Formulation of Stabilized Ultrasound Visible Nanoparticles for Thermal Sensitizer Delivery

R.H. Perera1, H. Wu1, P. Kota2 and A.A. Exner1

1Case Center for Imaging Research, Department of Radiology, 2Department of Biomedical Engineering, Case Western Reserve University, 11100 Euclid Ave, Cleveland, OH, 44106, USA
rhp26@case.edu

ABSTRACT SUMMARY
Stabilized ultrasound-sensitive theranostic nanoparticles (cross-linked poly(ethylene glycol) (PEG) nanobubbles or CL-PEG-NB) were developed by incorporating a thermal sensitizer, Pluronic, in the shell of lipid, perfluorocarbon filled bubbles stabilized with N,N-Diethylacrylamide1,2. The bubbles were less than 150 nm in diameter yet retained high echogenicity. The CL-PEG-NB were utilized to deliver Pluronic to colorectal cancer undergoing hyperthermia treatment. Due to improved uptake of payload via sonoporation of the cell membrane, the sensitivity of cells was enhanced to low grade hyperthermia by CL-PEG-NB compared to delivery of unencapsulated Pluronic and toxicity was increased.

INTRODUCTION
Ultrasound driven contrast agents have achieved much attention due to their ability to form acoustically induced pores in the cell membrane, which enhances the cellular drug uptake at the site of action3. Our prior studies showed that nanobubbles formulated by assimilating Pluronic into lipid shelled bubbles showed reduced size, increased stability and echogenicity, by interacting with the lipid membrane4. We also showed that the nanobubbles can serve as an effective delivery method for Pluronic, which has been shown to enhance tumor thermal ablation treatment efficacy both in vitro and in vivo5.

In the current approach, we focused on the formulation and characterization of stabilized Pluronic nanobubbles by introducing PEG and by adding interpenetrating cross-linking biodegradable polymer N, N-diethyl acrylamide (NNDEA) and N,N-bis(acryroyl) cystamine (BAC)6, to achieve longer circulation times for enhanced thermosensitizer delivery. Here we study the efficiency of cross-linked nanobubbles (CL-PEG-NB) as an ultrasound mediated theranostic sensitizer delivery system.

EXPERIMENTAL METHODS
To formulate Pluronic nanobubbles, lipids DPPC (1, 2-Dipalmitoyl-sn-Glycero-3-Phospho choline), DPPE (1, 2-dipalmitoyl-sn-glycero-3-phosphoethanolamine), DPPA (1,2 Dipalmitoyl-sn-Glycero-3-Phosphate), and mPEG-DSPE (1,2-Distearyl-phosphatidylethanol amine-methyl-poly ethylene glycol conjugate-2000 with a 4:1:1:1 mass ratio were dissolved in chloroform. The solvent was then removed by evaporation, which resulted in the formation of a lipid film. The film was hydrated by adding 1ml of 0.6 mg/ml Pluronic solution in 0.5% Irgacure 2959 (in PBS) in the presence of glycerol (50ml) at 75 °C for 30 min. Next, NNDEA and BAC (2:1 weight ratio) were added and air was removed from the sealed vials and replaced with octafluoropropane until the pressure inside the vial was equalized. Finally, the vial was shaken on a VialMix shaker for 45s and the bubble vials were irradiated at 254 nm using a UV lamp for 30 min.

Nanobubbles were characterized by dynamic light scattering (DLS) and gated Stimulated Emission Depletion (STED) imaging. The qNano platform from Izon Science was used to analyze the absolute diameter and the concentration of CL-PEG-NB. The qNano was equipped with the NP150 nanopore for the particles in the range of 70-200 nm. The optimized parameters of the qNano were adjusted (45 mm stretch, 0.7 V current) to obtain a stable baseline current with PBS buffer. Then, 40 μl of diluted CL-PEG-NB solution was loaded to the upper fluid cell and pressure (0.5kPa) applied to obtain the desired 500 particle count. The size and concentration distribution plots were created by Izon Control Suite software, version 2.1.

In vitro bubble stability was analyzed by imaging the nanobubbles using a Toshiba clinical ultrasound imaging system. The efficiency of Pluronic delivery was studied in vitro by immersing human colorectal adenocarcinoma (LS-174T) cells with nanobubbles (400 μl of 1:2 times diluted bubbles) with or without application of therapeutic US (3 MHz at 2W/cm2 power density and 20% duty cycle for 1 min) and heat (43°C for 20 minutes). Cell viability was measured with the WST-1 assay using TECAN plate reader.

Two-tailed unpaired Student’s t-test with unequal variants was used to determine the significance of the outcome where applicable. Data analysis was performed with Microsoft Excel.

RESULTS AND DISCUSSION
DLS and qNano measurements showed particle diameters of 74 ± 6 nm and 90 ± 3 nm, respectively. The size range is appropriate for imaging and delivery application via the enhanced permeation and retention (EPR) effect. STED confocal images confirm the size and spherical shape of CL-PEG-NB (Fig 1A). Concentration of CL-PEG-NB obtained from qNano was 2 x 10^{12} particles per ml.
The data obtained from in vitro bubble echogenicity studies showed a 38% lower decay rate of CL-PEG-NB compared to the decay rate of the uncross-linked PEG-NB (-0.104 dB/min compared to -0.144 dB/min) (Fig. 2A) over 1h.

In vitro ultrasound assisted bubble delivery studies showed a significant reduction (P<0.001) in cell viability when cells were treated with CL-PEG-NB in combination with ultrasound and heat (16 ± 7%), compared to nanobubbles without ultrasound and heat treatments (104 ± 17%), and free Pluronic with ultrasound and heat (35 ± 9%), indicating the ultrasound triggered release of sensitizer (Fig 2B).

CONCLUSION
Ultrasound-sensitive CL-PEG-NB can be used to deliver Pluronic to the targeted site. CL-PEG-NB showed enhanced ultrasound signal and low ultrasound decay rate compared to uncross-linked PEG-NB. In vitro studies confirmed successful delivery of Pluronic from CL-PEG-NB with the aid of ultrasound and enhanced uptake by cells due to sonoporation of the cell membrane, which lead to an improvement of tumor hyperthermia treatment.

REFERENCES

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