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MICROBUBBLES AS ULTRASOUND CONTRASTING AGENTS AND CURRENT CLINICAL APPLICATIONS: A REVIEW

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Abstract

Micro bubbles are small spherical type of bubble which consists of a gas and have size range of usually 1-100 micrometer. The advent of microbubble has increased the diagnostic sensitivity and specificity of Ultra sound Contrasting technique thus widening its clinical applications. Ultrasound imaging is clinically established for routine screening examinations of breast, abdomen, neck and other soft tissues, as well as for therapy monitoring. Microbubbles as vascular contrast agents improve the detection and characterization of cancerous lesions, inflammatory processes, and cardiovascular pathologies. The third generation of ultrasound contrast agents consist of sulphur hexafluoride microbubbles encased in a phospholipid shell. Taking advantage of the excellent sensitivity and specificity of ultrasound for microbubble detection, molecular imaging can be realized by binding antibodies, peptides, and other targeting moieties to microbubble surfaces. Molecular microbubbles directed against various targets such as vascular endothelial growth factor receptor, vascular cell adhesion molecule, intercellular adhesion molecule, selectins, and integrins were developed and were shown in preclinical studies to be able to selectively bind to tumor blood vessels and atherosclerotic plaques. Currently, microbubble formulations targeted to angiogenic vessels in prostate cancers are being evaluated clinically. Based on the relevant materials, the bioeffects, early successes with gene and drug delivery, and potential clinical applications are reviewed. Ultrasound imaging is widely used worldwide principally because it is cheap, easily available and contains no exposure to ionizing radiation.

INTRODUCTION:

Microbubbles are 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. 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. The liquid phase contains surfactants to control the surface properties as well as stability of the bubble. These bubbles are generated by various types of aerators now available in the market.

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 by improving 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 havean average size less than that of the size 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. They are used as diagnostic aids, as drug and gene carriers in combination with ultrasound for molecular imaging of various organs and even tumours. These are also proposed for drug and gene delivery to targeted regions in combination with various ligands. They are used in medical diagnostics as a contrast agent for ultrasound imaging.

The gas-filled, e.g., air or perfluorocarbon, microbubbles oscillate and vibrate when a sonic energy field is applied and may reflect ultrasound waves. This distinguishes the microbubbles from surrounding tissues. In practice, because gas bubbles in liquid lack stability and would therefore quickly dissolve; microbubbles must be encapsulated with a solid shell. The shell is made from either a lipid or a protein such as Optison-microbubbles which consist of

perfluoropropane gas encapsulated by a serum albumin shell.

TARGETED DRUG DELIVERY SYSTEMS:

Targeted drug delivery, sometimes called **smart drug delivery** is a method of delivering medication to a patient in a manner that increases the concentration of the medication in some parts of the body relative to others. The goal of a targeted drug delivery system is to prolong, localize, target and have a protected drug interaction with the diseased tissue. The conventional drug delivery system is the absorption of the drug across a biological membrane, whereas the targeted release system is when the drug is released in a dosage form.

The advantages to the targeted release system is the reduction in the frequency of the dosages taken by the patient, having a more uniform effect of the drug, reduction of drug side effects, and reduced fluctuation in circulating drug levels.

The disadvantage of the system is high cost which makes productivity more difficult and the reduced ability to adjust the dosages.

Targeted drug delivery systems have been developed which helps maintain the required plasma and tissue drug levels in the body. Therefore, avoiding any damage to the healthy tissue via the drug. The drug delivery system is highly integrated and requires various disciplines, such as chemists, biologist and engineers, to join forces to optimize this system.

APPROACHES FOR MICROBUBBLE TARGETED DRUG DELIVERY SYSTEMS:

Different Novel Drug Delivery Systems:

Delivery Vehicles[1-5]:

There are different types of drug delivery vehicles, such as, polymeric micelles, liposomes, lipoprotein-based drug carriers, nano-particle drug carriers, Implants, Pharmacosomes, dendrimers, niosomes, microspheres etc. An ideal drug delivery vehicle must be non-toxic, biocompatible, non-immunogenic, and biodegradable and avoid recognition by the host's defense mechanisms.

Routes of Administration:

Peroral Route, Parenteral Route, Subdermal implants, Buccal Administration, Occular Delivery, Transdermal delivery, Pulmonary

Drug DeliveryNasal delivery, Colon drug delivery.

Parenteral Controlled Release Systems:

A. Injectables- Solutions, Dispersions, Microspheres and Microcapsules, Nanoparticles and Niosomes, Liposomes and Pharmacosomes, Resealed erythrocytes

B. Implants

C. Infusion Devices- Osmotic Pumps (Alzet), Vapor Pressure Powered Pumps (Infusaid), Battery Powered Pumps.

MICROBUBBLES:

A contrast medium used with ultrasound, consisting of tiny bubbles of gas introduced into the vascular system intravenously. These are used as contrast agents for molecular imaging. These are small (typically 3 µm in diameter) gas filled bubbles that are usually injected intravenously. Injecting a gas into the circulation may seem potentially hazardous, but extensive clinical experience has shown that the tiny volume of air or gas given (under 200 µl) is not dangerous, and the safety of microbubbles compares well to that of conventional agents in radiography and magnetic resonance imaging. Although microbubbles were originally designed simply to improve conventional ultrasound scanning, recent discoveries have opened up powerful emerging applications.

Microbubbles 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 microbubbles produce has several special properties that can be exploited to improve diagnoses by producing overtones. Ultrasound scanners can be tuned to "listen" to these harmonics, producing strong preferential imaging of the microbubbles in an image. The selective excitation produced can also destroy microbubbles relatively easily, an effect that can be useful both in imaging and in emerging therapeutic applications.

Microbubbles increase the intensity of Doppler signals from blood for several minutes after their injection, and this effect can be prolonged by infusing them. They can thus rescue or improve an undiagnostic Doppler examination by raising the intensity of weak signals to a detectable level. They can be detected even in smaller vessels.

PROPERTIES OF MICROBUBBLES:

The ideal properties of microbubblescan be divided into two classes;

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**: Ultrasound-mediated microbubbles destruction has been proposed as an innovative method for non-invasive delivering of drugs and genes to different tissues. Microbubbles are used to carry a drug or gene until a specific area of interest is reached, and then ultrasound is used to burst the microbubbles, causing site-specific delivery of the bioactive materials.

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

b) distribution so as to avoid complications when injected into the body.

c) Density & compressibility difference between themselves & the surrounding body tissues to create acoustic impedance & to scatter ultrasound at a much higher intensity than the body tissues so as to be used as contrast agents.

d) Sufficient surface chemical properties to be modified for the attachment of various ligands to target them to specific tissues or organs

e) Uniformity of shell thickness.

COMPONENETS OF MICROBUBBLES:

Microbubbles basically comprise of three phases[6, 7, 8, 9]:

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 other components.

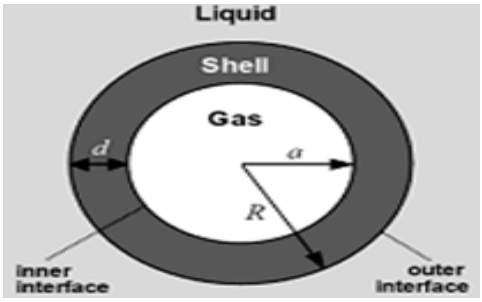

FIG. 1: COMPONENTS OF MICROBUBBLES.

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. First 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 and is used 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 for 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 membrane so as to achieve targeting of these to various other components, 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, the various types of shell materials that can be used are;

a. Proteins like albumin.

b. Phospholipids like phosphotidylcholine, phosphotidylethanolamine etc.

c. Biodegradable polymers like polyvinyl alcohol, polycaprolactone etc.

d. Surfactant Shells.

e. Polyelectrolyte Multilayer Shells.

a) Protein Shells[10, 11, 12, 13, 14]:

Albumin shelled microbubbles were a pioneering formulation used in contrast ultrasound imaging, mostly for formulations that could pass the lung capillaries and provide contrast in the left ventricle of the heart. The first albumin microbubbles formulation to be approved by the US Food and Drug Administration (FDA) was Albunex (GE Healthcare). An Albunex suspension consists of roughly 7x108 microbubbles/mL with a size range from 1 to 15 µm diameter. Albunex is stable upon refrigeration for at least two years.

These are formed by sonication of a heated solution of 5% (w/v) human serum albumin in the presence of air. During sonication, microbubbles of air are formed which become encapsulated within a 15-nm thick shell of aggregated albumin. Heating is necessary to denature the albumin prior to sonication and facilitate encapsulation. Biochemical analysis suggested that the shell is a monomolecular layer of native and denatured albumin in multiple orientations. The albumin shell is held together through disulfide bonds between cystein residues formed during cavitation. Covalent cross-linking may explain the relative rigidity of albumin shells observed during ultrasonic insonification.

d) Lipid Shells[15, 16, 17, 18]:

There are several commercially available lipid-coated microbubble formulations approved for clinical use in the US and abroad, including Definity (Lantheus Medical Imaging) and Sonovue® (Bracco Diagnostics). Phospholipids spontaneously self-assemble into a highly oriented monolayer at the air-water interface, such that their hydrophobic acyl chains face the gas and their hydrophilic head groups face

the water. Thus, the lipid monolayer will form spontaneously around a newly entrained gas bubble, just as for surfactants and proteins.

e) Polymer Shells[19, 20, 21]:

The term "polymer microbubble" typically refers to a special class of microbubbles that are stabilized by a thick shell comprising cross-linked or entangled polymeric species. The bulk nature of the polymer shell makes it more resistant to area compression and expansion than its lipid and albumin counterparts, which reduces the echogenicity and drug delivery activity.

The polymer microbubbles had a size distribution ranging from 1–20 µm. Optical microscopy and cryogenic transmission electron microscopy (cryo-TEM) showed that the microbubbles had elongated, crumpled shapes. The polymer shell was typically 150–200 nm thick.

Acoustic tests showed a dose-dependent increase in acoustic attenuation. In 1999, Nayaran and Wheatley reported on microbubbles formed by the biodegradable copolymer poly (D, L-lactide-co-glycolide) (PLGA). Also in 2005,Cavalieri*et al* ., described a method to create microbubbles coated with poly (vinyl alcohol) (PVA).

f) Surfactant Shell[22, 23]:

Microbubbles stabilized by mixtures of the synthetic surfactants SPAN-40 and TWEEN-40 were formulated by Wheatley *et al*. The SPAN/TWEEN solution was sonicated in the presence of air to form stable microbubbles. Surfactant derived from sonicated micro-bubbles was more stable (i.e., was capable of reaching higher collapse pressures on the Langmuir trough) than that used in the precursor solution.

g) Polyelectrolyte Multilayer Shells[24, 25, 26]:

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.

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.

3. Aqueous or Liquid Phase:

The external, continuous liquid phase 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.

- **Nonionic Surfactants**: Polyoxyehylenepolyoxy-propylene copolymers

Eg., Pluronic F-68,polyoxy-ethylene stearates, polyoxyethylene fatty alcohol thers,polyoxyethylatedsorbitan fatty acid esters, glycerol polyethylene glycol oxystearates, glycerol polyethylene glycol ricinoleate etc.

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

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.

METHODS TO PREPARE MICROBUBBLES[26-30]:

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 5˚C by adding HCl or H2SO4 as a catalyst, the cross linking reaction is stopped by neutralization of the mixture and microbubbles are then separated.

2. Emulsion Solvent Evaporation:

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.

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 these 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.

4. Sonication:

Sonication is preferred for formation of microbubbles, i.e., through an ultrasound 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.

CHARACTERISATION OF MICROBUBBLES:

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

A. 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 (SEM), Transmission Electron Microscopy.

B. 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.

C. Microbubble Concentration:

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

D. 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,

$$C_{air} = \rho_1 - \rho_2 / \rho_2 * 100$$

Where,

C_{air} is air content (%v/v);

ρ_1 (g/ml) density before elimination of encapsulated air;

ρ_2 (g/ml) density after elimination of encapsulated air.

E. 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.

MICROBUBBLES AND THEIR BIOEFFECTS: Potential Use For Drug Delivery.

Microbubbles have been proposed as a new vehicle for delivery of drugs and genes. Several properties of microbubbles make them a promising tool for drug and gene delivery to cells. Microbubbles have specific acoustic properties that make them useful for this goal. Whereas low and intermediate acoustic pressure results in linear and non-linear oscillations of microbubbles, respectively, high pressure ultrasound (MI O1:0) causes forced expansion and compression of microbubbles, leading to bubble destruction. Several studies have shown that destruction of microbubbles leads to permeability of cell membranes.

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

1) Incorporation of drug into Microbubbles:

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

- Unger[62] et al. described that drugs and genes can be attached to microbubbles(Fig. 2). Since microbubble shell constituents mainly exist from protein-, polymer- or lipid-based coatings, several ways of attachment of drugs to a microbubble have become available.

- Drug molecules can be incorporated within the bubble.

- Drugs can be conjugated to the microbubble membrane (shell) with the use of a charge dependent, noncovalent binding.

Ex: DNA that is a large negatively charged molecule is suitable for attachment to a

positively charged membrane.

- Another mechanism to load a bubble with drugs is to incorporate the drug in the shell (bubble membrane). This process is importantly influenced by the nature of the drug, in terms of lipophilicity and hydrophilicity. Although it seems difficult to enclose material within the microbubble itself, as it is a gasfilled microsphere, theoretically a drug containing powder may be attached to the inside of the bubble wall.

- Furthermore, drugs can be bound by ligands that are embedded in the membrane.

Ex: Avidin-biotin complex.

- Finally, even microbubbles with multiple layers can be constructed, in which drugs can be dissolved.

In conclusion, loading of microbubbles with a certain drug depends on several important factors like molecular weight, lipophilicity and charge. Recent experiments have shown that it is possible to create targeted microbubbles by incorporating monoclonal antibodies into the membrane.

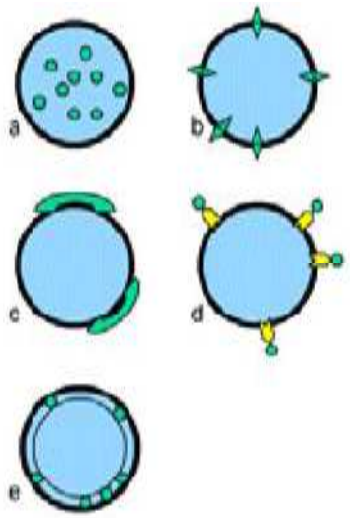

Figure 2: Several mechanisms to attach drugs to microbubbles.

a: Incorporation in the bubble; b: incorporation in the bubble membrane;

c: attachment to the membrane; d: attachment to a ligand;

e: incorporation in multilayer microbubble.

2) Drug release from Microbubbles:

- Skyba[63] et al. demonstrated that microbubble destruction during ultrasound exposure caused rupture of microvessels with extravasation of red blood cells.

- Price[64] et al. showed that microbubble destruction with ultrasound followed by infusion of 200 nm and 500 nm polymer microspheres resulted in red blood cells and polymer microspheres moving into the interstitium.Although various mechanisms by which microbubbles increase cell permeability have been proposed, the exact mechanism remains to be resolved. Ultrasound applied to fluid causes cavitation, i.e. the creation, vibration and collapse of small gas-filled bodies by the ultrasound beam. The effect of ultrasound alone has been studied and has been shown to increase cell permeability[65].on its own, without the addition of microbubbles. However, microbubbles in the presence of ultrasound with high acoustic pressure has an additional effect in increasing cell permeability[66].

- First, microbubbles, by acting as cavitation nuclei, can lower the threshold for cavitation. Stride and Saffari made an analysis of the conditions in the shell of the microbubble under influence of ultrasound and concluded that extremely high shell stresses and 'bubble like behaviour', including cavitation may be expected[67].In body tissue or blood, cavitation sets fluid in motion i.e., microbubbles oscillate and creates small shock waves (eddies) that give rise to microstreaming along the endothelial cell. Destruction of microbubbles may cause high-energy

microstreams, or microjets, that will cause shear stress on the membrane of an endothelial cell and increase its permeability(Fig. 3). This increase in permeability is probably due to transient holes in the plasma membrane and possibly the nuclear membrane.

- A second proposed mechanism, the generation of reactive oxygen species in endothelial cells under influence of ultrasound, was investigated by Basta et al[68].A significant, time-dependent increase in intracellular radical production after exposure to ultrasound was demonstrated. As the use of microbubbles together with ultrasound lowers the threshold for cavitation, this could possibly result in an increased production of free radicals, which are associated with cell killing in vitro and, as a consequence, may be also involved in enhancement of permeability of endothelial cell layers.

- A third interesting aspect is the rise in temperature in tissue following the application of high pressure ultrasound[69].Bubble collapse following high energetic ultrasound can create high velocity jet streams that may cause a local, transient increase in temperature. As a rise in temperature influences the fluidity of phospholipid bilayer membranes, cell membrane permeability could possibly be changed directly as a consequence of the increased bilayer fluidity.

- Fourth, endocytosis or phagocytosis, active membrane transport mechanisms, may also be involved in the uptake of the bubble, bubble fragments or material entrapped in microbubbles. Preliminary studies of our group showed increased uptake of fluorescent 20 nm nanospheres in the cells exposed to microbubbles loaded with these nanospheres. Even in absence of ultrasound, myocardial cells exposed to microbubbles loaded with nanospheres showed a significantly higher uptake of nanospheres per cell. This suggests that active processes like endocytosis or phagocytosis may be enhanced when microbubbles are used as a vehicle for nanospheres.

- A fifth mechanism by which the use of microbubbles could facilitate the deposition of drugs or genes in a cell is exchange or fusion of the phospholipid microbubble coating with the phospholipid bilayer of a

cell membrane. This could result in delivery of the cargo of the microbubble directly into the cytoplasm of the cell with the possibility of further uptake in endosomes or delivery to the cell nucleus.

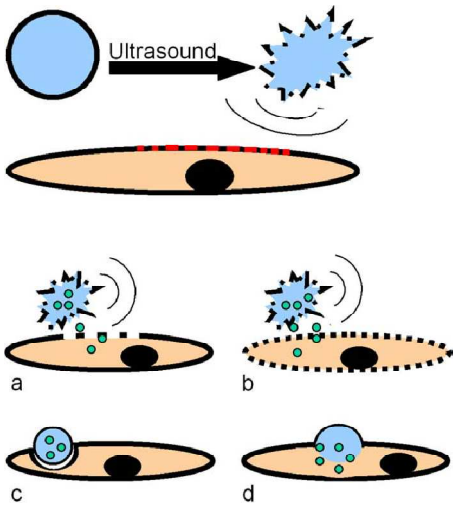

FIG 3: Destruction of microbubbles by ultrasound resulting in increased membrane permeability by shear stress, temperature rise and activation of reactiveoxygen species. Drug delivery by microbubbles by:

a: transient holes induced by shear stress; b: increase

in membrane fluidity; c: endocytosis of microbubbles;

d: fusion of the microbubble membrane with the cell

DESIGNING 'SMART' MICROBUBBLES: TARGETED MICROBUBBLES.

A recent and intriguing issue in contrast agents isthe development of targeted microbubbles. In combination with ultrasound, microbubbles have shown to be capable of delivering genes to specific tissue. As described earlier, microbubbles can be loaded with genes and injected into a vein, followed by localized ultrasound. In this way, microbubbles are aspecific and local delivery is controlled by the local application of ultrasound. However, as currently used microbubbles are relatively stable and circulate through the whole body,delivery of material could partly result in deposition of the contents of the microbubble in tissue that is not the target tissue, e.g. in the chest wall,or in the lungs, in which microbubbles with higher diameters are filtered. Therefore, it would be greatly desirable to have a microbubble which can be targeted to a specific tissue by using ligands and receptors that are incorporated in the bubble shell (Fig. 4). This would enable active attachment of microbubbles to target tissue and create furtherpossibilities for diagnostic imaging and therapy like local drug delivery to

target lesions. Targeted microbubbles have been developed into various types of tissue and processes, e.g., endothelial cells, thrombi, inflamed tissue and angiogenesis[49].

Author(s)/reference	Microbubbles	Target tissue	Receptor	Ligand	Outcome
Villanuevea et al.[28]	Perfluorobutane, lipid derived MB	Coronary artery endothelial cells	ICAM-1	Monoclonal antibody to ICAM-1	Significantly increased binding of MB with anti-ICAM-1
Schumann et al.[27]	Lipid-coated perfluorocarbon containing MB	Thrombus	GPIIbIIIa-receptor	Bioconjugate ligands	Higher affinity of targeted MB versus untargeted MB
Lindner et al.[26]	Lipid MB containing decafluorobutane	Inflamed tissue	Complement - mediated attachment to leucocytes	PS	Greater attachment of PS containing bubbles
Klibanov[49]	Perfluorobutane containing MB	Avidin coated culture plates	Avidin	Biotin	Increased attachment in contrast to control MB

MB, microbubble; ICAM-1, intercellular adhesion molecule-1; PS, phosphatidylserine.

These studies have described the first results in the assessment of targeted microbubbles. Identification of inflammation, preclinical atherosclerosis,

angiogenesis and vascular clots or thrombi is promising and creates options for diagnosis and treatment of cardiovascular diseases[30].

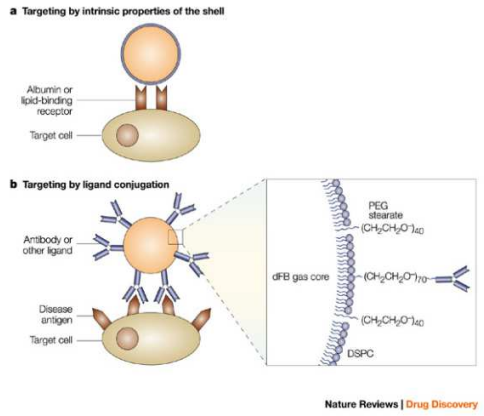

Fig 6: Targeted Microbubbles.

MECHANISMSFOR TARGET DRUG DELIVERY USING MICROBUBBLES[52-62]:

Two possible strategies for delivering drugs and genes with microbubbles are emerging. The first consists on the ultrasound-mediated microbubble destruction, which is based on the cavitation of microbubbles induced by ultrasound application, and the second is the direct delivery of substances bound to microbubbles in the absence of ultrasound. Different drugs and genes can be incorporated into the ultrasound contrast agents. It has already been demonstrated that perfluorocarbon-filled albumin microbubbles avidly bind proteins and synthetic oligonucleotides.

In a similar way, microbubbles can directly take up genetic material, such as plasmids and adenovirus and phospholipid-coated microbubbles have a high affinity for chemotherapeutic drugs. Furthermore, 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 microbubbles, which increases their deposition to activated endothelium.

The presence of microbubbles in the insonified field reduces the peak negative pressure needed to enhance drug delivery with ultrasound. This occurs because the microbubbles act as nuclei for cavitation, decreasing the threshold of ultrasound energy necessary to cause this phenomenon. The results of optical and acoustical studies have suggested the following mechanisms for microbubble destruction by ultrasound: 1- gradual diffusion of gas at low acoustic power, 2- formation of a shell defect with diffusion of gas, 3- immediate expulsion of the

microbubble shell at high acoustic power, and 4- dispersion of the microbubble 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. It has been reported that the application of ultrasound to contrast agents creates extravasation points in skeletal muscle capillaries, and this phenomenon is dependent on the applied ultrasound power.

- High intensity ultrasound (referred to as a high mechanical index) can rupture capillary vessels, resulting in deposit of protein and genetic material into the tissues. Skyba*et al*., demonstrated in an exteriorized spinotrapezius preparation that ultrasonic destruction of gas-filled microbubbles caused rupture of micro vessels with diameter ≤ 7 µm (capillaries), with local extravasations of red blood cells.

- Price[47]*et al*., have shown that polymer microspheres could be driven as much as 200 µm into the parenchyma with this method. The authors calculated that only a small number of capillary ruptures were required to deliver large quantities of the colloidal particles to the muscle. Using the same model of polymer microspheres bound to microbubbles and ultrasound, it has also been demonstrated that the ultrasound pulse interval and microvascular pressure influence the creation of extravasation points and the transport of microspheres to the tissue. Both were greatest when the pulse interval was around 5 seconds, which allows maximal microbubble replenishment within the micro-circulation after destruction by the ultrasound pulse.

- The formation of pores in the membranes of cells as a result of ultrasound-induced microbubble cavitation has been proposed as a mechanism for facilitating the drug deposition. Taniyama*et al*., demonstrated the presence of small holes in the surface of endothelial and vascular smooth muscle cells immediately after transfection of a plasmid DNA by ultrasound-mediated microbubble destruction, using electron microscopic scanning[47]. It was then postulated that these transient holes in the cell surface caused by microbubbles and

ultrasound resulted in a rapid translocation of plasmid DNA from outside to cytoplasm.

• Mukherjee[56] *et al.*, demonstrated by electron microscopy of a rat heart performed during application of ultrasound, that disruption or pore formation of the membrane of the endothelial cells occurred with acoustic power of 0.8 to 1.0 W/cm^2. However, it was a lower intensity of ultrasound (0.6 W/cm2) that caused more drug delivery with microbubbles. More recently, voltage clamp techniques were used to obtain real-time measurements of membrane sonoporation in the presence of albumin-coated microbubbles (Optison). Ultrasound increased the transmembrane current as a direct result of membrane resistance due to pore formation.

• Albumin-coated microbubbles 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 microbubbles could be selectively concentrated at the site of vascular injury in the presence or absence of ultrasound application.

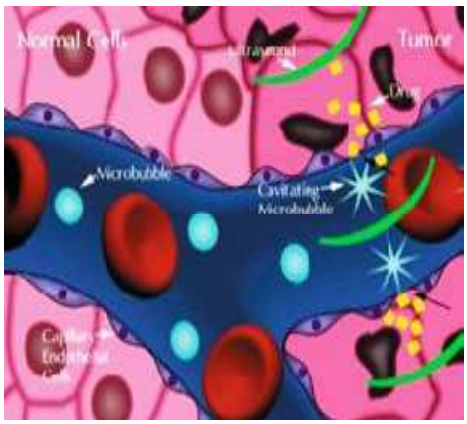

FIG. 4: **DRUG RELEASE FROM MICROBUBBLES.**

BIOMEDICAL APPLICATIONS:

1. Diagnostic Aids[27, 28, 29, 30, 34]:

Microbubbles are elastic and 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 Organ Edge Delineation, Blood Volume and Perfusion, Inflammation, Cancer, Liver and Gall bladder stone imaging, also used to scan the tumors arising in the body.

2. Gene Delivery[31, 34, 35, 36]:

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 they 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. 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. 7**).

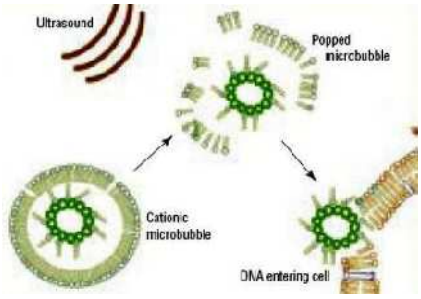

FIG. 7: ULTRASOUND SCAN OF LIVER USING MICROBUBBLES.

Levovist Microbubbles:

The clinical use of viral vectors for gene therapy is limited because viral proteins elicit an immune response within the target tissue 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.

In 1997, Bao*et al*., described the use of ultrasound and albumin-coated microbubbles to enhance the transfection of luciferase reporter plasmid in cultured hamster cells. Since then, many studies have confirmed the efficacy of ultrasound-mediated microbubble 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 microbubbles could be used to direct transgene expression to the heart *in vivo*.

Of note, transfection was not observed if the adenovirus was administered in the same dose without microbubbles, or if the adenovirus was administered with microbubbles 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.

3. Drug Delivery[37-46]:

Microbubbles have also been demonstrated an effective technique for targeted delivery of drugs and genes. Drugs can be incorporated into the microbubbles in a number of different ways, including binding of the drug to the microbubble shell and attachment of site-specific ligands.

As perfluorocarbon-filled microbubbles 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. Ultrasound applied over the skin surface can then be used to burst the microbubbles at this site, causing localized release of the drug. This technique then permits using lower concentrations of drugs systemically, and concentration of the drug only where it is needed. This improved therapeutic index may be extremely advantageous in cases of drugs with hazardous systemic side effects, like

cytotoxic agents. Albumin-encapsulated microbubbles have also demonstrated to adhere to the vessel walls in the setting of endothelial dysfunction [46]. This also may be a method of targeting delivery with microbubbles but without the application of ultrasound.

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.

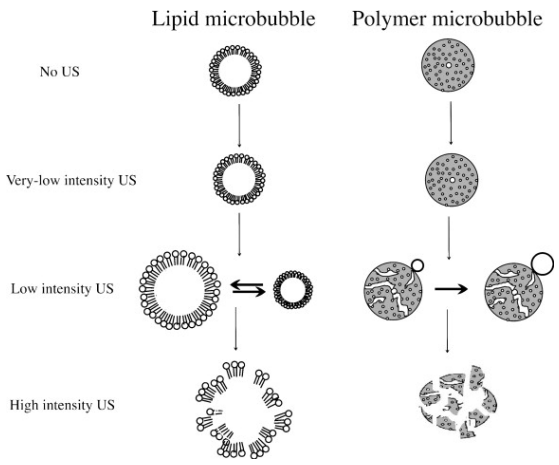

Fig 8 Various modes of Ultra sound application on the Microbubbles.

When driven by an ultrasonic pulse, these small gas bubbles oscillate with a wall velocity on the order of tens to hundreds of meters per second and can be deflected to a vessel wall or fragmented into particles on the order of nanometers. While single-session molecular imaging of multiple targets is difficult with affinity-based strategies employed in some other imaging modalities, microbubble fragmentation facilitates such studies. Similarly, a focused ultrasound beam can be used to disrupt delivery vehicles and blood vessel walls, offering the opportunity to locally deliver a drug or gene. Clinical translation of these vehicles will require that current challenges be overcome, where these challenges include rapid clearance and low payload. The technology, early successes with drug and gene delivery, and potential clinical applications are reviewed.

INCREASING APPLICATIONS OF MICROBUBBLES:

Now a day, Micro bubble drug delivery system is attracting researchers due to its wide scope and lots of applications.

- They are now used in areas in advanced and conventional science and technologies like heat removal, energy conversion,

cleaning and sterilization by shock waves etc.

- Microbubbles are applicable to a wide variety of field like medical field, Gene therapy.

- In medical field used 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 and in thrombolysis.

- The use of targeted microbubble has challenging therapeutic options, not only in CVS disease but also in treatment of inflammatory and malignant disease.

- These micro bubbles, being of extremely small size, are characterized by having electrical charges. They attract suspended floating particles very effectively. This particular property has been used in sludge treatment by using the micro bubbles to capture and float organic matters, thus decreasing the time required for the sludge treatment.

- The unique ability of microbubbles to respond to ultrasound makes them useful agents for contrast ultrasound imaging, molecular imaging of various organs and tumours, and targeted drug and gene delivery.

- Micro bubbles have also been introduced by the Japanese to market safe and good tasting oysters. Micro bubbles of concentrated oxygen containing about 2% ozone can be used to inactivate norovirus in shellfish and oysters. This norovirus is one of the major pathogens causing food poisonng in winter. This is a much more cost effective method compared to cultivating the oysters in sterile seawater and using chlorine-based germicide.

- Due to their 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 cleaning 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 can penetrate deeply into skin for a good scrub without the need for any shampoo or soap. This skin treatment has been introduced within some spas in Japan as well as shops specializing in bathing pets.

Needless to say, the baths are especially helpful for pets which have skin allergies to pet shampoos.

• Another emergent usage of micro bubbles is in the areas of cancer treatment. Scientists are in the process of developing a method of diagnosing cancer lesions by injecting micro bubbles into the blood stream. During the ultrasonic scan for cancer lesions, the micro bubbles contract and expand rapidly due to the pressures produced by the ultrasonic beam. Groups of the micro bubbles at cancerous tumours will show up very visibly on ultrasonic scans to indicate the presence of cancerous cells.

• Another important therapeutic property of microbubbles is their increased adherence to damaged vascular endothelium.

ADVANTAGES[70, 71, 72]:

• Ultrasound imaging can be performed by using microbubbles and ultrasound which allows real-time evaluation of blood flow.

• Ultrasonic molecular imaging is safer than molecular imaging modalities such as radionuclide imaging because it does not involve radiation.

• Since microbubbles can generate such strong signals, a lower intravenous dosage is needed, micrograms of microbubbles are needed compared to milligrams for other molecular imaging modalities such as MRI contrast agents.

• Targeting strategies for microbubbles are versatile and modular. Targeting a new area only entails conjugating a new ligand.

DISADVANTAGES[72, 73, 74]:

• Microbubbles don't last very long in circulation. They have low circulation residence times because they either get taken up by immune system cells or get taken up by the liver or spleen even when they are coated with PEG.

• Ultrasound produces more heat as the frequency increases, so the ultrasonic frequency must be carefully monitored.

• Microbubbles burst at low ultrasound frequencies and at high mechanical indices (MI), 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.

- Targeting ligands can be immunogenic, since current targeting ligands used in preclinical experiments are derived from animal culture.

- Low targeted microbubble adhesion efficiency, which means a small fraction of injected microbubbles bind to the area of interest.This is one of the main reasons that targeted contrast-enhanced ultrasound remains in the preclinical development stages.

CONCLUSION:

Microbubble ultrasound contrast agents offer a wide range of potential benefits for both diagnostic and therapeutic applications. As a result, they have become the subject of a broad and rapidly developing field of research. 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, in treatment of various diseases.

Microbubbles have rapidly evolved from a diagnostic adjuvant to a possible therapeutic agent. At present, however, their behaviour is by no means fully understood, and consequently their effectiveness has yet to be maximized. Moreover, while no definite evidence of harmful effects has been obtained, there remain some concerns as to their safety. As these questions are resolved by advances in the subject, it is anticipated that more and more of the benefits of contrast agents will become realizable.

REFERENCES:

1. Bertrand N, Leroux JC. (2011). "The journey of a drug carrier in the body: an anatomo-physiological perspective". Journal of Controlled Release. Doi: 10.1016/j.jconrel.2011.09.098

2. Scott, Robert C; Crabbe, Deborah; Krynska, Barbara; Ansari, Ramin; Kiani, Mohammad F (2008). "Aiming for the heart: targeted delivery of drugs to diseased cardiac tissue". Expert Opinion on Drug Delivery **5** (4): 459–70. Doi: 10.1517/17425247.5.4.459.

3. Torchilin VP. (2006)Adv Drug Deliv Rev. 2006 Dec 1; 58(14):1532-55.

4. Cobleigh, M; Langmuir, VK; Sledge, GW; Miller, KD; Haney, L; Novotny, WF; Reimann, JD; Vassel, A (2003). "A phase I/II dose-escalation trial of bevacizumab in previously treated metastatic breast cancer". Seminars in Oncology **30** (5 Suppl 16): 117–24. Doi:10.1053/j.seminoncol.2003.08.013.

5. Seidman, A.; Hudis, C; Pierri, MK; Shak, S; Paton, V; Ashby, M; Murphy, M; Stewart, SJ et al. (2002). "Cardiac Dysfunction in the Trastuzumab Clinical Trials Experience". Journal of Clinical Oncology **20**(5): 1215–21. Doi:10.1200/JCO.20.5.1215. PMID 11870163.

6. Rajesh Patel; Microbubble: An ultrasound contrast agent in molecular imaging, Pharma Times, May 2008; Vol. 40; 15.

7. DeepikaMaliwal; Microbubbles Contrast Agents Using Ultrasound; Research Journal of Pharmacy and Technology; Vol. 1; issue 03; July-Sept. 2008.

8. Eniola A.O. and Hammer D.A.; In vitro characterization of leukocyte mimetic for targeting therapeutics to the endothelium using two receptors; Biomaterials; 2005; Vol.26; 7136-44.

9. Eniola A.O., Willcox P.J. and Hammer D.A.; Interplay between rolling and firm adhesion elucidated with a cell-free system

engineered with two distinct receptor-ligand pairs; Biophys. J.; 2003; 85; 2720-31.

10. Christiansen C, Kryvi H, Sontum PC, Skotland T. Biotechnology and Applied Biochemistry. 1994; 19: 307–320

11. Myrset AH, Nicolaysen H, Toft K, Christiansen C, Skotland T. Biotechnology and Applied Biochemistry. 1996; 24: 145–153.

12. Grinstaff MW, Suslick KS. ProcNatlAcadSci U S A. 1991; 88: 7708–7710.

13. Dayton PA, Morgan KE, Klibanov AL, Brandenburger GH, Ferrara KW. IEEE Transactions on Ultrasonics Ferroelectrics and Frequency Control.1999; 46: 220–232.

14. Podell S, Burrascano C, Gaal M, Golec B, Maniquis J, Mehlhaff P. Biotechnology and Applied Biochemistry. 1999; 30: 213–223.

15. D'Arrigo JS. 'Stable Gas-in-Liquid Emulsions: Production in Natural Waters and Artificial Media'. New York, NY: Elsevier Science Pub. Co; 1986.

16. Notter RH, Wang ZD. Reviews in Chemical Engineering.1997; 13: 1–118.

17. Pattle RE. Nature.1955; 175: 1125–1126.

18. Verder H, Ebbesen F, Linderholm B, Robertson B, Eschen C, Arroe M, Lange A, Grytter C, Bohlin K, Bertelsen A, Danish-Swedish G. Multicentre Study. ActaPaediatr.2003; 92: 728–733.

19. Bloch SH, Wan M, Dayton PA, Ferrara KW. Applied Physics Letters. 2004; 84: 631–633

20. Epstein PS, Plesset MS. J Chem Phys. 1950; 18: 1505–1509.

21. Cavalieri F, El Hamassi A, Chiessi E, Paradossi G. Langmuir. 2005; 21: 8758–8764.

22. Singhal S, Moser CC, Wheatley MA. Langmuir. 1993; 9: 2426–2429.

23. Wang WH, Moser CC, Wheatley MA. Journal of Physical Chemistry.1996; 100: 13815–13821.

24. Shchukin DG, Kohler K, Mohwald H, Sukhorukov GB. AngewChemInt Ed. 2005; 44: 3310–3314.

25. Borden MA, Caskey CF, Little E, Gillies RJ, Ferrara KW. Langmuir. 2007; 23: 9401–9408.

26. Lentacker I, De Geest BG, Vandenbroucke RE, Peeters L, Demeester J, De Smedt SC, Sanders NN. Langmuir. 2006; 22: 7273–7278.

27. Klibanov A.L.; Targetted delivery of gas filled microspheres, contrast agents for ultrasound imaging : Adv. Drug Delivery Review; 1999; 37; 139-157.

28. Klibanov A.L.; Ligand carrying gas filled microbubble : Ultrasound contrast agents for targeted molecular imaging; Bioconjug. Chem.; Vol.16; 2005; 9-17.

29. Lindner J.R.; Microbubbles in medical imaging: Current applications and future directions, Nat Rev. Drug Discovery; Vol.3; 2004; 527-32.

30. McCulloch M.C., Gresser S., Moos J.; Ultrasound contrast physics: A series on contrast echocardiography; article 3; J Am SocEchocardiogy; 13; 959-67

31. Verma IM, Somia N: Gene therapy-promises, problems and prospects. Nature 1997, 389: 239-242.

32. Newman KD, Dunn PF, Owens JW, Schulick AH, Virmani R, Sukhova G, et al.: Adenovirus-mediated gene transfer into normal rabbit arteries results in prolonged vascular cell activation, inflammation, and neointimal hyperplasia. J Clin Invest 1995, 96: 2955-2965.

33. Felgner PL: Nonviral strategies for gene therapy. Sci Am 1997, 276: 102-106.

34. Porter TR, Iversen PL, Li S, Xie F: Interaction of diagnostic ultrasound with synthetic oligonucleotide labeled perfluorocarbon-exposed sonicated dextrose albumin microbubbles**.**

35. Taniyama Y, Tachibana K, Hiraoka K, Namba T, Yamasaki K, Hashiya N, et al.: Local delivery of plasmid DNA into rat carotid artery using ultrasound. J Am CollCardiol2003, 42: 301-308.

36. Shohet RV, Chen S, Zhou YT, Wang Z, Meidell RS, Unger RH, et al.: Echocardiographic destruction of albumin microbubbles directs gene delivery to the myocardium. Circulation 2000, 101: 2554-2556.

37. Annemieke van Wamel, KlazinaKooim ; Vibrating microbubbles poking individual

cells : Drug transfer into cells via sonoporation ; Journal of Controlled Release;Vol.112; 2006 Pg 149.

38. Ka-Yun &T.O.Matsunga; Ultrasond Mediated drug delivery; Wiley Publications.

39. Lindner J.R., Klibanov A.L., and Ley K.; Targetting inflammation, In: Biomedical aspects of drug targeting; 2002; 149-172.

40. Ying-Zheng Zhao Cui-Tao Lu; Factors that affect the efficiency of antisense oligodeoxyribonucleotide transfection by insonated gas-filled lipid microbubbles; J Nanopart Res (2008) 10:449–454.

41. Skyba DM, Price RJ, Linka AZ, Skalak TC, Kaul S: Direct in vivo visualization of intravascular destruction of microbubbles by ultrasound and its local effects on tissue. Circulation 1998, 98: 290-293.

42. Price RJ, Skyba DM, KAUL S ,Skalak TC: Delivery of colloidal particles and red blood cells to tissue through micro vessel ruptures created by targeted microbubble destruction with ultrasound. Circulation 1998, 98: 1264-1267.

43. Porter TR, Iversen PL, Li S, Xie F: Interaction of diagnostic ultrasound with synthetic oligonucleotide labeled perfluorocarbon-exposed sonicated dextrose albumin microbubbles. J Ultrasound Med 1996, 15: 577-584.

44. Main ML, Grayburn PA: Clinical applications of transpulmonary contrast echocardiography. Am Heart J 1999, 137: 144-153.

45. Wei K, Skyba DM, Firschke C, Jayaweera AR, Lindner JR, Kaul S: Interactions between microbubbles and ultrasound: in vitro and in vivo observations. J Am CollCardiol1997, 29: 1081-1088.

46. Unger EC, McCreery TP, Sweitzer RH, Caldwell VE, Wu Y: Acoustically active lipospheres containing paclitaxel: a new therapeutic ultrasound contrast agent. Invest Radiol1998, 33: 886-892.

47. Taniyama Y, Tachibana K, Hiraoka K, Namba T, Yamasaki K, Hashiya N, et al.: Local delivery of plasmid DNA into rat carotid artery using ultrasound. Circulation 2002, 105: 1233-1239.

48. Chen S, Shohet RV, Bekeredjian R, Frenkel P, Grayburn PA: Optimization of ultrasound parameters for cardiac gene delivery of adenoviral or plasmid

deoxyribonucleic acid by ultrasound-targeted microbubble destruction. J Am CollCardiol2003, 42: 301-308.

49. Shohet RV, Chen S, Zhou YT, Wang Z, Meidell RS, Unger RH, et al.: Echocardiographic destruction of albumin microbubbles directs gene delivery to the myocardium. Circulation 2000, 101: 2554-2556.

50. Mukherjee D, Wong J, Griffin B, Ellis SG, Porter T, Sen S, et al.: Ten-fold augmentation of endothelial uptake of vascular endothelial growth factor with ultrasound after systemic administration. J Am CollCardiol2000, 35: 1678-1686.

51. Villanueva FS, Jankowski RJ, Manaugh C, Wagner WR: Albumin microbubble adherence to human coronary endothelium: implications for assessment of endothelial function using myocardial contrast echocardiography. J Am CollCardiol1997, 30: 689-693.

52. Miller MW: Gene transfection and drug delivery. Ultrasound Med Biol2000, 26 Suppl 1:S59-S62. 48) Unger EC, McCreery TP, Sweitzer RH, Caldwell VE, Wu Y: Acoustically active lipospheres containing paclitaxel: a new therapeutic ultrasound contrast agent. Invest Radiol1998, 33: 886-892.

53. Fritz TA, Unger EC, Suthrland G, Sahn D: Phase I clinical trials of MRX-115. A new ultrasound contrast agent.Invest Radiol1997, 32: 735-740.

54. Lindner JR, Song J, Christiansen J, Klibanov AL, Xu F, Ley K: Ultrasound assessment of inflammation and renal tissue injury with microbubbles targeted to P-selectin. Circulation 2001, 104: 2107-2112.

55. Weller GE, Lu E, Csikari MM, Klibanov AL, Fischer D, Wagner WR, et al.: Ultrasound imaging of acute cardiac transplant rejection with microbubbles targeted to intercellular adhesion molecule-1. Circulation 2003, 108: 218-224.

56. Chomas JE, Dayton P, Allen J, Morgan K, Ferrara KW: Mechanisms of contrast agent destruction. IEEE.Trans UltrasonFerroelectrFreq Control 2001, 48: 232-248.

57. Chomas JE, Dayton P, May D, Ferrara K: Threshold of fragmentation for ultrasonic

contrast agents. J Biomed Opt 2001, 6:141-150.

58. Song J, Chappell JC, Qi M, VanGieson EJ, Kaul S, Price RJ: Influence of injection site, micro vascular pressure and ultrasound variables on microbubble-mediated delivery of microspheres to muscle. J Am CollCardiol2002, 39: 726-731.

59. Deng CX, Sieling F, Pan H, Cui J: Ultrasound-induced cell membrane porosity. Ultrasound Med Biol2004, 30: 519-526.

60. Porter TR, Hiser WL, Kricsfeld D, Deligonui U, Xie F, Iversen P, et al.: Inhibition of carotid artery neointimal formation with intravenous microbubbles. Ultrasound Med Biol2001, 27: 259-265.

61. Porter TR, Knnap D, Venneri L, Oberdorfer J, L of J, Iversen P, et al.: Increased suppression of intracoronary c-myc protein synthesis within the stent or balloon injury site using an intravenous microbubble delivery system containing antisense to c-myc: comparison with direct intracoronary injection. J Am CollCardiol2003, 41: 431A.

62. Unger EC, Hersh E, Vannan M, Matsunaga TO, McCreery T. Local drug and gene delivery through microbubbles. ProgCardiovasc Dis 2001;44(1):45e54.

63. Skyba DM, Price RJ, Linka AZ, Skalak TC, Kaul S. Direct in vivo visualization of intravascular destruction of microbubblesby ultrasound and its local effects on tissue.

64. Circulation 1998;98(4):290e3.

65. Price RJ, Skyba DM, Kaul S, Skalak TC. Delivery of colloidal particles and red blood cells to tissue through microvesselruptures created by targeted microbubble destruction with

66. ultrasound. Circulation 1998;98(13):1264e7.

67. Lawrie A, Brisken AF, Francis SE, Tayler DI, Chamberlain J, Crossman DC, et al. Ultrasound enhances reporter gene expression after transfection of vascular cells in vitro.

68. Circulation 1999;99(20):2617e20.

69. Wamel JET, Bouakaz A, Houtgraaf J, Cate FJ, de Jong N. Effects of diagnostic ultrasound parameters on molecular uptake

and cell viability. International ultrasonics symposium, proceedings; 2002.

70. Stride E, Saffari N. On the destruction of microbubbleultrasound contrast agents. Ultrasound Med Biol2003; 29(4):563e73.

71. Basta G, Venneri L, Lazzerini G, Pasanisi E, Pianelli M, Vesentini N, et al. In vitro modulation of intracellular oxidative stress of endothelial cells by diagnostic cardiac

72. ultrasound. Cardiovasc Res 2003;58(1):156e61.

73. Wu J. Temperature rise generated by ultrasound in the presence of contrast agent. Ultrasound Med Biol1998; 24(2):267e74.

74. Lindner, J.R. 2004. Microbubbles in medical imaging: current applications and future directions. Nat Rev Drug Discov. 3: 527-32.

75. Lindner, J.R., A.L. Klibanov, and K. Ley. Targeting inflammation, In: Biomedical aspects of drug targeting. (Muzykantov, V.R., Torchilin, V.P., eds.) Kluwer, Boston, 2002; pp. 149–172.

76. Klibanov, A.L. 1999. Targeted delivery of gas-filled microspheres, contrast agents for ultrasound imaging. Adv Drug Deliv Rev. 37: 139-157.

77. 73.Klibanov, A.L. 2005. Ligand-carrying gas-filled microbubbles: ultrasound contrast agents for targeted molecular imaging. Bioconjug Chem. 16: 9-17.

78. Takalkar, A.M., A.L. Klibanov, J.J. Rychak, J.R. Lindner, and K. Ley. 2004. Binding and detachment dynamics of microbubbles targeted to P-selectin under controlled shear flow. J. Contr. Release.96: 473-482.