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Microbubble and its applications

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Abstract

This review focuses on the characteristics of microbubbles that give them therapeutic properties and some important aspects of ultrasound parameters that are known to influence microbubbles-mediated drug delivery. In addition, current studies involving this novel therapeutic application of microbubbles will be discussed the microbubbles as drug carriers have an average size less than that of red blood cells .They are capable of penetrating even into the small blood capillaries and releasing drug and genes under the action of ultrasound field. Targeting ligands are attached to the surface of the microbubbles (i.e. targeted-microbubbles). Microbubbles dispersion method was investigated to improve oxygen transfer at low agitation rates and thus reduce power consumption and shear stress on the microorganisms. Myocardial contrast echocardiography is rapidly becoming a technique that can be utilized with intravenous with intravenous Microbubbles to detect myocardial perfusion abnormalities during stress echocardiography. Microbubbles destruction has been proposed as an innovative method for noninvasive delivering of drugs and genes to different tissues. Conventional flotation assisted with microbubbles (30–100 μm) finds application in the recovery of fine mineral particles (b13 μm) and flotation with these fine bubbles is being used as a solid/liquid separation to remove pollutants. Microbubble having boost utilization in formation of biofuel.Thus microbubbles having various applications in various fields.

Key-Words: Imaging, Ultrasound

Introduction

Microbubbles are small gas-filled microspheres that have specific acoustic properties that make them useful as a contrast agent in ultrasound imaging. First-generation microbubbles are room air microspheres[1]These microbubbles are capable of passing the pulmonary capillary bed, but cannot resist arterial pressure gradients. To increase stability of microbubbles further, second-generation contrast agents are filled with a heavy-molecular-weight gas like e.g. sulphur hexafluoride, which decreases solubility, thus improving survival and stability under higher pressure. Surfactants, sonicated albumin and (phospho) lipids are used to improve stabilization of the shell[1]The hydrogel network forms around the liposomes, protecting them from surrounding inflammatory cells and preserving their close proximity to co-suspended microbubbles, which might otherwise separate from them due to their different buoyancy. Colloidal bubbles (microbubbles) are emerging as important contrast agents for imaging and carriers for targeted drug delivery[2].

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Microbubbles are gas-filled lipid monolayers that have been used extensively as ultrasound contrast agents and recently as drug delivery vehicles [3, 4]. These agents can improve accuracy and imaging contrast for targeted sites of interest during ultrasound imaging. In the last 10 years, several site-targeted microbubbles have been developed, and have been used for molecular and cellular imaging in vitro and in vivo[5,6] as well as for ultrasound-assisted drug/gene delivery and triggered release.[7,8]. This review focuses on the characteristics of microbubbles that give them therapeutic properties and some important aspects of ultrasound parameters that are known to influence microbubble-mediated drug delivery .In addition, current studies involving this novel therapeutical application of microbubbles will be discussed (Jeane M Tsutsui et al., 2004).Recently, targeting ligands are attached to the surface of the microbubbles (i.e. targeted-microbubbles), which have been widely used in cardiovascular system and tumor diagnosis and therapy. In this paper, the characterization of novel targeted ultrasonic contrast agents or microbubbles and their potential applications in drug delivery or gene therapy are reviewed (Yiyao Liua, b, c, et al.,2006).With the continuous development of molecular biology, gene therapy for liver cancer has become a research hotspot and

direction[9] studies have shown that ultrasound-targeted microbubble destruction is a safe ,effective, non-invasive, and physical gene transfection technology, which brings a new hope for gene therapy in liver cancer [10,11].Flotation with microbubbles(30–100 μm) is the most utilized process removing a number of pollutants, among others, colloids, fines and ultra fines particles, precipitates, ions, micro organisms , proteins, dispersed and emulsified oils in water (Rubioet al., 2002a,b; Ross et al., 2003; Carissimi and Rubio,2005a; Matis and Lazaridis, 2002).Stability is enhanced by producing MBs using heavier than air gasses [12] with fluorocarbon gassessuch as octafluoropentane and n-decafluorobutane amongst those used most commonly. The primary medical use for MBs (only approved medical use in many countries) is as contrast agents for ultrasonic imaging.

Advantages

On top of the strengths of echocardiography, contrast-enhanced ultrasound adds these additional advantages: [13]

- The body is 73% water and, therefore, acoustically homogeneous. Blood and surrounding tissues have similar echogenicities, so it is difficult to clearly discern the degree of blood flow and perfusion, or the interface between tissue and blood, using traditional ultrasound.
- Ultrasound imaging allows real-time evaluations of blood flow.
- Ultrasonic molecular imaging is safer than molecular imaging modalities, such as radionuclide imaging, because it does not involve radiation.
- Alternative molecular imaging modalities, such as MRI, PET, and SPECT are very costly. Ultrasound, on the other hand, is very cost-efficient and widely available.
- Since microbubbles can generate such strong signals, a lower intravenous dosage is needed. Micrograms of microbubbles are needed to perform contrast-enhanced ultrasounds compared to milligrams for other molecular imaging modalities, such as MRI contrast agents.
- Targeting strategies for microbubbles are versatile and modular. Targeting a new area only entails conjugating a new ligand.
- Targeting ligands can be immunogenic, since current targeting ligands used in preclinical experiments are derived from animal culture. Ultrasound contrast agents can be used to improve imaging by introducing a material with different acoustic properties from that of tissues [14]
- It provides high spatialresolution in the range of 3 mm. Its minimal invasivenessresults from the very high frequency of ultrasound used,which does not generally cause damage or disturbance tothe cells [15]imaging, thus aiding in the

Disadvantages

Contrast-enhanced ultrasound suffers from the following disadvantages:

- Microbubbles don't last very long in circulation. They have low circulation residence times because they either get taken up by immune system cells or get taken up by the liver or spleen, even when they are coated with PEG.
- Ultrasound produces more heat as the frequency increases, so ultrasonic frequency must be carefully monitored.
- Microbubbles burst at low ultrasound frequencies and at high mechanical indices, which is the measure of the acoustic power output of the ultrasound imaging system. Increasing MI increases image quality, but there are tradeoffs with microbubble destruction. Microbubble destruction could cause local microvasculature ruptures and hemolysis.

Advantages and disadvantages of contrast-enhanced ultrasound in trauma

Contrast enhanced ultrasound (CEUS)CEUS does not harm the patients because it lac ks ionizing radiation and because the contrast agent is n ot nephrotoxic. The microbubbles are decomposed in t he liver and the gas is exhaled through the lungs. CEU S can easily be performed in the emergency room, the i ntensive care unit andeven in the operating room. With one bolus, it visualizes arterial, portal venous and paren chymal phases. However, in the severe injured patient, CEUS cannot be used as admission imaging modality, because it does not visualize sufficiently retroperitonea l structures and is useless for detection of fractures. Th e ideal patients groups in the trauma setting are therefor e [16] patients with isolated parenchymal trauma [17] patient s who cannot undergo computedtomography for injury evaluation for several reasons and [18] patients in follow-up after trauma.

Ultrasonic Microbubbles

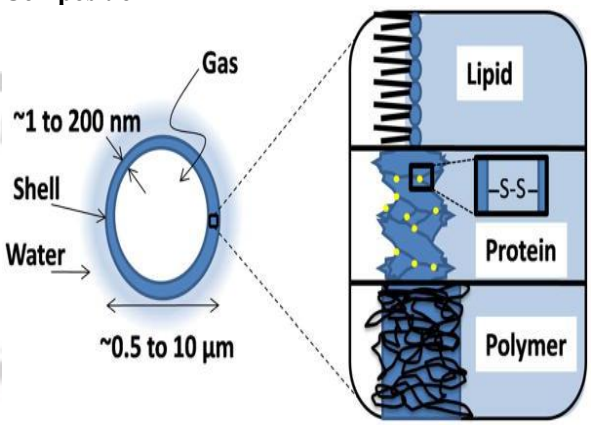
Medical ultrasound is now a well-established technique for clinical diagnostics and will continue to play an important role in the foreseeable future[19,20]. Ultrasound images however do not have a very sharp contrast and

sometimes the area being imaged is buried and shoed by tissues[21]. This problem can be resolved in part by using ultrasound contrast agents when imaging[22].Gas bubbles have an added advantage when being considered as ultrasound contrast agent because they can act as harmonic oscillators and resonate when insonated at their resonant frequency.The microbubbles which mostly contain oxygen or air can remain suspended in water for an extended period. Gradually, the gas within the microbubbles dissolves in to the water and the bubbles disappear. It is well known that air or gas microbubbles suspended in a liquid are exceptionally efficient ultrasound reflectors for echography Microbubbles is useful as ultrasonic contrast agents. For example, injecting suspensions of gas microbubbles (0.5-10µm in diameter) in a carrier liquid in to the blood stream will strongly reinforce ultrasonic echography visualization of internal organs for the detection of cardiovascular and other diseases[23,24]Microbubbles have a high degree of echogenicity, which is the ability of an object to reflect the ultrasound waves. The echogenicity difference between the gas in the micro-bubbles and the soft tissue surroundings of the body is immense.

Preparation approaches of encapsulated microbubbles:

Agents that could perform in a dual role as both target-specific contrast agent and drug delivery vehicle would be of significant advantage to the clinician. Contrast agents could be used as a vehicle to deliver macromolecular gene constructs for gene therapy treatment. Various techniques are using to incorporate drugs in microbubbles. These microbubbles undergo cavitation and release the drug locally into the tissues. Drug delivery can be monitored with ultrasound as the drug carrier themselves are in essence contrst agent. For targeted microbubble preparation, the microbubbles are attached to the ligand, that may be mono clonal antibody, carbohydrate ligand. The agents can be incorporated in different ways in microbubbles.The most common method for the preparation of lipid-coated microbubble suspensions is agitation and/or sonication. Although sonication enhances the structure of the surfactant monolayer and makes the microbubbles extremely stable, a high level of control over the microbubble size distribution cannot be achieved in this way (Wang *et al*. 1996; Borden *et al*. 2004*b*; Unger *et al*. 2004). Efforts have been made to prepare microbubbles suitable for medical applications using microfluidic devices (Talu *et al*. 2006; Pancholi *et al*. 2008)

Composition

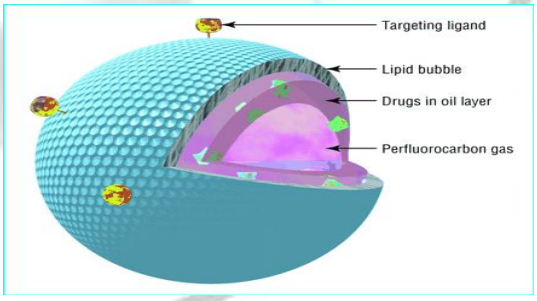


Protein Shells

Albumin shelled microbubbles were a pioneering formulation used in contrast ultrasound imaging. They paved the way for several subsequent formulations that could pass the lung capillaries and provide contrast in the left ventricle of the heart. The first albumin microbubble formulation to be approved by the US Food and Drug Administration (FDA) was Albunex (GE Healthcare). An Albunex suspension consists of roughly $7x10^8$ microbubbles/mL with a size range from 1 to 15 µm diameter

Surfactant Shells

Microbubbles stabilized by mixtures of the synthetic surfactants SPAN-40 and TWEEN-40 were formulated by Wheatley et al.[25,26] The SPAN/TWEEN solution was sonicated in the presence of air to form stable microbubbles. The surfactant derived from sonicated microbubbles was more stable (i.e., was capable of reaching higher collapse pressures on the Langmuir trough) than that used in the precursor solution, indicating that the sonication process modified the surfactant to form a more stable film.[26]



A targeted micro bubble: A gas microbubble is covered in a lipid membrane in which targetingligands have been incorporated.

Lipid Shells

Lipid-coated microbubbles are one of the most interesting and useful formulations used for biomedical imaging and drug delivery. The lipid shell is inspired by nature, as stable microbubbles found ubiquitously in the oceans and fresh waters of Earth are known to be stabilized by acyl lipids and glycoproteins.[27] There are several commercially available lipid-coated microbubble formulations approved for clinical use.lipid-coated microbubbles have exhibited favorable ultrasound characteristics, such as resonance with minimal damping and the ability to reseal around the gas core following fragmentation.[28, 29, 30]

Polymer Shells

The bulk nature of the polymer shell makes it more resistant to area compression and expansion than its lipid and albumin counterparts, which reduces the echogenicity and drug delivery activity. For example, polymer microbubbles have been observed to fracture during in sonification, thereby releasing their gas core via extrusion through the shell defect.[31]The resulting gas bubble was unstable and rapidly dissolved according to the classical Epstein and Plesset equation.[32]

Polyelectrolyte Multilayer Shells

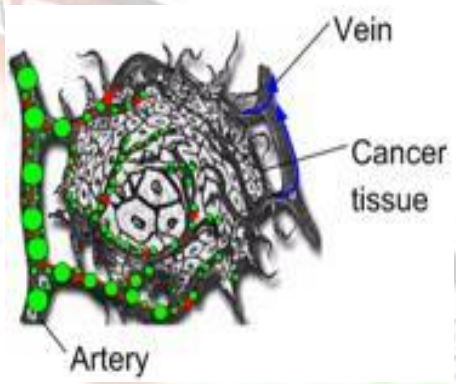
A new class of polymer-surfactant shell hybrids was recently introduced that involves polyelectrolyte multilayer shells on preformed microbubbles. The preformed microbubbles are coated with a charged surfactant or protein layer, which serves as a substrate for PEM deposition. The layer-by-layer assembly technique is used to sequentially adsorb oppositely charged polyions to the microbubble shell. Shchukin et a[33] were the first to report PEM deposition onto microbubbles. They used the polymers poly allylamine hydrochloride and polystyrene sulfonate for the polyion pair.

Properties

1. An interesting feature ofmicrobubbles is the specific acoustic properties they show in the presence of ultrasound, due to the encapsulated gas. This providesvarious possible methodsofincreasing cellpermeabilityby these ultrasound microbubbles.[34,35]

2. They can be targeted to specific tissue bythe incorporation ofligands into the shell. There areseveral ways in which drug, genes or ligands attach toa microbubble EspeciallynegativelychargedDNA can rather easily be attached to a positivelycharged bubble shell. Anotherway to loada bubble with drugs is to incorporate a lipophilic drug in the lipid membrane or to enclose thedrug within the microbubble itself .Furthermore, drugs can be bound by ligands that areembedded in the membrane .Whether abubble can be loaded with a certain drug depends onimportant factors such as molecular weight, lipophilicity and charge.[36]

Application of micro bubble in various fields



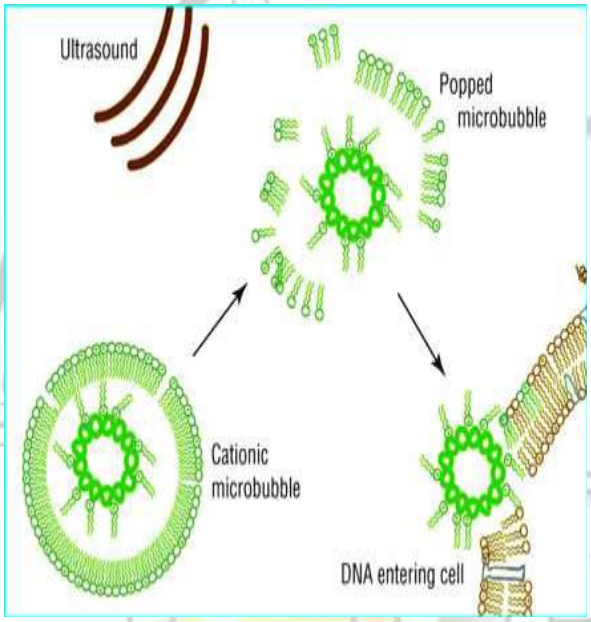
In Liver Cirrhosis

Contrast enhanced ultrasound is most effective when used for specific and well-designed indications in abdominal imaging. The major established application is for characterization of focal liver masses. Other abdominal organs that can be evaluated with CEUS include the kidney, aorta, prostate, spleen, bowel, and pelvic organs. The majority of incidentally detected liver masses in otherwise healthy patients are benign. CEUS is excellent for characterizing common benign masses such as hemangioma, focal nodular hyperplasia and focal fat deposits or sparing[37,38]Suspicious liver masses on CEUS can be referred to other imaging modalities or biopsy in a timely manner. Immediate performance of CEUS at the time of detection of the mass onroutine ultrasound examination leads to a reduced time to diagnosis, patient anxiety, and time to referral for other imaging examinations[39]Microbubbles make excellent contrast agents for ultrasound imaging. The strong echogenicity of microbubbles is a manifestation of their compressibility.[40,41] Solid and liquid particles, which are relatively incompressible, produce much less backscattered signal to transmitted ultrasound and are therefore not as effective as microbubbles for imaging. Thus, microbubbles stand alone as the main contrast agent for one of the most widespread, inexpensive, portable and safe imaging modalities.

In gene and drug delivery

Microbubbles have evolved rapidly, not only in diagnostic imaging but as possible therapeutic agentsaswell.[42] .Microbubbles are on average about2 to 5tmin diameter, which is relatively large compared with other carriers used in gene and drug delivery, such as the mentioned particles for magnetofection which

are only 10 to 20 nm." [43].Various ultrasound contrast agents are now commercially available with different gases and different types of shells. The usefulness of microbubbles in biomedical applications, more focus needs to be placed on designing more efficient drug carriers to promote intracellular uptake and drug transport. Some groups are beginning to investigate this area' [44, 45]



In oxygen transfer [46,47]

A microbubble dispersion method was investigated to improve oxygen transfer at low agitation rates and thus reduce power consumption and shear stress on the microorganisms. For an aerobic fermentation, the rate at which oxygen can be provided to the growing microorganisms determines the rate of fermentation. Oxygen can be provided by sparging, but the residence time of larger bubbles is quite short. Much of the filtered air introduced into fermentor exits without contributing to the growth of microorganism; it is wasted. Because of colloidal gas aphrons properties, it is hypothesized that the oxygen transfer rate can be improved by incorporating CGA dispersion instead of normal air sparging system. Kaster (1988) used the term micro bubble dispersion instead of CGA dispersion when he grew Baker's yeast in a 1-liter fermentor. His generator produced a mixture of CGA-size bubbles (20-70 µm) and some large bubbles (3-5 µm). Kaster (1988) modified the CGA generator to protect the foam from contamination the volumetric oxygen transfer coefficient (kLa) apparently increased when the micro bubble disperssiongenerator (operated at 100 RPM) was used to supply oxygen to a 1-liter fermentor. Hensirisak (1997) used the MBD generator to supply oxygen to a 20-liter fermentor and reported that the oxygen transfer rate increased relative to air sparging. For an anaerobic fermentation, Bredwell and Worden (1998) applied the MBD to increase the mass transfer of synthesis gas to produce ethanol and butanol.

Therapy for ocular diseases

Many hereditary and acquired ocular diseases have the great potential for benefit from gene therapy[48]Compared to most other tissues of the body, the eye is an excellent candidate for gene therapy as it is easily accessible and immune-privileged [49] In addition, the eye is a small organ, making the transfection of a significant proportion of the cells of interest a realistic possibility[50] ultrasoundtargetedmicrobubble destruction-mediated gene delivery to ocular tumorshas also been reported. A research group successfully demonstrated that enhanced green fluroscenceprotein gene could be transfected into retinoblastoma cells by using UTMD system and verified that transfection efficiency intoRB cellsmediated by UTMD was similar to that withlipofectamine 2000 [51]Although it still lacks in vivostudy, this result indicated the feasibility of UTMD-mediated gene therapy to ocular tumors.

Intravascular applications[52]

Angiogenesis

In addition to thrombosis, there are other intravascular targets for molecular imaging with ultrasound. Certain integrins such as AlphaVBetaIII are expressed inangiogenesis.Lindnerandothers have shown that microbubbles targeted to AlphaVBetaIII can be used to measure the temporal expression of this integrin in association with angiogenesis. A contrast agent targeted to endothelial- based markers of angiogenesis might be used to improve diagnosis and treatment of disorders affected by angiogenesis. Other endothelial-based targets such as P-selectin might be exploited to develop targeted imaging agents for detecting inflammation.

Vulnerable plaque

Another important intravascular target is vulnerable plaque. Vulnerable plaques are those that have been infiltrated by macrophages, and are undergoing inflammation (Bjørn Tore Gjertsen et al., 2002). Inflammation can lead to rupture of vulnerable plaque and formation of thrombus, as in stroke and myocardial infarct. Vulnerable plaques may lie hidden as unseen threats, liable to cause morbidity and sudden death. A noninvasive test is needed to detect vulnerable plaque.

Vulnerable plaque has been successfully detected using targeted microbubbles in combination with ultrasound.

Capture of particles by bubbles

Recent bench studies of flotation of different minerals; with injection of microbubbles (40 μm, mean diameter) to lab cells (in addition to the cell generated coarse bubbles) have improved separation parameters when compared to the mill standard (Yalcin et al., 2002; Rubio et al., 2003).The potential use of dissolved gas bubbles in mineral flotation was investigated using a copper–nickel ore (Inco Ltd. in Sudbury, Canada) (Yalcin et al., 2002).Such bubbles were generated by pressurizing (during1 min) the ore pulp in an air or argon atmosphere at276 kPa gauge (40 psig), and then releasing the pressureby discharging the pulp into a column where flotation took place. Based on the conclusions of an earlier work,dissolved gas bubbles were employed together with conventional bubbles, the latter being produced by a gas sparger located inside the flotation column.

Environmental applications of the flotation with microbubbles

Advantages of thedissolved air floatationprocess are the high volumeof the effluents being treated (100–20,000 m3h−1),smaller footprint, yields excellent treated water quality,generates thicker sludge, rapid start up and operation Dissolvedairflotation (DAF) has gained widespreadusage for the removal of contaminants and the recoveryof by-products from wastewater and other industrialprocess streams over the last 20 years. While considereda relatively simple technology, there have been signifi-cant improvements in the technology including operating parameters, bubble generation systems, and processdesign. There has also been an expansion of applications using DAF over the last several years in traditional andnon-traditional areas of water and industrial effluenttreatment (Capponi et al., 2006).

Microbubbles provide new boost for biofuel production[53]

The technique builds on previous research in which microbubbles were used to improve the way algae is cultivated.Algae produce oil which can be processed to create a useful biofuel. Biofuels, made from plant material, are considered an important alternative to fossil fuels and algae, in particular, has the potential to be a very efficient biofuel producer. Until now, however, there has been no cost-effective method of harvesting and removing the water from the algae for it to be processed effectively. A team has developed an inexpensive way of producing microbubbles that can float algae particles to the surface of the water, making harvesting easier, and saving biofuel-producing companies time and money.The major barrier to biofuel companies processing algae to use as fuel when we used microbubbles to grow the algae more densely. It turned out, however, that algae biofuels still couldn't be produced economically, because of the .difficulty in harvesting and dewatering the algae. We had to develop a solution to this problem and once again, microbubbles provided a solution."Microbubbles have been used for flotation before: water purification companies use the process to float out impurities, but it hasn't been done in this context, partly because previous methods have been very expensive.

Conclusion

Over the last decade, there has been phenomenal progress in designing innovative microbubble formulations for imaging and drug delivery. The development of lipid, protein, and polymer based microbubbles has shown potential for a wide variety of imaging and therapeutic applications. Engineered microbubbles are ideally suited as theranostic agents to enhance the imaging and therapeutic capabilities of ultrasound. In this article we review the applications which are most for our lifestyle. Firstly Specific acoustic and biological properties make microbubbles a promising tool as a vehicle for drug and gene delivery. , secondlythe use of targeted microbubbles has been a great step forward. Targeted microbubbles create various challenging therapeutic options, not only in cardiovascular disease but also in treatment of inflammatory and malignant diseases. Now the new approach of microbuble concept is biofuel production which can solve many question related with biofuel.

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52. The Faculty of Engineering at the University of Sheffield - the 2011 Times Higher Education's University of the Year - is one of the largest in the UK. Its seven departments include over 4,000 students and 900 staff and have research-related income worth more than £50M per annum from government, industry and charity sources. The 2008 Research Assessment Exercise (RAE) confirmed that two thirds of the research carried out was either Internationally Excellent or Internationally Leading.
53. The Engineering and Physical Sciences Research Council (EPSRC) is the main UK government agency for funding research and training in engineering and the physical sciences, investing £800 million a year in a broad range of subjects - from mathematics to materials science, and from information technology to structural engineering. www.epsrc.ac.uk.