

Current developments and clinical applications of bubble technology in Japan: a report from 85th Annual Scientific Meeting of The Japan Society of Ultrasonic in Medicine, Tokyo, 25-27 May, 2012

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Abstract

The potentials of bubble technology in ultrasound has been investigated thoroughly in the last decade. Japan has entered as one of the leaders in bubble technology in ultrasound since Sonazoid (Daiichi Sankyo & GE Healthcare) was marketed in 2007. The 85th Annual Scientific Meeting of The Japan Society of Ultrasonics in Medicine held in Tokyo from May 25 to 27, 2012 is where researchers and clinicians from all over Japan presented recent advances and new developments in ultrasound in both the medical and the engineering aspects of this science. Even though bubble technology was originally developed simply to improve the conventional ultrasound imaging, recent discoveries have opened up powerful emerging applications. Bubble technology is the particular topic to be reviewed in this report, including its mechanical advances for molecular imaging, drug/gene delivery device and sonoporation up to its current clinical application for liver cancers and other liver, gastrointestinal, kidney and breast diseases.

Keywords: microbubble, bubble liposome, contrast enhanced ultrasound, gene delivery, sonoporation.

Introduction

The purpose of The Annual Scientific Meeting of The Japan Society of Ultrasonics in Medicine is to accommodate researchers and clinicians from all over Japan in a Japanese forum to share and discuss annually the recent advances and new developments in ultrasound. Presentations encompassed both the medical and the engineering aspects of this science. Bubbles are used in contrast-enhanced ultrasonography (CEUS), which has immensely impacted the practice of ultrasound in medicine during the last decade by making detailed depictions of vascularity possible. Bubble technology has also shown potential in gene or drug delivery and microinjection. It

is thought clinical applications of ultrasound molecular imaging using bubble technology will become a reality in the very near future.

In this conference, the advanced research on the biological effects of the bubble including its application for diagnosis and treatment was discussed. This review will focus on reports involving basic and clinical aspects of bubble technology in ultrasound in Japan.

1. Mechanical advances in bubble technology

1.1. Bubble Liposome for molecular imaging and drug and gene delivery system

A group from the National Defense Medical College and Tokyo University reported the recent finding of bubble liposome applications, including molecular imaging and the gene and drug delivery system. Dr. Kohsuke Hagsawa reported the development of a gas cored molecular-targeted bubble liposome with a peptide sequence on its surface targeting glycoprotein IIb/IIIa (also known as integrin α IIb β 3), a marker of platelet activation and high-density integrated vascular thrombosis [1,2]. They developed a nano-size (120 nm) bubble liposome and

Received 15.03.2012 Accepted 30.03.2012

Med Ultrason

2013, Vol. 15, No 2, 140-146

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successfully imaged atherosclerotic lesions in a murine model *in-vivo*.

Using ultrasound exposure on a similar bubble liposome, Dr. Nobuhito Hamano described his successful delivery of Doxorubicin into human renal tumor cells *in vitro* as a Doxorubicin bubble liposome labeled with a peptide that binds to syndecan (a transmembrane co-receptor protein) with high selectivity named AG73. The combination of AG73-Dox with BLs and US did not enhance cellular uptake of Doxorubicin, but it did promote drug release in the cytoplasm (fig 1) [3]. Previously Dr. Hamano and his group were successful in developing AG73-PEG liposomes which can be a useful targeted gene delivery vehicle for syndecan-2 overexpressing cancer cells [4]. Dr. Yoichi Negishi introduced a novel perfluoropropane bubble liposome containing cationic lipid for ultrasound imaging and gene delivery [5,6]. The positively charged bubble liposome had increased binding affinity with nucleic acid, which is negatively charged. Combined with ultrasound application, they successfully transferred genes, including pDNA coding for the Luciferase reporter gene, into Cos-7 cells *in vitro*, which was proven by monitoring intracellular activity of these genes.

1.2. Sonoporation

A research group from Hokkaido University explored the new technique of sonoporation, which make possible microinjection to cells using microbubbles, possibly

for gene and drug delivery. Dr. Nobuki Kudo, one of the sonoporation experts [7] explained the mechanism of sonoporation as a transient increase in cell membrane permeability caused by cavitation phenomena during exposure to ultrasound [8]. He and his group demonstrated an increase in cytotoxicity of cisplatin to canine thyroid cancer cells under sonoporation. His colleague, Dr. Manabu Okuyama, developed optical light beam tweezers that can ‘grasp’ microbubbles and control the size and position of the microbubbles attaching to the cell surface [9]. This makes possible detailed research on sonoporation, including controlling the extent of cell membrane damage and the amount of drugs introduced into the cells. He demonstrated this by introducing propium iodide, a fluorescent substance, into human prostate cancer cells with 1 MHz short-pulsed ultrasound.

1.3. Controlling Microbubble Dynamics

Professor Yoshiki Yamakoshi, expert of microbubble dynamics from the Engineering Department of Gunma University [10,11] and his research group (including Mr. Takuya Kanai, Mr. Tomoyuki Ozawa and Mr. Tomoaki Isono) reported current and evolving approaches in the control of microbubble dynamics by acoustic radiation force (fig 2, fig 3). To study microbubble dynamics and the efficiency of micro-perforation, they exposed a blood-flow model to ultrasound waves, and captured the results with high-speed photography [12-14]. Professor Toshio Chiba, another expert in microbubble control [15] with

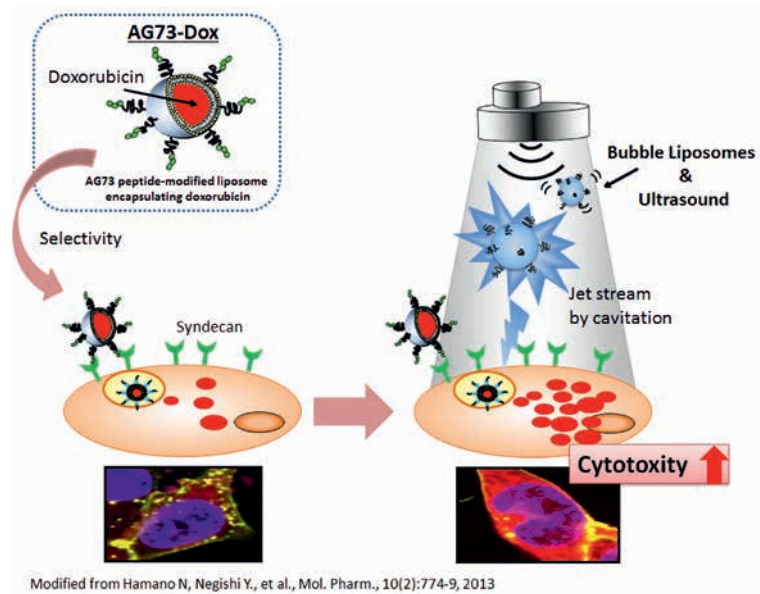


Fig 1. Enhancement of drug Release of AG73-DOX of bubble liposomes and ultrasound exposure

his team from Tokyo University of Agriculture and Technology reported on the dynamics of microbubbles within an artificial capillary network. Dr. Nobuhiko Shigehara reported that he succeeded in stimulating the aggregation of microbubbles to redirect blood flow in a model of an arterial branch [16,17]. In the same session, Dr. Ren Koda, utilizing the same artificial blood vessel network, demonstrated that a particular direction of ultrasound exposure could be used to redirect the streaming of microbubbles at speeds up to 50 mm/sec [18]. Dr. Takashi Azuma, an expert in high intensity focused ultrasound (HIFU) sys-

tems for therapy, from the School of Engineering of University of Tokyo, reported increased control of imaging and bursting of microbubbles by using a dual frequency bilaminar transducer (2 MHz 64 channel and 500 kHz 16 channel) [19]. Dr. Ken-ichi Kawabata from Hitachi's Medical System Research Development demonstrated that applying ultrasound to a nano-sized lipid precursor could form microbubbles in the desired target, calling this compound Phase Changed Nano Droplet (PNCD) [20].

The future of bubble technology in medicine was briefly summarized by Professor Katsuro Tachibana,

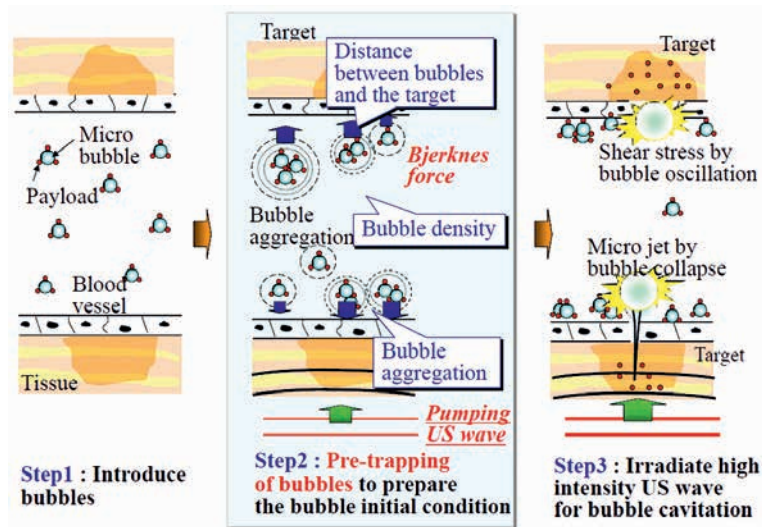


Fig 2. Pre-trapping of bubbles for preparing bubble initial condition

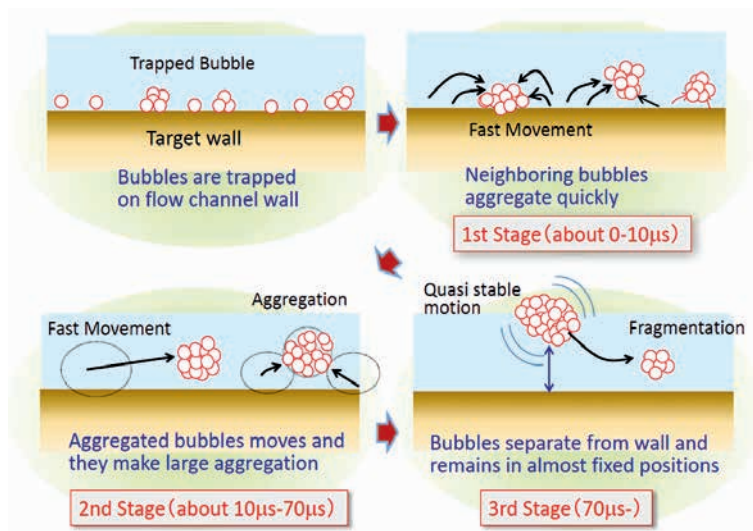


Fig 3. Summary of observations for bubble cloud cavitation

from Fukuoka University, closing the “Bubble” session [21]. He stated that in the near future, microbubbles will assume the main role in molecular imaging and therapy, especially with nano-level technology, which will move the boundary of diagnosis and treatment from the blood vessel wall to the actual cancer tissue. Adding polyethylene glycol (PEG) and subsequently the targeting moieties may direct nanobubbles to specific targets. This may also improve therapeutic outcomes, shorten treatment time, decrease the amount of drug administered and decrease side effects.

2. Current clinical applications of bubble technology

2.1. New Techniques for Liver Malignancy Detection

Dr. Ikutarou Tsukada from Kyorin University presented parametric microflow imaging (MFI) using Sonazoid contrast-enhanced ultrasonography (set to a value 0.2-0.3 MI) and its ability to depict detailed blood flow on first pass images, adding objectivity to sonographic diagnosis [22].

2.2. Hepatocellular carcinoma

Half a decade has passed since the contrast-enhanced ultrasonography using second-generation ultrasound contrast agent Sonazoid was introduced in Japan, and it is now in routine clinical use. Dr. Hitomi Nakamura from Nihon University Itabashi Hospital discussed the current status and diagnostic accuracy of contrast enhanced ultrasound (Sonazoid) for liver tumors, based on 803 cases from 2007 to 2011 [23]. Using contrast enhanced CT and MRI for confirmation, he showed that most hepatocellular carcinomas (HCCs) could be diagnosed with accuracy, but deeper lesions and hypoechoic lesions were problematic. Intrahepatic bile duct cancers were found to be very difficult to diagnose.

In many cases, hypoechoic lesions in the Kupffer phase have been reported to transform over time into highly malignant lesions. Dr. Kunio Suzuki from Saiseikai Senri Hospital compared the CEUS findings and the cell differentiation of HCCs in the histopathological data of 44 nodules from 32 patients with chronic liver diseases (from poorly differentiated HCC to well-differentiated HCC), and found their differences in the contrast enhancement patterns [24]. Dr. Fumiko Hori from Shinkokura Hospital used micro flow imaging (MFI) to visualize fine vascular structures in HCCs [25]. Based on her clinical experience with 33 patients (2009 to 2011) the concordance rate between CEUS MFI and immunohistology of blood vessels larger than 100 μm was as high as 87.3%. Dr. Yukinobu Watanabe from Nihon University School of Medicine stated a high frequency probe (9MHz) is preferred for evaluating small HCCs

(sometimes even for lesion less than 10mm) on CEUS and proved useful for early detection [26]. Dr. Keiko Korenaga from Kawasaki Medical School shared her similar experience with CEUS on high-frequency probes (6 to 7MHz) in the detection of metastatic liver lesions in the session for metastatic liver tumors in 127 cases [27].

2.3. Metastatic Liver Tumors

Dr. Hitomi Nakamura stated that diagnosis of metastatic liver cancer by ultrasound imaging alone is difficult, even with CEUS, giving an accuracy rate of only 70% [23]. On the other hand, Dr. Kenji Takeshima from Ogaki Municipal Hospital compared CEUS images from 675 cases obtained from the vascular phase (Kupffer phase) of liver metastases with their respective contrast enhanced CT images, comparing them for sensitivity and specificity, and found no significant differences between the two modalities. The sensitivity of CEUS was 86.3%, with specificity of 97.3%, positive predictive value and negative predictive value of 82.6% and 97.9%, respectively. More interestingly, the tumor measurements in the two modalities were similar. Average tumor size in CEUS image was 19.1mm while in contrast-enhanced CT it was 20.0 mm. CEUS was also able to detect tumor size as small as 2mm [28]. The potential of CEUS for monitoring therapeutic response during chemotherapy was studied by Dr. Yasuko Mizushima from Kurume University Medical Center [29]. While CT currently plays the main role in evaluating efficacy, the excellent temporal and spatial resolution of CEUS enables depiction of blood flow within tumors, which has potential in monitoring hemodynamic changes before and after chemotherapy.

2.4. Benign liver lesions

Dr. Yoko Ohyama from Akita Kumiai General Hospital discussed the period when contrast agent appears to disappear on CEUS, calling this the ‘manhole phenomenon’ and tried to elucidate its mechanism and diagnostic value [30]. She hypothesized that in hepatic hemangioma, which has slow blood flow, burst bubbles remain in the lesion, while in HCC, with its higher blood flow, the burst bubbles are quickly replaced with “fresh” ones. Dr. Tomoko Maeno and Dr. Kiyoshi Maekawa from Professor Masatoshi Kudo’s research group (Kinki University Faculty of Medicine) presented their comparative studies between CEUS and CE-CT and MRI to diagnose hemangioma and AP shunt. Dr. Maeno explained that in CEUS, defect reperfusion image was observed in 94.7% case (90 of 95 nodules) [31]. Dr. Maekawa reported a comparison study of 28 patients diagnosed as AP shunt on CEUS with CE-CT and EOB-MRI findings [32]. He found that CEUS is useful for AP shunt diagnosis, as it could reveal the different pattern in arterial phase compared to the two other modalities.

Focal nodular hyperplasia (FNH) is the second most frequent benign liver tumor and a common incidental finding. Dr. Tsukata Saitoh from Shimane University Hospital reviewed the usefulness of Sonazoid CEUS in the differential diagnosis of FNH, especially by using maximum intensity projection images, since finding in Kupffer phase of FNH is similar with the hypervascular liver tumor [33].

2.5. CEUS for diffuse liver disease

CEUS has been used to evaluate hemodynamic changes in liver cirrhosis and chronic hepatitis. It is known that as the disease progresses, portal vein blood flow decreases to compensate the arterial blood flow predominance in chronic liver disease. Dr. Hidehiko Waki from Meiwa Hospital explained the significance in measurements of arrival time of contrast in the hepatic artery, portal vein and hepatic vein in hepatic malignancy in predicting preoperative portal vein pressure [34].

In the previous JSUM conference, Dr. Yasushi Matsukiyo and his research groups from Toho University reported that in the acute phase of acute hepatitis, the parenchymal blood perfusion undergoes transient changes toward hepatic artery dominance. In this year's conference, Dr. Matsukiyo presented his Sonazoid CEUS findings that there was no significant decrease in portal vein blood flow, suggesting that a totally different mechanism from chronic liver disease might exist for acute hepatitis [35]. Dr. Noritaka Wakui presented the potential of Sonazoid CEUS by parametric imaging to estimate alcoholic intake in heavy drinkers [36;37]. By using arrival time parametric imaging (At-PI) and color mapping, alcoholic intake could be evaluated objectively without relying on self-report. Dr. Kazue Shiozawa presented the evaluation of hepatic and splenic parenchymal microbubble collapse distance from the liver surface in chronic liver disease using Sonazoid CEUS and flash replenishment sequence in vascular phase [38]. In alcoholic liver cirrhosis, the microbubble collapse distance was significantly deeper than in chronic hepatitis C, alcoholic liver disease and non-alcoholic steatohepatitis.

Dr. Akiko Saishoji from the Hyogo College of Medicine reviewed CEUS findings of non-alcoholic steatohepatitis (NASH) of Kupffer phase of both Levovist and Sonazoid [39]. On Levovist CEUS, NASH brightness was reduced and could be differentiated from fatty liver. This effect was limited in Sonazoid due to its phagocytosis by Kupffer cells.

2.6. Pancreatitis

Dr. Masumi Yasuda from Misyuku Hospital found CEUS with Levovist useful in differentiating pancreatic cancer from masses that were not neoplastic, such as obstructive pancreatitis, autoimmune cause, and chron-

ic pancreatitis [40]. Dr. Yosuke Nakamura and his colleagues from Nagoya University presented their clinicopathological study on 5 cases of pancreatic arteriovenous malformation (PAVM), collected from 2004 to 2011 [41]. PAVM is very rare and usually congenital. Dr. Nakamura found that endoscopic ultrasound is superior to conventional ultrasound, and that diagnostic accuracy improved further with contrast enhanced endoscopic ultrasound.

2.7. Other Gastrointestinal Cases

Dr. Tatsuya Miyake from Shimane University Hospital and Dr. Naohito Yamashita from Kawasaki Medical School Hospital reported Sonazoid CEUS was useful in detecting intraabdominal haemorrhage. Dr. Miyake presented two representative cases from his six cases: three HCC rupture, one HCC rupture after embolization therapy, one colon diverticular rupture and one hepatic subcapsular hematoma [42]. Dr. Yamashita found that CEUS might replace upper gastrointestinal endoscopy, colonoscopy, capsule endoscopy or double balloon endoscopy in 56 untreated emergency cases. Using a 3.5MHz convex probe or 6-7MHz linear probe, he found CEUS was superior to B-mode ultrasound in detecting gastrointestinal bleeding [43].

Dr. Masaharu Odo from Kumamoto Medical Center showed through a clinical study of 20 patients with acute abdomen that CEUS can be used to evaluate blood flow of the intestinal wall and diagnose life-threatening acute mesenteric ischemia without exploratory laparotomy [44]. One of the causes of emergency gastrointestinal bleeding is rupture of esophageal varices. Dr. Masahiro Yamahira from the Hyogo College of Medicine presented his findings that liver to spleen 'luminosity' ratio (LS ratio) as depicted in the Kupffer phase was useful in predicting the development of esophageal varices requiring treatment [45].

2.8. Kidney

Dr. Takako Watanabe, from Akita Kumiai General Hospital, explained the role of CEUS and volume data-based US in the diagnosis of renal cell carcinoma in 17 patients [46]. She found Sonazoid CEUS using second harmonic imaging and multiplane imaging useful in diagnosing renal cell carcinoma.

2.9. Breast

Dr. Yukio Mitsuzuka from Toho University presented the current advanced technique of tissue signal suppression in Sonazoid CEUS to diagnose breast cancer in 28 nodules from 26 patients, and found it helpful in differentiating malignant and benign breast masses. In Dr. Mitsuzuka's previous findings reported in the 18th Annual Scientific Meeting Japanese Breast Cancer Society, CEUS specificity was reported 62.5%, resulting in the correct diagnosis rate of 83.1%. With this new signal suppres-

sion method, 92.3% specificity was obtained [47]. Dr. Takashi Nakamura from Nara Medical University found CEUS helpful in both differentiating ductal carcinoma in situ (DCIS) from mastopathy and depicting the extent of intraductal spread of DCIS in 97 patients. Confirmation was made using contrast enhanced MRI and also pathologically [48].

Conclusion

Bubble technology is rapidly developing in both basic science and clinical applications. New innovations from the bench and continuous feedback from the bedside experience certainly will boost the pace. This Japanese conference shared new ideas and awareness of the huge potential of bubble technology and contrast enhanced ultrasound.

Conflict of interest: none

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