Review Article

Microubbble: Targated Drug Delivery System: A Review

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ABSTRACT

Micro bubbles are promising as important contrast agents for imaging and carriers for targeted drug delivery. Micro bubbles with assigned air or gas filled microspheres suspended in a liquid carrier phase which results from the introduction of air or gas. The liquid phase contains surfactants to control the surface properties as well as stability of the bubble. The micro bubbles have an average size (1-8µm) less than that of red blood cells, so they are capable of penetrating even into the small blood capillaries and releasing drug and genes under the action of ultrasound field after getting the specific site of action. Ligands which are targeted at sites are attached to the surface of the micro bubbles, such application widely used in cardiovascular system and tumor diagnosis therapy. Micro bubbles dispersion method was introduced and investigated to develop oxygen transfer at low agitation rates and thus decreases power consumption and shear stress on the microorganisms. Micro bubbles are having boost utilization in formation of bio fuel. Recently, Ultrasound-mediated micro bubbles destruction has been planned as a novel method for non invasive delivering of drugs and genes to different tissues. This review focuses on the synthesis, characteristics of micro bubbles that give them beneficial properties and some important aspects of ultrasound parameters that are known to enhance micro bubble mediated drug delivery. In addition, current studies involve discussion of novel therapeutically application of micro bubbles.

Keywords: Micro bubbles, Ultrasound, Targeted drug delivery, Ultrasound contrast agent, Cancer treatment.

INTRODUCTION

Micro bubbles are an extremely small bubble, usually only a few micrometers in diameter that can be uniformly suspended in a liquid such as blood. Micro bubbles was first introduced to importance in the mid-'90s, when the FDA cleared them for use in imaging applications. In very less time, they became a cheap alternative to costly scans all that's needed is a shot and a portable ultrasound machine. In addition to that, this technique is fast; micro bubbles can be imaged in minutes, while CTs and MRIs take hours.

Since then, scientists have established that small bubbles have another use i.e. delivering pharmaceutical payloads inside the body. Bubbles are coated with specific molecules ultimately bind to definite cellular receptors. Once they are attached to the receptors, a strong ultrasound burst is all that's needed to pop the bubbles and free what's inside. Delivering drugs directly to site of a tumor this way would allow for smaller doses no more bombarding the complete body with radioactive material thus radically decreases side effects. Gene therapy, which treats genetic diseases by using viruses to distribute DNA, could also be safer if the material were administered via micro bubble. Both techniques are being tested on animals.

The most impressive achievement in the micro bubble's case is the ability to get drugs across the blood-brain barrier. Small molecules like alcohol may get entry into the gray matter, but anything larger is typically kept out which is a characteristic that protects us but has severely inadequate treatment options for neurological conditions like Alzheimer's diseases. Over the last few years, a team of scientists from UC Davis continuously working on how micro bubbles can be "pushed" to the barrier and then exploded, opening up small pores through which drugs can pass. The result of this experiment may eventually give doctors a much larger area for the development of drugs for brain diseases.

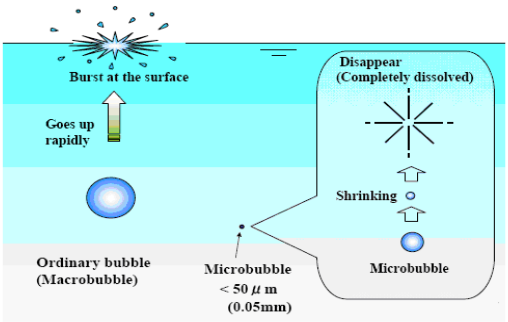

Figure 1: Basic difference between an ordinary bubble and a micro bubble

➢ **Fundamental properties of micro bubble**

1. Increase in interior gas pressure
2. Increase in ion concentration around the gas-water interface
    - Generation of free-radicals Ions

- Generation of Nano-bubble

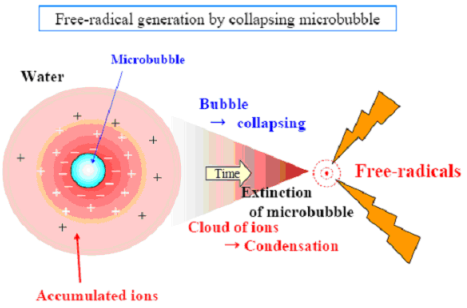

Figure 2: Free radical generation by collapsing micro bubble

Preparation of Micro bubbles

Method of preparation of micro bubble is using physical and chemical properties of the Ultrasound Contrast Agents (UCA). Traditional methods for the production of ultrasound contrast agents like sonication, it is difficult to have such particle properties. In this method, UCA will be prepared by emulsification process. Different techniques are used such as membrane, micro channel and micro sieve emulsification and that will be examined and compared for dedicated UCA production.

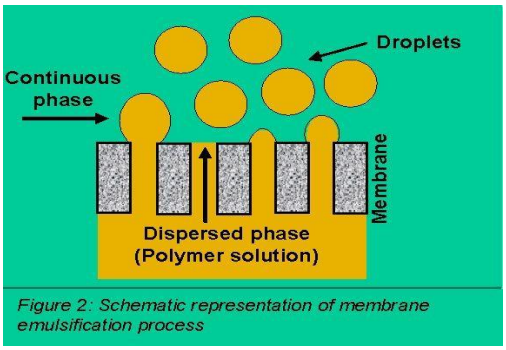

Figure 3: Cross flow membrane emulsification process.

How Micro bubbles work?

Micro bubbles are working by resonating in an ultrasound beam, quickly constricting and growing in response to the pressure changes of the sound wave. By a providential concurrence, they vibrate mainly strong at the high frequencies used for investigative ultrasound imaging. This makes them a number of thousand times more reflective than normal body tissues. In this way they improve both grey scale images and flow mediated Doppler signals. Being useful in itself, the speed that micro bubbles produce has numerous special properties that can be exploited to develop diagnoses. Just as with a musical instrument, multiple harmonic signals or overtones are produced, Ultrasound scanners can be tuned to "listen" to these harmonics, producing strong preferential imaging of the micro bubbles in an image. The selection of excitation produced may also destroy micro bubbles comparatively easily, an effect that can be useful both in imaging and in emerging therapeutic applications

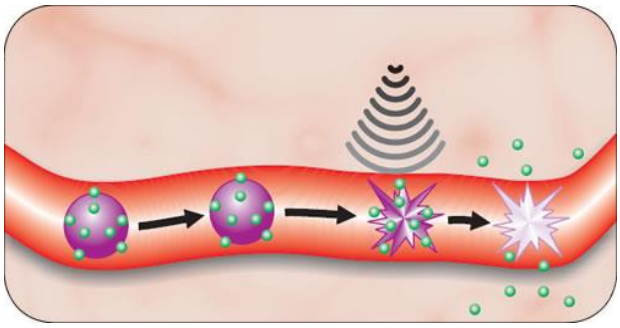

Figure 4: shows the working of Micro bubbles and the effect of ultrasound on it.

Micro bubbles in General Radiology

Micro bubbles increase the power of Doppler signals from blood for a number of minutes after their injection, and this effect can be prolonged by infusing them.[1]Doppler examination is done by raising the intensity of weak signals to a detectable level. For example, they can develop detection of flow in the intracranial arteries by transcranial Doppler in adults, where the skull greatly attenuates the ultrasound signal.[2] Another use is in detecting flow in smaller vessels, such as in the circulation of malignant tumours.[3]

Anticipated developments

- Robust methods are developed for detecting and measuring microcirculatory flow, allowing quantification of regional ischemia in the myocardium and other organs.
- Regular use of micro bubbles to improve imaging of the liver parenchyma, improving accuracy of ultra sonographic measurement and staging of cancer.
- Generation of micro bubbles can be for delivery of gene therapy and other treatments for target specific sites.

Micro bubble based methods are used for non-invasive clot lysis. Micro bubbles can also be administered into body cavities which are performed by simple functional tests. For example, vesicoureteric reflux in children can be exposed by injecting them into the bladder cavity and scanning the kidneys and ureters (fig. 4). Fallopian tube patency can be confirmed by observing and detecting an better signal after instilling micro bubbles into the uterine cavity.

Micro bubble Contrast Agents: A New Era in Ultrasound

Contrast agents are broadly used in imaging as well as in ultra sonography which was used recently. The concept of ultra sonography changed with the introduction of Micro bubbles small gas filled bubbles (typically 3 µm in diameter) that are typically injected intravenously. Injecting a gas with help of micro bubble into the blood stream may seem potentially dangerous, but broad clinical experience has shown that the small volume of air or gas given (under 200 µl) is not hazardous, and the

safety of micro bubbles compares well to that of traditional agents in radiography and magnetic Resonance imaging.[4] Although, micro bubbles were originally developed simply to improve conventional ultrasound scanning, recent discoveries have opened up powerful emerging applications.

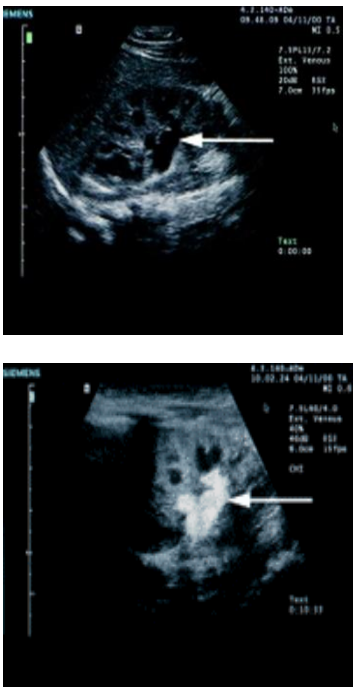

Figure 5: Use of micro bubbles to demonstrate Vesicoureteric reflux.

Top figure shows left kidney with a slightly dilated renal pelvis (arrow). Bottom figure shows the same area after Micro bubbles have been instilled into the Bladder. Bright echoes, representing micro bubbles can now be seen in the renal pelvis (arrow), indicating vesicoureteric reflux, which was confirmed on *x* ray micturating cystourethrogram.

(Images courtesy of Dr Thomas Albrecht,

Benjamin Franklin University,

Berlin, Germany)

Treatment with micro bubbles

Micro bubbles can helps in drug delivery by acting as "cavitation nuclei" and as agents to carry drugs for target site specific treatment. Their most exciting application is in the emerging area of gene therapy, where delivery of genetic material to a chosen site is difficult.[5]

Gas filled microspheres may be designed so that their interior is loaded with drug and gas. A stabilizing material, here a lipid, surrounds the perfluoro carbon bubble. Drugs may be incorporated by themselves or, if insoluble in water, in an oil layer. The microsphere may be targeted to specific tissue by incorporating protein ligands on the surface.

Ultrasound can improve drug delivery by creating temporary non-lethal perforations in cell membranes to assist entrance of large molecules and particles into the cells such process is known as "sonoporating". Generally, this requires very high acoustic power, significantly beyond that permitted for imaging, but the power needed is greatly reduced when micro bubbles are present. This is caused due to lower energy requirement for the micro bubbles necessary for cavitation, the process in which extreme oscillations induced by ultrasound pulses lead to micro bubble collapse.[6-8.] The potential of this in gene therapy has already been shown.[9] Cavitations of micro bubbles in capillary beds also increase capillary permeability, which improves local access of the released therapeutic agent.[10]

Micro bubbles as Drug Delivery Vehicles

Formulation of Micro bubbles may carry many therapeutic and clinical agents. Some micro bubbles based albumin with shells of charged lipids take up genetic material directly.[7, 11] Hydrophilic compounds can be covered within lipid membranes or polymeric shells that stabilize the micro bubbles. The circulation of these therapeutic agents loaded micro bubbles can be followed with ultrasound, and when they reach the target site. After that, they can be disrupted, releasing their therapeutic payload to the surrounding tissue (fig 5). A recent study showed that transfection of a reporter gene in a mouse heart model was augmented 10-fold using micro bubbles loaded with an adenovirus gene vector.[12]

Drug delivery to a target specific site can be supported by incorporating ligands into the membrane of the micro bubbles that target receptors on cell membranes. For example, inclusion of a surface ligand that binds to the GPIIB/IIIA receptors on activated platelets allows micro bubbles to bind to a thrombus and deliver thrombolytic agents.[10]

Mechanisms for Micro bubble Delivery

Considerably, Ultrasound based micro bubbles destruction has been considered as a new method for noninvasive delivering of drugs and genes to different tissues. Micro bubbles are used to bring a drug or gene until a specific area of interest is reached, and then

ultrasound is used to burst the micro bubbles, causing site-specific delivery of the bioactive materials. The probable mechanism to deliver drugs even in the absence of ultrasound is ability of albumin-coated micro bubbles to stick to vascular regions with glycocalix damage or endothelial dysfunction. The new advances in gene therapy and molecular biology have better the interest in methods of noninvasive delivery of therapeutic agents. Moreover the well-known application of micro bubbles as contrast agents for diagnostic ultrasound, micro bubbles have also been confirmed an effective technique for targeted delivery of drugs and genes[13-18].

Incorporation of drugs may be possibly into the micro bubbles by using different ways, including required of the drug to the micro bubble shell and attachment of site-specific ligands. As perfluoro carbon-filled micro bubbles are sufficiently stable for circulating in the vasculature as blood pool agents, they act as carriers of these agents until the site of interest is reached. Application of Ultrasound through the skin surface is used to burst the micro bubbles at this site, which causes localized release of the drug.[19-22] This technique then permits using lower concentrations of drugs systemically and concentration of the drug only where it is needed. This improvised therapeutic index of drug not only hazardous systemic side effects like cytotoxic agents but also may be greatly advantageous. Albumin-encapsulated micro bubbles have also demonstrated to adhere to the vessel walls in the setting of endothelial dysfunction.[23]This also may be a method of targeting delivery with micro bubbles but without the application of ultrasound.

Mechanisms for Target Drug Delivery Using Micro bubbles

Two probable strategy for delivering drugs and genes with micro bubbles are emerging. The first consists on the ultrasound-mediated micro bubble destruction, which is based on the cavitation of micro bubbles induced by ultrasound application, and the second is the direct delivery of substances bound to micro bubbles in the absence of ultrasound.

Different drugs and genes can be incorporated into the ultrasound contrast agents:

- Perfluoro carbon-filled albumin micro bubbles avidly bind proteins and synthetic oligonucleotides.
- Micro bubbles directly take up genetic material, such as plasmids and adenovirus.[24,25]
- Phospholipid-coated micro bubbles have a high affinity for chemotherapeutic drugs. [26]
- Specific ligands for endothelial cell adhesion molecules, such as P-selectin and leukocyte intercellular adhesion molecule 1 (ICAM-1), can be attached to both lipid- and albumin-encapsulated micro bubbles, which increases their deposition to activated endothelium. [27,28]

The mechanism by the delivery of drugs and genes from ultrasound facilitating that results from complex relationship between the therapeutic agent, the micro bubble characteristics, the target tissue, and the nature of ultrasound energy.

The presence of micro bubbles in the insonified field reduces the peak negative pressure needed to enhance drug delivery with ultrasound. This occurs because the micro bubbles act as nuclei for cavitations, decreasing the threshold of ultrasound energy necessary to cause this phenomenon.

The results of optical and acoustical studies have suggested the following mechanisms for micro bubble destruction by ultrasound:

- Gradual diffusion of gas at low acoustic power,
- Formation of a shell defect with diffusion of gas,
- Immediate expulsion of the micro bubble shell at high acoustic power,
- Dispersion of the micro bubble into several smaller bubbles.

Cavitation of the bubbles is characterized by rapid destruction of contrast agents due to a hydrodynamic instability excited during large amplitude oscillations, and is directly dependent on the transmission pressure.[29, 30]

The mechanism for facilitating the drug deposition by pores formation in the cells membranes resulting by ultrasound-induced micro bubble cavitations has been probable.

An additional important therapeutic property of micro bubbles is the increased adherence to damaged vascular endothelium. Albumin-coated micro bubbles do not adhere to normally functioning endothelium, but their adherence does occur to activated endothelial cells or to extra-cellular matrix of the disrupted vascular wall, and this interaction could be a marker of endothelial integrity. Because of this characteristic, the delivery of drugs or genes bound to albumin-coated micro bubbles might be selectively concentrated at the site of vascular injury in the presence[31] or absence of ultrasound application.[32]

Various Instruments Producing Micro bubble

Two diffusers produce micro bubble:

- Air & Oxygen Diffusers
- Micro bubble Ceramic Plate Diffusers

They are highly efficient and have economical oxygen consumption. Micro bubble ceramic plate diffusers produce a cloud of extremely fine bubbles, 100 to 500 microns. The smaller the bubble, the more efficient the absorption of the gas. The fine pore ceramic plate produces uniform bubbles across its entire surface. Ceramic is a clean, inert material.

Working pressure: 25 to 35 psi (1.7 to 2.4 bar). Must not exceed 50 psi**.**

Targeted Molecular Imaging and Therapy:

Benefits of micro bubble target delivery system include:

- Real-time imaging,
- Relatively short and efficient imaging protocols,
- Non-invasive with minimal patient discomfort and low operating costs.

Two possible approaches for targeted imaging are

- Passive targeting
- Active targeting

Active targeting relies on adhesive ligands, as markers of inflammation and thrombus, among other factors.

Micro bubbles today have different characteristics that allow them to be used as targeting materials, including various sizes and the types of gases, and shell materials. Under development are micro bubble agents, liposomal agents, and perflurocarbon emulsion nanoparticles.

The micro bubble is comprised of an outer shell, which can contain target ligands, and gases within the micro bubble. Drugs can be encapsulated within the micro bubbles, which can be targeted for drug release or enhancement of drug effects using Ultrasound. Ultrasound alone enhances drug delivery and efficiency. Thrombolysis can be enhanced by the combination of Ultrasound and thrombolytic agents. Tissue penetration can be enhanced by combining Ultrasound and transdermal drug delivery. Hall and colleagues demonstrated nanoparticles that adhere to fibrin and the nanoparticles can be visualized by Ultrasound. Another example is a micro bubble with GPIIb/IIIa receptor that adheres to thrombus, which had been demonstrated in an animal model. Lanza et al had shown that using nanoparticle accumulation and US imaging, it is possible to obtain enhanced imaging of balloon-injured arteries, whereas there was no enhancement in the control animals.

Applications

Specific Diagnostic Applications

Imaging the liver

This is perhaps the most promising clinical application of micro bubbles in radiology at present. The liver and spleen take up some, but not all, micro bubbles. The precise mechanism is unclear, but the reticuloendothelial system is probably involved. This liver phase lasts about 30 minutes with the licensed agent Levovist[33] and several hours with some new agents in clinical trials.[8] During this phase the liver is particularly well seen with micro bubble-specific imaging modes such as harmonic imaging. The main practical importance is that many focal liver lesions, particularly metastases and hepato cellular carcinoma, appear as defects, and their visibility is greatly increased with micro bubbles (fig 6).[34]

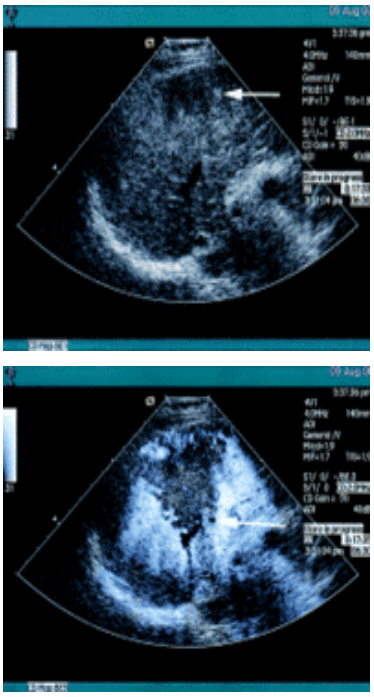

Figure 6:

Effect of liver-specific micro bubbles on the visualization of a hepato cellular carcinoma complicating chronic hepatitis B infection. Top figure shows the liver is heterogeneous with an ill-defined lesion (arrow). Bottom figure shows the presence of liver-specific micro bubbles (Levovist) administered 5 minutes earlier. A defect is clearly seen in the central right lobe of the liver, with several additional defects thought to represent additional satellite foci of hepatocellular carcinoma.

Early evidence from several studies suggests that this can substantially increase the sensitivity of ultrasound to metastatic disease.[34-37] It seems particularly useful in detecting small lesions (under 1 cm diameter), for which

all imaging methods lack sensitivity. A recently completed multicentre study showed an increased sensitivity to liver metastases from 71% to 88%, and the mean size of the smallest lesions detected decreased by 50% to under 1 cm.[38] It therefore seems likely that the sensitivity of ultrasound to liver metastases, and possibly to hepatocellular carcinoma, could be substantially increased. This mode of scanning has limitations, however, including the transience of the effect and the difficulty of accessing the whole of the liver. The advent of new, more durable, liver-specific agents currently in clinical trials will probably address at least the former concern.

Micro bubbles may also increase specificity in liver imaging since some lesions can be characterized by their enhancement patterns. For example, haemangiomas, common benign tumours that can mimic more aggressive pathology, show a characteristic globular or peripheral pattern of enhancement with centripetal fill-in, and their identification with ultrasound may avoid the need for further tests to characterize them.[39] Another study has shown that late uptake of micro bubbles is characteristic of many benign lesions.[40]

An analysis of the initial vascular enhancement produced by micro bubbles can also give much useful information. Changes in intensity (or loudness) of a spectral Doppler signal are proportional to micro bubble concentration. Thus, if a hepatic vein is scanned after a bolus injection of micro bubbles, analysis of the changes in Doppler intensity with time gives much useful information. Early enhancement is seen in cirrhosis and malignancy because of vascular shunting and arterialisation of the liver's blood supply.[41, 42] This simple technique may be particularly useful for distinguishing between different types of diffuse liver disease and diagnosing cirrhosis, for which traditional imaging findings are notoriously unhelpful so that biopsy is often required. Research is under way to study whether early enhancement of the ultrasound signal is also characteristic of “micrometastases” in colon cancer before they are detectable by conventional methods.

Imaging the heart

Micro bubbles can enhance Doppler flow signals in cardiac ultrasonography, and this can be useful in several situations, such as detecting valvular stenoses.[43] Of greater impact has been their role in improving delineation of the endocardial border. Micro bubble contrast agents highlight the left ventricular cavity and make the blood-tissue boundary much clearer, which helps in assessing regional abnormalities in wall motion, estimating ejection fraction, and detecting left ventricular thrombus. Evaluating left ventricular function is the key to the management of many cardiac conditions, and this procedure forms about 60% of the workload of an adult echocardiography department. Good endocardial definition is critical to this evaluation, and any improvement in definition is especially useful for the 10-20% of patients with technically difficult studies using standard techniques.[44] Some contrast agents have been shown to convert 74% of non-diagnostic images into diagnostic studies by improving endocardial visualisation.[45] They can assist in thrombus detection when imaging is difficult, which may be crucial in deciding whether to start anticoagulant treatment.

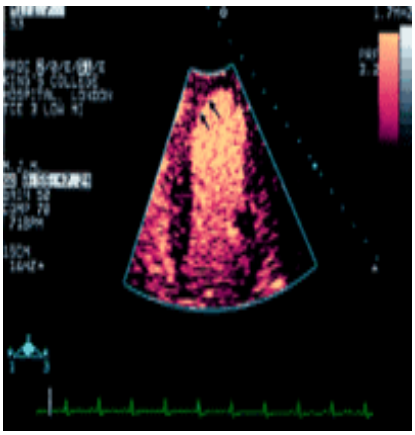

Figure 7:

Echocardiographic image of the left ventricle using real time imaging of perfusion and an intravenous micro bubble as contrast agent. The contrast agent fills the ventricular cavity, clearly delineating the endocardial border, and gives colour enhancement in the myocardium, showing perfusion of the apex and septum (arrows)

The excellent endocardial sharpness particularly critical when echocardiography is perform under physiological or pharmacological stress (“stress echocardiography”), and the utilization of contrast agents in this condition has been particularly valuable. In about 35% of stress echocardiographic studies some segments of the endocardium are poorly seen, and contrast agents can render most of these interpretable. Overall, such agents improve diagnostic reliability and accuracy and reduce inter observer variability.[45] Many centres perform at least 60% of their studies with the aid of contrast agents.

Emerging roles for micro bubbles in cardiology include assessing myocardial perfusion. For this, an understanding of the fragility of micro bubbles in an ultrasound beam is crucial. Until recently, detecting blood flow required intermittent imaging because of extensive destruction of micro bubbles when continuous scanning was used.[46] Now, however, contrast-specific technologies that use low acoustic power, and hence disrupt micro bubbles less, allow real time imaging of perfusion. This allows information about wall motion and myocardial perfusion to be obtained simultaneously (fig 7), offering great potential for diagnosing acute myocardial infarction, determining the area of myocardium at risk, and assessing the success of thrombolysis. Micro vascular integrity, and therefore myocardial perfusion, is essential for functional recovery of affected segments of heart muscle, and myocardial contrast echocardiography can accurately predict this[47].Real time perfusion imaging in stress echocardiography may offer a potent tool for assessing both resting and inducible ischaemia. The method can be extended further by applying intermittent high power pulses to destroy most of the micro bubbles in a scan plane and then watching refilling: the rate at which this occurs is a measure of microcirculatory flow speed.[48]

Micro bubbles Use for Gene Therapy

The clinical uses of viral vectors for gene therapy is limited because viral proteins elicit an immune response within the target tissue [49], and have been shown to cause an intense inflammatory activation of endothelial cells [50] .On the other hand, the non viral delivery of vehicles, such as plasmids and antisense oligonucleotides, has been associated with a lower transfection efficiency and transient expression of the gene product [51].

Micro bubbles Delivery of Insulin Gene to Pancreas

The method of ultrasound-targeted micro bubble destruction (UTMD) is used to deliver insulin genes to the pancreas of rats which showed a decrease in blood sugar levels. Tiny gas-filled bubbles coated with DNA encoding the insulin gene is injected into the bloodstream. Beams of ultrasound are then directed at the Islets of Langerhans, the region of the pancreas containing the insulin-producing beta cells. This causes the micro bubbles in nearby blood vessels to burst, releasing the insulin gene. The ultrasound beams also created pores in the beta cell membranes, through which the DNA can enter. Not only is the blood sugar lowered, but also there is no damage to the pancreas.

Intracellular Delivery of Peptides and siRNA's

Bioactive peptides and nucleic acid based molecules have a great potential for modifying the functions of cells. This interesting feature has great hope in the use for curing various diseases. One of the challenges, however, that prohibits the uses of these promising agents is the establishment for the method of intracellular delivery. Although many attempts have been made to make intracellular delivery of both peptides and short interfering RNAs (siRNAs) possible, many problems remain, such as efficiency, target selectivity and invasiveness to the host.

On the other hand, the development of ultrasound technology and micro-bubble based ultrasound contrast agents, brought up a completely new method for delivering foreign substances into cells, which is often mentioned as "sonoporation". By using this method, intracellular delivery of fluorescent markers or even naked plasmid DNA has been reported both in vitro and in vivo. Sonoporation method has advantage over conventional methods because it requires less invasive procedures and also makes it possible to localize the effect only within the target of interest.

It has been implicated by using ultrasound contrast agent OPTISON® to deliver

1) The BH3 peptide from Bak protein for enhanced cell killing and

2) Enhanced green fluorescent protein (EGFP) targeting siRNA for silencing both endogenous and exogenous EGFP expression.

Oligonucleotide delivery

To date drug delivery systems have been included drug carriers based upon proteins, polysaccharides, synthetic polymers, erythrocytes, DNA and liposomes. New generation biologicals such as monoclonal antibodies, gene therapy vectors, anti-cancer drugs such as Taxol, viral based drugs, and oligo and polynucleotide have presented several problems with regard to delivery. This problem is somewhat resolved by using new and improved pharmaceutical composition and method for delivery of therapeutic agents.

Early detection of cancers

A European research project is developing a highly sensitive ultrasound technique that could help visualize minute quantities of cancerous tissue in patients. The approach uses micro bubble-based ultrasound contrast agents that specifically target and bind to certain pathogenic cells in the body. When combined with enhanced ultrasound equipment and signal-processing capabilities, the scheme could help detect tumours in their earliest stages of development.

Improving the efficiency of gene transduction in skeletal muscle with reduced tissue damage.

Intramuscular injection of naked plasmid DNA is a safe approach to the systemic delivery of therapeutic gene products, but with limited efficiency. The use of micro bubble ultrasound to augment naked plasmid DNA delivery by direct injection into mouse skeletal muscle in vivo, in both young (4 weeks) and older (6 months) mice has been investigated. It was observed that the albumin-coated micro bubble, Optison (licensed for echocardiography in patients), significantly improved the

transfection efficiency even in the absence of ultrasound. The increase in transgene expression is age related as Optison improves transgene expression less efficiently in older mice than in younger mice. More importantly, Optison markedly reduces muscle damage connected with naked plasmid DNA.

Water Purification Using the Adsorption Characteristics of Micro bubbles

Recently, technology using micro bubbles has been studied for water purification. However, the mechanism and physical parameters of the purification process have not yet been sufficiently clarified. The purpose of this study is to clarify the physical parameters of micro bubbles that influence water purification.

There are various ways of purifying polluted water such as using microorganisms and supplying heat and so on, in the way. However, these methods do not have high efficiency or a stable performance. On the other hand, the technology of micro bubbles has been developed and purification systems using micro bubbles have been studied in recent years. However these studies were only on the performance of systems, and studies on the adsorption of micro bubbles having not been carried out. These extremely small sized micro bubbles, are characterized by having electrical charges. They attract suspended floating particles very powerfully. This exact property has been used in sludge treatment by using the micro bubbles to capture and float organic matters, thus falling the time required for the sludge treatment.

CONCLUSIONS

We summarize our results as follows.

1) The adsorption of the bubbles is determined by the chemical potential and equilibrium constant of the pollutant.

2) The rate of purification can be calculated using the equilibrium constant.

3) The most significant factor determining the adsorption is the surface area.

4) The surface tension of micro bubbles is not a significant factor in this study.

Micro bubbles Can Image Blood Vessel Growth in Tumors

Researchers have now been able to quickly detect and diagnose blood vessel growth in cancerous tumors, and even predict how fast the tumors might metastasize or spread. Researchers at the University of Virginia Health System are doing just that in animal models using millions of tiny micro bubbles injected into the bloodstream, coupled with contrast-enhanced ultrasound, an inexpensive and widely used technique using sound waves to "see" inside the body. Research shows that scientists can detect cancer using ultrasound contrast agents targeted to abnormal blood vessels that reside within tumor. By assessing how much new blood vessel growth there is, we can detect tumors and metastatic spread at a very early stage.

One of the first signs of tumor and metastasis is a remodeling of surrounding blood vessels in the normal tissue near a tumor. The tumor activates the process of growth of new blood vessels called angiogenesis, supplying nutrients and oxygen to the tumor and keeping it alive. To detect angiogenesis in and around a tumor, micro bubbles normally about half the size of a red blood cell and are composed of a gas surrounded by a shell. They are currently being used worldwide to image blood flow and heart function in patients.

Additional applications:

- Micro bubbles also introducing by the Japanese to market safe and good tasting oysters. Micro bubbles of concentrated oxygen contain about 2% ozone can be used to inactivate noro virus in shellfish and oysters. This Noro virus is one of the major pathogens causing food poisoning in winter. This is a much more cost-effective method compared to cultivating the oysters in sterile seawater and using chlorine-based germicide.
- Due presence of large surface area volume ratio, micro bubbles can penetrate deeply into a surface for effective cleaning. This cleaning effect of micro bubbles is used in clear out the inside of vegetables such as cabbage and radish sprout, as well as maintenance of freshness with vegetables in one particular vegetable processing center in Japan.
- On a more personal level, the micro bubbles be able to penetrate deeply into skin for a good scrub devoid of the need for any shampoo or soap. This skin treatment has been introducing inside some spas in Japan as well as shops specializing in bathing pets. Needless to say, the baths are particularly helpful for pets, which have produce skin allergies to pet shampoos.

Flotation with Microbubbles[52]

Advantages of the dissolved air floatation process are the high volume of the effluents being treated (100– 20,000 m3h−1), smaller footprint, yields excellent treated water quality, generates thicker sludge, rapid start up and operation Dissolved air flotation (DAF) has gained widespread usage for the removal of contaminants and the recovery of by-products from wastewater and other industrial process streams over the last 20 years. While considered a relatively simple technology, there have been significant improvements in the technology including operating parameters, bubble generation systems, and process design. There has extended an application using DAF over the last several years in traditional and nontraditional areas of water and industrial waste matter treatment[53]. Micro bubbles make available new boost for bio fuel production. The

technique builds on previous research in which micro bubbles were used to get better the way algae is cultivated. Algae produce oil which can be processed to create a useful biofuel. Biofuel, 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 efficient way to produce micro bubbles using float algae which is using particles on water surface for easier harvesting which is also saves time and money of biofuels producing companies. The major disadvantage is that companies a using processing alga which causes to grow more densely when use micro bubbles. The other way out is that algae biofuel still couldn't be produced inexpensively, here the role of micro bubbles once again important because of the difficulty in harvesting and dewatering the algae.

Animal Testing in Micro bubble Cancer Therapy Research

In order to determine the efficacy and the potential risks associated with the different Micro bubble technologies, many research teams utilize animal test subjects, in most cases mice, to test how the internal environment affects the effectiveness of the micro bubble drug delivery systems. Typically, the mice are injected with some form of cancerous cells, which are allowed to grow to a desired diameter or volume. This process was done by members of the Chongqing Medical University who purposely injected mice test subjects with tumorous cancer cells on the dorsal flank area in order to test the effectiveness of the 10-Hydroxycamptothecin-loaded micro bubbles previously mentioned, which can be seen in Figure 12.

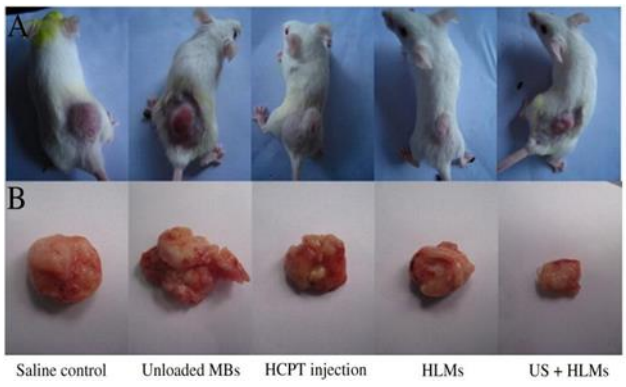

Figure 12: Mice Test Subjects with Tumors and Tumors Removed after 10-Hydroxycamptothecin Micro bubble Treatment

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