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# Development of Microbubble Contrast Agents for High Frequency Ultrasound Microscopy[1]

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**Purpose:** To develop optimal microbubble contrast agents (MBCAs) for performing ultrasound microscopy when examining small animals.

**Materials and Methods:** We prepared three types of MBCAs. First, a mixture of three parts of 40% dextran and one part of 5% human serum albumin were sonicated with perfluorocarbon (PFC) ($MB_1$-D40A5P). Second, three parts of 40% dextran and one part of 1% human serum albumin were sonicated with PFC ($MB_2$-D40A1P). Third, all parts of 1% bovine serum albumin were sonicated with PFC ($MB_3$-A1P). We measured the microbubbles' sizes and concentrations with using image analysis software. The acoustic properties of the microbubbles were assessed both *in vitro* and *in vivo*.

**Results:** The majority of the $MB_1$-D40A5Ps had a diameter of 2 - 5 μm, the mean diameter of the $MB_2$-D40A1Ps was 2.5 μm, and the mean diameter of the $MB_3$-A1Ps was less than 2.0 μm. Among the microbubbles, the $MB_1$-D40A5Ps and $MB_2$-D40A1Ps showed increased echogenicity in the abdominal vessels, but the duration of their contrast effect was less than 30 sec. On the contrary, the MB3-A1Ps exhibited strong enhancement in the vessels and their duration was greater than 120 sec.

**Conclusion:** A microbubble contrast agent consisting of all parts of 1% serum albumin sonicated with PFC is an effective contrast agent for ultrasound microscopy.

**Index words :** Ultrasound (US)
Ultrasound (US), contrast media
Microbubbles
Animals
Microsphere

High frequency ultrasound microscopy has recently been introduced as an important part of small animal research. Commercialized microbubbles have limitations for use with clinical ultrasound equipment with lower diagnostic frequencies (1 to 8 MHz), which is a frequency that microbubble contrast agents have been shown to resonate. Thus, microbubbles need to be improved for their use in high frequency ultrasound microscopy. The precise resonant frequency of each microbubble is dependent not only upon its own size and capsule composition, but also on the incident acoustic pressure that's exerted upon it. At higher diagnostic frequencies (>30 MHz), the acoustical properties of contrast agent microbubbles are not so clearly understood, and only limited reports have been published on the acoustic charac-

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terization of gas-filled contrast agents. Deng et al. studied microbubbles at three distinct concentrations in the frequency range from 15 to 50 MHz (1).

Experimental studies performed in animals under normal physiologic conditions have confirmed that air-filled microbubbles made from sonicated 5% human albumin are kinetically similar to erythrocytes and they pass unimpeded through the microcirculation (2, 3), thereby validating the use of this agent as a red blood cell tracer *in vivo* (4, 5). Kripfans et al. have shown that albumin-coated perfluoropentane microbubbles (1.5 to 4 μm) act as superheated liquid microbubbles and they only vaporize when perturbed by US (6). In addition, the microbubble's albumin shell acts as a stabilizer that reduces diffusion and also prevents droplets from coalescing. For this reason, the albumin-coated microbubbles are very successful for *in vivo* use.

We prepared three types of microbubbles, with different ratios of albumin, to find the optimal contrast agent microbubble at a frequency of 30 MHz with using a US microscope. The purpose of this manuscript is to present the preliminary results of the backscatter from three different microbubbles in an *in vitro* test. In addition, we determined the optimal condition that enhanced the echogenecity of the blood pool in a small animal model.

## Materials and Methods

### *Production of Microbubbles*

Based on a previous article (7), a method to generate dextran-albumin based microbubbles was modified by using either 5% or 1% albumin and 40% (g/L) dextran at a ratio of 1:3 (v/v); the mixture then underwent electromechanical sonication (Vibra Cell 600, Bioblock, Strasbourg, France) at a mechanical energy of 260 W. As a result, three types of MBCAs were prepared. The first was a mixture of three parts of 40% dextran and one part of 5% human serum albumin ($MB_1$-D40A5P), the second was three parts of 40% dextran and one part of 1% human serum albumin ($MB_2$-D40A1P), and the third was all 1% bovine serum albumin ($MB_3$-A1P) (Fig. 1)

To investigate the effect of the sonication time on the microbubble size and distribution, the samples were sonicated for 90 sec (s), 120 sec, 160 sec or 180 sec, respectively. During the sonication process, perfluorocarbon (PFC) gas was added to the mixture. The microbubble solution was then poured into a burette and the solution separated into three layers 30 minutes later. The upper layer was a white solution with larger microbubbles, the middle layer was a milky-white solution containing smaller microbubbles and the bottom layer was a heavy white solution containing the precipitates from the mixture of microbubbles. The smaller microbubbles were collected from the middle layer.

### *Determination of Microbubble Size*

The microbubbles produced from the different conditions were evaluated via optical microscopy (Olympus Optical Co., Tokyo, Japan). Samples from each vial were diluted with saline at a ratio of 1:10 or 1:100 based on the concentration of microbubbles, and an aliquot of the solution was placed on a hemocytometer slide and this was examined under an optical microscope at 400X magnification. The images were digitally captured using a CCD camera (Photometrics, AZ, U.S.A.). The sizes of the microbubbles were determined based on calibration measurements with using stage micrometer software (MetaMorph, Universal Imaging Corp., PA, U.S.A.). The density, i.e., the concentration was estimated from the volume of the fluid from which the microbubbles were counted by noting the area of the hemocytometer and determining the height of the fluid column in the hemocytometer (100 μm). With using the optically

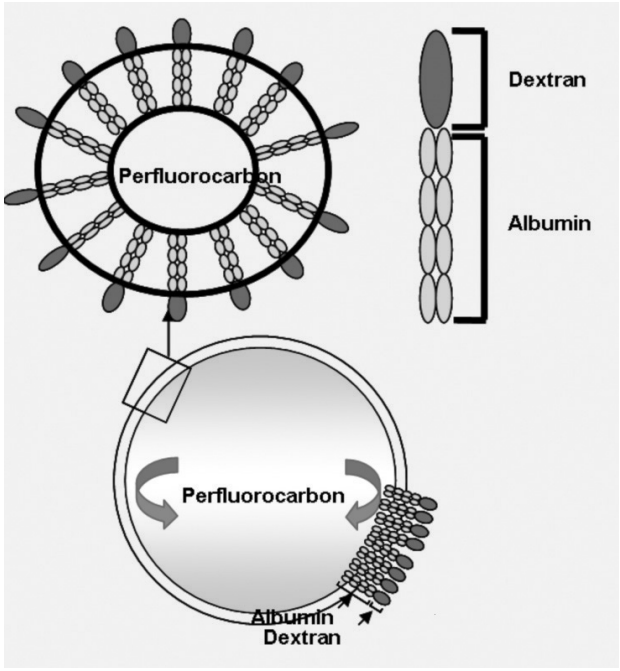


**Fig. 1.** A hypothetical model for preparing the US microbubble contrast agents designed in our study. Perfluorocarbon was used as the gas compound In the inner core of the microbubbles, and the outer surface was stabilized by albumin and/or dextran.

scanned volume ($V_{total}$), a dilution of 1 to 10 times, and the total volume to the volume of the counted droplets ($V_{droplet}$), the droplet volume fraction was then determined as $V = X \cdot (V_{droplet} / V_{total})$ (1).

#### *Enhancement Effect of Microbubbles on In vitro Testing*

After preparation, the microbubbles were diluted with saline. The contrast images of the microbubbles were taken using ultrasonic microscope equipment (Vevo 660 model; VisualSonics Inc., Toronto, Canada). The settings of the US equipment that was used for capturing the contrast images were as follows: applied frequency = 30 MHz, US spatial peak temporal average intensity = 47.2 mW/cm$^2$, total acoustic power = 0.091 mW and the frame rate = 32 Hz. The image on the monitor of the B-mode equipment was fed into the computer with using a built-in frame grabber. To examine the echogenicity, the three agents were diluted; 100 μl of each agent was introduced in a beaker containing 100 mL of sterile water, thereby creating approximately $1 \times 10^6$ microbubbles per mL of suspension, and then the suspension was mixed for 30 sec. This suspension of contrast agents was scanned at 30 MHz with using the ultrasonic microscope system. The transmit power ranged from 10% to 100% of the maximum output power, and the focal distance was 12.5 mm. To avoid the effect of a change in size of the microbubbles according to their time in sterile water, the measurements of their size and acoustic intensity (echogenicity) were carried out for 10 sec after placing the microbubbles in sterile water. For measuring the acoustic intensity, the ultrasonic images were analyzed for determining the average pixel video intensity (VI) at the region of interest in the suspensions. The VI was calculated using the image-processing software (Vevo660 version 1.3.8, VisualSonics Inc., Toronto, Canada) designed for the US equipment. These experiments were repeated four times to obtain the average result for each of the three kinds of microbubbles.

#### *Small Animal Imaging with the Microbubble Contrast Agents*

We used six-week-old male BALB/C mice for our *in vivo* imaging study. The mice ranged in weight from 20 - 25g. The mice were anesthetized with isofluorane that was maintained at 450 - 500 bpm. Their body temperature was kept at 37℃ by keeping them placed on a heating pad. The contrast agents were weighted and suspended in saline prior to injection. The agents were injected through a tail vein with using a 30-gauge needle. Immediately after injection, US examination was performed using the Vevo 660 system with a 30 MHz transducer. The US equipment settings, including gain (14.0 dB), the compression and the time-gain compensation, were fixed throughout the study. The real-time images were recorded. The intrahepatic portions of the IVC and the portal vein were observed after injection of $1 \times 10^8$ MBs.

To measure the echogenicity of the intrahepatic portion of the IVC and the portal vein, all the frames were obtained both before and after injecting the microbubbles. The sizes of the regions of interest (ROI) varied from 2.0 - 2.5 mm$^2$, and the contrast enhancement ratios (CER) were then calculated. The CER of a vessel between the precontrast ($C_0$) and postcontrast ($C_1$) images was calculated with using the following equation:

$$CER = (C_1 - C_0)/C_0 \cdot 100 \quad (2)$$

## Results

#### *Sonication Variations*

In order to use ultrasound MBCAs for intravascular applications, a defined size of the MBs must be maintained and it must generally be smaller than the size of a red blood cell. Three types of microbubbles were sonicated for 90s, 120s, 160s or 180s in order to determine if the sonication time has an effect on the mean bubble size. Table 1 shows the results of the size, stability and contrast enhancement ratio according to the three types of US MBCAs. A long sonication time results in smaller droplets as well as higher density (Fig. 2). The mean size of the $MB_1$-D40A5Ps was found to be 10.5 μm for 90s sonication, 4.5 μm for 2-minutes (min) sonication, 3 μm for 160s sonication and 2.6 μm for 3-min sonication (Fig. 2A). The average mean size of the $MB_2$-D40A1Ps was found to be 10.5 μm for 90s sonication, 4.25 μm for 2-

**Table 1.** The Results of the Size, Stability and Contrast Enhancement Ratio according to the Three Types of US Microbubble Contrast Agents

| | $MB_1$-D40A5P | $MB_2$-D40A1P | $MB_3$-A1P |
|---|---|---|---|
| Size (μm) | 2.6±1.31 | 2.0±0.82 | 1.92±0.60 |
| Stability (sec) | ≤10 | ≤30 | ≤150 |
| Enhancement ($CER_{Max}$) | 241.67±22.79 | 221.236±41.39 | 379.95±44.73 |

$CER_{Max}$: The maximum value of CER
$MB_1$-D40A5P: a mixture of three parts of 40% dextran and one part of 5% human serum albumin
$MB_2$-D40A1P: three parts of 40% dextran and one part of 1% human serum albumin
$MB_3$-A1P: all 1% bovine serum albumin

min sonication, 2.5 μm for 160s sonication and 2.0 μm for 3-min sonication (Fig. 2B). The average mean size of the $MB_3$-A1Ps was found to be 3.2 μm for 90s sonication, 2.5 μm for 2-min sonication, 2.25 μm for 160s sonication and 1.98 μm for 3-min sonication (Fig. 2C). In addition, the density, i.e., concentration of the microbubbles, was related to the sonication time (Fig. 2D). The mean concentration of the $MB_1$-D40A5Ps was $2\times10^8$ for 90s sonication, $4.2\times10^8$ for 120s sonication, $7\times10^8$ for 160s sonication and $8\times10^8$ for 180s sonication. The mean concentration of the $MB_2$-D40A1Ps was $6\times10^8$ for 90s sonication, $7.2\times10^8$ for 120s sonication and $1.2\times10^9$ for 160s and 180s sonication. The mean concentration of the $MB_3$-A1P was $8\times10^8$ for 90s sonication, $1.1\times10^9$ for 120s sonication and $1.2\times10^9$ for 160s and 180s sonication.

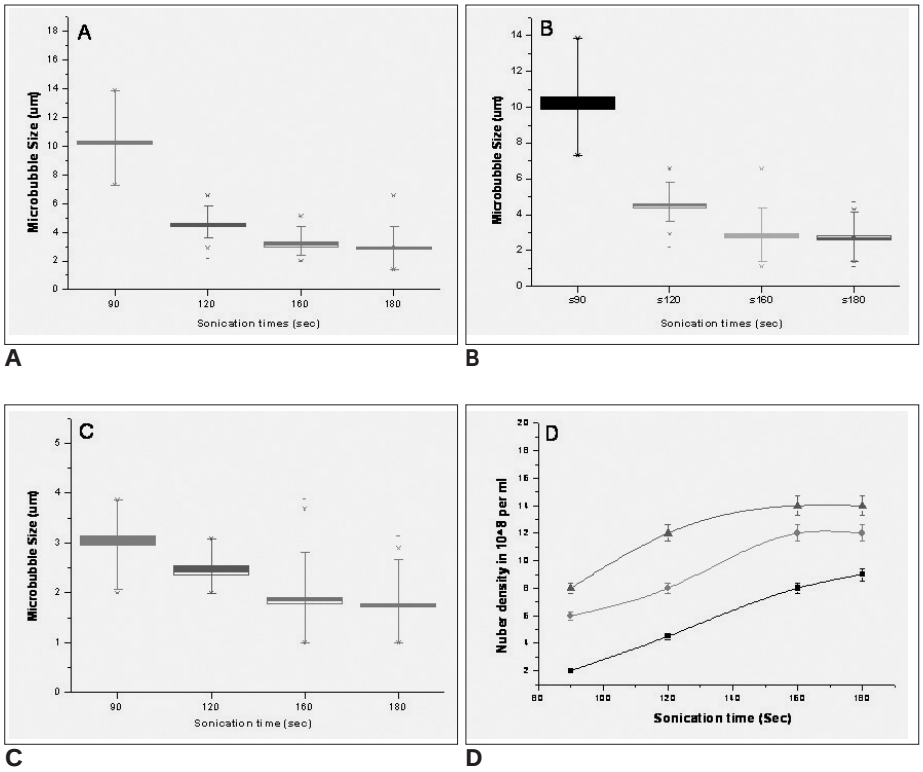


**Fig. 2.** Effects of sonication time on the mean diameter (**A - C**) and density (concentration) (**D**) of three types of microbubbles. Longer sonication results in smaller droplets as well as higher density. A: $MB_1$-D40A5P, B: $MB_2$-D40A1P, C: $MB_3$-A1P, D: concentration of $MB_1$-D40A5P (■), $MB_2$-D40A1P (●) and $MB_3$-A1P (▲).

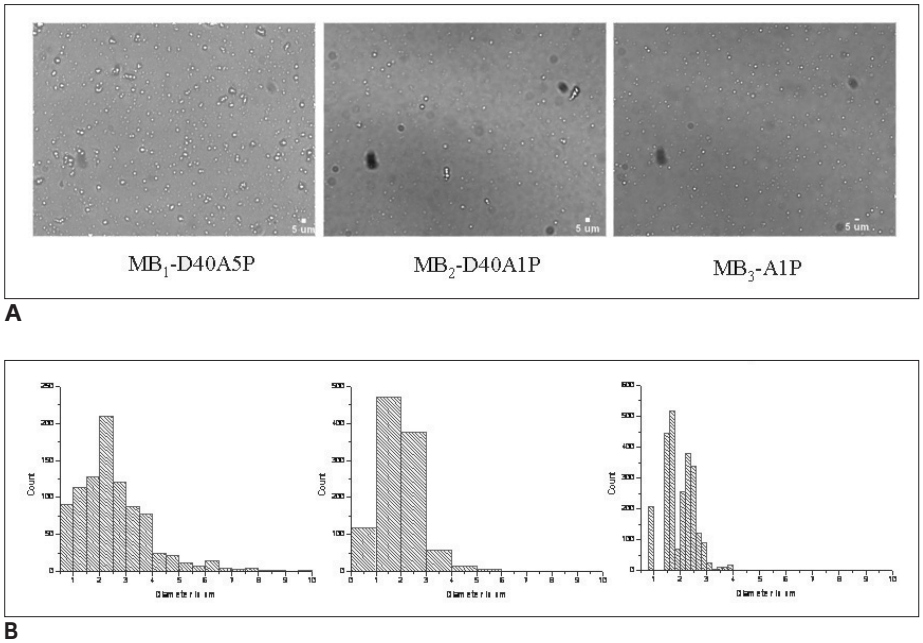


**Fig. 3.** The three microscope images show microbubble formation for $MB_1$-D40A5P, $MB_2$-D40A1P and $MB_3$-A1P. After 180s sonication, the microbubbles were observed by microscopy (× 400). The mean diameter of the $MB_1$-D40A5P was 2.6±1.31 μm, that of the $MB_2$-D40A1P was 2.0±0.82 μm and that of the $MB_3$-A1P was 1.92±0.60 μm. The mean diameter of the long axis of the microbubbles was measured by using the MetaMorph image analysis program.

Using the above mentioned optical methods, the size distribution for the microbubbles formed from the separated microbubbles is shown in Figure 3A. The microbubbles were measured using the MetaMroph image analysis program (Fig. 3B). From these results, the $MB_1$-D40A5P was less than 2.75 μm (71.75%), the $MB_2$-D40A1P was less than 2.25 μm (84.60%) and $MB_3$-A1P was less than 2.0 μm (74%).

### *Enhancement Effect of the Microbubbles*

Figure 4 shows the enhancement effect of the contrast agents, and this was evaluated by performing an *in vitro* beaker test. $MB_1$-D40A5Ps showed week enhancement and $MB_2$-D40A1Pd showed stronger enhancement than that of the $MB_1$-D40A5Pd. The $MB_3$-A1P had the strongest enhancement of the three types of microbubbles (Fig. 4A). According to the acoustic power of the US machine, the enhancement effect of the microbubbles varied. The acoustic intensity, i.e., the echogenicity was calculated according to the three types of microbubbles and the acoustic power at each of the regions of interest (ROIs), and this was represented as the average pixel videointensity (VI) (Fig. 4B). The VIs of the $MB_1$-D40A5P were 19.11±10.64 for the 100% power, 17.28 ±9.33 for the 30% power and 14.08±7.18 for the 10% power. The VIs of the $MB_2$-D40A1P were 16.89±9.69 for the 100% power, 19.32±11.32 for the 30% power and 13.53±6.2 for the 10% power. The VIs of the $MB_3$-A1P were 29.62±16.08 for the 100% power, 30.6± 17.97 for the 30% power and 22.24±12.04 for the 10% power.

### *Small Animal Imaging with using the Microbubble Contrast Agents*

The US echogenecity, within the intrahepatic portion of the IVC and portal vein after injecting the three types of microbubbles, was measured in order to determine the contrast effects on the mice. The echogenecity within the IVC showed increased enhancement at 1 sec after contrast injection of $MB_1$-D40A5Ps. However, in the portal vein, the echogenicity did not show significantly strong enhancement and there was only a short lifespan

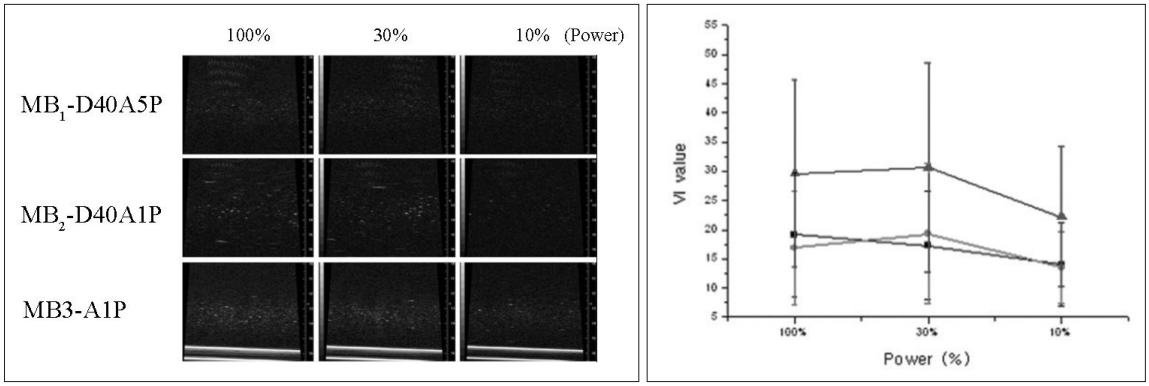


**Fig. 4.** Examples of US images acquired for the three types of microbubbles ($MB_1$-D40A5P (■), $MB_2$-D40A1P (●) and $MB_3$-A1P (▲)). The images were obtained according to the three types of identified microbubbles at each acoustic pressure (**A**). The average pixel videointensity (VI) values at different conditions were calculated (**B**).

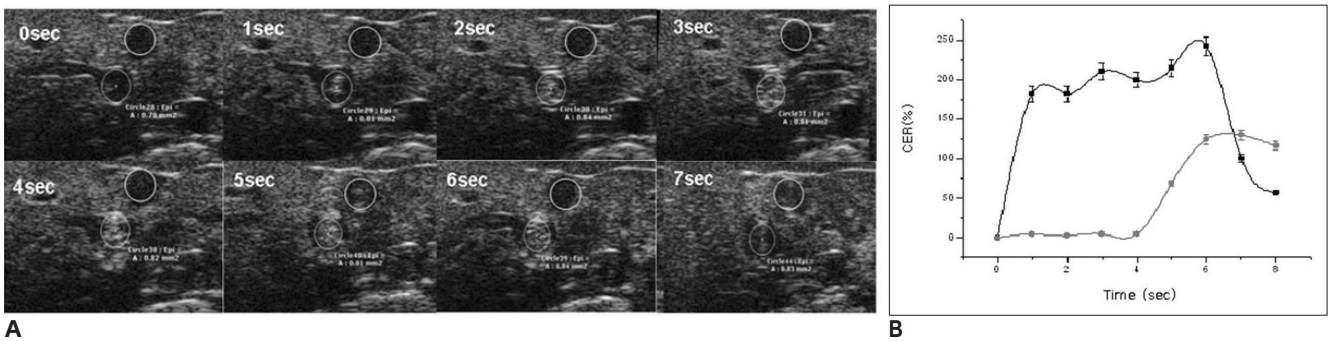


**Fig. 5.** The figure shows the in vivo imaging for the ultrasound contrast effect of the $MB_1$-D40A5P (**A**). The graph shows the mean value of the CER by microbubble injection into the IVC (■) and the portal vein (●) areas of the liver (**B**). The duration of the microbubble efficacy lasted for 10 sec.

of the contrast effect (Fig. 5). After $MB_2$-D40A1Ps were injected into the tail vein, the echogenicity in the IVC and the portal vein was still increased until 30 sec after injection (Fig. 6). In the case of $MB_2$-D40A1P injection, the CER value and the lifespan of the microbubble contrast effect were increased compared with those of the $MB_1$-D40A5Ps for enhancement of the US contrast effect in the vasculature of the liver. The dextran-based microbubbles used in our study were seen as prompt vascular enhancement; however, this disappeared within 1 min after injection. In addition, the contrast effects of these dextran-based microbubbles were limited in the relatively large hepatic vessels, but not in the capillaries. The $MB_3$-A1Ps used in this study visualized the small hepatic vessels for longer than 150 sec (Fig. 7). The maximum CER values were 241.67±22.79 for the $MB_1$-D40A5Ps, 221.236±41.39 for the $MB_2$-D40A1Ps and 379.95±44.73 for the $MB_3$-A1Ps.

## Discussion

The microbubble suspensions used as contrast agents for medical ultrasound imaging are currently undergoing significant development. Air, perfluorocarbons and sulfur hexafluoride are commonly used as gas compounds in these microbubbles (8, 9), and while free bubbles may work well as contrast agents, they are large and unstable in vivo, thereby diffusing into solutions in only a matter of seconds. However, when simple air bubbles are injected into the blood steam, they disappear within seconds through the combined effects of the Laplace pressure, the blood pressure and exposure to ultrasound energy. Due to their low solubility and diffusiveness, perfluorocarbon-filled microbubbles can be enhanced for a longer time in blood vessels than air bubbles can (10 - 12). This result has led to selection of perfluorocarbons with high molecular weights and a natural boiling point that's approximately body temperature. Several stabilizing materials for microbubbles have been

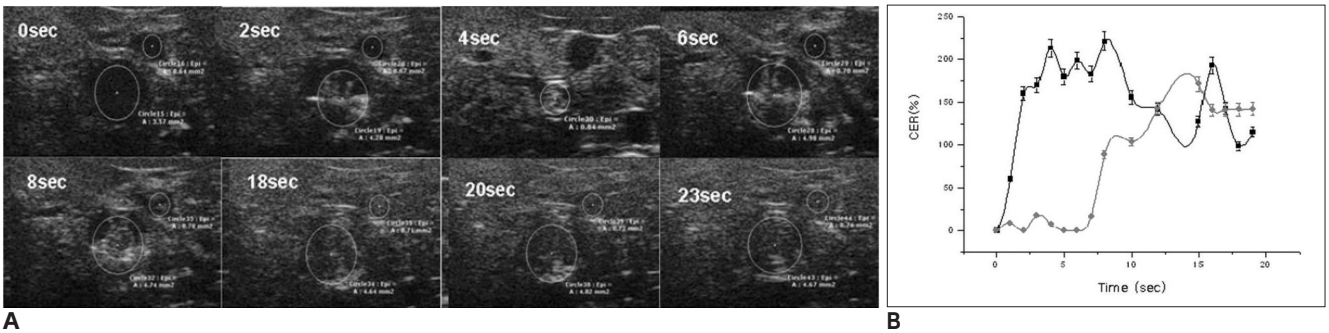


**Fig. 6.** The figure shows the in vivo imaging of the ultrasound contrast effect of the $MB_2$-D40A1P (**A**). The graph shows the mean value of the CER after MB injection into the IVC (■) and the portal vein (●) areas of the liver (**B**). The duration of the MB contrast effect lasted for 30 sec.

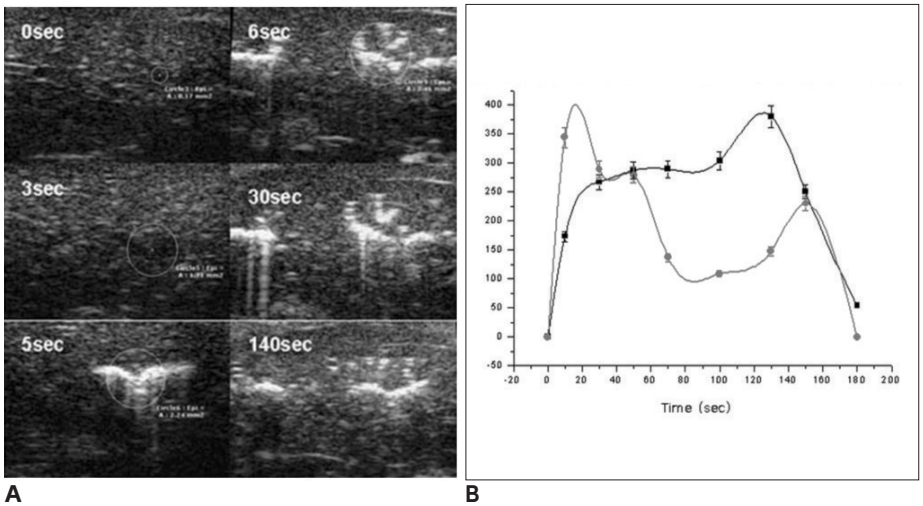


**Fig. 7.** The figure shows the in vivo imaging of the ultrasound contrast effect of the $MB_3$-A1P (**A**). The graph shows the mean value of the CER, which increased in the IVC (■) and in the portal vein (●) immediately after microbubble injection (**B**). The duration of the 1% AMB effect lasted for 150 sec.

developed in order to produce an encapsulated gas microbubble contrast agent media. These materials include albumin, gelatin, galactose microspheres, polyglutaminic acid, lipophilic monolayer surfactants and lipid bilayers (liposomes).

The MBCAs should not alter the blood flow, they should have a size restriction of less than 8 μm in order to pass unimpeded through the capillary circulation and they must be stable enough to perfuse the tissue and to last for the duration of imaging (~3 minutes) (13). A contrast agent circulates through blood vessels for a limited amount of time, depending on the properties of the agent. An end to the contrast effect may be due to one or more causes, e.g., the contrast agent may be destroyed in the process of imaging, the pressures imposed upon it by the body while in systemic circulation may destroy it, and the encapsulated air/gas may diffuse out of the agent. Natural processes also play a part in agent elimination as there is a rapid uptake of intravenously injected particles by the cells of the reticuloendothelial system (RES); these cells primarily include the Kupffer cells of the liver and the macrophages of the spleen and bone marrow (14, 15).

The first generations of contrast agents contained free air bubbles, whereas the newer generations contain a less diffusible gas core and they have very flexible and soft envelopes. It is also well known that contrast microbubbles disappear within a few minutes after injection. The disappearance of contrast agent microspheres occurs due to gas diffusion and dissolution in the lungs and other organs (via the Kupffer cells) (16). Microbubbles can also disappear relatively rapidly when they are destroyed by ultrasound waves that have appropriate parameters, e.g. their frequency, pulse length and pressure. Ultrasound contrast agents are the only contrast agents that undergo alterations after interaction with the imaging waves. This important property of contrast microbubbles has been utilized for various purposes, including diagnosis, and more recently therapy.

Albumin-encapsulated microbubbles have also been noted to adhere to vessel walls in the setting of endothelial dysfunction (17). Albumin-coated microbubbles have also been observed to bind to activated leukocytes and monocytes, which slowly roll along the injured venular endothelial cells (17). In 1996, Porter et al (18) demonstrated that perfluorocarbon-exposed, sonicated dextrose albumin (PESDA) microbubbles, unlike room air-containing sonicated dextrose albumin microbubbles, have bioactive albumin on their surface that can avidly bind synthetic antisene oligonucleotides and then release them in the presence of ultrasound. Furthermore, specific ligands for endothelial cell adhesion molecules, such as P-selectin and leukocyte intercellular adhesion molecule 1 (ICAM-1), can be attached to both lipid-encapsulated and albumin-encapsulated microbubbles, which increases their deposition onto activated endothelium (19, 20). Those targeting microbubbles may be a method for drug delivery in the field of applied ultrasound microscopy. As the ultrasound microscope has very high resolution and it can observe capillary regions in small animals, it could be a very useful tool for monitoring targeted drug delivery and gene therapy and for monitoring the expression of proteins when well-designed specific microbubbles are used.

Cachard et al. demonstrated that contrast agents could be visualized at frequencies far removed from their resonance frequencies (21), and Pavlin et al. very elegantly demonstrated that at 50MHz, contrast agents can be visualized and used with great effect to study eye structures (22). An additional area of interest for high-frequency contrast imaging is visualization of contrast microbubbles that have been modified to specifically bind to target sites such as vascular thrombi (23, 24). Such contrast microbubbles may also have a potential role for site-specific drug or gene delivery. High frequency ultrasound provides the opportunity to visualize these microbubbles either in the bloodstream or when they are bound to sites, at high resolution and in close proximity with the vascular wall.

We think that the basic equation for the resonance frequency ($f_0$) in kHz of a free bubble is Eq. (3), [13]:

$f_0 = 6500/d$ (3)

where d is the bubble diameter in μm. Therefore, at 30 MHz, bubbles less than 1 μm may significantly contribute to the high-frequency effects; as a consequence, the magnitude and variation in the mean backscatter effects from the three types of contrast agents may be attributed to their submicrometer bubble distribution.

The importance of the microbubble's size is reflected in their in vivo distribution. As microbubbles are typically smaller than red blood cells, they move freely in and out of the capillary beds. In order to extend the circulation time of the US contrast agents, we determined the conditions of microbubble preparation in order to reduce their diameter to less than 6 μm. The final choice of the process parameters will need to be a compromise of size, stability and the maximum achievable enhance-

ment. The results presented in this study show that the size of a microbubble can be determined by the albumin concentration and the sonication time. The lower the concentration of albumin, the smaller the microbubbles produced. In addition, the longer sonication that is applied, the smaller are the formed microbubbles.

From a medical point of view, safety issues such as their size distribution, their density and the lifetime of the bubbles produced, as well as the stability of the droplets in the absence of US, are very important. Therefore, we investigated the three types of contrast agents. The results with injecting microbubbles into the mouse tail vein show that $MB_1$-D40A5Ps and $MB_2$-D40A1Ps had a short duration (< 1 min) and low enhancement, but $MB_3$-A1Ps had very high enhancement (the CER values were > 300%) and a reasonable duration time of 3 min after injection.

In general, the behavior of the MBCAs depend on the shell and the gas core. The shell, which is designed to reduce diffusion into the blood, can be stiff (e.g. denatured albumin) or more flexible (phospholipid). Encapsulated microbubbles are highly echogenic due to their compressibility. From our result, we think that $MB_3$-A1Ps have higher compressibility than that of other two microbubbles even though we did not measure the compressibility (the compressibility of air is $7.65 \times 10-6$ m2/N, and the compressibility of water is $4.5 \times 10-11$ m2/N). In addition, the MBCAs showed a longer duration than that of other two microbubbles. We think that the MB3-A1Ps are not easily destroyed because their shell was stiff. However, there are currently several commercially available MBCAs, including Levovist®, Alunex®, Sonovue® etc. Compared with these MBCAs, our MBCAs showed a short duration of action even though the echogenicity was relatively acceptable. Therefore, we need to improve the MBCAs to resolve this problem by lengthening the duration time.

Further investigation will be needed so that a nano-sized bubble population can be produced via separation by choosing the separation parameters. Furthermore, other studies will be needed to achieve acoustic microbubble vaporization in vitro and for studying the extent to which blood vessels are thereby occluded from the resulting gas bubbles.

Our study showed that the $MB_3$-A1Ps less than Ø 2 μm have a duration time longer than 3 min under a 30 MHz US microscope. In conclusion, a microbubble contrast agent consisting of all parts of the 1% serum albumin sonicated with PFC is an effective contrast agent for performing ultrasound microscopy.

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# 고해상도 초음파현미경을 위한 미세기포초음파조영제개발[1]

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**목적:** 작은 동물의 초음파현미경영상검사에 이용할 수 있는 적절한 미세기포초음파조영제를 개발하고자 하였다.
**대상과 방법:** 세 가지 종류의 미세기포초음파조영제를 개발하여 실험하였다. 첫 번째는 40%의 덱스트란과 5% 혈청알부민을 perfluorocarbon (PFC)에 혼합한 제제 ($MB_1$ - D40A5P). 두 번째는 40%의 덱스트란과 1% 혈청알부민을 PFC에 혼합한 제제 ($MB_2$ - D40A5P). 세 번째는 1%의 혈청알부민에 PFC 에 혼합한 제제 ($MB_3$ - A1P)였다. 이들 세 가지 형태의 미세기포의 크기와 농도를 영상분석소프트웨어로 분석하였고, 초음파현미경을 이용하여 시험관 및 생체영상검사를 시행하였다.
**결과:** 미세기포조영제의 크기는 90%이상에서 $MB_1$ - D40A5P는 2 - 5 ㎛, $MB_2$ - D40A1P는 2.5 ㎛, $MB_3$ - A1P 2.0 ㎛ 이하였다. 이들 조영제중 $MB_3$ - A1P 이 다른 조영제보다 강한 조영증강효과를 보였다. $MB_1$ - D40A5P과 $MB_2$ - D40A1P는 주요복부혈관에서 증가한 조영효과를 보였으나. 조영증가효과가 30초 이내였다. 이에 비해 $MB_3$ - A1P는 같은 혈관에서 강한 조영증강효과를 보였으며, 120초 이상의 조영증가효과를 보였다.
**결론:** 초음파현미경용 미세기포조영제로서 1%의 혈청알부민에 PFC에 혼합한 제제($MB_3$ - A1P)가 효과적으로 이용할 수 있을 가능성이 있는 것으로 보인다.