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Nanobubbles: Fundamentals and recent drug delivery applications

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***Abstract*---**The emerging branch in pharmaceutical sciences known as pharmaceutical nanotechnology presents new tools, opportunities and

scope, which are expected to have significant applications, in disease diagnostics and therapeutics. There is a growing interest in nanobubble (NB) technology because of its wide range of potential applications in theranostics and targeted drug delivery including anticancer drug delivery, antibiotic delivery, gene delivery etc. Nanobubbles could offer several features in anticancer drug delivery, improving cellular uptake of chemotherapy drugs into cancer cell lines. Nanobubbles opened a new field of ultrasound imaging and were used as a diagnostic method. Generally, the delivery of drugs to organs and tissues is affected by two main ultrasound effects including the cavitation and sonoporation effects. This review describes the history of nanobubble, nomenclature, stability of nanobubble, physicochemical properties, characterization of nanobubble, method of preparation and applications. This article aims at highlighting the most recent and promising research trends including oxygen nanobubble, silica nanobubble and pluronicnanobubble and enlist the various drug delivery approaches involving the application of nanobubbles.

***Keywords*---**anticancer, cavitation, diagnostic tool, nanobubble, ultrasound imaging.

Introduction

Nano word is started from Latin word, which implies dwarf. The perfect size range offered by nanotechnology refers to one thousand millionth of a particular unit in this manner nanometer is one thousand millionth of a meter (i.e. 1 nm = 10^{-9} m)(Bhandari et al., 2018a). Nanobubbles (NB) are nanoscale cavities filled with vapour or any gas in liquid and have received growing attention due to their high potential for applications in various scientific and engineering fields(Arai et al., 2017). NBs are either made by heterogeneous nucleation (within two interphases (solid/liquid/gas) or prepared homogeneously under atmospheric conditions in the presence of gas and may also be generated by the coalescence of vacancies in the diameter less than 1μm.Experimental studies report that NBs majorly form on a hydrophobic solid surface which can alter interfacial properties such as lubrication surface forces, adsorption and thus stabilize the colloidal particles. These NBs are experimentally produced by pressure release, heating, solvent exchange and water electrolysis(Ayodele et.al,2017).

A major disadvantage of microbubbles as drug delivery systems is their moderately extensive size (1–10 μm), which is an issue for microbubbles which needs to penetrate through the epithelial cells of the vasculature to the target tissue. On intravenous injection, microbubbles are infused into the circulation and eventually become trapped in the lungs where gas exchange occurs. Therefore, drug-loaded microbubbles are mainly restricted to cardiovascular targets and tumour endothelium.To overcome this limitation, NBs with sizes smaller than 1 μm have been developed(Zhao et al., 2013b).NBs might be endowed with enhanced stability and longer residence time in the systemic circulation(Cavalli et al., 2016b).

Gas bubbles usually comprise with gas core and stabilizing shells. The high molecular weight and low-solubility filling gas such as sulphurhexafluoride (SF_6) or perfuoropropane (C_3F_8)are chosen as the gas component in the majority of ultrasound contrast agents (UCAs), which exhibit less susceptibility to outward loss than air. Coating materials of bubbles are always composed of lipid, polymer and/or protein since all these materials are assured of safety when intravenously administered. Phospholipids or proteins are often chosen as the thin soft shell of the bubbles, which is more flexible and highly sensitive to acoustic waves than the hard shells of the cross-linked or entangled polymers. Among them, the lipid shell offers the excellent characteristics of easy expansion, rupture, reseal, compress, buckle, or respread under ultrasound exposure. The formulations of commercially available UCAs, such as Definity, Imagent, Sonovue, and Levovist are all mainly composited by lipids. However, almost all the commercial available UCAs have diameters on the micrometer scale and, thus, are restricted to just enhance the visibility of blood vessels and cannot enter surrounding tissues or cells. Besides, microsized bubbles often exhibita short circulation half-life and are easily arrested by the liver and spleen. Nanobubbles, with sizes less than 1 μm, may be expected to have some priority in ultrasound molecular imaging. Through the enhanced permeability and retention (EPR) effects, nanobubbles could be transferred from vessels into surrounding tissues even cells to be potentially imaged by ultrasound after accumulation, which triggers researchers' great interest to develop nanoscaled bubbles for early diagnosis of extravascular lesions (Brutin et. al, 2015).More recently, they have also been studied concerning the drug, gene and gas delivery. Generally, NB can be called differently based on their size and position at the interface which is illustrated below in Figure 1.

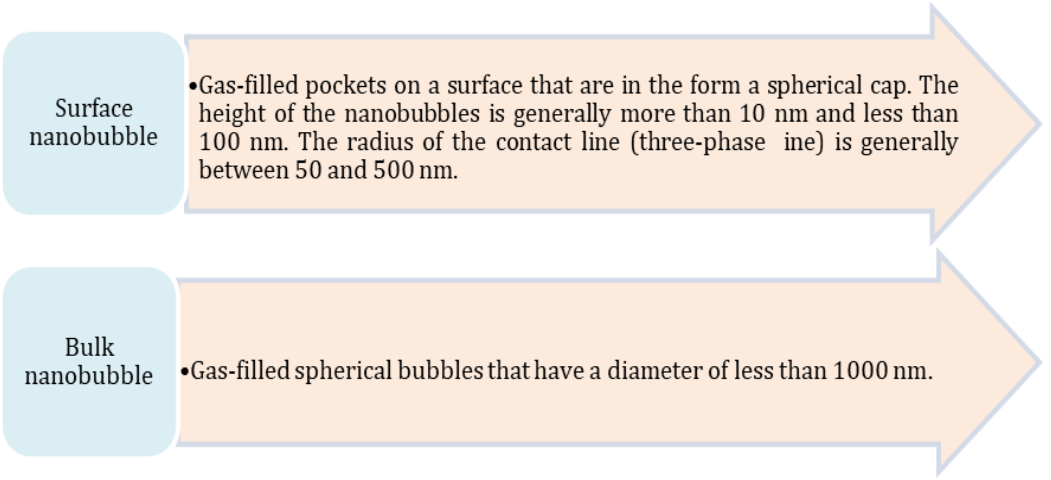

Figure 1. General nomenclature of the nanobubble

History of Nanobubbles

The first report of the presence of nanobubbles appeared in1994. The relationship between the force and the separation oftwo hydrophobic surfaces immersed in the liquid was studied and they found that the surface attraction as a function of separation presented clear steps.The brief development of the nanobubbles has been shown in Table 1(Alheshibri et al., 2016).

Table 1. Historic development of the Nanobubbles

Sr no.	Historical Development	Reference
1	The existence of nanobubblesis dismissed based on the expected lifetime.	(Hobbs et al., 1998)
2	The existence of surface nanobubbles was provided, using Fourier Transform Infrared spectroscopy (FTIR) also investigated the effects of wettability on nanobubbles.	(Miller et al., 2002)
3	The first images of surface nanobubbles recorded using atomic force microscopy (AFM) was published	(May et al., 2002)
4	The use of bulk nanobubbles as ultrasound contrast agents was reported.	(Marxer et al., 2011)
5	Replicas of bulk nanobubbles were imaged by cryo-scanning electron microscopy	(Zerbini et al., 2010)
6	The density of the bulk nanobubbleswas measured using a microresonator.	(Kobayashi, et al 2014)
7	Fluorescence microscopy proved surface nanobubbles to be in the gaseous form	(Wang et al., 2013)

Composition of Nanobubble

Two main types of nanobubble components are present with different physicochemical characteristics, namely the inner core and the outer shell. The shell mainly comprises surfactants, polymers or proteins, while the core air contains sulphur hexafluoride and perfluorocarbons.The composition of the shell also determines the stiffness of the bubbles, their resistance to rupture in the ultrasound pressure field, and the ease with which they are recognized and cleared by the reticuloendothelial system. The coreis a single low-density chamber that makes up the largest part of a particle's volume while the shell acts as a barrier between the encapsulated gas and the surrounding aqueous medium, preventing gas dissipation(Khan et al., 2018). A few common examples of core and shell are represented in Table 2.

The shell forms a protective layer around the gas to provide stability and protection from endogenous scavengers, and it reduces the rate of diffusion of the core gas into the surrounding media(Unger et al., 2004).The shell composition determines the stiffness, elasticity, gas exchange, half-life, resistance against the applied ultrasonic pressure, and the ease in excretion of the NBs from the body. If the shells are soft, they will break easily, while hard shells will not be able to oscillate in ultrasonic fields(Cavalli et al., 2016b). Shell composition is an important factor in the loading of drugs and genes. Therefore, it is important to choose appropriate shell materials for diverse applications of MNBs with various thicknesses, stiffnesses, charges, and functional groups.Chitosan was selected for the nanobubble shell because of its low toxicity, low immunogenicity, and excellent biocompatibility(Cavalli et al., 2012a). PLGA's high stability, biodegradability, and biocompatibility in vivo enabled it as a preferable choice for pharmaceutical carrier material(Zhang et al., 2014).

Table 2 Few common examples of core and shell material of Nanobubbles

Core	Shell
Air	Surfactant/cosurfactant
Oxygen	Lipid
Sulfur hexafluoride	polymer
Perfluorocarbon	protein
Octofluoropropane	Polyelectrolyte multilayer

Stability of Nanobubble

Defining the formulation design of nanobubbles is a challenging task because several parameters must be taken into account to obtain stable and safe systems. First, the critical role played by interfacial tension and Laplace pressure should be considered, besides the structures of the dispersed and continuous phases. Molecular interaction between the internal gas core and outer liquid medium is called as the surface tension at the interface of binary mixtures. Laplace pressure is the pressure difference between the inside and the outside of a bubble (or a droplet), given as:

$$\Delta P = Pinside - Poutside = 2\sigma/r$$

ΔP is the Laplace pressure, P inside is the pressure inside the bubble, P outside is the pressure outside a bubble, σ is the interfacial tension, r is the bubble radius. Since Laplace pressure is inversely related to the size of a bubble, smaller bubbles will have higher pressure values than larger ones. When the inner gas leaves the core, driven by the pressure gradient, the bubbles shrink and Laplace pressure increases, thereby accelerating the rate of gas dissipation and the resulting bubble shrinkage until the system ruptures. Since the surface tension generates a pressure that drives bubble dissolution by gas diffusion, the rate of dissolution of the bubble in vivo will primarily depend on the Laplace pressure.In terms of stability, experimental results indicated that nanobubbles could protect apomorphine from degradation. In vitro drug release was sustained over time, and was enhanced by insonation, demonstrating a possible drug-targeting effect(Cavalli et al., 2016b).

Based on the above premises, formulation criteria comprise various technological approaches to stabilize nanobubbles, including the presence of surfactants at the interface, reduction of the Laplace pressure difference, limitation of gas diffusion and control of the interfacial structure. Gas diffusion between two bubbles is related to Ostwald ripening due to the surface tension effect which generates pressure for the gas dissolution and affects the stability and preparation of an NB.The stability of NBs depends not only on the composition of surfactant and polymers at the interface but also on the size and a low-density gas in its core which is then stabilized by coating materials such as lipid and synthetic polymer. Following is the list of various theories that have been put forward to understand the stability of nanobubbles(Oeffinger& Wheatley, 2004a).

Stability theories of nanobubbles(Sun et al., 2016)

1. Contamination (impurity) theory
2. Dynamic equilibrium theory
2.1. Knudsen gas theory
2.2. Improved dynamic equilibrium theory
2.3. Contact line pinning effect
2.3.1. Unified mechanism: contact line pinning and supersaturation
2.3.2. Sequential changing process of pinning and depinning
2.4. Internal pressure theory
2.5. Other theories
2.5.1. Liquid height theory
2.5.2. Gas density theory
2.5.3. Line tension theory
2.5.4. Surface force between the interfaces of gas-liquid and gas-solid

The type of gas phase could act as a cosurfactant at the interface affecting the bubble. The effect of fluorocarbon gases on the properties of phospholipid monolayers was theorized by Krafft and coworkers (2015).Nanobubbles remain stable in liquids for long periods at a high concentration owing to their negatively charged surface and high internal pressure, whereas macrobubbles (>50 m in diameter), increase in size and rapidly burst at the surface of liquids(Takahashi et al., 2007).

Drug Delivery Application of Nanobubbles

NBs have recently attracted significant attention in drug delivery, especially as theranostics in cancer as well as gene delivery. The following table depicts a few recent examples in drug delivery with various core and shell materials.

Table 3. Salient examples of drug delivery application of Nanobubble

Drug	Shell	Core	Conclusion	Reference
Coumarin-6	Tween 80 1	Sulphur hexafluoride	A nanobubble formulation is a promising approach for both ultrasound imaging and drug delivery enhancement.	(Wang et al., 2010)
Doxorubicin	Poly(lactic-co-glycolic acid) (PLGA)	Carbon tetrafluoride	Doxorubicin nanobubble can be used as ultrasound contrast agent to enhance tissue imaging.	(Meng et al., 2016)
Gene delivery	Chitosan	Perfluoropentane	chitosan nanobubbles have the potential to be promising tools for ultrasound-mediated DNA delivery	(Cavalli et al., 2012b)
Oxygen delivery	Polysaccharide	Perfluoropentan	The oxygen-filled	(Cavalli et al.,

	2		nanobubble formulations might be proposed for therapeutic applications in various diseases	2009)
Oxygen delivery	Lecithin	Oxygen	Reducing Tumour Hypoxia via Oral Administration of Oxygen Nanobubbles	(Owen et al., 2016)
Biotinylated rabbit-IgG	1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)	Perfluoropropane	Enhancing macromolecular permeation through layers of the retina	(Thakur et al., 2017)
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.	(Song et al., 2017)
Pluronic	Lipid and Pluronic-shelled	perfluoropropane	can serve as an effective theranostic method for sensitization of tumours to radiofrequency ablation.	(Perera et al., 2014)
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	(Abdalkader et al., 2017)
Mitomycin-C	sodium carboxymethylcellulose	oxygen	enhance the efficacy of localization and targeting for reverting hypoxia in NMIBC (non-muscle invasive bladder cancer tumours)	(Bhandari et al., 2018b)
Methotrexate	poly(lactic-co-glycolic acid) (PLGA)	Perfluorocarbon	Methotrexate-loaded nanobubbles as a targeted drug carrier, an efficient ultrasound	(Zhang et al., 2014)

			contrast agent, as well as a synergistic agent for HIFU(High intensity focused ultrasound) ablation of choriocarcinoma	
Herceptin	Phospholipid	octafluoropropane	Targeted delivery of therapeutic drugs or genes.	(Jiang et al., 2016)
Camptothecin	DSPE-PEG2000	Perfluorobutane	safe and efficient drug delivery system for specific cancer treatment.	(Xie et al., 2016)
Apatinib	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 an ultrasound.	(Tian et al., 2018)
Apomorphine	Hydrogenated soybean phosphatidylcholine (SPC, Phospholipon180H)	Perfluoropentane	Apomorphine-loaded perflurocarbonnanobubble showed promising stability and safety. They were successful in sustaining apomorphine delivery.	(Hwang et al., 2009)
pDNA (pCMV–luciferase)	Distearoylphosphatidylcholine (DSPC)	Perfluoropropane	effective and safe intraperitoneal gene transfection using BLs with US irradiation in mice	(Nishimura et al., 2017b)
Paclitaxel(Chandan& Banerjee, 2018a)	DSPC:DSPE	Sulphur hexafluoride	the potential of PSPLBC as a promising noninvasive, pro-apoptotic, smart DDS for US-responsive, image-guided cancer therapeutics	(Chandan& Banerjee, 2018b)

Gene delivery

The development of nonviral gene delivery systems is one of the most intriguing topics in nanomedicine(Cavalli et al., 2012b). Nanoscale systems were originally designed as contrast agents, and only also been studied in relation to drug and gene delivery (Tan et al., 2021). Gene therapy, a promising therapeutic option for the treatment of genetic or acquired diseases, is based on the ability to introduce new genetic material into hosts. A major obstacle to gene transfer is the fact that

naked nucleic acids are not efficiently taken up by cells due to their negatively charged phosphate groups, relatively large sizes and hydrophilic nature. Moreover, they are very susceptible to degradation mediated by nucleases present in the blood. To overcome these limitations, a number of gene delivery vectors have been developed, falling into either viral or non-viral categories. nanobubbles provide a promising non-viral strategy for the site-specific delivery of genetic material; this is due to their potential to be 'activated' in the presence of ultrasound (US) and mediate the delivery of DNA to specific cell targets(Cavalli et al., 2013). Localised gene delivery using nanobubbles and a dual intensity ultrasound system has also been,which has shown an increase in localization of molecule delivered, helping genetherapy for bladder cancer.Viral vectors represent efficient carriers for gene transduction, but they also come with certain limitations, including toxicity and immunogenicity.Nonviral vectors have consequently attracted much interest as gene carriers to overcome these problems, but their transduction efficiency is very low, although many efforts have recently been directed towards improving this aspect(Suzuki et al., 2011a).

Liposomal Nanobubble

This formulation has demonstrated the effective and safe intraperitoneal gene transfection using bubble liposomes (BLs) with US irradiation in mice (Nishimura et al., 2017b).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). Horie et al. showed that perfluoropropane gas was trapped within the BLs (Horie et al., 2010b). Gene transfection by BLs with US irradiation is expected to be a useful method because BLs are more pharmaceutically stable than microbubbles due to the smaller particle size with PEGylation. BLs are also easily modified with targeting ligands in addition to PEGylation. Gene delivery systems using BLs with US irradiation enhance the gene transfection efficiency to targeted sites, such as the liver, kidney and tumors. Under the optimal condition of US intensity and irradiation time, highly efficacious, long-term transgene expression has been achieved.(Nishimura et al., 2017a).Established methods for incorporation of gas within liposomes include hydration of a lipid film, freezing in the presence of mannitol, lyophilizing, and rehydration.(Nguyen &Wrenn, 2014). In this study, based on the preprepared free gas nanobubbles aqueous solutions, the author developed a novel and controllable preparation technique to make lipid encapsulated nanobubbles. Results demonstrated that the structure of about 200 nm diameter nanobubbles was multilayer lipid encapsulation due to lipid assembly on the surface of free bubbles. Due to the gas core and the multilayer lipid loading capability, such gas-filled ultrasound-sensitive liposome (GU-Liposome) would be beneficial for extravascular ultrasound imaging and drug delivery in the future(Tian et al., 2015).

Silicon Nanobubble

There is still no practical method to control the diameter of bubbles. Liu et al. developed a new method to control the size by incorporating silicon hybrid lipids into the bubble membrane. The size of resulting NBs increased with the decrease in the amount of silicon hybrid lipids, indicating the diameter of NBs can be

regulated by modulating the ratio of silicon hybrid lipids in the bubble shell(Liu et al., 2017b).

Pluronic Nanobubble

Pluronic is effective in lipid bubble size control, and Pluronic Mw, hydrophilic-lipophilic balance (HLB), and Pluronic/ lipid ratio are critical determinants of the bubble size.In order to reduce bubble size into the nanometer range without affecting echogenicity, Krupka et al., proposed the addition of surfactants oramphiphilic polymers, such as pluronic, into the formulation of lipid bubbles. For this purpose, five types of pluronics, with different molecular weights(Mw 1100–4600 Da), were incorporated into the lipid bubble shell. The results demonstrated that Pluronic-lipid interactions lead to a marked reduction in bubble size Pluronics (also known as poloxamers), or polyethylene oxide (EOx)-polypropylene oxide (POy)-polyethylene oxide (EOx), are a family of nonionic triblock copolymers (Mw: 1100-14600) once classified as “inactive excipients” by the FDA. These amphiphilic surfactants are commonly used in industrial applications as anti-foaming agents, cosmetics, pharmaceuticals as emulsifiers, colloidal dispersion stabilizers, and supplements for cell culture media. Because of their relatively nontoxic nature, the applications of pluronics in experimental medicine and pharmaceutical sciences can be traced far back. In recent years, functional applications of pluronic as chemo and thermal sensitizing agent to cancer treatment have also been explored and shown to hold some promise in modifying biological cancer cell response (Krupka et al., 2010b).

Pluronic is effective in lipid bubble size control,its molecular weight, hydrophilic-lipophilic balance (HLB), and pluronic/ lipid ratio are critical determinants of the bubble size.Porphyrin-Loaded pluronic NBs is a new US-activated agent for future theranostic applications.This study aims to combine porphyrinsNBs to obtain an ultrasound-activated theranostic agent that exploits the sonodynamicactivity in vitro. Two porphyrin classes, exposing different hydrophobic side chains, were synthesized. NB size and encapsulation efficiency were markedly dependent on the porphyrin structure. The combination of these porphyrins and NBs resulted in a significant reduction in cell viability upon sonication in pilot studies performed on the LS 174T colorectal cancer cell line(Bosca et al., 2018).

Plasmonicnanobubble

The use of plasmonic nanobubbles, a novel cellular agent with a dual and tuneable mechanical and optical action, to obtain selective gene transfection.Plasmonic nanobubbles are transiently heated using a short laser pulse and, through the mechanism of plasmon resonance, vapour nanobubbles are formed around the plasmonic nanoparticle, producing a localised mechanical, non-thermal impact on the cell. Plasmonic nanobubbles can provide targeted gene delivery at the single cell level in a single pulse procedure that can be used for safe and effective gene therapy.This approach does not concern a nanobubble formulation, but it is included here to complete the scenario of possible nano-scale systems involved in gene transfer.(Lukianova-Hleb et al., 2012).

Oxygen delivery

Hypoxia, i.e. a reduction in dissolved oxygen concentration below physiologically normal levels, has been identified as playing a critical role in the progression of several diseases, including many types of cancer. The administration of therapeutic gases has been the focus of growing interest. Much research has centred on oxygen delivery, since many medical conditions, such as diabetes, burns, bedsores and wounds, are related to insufficient oxygen supply to the tissues. In addition oxygen deficiency, together with acidosis, is also the main hallmark of cancerous solid tumours. Moreover, targeted oxygen delivery could be a useful adjuvant for antibiotic therapy of anaerobic infections. Hence, these are potential fields of application of oxygen-filled nanobubbles, besides other approaches for oxygenation, such as the use of microbubbles, nanosponges or echogenic liposomes. Interestingly, perfluorocarbons can dissolve and store oxygen, a feature that is exploited by several formulations.

Ultrasound Contrast Agents

Ultrasound contrast agents (UCA) are small gas-filled bubbles with a stabilizing shell made from a variety of materials such as polymer, protein or lipid.Other than the traditional applications of these agents in diagnostic ultrasound imaging(Krupka et al., 2010a).Nanoscale ultrasound contrast-enhanced agents with various shells (polymers or phospholipids) and cores (gas, liquid, or solid) have been fabricated.(Yin et al., 2012).Ultrasound is an important local stimulus for triggering drug release at the target tissue. Ultrasound-responsive drug delivery systems (URDDS) have become an important research focus in targeted therapy. URDDS include many different formulations, such as microbubbles, nanobubbles, nanodroplets, liposomes, emulsions, and micelles. Drugs that can be loaded into URDDS include small molecules, biomacromolecules, and inorganic substances (Zhao et al., 2013b).Ultrasound consists of pressure waves at frequencies of 20 kHz or greater. Like optical and audio waves, ultrasonic waves can be focused, reflected, and refracted through a medium. As a mature medical technology, ultrasound imaging can be used repeatedly without concern about residual radiation.2 Therefore, ultrasound imaging is acceptable to most patients and the equipment involved is generally less expensive than that of other imaging technologies(Zhao et al., 2013a).

NBs opened a new field of ultrasound imaging,through the enhanced permeability and retention (EPR) effects, nanobubbles could be transferred from blood vessels into surrounding tissues and be imaged by ultrasound after accumulation.Ultrasound contrast agents are used to enhance the backscattered signal, improving resolution.The major principle behind all ultrasound contrast agents is an impedance mismatch between the surrounding medium (blood and soft tissue) and the agent (gas)(Liu et al., 2017a).

The ultrasound (US)-targeted nanobubble destruction (UTND) method has become a new trend for drug delivery to solid tumours. Compared with other drug delivery systems, UTND has multiple significant advantages. First of all, nanobubbles (NBs) are easily prepared by modified emulsification processes20 and used as US contrast agents to visualize tumours. In addition, NBs in combination with US

can induce acoustic cavitation, stimulating cell membrane permeabilization and improving drug uptake by tumour cells. Previous studies particularly paid attention to nontargeted NBs that are easily accumulated in the reticuloendothelial system, resulting in lower drug concentration at the tumour site. To increase therapeutic efficacy and reduce systemic toxicity, it is essential to construct targeted and drug-loaded NBs, carrying tumour-specific ligands such as antibodies and peptides.Ultrasound is a form of mechanical vibration of matter with a frequency beyond human audible range (i.e., above 20 kHz). The speed of sound is dependent on the medium: roughly 340 m/s in air, 1480 m/s in pure water, 1550 m/s in soft tissue and 3700 m/s in bone. The particle excursion in a sound wave is related to the instantaneous local pressure through the wave equation. The wave is damped with propagation distance due to tissue absorption and geometric effects.This behaviour induces mechanical effects in the medium which are amplified when inertial cavitation occurs. Inertial cavitation is the process of formation and subsequent collapse of bubbles driven by an acoustic field. Ultrasound is a frequency of mechanical vibrations or pressure waves which are equal to or above that of human hear (20 kHz) due to its compressional and rarefactional pressure fluctuations. Generally, the delivery of drugs to organs and tissues is affected by two main ultrasound effects including the cavitation and sonoporation effects. The cavitation effect results in the reduction of bubble size, whilst the sonoporation effect leads to the uptake of the reduced bubble(Delalande et al., 2012).

Cavitation Effect

In an unresectabletumour, radiofrequency (RF) ablation is an invasive procedure in treating tumour diseases. However; RF ablation has limitations on the size and location of the tumour (lesion) which produces variable results due to the flow of blood and act as heat sink to the tumour vessels. Pluronic is an effective thermosensitizer to increase the efficacy of RF ablation on MBs. Pluronic as an amphiphilic surfactant has the ability to decrease the size of MBs without reducing echogenicity.Cavitation effects and radiation force are the main driving forces behind improved extravasation and convection of drugs via the reduction of its carrier (delivery device) when exposed to the ultrasound as a result of pressure wave passing through the media. cavitation can become stable (non-inertial) if acoustic pressure amplitude would be above a threshold level, leading to the growth and subsequent explosion of the NBs. High temperatures and highly reactive radicals may occur during the explosion of the bubbles. In addition, cavitation can be transient (inertial) if the resulting oscillation which creates a circulating shear flow of bubble at the surrounding fluid would be proportional to the amplitude. The change in fluidity can also control the resonant response of NBs to the ultrasound irradiation, leading to an increment of bubble echogenicity(Huynh et al., 2016).

Sonoporation

Sonoporation is the ultrasound-assisted transient permeabilization of the plasma membrane. This permeabilization allows for the transfer of molecules between the intra- and extra-cellular medium.The sonoporation effect is a process in which ultrasound is used to alter the permeability of the cell plasma membrane and

provide a very specific and high concentration of drugs at the site of interest while minimizing the overall exposure of the drug to other parts of the body. Depending on the type of tissue surface, an asymmetrical collapse occurs near the surface which ejects a liquid at sonic speed towards the surface and then pierces the tissue surface. The collapsing bubbles can create transient holes in the cell membrane and large molecules such as nucleotides can be entered into the cells. Generally, this mechanism has been demonstrated by experiments and proven by a theoretical explanation. The collapsing bubble is not only dependent on the physical properties of the bubble but also on the higher intensity and lower frequency of the ultrasound which affects its cavitation process (Delalande et al., 2012).

Method of preparation of nanobubble

NBs are mainly prepared by thin-layer evaporation, sonication, high shear emulsification, mechanical agitation and coacervation or coalescence; techniques that have also been used in microbubble preparation.

Sonication method

Sonication involves the dispersion of gas or liquid in a suspension of a suitable coating material using high-intensity ultrasound. This process emulsifies gas or liquid to form a suspension of micro-droplets/bubbles onto whose surfaces a coat of, for example, protein or surfactant, is automatically adsorbed. Biocompatible nanobubbles were fabricated by ultrasonication of a mixture of Span 60 and polyoxyethylene 40 stearate (PEG40S), followed by differential centrifugation to obtain a nano-sized bubble population with a unimodal size distribution. NBs were prepared by sonication method. Briefly, DSPC:DSPE (4:1, molar ratio) were dissolved in chloroform:methanol (2:1, v/v). The solvents were evaporated using a rotary evaporator to form a thin film, followed by hydration in phosphate buffer saline (PBS) at 60 °C for 45 minutes. Subsequently, the suspension was sonicated (Model 3000 ultrasonic homogenizer, Biologics Inc. USA) with simultaneous gas purging for 30 seconds to form nanobubbles(Chandan& Banerjee, 2018b).

Microfluidic Techniques

Recently, nanobubble production via microfluidic devices has been investigated, as microfluidics enables precise control over bubble diameter and production rate, through the interaction of the gas pressure, liquid flow rate and device geometry. Microfluidic devices have the ability to synthesize MNBs with controlled size distributions. Flow rate, pressure, viscosity of the liquid solution, and the orifice size of the device can be controlled to determine the size and distribution of the MNBs [22,57]. To the two main methods for the fabrication of microfluidic devices are as follows: (1) Soft lithography techniques to produce flow focusing units; and (2) mechanically assembled units from capillaries assembled in a polymeric block. The gas and liquid flows into a T-junction in both cases The MNBs are then generated in the T-junction depending on the size of orifice and other parameters of the device being used (Dhanaliwala et al., 2015).

Emulsification method

This method is usually applied to synthesize polymer shell NB. In this process, water is formed in an oil emulsion with a carrier polymer, and this emulsion is further emulsified in a large volume of water. The solvent is evaporated or extracted to obtain a solid polymer shell, and lyophilized shells are refilled with core gas, such as PFCs. A high-shear emulsification method has been used to synthesize NBs with a broader size range. A membrane emulsification method can be used to generate NBs with a narrow size distribution. A porous membrane is used for this purpose. Gas bubbles permeate and disperse into a continuous phase flowing along the membrane surface. Emulsifiers are added to prevent coalescence (Lee et al., 2015).

Agitation Method

Mechanical agitation can improve the interface between the liquid phase that contains surfactants and the gas phase during the preparation of MBs or NBs.NBs, especially those having lipid shells, can be produced by agitating the liquid solution at several thousand oscillations per minute in a shaker. This will produce bubbles with a random size distribution. To encapsulate a given gas in an MNB, the container is filled with the desired coating material in the liquid phase and the gas is perfused from the top and then the container is mechanically agitated so that the shell material encapsulates the desired gas. Mechanical agitation is a promising method to produce MNBs on an industrial scale(Xing et al., 2010).

Ink-Jet Method

Microbubble synthesis has been performed using an ink-jet method, in which a polymer solution is forced through a piezo-driven ink-jet nozzle of desirable size, depending on the application. The piezoelectric crystals create pulses in the solution and the bubbles that are formed are removed from the nozzle. A similar method has also been applied to generate ultrafine oxygen nanobubbles from pure water and an oxygen supply by utilizing a high-pressure flow through the nozzle(Cavalli et al., 2016a).

Laser Ablation Method

The laser ablation method is also a stochastic method that can generate MNBs. An excimer laser of a particular wavelength can be focused onto aluminium oxide particles in water, which then form oxidized nanoparticles. During the process, bubbles will also be produced at the solid-liquid interface. The bubbles/interface are stabilized by the aluminium oxide nanoclusters(Yao et al., 2018).

Characterization of Nanobubble

Typical characterization is usually carried out for the NBs is appended below.

Table 4 Typical parameters for nanobubble characterization(Stride &Edirisinghe, 2008)

Parameter	Brief Detail
Bubble size	dynamic light scattering using photon correlation spectroscopy (PCS) at 25 °C
Zeta potential	Zeta Plus Analyzer, Surface potential Cytometry
Magnetic properties of magnetic nanobubble	Measured by using vibrating sample magnetometer(VSM)
Structure of nanobubble	Scanning electron microscopy (SEM)
	transmission electron microscopy (TEM)
Dissolved oxygen	Electrochemical sensing, fiber optic-based sensing, and fluorescence quenching
Bubble concentrations	hemacytometer

Nanobubble Targeting

Nanobubbles can be loaded with gases, small molecules and macromolecules, either hydrophilic or lipophilic ones.Drugs might be encapsulated within the core, or they might be incorporated within or just beneath the nanobubble shell. Additionally, encapsulation of the drug in a nanoparticle subsequently attached to the bubble surface is anotherapproach to loading. The main purpose of loading NBs with drugs and genes is to minimize the side effects associated with these bioactive substances, along with improving therapeutic efficacy by lowering the effective dosage and minimizing the interference while reaching the target site. NBs can be used for both passive and active targeting. Passive targeting refers to the tendency of the NBs to accumulate at tumour sites owing to the leaky vasculature. The effect is also known as enhanced permeability and retention (EPR). Tumour vasculature is irregular and contains large pores within the range of 300–700 nm. NBs in this size range have the benefit of EPR. Physical properties of NBs like elasticity, porosity, surface charge, size and shell composition and their interaction with the tumour microenvironment play a great role in the EPR effect. Higher EPR would translate into higher uptake, better biodistribution and more bioavailability of the drug, resulting in more effectiveness and better treatment. A higher cellular uptake of NBs owing to endocytosis makes them suitable for drug delivery applications(Horie et al., 2010a).

Functionalization of NBs

Surface modification of NBs is required for active targeting by attaching some targeting ligands. This can be achieved by attaching bioactive molecules to the shell of the NBs. Targeting NBs can be created by incorporating targeting ligands, such as biomarkers, antibodies, polysaccharides, or other active biomolecules. Three methods can be applied to the functionalization of the NBs (Shri Devi S. et al., 2022).

First, NBs can be synthesized with biomolecules/bioactive substances incorporated in the shell or inside the core of the NBs. Hydrophilic and amphiphilic biomolecules can be incorporated into the shell while hydrophobic drugs can be loaded into the core of NBs. The drug-loading capacity is dependent on the type of shell employed. Thin phospholipid shells are more echogenic and favourable for hydrophilic molecules while thick polymeric shells are preferred for hydrophobic drug loading in the core. Second, covalent and non-covalent techniques can be applied for the functionalization of the NBs by attaching targeting ligands to the protein, polymer, or lipid-based shells. This method is favourable for hydrophilic drugs for targeted delivery (Vantimitta, S. R., 2022). Biotin-avidin linkages can be incorporated in NBs for linking antibodies and proteins.NB shells can be made cationic to apply electrostatic interactions for gene delivery. This method facilitates gene therapy and various researchers have aimed at using NBs to enhance targeted gene delivery. Finally, NBs can be co-administered with bioactive substances, using high-intensity ultrasound to enhance cell permeability for a higher uptake of the bioactive molecules. The lipid bilayer of the liposomes provides extended space for hydrophobic interactions resulting in high encapsulation efficiency of the hydrophobic drugs. Therefore, liposomes were chosen as the drug carrier(Suzuki et al., 2011b).

Conclusion

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. This nanocarrier provides an innovative multifunctional drug delivery platform that is suitable for a scope of therapeutic applications and administration routes. The application of ultrasound (US) consolidated to nanobubbles might increase the therapeutic index of drugs, favouring their release on demand in the desired tissues. Moreover, US can be conveniently added to overcome biological barriers, such as the blood-brain barrier. Small particle size is an essential prerequisite for ultrasound contrast-enhanced agents that penetrate tumour blood vessel pores to allow for targeted imaging and therapy. In our opinion, nanobubbles will play an important role in future nanomedicine applications. Nanobubble's physicochemical properties might be customized to develop smart or intelligent systems that are responsive to endogenous stimuli in order to deliver the therapeutic molecule on-demand in the field of personalized nanomedicine.

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