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Fundamental Study on Micro Calcification Detection Using Twinkling Sign (TS): The Effect of Stiffness of Surrounding Tissue on the Appearance of TS*

Lei Liu, Kenichi Funamoto, Masayuki Tanabe, and Toshiyuki Hayase

Abstract— **The twinkling sign (TS) observed in ultrasound imaging (e.g., color flow mode and pulse Doppler mode) has been reported in previous researches as a potential phenomenon to detect micro calcification in soft tissue. However, the mechanism of the twinkling sign has not been clearly understood yet. The authors investigated the effect of stiffness of surrounding tissue on the appearance of TS using the soft tissue-mimicking phantoms and a medical ultrasound device. The author used Poly (vinyl alcohol) hydro (PVA-H) gel as the material of phantom and developed three phantoms with different PVA concentration; 8 %wt, 10 %wt and 15 %wt those correspond to Young's modulus (*E*) as 50 kPa, 100 kPa and 230 kPa, respectively. Micro glass and $CaCO_3$ particles were embedded in the phantoms as pseudo micro calcification. The authors observed TS in each phantom and analyzed the temporal average of TS. The temporal average of TS was largest in the 8 %wt (*E* = 50kPa) PVA-H gel phantom, and decreased with increasing the phantom stiffness. The result indicated that the micro oscillation of the particles had a close relationship with the occurrence of TS.**

I. Introduction

Breast cancer is a global healthcare issue. Micro calcification (average size under 500 μm) is a key for the detection of the breast cancer in a very early stage [1]. Mammography is the gold standard for breast cancer screening, however, mammography inevitably causes a certain degree of X-ray exposure to patients. Also it is recently reported that there is little significant effect of periodic mammography checkup for women aged 40 and under with high breast density (dense breast) by USPSTF (U.S. Preventive Services Task Force) [2]. The background indicates that to develop a new micro calcification method based on ultrasound technology will bring benefit to breast cancer care area. The ultrasound is a non-invasive technology, widely used in the breast cancer disease management process. It is also useful in diagnosing dense breast, however, the micro calcification detectability of conventional ultrasound scan application, e.g. B-mode, is known to be insufficient.

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Lei Liu is with the GE Healthcare Japan Corporation, 4-7-127 Asahigaoka, Hino, Tokyo, Japan (corresponding author to provide phone: 81-42- 585-5369; fax: 81-42-585- 5950; e-mail: 122liu@ ge.com).

Kenichi Funamoto and Toshiyuki Hayase are with Institute of Fluid Science, Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai, Japan (e-mail: funamoto@reynolds.ifs.tohoku.ac.jp, hayase@ ifs.tohoku.ac.jp).

Masayuki Tanabe is with Graduate School of Science and Technology, Kumamoto University, Kurokami 2-39-1, Chuo-ku, Kumamoto, Japan (e-mail: mtanabe@cs.kumamoto-u.ac.jp).

Previous clinical observations and experiment studies demonstrated that stones, calcification, and artificial materials in soft tissue show special enhancement to ultrasound scanning, e.g., color flow (CF) mode or pulse Doppler (PW) mode [3-12]. This phenomenon is known as "twinkling sign (TS)". Previous studies noted that the twinkling sign would be useful for detecting calcification in soft tissue, however, the mechanism of the TS occurrence has not been clearly understood yet.

There are two well-known hypotheses for the occurrence mechanism of the TS based on changes in ultrasound intensity or phase derived by the roughness of a reflector [5, 8]. Another trigger that possibly causes the TS is the motion of a reflector due to the ultrasound radiation force. Behnam *et al*. computationally showed that high-frequency oscillation with a small displacement of the scatter due to the ultrasound radiation force caused the TS [13]. Liu *et al*. have recently optically observed the micro particle in a tissue-mimicking phantom under an ultrasound scanning condition, and experimentally confirmed the micro oscillation of the particles caused by ultrasound radiation [14]. The result supports the study of Behnam *et al*.

Breast tissue is known to be complicated. The stiffness varies among its components [15]. The stiffness of surrounding tissue is considered to have an effect on the oscillation amplitude and oscillation characteristics of the micro calcification, namely the appearance of TS. However, there are few studies that confirmed the relationship between the TS appearance and the mechanical property of the sounding tissue.

In the present study, the author developed a tissue-mimicking phantom using Poly (vinyl alcohol) hydro (PVA-H) gel with different stiffness. Micro glass and $CaCO_3$ particles were embedded in the phantoms as pseudo micro calcification. Ultrasound scan experiment was conducted by using a medical ultrasound scanner to observe the effect of the surrounding tissue stiffness on TS appearance.

II. Method

A. PVA-H Tissue-mimicking phantom

PVA-H is known as a tissue-mimicking material for soft tissue. The elasticity and viscosity of PVA-H can be adjusted by changing the concentration, polymerization degree, and crystallinity of PVA. The elasticity and viscosity of native soft tissues were approximately reproduced in previous studies [16-18]. In terms of other physical features, it is also possible

to assign *in vivo* acoustic properties to PVA-H [19]. These features show that PVA-H is an ideal tissue-mimicking material for *in vitro* experiment of ultrasonic measurement.

The tissue-mimicking phantom had two PVA-H layers to sandwich the glass and $CaCO_3$ particles. The PVA solution was prepared by adding PVA powder (JF 17, JAPAN VAM & POVAL Co., Ltd., Osaka, Japan) to a solvent composed of 20 wt% water and 80 wt% dimethylsulfoxide (Toray Fine Chemicals Co., Ltd., Chiba, Japan). The fabrication process of the phantom followed our previous study [14]. The stiffness of the phantom was controlled by changing the concentration of PVA. The author developed three phantoms with different PVA concentration; 8 %wt, 10 %wt and 15 %wt which correspond to Young's modulus (E) as 50 kPa, 100 kPa and 230 kPa, respectively. The Young's modulus of the PVA-H gel was confirmed in the pilot study of the present study using a tensile test equipment (EZ-S, Shimadzu Co. Ltd., Kyoto, Japan). Three glass particles with 1 mm, 400 μm and 200 μm in maker-spec diameter (Tech Jam Co., Ltd., Osaka, Japan) and one micro $CaCO_3$ particle (specially-manufactured, NEW LIME Co., Ltd., Oita, Japan) were embedded in each phantom as pseudo micro calcification. The size of all phantoms were x: y: z = 70: 45: 8 mm (Fig. 1). The position of particles were placed at x = 30 mm and z = 4 mm. The optical micro scope images of PVA-H phantom (8 %wt) are also shown in Fig. 1. The optical observation was done by using a digital micro scope (VHX-900, Keyence Corporation, Osaka, Japan) after all the phantoms were completed. The measured sizes of the micro particles in each phantom are summarized in Table I The $CaCO_3$ particles were non-spherical thus the description in Table I was summarized as the long axis (x) and short axis (y) sizes. Also only the 200 μm glass particle in the 15 %wt PVA-H phantom was confirmed to have non-spherical geometry (two small particles united as one). The description follows that of $CaCO_3$ particle in Table I.

B. Ultrasound measurement and data analysis

Fig. 2 shows an overview of the experimental setup. Medical ultrasound diagnosis equipment (LOGIQ S8 pilot unit, GE Healthcare Japan, Hino, Tokyo, Japan) and ML 6-15-D probe (GE Healthcare Japan, Hino, Tokyo, Japan) were used for the ultrasound measurement of the PVA-H phantom with color flow (CF) mode. Ultrasound scanning conditions that were used to observe the twinkling sign, such as center frequency, pulse repetition frequency (PRF), and focus position, are summarized in Table II.

Table I Phantom and measured particle sizes

Phantom	**Measured particle size [μm]**			
	1 mm	***400 μm***	***200 μm***	***$CaCo_3$***
8 %wt	1146	402	242	Long: 322 Short: 369
10 %wt	1129	417	226	Long: 220 Short: 217
15 %wt	1011	434	Long: 272 Short: 238	Long: 186 Short: 171

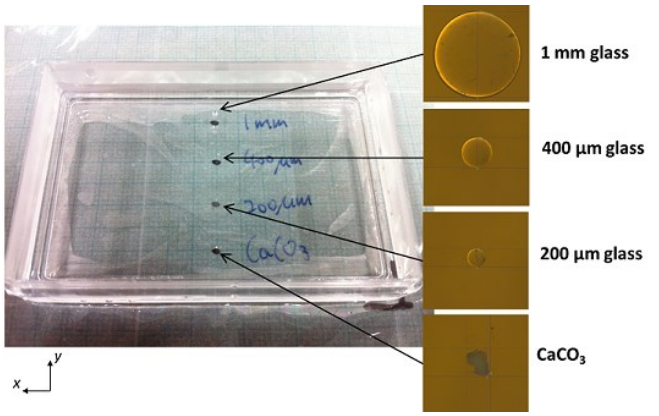

Fig. 1 PVA-H phantom (8 %wt, left image) and the optical micro scope images of micro particles which were embedded in the phantom (scaled up to 150 times, right images).

A TS image of the with 8 %wt PVA-H phantom is shown in Fig. 3.We scanned each phantom for 100 frames in the same condition (Table II) and stored the movie in AVI format. The white dashed area is the region of interest (ROI) for each particle to process analysis. TS from each particle was quantified by an original MATLAB (The MathWorks, Inc., MA, USA) program. In the analysis, we calculated the temporal changing of TS (total number of the color pixels in the ROI of each particle) and the temporal average of TS.

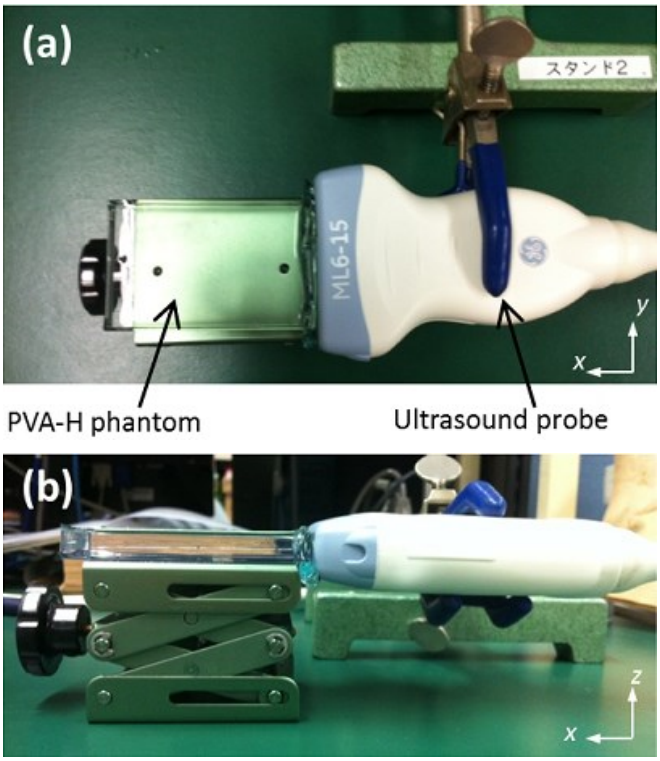

Fig. 2 Overview of the ultrasound scanning experiment. (a) shows the overhead view and (b) shows the side view. The space between the probe and the phantom was filled up with conventional ultrasound gel.

Table II Ultrasound scanning conditions

Scan mode	Color flow (CF) mode
Frequency (Fc)	5.0 MHz
Image depth	4.5 cm
Focus position	3.5 cm
Packet size	8
PRF	0.3 kHz
Wall filter	43 Hz
Frame rate	7 Hz
Acoustic output (displayed)	80 %

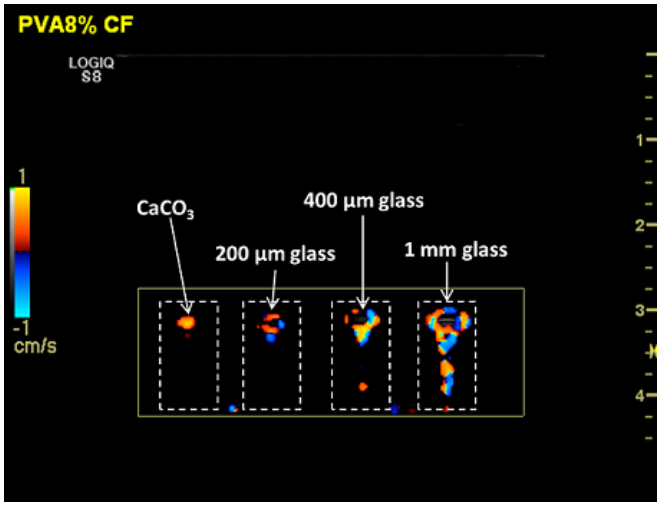

Fig. 3 TS image observed using the 8 %wt PVA-H phantom. The white dashed area is the region of interest for each particle to analyze the temporal changing of TS.

III. Result

Fig. 4 shows the relationship between the TS temporal average and the PVA-H phantom stiffness (PVA concentration of the phantom). The values were normalized by the result of 1 mm particle in 8 %wt PVA-H phantom. The temporal average of TS from all types of particles was the largest in the 8 %wt PVA-H gel phantom, and decreased with increasing the phantom stiffness. TS from $CaCo_3$ particle was not detected in the 15 %wt PVA-H gel phantom.

Fig. 5 shows the result of TS temporal average for each micro particle that obtained in three PVA-H phantoms. The temporal average of TS was plot versus corresponding particle, and the value was normalized by the result of 1 mm particle in each phantom. The analytical projection area (PA, equivalent to the cross-section area) of glass particles was plotted in Fig. 5 for reference. PAs was also normalized by 1 mm particle. The result of glass particle increased with increasing the size of particle, whereas the linearity varied between phantoms. The temporal average of TS of $CaCO_3$ particle was smaller than that of the 200 µm glass particle in the 8 %wt PVA-H phantoms, but larger than that of 200 µm glass particle in 10 %wt PVA-H phantom. The comparison of the 200 µm glass and $CaCO_3$ particles in 8 %wt and 10 %wt PVA-H phantoms is shown in Fig. 6. The size and geometry of two $CaCO_3$ particles were different (please refer to Table I for size).

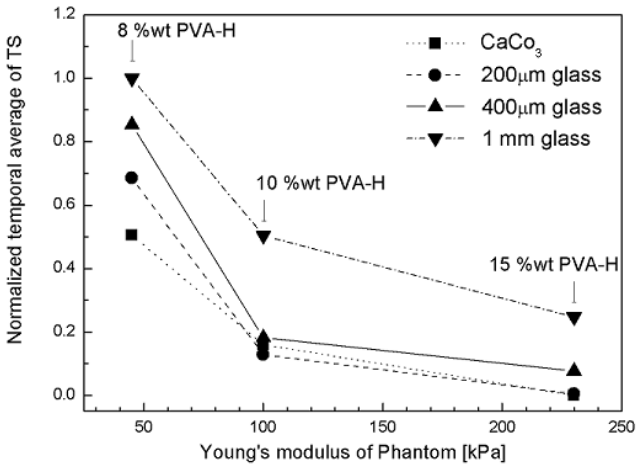

Fig. 4 Relationship between the changing of TS temporal average and the PVA-H phantom stiffness.

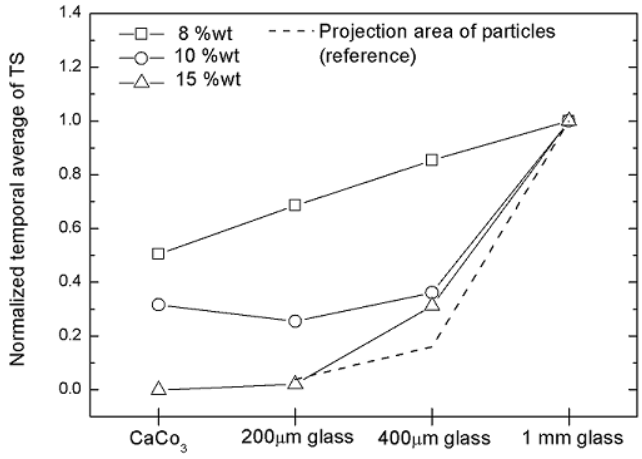

Fig. 5 Temporal average of TS for each micro particle in three phantoms.

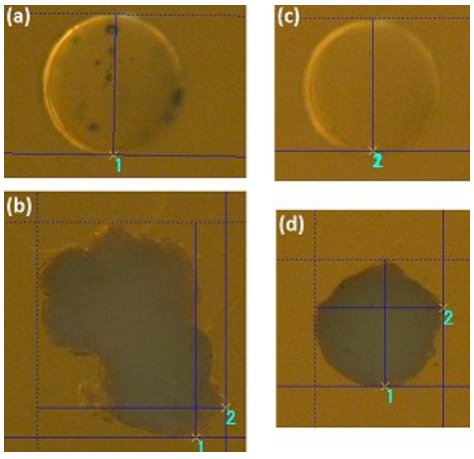

Fig. 6 Comparison of 200 µm glass and $CaCO_3$ particles in 8 %wt and 10 %wt PVA-H phantoms. (a) glass and (b) $CaCO_3$ were embedded in the 8 %wt PVA-H phantom. (c) and (d) were embedded in the 10 %wt PVA-H phantom.

IV. Discussion

The source of the twinkling sign observed in ultrasound CF imaging were both of the glass particle and the $CaCO_3$ particle (Fig. 3). It was confirmed that the temporal average of TS of glass particles was equivalent or larger than that of the $CaCO_3$ particle with the consideration of the particle size. In addition, the smooth the surface of the glass particles (Fig. 1) excluded one possibility of TS occurrence caused by surface roughness [5, 8]. These results showed that the surface roughness is not the dominant trigger of the TS.

Another possible trigger for the occurrence of TS is the ultrasound radiation force induced micro oscillation of the particles [13, 14]. The ultrasound scan condition including the acoustic output was fixed in the experiment except for the stiffness of the phantoms (Table I). The Young's modulus of the phantom can be considered as an index of the mobility of the micro particles. The result in Fig. 4 showed that the TS temporal average decreased with increasing the phantom stiffness, which indicated that the micro oscillation of the particles had a close relationship with the occurrence of TS.

In Fig. 5, linearity of temporal TS average was varied between phantoms. Moreover, the changing of the temporal TS average due to the particle size approached to that of analytical projection area (PA) of glass particles with increasing the stiffness of the phantoms. The signal strength of the reflected ultrasound (Echo) is considered to be affected by the particle size, namely PA. However it is difficult to explain this linearity changing only by the variance of PAs. This result indicated the character of oscillations of particles were possibly different between the phantom stiffness. The relationship between the size of 200μm glass particle, $CaCo_3$ particles and their TS temporal averages were conflicting (Table I, Fig.s 5 and 6). The result implies that the geometry of the particle has an effect on the TS appearance, and needing further investigation in detail.

Further researches are necessary to clarify the oscillation characteristics of the micro particles to understand the mechanism of the twinkling sign. Conducting optical observation with a soft tissue mimicking phantom, for example, using a high speed camera with an optical microscope to observe the oscillation of micro particles under the ultrasound scan is a reasonable approach. Developing a numerical model corresponding to the experiment and conducting a computer simulation will bring flexibility, and will enable a multidirectional approach to the present study. These items will be conducted in our future research.

V. Conclusion

The authors conducted an *in vitro* experiment to confirm the effect of stiffness of the surrounding tissue on the appearance of TS. The appearance and characteristics of TS changed due to the stiffness of the PVA-H phantoms. The result indicated that the micro oscillation of the particles had a close relationship with the occurrence of TS.

Conflict of Interest

The authors have declared no conflicts of interest.

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