Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 354 (2008) 49–55
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Mini review

# Effective gene delivery with novel liposomal bubbles and ultrasonic destruction technology

Ryo Suzuki [a], Tomoko Takizawa [a], Yoichi Negishi [b], Naoki Utoguchi [a], Kazuo Maruyama [a,*]

[a] *Department of Biopharmaceutics, School of Pharmaceutical Sciences, Teikyo University, 1091-1 Suwarashi, Sagamiko, Sagamihara, Kanagawa 229-0195, Japan*
[b] *School of Pharmacy, Tokyo University of Pharmacy and Life Science, Hachioji, Tokyo, Japan*

Received 28 July 2007; received in revised form 19 October 2007; accepted 22 October 2007
Available online 1 November 2007

**Abstract**

From the viewpoint of safety, non-viral vector systems represent an attractive gene delivery system for gene therapy. However, the transfection efficiency of non-viral vectors *in vivo* is generally very low. Previously, it was reported that microbubbles, utilized as imaging agents for diagnostic echocardiography, could promote gene delivery into cells when combined with ultrasound exposure. We therefore developed novel liposomal bubbles (Bubble liposomes) containing the lipid nanobubbles of perfluoropropane which is used as ultrasound imaging agent. These Bubble liposomes were smaller in diameter than conventional microbubbles and induced cavitation upon exposure to ultrasound. These results suggested that cavitation of these Bubble liposomes could be an efficient approach for delivering plasmid DNA into cells. In addition, in *in vivo* gene delivery, the combination of Bubble liposomes and ultrasound provided more effective gene delivery than conventional lipofection methods, further suggesting that Bubble liposomes could be effective as a non-viral vector system in *in vivo* gene delivery. In this review, we discuss the characteristics of Bubble liposomes and their potential utility as a gene delivery tool *in vitro* and *in vivo*.


*Keywords:* Gene delivery; Ultrasound; Cavitation; Bubbles; Liposomes

## Contents



## 1. Introduction

Ultrasound has been successfully utilized in *in vivo* imaging, destruction of renal calculus, and treatment of fibroids in the uterus. It has been reported that ultrasound increases the permeability of the plasma membrane and reduces the thickness of the unstirred layer at the cell surface, thus facilitating the entry of DNA into cells (Fechheimer et al., 1987; Miller et al., 1996). The first studies investigating ultrasound for gene delivery used frequencies in the range of 20–50 kHz (Fechheimer et al., 1987; Joersbo and Brunstedt, 1990). However, these frequencies, along with cavitation, are also known to induce tissue damage if not properly controlled (Miller et al., 2002; Guzman et al., 2003; Wei et al., 2004). To address this problem, many gene deliv-

* Corresponding author. Tel.: +81 42 685 3722; fax: +81 42 685 3432.
*E-mail address:* maruyama@pharm.teikyo-u.ac.jp (K. Maruyama).

0378-5173/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2007.10.034

Table 1
Examples of microbubbles (Lindner, 2004)

| Microbubble | Manufacturer | Shell | Gas | Mean size |
|---|---|---|---|---|
| Albunex | Molecular Biosystems | Albumin | Air | 4.3 μm |
| Optison | Mallinckrodt/Amersham | Albumin | Octafluoropropane | 2–4.5 μm |
| Definity | Bristol-Myers Squibb Medical Imaging | Lipid/surfactant | Octafluoropropane | 1.1–3.3 μm |
| Imagent | Imcor | Lipid/surfactant | $N_2$/perfluorohexane vapor | 6.0 μm |
| Sonovue | Bracco Diagnostics | Lipid | Sulfur hexafluoride | 2–3 μm |
| Levovist | Schering AG | Lipid/galactose | Air | 2–4 μm |
| Cardiosphere (BP127) | Point Biomedical | PLGA polymer/albumin | Nitrogen | 4 μm |
| AI-700 | Acusphere | PLGA polymer | Perfluorocarbon | 2.2 μm |
| Sonovist | Schering AG | Cyanoacrylate polymer | Air | |
| Sonazoid | GE Healthcare | Lipid (single layer) | Perfluorocarbon | 3 μm |
| Bubble liposomes | | Lipid (bilayer) | Perfluorocarbon | 950 nm |

PLGA: polylactide-*co*-glycolide.

ery studies have been conducted using therapeutic ultrasound, which operates at frequencies of 1–3 MHz and at intensities of 0.5–2.5 W/cm$^2$ (Kim et al., 1996; Tata et al., 1997; Duvshani-Eshet and Machluf, 2005). In addition, it has been reported that the combination of therapeutic ultrasound and microbubble echo contrast agents enhances gene transfection efficiency (Greenleaf et al., 1998; Shohet et al., 2000; Taniyama et al., 2002a,b; Li et al., 2003; Chen et al., 2006; Sonoda et al., 2006; Suzuki et al., 2007a,b, in press). Using this approach, DNA is effectively and directly transferred into the cytosol. Conventional microbubbles encapsulating ultrasound contrast agents, based on protein microspheres and sugar microbubbles, are commercially available; the diameters of these bubbles are between 1 and 6 μm (Table 1) (Lindner, 2004). However, although the mean diameter of, for example, Optison microbubbles is about 2.0–4.5 μm, bubbles of up to 32 μm in diameter are present in the preparation, suggesting that Optison is too large to reach peripheral tissues. Tsunoda et al. (2005) reported that several mice died immediately after i.v. injection of Optison, without ultrasound exposure, due to lethal embolisms in vital organs. The same problem has not been reported in humans, but the possibility exists that Optison cannot pass through capillary vessels. Therefore, microbubbles should generally be smaller than red blood cells, necessitating the development of novel bubbles which are smaller than conventional microbubbles.

Liposomes have several advantages as drug, antigen and gene delivery carriers (Maruyama et al., 1990, 2004; Ishida et al., 2001; Harata et al., 2004; Yanagie et al., 2004; Hatakeyama et al., 2007; Kawamura et al., 2006; Yanagie et al., 2006). For example, their size can be easily controlled, and they can be modified to incorporate a targeting function. Using liposome technology, it was expected that these novel bubbles would be smaller than conventional microbubbles. Thus, we attempted to develop novel liposomal bubbles containing the ultrasound imaging gas, perfluoropropane. And we have successfully developed liposomal bubbles in which the lipid nanobubbles of perfluoropropane with lipids derived from liposomes were encapsulated. We called these bubbles "Bubble liposomes", and confirmed that Bubble liposomes are smaller than Optison. In addition, 1 mg of Bubble liposomes (in terms of lipid amount) injected into the tail veins of mice did not result in any deaths, indicating that these novel liposomes might be safe for use *in vivo*. Moreover, we reported that Bubble liposomes could be used as a novel non-viral gene delivery tool by combining the bubbles with ultrasound exposure (Suzuki et al., 2007a,b, in press). The present review focuses on gene delivery systems utilizing Bubble liposomes in combination with ultrasound.

## 2. Characteristics of Bubble liposomes

Bubble liposomes are polyethyleneglycol-modified liposomes (PEG-liposomes) prepared by the reverse phase evaporation method. PEG-liposomes were placed in vials supercharged with perfluoropropane gas, then sonicated in a bath sonicator. The suspension of Bubble liposomes became cloudier than the original liposome suspension (Fig. 1(a) and (b)). Observation of both Bubble liposomes and Optison under a microscope equipped with a darklite illuminator (NEPA Gene Co. Ltd., Chiba, Japan) (Fig. 1(c) and (d)) showed that Bubble liposomes are smaller than Optison. The diameter of most Bubble liposomes is less than 2 μm, and the average diameter is about 950 nm (Suzuki et al., in press). In addition, we confirmed by means of transmission electron microscopy that perfluoropropane gas was in fact trapped within the Bubble liposomes (Fig. 1(e)). Interestingly, there were nanobubbles in the lipid bilayer, suggesting that Bubble liposomes differ from conventional microbubbles, in which an echo gas is encased by a lipid monolayer (Fig. 1(f)). Kodama et al. and Klibanov et al. described microbubbles prepared from distearoylphospatidylcholine and PEG-stearate (Leong-Poi et al., 2003; Takahashi et al., 2007). At the same time, perfluoropropane was entrapped within lipid micelles composed of DSPC and DSPE-PEG (2k), forming nanobubbles. These nanobubbles were encapsulated within the reconstituted liposomes, which were approximately 1 μm in diameter rather than the 150–200 nm of the original liposomes (Suzuki et al., in press). These Bubble liposomes were too large to use to passively target tumor tissues by the enhanced permeability and retention (EPR) effect. However, we expected that Bubble liposomes could penetrate tissues deeper than conventional microbubbles by means of blood circulating in organs.

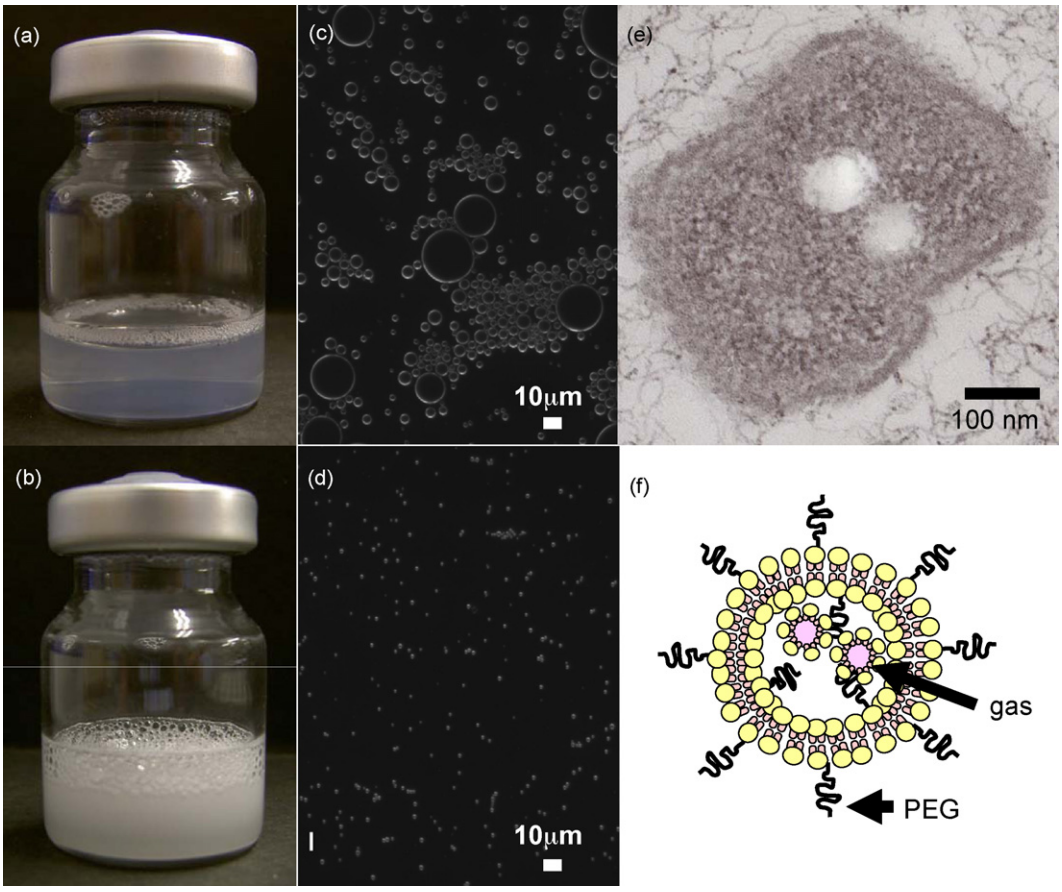


Fig. 1. Structure of Bubble liposomes. PEG-liposomes (a) were sonicated with supercharged perfluoropropane gas, generating Bubble liposomes (b). Optison (c) and Bubble liposomes (d) were observed with a microscope using a darklite illuminator. (e) Transmission electron microscopy (TEM) of Bubble liposomes. (f) Scheme showing the structure of Bubble liposomes.

To confirm the gas trapped in Bubble liposomes, we observed Bubble liposomes using ultrasound imaging (UF-750XT, Fukuda Denshi Co Ltd., Tokyo, Japan) as shown in Fig. 2(a). Echo signals were apparently enhanced in Bubble liposomes compared with conventional PEG-liposomes (Fig. 2(b) and (c)). Therefore, it is expected that Bubble liposomes can be utilized as ultrasound imaging agents, and indeed we succeeded in conducting echocardiography using Bubble liposomes (Suzuki et al., 2007b).

Conventional microbubbles can induce cavitation upon exposure to ultrasound (Tachibana and Tachibana, 1995). Cavitation supplies the energy required to deliver extracellular molecules into the cytosol (Taniyama et al., 2002a,b; Kinoshita and Hynynen, 2005a,b; Larina et al., 2005). We confirmed whether Bubble liposomes could induce cavitation by exposing Bubble liposomes to ultrasound generated using a Sonoporation Gene Transfection System (Sonopore, NEPA Gene Co. Ltd., Chiba, Japan). After ultrasound exposure, the strength of the ultrasound echo signals decreased markedly upon ultrasound imaging compared with Bubble liposomes not exposed to ultrasound (Fig. 2(d) and (e)). This result indicated that cavitation was effectively induced by the combination of Bubble liposomes and ultrasound exposure, suggesting that Bubble liposomes could be utilized as a gene delivery tool.

## 3. *In vitro* gene delivery with Bubble liposomes

Gene transfection by means of microbubbles has been previously reported (Taniyama et al., 2002a,b; Li et al., 2003; Chen et al., 2006; Sonoda et al., 2006). Li et al. (2003) compared the gene transfection efficiency of Albunex, Optison and Levovist into skeletal muscle cells and found that the efficiency of Optison was the highest. In addition, Machluf et al. reported enhanced transfection efficiency into kidney cells using a combination of Optison and ultrasound (Duvshani-Eshet et al., 2006). Under these conditions, cell surfaces became rougher, and showed depressions with diameters of 100–300 nm. Also using Optison in combination with ultrasound, Taniyama et al. (2002a,b) investigated transfection into vascular endothelial cells and smooth muscle cells. They reported small holes in the cells immediately following ultrasound exposure with Optison, but the holes disappeared after 24 h. Most gene delivery studies using microbubbles utilize commercially available microbubbles such as Optison; there are few reports regarding gene transfection with liposomal bubbles. Therefore, we examined the transduction of naked plasmid DNA into COS-7 cells by Bubble liposomes and/or ultrasound. Levels of luciferase expression were much higher after ultrasound treatment in the presence of Bubble liposomes compared to in their absence (Fig. 3). Interestingly, gene expres-

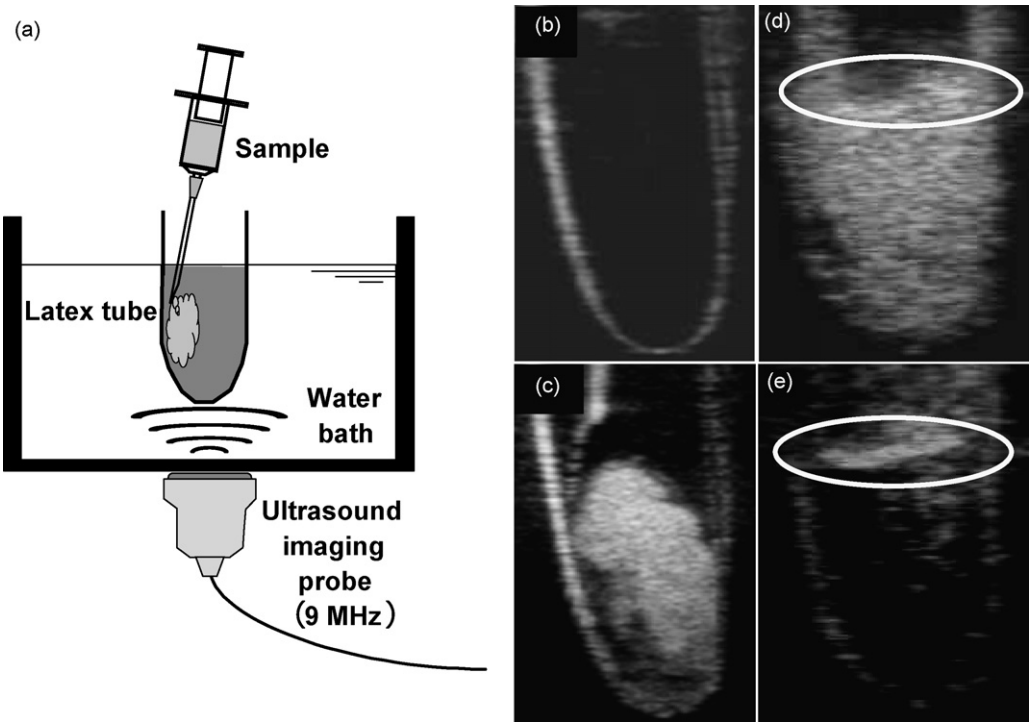


Fig. 2. Ultrasonography of Bubble liposomes. The ultrasonography method for observing Bubble liposomes is shown in (a). PEG-liposomes (b) or Bubble liposomes (c) were injected into a PBS-filled latex tube in a water bath and the samples were observed with ultrasonography. To confirm the disruption of the Bubble liposomes, the Bubble liposomes were observed with ultrasonography before (d) and after (e) ultrasound exposure (2 MHz, 2.5 W/cm$^2$, 10 s).

sion efficiency was very high following ultrasound exposures as short as 10 s. We also confirmed that Bubble liposomes could effectively deliver plasmid DNA into cells even after 1 s of ultrasound exposure (Suzuki et al., in press). Thus, Bubble liposomes are novel gene delivery agents that can almost instantaneously transfect extracellular plasmid DNA into cells.

Heat and jet streams are generally induced by cavitation, which might damage cells. We therefore examined the effects of ultrasound on cells both in the presence and absence of Bubble liposomes (Fig. 4). Cell viability was measured using the MTT assay (Mosmann, 1983) at 1 day following treatment. Ultrasound did not damage COS-7 cells in the absence of Bubble liposomes, and only slightly affected the cells even when

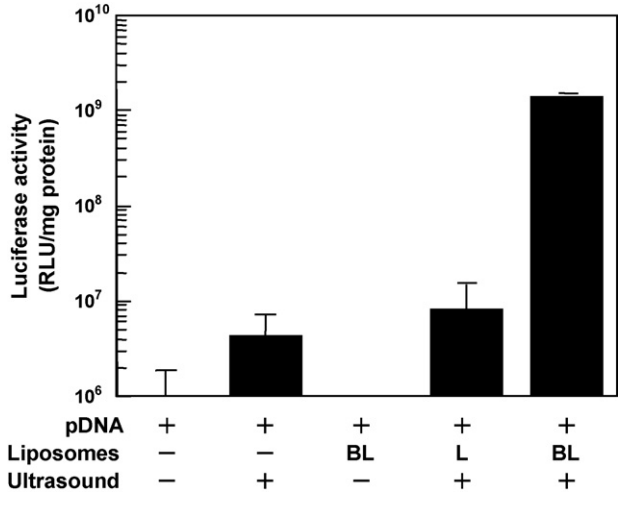


Fig. 3. Luciferase expression in COS-7 cells transfected with Bubble liposomes following ultrasound exposure. COS-7 cells ($1 \times 10^5$ cells/500 μL per tube) were mixed with pCMV-Luc (5 μg) and Bubble liposomes (60 μg). The cell mixture was exposed to ultrasound (frequency: 2 MHz; duty: 50%; burst rate: 2 Hz; intensity: 2.5 W/cm$^2$; time: 10 s). The cells were washed and cultured for 2 days, then luciferase activity was measured. Each data point represents the mean ± S.D. ($n = 3$). BL: Bubble liposomes; L: PEG-liposomes.

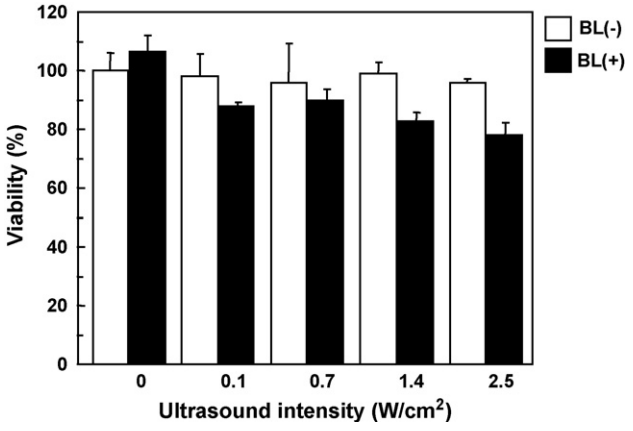


Fig. 4. Viability of COS-7 cells exposed to ultrasound and Bubble liposomes. COS-7 cells were exposed to each ultrasound intensity with or without Bubble liposomes, then the cells were cultured for 24 h and their viability was assessed using the MTT assay. Briefly, MTT (5 mg/mL, 10 μL) was added to each well and the cells were incubated at 37 °C for 4 h. The formazan product was dissolved in 100 μL of 10% sodium dodecyl sulfate (SDS) containing 15 mM HCl. Color intensity was measured using a microplate reader at test and reference wavelengths of 595 and 655 nm, respectively. Each data point represents the mean ± S.D. ($n = 3$). BL: Bubble liposomes.

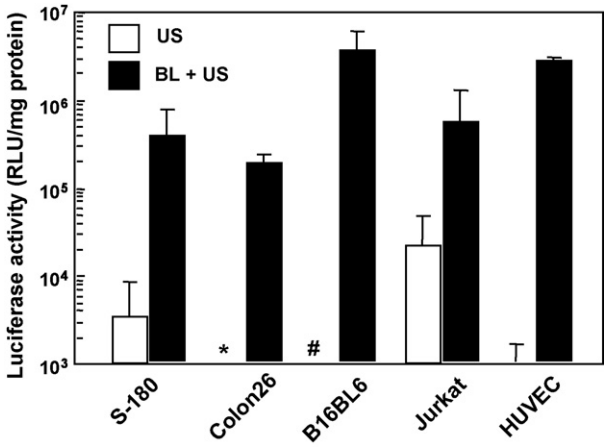


Fig. 5. Luciferase expression in various cell types transfected using Bubble liposomes and ultrasound. Cells ($1 \times 10^5$ cells/500 μL per tube) were mixed with pCMV-Luc (5 μg) and Bubble liposomes (60 μg). A portion of the cell mixture was exposed to ultrasound (frequency: 2 MHz; duty: 50%; burst rate: 2 Hz; intensity: 2.5 W/cm$^2$; time: 10 s). Exposed and non-exposed cells were washed and cultured for 2 days, then luciferase activity was measured. Each data point represents the mean ± S.D. ($n = 3$). BL: Bubble liposomes; US: ultrasound. *<$10^3$ RLU/mg protein, #<$10^6$ RLU/mg protein.

the amount of ultrasound administered was sufficient to induce cavitation of the Bubble liposomes. We also confirmed that the cavitation induced with Bubble liposomes did not damage plasmid DNA (Suzuki et al., 2007a). In addition, we assessed the feasibility of delivering genes into various types of cells such as mouse tumor cells, a human T cell line and human vessel endothelial cells. Fig. 5 shows that Bubble liposomes combined with ultrasound more effectively delivered plasmid DNA into all these cell types than did ultrasound alone. *In vivo* gene delivery with Bubble liposomes requires the delivery of plasmid DNA into cells in the presence of serum. Thus, we examined the effect of serum on gene delivery with Bubble liposomes (Fig. 6) and showed that gene expression following treatment

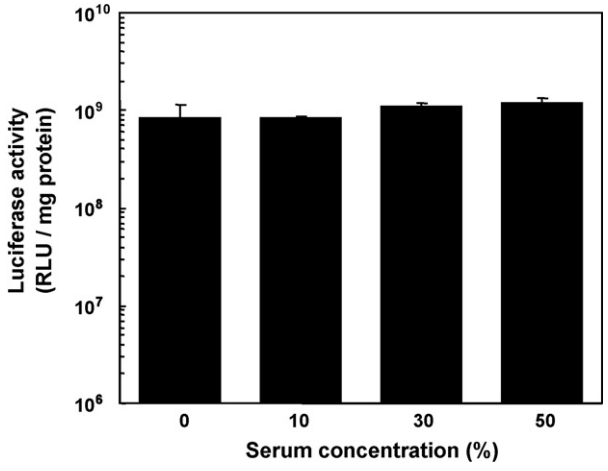


Fig. 6. Effect of serum on transfection efficiency of Bubble liposomes. COS-7 cells ($1 \times 10^5$ cells/500 μL) mixed with pCMV-Luc (0.25 μg) and Bubble liposomes (60 μg) were exposed to ultrasound (frequency: 2 MHz; duty: 50%; burst rate: 2 Hz; intensity: 2.5 W/cm$^2$; time: 10 s) in the absence or presence of serum (0, 10, 30 and 50%). The cells were washed and cultured for 2 days, then luciferase activity was measured. Data are shown as means ± S.D. ($n = 3$).

with Bubble liposomes was not affected even in the presence of serum.

## 4. *In vivo* gene delivery with Bubble liposomes

To further assess the potential of *in vivo* gene delivery using Bubble liposomes and ultrasound, the delivery of plasmid DNA into the femoral artery of mice was studied (Fig. 7). Bubble liposomes and plasmid DNA were injected into the femoral artery as ultrasound was transdermally applied downstream of the injection site. Additionally, using Lipofectamine 2000, gene expression efficiency using Bubble liposomes was compared to conventional lipofection (Fig. 7(a)). The combination of Bubble liposomes and ultrasound exposure was more effective than conventional lipofection in inducing gene expression in the femoral artery. Moreover, gene expression with Bubble liposomes and ultrasound exposure was observed only in the area exposed to ultrasound (Fig. 7(b)). These results suggest that Bubble liposomes quickly deliver plasmid DNA into the artery by cavitation even after only short contact between the Bubble liposomes and endothelial cells by means of the blood stream. It had been thought that plasmid DNA was delivered into endothelial cells lining the femoral artery because it was physiologically difficult for plasmid DNA and Bubble liposomes to extravasate from a normal artery. Mizuguchi et al. (1998) reported effective cancer gene therapy by locally introducing cytokine genes by gene delivery into arteries leading to the tumor, or delivery into arteries in the tumor tissue. Therefore, we anticipated that Bubble liposomes could be used to deliver genes into arteries in tumor tissue. In the footpad tumor bearing mouse model, Bubble liposomes and luciferase-encoding plasmid DNA were injected into arteries leading to the tumor while the tumor tissue was transdermally exposed to ultrasound. After 2 days, luciferase expression was observed only in the tumor tissue. In addition, the gene expression efficiency of this method was higher than that of the conventional lipofection method using Lipofectamine 2000 (Suzuki et al., in press). We therefore believe that this method has significant advantages for tumor gene therapy using non-viral vectors.

In another study, we compared transfection efficiency using Bubble liposomes and Optison. Green fluorescent protein (GFP)-encoding plasmid DNA was delivered into cultured rabbit corneal epithelial cells using ultrasound exposure in the presence of Bubble liposomes or Optison. Gene expression efficiency with Bubble liposomes vs. Optison was about 25 and 10%, respectively. In an *in vivo* study, GFP-encoding plasmid DNA was injected into rat subconjunctiva tissue using bubbles and ultrasound exposure. Bubble liposomes more effectively induced gene expression in the tissue compared with Optison (Yamashita et al., in press). These results show that Bubble liposomes are more effective than conventional microbubbles at delivering genes for ophthalmologic treatments.

It is believed that gene expression using Bubble liposomes is transient, and that in order to maintain extended gene expression it is necessary to inject the liposomes repeatedly. However, there are reports of accelerated blood clearance (ABC) of PEGylated liposomes after repeat injections due to the enhanced accumu-

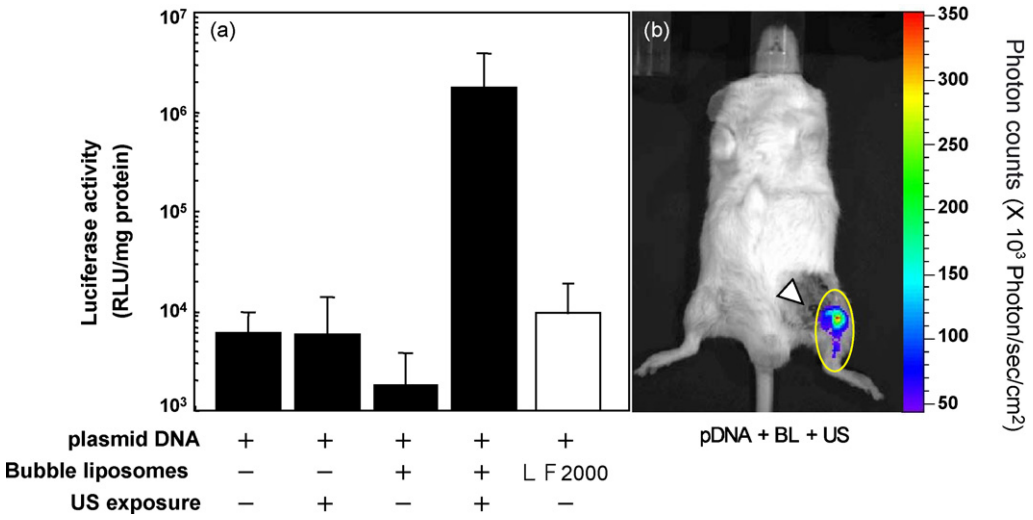


Fig. 7. Gene delivery to mouse femoral artery using Bubble liposomes. Each sample containing plasmid DNA (10 μg) was injected into the mouse femoral artery while ultrasound (frequency: 1 MHz; duty: 50%; burst rate: 2 Hz; intensity: 1 W/cm$^2$; time: 2 min) was applied downstream of the injection site. (a) Luciferase expression in the area of the femoral artery exposed to ultrasound 2 days after transfection. Data are shown as mean ± S.D. ($n$ = 5). (b) *In vivo* luciferase imaging 2 days after transfection of mice treated with plasmid DNA, Bubble liposomes and ultrasound exposure. The photon counts are indicated by the pseudo-color scales. The arrowhead shows the injection site and the circle shows the ultrasound exposure area. BL: Bubble liposomes; LF 2000: Lipofectamine 2000; US: ultrasound.

lation of PEGylated liposomes in the liver and thus its rapid clearance from blood circulation. Ishida et al. reported that IgM secreted in response to the first dose is involved in this clearance response (Ishida et al., 2006; Wang et al., 2007). In *in vivo* gene delivery using Bubble liposomes and ultrasound exposure, ultrasound is normally applied at the same time as the Bubble liposomes are injected, or soon after the injection. Bubble liposomes would therefore immediately deliver plasmid DNA into the cells upon ultrasound exposure *in vivo*. Therefore, it is thought that transfection efficiency will not be affected by the ABC phenomenon even in the presence of IgM against PEGylated liposomes after multiple injections, and that repeat injections of Bubble liposomes will not reduce the efficiency of gene delivery *in vivo*.

## 5. Conclusions

We prepared Bubble liposomes containing submicron-sized bubbles using a novel methodology. These novel liposomes induced cavitation upon exposure to ultrasound, which resulted in plasmid DNA transduction into cells both *in vitro* and *in vivo*. Gene delivery was complete within a very short period of time. Therefore, Bubble liposomes could effectively deliver plasmid DNA into an artery *in vivo*, even in the blood stream. In this study, a mixture of plasmid DNA and Bubble liposomes was injected into the femoral artery. Interestingly, the area of gene uptake using Bubble liposomes was limited to the area exposed to ultrasound, indicating that gene expression depends on the area exposed to ultrasound. Therefore, gene targeting should be easily achieved using this gene delivery system simply by changing the site of ultrasound exposure. Our future goal is to establish non-invasive and tissue-specific gene delivery with Bubble liposomes after systemic injection.

## Acknowledgements

We are grateful to Dr. Katsuro Tachibana (Department of Anatomy, School of Medicine, Fukuoka University) for technical advice regarding the induction of cavitation with ultrasound, to Ms. Kumiko Tanaka, Ms. Kaori Sawamura, Mr. Yusuke Oda, Mr. Eisuke Namai, Mr. Yuta Saito, Ms. Naoko Yamashita and Mr. Yosuke Suyama (Department of Biopharmaceutics, School of Pharmaceutical Sciences, Teikyo University) for excellent technical assistance, to Dr. Yoshio Nakano and Dr. Akinori Suginaka (NOF Corporation) for technical advice regarding lipids and for providing the lipids, and to Mr. Yasuhiko Hayakawa, Mr. Takahiro Yamauchi and Mr. Kosho Suzuki (NEPA Gene Co. Ltd.) for technical advice regarding ultrasound exposure.

This study was supported by an Industrial Technology Research grant (04A05010) in 2004 from the New Energy and Industrial Technology Development Organization (NEDO) of Japan, a Grant-in-Aid for the Encouragement of Young Scientists (160700392), an Exploratory Research grant (16650126) from the Japan Society for the Promotion of Science, and a Research on Advanced Medical Technology in Health and Labour Sciences Research grant (17070301) from the Ministry of Health, Labour and Welfare.

## References

Chen, S., Ding, J.H., Bekeredjian, R., Yang, B.Z., Shohet, R.V., Johnston, S.A., Hohmeier, H.E., Newgard, C.B., Grayburn, P.A., 2006. Efficient gene delivery to pancreatic islets with ultrasonic microbubble destruction technology. Proc. Natl. Acad. Sci. U.S.A. 103, 8469–8474.

Duvshani-Eshet, M., Machluf, M., 2005. Therapeutic ultrasound optimization for gene delivery: a key factor achieving nuclear DNA localization. J. Control. Rel. 108, 513–528.

Duvshani-Eshet, M., Adam, D., Machluf, M., 2006. The effects of albumin-coated microbubbles in DNA delivery mediated by therapeutic ultrasound. J. Control. Rel. 112, 156–166.

Fechheimer, M., Boylan, J.F., Parker, S., Sisken, J.E., Patel, G.L., Zimmer, S.G., 1987. Transfection of mammalian cells with plasmid DNA by scrape loading and sonication loading. Proc. Natl. Acad. Sci. U.S.A. 84, 8463–8467.

Greenleaf, W.J., Bolander, M.E., Sarkar, G., Goldring, M.B., Greenleaf, J.F., 1998. Artificial cavitation nuclei significantly enhance acoustically induced cell transfection. Ultrasound Med. Biol. 24, 587–595.

Guzman, H.R., McNamara, A.J., Nguyen, D.X., Prausnitz, M.R., 2003. Bioeffects caused by changes in acoustic cavitation bubble density and cell concentration: a unified explanation based on cell-to-bubble ratio and blast radius. Ultrasound Med. Biol. 29, 1211–1222.

Harata, M., Soda, Y., Tani, K., Ooi, J., Takizawa, T., Chen, M., Bai, Y., Izawa, K., Kobayashi, S., Tomonari, A., Nagamura, F., Takahashi, S., Uchimaru, K., Iseki, T., Tsuji, T., Takahashi, T.A., Sugita, K., Nakazawa, S., Tojo, A., Maruyama, K., Asano, S., 2004. CD19-targeting liposomes containing imatinib efficiently kill Philadelphia chromosome-positive acute lymphoblastic leukemia cells. Blood 104, 1442–1449.

Hatakeyama, H., Akita, H., Kogure, K., Oishi, M., Nagasaki, Y., Kihira, Y., Ueno, M., Kobayashi, H., Kikuchi, H., Harashima, H., 2007. Development of a novel systemic gene delivery system for cancer therapy with a tumor-specific cleavable PEG-lipid. Gene Ther. 342, 194–200.

Ishida, O., Maruyama, K., Tanahashi, H., Iwatsuru, M., Sasaki, K., Eriguchi, M., Yanagie, H., 2001. Liposomes bearing polyethyleneglycol-coupled transferrin with intracellular targeting property to the solid tumors in vivo. Pharm. Res. 18, 1042–1048.

Ishida, T., Ichihara, M., Wang, X., Kiwada, H., 2006. Spleen plays an important role in the induction of accelerated blood clearance of PEGylated liposomes. J. Control. Rel. 115, 243–250.

Joersbo, M., Brunstedt, J., 1990. Protein synthesis stimulated in sonicated sugar beet cells and protoplasts. Ultrasound Med. Biol. 16, 719–724.

Kawamura, K., Kadowaki, N., Suzuki, R., Udagawa, S., Kasaoka, S., Utoguchi, N., Kitawaki, T., Sugimoto, N., Okada, N., Maruyama, K., Uchiyama, T., 2006. Dendritic cells that endocytosed antigen-containing IgG-liposomes elicit effective antitumor immunity. J. Immunother. 29, 165–174.

Kim, H.J., Greenleaf, J.F., Kinnick, R.R., Bronk, J.T., Bolander, M.E., 1996. Ultrasound-mediated transfection of mammalian cells. Hum. Gene Ther. 7, 1339–1346.

Kinoshita, M., Hynynen, K., 2005a. Intracellular delivery of Bak BH3 peptide by microbubble-enhanced ultrasound. Pharm. Res. 22, 716–720.

Kinoshita, M., Hynynen, K., 2005b. A novel method for the intracellular delivery of siRNA using microbubble-enhanced focused ultrasound. Biochem. Biophys. Res. Commun. 335, 393–399.

Larina, I.V., Evers, B.M., Esenaliev, R.O., 2005. Optimal drug and gene delivery in cancer cells by ultrasound-induced cavitation. Anticancer Res. 25, 149–156.

Leong-Poi, H., Christiansen, J., Klibanov, A.L., Kaul, S., Lindner, J.R., 2003. Noninvasive assessment of angiogenesis by ultrasound and microbubbles targeted to alpha(v)-integrins. Circulation 107, 455–460.

Li, T., Tachibana, K., Kuroki, M., Kuroki, M., 2003. Gene transfer with echo-enhanced contrast agents: comparison between Albunex, Optison, and Levovist in mice—initial results. Radiology 229, 423–428.

Lindner, J.R., 2004. Microbubbles in medical imaging: current applications and future directions. Nat. Rev. Drug Discov. 3, 527–532.

Maruyama, K., Kennel, S.J., Huang, L., 1990. Lipid composition is important for highly efficient target binding and retention of immunoliposomes. Proc. Natl. Acad. Sci. U.S.A. 87, 5744–5748.

Maruyama, K., Ishida, O., Kasaoka, S., Takizawa, T., Utoguchi, N., Shinohara, A., Chiba, M., Kobayashi, H., Eriguchi, M., Yanagie, H., 2004. Intracellular targeting of sodium mercaptoundecahydrododecaborate (BSH) to solid tumors by transferrin-PEG liposomes, for boron neutron-capture therapy (BNCT). J. Control. Rel. 98, 195–207.

Miller, M.W., Miller, D.L., Brayman, A.A., 1996. A review of in vitro bioeffects of inertial ultrasonic cavitation from a mechanistic perspective. Ultrasound Med. Biol. 22, 1131–1154.

Miller, D.L., Pislaru, S.V., Greenleaf, J.E., 2002. Sonoporation: mechanical DNA delivery by ultrasonic cavitation. Somat. Cell Mol. Genet. 27, 115–134.

Mizuguchi, H., Nakagawa, T., Toyosawa, S., Nakanishi, M., Imazu, S., Nakanishi, T., Tsutsumi, Y., Nakagawa, S., Hayakawa, T., Ijuhin, N., Mayumi, T., 1998. Tumor necrosis factor alpha-mediated tumor regression by the in vivo transfer of genes into the artery that leads to tumors. Cancer Res. 58, 5725–5730.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63.

Shohet, R.V., Chen, S., Zhou, Y.T., Wang, Z., Meidell, R.S., Unger, R.H., Grayburn, P.A., 2000. Echocardiographic destruction of albumin microbubbles directs gene delivery to the myocardium. Circulation 101, 2554–2556.

Sonoda, S., Tachibana, K., Uchino, E., Okubo, A., Yamamoto, M., Sakoda, K., Hisatomi, T., Sonoda, K.H., Negishi, Y., Izumi, Y., Takao, S., Sakamoto, T., 2006. Gene transfer to corneal epithelium and keratocytes mediated by ultrasound with microbubbles. Invest. Ophthalmol. Vis. Sci. 47, 558–564.

Suzuki, R., Takizawa, T., Negishi, Y., Hagisawa, K., Tanaka, K., Sawamura, K., Utoguchi, N., Nishioka, T., Maruyama, K., 2007a. Gene delivery by combination of novel liposomal bubbles with perfluoropropane and ultrasound. J. Control. Rel. 117, 130–136.

Suzuki, R., Takizawa, T., Negishi, Y., Utoguchi, N., Maruyama, K., 2007b. Effective gene delivery with liposomal bubbles and ultrasound as novel nonviral system. J. Drug Target. 15, 531–537.

Suzuki, R., Takizawa, T., Negishi, Y., Utoguchi, N., Sawamura, K., Tanaka, K., Namai, E., Oda, Y., Matsumura, Y., Maruyama, K., in press. Tumor specific ultrasound enhanced gene transfer in vivo with novel liposomal bubbles. J. Control. Rel.

Tachibana, K., Tachibana, S., 1995. Albumin microbubble echo-contrast material as an enhancer for ultrasound accelerated thrombolysis. Circulation 92, 1148–1150.

Takahashi, M., Kido, K., Aoi, A., Furukawa, H., Ono, M., Kodama, T., 2007. Spinal gene transfer using ultrasound and microbubbles. J. Control. Rel. 117, 267–272.

Taniyama, Y., Tachibana, K., Hiraoka, K., Aoki, M., Yamamoto, S., Matsumoto, K., Nakamura, T., Ogihara, T., Kaneda, Y., Morishita, R., 2002a. Development of safe and efficient novel nonviral gene transfer using ultrasound: enhancement of transfection efficiency of naked plasmid DNA in skeletal muscle. Gene Ther. 9, 372–380.

Taniyama, Y., Tachibana, K., Hiraoka, K., Namba, T., Yamasaki, K., Hashiya, N., Aoki, M., Ogihara, T., Yasufumi, K., Morishita, R., 2002b. Local delivery of plasmid DNA into rat carotid artery using ultrasound. Circulation 105, 1233–1239.

Tata, D.B., Dunn, F., Tindall, D.J., 1997. Selective clinical ultrasound signals mediate differential gene transfer and expression in two human prostate cancer cell lines: LnCap and PC-3. Biochem. Biophys. Res. Commun. 234, 64–67.

Tsunoda, S., Mazda, O., Oda, Y., Iida, Y., Akabame, S., Kishida, T., Shin-Ya, M., Asada, H., Gojo, S., Imanishi, J., Matsubara, H., Yoshikawa, T., 2005. Sonoporation using microbubble BR14 promotes pDNA/siRNA transduction to murine heart. Biochem. Biophys. Res. Commun. 336, 118–127.

Wang, X., Ishida, T., Kiwada, H., 2007. Anti-PEG IgM elicited by injection of liposomes is involved in the enhanced blood clearance of a subsequent dose of PEGylated liposomes. J. Control. Rel. 119, 236–244.

Wei, W., Zheng-Zhong, B., Yong-Jie, W., Qing-Wu, Z., Ya-Lin, M., 2004. Bioeffects of low-frequency ultrasonic gene delivery and safety on cell membrane permeability control. J. Ultrasound Med. 23, 1569–1582.

Yamashita, T., Sonoda, S., Suzuki, R., Arimura, K., Tachibana, K., Maruyama, K., Sakamoto, T., in press. A novel bubble liposome and ultrasound-mediated gene transfer to ocular surface: RC:1 cells in vitro and conjunctiva in vivo. Exp. Eye Res.

Yanagie, H., Ogura, K., Takagi, K., Maruyama, K., Matsumoto, T., Sakurai, Y., Skvarc, J., Illic, R., Kuhne, G., Hisa, T., Yoshizaki, I., Kono, K., Furuya, Y., Sugiyama, H., Kobayashi, H., Ono, K., Nakagawa, K., Eriguchi, M., 2004. Accumulation of boron compounds to tumor with polyethylene-glycol binding liposome by using neutron capture autoradiography. Appl. Radiat. Isot. 61, 639–646.

Yanagie, H., Maruyama, K., Takizawa, T., Ishida, O., Ogura, K., Matsumoto, T., Sakurai, Y., Kobayashi, T., Shinohara, A., Rant, J., Skvarc, J., Ilic, R., Kuhne, G., Chiba, M., Furuya, Y., Sugiyama, H., Hisa, T., Ono, K., Kobayashi, H., Eriguchi, M., 2006. Application of boron-entrapped stealth liposomes to inhibition of growth of tumour cells in the in vivo boron neutron-capture therapy model. Biomed. Pharmacother. 60, 43–50.