



Microbubbles: Gas Core For Drug Delivery

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Accepted on: 27-11-2013; Finalized on: 31-01-2014.

ABSTRACT

Microbubbles are spherical colloidal bubbles which are composed of gas. There are very few systems which are composed of gas as drug delivery system. Microbubbles are defined as small spherical gas bubbles made up of phospholipids or biodegradable polymers that are approximately the size of red blood cell which allows it to display similar rheology in the microbubbles and capillaries throughout the body. Single gas or combination of gases can be used in microbubble preparation. Combination of gases consists of primary modifier also known as first gas. Air and nitrogen is used as primary modifier. The other gas is gas osmotic agent also known as second gas. Perfluorocarbon and sulphur hexafluoride can be used as osmotic agents. These microbubbles act in combination with ultrasound. Ultrasound has a strong future in diagnostic imaging. This review focuses on the properties, method of preparations, and mechanism of action and applications of microbubbles. In addition, current study also involves the future aspects of microbubbles.

Keywords: Gas drug delivery system, Microbubbles, Molecular imaging, Targeted drug delivery, Ultrasound.

INTRODUCTION

Bio medically microbubbles are defined as small spherical gas bubbles made up of phospholipids or biodegradable polymers that are approximately the size of red blood cell which allows it to display similar rheology in the microbubbles and capillaries throughout the body.¹ Microbubble must be encapsulated with a solid shell. The gas core comprises most of the particle volume and provides the mechanism for ultrasound backscatter and drug delivery.²

Microbubbles are spherical colloidal bubbles which are composed of gas. The size range of microbubble is usually 1-10 micrometer. The microbubble which mostly contains oxygen or air can remain suspended in the water for an extended period.

As gas in the microbubble dissolve into the water the bubbles when comes in contact with water, it gets disappear.³ The examples of filled gas are air, nitrogen, perfluorocarbon etc. Microbubble oscillate or vibrate when a sonic energy field is applied and may reflect ultrasound waves, this distinguishes the microbubbles from surrounding tissue because gas bubbles in liquid lack stability and would therefore quickly dissolve.

Gas bubbles of this size in aqueous media are inherently unstable owing to surface tension effect and therefore require a stabilising cell.⁴ The key to success for microbubble as drug delivery vehicle is their extreme activity when exposed to ultrasonic waves. The gas core expands during the rare function phase of pressure wave and contract during the compression phase.¹

Depending on the ultrasound parameter various phenomenons may occur, facilitate ultrasound backscatter or the release and local delivery of the drugs

from microbubble shell. These phenomenon is summarized below and range from subtle effect such as acoustic radiation, force to highly energetic event such as inertial cavitation. Combination of these phenomenon allow for imaging, targeting, controlled release and vascular permeability enhancement.¹

Properties of microbubbles

The properties of microbubbles are as follows:

- 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) **Injectability:** Since these microbubbles are to be injected into the body so as to exert their various actions they should be injectable. b) **Ultrasound Scattering Efficiency:** As these microbubbles act in combination with ultrasound they should have ultrasound scattering efficiency. c) **Biocompatibility:** Microbubbles interact with the vital organs of the body at cellular levels they should be biocompatible.³

2. Structural Properties

These refer to the structure or the physical properties of the microbubbles, 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 thickness³.

Composition of microbubbles

Microbubbles basically consist of (Figure 1);

- 1) Gas phase
- 2) Shell material enclosing the gas phase
- 3) Outermost liquid or aqueous phase

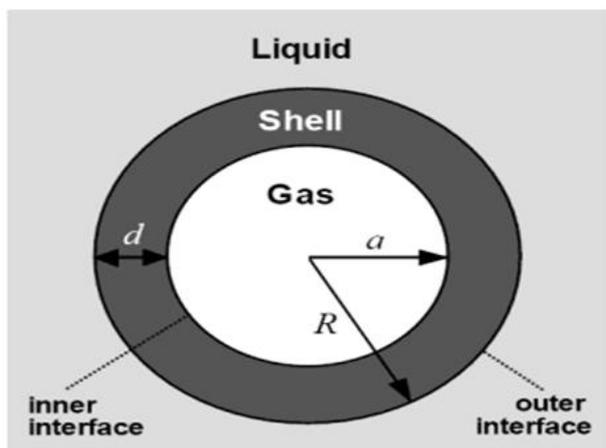


Figure 1: Components of Microbubbles

1. Gas phase

Single gas or combination of gases can be used. Combination gases cause differentials in partial pressure and to generate gas osmotic pressure which stabilize the bubbles. Combination of gases consists of primary modifier also known as first gas. Air is preferably used and sometimes nitrogen is also preferred as primary modifier. The combination of gases is selected so that, the vapour pressure of first gas is $(760-x)$ where x is vapour pressure of second phase. The other gas is gas osmotic agent also known as second gas. Gas osmotic agent is less soluble in blood and serum. Perfluorocarbon, sulfurhexafluoride can be used as osmotic agents.

2. Shell Material

The gas core is surrounded by protein, lipid, surfactant or biocompatible polymer shell (2-500nm thick).³

Advantages of shell material

- It improves stability against gas; less dissociation, microbubble coalescence.
- It produces more standard size distribution.
- Surfactant layer increases the half-life of microbubble.⁵

Shell material may be:-

Protein shells

Albumin is used for coating. The first albumin microbubble formulation to be approved by US FDA was

ALBUNEX (GE Healthcare) which consist of roughly 7x10⁸ microbubbles per ml with a size range from 1-15 micrometer diameter. Several proteins have been used for the preparation of microbubbles. Amphipathetic nature of protein makes them highly surface active. Example:

OPTISON (GE Healthcare)

Perfluorocarbon is a gas core which gives much longer circulation persistence in vivo. Cavalieri & co-worker used lysoenzyme to form microbubbles which were found to be stable and retain their enzymatic activity for several months. Korpanty et al used mixture of albumin and avidin incorporation of avidin into protein shells allowed biotin mediated coupling of antibodies for targeting vascular endothelium.¹

Surfactant shell

Synthetic surfactant SPAN-40 and TWEEN-40 were used to stabilize microbubble. Using a Langmuir trough they were able to establish correct ratio of SPAN to TWEEN (roughly 1:1) to use for maximum film stability.¹

Lipid shells

Phospholipids like phosphatidylcholine, phosphatidylethanolamine etc.³ Lipid coated microbubble shows favourable ultrasound characteristics such as resonance with minimal dampening and capacity to reseal around the gas core following fragmentation. Finally lipid coated microbubble may be used for drug delivery, molecular imaging or other purpose by incorporating different lipid head group species or post production bioconjugation.¹

Polymer shells

In 1990, Wheatley et al reported new polymer in which shell was formed by the ionotropic gelation of alginate. Biodegradable polymers like PVA and polycaprolactone etc were used.¹

Polyelectrolyte multilayer shells

A new class of polymer-surfactant shell hybrids was recently introduced that involves polyelectrolyte multilayer (PEM) 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 al were the first to report PEM deposition onto microbubbles. They used the polymers poly (allylamine hydrochloride) (PAH) and poly (styrene sulfonate) (PSS) for the polyion pair. This system gave a relatively uniform PEM coating that provided the microbubbles with remarkable stability. Borden et al.,⁴⁴ developed a PEM microbubble with phospholipid containing the cationic head group trimethylammonium propane (TAP) as the underlying shell and DNA and poly(L-lysine) (PLL) as the polyion pair. Interestingly, the PEMs formed as islands owing to phase separation of the phospholipid species in the shell. The formation of such islands may be useful for

surface compartmentalization for multi-functional microbubbles that require both ligands-receptor mediated adhesion and drug release through ultrasound-triggered fragmentation. Lentacker et al described a multilayer microbubble in which albumin microbubbles were coated with DNA and PAH, where the latter layer served to bind and protect the DNA from enzymatic degradation.¹

3. Aqueous or Liquid Phase

The external continuous phase in which the bubble includes surfactant or foaming agent. The persistence of microbubble is inversely proportional to Laplace pressure and directly proportional to the surface tension of bubble. Some basic examples are Block copolymers of polyoxypropylene, polyoxyethylene, sugar esters, fatty alcohols, aliphatic amine oxides, hyaluronic acid, esters and their salts, dodecyl poly (ethyleneoxy) ethanol etc.

Other components

Various components such as osmotic agents, stabilizers, chelators, buffers, viscosity modulators, air solubility modifiers, salts and sugars can be added to stabilize microbubbles for maximum shelf life and contrast enhancement effectiveness.³

METHOD OF PREPARATION

Various methods can be used to prepare microbubbles. These are as follows;

- 1) Cross linking Polymerization
- 2) Emulsion solvent evaporation
- 3) Atomization and Reconstitution
- 4) By microchannel
- 5) Sonication

1. Cross linking Polymerization

In this process polymer solution is vigorously stirred which results in formation of froth of polymer which act as a colloidal stabilizer. Polymer is then cross linked and microbubbles float on the mixture. Floating microbubbles are separated and extensively dialyzed against Milli Q water. Example 2% aqueous solution of telechelic PVA is vigorously stirred at room temperature for 3 hours 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 crosslinked at room temperature and at 5 C by adding hydrochloric acid or sulphuric acid as a catalyst. Cross linking reaction is stopped by neutralization of microbubble are then separated.³

2. Emulsion and solvent evaporation

Two solutions are prepared in which one is aqueous solution having an appropriate surfactant material which may be amphiphilic biopolymer such as collagen, albumin, globulin or gelatine. This becomes the outer continuous phase of emulsion system. The second is made from the dissolution of a wall forming polymer in mixture of two

water immiscible organic liquid. One of organic liquid is volatile and other is relatively non-volatile, nonsolvent for polymer. The polymer solution is added to aqueous solution with agitation to form emulsion.³

Polymeric microbubble manufactured by a double water-in-oil emulsion technique which forms a microsphere, precursor of microbubble with an internal water phase and external organic phase containing the dissolved polymeric example (lactide- co- glycolide) or poly (lactide- co- ethylene glycol). Organic solvent is removed by evaporation or extraction and water phase is eliminated by lyophilisation. Ideally in such a bubble single hollow core is present covered by polymeric shell. SEM or TEM reveals the internally porous structure with multiple voids. Volatile compound such as camphor or ammonium bi carbonate can be added to preparation to increase the porosity of particle.⁶

3. Atomisation and Reconstitution

A spray dried surfactant solution is formulated by atomising surfactant solution into a heated gas these results in formation of porous spheres of the surfactant solution with the primary modifier gas enclosed in it. These porous spheres are packaged into a vial is then filled with the second gas or gas osmotic agent. The vial is then sealed at the time of use it is reconstitution the primary modifier gas diffuses in, resulting size reduction. The microbubbles so formed remain suspended in saline solution.³

4. By Microchannel

Microbubbles can be prepared by using a microchannel with a squeezed T- junction. The mechanism of bubble generation was observed by developed ultra high speed micron resolution particle tracking velocimetry method. Bubbles of monodisperse 6.1 micrometer diameter were generated through suitable design of channel shape for generating smaller bubbles. The experimental result show that proposed technique enables generation of 2-70 micrometer diameter bubbles at frequency of 1-102 kHz. The results indicate that as liquid velocity increases, the diameter of generated bubble decreases the interfacial tension of gas liquid interface. The high speed observation indicates that the bubbles generation consists of two stages: a)- intruding stage and b)- squeezing stage. Different kinds of bubble are observed for these two stages.⁷

5. Sonication

The method of Sonication includes following steps:

Preparing the solution

The first step of process consists forming a solution having protein and saccharide and then saturating with gas.

Protein: The protein may be single or mixture of protein which may be natural, synthetic or recombinant. The concentration of protein- saccharide solution depends



upon the size or physical characteristics of the microbubble. In general the concentration of the protein in solution will be less than 20% (w/v). In preferred embodiment the concentration of protein may range from 1-15% w/v. In an exemplary embodiment the concentration ranges from 1-6% (w/v).

Saccharide: Numerous saccharides are suitable for use in the invention. The concentration of saccharide in the solution will be less than 20% w/v. In preferred embodiment 1-15% and in exemplary the concentration may range from 3-12% w/v. Both protein and saccharide stock solution are prepared and mix in the ratio of 2:1,1.5:1,1:1,1:1,1:2,1:3,1:4,1:5,1:6,1:7,1:8,1:9,1:10.⁸

Gas: The protein-saccharide solution is saturated with a gas such that upon sonication gas filled microbubble may be formed. The gas is layered over the surface of protein-saccharide or dissolved or interpresed into solution by hand agitation, rotation, rolling, vortex mixing or combination thereof.

Delivering Ultrasound energy

Ultrasound energy is provided to gas saturated protein-saccharide solution by a Fischer model 500 sonicator alternatively by sonicator sonic disruptor. Ultrasonic mixing will be performed at 20 kHz. The power level may range from 100W- 500W for a time period ranging from 1-200 second. Microbubble may be prepared using a one step, 2 step or multistep protocol. One step protocol gives microbubbles of 1 micro- meter or fewer diameters. Two step protocols give microbubbles of 1, 2,3,4,5 or 6 micron. One step sonication involves delivering US energy outside the solution by placing the tip of sonic probe at surface of solution positioning the tip of sonic probe.

Two step involves delivering first round of US energy within the solution by immersing the sonic probe in the solution (2) delivering second round of US energy outside the solution by placing the tip of sonic probe above the surface of solution. Multistep sonication involves three rounds of delivering US energy.⁸

Mode of Action

Several properties of microbubbles make them a promising tool for drug and gene delivery to cells. Microbubbles have specific acoustic properties whereas low and intermediate acoustic pressure results in linear and nonlinear oscillations of microbubbles respectively, while high pressure ultrasound (MI > 10) causes forced expansion and compression of microbubble leading to bubble destruction.⁹

Two possible mechanism for delievering drugs and genes with microbubble are studied. The first consists of the ultrasound mediated microbubble destruction which based on cavitation of microbubble induced by ultrasound application (fig 2) and second is directly delievering of substance bound to microbubble in the absence of microbubble.¹⁰ Ultrasound applied to fluid cause cavitation i.e. the creation, vibration and collapse

of small gas filled bodies of ultrasound beam (cavitation is the formation and/ or activity of gas filled bubbles in a medium exposed to ultrasound).¹¹

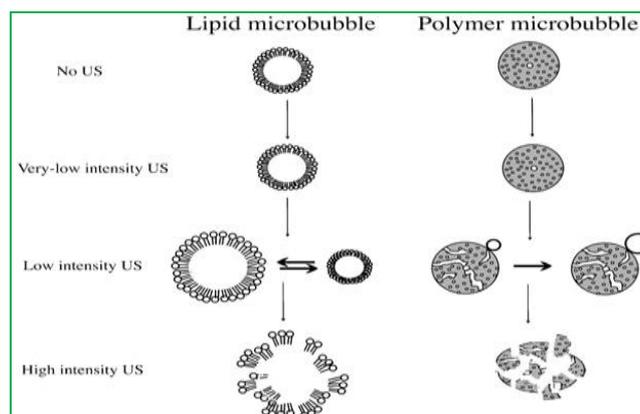


Figure 2: Schematic representation of lipid and polymer microbubble interaction with ultrasound of increasing intensity (top to bottom).⁵

First, microbubble by acting as cavitation nuclei, can lower the threshold for cavitation. In body tissue or blood, cavitation sets fluid in motion and creates small shock waves that give rise to microstreaming along the endothelial cell. Destruction of microbubble results in high energy microstreaming microjets that causes increase in shear stress on membrane of endothelial cell and increase its permeability.¹²

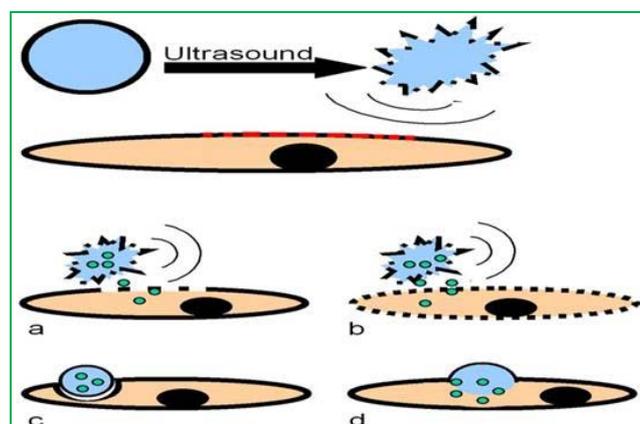


Figure 3: Destruction of microbubble by ultrasound resulting in increased membrane permeability by shear stress, temperature rise, activation of reactive oxygen species. Drug delivery by microbubble by: a- transient holes induced by shear stress; b- increase in membrane permeability; c- endocytosis of microbubble; d- fusion of the microbubble membrane with cell membrane.

Another proposed mechanism is generation of reactive oxygen species in endothelial cell under influence of ultrasound. Microbubble with ultrasound lower threshold for cavitation this results in increased production of free radical which is associated with cell killing in vivo (figure 3).

Third aspect is rise in body temperature results from the application of high pressure ultrasound, the bubble collapse and creates transient increase in body

temperature. It influences the phospholipid bilayer membrane, cell permeability has changed as a consequence of increased bilayer fluidity. Endocytosis, phagocytosis and active membrane transport mechanism. Fifth mechanism is the use of microbubble could facilitate the deposition of drugs or genes in a cell are exchange or fusion of the phospholipid bilayer of cell membrane. This could result in delivery of the cargo of microbubble directly into the cytoplasm of cell with further uptake in endosomes or delivery to the cell nucleus, drugs and genes can be attached to microbubbles.¹²

APPLICATIONS OF MICROBBLES

Ultrasound molecular imaging

The concept of ultrasound molecular imaging is so selectively adhere ligand bearing contrast agent particle to endothelia expressing a target receptor and by virtue of contrast agent accumulation and echo enhancement to quantify the extent to which vasculature expresses that target receptor.¹

Targeted microbubbles are injected i.v. and allowed to circulate and adhere to their target. After some dwell time, target tissue is scanned and contrast signal in the region of interest is determined. A destruction pulse is applied to fragment and dissolve all microbubble within the field of view. Free microbubbles are allowed to flow back into the field and again the contrast signal is observed. The signal from targeted microbubble is delineated from that of freely circulating microbubble and tissue movement by comparing the contrast signal before and after the destruction pulse.¹

Targeted Contrast Agent (figure 4)

Binding of these agents with selected cells, provide a more precise method of molecular imaging. The microbubble coated with certain material example-Phosphatidyl serine show improve phagocytosis and such agents targeting WBC can be used to image inflammation. The targeting ligands may be in form of modification in coating material or specific molecule attached to surface of microbubble. For targeting with in vasculature micron sized microbubble adequate and <500 nm diameter are adequate for beyond vasculature.¹³

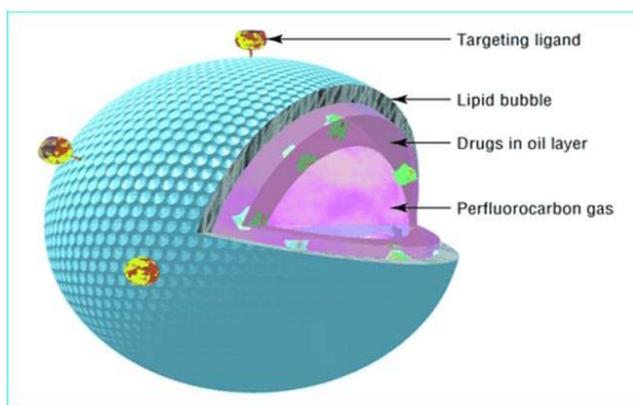


Figure 4: A targeted microbubble: a gas microbubble is covered with a lipid membrane in which targeting ligands have been incorporated.

MRX- 408 is recently developed microbubble incorporating biconjugates targeted to the GPIIb/IIIa receptor of activated platelets.

Intravascular Applications

Intravascular applications includes the following;

Angiogenesis

Certain integrins such as Alpha V Beta1 are expressed in angiogenesis, microbubble targeting to this integrin is used to measure 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.

Vulnerable Plaque

It has been successfully detected using targeted microbubble in combination with ultrasound.

Extravascular Application

When sufficient quantities of very small bubble are delivered to tissue, they can act as specular reflectors and greatly increase the backscattered signal.

Sonothrombolysis

Microbubble enhanced sonolysis may have potential clinical application (such as myocardial infarction, stroke and deep vein thrombosis) for rapid and safe treatment of vascular thrombosis. Sonolysis using ultrasound at 200 kHz with microbubble restored blood flow.¹³

Passing through blood brain barrier

Molecular targeted agents with ultrasound activation might be used to afford precise entry into the central nervous system by controlling the blood brain barrier at molecular level.

Increase effectiveness of cancer treatment

The bubble overlapping mode can increase the efficiency of cancer treatment by laser heated nanoparticle as a result of the large damage range. It was demonstrated that the formation of nanoclusters on the cell surface with sizes larger than the sizes of individual of laser treatment of cancer.

Prostate cancer detection

A new ultrasound technique involving colour enhanced Doppler imaging with microbubble contrast improves the accuracy of screening for prostate cancer. The microbubbles further enhance imaging by increasing the intensity of backscatter signal this allow physician to more accurately locate where biopsies should be taken.

Leukaemia treatment

Imaging modalities provides a thorough view of anatomical, physiological or molecular processes in vivo and allows quantitative measurement and visualization of processes in specific targeted organs or tissues.

Detection of blood vessel growth in tumours (figure 5)

Millions of tiny microbubble injected into blood stream with contrast enhanced ultrasound and imagine is being able to detect blood vessel growth in cancerous tumours and how fast tumour metastasizes.

Researchers at the University of California are developing an innovative technique that uses ultrasound and drug loaded microbubble to deliver concentrated chemotherapy drugs to inner lining of blood vessels.

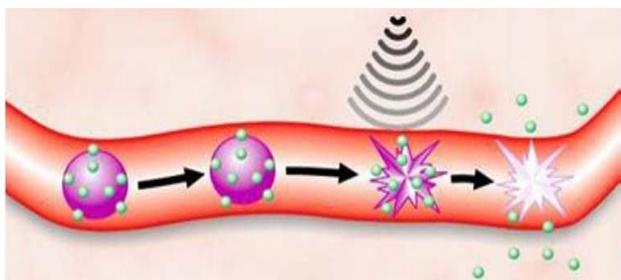


Figure 5: Gas filled microbubbles covered with a bioactive substance pass harmlessly and uneventfully through blood vessels until they are exposed to ultrasound. Then the bubble burst causing not only the release of bioactive substance but also the opening of the holes in the cells (sonoporation) that line the vessel.

Gene & drug delivery (figure 6)

The microbubble can be injected. Upstream of targeted region and then imaging is done at low intensity by the presence of bubbles, when imaging demonstrates ultrasound is focussed to target tissue, intensity is increased to create collapse cavitation. It permeabilize the vessel wall and provides pathway for extravasations of DNA that is freely floating along with microbubble.¹¹

Localized delivery, drug release and tissue penetration can be achieved by local application of ultrasound leading to microbubble disruption. In cell culture magnetic microbubble are sediment on target cells by magnetic force and bubble disruption and gene delivery is triggered by application of ultrasound of 1MHz.¹³

In identification & diagnosis of ocular spaceoccupying lesions

Ultrasound contrast examination and UCA entrance the ultrasonic detection signal shows the blood perfusion in vessels, increase the image contrast resolution and improve the lesion's detection.¹⁴

In ophthalmic targeted therapy

Contrast enhanced ultrasound and microbubble plays a greater role in diagnosis and treatment of ophthalmic disorder.¹⁵

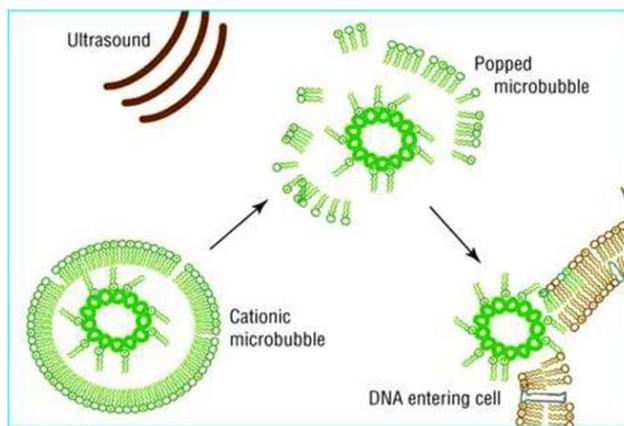


Figure 6: Gene delivery using microbubble and ultrasound.

Therapeutic agents (figure 7)

The microbubble employed in non-invasive delivery of therapeutic agents are < 10 micrometer in diameter and consist of gas stabilized by shell material thus allowing passage through pulmonary circulation.¹⁶

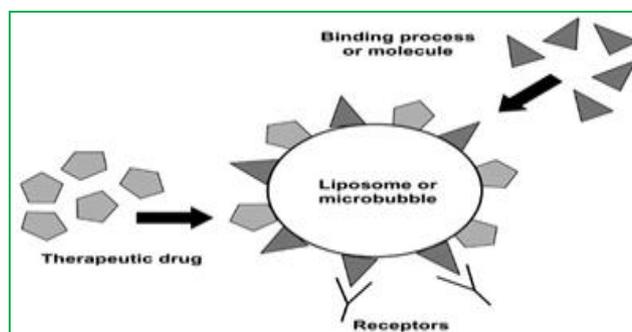


Figure 7: Scheme of ultrasound targeted microbubble composition employed in therapeutic agent delivery.

Microbubble carrying liposome using high affinity interaction between avidin and biotin can be used to produce an efficient therapeutic delivery vehicle¹⁶ (figure 8).

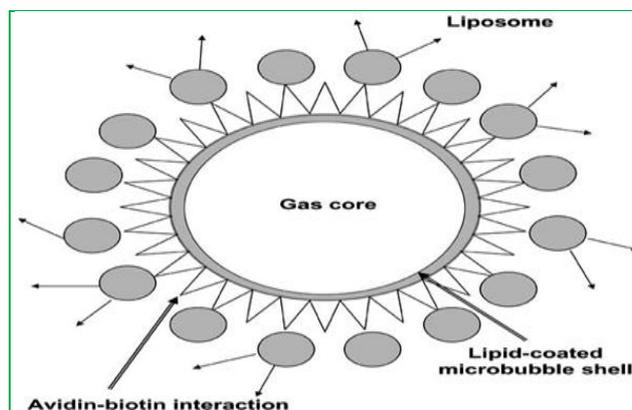


Figure 8: Scheme of microbubble carrying liposomes on its surface in order to expand the drug carrying capacity of contrast agent, while preserving the echogenicity and effective manipulation with of ultrasound energy of classical microbubble.

Future Aspects: Microbubble proved to be effective tool for drug delivery especially in oncology and vascular application. Most of the free drug often causes harmful side effects, their encapsulation in microbubble and subsequent local release, deposition and potentiation in the target tissue by ultrasound triggering will improve the therapeutic index, lower the incidence of adverse events and achieve successful therapy. Future decades will show which of these drugs and gene delivery approaches will be most useful in the practical clinical arena.

CONCLUSION

Microbubble improves drug penetration into tissues when combined with focussed ultrasound treatment. Drug substance including plasmid DNA, can be attached to or incorporated in the microbubble particle for ultrasound triggered release in insonated organs and tissues. Co-administration of ultrasound microbubble and thrombolytic enzyme results in rapid and minimally invasive clot lysis, target vessel recanalization such as treatment for stroke. Overall ultrasound assisted drug delivery combined with microbubble contrast agent will aid in treatment of debilitating disease.

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Source of Support: Nil, Conflict of Interest: None.

