to be reproduced regardless of the flow velocity of the solution.

1. INTRODUCTION

In Japan, lifestyle-related diseases such as diabetes, hypertension and hyperlipemia have been increasing due to aging and a change in eating habits. With the increase of lifestyle-related diseases, a variety of vascular diseases (e.g., cerebrovascular accident, myocardial infarction, angina pectoris, obstructive arteriosclerosis, and renal insufficiency) have, dramatically increased. Among various examinations for blood vessels, ultrasound is generally practiced. It is used for diagnosis of pulmonary embolus and deep vein thrombosis also known as economy class syndrome. During intravenous ultrasound examination of lower extremities or trans-esophageal echocardiography (TEE), spontaneous echo contrast (SEC) is often seen in blood vessels[1]. The image of the SEC is described as high density or high echo, showing a shape of a slight particle with various sizes. The main parts of the SEC are red corpuscle condensation and rouleaux formation, and the SEC is observed in certain situations such as for valvular disease and an arrhythmia [2,3]. Thus, a fall in the blood flow velocity and a turbulent flow are strong factors in the generating the SEC. As a result, the patient having the SEC shows increased levels of thrombin-antithrombin (TAT) and D-dimer[4]. Therefore, this strongly suggests that the SEC shows a thrombus preparatory state.

The SEC will disappear when a flow velocity increases by the improvement of the patient's heart rhythm, heartbeat, etc [5,6], and the SEC is not always observed even in the same patient. Moreover, the visibility of the SEC depends on the kind of ultrasound device used. For standardization of ultrasound equipment and the conditions for detecting the SEC, making an artificial SEC may be helpful. It has been recognized that the SEC will be reproducible in vitro using human blood [7-9]. However, one of the drawbacks of using human blood for experiments is that the observation conditions cannot be kept constant. In this study, we tried to

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Accepted：2014.2.24

create a solution for reproducing the artificial SEC using various solutions other than human blood.

2. METHOD

In this study, the SEC was defined as follows [8,9]:

a) It is described as an image in which smoke trails.
b) It is observed in the intravascular lumen.
c) It does not disappear, even if the gain in an ultrasound device is varied.
d) It is observed at a high echo level in the same luminosity filling the inside of the tube.
e) It can be observed, even if the bloodstream is in a stationary state.

One medical doctor and seven radiological technologists skillful in ultrasound examination performed a visual evaluation of the SEC.

2.1. Creation of artificial SEC

A circulation circuit was created with a circulating pump and 3 kinds of vinyl tubes: the enema teleflator CK-80 (Kaigen, Osaka, Japan), the elemental diet tube set (Fresenius Kabi Japan, Tokyo, Japan) and a common extension tube for intravenous drip (Fig.1). As an imitation blood vessel, the elemental diet tube was sunk in the chamber (i.e. a tank filled with distilled water) and both sides were fixed to the inner wall of the tank by tape. The inside dimension of the chamber was 25 x 40 x 30 cm. The imitation blood vessel passed through the center of the tank. We observed ultrasound images of several liquids which flowed through the tube by an ultrasound device, iU22 (Philips, Amsterdam, Netherlands) with an echo-transducer L12-5 (transmit frequency width: 5 - 12 MHz). The images were obtained at a fixed point in the chamber. The acoustic power output, time gain compensation, compression, depth, preprocessing, postprocessing settings, and the environment of observing the ultrasound images were constant throughout the experiment. Moreover, room temperature was set at a constant of between 20-23 ℃.

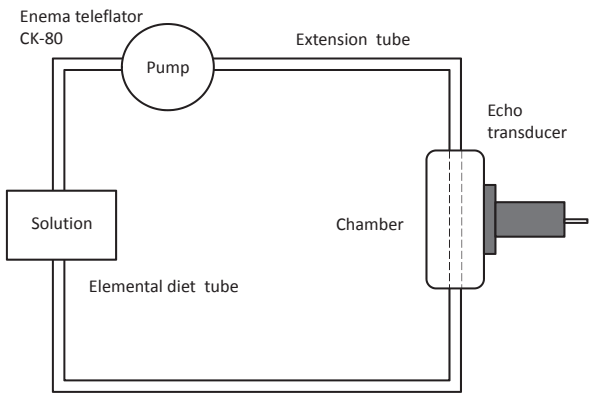


Fig.1 Illustration of the circuit model for reproduction of the SEC.

The candidates of solutions for reproducing the artificial SEC were chosen based on the following criteria.

a) There is no temporal change.
b) Washing of the pump and a circuit tube used for the experiment is easy.
c) An additional supplement is possible when the amount of the solution decreases.
d) Acquisition of the solution is easy and inexpensive.

Tap water, tomato juice (KAGOME, Nagoya, JAPAN), plastic bottled green tea (Nama-cha, KIRIN, Tokyo, JAPAN), canned sugar - free coffee (FIRE BLACK, KIRIN, Tokyo, JAPAN), plastic bottled milk tea (MILK TEA, KIRIN, Tokyo, JAPAN), instant café au lait (Blendy stick, AGF, Tokyo, JAPAN), hot water with bath salt (HYAKKA-RYOURAN, NIHON DETERGENT MFG.CO. Osaka, JAPAN), and India ink (SAKURA COLOR PRODUCTS CO. Osaka, JAPAN) were used for the experiment. Among them, we made different concentration solutions of the instant café au lait and the India ink. The single concentration solution was made from a standard quantity. Triple the amount of the concentration solution was made from a tripled amount of the standards.

2.2. A flow velocity, a vessel diameter, and the relation to artificial SEC

The flow velocity of the solutions that flows through an imitation intravascular lumen was changed, and the state of the SEC was observed. The ultrasound image was observed to experience a changing flow velocity of a circulation circuit by changing the pump setting. In order to measure the flow velocity in the circuit, the sample volume (SV) of a pulse Doppler was arranged and measured in the imitation blood vessel. The flow velocity was measured at several points. In an ultrasound examination, the peak velocity (PV) and mean velocity (MV) are measured by making the SV size 2/3 of the blood vessel diameter.

Then, the imitation blood vessel was changed to a tube having a different diameter, and the depiction of the state of SEC was similarly examined. Two kinds of elemental diet

tubes (with inside diameters of 8 mm and 5 mm) were used as the imitation blood vessel.

3. RESULTS

3.1. Creation of artificial SEC solution

Plastic bottled milk tea and single concentrated solution of instant café au lait showed an image that was similar to weak a SEC. However, they disappeared depending on the gain setup. The SEC was observed by 2 to 6 times the amount of a concentration solution of café au lait regardless of the gain setup (Fig.2). A higher concentration solution provided clearer SECs. With tomato juice, the SEC was observed immediately after the experiment. However, the tomato juice was not able to show a stable SEC image (Fig.3). The SEC was not shown in tap water, plastic bottled green tea, canned sugar - free coffee, hot water with bath salt, or either single and twice concentrated solutions of India ink (Table 1). A visual comparison was made between the clinical and the artificial SEC images. Then, we found that a triple concentration solution of instant café au lait was most suitable for use as an artificial SEC.

3.2. A flow velocity, a vessel diameter, and the relation to the artificial SEC

The maximum flow velocity in a circulation circuit changed depending on the inside diameter of the imitation blood vessels. When the circulating pump was set at as

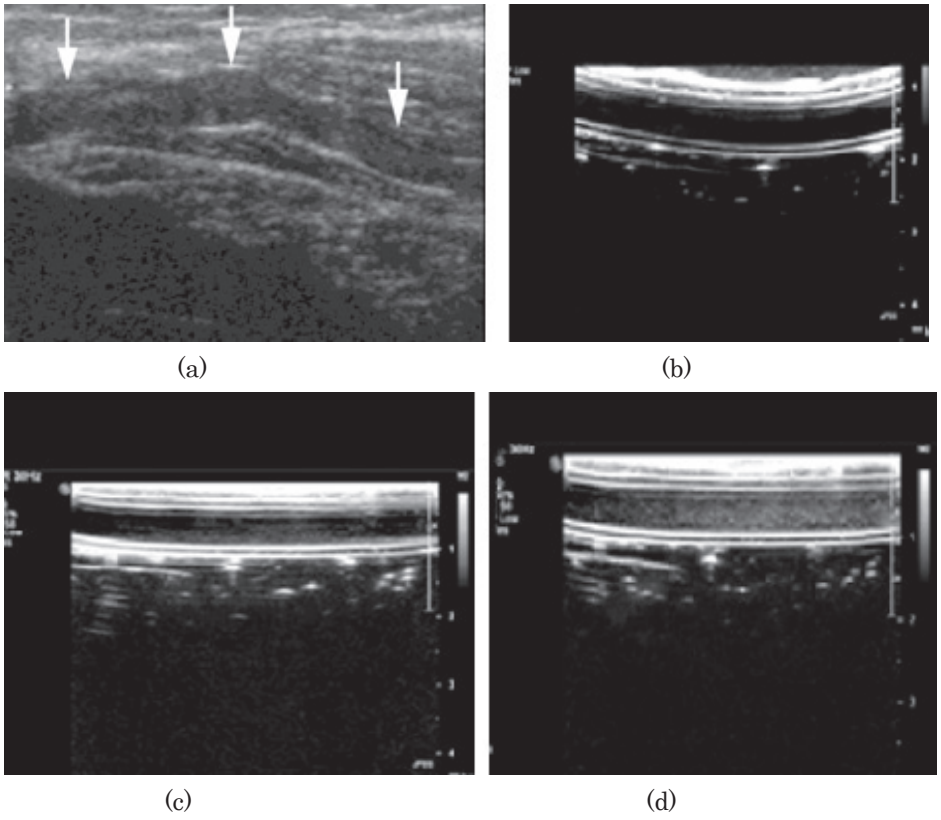


Fig.2 Comparison of the SEC image

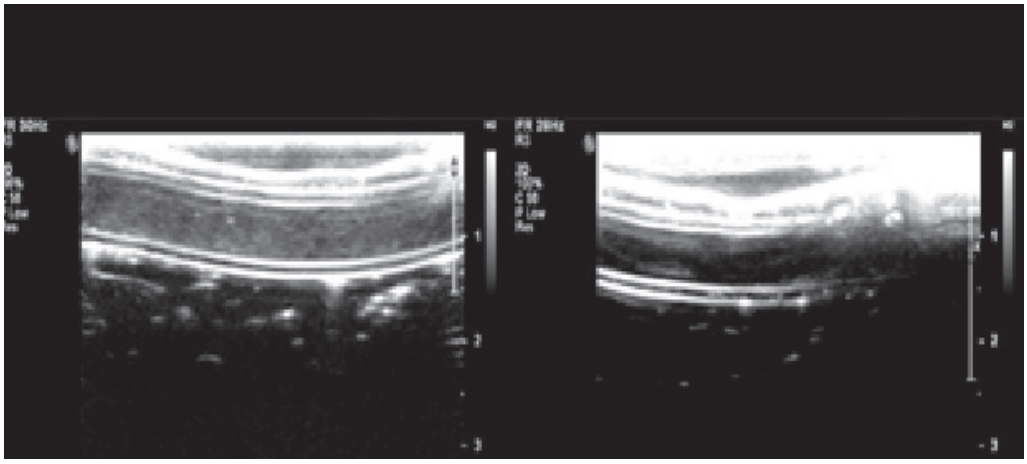
(a) A SEC image in human body. White arrow is the SEC image.

(b) A SEC image in single concentration solution of instant café au lait.

(c) A SEC image in triple concentration solution of instant café au lait.

(d) A SEC image in 4 times concentration solution of instant café au lait.

The image of the SEC is described as high density or high echo. As for café au lait in the amount of the standards (single con centration solution), the SEC was observed weakly. As for café au lait in the more amount of the standards (triple and 4 times concentration solutions), clear a SEC was observed. However, the SEC image in 4 times concentration solution of instant café au lait was too high density.



(a) (b)

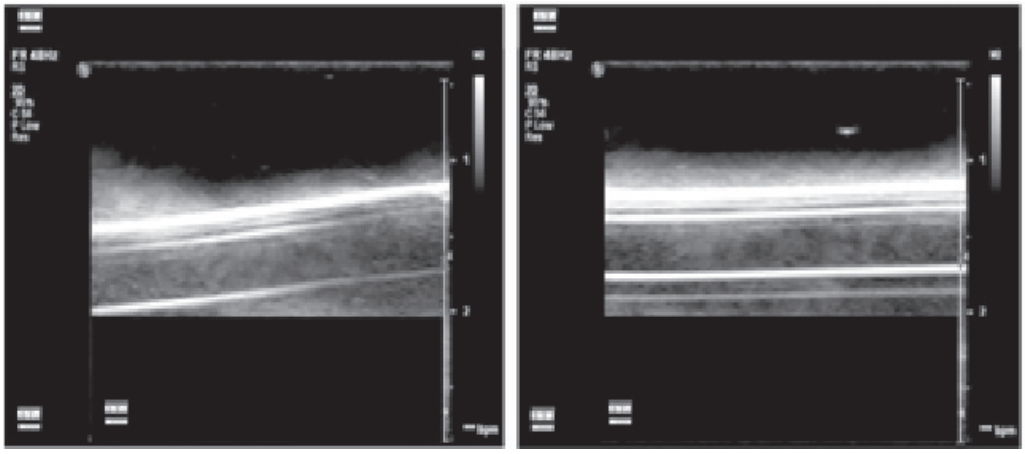
Fig.3 The SEC evaluation with tomato juice and India ink

(a) Tomato juice (b) India ink solution (twice concentration)

With tomato juice, the SEC was observed at the time of an experiment start. However, it could not stabilize. The SEC was not observed with India ink.

Table 1 Result of each solutions of the SEC created artificially

	SEC		SEC
India ink (single)	−	India ink (twice)	−
Tap water	−	Café au lait (single)	±
Green tea (plastic battle drink)	−	Café au lait (twice)	+
Sugar-free coffee (canned drink)	−	Café au lait (triple)	+
Milk tea (plastic battle drink)	±	Café au lait (4 times)	+
Hot water with bath salt	−	Café au lait (5 times)	+
Tomato juice	+	Café au lait (6 times)	+



(a) (b)

Fig.4 The SEC evaluation in the minimum flow velocity and the maximum flow velocity in a 5 mm tube

(a) Minimum flow velocity (Halt condition) (b) Maximum flow velocity (the peak velocity was 87.1 cm/sec)

The SEC was observed at both of the minimum and the maximum flow velocity.

maximum, the PV and MV were 87.1 cm/sec and 74.0 cm/sec in a tube with a 5 mm inside diameter. Similarly, PV and MV were 48.6 cm/sec and 41.1 cm/sec, respectively, in an 8 mm tube. We could change the PV and MV continuously from the maximum to a halt condition (i.e. a minimum flow velocity, below 1 cm/sec). Using the triple concentration solution of café au lait, the SEC images could always be observed even if the flow velocity was changed in both the 5 mm and 8 mm imitation vessels (Fig.4, Fig.5). It was confirmed that the artificial SEC could be shown regardless of the flow velocity and the diameter of the tubes.

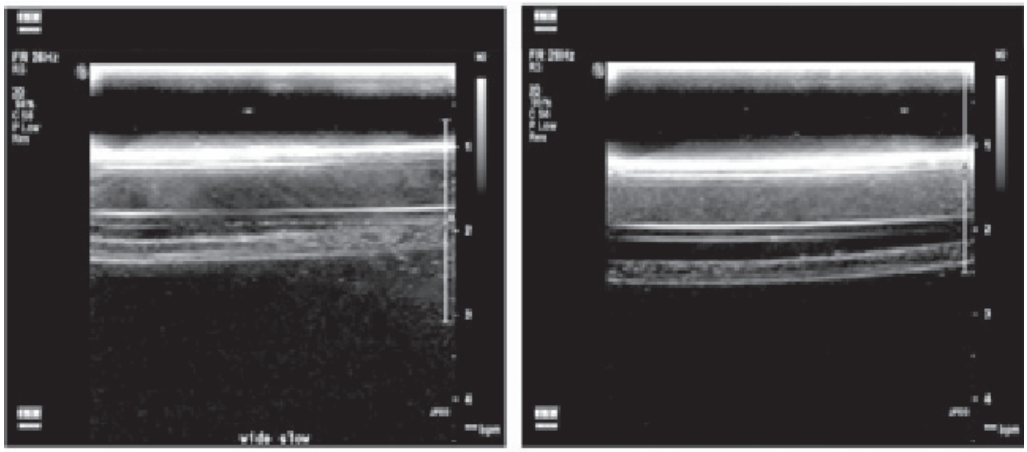
4. DISCUSSION

4.1. Necessity for standardization of ultrasonic condition for observing the SEC

The SEC is often observed in ultrasound examinations of several blood vessels such as a carotid artery, an upper arm artery, and a lower extremity vein. The appropriate conditions to detect the SEC need to be standardized. However, there have been no reports comparing the detectability of the SEC among different ultrasonic devices and among different settings of the condition. It has been confirmed that the SEC is reproducible in vitro using human blood. However, the experimental condition cannot be kept constant because the condition of the human blood may be different depending on the conditions (time and temperature) of blood sampling, and the same blood donor may not always be available. It is also widely known that the visibility of the SEC depends on the ultrasonic devices used, and the appropriate condition of the ultrasonic device for the SEC detection has not been found until now. One of the reasons for the lack of a standard method to detect the SEC may be the poor reproducibility of the SEC. Therefore, we attempted to reproduce the artificial SEC using various types of solutions other than human blood. Because the SEC is a special echo phenomenon occurring at low flow situations[3-5,7], the relationship between the type of the solution and the flow velocity was also examined.

4.2. Etiology of the SEC image

A high concentration dissolution of instant café au lait was appropriate for the SEC observation. The size of the fat globule that is considered to be a main echogenic material of instant café au lait is still unknown. However, the fat globule size of unprocessed milk is 2 - 6 μm, and after a uniform processing of making the commercial milk, the fat globule size was reduced to about 1 μm. This size is considered to be almost the same as that of café au lait. The size of the red corpuscles of human blood is 8.5 μm x 1.8 - 2.0 μm. Other than red corpuscles, there are white corpuscles and blood platelets in blood cells. Blood platelets are in the shape of a disk measuring 2 - 3 μm in diameter, and are the minimum elemental particle in blood cells. Therefore, these blood cell complexes are slightly larger than the size of the fat globule contained in the café au lait solution. If the SEC image is



(a) (b)

Fig.5 The SEC evaluation in the minimum flow velocity and the maximum flow velocity in an 8 mm tube.
(a) Minimum flow velocity (Halt condition)
(b) Maximum flow velocity (the peak velocity was 48.6 cm/sec)
The SEC was observed at both of the minimum and the maximum flow velocity.

made of particles of the same size as red corpuscle condensation and rouleaux formation, the etiology for the artificial SEC made of café au lait cannot be clearly explained.

4.3. Range resolution

The axial resolving power of the probe (L 12 - 5) on the ultrasound device used for the experiment was a 5 - 12 MHz transmit frequency width. The wavelength is denoted by the following formulas.

$\lambda = \nu / f$

Here, λ is the wavelength (μm), ν is the speed (m / s.), and f is the frequency (MHz). The shortest distance resolution of an ultrasound wave is determined by the wavelength. For example, a wavelength is set to 200 μm, when the speed of the ultrasound wave is set at 1,500 m / s, and center frequency is transmitted at 7.5 MHz. With the highest frequency (12 MHz), the wavelength becomes 125 μm which is the minimum distance resolution of this experimental system. Here, even if the size after red corpuscle condensation or rouleaux formation (not being in a clot state) amounts to 100 μm, the minimum resolution of the ultrasound device is much larger. Therefore, the clinical SEC image may not be created by only blood cell complexes. The image of the SEC may be observed as a total of the ultrasound wave reflected from the whole intravascular fluid. Ultrasound devices in recent years have a high resolution and wide dynamic range. By using a high-resolution ultrasound probe, the detectability of the SEC may vary.

4.4. A flow velocity and a vessel diameter, and the relation of artificial SEC

The flow created by a pump in the present experimental system had a pulsation wave. By the pulsation wave, the shear stress and normal stress that flow through the inside of a tube occurs. Therefore, a difference in the flow velocity of fluid appears between the wall and the center of the imitation blood vessel. Compared to the circulation system that creates the flow by rotation of a stirrer[7], the experimental environment we used is closely similar to the blood circulation dynamic state in a human body. In this experiment, we changed the flow velocity by changing the MV and PV acquired from the SV. With the triple concentration solution of the café au lait, we confirmed that the SEC could be observed even if the flow velocity was changed, and it did not depend on the diameter of the tubes. In the in vitro experiment using human blood, the depiction of the SEC was dependent on the fall of flow velocity. However, considering the result obtained in this experiment model, the mechanism of the SEC may not be solely related to the decrease of flow velocity.

5. CONCLUSION

The SEC was able to be reproduced with an artificial solution. It has become clear that the type and concentration of solution greatly influence the depiction of the SEC. Unlike the past in vitro experiment using human blood, the SEC was able to be reproduced without depending on the flow velocity.

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