



A Review on Microbubbles as a Boon for Novel Delivery System

Makne P.D¹, Shetkar M.A*¹, Patil.S.S², Poul B.N³

1. Maharashtra College of Pharmacy, Department Of Quality Assurance, Nilanga-413521, Dist. Latur (MS) India.

ABSTRACT

Microbubbles designate air or gas filled microspheres suspended in a liquid carrier phase which generally 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. Microbubbles have an average size less than that of RBC's i.e. they are capable of penetrating even into the smallest blood capillaries & releasing drugs or genes, incorporated on their surface, under the action of ultrasound. Microbubbles in general have a wide variety of applications. However in the biomedical field these are primarily used as diagnostic agents in combination with ultrasound for molecular imaging of various organs and even tumors. These are also proposed for drug and gene delivery to targeted regions in combination with various ligands. Most of the physicians today prefer imaging with ultrasound in combination with microbubbles compared to other diagnostic techniques for low cost and rapidity.

Keywords: Micro bubbles, diagnostic agents, ultrasound, drug delivery, gene delivery, ligands.

*Corresponding Author Email: madhav_shetkar@rediffmail.com

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INTRODUCTION

Micro bubbles are also small spherical bubbles comprising of gas, they remain distinct from each other or separate from each other i.e. do not agglomerate, also they have their size range in micrometers usually 1-100 μm . There has been a lot of research on micro bubbles in recent years. Micro bubbles are miniature gas bubbles of less than 50 microns diameter in water. The micro bubbles, which mostly contain oxygen or air, can remain suspended in the water for an extended period. Gradually, the gas within the micro bubbles dissolves into the water and the bubbles disappear. In the medical field micro bubbles have been used as diagnostic aids to scan the various organs of body and recently they are being proposed to be used as drug or gene carriers and also for treatment in cancer therapy. Microbubbles have been used in a variety of fields, these have been used to improve the fermentation of soil, used to increase the hydroponic plant growth, have been used to increase the aquaculture productivity, these have been also used to improve the quality of water, used in sewage treatment. Bio medically micro bubbles are defined as small spherical gas bubbles made up of phospholipids or biodegradable polymers, that are approximately the size of RBC's and are used as diagnostic aids, as drug and gene carriers in combination with ultrasound^{1,2}.

Advantage

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.

1. Ultrasound imaging allows real-time evaluations of blood flow.
2. Ultrasonic molecular imaging is safer than molecular imaging modalities, such as radionuclide imaging, because it does not involve radiation.
3. 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.
4. Since micro bubbles can generate such strong signals, a lower intravenous dosage is needed. Micrograms of micro bubbles are needed to perform contrast-enhanced ultrasounds compared to milligrams for other molecular imaging modalities, such as MRI contrast agents.
5. Targeting strategies for micro bubbles are versatile and modular. Targeting a new area only entails conjugating a new ligand.
6. 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.

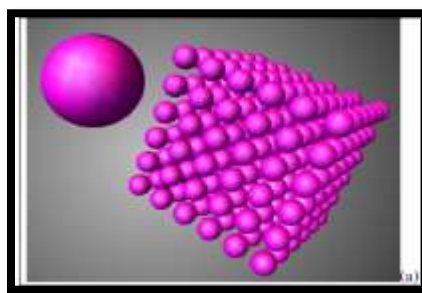
7. It provides high spatial resolution in the range of 3 mm. Its minimal invasiveness results from the very high frequency of ultrasound used, which does not generally cause damage or disturbance to the cells^{3,4}.

Disadvantage

1. Contrast-enhanced ultrasound suffers from the following disadvantages:
2. 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.
3. Ultrasound produces more heat as the frequency increases, so ultrasonic frequency must be carefully monitored.
4. 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^{3,4}.

The Benefit of Microbubbles^{5,6,7}

In many instances, miniaturization is sought for the purposes of convenience –smaller devices are more portable, or require fewer uses of resources. Why would microbubbles be a benefit? In the case of consumer products which use microbubbles, it might be the texture of the product (frequently a foam) is perceived better; possibly the processing of microfoams is better – with lower viscosity or better rheological features. Separations processes such as for minerals or biotech materials might be enhanced, or for the flotation or air-lift of wastes or oil recovery. A common thread among the benefits of microbubbles is in their transport behavior – mass, momentum, and heat transport at the interface of microbubbles is influenced by the interfacial surface area. Figure 1 depicts the key feature of high surface area to volume ratio.



(a)

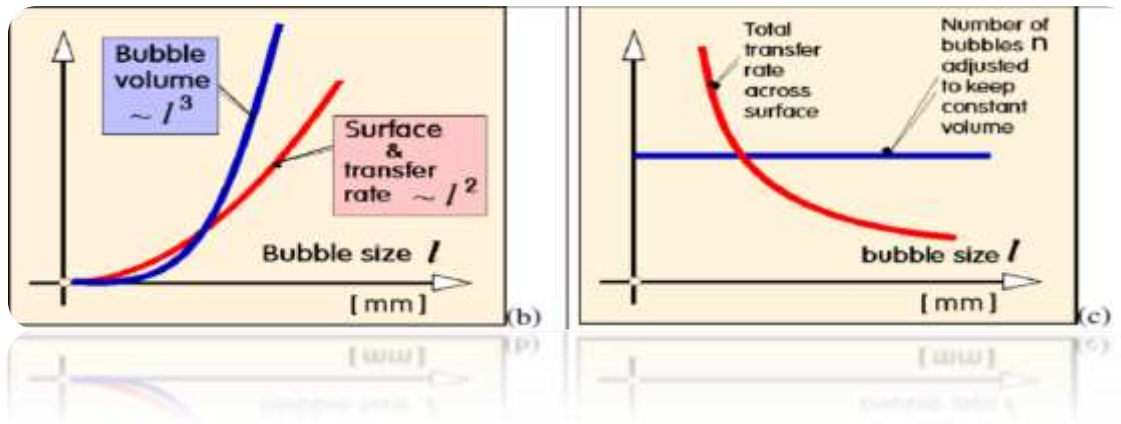


Figure 1: The transfer benefit of microbubble generation.

(a) Division of a volume into n smaller, equally sized objects, produces additional surface area that scales with the cube root of the dividing number. (b) For a single bubble, the surface area and transfer rate scale as the square of the bubble size l , but bubble volume scales with its cube. (c) Therefore, the total transfer rate with the number of bubble adjusted to keep the air phase volume constant, scales inversely with the bubble size l —smaller bubbles lead to greater transfer.

The argument given in Figure 2 for the benefit in transfer efficiency is typified by the common chemical engineering phenomenological description of interphase mass transfer flux J (moles per second):

$$J = KLa (c_g - c_l) \quad (1)$$

where,

Kl is the mass transfer coefficient (units of velocity),

a is the interfacial area, and c_g and c_l are molar concentrations. There is a direct analogy to heat transfer flux Q where the roles of the concentrations are played by temperature, i.e. Newton's Law of Cooling. What is not so intuitive, however, is that there is a similar transfer effect for momentum, where the role of J is taken by the force F in the vertical direction induced by velocity changes in the horizontal direction, which follows from Newton's law of viscosity:

$$F = -\mu a \frac{\partial w}{\partial x} \quad (2)$$

The interpretation of equation (2) is that the momentum transfer by a cloud of rising bubbles increases with the surface area of the cloud dragging more of the ambient liquid with it than a larger bubble with less surface area. This feature opposes the more intuitive feature that smaller bubbles rise less quickly than a single larger bubble that matches its volume. The rise velocity is a linear effect with bubble size as shown in Figure 3.

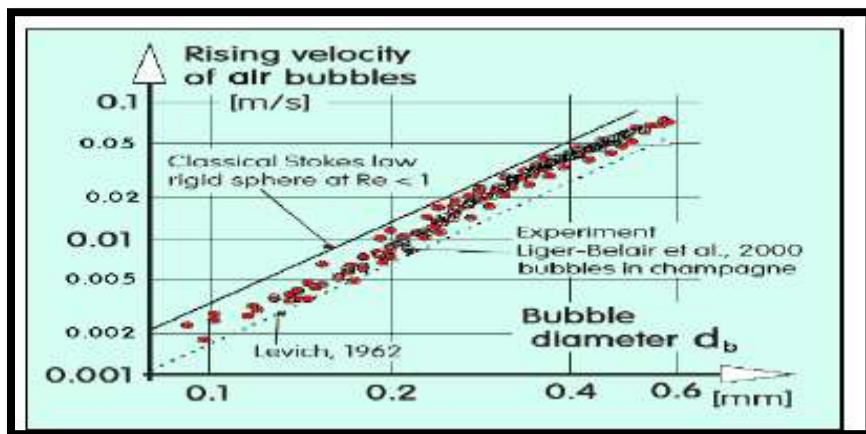


Figure 2. The rise velocity of bubbles as shown theoretically (Levich) and experimentally.

As transfer rates are shown to increase inversely proportionally to bubble size, but the velocity difference rises proportionally to bubble size, one would expect momentum transfer by the cloud of bubbles to be relatively constant. The total amount of momentum transferred, however, should be larger by the smaller bubbles due to the finite height of the liquid layer. The slope of the above graph shows that bubbles three fold smaller stay in the liquid ten fold longer, thus having longer time to transfer the same momentum rate. The “stirring effect” by a rising cloud of small bubbles, according to Figure 2, exceeds that of the passage of a single larger bubble if nonlinearity is neglected (Stokes regime). The canonical chemical engineering application for such microbubble dispersal is called surface aeration, frequently the most important process in bioreactors and fermentors (see Grammatika and Zimmerman, 1999). The paper is organized as follows. In section 2, the two classes of high power consumption formation of microbubbles – compression followed by release, and power ultrasound – are discussed, with an eye to the high value added products from the processes that utilize microbubbles. In section 3, the third class of low power consumption microbubble generation is discussed. In recent patents, this class has included the use of porous materials, flow focusing, and fluidic oscillation actuated microbubble generation. In section 4, speculations about current and future trends are discussed, particularly with regard to the future promise of miniaturization and power efficiency.

Properties of Microbubbles

The ideal properties of microbubbles can be divided into two classes,

- 1) Functional Properties
- 2) Structural Properties

1) Functional Properties

The functional properties are those which render them useful for performing their various functions these include,

- a) Inject ability: Since these micro bubbles are to be injected into the body so as to exert their various actions they should be inject able.
- b) Ultrasound Scattering Efficiency: As these micro bubbles act in combination with ultrasound they should have ultrasound scattering efficiency.
- c) Biocompatibility: Micro bubbles interact with the vital organs of the body at cellular levels they should be biocompatible.

2) Structural Properties

These refer to the structure or the physical properties of the micro bubbles, these are as follows,

- a) should have an average external diameter between the ranges of 1-10 μm , narrow size distribution so as to avoid complications when injected into the body
- b) density & compressibility difference between themselves & the surrounding body tissues to create an acoustic impedance & to scatter ultrasound at a much higher intensity than the body tissues so as to be used as contrast agents
- c) sufficient surface chemical properties to be modified for the attachment of various ligands to target them to specific tissues or organs
- d) uniformity of shell thickness.^{8,9}

Components of Microbubbles

Microbubbles basically comprise of three phases

- 1) Inner most Gas Phase
- 2) Shell Material Enclosing the Gas Phase
- 3) Outermost Liquid or Aqueous Phase

In addition to this the formulation may also comprise of various other components.

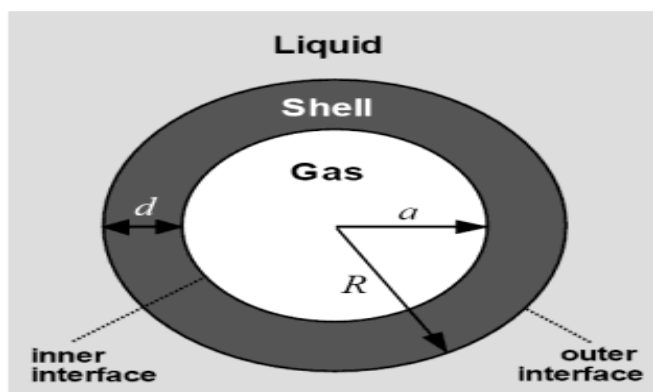


Figure 3: Components of Microbubbles

Gas Phase

The gas phase can be a single gas or a combination of gases can be used. Combination gases are used to cause differentials in partial pressure & to generate gas osmotic pressures which stabilize the bubbles. When a combination of gases is used two types of gases are involved one is the Primary Modifier Gas also known as first gas. Air is preferably used as primary modifier gas, sometimes nitrogen is also used as first gas. The vapor pressure of first gas is $(760 - x)$ mm of Hg, where x is the vapor pressure of the second gas. The other gas is Gas Osmotic Agent also known as second gas; it is preferably a gas that is less permeable through the bubble surface than the modifier gas. It is also preferable that the gas osmotic agent is less soluble in blood & serum. Gas osmotic agent is normally a gas at room temperature or liquid so long as it has a sufficient partial or vapor pressure at the temperature of use to provide the desired osmotic effect. Some examples of second gas are per fluorocarbons or sulfur hexafluoride^{10, 11}.

2) Shell Material

The shell material encapsulates the gas phase. It plays a major role in the mechanical properties of microbubble as well as diffusion of the gas out of the microbubble. The shell also acts a region for encapsulation of drug molecules also ligands can be attached to the shell membrane so as to achieve targeting of these microbubbles to the various organs or tissues. It accounts for the elasticity or compressibility of microbubbles. More elastic the shell material is more acoustic energy it can withstand before bursting or breaking up, this increases the residence time of these bubbles in body. More hydrophilic the shell material, more easily it is taken up by the body this decreases the residence time of these bubbles in the body^{12,13}.

Eg: The various types of shell materials that can be used are

- Proteins like albumin
- Carbohydrates like galactose.
- Phospholipids like phosphor ti dylcholine, phosphor tidylethanolamine etc.
- Biodegradable polymers like polyvinyl alcohol, poly caprolactone etc.

2) Aqueous or Liquid Phase

The external, continuous liquid phase in which the bubble resides typically includes a surfactant or foaming agent. Surfactants suitable for use include any compound or composition that aids in the formation & maintenance of the bubble membrane by forming a layer at the interphase. The foaming agent or surfactant may comprise a single component or any combination of compounds, such as in the case of co surfactants.

Also the persistence of microbubble in body is inversely proportional to La Place pressure which in turn is directly proportional to surface tension of bubble. In other words decrease in the surface tension acting on the bubble increases the persistence time of the bubble in the body.

Eg: Block copolymers of polyoxypropylene, polyoxyethylene, sugar esters, fatty alcohols, aliphatic amine oxides, hyaluronic acid esters & their salts, dodecyl poly (ethyleneoxy) ethanol, etc.

Nonionic Surfactants: Polyoxyethylene polyoxypropylene copolymers Eg. Pluronic F-68, polyoxyethylene stearates, polyoxyethylene fatty alcohol ethers, polyoxyethylated sorbitan fatty acid esters, glycerol polyethylene glycol oxystearates, glycerol polyethylene glycol ricinoleate etc¹⁴.

Anionic Surfactants: Fatty acids having 12 -24 carbon atoms Eg. Sodium Oleate.

3) Other Components

The various other components that may be incorporated in the formulation include osmotic agents, stabilizers, chelators, buffers, viscosity modulators, air solubility modifiers, salts & sugars can be added to fine tune the microbubble suspensions for maximum shelf life & contrast enhancement effectiveness. Such considerations as sterility, isotonicity & biocompatibility may govern the use of such conventional additives to injectable compositions.

How Microbubbles Works

Micro bubbles work by resonating in an ultrasound beam, rapidly contracting and expanding in response to the pressure changes of the sound wave. By a fortunate coincidence, they vibrate particularly strongly at the high frequencies used for diagnostic ultrasound imaging. This makes them several thousand times more reflective than normal body tissues. In this way they enhance both grey scale images and flow mediated Doppler signals. As well as being useful in itself, the resonance that micro bubbles produce has several special properties that can be exploited to improve 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 selective excitation produced can also destroy micro bubbles relatively easily, an effect that can be useful both in imaging and in emerging therapeutic applications (Martin JK Blomley *et al.*, 2001)^{15,19}.

Methods to Prepare Microbubbles

The various methods that can be used for the preparation of these microbubbles include:

- 1) Cross Linking Polymerization
- 2) Emulsion Solvent Evaporation

3) Atomization & Reconstitution

4) Sonication

1) Cross Linking Polymerisation

In this a polymeric solution is vigorously stirred, which results in the formation of a fine foam of the polymer which acts as a colloidal stabilizer as well as a bubble coating agent. The polymer is then cross linked, after cross linking microbubbles float on the surface of the mixture. Floating microbubbles are separated & extensively dialyzed against Milli Q water.

Eg: 2% aqueous solution of telechelic PVA is vigorously stirred at room temperature for 3 hrs at a pH of 2.5 by an Ultra Turrax T-25 at 8000 rpm equipped with a teflon coated tip, fine foam of PVA is formed. The PVA is then cross linked at room temperature and at 50C by adding HCl or H₂SO₄ as a catalyst, the cross linking reaction is stopped by neutralization of the mixture and micro bubbles are then separated.²¹

2) Emulsion Solvent Evaporation

In this method two solutions are prepared, one is an aqueous solution containing an appropriate surfactant material which may be amphiphilic biopolymer such as gelatin, collagen, albumin or globulins. This becomes the outer continuous phase of the emulsion system. The second is made from the dissolution of a wall forming polymer in a mixture of two water immiscible organic liquids. One of the organic liquids is a relatively volatile solvent for the polymer & the other is relatively nonvolatile nonsolvent for the polymer. The polymer solution is added to the aqueous solution with agitation to form an emulsion. The emulsification step is carried out until the inner phase droplets are in the desired size spectrum. It is the droplet size that will determine the size of the microbubble. As solvents volatilizes, polymer conc. in the droplet increases to a point where it precipitates in the presence of the less volatile nonsolvent. This process forms a film of polymer at the surface of the emulsion droplet. As the process continues, an outer shell wall is formed which encapsulates an inner core of nonsolvent liquid. Once complete, the resulting microcapsules can then be retrieved, washed & formulated in a buffer system. Subsequent drying, preferably by freeze-drying, removes both the nonsolvent organic liquid core & the water to yield air filled hollow microbubbles.²⁴

3) Atomisation and Reconstitution

A spray dried surfactant solution is formulated by atomizing a surfactant solution into a heated gas this results in formation of porous spheres of the surfactant solution with the primary modifier gas enclosed in it. These porous spheres are then packaged into a vial, the headspace of the vial is then filled with the second gas or gas osmotic agent. The vial is then sealed, at the time

of use it is reconstituted with a sterile saline solution. Upon reconstitution the primary modifier gas diffuses out & the secondary gas diffuses in, resulting in size reduction. The microbubbles so formed remain suspended in the saline solution & are then administered to the patient²².

4) Sonication

Sonication is preferred for formation of micro bubbles, i.e. through an ultrasound transmitting septum or by penetrating a septum with an ultrasound probe including an ultrasonically vibrating hypodermic needle. Sonication can be accomplished in a number of ways, for eg. A vial containing a surfactant solution & gas in headspace of the vial can be sonicated through a thin membrane. Sonication can be done by contacting or even depressing the membrane with an ultrasonic probe or with a focused ultrasound “beam”. Once sonication is accomplished, the micro bubble solution can be withdrawn from the vial & delivered to the patient. Sonication can also be done within a syringe with a low power ultrasonically vibrated aspirating assembly on the syringe^{17,18}.

Biomedical Applications

1) Diagnostic AIDS

Micro bubbles are elastic and compressible, these undergo compression and rarefaction thereby creating an acoustic impedance mismatch between biological tissues and fluids as these are efficient reflectors of ultrasound, hence used as contrast agents

These are used as diagnostic aids for:

- 1) Organ Edge Delineation
- 2) Blood Volume and Perfusion
- 3) Inflammation
- 4) Cancer
- 5) Liver
- 6) Also used to scan the tumors arising in the body.
- 7) Used for imaging the gall bladder stone.

2) Drug Delivery

On application of low frequency ultrasound, these microbubbles start oscillating & undergo a process of cavitations resulting in bursting or break up of the bubble, drug molecules if incorporated within the bubble are released by this process & these are useful in drug delivery²⁶.

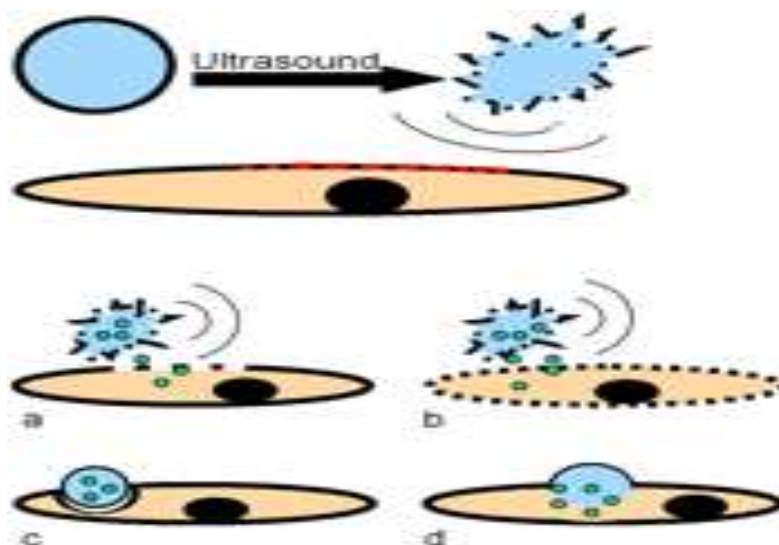


Figure 4: Drug delivery via Microbubbles

Two factors which are taken into account for drug delivery are:

- 1) Incorporation of drug into these micro bubbles
- 2) Drug release from these micro bubbles

1) Incorporation of drug into microbubbles

Drug molecules can be incorporated in a variety of ways within the microbubble as follows,

- a. Drug molecules can be incorporated within the bubble
- b. Drug molecules can also be incorporated within the bubble membrane or shell material of the microbubble.
- c. Drugs can also be attached to the shell of the microbubble (for eg. by noncovalent bonds) d. These can also be attached to the microbubble surface via a ligand (for eg. avidin-biotin complex).
- e. Also if the microbubble is made up of multiple layers it can also be incorporated within the various layers of these microbubble.

Targeted microbubbles are created by attaching a targeting ligand (such as a monoclonal antibody or a peptide) specific for the desired (endothelial) marker onto the shell of the microbubbles. Targeted ultrasound contrast agents have been used to assess vascular pathology associated with several intravascular markers, including P-selectin, ICAM-1, GpIIb/IIIa, the α v integrins and other markers of tumor angiogenesis.

2) Drug release from micro bubbles

Micro bubbles are also proposed to be carriers to be used in drug delivery. Micro bubbles on application of ultrasound undergo a process known as cavitation which results in bursting or breakup of the micro bubble on application of ultrasound. On cavitation the body fluids start

insonating creating acoustic cavitation. Further as the micro bubbles oscillate they then give rise to small eddies, these eddies give rise to micro streaming or micro jets resulting in increase in permeability of the cell membrane & facilitating drug transfer across the membrane. Sometimes the micro bubbles may also be phagocytosed by the cell membrane resulting in drug release. Another possible mechanism is fusion of the phospholipid microbubble with the phospholipid bilayer of cell membrane resulting in delivery of the drug or genes directly into the cytoplasm of the cell membrane. This is the proposed mechanism for gene delivery as it transfers the gene in close proximity of the nucleus. The following figure shows drug delivery via the micro bubbles

- a. Drug delivery by cavitation
- b. Drug release by cavitation as well as increasing the permeability of cell membrane
- c. Phagocytosis of the microbubble by cell membrane
- d. Fusion of microbubble with the cell membrane

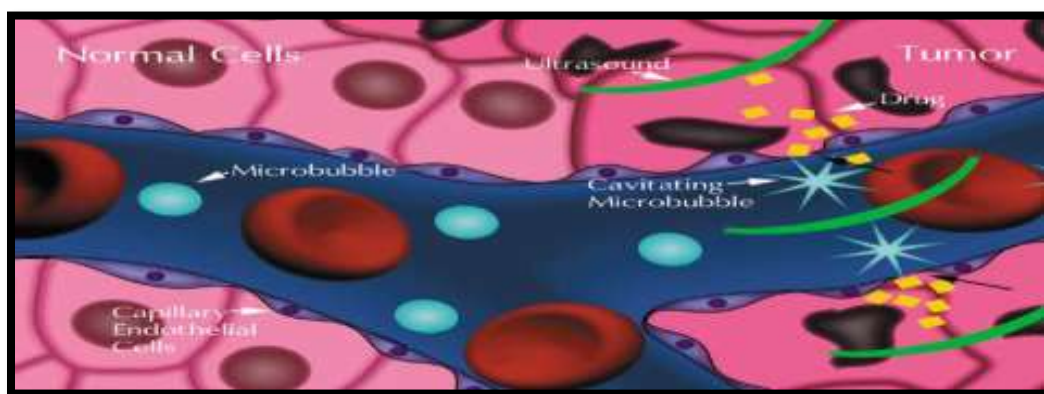


Figure 5: Drug Release from Microbubbles By Cavitation

Advantages of Using Microbubble for Drug Delivery

1. Since the microbubble delivers the drug in proximity to its target smaller dose of the drug is required as compared to conventional.
2. Also since the drug is released near its target & due to the small dose a decrease in the side effects is noticed especially for antineoplastic drugs.
3. By attaching various ligands these can be used for targeted drug delivery.

3) Gene Delivery

The next most promising application of these micro bubbles is these can be used as tools for gene delivery. The salient features of these micro bubbles which make them useful for gene delivery are as follows

1. Micro bubbles are metabolically inert
2. When injected into the body they do not produce any immune response

3. Also the gene encapsulated or attached to the micro bubble is carried to its target without getting digested by the various enzymes.

Charged drugs can be stabilized in or onto the surfaces of micro bubbles by virtue of electrostatic interactions lipid-coated micro bubbles to bind DNA. DNA, because of the sugar phosphate groups in the molecule, is a polyanion (i.e. negatively charged). DNA is avidly bound to cationic (positively charged) micro bubbles. The gene is released when ultrasound energy cavitates the micro bubble²⁷.

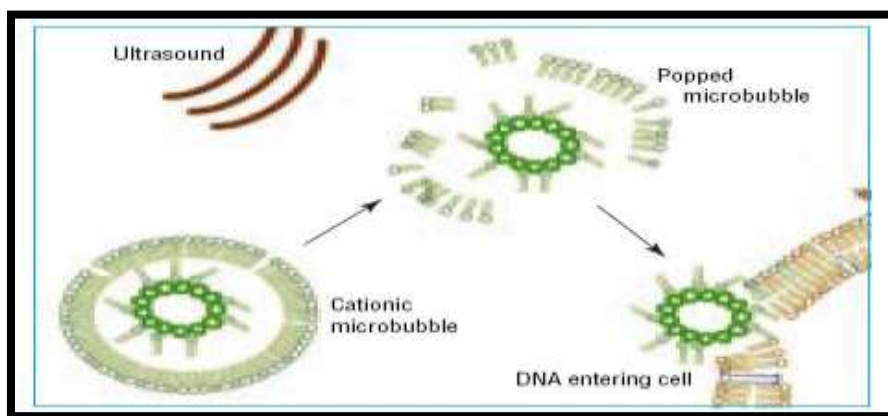


Figure: 6 Ultrasound Scan of Liver Using Levovist Micro bubbles

Levovist Micro bubbles:

The clinical use of viral vectors for gene therapy is limited because viral proteins elicit an immune response within the target tissue²³ and have been shown to cause an intense inflammatory activation of endothelial cells. On the other hand, the nonviral delivery of vehicles, such as plasmids and antisense oligonucleotides, has been associated with a lower transfection efficiency and transient expression of the gene product. The first published report of targeted DNA delivery was performed in 1996, using surface ultrasound and intravenously delivered micro bubbles carrying antisense oligo nucleotides. In 1997, Bao *et al*²⁶., described the use of ultrasound and albumin-coated micro bubbles to enhance the transfection of luciferase reporter plasmid in cultured hamster cells. Since then, many studies have confirmed the efficacy of ultrasound-mediated micro bubble destruction for drug and gene delivery, both *in vitro* and *in vivo*. Shohet *et al*, demonstrated for the first time with an adenovirus vector that the ultrasound-mediated disruption of gas-filled micro bubbles could be used to direct transgene expression to the heart *in vivo*. They showed that intravenously injected recombinant adenovirus vectors encoding a beta-galactosidase reporter gene were successfully delivered to normal rat myocardium using micro bubbles and transthoracic 1.3 MHz diagnostic ultrasound, at a mechanical index of 1.5, delivered at a burst of 3 frames of ultrasound every 4 to 6 cardiac

cycles. Of note, transfection was not observed if the adenovirus was administered in the same dose without micro bubbles, or if the adenovirus was administered with micro bubbles but in the absence of ultrasound. Importantly, using the same model the authors confirmed that plasmid transgene expression can be directed to the heart, with an even higher specificity than viral vectors, and that this expression can be regulated by repeated treatments. Taniyama *et al.*, have also shown effective transfection of a plasmid DNA to endothelial and vascular smooth muscle cells with albumin-coated microbubbles (Optison) and ultrasound. In vivo studies demonstrated that transfection of wild-type p53 plasmid DNA into balloon-injured blood vessels was effective and resulted in significant inhibition of the ratio of neointimal-to-medial area, as compared with transfection of control vector. In contrast, transfection of p53 plasmid DNA by means of ultrasound without microbubbles failed to inhibit neointimal formation in the rat carotid. In a recent study, Teupe *et al.*, have documented efficient transfer of plasmids encoding either beta-galactosidase or endothelial nitric oxide synthase to the endothelial cells of conductance arteries with preservation of the functional integrity of the transfected endothelial cell layer after ultrasound treatment^{28,30}.

CONCLUSION

The application of micro bubble with ultrasound which gives a synergistic effect for drug/DNA delivery is currently in its infancy. The use of targeted micro bubbles is a great step forward and has created various challenging therapeutic options, not only in cardiovascular disease but also in treatment of inflammatory and malignant diseases. Micro bubbles have rapidly evolved from a diagnostic adjuvant to a possible therapeutic agent. In the coming years, this promising technique needs further development to make it available for clinical applications.

Future Perspectives

The use of micro bubbles as a tool for drug delivery enhancement has an enormous clinical potential, especially in oncology and vascular applications. Whereas free drugs often possess harmful side effects, their encapsulation in micro bubbles and subsequent local release, deposition, and potentiation in the target tissue by ultrasound triggering will help improve the therapeutic index, lower the incidence of adverse events, and achieve successful therapy. Microbubbles combined with ultrasound offer a possibility to optimize the action of the currently approved drugs and drug delivery systems by improving their pharmacokinetics and delivery to the target.

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