**Introduction:** Osteosarcoma is the most frequent primary malignant bone tumor. The identification of effective neoadjuvant chemotherapy in combination with surgery for osteosarcoma patients has led to a significant improvement in patient outcome over the last several decades. However, there are still a certain number of non-responders who do not benefit from these improvements. It is therefore believed that alternative strategies for the treatment of osteosarcoma patient are necessary. Further improvements in the management of osteosarcoma may rely on a more individualized treatment strategy, as well as on the introduction of new drug. Recently, it was reported that microbubbles, which are ultrasound (US) contrast agents, improved the drug and gene delivery efficiency by cavitation with US exposure. However, microbubbles had problems with stability and targeting ability. To solve these problems, we focused on liposomes that had many advantages such as being stable and safe in vivo and easily modifying targeting ligand. We succeeded in preparing the liposomes(Bubble liposomes(BL)) entrapping perfluoropropane gas which was utilized for contrast enhancement in ultrasonography. In this study, we used doxorubicin(DOX) and designed the new drug delivery system which used BL enhanced US exposure and experiment an effect for murine osteosarcoma cell.

**Materials and Methods:** Liposomes composed of 1,2-distearoyl-sn-glycero-phosphatidylcholine (DSPC) and 1,2-distearoyl-sn-glycero-3-phosphatidyl-ethanolamine-methoxy-polyethyleneglycol (DSPE-PEG (2k)-OMe) (94:6 (m/m)) were prepared by reverse phase evaporation. Physiological saline was added into the lipid solution. After that, the mixture was sonicated and evaporated at 65°C. Bubble liposomes were prepared from liposomes and perfluoropropane gas. In brief, 5 mL sterilized vials containing 2 mL of liposome suspension were filled with perfluoropropane gas, capped and then pressured with 7.5 mL of perfluoropropane gas. The vial was placed in a bath-type sonicator for 5 min to form Bubble liposomes.

1x10³ cells for LM8 cells (murine osteosarcoma cell) in phosphate-buffered saline were injected subcutaneously in the back space of C3H female mice. Experiments were initiated approximately 7-10 days after injection when tumors reached the size of 5-7mm. This day was set as day 0, mice were injected DOX and Bubble liposome preparations administered i.v. via the tail vein. Immediately after injection, in the groups of needing sonically treatment, ultrasound was generated. (Evaluation of tumor volume) On day 0,2,4, mice were treated with different administration modalities. After treatment, growth of the tumors was monitored every 2 days by measuring tumor volume and evaluated by normalized tumor volume. (Experimental pulmonary metastasis assay) On day 0,2,4, mice were treated with different administration modalities. The mice were humanely euthanized on day 21. The lung was collected. Prepared sections were stained with hematoxylin-eosin (H&E). Visible colonies on preparation were counted. (Evaluation of side effect) In this study, mice which bearing LM8 tumors reached the size of 10mm were used. Mice were treated with different administration modalities. After 9 days, for measurements of red blood cell (RBC), white blood cell (WBC), hemoglobin (Hb), platelet (Plt), glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), mice were anesthetized, 800μL samples of blood were obtained from the inferior vena cava.

**Results:** (Evaluation of tumor volume) Measurement of tumor volume in mice bearing LM8 tumors demonstrates significant suppression of tumor growth by DOX(1mg/kg)+BL+US, compared with the control group. (Experimental pulmonary metastasis assay) The number of tumor colonies observed macroscopically in the whole lung is that treatment with DOX(1mg/kg)+BL+US can significantly suppress pulmonary metastasis. (Evaluation of side effect) Mice receiving DOX(1mg/kg)+BL+US showed no serious side effects, with no significant differences from the control group.

**Discussion:** We demonstrated that Dox with Bubble liposome and Ultrasound exposure showed anti-tumor activity in vivo in osteosarcoma cell lines. Our data suggest that DOX with Bubble liposome and Ultrasound exposure may be an option for the treatment of osteosarcoma that has poor prognosis.

**References:**
1.Ryo Suzuki, Gene delivery by combination of novel liposomal bubbles with perfluoropropane and ultrasound.