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Review Article

MICROBUBBLES: A NOVEL DELIVERY SYSTEM

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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: Microbubbles, diagnostic agents, ultrasound, drug delivery, gene delivery, ligands.

INTRODUCTION[1]:

Microbubbles 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 microbubbles 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 to used to increase the aquaculture productivity, these have been also used to improve the quality of water, used in sewage treatment .

Biomedically microbubbles 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.

PROPERTIES OF MICROBUBBLES [1]:

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) 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.

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 thickness

COMPONENTS OF MICROBUBBLES[1,2,3,4]:

Microbubbles basically comprise of three phases

1) Innermost 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

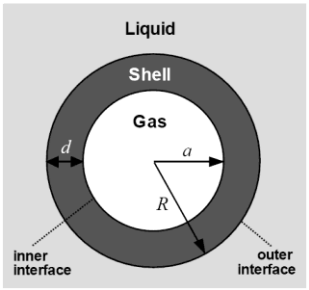


FIG.1 : Components of Microbubbles

1) 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.

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.

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

Proteins like albumin

Carbohydrates like galactose

Phospholipids like phosphotidylcholine, phosphotidylethanolamine etc.

Biodegradable polymers like polyvinyl alcohol, polycaprolactone etc.

4) 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.

3) 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: Polyoxyehylene 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 etc.

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

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[5]:

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 5°C by adding HCl or H_2SO_4 as a catalyst, the cross linking reaction is stopped by neutralization of the mixture and microbubbles are then separated.

2) EMULSION SOLVENT EVAPORATION[6]:

In this method two solutions are prepared, one is an aqueous solution containing an appropriate surfactant material which may be amphilic 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.

3) ATOMISATION & 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 patient.

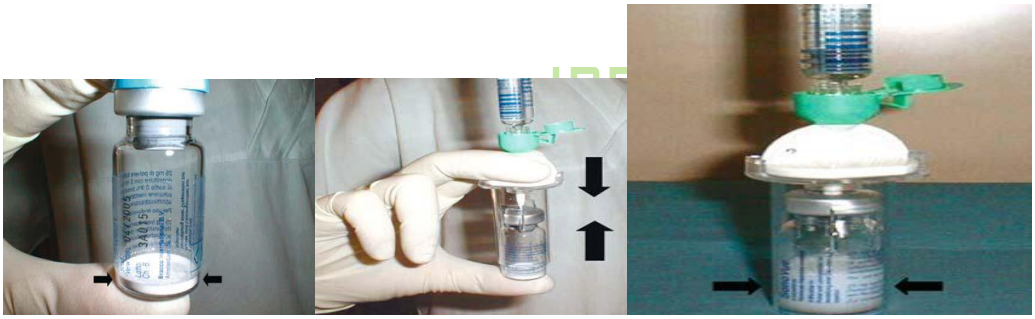


FIG.2 **Lyophilised or Spray dried powder** **Reconstitution with sterile saline solution**

Sonication is preferred for formation of microbubbles, 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 microbubble 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.

CHARACTERISATION OF MICROBUBBLES[6]:

Once prepared these microbubbles are characterized as per the following parameters,

1) Microbubble Diameter & Size Distribution:

The average diameter as well as size distribution of these microbubbles can be determined by Laser light Scattering, Scanning Electron Microscopy, Transmission Electron Microscopy.

2) Shell Thickness:

Shell thickness is determined by coating the shell with a fluorescent dye like Red Nile, this is then determined by Fluorescent Microscopy against a dark background.

3) Microbubble Concentration:

The microbubble concentration is determined by counting the no. of microbubbles per ml by using the Coulter Counter Machine.

4) Air Content by densitometry:

The content of air encapsulated within the microbubbles in the suspension samples is measured by oscillation U-tube densitometry with a DMA-58. The instrument is calibrated

with air and purified water prior to use. The density of the suspension is measured before and after elimination of encapsulated air. The complete removal of encapsulated air is achieved by 5 min high powered sonication in a sonicator.

The air content is calculated as,

$$Cair = \rho1 - \rho2/\rho2 * 100$$

Where, Cair is air content (%v/v)

ρ1 (g/ml) density before elimination of encapsulated air

ρ2 (g/ml) density after elimination of encapsulated air

BIOMEDICAL APPLICATIONS[7,8,9,10]:

1) DIAGNOSTIC AIDS:

Microbubbles 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

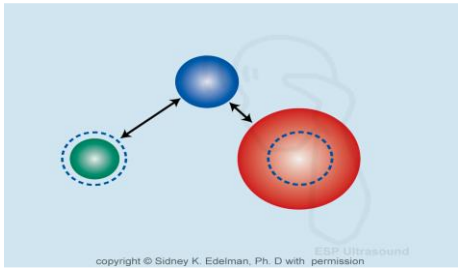
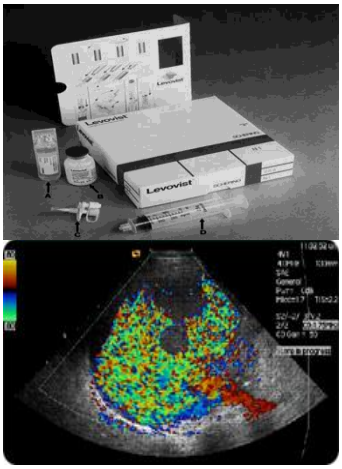


FIG.3 : Non Linear Behaviour Microbubbles Undergoing Compression And Rarefaction

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.



5) Ultrasound Reflectance Measurement:

Experimental set up consists of transducer, microbubble contained in a vessel consisting of metallic reflector and cellophane membrane, this vessel is in turn kept in another vessel containing water. The signals which are reflected are evaluated for the ultrasound reflecting capacity of these microbubbles.

FIG.4 : Ultrasound Scan Of Liver Using Levovist

ADVANTAGES:

1) Inexpensive as compared to other diagnostic agents.
2) No use of ionizing radiation.
3) Faster and accurate as compared to other diagnostic techniques.

DRUG DELIVERY[11,12,13,14]:

On application of low frequency ultrasound, these microbubbles start oscillating & undergo a process of cavitation 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 delivery.

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

1) Incorporation of drug into these microbubbles
2) Drug release from these microbubbles

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. 4. These microbubbles are proposed to cross the blood brain barrier.

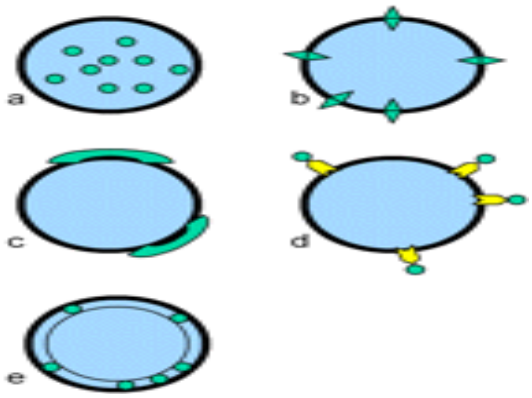


FIG.5 : Incorporation of drug into Microbubbles

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 αv integrins and other markers of tumor angiogenesis.

2) Drug release from microbubbles:
Microbubbles are also proposed to be carriers to be used in drug delivery. Microbubbles on application of ultrasound undergo a process known as cavitation which results in bursting or breakup of the microbubble on application of ultrasound.
On cavitation the body fluids start insonating creating acoustic cavitation. Further as the microbubbles 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 microbubbles may also be phagocytosed by the cell membrane resulting in drug release.

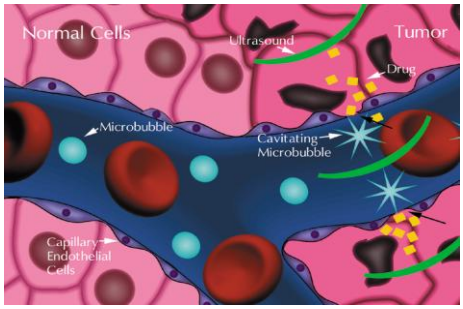


FIG.7 : Drug Release From Microbubbles By Cavitation

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 microbubbles
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

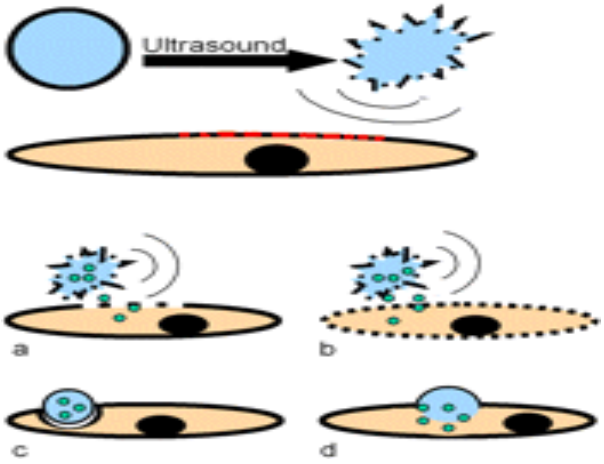


FIG.6 : Drug delivery via Microbubbles

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.

GENE DELIVERY[15]:
The next most promising application of these microbubbles is these can be used as tools for gene delivery. The salient features of these microbubbles which make them useful for gene delivery are as follows:
1. Microbubbles are metabolically inert
2. When injected into the body the do not produce any immune response
3. Also the gene encapsulated or attached to the microbubble is carried to its target without getting digested by the various enzymes.

FIG.8

Charged drugs can be stabilized in or onto the surfaces of microbubbles by virtue of electrostatic interactions lipid-coated microbubbles 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) microbubbles. The gene is released when ultrasound energy cavitates the microbubble.

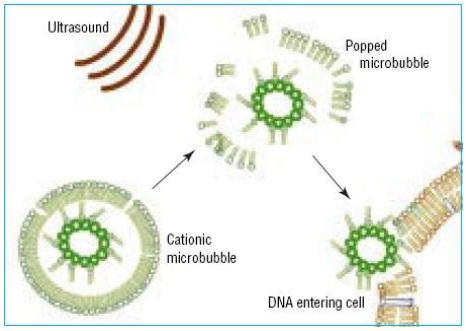


Fig:8 Ultrasound Scan Of Liver Using Levovist Microbubbles

CONCLUSION:

The application of microbubble with ultrasound which gives a synergistic effect for drug/DNA delivery is currently in its infancy. The use of targeted microbubbles 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

Microbubbles 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 microbubbles 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 microbubbles 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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