REVIEW ARTICLE

State of Art Review on Nanobubbles

Ajinkya Kailas Pote[1], Pooja Jayram Jadhav[2], Vishal Vijay Pande[3,*], Mahendra Ashok Giri[4], Shubham Rameshrao Pandit[2]

[1]Department of Pharmaceutics, Rajarshi Shahu College of Pharmacy, Buldana, Maharashtra 443001, India
[2]Department of Pharmaceutics (PG), Sanjivani College of Pharmaceutical Education and Research, Kopargaon, Maharashtra 423603, India
[3]R.S. M's N.N Sattha College of Pharmacy, Ahmednagar, Maharashtra, India
[4]Department of Pharmacology, Rajarshi Shahu College of Pharmacy, Buldana, Maharashtra 443001, India

*Corresponding author: E-mail: drvishalpande@gmail.com; Tel.: (+91) 9623443179

DOI: 10.5185/amlett.2021.031608

Nanotechnology has proved an emerging field in diagnostic in drug delivery. The current review has done special emphasis on the history of nanobubble, nomenclature, stability of nanobubble, physico-chemical properties, characterization of nanobubble, method of preparation and applications. The nanobubbles has now been explored for its antibiotic delivery, gene delivery, targeting drug delivery, anticancer drug delivery, improving cellular uptake of chemotherapy drugs into cancer cell lines. Nanobubbles (NBs) opened a new field of ultrasound imaging and used as a diagnostic method. The article explored a novel application like oxygen delivery through nanobubble which is highly beneficial in most of the diseases.

Introduction

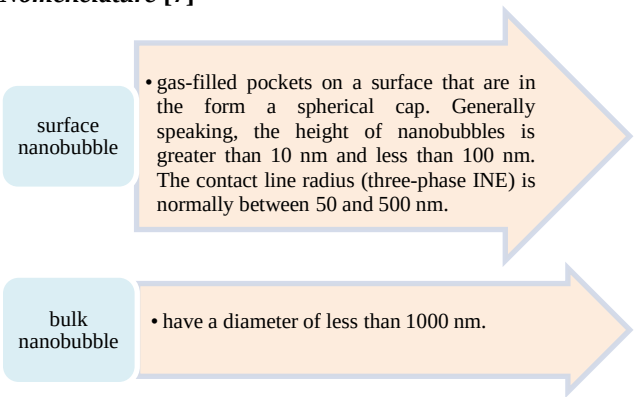
Gas in liquid or vapor entrapped nanoscale cavities are coined as Nanobubbles (NB). The NB had attracted attention of the researchers in the field of scientific and Engineering fields due to enhanced potential applications [**1**,**2**]. NBs are either formed by heterogeneous nucleation (within two interphases (solid / liquid / carbon) or homogeneously prepared in the presence of carbon under atmospheric conditions and may also be created by the coalescence of vacancies of less than 1μm in diameter. Experimental studies indicate that NBs primarily form on the solid hydrophobic surface that can change interfacial properties such as surface forces and adsorption of lubrication and stabilise the colloidal particles. These NBs are experimentally produced by pressure release heating solvent-exchange and water electrolysis [**3**].

Microbubbles offers a major disadvantage as drug delivery systems is their moderately extensive size (1–10 μm), it creates difficulty needing to penetrate through the epithelial cells of the vasculature to the target tissue. Drug-loaded microbubbles are mainly restricted to cardiovascular targets and tumor endothelium as the intravenous injection of microbubbles get stuck into lungs where gas exchange occurs. To get rid of these problems, nanobubbles with sizes smaller than 1 μm has come into existence [**4**]. Nanobubbles has offered enhanced stability and longer residence time in systemic circulation [**5**].

Gas bubbles typically consist of central gas and stabilising shells. In most UCAs, the elevated molecular weight and low solubility filling gas such as SF6 or C3F8 is selected as the gas component, which is less vulnerable to external loss than air loss. Lipid, polymer and/or protein compose of the coating materials since all these materials are safe when administered intravenously. Phospholipids or proteins are also picked as the bubble's thin soft shell, which is more stable and particularly susceptible to acoustic waves than the cross-linked or intertwined polymers' hard shells. Among them, under ultrasound exposure, lipid shell provides the outstanding characters of readily widening, rupture, reseal, compress, buckle, or respread. The commercially available UCA formulations, such as Definity, Imagent, Sonovue, and Levovist, are all primarily lipid-composited. Nearly all commercially available UCAs, however, have micrometre-scale diameters and are thus limited to merely enhancing the illumination of blood vessels and are unable to penetrate surrounding tissues or cells. In comparison, the short circulating half-life is also preserved by microsized bubbles and the liver and spleen are quickly arrested. In ultrasound molecular imaging, nanobubbles with sizes smaller than 1 μm can be assumed to have those priorities. Via the improved permeability and retention (EPR) results, nanobubbles could be transported from vessels into surrounding tissues even cells to be theoretically imaged by ultrasound after aggregation, which activates researchers' great interest to build nanoscale bubbles for early diagnosis of extravascular lesions [**6**]. More recently, they have also been researched with regard to the delivery of drugs, genomes and gases [**5**]. There were two mysteries associated with the stability of nanobubbles and the radical formation upon dissolution of nanobubbles. The former is justified by the dynamic equilibrium model while the latter is explained by numerical simulations. From the numerical simulations it is suggested that there

was no formation of OH radical on dissolution of nanobubble. It was concluded that the signals generated in the experiment were not in OH radical but was in H_2O_2 [**61**]. Rak *et. al.*, 2019, suggested that the there is no existence of bulk nanobubbles. They studied the addition of the organic compounds to water and the ultrasonic cavitation. They found out that the both the process lead to formation of the nano objects but which are not bulk nanobubbles [**62**]. However, Jadhav *et. al.* 2020, discussed the concept of bulk nanobubble and proved that the there is an existence of bulk nanobubbles and they are stable in nature. They provided various proofs like IR, Raman analysis, cryo SEM analysis, ICP−MS Analysis. Encapsulation of nanobubbles, TEM analysis and concluded that they posses long term stability, the amount of dissolved gas has direct relation with the number of nanobubbles generated, Cryo-SEM images, the nano entities show as cavities [**63**]. Yasuda and colleagues studied effects of ultrasonic power and frequency on nanobubble concentration and diameter. They found out that Nanobubble concentration decreased with increasing ultrasonic power and frequency. During the BBB opening with nanobubbles, Cheng and associates explore the influence of the ultrasound target position in the rat brain on the acoustic control quality. At each ultrasound pulse, temporary examination of the acoustic signals received revealed that stable nanobubble oscillation was present throughout the entire 10 ms ultrasound exposure. The auditory feedback control signals in rats is very sensitive to the spatial positioning of the brain. A shared pattern of acoustic regulation flexibility in the brain tends to occur among multiple species, indicating anatomical traits are an underlying cause. The results stress the significance of tuning acoustic feedback control algorithms to ensure optimum efficiency for particular rodent brain regions of interest [**65**].

***Nomenclature* [7]**



***History of nanobubbles** (7)*

The nanobubbles were first reported in 1994. The relationship between the force and the isolation of two hydrophobic surfaces submerged in the liquid was investigated and they observed that simple steps were taken to attract the surface as a function of isolation [**44**].

Survey of nanobubbles

Author	Content
Ljunggren, S.; *et. al.*, 1997	based on expected lifetime nanobubble existence dismissed.
Miller *et. al.*, 1999 (20)	Fourier transform infrared spectroscopy (FTIR) was used to show existence of surface nanobubbles. The effects of wettability on nanobubbles was determined.
Lou, S. T.; *et. al.*, 2000	atomic force microscopy (AFM) images of surface NB were published
Dube, N. K.; *et. al.*, 2003	The use of bulk nanobubbles as ultrasound contrast agents was reported.
Ohgaki, K.; *et. al.*, 2010	cryo-scanning electron microscopy were used to determine the similar bulk nanobubbles.
Kobayashi, H.; *et. al.*, 2014	Density of bulk nanometer measured using a microresonator.
Seo *et. al.*, 2015	Fluorescence microscopy proved surface nanobubbles to be gaseous

Composition of nanobubble

Basically, the nanobubble consists of two elements, namely the inner core and the outer layer, which have distinct physico-chemical characteristics. The shell consists mostly of surfactants, polymers or proteins, while sulphur hexafluoride and perfluorocarbons are found in the central air [**3**].

The stiffness of the bubbles, their rupture tolerance in the field of ultrasound pressure, clearance by the reticuloendothelial system and the ease with which they are recognized is mainly determined by the composition of the shell [**27**].

Core

Core is a single low-density chamber that makes up the main portion of a particle's volume [**8**]. It usually comprises of the medical gas. Upon application of the acoustic waves the nanobubbles oscillate due to the gas density and surrounding aqueous solution. In case when the acoustic pressures are low nanobubble oscillates frequently to cause a phenomenon of stable cavitation which increases the diffusion of core gas out of bubble. The gas present in the core is important parameter of the nanobubbles which must be tailored for serving the purpose of nanobubbles. Perfluorocarbons (PFC) and sulfur hexafluoride (SF6), in combination with the oxygen are used to enhance longevity & stability of the nanobubbles. Gases like NO has also been used in the core as an alternative for oxygen. Various applications of nanobubbles can be exploited by altering the gas in the core. For example, oxygen can be used in the core for reversal of hypoxemia in blood and improving blood oxygen levels [**25**].

Shell

The Shell serves as a barrier to the dissipation of gas between the encapsulated gas and the underlying aqueous medium [**8**]. A protective layer around the gas is formed by the shell to provide cohesion and protection from

endogenous scavengers, and it decreases the rate of diffusion into the surrounding media of the core gas [**45**]. The structure of the shell is not only determined by the density, elasticity, gas exchange, but also by the half-life, resistance to the ultrasonic pressure applied and ease of excretion of the NBs from the body [**30,47**]. Soft shells tend to crack quickly, although in ultrasonic environments, hard shells would not be able to oscillate [**30**]. In the NB system, the shell structure is an essential element in the loading of drugs and genes. For various applications of MNBs with different rigidities, charges, thicknesses, and functional groups, adequate selection of shell materials is critical [**54**]. For different uses, the MNBs' shells may be engineered. Chitosan is a material of choice for the nanobubble shell because of its low toxicity, low immunogenicity, and excellent biocompatibility [**30**]. MNBs can be bio-conjugated with different forms of drugs or proteins / DNA for selective delivery. High stability, biodegradability and in vivo biocompatibility allowed PLGA to make the preferred choice of pharmaceutical carrier material [**29**].

Stability of nanobubble

Design of nanobubble formulation is always a challenging task as number of parameters has been involved in development of stable and safe system. In addition to the mechanisms of the scattered and continuous stages, interfacial stress and Laplace pressure play a critical role.

The surface tension at the interface of binary mixtures determines the molecular interaction between the internal gas centre and the outer liquid medium.

$$\Delta P = P_{inside} - P_{outside} = 2\sigma/r$$

Laplace pressure is the pressure difference between the inside and the outside of a bubble (or a droplet), given as:

ΔP is the Laplace pressure
P inside is the pressure inside bubble,
P outside is the pressure outside a bubble,
σ is the interfacial tension,
r is the bubble radius.

Table 1. Characteristics of MNBs, preparation, application by various researchers.

Sr. No	Title	Core	Shell	Preparation method	Application	Ref
1	The Optimized Fabrication of a Novel Nanobubble for Tumor Imaging	Octafluoropropane	1,2-distearoyl-sn-glycerol-3-phosphatidylcholine (DSPC) and 1,2-distearoyl-sn-glycerol-3- phosphoethanolamine *N*-[biotinyl(polyethyleneglycol) 2000] (DSPE–PEG 2000)	Centrifugation	Tumor Imaging	66
2	Biogenic nanobubbles for effective oxygen delivery and enhanced photodynamic therapy of cancer	Oxygen	Dioleoyl phosphatidylcholine (DOPC)	Emulsification	Cancer Therapy	67
3	Ultrasound molecular imaging of acute cardiac transplantation rejection using nanobubbles targeted to T lymphocytes	anti-CD3 antibody	DSPC, DSPE-PEG2000 and biotinylated DSPE-PEG2000	Thin-film hydration method	Detect acute rejection in heart transplantation	68
4	Reducing Tumour Hypoxia via Oral Administration of Oxygen Nanobubbles	Oxygen	Lecithin	Sonication	Reduce tumour hypoxia in order to increase the efficacy of current cancer therapies	69
5	Development and characterization of nanobubbles containing paclitaxel and surviving inhibitor YM155 against lung canc	Oxygen	Chitosan	High Sher Mixing Ultrasonication	Lung Cancer	70
6	Molecular imaging of atherosclerotic plaque with lipid nanobubbles as targeted ultrasound contrast agents	Gas core	1, 2-Dimyristoyls-sn-glycerol-3-phosphocholine (DMPC)	Ultrasonic emulsion Method	Imaging of atherosclerotic plaque	71
7	Ultrasound-responsive nanobubbles for enhanced intravitreal drug migration: An ex vivo evaluation	Rhodamine-tagged Perfluoro propane gas	1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] and 5-carboxy-tetramethylrhodamine N-succinimidyl ester (5-TAMRA)	Emulsion method	Targeting molecules to posterior regions of the vitreous humor	72
8	Development of novel ST68/PLA-PEG stabilized ultrasound nanobubbles for Potential tumor imaging and theranostic	Perfluoro propane gas	PLA-PEG-NH2 block copolymers	Centrifugation	Tumour Imaging and Theranostic	73

Since the Laplace pressure is inversely proportional to the size of a bubble, higher pressure values would be given to the smaller bubbles. The bubbles shrink and Laplace pressure increases as the inner gas exits the core, powered by the pressure gradient, thus increasing the rate of gas dissipation and the subsequent bubble shrinkage before the device fails. Since the surface tension creates a pressure that drives the dissolution of the bubble by gas diffusion, the dissolution rate of the bubble in vivo depends primarily on the pressure of Laplace [**18,5**]. The experimental results have proved that the nanobubbles can protect drugs like apomorphine from degradation. A possible drug-targeting effect of nanobubbles has been demonstrated by the sustained *invitro* drug release which increases upon insonation [**14**]. On the basis of the aforementioned premises, the formulation requirements include separate technical methods for the stability of nanobubbles, including the inclusion of interface surfactants, the reduction of the Laplace pressure differential, the restriction of the diffusion of gases and the regulation of the interfacial structure [**5**]. Due to the surface tension effect that produces a strain for the gas dissolution and affects the stability and preparation of an NB, gas diffusion between two bubbles is linked to Ostwald ripening [**3**].

The stability of NB depends not only on the structure of the interface surfactant and polymers, but also on the size and low-density gas in its centre, which are then stabilized by coating materials such as lipid and synthetic polymer [**3**]. At the interface influencing the bubble, the form of gas phase could act as a cosurfactant. The effect of fluorocarbon gases on the properties of phospholipid monolayers was theorized by Krafft *et. al.,* [**5**]. The nanobubble remained stable in liquids for long periods at a high concentration due to the negatively charged surface and high internal pressure, while macrobubbles (> 50 m in diameter), increase in size and rapidly burst on the surface of liquids [**54,55**]. Researchers are proposing various hypotheses that indicate the stability of nanobubbles [**43**].

Table 2. Various drug, gene and oxygen delivery through nanobubble.

Drug	Shell	Core	Conclusion	Reference
Coumarin-6	Tween 80 1	sulphur hexafluoride	nanobubble formulation is a promising approach for both ultrasound imaging and drug delivery enhancing.	(28)
Doxorubicin	poly(lactic-co-glycolic acid) (PLGA)	carbon tetrafluoride	Doxorubicin nanobubble can be used as ultrasound contrast agent to enhance tissue imaging.	(29)
Gene delivery	Chitosan	perfluoropentane	chitosan nanobubbles have the potential to be promising tools for ultrasound-mediated DNA delivery	(30)
Oxygen delivery	Polysaccharid 2	Perfluoropentan	The oxygen-filled nanobubble formulations might be proposed for therapeutic applications in various diseases	(31)
Oxygen delivery	Lecithin	Oxygen	Reducing Tumour Hypoxia via Oral Administration of Oxygen Nanobubbles	(32)
biotinylated rabbit-IgG	1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)	Perfluoropropane	enhancing macromolecular permeation through layers of the retina	(33)
Paclitaxel	PLGA	Perfluoropropane	PTXUSPIO-HER-NBs have potential as a multimodal contrast agent and as a system for ultrasound-triggered drug release in breast cancer.	(34)
Pluronic	Lipid and Pluronic-shelled	perfluoropropane	can serve as an effective theranostic method for sensitization of tumors to radiofrequency ablation.	(35)
Gene transfection	1,2 distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2 distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino-(polyethylene glycol)-2000] (PEG2000-DSPE)	perfluoropropane	mechanical agitation method is a useful alternative for the development of stable NBs that can be used efficiently for in vivo gene transfection	(36)
mitomycin-C	sodium carboxymethylcellulose	oxygen	enhance the efficacy of localization and targeting for reverting hypoxia in NMIBC (non-muscle invasive bladder cancer tumors)	(37)
Methotrexate	poly(lactic-co-glycolic acid) (PLGA)	Perfluorocarbon	methotrexat-loaded nanobubbles as a targeted drug carrier, an efficient ultrasound contrast agent, as well as a synergistic agent for HIFU (High intensity focused ultrasound) ablation of choriocarcinoma	(38)
Herceptin	Phospholipid	octafluoropropane	Targeted delivery of therapeutic drugs or genes.	(39)
camptothecin	DSPE-PEG2000	Perfluorobutane	safe and efficiently drug delivery system for specific cancer treatment.	(40)
Apatinib (23)	DSPC and DSPE-PEG2000	perfluoropropane	GPC3-targeted and apatinib-loaded nanobubbles can be considered a novel chemotherapeutic approach for treating liver cancer in combination with ultrasound.	(54)
Apomorphine (24)	Hydrogenated soybean phosphatidylcholine (SPC, Phospholipon1 80H)	Perfluoropentane	Apomorphine-loaded perflurocarbon nanobubble showed promising stability and safety. They were successful in sustaining apomorphine delivery.	(14)
pDNA (pCMV–luciferase) (51)	Distearoylphosphatidylcholine (DSPC)	perfluoropropane	effective and safe intraperitoneal gene transfection using BLs with US irradiation in mice	(33)
Paclitaxel (12)	DSPC:DSPE	sulphur hexafluoride	the potential of PSPLBC as a promising noninvasive, pro-apoptotic, smart DDS for US-responsive, image-guided cancer therapeutics	(50)

Method of preparation of nanobubble

Sonication, high shear emulsification, mechanical agitation, micro fluidic method, and inkjet techniques that have also been used in the preparation of microbubbles are the major preparations of nanobubbles [**42**].

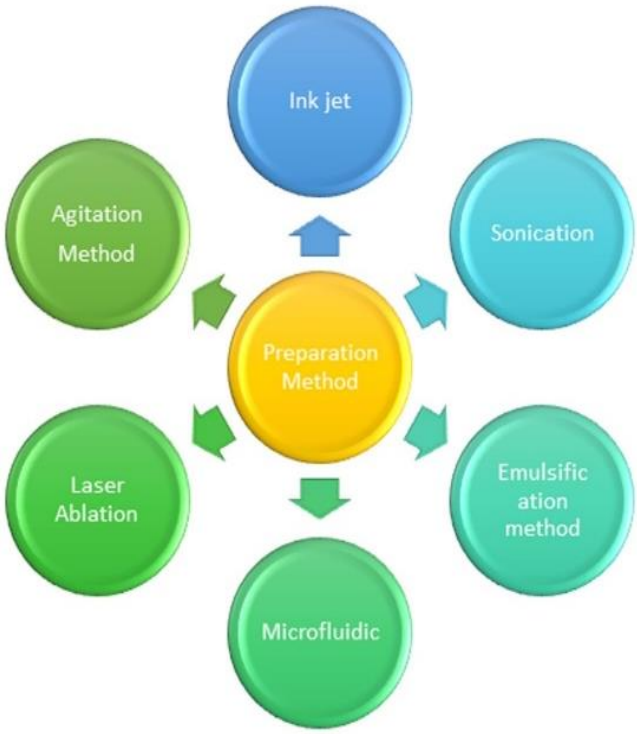


Fig 1. Method of Preparation of Nanobubbles

Sonication (28) (12)

In this process, using high intensity ultrasound, the gas or liquid is spread in a suspension of a suitable coating material. This process requires the emulsification of gas or liquid to create a suspension of micro-droplets / bubbles on the surface of which a coat of protein or surfactant, for example, is naturally adsorbed. Ultrasound of a mixture of Span 60 and polyoxyethylene 40 stearate (PEG40S), accompanied by differential centrifugation, results in the creation of a biocompatible, unimodally dispersed, nano-sized bubble population [**5**].

Microfluidics techniques

Recently, research studies have been performed into the preparation of nanobubbles using microfluidic devices, as microfluidics allow precise control of bubble diameter and production rate via the interaction of gas pressure, liquid flow rate and geometry of the system [**88,89**] (5).

Emulsification method (23)

Generally, to synthesise polymer shell NB, this procedure is used. Water is applied to an oil emulsion with a carrier polymer in the traditional synthesis process, and this emulsion is further emulsified into a large amount of water. In order to have a solid polymer shell, the solvent is evaporated or drained, and lyophilized shells are refilled with core gas, such as PFCs [**47**]. For the synthesis of NBs with a wider size spectrum, a high-shear emulsification approach was used [**48**]. To produce NBs with a narrow size distribution, a membrane emulsification approach may be used**.** For this reason, a porous membrane is used. Bubbles infiltrate and spread through the membrane surface into a continuous process. To avoid coalescence, emulsifiers are added [**48**].

Agitation method (36)

Mechanical agitation should be performed to boost the interaction between the liquid phase comprising surfactants and the gas phase during the preparation of MBs or NBs (36). By agitating the liquid solution at several thousand oscillations per minute in a shaker, lipid shells containing NB can be formed. With a random size distribution [**22,81**], this will create bubbles. The bottle is filled with the desired coating substance in the liquid stage in order to encapsulate a given gas in an MNB and the gas is perfused from the surface and then the bottle is mechanically agitated such that the desired gas is encapsulated by the shell material [**82**]. A promising technique for producing MNBs on an industrial scale is mechanical agitation [**29,41**].

Ink-Jet method

Microbubble synthesis has been carried out using an ink-jet process in which, depending on the application, a polymer solution is pushed through a piezo-driven ink-jet nozzle of a desirable scale. In the solution, the piezoelectric crystals produce pulses and the bubbles which are produced are extracted from the nozzle [**17,57**]. Using a high-pressure flow through the nozzle [**80**], a related approach was also used to produce ultrafine oxygen nanobubbles from pure water and an oxygen source.

Microfluidics techniques

Microfluidic instruments provide managed size distributions to the MNBs. The flow rate, friction, viscosity of the liquid solution and the orifice size of the system will affect the size and distribution of the MNBs. Around [**22,57**].

Laser ablation method

A stochastic process that can produce MNBs is also the process of laser ablation. A precise wavelength excimer laser may be concentrated on particles of aluminium oxide in water, which then become oxidised nanoparticles. Bubbles will also be produced at the solid – liquid interface during the process. Aluminum oxide nano-clusters [**57**] are used to stabilise the bubbles / interface.

Table 3. Characterization of nanobubble.

Parameter	Characterization	Ref
Bubble Size (9)	Dynamic Light Scattering Using Photon Correlation Spectroscopy (PCS) At 25 °C	**9**
Zeta Potential	Zeta Plus Analyzer Surface Potential Cytometry	**9, 29**
Magnetic Properties of Magnetic Nanobubble	Measured by Using Vibrating Sample Magnetometer (Vsm)	**34**
Structure of Nanobubble	Scanning Electron Microscopy (SEM) Transmission Electron Microscopy (TEM)	**34**
Measure Dissolved Oxygen (46)	Electrochemical Sensing, Fiber Optic-Based Sensing, And Fluorescence Quenching	
Bubble Concentrations (56)	Hemocytometer	

Typical applications of nanobubble

***Nanobubbles (NBs) opened a new field of ultrasound imaging* [13]**

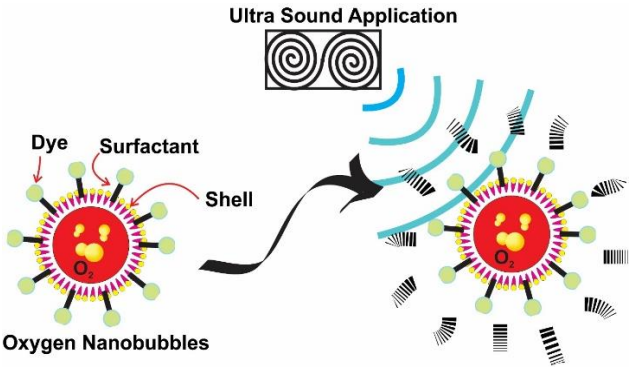


Fig. 2. Schematic Presentation of ultrasound and nanobubbles.

Nanobubbles may be moved from blood vessels into surrounding tissues via the improved permeability and preservation (EPR) effects and imaged by ultrasound after aggregation [**13**]. To improve the backscattered signal, ultrasound contrast agents are used, enhancing resolution [**22**]. An impedance imbalance between the surrounding medium (blood and soft tissue) and the agent (gas) is the core concept behind all ultrasound contrast agents [**22**].

A recent trend for drug delivery to solid tumours has been the ultrasound (US)-targeted nanobubble destruction (UTND) strategy [**16-19,23**].

UTND has several major benefits relative to other drug delivery technologies. First of all, by updated emulsification methods, nanobubbles (NBs) are conveniently prepared [**20**] and used to visualise tumours as US contrast agents. In addition, acoustic cavitation, inducing cell membrane permeabilization and enhancing drug uptake by tumour cells can be caused by NBs in conjunction with US. In specific, prior studies have paid attention to non-targeted NBs that are readily accumulated in the reticuloendothelial system, resulting in lower tumour-site drug concentrations. It is necessary to create targeted and drug-loaded NBs, carrying tumor-specific ligands such as antibodies and peptides, to improve therapeutic effectiveness and minimise systemic toxicity [**23**].

Two key ultrasound results, including cavitation and sonoporation, typically influence the delivery of medications to organs and tissues. The effect of cavitation results in the decrease of the size of the bubble, while the effect of sonoporation contributes to the reduced bubble being consumed [**3**].

Oxygen delivery (41)

Fig. 3. Oxygen delivery via Nanobubbles.

Hypoxia, i.e., a drop in the concentration of dissolved oxygen below physiologically normal levels, has been described as playing a vital role in the development of many disorders, including certain cancer types [**59**]. The administration of therapeutic gases, as recently reviewed by Borden *et. al.*, [**70**], has been a subject of increasing concern. Because many medical problems, such as asthma, burns, bedsores and wounds, are associated with inadequate supply of oxygen to the tissues, much study has focused on oxygen delivery. In comparison, oxygen deprivation is also the primary characteristic of cancerous solid tumours, along with acidosis. In addition, targeted oxygen delivery could be a useful adjuvant for the treatment of anaerobic infections with antibiotics. Therefore, in addition to other oxygenation methods, such as the use of microbubbles, nanosponges or echogenic liposomes, these are possible fields of use of oxygen-filled nanobubbles [**56**].

Drug, gene, gas loading

Gases, small molecules and macromolecules, either hydrophilic or lipophilic, may be filled with nanobubbles. [**5**].

Drugs may be encapsulated within the heart, or they may be embedded within the nanobubble shell or just underneath it. Additionally, another approach to packing is encapsulation of the drug in a nanoparticle eventually added to the bubble surface [**5**].

The primary goal of loading NBs with medications and genes is to mitigate the side effects associated with these bioactive agents, as well as to boost the therapeutic effectiveness by reducing the appropriate dose and decreasing the intervention at the target site [**49**].

For both passive and active targeting, NBs can be used. Passive targeting refers to the NBs' propensity to aggregate owing to the leaky vasculature at tumour sites. Enhanced permeability and retention (EPR) is also described as this effect. The vasculature of the tumour is abnormal and contains large pores within the 300-700 nm

region. NBs have the advantage of EPR in this size range. In the EPR effect, physical characteristics of NBs such as elasticity, porosity, surface charge, size and shell structure and their association with the tumour microenvironment play a major role. Higher EPR would result in higher medication penetration, improved biodistribution, and greater drug bioavailability, resulting in greater efficacy and better care. Due to endocytosis, greater cellular absorption of NBs makes them ideal for drug delivery applications.

For active targeting by adding some targeting ligands, surface modification of MNBs is important. By adding bioactive molecules to the shell of the NBs (41), this can be accomplished. By integrating targeting ligands such as biomarkers, antibodies, polysaccharides, or other active biomolecules into MNBs, targeting MNBs can be generated **(64).**

The functionalization of the NBs can be extended to three techniques.

Next, NBs can be synthesised in the shell or inside the heart of the NBs with biomolecules / bioactive substances added. Hydrophilic and amphiphilic biomolecules can be inserted into the shell, while the centre of NBs can be filled with hydrophobic drugs [**12**]. The ability of drug loading depends on the type of shell used [**29**]. Thin phospholipid shells are more echogenic and hydrophilic molecules are more desirable, whereas thick polymer shells are favoured in the heart for hydrophobic drug loading [**60,63**].

Secondly, covalent and non-covalent methods may be applied by adding targeting ligands to the enzyme, polymer, or lipid-based shells to functionalize the NBs. This approach is useful for selective delivery of hydrophilic drugs [**62,61**]. Biotin-avidin bonds can be integrated into NBs for antibody and protein binding [**60**].

To apply electrostatic interactions for gene distribution, NB shells can be made cationic [**30**]. This technique promotes gene therapy and the use of NBs to increase selective gene delivery has been selective by numerous researchers [**30**]. Finally, it is possible to co-administer NBs with bioactive substances, using high-intensity ultrasound to improve cell permeability for greater bioactive molecular absorption [**35,41**].

The lipid bilayer of the liposomes provides sufficient space for hydrophobic interactions, resulting in high hydrophobic drug encapsulation quality. Therefore, as the drug-carrier, liposomes were selected [**12**].

Gene delivery

One of the most intriguing issues of nanomedicine is the development of nonviral gene delivery systems (30) Nanoscale systems were initially developed as contrast agents and were only jointly tested of drug and gene delivery relevance [**22,25,26**] Gene therapy, a potential therapeutic alternative for the treatment of inherited or non-inheritable diseases, is focused on the power to incorporate n A big barrier to gene transfer is that, because of their negatively charged phosphate units, giant sizes and hydrophilic existence, naked nucleic acids do not appear to be easily concerned by cells. In addition, they undergo nuclease-mediated degradation present in the blood. A variety of gene delivery vectors have been created, falling into either viral or non-viral categories, to address these limitations. For the site-specific distribution of genetic material, nanobubbles offer a promising non-viral strategy; this is due to their ability to be 'activated' in the presence of ultrasound (US) and to mediate the distribution of DNA to specific cell targets. (8) There has also been a summary of localised gene transmission using nanobubbles and a dual strength ultrasound device [**10**].

Toxicity and immunogenicity are the key drawbacks of viral vectors used for gene transduction [**57**]. As a result, non-viral vectors have drawn a lot of interest as gene carriers to address these issues, but their transduction performance is very poor, although several attempts have recently been aimed at improving this feature [**58**]. Liposomal nanobubble demonstrated the effective and safe ip gene transfection using BLs with US irradiation in mice [**51**].

Polyethylene glycol (PEG)–liposomes with a US contrast gas, called ‘‘liposomal nanobubbles’’ (bubble liposomes; BLs), have been developed as nanosized gene transfection agents (Suzuki et al., 2008). Kodama et al. showed that perfluoropropane gas was trapped within the BLs (Kodama *et. al.,* 2010). BLs are more pharmaceutically stable than microbubbles due to the smaller particle size of PEGylation, gene transfection by BLs with US irradiation is supposed to be a useful technique. In addition to PEGylation, BLs with targeting ligands are also readily modified. Gene delivery systems with US irradiation using BLs improve the efficacy of gene transfection at targeted sites, such as the liver, kidney and tumours. (Un *et. al.,* 2010; Kurosaki *et. al.,* 2014; Suzuki *et. al.,* 2015). Under optimal condition of US intensity and irradiation time, highly efficacious, long-term transgene expression has been achieved (Kurosaki *et. al.,* 2014).

Hydration of a lipid film, freezing in the presence of mannitol, lyophilizing, and rehydration are established methods for incorporation of gas within liposomes [**52**].

The study showed that the prepared aqueous solutions of free gas nanobubbles established a special controllable preparation technique for the processing of lipid encapsulated nanobubbles. The findings revealed that the composition of nanobubbles with a diameter of around 200 nm was multilayer lipid encapsulation due to lipid assembly on the free bubble surface. These gas-filled ultrasound-sensitive liposomes (GU-Liposome) will be useful for extravascular ultrasound imaging and drug delivery in the future due to the gas core and multilayer lipid loading potential [**6**].

Conclusion & future prospective

The nanobubbles has proved its efficacy in many fields. These gasses filled bubbles are high performing material in diagnosis and drug delivery. The structural versatility of nanobubbles allows effective incorporation with a high payload of several active molecules, as it were, therapeutic gases, drugs, genes and biological molecules. The delivery of gaseous drugs can be facilitated by this novel nanotechnology-based solution which provides tremendous therapeutic effects as discussed in the oxygen delivery section. Many more applications of this nanocarrier provides an innovative multifunctional drug delivery platform that is suitable for a scope of therapeutic applications and administration routes. Moreover, US can be conveniently added to overcome biological barriers, such as the blood–brain barrier. A small particle size is an essential prerequisite for ultrasound contrast enhanced agents that penetrate tumor blood vessel pores to allow for targeted imaging and therapy Nanobubbles, in our view, would play an important role in future applications of nanomedicine. In order to produce the therapeutic molecule on-demand in the field of personalised nanomedicine, nanobubble physico-chemical properties could be modified to build smart or intelligent structures that are sensitive to endogenous stimuli. The nanobubbles has potential to cross the BBB and hence can be a theranostic agent in the future medicine. It has also ability to reach the posterior eye and hence can be utilized in the treatment of the eye related disorders/diseases. The complicated diseases like cancer can be diagnosed and treated with the nanobubbles.

Conflicts of interest

Authors declare that they have no conflict of interests.

Keyword

Nanobubble, oxygen delivery, ultrasound, theranostic.

Received: 05 May 2020
Revised: 29 October 2020
Accepted: 27 November 2020

References

1. Bhatiya, Saurabh; *Nat. Polym. Drug Deliv. Syst.* **2016**.
2. Noriyoshi, A.; Takahiro, K.; Toshikazu, E.; *Phys. Rev. E*, **2017**, *7*, 2253.
3. Ying, Z. Z.; Li, N. D.; Cui, Tao L. Y.; Shu, P.G., **2013**, *8*, 1621–1633.
4. Roberta, C.; Marco, S.; Monica, A.; *Ther. Deliv.*, **2016**.
5. Roberta, C.; Agnese, B.; David, L.; *Ijpharm*, **2013**, *456*, 437.
6. Sachiko, H.; Yukiko, W.; Rui, C.; Shiro, M.; Yashiro, M.; Tetsuya, K.; *Ultrasound Med. Biol*, **2010**, *36*, 1867.
7. Ekaterina, Y.; Lukianova, H.; Xiaoyang, R.; Debra, T.; Xiangwei, W.; Michael, E. K.; Dmitri, O. L., *Theranostics*, **2012**, *2*, 976.
8. Johan, U.; Misturu, H.; *Adv. Drug Deliv*, **2014**, *72*, 144.
9. Haleen, Dewitte; Sandra, V.L.; Carlo, H.; Kris, T.; Stefaan, C.; De, S.; Karine, B.; Ine L; *J. Control. Release*, **2014**, *194*, 28.
10. Hugh, D. V.; Soumya, R.; *J. Appl. Physiol*, **1997**, *82*, 2045.
11. James, R. T.; Seddon; Detlef, L.; *J. Phys.: Condens. Matter*, **2011**, *23*, 133001.
12. Miller, J.D.; Yuehua, H.; Veeramasuneni, S.; Yongqiang, Lu; *Colloids and Surfaces A: Physicochem. Eng. Aspects*, **1999**, *154*, 137.
13. Brian, E; Margaret, A.W.; *Ultrasonics*, **2004**, *42*, 343.
14. Tsong, H.; Yin, K.; Chin, H.; Huang, T.S.; Jia, Y.F.; *J. Pharm. Sci*, **2009**, *98*, 3735.
15. Ryo, S.; Yusuke, O.; Naoki, U.; **2010**, *77*, 430.
16. Wang, Y.; Xiang, L.; Yan, Z.; Pengyu, H.; Yuhong, X.; *ijpharm*, **2009**, *384*, 148.
17. Mingming M.; Jie G.; Chongchong W.; Xuan Z.; Xuefeng Z.; Xiangchun L.; Hong L.; Canghai W.; Hui S.; Kuiliang L.; Yadan W.; Xinying X.; Jing W.; *Tumor. Biol*, **2016**, *37*, 8673.
18. Roberta, C.; Agnese, B.; Michele, T.; Monica, A.; Andrea, C.; Manuela, D.; David, L.; *Int. J. Nanomedicine*, **2012**, *7*, 3309.
19. Joshua, O.; Conor, M.; Heather, N.; Phurit, B.; Raymond, A.; Mark, B.; Anthony, P. M.; John, F. C.; Eleanor, S.; *PLoS One*, **2016**, *11*, 2.
20. Weixiang, S.; Yindeng, L.; Yajing, Z.; Xinjie, L.; Jiannong, Z.; Jie Luo, Q. Z.; Haitao, R.; Zhigang, W.; Dajing, G.; *Nanomedicine*, **2017**, *12*, 991.
21. Reshani, H. P.; Luis, S.; Hanping, W.; Mihika, G.; Eric, S.; Christopher, H.; Pubudu, M. P.; Ann, M. B.; Agata, A. E.; *Pharm. Res*, **2014**, *31*, 1407.
22. Rodi, A.; Shigeru, K.; Johan, U.; Yuriko, H.; Ryo, S.; Kazuo, M.; Fumiyoshi, Y.; Mitsuru, H.; *Drug Deliv*, **2017**, *24*, 320.
23. Qiongchao, J.; Shaoyun, H.; Xiaoyun, X.; Jiyi, Y.; Bing, O.; Zizhuo, Z.; Fengtao, L.; Xin, P.; Baoming, L.; Hui, Z.; *Breast Cancer*, **2015**, *23*, 445.
24. Xiangyang, X.; Wen, L.; Hui, L.; Jianping, D.; Ying, C.; Hong, L.; Xudong, F.; Yang, Y.; *Drug Deliv*, **2015**, *23*, 423.
25. Muhammad, S.K.; Jangsun, H.; Kyungwoo, L.; Yonghyun, C.; K Hyung-J.K.; Jong, W.H.; Jonghoon, C.; *Molecules*, **2018**, *23*, 1.
26. Eleanor, S.; Mohan, E.; *Soft Matter*, **2012**, *4*, 2350.
27. Yujin, S.; Guangyuan, X.; Yaoli, P.; Wenching, X.; Jie, S.; *Colloids Surfaces A Physicochem. Eng. Asp*, **2016**, *495*, 176.
28. John, L. P.; Per, M.C.; *J. Phys. Chem.*, **1994**, *98*, 8468.
29. Evan, C. U.; Thomas, P.; William, C.; Rachel, L.; Terry, M.; Reena, Z.; *Adv Drug Deliv Rev.*, **2004**, *56*, 1291.
30. Ronald, X.X.; *Contrast Media Mol. Imaging*, **2011**, *6*, 401.
31. Mina, L.; Eun, Y.; Daeyeon, L.; Burn, J. P.; *Soft Matter*, **2015**, *11*, 2067.
32. Dijknas, P. A.; Juffermans, M.; Mustars, R. J., Wanol, A. V., Ten, F. J.; w. van G.; Visser, C. A.; Jong, N.; *Eur. J. Echocardiogr.*, **2004**, *5*, 245.
33. Koyo, N.; Shintaro, F.; Yuki, F.; Masayori, H.; Kazuo, M.; Shiger, K.; *Drug Deliv*, **2017**, *24*, 737.
34. Nguyen, T.; Steven, P. W.; *Nanomed Nanobiotechnol*, **2014**, *6*, 316.
35. Noriaki, M.; Shingo, I.; Takuji, I.; Osamu, N.; Motohiro, T.; Yoshihito, U.; Takami, Y.; *Eur Biophys J*, **2012**, *41*, 571.
36. Masayoshi, T.; Kaneo, C.; Pan, L.; *J. Phys. Chem. B*, **2007**, *111*, 1345.
37. Siddhesh, D. P.; David, G.R.; Diane, J.B.; *AAPS J.*, **2005**, 7.
38. Gregg, L.S., *N. Engl J. Med.*, **2012**, 365.
39. Samantha, M.F.; Mark, A. B.; Paul, A. D.; **2015**, *209*, 139.
40. Fabian, K.; Stanley, F.; Patrick, K.; Wiltrud, L.; Twan, L.; *J. Nucl Med*, **2012**, *53*, 345.
41. Alexander, L.K.; *Med Biol Eng Comput*., **2009**, *47*, 875.
42. Christian, R. M.; Nicolas, A. G.; Hugo, A. K.; Raffi, B.; *Expert Opin. Drug Deliv*, **2008**, *5*, 1021.
43. Cheery, C.C.; Mark, A. B.; *Biomaterials*, **2011**, *32*, 6579.
44. Gross, S.A.; Frizzell, L. A.; Dunn, F.; *Ultrasound in Med. & Biol*, **1979**, *5*, 181.
45. Alessandro, Z.; Massimo, P.; Diletta, L.; Guido, P.; Atim, M.; Elisa, N.; Simona, C.; Francosco, F.; Paolo, S.; Carlo, F.; Gabrile, M.; *Gastroentology*, **2010**, *138*, 1931.
46. Anthony, D.; Michiel, P.; Nathalie, M.; Patrick, M.; Chantal, P.; *Ther Deliv*., **2012**, *3*, 1199.
47. Jia, L.; Zhang, B.; Maotong, L.; Me; ijun Z.; Fei, L.; Xiuxian, H.; Min, P.; Xue, L.; Fei, Y.; *PLoS One*, **2017**, *12*, 1.
48. Tianyi, M. K.; Luis, S.; Robin, E.; Wil, S.; Hanping, W.; Nami, A.; Agata, A.E.; *Mol Pharm*, **2009**, *7*, 59.
49. Muidh, A.; Jing, Q.; Marie, J.; Vincent, S. J.; *Langmuir*, **2016**, *32*, 11086.
50. Rajeet, C.; Rinti, B.; *Sci. Rep.*, **2018**, *8*, 2624.
51. Sachin, S. T.; Micheal, S. W.; Amirali, P.; Nicole, B.; Flemming, M. P.; Nigel, L. B.; Harendra, S. P.; *PLoS One*, **2017**, *5*, 1.

52. Hozo, M.; Kosuke, E.; Hiroyuki, T.; Makoto, H.; Toru, I.; Takaaki, N.; Koji, S.; Shunsuke, N.; Tsuyoshi, M.; Hideki, Y.; *Int. J. Mol. Sci*, **2018**, *19*, 1395.
53. Xuemei, Z.; Yuanyi, Z.; Zhigang, W.; Shuai, H.; Chenn, Y.; Jiang, W.; Hua, Z.; Mingxia, D.; Qingshu, L.; Xioqui, X. L.; Zhibiao, W.; *Biomaterials*, **2014**, *35*, 5148.
54. Yuhang, T.; Zhao, L.; Zhang, L.; Zhang, J.; Xue, H.; Qiucheng, W.; Wen, C.; *Onco Targets Ther*, **2018**, *11*, 4785.
55. Tinghui, Y.; Ping, W.; Rongqin, Z.; Bowen, Z.; Cheng, D.; Xinling, Z.; Xintao, S.; *Int. J. Nanomedicine*, **2012**, *7*, 895.
56. Tianliang, L.; Zhou, J.; Chunlei, Z.; Xiao, Z.; Jiaqi, N.; Hualin, F.; Song, J.; Daxiang, C.; *NPG Asia Mater*, **2018**, *10*, 1.
57. Pushpak, B.; Gloriia, N.; Craig, J. G.; Joseph, I.; *Sci. Rep.*, **2018**, *8*, 3112.
58. Jilai, T.; Fang, Y.; Huating, C.; Ying, Z.; Xiaobo, R.; Ning, G.; *ACS Appl. Mater. Interfaces*, **2015**, *7*, 26579.
59. Federica, B.; Peter, B.; Agata, A. E.; Alessandro, B.; *Bioconjug. Chem.*, **2008**, *29*, 234.
60. R.cavalli, A.; Bisazza, A.; Glustetto, A.; Civra, D.; Lembo, C.; Trotta, M.; Trotta, P.; *Int. J. Pharm.*, **2009**, *381*, 160.
61. Erhua, Y.; Jinghong, C.; Xiaofang, Z.; Yinyan, Z.; Xianheng, W.; Fei, H.; Hecheng, H.; Ping, L.; Jianmin, L.; Fasheng, W.; Lianxing, L.; *Biomed Res Int*, **2018**, 1.
62. Kyuichi, Yasui; Toru, Tuziuti; Wataru, Kanematsu; *Ultrason. Sonochem.*, **2018**, *48*, 259.
63. Dmytro, Rak.; Michaela, Ovadova.; Maria´ n Sedla´ k´; *J. Phys. Chem. Lett.*, **2019**, *10*, 4215.
64. Ananda, J. Jadhav; Mostafa, Barigou; *Langmuir*, **2020**, *36*, 1699.
65. Keiji, Yasuda a; Hodaka, Matsushima a; Yoshiyuki, Asakura; *Chem. Eng. Sci.*, **2019**, *195*, 455.
66. Cheng, Bingbingm; Bing, Chenchenm; Chopra, Rajiv; *Sci. Rep.*, **2019**, *9*, 1.
67. Jiaqi, Zhang; Yihan, Chen; Cheng, Deng; Li, Zhang; Zhenxing, Sun; Jing, Wang; Yali, Yang; Qing, Lv; Wei, Han; Mingxing, Xie; *Front. Pharmacol*, **2019**, *10*, 1.
68. Lin, Song; Guohao, Wang; Xuandi, Hou; Shashwati, Kala; Zhihai, Qiu; Kin, Fung Wong; Fei, Cao; Lei, Sun; *Acta. Biomater*, **2020**, *108*, 313.
69. Jinfeng, Liu; Yihan, Chen; Guohua, Wang; Qing, Lv; Yali, Yang; Jing, Wang; Pingyu, Zhang; Jie, Liu; Yu, Xie; Li, Zhang; Mingxing, Xie; *Biomaterials*, **2018**, *162*, 200.
70. Owen, J.; McEwan, C.; Nesbitt, H.; Bovornchutichai, P.; Averre, R.; Borden, M.; McHale, A.P.; Callan, J.F.; *PLoS ONE*, **2016**, *11*, 168088.
71. Yücel, Başpınara; Gülşah, Erel-Akbabab; Mustafa, Kotmakçıa; Hasan, Akbaba; *Int. J. Pharm*, **2019**, *566*, 149.
72. Xiangyu, Zhang; Mingcong, Wu; Yu, Zhang; Jie, Zhang; Jin, Su; Chunrong, Yang; *Colloids Surfaces B Biointerfaces*, **2020**, *189*, 110861.
73. Sachin, S. Thakura; Ying-Shan, Chenb; Zachary, H. Houstonc; Nicholas, Fletcherc; Nigel, L. Barnettd; Kristofer, J. Thurechtc; Ilva, D. Rupenthalb; Harendra, S. Parekh; *Eur. J. Pharm. Biopharm*, **2019**, *136*, 102.
74. Mengmeng, Shanga; Kai, Wangb; Lu, Guoa; Sujuan, Duana; Zaijun, Lub; Jie Lia; *Ultrasonics*, **2019**, *99*, 105947.