A study of the effect of PEG-40 surfactant concentration on the stability of microbubbles post-injection through various needle sizes and its ultrasound imaging performance

Das, Dhiman; Villanueva, Kathlee K. A.; Pramanik, Manojit

2019

Das, D., Villanueva, K. K. A., & Pramanik, M. (2019). A study of the effect of PEG-40 surfactant concentration on the stability of microbubbles post-injection through various needle sizes and its ultrasound imaging performance. *Proceedings of SPIE - Progress in Biomedical Optics and Imaging 2019*, 10878, 1087834-. doi:10.1117/12.2508307

<https://hdl.handle.net/10356/142262>

<https://doi.org/10.1117/12.2508307>

Copyright 2019 Society of Photo-Optical Instrumentation Engineers (SPIE). One print or electronic copy may be made for personal use only. Systematic reproduction and distribution, duplication of any material in this paper for a fee or for commercial purposes, or modification of the content of the paper are prohibited.

Downloaded on 26 Apr 2023 23:25:22 SGT

A study of the effect of PEG-40 surfactant concentration on the stability of microbubbles post-injection through various needle sizes and its ultrasound imaging performance

Dhiman Das, Kathleen K.A Villanueva, and Manojit Pramanik*

School of Chemical and Biomedical Engineering, Nanyang Technological University, 62 Nanyang Drive, Singapore

ABSTRACT

Microbubbles stabilized by surfactant shells have been established as ultrasound contrast agents for the past several decades. The microbubbles often get destroyed as these are delivered to the region of interest using catheters with different needle sizes. Optimizing the concentration of the surfactants on its shell is crucial for minimizing microbubble destruction. In terms of shell material for this work; polyoxyethylene glycol 40 (PEG-40) stearate which is a non-ionic surfactant, polypropylene glycol and glycerol were used to stabilize the microbubbles with a nitrogen gas core. Presence of surfactants greatly influence the size and stability of the microbubbles and thus four different surfactant concentrations (2, 5, 10 and 15%) of PEG-40 and two different polypropylene glycol + glycerol (GPW) mixtures (10% and 15%) were examined. Nitrogen microbubbles were synthesized through high-shear rotor homogenizer and pushed through three different needle sizes (23, 27 and 30 gauge) using a syringe pump to examine their sensitivity to needle injection. A sample volume of 100 μ l containing microbubbles were collected at a constant flow rate of 43.63 μ l/min which is the maximum flow rate of the syringe pump used in our experiments. The microbubbles collected at the outlet of the needles were sandwiched between two glass slides for their stability characterization using optical microscopy. The results demonstrated that solution containing 10% PEG-40, 10% polypropylene glycol and 10% glycerol had the highest concentration of microbubbles post injection for all three needle sizes. Finally, phantom experiments were conducted to calculate the signal-to-noise (SNR) ratios of the microbubbles with the different surfactant concentrations using a clinical ultrasound system.

Keywords: microbubbles, surfactant concentration, needle size, ultrasound imaging, contrast agents, signal-to-noise (SNR) ratios.

1. INTRODUCTION

Ultrasound imaging is one of the most powerful, safest and least expensive biomedical imaging techniques for visualising internal body structures. It uses sound waves which are transmitted and received by a transducer. The images are generated from the reflected waves. Due to its acoustic impedance, microbubbles¹ shelled with surfactants or phospholipids, have been used as ultrasound contrast agents. It has been reported² that microbubble sizes with a larger diameter in the (4-5 and 6-8 μ m) show a higher contrast in the ultrasound images compared to smaller sizes (1-2 μ m). Similarly, a higher concentration (frequency or number of microbubbles)² of microbubbles also helps in enhancing the same. The stability of the microbubbles depends on the type and the concentration of these surfactants in addition to the type of the gas core. Parhizkar et.al³ reported that microbubbles coated with polyoxyethylene glycol 40 (PEG-40) stearate, a non-ionic surfactant, in a glycerol solution were the most stable and lasted for 150 days. Similarly, other works^{4, 5} have been published which reported the use of microbubbles coated with PEG-40, polypropylene glycol and glycerol as ultrasound contrast agents. These microbubbles are injected into the region of interest using catheters of different sizes. The role of PEG-40⁶ on the stability and morphology of microbubbles have also been studied. Talu et.al⁷ reported the impact of three needle sizes: 23G, 27G and 30G on the destruction rate of microbubbles at different flow rates using a syringe pump. As the needle size reduced from 23G to 30G and flow rate increased, the destruction rate of the microbubbles also increased. Injection of microbubbles using needles of smaller diameters⁸ is of significance because thinner needles reduces pain following cutaneous insertion and aids patient comfort

*Email: manojit@ntu.edu.sg

In this work, we investigated the durability of microbubbles after injection through three needle sizes: 23G, 27G and 30G for different concentrations of the PEG-40, polypropylene glycol and glycerol. Increasing the surfactant concentration, PEG-40, reduces the size of the microbubbles.³ Moreover, increasing the glycerol weight percentage increases the area of surfactant molecule at the air-solution interface.⁹ This increases the stability of the microbubbles while reducing the size of the microbubbles.⁴ While smaller microbubbles may survive needle injections with smaller diameters, it is not ideal because smaller microbubbles² show little enhancement in ultrasound signal amplitudes. It has been reported that post needle injection, the mean diameter of the microbubbles get reduced.⁷ In an ideal scenario, the mean diameter of the microbubbles should not get reduced after needle injection and the microbubbles destruction should be as minimal as possible. Hence, in this work, we have compared the size of the microbubbles post needle injection for different surfactant concentrations and tried to optimize the surfactant concentration.

2. METHODS

2.1 SYNTHESIS OF MICROBUBBLES

To examine the effects of surfactant concentration on the stability of microbubbles after needle injection, four different surfactant concentrations of PEG-40 stearate (2%, 5%, 10%, and 15%) in addition to a fixed weight percentage of 10% and 15% for both glycerol and polypropylene glycol were taken in deionized water. A clear solution is obtained after stirring it overnight at 1000 rpm using magnetic stirrer bars. 5 ml of this solution is then poured in a beaker into which the rotor blades of a high shear homogenizer (D160 Scilogex, USA) are inserted. Simultaneously, an outlet from a nitrogen gas cylinder (Leeden NOX, Singapore) fitted with a gas pressure regulator (Spectron, Singapore) and at a pressure of 1 bar was connected to the beaker. The blades were then rotated at a constant speed of 12400 rpm for 5 minutes to form the microbubbles as shown below.

Fig. 1 (a) 5 ml of clear solution containing PEG-40, glycerol and polypropylene glycol taken in a glass beaker (b) Synthesizing nitrogen microbubbles using rotor blade homogenizer (c) Nitrogen microbubbles in a beaker. The microbubbles are seen towards the bottom of the beaker.

2.2 STABILITY CHARACTERIZATION USING OPTICAL MICROSCOPY

Microbubbles from the various solutions were withdrawn gently into 1 ml syringes (BD plastic). Following this, sample volumes of 100 μ l containing microbubbles were collected on a glass slide (Corning microscope slides) at a constant flow rate of 43.63 μ l/min which is the maximum flow rate of the syringe pump used. The microbubbles were pumped through 23G (0.6 x 32mm), 27G (0.4 x 12mm) and 30G (0.3 x 13 mm) needle sizes (BD PrecisionGlide Needles, Singapore). Microbubbles, collected at the outlet, were then squeezed between two spaced glass slides with a gap of about 0.1875 mm. The gap was made by placing a scotch magic matte tape on the sides of the glass slide. The microbubbles were observed for an hour and images were captured for every 15 minutes to see the changes in size and frequency or the number of microbubbles.

2.3 ULTRASOUND IMAGING PERFORMANCE USING PHANTOM TUBES

Microbubbles shelled with 2%, 5%, 10%, and 15% PEG-40 surfactant concentrations and 10%, 15% polypropylene glycol + glycerol (GPW) mixture were injected through 23G, 27G, and 30G needle sizes into low density polyethylene (LDPE) tubes. In this work, 10% GPW mixture implies 10% weight for each of polypropylene glycol and glycerol in the mixture. Similarly, 15% GPW mixture implies 15% weight for each of polypropylene glycol and glycerol in the mixture. As mentioned previously, sample volumes of 100-1000 μ l containing microbubbles were collected at a constant flow rate

of 43.63 ul/min using a syringe pump. The 15% GPW mixture was not used as fewer microbubbles were observed during stability characterization [Fig. 2]. For the ultrasound imaging performance, a clinical ultrasound (E-Cube Alpinion) system was used. Images were saved in the beamformed datatype. A minimum of 10 images were exported to Matlab to calculate the mean value of signal to noise ratios (SNR). SNR is given in decibels (dB) by $SNR = 20 \times \log 10 (V/n)$, where V represents the ultrasound signal amplitude and n stands for the back-ground noise amplitude.

3. RESULTS AND DISCUSSIONS

For stability characterization, the size (diameter) and frequency (number) of microbubbles was quantified using optical microscopy. There was no strong correlation between the size of the microbubbles and the size of the needles (23G, 27G, and 30G) for 2%, 5%, 10%, and 15% PEG-40 in 10% GPW concentrations [Fig. 3 (a)]. Only the frequency (number of microbubbles) of microbubbles was affected the size of the needles and for all surfactant concentrations used in this work. However, as expected, the size of the microbubbles was reduced³ with increasing PEG-40 concentrations. With regards to frequency [Figs. 4(a-c)], it was observed that the frequency was maximum at 23G needle size compared to 27G and 30G. At 10% PEG-40 and 10% GPW mixture, the frequency of microbubbles was highest for all needle sizes at time, $t = 0$ mins. For 10% PEG-40 and 10% GPW, the frequency was 259 through 23G, 107 through 27G and 134 through 30G at time, $t = 0$ min. This could be explained due to the fact that at lower percentages (2% and 5%) of PEG-40, the microbubbles are bigger³ which would get ruptured during needle injection. On the other hand, at a higher concentration of 15% PEG-40, the microbubbles are too small which shrinks quickly due to its higher internal Laplace pressure.¹⁰ Other than the PEG-40 concentration, the concentration of GPW mixture was also varied. As glycerol increases the area of surfactant molecule at the air-solution interface,⁹ the microbubbles also get smaller. However, it increases the viscosity of the liquid solution which might have ruptured the microbubbles. Hence, increasing the GPW concentration did not help in improving the microbubbles durability during needle injection.

Fig. 2 Images of microbubbles shelled with 2%, 5%, 10% and 15% PEG-40 in 10, 15 % GPW mixture (glycerol and polypropylene glycol) injected through (a) 23G (b) 27G and (c) 30G needles.

Following stability characterization studies, ultrasound imaging performance was investigated using the clinical ultrasound system. The microbubbles were collected in LDPE tubes following injection needle of different sizes. The ultrasound signal amplitude is dependent on the size of microbubbles and concentration of the microbubbles.^{11, 12} The screenshots of the microbubbles within the LDPE tubes are shown in Fig. 4 (a) whereas the signal to noise ratio (SNR) values are shown in Figs. 4(b) and (c). From visual inspection, it was hard to compare the contrast enhance for different solutions. This is because as the microbubbles were pumped along the LDPE tubes, the microbubbles either adhered to the walls of the hydrophobic LDPE tubes, got burst under hydrodynamic pressure exerted by the syringe pump or coalesced with the neighbouring microbubbles (Ostwald ripening) to grow bigger in size. The bigger microbubbles have higher echogenicity as they can reflect more of the ultrasound waves and hence produced a stronger signal. Due to all

these effects, the impact of the various solutions on the ultrasound signal was compromised. The SNR values were almost similar for all three needle sizes: 23G, 27G, and 30G for all solutions due to the experimental constraints mentioned above. However, it is to be noted that the SNR values were slightly higher for the 10% GPW mixture compared to the 15% GPW mixture. This corroborated our stability characterization studies where we observed higher frequency and bigger size of microbubbles for the 10% GPW mixture.

Fig. 3 Microbubble sizes from different solutions of sample volume, 100 µl and observed immediately after injection through 23G, 27G and 30G needle sizes at a constant flow rate of 43.63 µl/min.

Fig. 4 Frequency (Number of microbubbles) for different surfactant solutions after injection through needle sizes of (a) 23G, (b) 27G and (c) 30G over time, t from 0 to 60 minutes.

Fig. 5 (a) Tube phantoms containing microbubbles shelled with different solutions obtained using the clinical ultrasound system. (b-c) Comparison of signal to noise ratios (SNR) of US signals for the various samples obtained with (b) 10% GPW mixture and (c) 15% GPW mixture.

4. CONCLUSION

This study was done to investigate the impact of fine changes in the amounts of PEG-40 concentration and glycerol + polypropylene glycol (GPW) weight percentage on the stability of nitrogen microbubbles. Four different concentrations (2%, 5%, 10%, and 15%) of PEG-40, a non-ionic surfactant, were tested with 10% and 15% GPW mixtures. Needles of three different sizes: 23G, 27G, and 30G were used to examine the durability of the microbubbles after injection through them at a constant flow rate of 43.63 ul/min using a syringe pump. Based on this work, we observed that the solution containing 10% PEG-40 and 10 % GPW mixture produced the most stable microbubbles as it contained the highest number of bubbles and is capable of contrast injection to smaller arteries and veins for diagnostic examinations *in vivo*. Ultrasound imaging experiments were also performed using phantom LDPE tubes to quantify the impact of the different solutions on the SNR values. However, the SNR values were compromised by the complex interactions between the microbubbles and the hydrophobic LDPE tubes as some of the microbubbles either got destroyed or adhered to the tube walls while being delivered into the tubes by the syringe pump.

5. ACKNOWLEDGEMENT

The research is supported by Tier 1 grant funded by the Ministry of Education in Singapore (RG48/16: M4011617). Authors have no relevant financial interests in the manuscript and no other potential conflicts of interest to disclose.

6. REFERENCES

- [1] E. Stride, and N. Saffari, "Microbubble ultrasound contrast agents: a review," Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine, 217(6), 429-47 (2003).
- [2] S. Sirsi, J. Feshitan, J. Kwan *et al.*, "Effect of microbubble size on fundamental mode high frequency ultrasound imaging in mice," Ultrasound in Medicine and Biology, 36(6), 935-48 (2010).
- [3] M. Parhizkar, M. Edirisinghe, and E. Stride, "The effect of surfactant type and concentration on the size and stability of microbubbles produced in a capillary embedded T-junction device," RSC Advances, 5(14), 10751-10762 (2015).

- [4] K. Hettiarachchi, E. Talu, M. L. Longo *et al.*, "On-chip generation of microbubbles as a practical technology for manufacturing contrast agents for ultrasonic imaging," *Lab on a Chip*, 7(4), 463-468 (2007).
- [5] D. Das, K. Sivasubramanian, C. Yang *et al.*, "On-chip generation of microbubbles in photoacoustic contrast agents for dual modal ultrasound/photoacoustic in vivo animal imaging," *Scientific Reports*, 8(1), 6401 (2018).
- [6] J. Owen, S. Kamila, S. Shrivastava *et al.*, "The Role of PEG-40-stearate in the Production, Morphology, and Stability of Microbubbles," *Langmuir*, (2018).
- [7] E. Talu, R. L. Powell, M. L. Longo *et al.*, "Needle size and injection rate impact microbubble contrast agent population," *Ultrasound Med Biol*, 34(7), 1182-5 (2008).
- [8] L. Arendt-Nielsen, H. Egekvist, and P. Bjerring, "Pain following controlled cutaneous insertion of needles with different diameters," *Somatosensory & Motor Research*, 23(1-2), 37-43 (2006).
- [9] G. D'Errico, D. Ciccarelli, and O. Ortona, "Effect of glycerol on micelle formation by ionic and nonionic surfactants at 25 degrees C," *J Colloid Interface Sci*, 286(2), 747-54 (2005).
- [10] M. Postema, A. Bouakaz, M. Versluis *et al.*, "Ultrasound-induced gas release from contrast agent microbubbles," *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control*, 52(6), 1035-1041 (2005).
- [11] S. Hernot, and A. L. Klibanov, "Microbubbles in Ultrasound-Triggered Drug and Gene Delivery," *Advanced drug delivery reviews*, 60(10), 1153-1166 (2008).
- [12] K. H. Martin, and P. A. Dayton, "Current status and prospects for microbubbles in ultrasound theranostics," *Wiley Interdiscip Rev Nanomed Nanobiotechnol*, 5(4), 329-45 (2013).