Comparison of Real-Time Two-Dimensional and Three-Dimensional Contrast-Enhanced Ultrasound to Quantify Flow in an *In Vitro* Model: A Feasibility Study

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

EF 1 Si-Min Ruan*
EF 2 Qiao Zheng*
B 1 Zhu Wang
C 1 Hang-Tong Hu
B 1 Li-Da Chen
B 1 Huan-Ling Guo
E 1 Xiao-Yan Xie
E 1,3 Ming-De Lu
AG 1 Wei Li
AEG 1 Wei Wang

1 Department of Medical Ultrasonics, Ultrasonics Artificial Intelligence X-Lab, Institute of Diagnostic and Interventional Ultrasound, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, P.R. China
2 Department of Medical Ultrasonics, Fetal Medical Center, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, P.R. China
3 Department of Hepatobiliary Surgery, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, P.R. China

* Si-Min Ruan and Qiao Zheng contributed equally to this work
Corresponding Authors: Wei Wang, e-mail: wangw73@mail.sysu.edu.cn, Wei Li, e-mail: liwei259@mail.sysu.edu.cn
Source of support: This study received funding from the National Nature Science Foundation of China (No: 81701719), and the Guangdong Natural Science Foundation (No: 2016A030310143)

Background: This feasibility study aimed to compare real-time two-dimensional contrast-enhanced ultrasound (2D-CEUS) and three-dimensional contrast-enhanced ultrasound (3D-CEUS) to quantify flow in an *in vitro* model.

Material/Methods: Five polyvinyl chloride (PVC) tubes were used for the perfusion models and used SonoVue ultrasound contrast agent with a perfusion volume ratio of 1: 2: 4: 8: 16. The contrast was injected at a constant speed to compare the raw quantitative data of 2D-CEUS and 3D-CEUS at angles of 0°, 45°, and 90°. The coefficient of variation (CV) of the peak intensity (PI) in the model were compared and the correlations between weighted PI and perfusion volume were analyzed.

Results: In the three angles used, real-time 3D-CEUS resulted in a more comprehensive view of the spatial relationships in the perfusion model. Using real-time 2D-CEUS, the mean CV was 0.92±0.36, and the mean CV in the real-time 3D-CEUS model was significantly less at 0.48±0.32 ($p<0.001$). Quantitative 3D-CEUS parameters showed a good correlation with those of 2D-CEUS with an r-value of 0.93 ($p=0.02$). The r-value of weighted PI and the perfusion ratio using 2D-CEUS was 0.66 ($p=0.23$) compared with values in 3D-CEUS of 0.84 ($p=0.08$).

Conclusions: The combination of real-time 3D-CEUS and quantitative analysis identified the spatial distribution of the changes in angle in the model, which was less influenced by sectional planes, and was more representative of the perfusion volume when compared with 2D-CEUS. Quantitative real-time 3D-CEUS requires in *vivo* studies to evaluate the potential role in the clinical evaluation of vascular perfusion of malignant tumors.

MeSH Keywords: Contrast Media • Imaging, Three-Dimensional • *In Vitro* • Ultrasonography

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/919160

📄 2420   🖾 4   📊 3   📖 24

Background

The development of ultrasound contrast agents with a low mechanical index (MI), which is correlated with the acoustic pressure, has advanced the imaging technique of contrast-enhanced ultrasound (CEUS). During minutes, CEUS can visualize dynamic enhancement patterns in real-time [1]. CEUS has become an effective standard diagnostic imaging method that is recommended and supported by several clinical guidelines [2–4].

However, the use of two-dimensional contrast-enhanced ultrasound (2D-CEUS) to evaluate the vasculature within a three-dimensional (3D) tissue, such as a tumor, can produce variable results. Interpretation of 2D-CEUS is based on one single 2D sectional plane, and tumor necrosis and vascularity may be heterogeneous in different 2D imaging planes. 2D-CEUS only shows the 2D layout of tumor blood vessels and not their complex spatial relationships [5]. Therefore, 2D-CEUS does not accurately reflect blood flow and perfusion of the whole lesion.

Three-dimensional contrast-enhanced ultrasound (3D-CEUS) combines the advantages of 2D-CEUS and 3D-US, and can objectively evaluate tumor vascularity by reconstruction of stereoscopic images [6]. Compared with 2D-CEUS, 3D-CEUS can faithfully reflect the contrast perfusion of the whole lesion and can precisely evaluate treatment response [7,8]. Static 3D-CEUS is a clinically valuable tool that can demonstrate the blood supply of tumors [9]. However, its drawback is that it may not visualize the dynamic perfusion of the lesion. Real-time 3D-CEUS solves this problem as there is continuous dataset acquisition [10]. Real-time 3D-CEUS has previously been studied in preclinical animal studies of tumor treatment response [8,11]. Further clinical evaluation has been carried out in disease diagnosis and the evaluation of therapeutic effects [7,12–14].

However, qualitative analysis of 3D-CEUS images is subjective and prone to interobserver variation, particularly by junior radiologists who may be inexperienced [15,16]. Combined with the used of on-line quantitative analysis software, 3D-CEUS can image the stereoscopic perfusion of contrast agents in real-time. Therefore, quantitative analysis of real-time 3D-CEUS is established to overcome imaging limitations and increase the objectivity of investigators when interpreting CEUS imaging information [17].

Currently, real-time 3D-CEUS quantitative analysis is still in the preliminary stage. Previous studies on the repeatability of quantitative real-time 3D-CEUS analyzed repeating pairs of acquisition data within a scan session under the same conditions [18,19]. A normalized and maneuverable model is required to demonstrate that quantitative 3D-CEUS is a stable and feasible method to analyze the fluid flow in lesions. Therefore, this feasibility study aimed to compare real-time
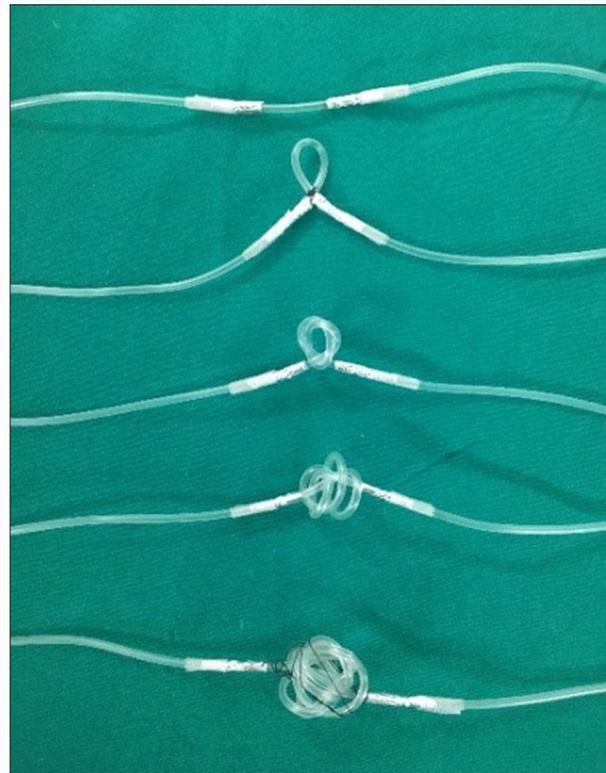
Figure 1. Perfusion models. The perfusion model is in the middle section of the tubes with lengths of 2 cm, 4 cm, 8 cm, 16 cm, and 32 cm. The perfusion model components are of different shapes, including tubular (2 cm), annular (4 cm and 8 cm), and spherical (16 cm and 32 cm).

two-dimensional contrast-enhanced ultrasound (2D-CEUS) and three-dimensional contrast-enhanced ultrasound (3D-CEUS) to quantify flow in an *in vitro* model.

Material and Methods

Establishment of the model

Five polyvinyl chloride (PVC) tubes with an outer diameter of 2 mm and an inner diameter of 1 mm were divided into three parts using tin foil, the inflow part, the perfusion model part, and the outflow part. The perfusion model was in the middle section of the tubes with lengths of 2 cm, 4 cm, 8 cm, 16 cm, and 32 cm, to represent different levels of blood supply of a tumor mass. The components of the perfusion model were formed into different shapes, including tubular (2 cm), annular (4 cm and 8 cm), and spherical (16 cm and 32 cm) (Figure 1). The tubes had no folds and no narrowing of the inner diameter. For this perfusion models, the volume ratio was 1: 2: 4: 8: 16.
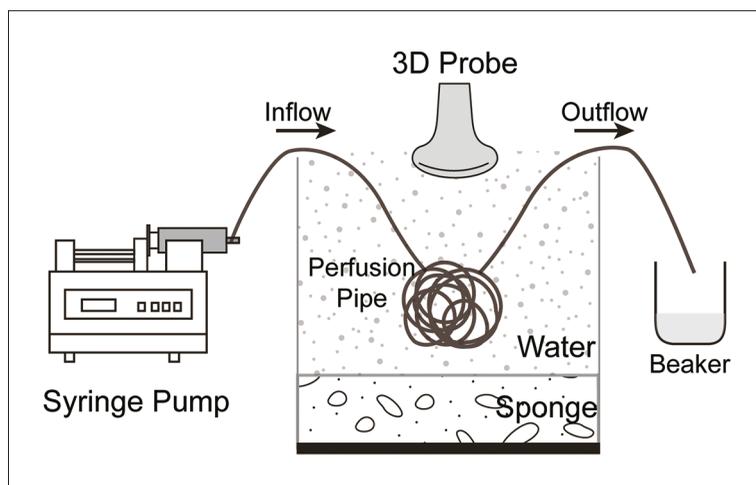
Figure 2. Construction of the *in vitro* model. This perfusion model is fixed in a water tank, which includes an acoustic absorption sponge with a thickness of about 2 cm on the top and bottom. The inflow end is connected to a syringe pump to ensure that the contrast agent perfuses at a constant speed. The outflow end is shown placed in a 1000 ml beaker.

This perfusion model was then fixed in a water tank, which included an acoustic absorption sponge with a thickness of about 2 cm on the sides and base. The inflow end was connected to a syringe pump (WZ-50C6) (Smiths Medical, Minneapolis, MN, USA) to ensure that the contrast agent was perfused at a constant speed (200.0 ml/h). The outflow end was placed into a 1000 ml beaker (Figure 2).

Image acquisition

Contrast-enhanced ultrasound (CEUS) was performed using an Aplio 500 ultrasound scanner (Toshiba Medical Systems, Tokyo, Japan) with a PVT375MV 3D imaging probe (frequency: 2–8 MHz) (Toshiba Medical Systems, Tokyo, Japan). The *in vitro* model was immersed in a water tank. The probe was fixed below the water surface and above the perfusion model using a rack. The largest sectional plane was regarded as the 0° plane, and then the probe was rotated to acquire the 45° plane and 90° plane images to compare the imaging stability of 2D-CEUS and 3D-CEUS. Real-time 2D-CEUS scans were performed using the settings of mechanical index=0.09, frame rate=10 fps, and acoustic power=2%. In the same session, real-time 3D-CEUS scans were performed using the settings of mechanical index=0.09, frame rate=1.5 fps, and acoustic power=2%. The other imaging conditions are shown in Table 1.

The ultrasound contrast agent used in this study was SonoVue® (Bracco Imaging, Milan, Italy), which is an ultrasound contrast agent consisting of sulfur hexafluoride microbubbles. The ultrasound contrast agent was reconstituted with 5 ml of 0.9% saline and gently shaken until it became a milky white suspension. One milliliter of contrast suspension was added to 50 ml of normal saline, resulting in a 1: 50 concentration dilution.

Two-dimensional ultrasound (2D-US) was performed in each plane, followed by real-time 2D-CEUS and 3D-CEUS. The ultrasound contrast agent was infused at a constant rate of 200 ml/h

Table 1. Imaging parameters of real-time two-dimensional (2D) and three-dimensional (3D) contrast-enhanced ultrasound (CEUS).

Parameter	2D-CEUS	3D-CEUS
Imaging technique	Harmonic wave	Harmonic wave
Mechanic index	0.09	0.09
Frequency (MHz)	3.5	3.5
Grey density (dB)	78	78
Dynamic range	60	60
Frame rate	10 fps	1.5 vps
Focal point	1	1
Focus location (cm)	7	7
Acoustic power output (%)	2	2

through an inner tube. The duration of each imaging session was 2 minutes, which was repeated six times. During the interval of each measurement, 0.9% saline was used to flush the tubes to remove the microbubbles from attachment to the tube wall, which might have affected the experimental results. The raw dynamic imaging data were stored for quantitative analysis.

Quantitative analysis of CEUS

The online software analysis package used was Aplio 500 version 3.7 (CHI-Q) (Toshiba Medical Systems, Tokyo, Japan). This study data analysis focused on the perfusion parameter of the peak intensity (PI), which was the maximum average peak intensity of flow perfusion in the region of interest (ROI). It has previously been reported that when the instrument set-up and
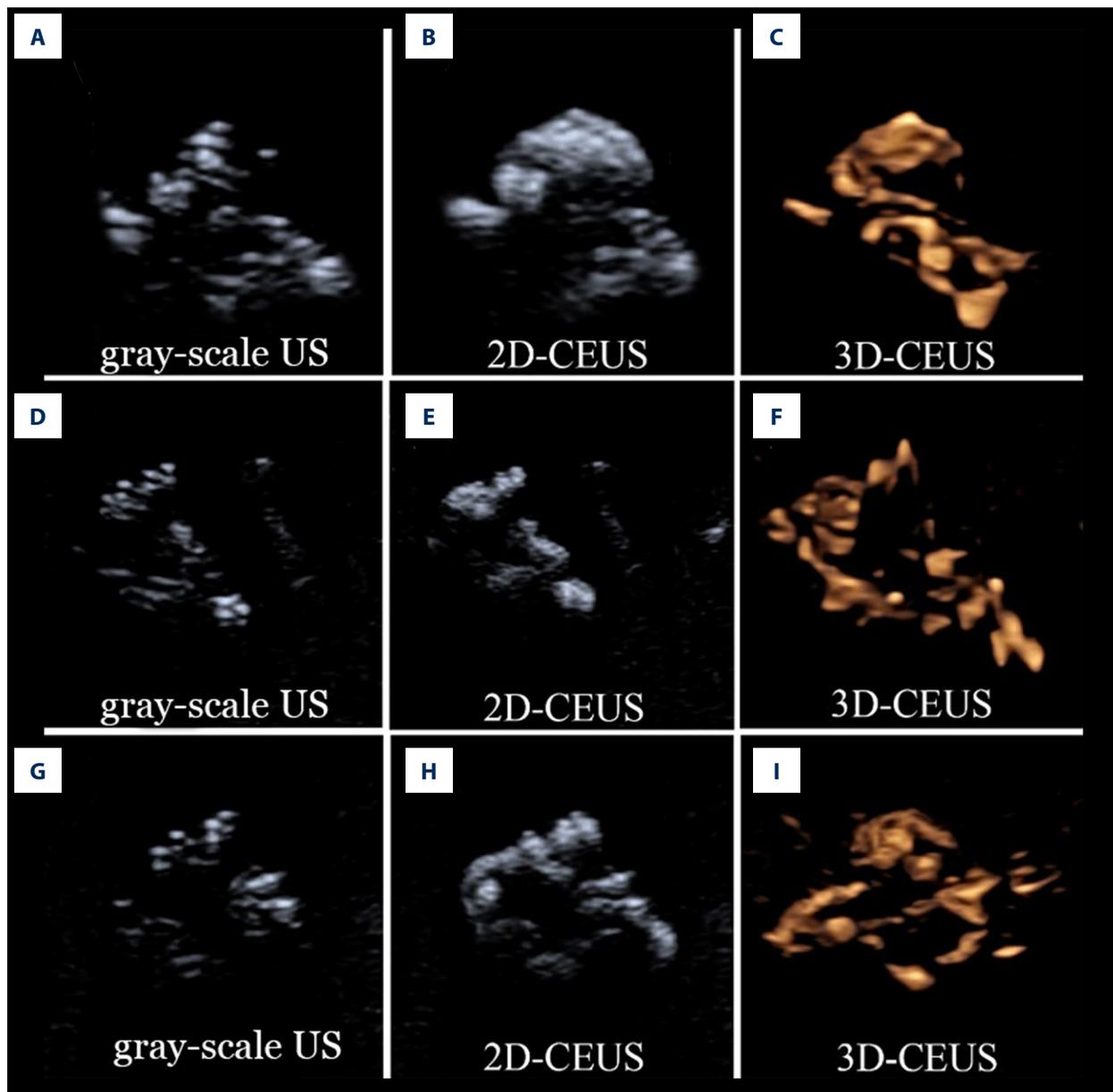
Figure 3. Ultrasound images of the perfusion model. The largest sectional plane is regarded as the 0° plane (**A–C**). The probe was spun to the 45° plane (**D–F**), and the 90° plane (**G–I**).

the dose of contrast agents remain constant, PI accurately reflects the blood volume of tissues [20].

Statistical analysis

Data were analyzed using SPSS version 19.0 software (SPSS Inc, Chicago, IL, USA). Normally distributed data were expressed as the mean±standard deviation. The coefficient of variation (CV) of different sectional planes in 2D-CEUS and 3D-CEUS were calculated and compared using the independent sample t-test. Correlation of the weighted PI (mean value of PI from three different sections) between two imaging methods, and correlation

of the PI weighted value and perfusion ratios (1: 2: 4: 8: 16), were determined by the r-value using Pearson's correlation analysis. A *p*-value <0.05 was considered to be statistically significant.

Results

The establishment of the perfusion model

The established model had a irregular shape in the imaging plane with structurally even tube diameters. There was no

Table 2. Comparison of the coefficient of variation (CV) between real-time two-dimensional (2D) and three-dimensional (3D) contrast-enhanced ultrasound (CEUS) in different length of the perfusion model and different sectional planes.

| Length of perfusion model (cm) | CEUS mode | PI (2D: AU×10⁻⁴; 3D: AU×10⁻⁶) | | | CV |
		0°	45°	90°	
2	2D	4.48±0.62	0.55±0.33	0.80±0.13	113.39%
	3D	1.84±0.05	0.98±0.05	3.78±0.59	65.21%
4	2D	4.89±0.89	0.16±0.04	0.47±0.04	143.70%
	3D	1.27±0.23	0.20±0.03	0.47±0.03	86.49%
8	2D	8.42±1.55	2.89±0.84	1.85±0.13	80.51%
	3D	2.53±0.47	3.26±0.21	3.47±0.23	16.06%
16	2D	12.75±0.77	9.86±0.60	3.79±0.38	51.96%
	3D	7.65±0.67	6.04±0.56	6.97±0.65	11.72%
32	2D	10.83±0.54	2.51±0.34	4.69±0.47	71.75%
	3D	11.09±1.00	3.50±0.34	5.38±0.05	59.36%

significant acoustic attenuation in the far field, and the imaging effect was satisfied. The model simulated a tumor with necrosis in the irregular shaped area. The images of two-dimensional ultrasound (2D-US), two-dimensional contrast-enhanced ultrasound (2D-CEUS), and three-dimensional contrast-enhanced ultrasound (3D-CEUS) in three different sections are shown in Figure 3. Real-time 3D-CEUS provided a more intuitive and comprehensive view of the spatial relationships of the perfusion model.

Variability of different sectional planes in quantitative CEUS

The peak intensity (PI) values of the different perfusion models are shown in Table 2. The PI of each model in different sectional planes was the average of six measurements and shown as the mean ± standard deviation (SD). Each coefficient of variation (CV) was calculated with the standard deviation and the mean of the PI of three different sectional planes in the same perfusion model and the CEUS mode. In each perfusion volume model, the CV of 3D-CEUS was always less than that of 2D-CEUS. The average CV of the five perfusion models using real-time 2D-CEUS was 0.92±0.36. The average CV using real-time 3D-CEUS was 0.48±0.32, which was significantly less than the average CV of 2D-CEUS ($p<0.001$). Therefore, the stability of the quantification of 3D-CEUS was better than 2D-CEUS in this *in vitro* model.

Table 3. Weighted peak intensity (PI) in real-time two-dimensional (2D) and three-dimensional (3D) contrast-enhanced ultrasound (CEUS).

| Ratio of perfusion model volume | Weighted PI (2D: AU×10⁻⁴; 3D: AU×10⁻⁶) | |
	2D-CEUS	3D-CEUS
1	1.94	2.20
2	1.84	0.64
4	4.39	3.09
8	8.80	6.89
16	6.01	6.66

Correlation between the perfusion volume and quantitative CEUS

The weighted PI of quantitative CEUS was the average PI of 0°, 45°, 90° planes in the same perfusion model, and the same CEUS modes. The results are shown in Table 3. The weighted PIs of real-time 2D-CEUS of these five perfusion models were 1.94, 1.84, 4.39, 8.80, and 6.01, while the corresponding values of real-time 3D-CEUS were 2.20, 0.64, 3.09, 6.89, and 6.66. As the weighted PIs were in accordance with normal distribution, Pearson's correlation analysis was computed to assess the correlation of the weighted PI of two imaging methods, as well as the correlation of the weighted PI and perfusion ratios (1: 2: 4: 8: 16) of the *in vitro* model.

Quantitative 3D-CEUS parameters showed a good correlation with those of 2D-CEUS with the r-value of weighted PI in 2D-CEUS compared with 3D-CEUS of 0.93 ($p=0.02$). The r-value

Table 4. Correlation of the weighted peak intensity (PI) value and perfusion ratio in real-time two-dimensional (2D) and three-dimensional (3D) contrast-enhanced ultrasound (CEUS).

CEUS mode	Weighted value of PI	
	r-Value	p-Value
2D	0.66	0.23
3D	0.84	0.08

of weighted PI and perfusion ratio by using 2D-CEUS was 0.66 (p=0.23), while that of 3D-CEUS was 0.84 (p=0.08) (Table 4). Quantitative real-time 3D-CEUS could reflect the actual perfusion volume of the lesion more precisely compared with quantitative real-time 2D-CEUS. The quantitative real-time 3D-CEUS was technically feasible and may be used for evaluation of the perfusion of tumors.

Discussion

Compared with two-dimensional contrast-enhanced ultrasound (2D-CEUS), instantaneous 3D-CEUS contrast-enhanced ultrasound (3D-CEUS) increases the perfusion imaging information in the coronal and sagittal planes. Therefore, 3D-CEUS can more effectively demonstrate the spatial structure of lesions and provide information that cannot be detected in 2D scanning, which may mean that it is and important supplement to real-time 2D-CEUS [21,22]. However, instantaneous 3D-CEUS is established on the static images reconstituted using computers, and cannot dynamically observe the time-intensity curve (TIC) of stereoscopic perfusion in lesions *in vivo*. Without TIC, it is possible to miss the key points in the perfusion process.

Real-time 3D-CEUS is established based on instantaneous 3D-CEUS together with the time vector, and can thoroughly and stereoscopically observe the perfusion characteristics of lesions with TIC. Meanwhile, the 'off-target' phenomenon and motion artifacts that result from motion decreased, which is another great advantage in ultrasound technology following real-time 2D-CEUS and 3D-CEUS [23,24]. As the present study has shown, real-time 3D-CEUS shows superiority to real-time 2D-CEUS and static 3D-CEUS in imaging lesion morphology, the spatial relationship of supplying blood supply vessels, and the distribution of internal blood vessels, which are important for the evaluation of flow perfusion with only subtle changes. The latest real-time 3D-CEUS technology can acquire the original volume raw data with a higher frame rate, and when combined with volume quantitative analysis software implanted on the instrument, it can more accurately reflect the perfusion of tumors [24].

In the present study, we used an *in vitro* model to demonstrate the stability and feasibility of quantitative real-time 3D-CEUS to evaluate the perfusion that might occur in solid tumors. Because malignant tumors are generally irregular in shape and contain areas of bleeding and necrosis, an irregular perfusion model was constructed to represent blood perfusion of a tumor. We performed comparative experiments under different sectional planes with the same concentration of ultrasound contrast agent to evaluate the stability of 3D-CEUS. As for the CEUS image of the same model, 3D-CEUS images were more similar to the actual structure of the model when compared with 2D-CEUS (Figure 3). The coefficient of variation (CV) was used to determine the variability of peak intensity (PI) of different sectional planes. The CV of PI was found to be relevant to the irregularity of the model. However, in the same model, the CV of 3D-CEUS was significantly lower than that of 2D-CEUS (p<0.01). The 3D-CEUS images are based on the x and y planes, respectively and reconstruction of a third axial plane (the z-axis plane) was based on the acoustic signals of the x and y axial planes, which synthesized a stereoscopic real-time contrast-enhanced image. 2D-CEUS could only obtain the acoustic signal of a single slice, and the difference between the signals of different planes were significant. This finding was particularly significant when the shape of the object was irregular, and the internal echo was heterogeneous, so the quantitative stability of 2D-CEUS was lower than for 3D-CEUS.

In this study, the r-value of PI between 2D-CEUS and 3D-CEUS was 0.9, which was a finding supported in the reported previously study by Cao et al. [17], which meant that 3D-CEUS had similar enhanced characters with 2D-CEUS. The weighted PI was used to adjust the quantitative results of different planes. Both real-time 2D-CEUS (r=0.66; p=0.23) and real-time 3D-CEUS (r=0.84; p=0.08) correlated quantitatively with the actual perfusion volume. However, real-time 3D-CEUS showed a better linear correlation with perfusion volume. Therefore, 3D-CEUS might more exactly reflect the actual perfusion volume compared with 2D-CEUS. This function has potential clinical application. A previously reported study showed that quantitative real-time 3D-CEUS could differentiate between early (within 24 hours) responders and non-responders in a mouse model of colorectal cancer [8]. Nam et al. showed that the PIs of tumor imaging from 3D-CEUS were significantly lower in the treatment complete responder group than in the incomplete responder group as early as one or two weeks after transhepatic arterial chemotherapy and embolization (TACE) [14].

This study had several limitations. Firstly, this feasibility study used an *in vitro* model with polyvinyl chloride (PVC) tubes representing blood vessels. Also, there were only five perfusion models in the study. However, because the lesions *in vivo* are variable, more models should be designed in future studies.

The ratio of weighted PI did not correspond with the ratio of the perfusion volume. The reasons might have been that the parts of the perfusion model were formed into different shapes, including tubular, annular, and spherical, with different planes. Although the PVC tubes had good acoustic ultrasound performance, the tube walls might have caused some acoustic attenuation. With the increased number of stacked wall layers, the effects of this attenuation on the quantitative results increased. Further developments to refine this model may be used in the future, such as improved ultrasound contrast agents and imaging systems to facilitate quantitative real-time 3D-CEUS.

Conclusions

This feasibility study aimed to compare real-time two-dimensional contrast-enhanced ultrasound (2D-CEUS) and three-dimensional contrast-enhanced ultrasound (3D-CEUS) to quantify flow in an *in vitro* model. The findings showed that the combination of real-time 3D-CEUS and quantitative analysis identified the spatial distribution of the changes in angle in the model, which was less influenced by sectional planes, and was more representative of the perfusion volume when compared with 2D-CEUS. Quantitative real-time 3D-CEUS requires *in vivo* studies to evaluate the potential role in the clinical evaluation of vascular perfusion of malignant tumors.

References:

1. Albrecht T, Blomley M, Bolondi L et al: Guidelines for the use of contrast agents in ultrasound. January 2004. Ultraschall Med, 2004; 25: 249–56

2. European Association for the Study of the Liver (EASL). EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. J Hepatol, 2018; 69(1): 182–236

3. Piscaglia F, Nolsoe C, Dietrich CF et al: The EFSUMB Guidelines and Recommendations on the Clinical Practice of Contrast Enhanced Ultrasound (CEUS): update 2011 on non-hepatic applications. Ultraschall Med, 2012; 33: 33–59

4. Korean Liver Cancer Association-National Cancer Center (KLCANCC). 2018 KLCA-NCC Korea Practice Guideline for the Managementof Hepatocellular Carcinoma. Available at [URL]: *http://livercancer.or.kr/study/guidelines.php*

5. Wagner S, Gebel M, Bleck JS, Manns MP: Clinical application of three-dimensional sonography in hepatobiliary disease. Bildgebung, 1994; 61(2): 104–9

6. Jia W-R, Chai W-M, Tang L et al: Three-dimensional contrast enhanced ultrasound score and dynamic contrast-enhanced magnetic resonance imaging score in evaluating breast tumor angiogenesis: Correlation with biological factors. Eur J Radiol, 2014; 83: 1098–105

7. Xu RX, Li YK, Li T et al: Real-time 3-dimensional contrast-enhanced ultrasound in detecting hemorrhage of blunt renal trauma. Am J Emerg Med, 2013; 31: 1427–31

8. Wang HJ, Hristov D, Qin JL et al: Three-dimensional dynamic contrast-enhanced US imaging for early antiangiogenic treatment assessment in a mouse colon cancer model. Radiology, 2015; 277: 424–34

9. Luo W, Numata K, Morimoto M et al: Three-dimensional contrast-enhanced sonography of vascular patterns of focal liver tumors: Pilot study of visualization methods. Am J Roentgenol, 2009; 192: 165–73

10. Lu Y, Liu B, Zheng Y et al: Application of real-time three-dimensional contrast-enhanced ultrasound using SonoVue for the evaluation of focal liver lesions: A prospective single-center study. Am J Transl Res, 2018; 10: 1469–80

11. Zhou J, Wang H, Zhang H et al: VEGFR2-targeted three-dimensional ultrasound imaging can predict responses to antiangiogenic therapy in preclinical models of colon cancer. Cancer Res, 2016; 76: 4081–89

12. Woźniak MM, Osemlak P, Ntoulia A et al: 3D/4D contrast-enhanced urosonography (ceVUS) in children – is it superior to the 2D technique? J Ultrason, 2018; 18: 120–25

13. Dong FJ, Xu JF, Du D et al: 3D analysis is superior to 2D analysis for contrast-enhanced ultrasound in revealing vascularity in focal liver lesions – A retrospective analysis of 83 cases. Ultrasonics, 2016; 70: 221–26

14. Nam K, Stanczak M, Lyshchik A et al: Evaluation of hepatocellular carcinoma transarterial chemoembolization using quantitative analysis of 2D and 3D real-time contrast enhanced ultrasound. Biomed Phys Eng Express, 2018; 4(3): 035039

15. Liang J, Huang X, Hu H et al: Predicting malignancy in thyroid nodules: Radiomics Score Versus 2017 American College of Radiology Thyroid Imaging, Reporting and Data System. Thyroid, 2018; 28(8): 1024–33

16. Rajpurkar P, Irvin J, Ball RL et al: Deep learning for chest radiograph diagnosis: A retrospective comparison of the CheXNeXt algorithm to practicing radiologists. PLoS Med, 2018; 15: e1002686

17. Tranquart F, Mercier L, Frinking P et al: Perfusion quantification in contrast-enhanced ultrasound (CEUS) – ready for research projects and routine clinical use. Ultraschall Med, 2012; 33(Suppl. 1): S31–38

18. Cao J, Dong Y, Fan P et al: Feasibility of dynamic three-dimensional contrast-enhanced ultrasound in focal liver lesions: Image quality evaluation and correlation of quantification with two-dimensional contrast-enhanced ultrasound. Clin Hemorheol Microcirc, 2019; 72(3): 305–16

19. El Kaffas A, Sigrist RMS, Fisher G et al: Quantitative three-dimensional dynamic contrast-enhanced ultrasound imaging: First-in-human pilot study in patients with liver metastases. Theranostics 2017; 7: 3745–58

20. Lassau N, Chami L, Benatsou B et al: Dynamic contrast-enhanced ultrasonography (DCE-US) with quantification of tumor perfusion: A new diagnostic tool to evaluate the early effects of antiangiogenic treatment. Eur Radiol, 2007; 17(Suppl. 6): F89–98

21. Prager RW, Ijaz UZ, Gee AH, Treece GM: Three-dimensional ultrasound imaging. Proc Inst Mech Eng H. 2010; 224(2): 193–223

22. Xu HX, Lu MD, Xie XH et al: Three-dimensional contrast-enhanced ultrasound of the liver: experience of 92 cases. Ultrasonics, 2009; 49: 377–85

23. Chen NG, Fowlkes JB, Carson PL, LeCarpentier GL: Rapid 3D imaging of contrast flow: Demonstration of a dual beam technique. Ultrasound Med Biol, 2007; 33: 915–23

24. Dietrich CF, Averkiou MA, Correas JM et al: An EFSUMB introduction into Dynamic Contrast-Enhanced Ultrasound (DCE-US) for quantification of tumour perfusion. Ultraschall Med, 2012; 33: 344–51