

MICROBUBBLES: A NOVEL APPROCH FOR TARGETED DRUG DELIVERY

Abstract

The harmful nature of chemotherapy agents is vital for them to destroy cancer cells. It too includes a source of side impacts experienced by patients. To minimize these side impacts, sound tissue presentation must be restricted by including the drug. And the drug ought to have potential for quick discharge inside the tumour. Microbubbles are that much smaller than the normal estimate of 1-10 μm than red blood cells. They are able to enter indeed the smallest blood capillaries and discharge drugs and genes beneath the activity of an ultrasound field after coming to a certain range of intrigues. The strategy of microbubble dispersion was presented and explored to create oxygen exchange at low development rate, to diminish control utilization and shear stress on microorganisms. Certain tissues can be focused on by joining protein ligands on the surface of microscopic bubbles.

Keyword: Microbubble, composition, types, method of preparation, diffusion mechanism, drug conveyance

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I. INTRODUCTION

These are the bubbles used for medicine both for imaging and for therapeutic application as well. Normally, if you think about putting bubbles into a human body, this is not a very good idea. They are associated for causing the bends for example, in scuba divers. But the bubbles that we deal with are much too small for that. They are almost a hundredth of a human hair in diameter. They can pass safely through human blood vessels without causing blockage. Gas embolism may be a known complication of different obtrusive strategies, and its administration is well established. The result of gas microemboli, microbubbles diagnosed less frequently than its occurrence and usually overlooked in day-by-day practice. We show the current information with respect to the pathophysiology of microemboli and their clinical results. Microbubbles begin basically in extracorporeal lines and gadgets like cardiopulmonary bypass and dialysis machines. May be endogenous in cases of decompression sickness or mechanical heart valves. Circulating within the bloodstream, microbubbles hold up within the capillary bed of different organs, basically the lungs. The microbubble obstructs blood stream within the capillary, which leads to tissue ischemia, taken after by inflammatory response and complement enactment. Accumulation of platelets or formation of clot happens as well, leading to advanced obstacles of microcirculation and tissue harm ^[2].

II. DEFINITION

Microbubbles are so tiny in size. The diameter of a microbubble is around 0.5um upto 10um and filled with gas. They are widely used in therapeutic imaging as differential specialists and as carriers for drug delivery ^[3].

A microbubble is a gas bubble that exists inside a fluid. Air, Nitrogen or Gas having higher molecular weight; for example, SF₆ or C₃F₈ can be used and the fluid is almost always water. Mostly 95% microbubbles are smaller than 10um and the average size of single microbubble is 3um. The stabilisation of microbubbles can be done by encasing them in a specific shell, that shell must me mead up of biocompatible components. Protein, polymer or lipids are some good examples of biocompatible materials. Microbubbles are used to differentiate operators in restorative ultrasound. ^[4].

Gas bubbles ordinarily begin in extracorporeal tubing and permeating with the liquids into the bloodstream. The bubbles may be shown whereas preparing the lines for utilize, or recently shaped as a result of turbulent stream within the vessel and at the vascular entrance. Contrasts in temperature is another conceivable cause for the bubble era in lines, since warming starts bubble arrangement, such as when a dynamic blood warming framework is used .

III. PROPERTIES

Ideal properties of microbubbles are basic properties and functional properties.

1. Basic properties

- Breadth of the miniaturized scale bubbles ought to be within the range of 1-10µm.
- Consistency of thickness of the shell.

- Uniform in size.
- Thickness and compressibility contrast ought to be able to diffuse with ultrasound.
- Ought to have adequate surface chemical properties to link the receptor for focusing on particular tissue or organs.

2. Functional Properties

- Small scale bubbles must be proficient to reply to the ultrasound.
- Infusion and Syringe ability must be appropriate to administer the micro bubbles systemic administration
- Biocompatible in nature .

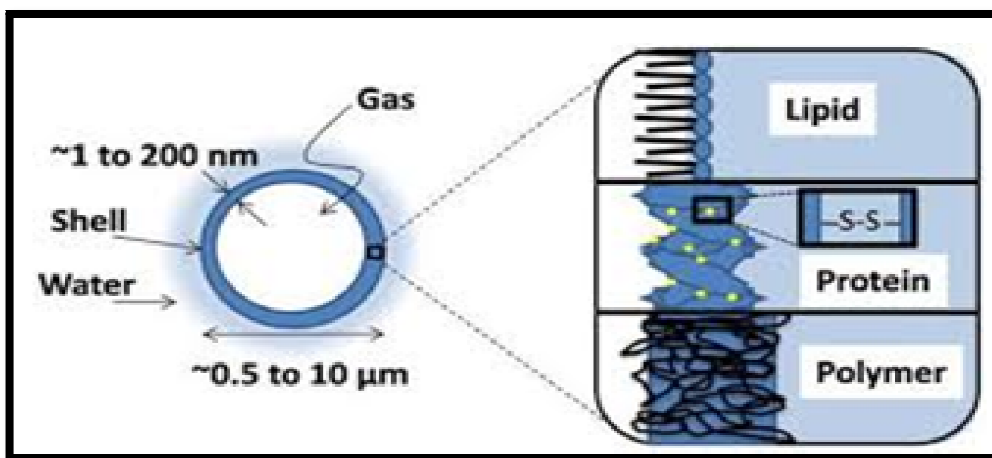


Figure 1: Component of Microbubbles

Micro bubbles contain essentially 3 stages in which internal, centre and outermost Figure 1. The following are the details:

Deepest Gas Phase

Shell Material Encasing the Gas Phase

Outermost Fluid or Watery Phase

- Internal most layers: gas phase:** It is commonly a layer of gas. In this gas phase it may consist of a single gas or the mixture of different gases. These mixtures of different gases are used to build fractional weight differentials and also produces gas osmotic pressure, which helps in stabilising the bubbles. In combination with the gas system there are two sorts of gases. Essential gas known as first gas. For the most part, it is air. At some point the first gas used is nitrogen and gas osmotic agents are secondary. The reason is they have lower solubility in blood and serum and a high enough vapour pressure at room temperature in order to achieve desired osmotic effects. ^[6,7,8,9,10,11,12]

- **Shell material:** The gas phase is covered by this shell material. The primary function of this layer is to serve as a container for medicate particles that are attached to a layer of shell and used to attack various tissue and organ components. Microbubble's flexibility or compressibility can be improved. In case of more versatile, It has the ability to withstand bursting and popping, and lengthening the time of these bubbles

to stay in the body. As a shell material, the material is used for example Proteins such as albumin, Carbohydrates such as galactose, Phospholipids such as phosphatidylcholine, phosphatidyl-ethanolamine etc., Biodegradable polymers such as polyvinyl alcohol, polycaprolactone etc. [6,7,8,9,10,11,12]

- **Fluid stage or watery phase:** The external continuous fluid stage in that bubble resides ordinarily is made up of surfactant (surface active agents) or foaming agent. Surfactants are any compounds or compositions which forms the layer at the interphase and helps to protect and organise the bubble membrane. The frothing agent, also known as a surfactant, is made up of a single ingredient or it can be a combination of compounds. For illustration, Block copolymers are polyoxyethylene, polyoxypropylene, sugar esters and fatty alcohols while Polyoxyethylene polyoxypropylene copolymers are non-ionic surfactants. Anionic Surfactants come in a variety of forms. Fatty acids with 12-24 carbon atoms are used (e.g., Sodium Oleate). [6,7,8,12]

IV. TYPES OF MICROBUBBLES

There are different types of microbubbles such as,

1. **Ultrasound microbubble:** When it affects the skin surface where it bursts and discharges the drug. It's used in a low-concentration form. It, too, adds to the number of therapeutic records. It is useful for medications that have risky and adverse side effects.
2. **Perfluorocarbon-filled microbubble:** Blood pools serve as carriers for perfluorocarbon-filled microbubbles, which are stable for circulating within the vascular system.
3. **Phospholipid-coated microbubble:** Which encompasses a high affinity or liking for chemotherapeutic drugs?
4. **Albumin-encapsulated microbubble:** Which follows and sticks to walls of vessels. [13]

V. FORMULATION METHOD OF MICROBUBBLES

Techniques that are used for the preparation of microbubble:

Atomization & Reconstitution
Sonication
Emulsion Solvent Evaporation
Cross Linking Polymerization

1. **Atomization & Reconstitution:** A spray dried surfactant solution is atomized in a vessel which contains hot gas, and forms porous spheres with the help of essential modifier gas enclosed in it. Synchronous pressing is to be done into the vials by filling the head space with inactive gas or osmotic operators. At that point the vial is fixed and after some time recently utilized vials are reconstituted by utilizing sterile saline solution. The vital modifier gas diffuses out and the auxiliary gas diffuses in after reconstitution, resulting in

a size reduction. The microbubbles are then administered to the patient after remaining suspended inside the saline arrangement. ^[10,14,15,17]

- 2. Sonication:** Sonication is the process which is used to generate microbubbles. In this method septum is injected through ultrasound device which has a special ultrasonically vibrating hypodermic needle or by an ultrasound transmission. A thin membrane may be used to sonicate the gas in the vial's headspace. Alternatively, an ultrasonic device or a concentrated ultrasound beam can be used to depress the membrane. The micro bubble solution can be pulled back from the vial and conveyed to the patient until sonication is completed. By deploying ultrasonically vibrated aspirating system on the syringe at a low power, sonication can also be achieved inside the syringe. ^[10,14,15,17]
- 3. Emulsion solvent evaporation:** In Planning of microbubbles there are two arrangements utilized. The First arrangement is a watery arrangement, it contains suitable surfactant. It can be amphiphilic biopolymer. Example of amphiphilic biopolymer- gelatine, collagen, albumin or globulins. This gets to be the outer continuous stage. The Second Arrangement contains a mixture of two. Water not miscible natural liquids. For the polymer, one is volatile and dissolvable, while the other is usually non-volatile and nonsolvent. To make an emulsion, combine the above second arrangement with the first solution. As the solvent in the droplet evaporator, the concentration of polymer rises up to the certain point where it can take involvement under the presence of nonsolvent that is less volatile. A polymer film on the emulsion droplet's surface is formed by this procedure. Further in this procedure, the formation of shell wall is done by encasing a non-solvent liquid inner core. By the result microcapsules can be recovered, cleaned and generated in a buffer complex. Following the drying process, Freeze-drying, ideally, eliminates all the water and a nonsolvent organic liquid core in order to produce a hollow microbubble which is packed with air. ^[9,10,14,15,16]
- 4. Cross linking polymerization:** The special apparatus named Ultra Turrax T-25 at 8000 rpm intensely stirs a 2% aqueous polymeric solution of telechelic PAV for 3 hours at a pH of 2.5 at room temperature. As a result, a fine foam forms, which serves as a colloidal stabilising agent and also behaves as a compound for bubble coating. Microbubbles are glide on the top surface of the mixture after polymers has been cross connected. Separated gliding microbubbles on the surface are widely purified in opposition to Milli Q water. ^[9,10,14,15,16]

VI. CHARACTERISATION OF MICROBUBBLES

These microbubbles are then characterised using the parameters mentioned down.

- 1. Microbubble breadth and measure distribution:** Scanning electron microscopy, Laser light scattering and Transmission electron microscopy can all be used to test it.
- 2. Shell thickness:** The estimation of shell thickness is done by applying fluorescent colour example, Ruddy Nile and shown against a dark backdrop under a microscope.
- 3. Microbubble concentration:** The number of microbubbles per each ml is being used to quantify the concentration of microbubbles with the help of Coulter Counter Machine.

- 4. Discuss substance by densitometry:** Wavering U-tube densitometer is used to measure the substance of suspension inside the microbubbles with a DMA-58 in the suspension tests.

In order to use this instrument, it must be normalised with filtered water and air. The evaluation of suspension thickness concluded twice, before and after encapsulated air is removed. In the sonicator, sonication with high power eliminates all of the encapsulated air within 5 minutes.

VII. MECHANISM OF DIFFUSION

Two diverse strategies are examined for conveying genes and drugs utilizing smaller scale bubbles are

1. The coordinate conveyance of substances bound to the micro bubbles within the non-attendance of ultrasound.
2. Ultrasound-mediated microbubble destruction (cavitation of miniaturized scale bubbles initiated by ultrasound application).

The micro sized bubbles have ultrasound differentiation properties where drugs and genes can be stacked into the centre for required pharmacological action. Micro bubbles are coated with phospholipid which have a great fondness towards the chemotherapeutic drugs. Albumin microbubbles can bind with the proteins and the manufactured oligonucleotides. The linking of leukocyte intercellular adhesion molecule 1 (ICAM-1) and P-selectin (both are specified ligands for endothelial cell adhesion) with lipid and microbubbles which are albumin encapsulated, raising their deposition for the activation of endothelium. Distinctive drugs and genes can be joined by the microbubbles which are the ultrasound differentiate agents. Plasmids and adenovirus sorts of genes which can be taken up directly by micro bubbles.

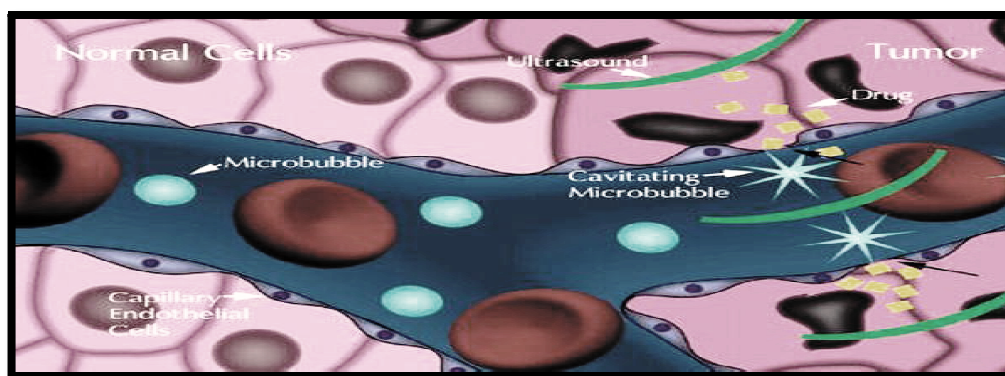


Figure 2: Mechanism of Diffusion

In this mechanism conveyance of drugs and genes are induced by ultrasound on the basis of complex interaction between therapeutic agents, the characteristics of microbubbles, the target tissue, and the essence of ultrasound energy. Peak negative pressure is decreased by the microbubbles in theinsonified field in result the conveyance of drug through ultrasound is increased. Since the microbubbles function as cavitation cores, reduction of ultrasound

energy required to create this situation. The outcomes of the optical and the acoustical considers have recommended the following instruments for microbubble pulverization by ultrasound:

- due to lower acoustic intensity, gas dispersal occurs,
- defection in shell development due to gas diffusion,
- as a result of high acoustic intensity, microbubble shell discharges immediately, and
- the micro bubble diffuses into a few little bubbles.

Specification of bubble's cavitation is based on quick destruction of targeting ligands which are induced by hydrostatic volatility that is triggered by high frequency oscillations and it is exactly related to the transmission intensity. The use of ultrasound with targeting ligands has been shown to induce extravasation levels in skeletal muscle capillaries. This effect is affected by the capacity of ultrasound. The ultrasound that is extremely intensive and also known as high mechanical index is able to burst small blood vessels (capillary veins) causing genetic material and proteins to be deposited in the tissues figure 2. [17,20-35]

VIII. DRUG CONVEYANCE

Micro bubbles are competent of binding with definite receptors within the body depending on the particular molecule coated the bubble. Once bubbles are joined to the receptors, a solid ultrasound burst is and all that's required to pop the bubbles and centre material is discharged for required pharmacological impact. As the drug is discharged at a targeted location no longer medicate can influence other body liquids, indeed organs, where side impacts due to the drug can be controlled.

Micro bubble medicate conveyance is most advantageous since smaller dosage of the medicate is adequate as compared to the conventional due to the discharge of drug focused on the site and side impacts are lower than other measurement forms due to the drug discharge, in case of against neoplastic drugs it is most beneficial. Micro bubbles begin swaying and experience the cavitation prepared on application of low frequency ultrasound. In this way the bubbles will burst or break up and drug atoms which are consolidated within the centre portion will be released in focus on location.

1. Consolidation of drug in to the centre material: Incorporation of drug particles into the bubble can be done by distinctive components of the bubbles.

- Joining of drugs within the internal most layer.
- Consolidation into the shell material.
- Medicate connected or cross connected to the shell by noncovalent bonds.
- Joining the medicate to the micro bubble surface through a ligand (ex: avidin-biotin complex).
- In the case of numerous layered micro bubbles. Medication can be consolidated inside the different layers.

2. Medicate discharge from microbubbles: For drug conveyance microbubbles acts as a vehicle, which carries the drug. When microbubbles are released by ultrasound, they

undergo a mechanism known as cavitation, which causes them to burst or breaks down. Cavitation occurs as body fluids begin to insonate, resulting in acoustic cavitation. Additionally, as the microbubbles vibrate, small vortex form. Now micro streaming is generated by these vortexes, which helps to improve cell membrane's penetrability. Medicate exchange through the membrane is also encouraged. The cell film can now phagocytose the micro bubbles in some cases, resulting drug release.

The phosphatide microbubble joins to the phosphatide bilayer of the cell membrane, ensuing drug or gene release directly inside cell membrane's cytoplasm. This is called gene delivery because in this mechanism gene is been shifted very near to the nucleus. The taking after figure appears medicates conveyance by means of the microbubbles.

- Medicate conveyance through the cavitation
- Discharge of the drug through cavitation while raising cell membrane permeability
- Interaction with the membrane
- Cell membrane phagocytes the microbubble

3. Medicate conveyance through the cavitation: The application of ultrasound bubbles experiencing a cavitation handle in this way comes about in the breakup or bursting of the bubble and concurrent discharge of medicate. After cavitation the body liquids begin insonating creating acoustic cavitation. Encouraged by the micro bubbles oscillates, at that point grant rise to little whirlpools. These permits miniaturized scale spilling or miniaturized scale planes leads to increase in porousness of the cell layer. Sometimes the micro bubbles may be phagocytosed by the cell layer.

4. Discharge of the drug through cavitation while raising cell membrane permeability: Conveyance of the medicate or genes specifically into the cytoplasm of the cell film is done by the combination of phospholipid of micro bubble with the phospholipid bilayer of cell layer. This mechanism is also used for gene delivery because it exchanges the genes in close nearness of the core.^[35-41]

IX. APPLICATIONS OF MICROBUBBLES

1. Gene therapy: In special cases of differential atomic imaging & improvement, the use of microbubbles as focused on conveyance vehicles is the foremost heightening investigated applications of ultrasound differentiate agents. Diffusion of bubbles in the field of ultrasound can increment the penetrability of an endothelial vasculature, permitting little particles to diffuse in tissue from the bloodstream, a method called as sonoporation^[42]. Sonoporation is still in question, but it followed jetting that caused during inertial cavitation^[43]. There are various comprehensive surveys on cavitation and ultrasound.^[42,45,46]

Hyperpolarization of the membrane occurs when microbubbles buzz near - edge layer of a cell^[47,48] that can stimulate the phagocytosis of macromolecules. This process in which insertion of macromolecules within the cell is frequently neglected in this research, but it is especially important in diagnostic and therapeutic applications by gene therapy involving smaller oligonucleotide or DNA molecules. Lentacker et al. studied the

absorption of lipoplexes in cultured cells after they were released from microbubbles during insonification in a new survey^[49]. Methyl β cyclodextrin which is an endocytic blocker was displayed to prevent endocytosis with cells. Conjugation of liposomes to the surface of microbubble and discharged by ultrasound within the medium, in any case, endocytosis was not the predominant mechanism of cellular entrance, as shown by the lack of impact of methyl β cyclodextrin on transfected stages.

Meijering et al.^[50] The reverse reaction has been found in current report that mainly decreases the potency of an ultrasound which induces macromolecule endocytosis. In the availability of microbubbles and ultrasound, fluorescently labelled dextran with less molecular mass around 4kDa as well as higher molecular mass around 400kDa were used to transfect cell grown under cell culture.

Lessened the reciprocity of dextran having lower molecular mass and no reciprocity of dextran with higher molecular mass by the use of caveolin and clatherin inhibitors as well as ATP diminution. It suggests that macromolecule internalisation is aided by cellular take-up via endocytosis. After dextran was integrated inside the cell, it migrated into the cytosol, at that point insonified with microbubbles, illustrated a transitory pore arrangement that happened that allowed inactive spillage of little particles. From this thought, as the molecular mass of the cargo enhances, the involvement of transient pore formation as the main method of entry decreases. This result appears to negate Lentacker's findings.^[49] In any case, the ultrasound intensity parameters utilised in this analysis and it's significant to note that they were less intense than the one mentioned by Lentacker et al.^[49] The functions of macromolecule absorption can be changed by the more intense cavitation.

The instruments of medicate take-up taking after discharge from the microbubble carrier have critical suggestions in the planning method of reasoning vectors for ultrasound activated medicate discharge. Several other drugs can spread across the bloodstream, but much more intense method of cell diffusion is needed in order to transmit their cargo by macromolecules. In circumstances where cavitation of disruptive microbubble is not needed, identifying an entrance where ultrasound having low intensity may be used to facilitate endocytotic action which can be a viable solution. Evidently, further more research is needed in this field.^[51]

Microbubbles exceptional potentiality to reciprocate to ultrasound intensity and to possibly lead to physiological reactions form them preferably suited for focusing on conveyance implementation. Over the final a few a long time, there have been various writing surveys that give a comprehensive outline on the subject of focused on drug/gene conveyance utilizing microbubbles.^[52,53-64] Whereas numerous thinks about have appeared effective location particular aggregation of a sedate, numerous of the microbubbles utilized in these thinks about are commercially accessible definitions that were created for differentiate picture improvement in echocardiography applications.

- 2. Using Microbubbles to improve AIDS:** The recently progressed strategies of non-invasive conveyance of restorative specialists are viable in quality treatment and atomic biology. The most useful implementation of microbubbles has been displayed a

successful strategy for focused on conveyance of drugs and genes and is additionally utilized as differentiate operators for demonstrative ultrasound^[65-70].

Microbubbles increment adherence to harmed vascular endothelium. As the viral proteins get a safe reaction inside target tissue the utilization of the viral vector is restricted in quality treatment. It has been seen that the viral vector causes an extreme swelling enactment of endothelial cells^[71].

By the administration of an ultrasound on the top layer of skin, microbubbles get burst which allows the medication to be discharged in a controlled manner^[72, 73, 74, 75]. Very less systemic drug concentration is mandatory in this procedure as a result improvement can be found in therapeutic ratios, and that is helpful in the context of medication that seem to have harmful severe side effects like those of cytotoxic agents^[76].

As to achieve maximum sound reflection, microbubbles produce acoustic obstruction among tissues and fluid substances. It is used to detect circulation of blood through tissues or to find fluids passing from space in such fields like cardiology and radiology. Microbubbles enhance the uptake of acoustic energy as well as the sound reflection^[77].

Extravasation i.e., leakage of fluid which irritates tissues, sort of spots in skeletal muscle capillaries are generated by using ultrasound with contrast media. Capillary vessels can be burst due to extremely intensive ultrasound thus inside the tissue some genetic materials and proteins are accumulated. For the transmission of colloidal parts to the muscles in a larger amount, just a tiny crack is enough^[78]. As a direct consequence of membrane defiance by cause of formation of pore, ultrasound enhances the current across the cell membrane^[79].

The carriers could then be disrupted by employing ultrasound cavitation also impacting on drug discharge. Sonodynamic is a therapy which is beneficial for the treatment of artery disease like the atherosclerotic plaques and also for tumour deletion. Targeting binding sites also known as ligands or coordination entity, were specifically designed to create targeted microbubbles that could be integrated inside the membrane to create a stable microbubble.^[80]

The targeted ligands are combines with the anchor inside the membrane, while the compiler provides sufficient space for the attachment of target with peptide-based targeting ligands. Platelet's receptor GP23B3A is enabled by peptides specifically thrombus peptides, these are tested for their potential to connect with platelets which are activated by prevention of platelet aggression.^[80]

An amphipathic fluid helps to stabilize the anterior layer. The main categories of the lipids have also been combined with bioconjugates. Cationic lipids help to keep the genetic element balanced. An electron dense particle is found inside the nanoparticles core is nothing but the compressed DNA, according to optical microscopy research. The broadness of these molecules is found approximately 100nm to 200nm^[81].

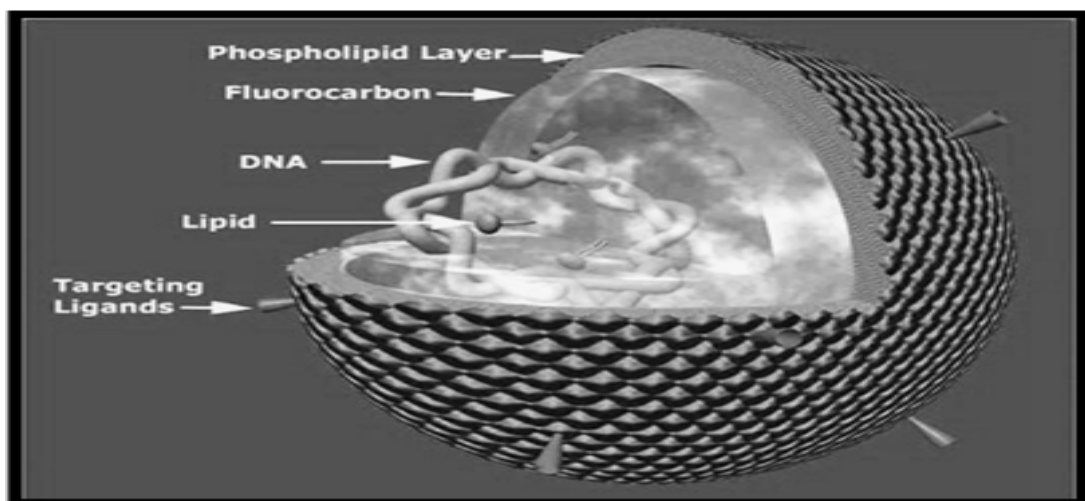


Figure 4: Microbubble as Gene Carrier

Lipid membranes have a lot of benefits. The hydrophobic acyl chains of the phospholipid resistance the gas, and the hydrophilic main groups resistance the water, when the space is reduced. As a result, a monolayer develops across a specially prepared bubble filled with gas. Underneath the ambient temperature, soluble diacyl phospholipids have a really poor surface pressure. Since surface tension at the circular layer creates a Laplace stresses, the centre of gas is compelled to dissolve. ^[82].

The single layer of lipid helps to balance microbubble at lower stress ^[83]. Due to the extreme enticing hydrophobic concentration among densely compacted acyl groups and van der waals force, mono layers of lipids have a solid like appearance and highly cohesive in character^[84]. These side effects may occur as, unlike proteins, the consistency of microbubbles throughout sonication isn't really reliant on formation of superoxide to promote disulphide bridging. As a result, lipids are ideal for different producing methods other than sonication, as currently defined by Stride and Endirisinghe^[85].

If the adenovirus was delivered through microbubble instead of ultrasound, the author reported how plasmid DNA transcription could be targeted to the heart with better accuracy than the tool called vectors, and that frequent therapies can control this expression ^[86]. While ultrasound was absent, microbubbles which are coated with an albumin substantially enhanced gene transcription in mouse skeletal muscle, according to Lu et al. ^[87]

X. DRAWBACKS OF MICROBUBBLES

A major drawback of microbubbles as medicate conveyance systems is their moderately huge estimate (1–10 μ m), This may be a problem for microbubbles trying to get through the vasculature's epithelial cells to the tissue which is been targeted. Microbubbles are administered into the bloodstream by injection and ultimately become stuck in the lungs. Hence, microbubbles are often restricted into tumour cell layered tissue and cardiovascular system. Nanobubbles and nanodroplets which dimensions are smaller than 1 μ m have been designed to eliminate this impediment. The majority of nanobubbles are generated by sonicating in the existence of a fluorinated gas material, for example perfluorocarbons or

sulphur hexafluoride. DNA Fragment, RNA interference, and coumarin have all been efficiently transmitted using this process. Fluorocarbon gas molecules can quickly vanish when exposed to the thermal gradients of ultrasound due to their lower vaporising point.

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