JUST AGRICULTURE
multidisciplinary e-Newsletter

Nanobubbles - A Review

Geo Thomas[1] and Adarsh M. Kalla[2]
[1]Food safety officer, ernakulam, kerala
[2]Assistant professor, Department of Dairy Engineering, Dairy science college, kalaburagi, KVAFSU, Bidar

ARTICLE ID: 42

Abstract

Nanobubble is a long-lasting gas-containing cavity in the aqueous solution and this unique property could be explained by the low internal pressure and surface tension, which may be due to the charged gas/liquid interface. The bubble stability decides the rate of bubble dissolution, so the dominant approach to understanding this is discussed. This paper also encompasses different methods for the production and characterization of nanobubbles, and theories for their stability. It also covers various applications of nanobubbles in different fields.

Keywords: Nanobubble, bubbles, dissolution, Microbubbles

Introduction

Generally, nanobubbles are considered as gaseous cavities of size less than a micron. As per ISO/TS 27687:2008, the term nano is associated with particles with lesser than 100 nm. Considering this fact, the particles of size less than 100 µm should be called 'fine' bubbles and sub-micron bubbles should be called 'ultrafine' bubbles. Bulk nanobubbles are highly stable gas containing bubbles (Seo et al., 2015) formed in aqueous solution. The first report regarding thepresence of nanobubbles was reported in by Thorpe et al. (1982). In the study conducted by Ultawoski et al. (2019), nanobubbles stable over weeks has been observed in distilled water and in open solution. These nanobubbles have negative zeta potential in the range of -25 to -40 mV.

The presence of nanobubbles in water is attributed long before to the discrepancy between the theortical and experimental cavitation threshold (it is the tensile strength of water, 1000 atm) of water. The experimentally determined cavitation threshold is more than one order of magnitude lower than the theoretical tensile strength of pure water. This huge difference can be due to the cavitation nuclei present, solid impurities and tiny gas bubbles. The cavitation threshold in air saturated water is about 1 atm. As the gas concentration in the bubble increases the cavitation threshold decreases. Fox and Herzfeld. (1954), suggested that the size of gas

JUST AGRICULTURE
multidisciplinary e-Newsletter

bubbles to be ranging from 100 nm to 10 µm. The long-range attraction between the hydrophobic surface is also attributed to the presence of nanobubbles. The study conducted by Attard. (1996) proposes that the long-range attraction between hydrophobic surfaces act through nanobubbles present. Due to its salient features nanobubbles are applied in various fields and new application methods are being developed for the efficient use of nanobubbles.

Stability of Nanobubbles

The stability of nanobubbles is explained using various models like skin model, Particle crevice model, electrostatic repulsion model and hydrodynamic model. Skin model suggested by (Fox and Herzfeld, 1954; Yount, 1979) explains that the shrinkage of bubble due to the dissolution of gas is prevented by the organic material or surfactant covering at the surface of the bubble. Particle crevice model (Atchely and Prosperetti, 1989) suggests that the gas bubble is trapped inside the crevice of a solid impurity present and since the interface of the trapped gas bubble is concave, the surface tension makes the internal pressure lower than the liquid pressure thus stabilizing the bubble. But this model is in contradiction with the resonant mass measurement experiment results. As per the Electrostatic repulsion model, the bulk nanobubble is negatively charged (Takahashi, 2005) in pure water pH ~7. The theory states that the electrostatic repulsion of the bubble surface may balance with the Laplace pressure preventing gas diffusion. However, the repulsive pressure is more than one order of magnitude smaller than that of the Laplace pressure (Yasui, 2018).

"Many body model" suggests that the liquid surrounding the nanobubble is supersaturated with gas preventing gas dissolution. This can be achieved if the concentration of nanobubbles is high such that the liquid is supersaturated with gas. This theory fails to explain the stability of nanobubbles at low concentrations. Hydrodynamic model is a more plausible theory in elucidating the stability of bulk nanobubble. As per the theory first proposed for a surface nanobubble on a hydrophobic surface by (Brenner and Lohse, 2008). Due to the water repulsion properties of hydrophobic substance, a depletion layer of thickness 0.2–5 nm is formed. In this depletion layer the density of liquid water reduces to 44-94% (Steitz et al., 2003) and the gas dissolved in the liquid water will be in trapped condition (Peng et al., 2013). The gas concentration in liquid water at hydrophobic surface is higher, hence the gas will diffuse into the bubble at the surface of hydrophobic material (Yasui, 2016). The dissolution of gas will be stopped when an equilibrium stage is reached between the gas influx at the

JUST AGRICULTURE
multidisciplinary e-Newsletter

surface of hydrophobic material and gas outflux from the uncovered part. However, it has been found that the size of nanobubbles stabilized by the hydrophobic material should be smaller than the experimentally determined size. This points out that the actual potential of hydrophobic attraction is lower than assumed. Also, the surface coverage of hydrophobic material should be more than 50% for the bubble to be stable (Yazui et al., 2018). In the study conducted by Sugano, Miyoshi, and Inazato, (2017) TEM image of nanobubbles were taken. Based on the analysis of the image it was found that the bubbles were stabilised by the partial coverage of the hydrophobic material. However, the surface coverage of the hydrophobic material was unclear.

Mechanism of dissolution of air from the nanobubble

The dissolution of a bubble of 100 nm takes around 75.36 μs. The temperature and pressure can increase upto 3000 K and 5 GPa, respectively at the final moment of the bubble dissolution. This increase in the temperature can be attributed to the pV work done by the surrounding fluid on the bubble. Due to this the temperature at the bubble surface can increase upto 85°C at the final moment of dissolution. This rise in temperature is not sufficient for thermal dissociation of water molecules and OH radical formation. Towards the end of dissolution only Nitrogen gas will be present in the bubble as the solubility of nitrogen gas is less. However, no nitrogen radicals will not be formed as the amount of N atom production inside a bubble is only in the order of 10^{-15} and is completely negligible (Yasui, 2016).

Manufacturing of nanobubbles

Bulk nanobubbles (ultrafine bubbles) are often produced using hydrodynamic cavitation by using Venturi-tube, swirling flow, injection of pressurized water containing gas, etc. In the initial phase due to production of microbubbles the water will turn milky, the microbubbles as result of buoyant force will move upwards gradually and disappear and the water will once again become transparent. When analysed with dynamic light scattering, laser diffraction scattering, and particle tracking analysis, bubbles of size range of 100–200 nm in diameter were found. These bubbles were stable for more than a month (Suagno et al., 2017; Maeda et al., 2014; Oh et al., 2015). Nanobubbles are developed using high speed agitation device, a tissue homogenizer, that can provide a three-dimensional multi directional motion. The gas (perfluoro-propane) and fluid is filled inside a container and is tightly sealed. The container tubes were shaken in a homogenizer at 6500 rpm for 60 seconds on ice with a 5-

JUST AGRICULTURE
multidisciplinary e-Newsletter

minute pause between each shaking phase. The samples were incubated at room temperature for one hour. The container was centrifuged (100 g for 10 minutes to extract same sized nanobubbles from microbubbles. The solution was preserved at 4°C (Lafond et al., 2018). Bubble liposomes are developed by taking 2 mL of prepared liposome in a 5mL sterilized vial followed by supercharging with 7.5 mL perfluoro-propane gas. It is then sonicated in a bath type sonicator for 5 minutes. The approximate size of liposomes was 500 nm (Suzuki et al., 2016).

Evaluation of nanobubble characteristics

Different methods are used with varied applicability to measure the size of the nanobubbles produced. Nanoparticle tracking analysis is a technique used for measuring the size of nanobubbles. Nanoparticle movement due to Brownian motion is used to analysis the size. Stokes-Einstein equation is used to calculate the size of nanobubbles. the suspension of nanobubbles is illuminated by using a 638 nm red laser and the particle movement is expressed as light scattering. This method can be used to in a size range of 10 to 1000 nm (Lofand et al., 2018). Dynamic light scattering is also used for calculating the size of nanobubbles. here also the light scattering caused by the Brownian motion of the bubbles is used. In this method the overall size distribution of the particles is analysed. In this method for an accurate calculation of the size distribution, the bubble concentration should be more than one billion nanobubbles per mL (Lofand et al.,2018). The number of nanobubbles generated can be estimated by flow cytometric analysis. Nanoparticles are detected by measuring the side scattering from ultraviolet laser. The signals are analysed to determine the number of nano particles. This method is also used for evaluating the stability of nanobubbles (Lofand et al., 2018).

Freeze fracture transmission electron microscopy was used for studying the structural and functional features of nanobubbles. This method is generally used for analysing the biological components. Initially the samples are fractured and a replica of the fracture plane is created by the vacuum deposition of platinum-carbon. The replica is then examined by TEM. In the study conducted by Uchida et al. (2011), 10 to 20 mm^3 of sample was taken in Au-coated Cu sample holder and rapid freezing was done using liquid nitrogen. The sample was fractured by applying vacuum of 10^{-4} to 10^{-5} Pa at 100 K. The replica was created by evaporating platinum-carbon. The replica thus developed was analysed using a field emission gun type TEM. Resonant mass measurement is used for the characterisation of nanobubbles based on its

JUST AGRICULTURE
multidisciplinary e-Newsletter

size. This consists of a cantilever with an embedded microfluidic channel. The change in the resonance frequency of the cantilever beam when the particles are passed through the microfluidic channel is measured. The passage of smaller density (less than density of water) particles will increase the resonance frequency while passage of larger density particles will decrease the resonance frequency. By measuring the change in the resonance frequency, mass of the particles is calculated. If the density of the particles, the volume (diameter of the particles is determined (Alheshibri and Craig, 2019).

The rheological parameters of nanobubbles can be investigated by applying bubble oscillation simulations using the information on cavitation thresholds. The surface tension and the corresponding dilatational viscosity are the key parameters calculated for developing a rheological model. The modelling was done using Rayleigh-Plesset-type equation. Using the rheological models' simulations of nanobubble behaviour can be developed and these simulations can be used to confirm the structure of the nanobubbles and activity of nanobubble. In the study conducted on the albumin nanobubbles, the simulated rheological models confirmed that albumin acts as a surfactant and the nanobubble behaves like an extremely soft-shelled bubble. This also points the possibility that a viscous surfactant can also be used for developing stable nanobubbles (Lofand et al., 2018). The dilatational viscosity of albumin nanobubbles are in the range 5.10−10 Ns/m to 1.10−9 Ns/m, which is much lesser than that of microbubbles. This lower dilatational viscosity of albumin nanobubbles explains the longer stability of nanobubbles along with its high sensitivity to ultrasound. This improves the applicability of nanobubbles in imaging and therapy applications (Lofand et al., 2018).

The mechanical effect generated and used in drug delivery, tumour treatment and gene transfection is the cavitation effect. Bubbles are used to reduce the large pressure levels required for the generation of desired cavitation effect as large pressure levels can cause safety concerns. Bubbles can act as the cavitation nuclei thereby inducing desired cavitation effect at lower pressure levels. The cavitation index is measured using an ultrasound emitter and an aligned in house PCD to receive the signal. The cavitation index of nanobubble and microbubble was calculated and was found that albumin nanobubbles can be used as an effective cavitation nucleus as the inertial cavitation threshold is under 2 MPa (Lafond et al., 2018).

Armoured nanobubbles

JUST AGRICULTURE
multidisciplinary e-Newsletter

Armoured nanobubbles is used to refer to coated bubbles of diameter less than 1000 nm. These armoured bubbles are used as contrast agents in ultrasound imaging. These bubbles are stable for long periods. The lipid coating acts a shell and provides mechanical resistance to the nanobubbles. On the application of external pressure, the reduction in size was considerably lesser than compared to a naked nanobubble. It was also observed that the condensation of gas inside the shell occurred at much higher pressures than the normal required levels. The shell also contributes to the stability of nanobubbles.The lipids used as shell material are hydrophobic and has low solubility in water, therefore it will reduce the surface energy at the interface resulting in negative Laplace pressure inside the bubble(Alheshibri and Craig, 2018). Additionally, the transport of gas across the interface is prevented by the lipid shell (Mountford et al., 2014). The concept of stability of armoured bubbles is consistent with the proposed idea of Ducker, (2009). According to his model the concentrated impurities accumulated at the interface will cause a reduction in the interfacial energy leading to the development of a negative surface energy.

Application of nanobubbles.

Bubbles are used as image contrasting agents in medical field as the acoustic properties of bubbles are different from tissues and fluids, so when bubbles are used enhanced image contrasts can be developed. In the medical field FDA approved microbubbles viz: Definity (Lantheus Medical Imaging) and Optison (GE Healthcare) preparations are already in use (Perera et al., 2015). To improve the lifetime of *in vivo* bubbles studies using nanobubbles are in progress (Wang et al., 2010; Perera et al., 2014; Yin et al., 2012). At low acoustic power microbubbles resonate symmetrically but when intermediate acoustic power is applied the expansion and contraction phases will exhibit nonlinear behaviour, at the same time tissues due to their incompressibility compared to bubbles will demonstrate a linear behaviour.

Microbubbles are also used as drug delivery agent. Usually, the bubbles will be coated with an external shell using specific lipids that will bind with the drugs. These bubbles can cluster in the desired area and the drug can be released either naturally or with the application of external stimulus such as ultrasound (Mestas et al., 2014). The application of microbubbles has some limitations due to the increased size, which have led to the necessity of an extensive study of nanobubbles. Ultrasound and the cavitation effect have large number of applications in the medical field. The bubbles are introduced as cavitation nuclei to reduce the negatory

JUST AGRICULTURE
multidisciplinary e-Newsletter

effects of large pressure caused by the cavitation. Bubbles can also be used as a medium to deliver the cavitation effect in the targeted region thereby reducing the risk of collateral cavitation in untargeted regions. The nanobubble can access regions which are not approachable by microbubbles. Also nanobubbles are less prone to clearance and thereby have a prolonged circulation time in blood. Nanobubble can be used as an efficient drug delivery agent to tumor (Narihira et al., 2017), nerve (Song et al., 2017), retina (Tahkur et al., 2017), vascular tissues (Sutton et al., 2013) and brain blood barriers (Huang et al., 2015). Gene transfection can also be done by the application of nanobubbles (Abdalkader et al., 2017). The application of nanobubbles have showed interesting developments in imaging applications (Yang et al., 2015) and theragnostic modalities (Sneider et al., 2017).

By using nanobubbles the lifetime of the bubbles can be extended. Nanobubbles prepared by Yin et al, (2012), demonstrated better contrast enhancement as compared to microbubbles. The enhancement lasted only 15 minutes in case of microbubbles where as it lasted to almost an hour in the case of nanobubbles. The study conducted by (Suzuki et al., 2016) shows benefits of combined application of ultrasound and bubble liposomes. The tumour temperature was 6°C higher when ultrasound and bubble liposome was applied together compared to the application of ultrasound alone. This is due to the localized heating of bubble interior. The necrosis of tumour tissues occurred at lower intensity when bubble liposome and ultrasound was applied together. The tumour was suppressed by approximately 45%. The combined application of bubble liposome and ultrasound induce damage to tumour cells by making pores in the cell membrane. This in turn will induce the release of tumour antigen to the blood stream which will prime the anti-tumour immune system. Micro and nanobubbles are also used in waste water treatment such as sewage treatment of wastewater by air floatation (Li and Tsuge, 2006) and removal of adsorbed proteins without detergent usage (Liu et al., 2008). Analysis of TEM images of bubbles created in waste water shows that insoluble impurities are collected on the surface of the bubbles. As the sweep area of the bubbles is limited, the effectiveness of application of bubbles for wastewater treatment depends on the concentration of the bubbles in the water and the residence time.

Future applications and further study in Nanobubble technology

Micro and nanobubbles are now employed in biological field as a functional material. Application of bubbles was found in accelerating the metabolism in vegetables. In the study

JUST AGRICULTURE
multidisciplinary e-Newsletter

conducted by Park and Kurata. (2019), lettuce was cultivated using deep flow technique hydroponics in which micro and nanobubbles were created. It was found that the weight of lettuce leaves increased upto 2.1 times. The application of microbubble can increase the gas-liquid mass transfer due to the increase in the interfacial area and it was found that when microbubble was used yeast can be cultivated with 1/100 to 1/10 of the gas flow required in a conventional sparger (Ago et al., 2006). Mixture of ozone and microbubbles was used to enhance the sterilization efficiency of ozone. Here also the enhanced mass transfer property of bubbles was utilized (Li and Tsuge, 2006).

The application and advantages of nanobubble in tumour treatment is already established/studied. However, the optimisation of concentration of nanobubbles required to have maximum effect has not yet been investigated. It is necessary to find the right concentration of nanobubbles required because a higher concentration of nanobubbles will disturb the ultrasound transmission and effective cavitation will not be induced (Suzuki et al., 2016). In the study conducted by Lofand et al. (2018) to evaluate the cavitation index of albumin nanobubbles, the mechanical effect produced was studied. However, the thermal effects and its implications on the cavitation threshold and surface tension were not studied. In the studies conducted by Alheshibri and Craig. (2018), the mechanical resistance and stability of armoured nanobubbles were investigated. Further investigations were conducted regarding the applicability of armoured nanobubbles and their implications such as the cavitation of water columns in xylem of plants (Schenk, 2015) and formation of bubbles and decompression sickness (Craig, 1996). The study conducted by Alheshibri and Craig. (2018), showed the inaptness of resonance mass measurement in characterising the lipid armoured nanobubbles. The dilution of the sample affected the lipid solubility which in turn affected the stability of armoured nanobubbles.

Conclusion

The nanobubbles are generally split into surface nanobubbles and bulk nanobubbles. The stability of nanobubbles is not yet clear however progress is being made and several hypotheses have been proposed to explain the observed long-term stability of nanobubbles. None of them succeed in describing all the experimental observations, but improvements in characterization techniques and a growing demand from industry for methods to make and control nanobubbles will continue to drive new research in the field.

JUST AGRICULTURE
multidisciplinary e-Newsletter

References

A.A. Atchley, A. Prosperetti, The crevice model of bubble nucleation, J. Acoust. Soc. Am. 86 (1989) 1065–1084.

Abdalkader, R. *et al*. The development of mechanically formed stable nanobubbles intended for sonoporation-mediated gene transfection. *Drug Deliv.* **24**, 320–327 (2017).

Alheshibri, M., & Craig, V. S. (2019). Armoured nanobubbles; ultrasound contrast agents under pressure. *Journal of colloid and interface science*, *537*, 123-131.

Attard, P. (1996). Bridging bubbles between hydrophobic surfaces. *Langmuir*, *12*(6), 1693-1695.

Cavalli, R., Soster, M. &Argenziano, M. Nanobubbles: a promising efficienft tool for therapeutic delivery. *Ther. Deliv.* **7**, 117–138 (2016).

D. Seo, S. R. German, T. L. Mega and W. A. Ducker, Phase state of interfacial nanobubbles, *Journal of Physical Chemistry C* **119** (2015) 14262-14266.

D.E. Yount, Skins of varying permeability: a stabilization mechanism for gas cavitation nuclei, J. Acoust. Soc. Am. 65 (1979) 1429–1439.

F.E. Fox, K.F. Herzfeld, Gas bubbles with organic skin as cavitation nuclei, J. Acoust. Soc. Am. 26 (1954) 984–989.

H. Peng, G.R. Birkett, A.V. Nguyen, Origin of interfacial nanoscopic gaseous domains and formation of dense gas layer at hydrophobic solid-water interface, Langmuir 29 (2013) 15266–15274.

H.J. Schenk, K. Steppe, S. Jansen, Nanobubbles: a new paradigm for air-seeding in xylem, Trends Plant Sci. 20 (2015) 199–205

Hernandez, C., Gulati, S., Fioravanti, G., Stewart, P. L. & Exner, A. A. Cryo-EM Visualization of Lipid and Polymer-StabilizedPerfluorocarbon Gas Nanobubbles - A Step Towards Nanobubble Mediated Drug Delivery. *Sci. Rep.* **7**, 13517 (2017).

Huang, H.-Y. *et al*. A multitheragnostic nanobubble system to induce blood–brain barrier disruption with magnetically guided focused ultrasound. *Adv. Mater.* **27**, 655–661 (2015).

K. Sugano, Y. Miyoshi, S. Inazato, Study of ultrafine bubble stabilization by organic material adhesion, Jpn. J. Multiphase Flow 31 (2017) 299–306.

JUST AGRICULTURE
multidisciplinary e-Newsletter

K. Sugano, Y. Miyoshi, S. Inazato, Study of ultrafine bubble stabilization by organic material adhesion, Jpn. J. Multiphase Flow 31 (2017) 299–306.

K. Ulatowski, P. Sobieszuk, A. Mróz and T. Ciach, Stability of nanobubbles generated in water using porous membrane system, *Chemical Engineering and Processing - Process Intensification*, **136** (2019) 62-71.

K. Yasui, Acoustic Cavitation and Bubble Dynamics, Springer, Cham, Switzerland, 2018.

K. Yasui, Mechanism for stability of ultrafine bubbles, Jpn. J. Multiphase Flow 30 (2016) 19–26 (in Japanese.

K. Yasui, T. Tuziuti, W. Kanematsu, Extreme conditions in a dissolving air nanobubble, Phys. Rev. E 94 (2016) 013106.

Lafond, M., Watanabe, A., Yoshizawa, S., Umemura, S. I., & Tachibana, K. (2018). Cavitation-threshold determination and rheological-parameters estimation of albumin-stabilized nanobubbles. *Scientific reports*, *8*(1), 7472.

Li P, Tsuge H: Ozone transfer in a new gas-induced contactor withmicrobubbles. J Chem EngJpn 2006, 39:1213-1220.

Li P, Tsuge H: Water treatment by induced air flotation using microbubbles. J Chem EngJpn 2006, 39:896-903.

Liu G, Wu Z, Craig VSJ: Cleaning of protein-coated surfaces using nanobubbles: an investigation using a quartz crystal microbalance. J Phys Chem C 2008, 112:16748-16753.

M. Takahashi, ζ potential of microbubbles in aqueous solutions: electrical properties of the gas-water interface, J. Phys. Chem. B 109 (2005) 21858–21864.

Mestas, J.-L. *et al*. Therapeutic efficacy of the combination of doxorubicin-loaded liposomes with inertial cavitation generated byconfocal ultrasound in AT2 Dunning rat tumour model. *J. Drug Target*. 688–697 (2014).

Narihira, K. *et al*. Enhanced cell killing and apoptosis of oral squamous cell carcinoma cells with ultrasound in combination with cetuximab coated albumin microbubbles. *J. Drug Target*. 1–11 (2017).

P.A. Mountford, S.R. Sirsi, M.A. Borden, Condensation phase diagrams for lipidcoatedperfluorobutane microbubbles, Langmuir 30 (2014) 6209–6218,

JUST AGRICULTURE
multidisciplinary e-Newsletter

P.S. Epstein, M.S. Plesset, On the stability of gas bubbles in liquid-gas solutions, J. Chem. Phys. 18 (1950) 1505–1509.

Park J-S, Kurata K: Application of microbubbles to hydroponics solutionpromotes lettuce growth. HortTechnology 2009, 19:212-215.

R. Steitz, T. Gutberlet, T. Hauss, B. Klosgen, R. Krastev, S. Schemmel, A.C. Simonsen, G.H. Findenegg, Nanobubbles and their precursor layer at the interface of water against a hydrophobic substrate, Langmuir 19 (2003) 2409–2418.

R.H. Perera, C. Hernandez, H. Zhou, P. Kota, A. Burke, A.A. Exner, Ultrasound imaging beyond the vasculature with new generation contrast agents, Wiley Interdiscip. Rev. Nanomedi. Nanobiotechnol. 7 (2015) 593–608.

R.H. Perera, L. Solorio, H. Wu, M. Gangolli, E. Silverman, C. Hernandez, P.M. Peiris, A.-M. Broome, A.A. Exner, Nanobubble ultrasound contrast agents for enhanced delivery of thermal sensitizer to tumors undergoing radiofrequency ablation, Pharm. Res. 31 (2014) 1407–1417, https://doi.org/10.1007/s11095-013-1100-x.

S. A. Thorpe, A. R. Stubbs, A. J. Hall and R. J. Turner, Wave-produced bubbles observed by side-scan sonar, *Nature*, **296** (1982) 636-638; Looking into the water, *Physics Bulletin*, **33** (1982) 199.

S. Maeda, H. Kobayashi, K. Ida, M. Kashiwa, I. Nishihara, T. Fujita, The effect of dilution on the quantitative measurement of bubbles in high-density ultrafine bubble-filled water using the light scattering method, Proc. SPIE 9232 (2014) 92320V.

S.H. Oh, J.G. Han, J.M. Kim, Long-term stability of hydrogen nanobubble fuel, Fuel 158 (2015) 399–404.

Sneider, A. VanDyke, D., Paliwal, S. & Rai, P. Remotely triggered nano-theranostics for cancer applications. *Nanotheranostics Syd. NSW* **1**, 1 (2017).

Song, Z., Wang, Z., Shen, J., Xu, S. & Hu, Z. Nerve growth factor delivery by ultrasound-mediated nanobubble destruction as a treatment for acute spinal cord injury in rats. *Int. J. Nanomedicine* **12**, 1717 (2017).

Sutton, J. T. *et al*. Ultrasound-mediated delivery of bioactive nanobubbles to vascular tissue. *J. Acoust. Soc. Am.* **134**, 4048–4048 (2013).

JUST AGRICULTURE
multidisciplinary e-Newsletter

Suzuki, R., Oda, Y., Omata, D., Nishiie, N., Koshima, R., Shiono, Y., ... & Kawakami, S. (2016). Tumor growth suppression by the combination of nanobubbles and ultrasound. *Cancer science*, *107*(3), 217-223.

T. Yin, P. Wang, R. Zheng, B. Zheng, D. Cheng, X. Zhang, X. Shuai, Nanobubbles for enhanced ultrasound imaging of tumors, Int. J. Nanomed. 7 (2012) 895–904,

T.M. Krupka, L. Solorio, R.E. Wilson, H. Wu, N. Azar, A.A. Exner, Formulation and characterization of echogenic lipid-Pluronic nanobubbles, Mol. Pharm. 7 (2010) 49–59.

Thakur, S. S. *et al*. Stably engineered nanobubbles and ultrasound-An effective platform for enhanced macromolecular delivery to representative cells of the retina. *PloS One* **12**, e0178305 (2017).

Uchida, T., Oshita, S., Ohmori, M., Tsuno, T., Soejima, K., Shinozaki, S., ... &Mitsuda, K. (2011). Transmission electron microscopic observations of nanobubbles and their capture of impurities in wastewater. *Nanoscale research letters*, *6*(1), 295.

V.S.J. Craig, Formation of micronuclei responsible for decompression sickness, J. Colloid Interface Sci. 183 (1996) 260–268

W.A. Ducker, Contact angle and stability of interfacial nanobubbles, Langmuir 25 (2009) 8907–8910

Wang, K. *et al*. Design of Ligands-Conjuncted Lipid Nanobubbles as Ultrasound Contrast Agents Targeted to Atherosclerotic Plaques. *J. Nanosci. Nanotechnol.* **16**, 7611–7616 (2016).

Wilson, K., Homan, K. &Emelianov, S. Biomedical photoacoustics beyond thermal expansion using triggered nanodroplet vaporization for contrast-enhanced imaging. *Nat. Commun.* **3**, 618 (2012).

Y. Wang, X. Li, Y. Zhou, P. Huang, Y. Xu, Preparation of nanobubbles for ultrasound imaging and intracelluar drug delivery, Int. J. Pharm. 384 (2010) 148–153, https://doi.org/10.1016/j.ijpharm.2009.09.027.

Yang, H. *et al*. Nanobubble–Affibody: Novel ultrasound contrast agents for targeted molecular ultrasound imaging of tumor. *Biomaterials* **37**, 279–288 (2015).

Yasui, K., Tuziuti, T., & Kanematsu, W. (2018). Mysteries of bulk nanobubbles (ultrafine bubbles); Stability and radical formation. *Ultrasonics sonochemistry*, *48*, 259-266.

JUST AGRICULTURE
multidisciplinary e-Newsletter

Yildirim, A., Chattaraj, R., Blum, N. T. & Goodwin, A. P. Understanding Acoustic Cavitation Initiation by Porous Nanoparticles: Toward Nanoscale Agents for Ultrasound Imaging and Therapy. *Chem. Mater.* **28**, 5962–5972 (2016).